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British Pharmaceutical Conference 1971 Science Papers and Communications presented at the 108th meeting, Glasgow, September 13—17, 1971

Volume 23 Supplement December 1971

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British Pharmaceutical Conference 1971

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Journal of Pharmacy and Pharmacology

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At the British Pharmaceutical Conference held annually under the auspices of the Pharmaceutical Society of Great Britain, both full length papers and communications of not more than 500 words in the sciences related to pharmacy are presented for discussion. The 1972 Conference is being held at Keele University, North Staffordshire, U.K., September 11-15. The closing date for papers is February 1, 1972 and for communications, July 3, 1972. Full details are available from the Honorary Secretary, Conference Science Committee, 17 Bloomsbury Square, London WC1A 2NN.

Journal of Pharmacy and Pharmacology

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Science Papers

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Science Papers

A comparison of granules prepared by pan granulation and by massing and screening

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Granules of lactose and calcium phosphate were prepared by pan granulation and by massing and screening. Capillary forces and the gentle action of tumbling in the pan were inadequate to compact the fine cohesive calcium phosphate but were highly effective with the less cohesive lactose where the absence of shear gave very high densification. Whereas massing and screening provided the necessary forces for consolidating calcium phosphate, with lactose a more open structure resulted which further dilated during screening. Increase in both moisture content and time of mixing increased granule density but the degree varied with both method and material as did granule shape, strength and compressibility.

The manifold physical advantages of a large powder aggregate or granule over the component particles has led to the adoption of granulation by many industries. Development of these methods, empirical until 1958, has attracted little systematic study although important scientific contributions have been made in the field of fertilizers by Newitt & Conway-Jones (1958), Capes & Dankwerts (1965) and ores by Kapur & Fuerstenau (1964), using pan granulation. The method used, together with large granule size and high liquid–solid ratio employed, renders the application of findings to a granulation produced by massing and screening somewhat uncertain. Although granulation by this last method has been somewhat neglected Neff & Morris (1968) studied the effect of granulation conditions on the dispersibility, bulk density and porosity of 'instantized' dried milk and demonstrated the susceptibility of these properties to liquid–solid ratio. Fonner, Banker & Swarbrick (1966) prepared granules by five different methods but variation in the binder concentration from method to method reduced the value of this work as a comparative study.

Little information is available on the influence of time of mixing, type of mixer, solid–liquid ratio or particle properties on the properties of granules or the tablets made from the granules, although inferences are often drawn from the behaviour of powder–liquid systems in pans (Pilpel, 1969).

A comparison of the pan granulation process and the conventional pharmaceutical method, using simple powder–liquid systems is therefore a first step in establishing the relation between granulation theory and pharmaceutical practice. Restricting the investigation to variation in solid–liquid ratio and mixing time, an examination of both process and product is made below.

MATERIALS AND METHODS

Materials

Lactose B.P. (Grade 350 A. Whey Products Ltd. Mean particle size 20 μm) was granulated using water as binder over the range 15–34% v/v.*

Calcium phosphate B.P.C. (Tricalcium phosphate. Albright and Wilson Ltd. Mean particle size 4 μm) was granulated with 10% w/v dextrose monohydrate B.P. solution, the quantity of binder varying between 117–191% v/v.

Pan granulation

A hemispherical pan, 40 cm in diameter inclined at 30° to the horizontal and rotated at 32 rev/min, was used. A charge of 2 kg of powder was placed into the rotating pan and the binding liquid sprayed onto the powder surface, at 5 ml s^{-1}, by means of a small atomizer. The pan was closed with a Perspex lid and after the prescribed process time the wet granules were discharged and tray dried, for 4 h at 70°, in a hot air oven.

Granulation by massing and screening

One kg of powder was massed in a z-blade mixer (Duplex Model 00, Morton Machine Co. Ltd., Wishaw, Scotland) with blades operating at 65 and 103 rev/min. The binding liquid was added as a continuous stream and after the specified time of mixing, half the mass was discharged through an oscillating granulator (Jackson Crockatt Model 6) equipped with a 16 mesh screen and the granules tray dried at 70°. The other half of the mass was dried unscreened. Both batches were dry granulated using a 12 mesh screen.

Characterization of granules

Particle size analysis. The particle size distribution of the granules was established by sampling and sieve analysis (B.S. 410 test sieves).

Tapped density. The tapped density of −12 +16 mesh granules was measured according to Neumann (1967). The measuring tube, 38·5 mm in diameter, was filled with 100 g of granules and dropped 200 times through a height of one centimetre.

Intragranular porosity. The internal porosity of the −12 +16 mesh granules was measured by the pycnometric method of Strickland, Busse & Higuchi (1956), the volume of mercury in the pycnometer being measured at intrusion pressures between 25 and 120 cm Hg.

Granule strength. The resistance of granules to fracture was measured by placing a −14 +16 mesh granule between the platens of a miniature press. The lower platen was driven upwards at 0·08 mm s^{-1} and the reaction of the granule measured by a sensitive load cell attached to the fixed upper platen. Electrical output from the load cell was fed to an ultraviolet recording galvanometer to give a continuous record of the signal. The test was repeated 20 times for each batch.

* The percentage moisture content is expressed as

$$\text{M.C. (\% v/v)} = \frac{\text{Vol of liquid}}{\text{Vol of dry solid}} \times 100$$

This method of expressing moisture content enables direct comparison to be made between results for different materials.

Granule shape. The shape of the −12 +16 mesh granules was estimated by measuring the ratio of the square of the perimeter to the area of enlarged photographic images (150×) using an opisometer and planimeter. Division of this value into that obtained for a circle, 12·57, gave a shape factor which approached unity as the granules became more spherical in shape. Measurement was made on five granules from each batch.

Granule compressibility. One g of −12 +16 mesh granules was placed in a die 19·05 mm in diameter closed at the lower end by a spigot. The instrumented upper punch was inserted and the assembly compressed, porosity of the granule mass being recorded as a function of pressure.

RESULTS

The particle size distribution of granules of lactose and calcium phosphate is given in Fig. 1.

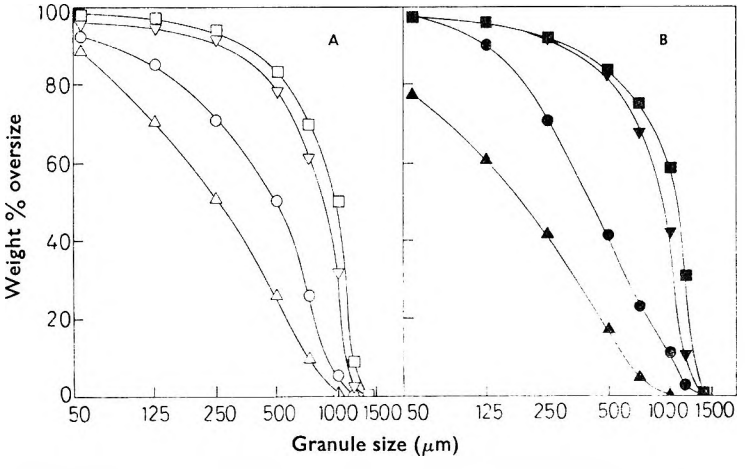

FIG. 1. Sieve analysis of granules prepared by massing and force screening. A. Lactose. Amount of binder added: △ 15·3% v/v, ○ 18·4% v/v, ▽ 23·0% v/v, □ 30·6% v/v. B. Calcium phosphate. Amount of binder added: ▲ 117% v/v, ● 125% v/v, ▼ 141% v/v, ■ 157% v/v.

Shape factors of typical massed and screened granules are compared to those of the equivalent pan granulated material in Table 1. Neither moisture content nor time of mixing had a significant effect on the shape factor.

Table 1. *Shape factors of granules prepared after* 60 *min processing.*

	Pan		Massed and screened	
	Shape factor	Standard deviation	Shape factor	Standard deviation
Calcium phosphate 157% v/v binder ..	0·93	0·04	0·78	0·05
Lactose 30·6% v/v binder ..	0·94	0·03	0·64	0·02

The intragranular porosity is derived from the weight and apparent volume of the granules in the pycnometer. The apparent volume of the material under test is the difference between the volume of the empty pycnometer and the measured

volume of mercury in the chamber with the granules present. As the external pressure is increased, mercury first fills the pores between the granules, completely enveloping the granules and then begins to enter the intragranular pores. The intragranular porosity is calculated from the volume before any internal pores are filled.

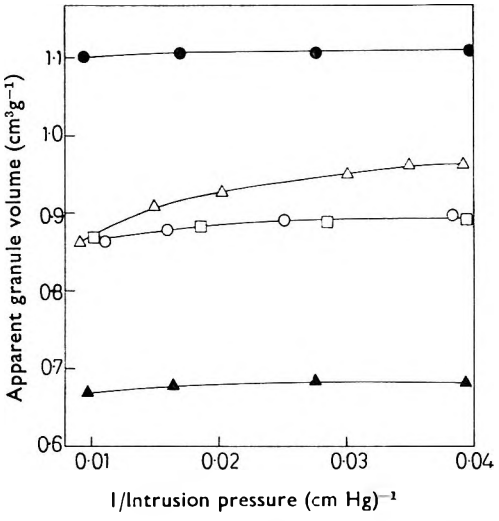

FIG. 2. Change in apparent volume of −12 +16 mesh granules during mercury penetration. Lactose, 30·6% v/v binder: ○ pan granulated, △ massed and screened, □ massed only. Calcium phosphate, 157% v/v binder: ● pan granulated, ▲ massed and screened.

Fig. 2 shows that there is no change in the apparent volume of granules of calcium phosphate, pan granulated lactose and lactose which had been massed but not screened, when the pressure on the enveloping mercury was increased from 25 to 100 cm Hg; this corresponds to a pore diameter range 56–14 μm. Virtually no pores are therefore present in this range and the distinction between intragranular and extragranular pores is therefore clear. With massed and screened lactose granules however, significant penetration occurred over this pressure range from which it can be calculated that 22% of the pores existed between 56 and 14 μm. The space which existed as voids greater than 50 μm was considered to be extragranular.

The total extragranular and intragranular porosities were derived from tap density and pycnometric measurements and are presented in Table 2.

A typical result of the strength test is given in Fig. 3. The test was considered complete at the last inflexion on the load curve, which corresponded to the last point of brittle failure. Little further work was then done on the plastic powder mass formed, the applied energy being almost completely transmitted to the load cell. The area under the curve represents the work done on the granules during crushing. Results for granules produced by the two methods of granulation are given in Table 3. The decrease in porosity when these granules are compressed in a die is given in Fig. 4.

Pan granulation

Increasing moisture content of calcium phosphate-dextrose solution mixes caused a slow but progressive increase in powder aggregation by layering; an increasing

Table 2. *Effect of moisture content on the porosities of* −12 +16 *mesh granules produced after* 10 *min processing.*

	Moisture content % v/v	Intragranular porosity %	Extragranular porosity %	Total porosity %
Pan				
Calcium phosphate	126	72·8	36·5	82·5
	157	69·7	40·0	82·0
	174	68·4	39·0	81·0
	191	56·8	material too large for test	
Lactose	23·0	32·6	39·0	58·0
	28·3	29·3	39·5	57·5
	30·6	27·1	41·0	57·0
	32·1	24·8	42·0	56·0
Massed and screened				
Calcium phosphate	117	51·6	45·0	74·5
	125	51·2	47·0	75·0
	157	51·1	42·5	71·5
Lactose	15·3	38·6	48·5	68·5
	18·4	37·6	49·5	68·5
	27·5	33·1	50·5	67·5
	30·6	32·0	50·0	66·0
	33·7	25·5	50·0	62·5

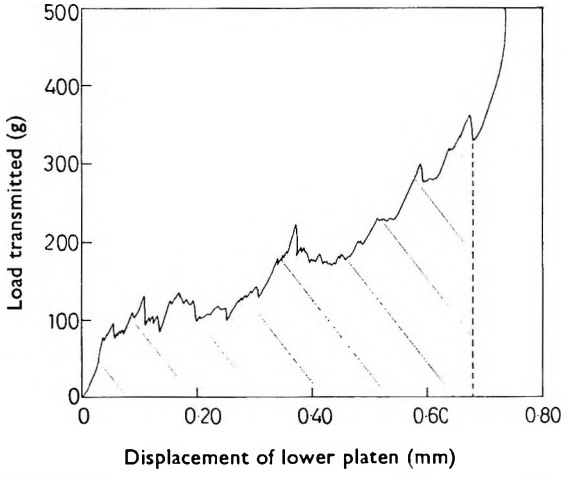

FIG. 3. Typical trace obtained from crushing a −14 +16 mesh granule. The shaded area is measured to calculate the work done.

Table 3. *Work done in crushing granules produced after* 10 *min processing.*

	Pan		Massed and screened	
	Work done J	s.d. J	Work done J	s.d. J
Calcium phosphate 157% v/v binder ..	$7·0 \times 10^{-5}$	$2·0 \times 10^{-5}$	$6·9 \times 10^{-4}$	$1·7 \times 10^{-4}$
Lactose 30·6% v/v binder ..	$5·1 \times 10^{-4}$	$2·1 \times 10^{-4}$	$7·1 \times 10^{-4}$	$1·6 \times 10^{-4}$

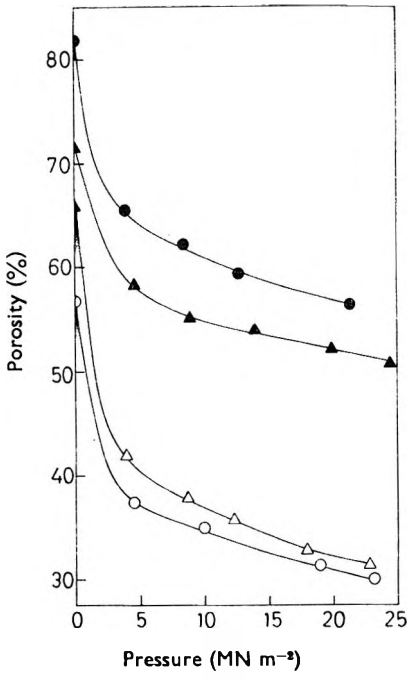

FIG. 4. The effect of pressure on the porosity of −12 +16 mesh granules. Lactose, 30·6% v/v binder: ○ pan granulated, △ massed and screened. Calcium phosphate, 157% v/v binder: ● pan granulated, ▲ massed and screened.

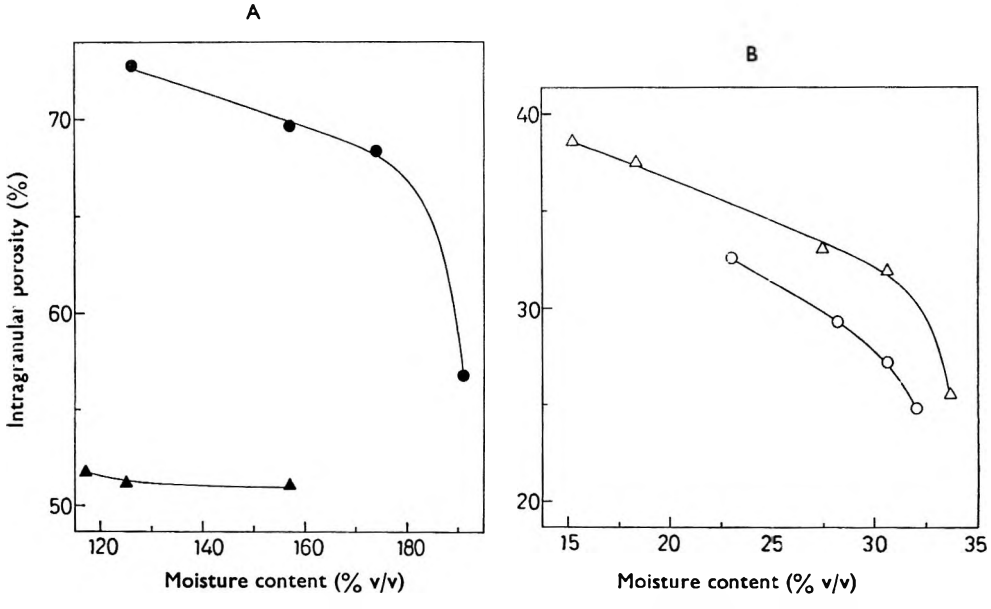

FIG. 5. A. The effect of moisture content on the porosity of −12 + 16 mesh calcium phosphate granules processed for 10 min. ● pan granulated, ▲ massed and screened.

B. The effect of moisture content on the porosity of −12 +16 mesh lactose granules processed for 10 min. ○ pan granulated, △ massed and screened.

percentage of the powder existing as balls with diameters greater than 1 mm until a moisture content of 174% v/v was reached. At this point very rapid ball growth suddenly occurred. This change in growth pattern only existed over a very narrow moisture content range (174–191% v/v). Above 191% v/v uncontrollable ball growth occurred.

Fig. 5A shows that the aggregation of powder over the moisture content range 126–174% v/v is accompanied by a slow increase in the packing density of the powder within the granule, the intragranular porosity falling from 72·8 to 68·4%. Conditions of rapid ball growth are also the conditions for sudden powder densification, the intragranular porosity falling from 68·4–56·8% when a further 17% v/v liquid was added.

Similar results were found with lactose. All the powder can be aggregated into granules by adding 8·5% v/v water. Controlled growth of these granules occurred provided the moisture content did not exceed 32% v/v. Above this level sudden ball growth occurred although Fig. 5B shows that the increased rate of densification was not as extensive as found with the calcium phosphate granules.

The effect of time on the density of the granules is shown in Fig. 6A. There is marked densification of the lactose over an hour in the pan and more gradual densification of the calcium phosphate.

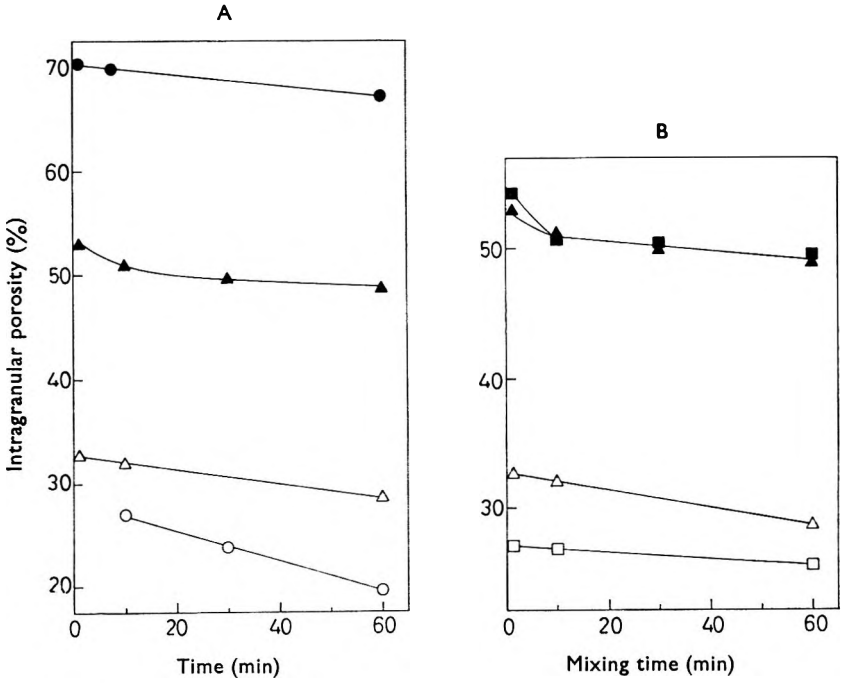

FIG. 6. A. The effect of time of processing on the porosity of −12 +16 mesh granules. Lactose, 30·6% v/v binder: ○ pan granulated, △ massed and screened. Calcium phosphate, 157% v/v binder: ● pan granulated, ▲ massed and screened.

B. The effect of wet screening on the porosity of −12 + 16 mesh granules. Lactose, 30·6% v/v binder: △ massed and screened, □ massed only. Calcium phosphate 157% v/v binder: ▲ massed and screened, ■ massed only.

Granulation by massing and screening

Granulation of calcium phosphate by this method produced suitable granules over a narrow moisture content range (117–157% v/v). Below this range the aggregates broke down to fines on screening and above it 'worms' were formed. Reference to Fig. 5A shows that within this range, moisture content had little effect on packing within the granules, the intragranular porosity in all conditions lying between 51 and 52%. Lactose, on the other hand, could be granulated over a wider range of moisture contents over which the intragranular porosity fell from 38·6 to 25·5% (Fig. 5B).

With both lactose and calcium phosphate, increase in moisture content led to the change in the particle size distribution given in Fig. 1 by elimination of the finer fractions. The limiting distribution reached with the wetter mixes was governed by the mesh used in wet screening.

Increase in the time of massing showed that the density of the wet mass increased over a period of 1 h. For lactose the porosity of the granules decreased from 32·5–28·5% (Fig. 6A).

Determination of the porosity of −12 +16 mesh granules formed with and without wet screening (Fig. 6B) showed that there was little effect on a wet mass of calcium phosphate although material massed for only 1 min was densified. The intragranular porosity of the lactose was increased.

DISCUSSION

A comparison of processes

A strict comparison of the data of pan granulation and granulation by massing and screening is not possible. This is because of the complex interaction of moisture content, the time for which the materials are in contact and the particular force system exerted on the mix during this time. Nevertheless, the following statements can be made.

Lactose

When water was added to lactose tumbling in a pan, granules typical of those used in tabletting processes were formed within the moisture range 15–30% v/v. Similar conditions were reported by Sherrington (1968) for agricultural granulation. A further increase in moisture causes very rapid ball growth and behaviour similar to that described for other materials by Newitt & Conway-Jones (1958), Kapur & Fuerstenau (1964) and Capes & Dankwerts (1965) is observed. The moisture range for ball growth is very limited because of the sudden fall in intragranular porosity. For any given moisture content, this densification will cause a larger proportion of the pores within the granules to become filled. Ultimately all voids become saturated, water appears on the surface of the balls and uncontrollable aggregation occurs These limiting conditions which occurred after 10 min tumbling when 32·1% v/v of water was added, gave dried aggregates with an intragranular porosity of 24·8%. Depending on the amount of lactose dissolved during tumbling and redeposited on drying, this porosity indicated that between 98 and 99% of the intragranular void space had been filled with liquid before drying.

Increased time of tumbling also causes densification of the mass and an increase in the fraction of the intragranular pores filled with liquid. This was most clearly

demonstrated when 31% v/v water was added. Granules were first formed but after 50 min tumbling saturation of the mass occurred and large balls were formed.

In the pan, the forces producing densification are capillary forces and the gentle repacking of particles due to rolling. Increased moisture content decreases the intragranular porosity because the capillary forces increase. Eventually an extremely close packed system is produced (intragranular porosity 20·1%) for material tumbled for 1 h This is far closer than any form of dry packing (tapped porosity 45%). Massing and screening, on the other hand, gave granules of higher intragranular porosity (Fig. 5B). The z-blade mixer employs a force system with a pronounced element of shear. In such a system, the deformation of closely packed aggregates must occur by making them less dense. The granules thus formed allowed slightly higher moisture contents to be tolerated before the mass became saturated. The additional shear encountered by the granules during screening causes further loss of density of the particles within the granules and as shown in Fig. 2 the pore size distribution within the granules is significantly widened over the range studied.

Calcium phosphate

Calcium phosphate is a very fine cohesive powder with a tapped porosity of almost 80%. It required much more liquid for granulation than lactose, and granules formed by tumbling had porosities of 68·4–72·8%. At this stage, the capillary forces appear to be far less effective in consolidating calcium phosphate than the coarser, less cohesive lactose. Even after the sudden densification which occurred when the moisture content exceeded 174% v/v, the limiting intragranular porosity was more than double that found for lactose.

When calcium phosphate was massed in the z-blade mixer and screened, the intragranular porosity of the dried granules was unaffected by change of moisture content.

Provided there was a mobile liquid layer to give capillary cohesion to the mass, mechanical agitation appeared to determine the degree of consolidation and much higher densification was obtained than in the pan. This closer packing limited the moisture content range over which granules could be successfully formed. This is in contrast to the behaviour of lactose where closest packing, and hence restriction of the range in moisture content, was found in the pan. Packing was not close enough, however, to produce the fall in density during additional shear, and screening had little effect on the porosity of the wet mass.

Granule shape, strength and compressibility

Pan granulated materials were almost spherical with shape factors approaching unity. Massed and screened lactose granules were irregular in shape, the calcium phosphate being much smoother with a higher shape factor. The almost spherical shape of the pan granulated material led to dense packing with extragranular porosities of 36–42%. Massed and screened lactose on the other hand packed with an extragranular porosity of 50%.

Massed and screened granules of calcium phosphate were much stronger than the equivalent pan granules. The strength of these aggregates is derived from the dextrose bonds formed at points of contact within the granule. The more dense massed and screened granules (porosity 51%) will have many more point contacts per unit area than the pan granules (porosity 70%).

Although pan granulated lactose was slightly more dense than granules that had been massed and screened, there was little difference in their strengths. Other factors, such as the rate of solution, may affect the strength of the bond formed by recrystallization.

The strength of the granules appears to play no part in the compressibility, and variation in this property is related to the amount of densification achieved in the granulation process. Thus the close packed granules of calcium phosphate formed by massing and screening are highly compressible, the component particles having already been moved to close juxtaposition so that compaction in the region investigated is largely the rearrangement of extragranular space by fragmentation—a relatively easy process. With pan granules, individual particles are less closely packed and more work must be done to achieve a compact of given porosity.

With lactose similar but less pronounced effects are found because intragranular porosities of the granules produced by the two methods differ by only 5%.

Acknowledgement

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REFERENCES

CAPES, C. E. & DANKWERTS, G. C. (1965). *Trans. Instn chem. Engrs*, **43**, T116–124.

FONNER, D. E., BANKER, G. S. & SWARBRICK, J. (1966). *J. pharm. Sci.*, **55**, 181–186.

KAPUR, P. C. & FUERSTENAU, D. W. (1964). *Trans. Am. Inst. Min. metall. Petrol. Engrs*, **229**, 348–355.

NEFF, E. & MORRIS, H. A. L. (1968). *J. Dairy Sci.*, **51**, 330–338.

NEUMANN, B. S. (1967). *Advances in Pharmaceutical Sciences*, Vol. 2, pp. 181–221. Editors: Bean, H. S., Beckett, A. H. & Carless, J. E. London and New York: Academic Press.

NEWITT, D. M. & CONWAY-JONES, J. M. (1958). *Trans. Instn chem. Engrs*, **36**, 422–442.

PILPEL, N. (1969). *Chem. Process Engng*, **50**, 67–72.

SHERRINGTON, P. J. (1968). *Chem. Engr, Lond.*, **46**, CE201–214.

STRICKLAND, W. A., BUSSE, L. W. & HIGUCHI, T. (1956). *J. Am. pharm. Ass. (Sci. Edn)*, **42**, 482–486.

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Solute migration during granule drying

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Spherical granules of magnesium carbonate, 12 mm diameter, have been dried under controlled conditions in a drying tunnel at 44°, the rate of moisture loss being continuously recorded. The granulating agent was polyvinylpyrrolidone (PVP). Granules were removed at various times for analysis. By controlled attrition, successive layers of powder were removed from the granules. The powder was analysed for PVP by infrared spectroscopy, and the friability, modulus of elasticity and Brinell hardness of the granules were measured as a function of depth from the granule surface. The radial distribution of the PVP and its physical effect on the granule properties were thus found.

The effective preparation of pharmaceutical tablets depends upon the material to be compressed being in a free-flowing form. The usual method of achieving this is to wet-granulate the powder and to dry the granules. Although the process of wet granulation is intended to produce granules of a uniform composition, it is well known that under certain drying conditions the outer layers may have a different composition from the central portion. In particular, any binder included in the mass may form a crust around the granule periphery and the resultant granule, during subsequent tabletting, may deform differently from a granule with a uniform distribution. A tablet produced from granules in which the binder is concentrated in the outside layers of the granules is liable to have localized areas of high cohesion, thus resisting subsequent disintegration and dissolution. Any accidental attrition of non-uniform granules will also give fines which have a different composition from the main bulk; the non-uniformity can, in some circumstances, extend to the active constituent.

The drying rate of a surface-dried particulate material has been shown to exhibit a number of distinct drying periods (Fisher, 1932). The material dries at a constant rate until a definite moisture concentration, the critical point, is reached, when a decrease in the drying rate occurs. The first falling rate period then ensues, the drying rate decreasing in a linear manner. In the second falling rate period the decrease in rate is non-linear and is usually an asymptotic, exponential approach to zero moisture content with respect to time.

Ridgway & Callow (1967) have shown that, in the falling rate periods, a linear relation exists between the logarithm of the drying rate and the liquid content. Drying rates have also been shown to be modified by the presence of solutes. Krevelen & Hoftijzer (1949) dried single granules of nitrochalk, and found that, during the constant rate period, the dissolved ammonium nitrate formed an impermeable crystalline film around the granule, so reducing the rate of drying.

Newitt & Papadopoulos (1959) examined the drying of fertilizer granules, and also of sand containing aqueous sodium chloride solution. They found that solutes were transported to the outside of the granule forming a crust, but they made no

quantitative analysis of the resultant solute distribution. Higuchi & Kuromoto (1954) showed that polyvinylpyrrolidone (PVP), which is used as a binder for tablet granules was able to form complexes with many pharmaceuticals, and more recently (Higuchi, Simonelli & Mehta, 1969) found that the rate of release of sulphathiazole from compressed tablets containing PVP was increased if the drug had been previously co-precipitated with PVP. It thus seems possible that the non-uniformity of distribution of at least some drugs in granules may parallel the non-uniform distribution of PVP.

METHODS

The powder used was heavy magnesium carbonate B.P. [$3MgCO_3,Mg(OH)_2.4H_2O$] (BDH Ltd.). The mean particle size was 20 μm, with 95% by weight being larger than 10 μm and 5% larger than 32 μm. Granules were made by mixing 290 g of powder with 170 ml of a solution containing 15 g of PVP (BDH Ltd.). The wet mass was passed through an 8 mesh sieve, remixed and repassed through the sieve. It was then rotated in an 11 inch diameter coating pan at 30 rev/min, 30° to the horizontal, to produce spherical granules. 25 of these, of the required size of 12 mm were selected for an experimental run.

The selected granules were placed in two concentric circles on a nylon platform, which was hung from the pan of an automatic recording balance in a drying tunnel (Ridgway & Callow, 1967), through which air at 44° was blown at a controlled velocity. After 30 min, 5 granules were taken from the tunnel, plunged into liquid nitrogen, and then dried in a laboratory freeze drier for 12 h. This procedure prevented any further migration of solute within the granule. Further groups of 5 granules were removed from the tunnel after 1½, 3, 4, and 24 h.

The indentation hardness and elastic recovery of the outer surface of the granules were measured with the ICI pneumatic micro-indentation apparatus and technique described by Ridgway, Aulton & Rosser (1970). The granules were then separately treated in the multiple attrition cell apparatus (Fig. 1) to remove their outer layers. Each granule is placed in one of five cylindrical chambers, and rests inside a stainless steel gauze cage. The cage has a central brass ring soldered to it. The ring rests on the inner flange of a duralumin canister, so that the cage can be both closed and held in position within the canister by means of a screwed rubber-lined lid and a spacer tube. The five duralumin canisters are held in a brass plate which replaces the sieve in a 4 inch deep 8 inch diameter brass cylinder suitable for clamping onto a Finex sifter (Russell Construction Co).

The granules are shaken for a measured time interval; they retain their spherical shape but are gradually worn away. The powder thus attrited from the granule is collected, weighed and analysed for PVP by infrared spectroscopy (Ridgway & Rubenstein, 1971). The core weight is recorded, the diameter is measured and the Brinell hardness number and elastic modulus are determined. The process is then repeated until the intact core weight is reduced to about 10% of the initial weight.

RESULTS AND DISCUSSION

The drying curve for 25 granules dried at 44° is shown logarithmically in Fig. 2. Three stages of drying can be seen. Initially the drying rate falls linearly to the critical moisture content. A further linear fall in rate occurs after 1½ h drying, and finally, there is a third fall to the equilibrium moisture content.

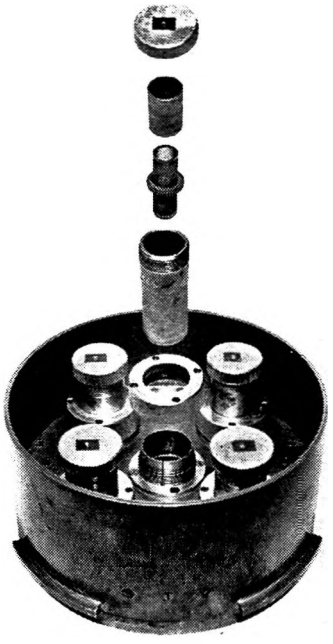

FIG. 1. The multiple attrition cell apparatus. Up to five granules can be held in closed gauze baskets, held inside the duralumin containers which are mounted in a brass plate and sieve surround so that the entire assembly can be vibrated on a sieve shaker. In the exploded view shown, the parts are, from the top downwards, the screwed rubber-lined lid, the spacer tube, the gauze cage, the canister itself, and the locking ring.

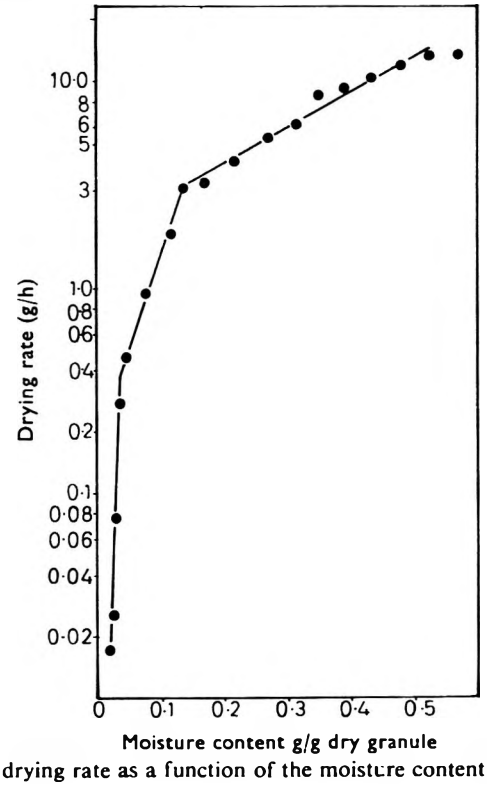

FIG. 2. The drying rate as a function of the moisture content of the granules.

In the initial stages of drying, PVP migrates to the surface of the granule. It is at the air-solid interface that evaporation takes place, with the result that PVP is deposited at the granule surface forming a crust. This deposited PVP blocks the interstices between the magnesium carbonate particles, thereby reducing the surface area available for evaporation and limiting further solution being drawn to the surface by capillarity. Thus a reduction in drying rate occurs and this accounts for the initial fall in drying rate to the critical moisture content (as distinct from the normal constant rate drying period which is found for pure liquids evaporating from beds of powder). The decline in drying rate continues for about 1½ h, when the critical moisture content is reached, and there is a sudden change in slope of the logarithmic drying rate line. At this point the surface of the granule is no longer wet enough to act as a free water surface.

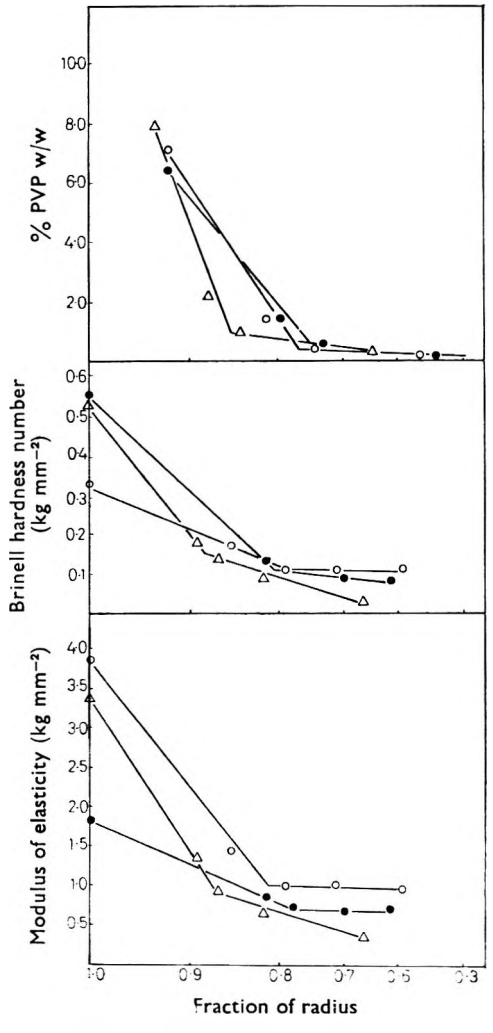

FIG. 3. Variation of % w/w PVP, Brinell hardness number and Young's modulus of elasticity with depth from the granule surface. △ after ½ h drying. ○ after 3 h drying. ● after 4 h drying.

The surface menisci then recede into the surface voids, with a linear fall of drying rate during the period from $1\frac{1}{2}$ to $3\frac{1}{2}$ h. This period will end when all the surface menisci have receded into the voids and the surface will effectively be dry. The final falling rate period occurs after $3\frac{1}{2}$ h drying. Since the surface is now dry the evaporative interface recedes towards the centre of the granule. Removal of water is by internal vaporization; vapour diffusion to the surface is the limiting factor.

The PVP distribution, Brinell hardness number and Young's modulus of elasticity against the radial distance is shown in Fig. 3 for three groups of granules dried for $\frac{1}{2}$, 3 and 4 h. The outer layers of the granules have a relatively high PVP content and their Brinell hardness number and Young's moduli are correspondingly high. Towards the middle of the granule the PVP content, Brinell hardness number and Young's modulus decrease linearly in value. A point is reached where no further decrease is observed and the three parameters remain constant. This point gives a measure of the crust thickness.

Fig. 4 shows how the PVP content, Brinell hardness number and Young's modulus at the surface and in the core of the granules, vary with decreasing moisture content. The top graph of PVP concentration against moisture content represents the total concentration of PVP: this is the sum of that deposited as well as that in solution. During the first $\frac{1}{2}$ h drying, the PVP concentration in the surface layer rises well above, and that in the core declines below, the initial uniform concentration (Fig. 4). Since there is now a greater concentration of PVP in the outer layers relative to the core, PVP must be migrating and concentrating in the outer surface of the granules. Subsequently, there appears to be a steady fall in the concentration of PVP in the surface layers as drying proceeds up to a point representing the end of the second falling rate period. The core concentration of PVP increases slightly over this period. This can be explained by considering that "back-diffusion" occurs. This is the diffusion of PVP from a point of higher concentration in the outer surface of the granule to a point of lower PVP concentration in the core. The movement of PVP is thus governed by evaporative and diffusional transport. In the initial stages of drying the evaporative rate is high and back-diffusion, although occurring, is negligible. As PVP is deposited and the drying rate is reduced, back diffusion of PVP in solution becomes more important; PVP moves under a concentration gradient into the interior of the granule from the surface layers—thus no more PVP will be deposited in the outer surface. Since PVP is soluble to the extent of more than 50% w/w, once a crust has been built up and the evaporative rate reduced, it is unlikely that more PVP will be deposited. In addition, the concentration gradient causing back-diffusion will also be large. Thus in Fig. 4 the graph of % w/w PVP in the core of the granule against moisture content shows a steady increase in concentration, due to back diffusion, until the end of the second falling rate period. The sudden rise in concentration during the third falling rate period is due to the deposition of PVP at the receding evaporative interface. Since that part of the granule between the outer surface and the receding evaporative interface is dry during the final falling rate period, diffusion of PVP cannot occur in this region, and it can be seen from Fig. 4 that the surface concentration of PVP does, in fact, remain constant during this final drying period.

Fig. 5 depicts the variation of crust thickness with moisture content. The thickness of the crust increases from 0·14 r to 0·22 r, where r is the radius of the granule, as PVP is deposited in the first falling rate period. After the end of the first drying

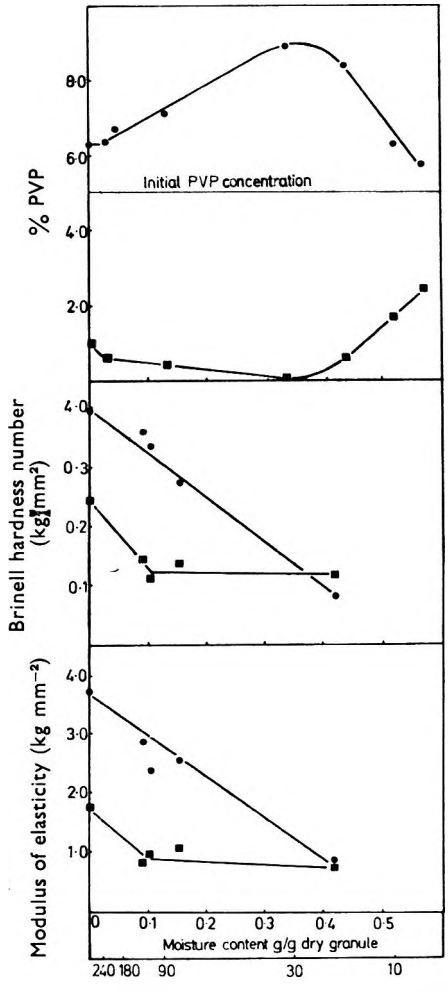

FIG. 4. The variation of PVP content, Brinell hardness and modulus of elasticity with granule moisture content. ● at the surface of the granules. ■ in the core of the granules.

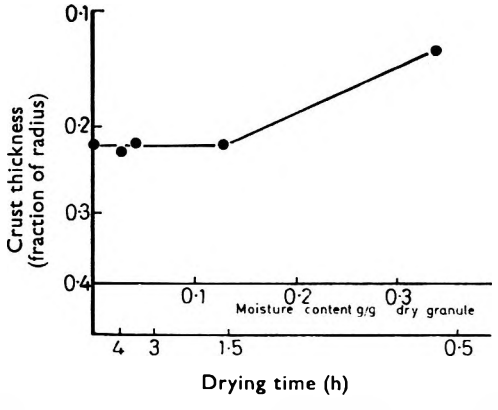

FIG. 5. Variation of crust thickness with drying time.

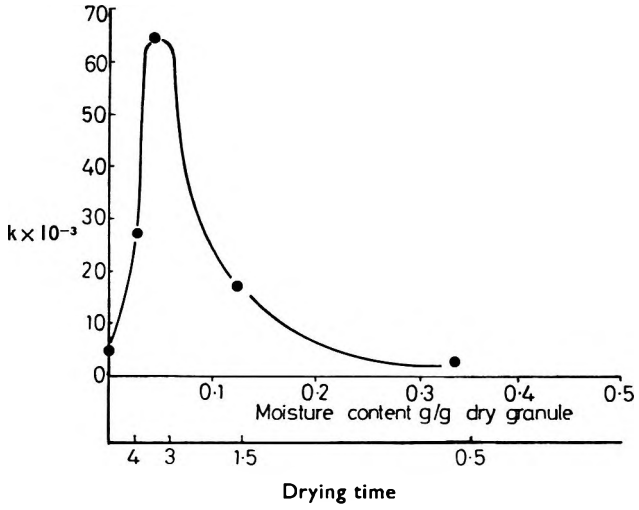

FIG. 6. The effect of moisture content on the granule friability. k is the gradient of the straight line representing the attrition time against the logarithm of the percentage weight intact, for granules shaken in the multiple attrition cell apparatus.

period no more PVP is deposited, since by the process of back diffusion PVP now moves to the central core of the granule. Therefore, the thickness of the crust after the critical moisture content should remain constant and it did.

For each granule a plot of attrition time against the logarithm of the percentage weight intact was determined for each granule shaken in the multiple attrition cell apparatus. These graphs produced straight lines and the average slope of the lines within each group was calculated. This gradient, k, was plotted against the corresponding moisture content to produce Fig. 6. The value of k is a measure of friability: as it increases, a granular solid becomes more friable, and vice versa. Initially the granules are wet and the solid particles are held together by liquid surface tension forces. Thus, the granules will be appreciably resistant to attrition. As drying proceeds, the resistance to attrition is reduced and the graph of k reaches a peak at the end of the second falling rate period, when the surface is just dry and the solid particles are held together by pendular liquid rings. After this point the surface becomes totally dry and as the interior of the granule becomes dry during the final falling rate period, the binding effect of PVP increases the attrition resistance and the k value falls away accordingly.

REFERENCES

FISHER, E. A. (1923). *Proc. Roy. Soc.*, **103A**, 139–161; 664–675.

HIGUCHI, T. & KUROMOTO, R. (1954). *J. Am. pharm. Soc.*, **43**, 393.

HIGUCHI, W. I., SIMONELLI, A. P. & MEHTA, S. C. (1969). *J. pharm. Sci.*, **58**, 538.

KREVELEN, D. W. VAN & HOFTIJZER, P. J. (1949). *J. Soc. Chem. Ind.*, **68**, 59–66.

NEWITT, D. M. & PAPADOPOULOS, A. L. (1959). *Proc. Fertil. Soc.*, **55**, 355.

RIDGWAY, K., AULTON, M. E. & ROSSER, P. H. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 70S 78S.

RIDGWAY, K. & CALLOW, J. A. B. (1967). *Ibid.*, **19**, *Suppl.*, 155S.

RIDGWAY, K. & RUBINSTEIN, M. H. (1971). *Ibid.*, **23**, 587–589.

J. Pharm. Pharmac., 1971, **23**, *Suppl.*, 18S–24S *Received January* 29, 1971

The effect of starch type, concentration and distribution on the penetration and disruption of tablets by water

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The effect of starch type, and its concentration and distribution, on the pore structure of tablets of aspirin and magnesium carbonate has been measured using air permeability and liquid penetration techniques. The addition of starch had no significant effect on the pore structure of the dry tablet but caused disruption and alteration of this structure when penetrated by water. When each starch was incorporated into the granules in wet massing the rate of disruption decreased in the order potato, maranta, wheat, corn, waxy corn and rice; but a more complicated pattern was produced when the starch was added to the granules as a pre-dried powder. Maximum break-up efficiency of magnesium carbonate tablets was produced when 10% potato starch was incorporated internally and the tablet compacted to a porosity of 28%.

The physical and chemical properties of starch, its components and derivatives, have been extensively studied (Hellman, Boesch & Melvin, 1952; Hofstee, 1953; Samec, 1953; Nemitz, 1962; Schierbaum & Taeufel, 1963; Montgomery, Sexson & others, 1964; Goudah & Guth, 1965; Shotton & Harb, 1965). The unique characteristics of these materials have led to their use in tabletting as binders, glidants, lubricants and distintegrants, although their most important role is that of tablet disintegrants.

Starch may be incorporated as a granulating agent (binder) either in the form of a paste or as a dry binder in direct compression (Kwan & Milosovich, 1966). It can be added to granules as a pre-dried powder (Higuchi, Elowe & Busse, 1954) and this will be referred to as "external" starch. Added thus its distribution in the final tablet is uneven and is controlled by the configuration of the granules during packing and compression. The incorporation of starch powder into the granules during the wet massing process—"internal" starch—is the most common method and presents an even distribution of starch throughout the tablet. However, in some tablet formulations a percentage of starch, added as disintegrant, is incorporated before massing and the remainder added after granulation. Nair & Bhatia (1957) have suggested that the external starch would break the compressed tablets, on disintegration, into granules and the internal starch would reduce these into particles with a size distribution similar to that of the original powder blend. Added concentrations of starch may vary from 5–20%, and, from disintegration studies, several authors agree that there is an optimum concentration of starch that can be added to a formulation to make disintegration efficient (Fakouhi, Billups & Sager, 1963; Bergman & Bandelin, 1965; Commons, Bergen & Walker, 1968; Nogami, Nagai & others, 1969). This optimum concentration is dependent on the physical properties of the

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tablet constituents. It therefore appears that distribution and concentration are essential criteria when stating the efficiency of starch as a tablet disintegrant.

Starches from different botanical sources have been used as disintegrants and because of its availability and cost, the most popular is corn (maize) starch. Properties such as vapour sorption and swelling have been studied, although these cannot be related to disintegrating efficiency (Billups & Cooper, 1964). Some starches such as potato, wheat, rice, yam, cassava and moriyo have been examined along with ultra amylopectin, amylose and gelatinized starch (Horsch, Martin & Roppert, 1958; Patel & Chikhlia, 1963; Saber, Rahman & others, 1963; Krebs, 1959; Patel & Paucholi, 1964; Mital & Ocran, 1968). In these comparative studies variations in disintegrating efficiency have variously been ascribed to amylose-amylopectin ratio, grain size, moisture absorption properties and to differences in fat content.

Although many starches have been examined as disintegrants, no conclusive agreement has been reached on their mechanism of action. Some authors (Fakouhi, Billups & Sager, 1963; Billups & Cooper, 1964; Patel & Hopponen, 1966) consider that swelling of the starch grains disrupts the tablet. Others (Curlin, 1955; Manudhane, Contractor & others 1969; Borzunov & Shevchenko, 1967; Ingram & Lowenthal, 1968) attribute the effect to improved capillarity of the starch-containing powder matrix. This inconclusive situation is partly due to the lack of control of the powder system as the starch type, concentration and distribution were varied. In this paper, these variables are allowed to affect powder aggregates prepared under closely controlled conditions.

MATERIALS AND METHODS

Materials and granulation

The starches chosen were potato, rice, wheat, maranta (arrowroot), corn (maize) and waxy corn. Some properties of these starches are given in Table 1. Their grain size ranges were measured microscopically (mean of n grains).

Table 1. *Some properties of various starches.*

Starch	Amylose content (%)	Grain size range (μm)	Grain shape
Potato	20	15–100	Egg-shaped
Rice	16	3–8	Polygonal
Maranta	20	25–50	Egg-shaped
Corn	24	5–25	Round
Wheat	22	2–35	Elliptical
Waxy corn	0	5–25	Round

Heavy magnesium carbonate and aspirin were used as granules and these were prepared by wet massing with 10% acacia solution and absolute ethanol respectively. Heavy magnesium carbonate granulations were prepared with 2, 5, 10 and 20% potato starch internally and 5% internally + 5% externally. It was also used in formulations to which each of the starches (10%) was added internally and in others to which the starches were added externally. Aspirin granulations were prepared with each of the starches (10%) internally.

All granular material was screened and the fraction passing a 1000 μm (16 mesh) sieve but retained on a 710 μm (22 mesh) sieve was selected for study.

Characterization of tablets

The compressibility, permeability and water penetration of the tablets were measured according to Ganderton & Selkirk (1970) and Ganderton & Fraser (1970). The time of disintegration was measured by the method of the British Pharmacopoeia 1968 using individual tablets.

RESULTS AND DISCUSSION

The physical properties of starches

Of the various samples studied, potato starch had the largest grain size and rice the smallest. The compressibility of the starch powders did not differ *greatly* with source although potato and waxy corn starches were most compressible giving compacts with a porosity of 11–12% when compressed at 100 MNm^{-2}. Other starches compacted to 15–17% porosity under these conditions. Fig. 1 shows that increasing grain size gave compacts of more open structure and higher permeability.

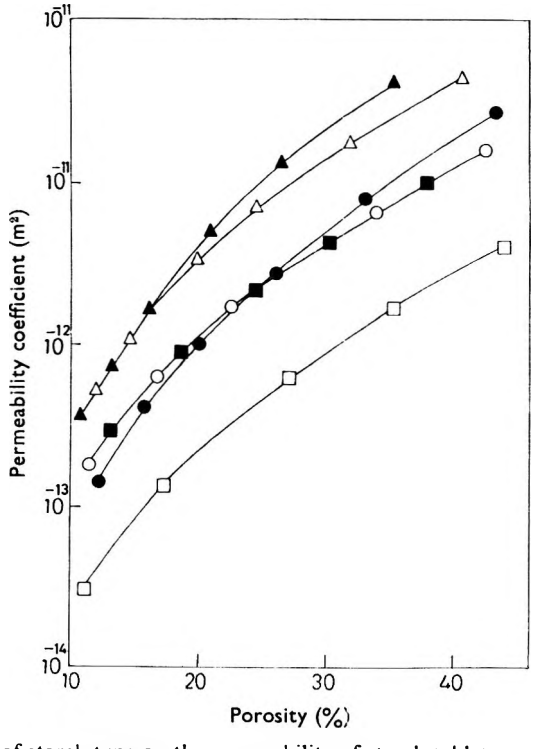

FIG. 1. The effect of starch type on the permeability of starch tablets. ▲ Potato. △ Maranta. ● Wheat. ○ Corn. ■ Waxy corn. □ Rice.

The porosity and permeability of tablets containing starch

Table 2 shows that the effect of starch on the porosity and permeability of tablets, though small, is largely additive. When 10% starch is added to fine, incompressible

Table 2. *The effect of starch type and distribution on the permeabilities and porosities of tablets compressed to* 100·0 MNm^{-2}.

Material	Mode of incorporation and starch type (10% added)	Porosity (%)	Permeability Coefficient (m^2)
Magnesium carbonate		36·4	$1·9 \times 10^{-13}$
Magnesium carbonate:	with internal potato	33·4	$2·6 \times 10^{-13}$
	" external "	33·8	$2·4 \times 10^{-13}$
	" internal wheat	34·8	$3·6 \times 10^{-13}$
	" external "	34·4	$2·5 \times 10^{-13}$
	" internal rice	34·8	$2·2 \times 10^{-13}$
	" external "	33·8	$2·0 \times 10^{-13}$
	" internal corn	35·6	$2·2 \times 10^{-13}$
	" external "	34·3	$2·3 \times 10^{-13}$
	" internal waxy corn	34·4	$2·6 \times 10^{-13}$
	" external " "	34·6	$2·8 \times 10^{-13}$
	" internal maranta	35·4	$2·3 \times 10^{-13}$
	" external "	35·3	$5·0 \times 10^{-13}$
Aspirin		6·0	$6·6 \times 10^{-15}$
Aspirin:	with internal potato	6·6	$1·1 \times 10^{-14}$
	" " wheat	6·0	$9·7 \times 10^{-15}$
	" " rice	6·4	$1·3 \times 10^{-14}$
	" " corn	6·0	$1·5 \times 10^{-14}$
	" " waxy corn	5·7	$9·5 \times 10^{-16}$
	" " maranta	5·2	$9·5 \times 10^{-16}$

magnesium carbonate, a tablet of higher permeability but lower porosity is produced. When the starch was added to compressible aspirin, the changes were even smaller. With neither material were statistically significant effects found that could be attributed to starch type or distribution. No structural changes which would greatly affect the penetration of a tablet by water are therefore apparent.

Aqueous penetration studies

The rate of uptake of water into compacts of pure starch was so fast that it could not be measured adequately. The effect of starch type on the pattern of water penetration into tablets containing starches of different botanical source is shown in Fig. 2. In the early stages of penetration, starch enhances penetration in all cases except rice, the intensity of this effect increasing with starch grain size. There follows a sudden increase in penetration rate that indicates severe disruption of the wetted portion of the tablet and the decline of the viscous resistance to entry. Since the uptake rate is the same for all materials, this phase of the destruction of the tablet is independent of starch type. However, the overall rate of disruption decreases in the sequence potato, maranta, wheat, corn (maize), waxy corn, rice, and this is also shown in the results of the disintegration test (Table 4).

The effect of starch distribution

The pattern of penetration of tablets containing external starch was similar to tablets containing internal starch. Table 3 shows, however, that although potato gave the fastest penetration and rice the slowest, the progressive effect of grain size is not followed: a similarly confused situation is found in the disintegration test (Table 4). Thus, simple comparison of the two methods of distribution is not

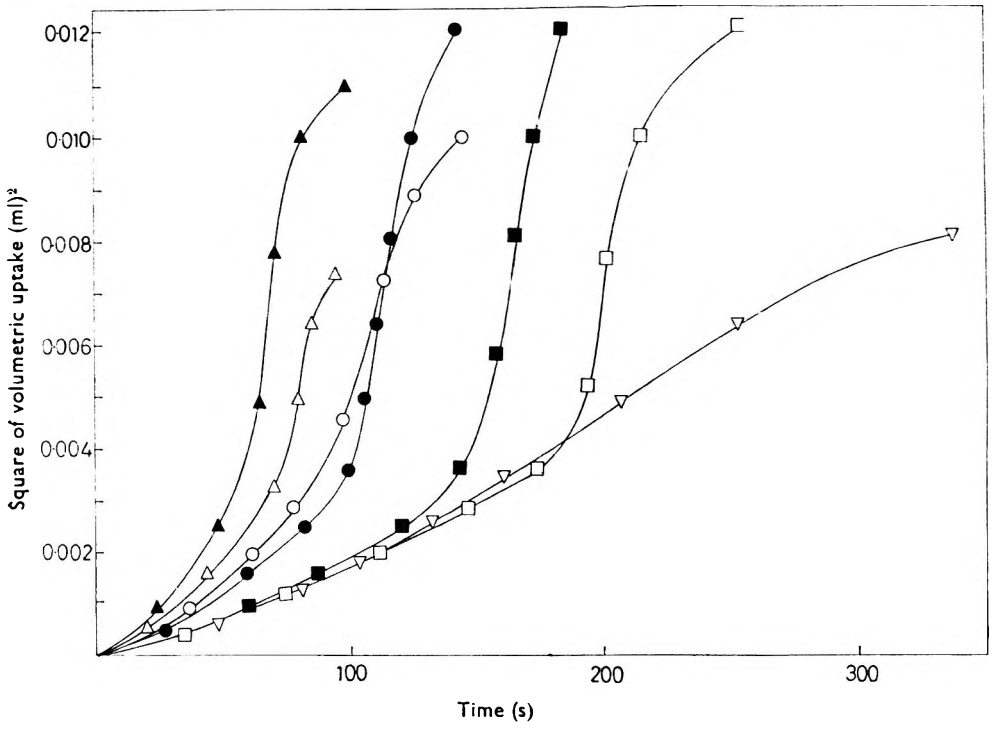

FIG. 2. The effect of starch type on the aqueous penetration of magnesium carbonate granules containing 10% internal starch compressed at 100 MNm^{-2}. ▲ Potato. △ Maranta. ● Wheat. ○ Corn. ■ Waxy corn. □ Rice. ▽ No starch.

Table 3. *Effect of starch type and distribution on the time for penetration of* 0·08*ml water into tablets compressed at* 100·0 MNm^{-2}. (*Figures are the means of* 8 *results.*)

Material	Time (s) Internal starch	External starch
Magnesium carbonate with 10% potato starch	67·5[1]	82·0[2]
" " 10% maranta starch	85·0	121·0
" " 10% wheat starch	111·0	133·8
" " 10% corn starch	112·0	98·6
" " 10% waxy corn starch	159·0	120·6
" " 10% rice starch	197·5	157·3
Magnesium carbonate with no starch		445·0[4]
Magnesium carbonate with 5% internal and 5% external potato starch		108·9[3]

1. s.d. 2·13, coefficient of variance (%) 3·2.
2. " 30·8 " " 37·6.
3. " 22·5 " " 20·7.
4. " 37·5 " " 8·4.

possible although in most cases *internal* distribution gives tablets that are penetrated and disrupted more quickly.

A clear difference in the consistency of disruption was, however, found. Table 3 also gives the variation in the time required for the uptake of 0·08 ml of water. The coefficient of variance for tablets containing external starch was over ten times that of a tablet containing internal starch. Tablets in which half the starch was

Table 4. *The effect of starch type and distribution on the disintegration time of tablets compressed at* 100·0 MNm^{-2}. *(Figures are the means of* 8 *results.)*

Material	Disintegration time (s) Internal	External
Magnesium carbonate with 10% potato starch	13·9	31·5
" " maranta starch	17·2	36·6
" " wheat starch	24·6	40·9
" " corn starch	29·5	32·9
" " waxy corn starch	34·0	43·1
" " rice starch	47·6	40·4
Magnesium carbonate with no starch		63·2
Magnesium carbonate with 5% internal and 5% external potato starch		43·6

added before and half after granulation showed an intermediate variability. This effect must be ascribed to the difficulty of mixing starches effectively after granulation. Segregation and variation in starch content are thus unavoidable and variation in tablet break-up results.

The effect of starch concentration

The variation of starch concentration between 0 and 10% showed profound effects on break-up. Further addition up to 20% was almost without effect. Addition of starch to magnesium carbonate reduced the disintegration time from 63 s at 0% concentration to 48 s at 2%, 39 s at 5% and 14 s at 10%. A further addition of 10% caused a further reduction of only 1 s. The mechanism of disruption is therefore fully realized at a concentration of 10%. It has been shown earlier, however (Ganderton & Fraser, 1970), that a suitably close packing of starch and drug is also necessary for proper tablet break-up. This is shown in Fig. 3 where

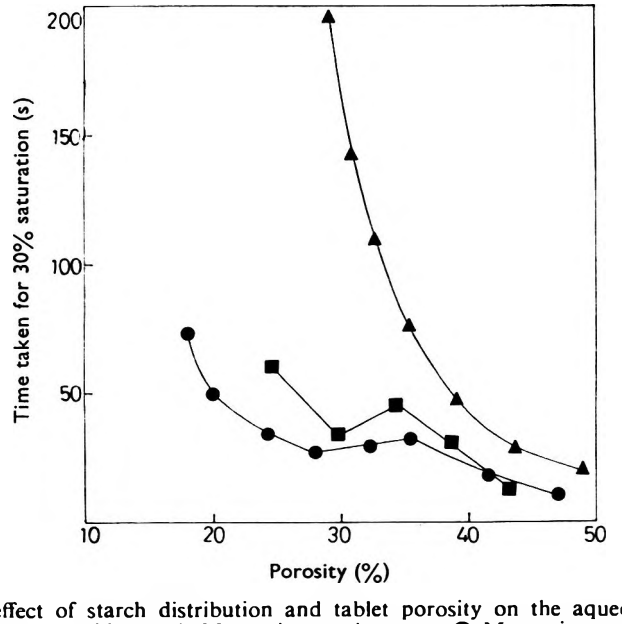

FIG. 3. The effect of starch distribution and tablet porosity on the aqueous penetration of magnesium carbonate tablets. ▲ Magnesium carbonate. ● Magnesium carbonate with 10% internal potato starch. ■ Magnesium carbonate with 10% external potato starch.

both internal and external starch show maximum penetration and break-up when tablets were compressed to a porosity of 28%. Here again, internal distribution was superior in producing disruption, and 10% starch added in this way and compressed to a porosity of 28% represents the optimum combination of variables for the efficient and reproducible break-up of magnesium carbonate tablets in water.

Acknowledgements

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REFERENCES

BERGMAN, L. A. & BANDELIN, F. J. (1965). *J. pharm. Sci.*, **54**, 445–447.

BILLUPS, N. F. & COOPER, B. F. (1964). *Am. J. Pharm.*, **136**, 25–37.

BORZUNOV, Y. Y. & SHEVCHENKO, S. M. (1967). *Farm. Zh. (Kiev)*, **22** (2), 45–48.

COMMONS, K. C., BERGEN, A. & WALKER, G. C. (1968). *J. pharm. Sci.*, **57**, 1253–1255.

CURLIN, L. C. (1955). *J. Am. pharm. Ass. (Sci. Edn)*, **44**, 16.

FAKOUHI, T. A., BILLUPS, N. F. & SAGER, R. W. (1963). *J. pharm. Sci.*, **52**, 700–705.

GANDERTON, D. & FRASER, D. R. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 95S–103S.

GANDERTON, D. & SELKIRK, A. B. (1970). *Ibid.*, **22**, 345–353.

GOUDAH, M. W. & GUTH, E. P. (1965). *J. pharm. Sci.*, **54**, 298–301.

HELLMAN, N. N., BOESCH, T. F. & MELVIN, E. H. (1952). *J. Am. chem. Soc.*, **74**, 348–350.

HIGUCHI, T., ELOWE, L. N. & BUSSE, L. W. (1954). *J. Am. pharm. Ass. (Sci. Edn)*, **43**, 685–689.

HOFSTEE, J. (1953). *Die Stärke*, **5**, 83–86.

HORSCH, W., MARTIN, R. & ROPPERT, W. (1958). *Pharm. Prax. Beilage Pharmazie*, No. 5, 50–52.

INGRAM, J. T. & LOWENTHAL, W. (1968). *J. pharm. Sci.*, **57**, 393–399.

KREBS, V. C. (1959). *An. Fac. Quim. Farm. Univ. Chile*, **11**, 204–210.

KWAN, K. C. & MILOSOVICH, G. (1966). *J. pharm. Sci.*, **55**, 340–343.

MANUDHANE, K. S., CONTRACTOR, A. M., KIM, H. Y. & SHANGRAW, R. F. (1969). *Ibid.*, **58**, 616–620.

MITAL, H. C. & OCRAN, J. (1968). *Pharm. Acta Helv.*, **43**, 493–496.

MONTGOMERY, E. M., SEXSON, K. R., DIMLER, R. J. & SENTI, F. R. (1964). *Die Stärke*, **16**, 345–351.

NAIR, A. D. & BHATIA, V. N. (1957). *J. Am. pharm. Ass. (Sci. Edn)*, **46**, 131–134.

NEMITZ, G. (1962). *Die Stärke*, **14**, 276–278.

NOGAMI, H., NAGAI, T., FUKUOKA, E. & SONOBE, T. (1969). *Chem. pharm. Bull., Tokyo*, **17**, 1450–1455.

PATEL, N. R. & HOPPONEN, R. E. (1966). *J. pharm. Sci.*, **55**, 1065–1068.

PATEL, R. P. & CHIKHLIA, D. T. (1963). *Indian J. Pharm.*, **25**, 220–224.

PATEL, R. P. & PAUCHOLI, J. M. (1964). *Ibid.*, **26**, 313–316.

SABER, A. H., RAHMAN, A. A., HUSEIN, A. M., KASSEM, A. A. & GIRGIS, E. H. (1963). *Bull. Fac. Pharm.*, **2**, 129–136.

SAMEC, M. (1953). *Die Stärke*, **5**, 105–108.

SCHIERBAUM, F. & TAEUFEL, K. (1963). *Ibid.*, **15**, 93–98.

SHOTTON, E. & HARB, N. (1965). *J. Pharm. Pharmac.*, **18**, 504–508.

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The modifying effects of a cationic surfactant on the rates of base catalysed hydrolysis of esters of different structures

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The effect of a cationic surfactant, cetyltrimethylammonium bromide (CTAB), on the hydrolysis rates of four esters of different structure has been investigated. The esters used were ethyl *p*-aminobenzoate (EPAB), *p*-nitrophenyl acetate (PNPA), ethyl *p*-nitrobenzoate (EPNB) and *p*-aminophenyl acetate (PAPA). Association between the esters and the CTAB occurs so that above its cmc the surfactant modifies the rates of hydrolysis, increasing those of EPNB and PNPA and decreasing those of EPAB and PAPA. It thus appears that the type of group on the *p*-position of the aromatic ring plays a major role in determining the kind of effect brought about by the CTAB. Possible reasons to explain the two different effects that CTAB has on ester hydrolysis are put forward.

Mechanisms of ester hydrolysis are well understood and the modifying influence of many agents on the rates of hydrolysis, including the effect of addition of surfactants, has been examined (see Fendler & Fendler (1970) for review). The interactions of surfactants and esters are of importance to Pharmacy, as the shelf lives of preparations containing esters may well be determined by the esters' susceptibility to hydrolysis.

Much of the published work on drug-surfactant interactions is not easily interpreted and the mechanisms of the modifying action exerted by surfactants are not fully understood. Among the simpler factors shown to affect the interaction are the ionic nature and chain length of both surfactant and drug, and whether the ester is hydrolysed by base or acid catalysis. Menger & Portnoy (1967), using the *p*-nitrophenyl esters of acetic, dodecandioic and octanoic acid, have found that anionic micelles *retard* and cationic micelles *enhance* the rate of base catalysed hydrolysis, and that the magnitude of the modifying effect depends on the chain length of the ester. Non-ionic surfactants always appear to retard hydrolytic reactions (Fendler & Fendler, 1970).

Drug-surfactant interactions occur particularly above the critical micelle concentration (cmc), and the effect on the hydrolysis rate, i.e. an *increase* or *decrease* may depend upon the site and orientation of the drug in the micelle. If the drug is in the hydrophobic interior of the micelle, then hydrolysis should be retarded, irrespective of the ionic nature of the surfactant. The structure and ionic nature of the drug may affect its ability to penetrate to the centre of the micelle and such penetration may be dependent upon the pH of the system and configuration and polarity of substituent groups of the drug molecule. If the drug is in the hydrophilic portion of the micelle, either close enough to the surface for electrostatic effects between the surface and ionic species of water to be operative, or at the surface itself, then the nature of the

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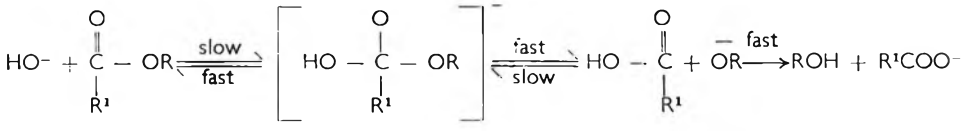
charge on the micelle could well be the major factor in determining the rate of hydrolysis. Simple electrostatic theory would predict that a positive charge on the micellar surface would attract hydroxyl ions and thus base catalysed ester hydrolysis should be enhanced in the presence of a cationic surfactant (cf. Menger & Portnoy, 1967; Romsted & Cordes, 1968; Fendler & Fendler, 1970). These authors have also suggested that the positive charge on the micelle stabilizes the negatively charged tetrahedral intermediate (I) formed according to the BAC 2 mechanism (Gould, 1959):—



(I)

and that this would also be expected to lead to an enhanced rate of hydrolysis.

Simple electrostatic theory cannot, however, account for the decrease in the rate of base catalysed hydrolysis of ethyl *p*-aminobenzoate, ethyl benzoate, diethyl phthallate and propyl benzoate (Mitchell, 1962, 1964) caused by concentrations of cetrimide B.P. above its cmc. It has also been reported (Riegelman, 1960) that the rate of base catalysed hydrolysis of ethyl *p*-aminobenzoate is *increased* by cetyltrimethylammonium bromide (CTAB) in a concentration double its cmc, whilst higher concentrations of CTAB *decrease* the rate to an extent that is proportional to its concentration.

The present work was initiated to investigate the mechanism by which ester structure determines the modifying effect produced by a cationic surfactant on the rates of ester hydrolyses.

MATERIALS AND METHODS

Esters. The following four esters were used: ethyl *p*-aminobenzoate (EPAB), *p*-nitrophenyl acetate (PNPA), ethyl *p*-nitrobenzoate (EPNB), *p*-aminophenyl acetate (PAPA).

The stability of EPAB and PNPA in the presence of CTAB had been investigated previously (Riegelman, 1960; Mitchell, 1962; Menger & Portnoy, 1967; Romsted & Cordes, 1968), and at concentrations above the cmc the rate of hydrolysis of EPAB was decreased and of PNPA was increased. EPNB and PAPA were chosen as the natural counterparts so that the importance of the type of groups in both the 1- and 4-positions of the aromatic ring in determining the nature of the modifying effect could be established.

EPAB was obtained from BDH Ltd., and was of reagent grade. It was twice recrystallized from 50% ethanol; m.p. 89° (lit. 88–90°, Merck Index, 1968).

PNPA was prepared from *p*-nitrophenol by the method of Chattaway (1931). The product was washed with water and recrystallized from 50% ethanol; m.p. 77° (lit. 77·5–78°, Bender & Nakamura, 1962).

EPNB was prepared by the method of Vogel (1951). The product was recrystallized twice from 70% ethanol; m.p. 57° (lit. 57°, Merck Index, 1968).

PAPA was prepared by a three stage reaction. Equimolar quantities of benzaldehyde and *p*-aminophenol were refluxed with dilute acetic acid as solvent for 15 min, to produce benzylidene *p*-aminophenol which was recrystallized from absolute

ethanol. This was then acetylated by the method of Chattaway (1931) and the product was recrystallized from benzene. *p*-Aminophenyl acetate was then prepared from the benzylidene derivative by the method of Galatis (1926). M.p. 75° (lit. 75°, Galatis, 1926).

The structure and purity of all the esters was confirmed by nmr spectroscopy.

Surfactant. This was cetyltrimethylammonium bromide (CTAB). To obtain a pure sample, cetyl bromide was reacted with anhydrous trimethylamine in "super dry" ethanol at 110° for 6 h in a high pressure reaction vessel (Baskerville Lindsay, U.K.). The crude CTAB formed was Soxhlet extracted for 12 h with light petroleum (b.p. 40–60°) and twice recrystallized from absolute ethanol. Nmr and mass spectroscopy were used to confirm the structure. No minimum was observed in the surface tension-log concentration curves that were obtained.

The four esters and CTAB were dried in a vacuum oven at 22 torr and 50° and subsequently stored in a desiccator over phosphorus pentoxide.

Buffer salts and analytical reagents were all of Analar quality.

Water was freshly distilled from an all glass still and had a specific conductivity of $< 10^{-7}$ ohm^{-1} cm^{-1} at 25°.

Buffer solutions. Two different systems were used: Sørensen's glycine (glycine 0·1M in 0·1M sodium chloride and 0·1N sodium hydroxide); Delory and King's carbonate bicarbonate (0·2M anhydrous sodium carbonate and 0·2M sodium bicarbonate). The appropriate standard solutions were prepared according to Documenta Geigy (1962).

pH measurements. These were made at the temperature of the kinetic run using a Pye Dynacap pH meter fitted with a Pye Ingold 405 combined electrode and 622 thermal resistor. The meter and electrode system were standardized using sodium tetraborate buffer (Manov, DeLollis & others, 1946).

Spectrophotometric measurements were made in a Unicam SP500 spectrophotometer using 1 cm cuvettes.

Estimation of the critical micelle concentration

The cmc of the CTAB was measured under all the experimental conditions used during the kinetic runs. The buffer constituents had a marked effect on the value obtained. The cmc was determined by both conductivity and dye solubilisation methods. At 50° in Sørensen's glycine at pH 10·39 the value was 1×10^{-5}M, while in Delory and King's buffer the values for all systems were between 1 and 2×10^{-4}M.

Kinetic studies

The appropriate buffer solution (100 ml) containing the desired concentration of CTAB was prepared and equilibrated to the required temperature. The pH of the solution was measured and if necessary adjusted slightly to the correct value. To 96 ml of this solution was added 4 ml of an aqueous solution, of 25 times the required ester concentration. The solution, after mixing, was assayed immediately and then at appropriate intervals throughout the run in order to follow the course of the hydrolysis.

EPAB and PAPA were hydrolysed in flasks kept in a lagged water bath (temperature fluctuations were ±0·1°). PNPA and EPNB were hydrolysed more rapidly and the degradation was followed on a single sample in a 1 cm cuvette kept in the jacketed cell compartment of the spectrophotometer.

EPAB, PNPA and EPNB were hydrolysed in solutions that were not controlled for degree of aeration. With PAPA under these conditions first order kinetics were not obeyed, owing to interference from the oxidation of *p*-aminophenol formed as a degradation product. Thus solutions of PAPA and CTAB were bubbled with oxygen free nitrogen (B.O.C. Ltd.) for 2 h before mixing and the bubbling was continued throughout the experiment.

Assay procedures

Only the principles of the methods used are given. Slight modifications were necessary to overcome problems that occurred when the surfactant and ester concentrations were altered, and in all cases the suitability and accuracy of the method employed was confirmed.

EPAB was assayed spectrophotometrically by a method similar to that of Higuchi, Havinga & Busse (1950) at the wavelength of maximum absorption (λ_{max}) of the ester of 286 nm.

With PNPA the hydrolysis was followed by measuring the production of *p*-nitrophenol at its λ_{max}, at the pH of the kinetic run, of 400 nm.

The concentration of EPNB was determined by a method similar to that employed for EPAB, at the λ_{max} of the ester of 265 nm.

PAPA was assayed by a modified Bratton-Marshall reaction (Tansey, 1969) at 550 nm. This was possible as the degradation product *p*-aminophenol does not form a coloured complex in this reaction.

Treatment of results

With one exception, solutions of the esters were found to degrade by apparent first order kinetics. The percentage residual concentration of ester was calculated for each period of heating. Values for the rate constant (k) were obtained from log% concentration-time data by means of a computerized least-squares regression analysis, which gave values of k, its associated standard error, and the correlation coefficient.

RESULTS AND DISCUSSION

The effect of a range of concentrations of CTAB on the rate of hydrolysis of EPAB in Sørensen's glycine buffer at a pH of 10·39 at 50° is seen from Table 1. The pre-

Table 1. *The effect of CTAB on the rate of hydrolysis of ethyl* p*-aminobenzoate (EPAB) at a concentration of* 1×10^{-3}M, *at pH* 10·39 *in Sørensen's glycine buffer at 50°.*

Surfactant concentration (mol litre^{-1})	Observed 1st order rate constant, k (min^{-1})	Standard error of k
0	$1{\cdot}280 \times 10^{-3}$	$1{\cdot}37 \times 10^{-5}$
5×10^{-5}	$1{\cdot}321 \times 10^{-3}$	$3{\cdot}15 \times 10^{-5}$
1×10^{-4}	$1{\cdot}177 \times 10^{-3}$	$1{\cdot}12 \times 10^{-5}$
2×10^{-4}	$1{\cdot}204 \times 10^{-3}$	$1{\cdot}16 \times 10^{-5}$
3×10^{-4}	$1{\cdot}152 \times 10^{-3}$	$9{\cdot}8 \times 10^{-6}$
4×10^{-4}	$1{\cdot}166 \times 10^{-3}$	$1{\cdot}41 \times 10^{-5}$
1×10^{-3}	$1{\cdot}077 \times 10^{-3}$	$1{\cdot}51 \times 10^{-5}$
2×10^{-3}	$9{\cdot}003 \times 10^{-4}$	$1{\cdot}29 \times 10^{-5}$
4×10^{-3}	$7{\cdot}388 \times 10^{-4}$	$1{\cdot}34 \times 10^{-5}$
1×10^{-2}	$6{\cdot}295 \times 10^{-4}$	$1{\cdot}30 \times 10^{-5}$
2×10^{-2}	$4{\cdot}630 \times 10^{-4}$	$4{\cdot}71 \times 10^{-6}$

cision of the experimental technique was confirmed by four replicate experiments in the absence of CTAB and four in the presence of 1×10^{-4}M CTAB. The calculated coefficients of variation were 1·39 and 0·87% respectively. With the exception of the lowest value of CTAB used (5×10^{-5}M) where the rate constant was not significantly different from the value obtained in the absence of surfactant (t calc. = 0·605, t tab. at $P = 0{\cdot}05 = 2{\cdot}18$), increasing the concentration of CTAB present resulted in a retardation of hydrolysis, the magnitude of which effect increased with surfactant concentration (Fig. 1). Thus, unlike Riegelman (1960), we did not observe

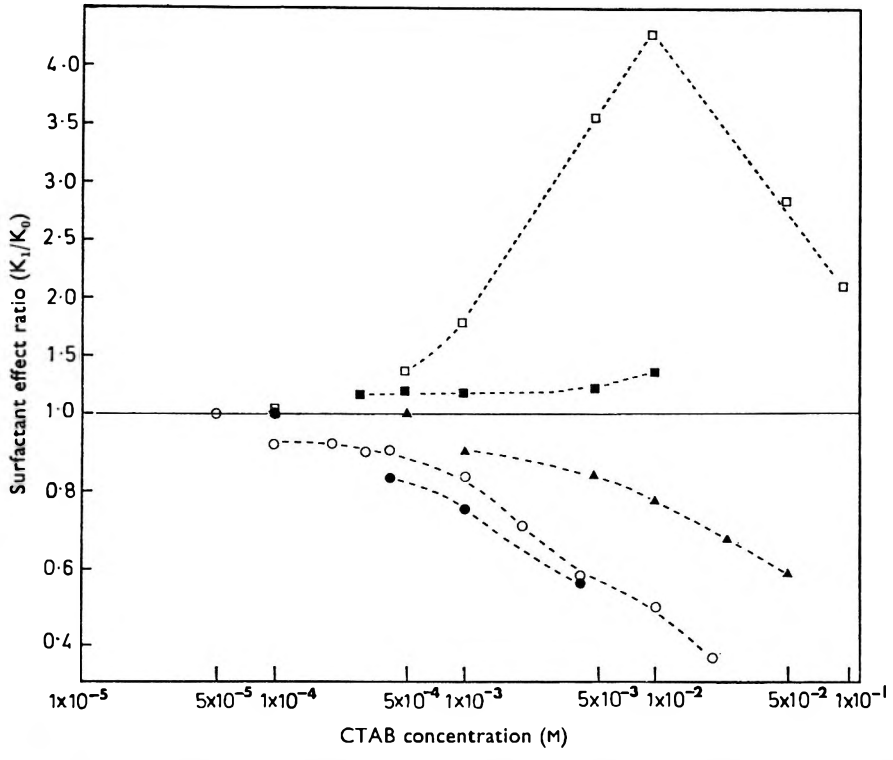


FIG. 1. The effect of CTAB on the hydrolysis of four esters expressed as a ratio (k_1/k_0) of the first order rate constants obtained in the presence (k_1) and absence (k_0) of the surfactant. ●, EPAB; ▲, PAPA; □, PNPA; ■, EPNB; all in Delory and King's buffer; ○, EPAB in Sørensen's glycine buffer.

CTAB, just above its cmc (1×10^{-5}M, in glycine buffer) to have a potentiating effect on this ester. The different concentration of ester used by Riegelman (6×10^{-5}M) does not explain the difference since we found reduced values of k to be produced by all concentrations of CTAB above the cmc when the experiment was repeated with an ester concentration of 4×10^{-5}M in bicarbonate buffer (Table 2). The surfactant effect appears to depend upon the formation of micelles, as a concentration of CTAB near the cmc (measured as $1{\cdot}0 \times 10^{-4}$ in bicarbonate buffer) showed no significant modifying action. The reason for the difference between this and Riegelman's findings is not known. Examination of his data suggests the possibility that the reported increase may not be statistically significant.

Table 2. *The effect of CTAB on the rate of hydrolysis of ethyl* p-*aminobenzoate (EPAB),* p-*nitrophenyl acetate (PNPA), ethyl* p-*nitrobenzoate (EPNB) and* p-*aminophenyl acetate (PAPA) at a concentration of* 4×10^{-5}M *in Delory and King's buffer.*

Surfactant concentration (mol litre^{-1})	EPAB k values (min^{-1}) pH 10·55, T = 50°	PNPA k values (min^{-1}) pH 9·2 T = 25°	EPNB k values (min^{-1}) pH 10·64 T = 25°	PAPA k values (min^{-1}) pH 10·64 T = 25°
0	$2·175 \times 10^{-3}$	$1·611 \times 10^{-2}$	$1·212 \times 10^{-2}$	$2·436 \times 10^{-2}$
$9·6 \times 10^{-5}$	$2·135 \times 10^{-3}$	$1·591 \times 10^{-2}$		
$2·9 \times 10^{-4}$			$1·399 \times 10^{-2}$	
$4·8 \times 10^{-4}$	$1·808 \times 10^{-3}$	$2·205 \times 10^{-2}$	$1·440 \times 10^{-2}$	$2·422 \times 10^{-2}$
$9·6 \times 10^{-4}$	$1·648 \times 10^{-3}$	$2·817 \times 10^{-2}$	$1·426 \times 10^{-2}$	$2·165 \times 10^{-2}$
$4·8 \times 10^{-3}$	$1·209 \times 10^{-3}$	$5·744 \times 10^{-2}$	$1·475 \times 10^{-2}$	$2·043 \times 10^{-2}$
$9·6 \times 10^{-3}$		$6·901 \times 10^{-2}$	$1·625 \times 10^{-2}$	$1·886 \times 10^{-2}$
$2·4 \times 10^{-2}$				$1·648 \times 10^{-2}$
$4·8 \times 10^{-2}$		$4·678 \times 10^{-2}$		$1·423 \times 10^{-2}$
$9·6 \times 10^{-2}$		$3·376 \times 10^{-2}$		

Preliminary experiments showed that the glycine buffer, while having no effect on EPAB, catalysed the hydrolysis of PNPA, and therefore all our subsequent experiments were performed in Delory and King's buffer. The results are in Table 2. For each ester the observed first order rate constant (k_1) was used to calculate the *surfactant effect ratio* k_1/k_0, where k_0 is the value in the absence of CTAB. The values obtained plotted against log CTAB concentration (Fig. 1) illustrate the effect of the surfactant. It is clear that CTAB alters the rate of hydrolysis of the four esters at all concentrations above its cmc. However it increases the rate of hydrolysis of PNPA and EPNB and decreases the rate for EPAB and PAPA.

The magnitude of the modification is much greater for PNPA than for EPNB, possibly because there may be a difference in the association of the two esters with the CTAB. Increasing the concentration of CTAB in the presence of PNPA increases the surfactant effect ratio to a maximum value of 4·2 at a surfactant concentration of $9·6 \times 10^{-3}$M. The subsequent fall in the ratio is probably attributable to an increase in counter ion (bromide) concentration causing displacement of hydroxyl ions from the area immediately surrounding the micelle surface, as postulated by Romsted & Cordes (1968). This effect, although operative throughout will become noticeable only after the maximum effect of surfactant has been obtained which, from our results, must lie between $9·6 \times 10^{-3}$ and 5×10^{-2}M. Menger & Portnoy (1967), using n-dodecyltrimethyl ammonium bromide and Romsted & Cordes (1968) using CTAB have also demonstrated the potentiating effect of cationic surfactants on the hydrolysis rate of PNPA. There are quantitative differences between all these results that are probably due to the different buffer solutions used. Unlike our results, no decrease in surfactant effect ratio was reported, probably because the other workers used concentrations of CTAB below that giving a maximum value.

The result obtained with PAPA is similar to that for EPAB but the effect was only noted with PAPA above 1×10^{-3}M CTAB which could reflect a difference in association constants of the esters with CTAB.

The nature of the ester grouping on the aromatic ring does not therefore determine whether CTAB increases or decreases the hydrolysis rates, although it is still possible that it could influence the magnitude of the response. Rather it is the group on the

p-position that appears to determine the kind of modifying effect, probably by determining the site of drug-surfactant interaction, or the orientation of the drug with respect to the micellar surface, or both.

Where CTAB increases the rate of hydrolysis, the site of association must be the micellar surface, or close enough to it, for the ester linkage to be in a region of high hydroxyl ion concentration such as will be found close to the Stern layer. It would seem logical that the δ^- charge associated with the nitro-group of PNPA and EPNB will cause these esters to orientate themselves with their NO_2 groups next to the polar head of the surfactant while the remainder of the molecule projects through the Stern layer into the hydroxyl ion enriched diffuse part of the double layer. Certainly it is unlikely that these esters will penetrate into the hydrocarbon centre of the micelle.

Replacement of the NO_2 group by the NH_2 group results in a reversal of the CTAB effect. Nmr spectroscopic measurements with a series of organic compounds of different structure (Eriksson & Gillberg, 1966) would suggest that EPAB and PAPA are bound close to the micellar surface, and a similar conclusion for EPAB was reached by Riegelman (1960) on the evidence of ultraviolet spectroscopy. If this is true, then the presence of the NH_2 group results, in some way, in the removal of the ester linkage from the region where hydrolysis can readily take place. The NH_2 group, with its $\delta+$ charge, will not interact with the micellar surface and it is feasible that it will penetrate into the micelle, thereby drawing the ester group into the viscous region surrounding the micelle into which hydroxyl ions find difficulty in diffusing. It is also feasible that these ester groupings could be brought into the viscous regions as a result of the molecule being adsorbed flat on to the surface of the micelle. An alternative explanation, one analogous to that given by Menger & Portnoy (1967) for the retardation of ester hydrolysis brought about by an anionic surfactant, is that protection is afforded the esters because they are adsorbed into the hydrocarbon interior of the micelles. Menger and Portnoy's conclusion was based on the results of an analysis that indicated that the rate of hydrolysis of the esters in the micelles was zero. In the absence of a similar analysis, which must await measurements on the association constants for micelle-ester interactions, it is not possible to reject a similar explanation for our results, but it would be surprising if the substitution of a NH_2 for a NO_2 group caused such a profound change in the penetration properties of the esters concerned.

It was necessary to ensure that the results obtained did not merely reflect a slight change in the experimental conditions used, since reported work has indicated that surfactant effects are complex and can be modified by many factors. For example the micellar effect on the base catalysed hydrolysis of *p*-nitrophenyl hexanoate (Romsted & Cordes, 1968) is influenced by the nature and presence of added salts, NO_3^- and Br^- ions changing a *potentiating* micellar effect to a *protective* one at high salt concentrations. In view of this, the effect of CTAB on the four esters was compared under identical conditions of pH, buffer strength and temperature (Table 3). At this lower pH the degradation of PAPA no longer followed first order kinetics. The effect, however, is qualitatively unequivocal and, in order to obtain an approximate quantitative measure of the effect, the initial rate was estimated and it is these values that are given in Table 3. The surfactant effect ratios show that the effect on the four esters is qualitatively the same as already described. However with EPAB, EPNB and PAPA, where the pH has been decreased, the magnitude of the surfactant effect has increased both in its potentiating and in its protective role. This could be

Table 3. *The effect of* $4{\cdot}8 \times 10^{-3}$M *CTAB on the rate of hydrolysis of the four esters at a concentration of* 4×10^{-5}M, *at pH* 9·2 *and* 25°.

Ester	k_0 value (min^{-1})	k_1 value (min^{-1})	Surfactant effect ratio ($k_1 : k_0$)
EPAB	$3{\cdot}739 \times 10^{-6}$	$1{\cdot}326 \times 10^{-6}$	0·355
PNPA	$1{\cdot}611 \times 10^{-2}$	$5{\cdot}744 \times 10^{-2}$	3·565
EPNB	$6{\cdot}023 \times 10^{-4}$	$8{\cdot}120 \times 10^{-4}$	1·348
PAPA	$1{\cdot}674 \times 10^{-3}$	$1{\cdot}040 \times 10^{-3}$	0·621

because of the change in hydroxyl ion concentration or it could reflect the different ionic constitution of the buffer solutions used.

These results show that stability studies with solubilized systems should be made on the individual preparations and that results for one compound should not be extrapolated even to similar compounds in systems that have not been fully characterized.

REFERENCES

BENDER, M. L. & NAKAMURA, K. (1962). *J. Am. chem. Soc.*, **84**, 2577–2582.

CHATTAWAY, F. D. (1931). *J. chem. Soc.* Part II, 2495–2496.

DOCUMENTA GEIGY (1962). Scientific Tables, 6th Edn, 315.

ERIKSSON, J. C. & GILLBERG, G. (1966). *Acta chem. scand.*, **20**, 2019–2027.

FENDLER, E. J. & FENDLER, J. H. (1970). *Advances in Physical Organic Chemistry*, **8**, 271–406. London: Academic Press.

GALATIS, L. (1926). *Ber. dt. Chem. Ges.*, **59**, 848–850.

GOULD, E. S. (1959). *Mechanism and structure in organic chemistry*, p. 316. New York: Holt, Reinhardt & Winston.

HIGUCHI, T., HAVINGA, A. & BUSSE, L. W. (1950). *J. Am. pharm. Ass. (Sci. Edn)*, **39**, 405–410.

MANOV, G. G., DELOLLIS, N. J., LINDVALL, P. W. & ACREE, S. F. (1946). *J. Res. natn. Bur. Stand.*, **36**, 543–558.

MENGER, F. M. & PORTNOY, C. E. (1967). *J. Am. chem. Soc.*, **89**, 4698–4703.

MERCK INDEX, 8th Edition (1968). Rahway, N.J., U.S.A.: Merck & Co. Inc.

MITCHELL, A. G. (1962). *J. Pharm. Pharmac.*, **14**, 172–176.

MITCHELL, A. G. (1964). *Ibid.*, **16**, 43–48.

RIEGELMAN, S. (1960). *J. Am. Pharm. Ass. (Sci. Edn)*, **49**, 339–343.

ROMSTED, L. R. & CORDES, E. H. (1968). *J. Am. chem. Soc.*, **90**, 4404–4409.

TANSEY, I. (1969). M.Sc. Thesis, Bath University.

VOGEL, A. I. (1951). *A Text Book of Practical Organic Chemistry*, p. 1001. London: Longmans.

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The effect of detergent concentration on monomer activity in a non-ionic detergent solution

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The mono-octyl ether of hexaoxyethylene glycol has been synthesized and carefully purified using foam fractionation in the ultimate stages of the purification. Surface tension measurements of solutions of the detergent in water using a Wilhelmy plate indicate a continuing decrease in surface tension with increasing detergent concentration above the critical micellar concentration. Vapour pressure osmometry using the Mechrolab 302 substantiated the interpretation of the results in terms of the law of mass action.

Two main theories of micellization are extant. One, the "pseudophase," treats micelle formation as the production of a separate but soluble phase commencing at the critical micellar concentration. This theory implies constant activity of the monomers at total detergent concentration values greater than the critical micellar concentration. The second theory considers micellization as the reversible formation of an aggregation of monomers with an equilibrium between the monomers and micelles that can be described by a law of mass action type equation. In this theory the activity of the monomeric species continues to change as the concentration of detergent is increased even above the critical micellar concentration.

Dialysis experiments (Harrap & O'Donnell, 1954; and Abu-Hamdiyyah, Mukerjee & Mysels, 1963) as well as surface tension measurements (Hudson & Pethica, 1964; Elworthy & Mysels, 1966) have shown that the activity of monomers continues to increase with detergent concentration. Elworthy, Gyane & Macfarlane (1969) discussed the effect of purity and contamination on the surface tension of aqueous solutions of a non-ionic detergent. We have now examined the monomer activity over a range of concentrations above and below the critical micellar concentration.

MATERIALS AND METHODS

Syntheses of glycol and detergent

3,6,9,12,15-Pentaoxaheptadecane-1,7-diol was synthesized as described by Elworthy & Macfarlane (1963).

3,6,9,12,15,18-Hexaoxahexacosane-1-ol (*hexaoxyethylene glycol mono-octyl ether*, C_8E_6). Sodium (0·25 mol) was dissolved in 3,6,9,12,15-pentaoxaheptadecane-1,7-diol (1 mol) under a layer of light petroleum (100–120°) (cf. Macfarlane, 1963). 1-Bromooctane (0·25 mol) was slowly added to the solution over 4 h and the reaction mixture held at 120° for a further 4 h. C_8E_6 was extracted from the cooled reaction mixture

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with diethylether, which was then evaporated and the residue re-extracted with light petroleum (60–80°). The light petroleum was evaporated and the extracted material was decolourized by gently stirring at 30° in a 30% suspension of charcoal in acetone (charcoal: extract ratio 1:2, by weight). The charcoal was filtered off and the solvent evaporated. 50 ml of a 20% solution of extract in absolute ethanol was then passed down a column of 120 ml of a strong anion-exchange resin. The eluate was recycled twice then a further 100 ml of absolute ethanol passed. The solvent was evaporated from the total eluate and the residue dried under vacuum in the presence of phosphoric oxide. Infrared spectra of the residue failed to detect any carbonyl impurities.

This crude product (2 g) was then adsorbed from benzene onto a column of Mallinckrodt silicic acid (25 g) mixed with celite (5 g). The column was washed with increasing concentrations of acetone in benzene. All pre-run was removed by 25% acetone in benzene while C_8E_6 was eluted by 30% acetone in benzene. The yield of this stage was approximately 65% of the adsorbed product.

Final purification was by foam fractionation* of a 0·35% aqueous solution of the compound until the solution was reduced to 60% of its volume. The foamed solution was then freeze dried in an all glass apparatus and the remaining water removed under vacuum at 30° in the presence of phosphoric oxide. Found: C, 61·2; H, 10·6; $CH_2.CH_2.O$, 66·9. Calculated for $C_{20}H_{42}O_7$: C, 60·9; H, 10·7; $CH_2.CH_2.O$, 67·0.

Materials

All organic solvents (Analar grade) were dried and redistilled immediately before use. Chromatographic materials were soaked in and thoroughly eluted with the appropriate solvents.

Water used was from the laboratory still, redistilled from alkaline permanganate then finally distilled in a seasoned and sterilized all glass still.

All glass joints were hand ground to obviate the need for vacuum grease.

