ISSN 0021-9673

VOL. 125 NO. 3 OCTOBER 27, 1976

THIS ISSUE COMPLETES VOL. 125

HED
IGHTLY

AL OF

HROMATOGRAPHY

ATIONAL JOURNAL ON CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
AMSTERDAM

CONTENTS

Printed in The Netherlands

RS Solvents for the analysis of pesticide residues

)n the recommendation of international organisations such as FAO and WHO, ıany nations throughout the world have felt the necessity to regulate the use and ıe control of pesticides in foodstuffs.
'he analysis of pesticide residues presents unusual problems because of the small mounts of substances to be determined and the large number of possible ıterfering substances which must be first eliminated. In order to determine extremely mall quantities, very sensitive analytical methods are required, which however :annot be applied directly to the substances under examination. The general ırocedure is:

- extraction of the pesticide from the sample;
- concentration of the extract by evaporation of the solvent;
- removal from the extract of naturally-occurring substances which would interfere with the pesticides;
- determination of the extracted pesticide.

n these operations large quantities of polar and non polar solvents are used.
Γhe usual Analytical Grade solvents, when subjected to a more detailed investigation (GLC e.c.d. and Na d.), show peaks due to impurities, whose positions coincide with those of the peaks of pesticides. The use of solvents from which these mpurities have been eliminated is therefore indispensable.
Carlo Erba RS solvents for pesticides have been studied and developed in order :o satisfy these requirements. Their main characteristic is that of having a greatly reduced quantity of any residue which may interfere with the analytical method. This has been attained by working under special conditions, with small batches which are controlled individually, and by special choice of packing materials, cleaning methods and bottle closure procedures. For chlorinated compounds, a maximum limit of 10^{-9}% as aldrin (GLC e.c.d.) is guaranteed, and for phosphorylated compounds a maximus limit of 10^{-8}% as parathion (GLC Na d.).
All these products are available in bottles of 1000 ml.

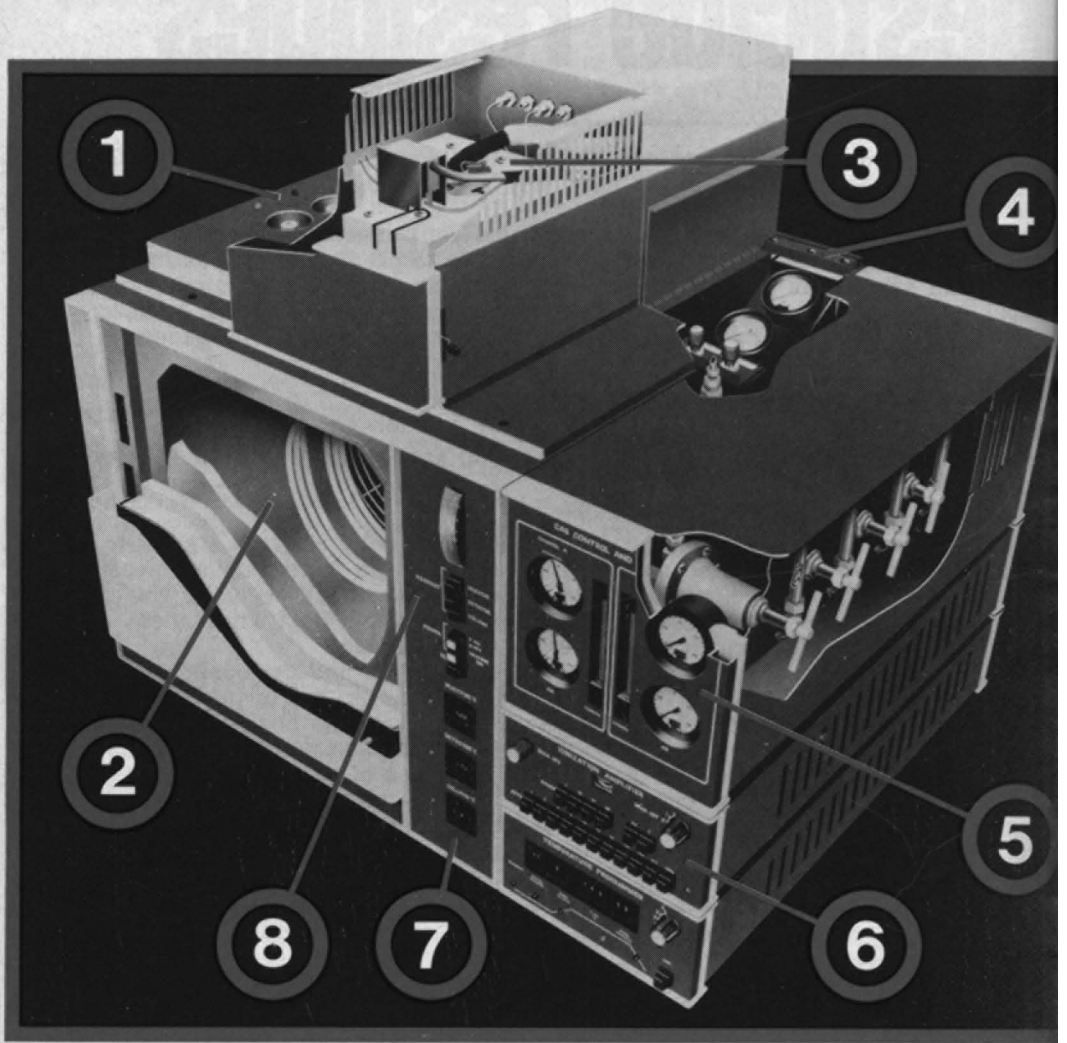
Acetone RS
Acetonitrile RS
Benzene RS
Chloroform RS
Cyclohexane RS
Dioxane RS
Ethyl acetate RS
Ethyl ether RS
n-Hexane RS
Isooctane RS
Isopropyl alcohol RS
Methanol RS
Methylene chloride RS
Petroleum ether 40°-60° RS
n-Pentane RS
Toluene RS

MONTEDISON GROUP
CARLO ERBA

CHEMICALS DIVISION
P.O. Box 3996/020159 Milano/Via Imbonati 24 (Italy)
Telex Erba Mi 36314/Tel. 6995

ref.no. 229

THE INSIDE STORY OF A GREAT NEW GAS CHROMATOGRAP



Pye Unicam L

York Street Cambridge England CB1 2PX
Cambridge (0223) 58866 Telex 817331

A member of the Pye of Cambridge Group

ref.no. 275a

Journal of Chromatography, 125 (1976) 455–469

CHROM. 9157

CELLULOSE IN BEAD FORM

PROPERTIES RELATED TO CHROMATOGRAPHIC USES

J. PEŠKA, J. ŠTAMBERG, J. HRADIL and M. ILAVSKÝ

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6 (Czechoslovakia)

(First received December 2nd, 1975; revised manuscript received February 25th, 1976)

SUMMARY

Cellulose in the form of regular beads was characterized by its swelling, porosity, deformability of the individual particles expressed as the penetration modulus, and the permeability of layers in chromatographic columns. The bead cellulose possesses sufficient mechanical strength while having a considerable porosity; the flow resistance is in good accord with the spherical shape of the particles, and the allowed flow stress lies within technically useful limits. Bead cellulose has been tested as a packing in gel chromatography, and it is expected that it can be used advantageously in other chromatographic methods also, not only in its basic unsubstituted state, but also in the form of derivatives.

INTRODUCTION

Most chromatographic sorbents and phase carriers are used in the form of regular beads of a particular size. Cellulose and its derivatives are exceptions to this generalization, as up to now they have been commercially available only in the fibrous or so-called microgranular form. Although several procedures for the preparation of bead cellulose have been described[1–3], they have been used only exceptionally as chromatographic materials[4].

In this paper, a cellulose in the form of regular beads of different structure is described, which was prepared by a new procedure[5]. Properties that may affect its uses in chromatography, particularly the content of solvents in the swollen state, deformation characteristics and the column behaviour, are dealt with in detail. The applicability of the new material in gel chromatography has been demonstrated on several examples.

EXPERIMENTAL

Material

Bead cellulose was prepared according to a new procedure[5] similar to that of

Determann and Wieland[2] in that it involves the solidification of the oil suspension of a cellulose solution. The main difference is that the solidification occurs at first by heat treatment and not by the action of chemicals. The preparation of bead cellulose as well as some further chemical and physical properties are dealt with in two other papers[6,7]. Various degrees of porosity of the product were obtained by various treatments of the suspension during solidification and by various treatments of the solidified product. Samples with volume fractions of pores ranging from $P = 0$ to 0.8 can be prepared by this procedure. Samples exhibiting the highest porosity were not completely stable during drying and were therefore stored in the swollen state with sodium azide added as a bacteriostat. Samples with low porosity can be dried without change.

Bead cellulose was thoroughly washed with water prior to measurements. Fractions of particular sizes were obtained by sieving in water. The separate samples were characterized by the bed volume of the swollen material relative to its weight in the dry state (ml/g). Microphotographs of the bead and fibrillar and microgranular cellulose were obtained with an Exacta apparatus with an extension for close photographing. The samples were dry and lit from above.

Swelling and porosity

Swelling was determined with centrifugal columns described elsewhere[8]. The samples were swollen to equilibrium in water or methanol, centrifuged (1400 *g*) and weighed. The procedure was repeated several times until constant values were obtained. On drying *in vacuo* at room temperature, the measurement was repeated and the sample was dried again. The results obtained were used to calculate the weight swelling in grams of the solvent per gram of the dry matter; for methanol, the weight swelling was re-calculated by dividing by the methanol density, which yielded a comparable quantity, the volume swelling in millilitres of the solvent per gram of the dry matter. In both instances the porosity was also calculated, *i.e.*, the volume fraction of the solvent in the swollen sample. The volume of the substance was calculated from the specific gravity (1.52) for the basic regenerated cellulose.

Deformation characteristics

A theoretical solution of the problem of stress distribution between two spherical bodies in contact with each other was first postulated by Hertz for the case of the validity of Hook's law between stress and strain. Assuming contact between two beads that have radii r_1 and r_2, the theory[9] predicts that the total pressure force, F, is given by

$$F = \frac{16}{3\,(x_1 + x_2)} \cdot \left(\frac{r_1\, r_2}{r_1 + r_2}\right)^{1/2} \cdot d^{3/2} \qquad (1)$$

where d is compression, *i.e.*, a change in the distances between parts of the beads undeformed by pressure, and $x_i = 4(1 - \mu_i^2)/E_i$ $(i = 1, 2)$, where μ_i and E_i are Poisson's constant and Young's modulus of the material, respectively.

From eqn. 1, in the application to our particular procedure, the deformation of the bead between two planes having a high Young's modulus ($r_1 = r$, $E_1 = E$, $x_1 = x$, $r_2 \to \infty$, $E_2 \to \infty$, $x_2 = 0$) gives

$$F = (16/3)\, A\, r^{1/2}\, (\Delta y/2)^{3/2} \qquad (2)$$

where $A = 1/x$ is the penetration modulus and $\Delta y = y_0 - y$ is a change in the distances of the deformation planes in the undeformed and deformed states. If the material is incompressible ($\mu = 1/2$), $A = G = E/3$, where G is the shear modulus. Eqn. 2 is similar to the relationship derived for the penetration of a bead with a diameter r and a high penetration modulus into an elastic plane[10,11], which is a consequence of the symmetry of eqn. 1 with respect to subscripts 1 and 2.

Deformation measurements on the individual beads compressed between two plates were carried out on a simple apparatus (Fig. 1). The force value was measured with an inductive force transducer connected with a bridge (KWS-2, Hottinger Baldwin Messtechnik, G.F.R.) and recorded with a recorder (accuracy $\pm$ 1 mN). The deformation was imposed by means of a screw and conveyed with a rod firmly connected with the force transducer; its value was read off using a deformation indicator with an accuracy of $\pm$ 0.001 mm. In the determination of the deformation value, a correction was made for the deformation of the membrane of the force transducer with increasing load. The surfaces in contact with the sample were made of PTFE in order to provide the best possible sliding of the sample surface on the bearing plate. Special care was taken to obtain the highest possible parallelism of the two PTFE surfaces. During measurements, the samples were surrounded by the solvent in which they were swollen to equilibrium; the measurement was carried out at constant temperature (20°).

Prior to the deformation measurement, the diameter of the bead was determined by means of an Abbé comparator with an accuracy of $\pm$ 0.002 mm, each time for at least three different positions of the bead. The mean of the three values was used in further calculations. The individual diameters were found to differ by only

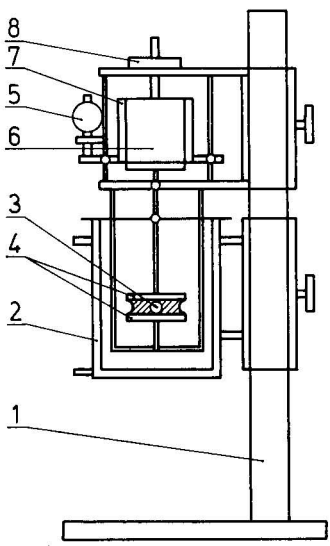


Fig. 1. Apparatus for measurement of mechanical characteristics. 1 = Holder; 2 = thermostating cylinder; 3 = sample; 4 = PTFE plates; 5 = deformation indicator; 6 = force transducer, 7 = thermostating cylinder for 6; 8 = micrometer screw.

1–2%, which suggests a good spherical shape of the samples. The bead was then transferred into a deformation apparatus and compressed to the lowest deformation y_1 and the change in the force F with time was recorded. After a chosen constant time of 2 min, the force F_1 corresponding to the compression y_1 was read off. The deformation was then increased and the whole process repeated. At least 20 values of y_i and F_i were measured on each sample, and at least two beads were taken for each system. However, the values of F_i cannot be regarded as the equilibrium values, because for samples with a low modulus in particular, a pronounced time dependence of F was observed, indicating a considerably viscoelastic character of deformation of such samples. Because of the purpose of this work, this time dependence was not studied in more detail.