Surface tension measurements

Surface tensions were measured using a light glass Wilhelmy plate (see Elworthy & Macfarlane, 1962). The difference in weight of the plate in the air–solution interface and in air was measured using a torsion balance of sensitivity of $\pm 0{\cdot}1$ mg. With the size of plates used this is equivalent to $\pm 0{\cdot}02$ dynes cm^{-1}. The solution flask was immersed in a thermostat bath at $25 \pm 0{\cdot}02°$.

A CI microbalance giving readings of better than $\pm 0{\cdot}01$ mg ($0{\cdot}002$ dynes cm^{-1}) was used in the study of the region above the cmc. The glass plate was suspended from the left arm of the balance and counterbalanced by a specially prepared counterweight and the zero adjustment circuit of the apparatus. Readings of surface tension were obtained by counterbalancing the greater part of the pull by a further set of specially prepared platinum weights to bring the force exerted by the balance within the range of the scale selected.

* *Foam fractionation.* The all glass apparatus consisted principally of a one litre round bottomed flask containing the solution, and a two-necked adapter that served as an inlet for the gas diffusion tube used as a bubbler, and as exit for the foam and point of attachment for a vertical Vigreux fractionating column. The column was connected through adapters to a receiver flask. (For fuller details see Gyane, 1970.)

By slow, controlled, bubbling of oxygen-free, water-saturated nitrogen into the solution, foam was formed which rose slowly up the Vigreux column. Drainage of the foam during this process effectively concentrated the more surface-active impurities which were carried over into the receiver flask. The rate of foaming was adjusted to give as dry a foam as possible (ideally black films).

By allowing the horizontal lower edge of the Wilhelmy plate to just touch the surface of the solution, buoyancy effects were minimized. To reduce evaporation and aerial contamination and to ensure a saturated atmosphere above the solution a glass cover, with only a small hole in the middle to allow passage of the suspending rod, was fitted to a flask containing the solution being measured. This flask was always cleaned with chromic acid, thoroughly rinsed with triple distilled water then rinsed with a very dilute foamed solution and drained before use. This procedure, apart from removing any undesirable substance from the glass that might affect the result, compensates for concentration errors due to adsorption of detergent onto the glass.

Vapour pressure osmometry

A range of concentrations of C_8E_6 was studied using a Mechrolab Model 302. Drop size was controlled using a telescopic measuring device (Deshmukh & Fleming, 1969). The apparatus was calibrated using sucrose and had a calibration factor of 45 over the concentration range studied.

RESULTS AND DISCUSSION

The surface tension as a function of concentration of solutions of C_8E_6 is illustrated in Fig. 1.

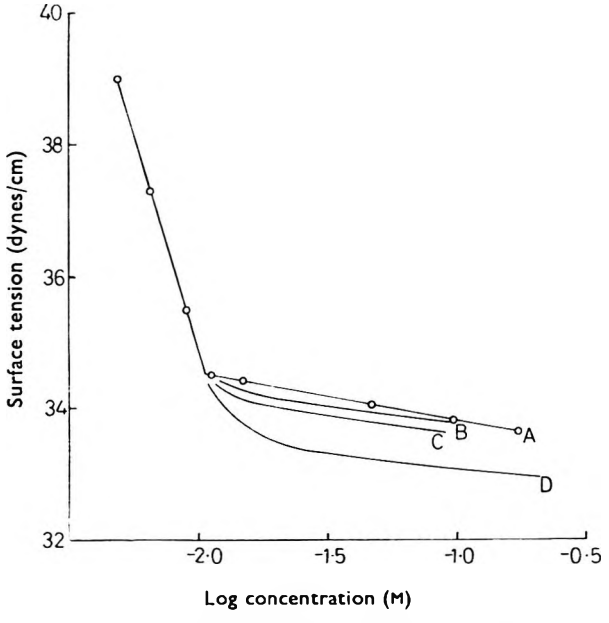

FIG. 1. Measured and theoretical values of surface tension *vs* concentration of aqueous solutions of C_8E_6. Plot A—measured; plots B, C and D were calculated from K equal to 10^{12}, 10^{11} and 10^{9} respectively.

While there is the normal change of gradient attributed to the formation of micelles, the plot does not show the usual pattern of a line parallel to the abscissa above the critical micellar concentration but the continuance of a negative slope. This is not a unique effect but it can be masked by inadequate purification or by contamination.

The continuing decrease in surface tension of these solutions with increasing solute

concentration above the critical micellar concentration would therefore appear to indicate that the activity of the monomers of C_8E_6 continues to increase in this region.

Calculation of critical micellar concentration

If the micelles formed by a non-ionic detergent are in equilibrium with the monomers present, then using the law of mass action and replacing activities by concentration, the system may be described by

$$K = \frac{m}{x^n} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where m is the concentration of micelles; x the concentration of monomers; n the number of monomers per micelle; and K the equilibrium constant.

The total concentration of solute in the system, C, may be described by:

$$C = x + nm \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Hence knowing n and assuming arbitrary values for K, the respective values for m and C can be calculated for a range of values of x.

To obtain theoretical values that are significant the calculated cmc requires interpretation in a manner that correlates with the practical measurements. Three methods of obtaining this value have been used:

(1) It was taken as the point of intersection of straight lines drawn through the pre- and post-micellar regions of a plot of x against C. As the plot is not straight above the critical micellar region, the line chosen depends on subjective judgment and the method was used to obtain only an *approximate* value for K.

(2) On the premise of Phillips definition (Phillips, 1955) that the cmc could be described by $d^3\phi/dC^3 = 0$, where ϕ is any colligative property of the system, the value of C corresponding to the maximum change of slope of dx/dC was calculated. dx/dC was obtained by combination of equations (1) and (2) and differentiation.

(3) Mathematically, where C becomes greater than x can also be taken as the cmc. This assumes that when micelles appear they make a measureable contribution to the physical properties of the system. This method requires a decision being taken about the point at which the presence of micelles is acknowledged in the calculated results.

Using a value of 36 for n, obtained from the work of Balmbra, Clunie & others (1964) values of K were adjusted until the critical micellar concentrations obtained by the respective methods coincided with that obtained practically.

The values of K used and cmc calculated are:

Calculated cmc (%)	K	Method	Equation 3 constant
0·403	10^{12}	1	8·352
0·403	10^{11}	2	8·579
0·403	10^{9}	3	8·720

Plots of monomer concentration as a function of total detergent concentration are plotted in Fig. 2. This shows that for a given method increasing the value of K for a given aggregation number decreases the cmc obtained.

Calculation of surface tension was made using the integrated form of Gibb's adsorbtion equation and can be expressed as

$$\gamma = -2{\cdot}303\ RT\Gamma_2 \log a + \text{constant} \quad .. \quad .. \quad .. \quad (3)$$

where Γ_2 is the surface excess of solute; a the solute activity and γ the surface tension of the solution.

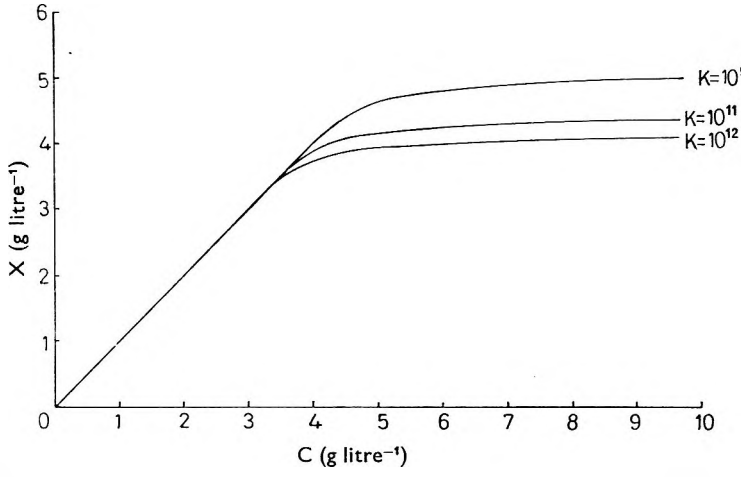

FIG. 2. Plot of C *vs* x calculated from an aggregation number of 36 and equilibrium constants of 10^9, 10^{11} and 10^{12} respectively.

Assuming an activity coefficient of 1, the surface excess was calculated from the slope of the surface tension graph just below the cmc. The calculated monomer concentration of and surface tension at the cmc was substituted with Γ_2 into equation (3) to obtain the appropriate values of the constant. These are also given in Table 1. The constants were used in equation (3) to calculate the theoretical surface tensions shown in Fig. 1.

Theoretical values obtained by methods 1 and 2 are in reasonable agreement with these obtained practically (light scattering aggregation number used in these calculations is subject to an error of around $\pm 10\%$).

The results from method (3) substantiate the inherent problem of differentiating between a theoretical and a practical critical micellar concentration.

Vapour pressure osmometry

A number of average molecular weight, M_n, can be obtained by this technique from

$$\int_{C_{c\to o}} \frac{\Delta R}{C} = \frac{H}{M_n} \qquad (4)$$

where ΔR is the resistance increment, and H the apparatus calibration factor.

For micellar systems this is modified to

$$\int_{C-\text{cmc}\to o} \frac{\Delta R - \Delta R_{\text{cmc}}}{C - \text{cmc}} = \frac{H}{M_n} \qquad (5)$$

to compensate for the effects of the monomers in the solution. Results for aqueous solutions of C_8E_6 in this form are shown in Fig. 3.

In the non-ionic detergent system studied a straight line graph would be expected if the activity of the monomer remained constant above the cmc. Results for aqueous solutions of C_6E_6 (unpublished observations) indicate that this system behaves ideally to a concentration of more than 2·5% of detergent. It is therefore reasonable to assume that the results obtained in a comparable concentration range with C_8E_6 are not due to non-ideality.

The detergent number average molecular weight for a system made up of monomers and micelles can be calculated from

$$M_n = \left(\frac{y + z}{ny + z}\right) Mn \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

where y and z are the concentrations of monomers and micelles respectively, and M is the monomer molecular weight.

Theoretical values for $\frac{\Delta R - \Delta R_{cmc}}{C - cmc}$ were calculated using equations (5) and (6) with the monomer and micellar concentrations previously obtained corrected for their respective cmc values. These are shown in Fig. 3.

The similar pattern between practical and theoretical results further endorses the view of a continuing increase in monomer activity above the cmc.

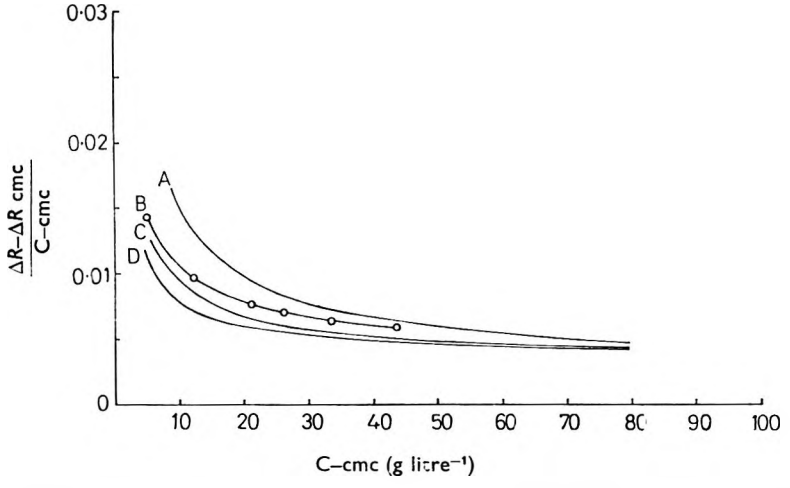

FIG. 3. Measured and calculated vapour pressure osmometry values plotted as a function of corrected concentration. B—measured; plots A, C and D were calculated from monomer and micellar concentrations corresponding to equilibrium constants of 10^{4}, 10^{11} and 10^{12} respectively.

REFERENCES

ABU-HAMDIYYAH, M., MUKERJEE, P. & MYSELS, K. (1963). *J. phys. Chem.*, **67**, 1943–1944.

BALMBRA, R. R., CLUNIE, J. S., CORKILL, J. M. & GOODMAN, J. F. (1964). *Trans. Faraday Soc.* **60**, 979–985.

DESHMUKH, A. & FLEMING, R. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 91S–97S.

ELWORTHY, P. H., GYANE, D. O. & MACFARLANE, C. B. (1969). *Ibid.*, **21**, 617–619.

ELWORTHY, P. H. & MACFARLANE, C. B. (1962). *Ibid.*, **14**, *Suppl.*, 100T–102T.

ELWORTHY, P. H. & MACFARLANE, C. B. (1963). *J. chem. Soc.*, 907–914

ELWORTHY, P. H. & MYSELS, K. J. (1966). *J. Colloid Inter. Sci.*, **21**, 331–347.

GYANE, D. O. (1970). Ph.D. Thesis, Strathclyde University.

HARRAP, B. S. & O'DONNELL, I. J. (1954). *J. phys. Chem.*, **58**, 1097–1099.

HUDSON, R. A. & PETHICA, B. A. (1964). *Proceedings of the Fourth International Congress on Surface Active Substances.* Gordon and Breach, New York, **2**, 631–639.

MACFARLANE, C. B. (1963). Ph.D. Thesis, Glasgow University.

PHILLIPS, J. N. (1955). *Trans. Faraday Soc.*, **51**, 561–569.

J. Pharm. Pharmac., 1971, 23, *Suppl.*, 39S–48S *Received January* 25, 1971

The solubility of some compounds in hexadecylpolyoxyethylene monoethers, polyethylene glycols, water and hexane

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The solubilities of a series of compounds of varying polarity, i.e. decane, benzene, sulphadiazine, *p*-hydroxybenzoic acid, ethyl and butyl *p*-hydroxybenzoate and methyl anisate in cetomacrogol 1000 and hexadecyl heptaoxyethylene ether solutions, have been determined. A comparison of these results with the solubilities of these compounds in water, n-hexane and polyethylene glycol solutions gives some evidence on the mechanism of solubilization. The results show that the solubilization of these compounds is not adequately described solely on the basis of solubility into the separate regions of the micelle. The micellar solubility of the compounds was dependent on the hydrocarbon solubility and the presence of groups in the molecule which can form hydrogen bonds. Butyl paraben was found to have a higher solubility in 10% cetomacrogol solutions than either ethyl paraben or methyl anisate. The hydrophil–lipophil characteristics of the detergents with the polarity of the solubilizate molecule also affect solubilization. A non-polar molecule such as decane has a greater solubility in the more lipophilic detergent and the reverse is true of *p*-hydroxybenzoic acid.

One of the main advantages in the use of non-ionic detergents in formulation is that the oxyethylene and hydrocarbon chain lengths can be tailored to provide any particular hydrophilic–lipophilic characteristic and thus it is possible to solubilize a wide range of compounds by adjusting these parameters.

Theoretically it should be possible to calculate the maximum additive concentration (M.A.C.) of a compound in a detergent solution from the saturation solubilities of the compound in the different regions of the micelle. This approach is valid for only a few compounds solubilized by ionic detergents (Hartley, 1938). Micelles of non-ionic detergents have an oxyethylene–water complex region of indeterminate composition and consequently the simple "bulk solubility" approach is not adequate. For a detergent of a given hydrocarbon chain length a minimum hydrophilic chain length is required for the detergent to be water soluble provided this occurs, the smaller the hydrophilic chain in a series of surfactants, the more hydrocarbon can be solubilized (Saito & Shinoda, 1967).

It has been found that the M.A.C. of benzoic acid in a series of polyoxyethylene hexadecyl ethers increased from 1·0 mol benzoic acid/mol $C_{16}n_{16}$ to 3·0 mol benzoic acid/mol $C_{16}n_{96}$ (Humphreys & Rhodes, 1968). [Detergents are abbreviated thus $CH_3(CH_2)_{15}\cdot O\cdot(CH_2CH_2O)_{16}H = C_{16}n_{16}$.]

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The solubilizate molecule may itself affect the M.A.C. Nakagawa & Tori (1960) showed that, in $C_{10}n_{10}$, decane and decyl chloride were solubilized less than some other more polar decyl compounds. Similar results have been found for substituted benzoic acids and esters (Chakravarty, Lach & Blaug, 1957).

MATERIALS AND METHODS

Heptaoxyethylene glycol monohexadecyl ether, prepared according to Elworthy & Macfarlane (1963), was chromatographed (Macfarlane, 1963) to yield white crystals m.p. 37·8–38·2°. (Elworthy & Macfarlane, 1963, give m.p. 38·5°.)

Cetomacrogol 1000 B.P.C. (Macarthy's Ltd., Romford) was used as received. It was assayed for oxyethylene units by the method of Siggia, Starke & others (1958). The mean result of two determinations was 79·61% of oxyethylene units in the molecule. (B.P.C. limits: 76·52–81·36%.) Assuming the hydrocarbon chain to be the hexadecyl chain, this gives the number of oxyethylene units as 21·5. For all subsequent calculations the molecular weight was taken as 1210.

Polyethylene Glycol 1000 (PEG) was B.D.H. Laboratory Reagent and was used as received. The oxyethylene content was not determined but the molecular weight was taken as 986 for all calculations, and corresponded to an oxyethylene chain of 22 units.

Hexaoxyethylene glycol (*Hexagol*), prepared according to Elworthy & Macfarlane (1963) was a pale straw coloured liquid n_D^{25} 1·4609. (Curme & Johnston, 1952, give n_D^{38} 1·4589.)

Benzene, crystallizable (W. Jarvie Ltd.) was distilled before use, n_D^{18} 1·5028. (Handbook of Chemistry and Physics 1966 gives N_D^{20} 1·5011.)

n-Decane, olefine free (Fluka AG. Purum grade) was used as received, n_D^{18} 1·4131. (Handbook of Chemistry and Physics 1966 gives N_D^{20} 1·4119.)

n-*Hexane* (BDH spectroscopic grade) was used as received.

Sulphadiazine B.P. (Macarthy's Ltd., Romford) was recrystallized twice from dimethylformamide–ethanol mixture (1 : 3) and dried in a vacuum oven at 40° over phosphorous pentoxide: m.p. 254° (decomp). (British Pharmacopoeia 1968 gives m.p. 255 decomp.)

p-*Hydroxybenzoic acid* (BDH Ltd., Laboratory reagent) was recrystallized from distilled water and dried overnight at 40° in a vacuum over phosphorous pentoxide; m.p. 214°. (Vogel, 1956, gives 212°.)

Ethyl p-*hydroxybenzoate* (*ethyl paraben*) (BDH Ltd., Laboratory reagent) was recrystallized from distilled water, then dried overnight at 40° in a vacuum oven over phosphorous pentoxide; m.p. 112°. (Vogel, 1956, gives 111–115°.)

n-*Butyl* p-*hydroxybenzoate* (*butyl paraben*) (BDH Ltd., Laboratory reagent) was recrystallized from water–ethanol mixture (3 : 1) then dried in a vacuum at 40° over phosphorous pentoxide; m.p. 68°. (Merck Index, 1968, gives m.p. 68–69°.)

Methyl p-*methoxybenzoate* (*methyl anisate*) was prepared by esterification of anisic acid (Koch-Light Laboratories Ltd., Puriss grade) in the usual way. It was recrystallized from a methanol–water mixture (6 : 4) then dried in a vacuum over phosphorous pentoxide; m.p. 48·0–48·5° not raised by further recrystallization. (Lifschitz & Girbes, 1924, give m.p. 49·0–49·5°.)

Water used for the preparation of solutions was tap water distilled once from glass.

Solubility measurements

A small excess of decane was equilibrated with "solvent" (water or detergent) by shaking at 20° until saturation was reached. The saturated aqueous phase was assayed for hydrocarbon by gas liquid chromatography on a 6 ft Carbowax column at 75° using n-butanol as the internal standard.

Benzene was equilibrated in the same way and the benzene in the aqueous phase determined by ultraviolet spectrophotometry ($\lambda_{max} = 254$ nm). Saturation solubilities of solid compounds were determined as described by Elworthy & Lipscomb (1968a). Aqueous phases as well as hexane solutions were assayed by ultraviolet spectrophotometry.

Phase diagrams were constructed according to Boffey, Collison & Lawrence (1959).

RESULTS AND DISCUSSION

Solubility in water

The most important properties of the solute molecules governing the solubility in water are the molecular volume and the presence of hydrophobic substituents requiring extensive structuring of the water molecules. The ability of substituent groups to participate in hydrogen bonding with the water molecules is less important. This is well illustrated by the results in Table 1.

Table 1. *The solubilities of compounds in pure liquids and the M.A.C.s in the detergent solutions (millimolal) at* 20°.

	Solute						
Solvent	Decane	Benzene	Sulpha-diazine	*p*-Hydroxy-benzoic acid	Ethyl paraben	Butyl paraben	Methyl anisate
Water	—	21·8	0·236	34·7	†4·91	1·065	3·87
n-hexane	∞	∞	0	0	1·04	4·48	636
1% w/v cetomacrogol	2·42	33·9	0·272	45·9	13·9	13·5	11·8
5% w/v cetomacrogol	9·72	125	0·535	90·8	45·1	58·3	29·6
10% w/v cetomacrogol	19·7	182	1·01	157	78·6	121	50·0
1% w/v $C_{16}n_7$	22·2	*261	—	45·0	15·9	—	12·0
2% w/v $C_{16}n_7$	38·2	—	—	56·9	27·6	—	20·5

* in 12·5% cetomacrogol solution.

† The standard error of the solubility of ethyl paraben in water was found to be ±0·06 milli molal.

Solubility in hexane

These results show the typical dependence of solubility in apolar solvents on the polarity of the solutes.

Solubility in PEG solutions

The solubilities of all the compounds was increased in polyethylene glycol solutions. If this increased solubility was dependent solely on the ability to form hydrogen bonds with the ether oxygen atoms it would be expected that solutions containing equal concentration by weight of the PEG 1000 and hexagol (PEG 300) would dissolve the same amount of solute.

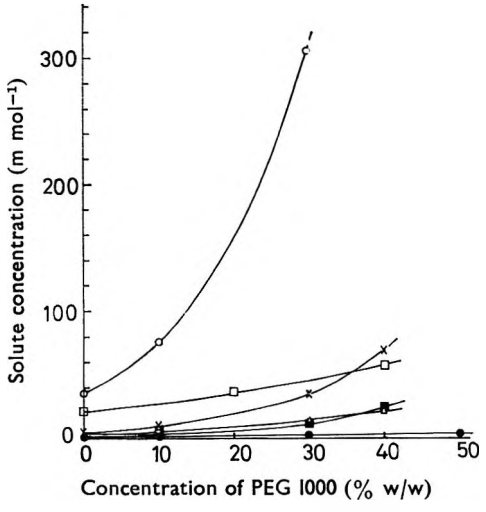

FIG. 1. The Solubility of compounds in PEG 1000 Solutions at 20° C. ○ *p*-Hydroxybenzoic acid. X Ethyl paraben. □ Benzene. △ Methyl anisate. ■ Butyl paraben. ● Sulphadiazine.

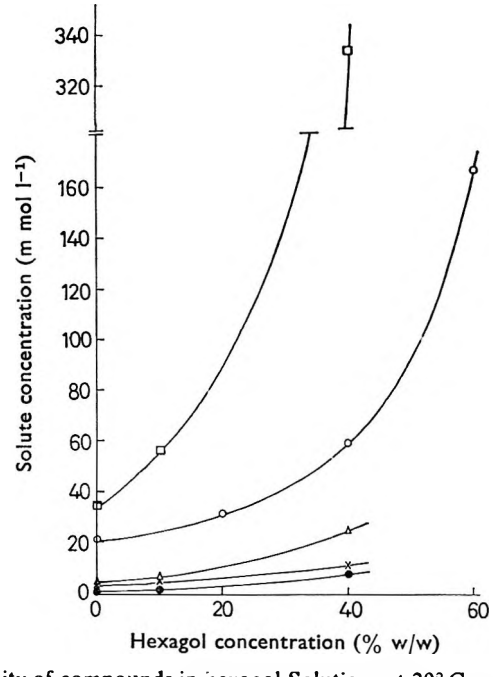

FIG. 2. The solubility of compounds in hexagol Solutions at 20° C. ⊏ *p*-Hydroxybenzoic acid. ○ Benzene. △ Ethyl paraben. X Methyl anisate. ● Butyl paraben.

This does not occur, except with benzene. As can be seen from Figs 1 and 2 the longer chain polymer (PEG 1000) has a greater capacity to solubilize than has hexagol. This effect has also been reported by Blaug & Ahsan (1961) and may be due to the long polymer chain forming a partially coiled structure around the solute and thus increasing the solubility.

Paruta (1969) has shown that the paraben esters have a high solubility in solvents whose dielectric constant is about 30. At 20° the dielectric constant of ethylene glycol is 37·7; (water = 80·4, n-decane = 2·0), and if we assume, as is reasonable, that the dielectric constants of the PEGs are of a similar magnitude to that of ethylene glycol it would be expected that as the concentration of PEG is increased, the solubility of the parabens would increase markedly.

Table 2. *The solubilities of compounds in PEG solutions (millimolal) at* 20°.

	Solute					
Solvent	Benzene	Sulpha-diazine	*p*-Hydroxy-benzoic acid	Ethyl paraben	Butyl paraben	Methyl anisate
10% w/v PEG 1000	20% PEG 36·1	— 0·719	— 75·6	— 10·5	— 2·54	— 8·59
30% w/v PEG 1000	40% PEG 57·2	— 2·11	— 304	— 35·0	— 11·0	— 14·6
40% w/v PEG 1000	60% PEG 154·3	50% PEG 4·20	— 559	— 69·3	— 22·1	— 21·5
80% w/v PEG 1000	611	—	—	—	—	—
10% w/v hexagol	20% n_6 31·9	— —	— 56·6	— 7·38	— 1·96	— 5·11
40% w/v hexagol	59·4	—	334	25·5	8·02	11·4
60% w/v hexagol	167	—	—	—	—	—

Solubilization in cetomacrogol solutions

The solubility of all compounds is greater in cetomacrogol solutions than in water when the concentration of cetomacrogol is much greater than the cmc of 0·007% (Elworthy, 1960). This increase is linear with increase in concentration of the detergent above the cmc (Fig. 3). Sulphadiazine has a very low solubility in water, in hexane and in PEG solutions: it is solubilized by cetomacrogol solutions least of all of the compounds investigated. This may be due to the large size of the sulphadiazine molecule. Elworthy & Lipscomb (1968b) found a correspondingly low solubility for griseofulvin in cetomacrogol.

p-Hydroxybenzoic acid is insoluble in hexane but its high solubility in PEG solutions results in the highest M.A.C. of all of the compounds in surfactant solutions.

The other compounds studied are soluble in water, PEG solutions and in hexane and so their M.A.C.s are, in part, a reflection of these properties.

Table 3 shows the solubilities of the compounds expressed as mol of solubilizate/mol of detergent (cetomacrogol or $C_{16}n_7$) and this is a function of the solubility of the compounds in the micelles. It can be seen that butyl paraben is as soluble in the

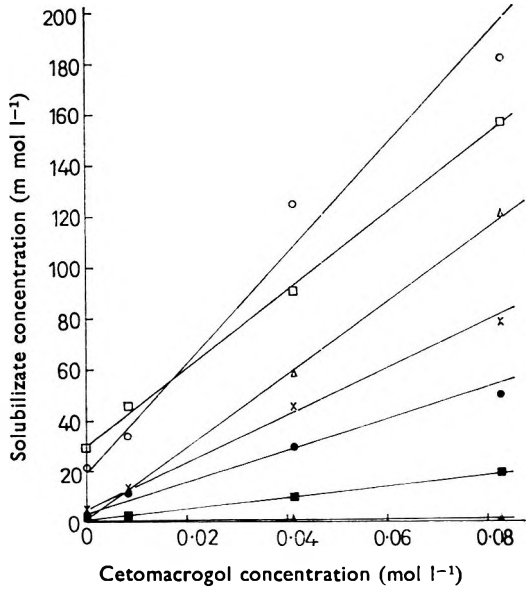

FIG. 3. The solubility of compounds in cetomacrogol solutions at 20° C. ○ Benzene. □ *p*-Hydroxybenzoic acid. △ Butyl paraben. X Ethyl paraben. ● Methyl anisate. ■ Decane. ▲ Sulphadiazine.

Table 3. *The solubilities of compounds per mol of each detergent at* 20°.

	$C_{16}n_7$		Cetomacrogol	
Solute	Solubility as mol/mol	Solubility as mol/oxyethylene unit	Solubility as mol/mol	Solubility as mol/oxyethylene unit
Decane	0·888	0·127	0·232	0·0106
Benzene	—	—	2·33	0·106
p-Hydroxybenzoic acid	0·616	0·088	1·460	0·0664
Ethyl paraben	0·630	0·090	0·911	0·0414
Butyl paraben ..	—	—	1·45	0·066
Methyl anisate ..	0·430	0·061	0·618	0·0281
Sulphadiazine	—	—	0·0099	0·00045

micelles as is *p*-hydroxybenzoic acid. This cannot be explained by summing the solubilities of these compounds in hexane and in polyoxyethylene-water mixtures, as, although the butyl ester is more soluble than the *p*-hydroxybenzoic acid in the hydrocarbon core, it is less soluble in the oxyethylene–water complex. Therefore, there may be a significant change in the micelle structure on solubilization of these two compounds.

The micellar solubility of the other compounds in both cetomacrogol and $C_{16}n_7$ (Table 3) shows a decrease in the order *p*-hydroxybenzoic acid > ethyl paraben > methyl anisate > decane. This is the order of *decreasing* solubility in water alone and in PEG solutions and of *increasing* solubility in hydrocarbon solvents. Blaug & Ahsan (1961) reported similar results for other paraben–non-ionic systems.

Benzene seems to have an anomalously high solubility in the cetomacrogol micelles (Fig. 1): it is much less soluble in PEG solutions than *p*-hydroxybenzoic acid, although it is miscible in all proportions with hydrocarbon solvents. These results all show

that the M.A.C.s of the compounds in cetomacrogol cannot be rationalized simply in terms of their solubility in typical constituent parts of the micelle. Changes in the structure of the micelles must also be important factors in the mechanism of solubilization.

Solubilization in $C_{16}n_7$ solutions

The solubility of decane, *p*-hydroxybenzoic acid, ethyl paraben and methyl anisate increases with increasing concentration of $C_{16}n_7$. Butyl paraben causes separation of $C_{16}n_7$ solutions into two immiscible phases at 20°; it was also found to coacervate with cetomacrogol solutions at temperatures higher than 22°. Butyl paraben has been reported to coacervate in solutions of Myrj 52 and Tween 80 (Blaug & Ahsan, 1961).

Table 3 shows that decane displays the greatest micellar solubility, whereas methyl anisate is less soluble in the micelles than either ethyl paraben or *p*-hydroxybenzoic acid.

Comparison of the M.A.C.s in cetomacrogol and $C_{16}n_7$ solutions

Solubilities in $C_{16}n_7$ solutions were always greater than in cetomacrogol solutions even when the differing sizes of the micelle core in the two cases was considered.

The hydration of the oxyethylene layer around the $C_{16}n_7$ micelles is significantly less than that of cetomacrogol in the simple detergent solutions, and is equivalent to that in an 80 and 30% solution of PEG respectively (Elworthy, 1960; Elworthy & McDonald, 1964).

Therefore, comparing solutions of the two detergents containing the same amount of oxyethylene chains, the $C_{16}n_7$ solution has a greater capacity for solubilization at all sites providing that the act of solubilizing a compound does not significantly change the aggregation number and hydration of the micelles. This is reflected in the data in Table 4.

Table 4. *Solubilities in different parts of the micelle*

	Solubility		*Solubility—proportion in hydrocarbon	
Compound	†0·01 mol litre^{-1} cetomacrogol	†0·03 mol litre^{-1} $C_{16}n_7$	0·01 mol litre^{-1} cetomacrogol	0·03 mol litre^{-1} $C_{16}n_7$
p-Hydroxybenzoic acid ..	48·3	52·7	48·3	52·7
Ethyl paraben	14·75	22·0	14·75	22·0
Methyl anisate	9·8	17·0	8·3	12·7

* The solubility of the compounds after subtracting the amount of the compound that dissolves in the relevant proportions of hydrocarbon in each detergent.

† A 0·01 molar solution of cetomacrogol contains the same quantity of oxyethylene chains as a 0·03 molar solution of $C_{16}n_7$.

For compounds which are solely present in the micellar core, the M.A.C. should be the same in solutions of equal molarity of each detergent. A study of Figs 3 and 4 shows that this is not true for any system, even the decane solutions. This must mean that some additional factors are present.

The differences between the solubilities in each detergent increase in the order, methyl anisate, ethyl paraben, *p*-hydroxybenzoic acid. This suggests that the

solubility of *p*-hydroxybenzoic acid is more dependent on the oxyethylene content or the surface area of the micelles, than is the solubility of methyl anisate. Table 3 also shows this. This points to a high solubility in the micelle core for methyl anisate.

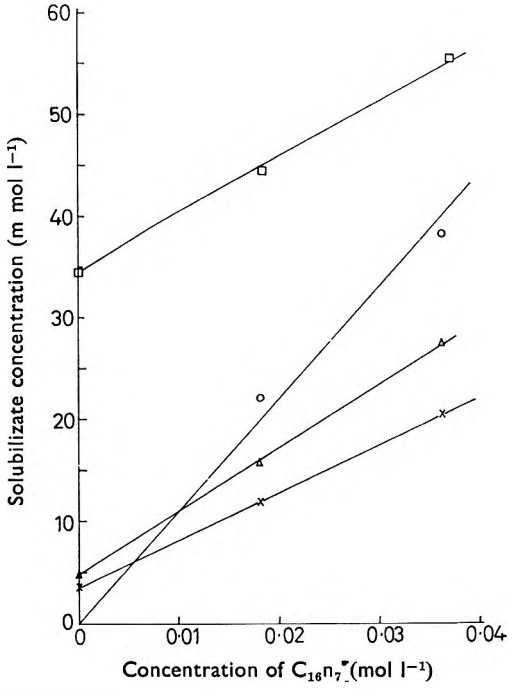

FIG. 4. The solubility of compounds in $C_{16}n_7$ solutions at 20° C. □ *p*-Hydroxybenzoic acid. △ Ethyl paraben. X Methyl anisate. ○ Decane.

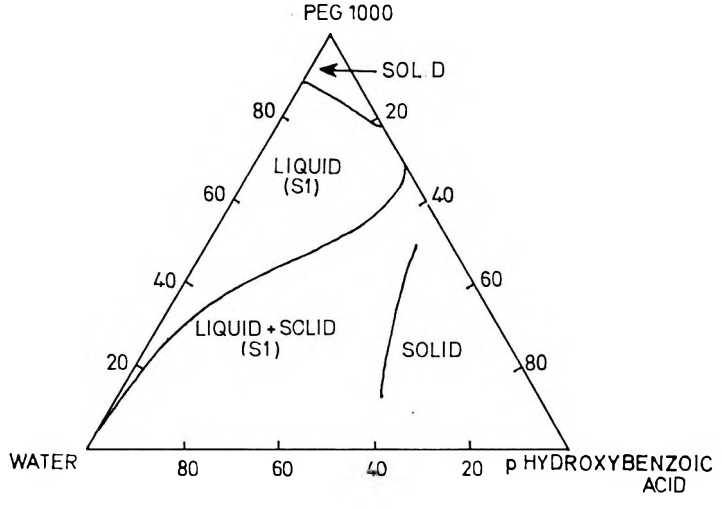

FIG. 5. Phase diagrams of *p*-hydroxybenzoic acid—PEG 1000-water mixtures. (S_1 = water miscible phase).

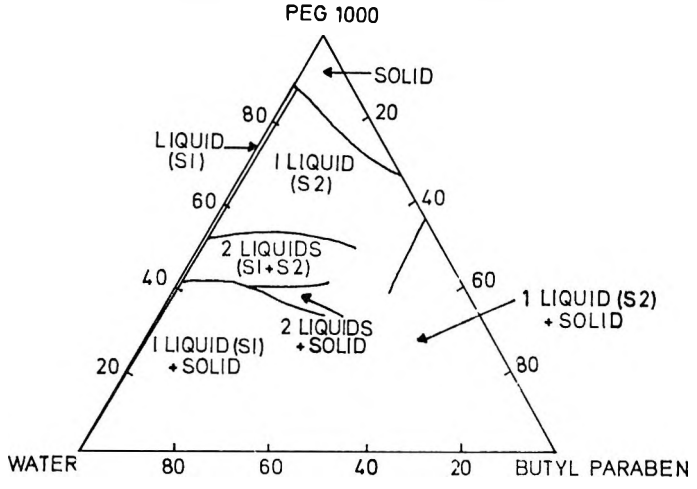

FIG. 6. Phase diagram of butyl paraben—PEG 1000-water mixtures. (S_1 = water miscible liquid, S_2 = water immiscible liquid).

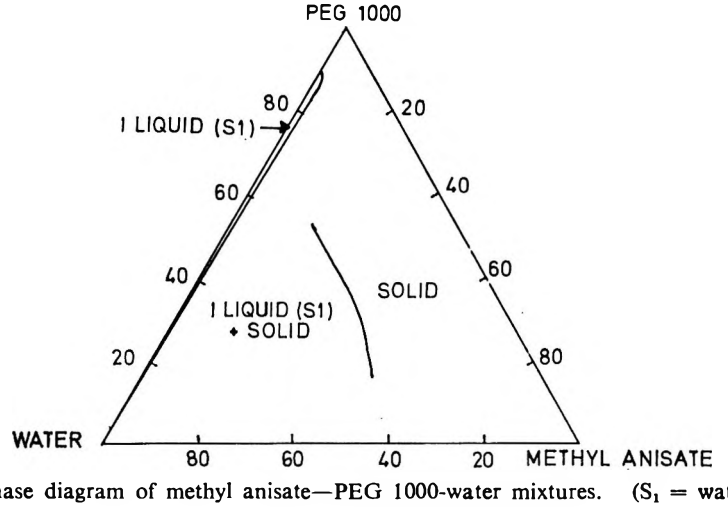

FIG. 7. Phase diagram of methyl anisate—PEG 1000-water mixtures. (S_1 = water miscible liquid).

Phase diagrams

The chief difference between the three diagrams (Figs 5, 6 and 7) is the presence of the water immiscible liquid in the butyl paraben system.

Comparison of the diagrams for *p*-hydroxybenzoic acid and methyl anisate shows the different degree of interaction between these two compounds and the PEG. The hydrogen bonding of the acid on to the oxyethylene chains results in a fluid system at much lower concentrations of water than in the methyl anisate system. The solubility of the acid in the PEG–water mixtures is much greater.

In the butyl paraben system the solubility of the ester in the PEG solutions is low but at low water concentrations and at the higher PEG concentrations, the second liquid phase appears. The probable difference between the two systems is that when a PEG molecule is associated with hydrogen bonded acid molecules it is still hydrophilic; when the ester molecule hydrogen bonds to a PEG molecule, the butyl chain

of the ester renders the complex hydrophobic and so a water-immiscible phase is formed. This provides an explanation of the coacervation noted in some of the butyl paraben systems in the solubility studies. There is no similar water-immiscible phase present in the methyl anisate system and this shows the lack of interaction between the ester and the PEG. The small increase in solubility of the ester which does occur at the higher PEG concentrations may be due to the solute molecules being trapped in the lattice of intermolecularly hydrogen bonded PEG chains.

Using this information it is possible to tentatively suggest the site of solubilization of the compounds investigated. Sulphadiazine and *p*-hydroxybenzoic acid are probably solubilized solely within the palisade layer, the acid being more concentrated in the deeper region of the layer. Ethyl and butyl paraben, methyl anisate and benzene are probably present throughout all regions of the micelle with increasing concentration in the core in the listed order above. Decane is probably nearly all solubilized within the core of the micelle.

Acknowledgement

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REFERENCES

BLAUG, S. M. & AHSAN, S. S. (1961). *J. pharm. Sci.*, **50**, 441–443.
BOFFEY, B. J., COLLISON, R. & LAWRENCE, A. S. C. (1959). *Trans. Faraday Soc.*, **55**, 654–661.
British Pharmacopoeia (1968). London: Pharmaceutical Press.
CHAKRAVARTY, D., LACH, J. L. & BLAUG, S. M. (1957). *Drug Stand.*, **25**, 137–140.
CURME, G. O. & JOHNSTON, F. (1952). *The Glycols*, p. 180. New York: Reinhold.
ELWORTHY, P. H. (1960). *J. Pharm. Pharmac.*, **12**, 260*T*–266*T*.
ELWORTHY, P. H. & LIPSCOMB, F. J. (1968a). *Ibid.*, **20**, 790–792.
ELWORTHY, P. H. & LIPSCOMB, F. J. (1968b). *Ibid.*, **20**, 817–824.
ELWORTHY, P. H. & MACFARLANE, C. B. (1963). *J. chem. Soc.*, 907–914.
ELWORTHY, P. H. & MCDONALD, C. (1964). *Kolloid-Z.*, **195**, 16–23.
Handbook of Chemistry & Physics (1966). 47th Edn, Editor: Weast, R. C. Cleveland: The Chemical Rubber Co.
HARTLEY, G. S. (1938). *J. chem. Soc.*, 1968–1975.
HUMPHREYS, K. J. & RHODES, C. T. (1968). *J. pharm. Sci.*, **57**, 79–83.
LIFSCHITZ, I. & GIRBES, G. (1928). *Chem. Ber.*, **61**, 1463–1491.
MACFARLANE, C. B. (1963). Ph.D. Thesis, Univerity of Glasgow.
The Merck Index (1968). 8th Edn. Rahway N.J.: Merck & Co. Inc.
NAKAGAWA, T. & TORI, K. (1960). *Kolloid-Z.*, **168**, 132–139.
PARUTA, A. N. (1969). *J. pharm. Sci.*, **58**, 364–366; 204–207; 216–219.
SAITO, H. & SHINODA, K. (1967). *J. Colloid Sci.*, **24**, 10–15.
SIGGIA, S., STARKE, A. C., GARIS, J. J. & STAHL, C. R. (1958). *Analyt. Chem.*, **30**, 115–116.
VOGEL, A. I. (1956). *Practical Organic Chemistry*, 3rd Edn. London: Longmans.

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Identification of the site of solubilization of various compounds by cetomacrogol

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Solubilization of some of the esters of *p*-hydroxybenzoic acid by cetomacrogol 1000 solutions has been investigated by ultraviolet spectrophotometry, nuclear magnetic resonance spectrometry and viscometry to provide evidence on the environment of each part of the solubilizate molecule as well as the detergent molecule. Hydration of the micelles calculated from the viscometry results, gave further evidence on the site of solubilization of the compounds. On the basis of these results and solubility results published previously it is suggested that the compounds are solubilized as follows: *p*-Hydroxybenzoic acid is wholly solubilized deep in the oxyethylene layer of the micelle. Ethyl *p*-hydroxybenzoate is solubilized mostly in the oxyethylene layer and is situated adjacent to the core: some solubilization occurs within the core. Butyl *p*-hydroxybenzoate is solubilized mainly at the oxyethylene-hydrocarbon junction, the phenyl ring in the oxyethylene layer and the butyl chain in the core: some solubilizate is wholly present in the core. Methyl *p*-methoxybenzoate: most of the compound is dissolved in the micellar core, but some is also present in the oxyethylene layer. Benzene is solubilized in a similar manner to methyl *p*-methoxybenzoate.

Physical methods provide invaluable tools in the study of solubilization. Ultraviolet spectrophotometry (Riegelman, Allawala & others, 1958; Donbrow & Rhodes, 1966; Tokiwa, 1968) and nuclear magnetic resonance spectroscopy (Donbrow & Rhodes, 1966; Eriksson & Gilberg, 1966) have both been used to identify the site of solubilization of many different compounds.

The size and shape of micelles can be inferred from measurements of the viscosity of systems and comparison of one system with another can yield evidence of the micellar structure. The hydration of the micelles can be calculated from the following:

$$\text{The volume of 1 micelle } V_h = \frac{1}{\mathcal{N}}[(n_2M_2v_2 + n_3M_3v_3) + w_1v_1(n_2M_2 + n_3M_3)] \quad (1)$$

where $\mathcal{N}$ = Avogadro's Number; n_1 = the number of water molecules bound in the micelle; n_2 = the number of detergent molecules bound in the micelle; n_3 = the number of solubilizate molecules bound in the micelle. (The subscripts always refer to the same three species.) M = molecular weight; v = specific volume of the bound compounds; w = weight of compound bound per g of detergent.

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The partial specific volume of the detergent in the solution is:

$$\left(\frac{dv}{dg_2}\right)_{T.P.\ g_1g_3} = w_2v_2 + w_1v_1 + w_3v_3 - w_1v_1^0 - w_3v_3^0 \quad .. \quad (2)$$
$$= \bar{v}_2$$

where v^0 = specific volume of the unbound compound, and where g = total weight of the compound in the solution. Substituting 2 into 1 and rearranging gives

$$v_h = \frac{1}{\mathcal{N}}[(n_2M_2 + n_3M_3)(\bar{v}_2 + w_3v_3^0 + w_1v_1^0)] \quad .. \quad .. \quad .. \quad (3)$$

If the total concentration of micellar substance is $c_2 + c_3$ g/ml the volume fraction, ϕ, of the micelles is:

$$\phi = (c_2 + c_3)(\bar{v}_2 + w_1v_1^0 + w_3v_3^0)$$

for spherical micelles: $\eta_{sp} = 2{\cdot}5\phi$

at infinite dilution $\left[\frac{\eta_{sp}}{(c_2 + c_3)}\right]_{c_2 + c_3 = 0} = [\eta]$

$$\therefore w_1 = \frac{[\eta] - \bar{v}_2 - w_3v_3^0}{2{\cdot}5v_1^0} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

METHODS

Ultraviolet absorption spectra were recorded at a path length of 1 cm using a Unicam SP800 recording spectrophotometer. The sample in the reference beam was either the corresponding solvent or detergent solution.

Proton magnetic resonance spectra of the solubilizates in 20% w/v cetomacrogol solutions were recorded using a Perkin Elmer R10 60 MHz spectrometer.

Viscosity of solutions was determined using a suspended level dilution viscometer (flow time for water 206 s) at 20° ± 0·1°.

RESULTS

Ultraviolet spectrophotometry

As expected, spectra of the compounds show an increased degree of fine structure in n-hexane compared with more polar solvents. The fine structure is also more noticeable for the less polar solubilizates. In cetomacrogol solutions, the wavelength maximum for each compound shifts to longer wavelengths as the concentration of cetomacrogol increases; this is probably due to the relative increase in the micellar phase and consequently in the amount of the compound solubilized (Table 1). For benzene, the wavelength maximum shifts from the water value in 0·1% cetomacrogol solution to the hexane value in 8% cetomacrogol solution indicating that the benzene is predominantly in a hydrocarbon environment in 8% cetomacrogol solution.

In contrast to benzene the spectra for *p*-hydroxybenzoic acid and its butyl and ethyl esters suggest that in the more concentrated cetomacrogol solutions these compounds are in an oxyethylene environment.

The wavelength maxima for methyl anisate in cetomacrogol solutions is shifted 1 nm towards longer wavelengths compared with the spectrum in aqueous solution,

Table 1. *The wavelengths of the absorption maxima for the solutions (nm).*

Compound	Solvent					
	Water	n-Hexane	PEG 1000 30% w/v	Cetomacrogol 0·1%	Cetomacrogol 2%	Cetomacrogol 8% w/v
Benzene	254·0	255·0	254·5	254·0	254·5	255·0
p-Hydroxybenzoic acid	255·5	—	257·0	255·5	257·0	257·5
Ethyl *p*-hydroxy benzoate	255·0	246·5	257·5	256·0	257·0	258·0
Butyl *p*-hydroxy benzoate	256·0	246·5	258·5	258·0	259·0	259·0
Methyl *p*-methoxy benzoate	257·0	253·0	258·5	257·0	257·5	258·0

with the concomitant small increase in the complexity of the spectrum in 8% cetomacrogol solution. This suggests that a large amount of the ester is solubilized in the hydrocarbon core. The nmr data also support this viewpoint.

Nmr spectroscopy

Fig. 1 shows the dependence of chemical shift of various protons (measured from the water reference line) on the mol ratio of solute to detergent. It has already been shown (Eriksson, 1963; Eriksson & Gilberg, 1966) that the water line moves only a very small amount, compared with an external reference, on solubilization of various compounds in aqueous cetyl trimethylammonium bromide and cetyl pyridinium chloride.

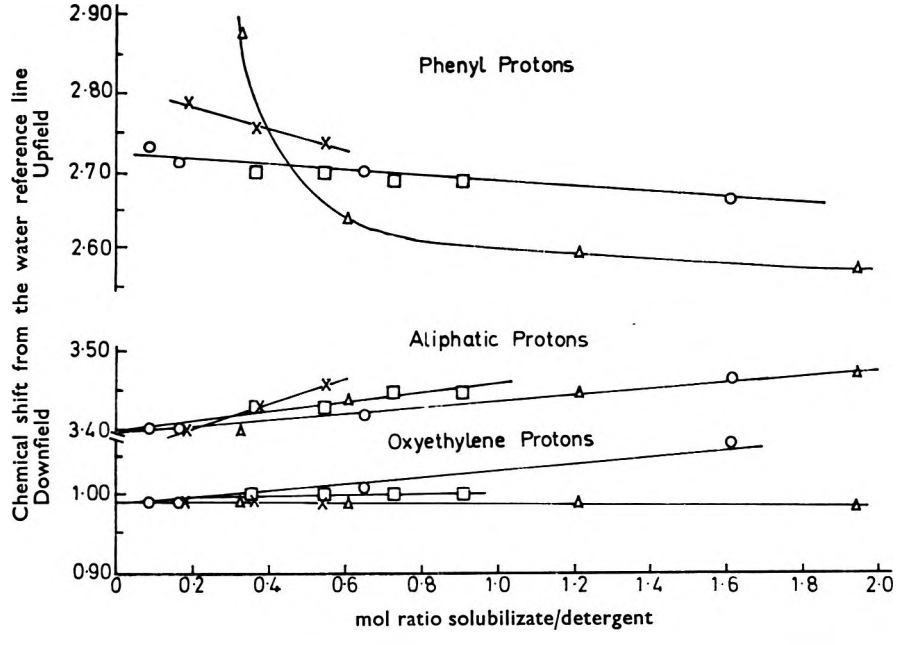

FIG. 1. The chemical shifts of the solubilized systems. ○ = *p*-Hydroxybenzoic acid. △ = Benzene. □ = Ethyl paraben. × = Methyl anisate.

The chemical shift of the oxyethylene protons is independent of the amount of solute solubilized by the surfactant except in the case of *p*-hydroxybenzoic acid where there is a concentration dependence. *p*-Hydroxybenzoic acid thus appears to be the

only compound to appreciably alter the structure of the oxyethylene water complex. The other compounds, may not be present in sufficient concentration in the oxyethylene complex to modify the environment of the protons and so alter the line position. The chemical shift of the aliphatic protons of the cetomacrogol is dependent on the mol ratio of solute to detergent whatever the solute involved. As the mol ratio increases, the environment of the aliphatic protons is continually changing. The slope of the curve in Fig. 1 for methyl anisate is greater than that for ethyl paraben and is probably due to the greater concentration of the former compound in the hydrocarbon core. Since *p*-hydroxybenzoic acid is insoluble in hydrocarbon solvents its effect on the chemical shift of cetomacrogol aliphatic protons is probably due to the solubilization of the acid molecules in the oxyethylene water complex, thus resulting in a changed configuration of the oxyethylene chains which modifies the structure of the hydrocarbon core.

Resonance lines for the phenyl protons of *p*-hydroxybenzoic acid and its ethyl ester both show the same shifts, probably indicating that both are in the oxyethylene layer. Since shifts for the phenyl protons of methyl anisate are different from those of the other two solids, this probably reflects the increased solubilization of the ester in the micellar core. The plot for benzene shows a large change in slope at about 0·5 mol/mol detergent suggesting that at very low concentrations of benzene the site of solubilization is different to that at higher benzene concentrations. It is suggested that benzene may be adsorbed on the micelle surface at less than 0·4 mol/mol detergent and only enters the core above 0·6 mol/mol. Eriksson & others (1966) have reported similar results for the solubilization of benzene by cetyl trimethylammonium bromide and cetyl pyridinium chloride.

Viscometry

The intrinsic viscosities and line slopes for plots of $\frac{\eta_{sp}}{c}$ vs. c are presented in Table 2.* Addition of *p*-hydroxybenzoic acid (or its ethyl ester) to solutions of cetomacrogol result in a small decrease in micellar hydration. This is probably due to simple displacement of some of the bound water by the large number of solubilizate molecules in the micelle.

Table 2. *The intrinsic viscosity and hydration of the saturated systems at* 20°.

	[η]			Hydration g water/g detergent (w_1)*
	PEG 1000	Hexagol	Cetomacrogol	
No additive	5·9	3·0	7·6†	2·2 ± 0·2
p-Hydroxybenzoic acid	5·3	2·8	6·4	1·9 ± 0·2
Ethyl paraben	5·6	2·4	5·8	1·6 ± 0·1
Butyl paraben	5·9	3·2	6·7	2·2 ± 0·2
Methyl anisate	5·6	2·7	7·2	2·1 ± 0·2
Decane	—	—	5·3	1·2 ± 0·1

* Applies to cetomacrogol only.
† The error in intrinsic viscosity determinations is approximately ±5% (Macfarlane, 1963).

* A computer program for this calculation can be obtained from the authors on request.

The decrease for the ethyl ester is greater than for the acid and is ascribed to the larger size of the ester molecule displacing more water molecules. The hydrophobic ester chain may also cause the oxyethylene chains to coil more tightly and thus squeeze out some of the trapped water molecules.

The values of w_1 for the butyl paraben and methyl anisate systems are close to that of cetomacrogol in the absence of solubilizate and this may be due to a balance of two factors. Although these solubilizates displace water from the polyoxyethylene/water layer of the micelle, *decreasing* w_1, they may also affect the structure of the micelle, expanding the hydrocarbon core, and *increasing* w_1.

The decane system has a very low hydration. If most of the solubilized decane enters the micellar core, then expansion of the micellar core would result. Provided the structure of the palisade has not been modified, the overall size of the micelle may remain constant. This would result in a decreased volume of the palisade layer and therefore a low degree of hydration.

The site of solubilization

Decane is miscible with the hydrocarbon core of the micelles and the only restriction on the magnitude of the M.A.C. is that which limits the volume of the micelle. This is the ability of the oxyethylene chains to orientate themselves to effectively cover the expanded core. It follows that the M.A.C. of decane is higher in non-ionic detergents than in ionic detergents (McBain & Richards, 1946; Corby & Elworthy, 1971).

Benzene has an appreciable solubility in the oxyethylene layer of the micelle (Corby & Elworthy 1971). which would result in dehydration of this layer and this is probably the limiting factor in the M.A.C. Evidence presented above suggests that only a very small amount of benzene is dissolved in the oxyethylene layer, most being solubilized in the hydrocarbon core. The limit is reached when the core becomes so large that the partially dehydrated oxyethylene–water complex cannot any longer hold the micelle in solution.

p-*Hydroxybenzoic acid and its derivatives.* Whereas the free acid appears to be wholly solubilized in the oxyethylene layer of the micelles, its ethyl ester is solubilized partly in the micelle core and partly in the palisade layer, but with most near to the core-palisade junction. Orientation of ester molecules at this junction is likely so that the ethyl chain is just within the hydrocarbon core. The corresponding butyl ester is similarly solubilized but the proportions of the two esters present at each site are different because of the different polarity of each compound. To account for the greater M.A.C. of the butyl derivative in cetomacrogol solutions, it is suggested that this compound modifies the micellar structure to a much greater extent than ethyl paraben.

Methyl anisate. Some solubilization of methyl anisate takes place within the oxyethylene layer with most present in the micellar core.

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REFERENCES

DONBROW, M. & RHODES, C. T. (1966). *J. Pharm. Pharmac.*, **18**, 424–428.
ERIKSSON, J. C. (1963). *Acta chem. scand.*, **17**, 1478–1481.
ERIKSSON, J. C. & GILLBERG, G. (1966). *Ibid.*, **20**, 2019–2027.
CORBY, T. C. & ELWORTHY, P. H. (1971). *J. Pharm. Pharmac.*, **23**, *Suppl.*, 39S–48S.
MCBAIN, J. W. & RICHARDS, P. H. (1946). *Ind. Engng Chem.*, **38**, 642–645.
MACFARLANE, C. B. (1963). Ph.D. Thesis, University of Glasgow.
RIEGELMAN, S., ALLAWALA, N. A., HRENOFF, M. K. & STRAIT, L. A. (1958). *J. Colloid Sci.*, **13**, 208–217.
TOKIWA, F. (1968). *J. phys. Chem.*, **72**, 1214–1217.

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The analysis of nicotine-1′-*N*-oxide in urine, in the presence of nicotine and cotinine, and its application to the study of *in vivo* nicotine metabolism in man

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A rapid quantitative assay for nicotine-1′-*N*-oxide in urine, in the presence of nicotine and cotinine, is reported. The urinary excretion of nicotine, cotinine and nicotine-1′-*N*-oxide was determined after nicotine had been administered in cigarette smoke, orally, or intravenously to subjects with either fluctuating, controlled acidic or controlled alkaline urinary pH. The urinary excretion of the *N*-oxide in 24 h from smokers under normal conditions was about half that of the cotinine excretion; more *trans*- than *cis*-diastereoisomer of nicotine-1′-*N*-oxide was excreted.

Cotinine is the major basic urinary metabolite of nicotine in man (McKennis, 1965); recently Booth & Boyland (1970) demonstrated nicotine-1′-*N*-oxide in human urine. A quantitative method of analysis for nicotine-1′-*N*-oxide in urine, in the presence of nicotine and cotinine has been developed and used to examine the excretion of cotinine and nicotine-*N*-oxide in man administered nicotine by various routes.

MATERIALS AND METHODS

Materials

(−)-Nicotine (base and hydrogen (+)-tartrate salt) and titanium trichloride solution, technical grade 12·5% w/v $TiCl_3$ in *ca* 15% w/v HCl (total) were obtained from BDH Ltd. (−)-Nicotine-1′-*N*-oxide* was synthesized according to Taylor & Boyer (1959), (−)-nicotine-di-*N*-oxide and (−)-nicotine aryl mono-*N*-oxide according to Johnson, King & Turner (1958), and (−)-cotinine to Bowman & McKennis (1959).

Nicotine and cotinine were extracted and analysed as described by Beckett & Triggs (1966) using a Perkin Elmer F 11 gas liquid-chromatograph with a flame ionization detector.

Determination of the diastereoisomeric (−)-nicotine-1′-N-oxides in urine in the presence of (−)-nicotine and (−)-cotinine

Nicotine-1′-*N*-oxide* was reduced to nicotine with titanium trichloride at room temperature.

Replicate urine samples (5·0 ml) containing added nicotine-1′-*N*-oxide (10·0 μg/ml) were placed in centrifuge tubes and 5N hydrochloric acid (0·2 ml) and titanium trichloride solution (0·2 ml) added (Brooks & Sternglanz, 1959). At intervals between 5 min and 24 h after the addition of titanium trichloride solution the reduction of each sample was stopped by the addition of 5N sodium hydroxide (0·5 ml).

* 7:3 mixture *trans*- and *cis*-diastereoisomers (Beckett, Gorrod & Jenner, to be published).

Replicate urine samples (5·0 ml) containing nicotine-1′-*N*-oxide (10·0 μg/ml) were reduced for 1 h as above and analysed for nicotine by g.l.c. (Beckett & Triggs, 1966). The peak height ratio obtained was compared with that for the theoretically equivalent amount of nicotine added to urine and assayed by the same procedure.

Using this procedure, a calibration curve for nicotine-1′-*N*-oxide between 0·1 and 10·0 μg/ml in urine was constructed. Again, the peak height ratios obtained were compared with those obtained for the theoretical equivalent of nicotine. Replicate urine samples (5·0 ml) containing either nicotine or cotinine (10 μg/ml) were similarly assayed but after a reduction time of 24 h.

*General procedure for the determination of the nicotine-*1′-N-*oxide, nicotine and cotinine content of urine*

Urine samples (4·0 ml) internal marker (1·0 ml; phendimetrazine 10 μg/ml) and 5N sodium hydroxide (0·2 ml) were placed in a centrifuge tube. Any nicotine present was extracted with diethyl ether (3 × 2·5 ml) and assayed by g.l.c. (Beckett & Triggs, 1966). The aqueous layer was washed with diethylether (2 × 2·5 ml) and then 5N hydrochloric acid (0·2 ml) added followed by titanium trichloride solution (0·2 ml). After 1 h, 5N sodium hydroxide (0·5 ml) and the internal marker (1·0 ml) were added and the solution assayed for nicotine as above. Cotinine was then extracted with dichloromethane (3 × 2·5 ml) after the addition of internal standard (1·0 ml; lignocaine 10 μg/ml) and assayed by g.l.c. (Beckett & Triggs, 1966). Blank samples of urine and standard solutions of nicotine-1′-*N*-oxide in urine (10·0 μg/ml) were assayed immediately by the above procedure and at intervals during two weeks.

*Detection of nicotine-*1′-N-*oxide in the urine of a smoker*

A 24 h urine collection from a heavy smoker (40 cigarettes/day) was concentrated to a small volume under vacuum at 70° using a rotary film evaporator and the residue freeze dried. The remaining viscous liquid was streaked across the origin of sheets of Whatmans 3MM paper and run in descending system overnight using n-butanol–n-propanol–2N ammonia (2 : 1 : 1 v/v) as solvent to separate the *cis*- and *trans*-diastereoisomers of nicotine-1′-*N*-oxide (Booth & Boyland, 1970). Reference samples of synthetic nicotine, cotinine, nicotine-1′-*N*-oxide, nicotine-di-*N*-oxide and nicotine aryl mono-*N*-oxide were also run. After elution, the papers were examined under ultraviolet light (Hanovia CHI 291) and compounds located by either Dragendorff or Iodoplatinate spray reagent (Smith, 1960). Papers were cut into strips according to the fluorescent bands present. These were shredded and then eluted with methanol containing 3% v/v ammonia (S.G. 0·880). The eluates were evaporated to dryness under vacuum at 40° using a rotary film evaporator. The residue was dissolved in distilled water (4·0 ml) and assayed for *N*-oxide metabolites.

Trials

The subjects were healthy males between 20 and 40 years. Smokers ceased smoking at least 36 h before the trial and the analysis of a "blank" sample of urine was used to demonstrate the absence of nicotine, nicotine-1′-*N*-oxide and cotinine.

Control of urinary pH. Urinary pH was maintained acidic (pH 4·8 ± 0·2) by prior administration of enteric coated ammonium chloride tablets (Beckett & Tucker, 1966). An alkaline urinary pH was maintained by an equivalent dosage regimen of sodium bicarbonate in aqueous solution.

Urine collection. Urine samples were collected at 30 min intervals for 4 h after drug administration, at 60 min intervals for a further 8 h, then at will until a final sample was collected at 24 h, unless otherwise stated. The pH and volume of each sample was measured immediately and all samples were stored at 4° until analysed.

Oral trials. (−)-Nicotine hydrogen (+)-tartrate in aqueous solution (6·14 mg ≡ 2·0 mg base) was swallowed by subjects with acidic, alkaline or fluctuating urinary pH.

Intravenous trials. Subjects, with controlled acidic urinary pH, were injected intravenously with (−)-nicotine hydrogen (+)-tartrate (3·07 mg ≡ 1·0 mg base) in sterile aqueous solution (5·0 ml) over 5 min.

Smoking trials. These involved three separate experiments. (i) A subject with controlled acidic urinary pH smoked one cigarette normally. (ii) The same subject, with fluctuating urinary pH, smoked a cigarette normally every half-hour during the course of two working days and urine samples were collected hourly for 36 h. (iii) Subjects with fluctuating urinary pH were allowed to smoke normally and 24 h urine samples were collected starting after the first passage of urine of the morning and continuing until the first passing of urine next morning.

RESULTS AND DISCUSSION

Nicotine-1′-*N*-oxide in urine was reduced quantitatively by titanium trichloride in 5 min at room temperature; a reduction time of up to 24 h did not reduce nicotine or cotinine. A reduction time of 1 h was adopted for the general procedure. Neither constituents in urine nor storage of urine led to interference with the assay procedure. The standard error (s.e. = ±2 standard deviations) of analysis results of replicate samples of nicotine-*N*-oxide in urine was $< \pm 4\%$; that over the calibration range for nicotine-1′-*N*-oxide was ±9·0% and that for nicotine and cotinine, ±5·0%.

Chromatograms of smokers' urine showed two bands corresponding to *cis*- and *trans*-nicotine-1′-*N*-oxide (Booth & Boyland, 1970) (R_F 0·42 and 0·51 respectively)* (Table 1A); more *trans*- than *cis*-diastereoisomer was indicated by visual inspection. Bands corresponding to nicotine (R_F 0·95) and cotinine (R_F 0·82) were also observed. No colour reactions were obtained in the regions where nicotine-di-*N*-oxide or nicotine-aryl-mono-*N*-oxide were located (Table 1A). Elution of bands 6, 7, 8, which span R_F values covering *cis*- and *trans*-nicotine-1′-*N*-oxide, followed by reduction,

Table 1. *R_F values of* (A) *nicotine and some possible metabolites and* (B) *fluorescent bands observed on chromatograms of concentrated smokers urine on Whatman 3MM paper using n-butanol, n-propanol* 2N *ammonium hydroxide* 2:1:1 (v/v) *as developing solvent.*

A		B	
Compound	R_F	Strip No.	R_F of leading edge
Nicotine di-*N*-oxide	0·20	1	0·04
cis-Nicotine-1′-*N*-oxide	0·42	2	0·17
trans-Nicotine-1′-*N*-oxide	0·51	3	0·22
Nicotine aryl mono-*N*-oxide	0·77	4	0·30
Cotinine	0·82	5	0·34
Nicotine	0·95	6	0·41
		7	0·42
		8	0·46
		9	0·53
		10	1·0

* *cis*-Nicotine-1′-*N*-oxide denotes *cis*-(methyl/pyridyl).

gave nicotine (Table 1B). Band 8 (R_F 0·46–0·53) which represented primarily *trans*-nicotine-1′-*N*-oxide gave more nicotine on reduction than did bands 6 and 7 (R_F 0·41–0·46) which represented the R_F value of the *cis*-diastereoisomer with some overlap of the *trans*-band. No other bands contained a substance reduceable to nicotine. Thus more *trans*- than *cis*-nicotine-*N*-oxide is excreted in human urine in agreement with the results of Booth & Boyland (1970).

The urinary excretion of nicotine, after oral administration, was pH dependent, that of cotinine slightly pH-dependent but also volume-dependent, while that of nicotine-1′-*N*-oxide was independent of urinary pH and volume—as expected for a highly polar water-soluble compound (Fig. 1 and Table 2). The rate of urinary

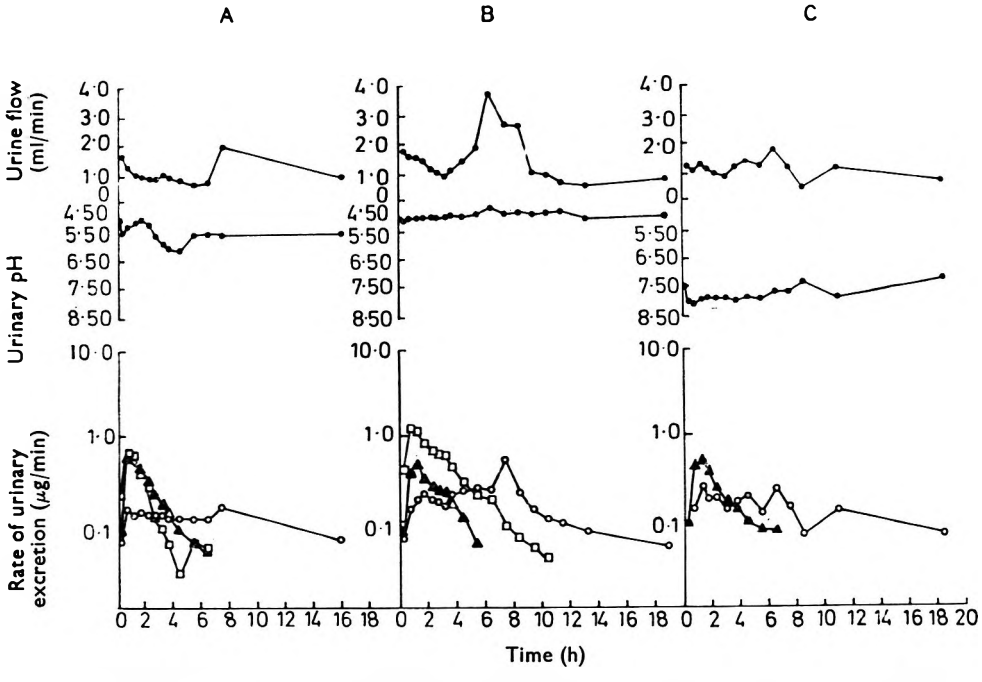

FIG. 1. Urinary excretion of nicotine, cotinine and nicotine-1′-*N*-oxide after oral administration of nicotine (2 mg) to a subject with (A) fluctuating, (B) controlled acidic and (C) controlled alkaline urinary pH. No nicotine excretion under alkaline conditions. □ Nicotine. ▲ Nicotine-1′-*N*-oxide. ○ Cotinine.

Table 2. *The urinary recoveries of nicotine, cotinine and nicotine-*1′-N-*oxide after oral and intravenous administration of nicotine.*

Route and dose	Subject	Urinary pH	% urinary recovery in 24 h		
			Nicotine	Cotinine	Nicotine-1-*N*-oxide
Oral	1	Uncontrolled	3·8	6·3	4·1
2 mg		Acidic	11·4	9·4	3·6
		Alkaline	0	7·9	4·1
	2	Uncontrolled	0·9	5·1	3·0
		Acidic	11·5	6·3	3·0
		Alkaline	—	—	—
Intravenous	1	Acidic	34·8	21·6	4·2
1 mg	2	Acidic	35·5	20·8	3·8
	3	Acidic	35·4	11·2	3·8

excretion of cotinine was lower than that of nicotine and of nicotine-1'-*N*-oxide but declined more slowly; the time for the cotinine excretion to fall to half its original value was 7 h compared with 2 h for nicotine and its *N*-oxide. Nicotine-1'-*N*-oxide excretion, under acidic conditions, was complete 6 h after nicotine administration and its peak rate of excretion and excretion profile was parallel to that of nicotine (Fig. 1A,B), while the cotinine excretion profile showed no distinct peak.

Table 3. *The urinary recoveries of nicotine, cotinine and nicotine-1'-N-oxide from smokers 24 h urine samples (fluctuating urinary pH).*

Subject	Recovery from 24 h urine sample (mg)			Ratio
	Nicotine	Cotinine	*N*-oxide	Cotinine/*N*-oxide
1	2·52	5·01	2·46	2·1 : 1
2	3·09	2·76	1·20	2·3 : 1
3	2·72	1·72	0·9	1·9 : 1
4	0·24	0·39	0·11	3·8 : 1
5	1·95	1·46	0·62	2·4 : 1
6	5·32	2·09	2·78	0·75 : 1
7	4·75	2·45	1·03	2·4 : 1
8	1·54	1·74	0·84	2·1 : 1

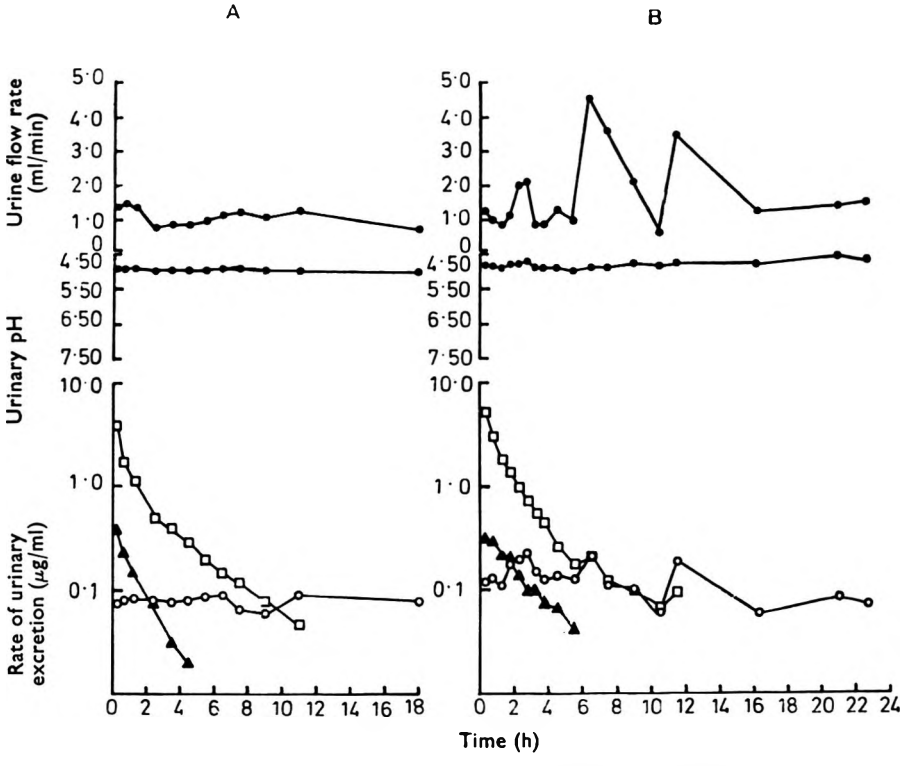

FIG. 2. Urinary excretion of nicotine, cotinine and nicotine-1'-*N*-oxide under conditions of controlled acidic urinary pH after (A) i.v. administration of nicotine (1 mg) and (B) smoking one cigarette. □ Nicotine. ▲ Nicotine-1'-*N*-oxide. ○ Cotinine.

With uncontrolled urinary pH, the urinary recovery of nicotine was greatly reduced (Fig. 1A) and under alkaline conditions was abolished (Fig. 1C). Urinary cotinine recoveries were altered only slightly by changes of urinary pH and *N*-oxide recoveries were unaffected even though, because of kidney tubular reabsorption, more extensive nicotine metabolism occurred than under acidic urine conditions.

A higher recovery of unchanged nicotine was obtained after intravenous nicotine administration than after oral administration but the recovery of nicotine-1′-*N*-oxide was unaffected (Table 2). Recoveries obtained after intravenous administration of nicotine, however, show inter-subject variation (Beckett, Gorrod & Jenner, 1971). After intravenous administration of nicotine the peak rate of excretion of nicotine and nicotine-1′-*N*-oxide occurred within 15 min; the excretion profiles of nicotine, cotinine and nicotine-1′-*N*-oxide had similar characteristics to those described after oral administration.

Similar urinary recoveries and excretion profiles of nicotine and its metabolites to those observed after intravenous administration have already been shown after rectal nicotine administration (Beckett, Gorrod & Jenner, 1970).

The urinary recovery of nicotine-1′-*N*-oxide, under conditions of acidic urinary pH, from a subject who smoked one cigarette, was small compared to that of nicotine

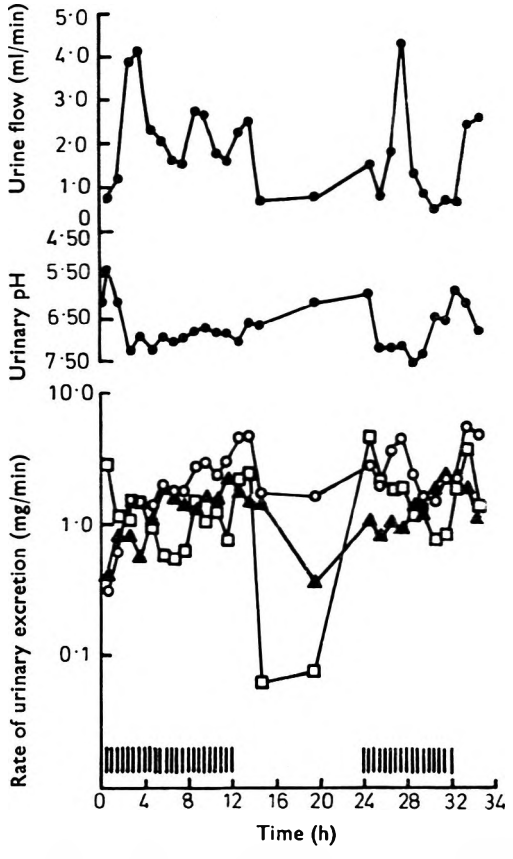

FIG. 3 The urinary excretion of nicotine, cotinine and nicotine-1′-*N*-oxide under conditions of normal fluctuating urinary pH in a subject smoking at regular intervals. □ Nicotine. ▲ Nicotine-1′-*N*-oxide. ○ Cotinine.

and cotinine (Fig. 2B). However, when one cigarette was smoked every 0·5 h for two working days, under conditions of fluctuating urinary pH, nicotine-1'-*N*-oxide was present in the urine in significant amounts (Fig. 3). Urinary recoveries and excretion profiles of nicotine, nicotine-1'-*N*-oxide and cotinine after intravenous nicotine and cigarette smoke are similar (cf. Fig. 2A and 2B; see also Triggs, 1967).

The amount of nicotine-1'-*N*-oxide excreted by smokers in 24 h under normal conditions was approximately half that of the cotinine excreted (Table 3). Inter-subject variations in the ratio of cotinine to nicotine-1'-*N*-oxide were small. *N*-Oxidation under these conditions, is an important route of metabolism of nicotine.

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REFERENCES

Beckett, A. H., Gorrod, J. W. & Jenner, P. (1970). *J. Pharm. Pharmac.*, **22**, 722.
Beckett, A. H., Gorrod, J. W. & Jenner, P. (1971). *Ibid.*, **23**. In the press.
Beckett, A. H. & Triggs, E. J. (1966). *Nature, Lond.*, **211**, 1415–1417.
Beckett, A. H. & Tucker, G. T. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 72S–75S.
Booth, J. & Boyland, E. (1970). *Biochem. Pharmac.*, **19**, 733–742.
Bowman, E. R. & McKennis, H. Jr. (1959). *Biochem. Prep.*, **10**, 31–38.
Brooks, R. T. & Sternglanz, P. O. (1959). *Analyt. Chem.*, **31**, 561–565.
Johnson, A. W., King, T. J. & Turner, J. R. (1958). *J. chem. Soc.*, 3230–3231.
McKennis, H. Jr. (1965). *Tobacco Alkaloids and Related Compounds:* 4th Wenner-Gren Centre International Symposium, **4**. Editor: Euler, U. S. von. Oxford: Pergamon.
Smith, I. (1960). *Chromatographic & Electrophoretic Techniques*, **1**. London: Heinemann.
Taylor, E. C. & Boyer, W. E. (1959). *J. org. Chem.*, **24**, 275–277.
Triggs, E. J. (1967). Ph.D. Thesis, University of London.

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The effect of smoking on nicotine metabolism *in vivo* in man

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Nicotine and a basic metabolite, cotinine, were determined in the urine by gas–liquid chromatography after intravenous administration of (−)-nicotine hydrogen (+)-tartrate to groups of male and female smokers and non-smokers in whom the urine was maintained at an acid pH. The urinary recoveries of nicotine and cotinine from male smokers fell in two groups. One showed a lower recovery of both alkaloids than was seen with male non-smokers. The other showed a similar recovery of nicotine but more cotinine than the male non-smokers. Female smokers excreted less nicotine but more cotinine than female non-smokers. More nicotine but less cotinine was excreted by female non-smokers than by male non-smokers. The results show sex dependent metabolism of nicotine occurs in non-smoking humans and that smoking causes alterations in nicotine metabolism.

The nature of tolerance to nicotine and tobacco smoking has received much attention (Dixon & Lee, 1912; Edmunds & Smith, 1915; Werle & Uschold, 1948; Yamamoto, Nagai & others, 1966; Wenzel & Broadie, 1966; Welch, Harrison & others, 1968, 1969; Stalhandske & Slanina, 1970; Ruddon & Cohen, 1970); evidence for the induction of hepatic microsomal enzymes as an explanation is equivocal since reports of inhibition as well as induction have occurred.

While it is known that there are sex differences in the drug metabolizing activity of rats and mice (Quinn, Axelrod & Brodie, 1968; Booth, 1966, 1967), evidence in men is lacking.

The observations of Beckett & Triggs (1967) have now been extended by an analysis of urine for nicotine and cotinine and an examination of the different nicotine metabolism between the sexes.

METHODS

Nicotine and cotinine were extracted and analysed by the method of Beckett & Triggs (1966) using a Perkin Elmer F11 gas chromatograph with a flame ionization detector. Phendimetrazine (Ayerst, McKenna & Harrison Ltd.) replaced chlorphentermine as the internal standard for nicotine (Rt nicotine 2·0, phendimetrazine 3·8 min). Lignocaine was used as the internal standard for the cotinine determination (Rt cotinine 6·0, lignocaine 4·2 min).

Sterile ampoules containing 3·07 mg (−)-nicotine hydrogen (+)-tartrate (BDH Ltd.) in 5 ml water (equivalent to 1 mg of nicotine base), were prepared. Randomly selected ampoules were assayed for nicotine content by gas–liquid chromatography.

Male and female volunteer smokers and non-smokers were injected intravenously with 5 ml of the above nicotine hydrogen tartrate solution: the urine of all subjects

was controlled at pH 4·3 ± 0·2 (Beckett & Tucker, 1966). Smokers ceased smoking at least 36 h before the injection and the analysis of a "blank" sample of urine before injection was used to demonstrate the absence of nicotine and cotinine. The injections were given over 5–10 min and the subjects reaction noted. Urine samples were collected at 30 min intervals for 4 h at 60 min intervals for a further 8 h, then at will until a final sample was collected at 24 h. The pH and volume of each sample was measured immediately using a pH meter and all samples were stored at 4° until analyses were complete. Duplicate experiments were carried out in two male smokers and one male non-smoker at intervals from 3 days to 15 months. Experiments where the pH of the urine was not controlled were carried out with two male non-smokers (Subjects 1 and 3). Nicotine was determined in each urine sample, cotinine was determined only in bulked 24 h samples.

RESULTS AND DISCUSSION

When the urine is adjusted to an acid pH the excretion of cotinine but not of nicotine is affected by changes in urinary output (Triggs, 1967). Although water-loading of the subject abolishes this effect, the resultant low concentrations of nicotine preclude its accurate measurement. In the present work, therefore, pH control without water-loading has been used and as a result cotinine recoveries exhibit greater inter- and intra-subject variation than recoveries for nicotine. Amounts of cotinine recovered in 24 h (*ca* 1600 ml urine) of less than 50 μg being too small to be measured accurately have been recorded as >5% cotinine in Table 1.

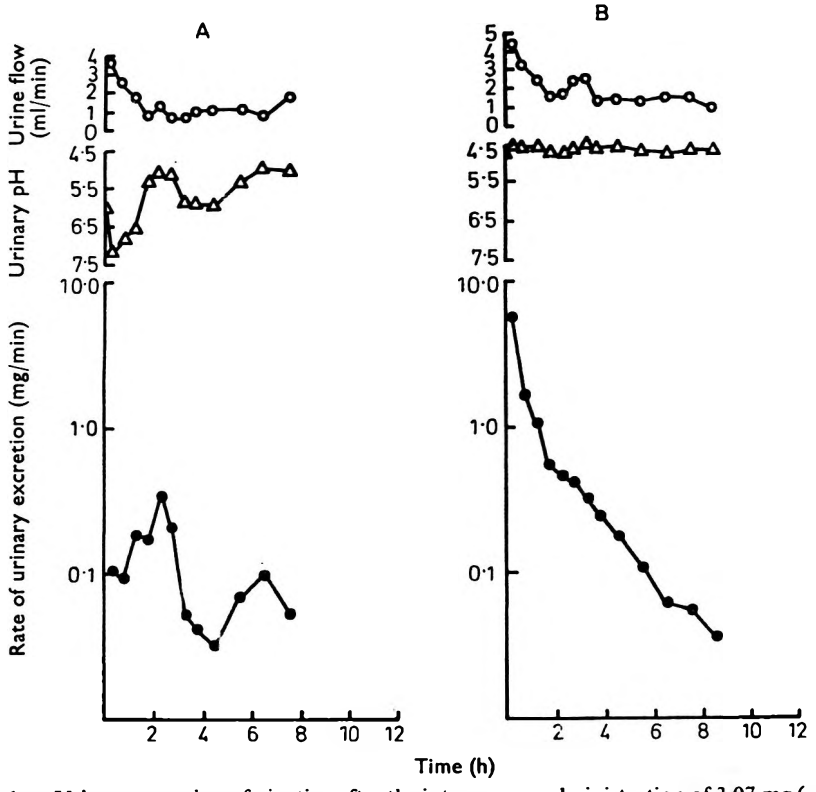

FIG. 1. Urinary excretion of nicotine after the intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate to a subject with, (A) fluctuating and (B) acidic controlled urinary pH.

Table 1. *Urinary recoveries of nicotine and cotinine from male and female smokers and non-smokers, with fluctuating or acidic urinary pH, after intravenous administration of* 3·07 *mg* (−)-*nicotine hydrogen* (+)-*tartrate.*

Subject	Age	Approximate number of cigarettes smoked daily	% Nicotine excretion in 12 h		% Cotinine excretion in 24 h		Urine volume 24 h
Male non-smokers							
1	23	—	31·0		ND		2822
1*	23	—	31·8		11·2		1497
2	34	—	31·7		7·3		3945
3	23	—	31·0		8·1		1701
4	23	—	35·5	Av.	ND	Av.	1683
5	51	—	36·8	33·5	ND	9·2	1754
6	22	—	32·1		ND		1394
7	23	—	35·5		ND		1386
8	22	—	34·5		9·2		1666
9	24	—	35·4		10·1		1180
1‡	23	—	4·8		<5		2132
3‡	23	—	4·7		<5		1846
Male smokers (low recovery)							
1	24	40	21·6		<5		1625
2	23	10	21·2		<5		1752
3	26	Pipe smoker	21·2	Av.	ND	Av.	1464
4	48	20	24·0	22·5	<5	<5	1426
5	24	30	23·6		ND		2170
6	24	30	23·1		<5		2470
Male smokers (high recovery)							
1	35	15	30·1		21·3		1384
1*	35	15	32·0		27·7		1353
2	40	40	34·8		21·6		1550
2*	40	40	35·5	Av.	20·8	Av.	1275
3	25	25	32·8	34·4	24·5	25·3	1537
4	21	25	34·8		24·5		1514
5	21	20	38·1		31·3		2935
6	23	20	37·4		30·4		1740
Female non-smokers							
1	22	—	44·0		<5		1890
2	23	—	49·3	Av.	<5	Av.	1969
3	22	—	44·7	45·4	<5	<5	1455
4	28	—	43·5		<5		2863
Female smokers							
1	23	20	34·0		22·5		1726
2	21	15	40·5	Av.	35·2	Av.	3408
3	23	25	37·3	35·9	23·4	26·4	982
4	21	15	38·4		24·5		1943
5	23	15	29·3		ND		1655

* Repeat after 15 month interval.
‡ Fluctuating urinary pH.

pH dependent fluctuations in the urinary excretion of nicotine under normal conditions (Fig. 1a) disappear when the urine is maintained at an acid pH (Fig. 1b) and a higher recovery of unchanged nicotine is then obtained (Beckett & Triggs, 1967) (Table 1). The elimination of nicotine does not appear to be a single first order process.

Typical urinary excretion patterns of male and female smokers and non-smokers (Fig. 2) show no significant differences in the time taken to eliminate half of the nicotine dose. The differences in nicotine recoveries arise from differences in the

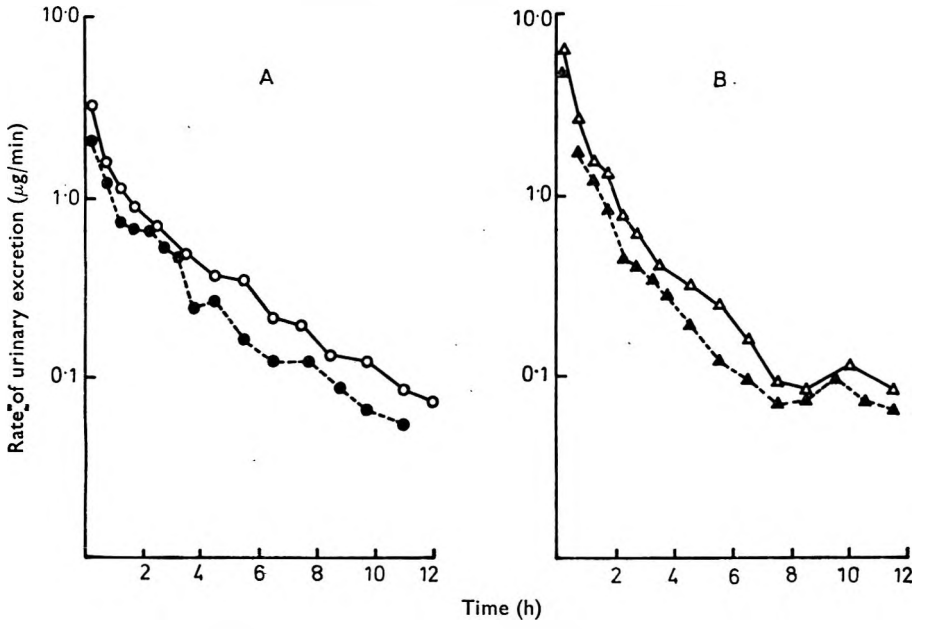

FIG. 2. Typical urinary excretion patterns of nicotine from (A) a male and (B) a female smoker and non-smoker after intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate under conditions of controlled acidic urinary pH. Open points—non-smokers. Closed points—smokers.

rate of excretion (μg/min) at any given time. The peak rate of nicotine excretion occurs within the first 30 min after injection; excretion is complete within 12 h in all groups.

Duplicate experiments in three male subjects—two smokers and one non-smoker—at intervals from three days to 15 months showed no variation in the urinary excretion pattern or in the percentage recoveries of nicotine and cotinine. This suggests that the enzyme activity in the individual is little changed over a long period.

None of the smokers reported any nausea from the nicotine injections; this was reported in varying degrees by all non-smokers, thus indicating some degree of tolerance to nicotine among smokers.

The urinary recoveries of nicotine and cotinine obtained after intravenous administration of nicotine to subjects with acidic urinary pH (Table 1; Fig. 3) show that subjects may be divided into distinct groups.

The urinary recoveries of nicotine and cotinine from male smokers fall in two groups. One showed a lower recovery of both alkaloids than was seen with male non-smokers (low recovery group). The other showed a similar recovery of nicotine but more cotinine (high recovery group) than male non-smokers. Female smokers excreted less nicotine but more cotinine than female non-smokers and gave a higher combined recovery of these compounds.

Possibly tobacco smoke may have caused the induction of nicotine and continine metabolism in "low recovery" male smokers. On the other hand, the results for "high recovery" male smokers indicates that smoking has either inhibited the metabolism of cotinine or emphasized the cotinine route of metabolism at the expense of an alternative route of nicotine metabolism.

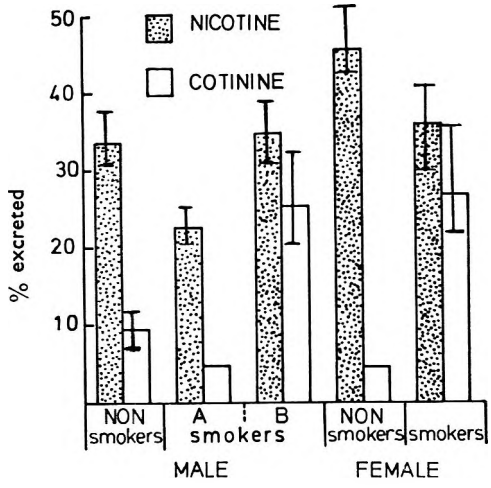

FIG. 3. Comparison of mean urinary recoveries of nicotine and cotinine in male and female smokers and non-smokers after the intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate. A, low recovery B, high recovery groups.

Smoking in females apparently caused increased total metabolism of nicotine and either increased cotinine formation or inhibition of the further metabolism of cotinine. In neither male nor female smokers is there a correlation between the urinary recoveries of nicotine and cotinine and the approximate number of cigarettes smoked daily before the trials were carried out.

The complex picture of human nicotine metabolism is in accord with many previously reported data where pretreatment with nicotine, tobacco smoke or other xenobiotics has led to either induction or inhibition of nicotine metabolism (or both) under similar experimental conditions (see Stalhandske & Slanina, 1970). However, tobacco smoke is a known inhibitor of many enzyme systems (Benedict & Stedman, 1968; Sato, Suzuki & Fayuyama, 1962; Schievelbein, 1967; Schievelbein, Werle & others, 1969) including dehydrogenases and an oxygenase, so that inhibition of the further metabolism of cotinine or the formation of nicotine-1′-*N*-oxide, recently observed as a metabolite of nicotine in man (Booth & Boyland, 1970), is possible.

Male non-smokers excrete less nicotine but more cotinine than female non-smokers (Fig. 3) showing that sex-dependent metabolism of nicotine occurs in humans. A comparison of nicotine metabolism in male and female smokers is complicated by the metabolic changes induced by smoking and the validity of such a comparison is dubious.

Acknowledgements

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REFERENCES

BECKETT, A. H. & TRIGGS, E. J. (1966). *Nature, Lond.*, **211**, 1415–1417.
BECKETT, A. H. & TRIGGS, E. J. (1967). *Ibid.*, **216**, 587.
BECKETT, A. H. & TUCKER, G. T. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 72*S*–75*S*.

BENEDICT, R. C. & STEDMAN, R. L. (1968). *Experientia*, **24**, 1205–1206.
BOOTH, J. (1966). *Brit. Emp. Cancer Campaign for Res. Ann. Report.*
BOOTH, J. (1967). *Ibid.*
BOOTH, J. & BOYLAND, E. (1970). *Biochem. Pharmac.*, **19**, 733–742.
BOWMAN, E. R. & MCKENNIS, H. Jr. (1959). *Biochem. Prep.*, **10**, 36–38.
DIXON, W. E. & LEE, W. E. (1912). *Q. Jl exp. Physiol. (Lond.)*, **50**, 373–383.
EDMUNDS, C. W. & SMITH, M. I. (1915). *J. Lab. clin. Med.*, **1**, 315–321.
QUINN, G. P., AXELROD, J. & BRODIE, B. B. (1958). *Pharmacologist*, **1**, 152–159.
RUDDON, R. W. & COHEN, A. M. (1970). *Toxic. appl. Pharmac.*, **16**, 613–625.
SATO, T., SUZUKI, T. & FUYUYAMA, T. (1962). *Br. J. Cancer*, **16**, 7–15.
SCHIEVELBEIN, H. (1967). *Abst. 1st Eur. Cancer Meeting*, Vienna, 50.
SCHIEVELBEIN, H., WERLE, E., SCHULZ, E. K. & BAUMEISTER, R. (1969). *Arch. exp. Path. Pharmak.*, **262**, 358–365.
STALHANDSKE, T. & SLANINA, P. (1970). *Acta pharmac. tox.*, **28**, 75–80.
TRIGGS, E. J. (1967). PhD. Thesis, University of London.
WELCH, R. M., HARRISON, Y. E., CONNEY, A. H., POPPERS, P. J. & FINSTER, M. (1968). *Science N.Y.*, **160**, 541–542.
WELCH, R. M., HARRISON, Y. E., GOMMI, B. W., POPPERS, P. J., FINSTER, M. & CONNEY, A. H. (1969). *Clin. Pharmac. Ther.*, **10**, 100–109.
WENZEL, D. G. & BROADIE, L. L. (1966). *Toxic. appl. Pharmac.*, **8**, 455–459.
WERLE, E. & USCHOLD, E. (1948). *Biochem. Z.*, **318**, 531–537.
YAMAMOTO, I., NAGAI, K., KIMURA, H. & IWATSUBO, K. (1966). *Jap. J. Pharmac.*, **16**, 183–190.

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A new buccal absorption model

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Buccal absorption tests indicate that loss of drug from the oral cavity cannot be accounted for solely in terms of passive diffusion into the buccal membrane. A model involving protein-binding is proposed, which satisfactorily explains the observed loss. Studies on two different physical simulations of buccal absorption confirm that the proposed model is consistent with the *in vivo* results.

A simple method of measuring the extent of buccal absorption (Beckett & Triggs, 1967) has led to the development of two models to describe buccal absorption (Beckett, Boyes & Triggs, 1968; Beckett & Moffat, 1970). Our studies of the physico-chemical properties of analgesics have suggested a reassessment of their models.

METHODS

Buccal absorption was measured by a modification of the method of Beckett & Triggs (1967). A solution containing drug (1 mg) in Clark and Lubs 0·2M phosphate buffer (pH 7·2) (25 ml) was circulated round the mouth 60 times/min for a given time, then expelled. The mouth was rinsed with buffer solution (pH 7·2) (10 ml), and the two solutions combined and made up to 100 ml with buffer, then filtered. The drug was extracted from a 5 ml aliquot with 3 × 3 ml ether. After evaporation of solvent, the residue in absolute ethanol (5 ml) was determined spectrophotometrically. This extraction procedure was satisfactory up to a drug concentration of about 6 mg in 25 ml. The minimal period between successive tests, for satisfactory repeat values to be obtained, was about 15 min after a 5 min contact time, and 50 min after a 10 min contact time.

Intra-subject variations are smaller than inter-subject variations (Beckett & Moffat, 1968): a single subject was therefore used in the buccal absorption tests. Each test was performed in duplicate, both solutions being analysed three times; each result is thus the mean of six values. Changes of pH during the test were never more than 0·2 pH units.

Physical simulation

(a) *Interface diffusion system.* The method of Perrin (1967) was modified (Fig. 1). Compartment A (995 ml) represents the oral cavity, and initially contained an appropriate concentration of "drug" (*p*-methylacetanilide was used) in 0·2M Clark and Lubs buffer (pH 7·2). Compartment B (475 ml), representing the buccal membrane, contained 1-octanol, and compartment C (480 ml), representing body fluids, contained 0·2M hydrochloric acid. The use of equimolar solutions in compartments A and C precluded the setting up of an osmotic gradient. Compartment D (100 ml) (which may be taken as representing protein-binding) also contained 1-octanol. Before use the aqueous phases were saturated with 1-octanol, and the 1-octanol with aqueous buffer (pH 7·2).

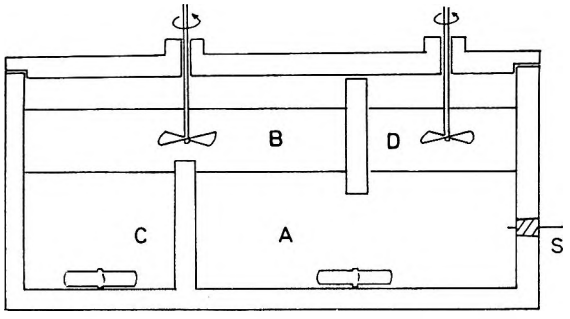

FIG. 1. Interface diffusion system used to simulate buccal absorption. The aqueous layers were stirred magnetically, the 1-octanol layers mechanically. Construction of the box was of 6 mm Perspex, the external dimensions being 24 × 11 × 14·5 cm high.

The test temperature was 22 ± 1°. Samples were withdrawn from and returned to compartment A with a syringe through a butyl rubber plug (S), and were analysed on a Unicam SP.500 spectrophotometer.

(b) *Hydraulic flow system.* The apparatus of Rowe & Morozowich (1969) was modified as shown in Fig. 2. Compartment E (100 ml) represents the oral cavity, and initially contained a solution of "drug" of appropriate concentration: salicylic acid was used, loss of which from E occurred *via* G to a spectrophotometer, whilst E was steadily replenished with water *via* H. Compartment F (100 ml), representing a compartment (such as protein-binding) tending to equilibrium with the oral cavity, initially contained water. The flow-rate of each pump was 35 ml min^{-1}. Spectrophotometric measurements were made on a Unicam SP.800 spectrophotometer, using a flow-through cell.

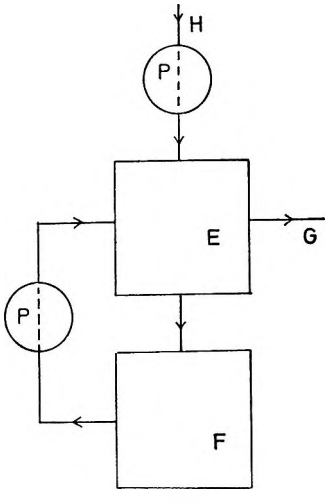

FIG. 2. Hydraulic flow system used to simulate buccal absorption. Peristaltic pumps (P) were used to produce the required flow.

RESULTS AND DISCUSSION

Inspection of the data of Beckett & Moffat (1968, 1970) indicates that the plot of buccal absorption against time is more sharply curved than can be accounted for by their two-compartment model, which invokes only first-order passive diffusion from

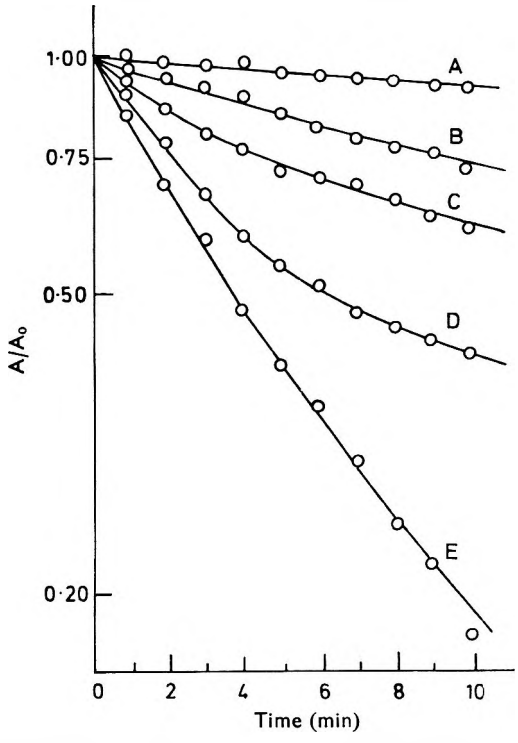

FIG. 3. The buccal absorption of a number of *p*-substituted acetanilides. The substituents are: (A) $-NH_2$; (B) $-OH$; (C) $-H$; (D) Cl; (E) I. A/A_0 is the fraction of drug unabsorbed, corrected for the effect of dilution on diffusion into a membrane (Dearden & Tomlinson, 1971). Over the time scale of a normal buccal experiment (5 min) the effect is small enough to be ignored.

oral cavity to buccal membrane. Inspection of our own results (Fig. 3) confirms that neither this nor a two-compartment model involving reversibility can adequately represent buccal absorption, since such a model must involve equilibrium. Our own and Beckett & Moffat's results show that this does not occur and a model involving at least three compartments must therefore be used.

The curvature of the results in Fig. 3 means either that saturation of the membrane is being approached or that drug is returning to the oral cavity. At the concentration of drug used, the former is unlikely (Beckett & Triggs, 1967), whereas support for the latter viewpoint is given by the observation that *some* drug may be recovered by rinsing the mouth after contact with the drug solution (Beckett, Boyes & Triggs, 1968). It may thus be inferred that the reversible step is between the oral cavity and an adjacent compartment.

In our view, this compartment is probably protein, which binds the drug. Rectilinear correlations between log (protein-binding equilibrium constant) and log (partition coefficient) for a wide variety of drugs have been reported by Penniston, Beckett & others (1969), and these authors point out that in considering the penetration of a molecule to its site of action, not only must passage through membranes be examined, but also adsorption to and desorption from macromolecules. Thus partition of a drug in the mouth may well involve its binding to protein.

Two arguments have been invoked against protein-binding in buccal absorption (Beckett & Triggs, 1967) (although Beckett & Moffat (1971) propose protein-binding

to explain the buccal absorption of some barbiturates)—firstly, that there is lack of stereoselectivity in buccal absorption: however, many proteins are non-stereoselective in any ligand binding (cf. Tucker, Boyes & others, 1970; Hansch, Steward & others, 1968).

Secondly, a rectilinear relation has been claimed between % drug absorbed and concentration, not only for single substances but also for mixtures of up to eight drugs. Examination of the data of Beckett & Triggs (1967), however, suggests that the curvature observed in the above relation is more consistent with some measure of protein-binding.

Three possible models of buccal absorption were therefore considered:

(i) oral cavity $\rightleftharpoons$ membrane $\rightarrow$ body fluids

(ii) oral cavity $\rightleftharpoons$ protein-binding $\rightarrow$ membrane $\rightarrow$ body fluids

(iii) protein-binding $\rightleftharpoons$ oral cavity $\rightarrow$ membrane $\rightarrow$ body fluids.

Model (i) is unlikely, because although reversibility between the oral cavity and the buccal membrane is possible, it cannot occur to any significant extent during absorption of compounds with reasonably high partition coefficients, provided that the membrane is not saturated. For example, Perrin (1967), using a physical model of a membrane comprising aqueous buffer (pH 2)–30% decanol in cyclohexane–aqueous buffer (pH 7·4), has shown that salicylic acid is lost from the acidic buffer solution according to a simple first-order non-reversible process.

Model (ii) (cf. Beckett, Boyes & Triggs, 1968) is tenable only if active transport of the drug by the protein, from the outer surface of the membrane to the lipid bilayer, is involved. Since this generally involves a specific binding mechanism, it is unlikely to be significant in buccal absorption, where many different types of compound are readily absorbed by the buccal membrane. Active transport is also a non-equilibrium process, and it has already been shown that reversibility must be involved in buccal absorption: drug must therefore be returned to free solution, or to a state approxi-

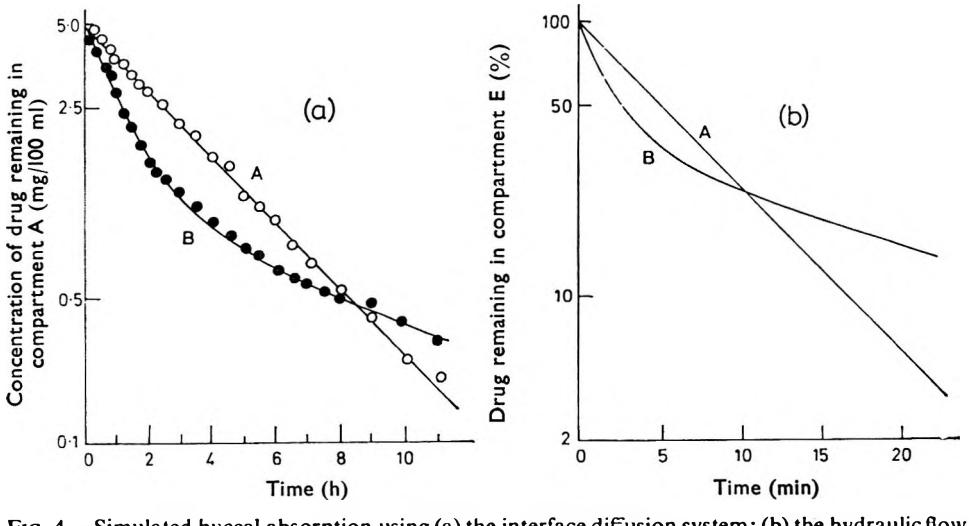

FIG. 4. Simulated buccal absorption using (a) the interface diffusion system; (b) the hydraulic flow system. Curves A are in the absence of, and curves B in the presence of, the compartments representing protein-binding.

mating it, before it can diffuse into the lipid bilayer. Thus model (iii) appears to us to be the best representation of buccal absorption. Other compartments, such as protein-binding *within* the membrane, may exist, but, because they are well-removed from the oral cavity, would influence but little the loss of drug from the oral cavity. Their chief effect would probably be to increase the capacity of the membrane to accept drug from the oral cavity.

Although the protein-binding proposed in model (iii) is not directly involved in buccal absorption, it does serve to concentrate the drug at or near the surface of the buccal membrane, and so increases the rate of absorption.

That our model gives results consistent with the buccal absorption results is shown by the behaviour of the two physical simulators of membrane processes. Fig. 4 shows the results obtained from both simulators. With each, first-order loss of "drug" occurs in the absence of that part of the system representing protein-binding. Inclusion of the "protein-binding" compartment results, in each case, in a more rapid initial loss of "drug", followed by a decrease in the loss rate as "drug" returns to the "oral cavity" from the "protein-binding" compartment. Non-rectilinear plots, similar to those shown in Fig. 3, are thus obtained.

The two physical simulators thus confirm that our proposed model of buccal absorption is entirely consistent with the results of buccal absorption tests.

Acknowledgements

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REFERENCES

BECKETT, A. H., BOYES, R. N. & TRIGGS, E. J. (1968). *J. Pharm. Pharmac.*, **20**, 92–97.
BECKETT, A. H. & MOFFAT, A. C. (1968). *Ibid.*, **20**, *Suppl.*, 239*S*–247*S*.
BECKETT, A. H. & MOFFAT, A. C. (1970). *Ibid.*, **22**, 15–19.
BECKETT, A. H. & MOFFAT, A. C. (1971). *Ibid.*, **23**, 15–18.
BECKETT, A. H. & TRIGGS, E. J. (1967). *Ibid.*, **19**, *Suppl.*, 31S–41S.
DEARDEN, J. C. & TOMLINSON, E. (1971). *J. pharm. Sci.* **60**, 1278—1279.
HANSCH, C., STEWARD, A. R., ANDERSON, S. M. & BENTLEY, D. (1968). *J. mednl Chem.*, **11**, 1–11.
PENNISTON, J. T., BECKETT, L., BENTLEY, D. L. & HANSCH, C. (1969). *Molec. Pharmac.*, **5**, 333–341.
PERRIN, J. (1967). *J. Pharm. Pharmac.*, **19**, 25–31.
ROWE, E. L. & MOROZOWICH, W. (1969). *J. pharm. Sci.*, **58**, 1375–1378.
TUCKER, G. T., BOYES, R. N., BRIDENBAUGH, P. O. & MOORE, D. C. (1970). *Anesthesiology*, **33**, 287–303.

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Buccal absorption as a parameter of analgesic activity of some *p*-substituted acetanilides

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The extent of buccal absorption of 16 *p*-substituted acetanilides is related parabolically to analgesic activity. The correlation is slightly better than that between log (partition coefficient) and analgesic activity. Thus, for this series of compounds at least, an *in vivo* partition test provides a slightly better parameter of biological response than does an *in vitro* test.

The effectiveness of sublingual administration of a drug was implied by Walton (1944) to be a function of the ability of the drug to penetrate the buccal mucosa. Recently, following the work of Beckett & Triggs (1967) in devising a quantitative assessment of buccal absorption, biological response has been discussed in this context (Flanagan, Broad & others, 1969; Tucker, Boyes & others, 1970). However, so far as we are aware, no attempt has been made directly to correlate a biological response with buccal absorption. The present study is concerned with such a relation, for a series of *p*-substituted acetanilides.

EXPERIMENTAL AND RESULTS

Buccal absorption and protein binding

Buccal absorption was determined as described by Dearden & Tomlinson (1971a), and protein-binding as described by Dearden & Tomlinson (1970).

Analgesic activity

Analgesic activities were measured by the abdominal constriction response method, using groups of 10 female albino mice (Tuck strain T.S.1), each of ~18 g, for each of four dose levels and for controls. Drugs were administered orally in 0·2% gum tragacanth suspension, and the challenge was by intraperitoneal acetylcholine. None of the drugs used showed any anti-acetylcholine activity, as indicated by a physostigmine lethality test (Collier, Dinneen & others, 1968). One compound, *N*-methylacetanilide, gave rise to some motor disco-ordination, and was therefore not included in regression analyses.

Partition coefficient (P)

The determination of partition coefficient was made using 1-octanol and Clark and Lubs 0·2M phosphate buffer, pH 7·2, as the solvent pair. Results of the partition measurements are expressed as Hansch's hydrophobic substituent constant, π (Fujita, Iwasa & Hansch, 1964).

Table 1 lists the results obtained for acetanilide and 15 *p*-substituted acetanilides. Buccal absorption values are uncorrected for dilution by saliva (Dearden & Tomlinson, 1971b), since the correction is very small over the duration of a normal 5 min test (e.g. 3% for *p*-chloroacetanilide).

Table 1. *The buccal absorptions, analgesic activities, π values and protein-binding constants of a series of* p*-substituted acetanilides.*

p-Substituent	% Buccal absorption in 5 min	ED50 (mmol kg^{-1}) (with 95% confidence limits)	π	ΔG of protein-binding at 19°* (kJ mol^{-1})
-H	26·9	0·390 (0·364–0·417)	0	−24·2
-Me	34·3	0·318 (0·273–0·369)	+0·239	−25·0
-OH	14·6	0·608 (0·578–0·640)	−0·360	−23·6
-OMe	23·3	0·404 (0·383–0·427)	−0·133	−23·5
-OEt	25·2	0·450 (0·425–0·477)	−0·098	−24·0
$-NH_2$	3·6	2·054 (1·912–2·205)	−1·076	−21·5
-F	33·6	0·234 (0·219–0·248)	+0·309	−25·0
-Cl	44·2	0·462 (0·430–0·496)	+0·714	−26·8
-Br	52·5	0·975 (0·929–1·024)	+1·130	−28·1
-I	57·5	1·392 (1·194–1·498)	+1·303	−28·8
-COOH	34·9	2·600 (2·194–3·081)	−0·605	−23·1
-CHO	7·6	0·457 (0·412–0·507)	+0·091	−24·7
$-NO_2$	42·4	0·195 (0·184–0·208)	+0·499	−26·2
>N-Me,-H	20·9	0·319 (0·300–0·339)	−0·187	−23·9
>N-Me,-OH	16·9	1·479 (1·296–1·687)	−0·549	−23·5
>N-Me,-OMe	19·4	0·977 (0·820–1·163)	−0·436	−23·6

* ΔG = Gibbs free energy of binding.

DISCUSSION

The work of Beckett and co-workers (e.g. Beckett & Moffat, 1969) and the work reported here shows that there exists a good rectilinear correlation of buccal absorption and log P or Hansch's hydrophobic substituent constant π (see Fig. 1). even when the compounds concerned cover a wide range of partition coefficients. Thus buccal absorption gives a measure of partition from the oral cavity into and on to the buccal membrane. We emphasize this point because the term "absorption" is usually taken to mean "passage through a membrane" (Wagner, 1968).

However, the good correlation of buccal absorption and π does not necessarily imply that passive partition between aqueous and lipid phases is the only factor governing buccal absorption. Dearden & Tomlinson (1971a) have shown that protein-binding probably plays a significant part in buccal absorption. This does not greatly perturb the relation between buccal absorption and π, however, since very good correlation also holds between π and log k, where k is the equilibrium constant of binding to bovine serum albumin ($F_{1,14} = 452{\cdot}8$; $F_{1,14}\,\alpha$, 0·001 = 17·14). Similar correlations have been observed for other series of drugs (Penniston, Beckett & others, 1969), and indeed are to be expected, since non-specific protein-binding is essentially a partition between a hydrophobic surface and an aqueous solution. It follows that there should also be a rectilinear correlation of buccal absorption and log k, and this is indeed observed for the present results ($F_{1,14} = 215{\cdot}5$; $F_{1,14}\,\alpha$, 0·001 = 17·14).

It has recently been demonstrated (Penniston & others, 1969) that the penetration of a drug *through* a series of lipid membranes is a parabolic function of log P. Thus compounds with very low partition coefficients are too hydrophilic to enter the lipid

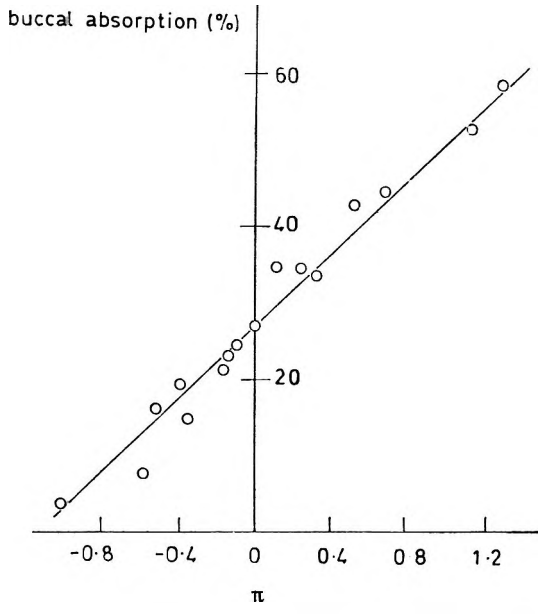

FIG. 1. Correlation of buccal absorption of *p*-substituted acetanilides with Hansch's hydrophobic substituent constant, π. The regression equation is: % absorption in 5 min = 23·73 (1·154) π + 27·37 (0·713). Figures in brackets are standard errors of the regression coefficients. $F_{1,14}$ = 427·6; $F_{1,14}$ α, 0·001 = 17·14.

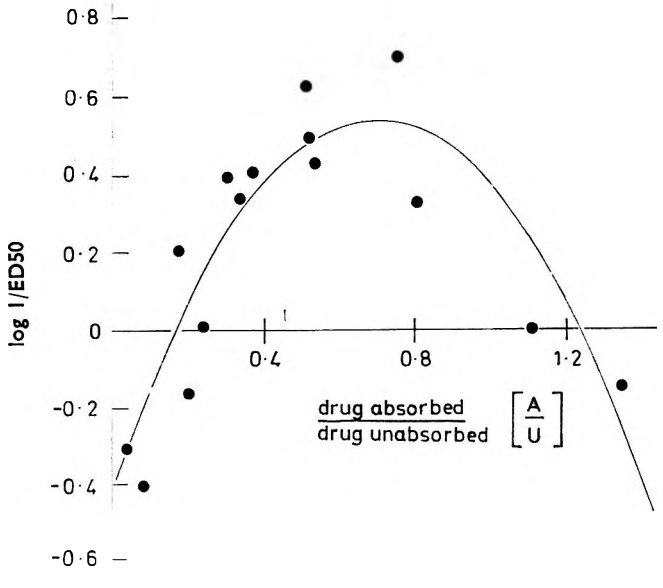

FIG. 2. Correlation of analgesic activtiy of *p*-substituted acetanilides with their buccal absorption. The regression equation is: log (1/ED50) = − 1·989 (0·318) $(A/U)^2$ + 2·800 (0·440) A/U − 0·439 (0·116), where A/U is the ratio of drug absorbed to drug unabsorbed in 5 min. Figures in brackets are standard errors of the regression coefficients. $F_{2,12}$ = 4·86; $F_{2,12}$ α, 0·05 = 3·89.

phase readily, those with very high lipid solubility tend to remain in the membrane rather than to pass through it.

We have found (unpublished observations) that, for the acetanilides studied here, a parabolic relation exists between analgesic activity and π; this is to be expected, since the biological activity of a compound must be a function of its ability to penetrate lipid membranes. Similar parabolic relations have been shown to exist with many different biological responses and types of compound (Hansch, 1969).

From the above correlations of buccal absorption and π, and between π and analgesic activity, it follows that there should be a parabolic relation between buccal absorption and analgesic activity. Fig. 2 shows that this is indeed the case. The correlation is slightly better than that between π and analgesic activity (for which $F_{2,12} = 3{\cdot}97$), and is similarly significant at the 95% level. It is perhaps surprising that an *in vivo* test does not correlate much better with biological activity than does an *in vitro* test. There are a number of possible reasons for this. Firstly, the reproducibility of buccal absorption measurements (mean % standard deviation = 3·7%) is not as good as that of the partition measurements (mean % standard deviation = 2·0%). Secondly, the enzymes present in saliva may cause degradation of a drug during its time in the mouth. Thirdly, after buccal absorption, the mouth is rinsed out to remove any drug solution still remaining; this could also remove some protein-bound drug, although in the present work the rinse-time was kept as short as possible (10 s) to minimize this. Finally, absorption can occur not only into the buccal mucosa, but also into the tongue, which has different, and probably specific, binding characteristics (Dastoli, Lopiekes & Price, 1968) and may thus absorb some members of a series of drugs more readily than others.

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REFERENCES

Beckett, A. H. & Moffat, A. C. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 144*S*–150*S*.

Beckett, A. H. & Triggs, E. J. (1967). *Ibid.*, **19**, *Suppl.*, 31*S*–41*S*.

Collier, H. O. J., Dinneen, L. C., Johnson, C. A. & Schneider, C. (1968). *Br. J. Pharmac. Chemother.*, **32**, 295–310.

Dastoli, F. R., Lopiekes, D. V. & Price, S. (1968). *Biochem.*, **7**, 1160–1164.

Dearden, J. C. & Tomlinson, E. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 53S—59S.

Dearden, J. C. & Tomlinson, E. (1971a). Ibid., **23**, *Suppl.*, 68S—72S.

Dearden, J. C. & Tomlinson, E. (1971b). *J. pharm. Sci.* **60**, 1278—1279.

Flanagan, T. H., Broad, R. D., Rubinstein, M. H. & Longworth, A. R. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 129*S*–134*S*.

Fujita, T., Iwasa, J. & Hansch, C. (1964). *J. Am. chem. Soc.*, **86**, 5175–5180.

Hansch, C. (1969). *Accts chem. Res.*, **2**, 232–239.

Penniston, J. T., Beckett, L., Bentley, D. L., & Hansch, C. (1969). *Molec. Pharmac.*, **5**, 333–341.

Tucker, G. T., Boyes, R. N., Bridenbaugh, P. O. & Moore, D. C. (1970). *Anesthesiology*, **33**, 287–303.

Wagner, J. G. (1968). *Drug intelligence*, **2**, 294–301.

Walton, R. P. (1944). *J. Am. Med. Ass.*, **124**, 138–143.

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Membrane osmometry of solubilized systems

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The solubilization of decane, ethyl *p*-hydroxybenzoate, methyl anisate and *p*-hydroxybenzoic acid by aqueous micellar solutions of cetomacrogol 1000 has been examined by membrane osmometry and viscosity techniques. The effect of the solubilizates on the number-average micellar molecular weight, M_n, has been related to their site of incorporation in the micelle. Decane and methyl anisate are solubilized in the hydrocarbon core of the micelles and both compounds produce an increase in M_n up to a maximum value of $2{\cdot}0 \times 10^5$, at a solubilizate concentration of approximately 80% of the saturation limit for each compound. This increase is shown to result from an increase in the number of molecules of both solubilizate and of surfactant per micelle. Further addition of solubilizate, to produce a saturation level in excess of 80%, results in a decrease in micellar size in both systems. The solubilization of ethyl *p*-hydroxybenzoate and *p*-hydroxybenzoic acid is thought to involve the oxyethylene region of the micelle and both solubilizates cause an increase in M_n owing to the inclusion of solubilizate into the micelle, the number of molecules of surfactant per micelle being unaffected by the solubilization process. Viscosity studies on the decane–cetomacrogol–water system are interpreted as indicating no change in either micellar symmetry or hydration as a result of solubilization. A spherical model for the micelles is shown to be consistent with the experimental data.

The effect of solubilizate on micellar size has been examined in only a few systems. In a light-scattering study of solubilization by hexadecyltrimethylammonium bromide (Hyde & Robb, 1964) it was shown that the incorporation of increasing amounts of the non-polar molecules, decane, octane and cyclohexane, caused a pronounced increase in the micellar molecular weight (mmw). This was due to increases in the numbers of solubilizate and surfactant molecules in each micelle. However, the solubilization of the polar molecule, octanol, although increasing the mmw, caused a decrease in the number of surfactant molecules in each micelle.

Nakagawa, Kuriyama & Inoue (1959, 1960) found the solubilization of decane and decanol by three methoxypolyoxyethylene decyl ethers to result in increases in the micellar weight of the micelles of these non-ionic surfactants. Each weight increase was a consequence of increases in the amount of solubilizate and surfactant per micelle. Viscosity and sedimentation studies of the solubilization of 1,2,4-trichlorobenzene and toluene by cetylpyridinium chloride (Smith & Alexander, 1957) have indicated an increase in mmw and in micellar asymmetry with increase in solubilizate concentration up to a maximum, after which further solubilizate promoted the formation of a more spherical micelle which existed in equilibrium with the rod-like micelles produced initially. In contrast, the solubilization of methyl cyclohexane by the same surfactant resulted in only a small regular increase in mmw and viscosity.

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In most cases, the differences in the effects of the various solubilizates on the micelle size and shape have been attributed to differences in the location of the solubilizate within the micelle. This paper reports an investigation of changes in the mmw of cetomacrogol micelles following the incorporation of four solubilizates for which the site of solubilization is known from previous studies (Corby, 1969, Corby & Elworthy, 1971) and correlates the effects produced with the location of the solubilizate within the micelle.

MATERIALS AND METHODS

Materials. Cetomacrogol 1000 B.P.C. in methanolic solution was deionized by passage through a column of 'Biodeminerolit' mixed bed ion-exchange resin and dried to constant weight over phosphorous pentoxide *in vacuo*.

n-Decane, olefine free (Fluka A.G. Purum grade) was used as received.

p-Hydroxybenzoic acid (BDH Ltd, Laboratory Reagent) was recrystallized from distilled water and dried at 40° over phosphorous pentoxide.

Ethyl *p*-hydroxybenzoate (BDH Ltd, Laboratory Reagent) was recrystallized from distilled water and dried at 40° over phosphorous pentoxide.

Methyl *p*-methoxybenzoate (methyl anisate) was recrystallized from a methanol—water mixture and dried at 30° over phosphorous pentoxide.

Preparation of solutions. Solutions were prepared by melting the solubilizate and cetomacrogol together at 40° then adding sufficient warm distilled water to produce the required total concentration. The weight of each solubilizate necessary to produce the required degree of saturation of the micelles was calculated from the solubility data of Corby (1969, Corby & Elworthy, 1971). All solutions were allowed to equilibrate at 30° (24 h) before measurements were made. Preliminary experiments indicated no measurable difference in the osmotic pressure of solutions prepared in this way and of corresponding solutions prepared by shaking for a period of three days. Hence it was concluded that the above procedure produced solutions in which solubilization to the required extent was complete.

Membrane osmometry. Measurements were made on a Hewlett-Packard 503 high-speed membrane osmometer at 30° ± 0·1°. B.19 cellulose acetate membranes (Schleicher and Schuell) were used since these had previously been shown to retain the micelles of cetomacrogol (Attwood, Elworthy & Kayne, 1969). Since the membranes are freely permeable to monomers and to free solubilizate it was necessary to achieve, as far as possible, an equal concentration of these species on either side of the membrane to reduce to a minimum their contribution to the osmotic pressure. Consequently a solution of cetomacrogol above the critical micelle concentration (cmc) and containing sufficient solubilizate to give the required degree of saturation was placed in the solvent compartment of the osmometer.

Viscosity measurements. Measurements were made at 30° ± 0·01° using a suspended-level dilution viscometer with a solvent flow time of approximately 200 s.

Density measurements. A Lipkin pycnometer of approximately 10 ml capacity was used. Measurements were made at 30° ± 0·01° according to the procedure of Bauer & Lewin (1960). Each density represents the mean of two measurements. The precision of repeat measurements was ±0·00001 g ml^{-1}.

RESULTS

Treatment of osmotic pressure results. Attainment of an osmotic pressure which remains constant for at least 2 h is indicative of complete retention of the micellar species by the osmometer membrane (Attwood, Elworthy & Kayne, 1969, 1970). This was shown to apply to all the solubilized systems investigated here. For such systems, the measured osmotic pressure, π, between a sample solution of concentration c g litre^{-1} and a 'solvent' of concentration c' g litre^{-1} is given by

$$\pi = RT(c - c')/M_n + RBT[(c - c')^2 + 2(c - c')(c' - \text{cmc})] \quad .. \quad .. \quad (1)$$

where B is the second virial coefficient and RT has the usual meaning (Coll, 1970). Hence the osmotic pressure data were plotted as $\pi/(c - c')$ against $(c - c')$ (Fig. 1)

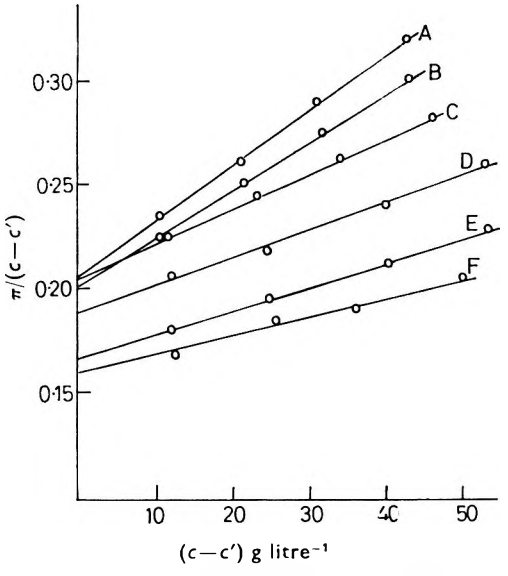

FIG. 1. Variation of reduced osmotic pressure with concentration for cetomacrogol micelles containing A 20%, B 30%, C 60%, D 80%, E 90% and F 100% of the saturation limit of *p*-hydroxybenzoic acid.

and extrapolated to $c = c'$. M_n was calculated from the intercept which is given by

$$\pi/(c - c')_{c=c'} = RT/M_n + 2RTB(c' - \text{cmc}) \quad .. \quad .. \quad (2)$$

In all the systems studied the correction term $2RTB(c' - \text{cmc})$ had a negligible effect on the value of M_n and hence accurate values of the cmc's and the second virial coefficients are not essential.

Variation of M_n with the degree of saturation of the micelles with each of the solubilizates is shown in Fig. 2. The numbers of molecules of solubilizate and of cetomacrogol per micelle were calculated as a function of the degree of saturation, assuming that all of the micelles contained the same amount of solubilizate. The results are shown in Figs 3 and 4.

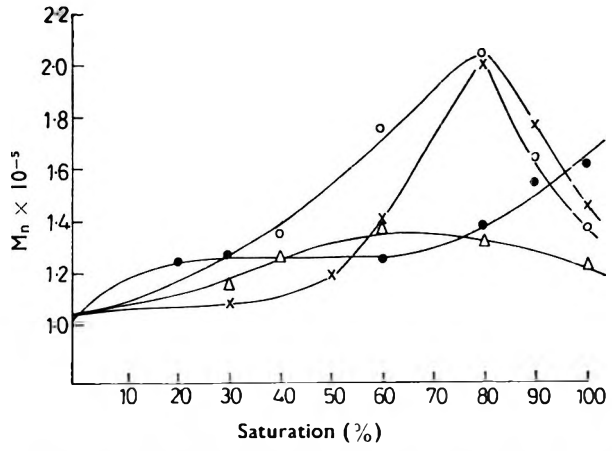

FIG. 2. Variation of micellar weight with the degree of saturation of cetomacrogol micelles with O decane; × methyl anisate; ● *p*-hydroxybenzoic acid and △ ethyl *p*-hydroxybenzoate.

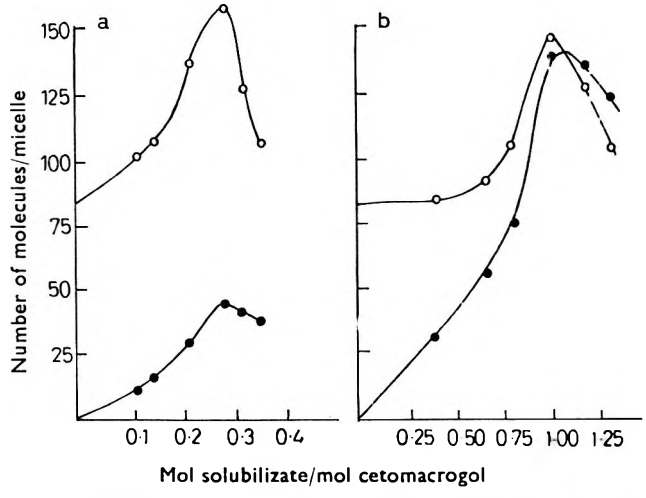

FIG. 3. The number of molecules per micelle of ○ cetomacrogol and ● solubilizate as a function of the molar ratio of solubilizate in the mixture, for the solubilization of cecane (a) and methyl anisate (b).

Viscosity results

Figure 5 shows the variation of reduced viscosity with total solute concentration for cetomacrogol micelles saturated to varying extents with cecane. Values of the limiting viscosity numbers, $[\eta]$, calculated by regression analysis are given in Table 1. The value of $[\eta]$ obtained for cetomacrogol alone is in reasonable agreement with that quoted by Corby (1969). However, the value for cetomacrogol micelles saturated with decane at 30° is higher than that found by Corby at 20° (5·30). This is unexpected since increase in temperature usually causes micellar dehydration and a consequent decrease in $[\eta]$. The extent of hydration of the micelles was estimated assuming micellar sphericity as w g of H_2O/g of total micellar solids using

$$[\eta] = 2{\cdot}5\,(\bar{V} + wV^{\circ}) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

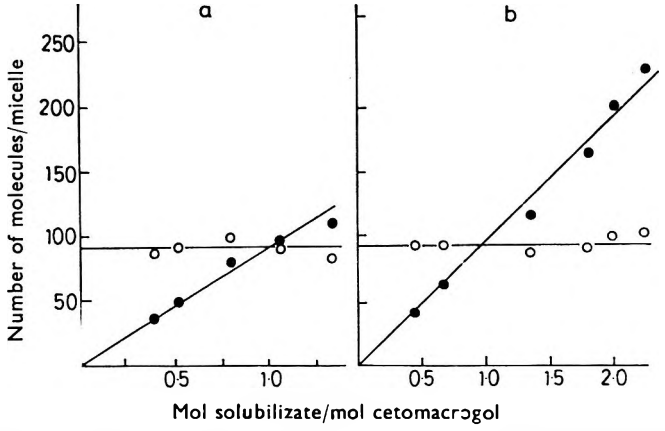

FIG. 4. The number of molecules per micelle of ○ cetomacrogol and ● solubilizate as a function of the molar ratio of solubilizate in the mixture, for the solubilization of ethyl *p*-hydroxybenzoate (a) and *p*-hydroxybenzoic acid (b).

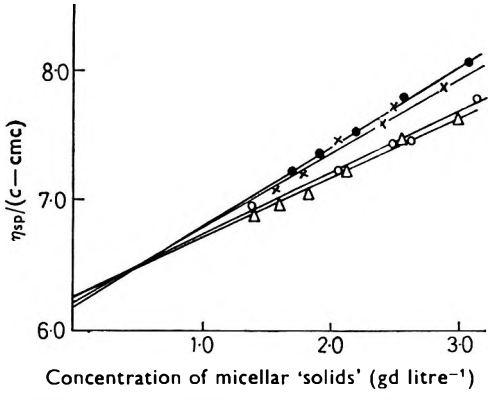

FIG. 5. Variation of reduced viscosity with concentration for cetomacrogol micelles containing ● 0%; × 20%; ○ 80% and △ 100% of the saturation limit of decane.

Table 1. *Viscosity, partial specific volume and hydration data for cetomacrogol micelles containing solubilized decane.*

Saturation %	Limiting viscosity number [η]	Partial spec fic Volume $\bar{V}$ ml g^{-1} of the micellar species	Hydration *w* g of H_2O/g of total micellar solids
0	7·14	0·9205	1·94
20	7·16	0·9240	1·94
40	—	0·9271	—
60	—	0·9309	—
80	7·19	0·9345	1·94
100	7·20	0·9370	1·94

where V° is the specific volume of the solvent. The partial specific volume of the micellar species, $\bar{V}$, was calculated from the slope of plots of density, ρ, as a function of the total solute concentration, c, using

$$\rho = \rho_0 + (1 - \bar{V}\rho_0)c \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where ρ_0 is the density of the solvent, i.e. water saturated with decane. Values of w and $\bar{V}$ are given in Table 1. $\bar{V}$ was a linear function of the percentage saturation of the micelles with decane. Identical values of w were obtained using an equation proposed by Corby (1969).

DISCUSSION

Because of its almost complete insolubility in water, decane is believed to be solubilized exclusively in the hydrocarbon core of the micelle. Fig. 3 shows that the observed increase in M_n up to a maximum value at 80% saturation of the micelles (Fig. 2) is caused by an increase in both the decane content of the micelles and the number of molecules of cetomacrogol per micelle. This increase in aggregation number is similar to increases reported from previous investigations and is probably a result of the need for more monomers to effectively cover the hydrophobic core, which has been expanded by the incorporation of decane. The subsequent decrease in M_n is similar to that reported by Smith & Alexander (1957) for the solubilization of trichlorobenzene and toluene by cetylpyridinium chloride. However, the viscosity data for the decane–cetomacrogol–water system indicate that if there is any increase in $[\eta]$ caused by a decrease in micellar symmetry, as was found by these authors, it is exactly cancelled out by a corresponding decrease in hydration. A more probable interpretation of the viscosity data is that no change occurs in the symmetry and this indicates a constant micellar hydration as shown in Table 1. The maximum value of M_n for both the decane and the methyl anisate systems is approximately $2{\cdot}0 \times 10^5$ and this may represent the maximum size the micelle can attain while still retaining its symmetry. Addition of more solubilizate, to produce a saturation level in excess of 80%, results in the production of more micelles of a smaller size rather than the expansion of the existing ones.

Macfarlane (1970) has shown that if the micelles of cetomacrogol are asymmetric it is unlikely that their axial ratio exceeds a value of 2. To determine whether a spheroidal model was consistent with the experimental data, calculations were made of the micellar dimensions, assuming the micelles to be perfectly spherical, for all degrees of saturation with decane. The volume of the micellar core, V_c, and hence the core radius, r_c, was calculated assuming that the decane and hexadecane composing the core had densities equal to those of these liquids in the pure state. The total hydrated radius, r_h, was calculated from the hydrated volume, V_h, using

$$V_h = \frac{M_n}{N}(\bar{V} + wV^\circ) = \frac{4}{3}\pi r_h^3 \quad .. \quad .. \quad .. \quad (5)$$

Thus the radius of the oxyethylene region, r_e, of the micelle could be estimated. If the hydration of the micelle is to remain constant during the micellar expansion, as indicated by the viscosity data, the extension of the oxyethylene chain should not vary to any significant extent, otherwise the amount of water mechanically trapped by these chains will also vary. Table 2 shows that r_e is reasonably constant, differing by a maximum of only 0·6 nm from its value in the pure cetomacrogol micelles. This

Table 2. *Micellar dimensions for cetomacrogol micelles containing solubilized decane, calculated assuming the micelles to be spherical.*

Saturation (%)	$V_h \times 10^{-2}$ (nm³)	r_h (nm)	$V_e \times 10^{-1}$ (nm³)	r_c (nm)	r_e (nm)
0	4·98	4·92	4·06	2·13	2·79
30	6·08	5·26	5·21	2·32	2·91
40	6·46	5·36	5·63	2·38	2·97
60	8·37	5·85	7·60	2·63	3·22
80	9·79	6·16	9·12	2·79	3·37
90	7·87	5·73	7·47	2·61	3·12
100	6·58	5·40	6·34	2·47	2·92

is not a significant difference in comparison with the total extended length of approximately 8 nm for a chain of 21 units, and hence a spherical model for the micelles is not an unreasonable one for this system.

The partial specific volume of a multicomponent micelle is given by the general equation (Kraemer 1940)

$$\bar{V} = \bar{V}_1 + \Sigma r_i \bar{V}_i/(1 + \Sigma r_i) \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

where $\bar{V}_1$ is the partial specific volume of the pure surfactant micelle and r_i is the number of g of the i^{th} component with partial specific volume $\bar{V}_i$ combining with 1 g of the surfactant. Application of equation 6 in the calculation of $\bar{V}$ for the cetomacrogol micelle saturated with decane, using the specific volume of decane (calculated from an experimental density of 0·722 g ml^{-1} at 30°) rather than the partial specific volume, gives a value of 0·9381 ml g^{-1} for $\bar{V}$. This agrees well with the experimental value, and supports the assumption that the decane is solubilized exclusively in the micellar core. Masterton (1954) has shown that the exposure of aliphatic hydrocarbons to an aqueous environment, as would be the case if the decane was incorporated within the oxyethylene region of the micelle, results in a significant decrease in the molar volume. Such an agreement between theoretical and experimental values of $\bar{V}$ might not then be expected.

Fig. 2 shows a similar increase in M_n following the solubilization of methyl anisate, a maximum M_n of $2{\cdot}0 \times 10^5$ being attained at approximately 80% saturation. As seen from Fig. 3 this is a consequence of an increase in both the number of solubilizate and cetomacrogol molecules per micelle, but unlike the decane system the increase in the latter is not apparent until the micelles are approximately 40% saturated. Nmr studies (Corby 1969) of the solubilization process have shown considerable disruption of the micellar core after an initial lack of effect. It was suggested that initial solubilization occurred in the oxyethylene region and then, as the concentration of the ester increased, the core took over as the main site of solubilization. A similar interpretation may be applied to the results reported here. Significant expansion of the micellar core, and therefore an increase in aggregation number, occurs only after 40% saturation, indicating a lack of involvement of this region in the initial stages of solubilization.

As seen from Fig. 4 the solubilization of *p*-hydroxybenzoic acid does not result in any increase in the number of cetomacrogol molecules per micelle, the increase in M_n observed in Fig. 2, being due solely to the inclusion of solubilizate into the micelle. Corby considers that *p*-hydroxybenzoic acid is solubilized deep within the

oxyethylene layer of the cetomacrogol micelle. Since the micellar core is not therefore expanded by the solubilization process, additional surfactant molecules are not required to shield the hydrophobic region and hence a lack of any increase in aggregation number might be expected.

A similar effect is produced by the solubilization of ethyl *p*-hydroxybenzoate. Fig. 4 indicates an almost linear increase in the number of solubilizate molecules per micelle which is the main cause of the increase in M_n shown in Fig. 2. Because of the solubility of ethyl *p*-hydroxybenzoate in hexadecane, it is thought possible that some molecules penetrate into the core, although the lack of any significant increase in the number of surfactant molecules per micelle indicates that any expansion of the micellar core caused by such penetration is not likely to be pronounced.

It is evident from this investigation that the site of solubilization within the micelle is an important factor in determining the effect a solubilizate has on the micellar weight of this non-ionic surfactant. Incorporation into the micellar core results in an increase in the number of surfactant molecules per micelle, whereas incorporation into the oxyethylene region of the micelle merely causes an increase in M_n which is due only to the presence of the solubilizate: the number of surfactant molecules per micelle being unaffected by the solubilization process.

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REFERENCES

ATTWOOD, D., ELWORTHY, P. H. & KAYNE, S. B. (1969). *J. Pharm. Pharmac.*, **21**, 619–620.

ATTWOOD, D., ELWORTHY, P. H. & KAYNE, S. B. (1970). *J. phys. Chem., Ithaca*, **74**, 3529–3534.

BAUER, N. & LEWIN, S. Z. (1960). In *Physical Methods of Organic Chemistry*, Vol. 1, 3rd Edn, p. 153. Editor: Weissberger. New York: Interscience.

COLL, H. (1970). *J. phys. Chem., Ithaca*, **74**, 520–528.

CORBY, T. C. (1969). Ph.D. Thesis, Strathclyde.

CORBY, T. C. & ELWORTHY, P. H. (1971). *J. Pharm. Pharmac.*, **23** *Suppl.* 45S–54S.

HYDE, A. J. & ROBB, D. J. M. (1964). *Proc. 4th Intern. Congr. Surface Activity.* London: Gordon & Breach, **2**, 743–750.

KRAEMER, E. O. (1940). In *The Ultracentrifuge*, p. 57. Editors: Svedberg & Pederson. Oxford University Press.

MACFARLANE, C. B. (1970). *Kolloid-Z. Z. Polym.*, **239**, 682–685.

MASTERTON, W. L. (1954). *J. chem. Phys.*, **22**, 1830–1833.

NAKAGAWA, T., KURIYAMA, K. & INOUE, H. (1959). *Symposium on Colloid Chem. (Chem. Soc. Japan), 12th Symposium*, p. 32.

NAKAGAWA, T., KURIYAMA, K. & INOUE, H. (1960). *J. Colloid Sci.*, **15**, 268–277.

SMITH, M. B. & ALEXANDER, A. E. (1957). *Proc. 2nd Intern. Congr. Surface Activity*, **1**, pp. 349 356. London: Butterworths.

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A laser light scattering apparatus

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The use of a low power helium-neon laser for light scattering measurements is described. The Rayleigh and Cabannes theories have been modified for polarized incident light. A new procedure is recommended for data when the solvent correction is relatively high. Using this method a precision of 4·7% was obtained for the molecular mass of bovine plasma albumin in water, compared with 6·3% for the more usual method.

The chief advantages of using a laser for light scattering are the high degree of monochromaticity and the well-defined collimated beam obtainable without the use of ancillary lenses slits and filters.

It has been shown (Kielich, 1968) that high intensity laser beams such as are obtained from ruby crystals, produce orientation of the scattering particles, and that the scattered intensity is not proportional to the incident intensity. For low energy neutral gas lasers these complications of the Rayleigh theory do not arise (Bothorel & Lalonne, 1968) and this has been confirmed using a helium-neon laser for scattering studies on liquids by Rudder & Bach (1967) and on polymer solutions by Block (1968) and by Anderson (1968).

In the visible spectrum the helium-neon gas laser giving light of 632·8 nm wavelength is generally used. The light output is plane polarized since the end windows of the tube are set at the Brewster angle to minimize reflection losses.

MATERIALS AND METHODS

The apparatus was similar to that of Attwood (1965) except that the mercury lamp, filters, slits and lenses for the incident light beam were replaced by a Scientifica-Cook B17/S laser (0·5 mW at 632·8 nm).

The scattered light intensity was measured by an E.M.I. 6097 B photomultiplier, which could be rotated horizontally for angular measurements; this was connected to a Techmation model 451NS digital voltmeter.

According to Heller (1965), Rayleigh's theory of light scattering applies to the scattering of unpolarized light of wavelength λ, by a particle of maximum dimension smaller than $\lambda/6$. The Rayleigh ratio is given by

$$R_\theta = \frac{ir^2}{I} = \frac{8\pi^2 p^2(1 + \cos^2\theta)}{\lambda^4} \quad \ldots \quad (1)$$

where i = intensity of scattered light at angle θ; I = intensity of incident light; r = distance of scattering particle from detector; p = polarizability of the particle; λ = wavelength of light.

In the bracketed term the 1 represents the *vertically* polarized component of the scattered light, which is independent of θ; the $\cos^2\theta$ term represents the *horizontally*

polarized output which is thus zero at $\theta = 90°$. In practice there is always some horizontally polarized scatter at 90° due to the anisotopic nature of the scattering particles and Cabannes & Rocard (1929) have shown that the observed value of R_θ must be multiplied by $(6 - 7d)/(6 + 6d)$ to give the true Rayleigh ratio where d, the depolarization at 90°, is the ratio of the intensity of horizontally polarized scattered light divided by the intensity of the vertically polarized component. This is measured using a polaroid sheet in front of the photomultiplier and measuring the ratio of photomultiplier output readings with the polaroid set for passing horizontally and then vertically polarized scattered light.

In practice $i \ll I$ and direct comparison is difficult. It is, however, possible to calculate the fraction of light scattered in terms of the scatter of a standard Perspex block calibrated by measurements of turbidities t, of solutions. The relation between turbidity and Rayleigh ratio is

$$t = 16\pi R_{90}/3 \quad \ldots\ldots\ldots\ldots \quad (2)$$

So that measurement of the turbidity using a spectrophotometer enables R_{90} to be calculated. Ludox, a colloidal preparation of silicic acid is used for this purpose since dilute sols act as Rayleigh scatterers provided large aggregates are removed by centrifugation. The quantity measured in the light scattering apparatus is S_θ defined as

$$S_\theta = \frac{\text{Scatter of solution at angle } \theta}{\text{Scatter of standard block of Perspex at } 90°}$$

and S_θ is directly proportional to R_θ

$$R_\theta = CS_\theta \quad \ldots\ldots\ldots\ldots \quad (3)$$

where C is the calibration constant for a given Perspex standard. In the case of He–Ne laser the incident light is polarised at ϕ to the horizontal. The vertically polarized component is proportional to $\sin^2\phi$, and the horizontal component to $\cos^2\phi$. The $(1 + \cos^2\theta)$ term of Rayleigh's theory then becomes

$$(\sin^2\phi + \cos^2\phi \cos^2\theta) = \sin^2\phi(1 + \cos^2\theta \cot^2\phi) \quad \ldots\ldots \quad (4)$$

in which ϕ is a constant for a given apparatus, and since R_θ is calculated during the calibration procedure, using the polarized light to measure scatter from a Perspex block, the $\sin^2\phi$ term becomes incorporated into the calibration constant C (Pugh, 1970).

Cabannes' factor must also be modified for the case of polarized incident light. The relation between the observed depolarization, $d(\phi)$ and the corresponding depolarization, d, for an unpolarized source is given by

$$d = 2\sin^2\phi(1 - 2\cos^2\phi + 1/d(\phi)) \quad \ldots\ldots\ldots \quad (5)^*$$

Equations (1), (2), (3), (4) show that S_θ is related to S_{90} by the equation

$$S_\theta = S_{90}(1 + \cos^2\theta \cot^2\phi) \quad \ldots\ldots\ldots \quad (6)$$

A correction must be applied however since the scattering volume viewed by the photomultiplier is proportional to $\sin\theta$. Equation (6) thus becomes

$$\text{or} \quad \begin{aligned} S_\theta &= S_{90}(1 + \cos^2\theta \cot^2\phi)/\sin\theta \\ S_\theta \sin\theta &= S_{90} + S_{90}\cos^2\theta \cot^2\phi \end{aligned} \quad \ldots\ldots\ldots \quad (7)$$

* Copies of the proof for this formula are available from the Editorial Department, Journal of Pharmacy and Pharmacology, 17, Bloomsbury Square, London, WC1A 2NN.

A plot of $S_\theta.\sin\theta$ against $\cos^2\theta$ should therefore give a straight line of intercept S_{90} and slope $S_{90}\cot^2\phi$. Ludox sols were used for testing this equation since they give scattering envelopes which are symmetrical about 90°. It was found that the plot was not exactly linear because of a small constant error in the angle scale markings, of the order of 10–20 minutes of arc. This was eliminated by taking the means of supplementary scatter readings on either side of 90° as being the true reading at exactly θ. This gave a good straight line relation, Fig. 1, from which ϕ was found to be 39° 45′.

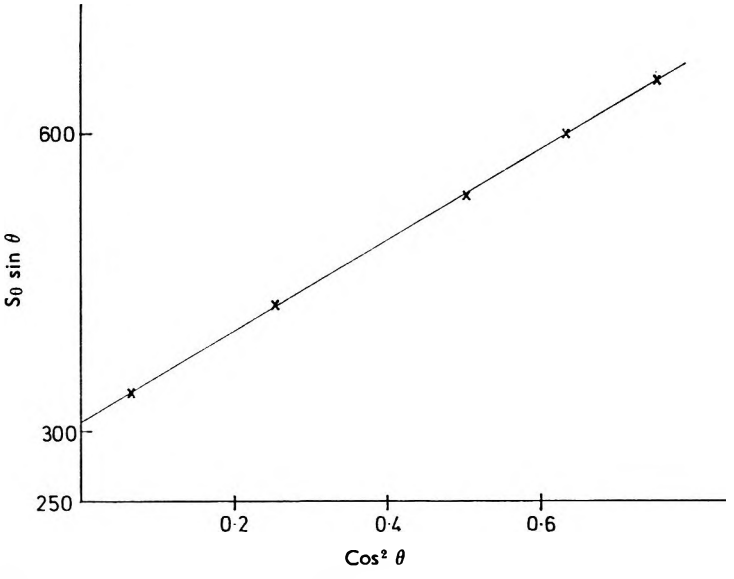

FIG. 1. Plot of $S_0 \sin\theta$ against $\cos^2\theta$ for Ludox.

Equation (1) has been developed by Debye (1947) and Zimm (1948) to enable calculation of molecular masses of solutes,

$$\frac{1}{M} = \frac{2\pi^2 n_0^2 (dn/dc)^2}{\lambda^4 N_A C}\left(\frac{c}{S}\right)_{c \to \text{zero},\ \theta \to \text{zero}} \quad .. \quad .. \quad .. \quad (8)$$

M = weight average molecular mass; n_0 = refractive index of solvent; c = concentration in g cm^{-3}; λ = wavelength of light *in vacuo*; N_A = Avogadro number; $S'_\theta = S_\theta \sin\theta/(1 + \cos^2\theta \cot^2\phi)$.

The extrapolation to zero angle is made to eliminate destructive interference.

For small particles it is only necessary to measure S'_{90}, S'_{45}, and S'_{135}. The ratio S_{45}/S_{135} is called the dissymmetry Z, and for a given particle shape a quantity $P^{-1}(\theta)$ can be calculated such that

$$S'_{90}\, P^{-1}(\theta) = S'_0$$

Tables of $P^{-1}(\theta)$ and Z have been given for various particle shapes by Beattie & Booth (1960).

Normally S_θ is measured by subtracting the scatter for pure solvent from the solution reading, this procedure introduces inaccuracies particularly when the scattering of solutions is low, the correction for solvent scatter then being relatively large and any error in the solvent scattering measurement is introduced into all the other measurements (Pugh, 1970). It is considered that a better result is obtained from a

plot of Δn against S'_θ at low concentrations. When, as with bovine plasma albumin, the plot is linear, the slope of the line gives the value of $\Delta n/(S'_{soln} - S'_{solv})$, otherwise the curve is fitted by a polynomial and the linear coefficient (the slope at low scatter) is taken as the measure of this quantity.

Substitution of $c = \Delta n/(dn/dc)$ in equation (8) for dilute solutions removes the need for squaring the (dn/dc) term

$$\frac{1}{M} = \frac{2\pi^2 n_0^2 (dn/dc)}{\lambda^4 NC} \left(\frac{\Delta n}{S'_\theta}\right) \qquad (9)$$

$\Delta n \to$ zero, $\theta \to$ zero

To use this second method values of S' are plotted as abscissa against refractive index differences between solvent and solution, Δn, determined with a Rayleigh refractometer at the same wavelength as the laser for each solution used in the scattering measurements, as ordinate.

The molecular mass of bovine plasma albumin in water was determined by both methods. The data are given in Table 1. The graph of Δn against S'_{90} was a straight line and so $(\Delta n/S'_{90})_{\Delta n \to 0}$ was simply the slope of this line.

Table 1. *Light scattering data for BPA in water.*

$\Delta n \times 10^3$	$c \times 10^3$ (c, g cm^{-3})	S'_{90}	$S'_{90} - 0{\cdot}419$	$\frac{c}{S'_{90} - 0{\cdot}419} \times 10^3$
3·207	17·240	9·390	8·971	1·922
1·703	9·156	5·337	4·918	1·862
0·977	5·253	3·194	2·775	1·893
0·816	4·387	2·432	2·013	2·179
0·457	2·457	1·704	1·285	1·912
0·457	2·457	1·622	1·203	2·042
0	0	0·419	0	—

$n_0 = 1{\cdot}332$; $\lambda = 6{\cdot}328 \times 10^{-5}$ cm; calibration constant, $C = 1{\cdot}595 \times 10^{-5}$; $dn/dc = 0{\cdot}1860$; $Z = 1{\cdot}042$; $d_0 = 0{\cdot}0328$; $\phi = 39° 45'$; $P^{-1}(\theta) = 1{\cdot}029$; Cabannes factor = 1·060.

The mean molecular mass was calculated using the normal procedure giving $M_w = 66\,100$ with $P = 0{\cdot}95$ limits of error of 6·3%; the modified method gave $M_w = 65\,700$ with $P = 0{\cdot}95$ limits of error of 4·7%. The statistical methods are described by Saunders & Fleming (1966). The mean results are in agreement with the value given by Riddiford & Jennings (1966).

REFERENCES

ANDERSON, R. J. (1968). *Diss. Abstr.* B, **28**, 4083.

ATTWOOD, D. (1965). *Ph.D. Thesis*, London University.

BEATTIE, W. H. & BOOTH, C. (1960). *J. phys. Chem., Ithaca*, **64**, 696–697.

BLOCK, A. M. (1968). *Diss. Abstr.* B, **28**, 3667.

BOTHOREL, P. & LALONNE, J. R. (1968). *J. Chim. phys.*, 1538– .

CABANNES, J. & ROCARD, Y. (1929). *La Diffusion Moleculaire de la Lumiere*, Le Presses Universitaires de Paris.

DEBYE, P. (1947). *J. phys. Colloid Chem.*, **51**, 18–32.

HELLER, W. (1965). *J. chem. Phys.*, **42**, 1609–1615.

KIELICH, S. (1968). *J. Colloid Inter. Sci.*, **27**, 432–441; **28**, 214–218.

PUGH, W. J. (1970). *Ph.D. Thesis*, London University.

RIDDIFORD, C. L. & JENNINGS, B. R. (1966). *Biochim. biophys. Acta*, **126**, 171–173.

RUDDER, R. R. & BACH, D. R. (1967). *U.S. Clearinghouse Fedn Sci. Tech. Inform.*, 663819.

SAUNDERS, L. & FLEMING, R. (1966). *Mathematics and Statistics*, London: Pharmaceutical Press.

ZIMM, B. H. (1948). *J. chem. Phys.*, **16**, 1093–1099.

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Adsorption of phosphatidylcholine at the benzene-water interface

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Interfacial tensions between benzene solutions of egg-phosphatidylcholine and aqueous bovine plasma albumin have been studied. The presence of the protein in the aqueous phase leads to a more rapid adsorption of the phospholipid at the interface, this being a result of interaction between the phospholipid and protein. The most probable mechanism for the interaction is discussed. The interfacial tensions between aqueous dispersions of egg-phosphatidylcholine and pure benzene have also been determined. The results clearly indicate the effect of ultrasonic treatment in producing a stable aqueous phospholipid dispersion.

The adsorption from benzene solution of egg-phosphatidylcholine (egg-PC) at the benzene-water interface is highly time dependent (Johnson & Saunders, 1968). The process is not entirely diffusion controlled but dependent upon the crossing of an energy barrier to adsorption. Mixed protein-egg-PC interfaces have been examined to determine the effect of introducing bovine plasma albumin (BPA) into the aqueous phase upon the adsorption process.

MATERIALS AND METHODS

Interfacial tensions were determined by the modified Wilhelmy plate technique described by Ruyssen (1946), using an electrical balance (Johnson & Saunders, 1968). A thin, depolished platinum plate 1·032 cm in length and approximately 0·5 cm in depth was used. The end correction, l_0, was determined by measuring the surface tension of a sample of water using two plates of different lengths (0·5 cm and 1 cm) but made from the same gauge of platinum (Padday, 1957). The value of l_0 (0·009 cm) was then added to the geometric length of the plate in subsequent calculations of the interfacial tension values. Double-distilled water was used, prepared from a seasoned all-glass still, only the middle third fraction being taken from the final distillate. Analar grade benzene (British Drug Houses) was used, without further purification.

Purified egg-PC was prepared from a commercial sample (Merck A.G.) by chromatographic treatment with alumina and silicic acid, as described by Saunders (1957). Confirmation of purity of the egg-PC was obtained from the phosphorus and nitrogen analysis giving an N:P ratio of 1·04. This value compares favourably with the results of Perrin (1962) and is close to the theoretical value of 1·0.

Bovine plasma albumin (BPA) (Armour; Fraction V) was purified by recrystallization from methanol (Cohn, Hughes & Weare, 1947).

Simple aqueous dispersions of egg-PC were prepared by dissolving the required amount of the material in ether. The ether solution was then added to a weighed

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quantity of water, and the ether removed under vacuum at 30°. The resulting milky dispersion was centrifuged to remove any coarse undispersed material, and the dispersion assayed by drying an aliquot to constant weight in a vacuum oven at 30°.

Sonicated dispersions of egg-PC were prepared by the method of Saunders, Perrin & Gammack (1962). A simple aqueous dispersion, after removal of the ether, was subjected to ultrasonic irradiation for 90 min, when the egg-PC had dispersed to give aggregates with a weight average molecular weight of 2×10^6 (see Attwood & Saunders, 1966).

All determinations of interfacial tension were made at 25°.

RESULTS

The interfacial tensions of pure benzene with aqueous solutions of Armour (Fraction V) BPA are plotted as a function of time in Fig. 1. The observed time dependence of the interfacial tensions is a well-known phenomenon with protein films (Cheesman & Davis, 1954).

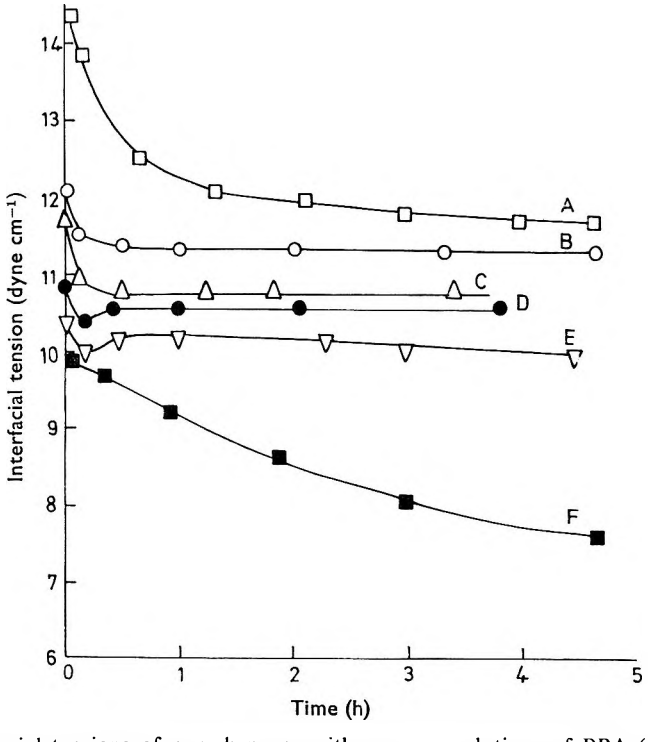

FIG. 1. Interfacial tensions of pure benzene with aqueous solutions of BPA (Armour) at 25°. A, $5{\cdot}0 \times 10^{-4}$% w/v; B, $5{\cdot}0 \times 10^{-3}$% w/v; C, $1{\cdot}0 \times 10^{-2}$% w/v; D, $5{\cdot}0 \times 10^{-2}$% w/v; E, $1{\cdot}0 \times 10^{-1}$% w/v; F, 1·0% w/v.

The occurrence of minima in some of the curves in Fig. 1 suggested competitive adsorption of some impurity in the protein solution, most probably fatty acid. Hence, the system was re-examined using the recrystallized protein, the results being given in Fig. 2.

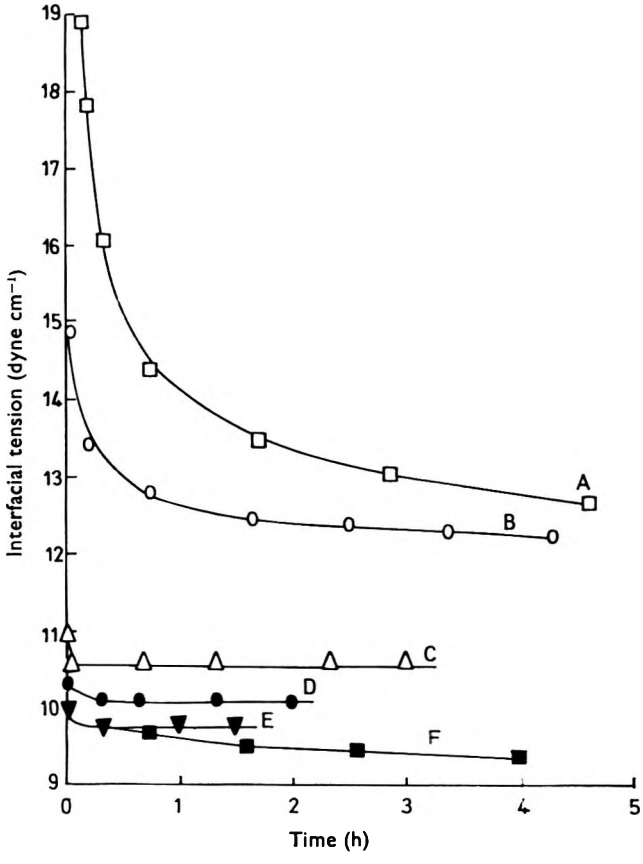

FIG. 2. Interfacial tensions of pure benzene with aqueous solutions of purified BPA at 25°. A, $1{\cdot}0 \times 10^{-3}$% w/v; B, $5{\cdot}0 \times 10^{-3}$% w/v; C, $1{\cdot}0 \times 10^{-2}$% w/v; D, $5{\cdot}0 \times 10^{-2}$% w/v; E, $1{\cdot}0 \times 10^{-1}$% w/v; F, 1·0% w/v.

The interfacial tension-time curves for the purified protein no longer exhibit minima, indicating that the impurity has been removed by the precipitation procedure.

In the concentration range 0·01 to 0·1% w/v the interfacial tensions of the purified BPA solutions exhibited only a small time dependence, equilibrium values being attained within 10 min of forming the interface (Fig. 2). Hence, for the study of mixed egg-PC-BPA interfaces, a constant protein concentration of 0·1% w/v BPA was used, thus avoiding superimposition of a large time effect due to the protein, upon that resulting from the adsorption of egg-PC from the benzene phase. The interfacial tension results between solutions of egg-PC in benzene and 0·1% w/v BPA aqueous solutions are given in Fig. 3.

For the interfacial tensions between simple aqueous dispersions of egg-PC and benzene, large time effects were observed although no clear dependence upon bulk concentration was found. In the case of ultrasonicated aqueous dispersions, a clear dependence upon the egg-PC concentration was observed. The interfacial tensions between pure benzene and the ultrasonicated dispersions are plotted as a function of time in Fig. 4.

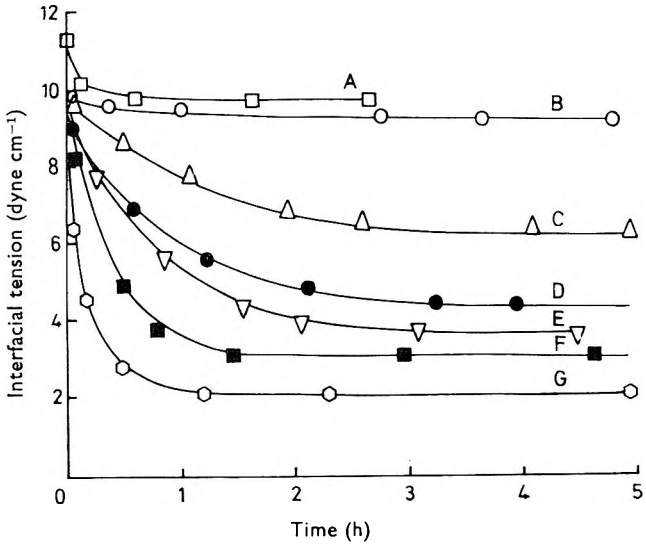

FIG. 3. Interfacial tensions for egg-PC in benzene with 0·1% w/v purified BPA aqueous solution at 25°. A, $1{\cdot}02 \times 10^{-5}$% w/w; B, $5{\cdot}52 \times 10^{-6}$% w/w; C, $8{\cdot}49 \times 10^{-5}$% w/w; D, $9{\cdot}84 \times 10^{-5}$% w/w; E, $1{\cdot}18 \times 10^{-4}$% w/w; F, $1{\cdot}68 \times 10^{-4}$% w/w; G, $2{\cdot}34 \times 10^{-4}$% w/w.

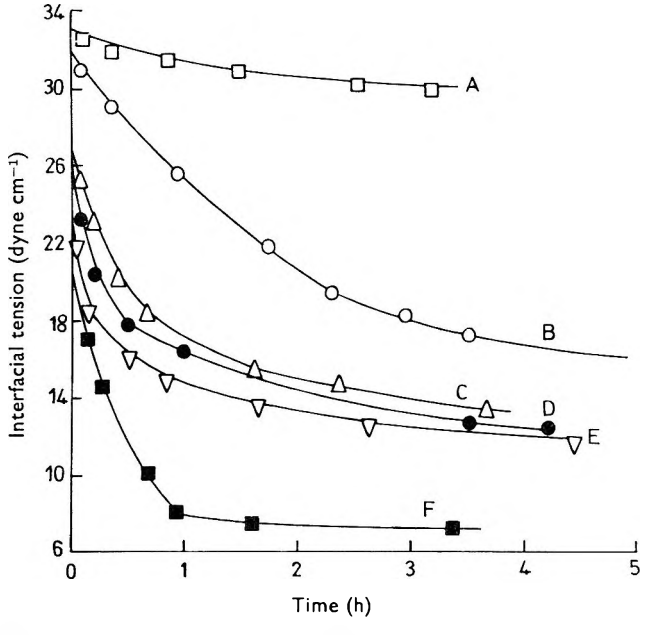

FIG. 4. Interfacial tensions for pure benzene with sonicated aqueous dispersions of egg-PC at 25°. A, $1{\cdot}10 \times 10^{-3}$% w/w; B, $5{\cdot}94 \times 10^{-3}$% w/w; C, $1{\cdot}87 \times 10^{-2}$% w/w; D, $2{\cdot}42 \times 10^{-2}$% w/w; E, $3{\cdot}17 \times 10^{-2}$% w/w; F, $4{\cdot}70 \times 10^{-2}$% w/w.

DISCUSSION

Previous experiments (Johnson & Saunders, 1968) have shown that the interfacial tensions between benzene solutions of egg-PC and pure water are highly time dependent, equilibrium values not being achieved even after 5 h. In contrast, the interfacial tensions of the egg-PC-BPA systems, while exhibiting a time dependence, attained equilibrium values after about 3 h in most cases (see Fig. 3).

Whilst adsorption of PC molecules from the benzene phase is not entirely diffusion controlled, it is an activated process, the phospholipid molecules requiring a certain activation energy for adsorption at the interface. The more rapid attainment of equilibrium at the interface in the presence of protein suggests that the interfacial protein is, in some way, reducing the adsorption activation energy of the phospholipid. This is probably the result of interaction between the protein and phospholipid.

Interactions between proteins and lipid monolayers at the air-water interface have been studied by injecting protein beneath the monolayer (Doty & Schulman, 1949; Eley & Hedge, 1956; Colaccio, 1969). The resulting increase in film pressure has been taken as a measure of the degree of interaction of the lipid and protein.

It has been found by Eley & Hedge (1956) and Colaccio (1969) that with phosphatidylcholine films. initially at low surface pressure, interaction with injected BPA and γ-globulin occurred, a large increase in film pressure being observed. Eley & Hedge (1956) concluded that the interaction of phosphatidylcholine with BPA was predominantly electrostatic between the charged groups of the lipid and the protein. By studying aqueous dispersions of egg-PC and BPA, O'Keeffe (1967) concluded that a complex of indefinite composition was formed, the most plausible mechanisms of binding being between the hydrophobic portions of the two species and electrostatic forces between the respective charged groups of the two compounds.

For the benzene-water interface, forces between the hydrophobic portions of the lipid and any protein protruding into the benzene phase, will be much reduced owing to the surrounding non-polar environment. The most probable interaction therefore is provided by electrostatic forces between the polar groups of the zwitterionic phospholipid protruding into the aqueous phase, and the corresponding oppositely charged groups of the protein amino-acid residues.

In the aqueous phase at pH 7, the protein carboxyl groups will be ionized, thus the interaction of these groups with the quaternary amino-groups of the PC is most probable.

In addition to a reduction in the adsorption activation energy, such interaction would result in the adsorbed phospholipid molecule achieving a lower potential energy at the interface due to the presence of the protein. The tendency of the phospholipid to diffuse back into the benzene phase would thus be reduced compared with the protein free interface; this would favour a more rapid equilibration.

For the aqueous dispersions of egg-PC at the benzene water interface, the effect of sonication which produces a stable aqueous dispersion of egg-PC, was clearly reflected in the observed concentration dependence of the interfacial tension results. The lack of dependence upon bulk concentration in the case of simple aqueous dispersions, is attributed to the unstable, highly polydisperse nature of these non-sonicated preparations.

REFERENCES

ATTWOOD, D. & SAUNDERS, L. (1966). *Biochim. biophys. Acta*, **116**, 108–113.
CHEESMAN, D. & DAVIES, J. T. (1954). *Adv. Protein Chem.*, **9**, 439–501.
COHN, E. J., HUGHES, W. L. & WEARE, J. H. (1947). *J. Am. chem. Soc.*, **69**, 1753–1761.
COLACCIO, G. (1969). *J. Colloid Inter. Sci.*, **29**, 345–364.
DOTY, P. & SCHULMAN, J. H. (1949). *Discuss. Faraday Soc.*, **6**, 21–27.
ELEY, D. D. & HEDGE, D. G. (1956). *J. Colloid Sci.*, **11**, 445–450.
JOHNSON, M. C. R. & SAUNDERS, L. (1968). *Proc. 5th Int. Congr. Surf. Activ.*
O'KEEFFE, A. (1967). Ph.D. Thesis, University of London.
PADDAY, J. (1957). *Proc. 2nd Int. Congr. Surf. Activ.*
PERRIN, J. H. (1962). Ph.D. Thesis, University of London.
RUYSSEN, R. (1946). *Recl. trav. chim. Pays-Bas. Belg.*, **65**, 580–584.
SAUNDERS, L. (1957). *J. Pharm. Pharmac.*, **9**, 834–840.
SAUNDERS, L., PERRIN, J. H. & GAMMACK, D. B. (1962). *Ibid.*, **14**, 567–572.

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The adsorption of crystal violet by kaolin

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The uptake of crystal violet on kaolin was examined over a pH range of 2·5 to 9·5, adsorption showing an increase with pH. The attainment of equilibrium between adsorbate and adsorbent is shown to be a two-stage process, the first stage being synchronous with the release of magnesium from the kaolin lattice. Evidence is presented that the major mechanism of adsorption is by electrostatic charges arising from cation replacement in the clay lattice, and that charges arising from the amphoteric nature of the aluminium atom of kaolin make little contribution to the overall adsorptive process.

The adsorption by kaolin of promazine has been reported by Sorby, Plein & Benmaman (1966) and of atropine by Ridout (1968a,b). These workers found the adsorption to be pH dependent. So that the mechanism of adsorption might be established, we have examined the uptake by kaolin of a basic substance, crystal violet, under a variety of experimental conditions.

MATERIALS AND METHODS

Crystal violet (reagent grade) was used without further purification.

Light kaolin (B.P. quality) was dried at 100° (3 h), mixed thoroughly and stored in tightly closed containers. The weight mean diameter of the kaolin was 3·3 μm (sedimentation balance), and the surface area 11·2 $m^2\ g^{-1}$ (nitrogen adsorption: Perkin-Elmer Shell sorptometer, Model 212D).

The effect of pH on the adsorption of crystal violet by kaolin

To flasks containing kaolin (2 g) was added 100 ml of a solution of crystal violet (50 mg/100 ml) in citrate–phosphate–borate buffers of varying pH (Teorell & Stenhagen, 1938). The flasks were equilibrated at 24° (12 h). Samples were removed from each flask and centrifuged at 10 000 rev/min (2 min) and the concentration of crystal violet determined spectrophotometrically at 590 nm. Beer's law was obeyed over the range of concentrations used.

Adsorption isotherms of crystal violet on kaolin were determined in the same way at a range of pH values and using crystal violet concentrations ranging from 40–60 mg/100 ml. Equilibration and determination of the dye in the supernatant were carried out as above and the adsorption isotherms constructed.

Leaching of inorganic ions from kaolin

Measured amounts of N/100 sodium hydroxide solution were diluted to 100 ml with water, the sodium content measured by flame photometry, kaolin (10 g) was added and the whole allowed to equilibrate overnight. The sodium content of the supernatant was then determined. The experiments were then repeated incorporating EDTA in the mixture and the supernatant assayed for aluminium by complexation with Alizarin S (Barton, 1948) and for magnesium and calcium by atomic absorption spectrophotometry.

RESULTS

Preliminary experiments showed that on prolonged shaking with water, kaolin released an unknown substance which absorbed light in the ultraviolet region of the spectrum. For this reason, crystal violet was chosen as adsorbate since it can be readily determined in the visible region of the spectrum with no interference from substances leached from kaolin. The colour of crystal violet fades gradually, particularly at high pH (Turgeon & LaMer, 1952), and this has been attributed to a reaction between the dye and hydroxyl ions to give the corresponding carbinol, which is appreciably less soluble in water than crystal violet. Though Turgeon and LaMer have described this as a second order reaction, the present work indicates that first order kinetics may be applied to a first approximation and that the rate of fading (k) at various pH values may be described by the equation:

$$\log k = -0{\cdot}392 - \frac{14.85}{pH}$$

All experimentally determined concentrations of crystal violet were thus corrected for time and pH effects by application of this equation.

Data obtained from adsorption experiments were shown to fit the Langmuir equation:

$$\frac{C}{x/m} = \frac{1}{ab} + \frac{1}{b} \cdot C$$

where x = weight of adsorbate (mg) adsorbed by m g of adsorbent; C = the equilibration concentration (mg/100 ml): a,b are constants. A plot of $\frac{C}{x/m}$ against C, gave a straight line with slope 1/b, changes in the value of which provided a useful indication of the extent to which adsorption changed with experimental conditions.

The increase in uptake of crystal violet on kaolin with increasing pH is shown in Fig. 1, and values of "1/b" (g/mg) derived from isotherms determined at pH values of 4·4, 5·8, 6·8, 7·4, 8·1 are: 0·0450, 0·0448, 0·0416, 0·0409, 0·0378 respectively. It has

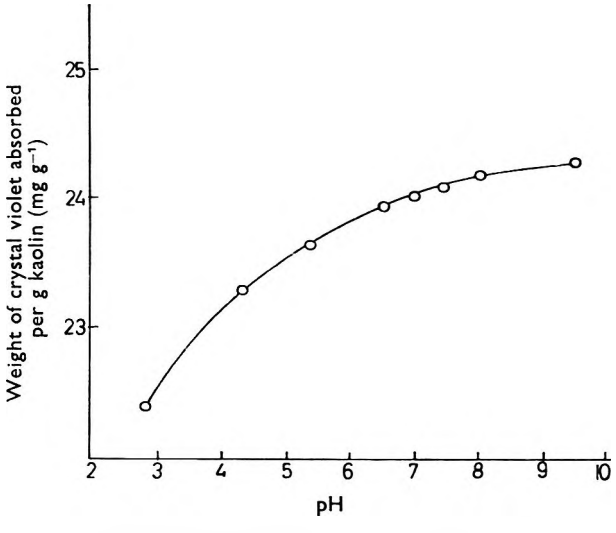

FIG. 1. The uptake of crystal violet on kaolin at a range of pH values.

been claimed (Ridout, 1968) that the uptake of atropine by kaolin can be represented by two straight lines of significantly different slopes because of the heterogeneous nature of the kaolin surface. No evidence of a two-stage Langmuir isotherm was found in the present work.

The rate of crystal violet uptake on kaolin shows a stepped curve before equilibrium is finally established after about 0·5 h (Fig. 2). No such deviation was reported (Ridout, 1968a) for the adsorption of atropine by kaolin and, since the adsorption curve before the "step" is non-linear, it is difficult to prove the validity of this step by standard statistical techniques. Nevertheless, numerous replicate determinations invariably showed the presence of this irregularity, and it is possible to explain it by reference to the molecular structure of kaolin.

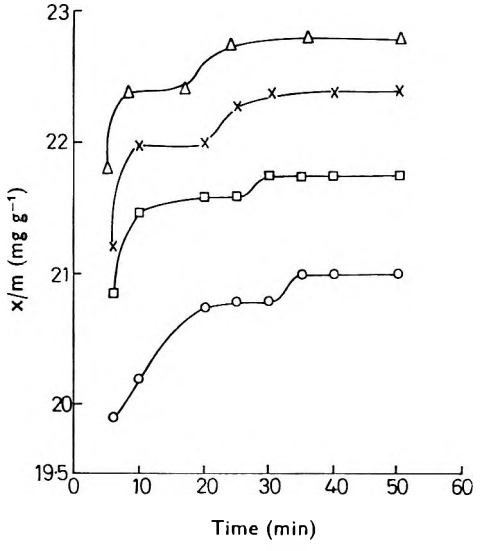

FIG. 2. The rate of uptake of crystal violet on kaolin. ○, pH 5·8; □, pH 6·6; ×, pH 7·7 △, pH 8·4.

DISCUSSION

The molecular structure of kaolin, $Al_4Si_4O_{10}(OH)_8$, is composed of two basic units (Brindley & Robinson, 1946; Brindley, 1951). One is an octahedron of oxygen atoms (or hydroxyl groups) surrounding a central aluminium atom, with several such octahedra sharing the oxygen atoms of identical adjacent structures, joined together to form a continuous sheet. The second unit is composed of four oxygen atoms situated at the corners of a regular tetrahedron, with a silicon atom in the centre. The atoms at the base of the tetrahedra are shared by neighbouring modules, and the two basic units are joined so that the apices of the tetrahedra and the top layer of the octahedra form a common plane (Gruner, 1932).

The adsorptive capacity of kaolin has been attributed to electrostatic charges which may arise by two different mechanisms. Grim (1953) has suggested that such charges may arise from broken bonds at the edge of the kaolin particle. Charges in the tetrahedral silicon layer will invariably be positive, irrespective of pH, while those in the octahedral aluminium layer will vary with pH, being positive at low pH and

negative at higher pH values. Thus at low pH, the entire edge carries a positive charge which will become less positive (and may even become negative) as the pH increases.

On the other hand, Schofield & Samson (1953) have suggested that the major site of electrostatic charges is on the face of the kaolin particle, caused by the isomorphous replacement of silicon and aluminium atoms in the tetrahedral and octahedral layers respectively by aluminium and magnesium (and to a lesser extent, calcium). Where the replacing atoms are mono or divalent, the resulting charges will invariably be negative and because the area of the faces is greater than that of the edges (Mering, Matthieu-Sicard & others, 1953), the overall charge on the particle is likely to be negative. Whatever the site of the charge, it is externally compensated by attraction of cations to the kaolin surface.

In the pH range studied, the molecular form of crystal violet is an ion bearing one positive charge (Finar, 1963), which suggests attachment to a negative site on the kaolin particle. The increase in the amount of adsorption with pH may then be due to two factors:

(i) An increase in the density of the negative charge on the edge of the kaolin due to the charge on the aluminium atoms at the edge changing from positive to negative. The pH at which the charge on hydrated alumina is reversed (the zero point of charge) has been reported by van Schuylenborgh & Sanger (1949) as ranging from pH 2·2 to 5·6, depending on the physical structure of the alumina. Though similar information for kaolin has not been published, electrophoresis and flocculation measurements (unpublished observations) indicate a zero point of charge in the range 4·9 to 5·0. This is unlikely to account for the marked increase in adsorption at higher pH values.

(ii) The increased concentration of sodium hydroxide in the system needed to achieve high pH values promotes replacement on the exterior of the clay lattice of the charge-compensating cations of magnesium, calcium and possibly aluminium, by sodium ions. This represents a cation exchange mechanism. The resulting electrical imbalance in the clay lattice increases the negative charge on the particle as a whole and consequently favours the adsorption of a positively charged ion such as crystal violet.

Though aluminium was considered to be the most likely atom to be replaced by sodium, kaolin is associated with appreciable amounts of magnesium and calcium by isomorphous replacement. Since it is probably easier for monovalent sodium to exchange with a divalent rather than a trivalent species, all the above possibilities of ion exchange were examined.

Fig. 3 shows the change in aluminium concentration with change in sodium hydroxide concentration. In this the amount of aluminium leached into the supernatant is negligible between pH 4 and pH 9.

By contrast the appearance of cations such as magnesium in the supernatant shows a marked increase with pH, and it is concluded that ion exchange is the main adsorptive process. Though it is recognized that this mechanism may involve several ionic species, the release of magnesium was examined because of the ease with which small changes in concentration of this ion can be determined. Fig. 4 shows that the attainment of a maximum magnesium concentration in the supernatant is synchronous with the first plateau of the adsorption curve of crystal violet on kaolin (Fig. 2). The second plateau of the latter is apparently unconnected with magnesium

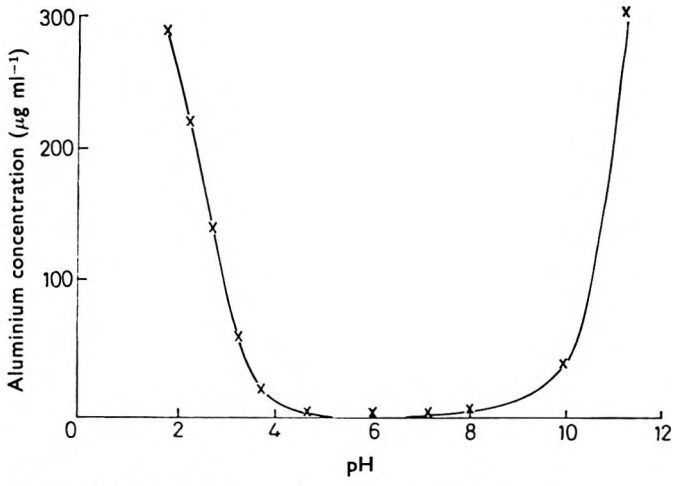

FIG. 3. Variation in supernatant concentration of aluminium with pH.

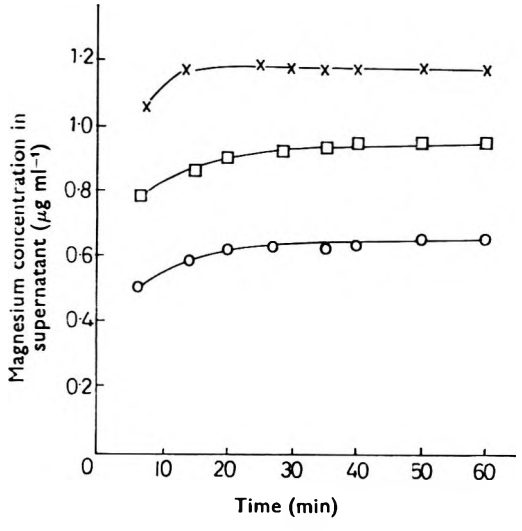

FIG. 4. The rate of leaching of magnesium from kaolin. ○, pH 5·8; □, pH 7·0; ×, pH 8·0.

exchange and marks completion of true physical adsorption by intermolecular attractive forces.

Ridout has suggested that the marked increase in the adsorption of atropine sulphate on to kaolin with increase in pH is due to the increase in the density of the negative charge on the edge of the kaolin particle, the amount of adsorption on the face of the particle being constant. However, this explanation must be viewed with caution since: (i) If the zero point of charge on the aluminium atom is in the region of pH 5, most of the aluminium atoms on the edge of the particle will bear a negative charge in the pH range 7 to 9, yet adsorption is still increasing at this point. (ii) The surface area of the edge of the kaolin particle is much smaller than the area of the faces. We have found that electron micrographs of shadowed kaolin indicate an edge to face area ratio of 1 to 14. (iii) The rate of uptake of crystal violet bears a

striking resemblance to the rate of release of magnesium from the clay by ion exchange.

Thus it is concluded that the major mechanism for the adsorption of bases on to kaolin is by electrostatic charges arising from cation replacement in the clay lattice. Such adsorption can take place either on the faces or the edge of the kaolin particle. It is considered that charges arising from the amphoteric nature of the aluminium atom make a relatively small contribution to the total adsorptive process.

REFERENCES

BARTON, C. J. (1948). *Analyt. Chem.*, **20**, 1068–1073.

BRINDLEY, G. W. (1951). *The kaolin minerals. X-ray identification and structure of clay minerals*, p. 32. Mineralogical Society of Great Britain Monograph.

BRINDLEY, G. W. & ROBINSON, K. (1946). *Nature*, **156**, 661–662.

FINAR, I. L. (1963). *Organic Chemistry*, Vol. 1, 4th Edition, p. 789. London: Longmans.

GRUNER, J. W. (1932). *Z. Krist.*, **82**, 75–88.

GRIM, R. E. (1953). *Clay Mineralogy*, p. 132. New York: McGraw-Hill.

MERING, J., MATTHIEU-SICARD, A. & PERRIN-BONNET, I. (1953). *Congr. Geol. Intern. Compt. Rend.*, **18**, 103–107.

RIDOUT, C. W. (1968a). *Pharm. Acta Helv.*, **43**, 42–49.

RIDOUT, C. W. (1968b). *Ibid.*, **43**, 177–181.

SCHOFIELD, R. K. & SAMSON, H. R. (1953). *Clay Minerals Bull.*, **2**, 45–50.

SORBY, D. L., PLEIN, E. M. & BENMAMAN, J. D. (1966). *J. pharm. Sci.*, **55**, 785–794.

TEORELL, T. & STENHAGEN, E. (1938). *Biochem. Z.*, **299**, 416–419.

TURGEON, J. C. & LAMER, V. K. (1952). *J. Am. chem. Soc.*, **74**, 5988–5995.

VAN SCHUYLENBORGH, J. & SANGER, A. M. H. (1949). *Recl. Trav. chim. Pays-Bas. Belg.*, **68**, 999–1010.

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The particle-size of sulphadiazine produced by a solvent-change method

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The factors affecting the particle-size of sulphadiazine produced by a solvent-change method of crystallization have been studied. The most important variables appear to be the degree of turbulence in the pipeline mixer used for the crystallization process, and the concentration of drug in the dimethylformamide injection solution. Mechanisms which can account for these, and other features of this crystallization method, are presented.

The *in vivo* absorption of a number of drugs is limited by their dissolution rate and with such materials reduction in particle-size can result in improved physiological availability (Fincher, 1968). Of the various methods used to obtain drugs in fine-particle form, the application of controlled crystallization brought about by solvent-change, has already been described (U.S. Patent Number 2,908,612, 1959; Lees, 1969). Factors affecting the particle-size of crystals produced by such methods, however, have received little attention and the report of Packter (1959), working with mechanically stirred solutions, appears to be the only one giving results for the crystallization of organic compounds. The solvent-change method has been adapted industrially as described in British Patent Number 899,667 (1962) for the production of fine-particle griseofulvin. In this case the mixing of solvent and non-solvent is carried out in a pipeline mixer and the process is termed "line-mixing". The present investigation reports studies on the factors affecting the particle-size of sulphadiazine produced by injection of a solution in dimethylformamide (DMF) into water flowing through a pipeline mixer.

MATERIALS AND METHODS

Materials. The sulphadiazine used was B.P. quality. Dimethylformamide was distilled under reduced pressure and gave $n_{25}^{D} = 1{\cdot}4283$ ($n_{25}^{D} = 1{\cdot}4294$, Dawson, Golben & others, 1952 and $n_{25}^{D} = 1{\cdot}4269$ Ruhoff & Reid, 1937). The water used in the pipeline mixer was deionized by passage through a mixed-bed ion-exchanger.

Apparatus

The apparatus (Fig. 1) consisted of a device giving a constant flow rate of water, a mixing tube and a means of injecting a solution of sulphadiazine in DMF, into the water flowing along the tube.

The constant-head device was a reservoir fitted with overflow and replenishment tubes, the latter carrying deionized water pumped from a bulk tank. Flow from the reservoir to the mixer was controlled by a tap fitted at the reservoir base.

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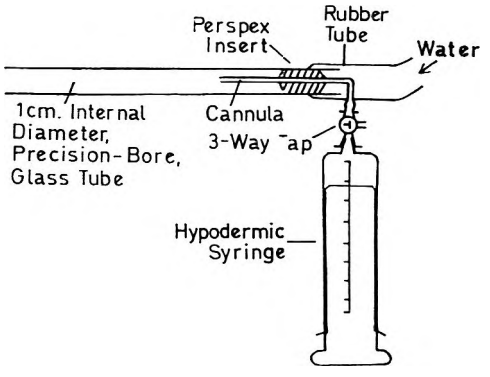

FIG. 1. Diagram of the pipeline mixer.

The mixing tube was 1 cm internal diameter, precision-bore, Pyrex glass tubing. The tubing was supported by brass angle-pieces secured to a movable horizontal beam which allowed the tube to be moved when the reservoir was raised or lowered. The beam was on a slide which was adjustable so that the tube was maintained horizontal. The tube assembly used for crystallization experiments made under turbulent flow conditions measured 193 cm in length from the position of the injector needle tip. That used for non-turbulent flow was 444 cm long.