For the direct treatment of the experimental data, eqn. 2 was applied in the form

$$F_i^{2/3} = \frac{1}{2}\left(\frac{16}{3} A r^{1/2}\right)^{2/3} (y_0 - y_i) = C - S y_i \tag{3}$$

where C and S are constants. The advantage of such a plot consists in its invariance with a shift of the origin at $y_i : y_0$ need not be known for determining A. Also, the absolute values of y_i are not important, so that the y_i values directly read off from the scale of the deformation indicator can be used. A was determined from the slope S according to the equation

$$A = 3(2S)^{3/2}/16r^{1/2} \tag{4}$$

The assumption used for deriving eqn. 2 about the high modulus of surfaces of the deformational apparatus was checked by direct measurement. The same time procedure as described above was used for the investigation of the penetration of a steel bead into a PTFE plate[10,11]. The penetration modulus for PTFE calculated from eqn. 2 was 190 MPa, that is, higher by one or two orders of magnitude than the moduli determined for the samples investigated.

Flow-rate

The hydrodynamic resistance of the layer in the column was measured in a flow of de-gassed distilled water. A sorbent column, 9 mm in diameter and 30–60 mm high, was supported by a network of stainless steel with holes of diameter 0.01 mm. The pressure above the column was measured with a metallic manometer of the Bourdon type; the flow-rate was calculated from direct measurements of time and the amount of water that had left the column. For higher flow-rates, the instantaneous flow-rate was read off from a rotameter placed behind the column. The readings were calibrated by direct measurements. The hydrodynamic resistance of an empty apparatus was also determined; it was found that the pressure losses in the empty apparatus at the flow-rates under investigation were negligible, so that no correction was needed.

Gel chromatography

A K-2 chromatograph provided with a refractive index detector (Waters R403) was manufactured in the workshops of the Institute of Macromolecular

Chemistry of the Czechoslovak Academy of Sciences. Stainless-steel columns (120 cm × 0.81 cm I.D.) were filled with an aqueous suspension of the sorbent at a pressure of 0.5–1 MPa. The test compounds were injected in doses of 2 ml of 1% aqueous solution. De-gassed distilled water was used as the eluent. The distribution coefficients, effectivities and the useful range of molecular masses were calculated from the results according to the literature[12].

RESULTS AND DISCUSSION

In Fig. 2, bead cellulose is compared with typical samples of commercial chromatographic cellulose. It can be seen that the shape of the particles resembles those of other chromatographic materials, ion exchangers and copolymers for gel and affinity chromatography.

The content of solvents in the swollen bead cellulose and the degrees of swelling and porosities derived therefrom were calculated from a number of measurements, illustrated by an example in Fig. 3. Fig. 3 shows that the weight of the dry sample is reproducible, which suggests that no extraction of soluble fractions occurred during washing. The results are summarized in Table I. It was found that the samples of bead cellulose dealt with in this work reached the same porosities as those of the dextran gels Sephadex G-25 and G-50. Consequently, they are much more porous than most of the usual macroreticular ion exchangers and chromatographic materials. Although the bead cellulose described here is a material that was not additionally chemically cross-linked, the samples can be cautiously dried. The measure of preservation of porosity after re-swelling can be seen from a comparison of the values I

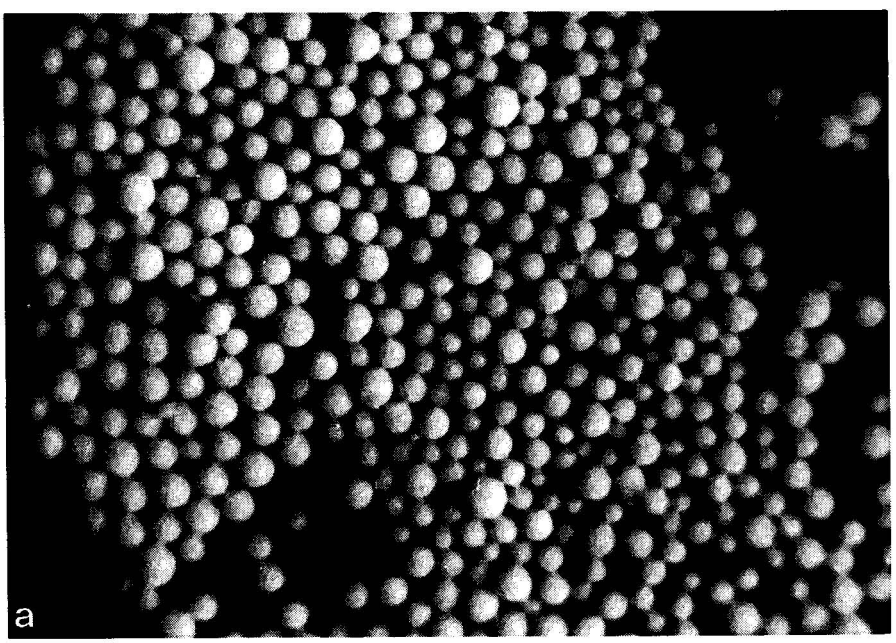


Fig. 2.

(Continued on p. 460)

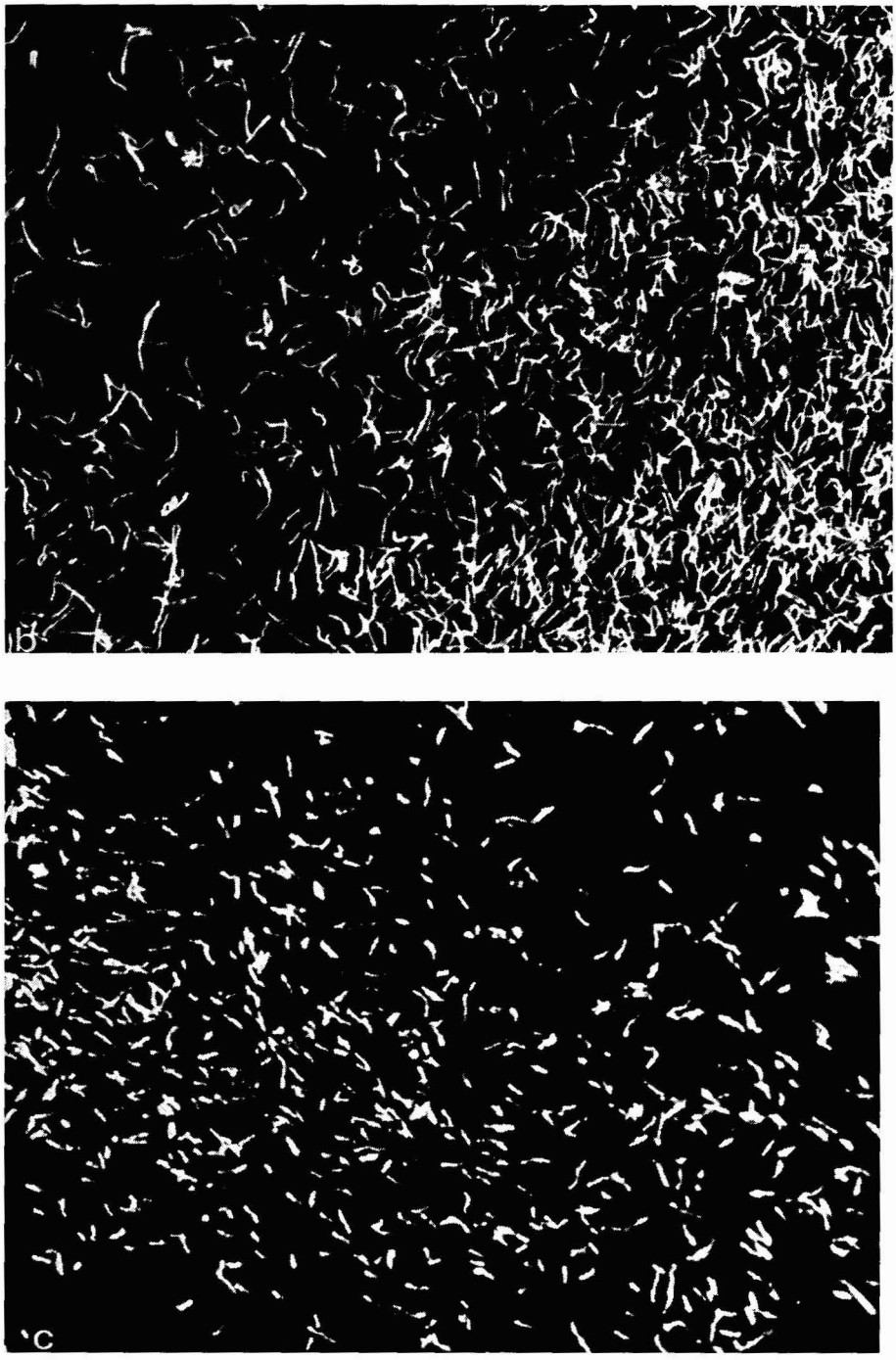


Fig. 2. Microphotographs of (a) bead cellulose No. 12, (b) standard-grade cellulose for chromatography and (c) microgranular cellulose.

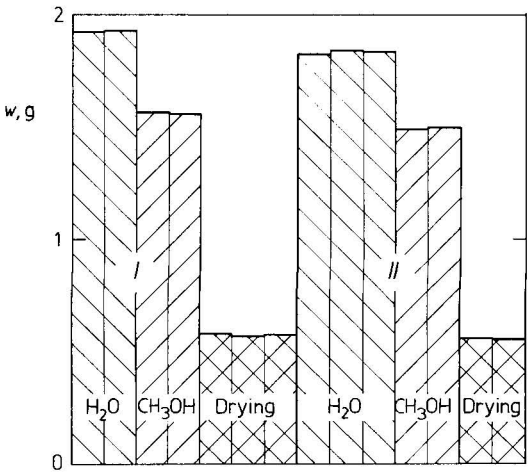


Fig. 3. Example of determination of swelling. Bead cellulose No. 1; sample weight (w) during determination of swelling.

and II (Fig. 3 and Table I). The character of the porosity can be estimated by comparing the swelling values in water and methanol (better and poorer swelling agent, respectively, for cellulose). In a limiting case of capillary porosity, one should expect the same swelling in both solvents, while the swelling porosity would be reflected by a very low swelling in methanol. The observed low decrease in porosity in methanol compared with that in water (Table I) indicates that both types of porosity are present, but that the capillary porosity predominates over the swelling porosity.

The deformation behaviour of the individual particles was characterized by the penetration modulus A defined by eqn. 2. A typical example of the reading of the modulus A is given in Fig. 4, where the dependence of $F^{2/3}$ on y is plotted, F being the

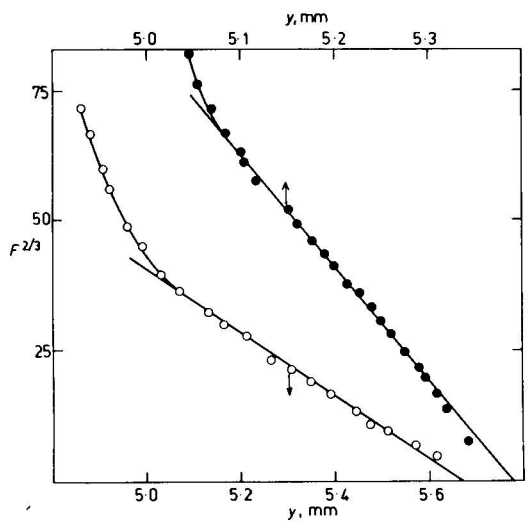


Fig. 4. Example of determination of penetration modulus. Deformation characteristic of bead cellulose No. 6 (●) and No. 10 (○) (bed volumes 4.1 and 10 ml/g, respectively). F, force (mN); Δy, deformation.

TABLE I

SWELLING OF BEAD CELLULOSE

No.	*Sample*		*Swelling I**			*Swelling II**			*Porosity I*		*Porosity II*	
	Bed volume (ml/g)	*Bead size (mm)*	*H_2O (g)*	*CH_3OH (g)*	*CH_3OH (ml)*	*H_2O (g)*	*CH_3OH (g)*	*CH_3OH (ml)*	*H_2O (%)*	*CH_3OH (%)*	*H_2O (%)*	*CH_3OH (%)*
1	5.0	0.25–0.16	2.34	1.71	2.15	2.28	1.66	2.10	78.1	76.6	73.6	76.2
2	7.1	0.25–0.16	3.36	2.58	3.25	2.16	1.58	1.99	83.6	83.2	76.7	75.2
3	6.9	0.25–0.16	3.48	2.55	3.22	2.19	1.53	1.93	84.1	83.0	76.9	74.6

* Swelling given is the amount of solvent per gram of dry sample; I = original sample, II = the same sample after drying to constant weight and swollen again to equilibrium.

force and Δy being the corresponding deformation. Fig. 4 shows that in the initial region of compression a linear dependence can be found, in agreement with the value predicted theoretically by eqn. 3; the penetration modulus was calculated from the slope of the linear part according to eqn. 4. The linear region is unexpectedly large and is about 40–50% of the total diameter of the bead for samples with low moduli. With increasing modulus A, its width decreases (Fig. 4). At higher compressions, one can observe a faster increase in $F_i^{2/3}$ with y_i, which means that A also increases with y_i. However, one may expect that in this region Hook's law, on which eqn. 2 is based, ceases to be valid. Another possible explanation of this deviation may consist in insufficient sliding of the bead surface on PTFE bearing plates.

In order to find the extent to which the values of the modulus A depend on the size of the sample, we measured beads of one system with radii ranging from 0.2 to 0.6 mm. Fig. 5 shows that the A values for $r > 0.4$ mm are virtually independent of the radius, while for $r < 0.4$ mm the values of the moduli increase with decreasing r. Similar results were found for the method involving the penetration of a solid bead into an elastic bearing plate[10], where the penetration modulus was found to increase with decreasing thickness of the plate and with increasing radius of the penetrating bead. In pressure tests on cylindrical samples it was also found that with a decreasing ratio of height to diameter of the cylinder the penetration modulus increases[13]. These results were explained by imperfect sliding of the sample surface on the plates. With respect to the above results, measurements on samples that have possibly identical radii, 0.5–0.7 mm, are included in Table II; these measurements are not affected by the bead size to any substantial extent (*cf.*, Fig. 5). The highest A values (for dry samples, Table II) can be subject to a *ca.* 10% error, because they are lower by only one order of magnitude than the modulus of PTFE used in the deformation apparatus.