The injector unit comprised a hypodermic syringe held in a Tufnol cradle, fitted with a screw drive propelled by a constant speed motor. The motor speed was controlled through a 50 KΩ helical wire-wound potentiometer fitted with a multi-turn indicating dial. The syringe was connected by way of a three-way tap to a cannula, which was bent to allow insertion into the inside of the mixing tube. The cannula had an internal diameter of 1·13 mm, and the portion within the tube was 13·5 cm long, measured from the point of entry, which was by puncture of the rubber tubing joining the mixing tube to the reservoir. The cannula was located centrally in the mixing tube by means of a small Perspex insert.

In operation, the water flow down the mixing tube was first established and this was followed by simultaneously operating the three-way tap (previously closed to prevent access of water to the syringe) and the syringe drive, to inject drug (sulphadiazine) solution into the mixing tube. The water flow rate was calibrated by collecting and measuring volumes issuing from the mixer over timed intervals. Calibration of injector flow rates was by weighing quantities of solutions collected over timed intervals, direct from the cannula which was withdrawn fom the mixing tube. The weights were converted to volumes using the density figures:—

% w/v sulphadiazine in DMF	3	5	6	7·5	9	10	12	15
Density (g/cm³) ..	0·9599	0·9672	0·9709	0·9763	0·9821	0·9856	0·9930	1·0036

Syringe flow-rate calibrations were made in duplicate over a range of potentiometer dial settings using 3, 6, 9, 12 and 15% w/v sulphadiazine in DMF solutions. The figures obtained showed a maximum variation about the mean of approximately 3% for a given dial setting, using different sulphadiazine concentrations, and the variation occurred randomly. Viscosity differences of the solutions did not appear to effect the flow rates.

To check that injector flow rates were not significantly modified when the cannula was positioned in the water stream of the crystallizer, a volume of mixed liquids was collected over a timed interval. The sulphadiazine concentration in the mixture was determined by dissolving the precipitated drug in a suitable volume of DMF and assaying an appropriately diluted sample spectrophotometrically. The calculated value for injector flow rate was in good agreement with that given by the calibration method described.

Collection and treatment of samples

Crystals were examined microscopically soon after collection from the mixer, 1 h after mixing and at later times. Except for cases of very low supersaturation arising from solutions of low concentration being injected, no significant change in the particle-size of crystalline suspensions obtained from the mixer occurred subsequent to storage for 1 h. Assays performed on suspension filtrates (passing an 0·2 μm membrane filter) at similar times indicated crystallization to be virtually complete after 1 h. Except where indicated, all crystal samples were obtained from slurries stored for 1 h before the commencement of filtration. Production and storage of slurries was at 20°.

Crystals were collected on 0·2 μm membrane filters using vacuum. This pore-size filter was chosen so that all particles, down to at least those just visible under the microscope (about 0·2 μm when using white light, Edmundson, 1967) would be retained. After washing with a small amount of water, the samples were sucked as dry as possible, and drying completed at 40° in a vacuum, over phosphorus pentoxide.

Particle-size measurement

The particle-size of crystalline products has been evaluated by a microscope method and by an air-permeability method. Because of the pronounced anisometry of the sulphadiazine crystals it was not possible to use the microscope method (British Standard 3406: Part 4: 1963) to obtain an estimate of the projected area of particles. Instead, the eyepiece graticule was used to size particles in terms of their length. Due to the hydrophobic nature of the drug, and the need to use a mountant of reasonable viscosity to prevent Brownian movement of fine particles, samples were suspended in light liquid paraffin containing 3% Span 80 (Honeywill-Atlas Ltd.). Sizing was carried out in other respects in accordance with the British Standard method: a minimum count of 1000 particles was made for each sample, with adjustment of the suspension concentration so that not less than 96 fields were counted for the most frequently occurring particle-size. The results have been used to give cumulative number-percent undersize-frequency curves and the arithmetic length-number mean, d_{ln} ($= \Sigma nd/\Sigma n$).

Surface area measurements were made on the crystalline samples using the Rigden air permeability method (Rigden, 1943) to the results of which a slip-flow correction was applied (Rigden, 1947). The Rigden apparatus was modified by use of an accurately machined powder cell allowing formation of plugs from 50 mg samples. Plugs, compressed to a porosity of 0·5, were prepared by applying even pressure on both plungers simultaneously, a process found to give the most reproducible results. Calculations were based on a density of sulphadiazine of 1·505, measured using a 50 ml density bottle.

Calculation of the theoretical degree of supersaturation

The theoretical degree of supersaturation, with reference to sulphadiazine, of pipeline mixtures after complete mixing was calculated as the ratio C'/C_s, where C' is the total concentration of sulphadiazine present and C_s is the equilibrium solubility of sulphadiazine in the pipeline solvent mixture. Values of C_s for solvent compositions in deionized water at 20° are:—

DMF (% v/v)	0	0·25	0·5	0·75	1·0
Sulphadiazine (% w/w) ..	0·00455	0·00475	0·00488	0·00510	0·00525

Values for intermediate solvent compositions were obtained by graphical interpolation.

RESULTS AND DISCUSSION

Crystallization conditions and particle-size data for water flow rates, of 6200, 3100, and 620 ml/min are given in Tables 1–3. The first part of the reference number used to identify each sample indicated the % w/v of sulphadiazine in the DMF solution injected.

Table 1. *Crystallization conditions and particle-size data.* Water flow 6200 ml min^{-1}.

Reference number*	Drug solution flow (ml min^{-1})	Theoretical degree of supersaturation,† SS	d_{1n}, μm, from microscope	Specific surface area from air permeability method $m^2\ g^{-1}$
15/1	4·3	2·27	$0·7_5$	insufficient sample
15/2	8·6	4·48	$0·9_1$	12·7
15/3	17·2	8·78	$0·9_1$	12·2
15/4	25·0	12·60	1·0	10·8
12/1	5·4	2·27	0·9	insufficient sample
12/2	7·7	3·22	1·0	10·3
12/3	10·8	4·48	1·2	8·9
12/4	15·4	6·32	1·2	9·5
12/5	21·6	8·73	1·2	9·6
12/6	25·0	10·00	1·5	8·7
9/1	7·7	2·41	2·0	6·4
9/2	10·8	3·36	2·5	6·3
9/3	15·4	4·74	3·0	5·7
9/4	21·6	6·55	3·4	5·6
9/5	25·0	7·52	3·3	5·6
6/1	10·8	2·24	4·1	insufficient sample
6/2	13·6	2·79	7·0	1·7
6/3	15·4	3·15	7·6	1·9
6/4	19·1	3·88	7·4	1·8
6/5	21·6	4·36	8·3	1·4
6/6	25·0	5·00	8·5	1·3
3/1	15·4	1·57		
3/2	21·6	2·18		See footnote†
3/3	25·0	2·50		

* The first part of the reference number is the % w/v of sulphadiazine in the injection solution.

† $SS = \dfrac{\text{Weight of sulphadiazine contained in 100 ml pipeline mixture before crystallization}}{\text{equilibrium saturation solubility of sulphadiazine in pipeline mixed solvent}}$

‡ Crystals collected after 24 h storage at 20° in case of sample 3/3. No crystals visible in 3/1 and 3/2 after 24 h storage.

The data, together with previously published information on the solubility of sulphadiazine in mixtures of water and DMF (Elworthy & Worthington, 1968), have been used to interpret the effects of turbulence and other factors on the particle-size of sulphadiazine produced by solvent change.

Table 2. *Crystallization conditions and particle-size data.* Water flow 3100 ml min^{-1}.

Reference number*	Drug solution flow (ml min^{-1})	Theoretical degree of supersaturation,† SS	d_{ln}, μm, from microscope	Specific surface area from air permeability method (m^2 g^{-1})
15/5	2·15	2·27	$0·9_5$	10·5
15/6	4·3	4·51	$0·9_5$	11·7
15/7	8·6	8·58	1·0	10·5
15/8	17·2	16·97	1·2	9·3
15/9	25·0	23·88	1·3	8·9
12/7	2·7	2·25	1·5	8·6
12/8	5·4	4·48	1·5	8·2
12/9	10·8	8·74	1·6	8·1
12/10	15·4	12·22	1·6	8·1
12/11	21·6	16·24	1·8	7·5
12/12	25·0	18·95	1·4	9·1
9/6	3·85	2·42	2·5	6·8
9/7	7·7	4·74	2·8	6·7
9/8	10·8	6·55	2·7	6·6
9/9	15·4	9·14	2·5	6·6
9/10	21·6	12·46	2·6	6·5
9/11	25·0	14·21	2·7	6·6
6/7	5·4	2·24	3·4	4·8
6/8	7·7	3·15	3·6	4·6
6/9	10·8	4·36	3·7	4·2
6/10	13·6	5·42	4·6	3·6
6/11	16·7	6·54	5·3	2·9
6/12	19·1	7·43	6·7	2·4
6/13	25·0	9·42	3·7	4·2
3/4	7·7	1·57		
3/5	10·8	2·17		
3/6	15·4	3·04		See footnote §
3/7	21·6	4·13		
3/8	25·0	4·69		

*† See Table 1.
§ Crystals collected after 24 h storage at 20°. Crystals too large for microscope sizing and insufficient in quantity for air permeability method.

Table 3. *Crystallization conditions and particle-size data.* Water flow 620 ml min^{-1}.

Reference number*	Drug solution flow (ml min^{-1})	Theoretical degree of supersaturation,† SS	d_{ln}, μm from microscope	Specific surface area from air permeability method (m^2 g^{-1})
15/10	0·5	2·64	1·7	8·0
15/11	1·1	5·71	1·6	8·6
15/12	2·5	12·56	1·8	7·6
9/12	1·1	3·41	3·2	5·4
9/13	2·5	7·53	3·8	4·3
9/14	5·4	15·17	4·4	3·4
3/9	2·5	2·51		See footnote §
3/10	15·4	12·02	8·1	1·5

*† See Table 1,
§ Crystals collected after 24 h storage at 20°. Crystals too large for microscope sizing and insufficient in quantity for air permeability method.

The effect of turbulence on particle size

The rate at which mixing of the two fluid streams takes place in the pipe must be largely influenced by the degree of turbulence existing in the fluids as they pass down the mixing tube. The principal consequence of mixing is that a fluid of changed

solvent power for sulphadiazine is produced, giving a mixture supersaturated with respect to the drug. The speed at which supersaturation increases will depend on the speed of mixing and on the rate of the competing process of crystallization, the latter bringing about relief of supersaturation. The effects of turbulence are seen by comparing samples derived from mixtures of similar theoretical supersaturation but prepared under different mixing conditions, as for example in Table 4, extracted from

Table 4. *The effect of turbulence on particle-size.*

Reference number	Water flow (ml min^{-1})	Drug solution flow (ml min^{-1})	Reynolds number	Theoretical degree of supersaturation after complete mixing	d_{in}, μm	Specific surface area ($m^2 g^{-1}$)
15/1	6200	4·3	13 155	2·27	$0·7_5$	—
15/2	6200	8·6	13 155	4·48	$0·9_1$	12·7
15/5	3100	2·15	6577	2·27	$0·9_5$	10·5
15/10	620	0·5	1315	2·64	1·8	7·6
6/3	6200	15·4	13 155	3·15	7·6	1·9
6/8	3100	7·7	6577	3·15	3·6	4·6
6/5	6200	21·6	13 155	4·36	8·3	1·4
6/9	3100	10·8	6577	4·36	3·7	4·2

Tables 1–3 for clarity. In the first four entries, the results for sample number 15/2 are included in Table 4 in support of the single size measurement available for sample number 15/1, for which a surface area figure in excess of 12·7 m^2g^{-1} is predicted. The results indicate that an increase in turbulence decreases the sulphadiazine particle-size. The size of the effect is not as large as was expected since, with non-turbulent flow conditions, materials of substantial specific surface area result. This may be explained by the large decrease in the solubility of sulphadiazine in passing from pure DMF, to DMF containing a relatively low percentage of water. As a result, only the partial mixing of the incoming sulphadiazine-DMF solution with the water stream gives rise to high supersaturations and in consequence high nucleation rates, causing production of fine-particle products. Classical crystallization theory indicates that the crystallization process is a balance of the consecutive and concurrent stages of nucleation and crystal growth. Nucleation shows a high order dependence on supersaturation, whereas crystal growth shows an approximately linear relation (see for example Van Hook, 1961; Schoen, 1961). High supersaturations therefore favour the formation of small particle-size products.

The same effect, a decrease in particle-size with increased turbulence, is shown with the 12% w/v sulphadiazine injection solutions. For 6% solutions the effect is reversed, as shown by the second four entries in Table 4. Here, for equivalent theoretical supersaturations, the system with the lower Reynolds number produced crystals of smaller particle-size. The difference in viscosity of sulphadiazine solutions of low and high concentration was thought to be a possible means of explaining this effect. Viscosities of drug-DMF solutions at 20°, measured using an M2 viscometer (British Standard 188: 1957), were found to be:—

Sulphadiazine in DMF (% w/v)..	0	3	9	15
Viscosity (cP)	$0·90_3$	$0·98_3$	$1·26_9$	$1·69_7$

Two levels of mixing are recognized (Brodkey, 1966): (i) "scale of segregation", which is a measure of the size of unmixed portions of the pure compounds, (ii)

"intensity of segregation", which describes the effect of molecular diffusion on the mixing process, and is a measure of the difference in concentration between the neighbouring portions of fluid. The increased solution viscosity (see above) of high concentrations of sulphadiazine in DMF may, by molecular diffusion, slow the reduction of intensity of segregation although this may not show as an effect on particle-size because of the high supersaturations achieved with partial mixing. The lower viscosity of weaker solutions enables complete mixing to be more rapidly achieved.

A more probable explanation for the reversed effect with 6% injection solutions and the less turbulent water flow lies in the relative speeds of mixing and nucleation. At the higher water flow, mixing may outpace the speed of nucleation. Lower degrees of supersaturation are reached as a result of dilution rather than by immediate crystallization. In these circumstances nucleation will be less favoured and larger crystals formed. With the slower mixing associated with the lower water flow of 3100 ml min^{-1}, more nucleation will be possible before dilution reduces supersaturation and smaller crystals will result.

With the intermediate strength, 9% w/v, sulphadiazine solution, the effect appears transitional between that for the concentrated solutions and that for 6% w/v sulphadiazine-DMF solution (Table 5).

Table 5. *The effect of turbulence on particle-size.*

Reference number	Water flow (ml min^{-1})	Sulphadiazine solution flow (ml min^{-1})	Reynolds number	Theoretical degree of supersaturation after complete mixing	d_{1n}, μm	Specific surface area (m^2 g^{-1})
9/4	6200	21·6	13 155	6·55	3·4	5·6
9/8	3100	10·8	6577	6·55	2·7	6·6
9/12	620	1·1	1315	3·41	3·2	5·4
9/13	620	2·5	1315	7·53	3·8	5·3

With 3% sulphadiazine-DMF solutions, crystallization was slow and gave large crystals. A significant yield of crystals could be obtained for sample 3/3, for example, only after 24 h storage. In this case, a theoretical degree of supersaturation of 2·5 existed and the effects contrast with those obtained for samples 15/1 and 15/5 where a slightly lower theoretical degree of supersaturation gave a yield of small crystals within 1 h of mixing. Owing to the sparse yield of crystals and the difficulty of applying the microscope method to large-particle materials, quantitative information is not available for the crystal size of most samples produced using 3% solutions. The rate of crystallization in some of these samples was monitored by assaying 0·2 μm membrane filtrates of pipeline mixtures that had been stored, and results are shown in Table 6. These suggest that for sample 3/1 the supersaturation conditions achieved at any stage are insufficient to promote nucleation. With sample 3/2 the supersaturation reached is either too transient or too low to allow more than slight nucleation: crystals are seen after 4 days. Conditions used for sample 3/3 gave crystals visible after 24 h. The effects are again explicable in terms of the relative rates of mixing, nucleation and crystal growth, and of the supersaturations possible during mixing. When low concentration solutions are used, the extent of mixing, in terms of the solvent composition, must be considerable before any supersaturation

Table 6. *The rate of decrease of sulphadiazine in solution in pipeline prepared mixtures using 3% w/v sulphadiazine in DMF and a water flow of 6200 ml min^{-1}.*

Reference number	Rate of injection of sulphadiazine solution (ml min^{-1})	Theoretical degree of supersaturation after complete mixing	0·2 μm membrane filtrate assays % w/w sulphadiazine 1 h	24 h	4 days	24 days
3/1	15·4	1·57	0·0072$_4$	0·0072$_1$	0·0072$_0$	0·0072$_0$
3/2	21·6	2·18	0·0102	0·0099$_7$	0·0085$_8$	0·0060$_0$
3/3	25·0	2·50	0·0119	0·0100	0·0070$_3$	0·0055$_7$

occurs. Because of this, the high local supersaturations possible in the early stages of mixing using high concentration solutions, cannot be realized. Consequently nucleation is limited, growth conditions prevail, and large crystals result.

A further factor, that may accentuate some of the effects described, is the phenomenon of "collision breeding" (Lal, 1966). By this means fresh nuclei are produced by collision of crystals under turbulent conditions. The effect is increased by an increase in supersaturation because of the decrease in critical cluster size with increase in supersaturation (Walton, 1965).

With laminar flow conditions, mixing takes place largely through molecular diffusion, but to some extent by convection. In the pipeline mixer a "core" of unmixed solution is evident for much of the length of the tube. Consequently, crystallization occurs in the region surrounding the core where diffusion has taken place to give a change in solvent characteristics. The core becomes coated with crystalline material which gradually separates into the bulk of the surrounding, largely aqueous, fluid. Concentrated solutions give materials of slightly larger particle-size than those produced at comparable theoretical degrees of supersaturation under turbulent flow conditions. However, the materials are small in particle-size compared to those resulting from injection of weaker solutions, even under turbulent conditions. It seems that because mixing is much less rapid with laminar flow, the 15% sulphadiazine solutions give reduced nucleation rates, although the high supersaturations resulting from small degrees of mixing still have an overwhelming influence. With the concentrated drug solution, material nucleated around the core may grow rapidly because of the higher concentration gradient from the underlying concentrated solution. With dilute solutions large crystals are produced, an effect again explicable by the low local supersaturations achievable with such mixtures. The absence of collision breeding will also emphasize the effect.

The effect of theoretical mixture supersaturations on particle-size

Numerous workers have shown the dependence of crystal number and size on the concentration of solute in excess of solubility. Although von Weimarn's work (von Weimarn, 1925) has been subject to some criticism (Kolthoff, 1932), the usefulness of the principles he deduced has been upheld by Cartwright, Newman & Wilson (1967). It is suggested that the theoretical mixture supersaturation figure is not reached in the pipeline because significant and often substantial crystallization occurs before mixing is complete. Nevertheless, the amount of drug available for crystallization per unit of volume of mixed fluids should be a factor influencing the overall process, and therefore the characteristics of the final crystalline product.

In a series of experiments in which water flow and strength of sulphadiazine solution are kept constant (for example samples 12/1 to 12/6, 9/1 to 9/5 and 9/12 to 9/14), the general trend is for an increase in average particle size, as final supersaturation increases. This result is the reverse of von Weimarn's findings; however von Weimarn was studying the precipitation of barium sulphate produced by reaction, rather than crystallization by solvent-change. In the present work, increasing theoretical mixture supersaturation was brought about by raising the speed of injection of sulphadiazine–DMF solution. This may affect the speed of mixing of the fluids in the tube; it will also affect the flow characteristics of the liquid leaving the cannula. Calculation of the Reynolds number for fluid flowing in the cannula, shows that this is streamline for all the injection rates used. It is possible that with small volumes, turbulent water flow breaks up the incoming fluid into smaller portions in a given time, than it does at faster injection rates. The lower scale of segregation would give higher supersaturations more rapidly, causing increased nucleation and smaller particle-size products.

Table 7. *The effect of the concentration of sulphadiazine in the DMF injection solution on particle-size.*

Reference number	% w/v sulphadiazine in DMF solution	Water flow (ml min^{-1})	Theoretical degree of supersaturation	d_{1n}, μm	Specific surface area (m^2 g^{-1})
15/2	15	6200	4·48	$0{\cdot}9_1$	12·7
12/3	12	6200	4·48	1·2	8·9
9/3	9	6200	4·74	3·0	5·7
6/5	6	6200	4·36	8·3	1·4
15/6	15	3100	4·51	$0{\cdot}9_5$	11·7
12/8	12	3100	4·48	1·5	8·2
9/7	9	3100	4·74	2·8	6·7
6/9	6	3100	4·36	3·7	4·2
15/12	15	620	12·56	1·8	7·6
9/13	9	620	7·53	3·8	4·3
9/14	9	620	15·17	4·4	3·4
3/10	3	620	12·02	7·9	1·5

The effect of the concentration of sulphadiazine in the DMF solution on particle-size

The effects on particle size of samples prepared under similar conditions of turbulence and theoretical mixture supersaturation, but using different strength solutions, are compared in Table 7. For any specified level of turbulence and theoretical mixture supersaturation, increasing the concentration of sulphadiazine solution brings about a decrease in particle-size: the effect is evident for both turbulent and non-turbulent fluid flow in the pipeline. With high concentration solutions, high local supersaturations exist in the earliest stages of mixing, causing high nucleation rates. Also the higher diffusion gradients existing with more concentrated solutions will facilitate more rapid mixing at the molecular level, and rapidly reduce the intensity of segregation. Table 7 also summarizes results for similar theoretical mixture supersaturations.

REFERENCES

BRODKEY, R. S. (1966). In *Mixing, Theory and Practice*, Vol. I, pp. 58. Editors: Uhl, V. W. & Gray, J. B. New York: Academic Press.

CARTWRIGHT, P. F. S., NEWMAN, E. J. & WILSON, D. W. (1967). *Analyst, Lond.*, **92**, 664–679.

DAWSON, L. R., GOLBEN, M., LEADER, G. R. & ZIMMERMAN, H. K. (1952). *J. electrochem. Soc.*, **99**, 28–29.

EDMUNDSON, I. C. (1967). In *Adv. pharm. Sci.*, Vol. II, pp. 121. Editors: Bean, H. S., Beckett, A. H. & Carless, J. E. London: Academic Press.

ELWORTHY, P. H. & WORTHINGTON, H. E. C. (1968). *J. Pharm. Pharmac.*, **20**, 830–835.

FINCHER, J. H. (1968). *J. pharm. Sci.*, **57**, 1825–1835.

KENNARD, E. H. (1938). *Kinetic Theory of Gases*, 1st edn, pp. 149. New York: McGraw-Hill.

KOLTHOFF, I. M. (1932). *J. phys. Chem., Ithaca*, **36**, 860–881.

LAL, D. (1966). Ph.D. Thesis, London, through Strickland-Constable, R. F. (1968). *Kinetics and Mechanism of Crystallization*, p. 117. London: Academic Press.

LEES, K. A. (1969). *Pharm. J.*, **202**, 415.

PACKTER, A. (1959). *Chimia*, **13**, 161–163.

RIGDEN, P. J. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 1–4.

RIGDEN, P. J. (1947). *Ibid.*, **66**, 130–136.

RUHOFF, J. R. & REID, E. E. (1937). *J. Am. chem. Soc.*, **59**, 401–2.

SCHOEN, H. M. (1961). *Ind. Engng Chem. ind. (int.) Edn*, **53**, 607–611.

VAN HOOK, A. (1961). *Crystallization, Theory & Practice*, pp. 97 & 115. New York: Reinhold.

VON WEIMARN, P. P. (1925). *Chem. Rev.*, **2**, 217–235.

WALTON, A. G. (1965). *Science, N.Y.*, **148**, 601–7.

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The kinetics of crystallization of potassium bromide from aqueous solution

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The densities of aqueous solutions of potassium bromide have been determined as a function of concentration and temperature up to saturation at temperatures between 10 and 50°. The saturation line and nucleation were studied, over the same temperature range, in a circulatory crystallizer with continuous concentration monitoring. Small quantities of seed crystals were grown under different degrees of supersaturation and at different temperatures, so that the mass transfer coefficients and the activation energy for deposition from solution could be measured. The activation energy of the crystallization process was 100·9 kJ mol^{-1}, indicating that the rate-determining step was the incorporation of KBr on to the crystal lattice. Rapidly-grown crystals might thus be expected to be softer, and this proved to be so.

Crystallization rates, particularly of pharmaceutically interesting materials from solution, have not been extensively studied, although the crystallization process is widely used in their manufacture. Rate data are relatively scarce yet are needed to test theories of deposition rate limitation, which may be governed by diffusion, viscosity or energy of incorporation into the crystal lattice: such data are also useful in crystallizer design.

Potassium bromide is used in the photographic industry, in pharmacy and in spectroscopy; it therefore seemed that measurements of the rate of growth of potassium bromide crystals from aqueous solution would be of some industrial interest. In addition, since the alkali halides have a simple crystal structure, it appeared likely that any lattice defects would produce macroscopic effects more easily observable than proved to be the case with aspirin crystals. Whereas the effect of growth rate upon crystal hardness could not be demonstrated with certainty for aspirin (Glasby & Ridgway, 1968), with potassium bromide it was felt that rapid growth rates might lead to lattice imperfections giving crystals with a measurably lower hardness. No data were found in the literature for the crystallization of potassium bromide beyond some solubility data for aqueous solutions (Linke, 1965).

APPARATUS AND METHODS

The continuously circulating crystallizer was of the type designed by Mullin & Garside (1968) (cf. Glasby & Ridgway, 1968); a small fluidized bed of seed crystals is held in a rising stream of supersaturated solution. The solution is heated to remove nuclei, then cooled to give the required supersaturation. Its concentration is monitored by continuous density measurement. A subsidiary heater prevented crystal deposition in the density meter sampling line; the meter compensator easily dealt with the 5° temperature rise so caused. Calibration was by using solutions of known density.

Material

The circulating solution was made from potassium bromide (B.D.H. Analar); used without further purification; the solution was filtered before pouring it into the crystallizer. Seed crystals were hand-picked from small batches recrystallized from water.

Density and solubility

Densities of solutions of known concentration were measured at temperatures between 50° and either 10° or the spontaneous nucleation temperature, whichever was the higher. The temperature was controlled, using a contact thermometer, to ±0·05° at 5° intervals over the range covered, and was maintained for at least 30 min at the appropriate temperature before the density determination was made. The range of concentration was 5 to 45% by weight of potassium bromide, the density being between 1·01 and 1·41 g cm^{-3}.

The saturation line between 10 and 50° ranges from 37 to 45 wt % of potassium bromide, and this composition range was examined in greater detail, using solutions increasing in concentration by 1 wt % increments, to give information about saturation and nucleation behaviour. The solution was brought to the correct strength in the crystallizer and cooled to a temperature of about 10° above the saturation temperature, which was already known approximately. The solution was then allowed to cool very slowly, density and temperature being recorded continuously, until nucleation occurred. This could be observed visually, and corresponded to the maximum recorded density. Crystals formed and the system was maintained at this constant temperature until there had been no density change, and hence no growth, for at least 1 h. This was considered to be saturation, reached from the supersaturated side. Saturation was then approached from the reverse direction. Most of the crystals that had been formed were removed, and the remainder were redissolved by heating. The solution was then re-cooled to just below the saturation temperature of the *original* solution. Thus the solution is now unsaturated because of the amount of potassium bromide that has been removed. When a steady state had been reached, this amount of bromide was replaced and the system left until the density ceased increasing, i.e., saturation had been reached from the unsaturated region by dissolution.

Crystal growth

The solution temperature was adjusted to 10° above the previously determined growth temperature, and the concentration also adjusted until the solution condition lay on the correct (extrapolated) density line for the required growth conditions. Solution was then circulated, cooled to the growth temperature, and allowed to stabilize for about 30 min, when it was ready for seeding.

Seed crystals were prepared by slow evaporation of an aqueous solution of potassium bromide at room temperature. They were removed from the solution when of the correct size, washed with acetone, dried and sieved into standard sieve fractions. The best cubic crystals were hand-picked under a binocular microscope and re-sieved, about 200 mg being used for a crystallization run. The small quantity is dictated, partly by the labour of obtaining good seed crystals, and partly so that the depletion of potassium bromide in solution in the crystallizer shall be small.

In a typical case, 200 mg were deposited on the seeds from the total of several kg in the circulating solution, so that the concentration driving force could be regarded as constant during a run.

The weighed quantity of seed crystals was allowed to remain in the crystallizer for a measured time under defined conditions, and was then removed, drained, washed with acetone, dried, and sieved using a set of 2 inch diameter sieves (Endecott Ltd.): the fractions were then weighed. Knowing the initial and final size distributions, and the weight of potassium bromide deposited, the coefficient of mass transfer operative during the run could be calculated.

The surface area on which the deposition occurred was found by determining the mean side length of a number of good cubic crystals by a projection microscope. For potassium bromide, which crystallizes in the cubic system, the dimensions of the unit cell are 0·328 nm; it has a density of 2·75 g cm^{-3} at 20°.

Surface hardness

Crystals from each batch were mounted on the stage of a pneumatic micro-indentation apparatus (Research Equipment (London) Ltd.), which gave a curve from which the Brinell hardness could be obtained by a technique described earlier (Ridgway, Aulton & Rosser, 1970).

RESULTS AND DISCUSSION

A summary of the experimental results is given in Table 1. The densities of aqueous solutions of potassium bromide at various concentrations are shown as a function of temperature in Fig. 1. More extensive determinations were made in the immediate neighbourhood of the saturation line to establish its position as accurately

Table 1. *Deposition rate of potassium bromide crystals from solution*

Temperature °C	Run	Growth concentration % w/w	Supersaturation ΔC % w/w	Percentage of supersaturation at growth	Weight of crystals (g) Initial	Final	Surface area of crystals (mm^2) Initial	Final	Deposition rate ng mm^{-2} s^{-1}	Mass transfer coefficient ng mm^{-2} s^{-1} $(\%w/w)^{-1}$	Brinell hardness number kg mm^{-2}
21·05	Saturation	(39·987)	(0)	(0)					—	3·90	
	21A	40·183	0·196	27	0·2914	0·3225	1620	2530	0·225		0·301
	21B	40·256	0·269	37	0·2101	0·2330	1345	1500	0·422		0·270
	21C	40·322	0·336	47	0·2354	0·2804	1016	1192	0·744		0·466
	21D	40·412	0·425	59	0·2057	0·3201	1480	1990	3·033		0·310
	21E	40·438	0·451	63	0·1957	0·4245	996	1737	93		0·216
	Nucleation	(40·70)	(0·72)	(100)					—		
29·97	Saturation	(41·982)	(0)	(0)					—	16·20	
	30A	42·233	0·251	38	0·2338	0·2575	1223	1385	0·698		0·354
	30B	42·302	0·320	49	0·6413	0·8494	2643	3193	2·328		0·268
	30C	42·369	0·387	59	0·3035	0·4758	1655	2632	2·542		0·432
	30D	42·435	0·453	69	0·3356	0·6395	1438	2285	15·2		0·151
	Nucleation	(42·64)	(0·66)	(100)					—		
40·00	Saturation	(43·584)	(0)	(0)					—	41·75	
	40A	43·719	0·135	35	0·1631	0·1808	928	982	1·45		0·282
	40B	43·815	0·231	60	0·1015	0·1494	515	755	4·15		0·396
	40C	43·877	0·293	76	0·3139	0·8314	1606	3825	7·25		0·321
	40D	43·918	0·334	86	0·2917	0·5828	1729	2444	454		0·164
	Nucleation	(43·97)	(0·39)	(100)					—		
47·57	Saturation	(44·545)	(0)	(0)					—	131·0	
	47A	44·695	0·150	39	0·2267	0·2775	1200	1369	2·60		0·481
	47B	44·753	0·208	54	0·1642	0·2570	779	1102	3·81		0·424
	47C	44·805	0·264	69	0·6329	1·1009	1317	3415	17·5		0·339
	47D	44·869	0·322	84	0·2406	3·1192	1451	6607	185		0·227
	Nucleation	(44·93)	(0·39)	(100)					—		

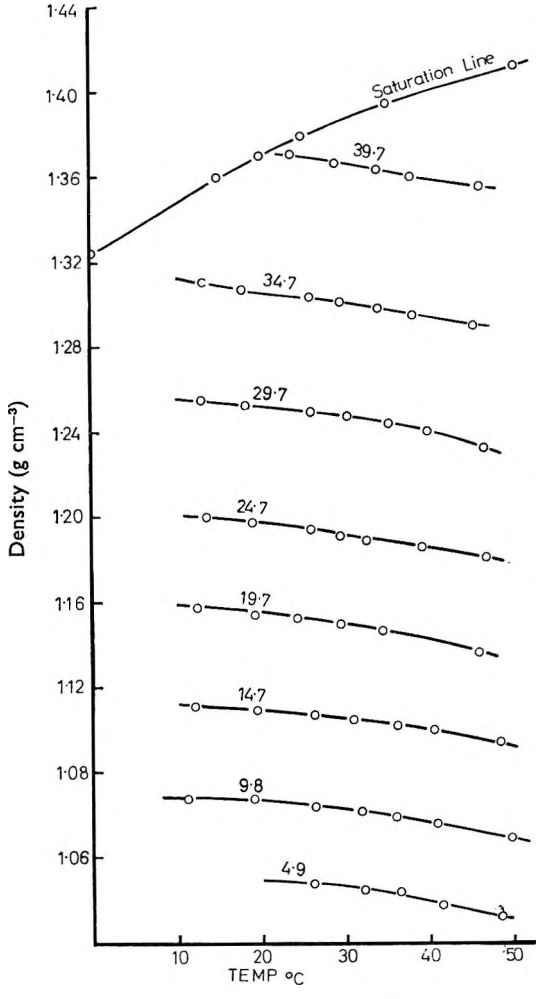

FIG. 1. Densities of aqueous solutions of potassium bromide of various concentrations, as a function of temperature. The numbers are the wt % KBr in the solution. The saturation line is plotted from published data (Linke, 1965).

as possible, and also the position of the nucleation line or limit of metastability, above which any solution will nucleate spontaneously under the working conditions of the crystallizer (Fig. 2). Since nucleation is a function of the degree and duration of agitation in the solution and of the presence of solid impurities which could act as nuclei, the scatter of the points defining nucleation is greater than that of the points defining the solubility line. The square points in pairs, one open and one filled, are the results obtained by approaching saturation from above and below respectively; these pairs bracket the saturation line closely.

In Fig. 3, a growth-rate determination is followed through in the form of a histogram. This series is for one of the higher temperature runs at which growth is rapid. In some of the slower runs, the change in sieve analysis was small, although the overall weight change was readily measured. This is why the weight rather than size change has been measured in most runs, and used to calculate the mass transfer coefficient. The median size increases as growth proceeds, although the range of

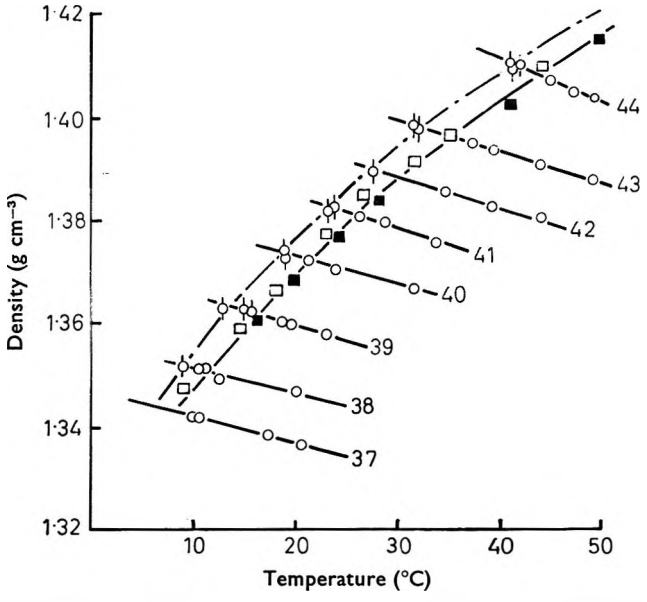

FIG. 2. The solubility line for potassium bromide in water, obtained by approaching the saturation condition from above and from below, the line being drawn between the points obtained by the two methods. □ approach from the supersaturated solution. ■ approach from the unsaturated solution. ○ density–temperature lines: the numbers on these lines refer to the wt % of KBr contained. Points with a vertical line through them indicate that nucleation occurred.

size does not broaden. In industrial practice, specific measures are taken to classify the product by size and to overcome any tendency of the size range to increase. This increase occurs (a) because of nucleation and attrition, both of which cause small crystals to form and (b) because large crystals tend to segregate to the bottom of a bed and meet incoming strong liquor, and thus grow even larger. In the circulating crystallizer used in this work, both effects appear to be absent. Any nuclei or small particles formed are removed from the crystal cage; also, the bed is small and well-mixed, so that there is no segregation of larger crystals within the cage.

The amount of potassium bromide deposited on the seed crystals is given in Fig. 4 for four temperatures and a range of supersaturations. After an initial short period of fast deposition in some cases all these plots of deposition against time are linear. This effect is usually ascribed (Mullin, 1961) to the fact that the seed crystal surface is microscopically rough and cracked due to attrition and storage: cracks are healed during the initial deposition, but the area for deposition is higher until this healing has taken place.

From the weight deposited plots of Fig. 4, the graphs of Fig. 5 are obtainable, where the data for each temperature are reduced to a single line, the slope of which is the mass transfer coefficient. This is the amount deposited on unit area of crystal surface in unit time, divided by the concentration difference causing the deposition. The slope of the lines increases with temperature, and all are approximately linear. They do not start from the origin, suggesting that a finite supersaturation is needed to start growth; such behaviour has been observed in other systems (Mullin, Amatavivadhana & Chakravorty, 1970), who showed that a degree of supersaturation was required in several salt-water systems to start growth. In dissolution, however,

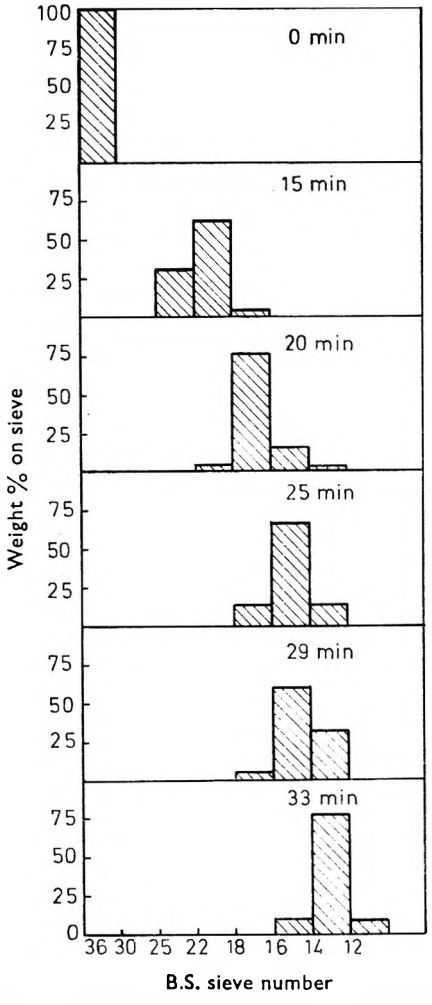

FIG. 3. Histogram showing the change of size distribution as growth proceeds.

it seems that the smallest reduction of concentration below the equilibrium solubility will cause the solid phase to dissolve. It is this effect which makes it necessary to approach the solubility line from both sides in order to fix its position. In this present work, at high supersaturations, the linearity of deposition with concentration driving force breaks down abruptly, since, even below the spontaneous nucleation point, growth occurred by rapid deposition of fresh small parasitic crystals on the seeds. Their surface became roughened, the deposition rate increased by a factor of 10–100 times, and the resultant crystals would have been regarded industrially as of very poor quality.

Mass transfer coefficients obtained from the slopes of the linear portions of the lines in Fig. 5 are strongly dependent upon the temperature. The dependence is of the Arrhenius form, so that a straight line is obtained in Fig. 6, where the logarithm of the mass transfer coefficient is plotted against the reciprocal of the absolute temperature: the slope of the line is -5260. The Arrhenius equation is

$$k = A \exp(-E/RT)$$

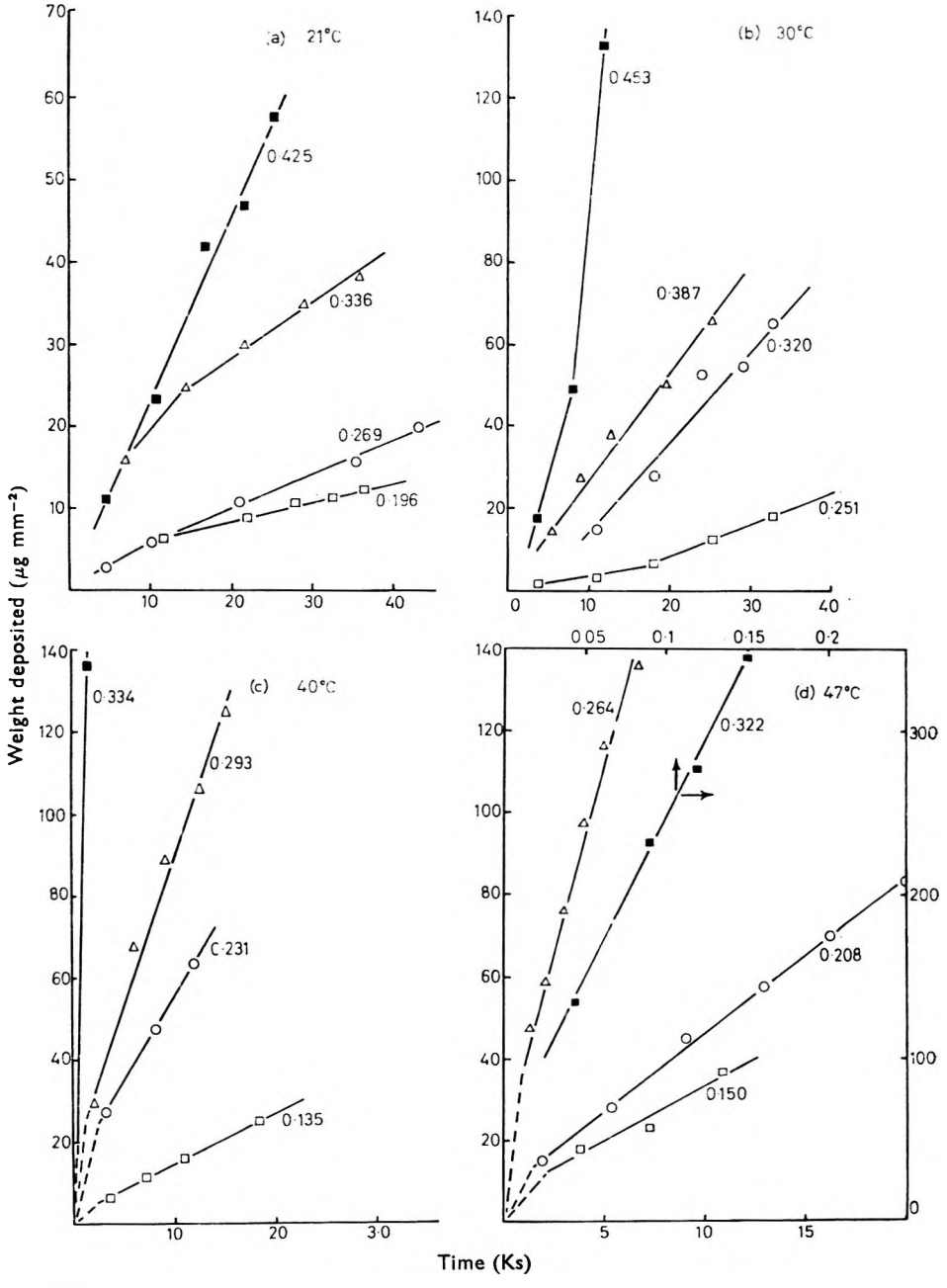

FIG. 4. The weight of potassium bromide deposited per unit area of crystal surface as a function of time, for four temperatures as indicated. The supersaturation, expressed as ΔC = (actual wt % concentration)—(saturated solution wt % concentration) is marked on each growth line.

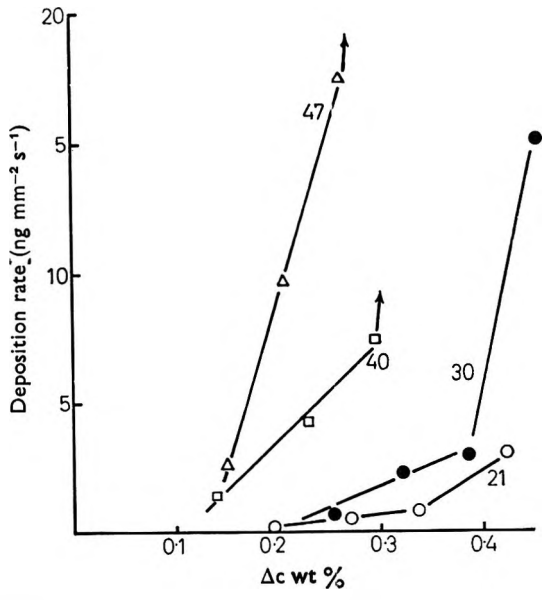

FIG. 5. The deposition rate of potassium bromide as a function of supersaturation at four temperatures. The initial portion of each graph is approximately a straight line, but a sudden rapid increase in deposition rate occurs at higher values of ΔC; the points are indicated, but the measured deposition rates are off the diagram at the scale used to show the other growth rates; their values are recorded in Table 1.

where k is a rate, A is a constant, E the activation energy for the rate process, R the gas constant and T the absolute temperature. Thus,

$$\log k = \frac{-E}{2{\cdot}303\ RT} + \log A$$

and the slope of the Arrhenius plot is $\frac{-E}{2{\cdot}303\ R}$: hence the activation energy is 100·9 kJ mol^{-1}. This may be compared with the value of 92·1 kJ mol^{-1} at 0° obtained by van Hook (1944) for the crystallization of sucrose from water, and with 91·2 kJ mol^{-1} obtained by Glasby & Ridgway (1968) for the crystallization of aspirin from ethanol. It is apparent from this value that the rate-controlling step is the incorporation of a molecule or ion-pair into the crystal lattice, and not the rate of diffusion to the crystal surface. Activation energies for diffusion are normally much lower, 20·9 kJ mol^{-1} being quoted for sodium chloride between 50 and 70° (Rumford & Bain, 1960). These authors' value for the activation energy of a diffusion-controlled crystallization agrees with van Hook's estimate of 27·2 kJ mol^{-1}, for sucrose, at the same elevated temperature. Rumford & Bain also suggested that below 50° the surface reaction step becomes controlling; no activation energies were published, however, for this region.

In the fluidized-bed crystallizer used in the present work, particularly with solutions of low viscosity, diffusion is rapid as boundary layers are thin. The viscosity of the solutions from which potassium bromide was deposited was about 1·1 cP despite the high concentration of the salt.

Fig. 7 suggests that there is a decrease in hardness with increase in rate of growth; rapidly grown crystals would be expected to contain more imperfections, built in

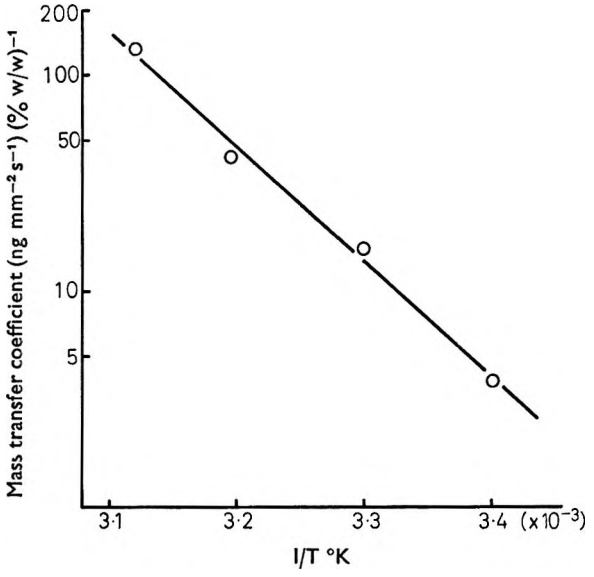

FIG. 6. Arrhenius-type plot of log k against 1/T. The line has a slope of −5260, giving an activation energy for the deposition process of 100·9 kT mol^{-1}.

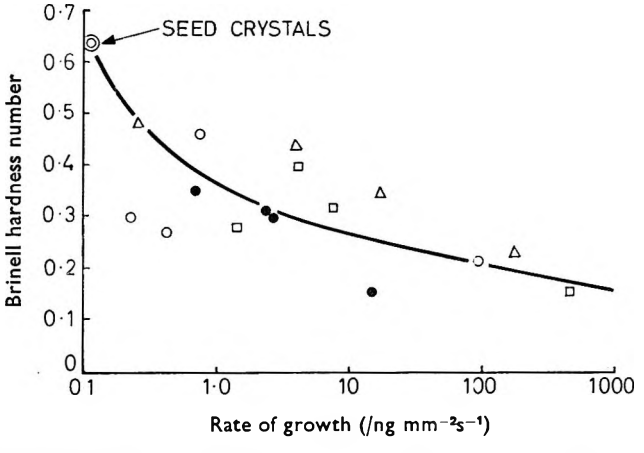

FIG. 7. Hardness as a function of growth rate. The symbols represent the growth temperatures as follows: ○ 21°, ● 30°, □ 40°, △ 47°.

during their deposition, and so, to be softer. The plot of hardness against the rate of growth for crystals made in the concentration range where the mass transfer coefficient was constant, gave an indication of the trend: the variability of the crystals within a batch, added to the experimental errors of hardness measurement, made it impossible to draw a line, but the trend towards softer crystals at higher growth rates is not in doubt; seed crystals moreover were harder than any grown in the circulatory crystallizer.

REFERENCES

GLASBY, J. & RIDGWAY, K. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 94S–103S.

LINKE, N. F. (1965). *Solubilities of inorganic and metal-organic compounds.* Am. chem. Soc., Washington, D.C., U.S.A.

MULLIN, J. W. & GARSIDE, J. (1968). *Trans. Instn. chem. Engrs*, **45**, 285–295.

MULLIN, J. W. (1961). *Crystallization.* London: Butterworths.

MULLIN, J. W., AMATAVIVADHAVA, A. & CHAKRAVORTY, M. (1970). *J. appl. Chem.*, **20**, 153–159.

RIDGWAY, K., AULTON, M. E. & ROSSER, P. H. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 70S–78S.

RUMFORD, F. & BAIN, J. (1960). *Trans. Instn. chem. Engrs*, **38**, 10–20.

VAN HOOK, A. (1944). *Ind. Engng Chem.*, **36**, 1042.

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The dissolution times of spherical particles

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The differential equation governing the rate of change of radius of a spherical particle dissolving or growing in a fluid has been numerically integrated by computer. The particle radius, as a function of time, can thus be calculated, and the dependence of the overall particle lifetime upon physical parameters obtained. The effects of high mass flux, change of solubility with particle size, and progressive saturation due to dissolution into a finite volume of liquid can all be taken into account during the integration process. Results are presented for salicylic, boric and citric acids dissolving in and crystallizing from water; these represent the behaviour of sparingly soluble, moderately soluble and very soluble compounds respectively.

The rate of dissolution of a particle in a liquid, the rate of growth of a crystal from a supersaturated solution, and the rate of evaporation of liquid from an atomized droplet in a spray drier or an aerosol spray, are all examples of high mass transfer rate processes. In this context high mass transfer rate means that the normal equations for the slow diffusion of a substance through a stagnant boundary layer do not apply, either because the movement of the transported substance itself disturbs the boundary layer, or because, as the phase change occurs, the surface of the particle recedes or advances relative to the instantaneous position of the interface between the phases. Ideally, for simulation calculation purposes, the particles should be spherical.

Differential equations governing the diffusion-controlled growth or dissolution of spheres in the absence of hydrodynamic instabilities have been derived by Readey & Cooper (1966). These authors assumed that the interface reaction was virtually instantaneous, i.e. that, for dissolution, the liquid adjacent to the surface was saturated with the solid. Composition-independent diffusion coefficients, and ideality of partial specific volumes of the solute and solvent with respect to concentration change, were also assumed.

Cable & Evans (1967) extended the work of the above authors and gave some computer solutions for dissolution by widening the ranges of the numerical factors, and by comparing their solutions with those of Scriven (1959) for growth from zero size. It became apparent that differences between the numerical computer solutions could easily arise, due to starting, iterative and discretization errors. Thus Duda & Vrentas (1969), who were interested in the growth and dissolution of bubbles, indicated that they disagreed in some quantitative respects with the results of Cable & Evans, whilst they in their turn have been criticized by Rosner (1969), for the same reason. Rosner has reviewed the earlier work, and has suggested an equation governing the rate of change of size; he has also obtained a solution for the overall particle lifetime, under dissolution conditions, in closed form for constant parameter

values. In a recent paper, Rosner & Epstein (1970) have included the effect of interface kinetic limitations, and have shown how to use the integral profile method (Goodman, 1964) in this field of non-linear moving-boundary problems.

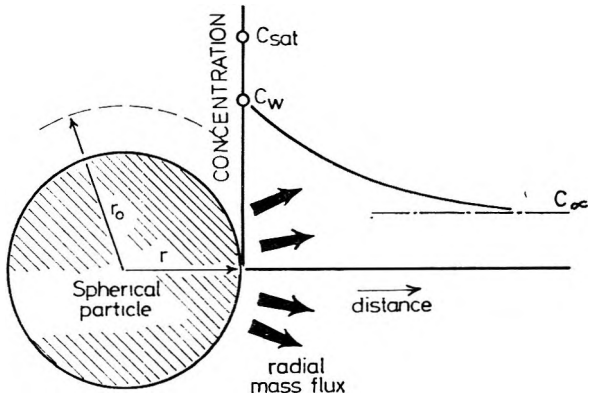

FIG. 1. Dissolution of a spherical particle. For explanation of symbols see text following equation (4).

Fig. 1 illustrates transfer of material, in dissolution, from the particle to the solvent phase (in growth, the transfer direction will be reversed). Dissolved material moves away into the bulk solution under the influence of the concentration gradient shown in the figure. If the rate of diffusional transport is large, the assumption of equilibrium at the solid–liquid interface may be incorrect, because dissolution rate may then become the controlling factor. Under such conditions Rosner (1969) has derived the equations which govern the behaviour of the particle. They are:

$$\frac{dr}{dt} = \frac{D\rho}{r\rho_s} \ln\left(\frac{1 - c_\infty}{1 - c_w}\right) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

and

$$k\,(c_{sat} - c_w)^p = \frac{D}{r} \ln\left(\frac{1 - c_\infty}{1 - c_w}\right) \quad .. \quad .. \quad .. \quad (2)$$

where r is the particle radius at any time t, D is the diffusivity of the solute, ρ is the density of the solvent and ρ_s that of the solute, k is the rate constant governing the interface kinetics, which may or may not be the same for dissolution and for growth, and p is the exponent on the mass transfer driving force, which has been shown to range from 1·0 to 1·8 (Strickland-Constable, 1968). Rosner dealt only with $p = 1{\cdot}0$.

The remaining symbols are the concentration terms: c_{sat} is the concentration of a saturated solution, c_w the concentration at the interface between solid and liquid, and c_∞ the bulk concentration in the liquid phase at a large distance from the dissolving particle.

The logarithmic term is the concentration driving force, in a form suitable for diffusional transport at high mass flux (Spalding, 1963). Both equations must be satisfied together, that is, the instantaneous rate of change of r with t must be as given by equation (1) with the appropriate value of r and of c_w; the value of c_w must satisfy equation (2). In addition, the value of c_{sat} may be changed during the integration due to the increase in solubility caused by diminishing particle size: this

is discussed more fully later. The value of c_∞ will also change if the volume of solvent is finite, and this also must be allowed for.

The major restriction on computer simulations of the type considered here is the lack of data: solubility, density and diffusivity in a particular solvent are rarely all available for any but simple substances. However, empirical correlations exist from which diffusivities can be estimated, and which are usually accurate to within 10%, at least for dilute solutions of non-dissociating solutes.

Wilke & Chang (1955) give perhaps the best such equation:

$$D = 7{\cdot}4 \times 10^{-8} \frac{(\psi M)^{\frac{1}{2}} T}{\mu V^{0{\cdot}6}} \qquad (3)$$

where D is the required diffusivity of the solute in $cm^2\ s^{-1}$, the molar volume of the solute being V cm^3, M is its molecular weight, T is the absolute temperature, μ is the solvent viscosity (in cP), and ψ is an "association parameter" for the solvent, which has the values 2·6 for water, 1·9 for methanol, 1·5 for ethanol and 1·0 for benzene and non-polar solvents generally.

There are several pharmaceutical examples of particulate mass transfer relevant to the technique reported here. Gwilt, Robertson & others (1963) reported increased rates of paracetamol absorption *in vivo* when sorbitol was added to the tablets; Walters (1968) showed that no complex was formed between paracetamol and sorbitol and suggested that a higher dissolution rate must therefore be responsible for the increase. Any rapidly-dissolving adjuvant should increase the dissolution rate of a drug, by increasing the overall mass transfer rate out of the tablet, and such aided dissolution could be simulated using the method of the present paper.

Although dissolution-limited absorption rates are usually reported only for sparingly-soluble drugs, dissolution rate can control the absorption of sodium *p*-aminosalicylate and acetylsalicylic acid, and for aspirin formulations at least, *in vitro* dissolution rates can be correlated with rates of absorption (Levy, Leonards & Procknal, 1967). For griseofulvin, absorption rates increase with the addition of lactose to the tablet formulation, the mechanism of increase being either the improvement of wetting or increased mass transfer (M. H. Rubinstein, personal communication).

The dissolution of fat globules in the gut by the action of lipases may well be mass-transfer limited; it is known to be assisted by the presence of bile salts, proteins and soaps, but the difficulty here is to obtain data for the physical properties of such a complex system.

The most promising field for calculations of particle lifetime is in the Ostwald ripening process, whereby crystalline precipitates of small particle size can be altered so that they contain fewer, but larger particles. In a slightly supersaturated mother liquor, the small crystals, which are more soluble, tend to dissolve, and the larger crystals, which are less soluble, to grow. The relation between size and solubility is due to Ostwald, but a modern discussion is given by Mullin (1961). The basic equation is

$$\ln \frac{c_{sat}}{c^*_{sat}} = \frac{2M\sigma}{\mathbf{R}T\rho r} \qquad (4)$$

where c_{sat} is the concentration of a solution in equilibrium with crystals of radius r, c^*_{sat} is the equilibrium solubility of large crystals, M is the molecular weight, σ = surface energy, $\mathbf{R}$ the gas constant, T the absolute temperature, and ρ is the density

of the solid. The effect of Ostwald ripening is enhanced by "temperature cycling". The temperature of the crystals and mother liquor is increased and decreased at a slow rate, keeping the mean temperature at about the level required for crystallization; the dissolution of small crystals and the growth of larger ones then occur at an increased rate. Rate determinations have been made for sulphathiazole by Carless & Foster (1966) and by Varney (1967) for the same drug and for oxyclozamide. Varney describes an automatic cycling device for implementing the method. Carless & Foster noted the peculiar fact that the rate of particle growth was decreased by the addition of cetomacrogol to the solution, despite the resultant increase in the solubility of the drug. This could be due to a diffusivity change.

Computation

Equation (1) was integrated by the Runge-Kutta method, the inaccuracy due to truncation error being checked by doubling the integration step length. Apart from this control, the step length was adjusted as the dissolution (or growth) proceeded, to provide output information at appropriate intervals. At each radius considered, the equilibrium solubility c_{sat} was calculated from equation (4); c_∞ was calculated by a mass balance, assuming for this purpose that all the dissolved solute was distributed uniformly through the liquid bulk—an assumption reasonable enough in most cases but open to question where a highly soluble solute is present in a severely restricted volume of liquid.

The interface concentration c_w was calculated for each radius as follows: Equation (2) may be expressed, for dissolution, in the form:

$$\phi = \frac{-kr}{D}(c_{sat} - c_w)^p + \ln\left(\frac{1 - c_\infty}{1 - c_w}\right) = 0 \quad .. \quad .. \quad (5)$$

a corresponding relation existing for the growth case where $c_{sat} < c_\infty$. By differentiation:

$$\left(\frac{\partial\phi}{\partial c_w}\right)_r = \frac{krp}{D}(c_{sat} - c_w)^{p-1} + \frac{1}{1 - c_w} \quad .. \quad .. \quad .. \quad (6)$$

and an estimate of c_w may be improved by the use of the recurrence formula:

$$[c_w]_{n+1} = [c_w]_n - \left[\phi_r \Big/ \frac{\partial\phi}{\partial c_w}\right]_{n,r} \quad .. \quad .. \quad .. \quad (7)$$

where n is the number of iterations. This formula was used repeatedly until both c_w and the relevant logarithmic term in equation (1) were constant within acceptable limits (a final relative correction $\leq 0{\cdot}0005$ was accepted). Convergence was good; typically 10 to 20 iterations were required to find c_w initially, then 2 or 3 iterations for each value of r as the latter changed progressively.

RESULTS AND DISCUSSION

The physical properties of the pure compounds are listed in Table 1: diffusivities given are those calculated by using the Wilke & Chang equation; the solubilities are experimental values obtained from the literature (Mullin, 1961).

Table 2 lists the results of calculations of particle dissolution lifetimes, and Table 3 the times taken to grow to a radius of 1 mm from an initial size of 1·1 times the critical radius (defined as the radius at which the particle is just in equilibrium with the

Table 1. *Physical properties of the three compounds studied.*

	Molecular weight	Density kg m^{-3}	Molar vol. m^3 kmol^{-1}
Boric acid	61·9	1435	0·0393
Citric acid monohydrate ..	210·1	1542	0·1723
Salicylic acid	138·1	1443	0·1350

	Acid	Temperature °C 10	20	30	40	60	80
Solubility mass fraction	Boric	0·0347	0·0476	0·0619	0·0800	0·1289	0·1922
	Citric	0·5921	0·6492	0·7073	0·7466	0·8037	0·8621
	Salicylic	0·00150	0·00200	0·00279	0·00418	0·00902	0·0221
Diffusivity $m^2 s^{-1} \times 10^9$	Boric	1·212	1·632	2·119	2·672	3·977	5·544
	Citric	0·499	0·673	0·873	1·101	1·638	2·284
	Salicylic	0·578	0·779	1·011	1·274	1·897	2·644
Viscosity $kg m^{-1} s^{-1} \times 10^3$	Water	1·308	1·005	0·801	0·656	0·469	0·357

Solvent association parameter for water = 2·6.

Table 2. *Particle lifetimes in dissolution for various values of the parameters which govern the dissolution rate.*

°C	$\dot{\kappa}$ (m s^{-1})	σ (J m^{-2})	p	Initial phase ratio	Lifetime Salicylic acid (s)	Lifetime Boric acid (s)	Lifetime Citric acid monohydrate (s)
10	25	0·2	1·0	infinite*	832 851	16 740	1722
20	25	0·2	1·0	infinite	463 807	9008	1094
30	25	0·2	1·0	infinite	255 341	5298	719
40‡	25‡	0·2‡	1·0‡	infinite‡	135 103‡	3219‡	510‡
60	25	0·2	1·0	infinite	41 989	1307	289
80	25	0·2	1·0	infinite	12 210	606	170
40	25	0·2	1·0	large*	202 011	4878	1007
40	25	0·2	1·0	medium*	463 325	11 357	2831
40	25	0·2	1·0	small*	1 250 312	30 941	8107
40	25	0·02–2·0	1·0	infinite	135 107–134 909	3219–3217	510–508
40	25	0·2	1·4	infinite	135 109	3219	510
40	25	0·2	1·8	infinite	135 293	3221	510
40	25	0·2	2·0	infinite	135 736	3225	511
40	25	0·2	3·0	infinite	161 372	3326	517
40	0·01	0·2	1·8	infinite	150 638	3374	525
40	0·01	0·2	2·0	infinite	171 103	3495	534
40	0·01	0·2	3·0	infinite	2 349 248	5155	604
40	0·10	0·2	2·0	infinite	145 502	3304	518
40	0·10	0·2	3·0	infinite	495 260	3974	554
40	0·50	0·2	3·0	infinite	275 713	3636	536
40	0·01–25	0·2	1·0	infinite	135 138–135 103	3221–3219	511–510

* The term infinite denotes that calculations were based on 10^6 kg of water per particle. Large, medium and small denote that the amount of water was such that, on complete dissolution of the particle, the water contained respectively 50%, 90% and 99% of the solute required to saturate it. At the start of the dissolution process the water was free of solute.

‡ "Pivotal" case.

Table 3. *Times taken by spherical particles to grow from* 1·1 × *critical size to a radius of* 1 *mm.*

					Salicylic acid			Boric acid			Citric acid monohydrate		
°C	k ms^{-1}	σ Jm^{-2}	p	Initial phase ratio	Critical radius m	Critical mass g	Growth† time s	Critical radius m	Critical mass g	Growth† time s	Critical radius m	Critical mass g	Growth† time s
10	25	0·2	1·0	infinite†	$8{\cdot}22 \times 10^{-7}$	$3{\cdot}36 \times 10^{-12}$	41 740 600	$3{\cdot}83 \times 10^{-7}$	$3{\cdot}39 \times 10^{-13}$	852 848	$2{\cdot}56 \times 10^{-6}$	$1{\cdot}09 \times 10^{-10}$	117 059
20	25	0·2	1·0	infinite	7·95 "	3·03 "	23 252 750	3·75 "	3·18 "	462 035	2·79 "	1·41 "	76 963
30	25	0·2	1·0	infinite	7·69 "	2·75 "	12 805 000	3·69 "	3·01 "	273 821	3·11 "	1·94 "	52 267
40‡	25‡	0·2‡	1·0‡	infinite‡	7·45 "	2·50 "	6 779 670‡	3·64 "	2·90 "	168 026‡	3·35 "	2·43 "	37 870‡
60	25	0·2	1·0	infinite	7·04 "	2·11 "	2 112 235	3·61 "	2·84 "	70 108	3·77 "	3·47 "	21 915
80	25	0·2	1·0	infinite	6·73 "	1·84 "	618 335	3·68 "	2·99 "	33 750	4·46 "	5·72 "	12 894
40	25	0·2	1·0	large†	7·45 "	2·50 "	8 819 050	3·64 "	2·90 "	218 516	3·35 "	2·43 "	45 958
40	25	0·2	1·0	medium†	7·45 "	2·50 "	14 610 800	3·64 "	2·90 "	361 704	3·35 "	2·43 "	59 603
40	25	0·2	1·0	small†	7·45 "	2·50 "	24 613 800	3·64 "	2·90 "	359 208	3·35 "	2·43 "	65 356
40	25	0·02	1·0	infinite	$7{\cdot}45 \times 10^{-8}$	$2{\cdot}50 \times 10^{-14}$	6 770 608	$3{\cdot}64 \times 10^{-8}$	$2{\cdot}90 \times 10^{-16}$	167 917	$3{\cdot}35 \times 10^{-7}$	$2{\cdot}43 \times 10^{-13}$	37 640
40	25	0·05	1·0	infinite	$1{\cdot}86 \times 10^{-7}$	$3{\cdot}91 \times 10^{-13}$	6 772 105	$9{\cdot}10 \times 10^{-8}$	$4{\cdot}53 \times 10^{-15}$	167 936	$8{\cdot}38 \times 10^{-7}$	$3{\cdot}80 \times 10^{-12}$	37 678
40	25	0·5	1·0	infinite	$1{\cdot}86 \times 10^{-6}$	$3{\cdot}91 \times 10^{-11}$	6 794 920	$9{\cdot}10 \times 10^{-7}$	$4{\cdot}53 \times 10^{-12}$	168 210	$8{\cdot}38 \times 10^{-6}$	$3{\cdot}80 \times 10^{-9}$	38 266
40	25	2	1·0	infinite	$7{\cdot}45 \times 10^{-5}$	$2{\cdot}50 \times 10^{-9}$	6 873 620	$3{\cdot}64 \times 10^{-6}$	$2{\cdot}90 \times 10^{-10}$	169 143	$3{\cdot}35 \times 10^{-5}$	$2{\cdot}43 \times 10^{-7}$	40 435
40	25	4	1·0	infinite	$1{\cdot}49 \times 10^{-5}$	$2{\cdot}00 \times 10^{-8}$	6 983 750	$7{\cdot}28 \times 10^{-6}$	$2{\cdot}32 \times 10^{-9}$	170 422	$6{\cdot}70 \times 10^{-5}$	$1{\cdot}95 \times 10^{-6}$	43 672
40	25	10	1·0	infinite	$3{\cdot}73 \times 10^{-5}$	$3{\cdot}13 \times 10^{-7}$	7 340 870	$1{\cdot}82 \times 10^{-5}$	$3{\cdot}62^{0} \times 10^{-8}$	174 460	$1{\cdot}68 \times 10^{-5}$	$3{\cdot}04 \times 10^{-5}$	54 648
40	25	0·2	1·4	infinite	$7{\cdot}45 \times 10^{-7}$	$2{\cdot}50 \times 10^{-12}$	6 780 620	$3{\cdot}64 \times 10^{-7}$	$2{\cdot}90 \times 10^{-13}$	168 046	$3{\cdot}35 \times 10^{-6}$	$2{\cdot}43 \times 10^{-10}$	37 874
40	25	0·2	1·8	infinite	7·45 "	2·50 "	6 834 350	3·64 "	2·90 "	168 621	3·35 "	2·43 "	37 970
40	25	0·2	2·0	infinite	7·45 "	2·50 "	7 008 500	3·64 "	2·90 "	170 033	3·35 "	2·43 "	38 180
40	25	0·2	3·0	infinite	7·45 "	2·50 "	$1{\cdot}26 \times 10^{8}$	3·64 "	2·90 "	276 211	3·35 "	2·43 "	48 905
40	0·01	0·2	1·8	infinite	7·45 "	2·50 "	12 536 700	3·64 "	2·90 "	220 868	3·35 "	2·43 "	46 598
40	0·01	0·2	2·0	infinite	7·45 "	2·50 "	33 167 200	3·64 "	2·90 "	307 832	3·35 "	2·43 "	57 825
40	0·01	0·2	2·5	infinite	7·45 "	2·50 "	$2{\cdot}38 \times 10^{9}$	3·64 "	2·90 "	47 413 800	3·35 "	2·43 "	2 133 995
40	0·10	0·2	2·0	infinite	7·45 "	2·50 "	11 585 100	3·64 "	2·90 "	203 127	3·35 "	2·43 "	43 169
40	0·10	0·2	2·5	infinite	7·45 "	2·50 "	$2{\cdot}53 \times 10^{9}$						
40	0·10	0·2	3·0	infinite	7·45 "	2·50 "	$2{\cdot}69 \times 10^{10}$	$3{\cdot}64 \times 10^{-7}$	$2{\cdot}90 \times 10^{-13}$	5 173 760	$3{\cdot}35 \times 10^{-6}$	$2{\cdot}43 \times 10^{-10}$	296 790
40	0·50	0·2	2·5	infinite	7·45 "	2·50 "	62 609 200						
40	0·50	0·2	3·0	infinite	7·45 "	2·50 "	$5{\cdot}41 \times 10^{9}$	$3{\cdot}64 \times 10^{-7}$	$2{\cdot}90 \times 10^{-13}$	1 371 872	$3{\cdot}35 \times 10^{-6}$	$2{\cdot}43 \times 10^{10}$	116 890
40	0·01	0·2	1·0	infinite	7·45 "	2·50 "	6 781 400	3·64 "	2·90 "	168 125	3·35 "	2·43 "	37 903
40	0·10	0·2	1·0	infinite	7·45 "	2·50 "	6 779 850	3·64 "	2·90 "	168 036	3·35 "	2·43 "	37 873
40	0·50	0·2	1·0	infinite	7·45 "	2·50 "	6 779 700	3·64 "	2·90 "	168 028	3·35 "	2·43 "	37 870
40	1	0·2	1·0	infinite	7·45 "	2·50 "	6 779 670	3·64 "	2·90 "	168 028	3·35 "	2·43 "	37 870
40	5	0·2	1·0	infinite	7·45 "	2·50 "	6 779 670	3·64 "	2·90 "	168 027	3·35 "	2·43 "	37 870
40	125	0·2	1·0	infinite	7·45 "	2·50 "	6 779 670	3·64 "	2·90 "	168 026	3·35 "	2·43 "	37 870

† In every case the solution from which growth took place contained initially 2% more solute than that required to saturate the water present. The term infinite denotes that calculations were based on 10^{8} kg of water per particle. Large, medium and small denote that the amount of solution was such that, on completion of growth to 1 mm radius, respectively 50%, 90% and 99% of the excess solute had been deposited. The growth time is that from a nucleus of radius 1·1 r_{crit} to 1 mm radius. ‡ "Pivotal" case.

supersaturated solution, so that it neither grows nor dissolves; the increase in solubility caused by the curvature of its surface is just equal to the degree of supersaturation of its environment). For all the growth cases, the amount of solute initially was 2% more than the amount needed to saturate the water present.

For all these data the conditions for dissolution are for a stationary particle surrounded by solvent, and no relative motion between the solid and liquid phases is assumed. This is not a restrictive condition when comparing one substance with another, but in predicting actual dissolution times the data here yield the longest time that dissolution could be expected to take. Any stirring or other movement would effectively increase the diffusivity from the molecular motion figure used here to a value appropriate to the eddying turbulent conditions caused by the stirring. This change in effective diffusivity could have been predicted, although not very accurately, by using known correlations based upon the Reynolds, Sherwood, Schmidt and Prandtl dimensionless groups (see for example Treybal, 1955). But since the change in diffusivity would be dependent upon the system fluid dynamics

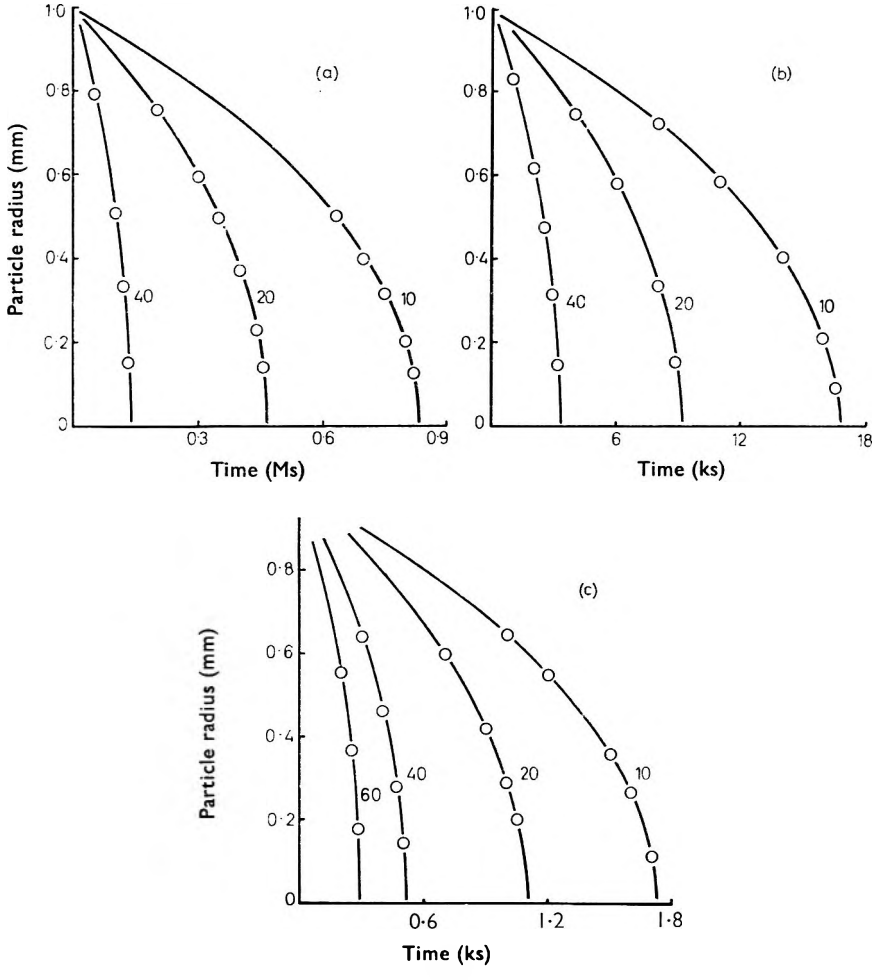

FIG. 2. Particle dissolution: (a) salicylic acid, (b) boric acid, (c) citric acid. Temperatures were as shown; other conditions "pivotal."

rather than upon what substances were being used, there would be little effect for comparative purposes. In consequence this parameter was not varied.

Because of the limited time available, all possible combinations of parameters were not inspected. For each substance, a "pivotal" case was chosen. This was at 40°, the middle of the temperature range, and the other factors were set at convenient levels in their ranges: σ at 0·2 J m^{-2}, k at 25 m s^{-1}, p at 1·0, c_∞ at zero for dissolution, and the phase ratio, the amount of solvent per unit mass of the solute, was made effectively infinite.

The dissolution rates of the three substances at various temperatures are plotted in Fig. 2, and Fig. 3 shows growth curves for boric acid only. The increased rate of reduction of radius near the end of the dissolution process is affected by the value of σ, the surface energy. This is not an easily-determined quantity for solids. It is analogous to the surface tension of a liquid, the presumption being that the surface tension does not disappear when a liquid solidifies, but is "locked in". The normal methods of surface tension determination are not applicable to solids, and the methods that are available are inaccurate. They are all based upon scratching, indentation or attrition. An account in detail is given by Kuznetsov (1957) who quotes values of a few hundred ergs cm^{-2} (mJ m^{-2}) for some substances, but rising to well over 1 J m^{-2} for results obtained by some of the methods. These are scarcely credible, as can be seen by an examination of the effects of varying σ, particularly in the case of the calculations performed for growth rates. In the present paper the initial size of the sphere considered has been taken to be a radius 10% greater than the critical size.

It was suggested by Strickland-Constable (1968), as a result of measuring the sublimation and deposition rates of benzophenone, that growth rates, and probably evaporation rates, were not first order processes. It is known (Mullin, 1961) that a

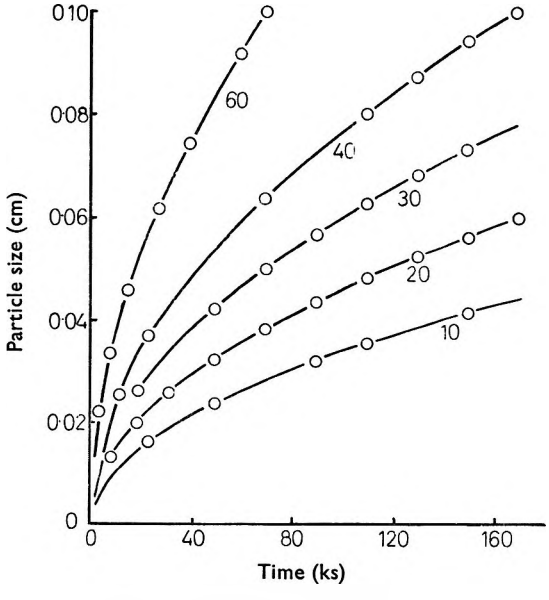

FIG. 3. Particle growth: spherical particle of boric acid in a 2% supersaturated solution. Radius of nucleus at $t = 0$ was 1·1 × the critical size. Temperatures were as shown; other conditions "pivotal."

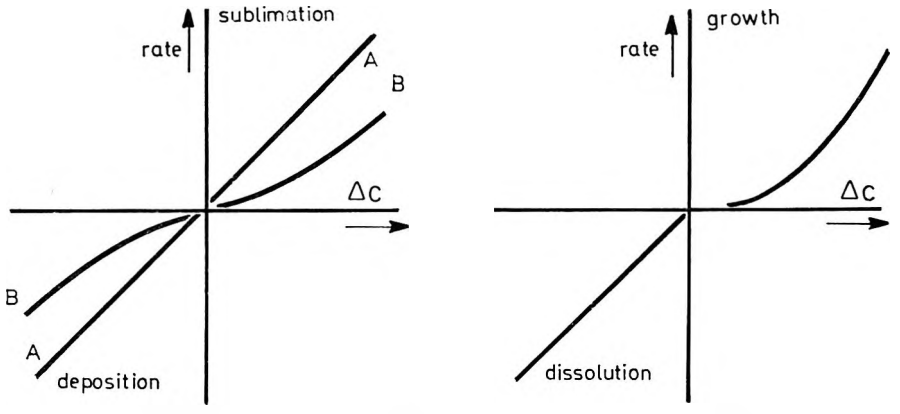

FIG. 4. The relation between growth or dissolution and concentration difference: (a) sublimation and deposition from the vapour phase (after Strickland-Constable)—AA linear (first-order) mechanism. BB higher-order mechanism. (b) dissolution, and growth from solution: dissolution is a first-order process, whereas solid deposition needs a finite concentration difference to begin, and then follows a power-law relation with exponent greater than unity.

finite degree of supersaturation is necessary to initiate crystal growth from solution, and that the growth rate is often proportional to the degree of supersaturation raised to a power of approximately 1·6.

These two types of behaviour lead to plots of the kind shown in Fig. 4. For sublimation and condensation on the Strickland-Constable model, the line AA in Fig. 4(a) shows the linear dependence of growth or evaporation upon concentration differences when first-order kinetics apply. For higher-order kinetics, curves such as BB are obtained. Crystallization behaviour usually resembles Fig. 4(b), where dissolution is a first-order process and yields a straight line, but the growth process requires a finite supersaturation for initiation; it then proceeds with a power-law relation in which the exponent is greater than unity. Both these types of behaviour are easily simulated by the numerical technique used in the present work, although such additions make it much more difficult to find an analytic solution to the differential equations which are being obeyed.

The effect of varying both the surface kinetics constant, k, and the exponent on the concentration difference term, p, is shown in Fig. 5. For values of k and p near the pivotal values of 25 m s^{-1} and 1·0, the curves of saturation concentration and interface concentration against particle radius follow one another closely down to the critical radius. With the higher values of p, however, there is a divergence, indicating that the rise in solubility at small particle size is less closely followed at the interface—as might be expected. When k also has a small value, the effect is enhanced. Even with the rise in solubility, interface concentration falls, because the surface kinetic step of removing a molecule from the crystal lattice into the solution is limiting. When k is reduced to 0·01, a value admittedly outside the normal range of 0·1–100, the effect of k overshadows that of p, so that the curves for $p = 2{\cdot}0$ and $p = 3{\cdot}0$ are less divergent from one another than both are from the c_{sat} curves.

Fig. 6 shows some plots of particle growth at extreme values of the parameters k and p. Those of Fig. 6(a) are for boric acid, and show the interface concentration and solubility as a function of particle radius during the growth process. Citric acid

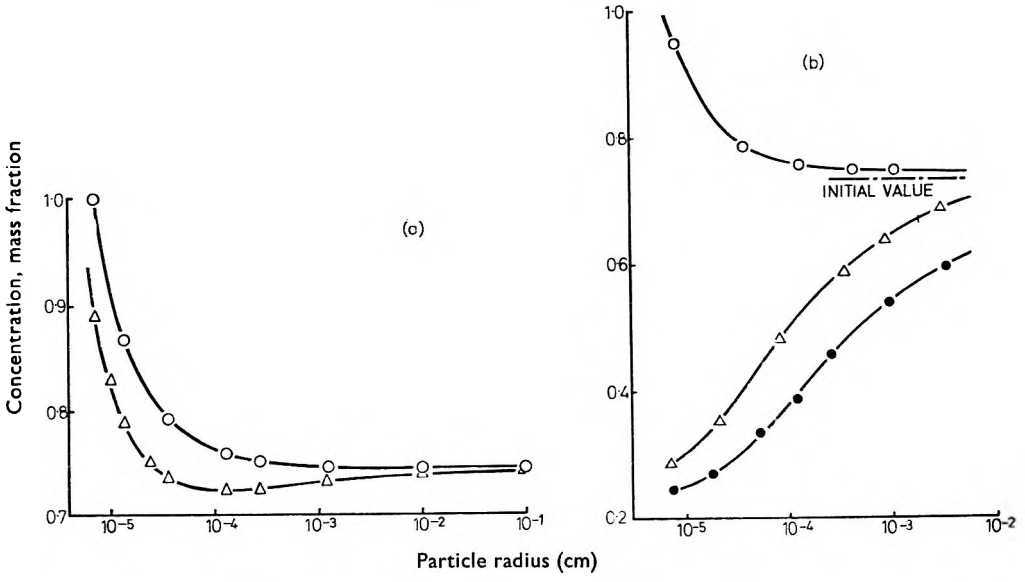

FIG. 5. Particle dissolution: solubility c_{sat} and interface concentration c_w *versus* particle radius. Conditions "pivotal" except where stated. (a) citric acid dissolution; $k = 25$ m s^{-1}, $p = 3{\cdot}0$—○ surface solubility, △ interface concentration. (b) citric acid dissolution; $k = 0{\cdot}01$ m s^{-1}—○ surface solubility, △ interface concentration, $p = 2{\cdot}0$, ● interface concentration, $p = 3{\cdot}0$.

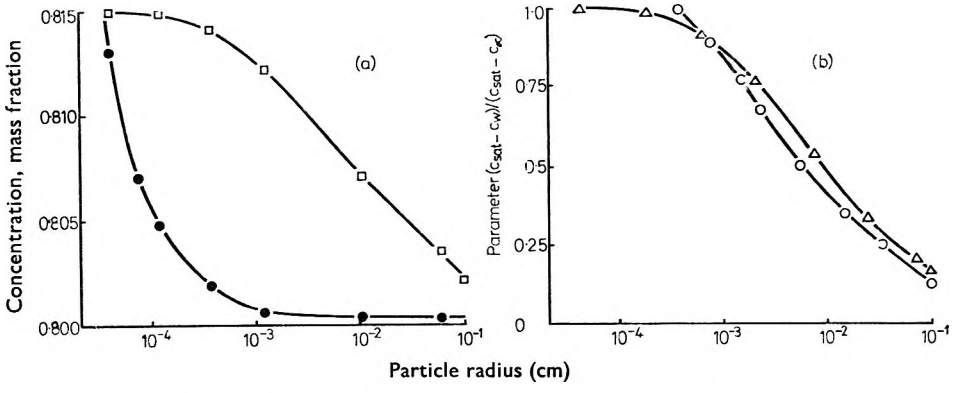

FIG. 6. Particle growth: conditions "pivotal" except where stated. (a) Solubility c_{sat} and interface concentration c_w *versus* particle radius; boric acid, $k = 1{\cdot}0$, $p = 3{\cdot}0$—● surface solubility, □ interface concentration. (b) The parameter $(c_{sat} - c_w)/(c_{sat} - c_\infty)$ *versus* particle radius. For values of the parameter near to unity, surface-kinetic control operates; for values near zero, diffusion is the limiting factor—○ citric acid, △ boric acid.

behaves similarly, but the solubility of salicylic acid is so small that for values of p approaching 3·0 the growth time becomes extremely large ($> 10^6$ years). This reflects the fact that, because solute molecules are present only in very low concentrations, any process which is governed by third-order kinetics and so depends upon the occurrence of intermolecular collisions is bound to be extremely slow.

The variation of the quotient $(c_{sat} - c_w)/(c_{sat} - c_\infty)$ with particle radius is shown in Fig. 6(b). This quotient measures the division of the driving force, for either growth or dissolution, between the immediate neighbourhood of the solid surface, which is the kinetically-controlled region, and the boundary layer, which is the

diffusion-controlled region. Values near unity indicate surface control, and values near zero, diffusional limitation. For the cases shown here, both the boric acid and the citric acid growth processes change their limiting mechanism as the particle radius increases.

In conclusion, it appears that the simulation algorithm reported here is reliable over a wide range of the main parameters which influence the rate of the crystallization and dissolution processes. It should be useful in evaluating experimental data for various substances, particularly for nucleation phenomena and particle growth at small radii; in addition, extensions of the technique may in future prove valuable in the study of Ostwald ripening.

REFERENCES

CABLE, M. & EVANS, D. J. (1967). *J. applied Physics*, **38**, 2899–2906.

CARLESS, J. E. & FOSTER, A. A. (1966). *J. Pharm. Pharmac.*, **18**, 697–708.

DUDA, J. L. & VRENTAS, J. S. (1969). *A.I.Ch.E. Jl.*, **15**, 351–356.

GOODMAN, T. R. (1964). *Advances in Heat Transfer*, **1**, 51–122. New York: Academic Press.

GWILT, J. R., ROBERTSON, A., GOLDMAN, L. & BLANCHARD, A. W. (1963). *J. Pharm. Pharmac.*, **15**, 445–453.

KUTTA, W. (1901). *Z. Math. Phys.*, **46**, 435–453.

KUZNETSOV, V. D. (1957). *Surface energy of solids*. London: H.M.S.O.

LEVY, G., LEONARDS, J. R. & PROCKNAL, J. A. (1967). *J. pharm. Sci.*, **56**, 1365–1367.

MULLIN, J. W. (1961). *Crystallization*. London: Butterworths.

MULLIN, J. W., AMATAVIVADHANA, A. & CHAKRAVORTY, M. (1970). *J. appl. Chem., Lond.*, **20**, 153–158.

READEY, D. W. & COOPER, A. R. (1966). *Chem. Engng Sci.*, **21**, 917–922.

ROSNER, D. E. (1969). *J. phys. Chem.*, **73**, 382–387.

ROSNER, D. E. & EPSTEIN, M. (1970). *Ibid.*, **74**, 4001–4004.

RUNGE, C. (1895). *Mathematische Annalen*, **46**, 167–178.

SCRIVEN, L. E. (1959). *Chem. Engng Sci.*, **10**, 1–13.

SPALDING, D. B. (1963). *Convective mass transfer*. London: Arnold.

STRICKLAND-CONSTABLE, R. F. (1968). *Kinetics and mechanism of crystallization*. London: Academic Press.

TREYBAL, R. E. (1955) *Mass transfer operations*. New York: McGraw-Hill.

VARNEY, G. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.*, 19S–23S.

WALTERS, V. (1968). *Ibid.*, **20**, *Suppl.*, 228S–231S.

WILKE, C. R. & CHANG, P. (1955). *A.I.Ch.E. Jl.*, **1**, 264–270.

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Alkaloids from two Nigerian species of *Fagara*

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Skimmianine, chelerythrine, nitidine, 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one and 1-hydroxy-3-methoxy-10-methylacridan-9-one have been isolated from the root and stem barks of *Fagara rubescens*. Using thin-layer chromatography and high voltage electrophoresis, a comparison of the chloroform-insoluble quaternary alkaloids of this species has been made with those from *F. leprieurii*. The distribution of the alkaloids within the barks and the chemotaxonomic significance of the alkaloids in the two species are discussed.

Fagara rubescens (Planch. ex Hook. f.) Engl. (syn. *Zanthoxylum rubescens* Planch. ex Hook. f.) is a small tree indigenous to Western Tropical Africa where the bark has been used as a toothache remedy: bark from *F. leprieurii* (Guill. et Perr.) Engl. (syn. *Zanthoxylum leprieurii* Guill. et Perr.) has been used similarly and for the treatment of coughs and colds (Oliver, 1960).

MATERIALS

The root and stem barks of *Fagara rubescens* and *F. leprieurii*, collected in Nigeria for the Tropical Products Institute, London, were authenticated at source.

The barks, from which all adhering epiphytes had been removed, were separately ground to coarse powders. In the case of the root bark of *Fagara rubescens* a quantity of loosely-adhering cork was removed and ground separately.

Thin-layer chromatography and high voltage electrophoresis. These were performed using the methods previously described (Calderwood & Fish, 1969), in the latter technique using a barbitone buffer of pH 7·4.

Identification of isolated compounds. Alkaloids were identified by comparative t.l.c. (3 systems), mixed melting points and comparison of ultraviolet and infrared spectra with those obtained for authentic samples (Calderwood, Finkelstein & Fish, 1970; Fish & Waterman, 1971a). Melting points (uncorrected) were determined on a Kofler hot stage, ultraviolet spectra (in ethanol) were recorded on a Unicam Stereoscan S.P. 800 and infrared spectra (Nujol mull) on a Perkin-Elmer 157B instrument.

METHOD

Isolation of the chloroform-soluble alkaloids of Fagara rubescens. The powdered barks (root bark 1 kg, stem bark 1 kg and cork 100 g) were separately extracted to exhaustion (48 h) in a Soxhlet with light petroleum (b.p. 40–60°) and then with chloroform. Each extract was concentrated under reduced pressure to a volume of 250 ml.

For each concentrate an aliquot (100 ml) was extracted successively with 2N hydrochloric acid (3 × 50 ml, 2 × 25 ml), the final acid wash being allowed to stand

overnight in contact with the original extract. The combined acid extracts were filtered and the precipitated base hydrochlorides subjected to column chromatography on alumina (Woelm, activity II) (20 g/g of crude base precipitate packed in chloroform–methanol (98:2). Elution with the same solvent gave chelerythrine in the first 50 ml, well separated from nitidine, which was eluted in the subsequent 150 ml. Chelerythrine was crystallized as the chloride from 2N hydrochloric acid–ethanol to give yellow needles, melting point 202° (Lit m.p. 202–203°, Cannon, Hughes & others, 1953). Nitidine was crystallized as the nitrate from 6N nitric acid–ethanol to give yellowish green needles, melting point 276–278° (Lit m.p. 277–278°, Gopinath, Khan & others, 1963). Yields are indicated in Table 1.

The bulked acid fractions from each initial extract were separately made alkaline with strong solution of ammonia and re-extracted with chloroform. Evaporation of the chloroform extracts under reduced pressure followed by crystallization from methanol gave (stem bark and the inner root bark) the furoquinoline alkaloid skimmianine, melting point 176–177° (Lit m.p. 176°, Deulofeu, Labriola & de Lange, 1942).

A second aliquot (100 ml) of each initial concentrate was subjected directly to column chromatography on Silica Gel G–Celite 545 (1:1) (250 g for each 100 ml of concentrate) packed in benzene–ethyl acetate (9:1). Elution with the same solvent gave 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one which was crystallized from ethyl acetate to give fine, yellow needles, melting point 175–176° (Lit m.p. 175–176°, Pakrashi, Roy & others, 1961). Elution with benzene–ethyl acetate (1:1) gave a second alkaloid 1-hydroxy-3-methoxy-10-methylacridan-9-one, crystallized from ethyl acetate to give yellow clusters, melting point 175–176° (Lit m.p. 174–175°, Drummond & Lahey, 1949) giving no depression on admixture with an authentic specimen.

The concentrations of the various alkaloids obtained from the barks are given in Table 1 together with yields previously reported from *Fagara leprieurii* (Fish & Waterman, 1971a).

Table 1. *Percentages of chloroform-soluble alkaloids present in the root and stem barks of* Fagara rubescens *and* F. leprieurii *and in the separated cork from the root bark of* F. rubescens.

	F. rubescens			*F. leprieurii*	
	Cork	Root, inner bark	Stem bark	Root bark	Stem bark
Skimmianine	absent	0·029	0·015	0·003	0·001
Chelerythrine	0·031	0·029	trace	0·017	trace
Nitidine	0·006	0·009	trace	0·004	trace
Acridone a*	absent	0·094	0·081	0·031	0·020
Acridone b*	absent	0·062	0·028	0·008	0·005

* a = 1-Hydroxy-2,3-dimethoxy-10-methylacridan-9-one.
b = 1-Hydroxy-3-methoxy-10-methylacridan-9-one.

Examination of quaternary alkaloids. The barks of *Fagara rubescens* and *F. leprieurii*, previously extracted with light petroleum and chloroform, were further extracted with methanol, the extracts concentrated under reduced pressure and

subjected to a partial purification process in which the concentrates were mixed with Amberlite IRC-50 ion exchange resin (75% Na^+ form) from which they were eluted with methanol containing hydrochloric acid (5%) (Albonico, Kuck & Deulofeu, 1964).

The concentrated extracts were then subjected to thin-layer chromatography (cellulose, 3 systems) and high voltage electrophoresis. The results obtained are given in Table 2.

Table 2. *Thin layer and electrophoretic separation of methanol-soluble alkaloids from* Fagara *barks.*

Reference compounds	Alkaloid movement	*F. rubescens* Root bark	*F. rubescens* Stem bark	*F. leprieurii* Root bark	*F. leprieurii* Stem bark
Magnoflorine	a 0·24 b 0·30 c 0·24 d 0·30	a 0·25 b 0·31 c 0·24 d 0·30	a 0·25 b 0·31 c 0·24 d 0·35	a 0·22 b 0·30 c 0·25 d 0·35	a 0·25 b 0·31 c 0·25 d 0·40
Alkaloid A		a, b, c ? ; d 7·70	a, b, c ? ; d 7·80	a, b, c, d −ve	a, b, c, d −ve
*Tembeterine	a 0·81 b 0·55 c 0·57 d 8·50	a 0·83 b 0·57 c 0·58 d 8·60	a 0·84 b 0·56 c 0·59 d 8·65	a 0·83 b 0·56 c 0·59 d 8·50	a 0·88 b 0·56 c 0·58 d 8·60
Candicine	a 0·90 b 0·45 c 0·47 d 14·70	a 0·91 b 0·45 c 0·47 d 14·50	a 0·90 b 0·45 c 0·47 d 14·60	a, b, c, d −ve	a, b, c, d −ve

(i) a = R_F in 0·1N HCl.
(ii) b = R_F in butanol–pyridine–water (6 : 4 : 3).
(iii) c = R_F in butanol saturated with 2N HCl.
(iv) d = distance travelled in high voltage electrophoresis (cm).
Alkaloid A may be either *N*-methylcorydine or *N*-methylisocorydine for both of which d = 7·75.
* For previous work on *F. leprieurii*, Calderwood & Fish, 1966.

DISCUSSION

The five chloroform-soluble alkaloids isolated from the bark of *Fagara rubescens* are identical with those previously reported from the bark of *F. leprieurii* (Fish & Waterman, 1971a). *F. rubescens* is thus the second African species of this genus shown to contain the acridone type of alkaloid, a type previously not reported from any other *Fagara* species.

The distribution of the alkaloids, indicating the presence of greater concentrations in the root bark than in the stem bark, confirms the results previously obtained for other members of this group (Calderwood & Fish, 1966; Fish & Waterman, 1971b). The presence of large quantities of chelerythrine, together with nitidine, in the cork of the root bark and the absence of all the other bases (including the methanol-soluble quaternary alkaloids) is noteworthy. These same two alkaloids are also present in the remainder of the root bark but almost absent from the stem bark although this contains appreciable quantities of the other alkaloids.

Information on alkaloid patterns in the genus *Fagara* may have chemotaxonomic significance since the present classification on African species, totalling about 40, is

considerably confused. The two species presently considered are morphologically very similar (cf. Oliver, 1868; Hutchinson & Dalziel, 1954). Chemical similarities obviously exist in that both species contain the dopamine-derived chelerythrine and nitidine (probably also tembetarine and magnoflorine) as well as the anthranilic acid-derived acridone alkaloids. To date these last have not been demonstrated in any other *Fagara* species. Differences are also shown by the preliminary work (Table 2) which suggests that the tyramine-derived candicine is present only in *F. rubescens*.

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REFERENCES

Albonico, S. M., Kuck, A. M. & Deulofeu, V. (1964). *Chemy Ind.*, 1580.

Calderwood, J. M., Finkelstein, N. & Fish, F. (1970). *Phytochemistry*, **9**, 675.

Calderwood, J. M. & Fish, F. (1966). *J. Pharm. Pharmac., Suppl.*, **18**, 119S–125S.

Calderwood, J. M. & Fish, F. (1969). *Ibid., Suppl.*, **21**, 126S–128S.

Cannon, J. R., Hughes, G. K., Ritchie, E. & Taylor, W. C. (1953). *Aust. J. Chem.*, 6, 86–89.

Deulofeu, V., Labriola, R. & de Lange, J. (1942). *J. Am. chem. Soc.*, **64**, 2326–2328.

Drummond, L. J. & Lahey, F. N. (1949). *Aust. J. scient. Res.*, **A2**, 630–637.

Fish, F. & Waterman, P. G. (1971a). *Phytochemistry*, in the press.

Fish, F. & Waterman, P. G. (1971b). *J. Pharm. Pharmac.*, **23**, 67–68.

Gopinath, K. W., Khan, M. S. Y., Kidwai, A. R. & Kohli, J. M. (1968). *Indian J. Chem.*, **1**, 99–100.

Hutchinson, J. & Dalziel, J. M. (1954). *Flora of West Tropical Africa*, Vol. 1, 2nd edn., revised by Keay, R. W. J., pp. 683–685. London: Governments of Nigeria, the Gold Coast, Sierra Leone and Gambia.

Oliver, B. (1960). *Medicinal Plants of Nigeria.* Ibadan: Nigerian College of Arts, Science and Technology.

Oliver, D. (1868). *Flora of Tropical Africa*, Vol. 1, pp. 304–306. London: L. Reeve & Co.

Pakrashi, S. C., Roy, S. K., Johnson, L. F., George, T. & Djerassi, K. (1961). *Chemy Ind.*, 464–465.

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Inactivation of resistant *Pseudomonas aeruginosa* by antibacterial combinations

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P. aeruginosa resistant to preservative concentrations of benzalkonium chloride, phenylmercuric nitrate and chlorocresol in nutrient broth was inactivated by using phenylethanol-antibacterial combinations. EDTA-antibacterial combinations also showed increased activity using benzalkonium and chlorocresol. *P. aeruginosa* resistant to 0·25% chlorbutol was inactivated using phenylethanol–chlorbutol combinations at concentrations which were ineffective alone. Similarly *P. aeruginosa* having an increased resistance to chlorhexidine was inactivated by phenylethanol–chlorhexidine combinations at concentrations that were ineffective alone. Phenylethanol showed a greater general usefulness than EDTA at the concentrations tested.

Pseudomonas aeruginosa, a common contaminant of ophthalmic solutions, can become resistant to a wide range of chemical agents (Richards, 1967a, b). It has been shown, however (Brown & Richards, 1964b, 1965), that polysorbate 80 and disodium ethylenediaminetetra-acetate (EDTA) are both capable of affecting the resistance of logarithmic phase cells of *P. aeruginosa* to inactivation by chlorhexidine, polymyxin and benzalkonium. Apparently, both polysorbate 80 and EDTA exerted their effects by modifying the permeability properties of the *P. aeruginosa* cells. Although polysorbate 80 is not suitable for use in combination with most chemical agents, since it inactivates antibacterial action, polymixin is an exception (Brown & Richards, 1964b).

The American N.F.XII (1965) recommends benzalkonium chloride as the most reliable antibacterial agent for the preservation of ophthalmic solutions and states that "Resistant strains of *Pseudomonas aeruginosa* have been made sensitive to benzalkonium chloride by the inclusion of 0·01 to 0·1 per cent. of disodium ethylenediaminetetra-acetate . . .".

Richards (1967b) concluded that further evaluation of antibacterial agents suitable for using to preserve ophthalmic solutions was needed with special reference to the activity of combinations against resistant bacteria.

Phenylethanol has been shown to affect the resistance of *P. aeruginosa* to inactivation by phenylmercuric nitrate (PMN) (Richards, Suwanprakorn & others, 1969) —work extended to other antibacterials by Richards & McBride (1971). Phenylethanol was first recommended for use as a preservative for ophthalmic solutions by Brewer, Goldstein & McLaughlin (1953) following a report that it was active against Gram-negative organisms (Lilly & Brewer, 1953). Silver & Wendt (1967) showed that phenylethanol exerted its antibacterial effect by modifying the permeability properties of the bacterial cell.

The investigations now described compare the efficiency of phenylethanol–preservative combinations with that of EDTA-preservative combinations in killing resistant *P. aeruginosa* cells contaminating nutrient broth.

MATERIALS AND METHODS

P. aeruginosa strain NCTC 6750, *Escherichia coli* strain NCTC 8196 and *Staphylococcus aureus* strain NCTC 6751 were grown on Oxoid nutrient broth No. 2 for liquid cultures and Oxoid nutrient agar for solid cultures: incubation was at 37°. The EDTA, PMN, chlorbutol, *p*-chloro-*m*-cresol and 2-phenylethanol were all BDH laboratory reagents. Chlorhexidine acetate B.P.C. was from ICI and the benzalkonium chloride B.P. from Macarthy Ltd., Glasgow. Cell numbers were estimated by colony counts. The counting procedure and inactivating broth were described by Richards & others (1969), and the maintenance of stock cultures by Brown & Richards (1964a).

Evaluation of native resistance

A series of six replicates of four dilutions of each chemical was prepared in 10 ml volumes of nutrient broth. 0·1 ml overnight culture of each organism was used as the inocula to give a final concentration of approximately 6×10^6 cells/ml for *P. aeruginosa* and *E. coli* and 2×10^6 cells/ml for *S. aureus*. The resulting reaction mixtures in duplicate were incubated for 7 days. Cultures showing no growth were subcultured 0·5 ml into 10 ml inactivating broth and incubated for a further 3 days.

Selection of resistant inocula of P. aeruginosa

The 7-day cultures growing in the presence of benzalkonium 0·02% (6×10^6)* and PMN 0·002% ($3{\cdot}8 \times 10^8$) were considered to be resistant cultures. Viable counts were made on the cultures and at the same time the cultures were used as sources of inocula for investigating the activity of preservative combinations.

The 7-day culture growing in the presence of 0·25% chlorbutol failed to grow in the presence of 0·5% chlorbutol and was recultured in the presence of 0·25% chlorbutol. This 7-day culture was counted ($3{\cdot}6 \times 10^7$) and used immediately as a source of inocula.

Cells showing an increased resistance to chlorhexidine were obtained using two methods. The first was to subculture in nutrient broth in the presence of increasing concentrations of chlorhexidine and incubate for 7 days. An inoculum of an overnight culture grew in the presence of chlorhexidine 0·001% and an inoculum from this grew in the presence of chlorhexidine 0·00143%. This culture was further subcultured in the presence of chlorhexidine 0·02% and the 7-day culture so obtained was counted ($1{\cdot}5 \times 10^8$) and used immediately as a source of inocula. The second method was to use 2 ml overnight culture to inoculate nutrient agar containing chlorhexidine 0·004% and incubate for 7 days. The surface culture was harvested, stored and used as a source of inocula as already described (Richards & others, 1969).

Cells growing in the presence of 0·05% chlorocresol were obtained by subculturing from a culture growing in the presence of 0·025% chlorocresol. The 7-day culture so obtained was also counted ($1{\cdot}4 \times 10^6$) and used immediately as a source of inocula in the next series of experiments.

Evaluation of antibacterial combinations

In this series of experiments each chemical was evaluated at two concentrations, both with EDTA and also with phenylethanol. [PMN was not tested with EDTA

* Figures in brackets indicate colony counts.

because PMN–EDTA combinations have no advantage over PMN alone (Brown, 1968).] A simultaneous test was made using a series of concentrations of the preservative, EDTA and phenylethanol as individual solutions in nutrient broth. The test procedure was the same as for "evaluation of native resistance" except that the sources of inocula were cultures having enhanced resistance to *P. aeruginosa*.

RESULTS AND DISCUSSION

Evaluation of native resistance

Chlorhexidine (0·0005–0·004%) showed growth at 0·001% with *P. aeruginosa* and at 0·0005% with all organisms.

PMN (0·00025–0·002%) showed growth at 0·00025% with *S. aureus* and at all concentrations with the other organisms.

Benzalkonium (0·005–0·02%) showed growth at all concentrations with *P. aeruginosa* and 0·001% with the other organisms.

Chlorbutol (0·05–0·5%) showed growth at 0·25% with all organisms.

Chlorocresol (0·01–0·10%) showed growth at 0·025% with *P. aeruginosa* and at 0·01% with the other organisms.

Therefore under the conditions of this experiment *P. aeruginosa* was resistant to concentrations of benzalkonium and PMN at concentrations recommended for the preservation of ophthalmic solutions. *E. coli* was likewise resistant to PMN. All three organisms showed a similar high resistance to chlorbutol and low resistance to chlorhexidine. *S. aureus* was sensitive to all the chemicals tested with the exception of chlorbutol.

Selection of resistant inocula of P. aeruginosa

The inocula obtained consisted of cells having either a native or cultivated resistance to the chemicals under test: colony counts/ml of *P. aeruginosa* have been given above.

Evaluation of antibacterial combinations

Table 1 shows chlorhexidine–phenylethanol combinations at all combinations of phenylethanol are more effective than either the chlorhexidine or phenylethanol alone. The chlorhexidine–EDTA combination showed no advantage over the chlorhexidine alone.

Benzalkonium 0·01% with EDTA 0·05% is more active than either benzalkonium or EDTA alone. However, no greater activity is shown with the benzalkonium–phenylethanol combinations than is shown by the phenylethanol alone. That phenylethanol alone at 0·4% is effective against the benzalkonium resistant cells indicates that there is no cross resistance between these two agents. The inactivation observed was apparently caused by the phenylethanol alone but Richards & McBride (1971), using a more sensitive technique, showed phenylethanol enhanced the action of benzalkonium against log phase *P. aeruginosa*.

PMN 0·001% with phenylethanol 0·4% and PMN 0·002% with phenylethanol 0·3% are more active against *P. aeruginosa* than either agent alone. These results agree with those of Richards & others (1969).

Chlorocresol 0·01% with phenylethanol 0·4%, and chlorocresol 0·05% with phenylethanol 0·3% are more active than either agent alone. Similarly, chlorocresol 0·01% with EDTA 0·05%, and chlorocresol 0·05% with EDTA 0·01% are more active than either agent alone.

Table 1. *Effect of simple solutions and combinations against resistant* P. aeruginosa. All determinations made in duplicate. Minimum concentrations effecting sterility determined by observing growth or no growth after incubation for 7 days. All apparent no growths were subcultured into inactivating broth for a further 3 days.

Simple solutions		Inoculum for simple solutions and combinations		Combinations		
				Principal antibacterial ("P")	Adjuvant antibacterial	
Antibacterial concentration (%)	Minimum concentration (%) effecting sterility	7-day culture in broth plus antibacterial concentration (%)	Cells/ml in reaction mixtures	Concentration (%)	Minimum concentration % phenylethanol plus "P" effecting sterility	Minimum concentration % EDTA plus "P" effecting sterility
Chlorhexidine 0·001–0·01	0·005	Chlorhexidine 0·02	$1·5 \times 10^6$	Chlorhexidine 0·001	0·2	None
Phenylethanol 0·2–0·6	0·4			Chlorhexidine 0·01	0·2	0·005
EDTA 0·005–0·1	None					
Chlorhexidine 0·001–0·01	0·002	7-day culture on *Agar* plus chlorhexidine 0·04	6×10^6	Chlorhexidine 0·001	0·2	None
				Chlorhexidine 0·002	0·2	0·02
Benzalkonium 0·001–0·01	None			Benzalkonium 0·001	0·4	None
Phenylethanol 0·2–0·6	0·4	Benzalkonium 0·02	6×10^4	Benzalkonium 0·01	0·4	0·05
EDTA 0·005–0·1	None					
PMN 0·001–0·002	None			PMN 0·001	0·4	Not done
Phenylethanol 0·2–0·6	0·5	PMN 0·002	$3·8 \times 10^6$	PMN 0·002	0·3	Not done
Chlorocresol 0·01–0·1	0·1	Chlorocresol 0·05	$1·4 \times 10^4$	Chlorocresol 0·01	0·4	0·05
Phenylethanol 0·2–0·6	0·5			Chlorocreso 0·05	0·3	0·01
EDTA 0·005–0·1	None					
Chlorbutol 0·1–0·5	0·3	Chlorbutol 0·25	$3·6 \times 10^4$	Chlorbutol 0·1	0·5	None
Phenylethanol 0·2–0·6	None			Chlorbutol 0·5	0·2	0·005
EDTA 0·005–0·1	None					

Chlorbutol 0·1% with phenylethanol 0·5% shows an increased antibacterial effect over either chlorbutol or phenylethanol alone. Chlorbutol-EDTA combinations did not show increased activity over the activity of the separate agents at equivalent concentrations. Cells grown in the presence of 0·25% chlorbutol would not grow in the presence of 0·3% chlorbutol but were able to grow in the presence of 0·6% phenylethanol. This suggests that it is difficult to produce cells resistant to chlorbutol and also that cells having some measure of resistance to chlorbutol are likely to show cross resistance to phenylethanol. The inoculum to a final concentration of $3·6 \times 10^5$ *P. aeruginosa* was not the highest used, but nevertheless it was the only inoculum to produce growth in the presence of phenylethanol 0·6%. [EDTA 0·02% reversed this resistance to phenylethanol 0·2% (Richards, unpublished observation)].

In addition to affecting cell permeability properties, EDTA can also affect cell growth by removing Mg, an essential nutrient, from the growth medium. The work of Weiser & others (1968 & 1969) and Neu & Winshell (1970) investigating synergism with EDTA-antibiotic combinations was criticized by Brown (1971) for not taking this effect on the growth medium into consideration in the evaluation of their results. In this present work no concentration of EDTA in simple solution effected sterilization of the contaminated broth, although growth was not always evident until after sub-culture in the inactivating medium. This sub-culture procedure therefore eliminated no growths occurring solely as the result of the effect of EDTA on the medium. In the conditions pertaining in the reaction mixture, however, EDTA could be enhancing the activity of the antibacterial agents both by an effect on cell permeability and by making conditions less favourable for growth by affecting the medium.

The results obtained show that, except for the three combinations noted above, the phenylethanol-antibacterial agent and EDTA-antibacterial agent combinations,

at selected concentrations, are more effective than either agent individually in overcoming resistant *P. aeruginosa* cells contaminating nutrient broth.

The concentrations proposed for using in combination with other antibacterial agents, when there are no contraindications, are 0·4% for phenylethanol and 0·05% for EDTA.

REFERENCES

BREWER, J. H., GOLDSTEIN, S. W. & MCLAUGHLIN, C. B. (1953). *J. Am. pharm. Ass. Sci. Edn*, **42**, 584–585.

BROWN, M. R. W. (1968). *J. pharm. Sci.*, **57**, 389–392.

BROWN, M. R. W. (1971). *Inhibition and Destruction of the Microbial Cell*, p. 354. London: Academic Press.

BROWN, M. R. W. & RICHARDS, R. M. E. (1964a). *J. Pharm. Pharmac.*, **16**, *Suppl.*, 41T–45T.

BROWN, M. R. W. & RICHARDS, R. M. E. (1964b). *Ibid.*, **16**, *Suppl.*, 51T–55T.

BROWN, M. R. W. & RICHARDS, R. M. E. (1965). *Nature, Lond.*, **207**, 1391–1393.

LILLY, B. D. & BREWER, J. H. (1953). *J. Am. pharm. Ass. Sci. Edn*, **42**, 6–8.

NEU, H. C. & WINSHELL, E. B. (1970). *Nature, Lond.*, **225**, 763.

RICHARDS, R. M. E. (1967a). *Aust. J. Pharm. Sci., Suppl.*, **55**, S86–S89.

RICHARDS, R. M. E. (1967b). *Ibid.*, **56**, S96–S101.

RICHARDS, R. M. E., SUWANPRAKORN, P., NEAWBANIJ, S. & SURASDIKUL, N. (1969). *J. Pharm. Pharmac.*, **21**, 681–686.

RICHARDS, R. M. E. & MCBRIDE, R. J. (1971). *Ibid.*, **23**, *Suppl.*, 141S–146S.

SILVER, S. & WENDT, L. (1967). *J. Bact.*, **93**, 560–566.

WEISER, R., ASSCHER, A. W. & WIMPENNY, J. (1968). *Nature, Lond.*, **219**, 1365–1366.

WEISER, R., WINPENNY, J. & ASSCHER, A. W. (1969). *Lancet*, **2**, 619–620.

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Phenylethanol enhancement of preservatives used in ophthalmic preparations

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The growth rates of *P. aeruginosa* in subinhibitory concentrations of antibacterial preservatives alone and in combination with phenylethanol were determined by an extinction method. Phenylethanol enhances the effect of benzalkonium chloride on growing cultures of *P. aeruginosa* and the results have been confirmed by viable counts. A similar effect is shown by phenylethanol in combination with chlorbutol, chlorhexidine diacetate, chlorocresol, merthiolate, methyl and propyl hydroxybenzoate mixture and phenylmercuric nitrate.

Phenylethanol has been shown to enhance the action of phenylmercuric nitrate (PMN) in the preservation of fluorescein solutions against the effects of contamination with *Pseudomonas aeruginosa* (Richards, Suwanprakorn & others, 1969). A permeability effect is indicated (Silver & Wendt, 1967), thus enabling concentrations of PMN, which, alone, could not effect penetration, to pass into the cell and exert an antibacterial effect. The present work was carried out to test the suggestion of Richards & others (1969) that phenylethanol might be used with advantage in combination with other antibacterial agents in preservation of pharmaceuticals.

MATERIALS AND METHODS

The test organism was *P. aeruginosa* NCTC 6750 and the counting procedure and inactivating broth used were described by Richards & others (1969). Stock cultures were maintained and logarithmic phase cultures prepared according to Brown & Richards (1964). Incubation was at 37°. The PMN, chlorbutol, *p*-chloro-*m*-cresol and 2-phenylethanol were all BDH laboratory reagents. The chlorhexidine acetate and propyl *p*-hydroxybenzoate were B.P.C. and the benzalkonium chloride B.P. Merthiolate (a 0·1% w/v thiomersal and 0·1% w/v monoethanolamine solution) was from Eli Lilly Limited and methyl *p*-hydroxybenzoate from MacFarlane Smith Limited. Cell numbers were estimated by colony counts. Growth rates of log phase cultures (shaken at 105 throws min^{-1} in a Mickle incubator) were determined by extinction measurements in 1 cm cells at 420 nm with a Unicam SP600 spectrophotometer.

Comparison of extinction measurements with viable counts

On three separate days, 1 ml quantities of log phase *P. aeruginosa* having an extinction of about 0·35 at 420 nm were used to inoculate 99 ml of prewarmed nutrient broth at 37° and the subsequent growth rate followed by means of viable counts and extinction measurements. The results were expressed in terms of doubling times with the 95% confidence limits. The relation between viable counts and extinction measurements is given in Fig. 1, where log extinction and log viable counts are plotted against time.

Effect of phenylethanol on P. aeruginosa *growing in subinhibitory concentrations of the different preservatives*

1 ml of log phase *P. aeruginosa* (extinction 0·35 at 420 nm) was added to each of four conical flasks, A, B, C and D, containing 99 ml of prewarmed nutrient broth (in A and B) or 99 ml of prewarmed nutrient broth containing benzalkonium chloride 30 μg/ml (in C and D). The flasks were maintained at 37° and samples taken for extinction measurements. When the growth rate had been established, prewarmed phenylethanol to a final concentration of 0·2% v/v was added to flasks B and D. At the same time an equal volume of prewarmed water was added to A and C. Subsequent extinction measurements were made at timed intervals as before. Fig. 2 shows the results obtained as graphs of log extinction against time. The regression coefficient (b) of the log extinction upon time and the 95% confidence limits were calculated for each line and the results expressed as doubling times or mean generation times (0·301/b) in minutes.

The following procedure was used to determine if the effect of the phenylethanol–benzalkonium combination was additive or not. The increase in doubling time, relative to the doubling of the control culture, was determined for phenylethanol and benzalkonium alone. The sum of these relative increases was compared with the relative increase in doubling time for the phenylethanol–benzalkonium combination.

Similar experiments were made using chlorhexidine, PMN, chlorocresol, chlorbutol, merthiolate and a mixture of methyl and propyl hydroxybenzoate, the results being given in Table 1.

Table 1. *The effect of ophthalmic preservatives and phenylethanol–preservative combinations on the growth of* Pseudomonas aeruginosa *NCTC 6750 in nutrient broth.*

Preservative	Concentration (%)	Nutrient broth containing preservative Doubling time (min)	Nutrient broth containing 0·2% v/v phenylethanol and preservative Doubling time (min)	Relative increase[a]
None	—	33·3	106·5 (103·4–109·8)	2·2
Benzalkonium chloride	0·003	37·4	1023 (481–lysis)	26·4
Chlorbutol	0·2	35·0	lysis	lysis
Chlorhexidine diacetate	0·000275	77·7	lysis	lysis
Chlorocresol	0·024	37·9	13 900 (855–lysis)	368
Thiomersal (as merthiolate)	0·0001	65·5	545 (429–750)	7·3
Methyl hydroxybenzoate and propyl hydroxybenzoate	0·023 0·0115	33·4 —	368 (292–496)	10
Phenylmercuric nitrate	0·0004	67·8	1683 (853–61 800)	23·8

[a] The increase in doubling time in comparison with that obtained in nutrient broth containing preservative but not phenylethanol.
The figures in parentheses are the 95% confidence limits.

This experiment was repeated to check whether extinction measurements were proportional to the viable counts when the preservatives were present in the culture

separately and in combination. The doubling times for each culture were determined by extinction measurements and concurrent viable counts.

Effect of different preservatives on P. aeruginosa *growing in subinhibitory concentration of phenylethanol*

1 ml of log phase *P. aeruginosa* (extinction 0·35 at 420 nm) was added to each of four conical flasks, E, F, G and H, containing 99 ml of prewarmed nutrient broth (in E and F) or 99 ml of prewarmed nutrient broth containing 0·15% v/v phenylethanol (in G and H). When the growth rate had been established by a sequence of extinction measurements, 0·5 ml of prewarmed benzalkonium chloride solution was added to flasks F and H to produce a final concentration of 35 μg/ml when the cultures had similar extinctions. At approximately the same time, 0·5 ml of prewarmed water was added to flasks E and G. The extinctions were then measured at intervals for a further period of time. Similar experiments were made with chlorhexidine and PMN. The results expressed as doubling times for each culture (Table 2).

Table 2. *The effect of the addition of benzalkonium chloride, chlorhexidine diacetate and phenylmercuric nitrate on the growth of* P. aeruginosa *NCTC 6750 in nutrient broth containing phenylethanol.*

Preservative	Concentration (%)	Nutrient broth containing 0·15% phenylethanol Doubling time (min)	Relative increase
None	—	42·7	—
Benzalkonium chloride	0·0035	94·9 (90·3–99·9)	1·22
Chlorhexidine diacetate	0·000275	52·5 (50·1–54·6)	0·22
Phenylmercuric nitrate	0·00075	824·6 (416–40 600)	18·3

The figures in parentheses are the 95% confidence limits.

RESULTS AND DISCUSSION

Extinction measurements compared with viable counts

The doubling times were 30·1 (26·6–34·5), 29·4 (23·7–38·7) and 30·6 (28·0–33·7) min as determined by the viable count and 27·6 (25·11–30·7), 28·3 (27·3–29·5) and 30·6 (28·7–32·7) were the corresponding times for the extinction method. These results indicate that there is no significant difference at the 95% level (the figures in parentheses are the 95% confidence limits) between the doubling times determined by extinction measurements and those by viable counts, but the extinction measurements gave the closer limits of error.

Fig. 1 shows that the growth rate of *P. aeruginosa* NCTC 6750 can be followed by extinction measurements over the concentration range 2×10^7 to 4×10^8 cells/ml. The difficulties of determining growth rates of *P. aeruginosa* NCTC 8203 by means of extinction methods (Brown & Richards, 1964) were not experienced with strain NCTC 6750.

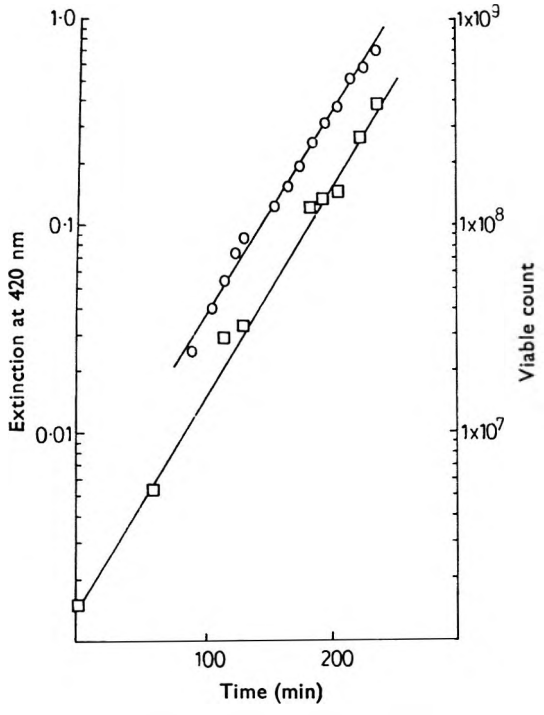

FIG. 1. Growth of *P. aeruginosa* NCTC 6750 in nutrient broth determined by measurement of extinction (○) and viable counts (□).

Effect of phenylethanol on P. aeruginosa *growing in subinhibitory concentrations of the different preservatives*

The growth rate of *P. aeruginosa* NCTC 6750 in nutrient broth containing 30 μg/ml of benzalkonium chloride does not differ markedly from that in nutrient broth alone (Fig. 2). Addition to similar media of sufficient phenylethanol to give 0·2% v/v concentration, however, gave different results. The growth rate in nutrient broth alone was reduced by phenylethanol, but that of the medium containing 30 μg/ml of benzalkonium chloride was almost stopped (Fig. 2). The sums of the relative increase in doubling time for each preservative and for 0·2% v/v phenylethanol were always less than the relative increase in doubling time for similar concentrations used in combination. Thus the combination appears to have a greater effect than addition.

Both the phenylethanol–chlorbutol and the phenylethanol–chlorhexidine combinations caused lysis of *P. aeruginosa*, while the phenylethanol–chlorocresol and phenylethanol–benzalkonium combinations were so effective that one of the 95% confidence limits indicated lysis.

The doubling times of both merthiolate and PMN alone are comparable. Merthiolate has a lower mercury content than PMN, the ratio being 1 to 2·6. Phenylethanol–merthiolate combination has a doubling time 3·2 times faster than phenylethanol–PMN combination, the ratio is similar to the mercury content of the two compounds. Thus the monoethanolamine in merthiolate appears either to enhance the activity of thiomersal or to modify the effect of the phenylethanol.

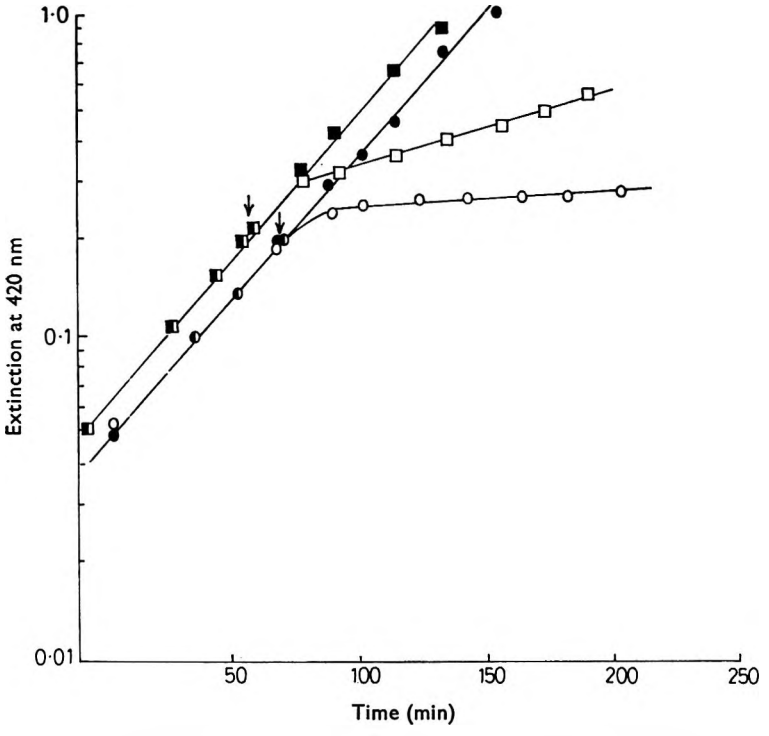

FIG. 2. The effect of 0·2% v/v phenylethanol, 0·003% w/v benzalkonium chloride, both alone and in combination on the growth of *P. aeruginosa* NCTC 6750. ■ *P. aeruginosa* grown in nutrient broth to which water was added after 58 min. □ *P. aeruginosa* grown in nutrient broth to which phenylethanol was added after 58 min. ● *P. aeruginosa* grown in nutrient broth containing 35 μg/ml of benzalkonium chloride to which water was added after 69 min. ○ *P. aeruginosa* grown in nutrient broth containing 35 μg/ml of benzalkonium chloride to which phenylethanol was added after 69 min.

Methyl and propyl hydroxybenzoates at the concentrations recommended by the B.P.C. 1959 in Solution for Eye-drops had no significant effect on the growth of *P. aeruginosa*. This is in agreement with the results of Hugo & Foster (1964).

In all of the experiments phenylethanol enhanced the effect of the preservative; no antagonism was observed. Richards (1971) has investigated the effect of phenylethanol–preservative combination against resistant *P. aeruginosa* by an end-point technique and obtained similar results. This supports the hypothesis that phenylethanol affects the permeability of the pseudomonas cell, allowing more effective penetration of the antibacterial agent (Richards & others, 1969).

The doubling times determined by means of viable counts were similar to those obtained from extinction measurements. Thus the presence of phenylethanol or benzalkonium chloride alone or in combination did not affect the extinction.

Effect of different preservatives on P. aeruginosa *growing in subinhibitory concentrations of phenylethanol*

Potentiation of the antibacterial effect of 0·15% v/v phenylethanol occurred with the three preservatives tested (Table 2), but the degree of enhancement was much reduced compared with the results for these preservatives, especially chlorhexidine, in Table 1. However, the preservatives were added to suspensions already containing

thirty times the number of viable organisms used in the previous test. The likelihood of a drop in effect is supported by the observation that the doubling time of *P. aeruginosa* in nutrient broth initially containing 0·15% phenylethanol is only slightly greater than in nutrient broth alone. *P. aeruginosa* cells selected by 0·2% phenylethanol being present in the broth before inoculation grew more than twice as fast as the cells in the log phase after addition of phenylethanol (Richards & McBride, unpublished observation). The phenylethanol–preservative combination is, however, more effective in reducing the growth rate than either phenylethanol or preservative alone.

In conjunction with previous work (Richards & others, 1969; Richards, 1971) the present results support the use of phenylethanol–preservative combinations because they are more effective in inactivating resistant contaminants than either preservative alone. The combinations also permit the use of lower individual preservative concentrations.

Acknowledgement

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REFERENCES

BROWN, M. R. W. & RICHARDS, R. M. E. (1964). *J. Pharm. Pharmac.*, **16**, *Suppl.* 41T–45T.

HUGO, W. B. & FOSTER, J. H. S. (1964). *Ibid.*, **16**, 209.

RICHARDS, R. M. E., SUWANPRAKORN, P., NEAWBANIJ, S. & SURASDIKUL, N. (1969). *Ibid.*, **21**, 681–686.

RICHARDS, R. M. E. (1971). *Ibid.*, **23**, *Suppl.*,136S–140S

SILVER, S. & WENDT, L. (1967). *J. Bact.*, **93**, 560–566.

J. Pharm. Pharmac., 1971, 23, *Suppl.*, 147S–155S *Received January* 26, 1971

The *in vitro* evaluation of gelatin coacervate microcapsules

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Microcapsules of sulphadiazine have been prepared by the simple gelatin coacervation technique, using sodium sulphate as coacervating agent. The free flowing microcapsular material was hardened with formalin. There is no direct relation between particle size and sulphadiazine concentration nor between different starting gelatin percentages. The effects on size of hardening time, temperature and sampling time are small. *In vitro* dissolution studies show that first order release characteristics are exhibited by all the hardened materials. Temperature and pH effects indicate the dissolution of the sulphadiazine itself to be the controlling step rather than the rate of diffusion through the microcapsule wall.

Although coacervation has been applied to the encapsulation of a wide variety of products (Green & Schleicher, 1956a,b; Phares & Sperandio, 1964; Luzzi & Gerraughty, 1964; Nixon, Khalil & Carless, 1968), few published data are available about the size distribution of microcapsules or the *in vitro* release of inclusions, particularly for simple coacervate systems such as gelatin–water–sodium sulphate (Phares & Sperandio, 1964; Nixon & others, 1968). Release of material from the complex coacervate produced by gelatin and acacia has been extensively studied by Luzzi & Gerraughty (1967a,b) and Bell, Berdick & Holliday (1966), as has that from nylon (Luzzi, Zoglio & Maudling, 1970).

Whilst the sizing of microcapsules has been performed by a number of methods (*Editorials* 1963, 1968; Luzzi & others, 1970; Shigeri, Koishi & others, 1970), a detailed examination of temperature and hardening effects has not been reported.

In the present work we have examined the comparative effects of pH, temperature, % sulphadiazine content and hardening on the *in vitro* release of sulphadiazine from simple coacervate microcapsules and we have recorded the effect of temperature, % sulphadiazine content, and hardening on their size.

MATERIALS AND METHODS

Gelatins: acid pre-treated material, deionized by the method of Janus, Kenchington & Ward (1951) was used throughout. This material had the following characteristics. Bloom No. 274, pH 4·2, viscosity ($6\frac{2}{3}$%, 40°) 6·2 cP, I.E.P. 9·2.

Sulphadiazine: (May & Baker) recrystallized from ethanol; m.p. 254·5–255·5°. Differential scanning calorimetry and infrared analysis showed the presence of Form 1 only.

All *electrolytes* were of A.R. grade; *water* was triple distilled from an all glass still.

Sulphadiazine was assayed by the method of Bratton & Marshall (1939).

Preparation of microcapsules. The method of Nixon & others (1968) was used with the following variations: a 10% w/w starting gelatin concentration and a 4:1

ratio of 7% cold sodium sulphate solution (7–9°). Batches required 300 g of gelatin solution as the starting material.

Dissolution studies. The rate of dissolution from the various microcapsules was examined by stirring the sample and dissolution medium in a flask. The microcapsules (sufficient to give 20 mg of sulphadiazine litre^{-1}) were prepared as a slurry, to prevent agglomeration, poured into 1 litre of McIlvaine buffer solution and stirred at 100 rev/min. The temperature was controlled to $\pm 0{\cdot}5°$. Samples were removed at suitable intervals and filtered through a Swinnex Millipore adaptor fitted to a syringe, before assay for sulphadiazine.

Particle size analysis. A suitable count level dispersion of the microcapsules was prepared by pouring a concentrated slurry, deflocculated by exposure to ultrasonics for 15 s, into 250 ml of 0·9% saline contained in a double walled Coulter cell. The temperature was controlled to $\pm 0{\cdot}5°$. Counts were taken at zero time and 30 min intervals using a Coulter model B calibrated with pecan pollen (42·3 μm diam.). The results were processed using a computer program first developed by Marshall & Ord Smith and adapted by Raymond (1968).

RESULTS

Particle size analysis

The results in Table 1 show that increasing the temperature, particularly for hardened microcapsules, causes only a slight decrease in size compared with the effect of changes in sulphadiazine content. However, from a study of other sulphonamide contents no direct correlation was immediately apparent between microcapsule size and sulphonamide content.

Table 1. *The effect of temperature and hardening time on the mean cumulative weight percentage oversize.*

Sampling time (min)	Temperature °C	% Sulphadiazine: 5 — Mean microcapsule size, μm, after hardening with formalin, at various times (min)					% Sulphadiazine: 30 — Mean microcapsule size, μm, after hardening with formalin, at various times (min)				
		0	30	60	120	180	0	60	120	180	240
0	10	84·5	85·5	84·5	86	86·7	—	43	37	38·5	35
30		84·4	85	—	87	88·7	—	43	37·5	38	34
0	20	80·5	86	85	83	83·5	28·5	41·5	33·5	35	36
30		78·5	85	86	84	84	28·5	37·5	32	33	35
0	30	—	83	82	83	83·5	18·5	28	28	27·5	26
30		—	82	82	83	82	12·0	26	27	27	26
0	40	—	82·2	80·9	81·3	80·5	—	—	26	—	26
30		—	81·3	82	80	80	—	—	26	—	—

Effects of hardening

Capsules containing 5% of the sulphonamide show little difference in size when hardened with formalin for up to 3 h. With 30% sulphadiazine the effect of hardening time is more marked; unhardened capsules were smaller than hardened ones. Hardening for 1 h with formaldehyde produces an increase in size which gradually decreases again as the hardening time is extended to 4 h. At temperatures below 30° there is no significant difference in capsule size up to 30 min after the

commencement of hardening. At 30° and above, the unhardened capsules were sensitive to sampling time, the hardened capsules were not.

The temperature at which the particle size analysis is made may cause size differences either by solution of the gelatin walls or the production of a diffuse hydrated layer. For both samples in Table 1 an increase in temperature up to 40° caused a fall in microcapsule size. With 5% sulphadiazine capsules (hardened) the effect is relatively small: for microcapsules containing 30% sulphonamide, particularly at 30–40°, the effect of temperature on the size was greater than that caused by the time of hardening.

The reaction between formaldehyde and the sulphonamide core material produces a soluble addition compound whose removal may cause a diminution in the microcapsule size. The other possible cause of particle size reduction could be dissolution from the microcapsules during the counting time.

Fig. 1, a typical series of dissolution data, shows that the initial release rate from the microcapsules is extremely rapid, but that after approximately 25% of the core material has been dissolved there is a gradual fall in the rate of release with the last 20% released very slowly. This pattern holds for all the dissolution rates investigated.

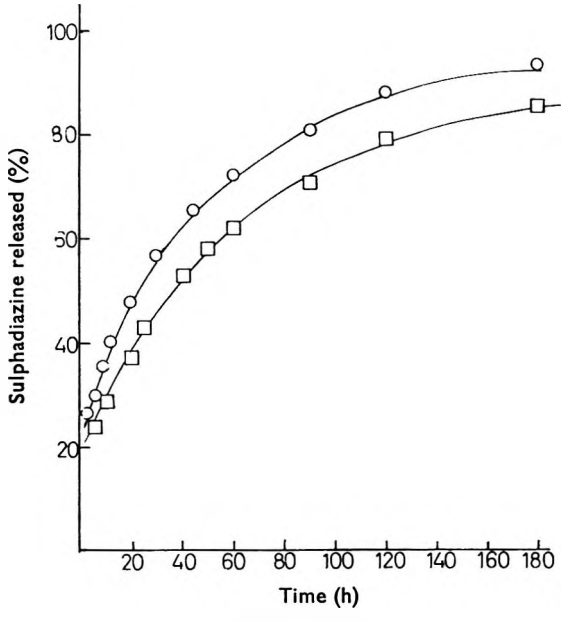

FIG. 1. Typical dissolution curves for sulphadiazine release from microcapsules. Temp. 20°, pH 6, Sulphadiazine ○, 5; □, 4%. 30 min hardening time.

The dissolution data have been examined by the methods of Gibaldi & Feldman (1967), Schwarz, Simonelli & Higuchi (1968) and Wagner (1969). Factors studied include the % sulphadiazine content of the microcapsules, the effect of hardening with formalin, the temperature of dissolution and the pH of the dissolution medium.

When the gelatin concentration was maintained constant, there was no apparent effect on the dissolution rate from an increasing sulphadiazine concentration in the microcapsules.

Time of hardening

The effect of hardening on the capsule size and the leaching effect of the formalin on the sulphonamide complicate the study of hardening effects on dissolution rates. Wagner plots (Fig. 2Ai) derived from hardened microcapsules no longer give straight lines over the whole range. As in all the dissolution experiments on this system, the initial release of drug was rapid and the amount released was slightly increased for longer hardening periods. Because of the cross linking effect of formaldehyde with the gelatin, the 50% release time was increased from that of unhardened capsules; hardening time to 50% release time (min) are: 0/13, 30/37, 120/37, 180/26. Higuchi plots (Fig. 2Aiii) show that hardening the microcapsules resulted in the dissolution obeying the Schwarz & others (1968) model.

The first order release plots (Fig. 2Aii) give straight lines for the hardened materials. As the hardening time was increased the initial rapid release of sulphadiazine was greater. Once the straight line portion of the curve is attained, the slope of the line is marginally dependent on the hardening time [Hardening time (min)/slope $\times 10^3$ are 30/–4·9, 120/–3·7, 180/–4·6]. Shorter hardening times still produce a faster dissolution rate; the unhardened sample gives a shallow curve with a faster release time than the formalin-treated material.

Unhardened gelatin microcapsules exhibit a tendency to disperse or dissolve above 30°. Hardening, as shown by Table 1, is effective in preventing solution of the capsule wall particularly with the large capsules of low sulphonamide content. Dissolution from unhardened microcapsules gave the usual straight line Wagner plots; above 30° solution occurred. An example of the effect of temperature on dissolution from microcapsules hardened for 30 min is shown in Fig. 2B.

At temperatures where the unhardened gelatin would not normally soften and dissolve, the plots are curvilinear, but at higher temperatures, where unhardened gelatin would dissolve, a straight line relation exists. At temperatures up to 60° the hardened microcapsules showed no tendency to disperse. A plot of the log mean release time against reciprocal temperature for this hardened sample gave a straight line.

Higuchi plots of the above data for hardened microcapsules produce no straightforward relation between temperature and dissolution rate.

First order release plots give a straight line relation, but when relatively small amounts of the sulphonamide core material remained to be dissolved the slope of the line changed to give a much slower release. An Arrhenius plot derived from the first order dissolution graphs gives a good temperature-rate relation (Fig. 3).

For the effect of the pH of the dissolution medium, Wagner plots gave straight lines at all pH values studied, although with low pH values there was a tendency at initial sampling times to depart slightly from the straight line. Higuchi plots were curvilinear and could not be readily interpreted; a plot of log % drug remaining against time showed a straight line down to 10% remaining, after which a slower dissolution rate occurred at low pH values. A plot of the slopes is shown in Fig. 4. At pH values between 2 and 6 there is little effect on the rate of dissolution, but once the pK_a of the NH group is passed and higher pH values are reached, the dissolution is extremely rapid. The McIlvaine buffer systems used do not go below pH 2·2 and the use of 0·1N HCl to give a pH of 1·5 produced a dissolution rate which did not fit the remainder of the data.

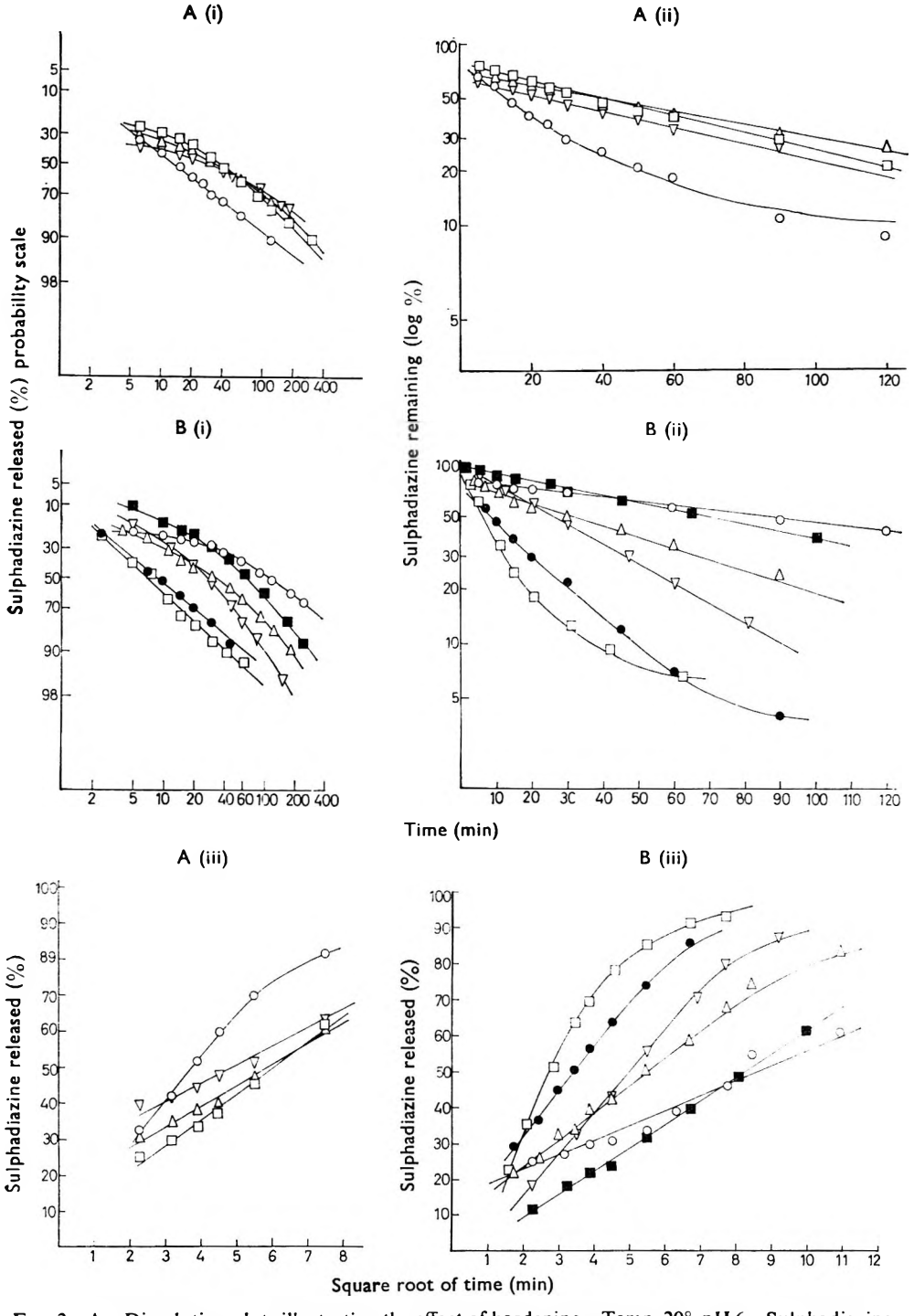

FIG. 2. A. Dissolution plots illustrating the effect of hardening. Temp. 20°, pH 6. Sulphadiazine 5%; (i) Wagner plots; (ii) First Order plots; (iii) Higuchi plots. Time of hardening: ○, unhardened; □, 30; △, 120; ▽, 180 min.

B. Dissolution plots illustrating the effect of temperature. Sulphadiazine 5%, 30 min hardening time, pH 6. (i) Wagner plots; (ii) First Order plots; (iii) Higuchi plots. Temperature: ○, 10; ■, 15; △, 20; ▽, 25; ●, 30; □, 40°.

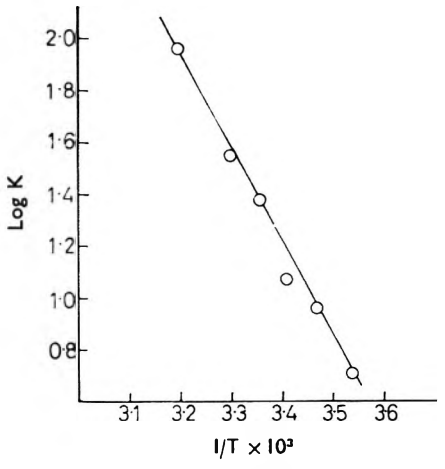

FIG. 3. Arrhenius plot of first order release rates from Fig. 2Bii.

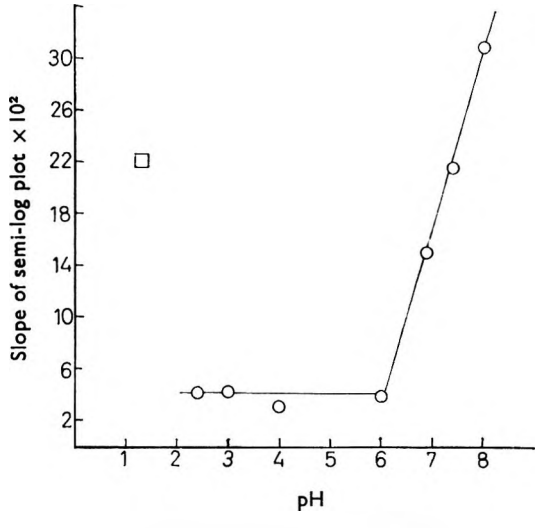

FIG. 4. Effect of pH on the slope of the first order dissolution plots. Sulphadiazine 5%, 30 min hardening time. Temp. 40°; ○, McIlvaine buffer; □, 0·1N HCl.

DISCUSSION

During the preparation of the microcapsules, before extraction and hardening and whilst the temperature is still high enough to keep the gelatin coacervate liquid, they are spherical. On hardening, the gelatin wall shrinks and the capsules tend to take on the shape of the crystals of sulphadiazine dispersed in them. Unlike microcapsules made from liquids or emulsified inclusions, which usually contain only one droplet, the individual capsules will be composed of a number of crystals which tend to be arranged towards the centre of the microcapsule.

The effect on unhardened microcapsules of temperatures over 30° may be interpreted by assuming solution of the gelatin wall. With both high and low sulphonamide:gelatin ratios the size of the capsules was relatively unaffected at lower

temperatures. Even at 10° or 20° a certain amount of solution appears to take place because there is an increase in size with capsules that have been hardened for up to 1 h. This effect is very much more pronounced at the higher temperatures where hardening with formalin renders the gelatin wall almost insoluble.

All capsules show an almost immediate hydration and swelling effect in water. Hardening reduces the degree of hydration and this is reflected by the slight fall in capsule size when longer periods of hardening are used. This decrease in size is greatest at 10° and 20° with the microcapsules containing 30% sulphadiazine.

One complicating factor in these particle size studies was the removal of sulphadiazine from the microcapsules in the form of a soluble compound with the formalin. Other hardening agents such as gluteraldehyde and acrolein reduced the sulphonamide content more rapidly and to a greater extent. The large microcapsules, with a small sulphonamide content, were far less susceptible to the dissolution of their contents than the smaller thin-walled capsules containing a relatively high proportion of inclusion (Table 2). With these thin-walled specimens, hardening times in excess of 1 h could not be used. Comparison of particle size data in Table 1 for 0 and 30 min sampling times does not suggest collapse of the capsules on the removal of the inclusion and the space occupied by the sulphadiazine appears to remain as a vacuole within the microcapsule.

Table 2. *The effect of hardening time on the sulphadiazine content of microcapsules at* 25°.

Starting % sulphadiazine	Sulphadiazine % after hardening with formalin for: 0 min	30 min	60 min	120 min	180 min	240 min
5	100	94	81·5	70	58	—
30	100	—	70	19	15·5	10

Dissolution of an encapsulated material will be controlled by a number of factors. The rate at which the solvent penetrates the wall material, p, the rate at which the drug dissolves in the solvent, s, and the rate at which the dissolved drug can penetrate the wall and disperse from the surface, p_1. The total rate of release, R, can thus be described as $R = f(p + s + p_1)$. It is not necessary that all these factors will have an equal importance and any may exert an overriding influence on the others.

Dissolution from the microcapsules is further complicated because they always contain a small proportion of sulphadiazine either unencapsulated or contained in cracked capsules (Nixon & others, 1968). This is termed "free sulphonamide" and accounted for approximately 2–10% of the total sulphonamide present: it is the material released rapidly during the dissolution studies. In the remainder of the inclusion contained in complete microcapsules, a certain amount will be associated with the wall material, but the bulk will exist as individual crystals in the centre of the microcapsules.

The dissolution and *in vitro* release of the drug from its formulation is open to treatment in different ways that are not always easy to correlate (Schwarz & others, 1968; Wagner, 1969; Langenbucher, 1969; Luzzi, Zoglio & Maudling, 1970). Further, the application of any one of these treatments to drug release from microcapsules presents a number of exceptions, no one model being able to adequately describe all release situations.

Wagner (1969) plots, because they tend to produce straight lines, allow easy comparison of the T50 release time and it has been suggested that this parameter, coupled with σ of the curve would serve to define the release pattern. His system, a tablet, had initially a low surface area which subsequently increased on disintegration. The method of calculation took this factor into account. The data here presented are not always amenable to this type of plot. In no case is a straight line obtained from the commencement of dissolution: there are other differences depending on hardening and temperature.

The Higuchi equation (Schwarz & others, 1968) was developed to define the release from wax matrices. These were tabletted and presented a rather low constant surface area throughout the course of dissolution, but because of removal of drug via the tortuous channels, which could present a constantly changing area for drug dissolution, porosity and "tortuosity" factors had to be introduced. In Higuchi's systems it was necessary to study slow release rates and the experiments did not, in general, proceed past 30% release in approximately 100 h.

Hardened microcapsules present an extreme case of the Higuchi model. The surface area is very large, but remains approximately constant throughout the experiment. Because of the coacervate nature of the original wall, it is possible that porosity and "tortuosity" will play an important part in the release of the inclusion. Even so, straight line relations were not obtained in all cases, though where these do occur, they remain linear to greater than 60% release. The fit of the data to this equation may be entirely fortuitous and with unhardened microcapsules a linear relation was never obtained, although the size remained little changed throughout the course of the dissolution.

As our system retained its gross particle size throughout the course of the experiment, the use of the Langenbucher (1969) cube root treatment for non-disintegrating granules might be expected to apply. Whilst this treatment adequately described his systems down to about 85% dissolution, application to microcapsules at no time produced a single straight line.

The best fit for the bulk of the data was a first order kinetics plot. The equation used, which applies in conditions of exponential change of surface area and under sink conditions was $\log (W^{\infty} - W) = \log M - \frac{k_s}{2{\cdot}303} (t - t_0)$ for $t \geqslant t_0$ where $W^{\infty} - W$ is the amount remaining undissolved, $M = (K/k_s)C_sS^{\circ}$ = intercept, t_0 is zero time, t is sampling time, S° is surface area available for dissolution at the commencement of the experiment, C_s is the equilibrium solubility and K and k_s are constants.

This gave straight lines down to approximately 10% sulphadiazine remaining, with hardened material for all parameters studied. An Arrhenius plot from hardened material was also a straight line. Even with this treatment, however, the unhardened microcapsules did not produce a straight line relation.

The effect of hardening is complicated by the relation between formalin and sulphonamides. Due to the leaching effect it is suggested that a gradient of sulphadiazine is set up in the microcapsule wall. The longer times of hardening produce more of this "wall material", which will be released faster than the crystalline inclusion at the capsule centre.

The nature of the cross linking process for hardening the capsules appears unable to significantly reduce further the rate of dissolution of the sulphadiazine for hardening

times greater than 30 min. The coacervating agent, sodium sulphate, will be rapidly dissolved from the microcapsule wall by the aqueous dissolution medium to leave pores through which the dissolution process can take place. The cross linking process cannot prevent this and the number of dissolution channels produced by removal of the sodium sulphate is probably the same irrespective of hardening time. The unhardened material does not exhibit first order release characteristics, probably due to changes in the ccacervate wall brought about by the gelatin forming a diffuse hydrated layer. Although no straight line relation exists, tangents to the curve give a greater slope, faster release rate, than any of the hardened materials.

The gelatin wall of unhardened microcapsules dissolved at temperatures above 30°, but with hardened samples there appears to be little effect on the size. All the plots show that at the higher temperatures the dissolution pattern changes slightly. Even so, down to about 35% dissolution there is a straightforward Arrhenius relation (Fig. 3): only at 40° is there a secondary slower release rate from the sample. Thus the effect of temperature appears to be only on the solubility of the sulphonamide and not on the permeability of the gelatin wall.

The effect of pH is straightforward. Until sulphadiazine becomes ionized there is no effect of pH on the rate of dissolution in McIlvaine buffer. Because the sodium salt is far more soluble the rate of dissolution at pH values in excess of 6 rises very rapidly. This appears to be simply a solubility effect.

It would appear that the *in vitro* dissolution of sulphadiazine from gelatin microcapsules is not amenable to any one type of treatment. Whilst first order rate kinetics allow the interpretation of most data it is necessary that other treatments of the results be applied to obtain the full picture.

REFERENCES

BELL, S. A., BERDICK, M. & HOLLIDAY, W. M. (1966). *J. New Drugs*, **6**, 284–293.

BRATTON, A. C. & MARSHALL, E. K. (1939). *J. biol. Chem.*, **128**, 537–550.

Editorial (1963). *Meas. and Control*, April, 142–147.

Editorial (1968). *Analyt. Chem.*, **40**, 110A–112A.

GIBALDI, M. & FELDMAN, S. (1967). *J. pharm. Sci.*, 56, 1253–1257.

GREEN, B. K. & SCHLEICHER, L. (1956a). *U.S. Patent* 2,730,456.

GREEN, B. K. & SCHLEICHER, L. (1956b). *U.S. Patent* 2,730,457.

JANUS, J. W., KENCHINGTON, A. W. & WARD, A. G. (1951). *Research, Lond.*, **4**, 247–248.

LANGENBUCHER, F. (1969). *J. pharm. Sci.*, **58**, 1265–1272.

LUZZI, L. A. & GERRAUGHTY, G. J. (1964). *Ibid.*, **53**, 429–431.

LUZZI, L. A. & GERRAUGHTY, G. J. (1967a). *Ibid.*, **56**, 634–638.

LUZZI, L. A. & GERRAUGHTY, G. J. (1967b). *Ibid.*, **56**, 1174–1177.

LUZZI, L. A., ZOGLIO, M. A. & MAUDLING, A. V. (1970). *Ibid.*, **59**, 338–341.

NIXON, J. R., KHALIL, S. A. H. & CARLESS, J. E. (1968). *J. Pharm. Pharmac.*, **20**, 528–538.

PHARES, R. E. & SPERANDIO, G. J. (1964). *J. pharm. Sci.*, **53**, 515–518.

RAYMOND, K. (1968). M.Sc. Thesis. London University.

SCHWARZ, J. B., SIMONELLI, A. P. & HIGUCHI, W. I. (1968). *J. pharm. Sci.*, **57**, 274.

SHIGERI, Y., KOISHI, M., KONDO, T., SHIBA, M. & TOMIOKA, S. (1970). *Can. J. Chem.*, **48**, 2047–2051.

WAGNER, J. G. (1969). *J. pharm. Sci.*, **58**, 1253–1257.

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Further studies on the effect of additives on the release of drug from hard gelatin capsules

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An experiment based on a 3^3 design has been undertaken to establish the effect on drug release from capsules produced by adding lactose (0, 10 and 50%), magnesium stearate (0, 1 and 5%), and sodium lauryl sulphate (0, 1 and 10%) to a 76–105μm particle size fraction of ethinamate filled into capsules at a high and low packing density. Statistical analysis of the results indicated the presence of 2nd order interaction at all time intervals and for both sets of capsule fill weights. This signifies that the effect produced by each additive is dependent on the presence and the level of the other two additives. The interaction limits the conclusions that can be drawn about the main factors of diluent, lubricant and wetting agent, but the indications are that (a) 10% diluent reduces drug release, whereas 50% produces enhanced release; (b) the presence of 1% sodium lauryl sulphate is sufficient to enhance drug release; (c) the additive effects are independent of the capsule packing density.

As a result of a preliminary experiment of restricted design (Newton, Rowley & Törnblom, 1971), a full three way analysis of variance of the factors diluent, lubricant and wetting agent has been made, to elucidate their influence on drug release from capsules. In the light of experience, the number of replicates was reduced from 8 to 4 and the time intervals were reduced from 5 to 3. The levels of the three additives were those used previously and thus the design can be represented by the scheme shown in Table 1.

MATERIALS AND METHODS

Materials

A single batch of ethinamate (1-ethynylcyclohexyl carbamate) U.S.N.F. was sieved to give a 76–105 μm size fraction. The lactose, magnesium stearate and sodium lauryl sulphate were of B.P. quality. All other chemicals were of BDH reagent grade.

Capsule filling

The way in which the different levels of the three additives were combined is set out in Table 1. The powder blends were filled into size 0 clear gelatin capsules as described by Newton & Rowley (1970) to give a series of capsules of low fill weight (95% of the theoretical value predicted from the maximum bulk density), and a high fill weight (that produced with a load of 45 kg on the loading plate). Twenty capsules were prepared from each blend and only those capsules within $\pm 5\%$ of the mean filling weight were tested further. By replicate assay on random sets of capsules, the mixing was found to provide capsules which contained within $\pm 5\%$ of the predicted mean drug content.

Table 1. *Scheme for testing the variables of diluent, lubricant and wetting agent.* D represents diluent, L, lubricant and W, wetting agent. The subscript represents the % of additive present.

Lubricant %	Wetting agent (%) 0			1			10		
	Diluent %								
	0	10	50	0	10	50	0	10	50
0	$D_0L_0W_0$	$D_{10}L_0W_0$	$D_{50}L_0W_0$	$D_0L_0W_1$	$D_{10}L_0W_1$	$D_{50}L_0W_1$	$D_0L_0W_{10}$	$D_{10}L_0W_{10}$	$D_{50}L_0W_{10}$
1	$D_0L_1W_0$	$D_{10}L_1W_0$	$D_{50}L_1W_0$	$D_0L_1W_1$	$D_{10}L_1W_1$	$D_{50}L_1W_1$	$D_0L_1W_{10}$	$D_{10}L_1W_{10}$	$D_{50}L_1W_{10}$
5	$D_0L_5W_0$	$D_{10}L_5W_0$	$D_{50}L_5W_0$	$D_0L_5W_1$	$D_{10}L_5W_1$	$D_{50}L_5W_1$	$D_0L_5W_{10}$	$D_{10}L_5W_{10}$	$D_{50}L_5W_{10}$

Dissolution testing

This was made according to Newton & Rowley (1970) with a multistirrer system driven by an electric motor fitted with a Kop Variator, to give a stirrer speed of 45 rev/min. After known time intervals 2 ml samples were removed through a filter by means of a syringe. The ethinamate in solution was hydrolysed with sulphuric acid at 90° and the ammonium salt formed with sodium phenate and sodium hypochlorite solution estimated, the whole process being carried out using a Technicon Autoanalyser. Statistical analysis of the multistirrer system proved the interchangeability of results between beakers. Four capsules from each batch were tested.

RESULTS AND DISCUSSION

The percentage of drug content of the capsules which appears in solution 5, 20 and 40 min after commencing the dissolution test is shown in Table 2. The analysis of variance of the results is summarized in Table 3 where the complexity of the effects reported by Newton & others (1971) is confirmed. An important feature of the results is the presence of 2nd order interactions (Table 3), which signifies that the effect produced by each additive is dependent on the presence and the level of the other two. It is thus not possible to predict from a knowledge of the levels of the individual factors how combinations of the three additives will influence drug release. This is illustrated by Fig. 1a, b and c. Here the effect of lubricant concentration on the drug release is shown for each level of diluent, at each level of wetting agent for high capsule fill weight after 40 min. If there were no interactions, for each diluent level each curve for the 3 wetting agent levels would have the same general shape, and these would be repeated at each diluent level. The complexity of shapes, within and between diluent levels illustrates the presence of 2nd order interactions.

The nature of the interactions is a matter for speculation. The % of the drug which dissolves during the test is a complex function of numerous factors, such as rate of solution of the gelatin shell, rate of penetration of water into the powder mass, the rate of de-aggregation of the powder mass and the characteristics of the powder mass after break up. The analysis of variance has confirmed the complexity of these events.

In spite of the presence of 2nd order interactions, the analysis of variance (Table 3), shows that diluent and wetting agent have a statistically significant effect on drug release. Assessment of the magnitude of the effects of each additive is made by averaging the results for a given level of a factor, irrespective of the other factors.

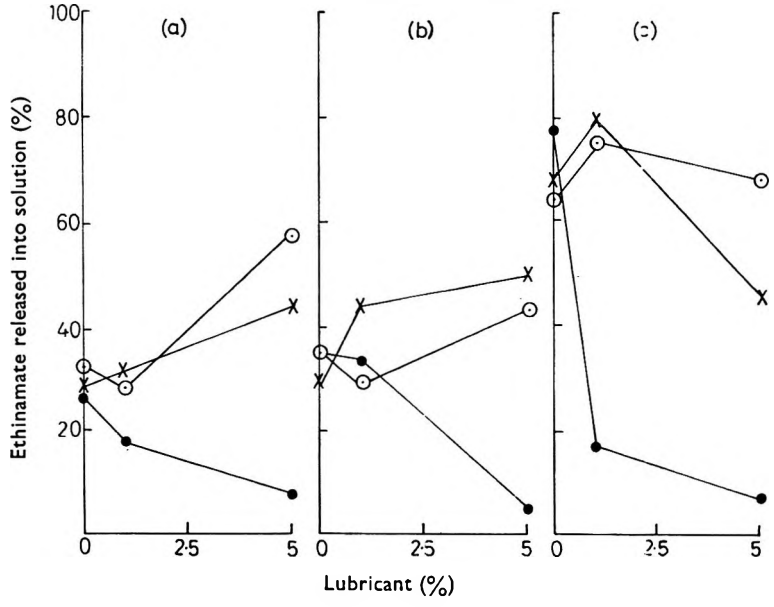

FIG. 1. The effect of lubricant content on the release of ethinamate from high fill weight capsules after 40 min at zero wetting agent (●), low wetting agent (×) and high wetting agent (○) content for (a) zero diluent, (b) low diluent, (c) high diluent.

For example, the effect of a 10% diluent level is obtained by averaging the results for the nine experiments in which 10% diluent was present, i.e. the results for all the systems D_{10} of Table 1. When treated in this way the results for the three levels of each additive for the low capsule fill weights are depicted in Fig. 2. These results

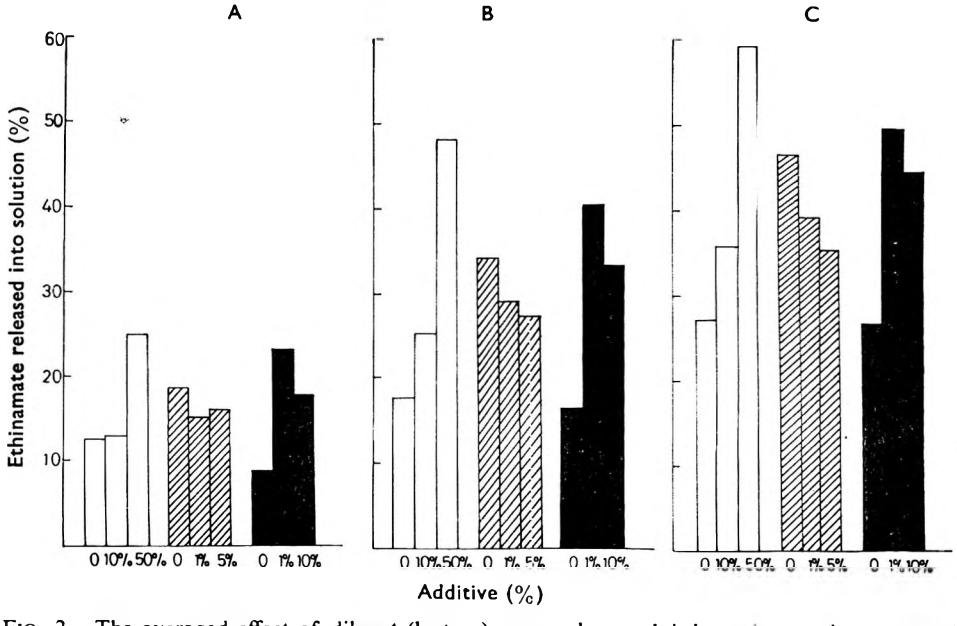

FIG. 2. The averaged effect of diluent (lactose) open columns, lubricant (magnesium stearate) hatched columns and wetting agent (sodium lauryl sulphate) solid columns on the release of ethinamate from capsules after (A) 5, (B) 20, (C) 40 min.

show that the significant effect of diluent is to produce an increase in drug release, especially at the 50% level. Lactose, being water soluble, will produce this effect by changing the powder bed from one which is hydrophobic to one which is more hydrophilic. A change in the hydrophobic character of the bed will also be produced by the wetting agent, hence the increase in drug release, Fig. 2. The effect, however, appears somewhat less at the high wetting agent concentration (10%), which implies that wetting is not the only factor involved in drug release and agrees with our previous findings using liquid penetration as an assessment of drug release from capsules (Rowley & Newton, 1970).

The finding that the presence of magnesium stearate does not significantly affect drug release is somewhat surprising in view of earlier results (Rowley & Newton, 1970). In the present experiments, when magnesium stearate is the only additive, drug release is clearly reduced (Table 2, Fig. 1a). In the absence of wetting agent

Table 2. *Mean percentage of drug released from capsules in dissolution tests.* Each block of figures represents the design of Table 1 and each entry is the mean of four determinations.

Time (min)									
				High capsule fill weight					
5	15·75	12·45	37·18	10·88	21·18	33·05	11·45	11·75	30·38
	4·25	3·48	4·75	8·85	18·75	42·80	7·30	9·73	34·05
	2·70	1·78	5·40	11·13	14·33	17·00	14·63	14·60	21·43
20	17·78	23·08	16·53	17·45	22·68	59·30	20·05	22·48	54·85
	11·38	13·93	7·65	24·55	35·90	72·60	16·18	19·80	65·93
	3·90	2·40	5·58	28·83	41·40	36·95	20·90	32·70	57·05
40	26·35	34·73	79·15	28·03	29·13	68·33	32·03	34·38	64·18
	17·58	28·08	17·13	31·20	43·93	79·50	27·98	29·00	75·18
	7·60	4·93	7·03	43·78	50·03	46·05	57·03	43·20	68·03
				Low capsule fill weight					
5	6·93	12·65	34·75	11·30	12·05	38·73	8·78	13·95	27·05
	3·95	2·55	4·73	16·15	20·58	31·58	7·38	14·78	33·40
	3·40	2·15	6·23	39·50	22·60	14·38	11·95	10·73	31·40
20	14·08	25·03	65·35	15·18	20·78	69·85	16·65	27·08	55·95
	8·90	12·73	6·60	24·68	42·52	72·40	20·03	17·83	55·98
	5·40	3·73	6·93	27·13	48·05	44·75	26·25	27·25	55·70
40	24·93	38·38	89·38	22·68	31·03	81·73	26·93	41·03	62·90
	20·63	25·13	17·45	29·80	52·90	78·73	31·70	27·10	69·50
	8·50	7·33	8·50	39·50	57·13	55·05	36·40	39·68	67·00

but the presence of diluent, the addition of magnesium stearate again causes a reduction in drug release (Fig. 1b, c) but the inclusion of both wetting agent and diluent provides the non-predictable pattern observed in Fig. 1b, c and the small differences in average affect seen in Fig. 2. Thus the overall effect of lubricant is overcome by the presence of the other additives and, in terms of statistics, contributes mainly to the interaction term.

Comparison of the results for each combination of additives at the two capsule fill weights indicates that similar quantities of drug are released for both low and high capsule filling densities (Table 2). The magnitude of the effects of each factor, shown for low capsule fill weights in Fig. 2 are, therefore, very similar. The variance

Table 3. *Statistical significance of results for drug release from capsules.*

Source of error	5 min Mean square S^2	5 min F ratio	20 min Mean square S^2	20 min F ratio	40 min Mean square S^2	40 min F ratio
			Low capsule fill weight			
Dilent D	1844·28	8·30‡	7843·2	77·95†	9935·96	16·93†
Lubricant L ..	117·68	*	503·3	5·00‡	1146·68	1·90
Wetting agent W ..	1908·47	8·60‡	5521·91	54·80†	5318·16	9·06†
D × L	514·49	2·32	1659·47	16·19†	1297·02	2·21
D × W	195·43	*	1231·88	12·24†	440·2	*
L × W	389·73	1·76	1510·96	15·02†	2057·59	3·51
D × L × W ..	221·66	8·51†	100·61	2·49‡	586·89	12·27†
Residual	26·05		40·30		47·84	
			High capsule fill weight			
Diluent D	2497·62	19·15†	8831·86	10·65†	7242·8	12·99†
Lubricant L ..	743·89	5·70‡	632·98	*	542·42	*
Wetting agent W ..	979·2	7·51‡	4460·99	5·37‡	6120·89	10·98†
D × L	281·22	2·16	828·52	1·0	1278·9	2·29
D × W	154·43	1·18	449·75	*	410·58	*
L × W	430·59	3·30	1158·09	1·40	2665·35	4·78‡
D × L × W ..	130·42	4·39†	829·25	25·60†	557·66	14·5†
Residual	29·69		32·29		41·32	

The F ratio for the interaction error D × L × W is the ratio mean square D × L × W: mean square residual. As these are always significant at the 5% level, the F ratio for all other factors is the ratio mean square for the factor: mean square D × L × W.

† F values significant at 1% level.

‡ F values significant at 5% level.

* The value of the mean square for this source of variation is less than that for the interaction, hence the F ratio is less than 1—indicating that the variation in question is probably of a random nature.

ratios (Table 3) also show a similar pattern of significance and hence the effect of additives can be considered independent of capsule filling conditions.

The failure to exclude interactions from the complete 3 way analysis of variance, suggests that the use of this approach to evaluate the effect of combined additives on the release of drug from capsules is not satisfactory if but three levels are used. Increasing the number of levels within the same range of concentration of additives would define the main effects in greater detail and also elucidate the contribution of the different factors to the interactions. Unfortunately, increasing the number of levels increases the number of experiments to be made, e.g. the use of 5 levels requires 125 different combinations of factors compared with the 27 of the present experiment. Reduced forms of designed experiments can, however, be used and measurements of factors other than drug release should also be considered when optimum levels of additives are sought.

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REFERENCES

NEWTON, J. M. & ROWLEY, G. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 163S–168S.
NEWTON, J. M., ROWLEY, G. & TÖRNBLOM, J-F. V. (1971). *Ibid.*, **23**, 452–453.
ROWLEY, G. & NEWTON, J. M. (1970). *Ibid.*, **22**, 966–967.

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Hydrophobic bonding in soap-stabilized emulsions

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The rheological properties of emulsions stabilized by potassium laurate have been measured at different temperatures and in the presence of urea to assess the validity of the concept of aggregation through hydrophobic interactions. The elasticity of the systems, as measured by a Rheogoniometer, increased with increased temperature and decreased with added urea. These results are entirely consistent with hydrophobic bonding. The mechanism of bonding is discussed in terms of interfacial hydrolysis, complex condensed films of acid soap, and polymolecular adsorption of surfactant at the oil–water interface.

Recently the aggregation behaviour of liquid paraffin, oil-in-water emulsions stabilized by potassium laurate was examined by Shotton & Davis (1968a). A maximum state of aggregation in the region of 5% surfactant could not be explained by the classical DVO theory of colloid stability (Derjaguin, 1940; Verwey & Overbeek, 1948). Instead, polymolecular adsorption of surfactant at the oil–water interface, followed by hydrophobic bonding was suggested as a possible alternative. Similar processes of polymolecular adsorption, micellar adsorption or hydrophobic bonding interactions (or both) have been proposed previously for the air-water interface (Dixon, Judson & Salley, 1954; Ross, 1945; Moilliet, Collie & Black, 1961), solid–water interface (Matijevic & Ottewill, 1958; Ottewill & Watanabe, 1958; Somasundarin, Healy & Fuerstenau, 1966), emulsions (Cockbain, 1952; Osipow, Birsan & Snell, 1957; Riegelman, 1962) and surfactant protein interactions (Nemethy, 1967).

Cockbain's original concept of hydrophobic bonding as an effective mechanism of aggregation has been thought improbable (Higuchi, Rhee & Flanagan, 1965; Becher, 1965) and some authors have preferred to restrict their conclusions to vague statements such as "film–film interactions" (Moore & Lemberger, 1963; Lemberger & Mourad, 1965) or "weakly adsorbed, thick hydrophilic layers" (Higuchi & others, 1965).

The liquid paraffin emulsions we used were pseudoplastic in their flow properties. This decrease in viscosity with increased shear rate can be explained in one of two ways. The link formation concept of Goodeve (1939) or the entrappment of continuous phase approach of Mooney (1946). Furthermore, any aggregated emulsion will be sensitive to changes in temperature. The entrappment theory predicts that an increase in temperature will cause partial destruction of aggregate structure and a subsequent fall in relative viscosity (Reiner, 1961). On the other hand, aggregates stabilized by hydrophobic bonds would be expected to increase in stability as the temperature rises due to the endothermic nature of hydrophobic bond formation (Nemethy, 1967). The relative viscosity would therefore increase. Likewise the

addition of a hydrophobic bond breaker, such as urea, should tend to decrease the relative viscosity.

Experiments of this nature have been carried out in the present work, to assess the validity of our suggested mechanism for aggregation.

MATERIALS AND METHODS

Materials and methods were as previously described (Shotton & Davis, 1968a, b; Davis, 1971). Emulsions of 60% liquid paraffin dispersed in potassium laurate of differing concentration, were prepared by initial mixing with a high speed stirrer, followed by homogenization. All emulsions were stored for one month before examination to allow for interfacial equilibrium, release of entrapped air, and possible changes in aggregation state (Sherman, 1967). Particle size distributions were characterized using a Coulter Counter (Model A—Industrial) (Shotton & Davis, 1968b). Rheological studies were performed with a Ferranti-Shirley viscometer and a Weissenberg Rheogoniometer. Bulk solution viscosities were measured by U-tube viscometers.

RESULTS AND DISCUSSION

Continuous phase

The viscosity of soap solutions varies exponentially with concentration:

$$\eta = e^{KC}$$

where K is a constant and C the soap concentration (Davis, 1967). The exponential shape of the curve is due to the association of soap molecules into micellar aggregates (Booij, 1949) and in the region of the critical micelle concentration there is a break. An increase in temperature or added solute would be expected to disturb the equilibrium between micellar and monomeric species and thereby affect viscosity.

Temperature. The viscosity of potassium laurate solutions falls monotonically with increased temperature over the range 25–40° (Fig. 1). This is qualitatively in

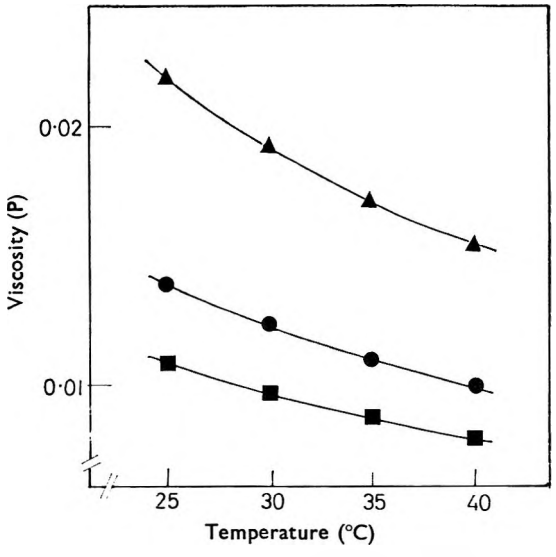

FIG. 1. Viscosity temperature relations for potassium laurate solutions above the critical micelle concentration. Potassium laurate concentration (% w/w). ■ 2·5. ● 5. ▲ 10.

accord with an empirical relation analogous to the Arrhenius equation of chemical kinetics:

$$\eta = Ae^{E/RT}$$

where A is a constant and E is the activation energy (Martin, Swarbrick & Cammarata, 1970). The curves are not truly exponential because the concentrations of the two dissolved species, monomer and micelle are highly dependent on temperature In general, for ionized surfactants such as potassium laurate, an increase in temperature results in an increase in the number of monomeric species and a reduction in the size and number of micelles (Shinoda, Nakagawa & others, 1963). A slight concomitant fall in viscosity would therefore be expected.

Urea. Urea produces a rise in viscosity and above a concentration of 2M there is a linear relation between viscosity and urea concentration (Fig. 2). The addition

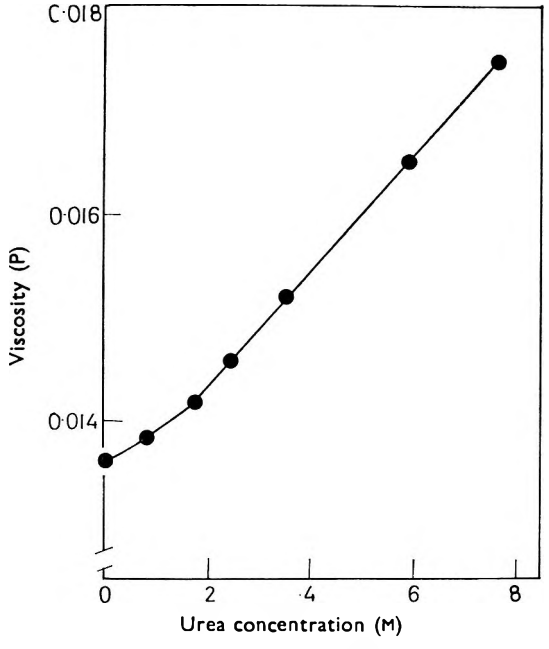

FIG. 2. The effect of urea on the viscosity of 5% potassium laurate (25°).

of urea to a soap solution above its c.m.c. will have two opposing effects on viscosity. It will raise the dielectric constant of water (Wyman, 1936) and should therefore promote micelle formation by decreasing the repulsive forces between the ionic heads of the laurate molecules. However urea has been shown to break up micellar structure due to its effect on hydrophobic bonds (Emerson & Holtzer, 1967; Bruning & Holtzer, 1961; Mukerjee & Ray, 1963), and would therefore be expected to reduce solution viscosity. On the other hand the addition of a solute, at high molar concentrations, will increase the viscosity on simple hydrodynamic grounds. The result of these two opposing effects is clearly shown in Fig. 2. At low concentrations (below 2M) micellar breakdown is occurring, and the gradient of the viscosity concentration relation is smaller than that at high urea concentrations where added urea is having a hydrodynamic effect only.

The effect of temperature on emulsion stability

Cone and plate viscometer. Samples of emulsion, stabilized by different concentrations of potassium laurate, were examined at temperatures in the range 25–40°. In all cases the flow curves were pseudoplastic and there was no evidence of thixotropy. The instrument was programmed to effect a compromise between instrumental effects at short sweep times and evaporation at longer sweep times (Davis, Shotton & Warburton, 1968). Rheograms were characterized by *apparent viscosity* (ratio of shear stress to shear rate at the highest shear rate of 1700 s^{-1}) and *limiting viscosity* (calculated from gradient of linear portion of flow curve). Both parameters fell with increase in temperature but the calculated relative viscosities increased with temperature. Relative apparent viscosity values are shown in Fig. 3. The relative limiting viscosities were similar.

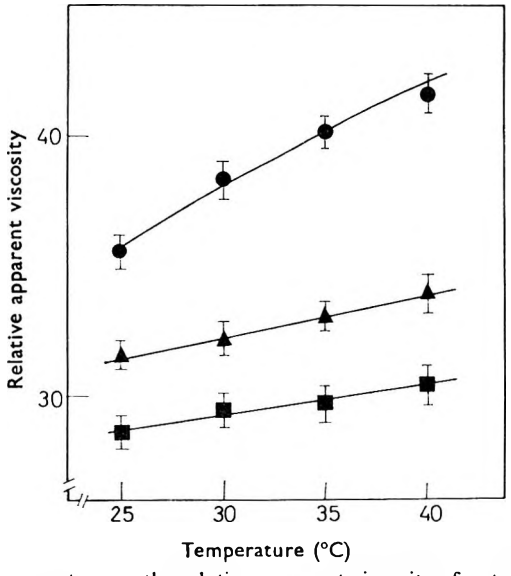

FIG. 3. The effect of temperature on the relative apparent viscosity of potassium laurate stabilized liquid paraffin emulsions (phase volume = 60%). Potassium laurate concentration (% w/w). ■ 2·5. ● 5. ▲ 10.

The rise in relative viscosity suggests that hydrophobic bonding interactions are indeed probable. Since the Ferranti-Shirley Viscometer is known to suffer from spurious effects due to evaporation, even when fitted with the manufacturers anti-evaporation unit (Davis & others, 1968), a further method of testing was selected.

Weissenberg Rheogoniometer. The emulsions under study were highly aggregated systems that demonstrated measurable elasticity when tested at very low shear rates. For a linear viscoelastic material undergoing forced harmonic oscillation of small amplitude

$$\sigma = 2\eta^* \dot{\gamma}$$

where σ is the shear stress, $\dot{\gamma}$ the shear rate, and η^* the complex dynamic viscosity which is a function of the frequency of oscillation (W rad. s^{-1}) (Walters, 1968). We can express η^* as real and imaginary parts

$$\eta^* = \eta' - i\,(G'/W)$$

where η' is the dynamic viscosity, G′ the dynamic rigidity, and $i = \sqrt{-1}$.

Samples of emulsion stabilized by 5% potassium laurate (the most highly aggregated system) were contained in parallel plate geometry and oscillated at small strain amplitude and low frequency (shear rate). The measured data, phase angle between stress and strain sine waves, and amplitude ratio were obtained using a Transfer Function Analyser (Warburton & Davis, 1969). These data were subsequently treated by digital computer using the equations of Walters & Kemp (1968).

A frequency of 0·5 rad. s^{-1} and an accurately known gap of around 0·06 cm were used for all experiments. The maximum shear rate was $8{\cdot}5 \times 10^{-3}\,s^{-1}$ and the maximum shear strain 2×10^{-2}. All experiments were performed as quickly as possible to avoid evaporation effects. Initially, viscoelastic properties were measured at 25° at weekly periods to monitor any storage change in G′ and η' (Sherman, 1967). When constant reproducible results had been obtained, measurements were made over the temperature range 22·5–35°. Each temperature was held constant for three days before testing. Finally the system was re-examined at 25°.

The elasticity (G′), and (η') increased with temperature over the whole range studied (Fig. 4). This was a reversible increase since on returning to the original

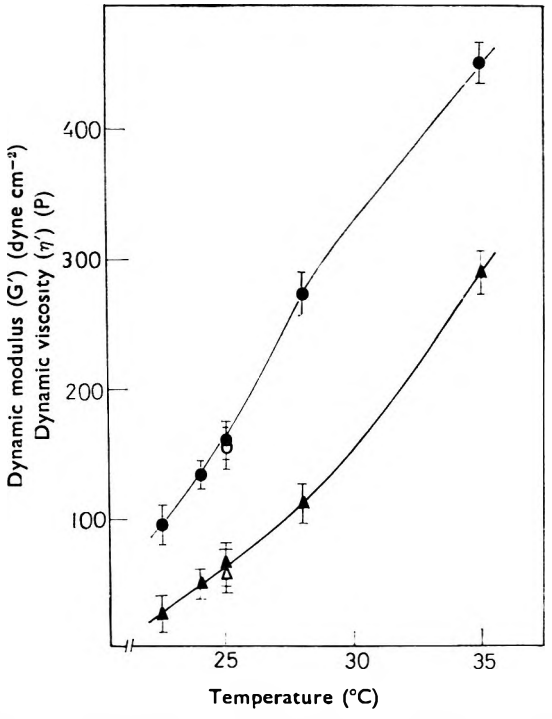

FIG. 4. The effect of temperature on the viscoelastic properties of a potassium laurate stabilized liquid paraffin emulsion (phase volume = 60%, soap concentration = 5% w/w). ● Dynamic viscosity. ▲ Dynamic modulus. Open symbols refer to repeat test after temperature cycle completed.

temperature, the two parameters had not changed significantly. The particle size distribution remained effectively the same throughout the tests. [Potassium laurate emulsions, although highly aggregated are known to be extremely stable to changes in particle size and particle size distribution (Shotton & Davis, 1968a, b)]. The results with the rheogoniometer confirm that the emulsions become more highly

aggregated as the temperature is increased. Using the Goodeve theory of pseudo-plastic flow we can consider that the interparticulate linkages become stronger with increase in temperature.

The process of hydrophobic bond formation (Nemethy, 1967; Kauzmann, 1959; Nemethy & Scheraga, 1962) has been compared thermodynamically with the solution of hydrocarbons in water (Kauzmann, 1959; Nemethy & Scheraga, 1962) and at room temperature has the following properties: $\Delta F_H < 0$, $\Delta S_H > 0$, $\Delta H_H > 0$.

The unfavourable enthalpy of formation is more than counterbalanced by the positive entropy term which results in a favourable free energy. Entropy is the most important factor in bond formation which can be profitably discussed in terms of water structure and the Frank & Evans (1945) concept of "ice-berg" formation around non-polar regions of dissolved species. The endothermic nature of the process causes the bonds to become stronger as the temperature rises to approximately 60°.

The effect of urea on emulsion stability

Quantities of high purity urea were added at 25° to emulsions stabilized by 5% potassium laurate. The systems were agitated for 2 h and then G′ and η' measured as before. Both parameters were reduced by urea, the major effect being in the concentration region 0–2M (Fig. 5).

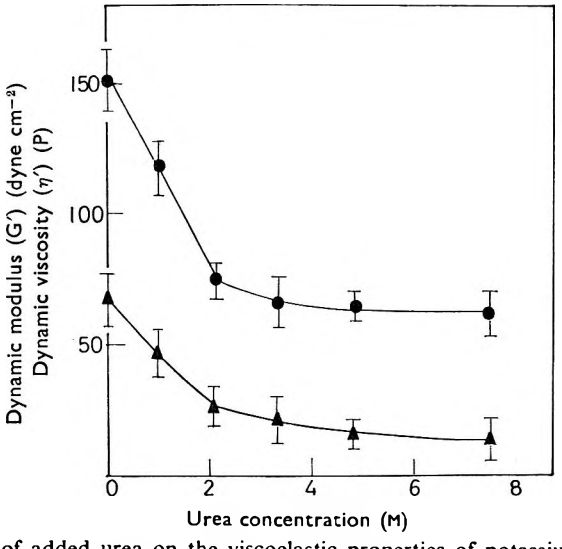

FIG. 5. The effect of added urea on the viscoelastic properties of potassium laurate emulsion in Fig. 4 (25°). ● Dynamic viscosity. ▲ Dynamic modulus.

Urea is known to break hydrophobic bonds (Kauzmann, 1959; Nemethy & Scheraga, 1962), although the exact mechanism is not clear. Frank & Franks (1968) and Mukerjee & Ray (1963) have pointed out that urea can hydrogen bond strongly with water and will therefore alter the water structure that presumably forms around hydrocarbon groups. That is, the "ice-berg" structures will be modified and hydrophobic interactions will be reduced. Kauzmann (1959) has also suggested the formation of urea–hydrocarbon clathrates as a possible mechanism. Urea concentrations in the range 0–2M are sufficient to cause breakdown of most of the hydrophobic bonds in the emulsion systems as well as the laurate micelles (Fig. 2).

Hydrophobic bonding in emulsions

The experiments on temperature effects and added urea are consistent with the premise that emulsions stabilized by potassium laurate are aggregated through a hydrophobic bonding mechanism. Cockbain's (1952) suggestion that aggregation could be accounted for by hydrophobic bonding was based on the assumption that a secondary layer of surfactant was adsorbed at the interface at concentrations just above the cmc. Single surfactant molecules would then be adsorbed with their hydrocarbon chains orientated towards the aqueous phase. Such particles in an aqueous medium would be hydrophobic and aggregation would occur through hydrophobic bonding. However, polymolecular adsorption of surfactant at interfaces is thought to occur only when two or more surface-active materials react to form a complex film (Moilliet & others, 1961). Indeed, Riegelman (1962) has proposed micellar adsorption of surfactant at the oil–water interface when a co-emulsifier is present. Polymolecular adsorption with "pure" surfactants can be considered improbable. The problem remains one of defining a pure surfactant when dealing with fatty acid soaps. The stability of soap emulsions has been attributed to the formation of acid soap by hydrolysis at the interface to give a complex condensed film (Martin & Hermann, 1941; Davis & Bartell, 1943). Such interfacial hydrolysis can occur even at high pH when bulk hydrolysis is negligible (Cook & Talbot, 1952; Eagland & Franks, 1957) so that the emulsions in the present study can be assumed to have complex interfacial films of ionized soap and free lauric acid. This type of film will have a greater stability than than a single component film. The mechanism of interaction between the two components is still uncertain, as is its stoichiometry (Goddard, Smith & Kao, 1966). Three alternative suggestions can be made: (i) Van der Waals attraction between hydrocarbon chains and the repulsive forces between charged heads screened by intervening carboxylic acid groups (Spink, 1963), (ii). The negative character of the carboxylate ion leads to strong hydrogen bonding interactions (Alexander, 1941–42) and (iii) Ion-dipole interaction between charged and uncharged head groups (Marsden & Schulman, 1938). As yet the position is unresolved but nevertheless it is clear that complex films are more stable than their one component counterparts and that coulombic repulsion between charged heads is considerably suppressed.

At this stage we can propose a tentative model for polymolecular adsorption and hydrophobic bonding based on interfacial hydrolysis of potassium laurate and the formation of a complex condensed film of soap and free acid (Fig. 6). Similar mechanisms could apply to other "pure" ionic surfactants such as sodium dodecyl sulphate. Here the mixed film would be available from either interfacial hydrolysis or incomplete sulphation in manufacture.