For comparison, Table II also includes the modulus values for samples of some commercial ion exchangers. It can be seen that the swollen bead cellulose exhibits modulus values of the same order of magnitude as those for the swollen

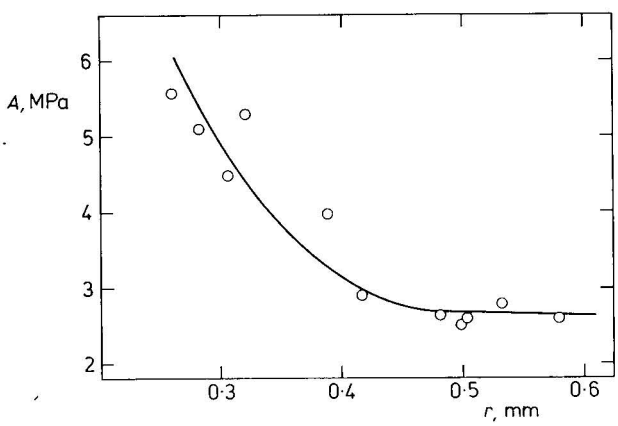


Fig. 5. Dependence of penetration modulus (A) on particle diameter (r). Bead cellulose No. 6 (bed volume 4.1 ml/g).

TABLE II

MECHANICAL CHARACTERISTICS OF BEAD MATERIALS

Swollen bead cellulose			*Dry bead cellulose*			*Swollen commercial ion exchangers*		
No.	*Radius, r (mm)*	*Penetration modulus, A (MPa)*	*No.*	*Radius, r (mm)*	*Penetration modulus, A (MPa)*	*Name*	*Radius, r (mm)*	*Penetration modulus, A (MPa)*
6*	0.533	2.80	8	0.347	24.73	Zerolit***	0.535	1.44
	0.503	2.56		0.332	25.84		0.605	1.50
	0.500	2.46	9	0.419	17.21		0.700	1.35
	0.483	2.63		0.408	20.74		0.638	1.30
7**	0.610	0.44				Zerolit§	0.692	0.28
	0.605	0.44					0.834	0.27
	0.615	0.34				Amberlite§§	0.510	27.62
	0.590	0.41					0.473	26.92
	0.595	0.59						

* Bed volume 4.1 ml/g.
** Bed volume 8.45 ml/g.
*** Carboxylic cation exchanger Zerolit 226 (H^+).
§ Zerolit 226 in equilibrium with 0.01 *N* NaOH.
§§ Strongly acid styrene sulphonic cation exchanger Amberlite IR-120 (H^+).

carboxylic cation exchanger; the values found for dry bead cellulose are comparable with those for swollen styrene ion exchangers, represented in this instance by Amberlite IR-120. For two samples of swollen cellulose, important differences in the values of the modulus A can be observed, which in Table II are related to the bed volume. The latter reflects the equilibrium swelling or porosities. As expected, a lower modulus was found for the more swollen sample.

The behaviour of sorbents in a flow of water is shown in Figs. 6 and 7 as a dependence of the linear flow rate, U (m/h) (flow-rate relative to an empty cross-section of the column), on the pressure gradient B (cm H_2O/cm). Curve 2 (Fig. 6), obtained with dextran gel, is typical of materials subjected to deformation. At low pressure gradients, there is a linear region characterized by the slope K_0 which is sometimes referred to as the specific permeability. At higher pressure gradients, the linear relationship ceases to hold, because the particles become deformed and the free space in the column is reduced, thus reducing the permeability of the layer. In general, the curves pass through a maximum, and in soft sorbents the flow-rate decreases to zero, which happens when the column becomes clogged owing to the destruction of particles. The slope of the linear part is determined only by the particle size and form, while the region of non-linear behaviour is determined predominantly by the deformability of the material.

In Fig. 6, bead cellulose is compared with some dextran gels. Curve 1 relates to bead cellulose, while curve 2 relates to bead dextran of comparable bead size. The full line indicates the initial slope calculated for a given particle size according to the literature[14]. It can be seen that bead cellulose and bead dextran differ markedly, especially in the non-linear part. The effect of the bead form is stressed if a comparison is made with earlier gels with an irregular shape. Their behaviour is illustrated by the individual points in the bottom part of Fig. 6. Although their particle size is

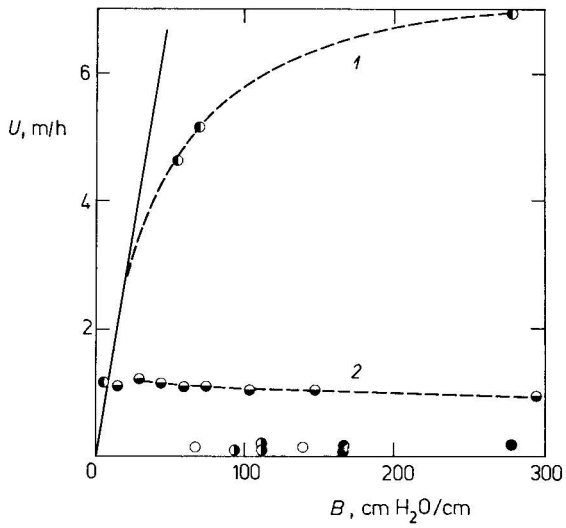


Fig. 6. Flow characteristics: comparison of bead cellulose with dextran gels. ◐, Bead cellulose No. 11 (bed volume 7.9 ml/g, bead size 20–60 μm); ◒, bead dextran gel (bed volume 10 ml/g, bead size 20–100 μm) (Sephadex G 50 Superfine). Dextran gels of irregular shape: ○, bed volume 5 ml/g, bead size 40–130 μm; ●, bed volume 5 ml/g, bead size 90–240 μm; ◑, bed volume 13 ml/g, bead size 110–390 μm. *U*, Linear flow-rate; *B*, pressure gradient.

'arger than that of the two other sorbents and the degrees of swelling are not too different, their permeabilities are very low. Fig. 7 illustrates the effect of the bead size of the sorbent. Curve 1 is the same as curve 1 in Fig. 6 and corresponds to a

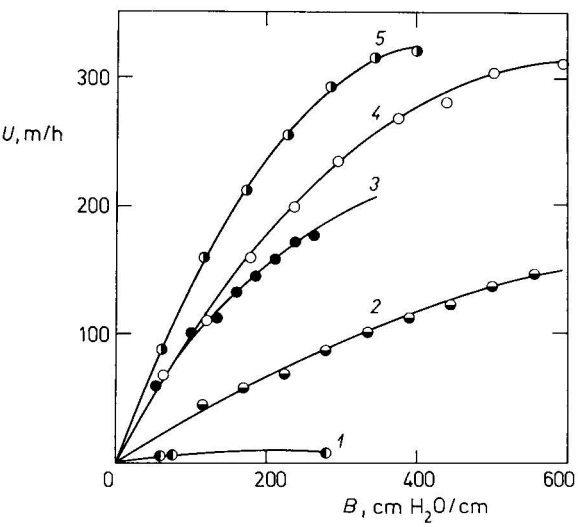


Fig. 7. Flow characteristics of bead cellulose; effect of bead size. ◑, No. 1 (bed volume 5.0 ml/g, bead size 140–250 μm); ○, No. 3 (bed volume 6.9 ml/g, bead size 140–250 μm); ●, No. 2 (bed volume 7.1 ml/g, bead size 140–250 μm); ◒, No. 2 but after being subjected to a gradient of 1000 cm H_2O/cm for 2 h; ◐, No. 11 (bed volume 7.9 ml/g, bead size 20–60 μm) (the same data as the upper curve in Fig. 6 on a different scale). *U*, linear flow-rate; *B*, pressure gradient.

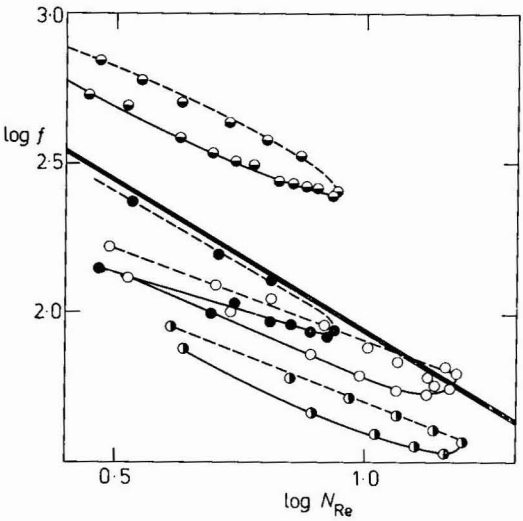


Fig. 8. Flow characteristics of bead cellulose in Fannig's correlation. Log–log plot of frictional coefficient (f) as a function of Reynolds number (N_{Re}). Behaviour after release of tension (reverse dependences) denoted by broken lines. ◑, No. 1 (bed volume 5.0 ml/g, bead size 140–250 μm); ○, No. 3 (bed volume 6.9 ml/g, bead size 140–250 μm), ●, No. 2 (bed volume 7.1 ml/g, bead size 140–250 μm); ◒, No. 2, but after being subjected to a gradient of 1000 cm H_2O/cm for 2 h.

finely granulated bead cellulose. Curves 3–5 are related to samples of bead cellulose with coarser particles. Of these, the softest sample was selected and loaded with a pressure gradient of 1000 cm H_2O/cm for several hours. A comparison of curve 3 (before load) and curve 2 (after load) indicates some decrease in permeability. With soft dextran gels, the column is completely clogged under such conditions.

The deformation of particles and the departure from linear behaviour due to it are represented better by Fanning's correlation[14]. In Fig. 8, the logarithm of the non-dimensional frictional coefficient, f, is plotted against the logarithm of the modified Reynolds number, N_{Re}, where

$$f = D\,\Delta p/2\,L\varrho U^2\alpha$$
$$N_{Re} = DU\varrho/\mu \tag{5}$$

where ϱ and μ are the specific mass and viscosity of the flowing liquid, respectively, U is the linear flow-rate, Δp is the pressure drop, L is the layer height, D is the nominal diameter of the particles and α is the wall factor. For non-deforming spheres of uniform size, the empirical relationship

$$\log f = 2.93 - \log N_{Re} \tag{6}$$

holds over the whole range of laminar flow[14]. This dependence is represented in Fig. 8 by the heavy line. In this correlation, deformation and a decrease in the flow-rate are reflected in an increase in $\log f$ and in an increase in slope in eqn. 6 above the

theoretical value of −1 up to positive values. Fig. 8 also involves the reverse curves, obtained by decreasing the pressure gradient (broken lines). In general, there are two limiting cases. Lasting deformation causes the linear reverse shoulder to shift towards higher log f values, while the theoretical slope −1 remains unchanged. Elastic deformation results in a return to the original values. Fig. 8 shows that the samples of bead cellulose described here possess both deformational components, and that the viscoelastic component is greatly dependent on time.

Thus, the flow properties give evidence about the deformability of particles in a similar manner to the penetration modulus. However, no direct comparison is feasible, because the flow properties were measured on samples other than that for the modulus; the samples used for the determination of the modulus had to be coarse and therefore required separate preparation. The connecting quantity for both determinations is the bed volume. As expected, samples with a small bed volume have the highest penetration modulus (Table II) and are those least deformed at pressure loads in the column (Figs. 7 and 8).

The gel chromatographic properties of bead cellulose were investigated on two types of samples, with different bed volumes. Data characterizing the samples and the separation effectivity are summarized in Table III. The resolving power of the gel is expressed in terms of the coefficient $\beta = \Delta K_{av}/\Delta \log M_W$, which describes the gel property independent of the geometry of the columns. The type A samples (Nos. 4 and 13), having higher porosity, appear to be a universal gel for a wide range of molecular weights; on the other hand, this is accompanied by a lower resolving power. The resolving power of the type B samples (Nos. 5 and 12) is higher; the separation occurs within a narrower range of molecular weights. Table III also shows that the heights equivalent to a theoretical plate depend mainly on the bead size. This could be further reduced only by introducing finer particle separation techniques than separation of the adsorbent on wet sieves. The measurements were reproducible during 1 month. As follows from the elution volumes of strongly polar compounds (Table IV), bead cellulose did not exhibit non-specific sorption that would be reflected in an increase in the elution volumes above the expected value, or

TABLE III

GEL CHROMATOGRAPHIC PROPERTIES OF BEAD CELLULOSE

Sample			*Exclusion limits*		*Outer volume,*	*Total volume,*	*Coefficient of resolving power,*	*Column efficiency**, HETP (cm)*	
No.	*Bed volume (ml/g)*	*Bead size (mm)*	*Upper,* M_{W_0}	*Lower,* M_{W_1}	V_0 *(ml)*	$V_0 + V_i$ *(ml)*	β*	*D-2000*	CH_3OH
4	10	0.31–0.12	$5 \cdot 10^5$	300	26.63	38.06	0.13	7.5	6.5
5***	5.8	0.15–0.07	$2 \cdot 10^5$	$2.5 \cdot 10^3$	20.93	41.87	0.27	2.2	2.2
5§	5.8	0.15–0.07	$2 \cdot 10^5$	$2.5 \cdot 10^3$	20.93	39.95	0.27	2.2	1.4
12	6	0.08–0.02	$5.6 \cdot 10^5$	$2 \cdot 10^4$	5.00	7.26	0.61	3.4	1.0
13	10	0.05–0.03	$5 \cdot 10^5$	500	24.24	38.29	0.124	1.7	0.5

* $\beta = \Delta K_{av}/\log M_W$.

** Calculated according to ref. 12 for dextran D-2000 or methanol.

*** First measurement.

§ Second measurement after 1 month.

TABLE IV

ELUTION VOLUMES OF POLAR COMPOUNDS ON BEAD CELLULOSE

Compound	*Sample 4*			*Sample 5*		
	Elution volume, V_e (ml)	K_{av}	*H (cm)*	*Elution volume, V_e (ml)*	K_{av}	*H (cm)*
Ethanolamine	38.06	0.329	4.8	36.15	0.376	9.2
Tetramethylammonium hydroxide	39.96	0.383	5.5	39.96	0.470	3.8
Pyridine	43.76	0.493	7.5	47.86	0.512	3.0
Acetic acid	41.86	0.438	6.8			
Glucose	41.86	0.438	5.6	41.86	0.517	3.9
Ethylene glycol	39.96	0.383	4.9	41.86	0.517	2.2
Methanol	39.96	0.383	4.1	39.96	0.470	1.7

in excessive tailing. Fig. 9 shows the dependence of the distribution coefficient, K_{av}, on the molecular weight of a number of polydextrans and low-molecular-weight compounds.