The process of bond formation can be considered similar to the dimerization of long chain surface active ions described by Mukerjee, Mysels & Dunlin (1958). The structure of the dimer is visualized as one in which the ionic heads are far apart and the flexible chains intertwined. The reduction in the amount of hydrocarbon surface exposed to water is estimated as being approximately equivalent to the removal of $8CH_2$ groups from aqueous environment. At 25° the change in free energy upon transfer of one CH_2 group from a non-polar organic phase to water is in the region of 3·6 kJ mol^{-1} (0·85 kcal mol^{-1}) with $\Delta H = 1{\cdot}7$ kJ mol^{-1} (0·4 kcal mol^{-1}) (Davis & Higuchi, unpublished observation). Such values can be used to calculate the energetics of hydrophobic bond formation (Nemethy, 1967) if we bear

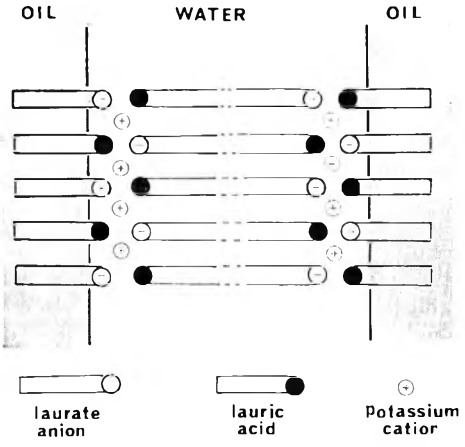

FIG. 6. A model of the mechanism for hydrophobic bonding aggregation in emulsions stabilized by potassium laurate.

in mind that the CH_2 groups in the bond will be much more constrained than in the liquid hydrocarbon and have a lower entropy (Mukerjee, 1967). A more realistic ΔF value for the CH_2 group is obtained from the thermodynamics of micellization of non-ionic surface active agents [$\Delta F_{CH_2} = 2{\cdot}9$ kJ mol^{-1} (0·7 kcal mol^{-1})]. Hence at 22·5° $\Delta F_H = -23{\cdot}0$ kJ mol^{-1} ($-5{\cdot}5$ kcal mol^{-1}).

We are currently exploring the possibilities of using aggregated soap emulsions as model systems for the systematic study of hydrophobic interactions. The hydrophobic bond is known to be of great influence in determining secondary and tertiary structure of protein molecules and other polymers as well as in the non-specific interaction of drugs at enzyme surfaces and with receptor sites (Belleau & Lacasse, 1964; Hansch, 1968).

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REFERENCES

ALEXANDER, A. E. (1941–1942). *Proc. Roy. Soc.* (*Lond.*), **A179**, 486–499.
BECHER, P. (1965). *Emulsions Theory and Practice*, 2nd edn., p. 49. New York: Reinhold.
BELLEAU, B. & LACASSE, G. (1964). *J. mednl. Chem.*, **7**, 768–775.
BOOIJ, H. L. (1949). *Colloid. Sci.*, Editor: Kruyt, H. R. Vol. **2** pp. 681–722. Amsterdam: Elsevier.
BRUNING, W. & HOLTZER, A. (1961). *J. Am. chem. Soc.*, **83**, 4865–4866.
COCKBAIN, E. G. (1952). *Trans. Faraday Soc.*, **48**, 185–196.
COOK, M. A. & TALBOT, E. L. (1952). *J. phys. Chem.*, **56**, 412–416.
DAVIS, J. K. & BARTELL, F. E. (1943). *Ibid.*, **47**, 40–50.
DAVIS, S. S. (1967). Ph.D. Thesis, London University.
DAVIS, S. S. (1971). *J. Colloid Inter. Sci.*, **35**, 665–674.
DAVIS, S. S., SHOTTON, E. & WARBURTON, B. (1968). *J. Pharm. Pharmac.*, **20**, 157S–167S.
DERJAGUIN, B. (1940). *Trans. Faraday Soc.*, **36**, 203–215, 730–731.
DIXON, J. K., JUDSON, C. M. & SALLEY, D. J. (1954). *Monomolecular layers*, pp. 63–106. Editor: Sobotka, K. A.A.A.S.

EAGLAND, D. & FRANKS, F. (1957). *Proc. 3rd Intern. Cong. Surface Activity*, **2**, 539–544.
EMERSON, M. F. & HOLTZER, A. (1967). *J. phys. Chem.*, **71**, 3320-3330.
FRANK, H. S. & EVANS, M. W. (1945). *J. chem. Phys.*, **13**, 507–532.
FRANK, H. S. & FRANKS, F. (1968). *Ibid.*, **48**, 4746–4757.
GODDARD, E. D., SMITH, S. R. & KAO, O. (1966). *J. Colloid Inter. Sci.*, **21**, 320–330.
GOODEVE, C. F. (1939). *Trans. Faraday Soc.*, **35**, 342–358.
HANSCH, C. (1968). *Il. Farmaco, Sci. Ed.*, **23**, 293–320.
HIGUCHI, W. I., RHEE, T. O. & FLANAGAN, R. (1965). *J. pharm. Sci.*, **54**, 510–513.
KAUZMANN, W. (1959). *Adv. Protein Chem.*, **14**, 1–63.
LEMBERGER, A. P. & MCURAD, N. (1965). *J. pharm. Sci.*, **54**, 229–232.
MARSDEN, J. & SCHULMAN, J. H. (1938). *Trans. Faraday Soc.*, **34**, 748–758.
MARTIN, A. N., SWARBRICK, J. & CAMMARATA, A. (1970). *Physical Pharmacy*, 2nd edn. Philadelphia: Lea & Febiger.
MARTIN, A. R. & HERMANN, R. N. (1941). *Trans. Faraday Soc.*, **37**, 25–38.
MATIJEVIC, E. & OTTEWILL, R. H. (1958). *J. Colloid Sci.*, **13**, 242–256.
MOILLIET, J. L., COLLIE, B. & BLACK, W. (1961). *Surface Chemistry*, p. 64. London: Spon.
MOONEY, M. (1946). *J. Colloid Sci.*, **6**, 162–170.
MOORE, A. & LEMBERGER, A. P. (1963). *J. pharm. Sci.*, **52**, 223–226.
MUKERJEE, P. (1967). *Adv. Colloid Inter. Sci.*, **1**, 241–275.
MUKERJEE, P., MYSELS, K. J. & DULIN, C. I. (1958). *J. phys. Chem.*, **62**, 1390–1396.
MUKERJEE, P. & RAY, A. (1963). *Ibid.*, **67**, 190–197.
NEMETHY, G. (1967). *Angew. Chem. Internat. Edn.*, **6**, 195–206.
NEMETHY, G. & SCHERAGA, H. A. (1962). *J. phys. Chem.*, **66**, 1773–1789.
OSIPOW, L., BIRSAN, S. & SNELL, F. D. (1957). *J. Am. Oil Chem. Soc.*, **34**, 34–38.
OTTEWILL, R. H. & WATANABE, A. (1958). *Kolloid Z.*, **170**, 132–139.
REINER, M. (1961). *Deformation, strain and flow*, p. 220. London: H. K. Lewis.
RIEGELMAN, S. (1962). *Am. Perfum.*, **77**, 59–62.
ROSS, S. (1945). *J. Am. chem. Soc.*, **67**, 990–994.
SHERMAN, P. (1967). *J. Colloid Inter. Sci.*, **24**, 97–107.
SHINODA, K., NAKAGAWA, T., TAMAMUSHI, B. I. & ISEMURA, T. (1963). *Colloidal Surfactants*. New York: Academic Press.
SHOTTON, E. & DAVIS, S. S. (1968a). *J. Pharm. Pharmac.*, **20**, 439–449.
SHOTTON, E. & DAVIS, S. S. (1968b). *Ibid.*, **20**, 430–438.
SOMASUNDARIN, P., HEALY, T. W. & FUERSTENAU, D. W. (1966). *J. Colloid Inter. Sci.*, **22**, 599–605.
SPINK, J. A. (1963). *J. Colloid Sci.*, **18**, 512–525.
VERWEY, E. J. W. & OVERBEEK, J. Th. G. (1948). *Theory of Stability of Hydrophobic Colloids*, II, pp. 66–133. Amsterdam: Elsevier.
WALTERS, K. (1968). *Basic Concepts and Formulae for the Rheogoniometer*. Bognor: Sangamo Controls.
WALTERS, K. & KEMP, R. A. (1968). *Rheol. Acta*, **7**, 1–8.
WARBURTON, B. & DAVIS, S. S. (1969). *Ibid.*, **8**, 205–214.
WYMAN, J. (1936). *Chem. Rev.*, **19**, 213–239.

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The determination of residual ethylene oxide and halogenated hydrocarbon propellants in sterilized plastics

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A specific and sensitive method is described for the determination of residual ethylene oxide, dichlorodifluoromethane and trichlorofluoromethane in sterilized plastic surgical equipment. Residues are isolated from the plastics by high vacuum distillation and analysed by gas chromatography using an exponential dilution apparatus, to avoid the use of solvents. Under the conditions specified, a lower limit of 1 ppm of each component could be detected in blood giving sets of which the main plastics material was PVC. The rate of loss of each component has been studied, to indicate the necessary holding time of the material before it can be considered safe to use.

Undesirable reactions *in vivo* have been reported from the use of surgical equipment sterilized with and containing residues of ethylene oxide (Freeman & Barwell, 1960; Clarke, Davidson & Johnson, 1966). There is thus a need for a specific and sensitive method for the determination of ethylene oxide. Many commercial sterilizing gases also contain halogenated hydrocarbon propellants but as yet no residue study has been made on these.

Chemical methods proposed for residual ethylene oxide (Gunther, 1951; Critchfield & Johnson, 1957; Belman, 1963; Sawicki, Stanley & Pfaff, 1963) either suffer from lack of sensitivity and specificity or require rigorous control of experimental technique. None is adaptable to propellant determination. Gas chromatography has also been used but the methods proposed (Kulkarni, Bartak & others, 1968; Ben-Yehoshua & Krinsky, 1968; Mokeeva & Tsarfin, 1968) suffer from the difficulties associated with leaching or solution of the plastic in a suitable solvent before analysis.

This investigation has been made to determine concomitantly the rate of loss of ethylene oxide and propellants from sterilized plastic materials, so that the storage times necessary to reduce their concentration to an insignificant level may be predicted. To achieve this it was necessary to develop a method which would be adaptable to a wide range of sample sizes, eliminate the use of solvents for residue removal and preferably be capable of application to the control analysis of purchased supplies of sterilizing gas mixtures.

METHODS

The method is based on distillation under high vacuum with a distillate trap cooled in liquid nitrogen. The volatile components in the plastic are thus removed in under 30 min and trapped without the use of a solvent. They are then expanded into an evacuated "exponential gas dilution" vessel (Lovelock, 1961) and raised to atmospheric pressure. The contents of the vessel are then diluted exponentially

and samples analysed by gas chromatography at timed intervals. The peak height-time plot is compared with individual standard dilution plots of ethylene oxide, and propellants diluted in the same way. From a comparison of the time taken for sample and standard to reach an equivalent peak height using the same gas chromatography amplifier sensitivity setting, the initial concentration of residues in the plastic can be calculated.

Extraction technique

Using the apparatus shown in Fig. 1 the residues are extracted as follows: (*a*) Weigh the complete plastic sample and place in test tube A: open tap 1 to connect test tube to trapping coil. (*b*) Cool the trapping coil in liquid nitrogen and apply vacuum source (0·1 mm) to outlet (x) of tap 2. (*c*) Heat the test tube containing the sample to 130° (30 min). (*d*) Isolate trapping coil with tap 1. (*e*) With taps 3 and 4 closed to isolate the exponential dilution vessel, adjust tap 2 to evacuate this vessel. (*f*) Adjust tap 2 to connect the trapping coil to the dilution vessel isolating the vacuum source. (*g*) Replace the liquid nitrogen bath with a water bath at about 60° and allow the volatile condensate in the trap to expand into the dilution vessel. (*h*) Using tap 1 allow pressure in the dilution vessel to return to atmospheric or other fixed pressure, then close tap 1. (*i*) Isolate dilution vessel with tap 2.

At this stage the dilution vessel contains in the gaseous state and at the predetermined pressure, the volatile components from the plastic. Exponential dilution and subsequent gas chromatographic analysis may then be carried out as follows: (*k*) Simultaneously open tap 3 and connect the nitrogen inlet to the dilution vessel by

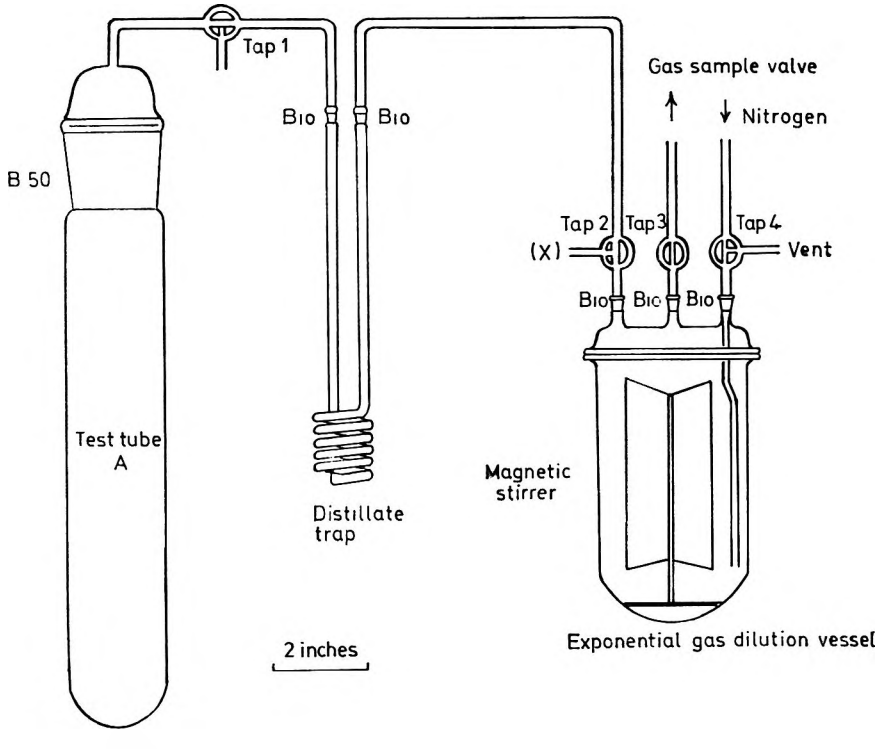

FIG. 1. Extraction apparatus (see text for description).

tap 4. (*l*) Sample the dilution vessel effluent at timed intervals with a gas sampling valve attached to the gas chromatograph. (*m*) Record peak heights, time of injection and sensitivity setting for each peak.

Standardization

Known concentrations of the individual standards are prepared in an exponential dilution vessel either by volume or by weight. Although standards may be prepared by volume using gas-tight syringes, correction must be made for deviation from N.T.P. Standards prepared by weight are more convenient but their initial preparation requires careful experimental technique. Individual components are sealed into preweighed lengths of capillary tubing cooled in liquid nitrogen and reweighed. The weighed tube is then placed in a sampling tube, and the exponential dilution flask evacuated. The sealed capillary is broken in a suitable manner, allowing the sample to vaporize into the dilution vessel, which is then raised to atmospheric pressure.

Calculation

Lovelock (1961) and Williams & Winefordner (1966) have previously demonstrated the exponential dilution of permanent gases in the type of dilution vessel used. The dilution follows the relation:

$$C = C_o \exp -\frac{Ut}{V} \quad \text{or} \quad 2{\cdot}303 \log C = 2{\cdot}303 \log C_o - \frac{Ut}{V}$$

where V = volume of flask (cc). U = flow rate of diluting gas (cc/min). t = time (min). C_o = initial concentration of sample gas. C = concentration at any time t. Therefore a plot of C versus time will give a slope of $-U/V$ and intercept of 2·303 Log C_o.

Since the major contribution to non linearity in the system probably comes from the gas chromatographic detector response and amplifier, it is preferable to compare the times taken for sample and standard to reach an equivalent peak height on identical amplifier sensitivity ranges. Then for both sample and standard, equation (1) applies.

$$\log_e C = \log_e C_o - \frac{Ut}{V} \qquad \ldots \qquad \ldots \qquad \ldots \qquad (1)$$

When peak heights for both sample (sm) and standard (Std) are equal, then

$$\log_e C_o\,(\text{sm}) - \frac{Ut}{V}\,(\text{sm}) = \log_e C_o\,(\text{std}) - \frac{Ut}{V}\,(\text{std})$$

$$\therefore \log_e C_o\,(\text{sm}) = \log_e C_o\,(\text{std}) - \frac{U}{V}\,(t_{std} - t_{sm})$$

or

$$\log_{10} C_o\,(\text{sm}) = \log_{10} C_o\,(\text{std}) - \frac{U(t_{std} - t_{sm})}{2{\cdot}303\ V} \qquad \ldots \qquad \ldots \qquad (2)$$

From equation (2), it is possible to calculate the initial concentration of 'residual' gas in the dilution vessel and hence the amount present in the original plastic sample.

Equipment. Pye 104 gas chromatograph equipped with flame ionization detector. 5 ft × 4 mm i.d. glass column packed with Phasepak Q (Phase Separations Ltd); column temperature: 150° isothermal; carrier gas: nitrogen 80 ml/min; sample injection: Pye gas sampling valve fitted with 0·14 ml sample loop.

Reagents. Ethylene oxide supplied by BDH. Dichlorodifluoromethane and trichlorofluoromethane supplied by ICI.

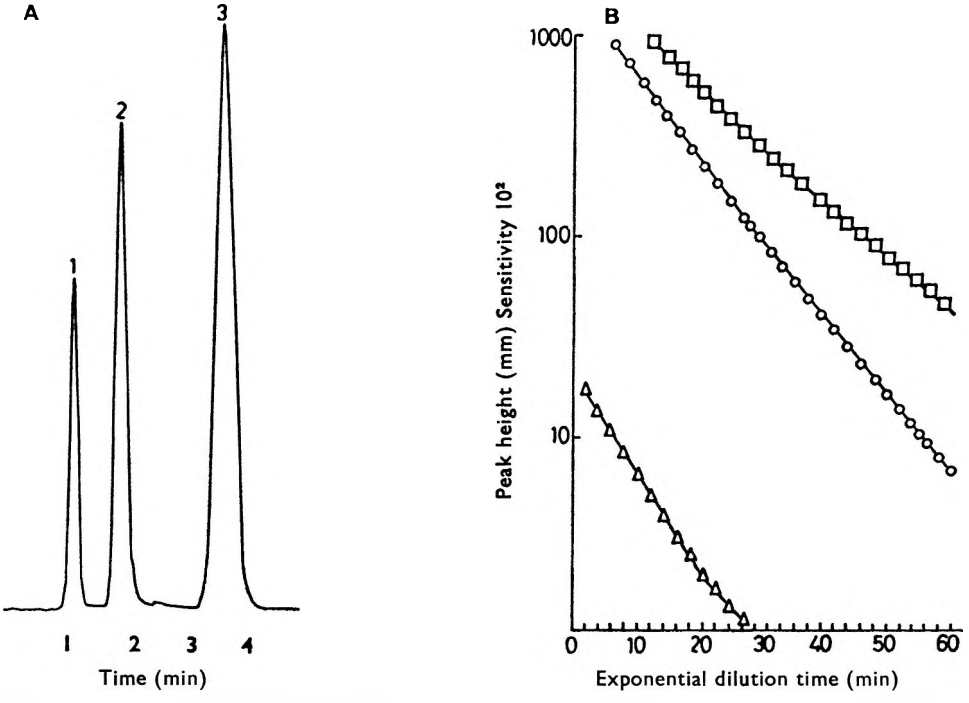

FIG. 2. A. A typical gas chromatographic separation of dichlorodifluoromethane (1), ethylene oxide (2), and trichlorofluoromethane (3).

B. Graph of log peak heights against time. △ Trichlorofluoromethane. □ Dichlorodifluoromethane. ○ Ethylene oxide.

RESULTS

Separation of propellants and ethylene oxide

Fig. 2A shows the separation achieved under the conditions stated. A porous polymer column with no liquid phase was chosen for this separation to achieve greater stability on high sensitivity; different conditions may be necessary for other propellants.

Linearity of the gas chromatographic system to exponential dilution

The complete amplifier range was checked for response to ethylene oxide, dichlorodifluoromethane and trichlorofluoromethane during exponential dilution from 0·33% v/v to the minimum detectable level. The graph of log peak height *vs* time shows a series of straight lines for each range step on the amplifier; Fig. 2B shows a typical example. When the amplifier range is changed the slope of this line changes to a new value. Hence, it is important to measure peak heights of sample and standard on identical ranges.

Effect of extraction time and temperature

Treatment at 130° (30 min) was found satisfactory for distillation of ethylene oxide and propellants from the PVC of blood transfusion equipment. Temperatures in excess of 150° tended to cause pyrolysis of the PVC leading to numerous additional chromatographic peaks.

Study of residue content of sterilized equipment

Samples of the blood transfusion equipment sterilized in a 200 ft^3 water jacketed sterilizer, were stored at room temperature under normal warehouse conditions and examined periodically by this technique to determine the rate of loss of ethylene oxide and propellants. The sterilization cycle was at 135° F ± 5° F. The chamber pressure was reduced by 12·77 p.s.i. and the chamber humidified for 1 h at a relative humidity in the range 50–65%. The sterilizing gas consisting of ethylene oxide 11% w/w, dichlorodifluoromethane 35% w/w and trichlorofluoromethane 54% w/w was then admitted to a pressure of 14 p.s.i. and maintained for 5 h, after which an air flushing cycle of 2 h was used before removal of samples.

The blood giving sets consisted mainly of PVC with some parts in high and low density polyethylene, nylon, polycarbonate, modified acrylics and latex rubber. The sets were stored in their normal cardboard trays and sleeves. No plastic covering is used in this packaging. For each estimation a complete blood set weighing about 40 g was extracted. Fig. 3 shows the residue levels obtained over 42 days. After this period the levels of ethylene oxide and dichlorodifluoromethane fell below 1 ppm whereas trichlorofluoromethane was still present to the extent of about 5 ppm.

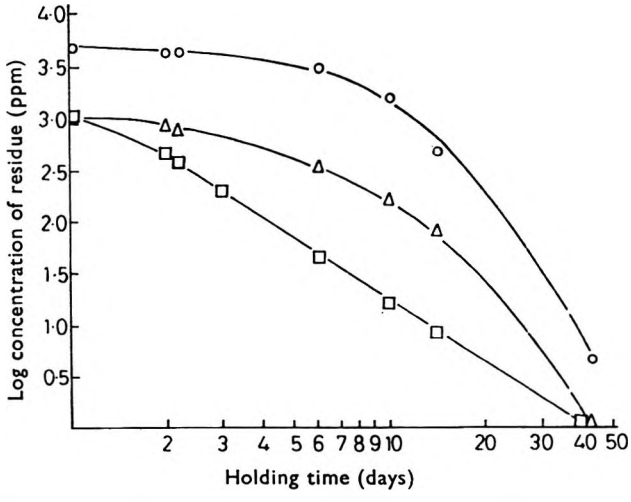

FIG. 3. Residue concentration with holding time of plastic blood giving sets. ○ Trichlorofluoromethane. △ Dichlorodifluoromethane. □ Ethylene oxide.

DISCUSSION

The use of vacuum distillation for removal of residues in an analysis of this kind has many advantages over solution techniques which require solvents of high purity and suffer severe limitations in the sample to solvent ratios that can be used. The use of an exponential dilution system enables a number of chromatograms to be

obtained from a single sample so that suitable sensitivity settings can be obtained as the dilution proceeds. Extension of this method to the analysis of sterilizing gas mixtures can be made by sealing samples of the liquified mixture into capillaries as detailed under the section on standardization. An exponential dilution plot of the constituents of this mixture can then be compared to standard dilution plots. With little modification the method may be used for other volatile residues or extended below the lower limit of 1 ppm described in this paper.

REFERENCES

BELMAN, S. (1963). *Analytica chim. Acta*, **29**, 120–126.

BEN-YEHOSHUA, S. & KRINSKY, P. (1968). *J. Gas Chromat.*, **6**, 350–351.

CLARKE, C. P., DAVIDSON, W. L. & JOHNSON, J. B. (1966). *Aust. N.Z. J. Surg.*, **36**, 53–56.

CRITCHFIELD, F. E. & JOHNSON, J. B. (1957). *Analyt. Chem.*, **29**, 797–800.

FREEMAN, M. A. R. & BARWELL, C. F. (1960). *J. Hyg., Camb.*, **58**, 337–345.

GUNTHER, F. A. (1965). *Analyt. Chem.*, **37**, 1172–1173.

KULKARNI, R. K., BARTAK, D., OUSTERHOUT, D. K. & LEONARD, F. (1968). *J. biomed. Mater. Res.*, **2**, 165–171.

LOVELOCK, J. E. (1961). *Analyt. Chem.*, **33**, 162–178.

MOKEEVA, R. N. & TSARFIN, Ya. A. (1968). *Plast. Massy*, **9**, 60–61.

SAWICKI, E., STANLEY, T. W. & PFAFF, J. (1963). *Analytica chim. Acta*, **28**, 156–163.

WILLIAMS, H. P. & WINEFORDNER, J. D. (1966). *J. Gas Chromat.*, **4**, 271–272.

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A polarographic method for the determination of flurandrenolone

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A simple fast scan polarographic method has been developed for the determination of the Δ^4-3 ketosteroid flurandrenolone in pharmaceutical formulations. The polarographic peak current due to steroid reduction is measured after ointments have been subjected to a preliminary extraction procedure and creams have been treated with tannic acid to precipitate interfering excipients. The method enables concentrations down to 0·01% w/w of the steroid to be measured. Neomycin sulphate does not interfere and clioquinol is readily removed using an ion exchange resin. Evidence is presented to show that the carbon-fluorine bond rather than the enone is primarily reduced. The method is faster than a colorimetric method using tetrazolium blue and has a similar accuracy and precision.

Flurandrenolone, 6α-fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione-16,17-acetonide is used for the topical treatment of local inflammatory conditions and because of the low concentrations employed in formulations, previously described analytical methods meet problems owing to interference from excipients. Lengthy extraction or chromatographic procedures are often necessary to remove this interference (Jakovljevic, Hartsaw & Drummond, 1965; Bailey, Holbrook & Miller, 1966; Görög, 1968).

The polarographic behaviour of Δ^4-3-ketosteroids has been well documented (Milner, 1957; Brezina & Zuman, 1958; Kabasakalian & McGlotten, 1962; Cohen, 1963; Zuman, 1967), but there are few references to the use of this technique in the quantitative analysis of formulated products. Gantés & Juhasz (1966) have described a polarographic method for the determination of hydrocortisone in ointments, but their method applies only to concentrations much in excess of those likely to be encountered using fluorinated steroids.

MATERIALS AND METHODS

Apparatus

Because of the low levels of fluorinated steroid involved, a cathode ray polarograph type A1660 manufactured by Southern Analytical Ltd., Camberley, Surrey, was used. Two synchronized dropping mercury electrodes (subtractive polarography) were found necessary for the determination of flurandrenolone in creams. In all cases measurements were made with a mercury pool as the reference electrode.

Reagents

Unless otherwise stated, all chemicals are of analytical reagent grade.

n-Heptane—M & B laboratory reagent. 50% v/v aqueous methanol containing 0·1% v/v concentrated hydrochloric acid. 50% v/v aqueous methanol containing 10% tannic acid (B.D.H. laboratory reagent).

† Present address: Hewlett Packard S.A., No. 7 rue du Bois du Lan, 1217 Meyrin, No. 2 Switzerland.

Supporting electrolyte. 50% aqueous methanol 0·5M with respect to potassium chloride and M with respect to hydrochloric acid.

Stock standard solution. Accurately weigh about 50 mg of flurandrenolone analytical standard and dissolve in 50·0 ml of methanol.

Working standards 1 and 2. Solutions containing 0·1 mg and 0·2 mg/ml of flurandrenolone (analytical standard) in 50% v/v aqueous methanol.

Preparation of samples for polarography

Ointments containing 0·0125% w/w of flurandrenolone. Disperse the ointment (4·0 g) in n-heptane at about 40° and extract the flurandrenolone with 20 ml, then 15 ml and finally 5 ml methanol-hydrochloric acid (see reagents): add supporting electrolyte (5·0 ml) and dilute to 50·0 ml with methanol-hydrochloric acid.

To 20·0 ml of the above solution add working standard 1 (2·0 ml).

Ointments containing 0·05% w/w of flurandrenolone. These are prepared as above but a 2·0 g sample of ointment is used and working standard 2 (2·0 ml) added to the final extract.

Creams containing 0·0125% w/w flurandrenolone. Dissolve 8·0 g of cream sample in a stoppered erlenmeyer flask containing methanol (25 ml) at 50°, add water (20 ml) mix well and cool to room temperature. Add tannic acid solution (10 ml) and shake vigorously (1 min). Add celite 545 (filter aid) (4 g) and again shake then filter the sample through a porosity 3 sintered funnel (low vacuum) into a 100 ml volumetric flask containing supporting electrolyte (10·0 ml). Wash through and dilute to volume with several small amounts of 50% v/v aqueous methanol.

Using 8·0 g of cream sample without steroid prepare a blank solution in an identical manner.

To the sample solution (20 ml), add 2·0 ml of working standard 1. This is the sample plus standard solution.

Creams containing 0·05% w/w flurandrenolone. The method is as for the 0·0125% cream but only 4·0 g of sample and blank are used with working standard 2 (2·0 ml).

Polarography of samples

Ointments. Polarograph the solutions of sample alone and sample plus standard at 20 ± 0·1° and determine the flurandrenolone peak currents at about −0·9 V for both solutions. All solutions should be degassed with oxygen-free nitrogen (saturated with 50% aqueous methanol).

Creams. Place the sample solution and blank solution under each of two synchronized dropping mercury electrodes. Polarograph and determine the flurandrenolone peak current at about −1·0 V with the instrument in the subtractive mode. Determine the sample plus standard peak current in the same manner.

$$\% \text{ flurandrenolone} = \frac{\text{Pa}}{1{\cdot}1\ \text{Pas} - \text{Pa}} \times \frac{\text{Ws}}{\text{Wa}} \times \text{F}$$

Where Pa = peak current of sample; Pas = peak current of sample plus standard; Ws = weight of standard (mg) in 50 ml of stock standard; Wa = weight of sample (mg); F = dilution factor = 1 for 0·0125% ointments, 2 for 0·0125% creams and 0·05% ointments and 4 for 0·05% creams.

RESULTS

The results obtained using the polarographic procedures for the analysis of cream and ointment samples, together with comparative results obtained by the colorimetric method using tetrazolium blue are summarized in Table 1.

Table 1. *Results for the analysis of cream and ointment samples using the polarographic and colorimetric assays.*

Flurandrenolone content claimed (mg/g)	No. of determinations	Flurandrenolone found (mg/g)* Polarographic assay	Colorimetric assay
Ointment (0·5)	12	0·515 ± 0·005	0·517 ± 0·010
Ointment (0·125)	12	0·130 ± 0·003	0·127 ± 0·005
Cream (0·5)	9	0·482 ± 0·007	0·484 ± 0·008
Cream (0·125)	12	0·125 ± 0·004	0·125 ± 0·003

* Average ± standard deviation.

DISCUSSION

Cathode ray polarography shows that flurandrenolone in 50% aqueous methanol is reduced in a variety of buffered systems having pH values within the range of 1–11. In acid solutions the major reduction peak is sharp and well defined but becomes broader at higher pH values indicating a slower reduction. The most satisfactory polarograms are obtained at pH 1 using a supporting electrolyte of potassium chloride and hydrochloric acid. At this pH, the flurandrenolone peak occurs at −0·79 V versus the mercury pool anode and is most probably due to reduction of the fluorine atom rather than to the enone system in ring A. This was shown by controlled potential electrolysis (−0·8 V for 48 h) after which the flurandrenolone solution still retained the typical absorption of an $\alpha\beta$-unsaturated ketone in λ_{max} from 238 nm to 242 nm. The infrared spectrum (KBr disc) of the residue obtained from this experiment shows little change in the carbonyl stretching region (1700 cm^{-1}) but an absence of the C–F stretching frequency present in flurandrenolone itself. Reduction of the carbonyl in addition to the C–F group can be accomplished by working with a more negative potential (−1·22 V) at which a second poorly defined reduction peak occurs. At this potential a large drop in absorbance of the ≈240 nm band is observed.

Calibration graphs of flurandrenolone at concentrations from 0 to 100 ppm against peak current on the cathode ray tube show deviation from linearity above 20 ppm. This has been tentatively ascribed to adsorption of the flurandrenolone on the surface of the mercury cathode drop (McIver & Rooney, 1962). Such adsorption effects, common in organic polarography, can be prevented by the addition of a small amount of a non-ionic surface-active agent to the solution to be electrolysed.

In flurandrenolone ointments which do not contain surfactants, therefore, the polarographic peak current at −0·79 V for such ointment samples is best compared directly with that of a standard in the region of linearity (curve A, Fig. 1).

On the other hand, where creams and ointments do contain non-ionic surface-active agents, calibration graphs show linearity up to at least 100 ppm of flurandrenolone. Some of the surface active agent is carried through in the method of analysis to the final solution to be electrolysed and probably prevents adsorption

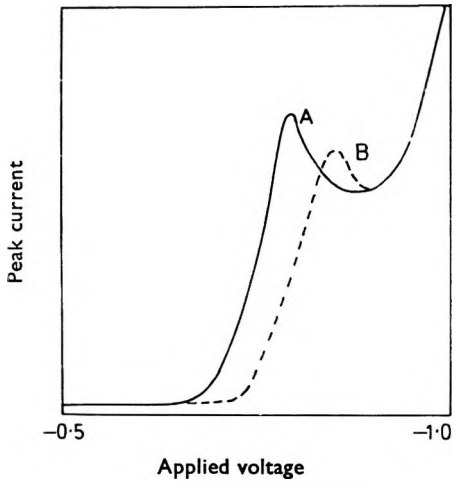

FIG. 1. Polarographic peak displayed by flurandrenolone after extraction from an ointment not containing surface-active agents (curve A), and one containing surface-active agents (curve B).

effects from taking place. In ointments of this type, however, the presence of non-ionic surface active agent, and possibly other excipient, in the final solution to be electrolysed, results in a shift of peak potential for flurandrenolone from −0·79 V to approximately −0·88 V. As no adsorption of flurandrenolone occurs on the mercury drop surface, a reduction in peak current is also apparent (curve B, Fig. 1). These two effects make the direct comparison of sample and standard peak current values impossible and the technique of standard addition to the sample may be adopted.

The non-ionic surface active agents (and possibly other excipients) in creams, which remain in solution after tannic acid precipitation, shift the peak potential for flurandrenolone very close to that of the hydrogen ion reduction wave (Fig. 2, curve

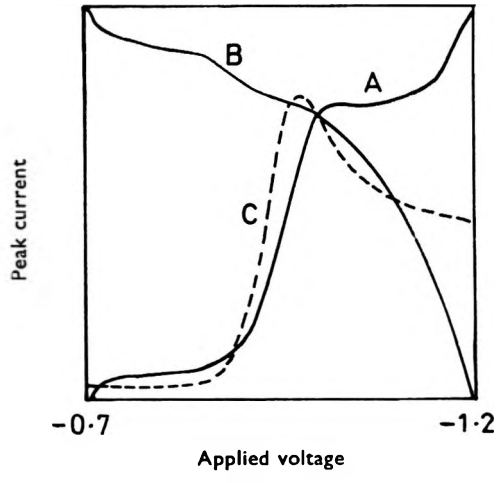

FIG. 2. Curve A—single wave displayed for flurandrenolone after extraction from cream excipients. Curve B—inverted single cell wave displayed by a solution resulting from treating a blank cream in the same manner as the sample. Curve C—the resultant wave obtained by subtraction of B from A.

A). Although the flurandrenolone peak and hydrogen wave can be resolved using derivative polarography, great loss in instrument sensitivity occurs and a more satisfactory result is achieved using subtractive polarography. Fig. 2, curve C shows the resultant wave which is the subtraction of the wave for the blank solution (Fig. 2, curve B) from the wave for the sample solution. Standard readings for creams are obtained using the technique of standard addition to the sample.

Neomycin sulphate which may be present in some formulations only gives a reduction wave in 50% v/v aqueous methanol when the pH is greater than 3. Consequently, no interference occurs due to the presence of this antibiotic under the conditions for determining flurandrenolone.

Clioquinol gives a reduction peak on the polarogram which completely masks that of flurandrenolone. The interference can be overcome by removal of the clioquinol from 50% v/v aqueous methanol solutions using Dowex 50W-X12 (a sulphonated ion exchange resin).

Comparison of the polarographic with the colorimetric assay (tetrazolium blue) shows that the accuracy and precision is similar in both (Table 1). The main advantage of the polarographic method lies in the speed at which a particular determination can be made. This makes it particularly suitable for the rapid checking of content and homogeneity of production samples.

Acknowledgements

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REFERENCES

BAILEY, F., HOLBROOK, A. & MILLER, R. J. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 125–165.

BREZINA, M. & ZUMAN, P. (1958). *Polarography in Medicine, Biochemistry, and Pharmacy*, p. 434. New York: Interscience.

COHEN, A. I. (1963). *Analyt. Chem.*, **35**, 128–131.

GANTÉS, P. & JUHASZ, J. P. (1966). *Annls Pharm. Fr.*, **24**, 687–692.

GÖRÖG, S. (1968). *J. pharm. Sci.*, **57**, 1737–1741.

JAKOVLJEVIC, I. M., HARTSAW, P. E. & DRUMMOND, G. E. (1965). *Ibid.*, **54**, 1771–1773.

KABASAKALIAN, P. & MCGLOTTEN, J. (1962). *Analyt. Chem.*, **34**, 1440–1442.

MCIVER, P. J. & ROONEY, R. C. (1962). *Analyst*, **87**, 895–899.

MILNER, G. W. C. (1957). *The Principles and Applications of Polarography*, pp. 609–615. London, New York: Longmans Green.

ZUMAN, P. (1967). *Substituent Effects in Organic Polarography*, pp. 312–365. New York: Plenum Press.

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Digital filters in the evaluation of titration curves

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The shape of a titration curve contains useful information, which may be extracted in terms of orthogonal polynomial coefficients. In this way, it is possible to (i) distinguish a monobasic acid from a polybasic acid, having groups of closely similar strength; (ii) detect acid-base impurities in acids and bases, and (iii) study medium effects. The choice of polynomial, range, number of points and experimental conditions are discussed. Using twelve point polynomials and equipment of modest performance, the P_I coefficient (for the central half of a monobasic acid titration curve) can be measured with a relative standard deviation of 0·58.

Instrumental methods have led to a substantial increase in the quantity of numerical data, which may be ascertained for a given sample. Whereas a pair of numbers (e.g. the volume of acid required to neutralize a given volume of sample) emerges from each gravimetric or volumetric assay, a mathematical *function* (e.g. a graph of pH against volume) may emerge from an instrumental method, like potentiometric titration. Furthermore, when extracting a maximum of chemical information, a function necessarily requires more complicated arithmetic than does a pair of numbers. Hence, when dealing with a function, the use of simple arithmetic, entirely adequate to gravimetric or volumetric results, will normally lead to much loss of information. Such a loss is bound to occur, in fact, if the analyst selects a maximum or minimum value from an instrumental curve and ignores all other values, when calculating the result. This loss is wholly concerned with the shape of the function and so certain principles of curve fitting (Draper & Smith, 1966) are bound to play an important role in any attempt to increase the yield of information. Nevertheless, we must emphasize that, *despite the use of curve fitting arithmetic, the present work is not concerned with curve fitting in the usual sense.*

PRINCIPLES OF CURVE FITTING

(a) *Summation of curves*

In Fig. 1a, f(v) is a function of the abscissa variable, v, and is reproduced by summing the three functions in Fig. 1b. Thus, for a given abscissa value, v_k, we can write:

$$f(v_k) = A_0(v_k) + A_1(v_k) + A_2(v_k) \quad .. \quad .. \quad .. \quad (1)$$

This means that the value, $f(v_k)$, in Fig. 1a is obtained by taking a sum of the values ($A_0(v_k)$, $A_1(v_k)$ and $A_2(v_k)$), read from the three curves in Fig. 1b (all these ordinate values referring to the same abscissa value, v_k). By repeating this process at many points on the abscissa, we could construct the entire curve, f(v), by summing

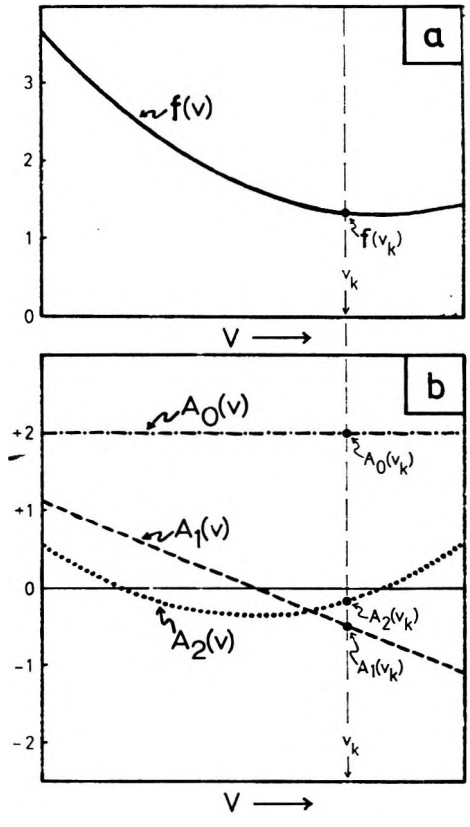

FIG. 1. Summation of component curves.

the three curves ($A_0(v)$, $A_1(v)$ and $A_2(v)$) in Fig. 1b. Hence, in more general terms, equation (1) becomes

$$f(v) = A_0(v) + A_1(v) + A_2(v) \quad .. \quad .. \quad .. \quad (2)$$

where $A_0(v)$, $A_1(v)$ and $A_2(v)$ are *mathematical components* of the function, f(v), and equation (2) is a mathematical *model* thereof.

These mathematical components evidently determine the shape of the curve f(v). Thus, if $A_2(v)$ were absent, f(v) would become a straight line. In a similar way, the overall slope of f(v) depends upon the component, $A_1(v)$. Moreover, we can claim to have *fitted* the curve, f(v), as soon as its mathematical components have been calculated by the method detailed below and tested for statistical significance, where necessary.

(b) *Standard mathematical components*

The above exercise would be trivial if the mathematical components were arbitrary and so curve fitting always proceeds on a basis of particular mathematical functions, which in the present context, may be regarded as no more than a set of standard shapes. For most experimental purposes, orthogonal polynomials (Fig. 2) afford the most useful set (Milne, 1949; Davies, 1958; Buckingham, 1962).

The curves in Fig. 2 were constructed from tables of orthogonal polynomials (Fisher & Yates, 1957). These give ordinate values of the polynomial at a specified

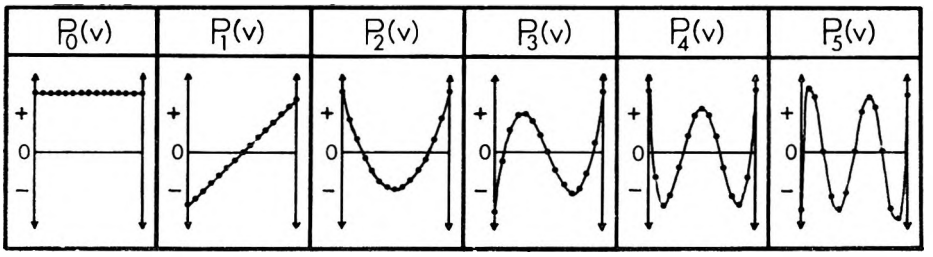

FIG. 2. Orthogonal polynomials. In each diagram, $P_j(v)$ is plotted against a set of *equally spaced* abscissa values, $v_0, v_1, v_2 \ldots . v_n$, the same set being used in all six diagrams. Each set of points represents a fundamental curve shape, whose general characteristics are indicated by the line which joins them.

number of points, equally spaced on a generalized abscissa scale ("v" in this paper); for example, one such table gives $P_1(v)$ for 12 equally spaced values of v. For any given polynomial, the number of points varies from table to table and, in the present case, Fig. 2 was constructed from "15 point polynomials".

We can now reveal that the three curves in Fig. 1b were obtained by using coefficients (p_0, p_1 and p_2) to adjust the scale of the curves, $P_0(v)$, $P_1(v)$ and $P_2(v)$, in Fig. 2. Thus,

$$A_0(v) = p_0P_0(v),\ A_1(v) = p_1P_1(v) \text{ and } A_2(v) = p_2P_2(v)$$

so that

$$f(v) = p_0P_0(v) + p_1P_1(v) + p_2P_2(v) \quad .. \quad .. \quad .. \quad (3)$$

The shape of f(v) is thus determined by the magnitudes of coefficients p_0, p_1 and p_2: if f(v) were more curved, p_2 would be greater, whilst if it possessed no overall slope, p_1 would be zero.

(c) *Calculation of coefficients*

Calculation of the mathematical components of f(v) is greatly simplified by the use of polynomials, which are orthogonal. Thus, to obtain coefficients of 12 point polynomials for the curve in Fig. 1a, it is only necessary to:

(i) note the ordinate values of f(v) at 12 *equally spaced* points on the abscissa (v).

(ii) multiply each ordinate value by the appropriate integer in the Table of orthogonal polynomials (Fisher & Yates, 1957, see Table 1) (the first ordinate value times the first integer in the Table, the second ordinate value times the second integer in the Table, and so on).

(iii) sum the products obtained in (ii) and then divide by the normalizing factor, given at the bottom of the Table (and in brackets in Table 1).

Such a calculation is set out in Table 1, wherein the data (used to plot Fig. 1a) appears in the first two columns, 12 point polynomials in the next four columns and the necessary products in the last four columns.

Each product is obtained by multiplying the value of f(v) in the same row by the appropriate value of the polynomial, also in the same row. For example, in the first row, the product, $-40{\cdot}15$, is equal to $3{\cdot}65 \times (-11)$.

In clarifying the arithmetic, Table 1 gives a false impression of the real arithmetical labour. An electric desk calculator, which is suitable for statistical work, sums the products automatically, whilst with a desk top computer, values of the orthogonal polynomials can often form part of the program, in which case, it is only necessary

for the operator to insert the correct program and enter the values of f(v) in numerical order.

Table 1. *Calculation of orthogonal polynomial coefficients.*

Data		Orthogonal Polynomials				Products			
v	f(v)	$P_0(v)$	$P_1(v)$	$P_2(v)$	$P_3(v)$	$f(v)P_0(v)$	$f(v)P_1(v)$	$f(v)P_2(v)$	$f(v)P_3(v)$
0·25	3·65	+1	—11	+55	—33	+3·65	—40·15	+200·75	—120·45
0·50	3·15	+1	—9	+25	+3	+3·15	—28·35	+78·75	+9·45
0·75	2·71	+1	—7	+1	+21	+2·71	—18·97	+2·71	+56·91
1·00	2·33	+1	—5	—17	+25	+2·33	—11·65	—39·61	+58·25
1·25	2·01	+1	—3	—29	+19	+2·01	—6·03	—58·29	+38·19
1·50	1·75	+1	—1	—35	+7	+1·75	—1·75	—61·25	+12·25
1·75	1·55	+1	+1	—35	—7	+1·55	+1·55	—54·25	—10·85
2·00	1·41	+1	+3	—29	—19	+1·41	+4·23	—40·89	—26·79
2·25	1·33	+1	+5	—17	—25	+1·33	+6·65	—22·61	—33·25
2·50	1·31	+1	+7	+1	—21	+1·31	+9·17	+1·31	—27·51
2·75	1·35	+1	+9	+25	—3	+1·35	+12·15	+33·75	—4·05
3·00	1·45	+1	+11	+55	+33	+1·45	+15·95	+79·75	+47·85
		(12)	(572)	(12,012)	(5148)				
Sums of products						+24·00	—57·20	+120·12	0·00
Orthogonal polynomial coefficients						2·00	—0·10	+0·01	0·00

From the coefficients at the bottom of Table 1, the curve in Fig. 1a evidently contains constant (P_0), linear (P_1) and quadratic (P_2) components but no cubic (P_3) component.

The values of f(v), used to plot the curve in Fig. 1a and provide data for Table 1, were deliberately chosen to obtain an exact fit from equation (3). Thus, on substituting the above coefficients, equation (3) becomes

for v = 0·25,

$$[(2{\cdot}00) \times (+1)] + [(-0{\cdot}10) \times (-11)] + [(+0{\cdot}01) \times (+55)] = 3{\cdot}65 = f(0{\cdot}25)$$

for v = 0·50,

$$[(2{\cdot}00) \times (+1)] + [(-0{\cdot}10) \times (-9)] + [(+0{\cdot}01) \times (+25)] = 3{\cdot}15 = f(0{\cdot}50)$$

for v = 0·75,

$$[(2{\cdot}00) \times (+1)] + [(-0{\cdot}10) \times (-7)] + [(+0{\cdot}01) \times (+1)] = 2{\cdot}71 = f(0{\cdot}75)$$

For each abscissa point, these expressions calculate the values of $A_0(v)$, $A_1(v)$ and $A_2(v)$ in Fig. 1b and sum them to obtain the corresponding value of f(v) in Fig. 1a. Such an exact fit is most unlikely to occur in practice and, if f(v) were derived from experiment, observational errors would almost certainly lead to a non-zero value of p_3. Hence, some coefficients are devoid of statistical significance (Davies, 1958).

(d) *Simplicity of calculation*

From the above example, it is evident that, if measurements are equally spaced on the abscissa scale, it is very easy to specify the shape of an experimental curve by calculating one or more orthogonal polynomial coefficients. In other words, it is very easy to extract most of the information, contained in an experimental function.

DIGITAL FILTERING

With increased availability of computers, the need to extract more information from instrumental techniques has been increasingly recognized (Fenwick, 1932; Gran,

1952; Feates & Ives, 1956; Sternberg, Stillo & others, 1960; Jones, Seshadri & others, 1963; Katakis, 1965; Fraser & Suzuki, 1966; Wernimont, 1967; Westerberg, 1969; Anderson, Gibb & Littlewood, 1970).

The present paper follows earlier work (Glenn, 1963; Glenn, 1967; Agwu & Glenn, 1967) in using a technique, more recently known as *digital filtering* (Blackburn, 1969) which is no more than a computer analogue of the communication engineer's well known technique of electrical filtering. In digital filtering, electrical filters are replaced by the arithmetic of curve fitting, but, as we shall argue below, digital filters should not be restricted to the usual objectives of curve fitting, for in the hands of communications engineers, electrical filters have been used with greater imagination!

The aim of filtering, whether electrical or digital, is to reject some part of the experimental function and so obtain a more reliable and useful conclusion. A pertinent analogy would concern a communications engineer asked to measure the intensity of sound emitted by a whistle, when blown at a busy traffic junction during the rush hour. In these circumstances, a huge positive error would emerge from a straightforward measurement of the gross intensity of sound and so special apparatus would be essential.

Fig. 3 shows the kind of apparatus, which would be suitable for a whistle, having a fundamental of 4KHz. The microphone and amplifier convert the total sound input to a measurable electrical signal, having a complicated waveform (A). This signal then passes through a 4KHz filter, when all frequencies which lie outside the passband of the filter are absorbed and only a small range of frequencies (B) are allowed to reach the A.C. meter. After suitable calibration, the latter would give a greatly improved estimate of the desired sound intensity.

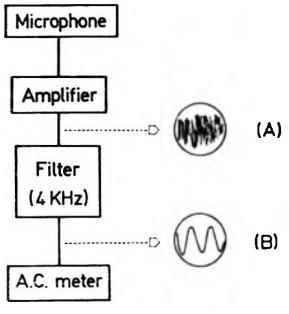

FIG. 3. Isolation of a signal from a noisy background.

The ability of the filter to reject unwanted sound is inversely related to the width of its passband; that is, assuming average conditions, wherein the unwanted sound is well distributed throughout the frequency range, 0–15 KHz (serious error might, however, result from a small boy with a similar whistle!).

The characteristic sound of a whistle resides more in its harmonics than in its fundamental frequency. Hence, if the filtered signal were applied to a loudspeaker instead of the A.C. meter, a somewhat characterless note would emerge. Nevertheless, the use of additional filters (at 8KHz, 12KHz and 16KHz) to measure the intensity of these harmonics (and so extract the full character of the whistle) would multiply the risk of interference from unwanted sound. In other words, additional filters would merely provide additional channels, through which the unwanted sound

could affect the meter. On average, it is unwise to use more than one channel in this kind of application.

The above example demonstrates two points:

(1) For some purposes, an experimental function can be adequately represented by only one of its mathematical components. This applies to the above example, in which the whistle was adequately represented by its 4KHz component. It also applies to the present work, wherein the central half of a titration curve is represented by just one component, other statistically significant components being unnecessary.

(2) For some purposes, it may be very unwise to represent an experimental function by *more than* one of its mathematical components. This applies to problems of background interference, as in the above example and also in spectrophotometric analysis (Wright, 1941; Glenn, 1963). Nevertheless, although relevant to the general problem of digital filtering, this point does not apply to the present analysis of titration curves, wherein the use of one component is a matter of convenience rather than necessity.

These two points justify an earlier statement, namely that, despite the use of curve fitting arithmetic, *the present work is not concerned with curve fitting in the usual sense.*

Curve fitting is usually concerned with *faithful models* of experimental functions and is mainly directed towards the elimination of observational error. Such an approach is equivalent to a communications engineer's use of a low pass filter to remove high frequency electrical noise, which often represents a high proportion of the total error of observation. Nevertheless, electrical filters can be used in other ways (p. 185S) and the same is also true of digital filters (or curve fitting arithmetic).

As in earlier work, this paper is concerned with useful *working models* of experimental functions and, in deriving such models, a number of statistically significant mathematical components are usually omitted. In consequence, one of our working models may differ very greatly from the corresponding faithful model, but this may be turned to advantage in some cases (p. 185S) and may constitute no material disadvantage in others.

In spectrophotometric analysis, the well known "base-line" method (Mulder, Spruit & Keuning, 1963) is an apt example of digital filtering, even as a purely graphical operation. It extracts the quadratic (P_2) component and rejects the constant (P_0) and linear (P_1) components of the absorption curve. For assay purposes, the quadratic component is a good working model of the compound's absorption curve.

Information obtainable from the shape of a titration curve

It is common practice to ignore the shape of a titration curve and confine attention to one or two abscissa points, such as the equivalence point and pH at half neutralisation, in the case of a monobasic acid. Although the shapes of small segments of a titration curve are important to the evaluation of equivalence points by derivative methods, the latter can hardly be said to evaluate shape in the present context.

By evaluating the shape of a titration curve, or a substantial segment thereof, we take account of more data than hitherto and, therefore expect an improvement, either in the quality or quantity of information produced. This is certainly true of a derivative method, which takes account of an appreciable segment of the curve (Fenwick, 1932). Moreover, in the authors' experience, the accuracy of a poorly defined equivalence point is greatly improved when the derivative is obtained by a

convolution process (Savitzky & Golay, 1964), which spans a reasonably large segment of the curve.

The scope of potentiometric titration is currently limited by the fact that even Savitzky & Golay's approach has difficulty in detecting an equivalence point between two stages of neutralization, which are associated with a difference of less than 2 units in pK_a. Nevertheless, from results obtained in the present work, evaluation of orthogonal polynomial coefficients would seem to offer good prospects for tackling this kind of difficulty in cases where the two stages can be separately characterized. Thus, even in the case of a mixture of two monobasic acids, which differ by as little as 0·5 unit in pK_a, the P_1 coefficient is still fairly sensitive to the composition of the mixture.

By evaluating p_1 for a substantial segment, it is relatively easy to distinguish between the curve of a monobasic acid and a curve which relates to a mixture of monobasic acids or to the overlapping stages of a polybasic acid. Such an approach may, therefore, be useful to investigations of molecular structure, where knowledge of the number of individual acid-base groups in a molecule is not only valuable in its own right, but is also essential to the calculation of a molecular weight from a titration curve. The same approach may also prove useful to the quality control of acid-base solutions, particularly in view of results obtained in the present work.

For quantifying solvent effects, acid-base interactions and association (King, 1965), there is much to be said for the use of orthogonal polynomial coefficients, based upon a substantial segment of the curve. In this respect, an accurate potentiometric titration constitutes a rapid method for detecting and quantifying the non-ideal behaviour of an acid-base system (see p. 192S).

When using a given polynomial (e.g. P_1) to quantify such effects,

$$p_1(\text{effect}) = p_1(\text{observed}) - p_1(\text{theoretical})$$

where p_1(effect) is a measure of non-ideal behaviour and p_1(theoretical) is obtained by substituting species concentrations into the appropriate acid-base equation (Ricci, 1952) and then calculating p_1, as in Table 2.

The present paper is concerned with an initial study of the potentialities of orthogonal polynomials in evaluating titration curves. To this end, it is essential,

Table 2. *Theoretical values of* $p_1 \times 10^5$ *for monobasic acids.*
[0·25–0·75 neutralization; 12 point polynomial (n = 11)]

pK_a	1·0 molar	0·1 molar
1 (or 13)	3222·6	2316·6
2 (or 12)	4010·7	3222·6
3 (or 11)	4190·2	4010·7
4 (or 10)	4211·2	4190·2
5 (or 9)	4213·4	4211·2
6 (or 8)	4213·6	4213·4
7	4213·8	4213·8

Values of $p_1 \times 10^5$ in Table 2 were based on pH's calculated from the following expression:

$$[H^+] = K_a\left(\frac{c(1-x)-y}{xc+y}\right)$$ where x is the fraction of neutralization,

and c, the stoichiometric molarity of acid. For $pK_a \leqslant 6$, $y = [H^+]$ and for $pK_a \geqslant 8$, $y = -[OH^-]$. For $pK_a = 7$, $y = 0$.

Deviation of the pK_a from 7 and reduction of concentration both produce a decrease in the magnitude of p_1.

not only to choose a suitable function, range of application and number of points, but also, to estimate the order of accuracy with which chosen coefficients can be measured by normal chemical procedures.

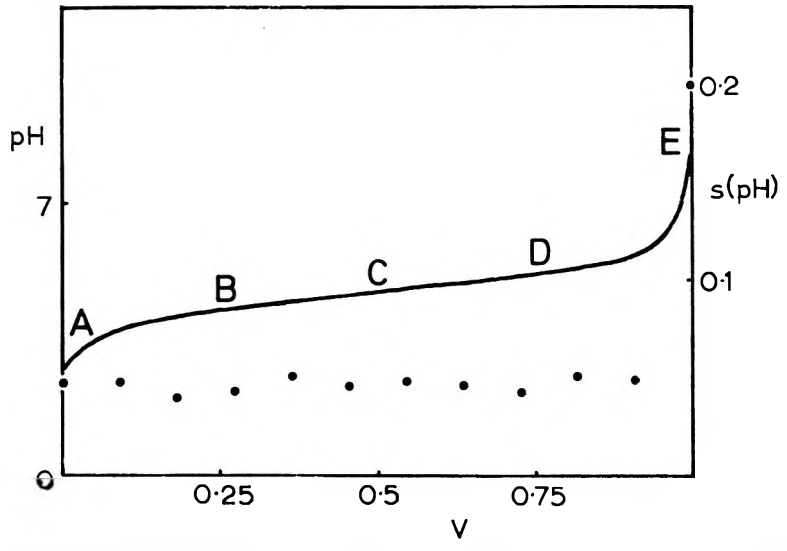

FIG. 4. Monobasic acid titration curve: standard deviation of pH measurement at different fractions of neutralization. ● = s(pH).

Choice of function and range

The typical weak monobasic acid curve of Fig. 4 provides a useful basis for the choice of function and range. Bearing in mind the principles outlined on p. 182S, the entire curve, A—E, can be seen to originate mainly from the mathematical components, $P_0(v)$, $P_1(v)$ and $P_3(v)$. Moreover, in view of the relation between the shape of a monobasic acid curve and the pK of the acid, it is evident that coefficients, p_0 and p_3, are highly dependent upon pK, p_0 bearing a substantially linear relationship thereto, whilst p_3 is a more or less linear function of $|pK - pH(\text{neutrality})|$. Apart from such high dependence upon pK, there is an added disadvantage that the poorly buffered regions, AB and DE, must inevitably provide major contributions to the error of the calculated coefficients. Similar objections apply to regions, AB, AC, CE and DE, but not to the region, BD, which therefore represents a good choice for most purposes. The further decision to assign B to 25% and D to 75% neutralization throughout the present work reflects a desire for the safety of a well buffered region.

Between 25 and 75% neutralization, p_0 and p_1 are the only significant coefficients. Moreover, the usefulness of p_0 in the study of solvent effects is underlined by the fact that $\Delta p_0 = \Delta pK$ for small values of ΔpK.

The number of points

This part of the discussion refers to a general form of equation (3), namely,

$$f(v) = p_0P_0(v) + p_1P_1(v) + p_2P_2(v) + \ldots + p_nP_n(v) \quad \ldots \quad \ldots \quad (4)$$

Perfect representation of a *continuous* experimental function, such as a titration curve, may demand an infinite number of terms in equation (4), in which case, one would

need to measure f(v) at infinitesimal intervals of v. In practice, however, one can never do more than sample the continuous function at a discrete set of abscissa values. Thus, in view of restrictions, such as the limits of reading a chart or the inevitable truncation of a digital output, all experimental variables are granular.

The effect of taking too small a sample is evident from the simple example in Fig. 5, wherein two different continuous functions, $f_1(v)$ and $f_2(v)$ give identical values of a given coefficient for the set, (v = 1, 3, 5), but two different values of the same coefficient for the set, (v = 1, 2, 3, 4, 5). In other words, $f_1(v)$ and $f_2(v)$ are indistinguishable when sampled at v = 1, 3 and 5, this phenomenon being known as "aliasing" (Blackburn, 1969).

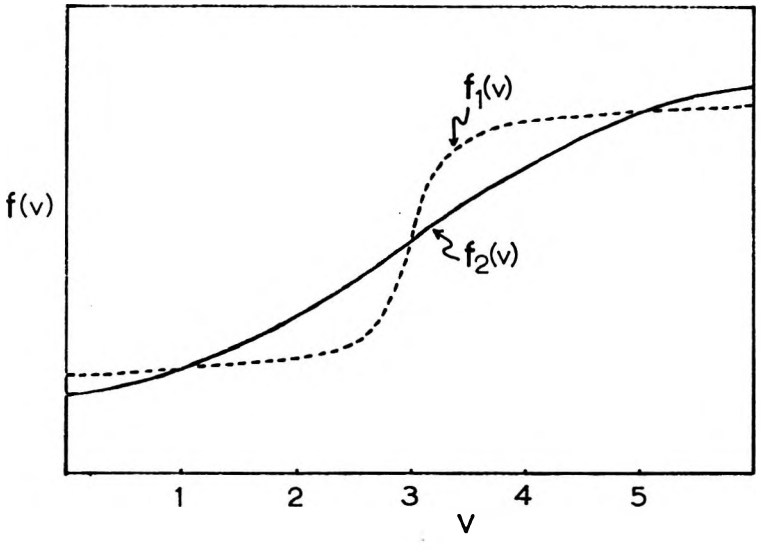

FIG. 5. The number of points.

Sudden fluctuations in f(v), arising from features such as a spike, hump (or step in the case of a titration curve), contribute to the coefficients of higher terms in equation (4). Moreover, the number of terms in (4) is limited to the number of points, (n + 1) and so n must always exceed the order of the highest term, which is statistically significant (n should, in fact, exceed this lower limit by a comfortable margin, in view of the tendency for observational errors to move from lower to higher terms as n increases). Hence, in determining the coefficients of lower polynomials, an adequate number of points is often a good deal larger than the bare minimum required to calculate the coefficient in question (e.g. 2 points for p_1).

In comparing coefficients obtained from equivalent segments of different titration curves, it is essential to adhere to a constant number of points. The latter must, therefore, be chosen in the light of that curve which contains the largest fluctuation in f(v) within the segment analysed.

An increase in the number of points also affects the reproducibility of the observed coefficients and in view of special circumstances, may be shown to reduce the relative standard deviation of the P_1 coefficient, used throughout the present work.

Substituting pH for f(v) and using s(x) to denote the estimated standard deviation of quantity, x, $s(p_1)$ is approximately equal (Davies, 1958) to $s(pH)/N_1^{\frac{1}{2}}$, where N_1

is the normalizing factor for $P_1(v)$; that is, 572 for 12 points. s(pH) refers to the observational error of pH measurement, which to validate the expression for $s(p_1)$, must be homogeneous over the set of points used to calculate p_1. Nevertheless, apart from small regions at the start and finish of a monobasic acid titration curve, there should be no difficulty in achieving reasonable homogeneity of s(pH), which can only depend upon errors in the measurement of pH and v respectively. Of these two contributions, the one due to pH measurement should be constant for measurements in aqueous solvents which avoid extremes of pH, whilst that due to v should also be constant if the curve undergoes no great variation in slope over the segment analysed. From the results in Fig. 4, the anticipated good homogeneity of s(pH) was evidently achieved over most of the curve for acetic acid, s(pH) at the equivalence point being the sole exception.

The present work involved analysis of an approximately linear segment of titration curve, spanning about 1 pH, and in these circumstances,

$$p_1 \approx \sum_{i=0}^{i=n} (i/n)P_1(v_i)/N_1 \text{ so that r.s.}(p_1) \approx \frac{100\, s(pH)\, N_1^{\frac{1}{2}}}{\sum_{i=0}^{i=n} (i/n)P_1(v_i)} \qquad \ldots \quad (5)$$

where $i = 0, 1, 2, \ldots . n$ and r.s. denotes relative standard deviation (or coefficient of variation, which term might confuse the reader in present circumstances).

Theoretical values of $r.s.(p_1)$, obtained by substituting the convenient value, $s(pH) = 0{\cdot}01$, into the last formula, are shown in Fig. 6. There is evidently a useful reduction in $r.s.(p_1)$ to be gained by increasing n, over and above the bare minimum, $n = 1$.

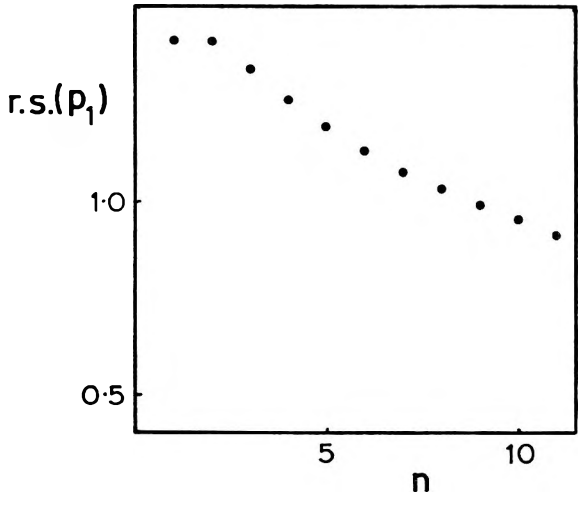

FIG. 6. Relative standard deviation of p_1 and the number of points.

Instrumentation and technique

The preliminary investigation now reported was carried through with a pH meter of modest performance and with no more than the usual technique for producing titration curves, accurate by present day standards. In consequence, it was only by extensive replication that the smaller differences between mean coefficients in Table 3

achieved significance. Improved instrumentation and technique on the other hand might well have reduced the number of replicates required to discriminate a given difference.

The accuracy and precision of coefficients depends not only upon operator technique but also upon maintenance of a linear pH/e.m.f. relationship of constant slope. Furthermore, although electrode standardization must remain constant throughout the measurement of a given coefficient, its accuracy is unimportant to the determination of any coefficient other than p_0. With a slide wire linearity of 0·01 % and ability to discriminate $\pm$0·0005 pH, a modern pH meter will achieve these requirements to within $\pm$0·0005 pH over a range of 1 pH unit, particularly when equipped with a single helical potentiometer which eliminates switch contact potentials from the slide wire system. In these circumstances, attainment of the desired pH/e.m.f. characteristics is effectively determined by the electrode system.

Under the dynamic conditions of an ordinary potentiometric titration, deficiencies of the electrode system mainly arise from hysteresis (Beck, Caudle & others, 1963) of the glass electrode and from the temperature coefficients of both electrodes. Some glass electrodes require a long time to reach the pH of the surrounding solution and in a titration of reasonable duration, the observed pH may lag behind the true value by as much as 0·05 pH. Moreover, the isothermal condition so necessary to meaningful pH measurement is destroyed by the heat of neutralization, which can raise the temperature by 1° in the course of titration, so affecting the pH/e.m.f. relationship as well as the potentials of reference electrode and inner half-cell of the glass electrode. The slope of the pH/e.m.f. relationship also depends upon other factors such as the electromotive efficiency (British Standards, 1965) of the glass electrode and the efficiency of the guard ring (Parker, 1950) both of which may fluctuate from day to day.

The last factors can be remedied by calibration, which for the limited purpose of quality control may also reduce the adverse effects of glass electrode hysteresis and thermal shifts. For this purpose, bracketing a given titration by titrations of a pure reference acid would seem to offer a powerful though laborious calibration procedure. For other purposes however, such as the study of solvent effects, it would seem advisable to (i) allow adequate time for electrode equilibration; (ii) stabilize both temperature and ionic strength and (iii) control factors such as electromotive efficiency by calibration with two buffers.

Using a free piston burette, titrant volume can be controlled to a limit (e.g. 0·01 ml in 20 ml) equivalent to about 0·0005 pH over the range of neutralization employed in the present work. Titrant volumes can be made integral by taking a suitable weight of titrand, based on a prior titration to complete neutralization. Coulometric generation of titrant would however be more attractive in view of the possibility of automating the process in conjunction with a pH meter giving a digital print out.

Experimental procedures

Organic acids were lab. grade, except for acetic acid (Analytical Reagent). In all titrations, 0·5M NaOH aq., contained in a Jencon free piston burette, was added to 40 milliequivalents of acid in 100 ml of water. Titrant volumes necessary to produce 12 readings, equally spaced on the abscissa scale, between 0·25 and 0·75 of total neutralization, were calculated from a prior titration. pHs were measured on a Cambridge Bench Type pH meter (discrimination: approximately 0·01 pH) using

glass (General Purpose) (British Standard, 1965) and saturated calomel electrodes, the temperature compensator being set to lab. temperature (19·6 ± 2°). Each titration was completed in about 30 min during which time the reading on M/20 potassium hydrogen phthalate drifted by less than 0·02 pH, usually toward higher values. The electromotive efficiency of the glass electrode, checked with phthalate and borate buffers at 2 hourly intervals, was never less than 0·996.

Discussion of results

In Table 3, each block (except *c*) refers to a set of replicate titrations of a given sample of acid, carried through in the course of two or three days. Trends between blocks were probably reduced, but not eliminated, by the use of the same glass electrode throughout all blocks. In block *c*, two samples of propionic acid were titrated alternately, in order to minimize the effect of trends.

Table 3. *Titration of mono- and di-carboxylic acids: mean values of orthogonal polynomial coefficients* (0·25–0·75 *neutralization; n* = 11) *and relative standard deviations thereof.*

Block	Acid	pK_a (25°C)	Mean value of coefficient × 10^5, relative standard deviation, degrees of freedom	
			p_1	p_3
a	Acetic	4·76	4219, 0·59%, 9 (0·00%)	—
b	Propionic	4·88	4136, 0·61%, 9 (—1·97%)	—
c	Propionic Fraction I	—	4125, 0·59%, 9 (—2·23%)	—
	Propionic Fraction V	—	4084, 0·83%, 9 (—3·20%)	—
d	Acetic + Propionic Equimolar proportions	4·76, 4·88; 0·12	4162, 1·46%, 8 (—1·35%)	—
e	Oxalic	1·23, 4·19; 2·96	11 090, 0·49%, 9 (+163%)	—399, 0·92%, 9
f	Malonic	2·84, 5·70; 2·86	12 700, 0·44%, 9 (+201%)	—502, 1·30%, 9
g	Succinic	4·19, 5·48; 1·29	5874, 0·39%, 7 (+39·2%)	19, 21%, 7

The relative standard deviations given in Table 3 for all blocks, other than *d*, relate to variances, which, according to Bartlett's test, form a homogeneous set whose mean corresponds with $s(p_1) = 24{\cdot}3 \times 10^{-5}$. This value is approximately equivalent (p. 189S) to s(pH) = 0·0058, which is consistent with the equipment and technique employed.

To assist comparison, the percentage deviation from the mean value of p_1 for block *a* is given beneath each mean value of p_1 in Table 3. Small statistically significant differences between means should be regarded with caution in view of possible trends between blocks. The difference between means (t = 10·1) for block *a* and Fraction V of block *c* probably reflects the tendency, in aqueous solution, for acetic acid to dimerize to a smaller extent than propionic acid. Thus, whereas acetic acid gave reasonable agreement between observed and theoretical values (Table 2), propionic acid showed a greater divergence on account of its larger association constant (Nash & Monk, 1957).

Differences between the means for blocks *a*, *b* and *d* are relevant to the distinction of mono- from polybasic acids, the pK_a difference between acetic and propionic acids being less than the theoretical minimum (0·3) for the difference in pK_a between consecutive stages of a polybasic acid. The statistical significance ($t = 2{\cdot}63, \phi = 10$) for the small difference between mean values of p_1 for blocks *a* and *d* suggests that in view of the much larger ΔpK_a likely to arise between consecutive stages of a polybasic acid, a valid distinction would require rather less than the 12 measurements employed throughout the present work. The absence of a significant difference between the mean values of p_1 for blocks *b* and *d* probably arose from a loss of degrees of freedom which resulted from the unusually large variance associated with block *d*. p_1 for succinic acid, whose titration curve exhibits no noticeable inflexion at the first equivalence point, is distinguishable from p_1 for a monobasic acid on the basis of only one titration. For the same acid, p_3 is too small for precise determination by the equipment and technique of the present work. Oxalic and malonic acids on the other hand exhibit P_3 coefficients large enough for precise determination.

To obtain some indication of the potentialities of orthogonal function coefficients in quality control, a sample of propionic acid was separated into five portions by fractional freezing. The first and fifth fractions gave significantly different ($t = 3{\cdot}09$) mean values of p_1 in an experimental design from which trends in experimental conditions were virtually eliminated. Moreover, as p_1 for a mixture of two acids of similar pK_a should be greater than that for either acid alone, the means show the anticipated relationship, p_1 for the first, presumably less pure, fraction being larger than that for the fifth fraction.

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REFERENCES

AGWU, I. U. & GLENN, A. L. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.*, 76*S*–87*S*.

ANDERSON, A. H., GIBB, T C. & LITTLEWOOD, A. B. (1970). *J. chromat. Sci.*, **8**, 640–646.

BECK, W. H., CAUDLE, J., COVINGTON, A. K. & WYNNE-JONES, W. F. K. (1963). *Proc. chem. Soc.*, 110–111.

BLACKBURN, J. A. (1970). *Spectral Analysis*. New York: Marcel Dekker.

BRITISH STANDARDS INSTITUTION (1965). *Glass electrodes for Measurement of pH*, B.S. 2586, p. 6.

BUCKINGHAM, R. A. (1962). *Numerical Methods*, p. 313. London: Pitman.

DAVIES, O. L. (1958). *Statistical Methods in Research & Production*, 3rd edn, p. 245. London: Oliver & Boyd.

DRAPER, N. R. & SMITH, H. (1966). *Applied Regression Analysis*. New York: John Wiley.

FEATES, F. S. & IVES, D. J. G. (1956). *J. chem. Soc.*, 2798–2812.

FENWICK, F. (1932). *Ind. Engng Chem. Anal. Edit.*, **4**, 144–146.

FISHER, R. A. & YATES, F. (1957). *Statistical Tables for Biological, Agricultural & Medical Research*, 5th edn, p. 90. London: Oliver & Boyd.

FRASER, R. D. B. & SUZUKI, E. (1966). *Analyt. Chem.*, **38**, 1770–1773.

GLENN, A. L. (1963). *J. Pharm. Pharmac.*, **15**, *Suppl.*, 123*T*–130*T*.

GLENN, A. L. (1967). *Proc. Soc. Analyt. Chem.*, **4**, 116–119.

GRAN, G. (1952). *The Analyst*, **77**, 661–671.

JONES, R. N., SESHADRI, K. S., JONATHAN, N. B. W. & HOPKINS, J. W. (1963). *Can. J. Chem.*, **41**, 750–762.

KATAKIS, D. (1965). *Analyt. Chem.*, **37**, 876–878.

KING, E. J. (1965). *Acid-Base Equilibria*. Oxford: Pergamon.

MILNE, W. E. (1949). *Numerical Calculus*, p. 265. Princeton: Princeton Univ. Press.
MULDER, F. J., SPRUIT, F. J. & KEUNING, K. J. (1963). *Pharm. Weekblad.*, **98**, 745–753.
NASH, G. R. & MONK, C. B. (1957). *J. chem. Soc.*, 4274–4280.
PARKER, P. (1950). *Electronics*, p. 829. London: Edward Arnold & Co.
RICCI, J. E. (1952). *Hydrogen Ion Concentration.* Princeton: Princeton Univ. Press.
SAVITZKY, A. & GOLAY, M. J. E. (1964). *Analyt. Chem.*, **36**, 1627–1639.
STERNBERG, J. C., STILLO, H. S. & SCHWENDEMAN, R. H. (1960). *Ibid.*, **32**, 84–90.
WERNIMONT, G. (1967). *Ibid.*, **39**, 554–562.
WESTERBERG, A. W. (1969). *Ibid.*, **41**, 1770–1777.
WRIGHT, N. (1941). *Ind. Engng Chem. Analyt. Ed.*, **13**, 1–8.

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Computer analysis of the relation between tablet strength and compaction pressure

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The load necessary to fracture lactose monohydrate tablets under diametral compression has been determined using an Instron physical testing instrument, so that true tensile failure was obtained in all cases, leading to improved reproducibility. Four ranges of tablet thickness were examined at 12·7 mm diameter. All tablets gave a linear increase of breaking load with compaction pressure up to 310 MN/m². Expressing the tablet strength as the breaking load gave a separate regression line for each range of tablet thickness, whereas the use of tensile strength provided a common regression line, within given statistical limits, for all but the lowest range of tablet thickness. The fact that such a correlation is possible shows that the tensile strength is a property of the "as compacted" material and provides a new and useful parameter to maintain constancy of properties when tablet size is changed.

Pharmaceutical tablets, when compressed diametrically, as in any of the tablet crushing tests normally applied, may fracture in any of the five ways shown in the upper part of Fig. 1. Failure by any of the first four mechanisms (a–d) will lead to greater variability in the crushing strength measurements than will failure by mechanism 1 (e). This is purely tensile fracture, giving a straight crack dividing the tablet into two semi-circular parts. It occurs only when the force applied to break the tablet is carefully controlled: in such circumstances the stress distribution within the tablet is calculable (Frocht, 1948) and is as shown in the lower part of Fig. 1. The vertical stress component σ_y varies along the vertical diameter, as does the maximum shear stress τ. The horizontal stress component σ_x, however, is virtually constant along the vertical diameter and tends to split the tablet into two equal halves. The value of this uniform horizontal stress is, at failure, the ultimate tensile strength of the "as compacted" material forming the tablet, and is given by

$$\sigma_x = \frac{2P}{\pi Dt}$$

where P is the load necessary to cause fracture, D is the tablet diameter and t is its thickness.

The various versions of the crushing strength tests have been compared by Ridgway (1970). To ensure correct load application, it is sometimes necessary to have packing pieces between the tablet and the loading platens, and experiment appears to be the only method of assessing the nature and quantity of padding required. Recognition of tensile failure is, however, readily made by inspection of the tablets after fracture.

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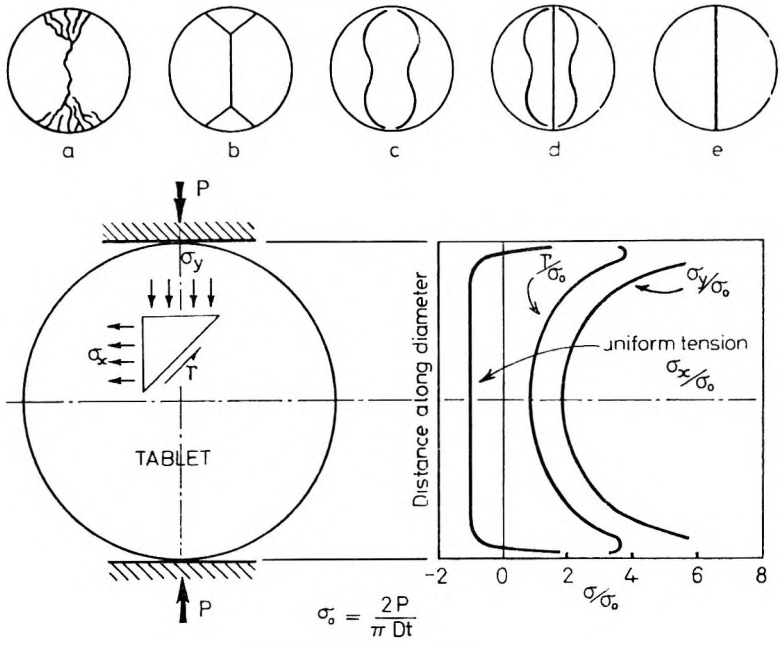

FIG. 1. Failure of tablets subjected to diametral compression:—(*a*) Compression failure locally at the loading points. (*b*) Failure under local shear at and near the loading points. (*c*) Failure along maximum shear loci when point loading applied. (*d*) "Triple-cleft" fracture due to transfer of load to each half-disc after breakage along the vertical diameter. (*e*) Ideal tensile failure.

The lower part of the figure illustrates the stress conditions in a tablet which are present when ideal tensile failure occurs.

The tensile stress (σ_x) on the vertical diameter is constant at $2P/\pi Dt$ over most of the graph. The compressive stress on the same diameter is σ_y. If ideal point loading was obtained this would tend to infinity. The shear stress is τ. The tablet material must be eight times stronger in compression and six times stronger in shear than it is in tension if the ideal tensile failure is to be obtained.

Failure in tension reduces the variability of the breaking load (Fell & Newton, 1970). The tensile strength is a fundamental property of the compressed tablet material and could, therefore, be a possible parameter for the characterization of tablets of different dimensions. Rees & Shotton (1969) showed that the strength of sodium chloride tablets of different dimensions could be compared by the expression $\left(\frac{P}{Dt_0}\right)$ where t_0 is the tablet thickness at zero porosity. They suggested that this expression could be considered as a "stress" (their inverted commas). It differs from the tensile strength defined above only by a multiplying constant, and by the use of the zero porosity thickness, which is constant, instead of the actual tablet thickness, which varies with the compaction pressure. Thus it neither represents the true stress that is acting, nor is it quite proportional to it, though it becomes more closely so as the compaction pressure increases. Thus, the use of the tensile strength proper (σ_x) appears likely to give an improved assessment of tablet strength. Derivation of the basic equation for tensile stress assumes that the compacted tablet is homogeneous throughout, whereas in fact tablets show an internal distribution of both density (Train, 1956) and hardness (Ridgway, Aulton & Rosser, 1971); these facts may limit the range of applicability of tensile strength methods.

MATERIALS AND METHODS

Materials

The powder used was lactose monohydrate B.P. supplied by Whey Products Ltd. (Crewe). The particle size distribution was Gaussian, with a median value of 67 μm s.d. 41 μm, as determined by an Alpine Air-Jet Sieve. The lactose was dried at 90° for 24 h and stored over silica gel.

Methods

Tablet preparation. Tablets were made on a Manesty F3 tablet machine instrumented by four foil strain gauges (Showa Sokki Kenkyusko, Japan, type 2b) connected as a bridge on the shank of a 12·7 mm diameter flat-faced upper punch. Another four strain gauges were similarly placed on the lower punch holder. The output from both bridges was fed into a signal conditioning unit, type MR701 (Data Acquisition Ltd., Stockport) and the amplified signal was recorded on a U.V. recorder, type 2005 (SE Laboratories (Engineering) Ltd., Feltham), fitted with type B160 moving coil galvanometers. The die walls and punch faces were thoroughly cleaned and then lubricated with a suspension of magnesium stearate in carbon tetrachloride. Tablets were prepared using a range of upper punch pressures at a machine speed setting of 42 tablets/min, for die fill weights of 0·4, 0·6, 0·8 and 1·0 g. The mean compaction pressure was calculated as the average of the upper and lower punch pressures. The weight of each tablet was determined to $\pm$0·0001 g. The diameter and thickness were determined to $\pm$0·005 mm.

Tablet strength. This was determined by diametral compression on an Instron physical testing instrument (Fell & Newton, 1968). The loading rate was 0·1 cm/min. No padding was used between the platens and the tablets, and all the tablets fractured in the fashion shown in Fig. 1 (e).

ANALYSIS OF RESULTS

For the large number of tablets examined the weight of fill and the compaction pressure could not be controlled exactly. The analysis was therefore designed to treat each tablet as an individual item. The calculations made enable the following tasks to be performed; regression lines are fitted to sets of data, associated in pairs, such as tablet breaking load and the corresponding mean compaction pressure P_m. The gradient, intercept and confidence ranges are calculated for each regression line. These linear regressions can then be compared, two at a time, and the significance level of the apparent differences between them determined. Such differences are examined in terms of the gradients and intercepts of the lines and of the quality of fit of the points to them. Comparison in pairs, although a little unorthodox (a more conventional approach would be a multiple regression analysis on all sets of data taken together), was preferred because the variations due to different tablet weights were expected to be substantial and not necessarily linear. Also this approach seemed to offer less difficulty in interpretation, whilst the computer programs* produced seemed likely to be of more general future value. The statistical methods are not generally presented in the standard textbooks in this form, but the underlying principles are well-documented (see, for example, Kendall & Stuart, 1951; Kenny & Keeping, 1951; Johnson and Leone, 1964).

* These are generally available through the University of London **Descriptive Programme** Index.

DISCUSSION

The compaction of 0·4, 0·6, 0·8 and 1·0 g quantities of lactose monohydrate at mean compaction pressures up to 310 MN/m² produced tablets with thickness: diameter ratios of 0·170–0·224, 0·250–0·330, 0·340–0·466 and 0·440–0·540 respectively. Analysis of the results for the relation between tablet breaking load and the mean compaction pressure resulted in the regression lines of Fig. 2. Statistical evaluation proved that each set of tablet weights yielded a significantly different relation (Table 1). Not unexpectedly, the results show that, as the quantity of lactose present increases, the breaking load of the tablets compacted with the same mean compaction pressure also increases.

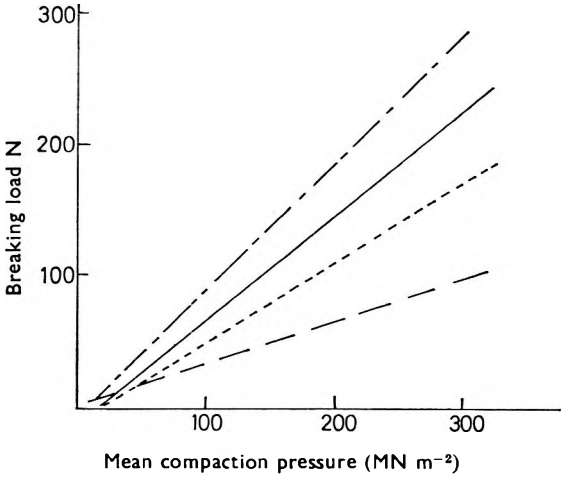

FIG. 2. Regression lines for the relation between the breaking load P, of the tablets and the mean compaction pressure P_m. The equations of the lines, with the residual variance of the P values given in parentheses are: 0·4 g tablets — — $P = 3{\cdot}29 \times 10^{-7} P_m + 2{\cdot}75$ (2·00) 49 points. 0·6 g tablets ------- $P = 6{\cdot}33 \times 10^{-7} P_m - 11{\cdot}57$ (0·80) 48 points. 0·8 g tablets —— $P = 8{\cdot}49 \times 10^{-7} P_m - 19{\cdot}61$ (1·50) 87 points. 1·0 g tablets — · — $P = 10{\cdot}01 \times 10^{-7} P_m - 13{\cdot}73$ (2·86) 70 points.

When the breaking loads are converted to tensile strengths, however, a different picture (Fig. 3) is obtained. Here the line for 0·4 g tablets is quite distinct from those for 0·6, 0·8 and 1·0 g tablets which are closely similar. Two of the differences within this set are only significant statistically at the 0·01% level despite the 48–87 points represented by each line; the third difference is totally insignificant (cf. Table 1). A common regression line was calculated for all 205 points from the 0·6 to 1·0 g experiments, yielding:

$$\sigma_x = 0{\cdot}0098\ P_m - 0{\cdot}33$$

with residual variance 2·58. It appears from the analysis that over the range of tablet weights 0·6 to 1·0 g the tensile strengths of 12·7 mm diameter lactose tablets are well correlated with compaction pressure by this common regression. Thus, in spite of the possible variation in the distribution of hardness within the tablet, the resultant tensile strength for the 0·6, 0·8 and 1·0 g tablets is the same, and the tensile strength is a linear function of the compaction pressure for all the tablets of these quantities studied. One allowance that can be made for the non-correlation of the

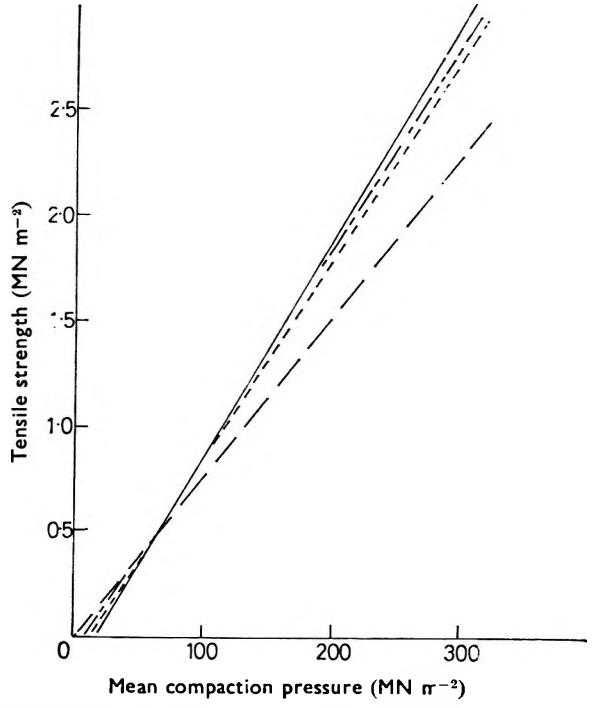

FIG. 3. Regression lines for the relation between the tensile strength σ_x, of the tablets and the mean compaction pressure P_m. The equations of the lines with the residual variance of the σ_x values given in parentheses are: 0·4 g tablets — — $\sigma_x = 0{\cdot}0075\ P_m - 0{\cdot}067$ (7·57) 49 points. 0·6 g tablets ------- $\sigma_x = 0{\cdot}0096\ P_m - 0{\cdot}221$ (1·30) 48 points. 0·8 g tablets —— $\sigma_x = 0{\cdot}0101\ P_m - 0{\cdot}267$ (3·42) 87 points. 1·0 g tablets — - — $\sigma_x = 0{\cdot}0096\ P_m - 0{\cdot}192$ (2·29) 70 points.

Table 1. *Statistical comparison of the gradients and intercepts of the regression lines of tablet strength and mean compaction force.*

Tablets weight compared	Significance level (as a %) of the hypothesis that the parameters of the regression lines differ					
	Regression lines for breaking load		Regression lines for tensile strength		Regression lines for tensile strength corrected for voidage	
	Gradient	Intercept	Gradient	Intercept	Gradient	Intercept
0·4 : 0·6	<0·01	—	0·01	—	0·01	—
0·4 : 0·8	<0·01	—	<0·01	—	0·01	—
0·4 : 1·0	<0·01	—	<0·01	—	0·01	—
0·6 : 0·8	<0·01	—	8·5	35	5*	34*
0·6 : 1·0	<0·01	—	97	22–25	99	26
0·8 : 1·0	0·2	—	10·8	84–86	7	93–94

* Thus for example the gradients of the estimated regression lines for the tensile strengths, corrected for voidage, of 0·6 and 0·8 g tablets differ marginally, the difference being statistically significant at the 5% level. On the hypothesis that the true gradients are identical, the intercepts differ only at a 34% significance level, i.e. negligibly.

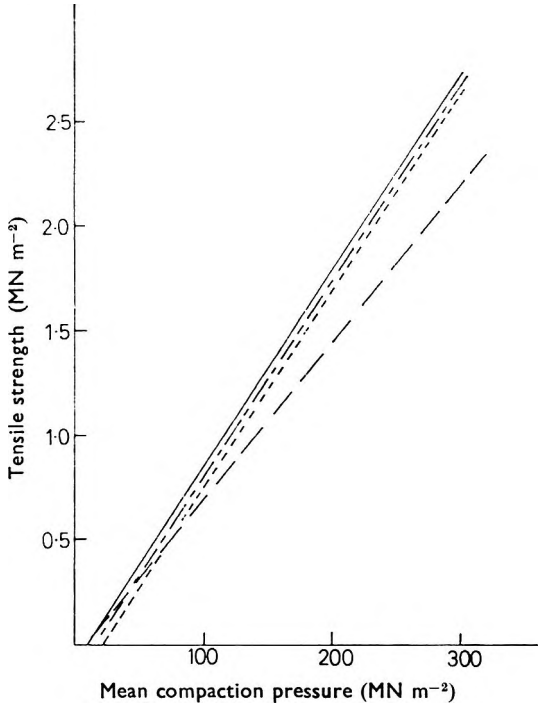

FIG. 4. Regression lines for the relation between the tensile strength, corrected for voidage $(\sigma_x)_c$, of the tablets, and the mean compaction pressure P_m. The equations of the lines, with the residual variance of the $(\sigma_x)_c$ values given in parentheses are: 0·4 g tablets — — $(\sigma_x)_c = 0{\cdot}0077\ P_m + 0{\cdot}0018$ (8·89) 49 points. 0·6 g tablets ------- $(\sigma_x)_c = 0{\cdot}0100\ P_m - 0{\cdot}159$ (1·28) 48 points. 0·8 g tablets —— $(\sigma_x)_c = 0{\cdot}0106\ P_m - 0{\cdot}218$ (3·90) 85 points. 1·0 g tablets — · — $(\sigma_x)_c = 0{\cdot}0100\ P_m - 0{\cdot}13$ (2·75) 70 points.

0·4 g tablets is to correct for the voidage. Since the fraction of the cross-sectional area occupied by solid is $(1 - e)$, where e is the fractional voidage, the tensile strength corrected for voidage will be $\frac{2P}{\pi Dt\,(1 - e)}$. The regression lines of this quantity upon the mean compaction pressure are given in Fig. 4 where the line for the 0·4 g tablets is still distinct from that for the other three weights. This is confirmed by calculation (Table 1). However, the above correction for a voidage effect is only an average correction that cannot allow for local differences in voidage. The 0·4 g tablets have the highest surface to volume ratio and hence are subjected to greater surface friction and shearing during compaction. Rees & Shotton (1969) reported that for short compacts, relatively large deviations from the relation between compaction pressure and breaking "stress" occurred, particularly at higher pressures. The deviations, as in the present case, resulted in lower values of tablet strength than would have been expected by comparison with the thicker tablets. It is also noteworthy that the residual variance in the correlation on a tensile strength basis for 0·4 g tablets is greater than it is on a breaking load basis (7·57 as compared with 2·00). The thinner tablets are thus intrinsically more variable: this may be due to maldistribution of powder within the die, which is more likely to occur where the amount of powder fill is small.

The practical significance of the present work is in the preparation of tablets of different dimensions from the same formulation. If the tablets are compacted to give the same crushing force, in kg, on the testing machine, different compaction pressures will be required and their true strengths will be different, as will their friability resistance and disintegration time. Compaction to the same tensile strength will provide tablets of more nearly identical properties. Because of the common regression line, tablets of the same tensile strength can be prepared by ensuring that the same mean compaction pressure is applied, provided that the frictional effects are not so great that deviations from the common regression line occur. Linking the present findings with the correlation of the tensile strength of mixed component tablets reported by Fell & Newton (1970), the manufacture of tablets of known strengths containing different ingredients and of different dimensions becomes a feasible proposition.

Acknowledgements

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REFERENCES

FELL, J. T. & NEWTON, J. M. (1968). *J. Pharm. Pharmac.*, **20**, 657–659.

FELL, J. T. & NEWTON, J. M. (1970). *Ibid.*, **22**, 247–248.

FELL, J. T. & NEWTON, J. M. (1970). *J. pharm. Sci.*, **59**, 688–691.

FROCHT, M. M. (1948). *Photoelasticity*, Vol. 2, p. 121. New York: John Wiley.

JOHNSON, N. L. & LEONE, F. C. (1964). *Statistics and Experimental Design in Engineering and the Physical Sciences*. New York: John Wiley.

KENDALL, M. G. & STUART, A. (1951). *The Advanced Theory of Statistics*, 3rd Edn. London: C. Griffin.

KENNEY, J. F. & KEEPING, E. S. (1951). *Mathematics of Statistics*, 2nd Edn. Princeton, N.J.: Van Nostrand.

REES, J. E. & SHOTTON, E. (1969). *J. Pharm. Pharmac.*, **21**, 731–743.

RIDGWAY, K. (1970). *Pharm. J.*, **205**, 702–712.

RIDGWAY, K., AULTON, M. & ROSSER, P. H. (1970). *J. Pharm. Pharmac.*, **22**, 70S–78S.

TRAIN, D. (1956). *Ibid.*, **8**, 745–761.

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The application of photoelastic techniques to a rotary tabletting machine

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Powders have been compacted on a rotary tabletting machine, using a Perspex die mounted above the die table, with an extended lower punch and a shortened upper punch. By high speed cine photography in polarized light, the fringes due to the radial stress in the die wall were recorded during compaction. At the same time, the upper and lower punch forces were monitored by instrumented pressure rollers coupled to a recording oscilloscope. The total information thus obtained was sufficient to enable compression cycles to be determined for the first time on a rotary machine.

Much fundamental work on the compaction of powders has been carried out since the development of the instrumented single punch tablet machine, and most of the available data relate to such machines, or to compression in a hydraulic press. Shlanta & Milosovitch (1964) pointed out that non-equilibrium data alone have physical meaning and relevance to tablet production, where the compaction process is much more rapid. Because of this commonly recognized fact, various workers have instrumented rotary tabletting machines to obtain data at normal operating speeds.

A Manesty type D3 machine was instrumented by Shotton, Deer & Ganderton (1963) by bonding strain gauges to the punches. The strain gauge signals were transmitted by a radiotelemetric system to the chart recorder. A comparison between the applied pressure and the tablet crushing strength was made for rotary and single punch machines. The upper and lower punch pressures were much more nearly equal in the rotary machine, the lower punch force being the smaller because the lower roller was sprung, the upper being rigidly supported. Sodium chloride tablets when produced by the rotary machine were stronger than when made by the single punch machine at the same mean pressure, but this difference was not found with aspirin tablets.

Knoechel, Sperry & others (1967) fitted strain gauges to the compression screws interposed between the arm holding the moveable axis of the pressure wheel and the spring used to adjust the compression force applied. Deflection measured by the gauges was proportional to the force applied to the punch. Modification of one of the supporting bolts for the ejection cam allowed the ejection force to be measured. The compression force was approximately linearly related to the tablet weight, when all other machine settings were held constant. The apparent density of the tablets produced increased to an asymptotic value as the compaction force was increased, at constant tablet weight. In correlating the physical properties of the tablets with the forces applied to them, Knoechel & others (1967) distinguished two types of property:

(i) thickness, apparent density, ejection force and compression forces were thought to depend on the machine rather than upon the material being compressed.

(ii) hardness, friability, disintegration and dissolution rates varied with compaction pressure, but this variation depended on the material being pressed. These properties were thought to be affected by the formulation at least as much as by the compressional forces used in the manufacture of the tablets.

Similar studies have been carried out by Wray (1967, 1969) who improved the measurement of the ejection force, finding it to be linear with percentage of added lubricant over a narrow range. He confirmed the equality of upper and lower punch forces to within about 3%.

In the work presented here the photo-elastic technique of Ridgway (1966) has been developed for use on a Manesty type D3 rotary tablet machine. The axial loads applied to the compact were monitored using instrumented compression rolls as described by Deer, Ridgway & others (1969) and the radial pressures transmitted to the die-wall were determined photoelastically as described by Ridgway (1966). Compression cycles were thus obtained for seven substances of pharmaceutical interest. These were the same seven used for compression cycle measurement using a Perspex die in a static hydraulic press (Ridgway, Glasby & Rosser, 1970). These static measurements showed the radial force to be dependent upon the surface hardness of the crystals in the die, confirming quantitatively the qualitative suggestion of Higuchi, Shimamoto & others (1968) that such a relation appeared to hold. They found that the lowest radial pressure was given by lactose and the highest by stearic acid, which were the hardest and softest materials which they compressed.

The work done by a punch during compression is $\int F.dx$ where F is the force and x is the distance moved by the punch face. Higuchi, Nelson & Busse (1955) measured this force in a single punch tablet machine as a function of punch displacement. The area under the curve of displacement against applied load for a sulphathiazole granulation then gave the work done. We have been able to make similar calculations, and have used the energy input to calculate the expected temperature rise for comparison with the results of Travers & Merriman (1970). Their method, implanting a thermocouple in a tablet during compression, cannot be extended easily for measurements on a rotary machine.

MATERIALS AND METHODS

A Manesty D3 rotary tabletting machine, capable of producing 500 tablets per minute in normal operation, was modified to enable the required measurements to be made. The general arrangement of the measuring equipment fitted to the machine is shown in Fig. 1 (for fuller details of technique and results, cf. Rosser, 1970). Fifteen of the stations were blanked off, and the Perspex die fitted at the remaining station on the die table with its associated shortened upper punch and lengthened lower punch. Brackets were bolted to the machine frame to carry the equipment for photoelastic measurement. Light from an Atlas 100 W projector bulb was passed through a 10 cm diameter condenser lens, focal length 25 cm, to give a parallel beam. This beam then passed through a polaroid filter, a first quarter-wave plate, the Perspex die, second quarter-wave plate, analysing polaroid and into the camera, a Hycam (Red Lake Laboratories, Inc., California) capable of a framing rate of up to 10 000 pictures s^{-1} on 16 mm film (Ilford Mk V, using a green filter for maximum fringe definition).

The die had a lower section which fitted into the die table and was held in position by two 4 B.A. screws. The upper section, 57 mm diameter and 32 mm deep, had a

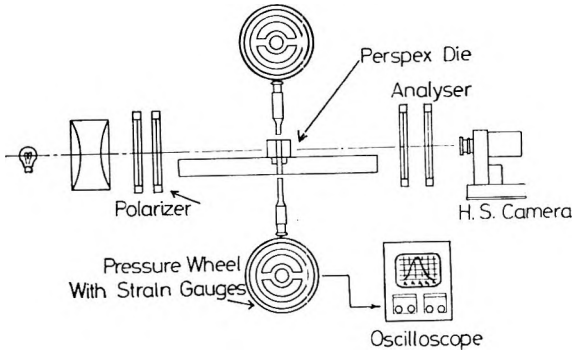

FIG. 1. General arrangement of the apparatus. Light passes through the condenser lens and is polarized by the polaroid filter A. It passes through the die and the second polaroid B to the camera. Upper and lower punch pressures are measured by the instrumented pressure rollers, the values being recorded on the oscilloscope or the chart recorder. The camera and oscilloscope are triggered by the contact wire, with timing marks being derived from the stroboscope and an initiating flash from the flash bulb.

12 mm bore. Two flat surfaces were cut at the front and rear for viewing purposes, and a graticule was ruled on the front surface.

The normal pressure rolls were replaced by a pair of the type mentioned earlier (Ridgway, Deer & others, 1969). These had two central spokes which deflect slightly under load. The original method of using two moire fringe plates to measure the deflection, and hence the applied force, was changed; strain gauges were fitted to the spokes as they gave greater precision. Four C6-121 foil gauges (Automation Industries Ltd., Camberley) were fitted to each pressure wheel. The wheels were mounted with their spokes horizontal, and a strain gauge was cemented to the upper and lower side of each spoke. By suitably connecting the four gauges into a Wheatstone bridge network, it was possible to make the assembly respond only to forces in the vertical direction, horizontal forces and twisting couples exerted on the wheel giving changes in the gauges which cancelled out.

The bridge output was fed to a differential D.C. amplifier (ZLD 2U. RC silicon integrated circuit, Ferranti Ltd.) and could be recorded by a Tektronix type 564 double-beam storage oscilloscope. The wheels and force-measuring equipment were calibrated by pressing in a hydraulic press along with a standardized load pillar of known characteristics.

For a tabletting run, in addition to the camera and the oscilloscope, a stroboscope (1209B, Dawe Instruments Ltd.) was operated so that it flashed light at 6000 flashes/min through a mirror to one part of the camera field of view, and also sent an electrical pulse at the same time to the second channel of the oscilloscope. This synchronizes the time for film and oscilloscope, and also gives a time interval calibration. As the die comes round to the compression station, it makes an electrical contact which fires a flash bulb as well as triggering the single sweep of the oscilloscope. The starting instant of the oscilloscope sweep can thus be correlated with the light flash appearing on the film. The filming speed was usually 1000 frames s^{-1}.

The net result of the technique is that a photographic record of the fringe pattern in the die wall during a compression is obtained, the film carrying markers which enable each frame to be calibrated for time and correlated exactly with the oscilloscope deflection which measures the tabletting force applied. Normally about 20 separate

frames during a compression were enlarged and printed for scrutiny. The calibration factor between radial load and the number of fringes obtained was determined by compression of a rubber plug which gives a hydrostatic pressure distribution under applied punch pressure. Punch displacement during a compression could also be obtained from the film or from a large scale drawing of the pressure roll and punch head profiles.

Weighed amounts of powder were loaded by hand into the die, since the feed frame had to be removed as it would have fouled the Perspex die. Ejection force was not measured because (a) the tablet was gripped more firmly for a given residual stress than it would have been in a steel die, because of the yielding of the wall and (b) the die was held in position by two small screws fitting into tapped holes in the die base, and the strength of these fixings was only just adequate to withstand the greater ejection force.

RESULTS AND DISCUSSION

Since the major remaining difference in compaction conditions between the present work and normal industrial practice is that the die is made of Perspex instead of steel, two sets of aspirin tablets were made at a range of compaction pressures in two dies of the same diameter, one steel and the other Perspex. Their diametral crushing strengths are shown in Fig. 2. Tablets made in the Perspex die are some 30% stronger on average than those of the tablets made in the steel die. This is because the wall of the Perspex die yields more than that of the steel die; this allows more shear to occur, which increases the tablet strength. The important point for the purpose of the present work is that it does not appear that compaction in Perspex

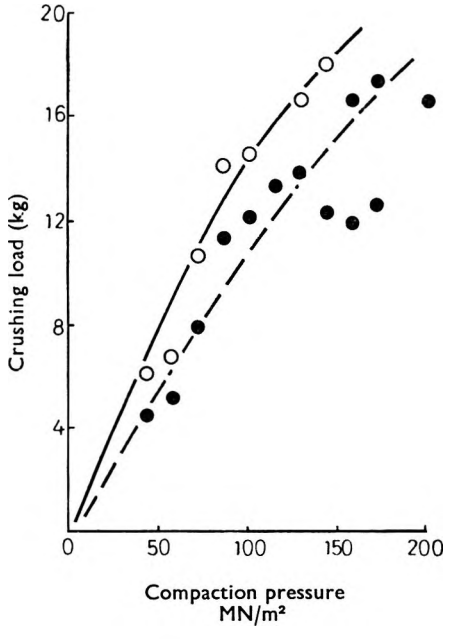

FIG. 2. Crushing load for aspirin tablets as a function of applied compaction pressure ○ tablets made in Perspex die. ● tablets made in steel die.

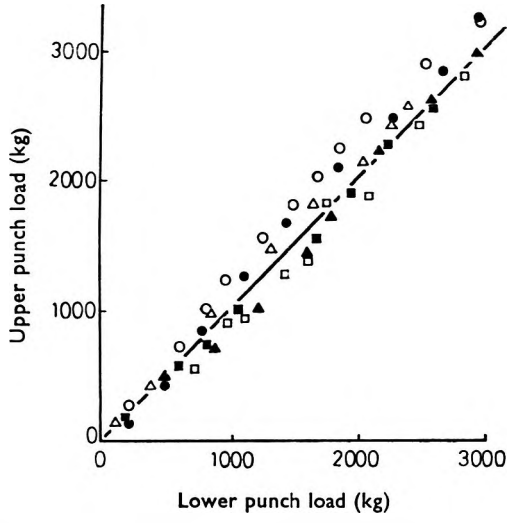

FIG. 3. Upper punch force as a function of lower punch force for a number of substances: ○ aspirin, ● hexamine, △ sucrose, ▲ urea, □ salicylamide, ■ sodium chloride.

is fundamentally different from compaction in steel, so that photoelastically-obtained results should be valid generally.

In Fig. 3 the experimentally-determined relation between upper and lower punch load is plotted for seven substances. From elementary statics, upper and lower punch forces should be equal. The only force which has a vertical component, other than those applied by the punches, is the shear between the tablet and the die wall, and even this should be fairly small. Indeed, for loading equally from top and bottom, it should be zero by symmetry. Inequality of upper and lower punch forces is thus

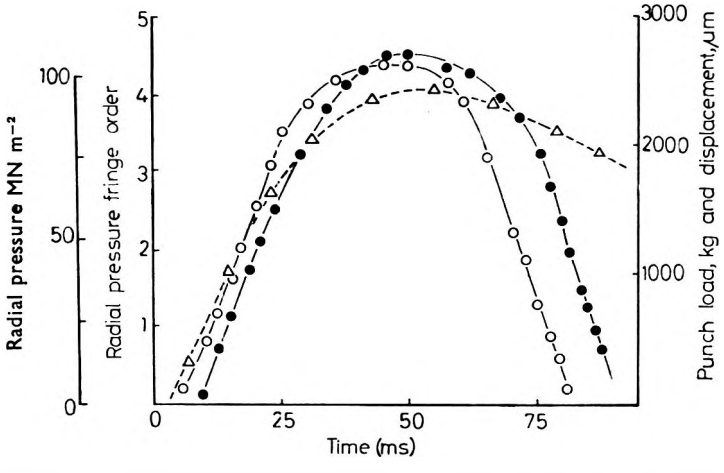

FIG. 4. Applied pressure, radial pressure and punch displacement as a function of time as derived from the photographic and electrical records. ○ punch load, ● radial pressure, △ punch displacement.

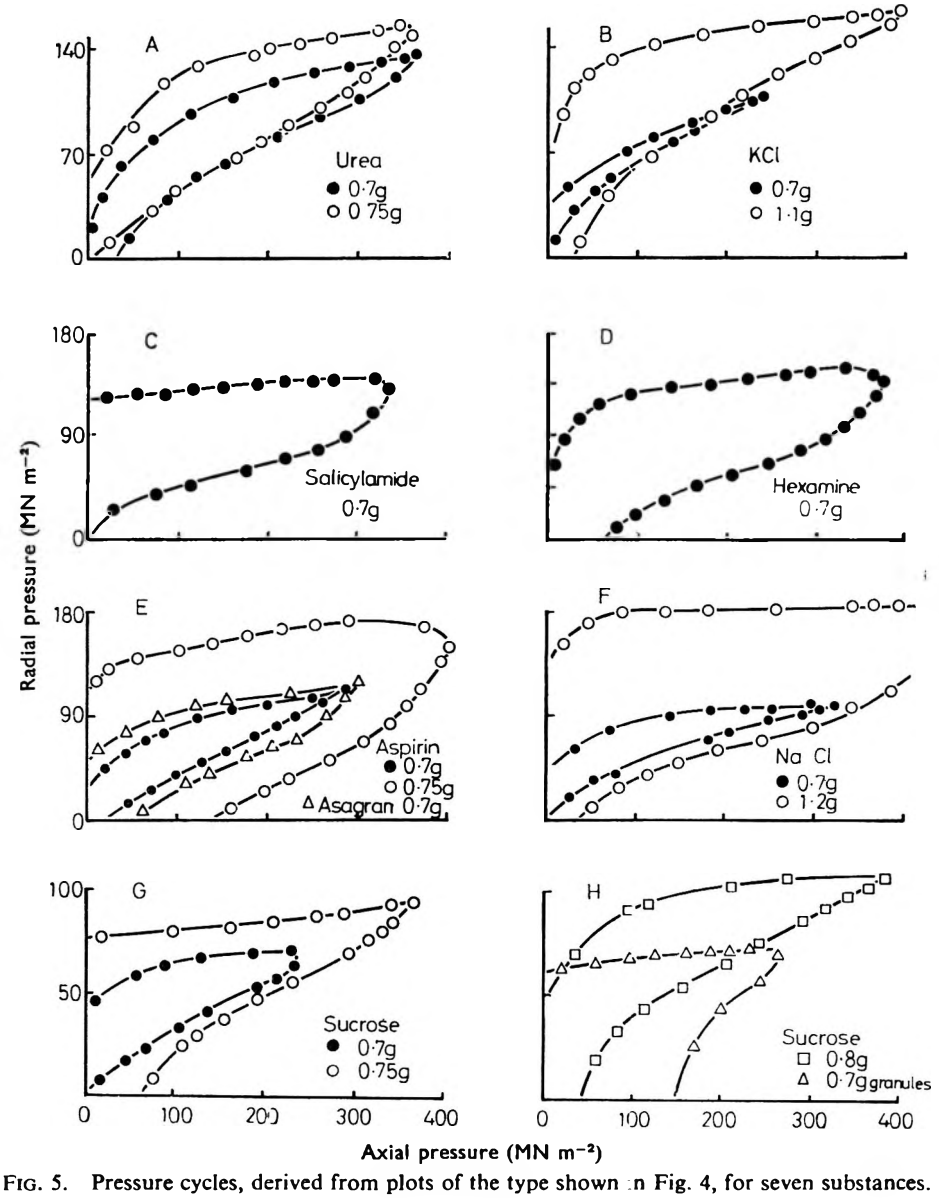

FIG. 5. Pressure cycles, derived from plots of the type shown in Fig. 4, for seven substances.

caused by dynamic factors, is probably machine-dependent, and is relatively unimportant. The greatest average difference in this work between upper and lower punch forces is 8%, for aspirin, but with no apparent correlation with substance properties. Shotton & others (1963) found a 10% difference, but in the opposite sense to that found in the present work (on the same machine). It seems certain that such force differences are due to different accelerations of the punches, either due to one being heavier and therefore more sluggish, or to slight relaxation of the sprung lower roller.

A typical result of a compaction run is shown in Fig. 4, where the applied pressure, the radial pressure on the die wall, and the punch displacement are shown as a

function of elapsed time from the zero marked by the flash bulb and the electrical contact. All three quantities increase to a maximum and decrease again, the radial pressure lagging behind the other two. The curve of punch load is a flat-topped parabola, the flatness reflecting the "dwell time" due to the shape of the punch head. The applied pressure is above 80% of its peak value for more than a third of the compaction event.

If the applied punch load is plotted against the radial force exerted at the same instant, pressure cycles may be obtained, and these are shown, for each of the substances examined, in Fig. 5. This method of plotting is due to Long (1960). The cycles in Fig. 5 may be compared to those resulting from slow compression in a hydraulic press (Ridgway & others, 1969). The chief difference is that in the static case, the force transmitted to the die wall is proportional to the hardness of the material being compacted, whereas in the rotary machine, no such correlation is immediately apparent, and it seems that under fast dynamic loading the radial force is more machine-dependent than substance-dependent.

However, some regularities can be seen which do reflect substance properties. Sodium and potassium chlorides both have elongated narrow cycles, pointed at the upper right extremity, indicating good recovery from the applied stress with a rapid deformation under load, as might be expected from an ionic crystal. As soon as the punch force decreases, recovery begins because the substance is capable of following the stress change applied to it.

The softer substances, aspirin, salicylamide and hexamine, show rounded extremities to the cycles. The two latter substances tend to have a horizontal upper contour as the punch force is removed, leaving a higher residual radial stress within the die at zero axial pressure.

The energy put into the tablet by the compression can be estimated, since the force exerted by the punch is known as a function of its instantaneous position. A top punch force versus displacement curve is shown in Fig. 6. The area under the upper part, up to A, is the work done in compressing the powder into a tablet. The area between the line AB and the punch displacement axis is the work done by the tablet

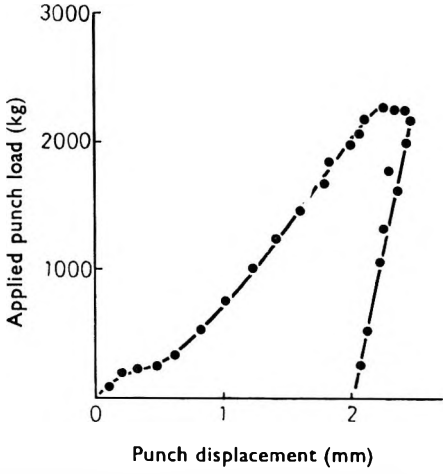

FIG. 6. Load applied by the upper punch as a function of punch displacement. The area inside the curve is the net work done on the tablet in a compaction. Equal work is done by the lower punch.

on the punch as it is withdrawn, and thus represents elastic recovery. The difference between these two quantities is the area of the cycle, and is the net work done on the tablet, $\int F.dx$. It is absorbed as particle fracture energy, plastic deformation, cold welding, wall shear and friction, and appears as heat. Taking upper and lower punch pressures to be equal and opposite, this cycle area is half the total work done on the tablet.

The cycle area in Fig. 6 is 2000 kg-mm, which is 2 kg-m or 18·6 Nm = 18·6 J. The tablet weight was 0·7 g of Asagran, specific heat 1·8 J/g, so that a temperature rise of perhaps 12° would be expected. The net work agrees with the value obtained by Higuchi & others (1955) for a sulphadiazole granulation on a single punch machine, and the temperature rise agrees with the measurements of Travers & Merriman (1970) for Asagran and other materials, also on a single punch machine: values between 10 and 15° on compression were obtained by these authors.

REFERENCES

Deer, J. J., Ridgway, K., Rosser, P. H. & Shotton, E. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 182S–184S.

Higuchi, T., Nelson, E. & Busse, L. W. (1955). *J. Am. Pharm. Ass. (Sci. Edn.)*, **44**, 223–225.

Higuchi, T., Shimamoto, T., Eriksen, S. & Yashiki, T. (1965). *J. pharm. Sci.*, **54**, 111–118.

Knoechel, E. L., Sperry, C. C., Ross, H. E. & Lintner, C. J. (1967). *Ibid.*, **56**, 109–130.

Long, W. M. (1960). *Powder Metallurgy*, **6**, 73–86.

Ridgway, K. (1966). *J. Pharm. Pharmac.*, **18**, 176S–181S.

Ridgway, K., Glasby, J. & Rosser, P. H. (1969). *Ibid.*, **21**, *Suppl.*, 24S–29S.

Rosser, P. H. (1970). Ph.D. thesis, University of London.

Shlanta, S. & Milosovitch, G. (1964). *J. pharm. Sci.*, **53**, 562–564.

Shotton, E., Deer, J. J. & Ganderton, D. (1963). *J. Pharm. Pharmac.*, **15**, 106–114.

Travers, D. N. & Merriman, M. P. H. (1970). *Ibid.*, **22**, *Suppl.*, 11S–16S.

Wray, P. E. (1967). 6th Annual Midwest meeting of A.P.L.A., Chicago, Illinois.

Wray, P. E. (1969). St. Johns University Pharmacy Congress.

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The effect of granule shape on bulk density, shear properties and tablet weight variation

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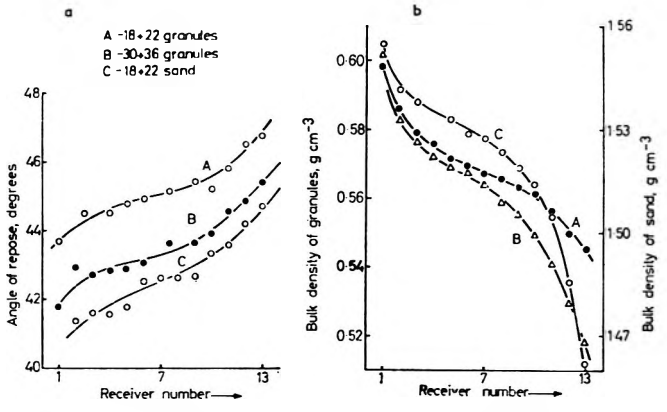
Two size fractions of a commercial lactose granulation (Thomas Kerfoot & Co. Ltd.) have been shape sorted on a vibratory table as described by Ridgway & Rupp (1969). They were 18–22 mesh (710–850 μm) and 30–36 mesh (425–500 μm) and when sorted, yielded 13 fractions varying from about 7·5 to 15·0 in Heywood shape factor. The poured bulk density and crater angle of repose were determined for three shape fractions in each size range. Figs 1a and b show the variation of these two parameters with particle shape: some earlier results for sand are included for comparison.



A new type of annular shear cell was developed which enabled the granules to be sheared under constant volume conditions. This means that the normal force exerted by the granules as they attempt to dilate under shear can be measured, as distinct from the more normal method of allowing the dilation to occur against a known constant applied load. The upper cup was held in an air bearing, and the lower cup was supported by a square array of steel strips carrying strain gauges, so that the vertical force exerted either by external loading or by the granules, could be measured.

Batches of about 150 tablets were made, from three shape fractions from each size range of granules, using an automatically-controlled instrumented Betapress rotary tabletting machine (Manesty Machines Ltd.) (Ridgway, Deer & others, 1971). All tablets were made at a machine speed of 700 tablets per minute, at which rate it proved possible to collect the tablets serially in a long glass tube of suitable diameter. The applied pressure for each tablet was determined from a chart record; the tablets were weighed individually, and their thicknesses measured. It is believed that this is the first time that a one-to-one correlation of such measurements has been made.

It was thus possible to analyse the variance in weight due to (a) the variation in punch lengths, eccentricity of the pressure rolls etc. in one revolution of the machine, (b) repeated compactions of the same pair of punches at one station in the machine and (c) the effect of granule size and shape. The intention was to put into a practical context the results obtained with shape-sorted sand particles on a hand-operated die simulator (Ridgway & Scotton, 1970).

REFERENCES

RIDGWAY, K. & RUPP, R. (1969). *J. Pharm. Pharmac.*, **21**. *Suppl.*, 30S–39S.
RIDGWAY, K., DEER, J J., FINLAY, P. L. & LAZAROU, C. (1971). *Ibid.*, **23**, *Suppl.*, 214S.
RIDGWAY, K. & SCOTTON, J. B. (1970). *Ibid.*, **22**, *Suppl.*, 24S–28S.

Automatic weight-control in a rotary tabletting machine

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A Manesty Betapress 16 station rotary tabletting machine has been fitted with an automatic weight control system. With the control system in operation, constancy of tablet weight is maintained in the face of changes in machine operating speed and granule size or shape distribution; gradual drift due to wear is also held in check. An adjustable upper and lower weight limit can be set, and any tablet falling outside the limits (despite the constancy of the mean tablet weight) can be directed by a small air-blast onto a rejection chute.

The basis of the system is as follows:

(a) the pressure rolls have been modified by machining cavities in the axles so that piezo-electric load washers (Kistler Ltd., type 903A) can be mounted inside the axle body. As each punch head contacts the pressure roll, an electrical pulse is produced from the load washer. The pulse voltage is proportional to the pressure applied to the powder in the die by the punch.

(b) the pulses are amplified and fed to a discriminator unit. This unit inspects the voltage of each pulse. If it is within the acceptable limits for the tablet being made, nothing is done. If the pulse voltage is too high or too low, a signal is sent to a stepping motor which drives, through appropriate gearing, the fill adjusting screw on the machine. This screw sets the level to which each punch drops as it passes under the powder feeding frame, and thus the amount of powder entering the die. For a pulse which is too large, the screw is raised by one step of the stepping motor, so that the amount of powder is reduced. For a pulse which is too small, the reverse happens. Thus the machine is kept at and near a preset mean tablet weight.

(c) when a pulse is too high or too low, a signal is also sent to a small rapid-acting solenoid valve which controls a compressed-air supply. This signal is sent some four compaction events later, at the time when the out-of-specification tablet is just leaving the die table. The tablet is deflected by a jet of air so that it enters a chute for reject tablets, separate from the chute carrying the bulk of the within-specification tablets. Because the pulse discriminator locks onto the operating speed of the machine, change in machine speed makes no difference to the efficiency of the rejection system. The high and low levels for rejection can be set by the operator.

(d) the amplified pulses are available for recording either by a high-speed u.v. chart recorder, or by a storage oscilloscope. Both methods have been used. If provision is made to collect the tablets serially as they come from the machine, it is possible to produce individually weighed tablets, with a record of their weight, at the full output rate of the tabletting machine, 1500 tablets per minute.

(e) the force to eject each tablet has also been monitored and a signal obtained, though currently nothing is done with this information.

A number of test runs have been carried out with the system, to determine what the control limits are. The ultimate limit is, of course, the quality of the granulation used to feed the machine.

The properties of tablets made from direct-compression bases on an automatically controlled rotary machine

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Four direct-compression bases, Celutab in the hydrous and the anhydrous form, Emcompress special and spray-dried lactose, have been compared with a traditional lactose granulation with respect to the initial physical properties of the powder, their tabletting performance and the characteristics of the tablets produced. The tablets were made on an automatically-controlled instrumented machine, a Manesty Betapress, so that compression force was continuously monitored. The following tablet properties: tensile strength by diametral crushing, porosity, weight, coefficient of variation of weight, surface microindentation hardness and

disintegration time were assessed for 180 batches of several hundred tablets. They were correlated with changes in compaction pressure, machine speed and tablet thickness.

Tablets were made from each material in three thicknesses: 3, 4 and 5 mm at a constant diameter of 12 mm using flat-faced punches. At each thickness, compaction pressures of approximately 90, 180, 260 and 330 MN m^{-2} were used, and at each pressure the tabletting machine was run at 700, 1100 and 1500 tablets/min.

Because Emcompress special (calcium dihydrogen phosphate) required a lubricant, 1·5% by wt of magnesium stearate was blended in with it, and with the other bases also in order to give comparability. In all cases the tap density of the powder was slightly increased by the addition of the lubricant. All the materials flowed well, and compressed without any sign of capping or difficulty in ejection, except for an initial test on unlubricated Emcompress special, when ejection difficulty was experienced.

Weight variation was small for all materials (coefficient of variation about 0·4%) but tended to increase with increasing machine speed and with decreasing tablet thickness. At the highest speed for 5 mm tablets, there was a great increase in coefficient of variation to above 2% due to difficulty in getting the powder to flow into the dies sufficiently freely.

Surface hardness and elasticity were independent of compaction force for all five materials, once a sufficient pressure had been applied to make a good tablet (usually 180 MN m^{-2}). The hardness was greater at the centre of the face than at the periphery.

The tensile strength decreased in the order Celutab hydrous, Celutab anhydrous, Emcompress, spray-dried lactose, lactose granulation. Machine speed had little effect. Plots of tensile strength against compaction pressure for lactose, both spray-dried and in granules, were linear, as reported by Fell & Newton (1970). This linearity cannot extend indefinitely, of course, but it does appear to cover the normal range of compaction pressures.

Mean disintegration times depended upon tablet thickness and upon compaction pressure. Celutab dissolved rather than disintegrated, the anhydrous form the more rapidly. Spray-dried lactose tablets dissolved to about 40% of their initial bulk, then fragmented. Emcompress tablets require a disintegrant and only a few were tested to check that they remained unaffected by two hours immersion in water at 37°. Lactose granulation tablets took the same time to pass the mesh as did Celutab anhydrous.

REFERENCE

FELL, J. T. & NEWTON, J. M. (1970). *J. pharm. Sci.*, **59**, 688.

Contribution of slip and Knudsen flow to tablet permeability measurements

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A cell was constructed to measure the air permeability of tablets after ejection from the die. Measurements were made either by drawing or by blowing dry air through the tablet.

The values of the specific permeability, B_0, were found to be a function of the pressure drop across the tablet. The permeability equation of Carman and Malherbe (1950), which allows for slip flow, includes a pressure dependent term. Using a modified permeability,

$$B = e^2/k\,(1-e)^2 S_0^2,$$

Carman's equation can be written as a quadratic equation in $\sqrt{B}$, i.e.,

$$\alpha B + \sqrt{B} = \beta,$$

where α and β are functions of the experimental variables and atmospheric pressure. The terms in B and $\sqrt{B}$ depend upon the contributions of slip and viscous flow respectively. Ignoring the slip flow term, B_0 can be calculated from $B_0 = e(\beta/\alpha)$, suggesting that the correct permeability should be obtained from an expression of the form, $e(B + \sqrt{B}/\alpha)$. This is a

function of pressure, but by inserting zero flow conditions at standard atmospheric pressure a non-pressure dependent permeability can be calculated, i.e.,

$$B^* = e(B + 0{\cdot}2268 \times 10^{-6}.CON.\sqrt{B}),$$

where CON is a constant dependent in part upon the Kozeny constant.

Table 1 compares values of B_0 and B^* and the contribution of slip and viscous flow to B^* at different flowrates and porosities for lactose tablets. The contribution of slip flow to the total flow is of the same order or greater than the contribution of viscous flow. This will lead to large errors in calculated values of S_0. The error in B_0 is sufficiently great at low porosities to require the computation of B^*.

Table 1

e	$B_0.10^{15}(m^2)$	$B^*.10^{15}(m^2)$	Viscous flow	Slip flow
0·1035	0·1141	0·1285	0·0185	0·1100
0·1035	0·1324	0·1265		
0·1686	0·6078	0·6445	0·1924	0·4521
0·1686	0·6889	0·6416		
0·2809	5·667	5·731	3·310	2·421
0·2809	5·843	5·736		

The authors acknowledge the assistance of Mr. I. Boyd in preparing the computer program.

REFERENCE

CARMAN, P. C. & MALHERBE P. le R. (1950). *J. Soc. chem. Ind.*, **69**, 134–143.

The compaction properties of potassium bromide with particular reference to infrared spectroscopy

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Potassium bromide in a range of particle sizes has been compacted in a vacuum die at various applied pressures to produce flat discs. The infrared transmittance of the discs has been measured and related to the compaction mechanism.

The material used was potassium bromide (Analar grade: B.D.H. Ltd.). It was milled, and the following size ranges (μm) were prepared by sieving: 53–90 (mean 71), 90–140 (mean 110), 355–420 (mean 388) and 500–600 (mean 550). All of these fractions were then used to make flat discs or tablets, 13 mm diameter, in a vacuum die. Compaction pressure was applied and measured by a small calibrated hydraulic press; the pressure range covered was 100–1000 MN m^{-2}. The infrared transmittance of each disc was determined on a Pye SP100 spectrophotometer, and its tensile strength was measured by diametral compression using the apparatus of Shotton & Ganderton (1960).

Lambert's law was obeyed by all the discs: plots of log (absorbance) against thickness were straight lines, plotting at constant compaction pressure. All the lines had the same slope, with the exception of the thinnest tablets at the largest particle size, where the thickness was only one or two particle diameters.

Over practically the whole pressure range, the 110 μm material gave higher transmittance. The 71, 388 and 550 μm particle sizes all scattered a greater proportion of the incident radiation. At any one particle size, the transmittance increased fairly rapidly with increasing compaction pressure, usually reaching a maximum at or about 400 MN m^{-2}. Thereafter, there was a slight fall, followed by a less-pronounced rise in the region of 1000 MN m^{-2}. This behaviour reflects the fact that in the initial compaction stages the crystals are being forced into contact and welded together: the relative density reaches about 0·99 at 400 MN m^{-2}. Additional compaction force introduces flaws into the crystals which act as scattering centres for radiation and reduce the transmittance. At 1000 MN m^{-2} these tend to heal. This is confirmed by the behaviour of the tensile strength of the discs, which also rises, for 0·5 g

tablets made from the 71 μm material, from 3 MN m^{-2} at 100 MN m^{-2} compaction pressure to 7·6 at 800 MN m^{-2}, subsequently falling to 6·9 at 1000 MN m^{-2}. Thicker tablets, weighing 1·0 g of the same material, had lower tensile strengths. So also did tablets made from the larger particle size material.

It thus seems that at about 110 μm particle size, the initial crystal size is such that intergranular scattering and flaw scattering are minimized over the whole pressure range at the 13 mm die diameter used here.

REFERENCE

SHOTTON, E. & GANDERTON, D. (1960). *J. Pharm. Pharmac.*, **12**, *Suppl.*, 87T–92T.

A stable free radical for the investigation of hydrogen abstraction reactions in aqueous solution

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A large number of biological and pharmaceutical reactions involve the abstraction of hydrogen, often as a primary step in the reaction sequence. An established method of examining hydrogen abstraction reactions is by use of a hydrogen-deficient, stable free radical, such as α,α-diphenyl-β-picrylhydrazyl (DPPH) (Dearden, 1971). However, the characteristics that bestow stability on a free radical generally tend also to bestow water-insolubility; whilst certain of the nitroxides are reasonably soluble in water, they are poor hydrogen abstractors.

In a search for a stable water-soluble free radical, we examined a number of ionic derivatives of DPPH, and found that the potassium salt of α,α-diphenyl-β-2,4-dinitro-6-sulphophenylhydrazyl (DDSH), first prepared by Ikrina & Matevosyan (1962), is reasonably stable in aqueous solution, and abstracts hydrogen readily from –OH, $-NH_2$ and >NH groups, but not in general from –COOH. We prepared the radical by sulphonation and subsequent nitration of *p*-chloronitrobenzene, followed by reaction with 2,2-diphenylhydrazine; treatment with lead dioxide then gave the free radical, which is deep purple in solution. The stability of DDSH in Clark and Lubs phosphate buffer is as follows:

pH	% decrease in absorbance (525 nm) in 1 h
5·6	0·7
6·5	0·6
7·4	0·8
8·6	1·1
9·8	1·9

These stabilities can be increased appreciably by de-gassing. The radical appears to be stable indefinitely in the solid form. The kinetics of hydrogen abstraction may be followed by either u.v. or e.s.r. spectroscopy. In the former case, a correction must be applied for absorption by the corresponding hydrazine formed as hydrogen abstraction proceeds, and a computer program has been written for this purpose.

We have so far investigated hydrogen abstraction from amino-acids and dipeptides in Clark and Lubs phosphate buffer (pH 7·4), and have obtained the following results: Glycine, $k = 0{\cdot}0048$ l $mol^{-1}s^{-1}$; L-alanine, $k = 0{\cdot}0025$ l $mol^{-1}s^{-1}$; L-proline, $k = 0{\cdot}015$ l $mol^{-1}s^{-1}$; glycylglycine, $k = 0{\cdot}031$ l^2 $mol^{-2}s^{-1}$; L-alanylglycine, $k = 0{\cdot}011$ l^2 $mol^{-2}s^{-1}$; glycyl-L-alanine, $k = 0{\cdot}024$ l^2 $mol^{-2}s^{-1}$; glycyl-L-proline, 0·023 l.$mol^{-1}s^{-1}$; L-alanyl-L-proline, 0·013 l $mol^{-1}s^{-1}$. The rate constants are clearly sensitive to changes in molecular structure, and may thus be explained in terms of electronic and steric effects within a molecule. In addition to its role in studies of reaction mechanisms, DDSH can also be used in quantitative analysis of pharmaceuticals (Papariello & Janish, 1965).

REFERENCES

DEARDEN, J. C. (1971). *J. chem. Soc.* In the press.
IKRINA, M. A. & MATEVOSYAN, R. O. (1962). *Zhur. Obs. Khim.*, **32**, 3952–3957.
PAPARIELLO, G. J. & JANISH, M. A. M. (1965). *Analyt. Chem.*, **37**, 899–902.

The effect of pH on the aerobic degradation of ascorbic acid solutions

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The stability of ascorbic acid in aqueous solution at 25° in the presence of excess oxygen has been studied in citric acid—phosphate buffer solutions in the pH range 2·2 to 7·0. The residual ascorbic acid was determined by potentiometric titration with ceric ammonium sulphate in the presence of iodide. At each pH, the logarithm of the concentration (C) of ascorbic acid is a rectilinear function of time, and the standard errors are about 2·5% of the regression coefficients. The degradation rate shows a maximum near pH 4 and a minimum near pH 5·6.

Finholt & others (1963) reported that the maximum rate of anaerobic degradation of ascorbic acid at 96° was at pH 4 and attributed this to a 1:1 complex of ascorbic acid molecules (A) and hydrogen ascorbate ions (A^-) which would be present at a higher concentration near pH 4 than at any other pH. Kassem & others (1969) found that both on storage at 50° and during autoclaving, the maximum loss was at pH 4 and the minimum loss at pH 6·5, but they gave no explanation.

$$C = [A] + [A^-] + [A^{2-}] + 2[A.A^-] = 5{\cdot}66 \times 10^{-3}\ \text{M}$$

$$K_1 = [H^+][A^-]/[A] = 9{\cdot}16 \times 10^{-5}\ \text{M} \qquad K_2 = [H^+][A^{2-}]/[A^-] = 4{\cdot}57 \times 10^{-12}\ \text{M}$$

$$A + A^- \leftrightharpoons A.A^- \qquad K_8 = \frac{[A.A^-]}{[A][A^-]}$$

The degradation rate has been found to depend mainly on the concentrations of the charged species A^-, A^{2-} and $A.A^-$, thus:

$$-dC/dt = kC = k_1[A^-] + k_2[A^{2-}] + k_3[A.A^-]$$

The first-order rate constants k_1, k_2 and k_3 contain the concentration of oxygen, which is a constant at constant temperature. The anaerobic breakdown of ascorbic acid is very slow at 25° and it can be ignored in the presence of excess of oxygen (Yacomeni, 1968).

It has been found that the experimental pH-rate profile is matched quite closely with the following values for the stability constant of the complex and for the rate constants:

$$K_8 = 500\ \text{litre/mol}$$

$$k_1 = 5{\cdot}7 \times 10^{-6}\ \text{s}^{-1} \qquad k_2 = 1{\cdot}7\ \text{s}^{-1} \qquad k_3 = 7{\cdot}4 \times 10^{-5}\ \text{s}^{-1}$$

If a preparation of ascorbic acid develops acidity on storage and if its initial pH is in the range 5 to 5·6, the rate of degradation will increase as the pH falls. An initial pH in the range 5·6 to 6 is recommended.

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REFERENCES

FINHOLT, P., PAULSSEN, R. B. & HIGUCHI, T. (1963). *J. pharm. Sci.*, **52**, 948–954.
KASSEM, M. A., KASSEM, A. A. & AMMAR, H. O. (1969). *Pharm. Acta Helv.*, **44**, 611–623, 667–675.
YACOMENI, J. A. (1968). M.Sc. Thesis. Heriot-Watt University, Edinburgh.

The effect of the pore size of the inhaler support upon the concentration of volatile drug emerging in the air stream from a nasal inhaler

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An investigation of the physico-chemical factors influencing the release of a volatile drug from a nasal inhaler (Armstrong, Carless & Enever, 1970, 1971) indicated that the pore size of the inhaler support into which the drug is impregnated may affect the concentration of the drug in the emergent airstream.

The phenomenon has been studied by using sintered glass plugs (7 mm diameter by 25 mm long) ranging in maximum pore diameter from 60 to 240 μm, in place of the inhaler support. Methylamphetamine (0·1 ml) was impregnated into the plugs and, using an air flow rate of

0·95 litre min^{-1}, the concentration of drug emerging from an inhaler system was determined over the temperature range of 15 to 35°.

The results for two of the glass plugs are shown in Fig. 1 in the form of a graph of logarithm of drug concentration *vs* reciprocal of absolute temperature. It can be seen that, the smaller the maximum pore diameter, the lower the concentration of drug in the air stream.

Vapour pressure values have been derived from the concentrations of methylamphetamine at 25° for the various sintered glass plugs. Fig. 2 shows the plot of logarithm of derived vapour pressure against reciprocal of maximum pore radius is a shallow curve rather than the straight line that would be expected if the Kelvin equation were obeyed (Gregg, 1961).

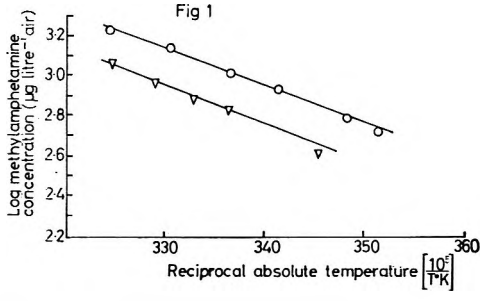

FIG. 1. Log concentration of methylamphetamine plotted against reciprocal absolute temperature. Maximum pore diameters ○ 206μm, ▽ 109μm.

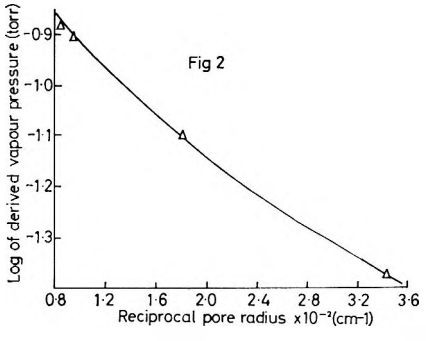

FIG. 2. Log derived vapour pressure of methylamphetamine at 25° plotted against reciprocal pore radius.

REFERENCES

ARMSTRONG, P. A. M., CARLESS, J. E. & ENEVER, R. P. (1970). *J. mond. Pharm.*, **13**, 5–13.
ARMSTRONG, P. A. M., CARLESS, J. E. & ENEVER, R. P. (1971). *J. Pharm. Pharmac.*, **23**, 473–481.
GREGG, S. J. (1961). *The surface chemistry of solids*, 2 edn p. 68. London: Chapman & Hall.

The sorption of benzocaine by nylon 6 (polycaprolactam)

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The sorption of drugs and formulatory adjuvants by plastics can present considerable problems when these materials are used as packaging media. These problems are aggravated by the lack of specific information regarding the individual contributions of the polymer resin and additives such as plasticizers and stabilizers to the product-plastics interaction. Here we report preliminary findings on the interaction of benzocaine in aqueous solution with pure nylon 6 (polycaprolactam) resin.

Powdered nylon 6 (specific surface by krypton adsorption, 7·0 m^2g^{-1}) was prepared from nylon chips by a precipitation process. Infrared spectra were consistent with the α form of nylon 6 containing very little monomer. Sorption was determined by shaking the powder with standard benzocaine solutions for one hour at a constant temperature and assaying the supernatant spectrophotometrically.

Uptake of benzocaine by powdered nylon 6 is rapid reaching equilibrium in less than 30 min and follows a C_1 type partition isotherm (Giles, MacEwan & others, 1960) which is linear over the concentration range studied (0–6 $\times$ 10^{-3}M). No plateau was observed. A similar result was obtained for benzoic acid. This contrasts with the finding of Kapadia, Guess & Autian (1964) who reported that the sorption of benzoic acid by commercial nylon 610 film followed a Langmuir isotherm.

The C_1 isotherms may be described by the expression, $C_n = KC_w$, where C_n is the uptake by nylon (mol kg^{-1}), C_w is the molar concentration in the aqueous phase at equilibrium and K is the equilibrium constant which characterizes the extent of adsorption for the system.

For benzocaine in water at 30°, K = 1·94 $\times$ 10^3 which increases to 2·53 $\times$ 10^3 in the presence of 0·5M potassium chloride. K values also vary with temperature and pH.

K decreases with increase in temperature and a plot of log K against $1/T_{abs}$ is linear, leading to a value of $-12{\cdot}3$ KJ mol^{-1} for the standard enthalpy of adsorption.

K values determined in buffer at ionic strength 0·5M and 30° show that benzocaine is only slightly adsorbed at very acid pH (0·69) but as the pH is increased sorption becomes more extensive, rising to a maximum around pH 5 and thereafter remaining constant. The extrapolated K value of 1·40 $\times$ 10^3 at the pK_a value for benzocaine in 0·5M KCl (2·5) where the drug is 50% ionized is almost exactly midway between the maximum and minimum K values, 2·54 $\times$ 10^3 and 0·2 $\times$ 10^3 respectively.

These results are consistent with the adsorption of a monofunctional solute by a polymeric substrate containing regions of varying crystallinity. It seems likely that principal interaction sites are the amide groups of the polymer which probably form weak hydrogen bonds with the amino group of the free benzocaine base.

REFERENCES

GILES, C. H., MACEWAN, T. H., NAKHWA, S. N. & SMITH, D. (1960). *J chem. Soc.*, 3973–3993.
KAPADIA, A. J., GUESS, W. L. & AUTIAN, J. (1964). *J. pharm. Sci.*, **53**, 28–35.

***In vitro* and *in vivo* studies of the metabolism of phenylbutazone in the alloxan rat and rabbit**

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In *in vitro* experiments, liver microsomal preparations of normal and alloxan diabetic rats and rabbits were incubated with phenylbutazone in the presence and absence of appropriate cofactors. These preparations were then assayed for unchanged drug and its metabolites at different intervals. In some of these experiments, preformed NADPH and/or a generating system for it were also incorporated in the incubation milieu. In separate experiments microsomal preparations from diabetic animals pretreated with insulin were similarly used.

In *in vivo* experiments the drug was administered to the normal and diabetic animals. Urine was collected periodically and analysed for unchanged drug and its metabolites. The

level of these substances was concurrently determined in the blood. Moreover, the above mentioned determinations were extended to urine and blood samples obtained from animals treated with insulin.

The results of the *in vitro* and *in vivo* experiments indicated that there is a significant difference in the rate of phenylbutazone metabolism in the normal and alloxan diabetic rats and rabbits, being slower in the alloxan-treated group. Because NADPH is involved in drug metabolism by microsomes it was speculated that the nucleotide may be deficient in the diabetic anaimals. To probe this possibility a specific micro assay procedure for NADPH in biological material was developed and applied to livers of normal and alloxan diabetic animals. The results indicated a very sharp drop in hepatic NADPH in the latter group as compared to the controls.

Metabolism of 5,8,11,14-eicosatetraynoic acid in the rat

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Administration of 5,8,11,14-eicosatetraynoic acid (I) (2g/day for 3 to 4 months) to patients suffering from acne decreased sebum production by up to 40% over control values (Strauss, Pochi & Whitman, 1967), indicating a potential use as an anti-acne agent. The metabolism of this compound is not known, but the disubstituted acetylene group in a number of drugs is apparently biologically stable.

$$CH_3{\cdot}(CH_2)_4{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}(CH_2)_3{\cdot}COOH....I$$

Intravenous administration (0·7 mg/kg) of Δ^{5-6}-^{14}C–I to the rat resulted in an initial rapid concentration of radioactivity in the liver (maximum of 40% of dose at 15 min to 1 h). This activity was excreted almost exclusively as metabolized drug via the bile to give about 60% of the radioactivity in the intestinal contents in 6 h. However it was only after 4 to 5 days that this amount of activity was excreted in faeces following i.p. administration of a similar quantity of radiolabelled I. Also, in the bile-duct-cannulated rat about 90% of administered activity was secreted in bile in 24 h, while in the intact animal only 40% appeared in faeces and 3% in urine in 18 h. Thus extensive reabsorption and entrohepatic cycling of this material was occurring. Within 5 days of an i.p. injection most of the radioactivity had been excreted with 65–68% in faeces, 8–17% in urine and 2–3% expired as $^{14}CO_2$. The latter would indicate a minor metabolic route by oxidative attack on the Δ^{5-6}-acetylene bond, probably following initial β-oxidation. The activity remaining in the body at 5 days (14–22%) was concentrated mainly in skeletal muscle (7·7%), skin (4·3%) and fat (3·8%).

Analysis of bile by radio-t.l.c. indicated exclusive incorporation of the radiolabel in phospholipids. Hydrolysis released a dicarboxylic fatty acid fraction as the main radioactive area. Further analysis indicated the presence of two non-endogenous compounds corresponding (by carbon number correlation on g.l.c.) to C-18 and C-16 dicarboxylic acids. In addition, examination of urine and faeces also indicated the presence of [^{14}C]dicarboxylic fatty acids. The finding of these products would suggest that both β- and ω-oxidative processes are important in the metabolism of I. Corroboration of the β-oxidation pathway was obtained from a similar study with 1-^{14}C-I. Within 5 days of an i.p. injection, 40% of the radioactivity was expired as $^{14}CO_2$ and the activity secreted in bile was associated mainly with the sterol/bile acid fraction, little remaining in the fatty acids.

The authors gratefully acknowledge the gift of radiolabelled 5,8,11,14-eicosatetraynoic acid from Roche Products, Ltd.

REFERENCE

STRAUSS, J. S., POCHI, P. E. & WHITMAN, E. N. (1967). *J. invest. Derm.*, **48**, 492–493.

Metabolism, distribution and excretion of orphenadrine in man

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A quantitative g.l.c. method was developed for the routine analysis of orphenadrine in urine and blood plasma, and its mono-*N*-demethylated metabolite and *N*-oxide [after reduction (Beckett, Mitchard & Shihab, 1971)] in urine.

The total *o*-tolyl phenyl methyl moiety excreted was determined quantitatively by oxidation, with alkaline $KMnO_4$, of the unchanged drug and its metabolites to *o*-methyl benzophenone which was then assayed by g.l.c.

Preliminary investigation showed that the excretion of orphenadrine in urine was dependent upon urine output as well as pH. Seven healthy male volunteers were given the drug while the urine was maintained at pH 5 ± 0·5 (Beckett & Tucker, 1966) and the intake of fluid increased to give a steady urine output (Beckett & Wilkinson, 1965). Under these conditions, the reabsorption of orphenadrine and its basic metabolites in the kidney tubules was reduced and the fluctuations in the excretion rates of orphenadrine virtually eliminated. Inter and intra subject variations were then minimal and therefore the excretion data could be used to study the absorption distribution and metabolism of orphenadrine from different preparations and from different routes of administration.

Under these controlled conditions, less than 30% of the drug after an oral dose was excreted unchanged while the mono-*N*-demethylated metabolite represented about 5% and the *N*-oxide about 4% of the administered dose. The *o*-tolyl phenyl methyl moiety excreted in the urine intact accounted for about 50% of the dose indicating that metabolism involving the aromatic rings was a major route for orphenadrine.

Under the controlled conditions, there was a direct relation between concentration of drug in plasma and the urinary excretion rates. Urinary excretion data was therefore used to propose a three-compartmental mathematical model to describe the kinetics of absorption distribution and metabolism of orphenadrine, the validity of which was examined using an analogue computer.

REFERENCES

BECKETT, A. H. & WILKINSON, G. R. (1965). *J. Pharm. Pharmac.*, **16**, 256–257.
BECKETT, A. H. & TUCKER, G. T. (1966). *Ibid.*, **18**, *Suppl.*, 72S–75S.
BECKETT, A. H., MITCHARD, M. & SHIHAB, A. A. (1971). *Ibid.*, **23**, 347–352.

Metabolism and excretion of guanoxan in man

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Guanoxan, 2-guanidinomethyl-1,4-benzodioxan, is an antihypertensive drug, the metabolism of which in man has not been reported. In urine samples (24 h) from 6 hypertensive patients receiving guanoxan (20 to 200 mg/day orally) only free guanoxan or 7-hydroxyguanoxan was detected. The level of 7-hydroxyguanoxan varied from 12 to 53% with little correlation with administered dose. Only in urine from the patient on the highest dose (200 mg/day) was a trace of guanoxan also found. Exceptionally, urine from one severely hypertensive female patient (50 mg/day dose) consistently contained no 7-hydroxyguanoxan and only guanoxan (39%). In the absence of faeces samples from this patient it is not possible to conclude the reason for this difference. Rapid metabolism and excretion, however, was generally evident as in the 24 h after an initial dose of guanoxan, up to 43% was found in the urine as free 7-hydroxyguanoxan.

The relation of urinary with faecal excretion was studied in the 24 h excreta from a non-hypertensive male subject following a single oral dose (20 mg). In urine no guanoxan was detected and 7-hydroxyguanoxan excretion (18%) was complete in 8 h. In faeces, guanoxan excretion was low (7·8%) and protracted (over 48 h) while 7-hydroxyguanoxan (4%) was

excreted only in the second 24 h. As the total recovery was only 30% a further study was made. The same subject took guanoxan (20 mg daily) for 7 days and collected excreta during a 24 h period following the final dose. A recovery of 59·5% based on one dose was obtained and as with hypertensive patients only 7-hydroxyguanoxan (51·5%) was excreted in urine. Faecal excretion was again low, the guanoxan concentration falling to 2% with 7-hydroxyguanoxan at 6%.

Despite the strong basicity of the guanidine group, guanoxan readily crosses body membranes and is extensively hydroxylated in the aromatic ring in man. Other work in this laboratory has shown guanoxan to be similarly hydroxylated by animal liver preparations. There was no indication of conjugation of guanoxan or 7-hydroxyguanoxan and the renal rather than biliary clearance of the latter would be anticipated. Although not all of an oral dose was accounted for, there was no evidence in excreta for degradation of the guanidine moiety of guanoxan. This is in agreement with the generally observed biological stability of the guanidine group in non-endogenous compounds, but in contrast to the major route of metabolism of guanoxan in the dog (Cañas-Rodriguez, 1966), in which the guanidine group underwent transamidination.

The authors gratefully acknowledge the gift of guanoxan and related compounds from Pfizer Ltd.

REFERENCE

CAÑAS-RODRIGUEZ, A. (1966). *Experientia*, **22**, 472–473.

Interactions between phosphatidylethanolamine monolayers and phenols in relation to antibacterial activity

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Considerable evidence already exists suggesting that damage to the bacterial cytoplasmic membrane occurs in the presence of phenols. This evidence has been reviewed by Proudfoot (1971). One question has not so far been conclusively answered:—is cytoplasmic membrane damage a direct or an indirect effect of phenols? The answer may be sought by determining whether phenols affect those molecular interactions which maintain the integrity of the cytoplasmic membrane.

To determine whether phenols do so affect lipid-lipid interactions the effects of phenol, *o*-cresol, *p*-cresol and 2,6-xylenol on monolayers of phosphatidylethanolamine (obtained from *E. coli*) at the liquid-gas interface have been examined using a film balance technique.

One observed effect of the phenols was a reduction in the total lateral cohesion between adjacent phosphatidylethanolamine molecules. For example, a subphase concentration of $4{\cdot}2 \times 10^{-3}$ mol/dm^3 phenol was found to reduce the total lateral cohesion of a phosphatidylethanolamine monolayer to the point where desorption of phosphatidylethanolamine molecules and disruption of the monolayer occurred at surface pressures greater than 15·2 mN/m.

The relative abilities of four phenols to disrupt monolayers of phosphatidylethanolamine was assessed by noting the mean highest surface pressure to which the lipid monolayers spread on sub-phases containing $2{\cdot}1 \times 10^{-3}$ mol/dm^3 of each phenol could be compressed before disruption occurred. On this basis, the relative disruptive abilities of phenol, *o*-cresol, *p*-cresol and 2,6-xylenol were 1·0, 2·0, 2·2 and 3·5 respectively. These values have the same order and relative magnitudes as the published phenol coefficients of these chemicals. If lipid-lipid interactions contribute to the maintenance of the integrity of the bacterial cytoplasmic membrane, the experimental observations suggest that phenols are capable of physically impairing this integrity. The observed similarity between the relative disruptive effects of the phenols and their phenol coefficients suggests that the antibacterial action of phenols involves disorganization of phospholipid molecules present in bacterial cytoplasmic membranes.

REFERENCE

PROUDFOOT, S. G. (1971). Ph.D. Thesis. University of Leeds.

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Anti-anginal drugs and the vasodilator response to myocardial hypoxia

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Anti-anginal drugs decrease the work of the heart (Petta & Zaccheo, 1971) and promote the formation of a collateral circulation (Russell Rees & Redding, 1969). They may also have a beneficial effect on myocardial metabolism (Parratt, 1969). It is possible that their overall spectrum of activity is influenced by yet another action—the modification of the normal vasodilator response to hypoxia. This could occur with several of the more recently introduced anti-anginal drugs, since they have been shown to potentiate the dilator effects of adenosine (Raberger & Kraupp, 1971), which itself may be a mediator of physiological dilatation (Rubio, Berne & Katori, 1969).

Myocardial blood flow was measured in anaesthetized cats using a heat clearance technique (McInnes & Parratt, 1969). Vasodilator responses were obtained to reactive hyperaemia, systemic hypoxia and by intravenous infusions of adenosine (0·25 mg/kg min). Reactive hyperaemia was produced by applying tension to a loose snare round the anterior interventricular artery for 10 or 30 s. After release of the snare, blood flow remained elevated for about 2 min. Systematic hypoxia was induced by artificial respiration with 5–10% oxygen in nitrogen. It was found that marked increases in blood flow occurred when the arterial pO_2 fell below 40 mmHg.

Dipyridamole (1 mg/kg, i.v.) itself produced a shortlasting increase in myocardial blood flow, which returned to control levels after 5 min. After dipyridamole, the vasodilator effects of adenosine were markedly potentiated. This effect lasted about 45 min. No changes were observed in the dilator effects of systemic hypoxia or of reactive hyperaemia.

These results do not support the suggestion that anti-anginal drugs with a dipyridamole-like action would influence the normal vasodilator response to myocardial hypoxia.

REFERENCES

McInnes, L. & Parratt, J. R. (1969). *Br. J. Pharmac.*, **37**, 272–282.
Parratt, J. R. (1969). *Prog. mednl. Chem.*, **6**, 11–66.
Petta, J. M. & Zaccheo, V. J. (1971). *J. Pharmac. exp. Ther.*, **176**, 328–338.
Raberger, G. & Kraupp, O. (1971). *Europ. J. Pharmac.*, **13**, 312–319.
Rubio, R., Berne, R. M. & Katori, M. (1969). *Am. J. Physiol.*, **216**, 56–62.
Russell Rees, J. & Redding, V. J. (1969). *Am. Heart. J.*, **78**, 224–228.

The effects of quazodine on myocardial blood flow in developing myocardial infarcts

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Acute ligation of a major branch of the left coronary artery in dogs markedly decreases local blood flow in the area supplied by the ligated vessel; this is partly the result of the conversion of the normal local vasodilator effect of adrenaline to vasoconstriction (Grayson, Irvine & others, 1968). In cats, the effect of noradrenaline on myocardial blood flow is much reduced following coronary artery ligation whereas the effect of isoprenaline is unchanged (Moore & Parratt, 1971). Quazodine (MJ 1988; 6,7-dimethoxy-4-ethylquinazoline) which possesses a spectrum of pharmacological activity similar to that of the β-adrenoceptor stimulants and the methylxanthines, markedly increases myocardial blood flow and contractility both in dogs (Carr, Cooper & others, 1967) and in cats (Parratt & Winslow, 1971). The purpose of this study was to determine if these effects were also present in the ischaemic myocardium and in the early stages of experimental cardiac failure.

Myocardial blood flow was assessed, in cats anaesthetized with sodium pentobarbitone, by a heat clearance technique (McInnes & Parratt, 1969). The effects of intravenous infusions of quazodine (0·5 mg/kg min^{-1}) on systemic blood pressure, heart rate, cardiac output and myocardial blood flow were determined up to 4 h after acute ligation of the anterior descending branch of the left coronary artery. In normal animals quazodine decreased systolic pressure by a mean of 7 ± 2 mmHg (from a mean control level of 135 ± 7 mmHg) and diastolic blood pressure by 14 ± 2 mmHg (from a mean control level of 92 ± 5 mmHg).

Heart rate was increased by 28 ± 3 beats/min (from 196 ± 7 beats/min), cardiac output by 44 ± 5 ml/kg body weight min (from 168 ± 16 mg/kg min) and local myocardial blood flow by a mean of 220%. Quazodine had similar effects in the infarcted animals, decreasing systemic pressure by 13 ± 3 mmHg (from 124 ± 6 mmHg) and diastolic pressure by 20 ± 3 mmHg (from 33 ± 6 mmHg). Heart rate was increased by 30 ± beats/min (from 201 ± 12 beats/min), cardiac output by 22 ± 5 ml/kg body weight min (from 133 ± 20 ml/kg body weight min) and myocardial blood flow by a mean of 34%. In the infarcted animals therefore quazodine produced a more marked decrease in systemic arterial pressure but the effects on cardiac output and on myocardial flow were considerably less than those observed in normal cats. Nevertheless, the degree of myocardial stimulation and the increase in coronary perfusion induced by quazodine, in animals with an ischaemic myocardium, suggest that it warrants further investigation in experimental cardiogenic shock.

REFERENCES

CARR, P. W., COOPER, T., DAGGETT, W. M., LISH, P. M., NUGENT, G. G. & POWERS, P. C. (1967). *Br. J. Pharmac. Chemother.*, **31**, 56–65.
GRAYSON, J., IRVINE, M., PARRATT, J. R. & CUNNINGHAM, J. (1968), *Cardiovasc. Res.*, **2**, 54–62.
MCINNES, L. & PARRATT, J. R. (1969). *Br. J. Pharmac.*, **37**, 272–282.
MOORE, G. & PARRATT, J. R. (1971). *J. Pharmacologie*, **2**, 188–189.
PARRATT, J. R. & WINSLOW, E. (1971). *Br. J. Pharmac.*, **42**, 193–204.

Muscle tremor produced by sympathomimetic bronchodilators

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Adrenaline, stimulation of the splanchnic nerves, and a variety of procedures that cause the reflex release of catecholamines from the adrenal medullae produce a decrease in the tension and an increase in the rate of relaxation of the maximal twitches of the slow-contracting soleus muscle of the anaesthetized or decerebrate cat. The effect is the result of a direct action on the muscle fibres, and is independent of concomitant cardiovascular changes (Bowman & Zaimis, 1958). The increased rate of relaxation means that the overall duration of the twitch is reduced, and this effect results in a pronounced decrease in the tension and degree of fusion when subtetanic contractions are evoked at frequencies of stimulation (5–15 Hz) that include the physiological range for this muscle. Adrenaline was effective in doses as low as 0·01 μg/kg intravenously. In different animals, noradrenaline was 50 to 200 times less potent.

Several sympathomimetic bronchodilators (isoprenaline, salbutamol, orciprenaline, terbutaline) have been shown to produce the same effect, and the use of relatively selective agonists and antagonists (sotalol, butoxamine, practolol) indicated that the adrenoceptors involved are β-receptors and that they resemble those of the bronchi (β_2 receptors) more than those of the heart (β_1 receptors). The same effect, occurring in the slow-contracting units of human muscles (Marsden & Meadows, 1968), probably accounts for the tremor that occurs in patients with phaeochromocytoma and that often accompanies the use of sympathomimetic bronchodilators. The cyclic AMP phosphodiesterase inhibitors, 3-acetamido-6-methyl-8-n-propyl-*syn*-(4,3-a) pyrazine (ICI 58,301), 3-acetamido-5-methyl-8-n-propyl-*syn*-triazolo (4,3-a) pyrazine (ICI 61,129), and 2-amino-6-methyl-7-oxo-8-n-propyl-*syn*-triazolo (4,3-) pyrazine (ICI 63,197), potentiated adrenaline and isoprenaline in their actions on the soleus muscle. ICI 63,197, effective in a dose of 50 μg/kg intravenously, was the most potent in this respect, whereas ICI 61,129, even in doses up to 10 mg/kg, was only very weakly effective. These compounds show the same rank order of potency in phosphodiesterase inhibiting activity (Somerville, Rabouhans & Smith, 1970), and the results are therefore compatible with the possibility that the effects of β-receptor agonists on the soleus muscle are mediated by cyclic adenosine 3′,5′ monophosphate, which in turn may be involved in the relaxation mechanism of the muscle.

REFERENCES

BOWMAN, W. C. & ZAIMIS, E. (1958). *J. Physiol. (Lond.)*, **144**, 92–107.
MARSDEN, C. D. & MEADOWS, J. C. (1968). *Ibid.*, **194**, 70P.
SOMERVILLE, A. R., RABOUHANS, M. L. & SMITH, A. A. (1970). *Biochem. J.*, **120**, 11P.

Actions of the sympathomimetic bronchodilator, AQL208, on the cardiovascular, bronchiolar and skeletal muscle systems of the cat

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Trimetoquinol is a sympathomimetic bronchodilator first described by Yamato, Hirakura & Sugasawa (1966).

It was reported to be about 10 times more potent by weight than isoprenaline in producing relaxation of the guinea pig tracheal chain, but only one fifth as potent as isoprenaline in stimulating the perfused heart (Iwasawa & Kiyomoto, 1967). In preliminary experiments in this laboratory, the active (−)-isomer of trimetoquinol (AQL208) was found to be about half as potent as (−)-isoprenaline in decreasing fusion of incomplete tetanic contractions of the cat soleus muscle. These results, on tissues from different species, suggested that AQL208 might be relatively selective for β-receptors in the lung compared with those in the heart and in skeletal muscle. Such a drug would be valuable in the symptomatic relief of asthma because it would be less likely to produce muscle tremor and unwanted cardiac stimulant effects.

Further experiments have now been performed in order to assess the effects of AQL208 on the cardiovascular and bronchial systems and on skeletal muscle of the chloralose-anaesthetized cat under identical *in vivo* conditions. Effects on myocardial blood flow and general haemodynamics were studied by the methods described by McInnes & Parratt (1969), and effects on lung compliance and resistance parameters by a modification (for the cat) of the method described by Amdur & Mead (1958). Effects on incomplete tetanic contractions of the soleus muscle were studied using the method described by Bowman & Nott (1970) (reference to which is made in the previous abstract).

Intravenous infusions of AQL208 (0·05 to 0·25 μg/kg min) were found to be about equipotent with (−)-isoprenaline in lowering general arterial blood pressure and in increasing left ventricular pressure, left ventricular dp/dt, myocardial blood flow, pulmonary artery pressure and heart rate. Intravenous injections of AQL208 were found to be about half as potent as (−)-isoprenaline in decreasing fusion of soleus contractions. In their abilities to antagonize 5-hydroxytryptamine-induced bronchospasm, AQL208 and (−)-isoprenaline again did not differ markedly in potency, although the dose-response curve for AQL208 was shallower than that for (−)-isoprenaline and therefore accurate comparisons of potency could not be determined. In all tests, the effects of AQL208 were 2–5 times longer lasting than those of (−)-isoprenaline when responses of equal magnitude were compared.

Thus the results emphasize the obvious importance of determining the various effects of a drug in the same species, and they show that, at least in the cat, AQL208 does not exhibit any marked selectivity for β-receptors in particular tissues. The results, therefore, suggest that if the cat is a reliable test animal, AQL208 may not be devoid of side effects on the cardiovascular and skeletal muscle systems in man.

REFERENCES

AMDUR, M. O. & MEAD, J. (1958). *Am. J. Physiol.*, **192**, 364–368.
BOWMAN, W. C. & NOTT, M. W. (1970). *Br. J. Pharmac.*, **38**, 37–49.
IWASAWA, Y. & KIYOMOTO, A. (1967). *Jap. J. Pharmac.*, **17**, 143–152.
MCINNES, L. & PARRATT, J. R. (1969). *Br. J. Pharmac.*, **37**, 272–282.
YAMATO, E., HIRAKURA, M., & SUGASAWA, S. (1966). *Tetrahedron Suppl.*, 8 Part I 129–134.

The hyperglycaemic effect of the diuretic chlorthalidone

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Chlorthalidone, like the benzothiadiazine diuretics, has been reported to impair glucose tolerance in some patients (Reutter & Labhardt, 1961; Carliner, Schelling, Russell, Okun & Davis, 1965) and to produce hyperglycaemia in the rat following single large doses of the drug dissolved in alkali or administered as a suspension (Tabachnick, Gulbenkian & Yannell, 1965; Foy, 1967; Wales, Grant & Wolff, 1968). The work presented in this communication was performed in an attempt to elucidate the mechanism of the hyperglycaemic effect.

Chlorthalidone, administered intraperitoneally in single doses up to 200 mg/kg, exerted no significant effect ($P > 0.05$) on rat blood sugar at 1 or 2 h after injection when compared with an alkaline saline control. The low solubility of chlorthalidone necessitated the use of high concentrations of alkali (pH 12) to dissolve the drug in sufficient concentration to administer the large doses employed. A similar control solution was shown to produce a statistically significant ($P < 0.05$) hyperglycaemic response when injected intraperitoneally. Two hours after injection of chlorthalidone (200 mg/kg, i.p.) the intravenous glucose tolerance, as measured by the rate of disappearance of an intravenous glucose load (1 g/kg), was not significantly different from that of the controls ($P > 0.05$). In concentrations up to 200 μg/ml, chlorthalidone did not diminish glucose uptake by rat diaphragm muscle or epididymal adipose tissue incubated *in vitro*. Oral treatment with chlorthalidone (100 mg/kg day) for 28 days produced no deterioration of intravenous glucose tolerance when compared with pair-fed controls.

It is concluded that chlorthalidone is not hyperglycaemic in the rat in single, large doses when compared with a suitable alkaline control solution and does not influence the glucose tolerance of rats so treated, or treated orally for 28 days with large doses of the drug.

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REFERENCES

CARLINER, N. H., SCHELLING, J. L., RUSSELL, R. P., OKUN, R. & DAVIS, M. (1965). *J. Am. med. Ass.*, **191**, 535–540.
FOY, J. M. (1967). *Life Sci., Oxford*, **6**, 894–902.
REUTTER, F. & LABHARDT, A. (1961). *Helv. med. Acta.*, **28**, 487–495.
TABACHNICK, I. I. A., GULBENKIAN, A. & YANNELL, A. (1965). *Life Sci., Oxford*, **4**, 1931–1936.
WALES, J. K., GRANT, A. M. AND WOLFF, F. W. (1968). *J. Pharmac. exp. Ther.*, **159**, 229–235.

Mechanism of action of neostigmine at the neuromuscular junction

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A study has been made, in cats under chloralose anaesthesia, of the relative abilities of a number of acetylcholine antagonists (hexamethonium, benzoquinonium, tubocurarine, gallamine and pancuronium) to inhibit the various facets of neostigmine's activity at the neuromuscular junction of the soleus muscle.

Neostigmine (150 μg/kg intravenously) increased the amplitude of the maximal twitch and gave rise to muscle fasciculations that were independent of the nerve stimulation. These effects were associated with repetitive action potentials both in the muscle and in the motor nerve, the latter being recorded antidromically in the soleus ventral rootlets (L7 and S_1).

Although, in large enough doses injected close-arterially into the muscle, all of the acetylcholine antagonists abolished all the effects of neostigmine, it was possible, by careful dosage with the different drugs, to dissociate muscle fasciculations and repetitive firing in the nerve from twitch augmentation and repetitive firing in the muscle. Hexamethonium depressed muscle fasciculations and repetitive firing in the nerve in doses slightly smaller than those necessary to diminish the augmented twitches and muscle repetition. On the other hand, gallamine and pancuronium depressed the augmented twitches and the muscle repetition in doses that allowed fasciculations and nerve repetition to continue. With both benzoquinonium and tubocurarine, it was not possible to demonstrate selectivity for any aspect of neostigmine's action, all effects being depressed simultaneously. The results indicate that muscle fasciculations and nerve repetitive firing are related events, as are twitch augmentation and muscle repetition. It is now known that acetylcholine can depolarize motor nerve endings as well as the post-junctional membrane of the muscle endplate (Hubbard, Schmidt & Yokota, 1965). Hexamethonium is relatively more active as a ganglion blocking drug than as a neuromuscular blocking drug, indicating its selectivity for neuronal receptors. Gallamine and pancuronium, however, have little ganglion blocking activity and are relatively selective for muscle receptors. Tubocurarine and benzoquinonium block both ganglionic and muscle receptors in comparable doses. It is concluded that muscle fasciculations and repetitive firing

in nerve produced by neostigmine are primarily due to the action of preserved transmitter acetylcholine on motor nerve endings, whereas twitch augmentation and muscle repetition are primarily post-junctional events. The finding that these actions may be separated by acetylcholine antagonists indicates that slight differences exist between the cholinoceptors on the motor nerve endings and those on the post-junctional muscle endplate membrane. However, the difference is by no means as marked as that between ganglion and muscle receptors. The slight differences between the two populations of cholinoceptors at the pre- and post-junctional sides of the neuromuscular junction may be explained by the concept of "iso-receptors" as described by Jack (1970).

REFERENCES

HUBBARD, J. I., SCHMIDT, R. F. & YOKOTA, T. (1965). *J. Physiol.*, **181**, 810–829.
JACK, D. *Pharm. J.*, **205**, 237–240.

The actions of depolarizing drugs and antagonists on developing muscle fibres *in vitro*

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The stages in development of skeletal muscle fibre up to and after the point of innervation may be successfully studied using cultured cells. With this system, not only the morphological development may be studied, but also the pharmacological evolution of drug receptors, thus presenting an additional source of information into the details of drug action. The development of muscle fibres can be divided into three stages: the myoblast, a mononuclear cell containing no organized contractile elements; the myotube, formed by fusion of the myoblasts and consisting of a multinuclear tube, within the cytoplasm of which are appearing those formed elements characteristic of striated muscle. Contraction is usually shown midway through this stage. The final stage is the muscle fibre as is conventionally recognized. Contractures in response to acetylcholine, carbachol and decamethonium have been evinced from young myotubes (Dryden, 1970) and membrane depolarization resulting from Ach application to myotubes has also been shown (Fischbach, 1970). However, evidence of earlier receptor presence has not hitherto been reported.

Using conventional microelectrode techniques, the resting membrane potential of chick embryo myoblasts in primary culture was found to be $-8{\cdot}3$ mV (s.e. $\pm$ 0·2 mV). After fusion the resting potential rises over successive days in culture to a level of -46 mV (s.e. $\pm$ 2·8 mV). Application of acetylcholine to myotubes at all stages of development resulted in depolarization of the membrane. The minimum effective dose was 10^{-4}M but this decreased with development of the fibre. The response could be inhibited by prior addition of 100 μg/ml (+)-tubocurarine to the culture dish. A similar depolarizing response, also blockable by curare, was found with TMA, but not with bethanecol. The response of the mononuclear cells to the addition of acetylcholine was different. Instead of depolarization to 0 mV, a variable change in membrane potential was found. Dependent on the resting potential, either a depolarization or a hyperpolarization to an equilibrium value of -7 mV was observed. This response also could be blocked by the addition of (+)-tubocurarine.

It is postulated that the changes in myotube sensitivity reflect increasing permeability to ions which begin after fusion of the myoblasts. A nicotinic receptor, however, is present on the membrane of the myoblast before any other morphological or physiological development has occurred.

These results are in partial contrast to those of Harris, Heinemann & Tarakis (1971) who found that a cloned line of rat myoblasts had a resting potential of -70 mV and depolarized on application of Ach. The differences in findings are thought to be attributable either to species difference or, more likely, to partial maturation of the rat myoblasts after several passages in culture.

REFERENCES

DRYDEN, W. F. (1970). *Experientia*, **26**, 984–986.
FISCHBACH, G. D. (1970). *Science*, **169**, 1331–1333.
HARRIS, A. J., HEINEMANN, S. & TARAKIS, H. (1971). *Nature, Lond.*, **231**, 296–301.

The influence of cellular metabolism on the α- and β-adrenoceptor responses of the rabbit small intestine

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The relaxation of the rabbit small intestine produced by sympathomimetics involves (depending on the drug used) interaction with α- and/or β-adrenoceptors (Bowman & Hall, 1970). These two receptor systems are not necessarily coupled to the same intracellular mechanisms.

Manifestation of the β-adrenoceptor effect on the rabbit small intestine was found to be dependent on the integrity of cellular metabolism. Partial glycogen depletion using the methods described by Bueding, Bülbring & others (1967) or treatment with the glycolysis inhibitor iodoacetate (5 mM) abolished the β-adrenoceptor effects of isoprenaline but not the α-adrenoceptor effects of phenylephrine or the α-adrenoceptor-like effects of ATP. Andersson & Mohme-Lundholm (1969, 1970) have recently obtained similar results after glycogen depletion of the rabbit colon and taeniae coli. In the continued presence of iodoacetate the isoprenaline effect was restored when the block was by-passed by adding pyruvate (8 mM) to the organ bath. Although iodoacetate or glycogen depletion did not reduce the extent of the inhibitory response produced by phenylephrine or ATP, they did hasten the onset of recovery from the inhibitory response to these two drugs and did augment the overshoot on washout which is found with these two drugs (Bowman & Hall, 1970). Thus it appears that these two conditions can block the β-adrenoceptor mediated inhibition of the rabbit small intestine and also potentiate the stimulatory phase of the α-adrenoceptor mediated response or of the response to ATP.

The conditions used in these experiments never abolished the spontaneous activity of the rabbit gut, nor did they diminish the inhibitory response of drugs acting on α-adrenoceptors. However the inhibitory effects of β-adrenoceptor agonists were reduced by glycogen depletion and by iodoacetate. Thus it would seem that one of the intermediary products of glycogenolysis is necessary for the expression of β-adrenoceptor mediated effects in the rabbit small intestine, whereas α-adrenoceptor mediated effects utilize an entirely different mechanism. This provides an explanation for the observation that towards the end of a prolonged experiment on an isolated tissue, or with tissues that have been lying on the bench for several hours in a beaker of oxygenated Krebs solution, β-adrenoceptor mediated responses were found to gradually diminish in size. It was shown that glycogen stores are significantly decreased under the latter conditions.

REFERENCES

ANDERSSON, R. & MOHME-LUNDHOLM, E. (1969). *Acta physiol. scand.*, **77**, 372–384.
ANDERSSON, R. & MOHME-LUNDHOLM, E. (1970). *Ibid.*, **79**, 244–261.
BOWMAN, W. C. & HALL, M. T. (1970). *Br. J. Pharmac.*, **38**, 399–415.
BUEDING, E., BÜLBRING, E., GERCKEN, G., HAWKINS, J. T. & KURIYAMA, H. (1967). *J. Physiol., Lond.*, **193**, 187–212.

The extraction and estimation of histamine from *Gossypium* species

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This investigation was a development of work by Lloyd & Nicholls (1964) and others on the constituents of cotton mill dusts likely to cause byssinosis in cotton workers. In this case, authenticated material from *Gossypium hirsutum* L., the Upland cotton, and *G. arboreum* L., tree cotton, was used and the experimental work concerned with the histamine content of the cotton plant.

Histamine was estimated fluorimetrically using a method by Shore, Burkhalter & Cohn (1959) based on a fluorogenic reaction between histamine and *ortho*-phthalaldehyde, (oPT). oPT was known to form a fluorescent complex with substances other than histamine which frequently occurred in biological materials (Turner & Wightman, 1968), and it was found that most of the extraction procedures which had been used to recover histamine from animal tissues also recovered interfering substances from the cotton plant material.

An extraction procedure was devised which excluded the interfering substances but gave a low recovery of histamine. This involved a combination of ion exchange (Huff, Davis & Brown, 1966) and solvent extraction (Shore, Burkhalter & Cohn, 1959): plant material was extracted with N trichloroacetic acid, the extract adjusted to pH 7·5 and passed through Amberlite CG 50 anionic exchange resin, the eluate was subjected to solvent extraction before the fluorogenic reaction with oPT.

The histamine content of fresh tissue of *Gossypium* species was estimated by this procedure: *G. hirsutum*, old leaf, 87 μg histamine/g fresh leaf; *G. hirsutum*, young mature leaf, 101 μg/g; *G. arboreum*, young mature leaf, 113 μg/g. Fresh mature bracts of *G. hirsutum* were estimated to contain 26 μg histamine/g of *G. arboreum*, 6 μg histamine/g.

By comparison, dried leaf of *G. hirsutum* was estimated to contain 1760 μg histamine/g which corresponded to 330 μg histamine/g fresh leaf.

REFERENCES

HUFF, J. A., DAVIS, V. E. & BROWN, H. (1966). *J. Lab. clin. Med.*, **67**, 1044–1047.
LLOYD, G. R. & NICHOLLS, P. (1964). *J. Physiol. Lond.*, **172**, 56–57P.
SHORE, P. A., BURKHALTER, A., & COHN, V. (1959). *J. Pharmac. exp. Ther.*, **127**, 182–186.
TURNER, T. D. & WIGHTMAN, S. L. (1968). *J. Chromat.*, **32**, 315–322.

The estimation of rauwolfia alkaloids by quantitative thin-layer chromatography

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Previous work on the estimation of rauwolfia alkaloids (Harris, Stewart & Court, 1968; Los & Court, 1969) involved thin-layer chromatographic separation followed by ultraviolet spectrophotometric estimation of the eluted alkaloids. Substances extracted from the adsorbent are known to interfere with the spectrophotometric measurements (Harris, 1966) and coloured complexes of the alkaloids can be formed and measured at wavelengths such that interference is minimal (Court, 1968).

Table 1. *Alkaloid content of* Rauwolfia *root bark.*

	R. caffra		*R. vomitoria*	
Alkaloid	Mean percentage	Coefficient of variation	Mean percentage	Coefficient of variation
Ajmalicine	0·016	2·20	—	—
Ajmaline	0·239	1·17	0·090	3·33
Rescinnamine	0·013	1·43	0·105	1·33
Reserpiline	—	—	1·090	1·01
Reserpine	0·016	1·97	0·218	0·46
Serpentine	0·180	1·24	—	—
	(based on 5 determinations)		(based on 10 determinations)	

In this work 10 rauwolfia alkaloids were separated using various chromatographic systems employing silicagel G layers 250 μm thick. The individual alkaloids were recovered by elution in alkaline chloroform and complexed with iodine in citrate-phosphate buffer (pH 4·1). The absorption measurements of the alkaloid-complex solutions were recorded at appropriate wavelengths in the range 365–396 nm. Results were calculated from compensated standard curves.

Recovery of the alkaloids by elution from the plates was investigated and the method was applied to samples of *Rauwolfia caffra* and *R. vomitoria* root barks (Table 1).

The method is more rapid and accurate than the earlier method and yields lower results due to reduced interference.

REFERENCES

COURT, W. E. (1968). *Quantitative Paper and Thin-Layer Chromatography*, p. 29–49. Editor: Shellard, E. J. London: Academic Press.
HARRIS, M. J. (1966). M.Sc. thesis, University of Manchester.
HARRIS, M. J., STEWART, A. F. & COURT, W. E. (1968). *Planta Med.*, **16**, 217–223.
LOS, C. W. & COURT, W. E. (1969). *Ibid.*, **17**, 164–169.

The titrimetric assay of solasodine, a spirosolane of commercial importance from *Solanum laciniatum* Ait.

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Solasodine is a starting material for the steroid industry. It occurs in *Solanum laciniatum* as its 3-β-*O*-glycosides. These are most abundant in the green, unripe fruits. Existing assays for solasodine involve solvent extraction of the glycosides from dried plant material, after which, because the glycosides are not readily estimated, they are hydrolysed and the aglycone is separated and determined titrimetrically or colorimetrically (Birner, 1968). It has been shown that aqueous incubation of sapogenin affording plant material alone and with additives increases sapogenin yield (Hardman & Brain, 1971; Hardman & Wood, 1971). Therefore our assay has been designed to process powdered plant material in water. Extraction of glycosides from aqueous media presents problems; their hydrolysis *in situ* by the addition of HCl to the media was hence the first stage in the assay. Preliminary results are now reported.

Dried unripe fruit, 2·5 g, which had been partially defatted by 24 h continuous extraction with light-petroleum (40–60°), was refluxed for 3 h with 50 ml 2N HCl and cooled to 80°. Solution of ammonia (2 × 20 ml, s.g. 0·880) was added. After further cooling the mixture was filtered and the residue washed and then dried overnight at 60°. The residue with filter paper was extracted with $CHCl_3$ for 24 h in a soxhlet and the extract adjusted to 100 ml with solvent. Aliquots of this solution were titrated automatically with 0·01N $HClO_4$ in dioxan using a recording potentiometric titrator.

Preliminary experiments have shown that 65·5% of the solasodine liberated during the hydrolysis is dehydrated to solasodiene. The end point potentials of this and of solasodine are very similar and the titration does not distinguish between them. The results are therefore expressed as solasodine.

Using this method we have made preliminary investigations of the effects of fine powdering and of partial defatting on the assay and its reproducibility (Table 1).

Table 1. *Replicate assays of dried, unripe fruits of* S. laciniatum.

Undefatted powder; 60% retained by a No. 30 sieve	Undefatted fine powder; 98% passed through a No. 30 sieve	Partially defatted fine powder	Light-petroleum extractive*
2·34	3·60	3·00	0·32
2·61	3·47	3·01	
2·55	3·31	2·98	
		3·00	
		2·98	

Results expressed as % base calculated as solasodine with reference to the dried fat-containing fruits (moisture, 7·5% fat, 6·4%*).

REFERENCES

BIRNER, J. (1968). *J. pharm. Sci.*, **58**, 258–259.
HARDMAN, R. & BRAIN, K. R. (1971). *Phytochem.*, **10**, 519–523.
HARDMAN, R. & WOOD, C. N. (1971). *Ibid.*, **10**, 757–763.

The determination of diosgenin and yamogenin in fenugreek seed by combined column chromatography and infrared spectrometry

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Acid hydrolysis of Moroccan seed of *Trigonella foenumgraecum*, L. (fenugreek), followed by extraction with light-petroleum 40–60° affords a mixture of the epimers diosgenin and yamogenin, 1%, with fixed oil, 6% and free sterol, sterol esters, spirostadienes, and gitogenin. We have separated diosgenin from yamogenin by preparative-t.l.c. and have described how they

may be determined individually in the presence of one another in all proportions by infrared spectrometry (Jefferies & Hardman, 1971). The degree of accuracy attained in the method can be achieved only in the absence of the fixed oil and other components of the crude extract, and we now have a routine procedure for their removal by column chromatography.

Silicagel for adsorption (Woelm), activity II, 6 g per 15 cm × 1 cm column was packed in hexane–ethyl acetate (9:1). The extract was prepared by refluxing 2·5 g whole seed with 100 ml 2N HCl for 2 h. The mixture was cooled and filtered and the residue was made alkaline with dilute ammonia solution before it was dried overnight at 70°. This material was extracted in a soxhlet for 24 h using light-petroleum and the solvent removed in a vacuum. The oily residue was transferred to the column using a total of 10 ml hexane–ethyl acetate (9:1). At a flowrate of 1 ml/min, 90 ml of the same solvent system was used to collect 85 ml (which contained all the unwanted material) and 3 × 5 ml fractions for a t.l.c. check. Then 55 ml hexane–ethyl acetate (3:1) was used to collect 40 ml containing diosgenin and yamogenin together, followed by 3 × 5 ml fractions for a t.l.c. check. All diosgenin and yamogenin residues from one column were then dissolved in 4 ml Analar $CHCl_3$ for infrared assay.

This procedure is suitable for up to 75 mg diosgenin and yamogenin sapogenins in the presence of up to 600 mg fixed oil, approximately three times the extractive from 2·5 g fenugreek seed. The recovery of diosgenin plus yamogenin sapogenin from the column was tested by using mixtures of pure sapogenin (30 mg) with 2N acid-treated fixed oil (250 mg) and gave recoveries of ±4% [coefficient of variation (c.v.) = 2·6%]. The reproducibility was tested by analysis of a crude extract (1% diosgenin plus yamogenin, ratio = 6:4) by twelve columns, and gave c.v. = 2·5% (diosgenin plus yamogenin), 3·3% (diosgenin) and 4·5% (yamogenin). Analysis of twelve × 2·5 g seed gave c.v. = 2·8% (diosgenin plus yamogenin), 2·2% (diosgenin) and 6·8% (yamogenin). For duplicate 2·5 g seed assays (t at $P = 0.05$), the range of error was found to be ±4·4% (total sapogenin), ±3·5% (diosgenin) and ±10·6% (yamogenin).

REFERENCE

JEFFERIES, T. M. & HARDMAN, R. (1971). *Planta Med.* In the press.

The composition and distribution of phytosterols in *Digitalis purpurea* L.

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A comparative study of the distribution of phytosterols of the lipid and glucoside fractions of mature flowering plants was undertaken. The plants were from the same clone, were harvested together and divided into leaf, flower, fruit, stem and root samples.

The phytosterols were generally distributed throughout the plants and small quantities of 7-ene precursors were also present in the lipid fractions, indicating that phytosterol biosynthesis is carried out in all tissues. The leaves were divided into three types according to size. It was evident that young leaves from beneath the inflorescence accumulated glucoside sterols, whilst mature leaves from the basal rosette contained greater quantities of lipid sterols. The proportions of 7-ene sterols were also greatest in mature leaves, indicating that these are the primary site of phytosterol biosynthesis.

In the flower buds and developing flowers high concentrations of both lipid and glucoside sterols were found. The quantities of lipid fraction sterols increased during maturation of the flowers, whilst the glucoside sterols remained at a constantly high concentration. In developing fruits phytosterols continued to accumulate, particularly during fruit ripening. The amounts of 5-ene and 7-ene sterols isolated from the stems and root were comparatively small. However, the amount of glucoside sterols isolated from the stems was higher than that of the roots, and was similar to the young leaves.

The 5-ene sterols of both fractions were found by g.l.c. to consist of β-sitosterol, stigmasterol, campesterol and cholesterol. Certain of the samples from the inflorescence also contained 24-methylene-cholesterol. β-Sitosterol at all times was found to be the major

component of the glucoside and the lipid fractions, but tended to accumulate in mature tissues. Stigmasterol, the C-22 unsaturated isomer, accumulated in young leaves and immature flower buds. The proportions of campesterol were generally similar in most organs with the exception of the roots where larger quantities were detected. It was interesting to note that concentrations of cholesterol glucoside were highest in the younger organs, and it is possible that these tissues are the sites of sterol metabolism to the cardenolides and sapogenins.

It is evident that biosynthesis of sterols occurs in the free form, and that either glucoside or ester formation occurs selectively at a stage when biosynthesis is complete, suggesting that a segregation of biological roles could lie behind this enzymatic selection. Free phytosterols have been implicated in the structure of cell and organelle membranes in association with phospholipids (Ansell & Hawthorne 1964; Evans 1971), and it has been suggested by Kemp, Goad & Mercer (1967) that ester sterols represent an intercellular transportation form. The areas of sterol requirement in the mature plant are the actively growing areas, and the export of sterols from mature leaves at the base of the plant could satisfy a heavy sterol requirement. This would involve the phloem transportation of sterols, possibly as the more hydrophilic glucosides, via the stem to the young leaves and developing inflorescence. The high phytosterol glucoside concentrations of these organs add weight to this suggestion.

REFERENCES

ANSELL, G. B. & HAWTHORNE, J. N. (1964). *Phospholipids, Chemistry, Metabolism and Function;* chapt. 10, Amsterdam: Elsevier.
EVANS, F. J. (1971). Ph.D. Thesis, University of London.
KEMP, R. J., GOAD, L. J. & MERCER, E. I. (1967). *Phytochem.*, **6**, 1609–1615.

The characterization of alkaloid D, a new alkaloid from *Euonymus europaea* L., as armepavine

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Five new alkaloids have recently been isolated from *Euonymus europaea* L. (Celastraceae) growing in Poland (Bishay & Kowalewski, 1971). These alkaloids are not identical with those reported to be present in *E. europaea* (Doebel & Reichstein, 1949; Pailer & Libiseller, 1962; Libiseller & Preisinger, 1962) and have been named alkaloid A, alkaloid B, alkaloid C, alkaloid D and alkaloid E.

A study of ultraviolet, infrared, nmr and mass spectrometric data suggested the possible identity of alkaloid D with the known alkaloid armepavine [1-(4′-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydro-6,7-dimethyoxyisoquinoline]. The R_F values of alkaloid D and an authentic sample of armepavine were found to be identical in several t.l.c. systems and the m.p. of authentic armepavine oxalate was not depressed by admixture with the oxalate of alkaloid D. Hence this alkaloid is considered to be identical with armepavine. The ORD curve of the alkaloid shows that it is *R*-(−)-armepavine.

REFERENCES

BISHAY, D. W. & KOWALEWSKI, Z. (1971). *Herba Polonica*, **17**. In the press.
DOEBEL, K. & REICHSTEIN, T. (1949). *Helv. chim. Acta*, **32**, 592–597.
LIBISELLER, R. & PREISINGER, A. (1962). *Mh. Chem.*, **93**, 417–419.
PAILER, M. & LIBISELLER, R. (1962). *Ibid.*, **93**, 403–416.