Samples of type B (bed volume *ca.* 6 ml/g, Table III) resemble the dextran gel Sephadex G-100 in their exclusion limits (bed volume 15–20 ml/g)[15]. The considerable difference in the bed volumes indicates the different character of the porosity of the two types of samples. Bead cellulose resembles macroporous materials, while the dextran gel exhibits only swelling porosity and consequently must swell three times as much to reach the same pore size. The higher swelling of the dextran gel leads to a correspondingly higher deformability and to a lower admissible pressure gradient, in accordance with what has been said earlier.

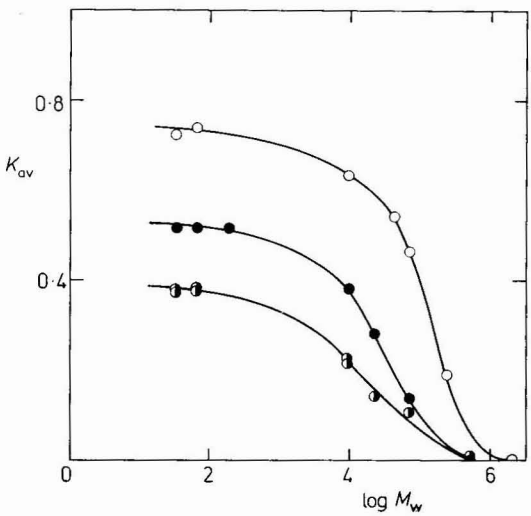


Fig. 9. Dependence of distribution coefficient (K_{av}) on log M_W of the test series of polydextrans and low-molecular-weight compounds. K_{av} calculated according to the literature[12]. ◑, Samples No. 4 and 13; ●, sample No. 5; ○, sample No. 12.

CONCLUSION

The bead cellulose described here is a new chromatographic material with various porosities, a low resistance in the column and suitable for use in gel chromatography. As reported elsewhere[6], bead cellulose can be chemically transformed into ion-exchange derivatives and other special adsorbents without damaging the spherical shape of the particles; consequently, similar advantages can be expected for its application in other chromatographic procedures, *e.g.*, in ion-exchange and partition chromatography.

REFERENCES

1 J. J. O'Neill, Jr., and E. R. Reichardt, *U.S. Pat.*, 2,543,928 (1951); *C.A.*, 45 (1951) 4927c.
2 H. Determann and T. Wieland, *Fr. Pat.*, 1,575,419 (1969); *C.A.*, 73 (1970) 16545z; *U.S. Pat.*, 3,597,350 (1971); *C.A.*, 75 (1971) 130959d; *Makromol. Chem.*, 114 (1968) 263.
3 K. Yasui, Y. Isome, K. Sugimori and S. Katsuyama, *Jap. Pat.*, 73-60,753 (1973); *C.A.*, 80 (1974) 15951m; K. Yasui and S. Katsuyama, *Jap. Pat.*, 73-21,738 (1973); *C.A.*, 79 (1973) 55153p.
4 K. Chitumbo and W. Brown, *J. Chromatogr.*, 80 (1973) 187.
5 J. Peška, J. Štamberg and Z. Blače, *Czech. Pat. Appl.*, PV 3858-74 (1974).
6 J. Peška, J. Štamberg and J. Hradil, *Angew. Makromol. Chem.*, in press.
7 J. Baldrian, J. Peška, J. Štamberg, J. Ludwig and D. Paul, *Symp. on Advances in the Chromatographic Fractionation of Macromolecules, Birmingham, 1976*, to be presented.
8 J. Štamberg and S. Ševčík, *Collect. Czech. Chem. Commun.*, 31 (1966) 1009.
9 Z. Horák, F. Krupka and V. Šindelář, *Technická Fyzika*, SNTL, Prague, 1961, p. 314.
10 N. E. Waters, *Brit. J. Appl. Phys.*, 16 (1965) 557.
11 D. J. Walsh, G. Allen and G. Ballard, *Polymer*, 15 (1974) 336.
12 H. Determann, *Gelová Chromatografie*, Academia, Prague, 1972, pp. 78, 83, 84.
13 J. Hasa, *Collect. Czech. Chem. Commun.*, 39 (1974) 1029.
14 J. H. Perry, *Chemical Engineering Handbook*, McGraw-Hill, New York, 3rd ed., 1950, p. 393.
15 *Sephadex, Gel Filtration in Theory and Practice*, Pharmacia Fine Chemicals, Uppsala, 1974.

Journal of Chromatography, 125 (1976) 471–485

CHROM. 9185

A NEW, GENERALLY APPLICABLE PROCEDURE FOR THE PREPARATION OF GLASS CAPILLARY COLUMNS

K. GROB and G. GROB
GC-Laboratory, ETH Zürich, EAWAG, 8600 Dübendorf (Switzerland)
(Received March 8th, 1976)

SUMMARY

A new procedure for the preparation of the inner surface of glass capillaries for subsequent coating with organic liquids is presented. This procedure is based on the production of a layer of barium carbonate crystals, grown from nuclei on the glass surface. In comparison with other methods it has the following merits: it greatly reduces dependence on glass type; it requires no difficult manipulations or materials; it gives high reproducibility and probability of success, producing almost neutral, thermostable columns with high resistance to rough usage, with wide applicability, and showing good, though not the highest, separation efficiency. This procedure is recommended in order to assist the numerous laboratories that wish to make their own glass capillary columns, without, however, needing to spend many months or even years in becoming specialized in this field. A simple, quick and almost universal method of preparation is needed to enable the bulk of their analytical problems to be handled.

A detailed description of the basic procedure alone is given. Various refinements are feasible, for example to increase separation efficiency or to include acidic columns by first transforming barium carbonate into barium sulphate.

INTRODUCTION

Laboratory-made versus commercial columns

As we have discussed this subject recently[1,2], it is intended to summarize here only the most important features, which concern the quality and the economic aspects of column making as well as an essentially psychological factor related to column handling.

Making glass capillary columns that fulfil high-quality requirements such as those described previously[3], is still a difficult task, demanding a considerable degree of specialization. A better understanding of the processes involved may finally lead to simplification of the production methods; however, it remains doubtful whether a scientist interested in using, rather than in making, glass capillary columns should spend weeks, or even months, as is now necessary, in preparing high-quality columns.

Economically, it is reasonable for laboratories to make their own capillary columns only when the working hours involved are considered to be non-accountable.

On the other hand, a strong case can be made for the individual preparation of the columns. A non-specialized analyst considers a purchased glass capillary column to be an extremely friable and delicate object that should not be tampered with unless absolutely necessary. This unjustified fear of damaging the column leads to the use of an unsuitable application technique. It seems that the analyst who can rely on his own ability to replace a ruined column has far better pre-conditions for acquiring a correct technique.

It is the increasing importance we attribute to this last consideration that prompts us to assist those who wish to prepare their own columns, but does not, however, contradict our previous[1,2] recommendation that a beginner should start by using one or more purchased columns of guaranteed quality.

Requirements for a general preparation procedure

Two of the above aspects (quality and economics) conflict with the third (the psychological factor). The solution to the problem must therefore be a compromise. The object is to introduce relatively simple preparation procedures that offer high probability of success after a few brief trials. The products may, on the other hand, be of good, if not the highest, quality in certain respects. The following fundamental requirements are considered in greater detail.

Lack of dependence on glass types. The controversy about the relative merits of published procedures, being successful in one laboratory and failing in another, is caused primarily by the high dependence on the raw material used. A procedure yielding excellent results may fail completely when glass with even minute structural variations is used.

Low sophistication. The procedure should not include very critical requirements, such as difficult manipulations, critical composition of reagents or sophisticated equipment design.

Wide application range. The product should be suitable for almost any kind of coating material and should, therefore, cover most analytical requirements. This universality includes polarity of liquid phases, temperature range, low adsorptive and catalytic activity, and controllable acid–base characteristics.

Interpretation of current experience

In recent years the importance of pre-treating the glass surface has become generally recognized[4–8]. Confusion has, however, arisen, about the use of terms such as "wettability", "deactivation" and "etching". Our interpretation of present knowledge and experience may be summarized as follows.

Two principles of wetting. Permanent spreading of a liquid over a solid surface may be brought about by two independent factors. A liquid film can be stabilized on a smooth (*i.e.*, degree of roughness far smaller than film thickness) surface by intermolecular forces that attract the liquid to the solid. This is the situation when untreated glass is coated with apolar polysiloxane phases. The second stabilizing factor is the so-called micro-roughness (degree of roughness of the same order of magnitude as film thickness). Both factors may operate to some extent in combination[9]. As has recently been confirmed[7], the first wetting mechanism produces higher

separation efficiencies as a consequence of forming a more homogeneous liquid film. Thus for apolar coatings unnecessary roughening of the surface should be avoided.

In contrast, polar liquids spread almost exclusively on roughened surfaces only, whereby the necessary degree of roughening, together with a corresponding loss of film homogeneity, is a complex function of molecular structure, as partly described by polarity or surface tension.

While this interpretation may be widely accepted, confusion sometimes arises when deactivation is also to be included. The most efficient method of deactivation known is the heat treatment with polar phases according to the method of Aue *et al.*[10], which leads to a paradoxical situation. Deactivation is most important for apolar coatings that are stable on smooth surfaces. However, the production of a coherent non-extractable layer of a polar phase before coating is feasible only on a roughened surface. Consequently, a perfectly deactivated apolar column requires a glass surface prepared for polar coating.

Evaluation of existing procedures

In considering mainly their suitability for general application, the procedures to be recommended consist of two groups. The procedures of the first group produce micro-roughness on the glass surface by etching or by effecting the growth of crystals or both. Those of the second group involve the introduction of extraneous small particles that act principally in the same way as a roughened surface.

The most promising procedure of the first group is etching with gaseous hydrogen chloride which, as Alexander and co-workers[11–13] have shown, simultaneously yields a layer of sodium chloride crystals. Without disregarding the elegance, simplicity, and the high merits of this method, as proved in many special applications[4–6], a few limitations must be mentioned, which might affect its general applicability. Firstly, crystal growth seems to depend on the glass structure to such an extent that reproduction of an ideal degree of micro-roughness may present a problem. Secondly, the procedure is limited to soft glass, from which more or less only alkaline columns can be made. A further limitation is the solubility of the crystals in polar liquids.

The second group, introduced first by Kaiser[14], had achieved prominence when Horning and co-workers[15,16], as well as Blumer[17], Nikelly and Blumer[18], and Schulte and Acker[19], independently of each other, were successful in introducing fine silica particles in different ways. These procedures are virtually independent of the variety of glass used. They are, however, limited by the necessity of using relatively thick liquid films, which leads to lowered separation efficiency. Furthermore, it is our experience that the silica surface is catalytically active with certain liquid phases (*e.g.*, polyglycols) as well as with certain solutes. This activity proves to be a limiting factor for some high-temperature applications.

It is very difficult to judge from experience gained in our laboratory whether or not the procedure described in this paper shows fewer limitations for general use.

SELECTION OF ROUGHNESS-PRODUCING MATERIAL

Sodium chloride as an interlayer between the glass surface and the liquid film exhibits the following characteristics, thus favouring wide application: it is easily

produced with suitable density and size of particles; the crystals adhere firmly to the glass surface and they are chemically inert.

An ideal interlayer material should show in addition the following characteristics. It should be produced by reagents introduced externally, *i.e.*, glass should not be one of these reagents. The structure of the interlayer (the degree of roughening) should be predictable and reproducible. Finally, the material should be insoluble in water.

The most universal interlayer material we have found is barium carbonate. This water-insoluble substance can easily be formed by dynamically coating the glass surface with barium hydroxide solution. Carbon dioxide is used as the gas that pushes the plug of hydroxide solution. Simultaneously, it acts as the second reagent, producing barium carbonate before the liquid film starts to break up into droplets. This process can easily be conducted in such a way that the barium carbonate is formed exclusively on the glass surface, *i.e.*, without precipitation in the liquid phase, thus ensuring a perfectly even layer that is permanently bound to the glass surface. The density of this layer is easily controlled and reproduced by regulating the composition of the coating solution and the speed of coating. The process works on any kind of glass, although on borosilicate glass a micro-structure is obtained that is markedly different from that on soft glass. Variations usually observed within a selected type of glass, *e.g.*, Pyrex, can be neglected. As with HCl-treated columns, our capillaries are immediately coated with polar phases or deactivated before apolar coating. For every kind of coating the appropriate density of barium carbonate is selected empirically, over a range of approximately 100:1.

This brief survey establishes that treatment with barium carbonate possesses none of the weaknesses that are typical of the HCl treatment. Its only drawback is its lower chemical inertness. In the hydrated state, carbonate ions show moderate base strength, and the barium ion is a weak Lewis acid. Both effects are drastically reduced in the crystalline state, as both are basically involved in the formation of the very stable crystal. While we have not been able to detect any activity attributable to the barium ion, acids with a pK_s value lower than about 6, *i.e.*, most free fatty acids, have difficulty in passing over the carbonate surface. It should be emphasized, however, that barium carbonate treated Pyrex glass is much less basic than HCl-treated soft glass.

An aspect that requires further investigation should be mentioned here. We understand that the HCl treatment leaves the glass surface between the sodium chloride crystals uncovered. In contrast, we have obtained evidence indicating that barium carbonate covers the entire glass surface. Direct analytical proof is difficult to obtain because of the great difference between the barium-ion concentration in the well formed crystals and that in the very thin layer that is assumed to cover the glass between the crystals. One of our indirect arguments in favour of such a layer is the lack of dependence of the weak column basicity on the barium carbonate load in Pyrex columns. If the slightly acidic Pyrex glass surface between the basic crystals were bare, then a decreasing barium load should shift the column characteristics in the acidic direction. We interpret the fact that this shift cannot be observed by assuming that the barium carbonate treatment produces a coherent barium carbonate surface.

In order to eliminate the remaining base effect, and to prepare acidic columns,

barium carbonate has to be transformed *in situ* into barium sulphate, the crystals of which show complete chemical inertness. This step requires an additional treatment that is not dealt with in this paper.

We have, of course, examined numerous substances with respect to their suitability as an interlayer material. We were, however, not able to find any reasonable alternative to barium carbonate.

PROCEDURE

Crystallisation process

The formation of barium carbonate crystals very probably occurs as follows. Barium ions are adsorbed on the glass surface, where they act as crystallisation nuclei. Under favourable conditions, *e.g.*, thin liquid layer, high basicity and elevated temperature, crystallisation at these points proceeds so rapidly that not even traces of precipitation in the free liquid phase can be observed. The scanning electron micrographs (Fig. 1) show clearly that the crystals have been grown on the glass surface, to which they firmly adhere. According to Stumm and Hohl[22], the crystallisation nuclei are understood to be complexes of barium ions bound to two silanol groups, their stability increasing with increasing basicity of the solution. The surface of freshly fused glass probably contains such complexes, formed by a variety of ions, in relatively high density. It is essential, therefore, to free the silanol groups from undesirable metal ions by rinsing with acid, which eliminates deposits of carbonates that would otherwise cause premature precipitation of barium carbonate.

Barium salt solution

Approximately 10 g of reagent-grade barium hydroxide [$Ba(OH)_2 \cdot 8\ H_2O$] are shaken with 100 ml of distilled water in a polyethylene or PTFE stoppered flask. After sedimentation, aliquots of the saturated barium hydroxide solution are pipetted out in order to prepare coating solutions.

All dilute barium salt solutions (the saturated solution is sufficiently alkaline) are made up with water and 1 *M* potassium hydroxide so as to be 0.2 *M* in potassium hydroxide. Besides creating conditions sufficient for stability of the complexes, this alkalinity ensures rapid uptake of gaseous carbon dioxide, as well as suppression of HCO_3^- ions in the HCO_3^-–CO_3^{2-} equilibrium.

To all barium salt solutions, 0.5‰ of a non-ionic detergent is added. We used Dehydrophen D (Dehydag GmbH, Düsseldorf, G.F.R.), which can, however, be replaced by an equivalent product. The concentration is not critical; we observed identical effects with 0.1–2‰. Without detergent, however, the barium salt solution does not spread on the glass surface, thus causing irregular deposits of barium carbonate. When the solutions are prepared, minute precipitates are normally formed. To allow complete sedimentation we do not use a fresh solution before the next day.

Our standard barium salt solutions, with different barium hydroxide concentrations, are as follows: saturated and diluted 3, 10, 30 and 100 times, with the corresponding notation 1, 03, 01, 003 and 001, respectively.

Principles of the technique

Fig. 2 shows, schematically, the composition and functioning of the reaction

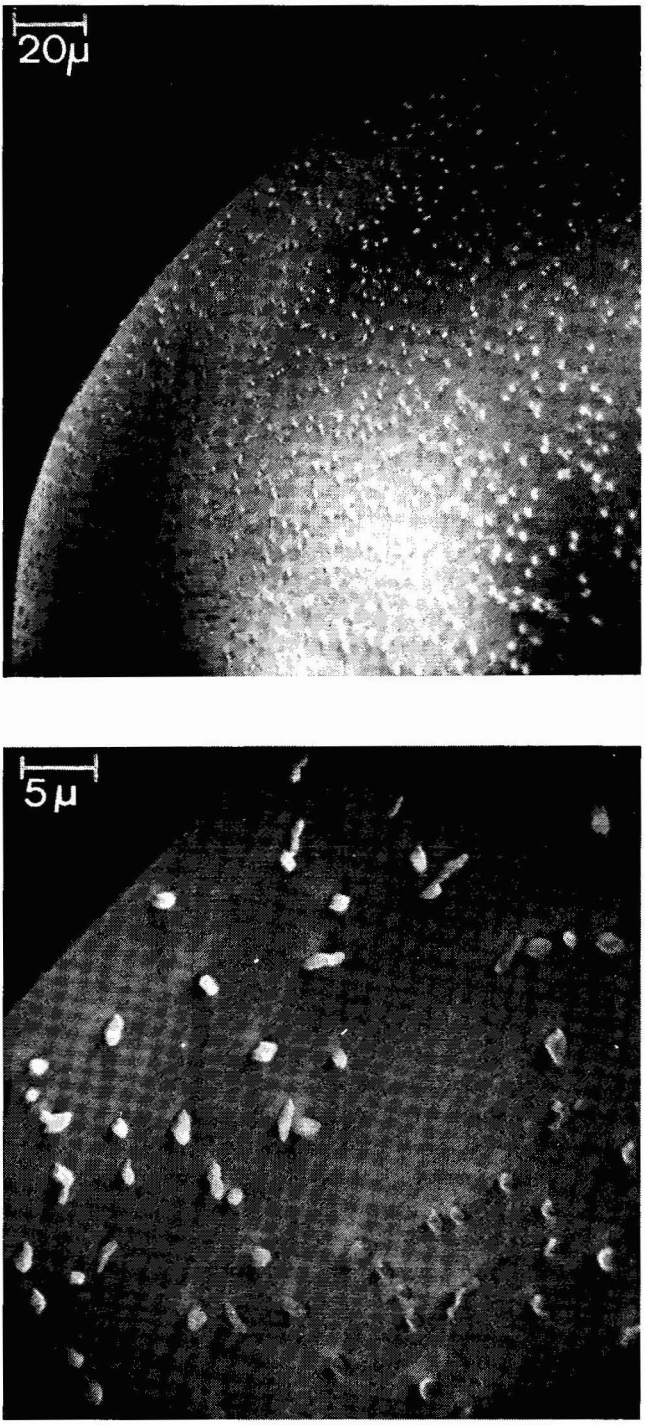


Fig. 1. Scanning electron micrographs of the inner wall of a Pyrex glass capiljary after barium carbonate treatment 03, taken at an angle of 40° from the tube axis (Pretorius and co-workers[20,21]). The crystals show the known shape of witherite (barium carbonate) and appear to have grown on the glass surface (micrographs by Department of Inorganic Chemistry, University of Zürich using a Cambridge Instruments Stereoscan 5 4).

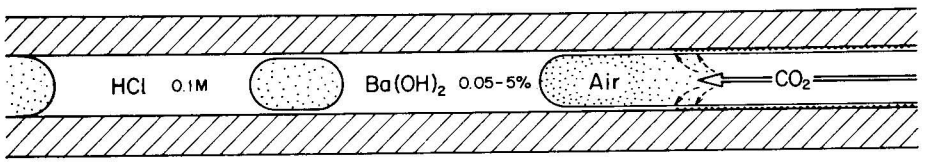


Fig. 2. Principles of the barium carbonate treatment. The glass surface is cleaned by washing with acid. Simultaneously free silanol groups are produced, which adsorb barium ions from the $Ba(OH)_2$ solution. A short air plug ensures formation of a homogeneous aqueous film, the barium ions of which are then precipitated by carbon dioxide, which functions as a moving gas. Growth of barium carbonate crystals starts from the glass surface only.

zone, which, in one combined step, produces the permanently modified surface when it moves through the untreated glass capillary. As a rough rule we adjust the lengths of the single liquid plugs to about one tenth of the column length if the capillary has an approximately 0.3 mm I.D. For wider capillaries the relative plug lengths have to be increased to account for more efficient mixing of the two moving phases. For more narrow tubing the lengths can be reduced. The appropriate length is easily checked at the outlet of the column. At least two thirds of the second plug should still be strongly alkaline. The end of the second plug has to be separated from the carbon dioxide by a small zone of air, about 5 cm long for a capillary of, 0.3 mm I.D., independent of column length. Without this insulating zone, barium carbonate would be precipitated in large particles in the end of the liquid plug, yielding very irregular deposits on the column wall. The short air zone ensures the formation of an undisturbed liquid layer behind the liquid plug before the reaction with carbon dioxide starts. It may be surprising that, regardless of the intense diffusion between air and carbon dioxide, the short zone adequately fulfils this task. This is because the carbon dioxide that diffuses into the air plug is rapidly and totally absorbed by the alkaline coating. The suggested length of 5 cm is a compromise. A shorter air zone may not have sufficient insulating effect and a longer zone is undesirable because during the prolonged period between film formation and reaction the film may start to break up, in spite of the relatively high concentration of detergent. Again, the appropriate length is easy to check. The rear side of the moving hydroxide plug has to remain absolutely transparent the whole time. Inert impurities in the carbon dioxide will automatically accumulate in the air zone. Therefore, this zone always tends to increase, not decrease.

According to our experience, the regularity of the crystal layer increases with increasing reaction temperature. Empirically, we find the optimum temperature to be 80°. A higher temperature may cause difficulty for two independent reasons. Degassing of the liquids may disturb film formation because of the formation of foam plugs. In addition, excessive reaction of the liquids with glass may cause precipitation, especially within the alkaline plug. For soft glass with a particularly low chemical resistance, a reaction temperature below 80° may be advisable.

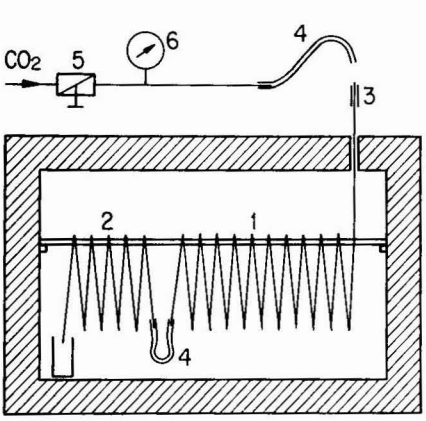
Practical directions

Straighten one end of the untreated glass capillary (length of end depending on oven geometry) to allow for easier application of solutions, and fill approximately one tenth of the column length with 0.1 *M* HCl through the straightened end (using

suction or pressure). After making a short air plug for visual control, introduce about the same length of the selected barium salt solution (concentration depending on proposed application). To avoid interference by ambient carbon dioxide, filling by suction is preferred. Disconnect the vacuum pump, and leave the straightened end immersed in the barium salt solution until the liquid stops moving. Withdraw the inlet end and immediately close it with a hermetically closing cap made from PTFE shrink tubing or equivalent material. Connect the outlet end to a buffer capillary with same I.D. and at least one fifth of the length. Place both capillaries in an oven with a glass door, whereby the straightened inlet end is pushed through a hole in the oven roof (Fig. 3). Adjust the temperature of the closed oven to 80°. Wait until liquid stops moving, which indicates pressure equalization between column and oven, and withdraw the cap from the column inlet. Wait until the plug end of the barium salt solution has fallen to the desired distance from the column end (3–10 cm, which is not critical). Then connect the column inlet to the carbon dioxide supply, ensuring that dead volumes of the carbon dioxide line (valves, manometer, etc.) have been flushed thoroughly before connecting, and adjust the desired moving rate to 1–2 cm/sec.

If no oven with a glass door is available, an alternative system, as indicated in Fig. 4, can be used. The flow-rate is determined by observing the movement of a short plug of, for example, toluene (a liquid with relatively low viscosity and volatility) in the buffer capillary. The longer the column, the longer the initial time during which exact determination is not possible. When the toluene plug reaches the end of the buffer capillary, disconnect the capillary and connect the opposite end. For repeated use an oven with a glass door is clearly preferable.

A few minutes after the barium salt solution has left the main column, open the door and disconnect the buffer capillary. If desired, push the liquids back and check that the remaining length gives an alkaline reaction with pH paper. Dry the



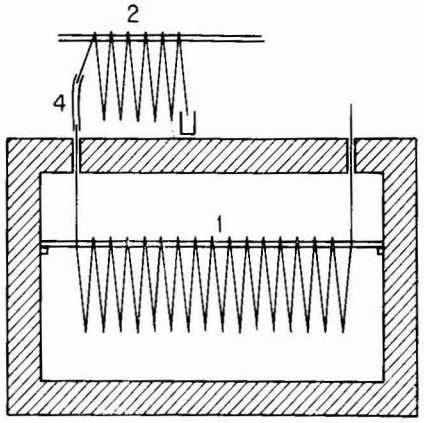


Fig. 3. Equipment for barium carbonate treatment including an oven with glass door. 1 = Glass capillary under treatment; 2 = buffer capillary; 3 = cap; 4 = elastic tubing; 5 = pressure regulator; 6 = manometer.

Fig. 4. Equipment including an oven without glass door. 1 = Glass capillary to be treated; 2 = buffer capillary containing a short plug of a liquid such as toluene; 4 = elastic tubing.

TABLE I

LIQUID PHASES AND THEIR PRE-TREATMENTS

Valuable for Pyrex glass, barium carbonate treatment at 80°, moving rate 1–2 cm/sec.

Liquid phase	*Suitable pre-treatment*			
Polyethylene glycol 20 000 (PG 20 M)	1	03		
Emulphor, Triton, Tergitol	1	03	01	
OV-225, Silar 5CP, 7CP, 10C*	1	03		1 × PG**
OV-17, OS-124	03	01		1 × PG**
Ucon LB, Ucon HB	01	003		
SE-52, SE-54, OV-1, SE-30	01		2 × PG, 2 × E**	
	003		1 × PG, 1 × E**	

* The polar silicones, particularly Silar 10C, can be applied without PG pre-treatment.
** See under *Deactivation*.

treaded column by successively passing through it acetone and diethyl ether, each about one third of the column length. Evaporate off the ether with a flow of dry air. The column is now ready for immediate polar coating.

Liquid phase and suitable pre-treatment

Every organic liquid phase requires a certain degree of surface roughening for optimum film formation. Table I gives some information concerning this relationship for some liquid phases, selected to cover the entire range of polarity as well as the most common molecular structures. This information has been gained exclusively from experiments carried out in our laboratory. A simple rule is that more polar phases require a higher degree of roughening. In detail, however, the information becomes much more complex. For most liquid phases we find a wide range of suitable degrees of pre-treatment. It should not be concluded that the average degree is the most suitable, because by varying the pre-treatment it is possible to adapt the column characteristics for analytical purposes. As a further simple rule, it can be said that the highest degree of roughening is recommended in order to obtain the most durable column, *i.e.*, maximum thermostability and minimum sensitivity for heavy overloading, strong polarity, heavy sample by-products and high water content in the sample. The least amount of roughening, on the other hand, yields the best preconditions for high separation efficiency. This is a simplified rule; more detailed information can only be obtained from the individual phases.