Polyphenolases in the 1000 *g* fraction of *Papaver somniferum* latex

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Work by Fairbairn, Palmer & Patterson (1968) and Fairbairn & Djote (1970) has shown that morphine can by synthesized by the latex from tyrosine and dopa and that this is associated with the latex fraction which sediments at 1000 g. Reports of the occurrence of polyphenolase in latex (Meissner, 1966a,b) together with the fact that various workers (Barton & Cohen, 1957) have considered the enzyme complex as possibly responsible for the oxidative coupling reactions involved in the biosynthesis of the alkaloids, have resulted in the present work on polyphenolase in poppy latex and its association with alkaloid biogenesis.

Poppy latex was separated into a 1000 *g* fraction (A), an 11000 *g* fraction (B) and the supernatant (C). The whole of the detectable polyphenolase activity resided in the 1000 *g* fraction. Treatments used designed to rupture the membranes of the organelles were (1) lowering of the osmotic pressure of the solution of organelles below 0·3M (2) freeze/thawing (3) sonication and (4) solubilization with 0·1% Triton X-100. The oxidation of the phenolic substrate (catechol) increased with increased fragmentation of the organelles, the greatest activity being observed with the use of Triton X-100. These experiments also showed that both enzyme and substrate occur within the organelle and therefore indicate compartmentalization within the organelle. The activity of the polyphenolase was inhibited with KCN and DIECA at concentrations of 10^{-4}M. Experiments indicated that up to 50% of the enzyme activity was strongly membrane bound. The substrates oxidized by latex polyphenolase at pH 8·0 were caffeic acid, catechol, *p*-coumaric acid, *p*-cresol, dopa, hydroquinone, hydroxytyramine and tyrosine. No oxidation was observed with ferulic acid, guaiacol, *p*-hydroxybenzoic acid, 2,6,methoxyphenol, ± reticuline, salutaridinol and vanillic acid. These results show that the latex 1000 *g* organelles contain catechol oxidase (Ec. 1.10.3.1) and also give evidence of both tyrosinase and laccase activities. Since this enzyme will not oxidize phenols containing a methoxy-group in the *ortho*-position, it is perhaps not surprising that the intermediates of morphine biosynthesis, (±)-reticuline and salutaridinol were not oxidized. The present evidence also indicates that the 1000 *g* organelles of poppy latex are not lysosomes (de Duve, 1959, Pujarniscle, 1968) nor are they similar to the peroxisomes of Tolbert, Oeses & others (1968) or the glyoxosomes of Cooper & Beevers (1969).

REFERENCES

BARTON, D. H. R. & COHEN, T. (1957). Festschrift A. Stoll, Basel. 117.
COOPER, T. G. & BEEVERS, H. (1969). *J. biol. Chem.*, **244**, 3507.
DE DUVE (1959). *Subcellular Particles.* p. 128–157. Ronald Press Co., N.Y.
FAIRBAIRN, J. W. & DJOTE, J. (1970). *Phytochem.*, **9**, 739.
FAIRBAIRN, J. W., PALMER, J. M. & PATERSON, A. (1968). *Phytochem.*, **7**, 2117.
MEISSNER, L. (1966a). *Flora Abt. A. Bd.*, **157**, 1–26.
MEISSNER, L. (1966b). *Ibid.*, **156**, 634–654.
PUJARNISCLE, A. (1968). *Physiol. Veg.*, **6**, 27–46.
TOLBERT, N. E., OESES, A., KISARKI, T., HAGEMAN, R. H. & YAMAZAKI, R. K. (1968). *J. biol. Chem.*, **243**, 519.

The preservation of ophthalmic solutions with antibacterial combinations

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Preserved solutions of pilocarpine hydrochloride (1·0%) and of atropine sulphate (1·0%) were sterilized by autoclaving at 115° for 30 min while preserved solutions of physostigmine sulphate and of salicylate (0·25%) were sterilized by heating at 98–100° for 30 min. Preservatives used were benzalkonium (0·01%), chlorhexidine (0·01%), phenylmercuric nitrate (PMN) (0·002%), chlorocresol (0·05%) and chlorbutol (0·5%) as simple solutions, and also as combinations with either phenylethanol (0·4%) or disodium edetate (EDTA) (0·05%). Solutions were contaminated on two separate occasions with 10^6–10^7 cells/ml from overnight cultures of *Pseudomonas aeruginosa* NCTC 6750.

Phenylethanol-antibacterial combinations killed the inoculum within 15 min except for one formulation with chlorhexidine and physostigmine salicylate (30 min), and all formulations with PMN (45–90 min). Nevertheless simple solutions of antibacterial alone in these formulations had much slower sterilization times than the phenylethanol combinations.

Benzalkonium with pilocarpine and with physostigmine sulphate had sterilization times within 15 min, but with atropine the time was 60 min. Benzalkonium is either less effective in atropine solutions than in solutions of the two other alkaloids or the inoculum into the atropine had higher intrinsic resistance. The phenylethanol-benzalkonium and EDTA-benzalkonium combinations, however, were both effective within 15 min.

Chlorbutol in simple solution has slower sterilization times with physostigmine salts (30 and 45 min) than with pilocarpine and atropine (15 min). This can be explained in terms of pH. The pH values of the autoclaved solutions are in the range 2·2–2·4 but the range for the steamed solutions is 3·1–3·6.

EDTA-PMN, EDTA-chlorbutol and EDTA-chlorocresol combinations show no clear advantage over the antibacterials in simple solution. The mode of action of the antibacterial agent and the state of resistance of the *P. aeruginosa* may determine whether EDTA enhances antibacterial activity or not.

Chlorhexidine had a slow sterilization time of 180 min with physostigmine salicylate. The sodium metabisulphite in the preparation may be reducing the effectiveness of the chlorhexidine. Phenylethanol-chlorocresol and phenylethanol-chlorbutol combinations sterilize physostigmine salicylate within 15 min.

These results, in conjunction with previous work (Richards, Suwanprakorn & others, 1969; Richards & McBride, 1971a,b; Richards, 1971) support the use of phenylethanol 0·4% in combination with other antibacterial agents in the preservation of ophthalmic solutions against contamination with *P. aeruginosa*.

REFERENCES

RICHARDS, R. M. E. (1971). *J. Pharm. Pharmac.* In the press.
RICHARDS, R. M. E. & MCBRIDE, R. J. (1971a). *Brit. J. Ophthal.* In the press.
RICHARDS, R. M. E. & MCBRIDE, R. J. (1971b). *J. Pharm. Pharmac.* In the press.
RICHARDS, R. M. E., SUWANPRAKORN, P., NEAWBANIJ, S., & SURASDIKUL, N. (1969), *Ibid.*, **21**, 681–686.

Effects of drying on polymyxin sensitivity of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa cultures dried over P_2O_5 during studies on cell wall composition were about 200-fold more resistant to polymyxin than before desiccation. Webb (1967) has reported possible mutagenic effects of desiccation on *Escherichia coli*.

P. aeruginosa strains NCTC 6750 and 1999, NCIB 8625 and several laboratory strains were cultured at 37° in nutrient broth either 100 ml in flasks in a shaking water bath or in 8 litre stirred magnetically. Cells were harvested by centrifugation, unwashed or washed three times in 0·9% NaCl, and the pellets stored in a vacuum desiccator over P_2O_5. The minimum inhibitory concentration (MIC) of polymyxin B sulphate (units/ml) in broth using inocula of 10^6 (total count) in final volume of 5 ml was measured before and after drying. The MIC increased from about 10–20 units/ml to over 2000 units/ml. These increases in resistance occurred with all strains on several occasions. With *P. aeruginosa* NCTC 6750 several consecutive attempts to increase resistance by vacuum drying were unsuccessful although previous and subsequent attempts using the same procedures were successful with this strain.

The resistance persisted through repeated subculture and was associated with colonial variants. These were small cream-yellow colonies similar in appearance to polymyxin resistant mutants obtained by selection. Colonies of both kinds occurred after drying and were picked off the surface of agar plates, diluted and standardized by optical density and the MIC measured. Typical green colonies were polymyxin sensitive and cream-yellow colonies were resistant. Colony plate counts and most probably number estimations in broth showed

between about 99 and 99·9% kill on vacuum drying. Differential colony counts and MIC determinations with varying sized inocula indicated that a high proportion of bacteria surviving vacuum drying were polymyxin resistant. Comparison was made with cultures freeze dried in Stamp's (1947) medium. Freeze drying reduced the count only to about 70% of the original and sensitivity was unaltered.

Vacuum dried whole cells were used as inocula for 8 litre nutrient broth cultures. Whole cell and cell wall preparations were analysed for readily extractable lipid (REL), calcium and magnesium (Brown & Watkins, 1970). The greatest difference was in wall phospholipid and wall Mg which were several-fold less in preparations from vacuum dried inocula.

These results support the hypothesis that drying had mutagenic effects.

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REFERENCES

BROWN, M. R. W. & WATKINS, W. M. (1970). *Nature (Lond.)*, **227**, 1360–1361.
STAMP, Lord (1947). *J. gen. Microbiol.*, **1**, 251.
WEBB, S. J. (1967). *Nature (Lond.)*, **213**, 1137–1139.

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Effect of slime on the sensitivity of *Pseudomonas aeruginosa* to EDTA and polymyxin

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The slime of *Pseudomonas aeruginosa* has been implicated in its resistance to chemotherapy (Brown & Richards, 1964). We have been unable to find published work investigating the rôle of slime in the resistance of this organism. We report *in vitro* studies with ethylene-diaminetetra-acetic acid (EDTA) and polymyxin B sulphate using slime producing gluconate cultures and non-slime producing glucose cultures of *P. aeruginosa* in chemically defined media (Brown, Scott Foster & Clamp, 1969). Sensitivity was measured using methods described by Brown & Melling (1969).

Inocula from 6 day, slimy, stationary phase cultures were incubated in fresh gluconate media and challenged with EDTA and polymyxin immediately they entered the exponential phase. Comparison was made both with 6 day non slimy glucose cultures treated in this way, and also with cultures inoculated with cells in the exponential phase in both media.

Early exponential cultures derived from 6 day stationary phase inocula were more sensitive to both agents than were cultures derived from log phase inocula. Slime slightly enhanced resistance to both agents, especially to polymyxin.

Stationary phase glucose and gluconate cultures incubated for 2 and 7 days were tested for lysis by EDTA and polymyxin. Slime had little effect on polymyxin sensitivity; 2 day cultures were the most sensitive. Slime had a significant effect in reducing EDTA sensitivity.

In general, these *in vitro* results suggest that slime has only a minor rôle in sensitivity to the agents tested. A significant rôle *in vivo* is not excluded.

REFERENCES

BROWN, M. R. W. & MELLING, J. (1969). *J. gen. Microbiol.*, **59**, 263.
BROWN, M. R. W. & RICHARDS, R. M. E. (1964). *J. Pharm. Pharmac.*, **15**, *Suppl.* 51T.
BROWN, M. R. W., SCOTT FOSTER, J. H., & CLAMP, J. R. (1969). *Biochem. J.*, **112**, 521.

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Metabolism of 3-deoxy-3-fluoro-D-glucose by *Pseudomonas aeruginosa*

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As part of a program of investigation concerned with the metabolic effects of monofluorinated deoxysugars on yeast (Woodward, Taylor & Brunt, 1969) and certain micro-organisms, including *Pseudomonas fluorescens* (White & Taylor, 1970), we have investigated the effect of 3-deoxy-3-fluoro-D-glucose (3 FG) on *Pseudomonas aeruginosa*.

P. aeruginosa was incubated in a mineral salts medium with glucose, glucose + 3 FG, and 3 FG as carbon sources at 37° on an orbital shaker. At first growth only occurred where glucose was present as the carbon source, but subsequently the organism was induced to grow on 3 FG as the sole carbon source. Subsequent experiments showed that growth rates, utilisation of carbon source, and final cell density were similar for both glucose and 3 FG. In addition, after an initial lag, fluoride ion (F^-) was released quantitatively. 3-deoxy-3-fluoro-D-gluconic acid (3 FGA) and 3-deoxy-3-fluoro-2-keto-D-gluconic acid (3 F2KGA) also served as sole carbon sources, F^- being quantitatively released after a lag period. No F^- was detected when *P. aeruginosa* was incubated with β-fluoro-pyruvate.

Oxygen uptake was studied using Warburg respirometers (Umbreit, Burris & Stauffer, 1964) and it was found that during the lag phase when F^- was not released two atoms of O_2 were consumed per mol of 3 FG and one atom of O_2 per mol of 3 FGA ozidized.

Using Eastman precoated silica gel t.l.c. sheets and a solvent system composed of acetic acid–ethyl acetate–water (3:3:1), 3 FGA was detected in the concentrated culture filtrate of lag phase 3 FG cultures. The spots were visualized with *p*-anisidine. 3 F2KGA was not identified positively using this system, but previous work with gas-liquid chromatography has suggested that it was present in trace amounts.

Work with fractionated, disrupted, cell suspensions (Watkins, 1970) showed that the cell envelope fraction released F^- from 3 FG and 3 FGA at approximately ten times the rate as did the cytoplasmic fraction.

These results suggest that *P. aeruginosa* metabolized 3 FG by the Entner-Douderoff pathway in a similar manner to glucose. The oxidation proceeded as far as 3 F2KGA with the consumption of two O_2 atoms per mol of 3 FG, at which stage the F^- is lost when the 6 carbon compound is cleaved to two 3 carbon compounds. The enzyme system responsible for the C–F bond cleavage is probably located in the cytoplasmic membrane.

REFERENCES

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1964). *Manometric Techniques*. pp. 1–61. Minneapolis: Burgess.
WATKINS, W. W. (1970). Ph.D. Thesis, Bath University.
WHITE, F. H. & TAYLOR, N. F. (1870). *FEBS Letters*, **11**, 268.
WOODWARD, B., TAYLOR, N. F. & BRUNT, R. V. (1969). *Biochem. J.*, **114**, 445.

The inactivation of phenylmercuric nitrate by sodium metabisulphite

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During work in this School concerning the uptake of phenylmercuric nitrate from solution by rubber closures, a greater rate of fall in phenylmercuric nitrate concentration was noted in the presence of sodium metabisulphte than in its absence. Aqueous solutions containing both phenylmercuric nitrate and sodium metabisulphite were therefore examined to assess any effect the latter substance might have on the phenylmercuric nitrate concentration under normal conditions of storage and sterilization. Phenylmercuric nitrate concentrations were determined by a polarographic method (Porter, 1968); antibacterial activity was monitored by the cup plate method with *Staphylococcus aureus* (N.C.T.C. 7447), as test organism.

Storage experiments. Ampoules containing a solution of phenylmercuric nitrate (20·0 μg/ml) with sodium metabisuphite (1·0 mg/ml) were kept at laboratory temperature and assayed weekly. Both phenylmercuric nitrate content and antibacterial activity fell steadily;

after five weeks the phenylmercuric nitrate concentration had fallen to 10·0 μg/ml with a corresponding reduction in antibacterial activity. Control solutions containing phenylmercuric nitrate only, showed no reduction in concentration or activity.

Heat experiments. Ampoules containing a solution of phenylmercuric nitrate (20·0 μg/ml) with sodium metabisuphite (1·0 mg/ml) were assayed after (a) exposure to a temperature between 98° and 100° for increasing periods of time and (b) autoclaving (115°, 30 min). The phenylmercuric nitrate concentration in (a) fell rapidly; after thirty minutes only 5·0 μg/ml remained and the antibacterial activity had diminished. In case (b) the presence of neither phenylmercuric nitrate nor antibacterial activity could be demonstrated. Controls in both cases showed no reduction in concentration or activity.

The use of phenylmercuric nitrate as a preservative has been criticized on other grounds (Brown & Norton, 1965; Norton, private communication), and the above results give further weight to the argument for its discontinuance. More disquieting, however, were the results obtained in examining heat-sterilized official eye drops and injections formulated to contain both phenylmercuric nitrate and sodium metabisulphite. Whether prepared at this School or purchased from reputable manufacturers, only negligible amounts (<1·0 μg/ml) of phenylmercuric nitrate could be detected, and no antibacterial action could be demonstrated.

No official test is specified to ensure the antibacterial activity of added preservation in eye drops or injections. This may explain why the inactivation of phenylmercuric nitrate by sodium metabisulphite has not hitherto been reported.

REFERENCES

BROWN, M. R. W. & NORTON, D. A. (1965). *J. Soc. Cosmetic Chemists*, **16**, 369–393.
PORTER, G. S. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 43S–44S.

Progress towards a standard to limit particulate contamination in intravenous fluids

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Instrumental methods for detecting unwanted undissolved solid particles in intravenous solutions are more objective than the visual inspection methods at present in use.

Following the observation that there is usually a log-log relation between particle size and cumulative number, Groves (1969) proposed a standard written in the form-specific value (S) = (log N_{10}–2·5)/M, where N_{10} is the estimated number of particles at a threshold of 1·0 μm, and M is the slope of the log-log distribution. This takes into account the fact that the numbers of particles and the slopes of the distributions varied widely from container to container, even those from the same batch, and is not confined to one instrumental method or principle of detection.

The response of a number of Coulter machines to both a square wave generator and to a standardized suspension of a polystyrene-DVB latex (mean diameter 8·25 μm) was measured. The standard error was ±0·31% of the mean response to the square wave generator for eleven machines, and ±1·96% of the mean count on the suspension for twelve machines. Provided suitable calibration materials were available it is suggested that the main obstacles to a collaborative trial of this instrument could be overcome.

The validity of the log-log relation between the numbers of contaminating particles and particle size was confirmed up to 30 μm using a HIAC Model P305-SST Automatic Particle Counter (Carver, 1969). The HIAC instrument was unable to count at levels exceeding 3000/ml. Since, in good quality intravenous solutions encountered in practice, counts rarely exceed 50 particles per ml at a threshold of 5·0 μm (e.g. Appino & Robinson, 1969) this limitation on counting rate is unlikely to constitute a problem.

Four bottles rejected from a hospital-made batch of Sodium Chloride Injection B.P. because of the presence of visible particles were examined using both the Coulter Counter and the HIAC instruments. Coulter counts were made using the device described earlier (Groves, 1969). The bottle was then disconnected and attached to the receiver of the HIAC. Replicate counts were taken on successive 40 ml volumes until the bottle contents had been used up.

Specific values measured were as follows:—

Bottle number	1	2	3	4
Coulter	0·70	0·62	0·62	0·64
HIAC	0·68	0·62	0·61	0·68

It is concluded that the two instruments are broadly comparable, and are suitable for the purpose of counting particulate contamination in intravenous solutions.

Collaboration between different laboratories is required to establish the utility of this approach to the problem of assessing contamination, and to devise a realistic standard of cleanliness.

I am grateful to Mr C. A. Johnson of the British Pharmacopoeia Commission for valuable discussions on this topic.

REFERENCES

APPINO, J. B. & ROBINSON, R. U. (1969). *Annals New York Acad. Sci.*, **158** (3), 680–689.
CARVER, L. D. (1969). *Ibid.*, **158**, 710–721.
GROVES, M. J. (1969). *Analyst*, **94**, 992–1001.

Some investigations of the effect of a non-ionic surfactant on the diffusion of hydrocortisone across a cellulose acetate membrane

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The authors report further studies of the effects of non-ionic surfactants upon the diffusion of steroids across a cellulose acetate membrane. Diffusion rates have been measured using the 'short' and 'ultra-short' time methods recently developed (Short, Abbs & Rhodes, 1970; Short, Jenkins & Rhodes, unpublished); the apparatus was also described in these publications. The temperature dependence of the diffusion rate has been examined in systems with and without n-alkyl polyoxyethylene surfactant. In all cases the Arrhenius equation was obeyed, e.g. correlation coefficient for five temperatures 0·997. The diffusional energies, ΔH_d, and entropies, ΔS_d, have been calculated, following the method of Barrer (1939). In distilled water the values were estimated to be $36{\cdot}0 \times 10^3$ J mol^{-1} and $-3{\cdot}4 \times 10^2$ J deg^{-1} mol^{-1} respectively.

The enthalpy, ΔH_b, and entropy, ΔS_b, of micellar binding, calculated by previously described methods (Molyneux, Rhodes & Swarbrick, 1965; Humphreys & Rhodes, 1968; Molyneux & Rhodes, unpublished), were found to be $-17{\cdot}6 \times 10^3$ J mol^{-1} and -22 J deg^{-1} mol^{-1} respectively.

Interpretation of the diffusion and micellar binding results has led to the development of a simple thermodynamic hypothesis. This can explain how surfactants can both increase and decrease diffusion rates even when, as in the systems investigated in this work, the surfactants do not modify membrane permeability by direct interaction with the membrane. From the micellar binding data values of ΔH_d and ΔS_d, with 2% $C_{16}E_{30}$ solutions on the donor side of the membrane, have been predicted. The agreement between the predicted and experimental values is good, ΔH_d 53·9 (expt 50·9) $\times 10^3$ J mol$^{-1} \times 10^3$, ΔS_d $-3{\cdot}1$ (expt $-2{\cdot}9$) $\times 10^2$ J deg^{-1} mol^{-1}.

REFERENCES

BARRER, R. M. (1939). *Trans. Farad. Soc.*, **35**, 628–643, 644–656.
HUMPHREYS, K. J. & RHODES, C. T. (1968). *J. pharm. Sci.*, **57**, 79–83.
MOLYNEUX, P., RHODES, C. T. & SWARBRICK, J. (1965). *Trans. Farad. Soc.*, **61**, 1043–1052.
SHORT, P. M., ABBS, E. T. & RHODES, C. T. (1970). *J. pharm. Sci.*, **59**, 995–998.

Kinetics of structure build-up in self-bodied emulsions stabilized by mixed emulsifiers

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The semi-solid properties of emulsions prepared with surfactant-long chain alcohol mixed emulsifiers are due to viscoelastic networks present in the continuous phases. These networks have similar properties to ternary systems formed by dispersing the mixed emulsifiers in water (e.g. Barry & Saunders, 1970). In this work, the differences between the mechanisms which operate to form ionic and non-ionic networks were investigated.

Ternary systems and emulsions prepared with anionic (sodium dodecyl sulphate), cationic (cetrimide) or non-ionic (cetomacrogol) surfactants and cetostearyl alcohol were examined in creep and in continuous shear rheometry at a storage temperature of 25°. Systems were tested frequently during the first 24 h after preparation, and then regularly for 3 months. Short time creep tests were analysed to derive continuous spectra of retardation times, using a second order approximation method (Leaderman, 1958), equation 1,

$$L(\tau) \sim \frac{d}{d/nt}\left[J(t) - \frac{dJ(t)}{d/nt}\right] \Bigg| \; t = 2\tau \qquad 1$$

where $L(\tau)$ is the value of a retardation spectrum, which is of the nature of a distribution function, although it has the dimension of compliance, and J(t) is the total compliance at time t. For each surfactant the results obtained were correlated with the mechanisms by which gel networks formed.

With age, anionic and cationic ternary systems and emulsions behaved similarly with respect to total compliance, retardation spectra and continuous shear parameters. Additional structure, although not extensive, formed within the first 2 h storage. This was indicated in creep by initial falls in total compliance values and decreases in spectral heights, and in continuous shear by corresponding increases in apparent viscosities and hysteresis loop areas. Thereafter negligible new structure formed, and thus spectra for systems tested after 2·5 and 24 h storage were similar, and there was little variation in total compliances, apparent viscosities and loop areas. The essentially constant position of spectral maxima indicated that the high temperature interaction which occurred during the preparation of ionic systems was the major mechanism which operated to form gel networks. The low temperature interaction occurring during the first 2 h storage was insignificant.

Cetomacrogol systems behaved differently. Structure built up rapidly during the first 24 h storage. This was shown in creep by marked reductions in total compliances and spectral heights, and in continuous shear by changing complex flow properties. The movement of maxima in the spectra to longer times indicated that mechanisms which operated to form gel networks at the storage temperature produced structures which differed at a molecular level from those formed at high temperatures.

REFERENCES

BARRY, B. W. & SAUNDERS, G. M. (1970). *J. Colloid Inter. Sci.*, **34**, 309–315.

LEADERMANN, H. (1958). In *Rheology, Theory and Applications*. Editor: Eirich, F. R. Vol. 2, 1–61, London: Academic Press.

The solubilization of salicylic acid by a series of non-ionic surfactants

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Solubilization may be used to increase the solubility of otherwise poorly soluble drugs. Recent work has shown that the bioavailability of solubilized drugs is not necessarily increased. Since only the unionized form of weak acids and bases is absorbed from the gastrointestinal tract, it would be useful to differentiate between the interactions of ionized drug molecules and of unionized drug molecules with surfactant micelles. The extent of drug/surfactant interactions can be conveniently expressed as a distribution ratio of the drug between micellar and non-micellar phases.

The distribution ratios of the ionized and unionized molecules of salicylic acid have been determined between water and micelles of the following polyoxyethylene (20) sorbitan esters: monolaurate (polysorbate 20); monopalmitate (polysorbate 40); monostearate (polysorbate 60) and monooleate (polysorbate 80).*

Excess salicylic acid was added to solutions of surfactant at different pH's. The solutions were shaken in a constant temperature bath for 48 h at 25° and pH was adjusted using a Radiometer pH stat. Filtered diluted samples were assayed spectrophotometrically for salicylic acid.

Plots of D_T/D_{H_2O} as a function of volume fraction, M, of polysorbate at several pH values, where D_T and D_{H_2O} are the solubilities of salicylic acid in polysorbate solutions and water respectively, are linear. The slopes, S, of these lines at each pH, plotted against the percentage of unionized salicylic acid present at that pH, are also linear. The values of S obtained by extrapolation to 0 and 100% unionized salicylic acid, are the distribution ratios K_i and K_u, of the ionized, and unionized molecules of salicylic acid respectively (Rippie, Lamb & Romig, 1964).

The distribution ratios of ionized molecules are zero indicating that the molecules do not partition into polysorbate micelles, supporting the conclusions of Hurwitz, Deluca & Kostenbauder (1963) that organic ions must have a large hydrophobic group to enable them to be solubilized by non-ionic surfactants. The distribution ratio of unionized salicylic acid increases as the alkyl chain length of the surfactant increases. It may thus be expected that the amount of unionized acid available for absorption from surfactant solutions will be dependent upon the nature of the surfactant.

REFERENCES

HURWITZ, A. R., DELUCA, P. P., & KOSTENBAUDER, H. B. (1963). *J. pharm. Sci.*, **52**, 893–897.
RIPPIE, E. G., LAMB, D. J. & ROMIG, P. W. (1964). *Ibid.*, **53**, 1346–1348.

* Tween 20, 40, 60 and 80 respectively, supplied by Honeywill-Atlas Ltd.

Prediction of the micellar molecular weight and thermodynamics of micellization of mixtures of alkyltrimethylammonium salts

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Shinoda's (1954) equation for the critical micelle concentration (cmc) of a soap mixture and published data for pure surfactants were used to derive a theoretical expression for the micellar molecular weight (M) of a surfactant mixture.

$$\Sigma M_{mi}^{-\frac{1}{2{\cdot}303D}\frac{\omega}{(kT)}\frac{x_i' \exp(m_i\,\omega/kT)}{\Sigma x_i' \exp(m_i\,\omega/kT)}} = M_{mix}^{-\frac{1}{2{\cdot}303D}\frac{\omega}{(kT)}} \qquad (1)$$

where ω is the energy change per methylene radical in passing from the aqueous phase to the interior of the micelle, k is the Boltzmann constant and T is the absolute temperature. Mm_i is the micellar molecular weight of a surfactant i of chain-length m, and x is the mol fraction of the surfactant i in a state of molecular dispersion. D is the regression coefficient of the straight-line plot of log M *vs* alkyl chain-length for pure surfactants. Experimental values of M for

some commercial samples of quaternary ammonium bromides containing up to seven components (Barry, Morrison & Russell, 1970) were measured by light scattering. Table 1 shows good agreement between theoretical and experimental values of M.

Table 1.

Commercial surfactant	Temperature °C	M from light scattering data $\times 10^{-4}$	M from eqn (1) $\times 10^{-4}$
Cetrimide B.P.	25	2·53	2·68
Dodecyltrimethylammonium bromide	25	2·09	2·15
Tetradecyltrimethylammonium bromide	25	2·73	2·60
Hexadecyltrimethylammonium bromide	30	3·33	3·62

The temperature dependence of the cmc of these mixtures was determined and an estimate of the effect of temperature upon the degree of counterion binding to the micelle was deduced. These values were used to calculate the thermodynamic parameters ΔG, ΔH, ΔS, and ΔC_p at different temperatures from equations based on the phase separation and mass action models of micellization. Values obtained from the former model were more negative since this model does not consider the extent of counterion binding to the micelle. Trends in all parameters were explained with regard to the structural changes in water. Results showed that the thermodynamic parameters of micellization of mixtures of surfactants of known composition yield as valuable information as those of single surfactants which are often difficult to prepare pure.

REFERENCES

BARRY, B. W., MORRISON, J. C. & RUSSELL, G. F. J. (1970). *J. Colloid Int. Sci.*, **33**, 554–561.
SHINODA, K. (1954). *J. phys. Chem.*, **58**, 541–544.

Monomer concentrations in micellar drug systems

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Some drugs in the local anaesthetic, tranquillizer and antibiotic classes are surface-active and may exert their action by interaction with membranes. Many of these surface-active drugs form micelles (Florence, 1968) and if the active species is the monomer, it is important that the concentration of monomer in the micellar system is known. Monomer concentrations can be obtained by interpretation of concentration dependent shifts of nuclear magnetic resonance (nmr) spectra (Corkill & others, 1969). The method is relatively simple and requires no assumptions about micellar charge or size, but the method requires independent means of verification.

The Law of Mass Action was applied to four systems containing phenothiazines in an attempt to confirm nmr data obtained previously (Florence & Parfitt, to be published). Application of the Law to the micellization process,

$$jD^+ + (j-Z)X^- \rightleftharpoons M^{z+}$$

where the phenothiazine micelle consists of j monomers (D^+) with j-Z firmly bound anions necessitates a knowledge of j and Z. Aggregation numbers for chlorpromazine, promazine, promethazine and thioridazine hydrochlorides in aqueous solution have been obtained by light-scattering. These are in the range 8 to 11. The number of unit charges per micelle determined by dye-tracer electrophoresis and also by conductivity techniques are high (6 to 8) indicating that few anions are tightly bound to the micelle surfaces. Using this experimental information in the mass-action calculations of monomer concentration, good agreement was obtained with the values derived from nmr, thus substantiating the validity of the latter method and the findings i.e. that at any given phenothiazine concentration above the cmc the amount of monomers in the system can vary by a factor of 5 in the series studied, being lowest for thioridazine and highest for promethazine. In all systems studied the concentration of monomers does not change appreciably above the cmc.

Determinations of the rate of dialysis of chlorpromazine across Visking membranes qualitatively agree with these observations in that the rate of transport decreases as the drug concentration is increased above the cmc. This phenomenon may have applications in sustained release technology.

REFERENCES

CORKILL, J. M., GOODMAN, J. F., WALKER, T. & WYER, J. (1969). *Proc. Roy. Soc.*, A **312**, 243–255.

FLORENCE, A. T. (1968). *Adv. Colloid Inter. Sci.*, **2**, 115–149.

The use of methylene chloride and chloroform for the extraction of tertiary alkaloids from *Strychnos* species

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When strychnine is extracted with chloroform a crystalline quaternary salt may separate out (Von Klemperer & Warren, 1955; Caws & Foster, 1956). Chloroform itself does not react with strychnine and it has been thought that methylene chloride, which is present as an impurity, is responsible (Caws & Foster, 1957). Strychnine refluxed with methylene chloride for 10 h gives the chloromethochloride which is not identical with the quaternary salt formed when chloroform is used. This salt is strychnine chloromethobromide formed from chlorobromethane another impurity in chloroform (Caws & Foster, 1957).

Chloroform and methylene chloride are both useful solvents for strychnine and related alkaloids which tend to be relatively insoluble in many common organic solvents. During the screening of strychnos material it was noted that a few alkaloid-rich extracts gave needle crystals which proved to be quaternary salts formed from strychnine-type alkaloids and methylene chloride. Although the indications from the literature (Caws & Foster, 1957) are that this is a slow reaction (see above) the present work shows that strychnine and brucine solutions in methylene chloride can form heavy crops of quaternary salt crystals within 2 h at laboratory temperature. Each of these salts gives two major spots on t.l.c. In order to study the behaviour of these compounds more closely the following quaternary salts of strychnine and brucine were prepared:—(a) chloromethochloride, (b) chloromethobromide, (c) bromomethochloride, (d) bromomethobromide.

T.l.c. of the mother liquors of the quaternary salts formed on allowing strychnine to stand in chloroform reveals the presence of three other major alkaloidal constituents. Two of these have been characterized as strychnine *N*-oxide and pseudostrychnine. Although this may cast doubt on whether these compounds occur naturally, control experiments have indicated that during normal extraction procedures only very small amounts of these compounds are formed.

Pseudostrychnine, pseudobrucine, icajine, novacine and vomicine do not appear to form quaternary salts with chloroform or methylene chloride.

It is clear from the work discussed above and from preliminary experiments with other alkaloids that the use of chloroform and methylene chloride may lead to considerable changes in the nature of alkaloids and their extracts. Hence when these solvents are used for alkaloid extraction care should be taken to determine whether or not the alkaloids remain chemically unchanged.

REFERENCES

CAWS, A. C. & FOSTER, G. E. (1956). *J. Pharm. Pharmac.*, **8**, 790–799.

CAWS, A. C. & FOSTER, G. E. (1957). *Ibid.*, **9**, 824–833.

VON KLEMPERER, M. E. & WARREN, F. L. (1955). *Chemy Ind., Lond.*, 1553.

The tertiary alkaloids of some Asian species of *Strychnos*

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In our screening program for alkaloids, the extracts from more than 200 samples mostly from herbarium collections, belonging to 34 Asian *Strychnos* species, have been examined by t.l.c. and g.l.c. methods. The results obtained with *S. nux-vomica* L. and *S. wallichiana* Steud. ex DC. (*S. colubrina* L.) are particularly interesting in that:—

1. The alkaloid composition of the leaf and seed, irrespective of age (up to 300 years old) appeared to be unchanged.

2. Both species contained alkaloids of the following types:—Normal series: strychnine, brucine, strychnine *N*-oxide, brucine *N*-oxide; pseudo series: pseudostrychnine, pseudobrucine; *N*-methyl-pseudo series: icajine, vomicine, novacine.

3. Examination of different plant parts of the two species showed that in the root bark and root wood alkaloids of the normal series tend to predominate; in the stem bark pseudo and *N*-methyl-pseudo alkaloids are the most important; in the leaves the main alkaloids belong to the *N*-methyl-pseudo series (cf. Maier & Groger, 1968; Sefcovic, Dubravkova & Torto, 1968); and in the seeds again normal seriesbases predominate. There is evidence that in *S. nux-vomica* the normal bases are formed in the roots (Schlatter, Waldner & others, 1969). Our data from *S. nux-vomica* and *S. wallichiana* suggest that as the alkaloids are transported up the plant through the wood they are gradually converted from bases of the normal series to bases of the pseudo and *N*-methyl-pseudo series, so that when they reach the leaves the *N*-methyl-pseudo alkaloids predominate. It is possible that the reverse process may be taking place if the alkaloids descend from the leaves through the bark.

Among the other species screened were:—

1. *S. ignatii* Berg., seed samples of which gave results very similar to those of *S. nux-vomica*.

2. *S. nux-blanda* A. W. Hill, leaf and seed samples of which contained small amounts of alkaloids similar in composition to those of *S. nux-vomica* except for the frequent occurrence of diaboline.

3. *S. potatorum* L.f., which contained diaboline as the major alkaloid in the leaves, seeds, and bark.

REFERENCES

MAIER, W. & GROGER, D. (1968). *Pharm. Zentralhalle*, **107**, 883–885.
SCHLATTER, C., WALDNER, E. E., SCHMID, H., MAIER, W., & GROGER, D. (1969). *Helv. chim. Acta*, **52**, 776–789.
SEFCOVIC, P., DUBRAVKOVA, L. & TORTC, F. G. (1968). *Planta Med.*, **16**, 143–146.

The conversion of *pseudo* heteroyohimbine alkaloids to oxindole alkaloids

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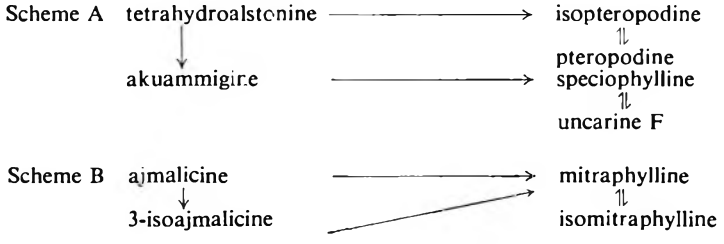
In the hypothesis put forward by Shellard, Phillipson & Gupta (1969) regarding the origin of oxindole alkaloids in the genus *Mitragyna*, the possibility that *pseudo* indole alkaloids could be involved was discounted because of the instability of the corresponding *pseudo* oxindole alkaloids. However, in some species of *Mitragyna* there is evidence that while *normal* oxindoles are present, the corresponding *normal* indoles do not occur although the corresponding *pseudo* indoles are present. This has led to a reconsideration of the hypothesis.

Employing the methods of Finch & Taylor (1962) and Shavell & Zinnes (1962) the *pseudo* mitrajavine has been converted to the *normal* javaphylline and isojavaphylline and the *pseudo* mitraciliatine has been converted to the normal rhynchociline and ciliaphylline. These *in vitro* conversions encouraged attempts to obtain similar *in vivo* conversion of *pseudo* indole alkaloids to *normal* oxindole alkaloids.

Young plants of *Mitragyna parvifolia* (Roxb.) Korth grown from seeds obtained from Ceylon

and containing the alkaloidal system shown in Scheme A were used. By means of the wick method of feeding through the xylem both ajmalicine (*normal*, closed E ring) and 3-isoajmalicine (*pseudo*, closed E ring) were introduced to the plant. In both cases after 24 h the corresponding *normal* closed E ring oxindoles, mitraphylline and isomitraphylline were present in the leaves. Since 3-isoajmalicine could not be detected when ajmalicine was fed into the plant there is some doubt about the hypothesis that this alkaloid is formed by conversion of the C(3)H-α ajmalicine to C(3)H-β. These results support the modified proposals that *normal* oxindoles may be derived from corresponding *pseudo* oxindoles (Scheme B).



REFERENCES

FINCH, N. & TAYLOR, W. L. (1962a). *J. Am. chem. Soc.*, **84**, 1318; **85**, 3871.
SHAVELL, J. & ZINNES, H. (1962). *Ibid.*, **84**, 1320.
SHELLARD, E. J., PHILLIPSON, J. D. & GUPTA, D. (1969). *Planta Medica*, **17**, 146.

The distribution of alkaloids in *Mitragyna parvifolia* (Roxb.) Korth in young plants grown from Ceylon seed

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Initial investigations of the alkaloidal pattern in *Mitragyna parvifolia* obtained from Ceylon showed the leaves to contain the closed E ring alkaloids, tetrahydroalstonine, akuammigine, pteropodine, isopteropodine, speciophylline and uncarine F while the trunk bark contains the open E ring alkaloids isorhynchophylline and rhynchophylline in addition. The root bark contained isorhynchophylline and rhynchophylline only (Shellard & Houghton, 1971).

A more detailed examination of all parts of a young plant grown from seed and of the seeds and seedlings has revealed an interesting distribution of alkaloids throughout the plant. The root xylem and phloem both contain isorhynchophylline, rhynchophylline and corynoxeine (Δ^{18}-rhynchophylline). This latter alkaloid occurs in large quantities in the root tips and is also present in the seeds and seedlings as the main alkaloid. This group of alkaloids also occurs in the stem xylem at all positions in the stem but could not be found in the petioles, leaves or stipules. Hirsutine and hirsuteine (Δ^{18}-hirsutine) also occur in the root phloem. The alkaloids in the stem phloem vary according to the position in the stem. No indole alkaloids were detected and the oxindole alkaloids present in the leaves also occur in the stem phloem in the upper part of the stem although the amounts present decrease to zero in a zone just above the hypocotylar region. This region contains isorhynchophylline, rhynchophylline and corynoxeine but in a narrow zone just above this region there are traces of an alkaloid which appears to correspond to mitraphylline on many t.l.c. systems. The stipules contain the same oxindole alkaloids as in the leaves with speciophylline predominant.

This distribution of alkaloids can lead to an interesting speculation regarding their movement and possible role in the plant.

REFERENCE

SHELLARD, E. J. & HOUGHTON, P. J. (1971) *Planta Med.*, **20**, 82–89.

A study of bioavailabilities and dissolution rates of commercial tablets of nitrofurantoin

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An investigation was undertaken to compare the bioavailabilities (BA) of a number of tablet formulations (100 mg) of the urinary antibacterial nitrofurantoin (NFT) with dissolution data on the same lots.

Preliminary studies showed that (1) An aqueous suspension of NFT of particle size 180–75 μm was a well absorbed and reproducible control dose. (2) Blood concentration profiles (8 sampling times in 6 h) reflected urinary excretion curves and (3) No drug was detected in the urine after 14 h. Peak blood concentrations occurred within 1 h after ingestion and were in the range 0·7 to 1·5 μg/ml. Each of 6 tablet formulations B through G and the control suspension A were given at weekly intervals to 7 volunteers. A standard regimen was adhered to before and during the trial and the sequence of administration followed a 7 × 7 latin square design. Urine was voided at 1, 2, 3, 4, 5, 6, 8, 10 and 14 h after the drug was taken and analysed for NFT by the method of Conklin & Hollifield (1963). The ranking of BA relative to control A demonstrated that formulation B was lowest (Tukey's allowable difference at 95% confidence level). Formulation D war lower than highest ranked G. For the first 6 h after administration formulation D gave significantly lower (95% confidence level) NFT excretion than control, while that of C was lower for 5 h. The observed urinary excretion rate constant was about 1·0 h^{-1}, $t_{\frac{1}{2}} = 0{\cdot}7$ h and the mean excretion curves for each formulation reflected the individual data. Approximate absorption rates were calculated using the Wagner-Nelson equation (1964) for a single compartment model (which appeared to fit the data). Compared to control, formulations B, C and D were more slowly absorbed (A, mean $t_{\frac{1}{2}}$ abs. = 0·5 h; B, mean $t_{\frac{1}{2}}$ abs. = 1·2 h).

The urinary NFT concentration of 30 μg/ml has been cited in clinical reports as sufficient to eradicate most sentive strains of infecting organisms while less sensitive strains may respond to 75 μg/ml. A comparison of the time required to attain and maintain 30 and 75 μg/ml indicated that formulation B was poorest.

The USP XVIII methods for disintegration and dissolution were used to obtain data for each formulation. The stated limit is not greater than 30 min for disintegration and the time required for 60% (T 60%) of NFT to dissolve should not be *less* than 1 h. The formulations of higher BA than control, E, F and G released the drug rapidly and gave T 60% less than 60 min. This dissolution standard was apparently designed to prevent rapid drug release in efforts to reduce nausea associated with NFT which may be accentuated with rapid absorption. The test may lead to formulations exhibiting lower bioavailabilities than control; B and D are two such dosage forms. B has a T30% of 55 min and a disintegration time of 7 min and D, a T60% of 140 min and a disintegration of 1·3 min. These experiments suggest that formulation of NFT is difficult and a suitable test that reflects BA is required.

REFERENCES

CONKLIN, J. D. & HOLLFIELD, R. D. (1953). *Clin. Chem.*, **11**, 925–931.
WAGNER, J. G. & NELSON, E. (1964). *J. pharm. Sci*, **53**, 1392–1403.

On the effect of pharmaceutical formulation on thioridazine absorption

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Mellinger (1965) showed that when thioridazine was given orally the resultant blood concentions were affected by the type of preparation administered, a syrup giving higher blood concentrations than a crushed tablet. Ingredients of an oral liquid preparation can affect absorption of a drug from the gastrointestinal tract (Wagner, 1968). Male Wistar rats (body weight 100–250 g) were given thioridazine (10 mg base/150 g body weight) by stomach tube as a soluble salt in a syrup or as a suspension of the insoluble base. The syrup contained 2·25% thioridazine, calculated as base. The suspension (I) contained 2·25% micronized thioridazine base, polysorbate 80, sucrose and water. Mean blood concentrations (determined according

to Pacha, 1969) were compared after appropriate time intervals by the *t*-test. Only at 1 h after administration, did suspension (I) give different (higher) blood concentrations than did the syrup. To obtain information on the absorption of thioridazine oral preparations in man, a single dose, 3-way cross-over study was carried out with six male adults. The preparations used were the syrup, suspension (I), and suspension (II) which contained 2·25% micronized thioridazine base and pharmaceutical formulating agents (Patent application no: 118–3076). The three preparations were given in a latin square order, with three weeks between successive administrations for elimination of thioridazine. Blood samples were taken at appropriate time intervals. Differences in thioridazine blood concentrations between subjects after the same preparation were greater than differences in a single subject after the three preparations. A modified *t*-test (Moroney, 1953) was therefore used to compare the differences in individuals after the three preparations. There was no significant difference between the blood concentrations after suspension (I) and suspension (II), but both these were consistently higher ($P < 0{\cdot}01$) than those after the syrup. Adjuvants added to suspension (II) had not reduced absorption. Thioridazine is a base and would be expected to be well-absorbed from the intestine and poorly absorbed from the stomach. The free base in suspension (I) and (II) will dissolve in the stomach. If solution is rapid and neither syrup nor suspension formulation affects absorption, the drug should be equally well absorbed from syrup and suspension. The superior absorption found for the suspensions could be due either to an ingredient included in both suspensions (but not the syrup) that increased thioridazine absorption, or to an ingredient in the syrup that reduced thioridazine absorption.

REFERENCES

MELLINGER, T. J. (1965). *Am. J. Psychiatr.*, **121**, 1119–22.
MORONEY, M. J. (1953). *Facts from Figures*, 2nd edn, p. 228. Harmondsworth: Penguin.
PACHA, W. L. (1969). *Experientia*, **25**, 103.
WAGNER, J. G. (1968). *Drug Intelligence*, **2**, 30–34.

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Human volunteer studies of the antitussive activity of dropropizine

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The production of cough and its assessment is possible with reasonable accuracy in laboratory animals but it is much more difficult in human subjects. A series of experiments was designed to induce cough in order to measure the effects of antitussives. The purpose was the verification of the antitussive property of 1-(2,3-dihydroxypropyl)-4-phenylpiperazine (dropropizine). In this study cough was induced by inhalation of citric acid aerosol using a modification of the method of Bickerman, German, Cohen & Itkin (1957).

In the first phase of the experiment, healthy volunteers were screened for response to citric acid inhalation using concentrations from 2% to 16%. The apparatus consisted of Rybar inhalers attached to a constant air supply of 14 p.s.i. so that each would deliver 0·175 ml/min of aqueous solution in atomized form. Initial screening was of 25 volunteers of ages ranging from 19 to 48 years and including both smokers and non-smokers. Of these, 14 were selected as positive cough responders on the basis of inability to tolerate a given threshold concentration of citric acid aerosol for 30 s or following 30 s inhalation to cough persistently for at least 30 s. The selected volunteers were rechecked to establish constancy of tussigenic threshold.

In the second phase, 9 of the selected volunteers were used in a double-blind study comparing a single dose of 20 mg codeine phosphate with a single dose of 60 mg dropropizine. After verification of the sensitivity threshold to citric acid inhalation for each volunteer, the first capsule was administered and the threshold redetermined 1½ h later. 4 h after ingestion of the first capsule, the second capsule was given and a further redetermination of the threshold made after 1½ h. The selection of medication was radomized and the subjects were unaware of the concentrations of citric acid that they were inhaling. Results showed that after dropropizine 6 subjects showed an increased threshold of response while after codeine phosphate 7

showed increase though none as great as with dropropizine. Enquiry elicited no adverse side-effects.

In the third phase, 11 of the selected volunteers were used in a double-blind comparison of a single dose of 90 mg dropropizine with placebo. The method was as in phase two. Results showed that dropropizine raised the threshold of sensitivity of 8 subjects by a greater amount than the placebo while in 2 cases the placebo effect slightly exceeded that of dropropizine. One subject showed no change in threshold. Again there were no adverse effects.

These results emphasize the importance of the psychological factor in antitussive assessment in humans, but the method was found to give consistent results for individual volunteers with remarkable constancy of the threshold baseline on repeated measurement. This would therefore seem to offer a simple, non-hazardous method of comparing antitussive potencies.

REFERENCES

BICKERMAN, H. A., GERMAN, E., COHEN, B. N. & ITKIN, S. E. (1957). *Am. J. med. Sci.*, **234**, 191–205.

BICKERMAN, H. A. (1962). *Clin. Pharmac. Ther.*, **5**, 353–368.

NICOLIS, F. B. & PASQUARIELLO, G. (1962). *J. Pharmac. exp. Ther.*, **136**, 183–189.

Extrinsic circular dichroism resulting from the interaction of sulphonamides with plasma albumin

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The effects of protein binding of sulphonamides on their duration of action and their metabolism are well known (Anton & Boyle, 1964). The mechanism of binding is imperfectly understood (see, e.g., Jardetzky & Wade-Jardetzky, 1965). The use of circular dichroism (CD) in investigating the interactions of drugs with proteins has been indicated by Chignell (1970).

Strong extrinsic CD bands were generated between 260 and 320 nm when solutions of crystalline bovine serum albumin (BSA, $2{\cdot}5 \times 10^{-6}$ M, pH 7·4) interacted with a number of optically inactive sulphonamides (5×10^{-5} M) having the general structure $H_2N\text{-}C_6H_4\text{-}SO_2NHR$. These included the highly lipid-soluble, strongly-bound compounds: sulphasomizole, sulphadimethoxine and sulphamethoxypyridazine. Sulphanilamide, sulphadiazine, sulphamerazine, sulphapyridine, sulphasomidine and sulphathiazole generated only weak effects or none at all. Similar, though not identical, results were obtained with human serum albumin.

Difference CD spectra of mixtures of BSA ($2{\cdot}5 \times 10^{-5} - 1{\cdot}0 \times 10^{-3}$ M) and sulphasomizole (molar ratio sulphonamide/protein 0·2–5·0) showed positive and negative peaks at 290 and 260 nm respectively. Graphs of difference ellipticity [$\Delta\psi_\lambda = \psi_\lambda$ (protein + sulphonamide) $- \psi_\lambda$ (protein)] against the number (r) of sulphonamide molecules bound per protein molecule, up to r = 2·6 (determined by equilibrium dialysis) fell into two linear regions with a discontinuity at r = 1. Interaction with the first binding site (Class I) is thus qualitatively different from binding to the second and third sites (Class II). The derived molecular extrinsic CD spectrum of sulphasomizole bound to Class I sites ($K_{assoc.} = 5 \times 10^4$ litre mol^{-1}, approx.) had peaks at 295 nm (molecular ellipticity = $[\theta]_{max} = +5{\cdot}6 \times 10^4$ deg cm^2 dmol^{-1}; dissymmetry factor = $g = [\theta]_{max}/3{,}300\epsilon = +1{\cdot}54 \times 10^{-3}$) and 260 nm($[\theta]_{max} = -5{\cdot}6 \times 10^4$ deg cm^2 dmol^{-1}; $g = -1{\cdot}13 \times 10^{-3}$). The positive peak is due to the heterocyclic ring and the negative peak is probably due to the *p*-aminobenzenesulphonic acid moiety. The results indicate that sulphasomizole is bound to Class I sites in such a way that both chromophores are subjected to asymmetric perturbations of similar magnitude by the protein. Sulphonamide bound to Class II sites ($K_{assoc.} = 10^3$ litre mol^{-1}, approx.) appears to have an extrinsic CD spectrum of lower magnitude ($\lambda_{max} = 275$ nm; $[\theta]_{max} = -1{\cdot}8 \times 10^{-4}$ deg cm^2 dmol^{-1}). The peak does not coincide with a peak in the absorption spectrum of the drug but may be due to perturbation of the tertiary structure of the protein. Determination of CD spectra between 200 and 250 nm indicated that none of the sulphonamides studied affected significantly the protein secondary structure.

Similar results were obtained with sulphadimethoxine or sulphamethoxypyridazine but

detailed interpretation is complicated by the close proximity of the absorption bands of the sulphonamide and protein chromophores.

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REFERENCES

ANTON, A. H. & BOYLE, J. J. (1964). *Canad J. Physiol. Pharmac.*, **42**, 809.
CHIGNELL, C. (1970). *Adv. Drug Research*, **5**, 55.
JARDETSKY, O. & WADE-JARDETSKY N. G. (1965). *Molec. Pharmac.*, **1**, 214.

2-Phenylisatogen as an electron acceptor for mitochondrial NADH dehydrogenase

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Previous investigations have shown that 2-phenylisatogen, a potentially useful anti-mycoplasma agent (Bond, 1969), inhibited ADP-stimulated succinate oxidation and uncoupler stimulated ATPase in tightly coupled rat liver mitochondria at a concentration of 12·5 n mol/mg mitochondrial protein (Sweetman, Green & Hooper, 1971). We have proposed that the site of action of 2-phenylisatogen is similar to that of the antibiotics oligomycin and aurovertin, which have been shown to inhibit the mitochondrial energy-transfer system (Roberton, Holloway & others, 1968; Lee & Ernster, 1968). We now wish to report a second action of 2-phenylisatogen on rat liver mitochondria obtained at higher concentrations with NADH as substrate.

Spectrophotometric determination of NADH oxidation at 340 nm showed that there was a forty-fold stimulation of NADH oxidation in the presence of $8{\cdot}3 \times 10^{-5}$ M 2-phenylisatogen. The stimulated respiration was not inhibited by respiratory chain inhibitors such as rotenone, sodium amylobarbitone, antimycin A and potassium cyanide. The reaction was inhibited by *p*-chloromercuribenzoate. This inhibitor specificity suggests that 2-phenylisatogen interacts with the NADH dehydrogenase system of the mitochondria at site 2, according to the scheme put forward by Ruzicka & Crane (1970). These workers have shown that quinones of the menadione type are reduced to their quinol forms by the respiratory chain-linked NADH dehydrogenase in the presence of NADH. If 2-phenylisatogen was being reduced by a similar mechanism then a possible reduction product would be 2-phenylindolone (see Bunney, 1970). We have obtained preliminary evidence for this possibility by our detection of 2-phenylindolone using a combination of extraction and thin-layer chromatographic techniques, after incubation of 2-phenylisatogen with mitochondria in the presence of NADH. When mitochondria were incubated with 2-phenylisatogen in the absence of NADH no 2-phenylindolone was detected.

Bunney (1970) has shown that 2-phenylisatogen reacts with 1,4-dihydrobenzylnicotinamide, a model compound for NADH, to produce 2-phenyl indolone and 2,2′-diphenyl-2,2′-diindoxyl.

We propose that 2-phenylisatogen interacts with the NADH dehydrogenase system of rat liver mitochondria, in a manner analagous to that found with quinones, to form the reduced compound 2-phenylindolone.

REFERENCES

BOND, C. C. (1969). Ph.D. Thesis, C.N.A.A.
BUNNEY, J. E. (1970). Ph.D. Thesis, London University.
LEE, C. P. & ERNSTER, L. (1968). *Europ. J. Biochem.*, **3**, 391.
ROBERTON, A. M., HOLLOWAY, C. T., KNIGHT, I. G. & BEECHEY, R. B. (1968). *Biochem. J.*, **108**, 445.
RUZICKA, F. J. & CRANE, F. L. (1970), *Biochim. biophys. Acta*, **223**, 71.
SWEETMAN, A. J., GREEN, A. P. & HOOPER, M. (1971). *FEBS Lett.*, **14**, 306.

Impurity profiles of pharmaceutical colourants

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Regulations governing the use of colourants in foods and in pharmaceuticals vary throughout the world with many differences from country to country in the dyes permitted. Hard gelatin capsules are marketed on a world-wide basis and a problem arises in the selection of colouring materials of international acceptability. In view of the nature of the compounds and the manufacturing procedures commerical dye materials are inevitably contaminated with impurities. These subsidiary impurities are usually closely related to the main component and are sometimes excluded dyes in their own right.

The aim of the present investigation was to examine a range of the commercial dye products available as a standard colour with the dual objects of assessing the quantities of subsidiaries present and of obtaining a qualitative 'impurity profile' for identification purposes. Amaranth (trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3,6-disulphonic acid) and Sunset Yellow FCF (disodium salt of 1-p-sulphophenylazo-2-naphthol-6-sulphonic acid) were selected and thirteen different commercial samples of the former and twelve of the latter were chromatographed on prespread films of cellulose (Macherey Nagel CEL 300 0·2 mm) using the solvent systems specified in the appropriate British Standard. The light absorption of the dried chromatograms at 525 nm was measured by scanning the films by transmission in a Vitatron TLD 100 flying spot densitometer in the log mode at a scanning speed of 1 cm/min using a 0·25 mm aperture and a strike length of 14 mm. Peak areas were estimated directly by means of a Vitatron integrating recorder and related to the concentrations of components by reference to standards.

The Amaranth samples showed between 78·4 and 99·7% of the total absorption in the major peak with some variation in the subsidiaries. Of the subsidiaries Fast Red E is permitted in Britain but not in the E.E.C. (although the French National Standard contains it). The Sunset Yellow samples had a lower range of major peak absorption of 93·6 to 98·6 per cent but the distribution of the subsidiaries was more varied. The subsidiary Orange II is no longer permitted in any country. Each sample showed a characteristic impurity profile which enabled the manufacturing source to be identified.

REFERENCES

B.S. 3341 (1961). Amaranth for use in foodstuffs.
B.S. 3340 (1961). Sunset Yellow FCF for use in foodstuffs.

The soda lime pyrolysis of saccharin

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We have repeatedly observed that a primary aromatic amine, detected by the Stenhouse reaction (Stenhouse, 1870), is formed when saccharin, or its sodium salt, is subjected to soda lime pyrolysis. In an effort to characterize the products formed in this common, semi-micro qualitative test, the pyrolysis was carried out on a larger, test-tube scale and the distillate was collected and dried. The distillate was then subjected to gas-liquid chromatography using a column packed with 10% polyethylene glycol adipate on celite, and to column chromatography on neutral alumina. The products were identified by comparison of their physical characteristics with authentic samples, and, in some cases, by the preparation of suitable derivatives. In this way saccharin was found to give rise to benzene, aniline, benzonitrile, biphenyl, diphenyl sulphide, carbazole and ammonia. Examination of the carbonized residue remaining after pyrolysis showed that it contained some benzenesulphonamide, together with the anions S^{-}, SO_3^{-} and a trace of SO_4^{-}.

A similar soda lime fusion of benzenesulphonamide resulted in the formation of the same products, with the exception of benzonitrile.

The formation of this complicated mixture of products under the drastic conditions of the

test makes the assignment of clear reaction pathways impossible. However, a number of suggestions can be made concerning the mode of formation of some of the products.

(i) Benzenesulphonamide, but not benzoic acid, is formed indicating that decarbonylation of saccharin (or decarboxylation of a derivative of it) takes place.

(ii) Benzonitrile may be formed either by dehydration of the imide group, or by attack of some aromatic species by CN^- formed *in situ*. The former is favoured since no CN^- was detected in the residue, and both benzamide and nicotinamide were converted into their nitriles by soda lime pyrolysis.

(iii) It is possible that aniline is formed through attack by NH_2^- on an aryne intermediate (i.e. by elimination-addition) but more probable that direct nucleophilic displacement of a sulphonate (or related) group occurs. Jackson & Wing (1886) have shown that the fusion of sodium amide with salts of aromatic sulphonic acids produces primary aromatic amines in low yield.

(iv) Carbazole is probably formed from aniline via the intermediate diphenylamine, since Braun & Grieff (1872) showed that distillation of either of these with lime gave carbazole.

(v) Under the reductive conditions of the test it is likely that benzene-sulphonamide is deaminated and converted into thiophenol, which may dimerize to form diphenyl disulphide. The latter would readily decompose on heating to diphenyl sulphide in a manner similar to that described by Heldt (1965) for *p*-tolyl disulphide.

(vi) The formation of benzene and biphenyl can be accounted for by assuming that phenyl radicals or ions are liberated which either combine with hydrogen or couple together. Reasonably good yields of *p*-bitolyl were obtained by Heldt (1965) from *p*-tolyl sulphide upon pyrolysis over metal oxide catalysts.

REFERENCES

BRAUN, R. & GRIEFF, P. (1872). *Ber.*, **5**, 276–277.
HELDT, W. Z. (1965). *J. org. Chem.*, **30**, 3897–3902.
JACKSON, C. L. & WING, J. F. (1886). *Ber.*, **19**, 902–903.
STENHOUSE, J. (1870). *Ann.*, **156**, 50–59.

Effect of sodium carboxymethylcellulose and compound tragacanth powder on the sedimentation and redispersal of sulphadimidine mixture, paediatric B.P.C. 1968

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This communication is a report on the physical stability of Sulphadimidine Mixture, Paediatric B.P.C. 1968, when prepared with sodium carboxymethylcellulose 50 or, extemporaneously, with compound tragacanth powder. Previous studies on the evaluation of factors controlling the physical stability of sulphonamide suspensions were conducted mainly on model systems (Haines & Martin, 1961; Wilson & Ecanow, 1963; Ecanow, Grundman & Wilson, 1966; Jones, Matthews & Rhodes, 1970).

Sulphadimidine Mixture, Paediatric B.P.C. 1968 was prepared using 1% sodium carboxymethylcellulose 50 or 4% compound tragacanth powder, as described in the British Pharmaceutical Codex (1968). Care was taken to minimize air entrainment into mixtures. Sedimentation heights were recorded as suggested by Martin (1961). Samples for particle size analysis were withdrawn at a fixed depth at the midpoint of settling mixtures, diluted 1:50 with filtered 1% sodium chloride solution and assayed using a Coulter Counter "Model B" Industrial, fitted with a 400 μm orifice tube (Coulter Electronics Limited, Dunstable). Redispersibility was measured as described by Matthews & Rhodes (1968). Mixtures were stored for periods up to 21 days in a constant temperature room at 20° ± 1°.

The initial sedimentation rate of the mixture containing 4% compound tragacanth powder was more rapid than for the mixture containing 1% sodium carboxymethylcellulose 50. However, as the period of storage was extended beyond 8 h the reverse effect occurred. Falling sphere viscosity measurements indicated that during this storage period constituents of the compound tragacanth powder settled, gradually forming a viscous sol. The latter initially produced a viscosity gradient in the mixture which increased progressively with depth. These findings would contribute to an explanation of the observed sedimentation rates. Size

analysis of particles over 40 μm showed that at equivalent times the diameter corresponding to a cumulative % oversize (volume), except 100%, was greater for the compound tragacanth mixtures. Photomicrographs taken at these times showed evidence of sulphadimidine—compound tragacanth aggregates sedimenting, which would account for the difference in size distributions obtained. At equivalent sampling times, the mixture containing the compound tragacanth powder was increasingly more difficult to redisperse to homogeneity than the mixture containing sodium carboxymethylcellulose 50.

The rapid appearance of a large supernatant layer and the caking tendencies of both suspensions during storage are undesirable. Studies are in progress on the use of agents to aid the optimum formulation of sulphadimidine suspension.

REFERENCES

British Pharmaceutical Codex (1968). pp. 1189–1190, London: The Pharmaceutical Press.
ECANOW, B., GRUNDMAN, R. & WILSON, R. (1966). *Am. J. Hosp. Pharm.*, **23**, 404.
HAINES, B. A. & MARTIN, A. N. (1961). *J. pharm. Sci.*, **50**, 228–232; 753–756; 756–759.
JONES, R. D. C., MATTHEWS, B. A. & RHODES, C. T. (1970). *Ibid.*, **59**, 518–520.
MARTIN, A. N. (1961). *Ibid.*, **50**, 513–517.
MATTHEWS, B. A. & RHODES, C. T. (1968). *Ibid.*, **57**, 569–573.
WILSON, R. & ECANOW, B. (1963). *Ibid.*, **52**, 757–762.

A possible mechanism for the action of dimethyl sulphoxide on percutaneous absorption

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In vitro experiments have been carried out to assess the effect of DMSO on the physicochemical properties of hyaluronic acid and chondroitin sulphate in aqueous solution. Hyaluronic acid and chondroitin sulphate are both present in skin. Day (1952) and others (Laurent & Petruszkiewicz, 1961) have shown that the former plays an important part in the resistance of flow through connective tissue. It exists in solution as a meshwork of long molecules which can impede the passage of even small molecules through the solution. It is therefore feasible that hyaluronic acid plays a part in resisting the percutaneous transport of large and small drug molecules.

A three-compartment cell was used. The aqueous solution of sodium hyaluronate or chondroitin sulphate was placed in the central compartment between two membranes separating the donor and recipient compartments. The rate of diffusion of the methylene blue and salicylic acid into the recipient cell was noted in the presence and absence of DMSO (see Table).

Table 1. *Rates of diffusion on methylene blue and salicylic acid through hyaluronic acid*[1] *and chondroitin sulphate*[2] *gels.*

Solvent	10^4K (g cm^{-2} min^{-1}) Methylene Blue - hyaluronic acid* (1 mg ml^{-1})	10^4K (g cm^{-2} min^{-1}) Methylene Blue - chondroitin sulphate (20 mg ml^{-1})	10^4K (g cm^{-2} min^{-1}) Salicylic acid - hyaluronic acid* (1 mg ml^{-1})
Water or buffer	2	4	7·3
10% DMSO	4	5·6	15·0

* Molecular weight, from viscosity measurements, $8{\cdot}9 \times 10^5$.

[1] Sigma grade III P. [2] Sigma grade II mixed isomers.

10% DMSO reduces the intrinsic viscosity of hyaluronic acid solutions from 1550 to 900 ml g^{-1}, which implies a reduction in the axial ratio of the hyaluronic acid from 156 to 120.

The experimental data support the view that DMSO exerts its absorption-enhancing effects by decreasing the microscopic viscosity of the barrier layers, thereby decreasing the resistance offered to diffusing solute molecules.

REFERENCES

DAY, D. T. (1952). *J. Physiol.*, **117**, 1.
LAURENT, T. C. & PIETRUSZKIEWICZ, A. (1961). *Biochim. Biophys. Acta*, **49**, 258.

Sensory testing of spreadability; investigation of the rheological conditions operative during application of topical preparations

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A method was developed which related sensory impressions of spreadability of ointments and creams to instrumental rheological analysis. The method may be used in routine industrial control procedures, and as a spreadability screening test for laboratory use prior to field trials in innovative work.

The mean temperature of the skin of the inner surfaces of the forearms of a test panel was approximately 34°. Instrumental rheograms for a series of test materials were determined at 34° using a Ferranti Shirley viscometer; two methods, modifications of that due to Wood (1968), were employed. Panel members were asked to compare test samples with Newtonian silicone oils by spreading them on the skin, and to select the Newtonian material most similar to the test sample. The intersection of the rheograms of the test sample and the selected Newtonian oil indicated the approximate rheological conditions during spreading on the skin.

A Master Curve of the approximate rheological conditions operative during spreading was determined for a series of lipophilic materials ranging from stiff semisolids to mobile fluids. Rates of shear varied approximately from 400 to 2500 s^{-1}, shear stresses varied approximately from 40 to 6000 $Nm.^{-2}$

The panel members assessed the test samples using Ordinal, Preference and Ratio scaling procedures (Torgerson, 1965). Logarithmic and double logarithmic plots of mean panel score against apparent viscosity or shear stress (at the rate of shear indicated by the Master Curve) were linear with one exception.

The Preference test data, in conjunction with the Master Curve, were employed to determine the rheological conditions which yielded maximum consumer acceptance of the spreadability of a product. The preferred region of the Master Curve was bounded approximately by 400 to 700 s^{-1} and 200 to 700 Nm^{-2}.

The panel was asked to spread Newtonian silicone oils of known viscosity between marks 10 cm apart on the skin of the forearm. An estimate was made of the number of strokes per unit time. The relative viscosity, V cm s^{-1}, between the skin surfaces was thus determined approximately. The rate of shear, $\dot{\gamma}$ s^{-1}, during spreading of each oil was determined by reference to the Master Curve. Using the equation for plane laminar flow between parallel plates

$$\dot{\gamma} = V/d$$

where d is the plate separation in cm, the approximate thickness of the oil film on the skin was calculated. The data indicated a *dynamic* relation between $\dot{\gamma}$, V and d, which may explain former difficulties and variation in results obtained in the evaluation of the shear conditions operative during spreading of topical applications (Langenbucher & Lange, 1970).

REFERENCES

LANGENBUCHER, F. & LANGE, B. (1970). *Pharm. Acta Helv.*, **45**, 572–582.
TORGERSON, W. S. (1965). *Theory and Methods of Scaling*, New York: Wiley.
WOOD, F. W. (1968). *S.C.I. Monograph No.* 27, p. 40–49.

The effect of constituents in white soft paraffin on the efficacy of corticosteroid ointments in the vasoconstrictor test

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The vasoconstrictor test developed by McKenzie & Stoughton, is widely accepted as one of the most useful methods for assessing the activity of a topical corticosteroid. Recently, modifications have been introduced to the test in order to examine the effect of formulation on the response of the steroid. The test now enables numerous formulation effects to be evaluated which would previously have required large-scale, and therefore costly, clinical trials. Four white soft paraffin bases (A, B, C and D) each meeting the B.P. specifications were

prepared with differing proportions of constituents so that the degree of waxiness increased in the order A < B < C < D. These bases were then used to prepare placebo ointments containing 5% propylene glycol and active ointments containing 0·025% fluocinolone acetonide (Synalar) dissolved in 5% propylene glycol. In spite of the similarity of the preparations the responses varied considerably in the vasoconstrictor test. Fig. 1 shows the variation in response among the fluocinolone acetonide ointments. It can be seen that the overall response in the test increases in order A < B ≃ C < D. The responses of the placebo preparations are shown in Fig. 2. These differ appreciably at the first reading increasing in the order A < B < D < C and then fall to much lower levels during the next 1½ h.

It is suggested that these results are due to the degree of occlusion that such preparations present to the skin. For some time ointments have been thought to be such effective corticosteroid formulations because they induce a degree of occlusion to the treated site and so increase the penetration of the drug through the skin in the same way as an occlusive plastic film. Conceivably the placebo response is really a whitening of the skin due to the increased hydration of the tissues under the application site. The fact that this response seems to dissipate quickly once the ointment has been removed tends to support this assumption.

The different responses of the active ointments can also be reasonably explained in similar terms. Those bases which cause a high degree of hydration of the skin can be expected to favour better penetration of the drug. Consequently the most waxy bases C and D deliver more steroid to the skin and therefore they are the most effective.

The experiment shows clearly how an alteration in the nature of the formulation can appreciably change the effectiveness of the preparation.

Figure 1. Responses of Different Ointment Bases Containing 0.025% Fluocinolone Acetonide at Different Time Intervals.

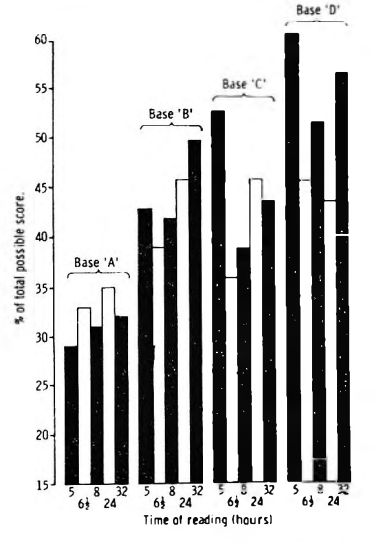
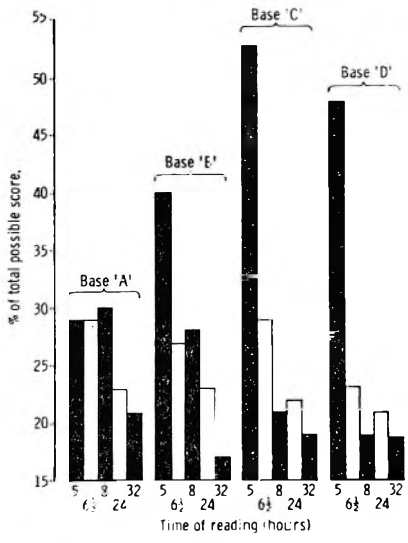


Figure 2. Responses of Different Ointment Bases at Different Time Intervals.



REFERENCE

McKenzie, A. W. & Stoughton, R. B. (1962). *Arch. Derm.*, **86**, 608.

Correlation of bactericidal properties of the α,α,α-trifluoromethylphenols with physico-chemical parameters

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Although many studies have been made of bactericidal activities of ring-halogenated phenols, there appears not to have been any study of side-chain-halogenated phenols. We have investigated the bactericidal effects of the isomeric trifluoromethylphenols on *Escherichia coli*, by the method of viable counts.

Physico-chemical parameters determined were partition coefficients, pKa values, dipole moments and infrared shifts. Rank-order correlations were found between bactericidal activity and all these parameters.

Of particular importance are the correlations with partition coefficient. Three systems were examined: oleyl alcohol, methyl oleate and cyclohexane, all against McIlvaine's buffer, pH 5. Fig. 1 shows the relationship between bactericidal activity and partition coefficients in the three systems. Good correlations are obtained with oleyl alcohol and methyl oleate as

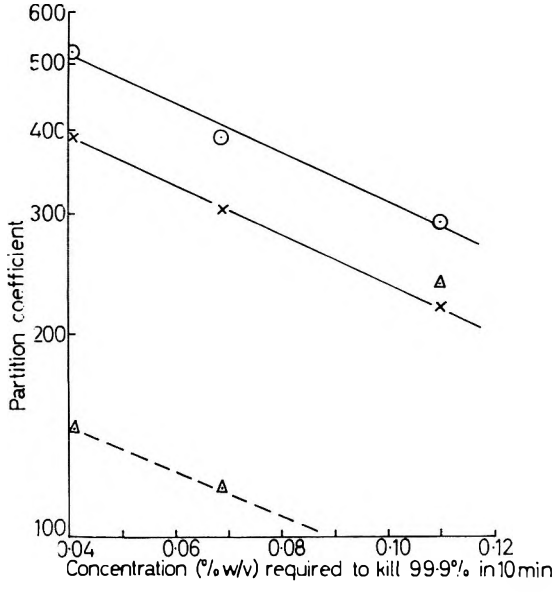

FIG. 1. Correlation of bactericidal activity and partition coefficient of the trifluoromethylphenols. Partition coefficients determined with oleyl alcohol (●), methyl oleate (×), and cyclohexane (▲). Partition coefficients in cyclohexane–buffer are shown × 100.

non-aqueous phases, but in the cyclohexane–buffer system, the partition coefficient of the *o*-isomer appears to be too high by a factor of three. It is suggested that this is due to intramolecular hydrogen bonding in this isomer; this reduces the solubility in water, giving an anomalously high partition coefficient. "Normal" partition coeficients are observed when polar solvents such as oleyl alcohol are used, because the intramolecular hydrogen bond is ruptured in such solvents. The above hypothesis is confirmed by an examination of the partition coefficients of the cresols (the *o*-isomer of which cannot form an intramolecular hydrogen bond), which do not show the above anomaly. Thus, in partition systems representing body membranes, the lipophilic solvent should be polar.

The heat resistance of bacterial spores after different vacuum drying treatments

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The resistance of bacterial spores to high temperatures of around 120° has been used in this work as a means of assessing damage induced in the spores by different vacuum drying treatments. The vacuum apparatus used enabled simultaneous measurements to be made, of pressure, sample weight and sample temperature changes, and allowed for correlation between physical measurements and biological response.

The effect of different drying treatments was assessed by exposing samples to a constant elevated temperature for different times and estimating the viability. Under all conditions it was found that the log surviving fraction (N/N_0)/heating time (t) curves exhibited a shoulder at high survival levels, but were linear below a surviving fraction of 0·1. The linear portion is described by $N/N_0 = ae^{-kt}$ where "a" is the intercept of the curve with the "y" axis. Two parameters have been used to characterize the response; firstly, the slope of the curve "k", and secondly, a shoulder constant "s" which is the heating time required to reduce the surviving fraction to 0·1 i.e. s = t, when $N/N_0 = 0{\cdot}1$.

The usefulness of the constants "k" and "s" in deducing possible lethal mechanisms relies on them changing with heating temperature in a meaningful way. Therefore the characteristics of the heat response of spores was investigated in aqueous suspension, and also after being subjected to sublimative low vacuum drying, and to high vacuum drying, where additional water is removed by isothermal desorption. Both "k" and "s" were found to be directly related to the heating temperature, and were shown to vary systematically and independently with the drying treatment.

When "k" values were treated according to the Arrhenius relationship $k = Ae^{-Ea/RT}$ it was found that the activation energy for the lethal mechanism (Ea) did not change with different drying treatments, being 155 kJ mol^{-1} (34 k cal mol^{-1}) in all cases. The susceptibility of the spore to these mechanisms, as indicated by the frequency factor (A), was, however, dependent upon the drying treatment, the value being 1000 times smaller after sublimative drying than in aqueous suspension, and 20 times greater after high vacuum than after sublimative drying.

A treatment of "s" in a similar way showed that the size of the shoulder decreased with increasing temperature, but that removal of water by sublimative drying caused an increase in "s" by a factor of 1000, the value after isothermal drying being 100 times lower than this.

A study of the variation of "k" and "s" under different conditions would lead us to doubt that they are representative of one mechanism. Rather we would postulate that "s", the shoulder constant, represents a lag time during which structural changes occur within the spore that cause it to become susceptible to the lethal mechanism represented by "k". The effects of different drying treatments have been analysed on this basis.

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Recognizing sporogenous yeast genera

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It is often important to the clinician that yeasts isolated from vaginal swabs are accurately identified. Conformation with Kochs postulates for pathogenicity has been established for several species of the imperfect genus *Candida* (Hurley, 1967), but not for any perfect yeast species. Some perfect species are distinguishable from *Candida* species only in their ability to form ascospores. I believe that the perfect yeasts are commensals. If an isolated yeast is established to be a commensal organism it will not mask the true cause of disease.

Isolates of four perfect yeast species, with postulated imperfect *Candida* stages, were used for this investigation. They were *Saccharomyces cerevisiae* (3 isolates), *Hansenula anomala* (2 isolates), *Pichia membranefaciens* (3 isolates) and *Kluyeromyces fragilis* (5 isolates). At

least ten single cell cultures of each isolate were examined. The formulae of the three agar solidified sporulation media that were investigated have been described (Merritt & Hurley, 1971). Slopes were made of 5 ml volumes of media in 20 ml screw capped glass bottles. Inoculated slopes were incubated at 25°. Samples were taken from each slope at regular intervals, heat fixed to a glass slide and stained with 0·5% safranin. A count was made of at least 500 structures to find the percentage of cultures in which at least 1% of the structures were asci. The ascospores of *K. fragilis* are released from the ascus as they mature, so the number of ascospores could be determined, but not the number of asci. The results for *K. fragilis* are for the percentage cultures in which at least 1% of the structures are ascospores.

Of the three media studied, the sodium acetate medium allowed optimum sporulation of all four species. The 6% *S. cerevisiae* cultures which were apparently nonsporing, all contained some asci, but not to 1% of the structures. The asci production on this medium was predictable and large variations in asci yields between replicates was unusual. The Gorodkowa medium was of little value for three of the species studied, although some of the cultures contained a few asci. The V8 medium was useful and occasionally gave relatively higher yields of asci than the sodium acetate medium. However, the medium was unreliable because a few cultures would fail to contain any asci when replicates were sporing freely.

For the yeasts of medical importance that I have investigated the sodium acetate medium is very suitable for inducing sporulation. The small amount of nutrient included in the formulation allows some cell division and compensates for some variation in inoculum levels, although this may delay the onset of sporulation by 1–2 days. I can recommend this unbuffered sodium acetate sporulation medium for routine isolates of yeast-like fungi which may be *Candida* species.

REFERENCES

HURLEY, R. (1967). *Rev. med. vet. Mycol.*, **6**, 159–179.
MERRITT, A. E. & HURLEY, R. (1971). *J. Med. Mic.* In the press.

The effect of cetyltrimethylammonium bromide on the cytochrome system of *Escherichia coli*

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The effect of a number of agents on cytochrome difference spectra of cells of *Escherichia coli* NCTC 1093 have been studied. Cells were grown as described by Rye & Wiseman (1966). harvested from the exponential phase of growth by membrane filtration and suspended in glucose-free medium to give a cell concentration of between 15 and 20 mg/ml. Up to 0·2 ml volumes of water or of solutions of substrates or reagents were added to 4 ml aliquots of these suspensions and difference spectra between pairs of them measured using a Unicam SP 700 recording spectrophotometer.

The spectra obtained between blanks of washed aerated cells and similar test suspensions to which had been added sodium succinate showed absorbancy peaks in the visible light region at 533, 560, 593 and 630 nm corresponding to those reported for *E. coli* by Smith (1954). The addition of potassium cyanide to the succinate respiring cells in such pairs of suspensions resulted in the elimination of the 630 nm cytochrome a_2 peak whilst the other peaks remained unaffected. This indicates that the terminal cytochrome had become oxidised, being no longer reducible by the remainder of the electron transport chain when complexed with cyanide.

In the spectra obtained between blanks of washed aerated cells and test suspensions of succinate respiring cells treated with some concentrations of CTAB the 630 nm cytochrome a_2 peak was again eliminated, with the other peaks unaffected. This indicated that CTAB is capable of specifically uncoupling the terminal cytochrome from the remainder of the electron transport chain. Similar elimination of the 630 nm peak also followed treatment of respiring cells with chlorhexidine diacetate. The final concentrations of CTAB and of chlorhexidine having these effects were 300 to 400 and 100 to 200 μg/ml respectively; lower concentrations had no effect on the cytochrome spectra whilst higher concentrations caused

the elimination of all peaks and agglutination of the suspensions. On extrapolation to the appropriate cell densities these concentrations approximate to those that would be expected to partially inhibit the growth of cultures of this organism and it seems possible that inhibition of bacterial growth by low concentrations of these membrane active agents results from the reversible uncoupling of the terminal cytochrome from the electron transport chain without any gross membrane damage or penetration of agent into the cells.

REFERENCES

SMITH, L. (1954). *Bact. Rev.*, **18**, 106.
RYE, R. M. & WISEMAN, D. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 114S.

Preliminary compaction studies using a device to simulate a rotary tabletting machine

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Rotary compressing machines commonly used for the manufacture of pharmaceutical tablets compress powders in a die between two moving punches. However, preliminary compaction studies are often made using hydraulic presses or eccentric compressing machines, in which one moving punch compresses the material against a second stationary punch. Since the frictional conditions at the die wall, and the stress distribution within the compact differ between these two types of compression it is often difficult to relate the results of preliminary studies to the subsequent behaviour of the material on a rotary machine. Nevertheless it is inadvisable to use precision equipment such as a rotary tabletting machine for initial compression studies with unlubricated powders or with formulations having ill-defined compaction properties.

A device has therefore been developed to simulate the compression conditions on a rotary machine. We have used the apparatus in conjunction with a universal testing instrument ("Instron") but the principle could also be applied to a hydraulic press or an eccentric tabletting machine. The lower punch is supported by a load cell located on the fixed platten of the universal testing instrument and the upper punch is attached to the movable crosshead. As the upper punch compresses the powder, the movement of the crosshead is translated to the die which also begins to move downwards but at a slower rate than the upper punch. The relative rate of movement of the die and the upper punch is adjustable, and the instant at which the die begins to move can be controlled.

Using this system with plane-faced punches of 33 mm diameter, the compaction properties of 40–60 mesh fractions (250–420 μm) of crystalline sodium chloride, potassium chloride, potassium citrate and lactose were investigated. Deformation of the material was measured at a range of applied loads up to 49·0 kN. For a range of maximum loads, when samples of each material had been compressed the upper punch movement was stopped. Decay in the load on the compact at constant strain, due to continuing deformation of the material, was studied. The effect of load on ejection force and compact strength was determined.

Differences in the compaction behaviour and the properties of the compacts of the four materials studied indicate differences in the mechanism of consolidation. Evidence of plastic deformation of sodium chloride and potassium chloride is shown by large stress relaxations, high ejection forces and high strength values. This effect is most apparent with potassium chloride. Conversely, lactose and potassium citrate exhibit much less stress relaxation and produce far weaker compacts. Differences between potassium citrate and lactose are explained by more extensive size reduction of lactose by fragmentation during compaction.

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The influence of the surface tension of pendular bonds on the tensile strength of moist beds of bulk solids

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The tensile strength of a bed of fine particle sized material depends upon a number of factors of which the main are: particle size and distribution, particle shape, surface roughness, chemical constitution and moisture (Eaves, 1971).

For non-cohesive materials it was found that increasing the moisture content increased the tensile strength of a bed at a fixed state of packing to a plateau where it is recognized that the moisture exists as pendular bonds between adjacent particles.

In this state, the tensile strength of an ideal system of monosize spheres may be estimated from an equation reported by Pietsch (1968).

For glass powder, which possessed negligible tensile strength when dry, a reduction in the surface tension of the wetting fluid produced a reduction in tensile strength of packed beds at fixed porosities over a range of liquid contents. The tensile strength at constant liquid content and packing density increased linearly with surface tension, consistent with the previously mentioned equation.

For materials with inherent tensile strength at dryness, increasing the moisture content was found to increase the strength of the bed at a fixed packing density to a maximum and thereafter to cause a decrease in tensile strength due to disruption of the inherent cohesive forces.

Beds of fine particle sized sodium chloride wetted with a fluid of lower surface tension than saturated sodium chloride solution showed a similar pattern of tensile strength changes but non quantitative reduction in values. This is attributed to a change in the location of the pendular bonds.

For calcium phosphate, however, liquid contents of up to about 50% by weight and of varying surface tensions were shown to produce little or no change in tensile strength and this is attributed to the location of liquid in the intraparticle voids where it is less likely to affect particle-particle interactions.

The bulk solids used were powders characterized in terms of size, size distribution, shape and density. The wetting fluids consisted of 5% w/w Tween 80 in either distilled water or a saturated solution of the solid being investigated. All measurements were made on pre-dried beds of bulk solids in a controlled temperature and humidity environment (Eaves & Jones, 1970) using tensile testing apparatus similar in design to that of Ashton, Farley & Valentin (1964).

REFERENCES

ASHTON, M. D., FARLEY, R. & VALENTIN, H. F. H. (1964). *J. Sci. Instrum.*, **41**, 763.
EAVES, T. (1971). Ph.D. thesis, University of Nottingham.
EAVES, T. & JONES, T. M. (1970). *J. Pharm. Pharmac.*, **22**, 594.
PIETSCH, W. B. (1968). *Nature, Lond.*, **217**, 736.

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Studies on mixing cohesive powders

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A tablet of total weight 100 mg, containing 0·1 mg of powdered drug, poses a mixing problem. Theoretical studies show that very fine powders are necessary to achieve satisfactory homogeneity, but give little information about either the rate of mixing or the distribution of minor component if a random mix is not achieved. A practical approach to the problem is reported.

Lactose represents the substance to be assayed, and calcium carbonate or heavy magnesium carbonate the diluent. These were classified by size into very cohesive, cohesive and free flowing fractions of lactose, very cohesive and cohesive fractions of calcium carbonate, and a free flowing heavy magnesium carbonate. A Lodige Morton mixer was used. Mixing was assessed at times between 4 and 50 000 s on 20 spot samples, each of about 100 mg. Random sampling of the mix was by a thief sampler designed for cohesive powders. Subsequent analysis gave the percentage of lactose in each spot sample and the degree of mix expressed as the coefficient of variation (C) on 20 spot samples. The coefficient of variation for a random mix (C_R) was computed by the method of Poole, Taylor & Wall (1964).

Results for mixing cohesive lactose, in concentrations 0·1, to 50%, with cohesive calcium carbonate agree with those of Poole & others (1964), in that, for increasing concentration of minor component, the time taken for C to approach C_R increased, whilst the time taken for C to reach an arbitrary degree of mix, C_A (constant for all mixes), decreased. For 0·1% mixes, both the time taken for C to approach C_R and for C to reach C_A decreased with increasing mobility of the major component, and increased with increasing cohesiveness of the minor component. Mixing 0·1% lactose with very cohesive calcium carbonate resulted in gross inhomogeneities due to compaction of the powder.

Unlike free flowing lactose, agglomerates occur in very cohesive and cohesive lactose which are not readily dispersed into component particles. It is suggested that the rate determining step in mixing these powders is the breakdown of agglomerates into individual particles, and not subsequent randomization of the particles. If this occurs in practice, a random mix will not be achieved until all agglomerates are dispersed. The distribution of minor component for 0·1% mix of very cohesive lactose in cohesive calcium carbonate was shown, on a basis of 120 spot samples, to be positively skewed. Early on in mixing, skewness is most marked, with a few very high lactose contents. Even after 50 000 s the distribution was slightly positively skewed and C_R had not been reached. It is suggested that the skewness was due to agglomeration of the lactose, and such powders should be avoided in pharmaceutical mixes. With free flowing heavy magnesium carbonate as major component, mixing was much faster; this could be due to rapid dispersion of agglomerates by free flowing particles.

REFERENCE

POOLE, K. R., TAYLOR, R. F. & WALL, G. P. (1964). *Trans. Instn Chem. Engrs.*, **42**, T305.

The mixing of micronized sodium bicarbonate with sucrose crystals

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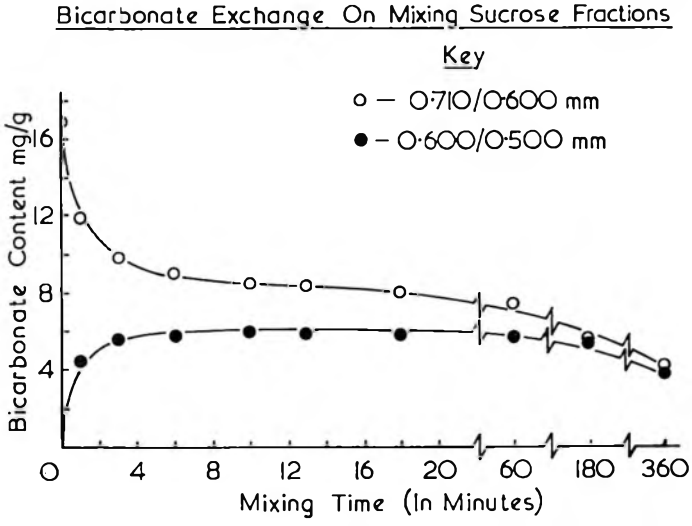
The mixing of a fine powder with granular material is important in dry granulation techniques but very little work has been done on such mixtures. In the mixing of 3% w/w of sodium bicarbonate (about 5 μm) with sieved sucrose crystal fractions (range 0·710–0·420 mm mesh), adsorption on the host crystals prevented the segregation normally accompanying differences in particle size (Coulson & Maitra, 1950). Prolonged sieving (1 h) reduced the bicarbonate to about 1% w/w. Electron microscopy revealed crystal indentations and irregularities which acted as adsorption sites.

Very little bicarbonate could be eluted by washing with chloroform, indicating that electrostatic adsorption forces are either absent or weak (cf. Jones & Pilpel, 1965).

Bicarbonate exchange between sucrose crystals was studied by mixing a bicarbonate rich

sucrose fraction (○) with a larger or smaller sized fraction of pure sucrose (●). Samples were withdrawn at intervals and the bicarbonate estimated. A typical result is shown below.



The initial rapid exchange between the two fractions suggests the existence of strong and weak sites on each crystal. The strong sites on the pure fraction then rob the weak sites on the rich fraction when interfacial contact occurs on mixing. The increase in interfacial contact follows an exponential law (Coulson & Maitra, 1950) consistent with the initial shape of the plots.

A study of the bicarbonate distribution on 0·710/0·600 mm crystals was made by analysing several series of up to 30 samples of the same weight taken from a bulk mix. The coefficient of variation was 1·49% for 1·0 g and 2·82% for 0·1 g samples (30 of each). The value for the smaller weight is below that predicted from mixing theory (circa $\sqrt{10} \times 1{\cdot}49\% = 4{\cdot}7\%$, Lacey, 1943). Uniform distribution and absence of segregation may make this type of mix useful in practical tabletting.

REFERENCES
COULSON, J. M. & MAITRA, N. K. (1950). *Indust. Chem. Mfr*, **26**, 55–60.
JONES, T. M. & PILPEL, N. (1965). *J. Pharm. Pharmac.*, **17**, 440–448.
LACEY, P. M. C. (1943). *Trans. Instn chem. Engrs, Lond.*, **21**, 53–59.

An evaluation of five commercially available tablet disintegrants for possible use in insoluble direct compression systems

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An investigation has been made of the properties of five disintegrants; corn starch, sodium starch glycolate (Primogel), calcium sodium alginate (the recently introduced alginate F417), a cation exchange resin (Amberlite IRP88), and sodium carboxymethyl cellulose. Evaluation was made in insoluble tablet matrices since this area had not apparently been fully investigated previously. The excipients used were: dicalcium phosphate dihydrate and a calcium-phosphato-carbonate complex (Calfos, edible bone powder), both with the same original particle size distribution. Disintegrants used were below 100 mesh size and were studied over the concentration range of 2·5% to 20% w/w. The following tests were employed: disintegration test using the B.P. method, dissolution rate measurement using amaranth as a tracer as previously described by the present authors (1971), hardness and friability determinations, apparent tablet density and particle size distribution from tablets after disintegration. Some of the results obtained are shown overleaf.

Disintegrant 10% w/w	Dicalcium phosphate dihydrate system		Calcium-phosphato-carbonate complex system	
	Dist. time min	Dissolution time min*	Dist. time min	Dissolution time min*
Corn starch	30	15	>120	>30
Sodium carboxyl methyl cellulose	56	>30	90	>30
Calcium sodium alginate	5	10	42	>30
Cation exchange resin	1·4	15	2·5	25
Sodium starch glycolate	0·4	4·5	26	>30

* 50% dissolution.

The effect of concentration of disintegrants on disintegration times of different systems is interpreted in terms of the differing mechanisms by which these substances act as disintegrants. It is suggested that tablet hardness and density measurements may provide some indication of the mechanism by which different disintegrants modify the compression process. It is concluded that any pharmaceutical scientist developing a new direct compression tablet system should seriously consider the possibility of using either sodium starch glycolate or the cation exchange resin as disintegrants.

REFERENCE
KHAN, K. A. & RHODES, C. T. (1971). *Pharm. Acta Helv.*, In the press.

Effect of compaction pressure on dissolution times of some direct compression systems

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The effect of compaction pressure upon the dissolution profiles obtained from a variety of direct compression tablet systems has been investigated. Two techniques have been used for compaction; compacts were prepared using a laboratory hydraulic press and tablets were made using a Manesty single punch machine type F3. The systems were kept as simple as possible, 1% w/w amaranth was included as tracer in all systems (Manudhane, Contractor & others, 1969) and 1% magnesium stearate was used as a lubricant. A cation exchange resin (Amberlite IRP88) was added as a disintegrant for dicalcium phosphate dihydrate and similar systems. Microcrystalline cellulose (Avicel) systems, however, did not require any disintegrant. Tablet hardness was measured using an Erweka tablet hardness tester, disintegraton time was determined using the B.P. method. Apparent tablet densities were obtained from thickness and weight measurements. The compaction process has also been examined by photomicrographic technique. Some of the results are shown in the following Table.

Dicalcium phosphate tablets (containing 2% w/w Amberlite).

Table pressure increasing 1 to 4 units machine setting	Tablet hardness Erweka	Apparent tablet density g cm³	Disintegration time (min)	Dissolution time (min)		
				t50%	t75%	t90%
P1	1·5	1·812	>120	>30	>30	>30
P2	7·0	1·930	15	8	29	>30
P3	9·0	1·954	10	4	13·5	>30
P4	9·5	1·955	8	4	8	15

The results for the effect of compaction pressure on dissolution for dicalcium phosphate dihydrate systems showed that increase in the pressure and the hardness of tablets enhanced the dissolution rate. The results obtained with the microcyrstalline cellulose systems were quite different from those shown in the above Table. Increase in pressure for the Avicel systems caused a significant decrease in the dissolution rate.

REFERENCE
MANUDHANE, K. S., CONTRACTOR, A. M., KIM, H. Y. & SHANGRAW, R. F. (1969). *J. pharm. Sci.*, **58**, 616–620.

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