The relationships given in Table I become even more relative when the following observations are included. It is possible to obtain perfectly stable columns, with excellent characteristics, outside the indicated range, as an example may show. A Pyrex capillary (Fig. 5) pre-treated with 30-fold diluted barium hydroxide solution (003) yields a very poor column after being coated with PG 20 M. This column, nevertheless, is conditioned during a few days at its normal temperature limit of *ca.* 250°. After rinsing and re-coating, it appears markedly improved, and the third coating may produce an excellent column. An analogous observation can be made when a Ucon phase is coated on a 03 or 1 pre-treated capillary. It is to be noted that silicone phases cannot be re-coated because of their autophobic behaviour.

It should be repeated that the above information has been obtained by using

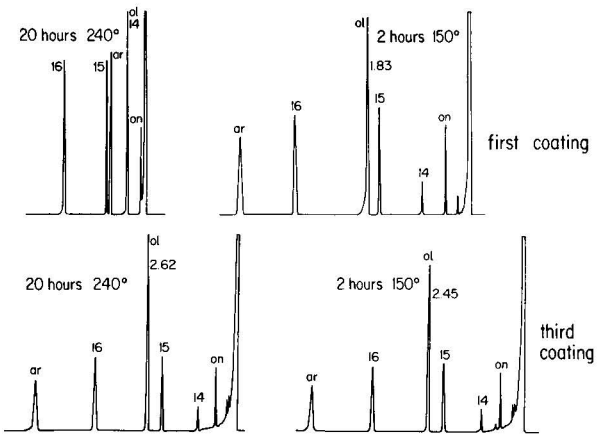


Fig. 5. Influence of repeated re-coating in the case of non-ideal surface pre-treatment. Pyrex capillary (18 m × 0.34 mm I.D.) barium carbonate treated 003 (suitable treatment 1, 03). Isothermal runs at 100°; 2.2 ml H_2/min. Substances: on = 5-nonanone; ol = 1-octanol; ar = naphthalene; 14–16 = *n*-tetradecane–*n*-hexadecane. Coating: 12% PG 20 M in dichloromethane, mercury plug, 0.3 cm/sec. Discussion see *Liquid phase and suitable pre-treatment*.

Pyrex glass. A change of glass type may markedly shift the indicated ranges. For instance, for soft glass considerably less roughening is needed.

Deactivation

The method used by Aue *et al.*[10] to inactivate a solid surface by producing a non-extractable thin film of a highly polar polymer is widely accepted and has been applied to glass capillaries[23]. The method we have used for several years is different in the following respects. It is our experience that a surface on which a polar liquid does not spread when applied in bulk amounts (*i.e.*, dynamic coating with a 1% or more concentrated solution), can ideally be covered by Aue *et al.*'s non-extractable film, when the deactivating agent is used in concentrations of 1‰ or less. The substance present in this dilute solution does not form a liquid layer after evaporation of the solvent. It is just sufficient to mask the most active sites. After heating it for 20–30 min, we repeat the treatment in order to cover remaining blanks. It seems that the film can be extended and completed in several steps, starting from the initially formed clusters. Extraction of non-adsorbed substance after heating is not necessary, as this is effected automatically during the subsequent coating.

Deactivation is only part of the above treatment. Wettability is also increased. Both effects are accomplished by the polyglycol treatment before coating with some polar phases, as indicated in Table I. A special case is the treatment we use before applying apolar coatings; in this treatment PG 20 M is used as an ideal deactivating agent, which greatly reduces adsorption of polar solutes on the column after apolar coating. However, the strongly polar PG is poorly wettable by apolar phases. Subsequent treatment with Emulphor (E in Table I) eliminates this problem, as this substance easily spreads on a PG layer due to the PG section of its molecule, while the hydrocarbon section, which is directed away from the solid surface, ensures

spreading of apolar liquids. In some instances treatment with Emulphor alone can yield sufficiently both effects.

The practical procedure is as follows. Solutions containing 0.1–1‰ of PG 20 M or Emulphor in methylene chloride (more concentrated solutions are used for more intense barium carbonate treatments) are passed through the capillary at normal coating rates of *ca.* 0.5–1 cm/sec. After quickly drying it by applying a vacuum to the outlet end, the column is mounted as usual in the oven of a gas chromatograph. It is flushed at room temperature for a few minutes with a high carrier gas flow. The flow is then reduced almost to zero and the oven is quickly heated and, with PG 20 M, is maintained for 20 min at 280°; the corresponding temperature for Emulphor is 240°. Before the heating is stopped, the carrier gas flow is increased again so as to prevent air from entering the column in the first moments of cooling. The cooled column is immediately ready for coating or for an additional PG or E treatment.

If a capillary is treated with barium carbonate with the intention of making a moderately polar or apolar column, then ether, which normally follows acetone the drying step, is replaced by a PG solution. In this event, a sufficient amount of acetone is entroduced to ensure complete drying. (Ether has some drying effect, which is not true for methylene chloride).

As indicated in Table I (bottom), more PG and E treatments are required for a more roughened surface (*e.g.*, two PG and two E treatments on a 01 loaded surface). The additional work is however, worthwhile, as decreased adsorption and increased stability may be obtained.

Dynamic coating

Most liquid phases yield columns of almost identical quality after dynamic or static coating. As the dynamic procedure is time saving, we reserve static coating for those phases which, because of high viscosity, cannot be applied dynamically.

The mercury-drop method, developed by Schomburg *et al.*[24], effectively increases the reproducibility and safety of dynamic coating. It permits the use of about a two-fold concentration of the liquid phase, *i.e.*, only one half the amount of solvent has to be evaporated. This almost totally eliminates difficulties from plug formation, local accumulation of liquid phase, etc. We use low-viscosity phases, such as Emulphor, the Ucones, OS-124, etc., in concentrations of 16–20%, with dichloromethane as the preferred solvent. The coating rate is *ca.* 0.5 cm/min. PG 20 M, as an example of high viscosity, is applied in 12% solution at the rate of 0.3 cm/min. It has to be noted that rather thin films, only slightly more than 10^{-4} mm thick, are obtained in this way.

For columns with not less than 0.25 mm I.D., the practical procedure is simple. The coating solution is introduced by the suction method. When 6–8% of the column length is filled, the motion of the liquid is slowed down, so that it can barely be observed. This is effected by correspondingly reducing the vacuum applied at the column outlet. The inlet end is quickly withdrawn from the coating solution and dipped into a drop of mercury in a short, 2–3 mm wide glass tube. When a 2–3 cm long mercury plug has entered the column, normal dynamic coating is started. It is important that mercury that has been used for coating or been in contact with a coating solution should not be re-used before being carefully cleaned. Also air zones between mercury and coating solution that are longer than 10–15 mm should be

avoided. A longer air zone is too elastic and may therefore cause the mercury plug to jump.

Static coating

Viscous phases such as OV-1, SE-52, etc. are exclusively handled by static coating, as developed by Bouche and Verzele[25]. For columns with 0.25–0.3 mm I.D., we use *ca.* 2‰ and for those with 0.3–0.4 mm I.D. *ca.* 1.5‰ solutions in methylene chloride. We still prefer to close one column end by sucking in *ca.* a 1-cm length of concentrated water glass and to start applying a vacuum at the opposite end after at least 6 h have elapsed.

The coating of the end sections, after dynamic or static coating, may be more or less irregular. We therefore rinse *ca.* 15 cm at each end with 0.5‰ PG 20 M (*i.e.*, a solution used for deactivation) in methylene chloride, connecting a 25-μl syringe to one end by a short piece of flexible tubing, while applying a low pressure of inert gas to the other end.

Besides these major aspects, further information regarding specific manipulations, tools and aids should be described. We feel, however, that practical demonstration is more effective.

Testing and evaluation

After drying the freshly coated columns, they are flushed with 2–3 times the normal carrier gas flow in the closed oven, without heating (40–50°), for *ca.* 20 min. The oven temperature is increased within 1 h to 150°. After conditioning for 1 h at 150°, the routine tests for polarity/adsorption, acidity and separation efficiency are carried out, as described earlier[26]. The results obtained indicate the characteristics of the column when it is fresh. Within a further 2–3 h the temperature is increased up to the limit (maximum temperature for continuous use) and this temperature is maintained overnight. With phases that are known to show moderate or high bleeding during initial conditioning, the column is disconnected from the detector. Before repeating the tests the next day, the outlet sections of these latter columns are again rinsed. Comparison of the two tests is in most instances strongly indicative of the column quality. The columns are then maintained at the upper temperature limit for 1–3 days in order to obtain information about long-term behaviour. This prolonged treatment is worthwhile only for columns showing reasonable results after the first night. After prolonged heating, valuable information is obtained from the bleeding test, carried out before cooling them down to test temperature.

Besides being dependent on the liquid phase, the temperature limit, as mentioned above, greatly depends on the character of the column surface. For Pyrex/barium carbonate columns we use the following temperature limits: OS-124, 200°; Ucon HB 5100 and Emulphor, 230°; PG 20 M and OV-17, 240°; OV-1 and SE-52, 250°. It must be emphasized that all types of column continuously withstand temperatures that are 20–30° higher without loss of separation efficiency. As stated earlier[3], other, more critical, column characteristics can be determined, especially when long-term routine use is intended.

A few practical examples may elucidate these indications. Fig. 6 shows some results from the first conditioning period of an Emulphor column. Emulphor ON 870 (Applied Science Labs., State College, Pa., U.S.A.) is slightly acidic, probably due to oxidation processes. After 2 h of conditioning at 150°, this acidic character can still

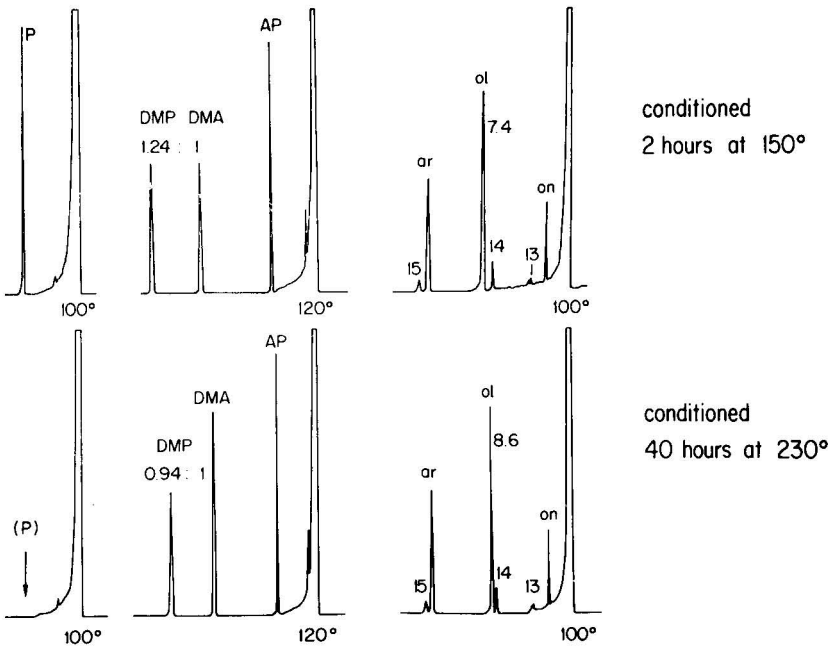


Fig. 6. Typical behaviour of a correctly prepared Emulphor column during conditioning. Pyrex capillary (20 m × 0.34 mm I.D.) barium carbonate treated 01, coated with 16% Emulphor ON 870 in dichloromethane, mercury plug, 0.4 cm/sec. For substances, see Fig. 5, plus acetophenone (AP), 2,6-dimethylaniline (DMA), 2,6-dimethylphenol (DMP), propionic acid (P), and *n*-tridecane (13). For discussion, see *Testing and evaluation*.

be observed from the 2,6-dimethylphenol (DMP)–2,6-dimethylaniline (DMA) ratio 1.24:1. The same observation indicates that the basic character of the column surface is weak. After prolonged conditioning at 230°, the ratio becomes 0.94:1, showing a slightly basic character. As a consequence of this shift, propionic acid exhibits drastically changed behaviour. A less drastic but very typical change is seen in the polarity test. Chromatographically, the alcohols exhibit weakly basic characteristics. Therefore, octanol shows increased retention on the acidic column (see distance from tetradecane before and after heating). Naphthalene, as a slightly acidic substance, clearly shows the opposite tendency, being more retained on the basic column, that is, after heating. The improved ratio of octanol to tetradecane (8.6 after heating) may be a consequence of the same shift. Further, exact measurement showed a reduction in retention of 7% after heating.

Fig. 7 shows the effect of inefficient deactivation on the adsorption characteristics after prolonged heating. While the separation efficiency remains unchanged, the 1-octanol peak indicates strongly increased adsorption. When the 01 barium carbonate layer, deactivated with two PG 20 M treatments, is followed by two rinsings with E and heating to 240° (see under *Deactivation*), the octanol peak keeps its perfect shape after several weeks of continuous heating.

In order to be considered satisfactory, a column has to show the following results after being maintained for several days at the temperature limit. No significant

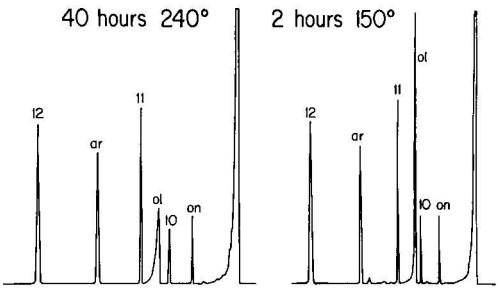


Fig. 7. Typical behaviour of an inefficiently deactivated column during conditioning. Pyrex capillary (20 m long, 0.34 mm I.D.) barium carbonate treated 01. Deactivation with 2.0‰ and 0.5‰ PG 20 M in methylene chloride, followed by maintaining at 280° for 30 min. Static coating with 1.5‰ SE-52 in methylene chloride. Isothermal runs at 75°. For substances, see Fig. 5, plus decane (10), undecane (11) and dodecane (12). For discussion, see *Testing and evaluation.*

increase in adsorption (no change in peak shape of 1-octanol, no change in peak-height ratio between 1-octanol and the next *n*-alkane); no significant change in polarity; no change in acid–base characteristics after the second day (a slight change during the first conditioning is normal); and no loss of retention after the second day (some column types show reduced retention after the first conditioning).

CONCLUDING RECOMMENDATIONS

When using the barium carbonate procedure for the first time, we recommend that a 30–50 m long and *ca.* 0.3 mm wide Pyrex capillary be treated with a 03 coating solution (a mixture of 10 ml of saturated barium hydroxide solution, 15 ml of distilled water, 5 ml of 1 *M* potassium hydroxide and 20 mg of non-ionic detergent), and coated, immediately after drying, with PG 20 M, Emulphor or Triton. This example requires minimum experience while yielding a column of wide applicability.

When some experience has been acquired, we recommend the use of apolar columns whenever it is clear that polar phases are not needed. Apolar columns, we specially recommend SE-52 and SE-54, exhibit a wider practical temperature range, less bleeding, less long-term polarity shift and longer life time, combined with higher separation efficiency. These conditions, however, hold only when adequate deactivation and wetting have been effected.

Although the barium carbonate treatment works on any kind of glass, we recommend the use of Pyrex or other borosilicate glass because it is more easily handled (drawing, straightening, etc.), more easily treated (no tendency for precipitation to occur and wider range of suitable treatments) and gives almost neutral columns.

In conclusion, it is necessary to emphasize again the essential role of the application technique, which is quite different from that suitable for packed columns, and which is less easily acquired than many analysts suppose. Weaknesses in the application technique will negate the effects of the best column.

ACKNOWLEDGEMENTS

Part of the experimental work on apolar coatings has been carried out by K. Grob, Jr. This work has again been generously sponsored by F. J. Burrus & Cie., Boncourt, Switzerland. Mrs. J. Davies kindly read the manuscript.

REFERENCES

1 K. Grob and G. Grob, in *Identification and Analysis of Organic Pollutants in Water*, Ann Arbor Science Publishers, Ann Arbor, Mich., in press.
2 K. Grob, *Chromatographia*, 8 (1975) 423.
3 K. Grob, *Chromatographia*, 5 (1974) 3.
4 H. T. Badings, J. J. G. van der Pol and J. G. Wassink, *Chromatographia*, 8 (1975) 440.
5 J. Roerade, *Chromatographia*, 8 (1975) 511.
6 G. Schomburg and H. Husmann, *Chromatographia*, 8 (1975) 517.
7 L. Blomberg, *J. Chromatogr.*, 115 (1975) 365.
8 F. W. Karasek, *Res. Develop.*, 26 (1975) 40.
9 M. Nečasová and K. Tesařík, *J. Chromatogr.*, 79 (1973) 15.
10 W. A. Aue, C. R. Hastings and S. Kapila, *J. Chromatogr.*, 77 (1973) 299.
11 G. Alexander and G. A. M. F. Rutten, *Chromatographia*, 6 (1973) 231.
12 G. Alexander, G. Garzó and G. Pályi, *J. Chromatogr.*, 91 (1974) 25.
13 G. Alexander and G. A. M. F. Rutten, *J. Chromatogr.*, 99 (1974) 81.
14 R. E. Kaiser, *Chromatographia*, 2 (1968) 34.
15 A. L. German and E. C. Horning, *J. Chromatogr. Sci.*, 11 (1973) 76.
16 A. L. German, C. D. Pfaffenberger, J.-P. Thenot, M. G. Horning and E. C. Horning, *Anal. Chem.*, 45 (1973) 931.
17 M. Blumer, *Anal. Chem.*, 45 (1973) 980.
18 J. G. Nikelly and M. Blumer, *Amer. Lab.*, 6 (1974) 12.
19 E. Schulte and L. Acker, *Z. Anal. Chem.*, 268 (1974) 260.
20 J. D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 112 (1975) 97.
21 J. D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 114 (1975) 190.
22 W. Stumm and H. Hohl, personal communication.
23 D. A. Cronin, *J. Chromatogr.*, 97 (1974) 263.
24 G. Schomburg, H. Husmann and F. Weeke, *J. Chromatogr.*, 99 (1974) 63.
25 J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.
26 K. Grob and G. Grob, *Chromatographia*, 4 (1971) 422.

Journal of Chromatography, 125 (1976) 487–494

CHROM. 9158

FLÜSSIGCHROMATOGRAPHISCHE PARAMETER HERBIZIDER WIRKSTOFFGRUPPEN

I. HARNSTOFFHERBIZIDE

JOSEF PRIBYL und FRITZ HERZEL

Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes, Corrensplatz 1, 1 Berlin 33 (B.R.D.)

(Eingegangen am 8. Dezember 1975; geänderte Fassung eingegangen am 1. März 1976)

SUMMARY

Liquid chromatographic parameters of groups of herbicidal active substances. I. Urea herbicides

Because of the polarity of herbicidal urea derivatives, liquid chromatography is the most suitable method for their determination. The separation of a number of active substances is described. A versatile applicable ternary solvent mixture is used as the mobile phase. Several columns are tested for their separation performances and their capability to retain active substances and some known degradation products. To fully utilize the sensitivity of the photometric detector the UV spectra of the tested compounds are determined.

PROBLEMSTELLUNG

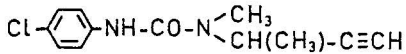
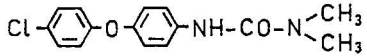
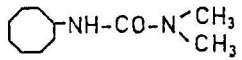
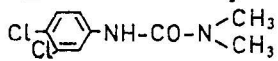
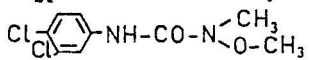
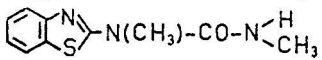
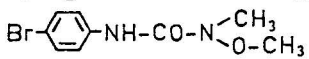
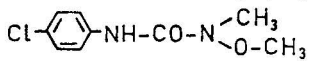
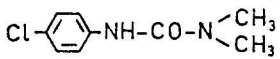
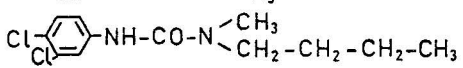
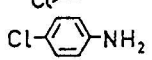
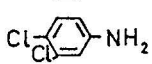
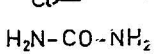
Im Gegensatz zu anorganischen oder metallorganischen Verbindungen, die sich anhand charakteristischer Eigenschaften im allgemeinen recht gut mit Hilfe der Atomabsorptionsspektrometrie, zum Teil sogar im Gemisch analysieren lassen, gelingt dies bei organischen Stoffen aufgrund geringer Unterscheidungsmerkmale meist nicht direkt. Sollen kleinste Konzentrationen aus Umweltproben (Boden- oder Wasserextrakte, Luftadsorbate) erfasst werden, so gilt es, nach einer Vorreinigung zunächst eine Trennung des Stoffgemisches herbeizuführen und möglichst zugleich auch die quantitative Bestimmung. Als Verfahren, das diesen Zweck erfüllt, kommt somit lediglich die Chromatographie in Frage.

Die meisten Insektizide sind aufgrund ihres relativ stark lipophilen Charakters auf dem Wege der Gas–Flüssigchromatographie bestimmbar; hinzu kommt, dass hierfür sehr empfindliche und zudem teilspezifische Detektoren zur Verfügung stehen, wie z.B. der Elektroneneinfangdetektor für Organochlorinsektizide sowie phosphorspezifische Detektoren. Von den herbiziden Wirkstoffen lassen sich viele nicht ohne vorherige Derivatisierung unzersetzt gaschromatographisch (GC) bestimmen; ihre Flüchtigkeit ist infolge höherer Polarität oder intramolekularer Wasserstoffbrücken-

TABELLE I

UNTERSUCHTE VERBINDUNGEN UND DEREN WICHTIGSTE PHYSIKALISCHE EIGENSCHAFTEN

Abkürzungen: MG = Molekulargewicht; F = Schmelzpunkt (°C); L = Wasserlöslichkeit bei 25° (ppm); Max = Absorptionsmaximum (nm); ε_{max} = Extinktionskoeffizient im Absorptionsmaximum (cm²/mg); ε_{254} = Extinktionskoeffizient bei 254 nm (cm²/mg).

Verbindung	*Strukturformel*	*MG*	*F*	*L*	*Max*	ε_{max}	ε_{254}
Buturon		236.7	146	35	246	48	37
Chloroxuron		290.8	151	4	247	36	33
Cycluron		198.3	138	155	226	2	0
Diuron		233.1	152	42	248	32	30
Linuron		249.1	93	75	248	42	40
Methabenzthiazuron		221.3	120	59	268	87	50
Metobromuron		259.1	95	336	247	39	37
Monolinuron		214.7	76	580	246	80	53
Monuron		198.7	177	230	245	75	53
Neburon		275.2	102	5	251	34	33
4-Chloranilin		127.6	70		244	32	26
3,4-Dichloranilin		162.0	62		249	30	26
Harnstoff		60.1	132		226	2	0

bildung begrenzt. Hier bietet sich die Flüssigchromatographie (Flüssig–Flüssig- und Flüssig–Festchromatographie) als sehr leistungsfähige Methode an.

Publikationen über die Trennung und Bestimmung von Harnstoffherbiziden mittels Flüssigchromatographie sind äusserst spärlich. In Fachzeitschriften ist u.W. bisher lediglich von Kirkland[1] eine entsprechende Arbeit bekannt. Darüber hinaus fanden wir noch ein ähnliches Trennbeispiel von vier herbiziden Harnstoffderivaten in einer Informationsschrift der Fa. Siemens[2].

Die im Rahmen dieser Arbeit untersuchten Wirkstoffe und Derivate sind in Tabelle I aufgeführt.

PARAMETER

Mobile Phase

An das Elutionsmittel müssen im vorliegenden Falle die folgenden Anforderungen gestellt werden:

(1) Es wurde grundsätzlich angestrebt, die zwar universell einsetzbare, meist jedoch langwierigere, schwerer beherrschbare und apparativ komplizierte Gradientelution zu umgehen. Die Elution mit Lösungsmitteln bzw. -gemischen konstanter Zusammensetzung besitzt zudem den Vorzug einer besseren Reproduzierbarkeit.

(2) Das Lösungsvermögen soll den zu trennenden Substanzen angepasst sein, so dass man zu vernünftigen Elutionszeiten kommt.

(3) Soweit der in der Flüssigchromatographie vielfach verwendete UV-Detektor zum Einsatz kommt, dürfen die Lösungsmittel keine Absorption in dem für die Messung vorgesehenen Spektralbereich aufweisen. Meist gilt das für Wellenlängen von mehr als 245 nm.

(4) Im Routinebetrieb fallen Lösungsmittelmengen an, die nicht nur aus Umweltgesichtspunkten, sondern auch aus wirtschaftlichen Erwägungen eine Wiederverwendung sinnvoll erscheinen lassen. Das Lösungsmittelgemisch sollte sich daher möglichst einfach wieder aufbereiten lassen, z.B. durch Destillation.

(5) Schliesslich sollte das Elutionsmittel keine korrosiven Eigenschaften gegenüber den Werkstoffen besitzen, mit denen es in Berührung kommt. Diesem Problem kommt —besonders beim Einsatz von Elutionsmitteln, die halogenhaltige Lösungsmittel enthalten— höhere Bedeutung zu als man zunächst angenommen hatte.

In den beiden vorgenannten Publikationen werden Äther, z.T. mit Isooctan gemischt, als Elutionsmittel verwendet. Wir haben mit diesen Lösungsmitteln gearbeitet und gefunden, dass sie sowohl hinsichtlich Handhabbarkeit als auch Reproduzierbarkeit namentlich für einen längeren Einsatz nicht allzu zweckmässig sind. Als geeigneter erwiesen sich für den vorliegenden Zweck Gemische aus Dichlormethan und Hexan mit einem geringfügigen Alkoholzusatz. Nach eingehender Prüfung verschiedenster Mischungen stellte sich das auch von Toth[3] verwendete Verhältnis 79:20:1 von Dichlormethan, Hexan und Äthanol als optimal heraus. Es erfüllt ausserdem die Bedingung der Wiederverwendbarkeit sehr gut, da sich das Gemisch relativ einfach destillativ trennen und reinigen lässt.

Säulenfüllungen

Es wurden die verschiedensten stationären Phasen auf ihre Leistungsfähigkeit getestet. Daten über Art und Herstellung der Säulen sind in Tabelle II zusammen-

TABELLE II

EINGESETZTE TRENNSÄULEN

Säule			*Träger*			*Belegung*			*Packungs-technik*	*Säulenvordruck (in bar) bei Durchflussgeschwindigkeit (ml/Std.)*				
Nr.	*Material*	*Masse*	*Art*	*Mittlerer Durchmesser (μm)*	*Herkunft*	*Art*	*%*	*Herkunft*		*10*	*20*	*30*	*50*	*100*
1	Glas	25 cm × 3 mm I.D.	Lichrosorb SI-60	10	Merck* 9307	vom Hersteller silanisiert			Slurry	12	25	40	68	125
2	Glas	25 cm × 3 mm I.D.	Lichrosorb SI-60	5	Merck 9388	—	0	—	Slurry	12	25	40	70	130
3	Glas	25 cm × 3 mm I.D.	Lichrosorb SI-60	5	Merck 9388	Fraktonitril III	10	Merck 9787	Slurry	15	30	50	75	145
4	Glas	50 cm × 3 mm I.D.	Lichrosorb SI-60	5	Merck 9388	—	0	—	Slurry	18	40	70	125	—
5	Glas	25 cm × 3 mm I.D.	Perisorb A	30–40	Merck 12431	vom Hersteller belegt			trocken mit Vibrator	8	12	18	25	42
6	Glas	25 cm × 3 mm I.D.	Polyamid 6	5–20	Macherey, Nagel & Co.**	—	0	—	Slurry	10	16	20	30	50
7	Glas	50 cm × 3 mm I.D.	Bio-Beads SX-12	40	Bio-Rad Labs.*** 1523650	—	0	—	Slurry	3	6	10	16	—
8	Stahl	25 cm × 2 mm I.D.	Mikropak CN	10	Varian§ 07 000 11000	fertig bezogen				12	24	37	65	125

* Darmstadt, B.R.D.
** Düren, B.R.D.
*** München, B.R.D.
§ Palo Alto, Calif., U.S.A.

gestellt. Wir verwendeten Stahlsäulen von 25 cm Länge und einem Innendurchmesser von 2 mm, vielfach jedoch auch Glassäulen (im Stahlmantel) von gleicher Länge und einer lichten Weite von 3 mm. Letztere sind nur bis zu 130 atü belastbar.

Die Beschickung der Säulen mit stationärer Phase geschah z.T. durch Einrütteln des trockenen Materials mit Hilfe eines Vibrators und der Wasserstrahlpumpe, zum grösseren Teil jedoch nach verschiedenen Slurry-Techniken. Es gelang uns selbst bei absolut gleichbleibenden Verfahren nicht, stets Säulen mit gut reproduzierbaren Trenneigenschaften zu erhalten. Es wurden deshalb von jedem Säulentyp prinzipiell zwei oder mehr Exemplare hergestellt. Für die Ermittlung der chromatographischen Daten wurde dann die leistungsfähigste und damit für die Charakterisierung ihrer Füllung typischste Säule ausgewählt.

Zum Unterschied zur GC zeigte sich, dass die Trennstufenzahl einer Säule viel stärker substanzabhängig ist. Die Berechnung der Trennstufenzahl (Tabelle III) erfolgte nach einem der üblichen Verfahren[4,5].

Geräte

Zu den Untersuchungen wurde der Flüssigchromatograph 8500 der Firma Varian verwendet mit dem Spektralphotometerdetektor 635. Die beiden Küvetten haben bei einer Schichtlänge von 1 cm ein Fassungsvermögen von 8 μl. Messungen unter 245 nm wurden möglich, da das Photometer als Zweistrahlgerät nach dem Kompensationsprinzip arbeitet.

Fig. 1 zeigt ein typisches Chromatogramm. Um die Nachweisempfindlichkeit zu optimieren, wurden die UV-Spektren der verschiedenen Verbindungen aufgenommen. Sie sind in Fig. 2 wiedergegeben. Die Extinktionskoeffizienten beim Absorptionsmaximum (ε_{max}) sowie bei der sehr gebräuchlichen Wellenlänge 254 nm (ε_{254}) enthält Tabelle I.

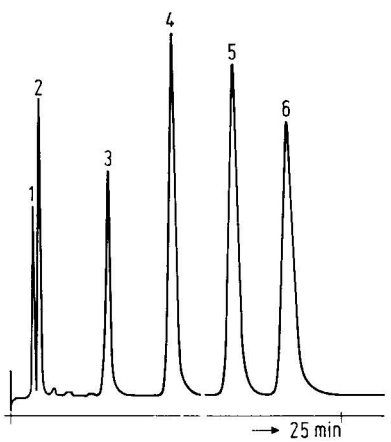


Fig. 1. Typisches Chromatogramm von Harnstoffherbiziden. Varian-Gerät 8500 mit Spektralphotometer 635; Messung bei 247 nm; stationäre Phase, Lichrosorb SI-60; mobile Phase, Hexan–Dichlormethan–Äthanol (20:79:1); Fliessgeschwindigkeit, 20 ml/Std. 1 = Metobromuron (0.5 μg); 2 = 4-Chloranilin (0.5 μg); 3 = Methabenzthiazuron (1.5 μg); 4 = Diuron (1 μg); 5 = Monuron (1 μg); 6 = Fenuron (1.5 μg).

TABELLE III

TRENNPARAMETER DER VERWENDETEN SÄULEN

(a) Trennstufenzahl (bei Durchfluss 30 ml/Std. und Dosierung 500 ng/2 μl); (b) Retentionszeit bei Durchfluss 30 ml/Std.

Verbindung	*Säule (Nr. aus Tabelle II)*															
	1		*2*		*3*		*4*		*5*		*6*		*7*		*8*	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Buturon	350	1.7	680	3.3	1800	2.6	400	6.2	250	1.6	80	2.3	25	4	800	1.8
Chloroxuron	340	2.8	1200	10.0	1800	9.0	680	19.8	700	2.5	80	2.8	25	4	740	3.8
Cycluron	340	2.0	750	6.8	800	5.6	450	13.3	150	1.1	80	2.4	25	4	750	2.3
Diuron	340	2.8	1000	9.6	1800	8.6	500	19.2	600	2.3	80	2.7	25	4	740	3.6
Linuron	290	1.6	800	2.9	900	2.4	360	5.9	250	1.6	80	2.2	25	4	500	1.6
Methabenzthiazuron	290	2.0	1200	6.3	1100	5.5	650	12.5	350	1.8	80	2.6	25	4	450	2.2
Metobromuron	290	1.6	800	3.6	700	2.5	430	6.8	250	1.6	80	2.2	25	4	450	1.6
Monolinuron	300	1.7	800	3.1	800	2.5	430	6.1	250	1.5	80	2.2	25	4	600	1.7
Monuron	310	3.1	1250	11.8	2200	10.9	700	22.3	700	2.8	80	3.0	25	4	650	4.3
Neburon	500	1.9	1300	3.3	3000	2.7	640	6.5	450	1.2	80	2.3	25	4	1500	1.9
4-Chloranilin	290	1.9	900	3.4	900	2.8	430	6.7	200	1.5	80	2.3	25	4	560	1.9
3,4-Dichloranilin	370	1.6	900	3.0	1900	2.4	430	5.8	200	1.5	80	2.2	25	4	1000	1.7
Harnstoff	390	2.5	700	6.1	2100	6.0	400	11.9	200	1.4	80	2.5	25	4	1000	2.6

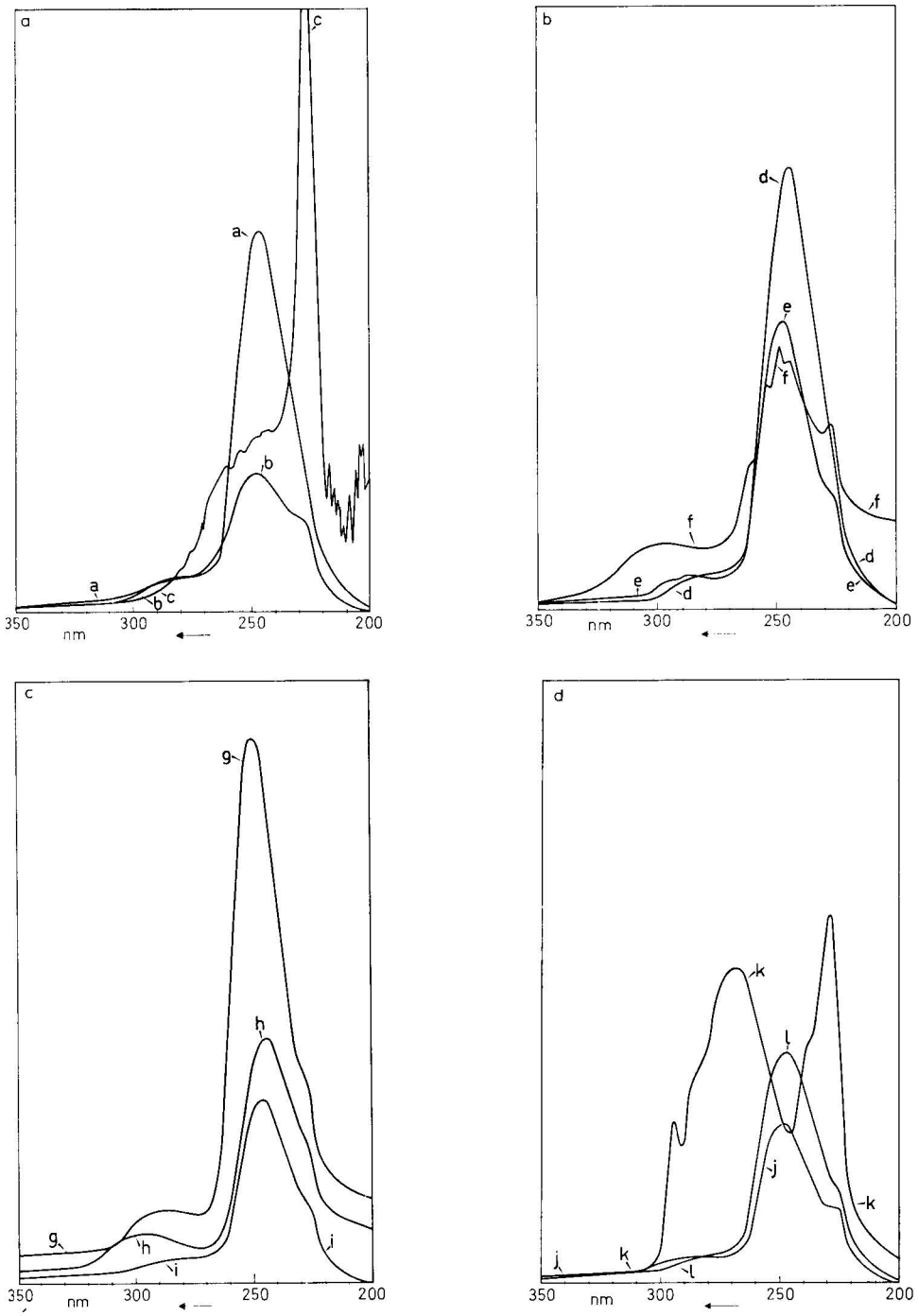


Fig. 2a–d. UV-Spektren einiger Harnstoffherbizide. a = Buturon; b = Chloroxuron; c = Cycluron; d = Monolinuron; e = Linuron; f = 3,4-Dichloranilin; g = Neburon; h = 4-Chloranilin; i = Monuron; j = Diuron; k = Methabenzthiazuron; l = Metobromuron.

Reproduzierbarkeit

Für Buturon ist der Detektor bis etwa 800 ng linear. Aus der Streuung der Messwerte errechnete sich eine relative Standardabweichung von 4%. Die Nachweisgrenze für Buturon liegt bei etwa 15×10^{-12} Mol.

DANK

Dem Bundesminister für Forschung und Technologie sei an dieser Stelle für die finanzielle Unterstützung der Arbeit gedankt.

ZUSAMMENFASSUNG

Für die Bestimmung der herbiziden Harnstoffderivate wird aufgrund deren Polarität zweckmässigerweiser die Flüssigchromatographie herangezogen. Es wird die Trennung einer Reihe von Wirkstoffen beschrieben. Als mobile Phase dient ein vielseitig geeignetes ternäres Lösungsmittelgemisch. Verschiedene Säulen werden auf ihre Trennleistung geprüft und auf ihr Retentionsverhalten gegenüber den Wirkstoffen sowie einigen bekannten Abbauprodukten. Um die Empfindlichkeit des Photometerdetektors voll auszuschöpfen, wurden die UV-Spektren der geprüften Verbindungen aufgenommen.

LITERATUR

1 J. J. Kirkland, *J. Chromatogr. Sci.*, 7 (1969) 7.
2 Siemens AG, *Analysentechn. Mitt.*, Nr. 85, Bild 25.
3 L. Toth, persönliche Mitteilung.
4 E. Cremer und L. Roselius, *Angew. Chem.*, 70 (1958) 42.
5 A. B. Littlewood, in D. H. Desty (Herausgeber), *Gas Chromatography*, Butterworths, London, 1958, S. 23.

Journal of Chromatography, 125 (1976) 495–502

CHROM. 9159

ANALYSIS OF SULPHUR-CONTAINING CARBAMATES BY FORMATION OF DERIVATIVES IN THE GAS–LIQUID CHROMATOGRAPH USING TRIMETHYLPHENYLAMMONIUM HYDROXIDE

R. H. BROMILOW and K. A. LORD

Chemical Liaison Unit, Rothamsted Experimental Station, Harpenden, Herts. (Great Britain)

(First received December 8th, 1975; revised manuscript received March 3rd, 1976)

SUMMARY

Carbamates injected into the gas–liquid chromatograph with trimethylphenylammonium hydroxide react to give derivatives with good gas–liquid chromatographic properties. Oximecarbamates yield methoximes by this procedure, and substituted-phenyl N-methylcarbamates yield anisoles. Yields over a range of conditions were generally better than 90%, except from aldoximecarbamates where competing reactions intervened. Although only sulphur-containing carbamates were studied, it should be possible to extend the reaction to other carbamates.

INTRODUCTION

Carbamate pesticides present difficult analytical problems due to their instability and low volatility in gas–liquid chromatography (GLC) columns. Recent approaches to these problems have been reviewed by Ruzicka[1], Thornburg[2] and Dorough and Thorstenson[3]. Cochrane[4] has reviewed the preparation of derivatives from carbamates for estimation by GLC or fluorimetry.

Direct GLC of methomyl[5], an oximecarbamate, and of several other carbamates including substituted-phenyl N-methylcarbamates[6–8] has been reported, but exacting chromatographic conditions are required to prevent decomposition. Aldicarb, an aldoxime-N-methylcarbamate, and its oxidation product, aldicarb sulphone, may be assayed as their corresponding nitriles produced by thermal degradation in the heated injection ports of GLC systems[9]. The hydrolysis products of carbamates also have been subjected to GLC directly: examples include the oxime from methomyl[10] and the phenols from methiocarb and its oxidation products[11]. Moye[12] has reported a novel procedure involving injection of N-methylcarbamates with methanol containing a little aqueous sodium hydroxide solution, whence transesterification occurs yielding methyl N-methylcarbamate.

Alkylation of compounds with –NH groups yields derivatives with improved stability and GLC characteristics. Methylated derivatives of several classes of such compounds including carbamates have been prepared by base-catalysed reaction

with methyl iodide[13]. The potentially simpler procedure of on-column methylation using trimethylphenylammonium hydroxide (TMAH) has been applied to barbiturates[14,15], cannabis metabolites[16], diphenylhydantoins[17,18], fatty acids[19], and phenylurea herbicides[20]. We have found that injection of the oximecarbamate oxamyl with TMAH results in loss of the carbamate group with formation of the corresponding methoxime in high yield, this derivative also having good GLC properties. This paper reports on the applicability of this technique to the GLC analysis of a series of sulphur-containing oximecarbamate and substituted-phenyl N-methylcarbamate insecticides and nematocides.

EXPERIMENTAL

Materials and reagents

The nitriles, some oximes and phenols and the carbamates were analytical grade supplied by the respective manufacturers; the other oximes and phenols were prepared by hydrolysis of the corresponding carbamates with 1 *N* aqueous sodium hydroxide. Stock solutions of test substances (approximately 1 mg/ml) were prepared in analytical-grade acetone or ethyl acetate and stored at −16°; diluted solutions were prepared immediately prior to use. TMAH was purchased as a 0.1 *M* solution in methanol from Eastman-Kodak (Rochester, N.Y., U.S.A.). Thin-layer chromatography (TLC) was carried out on pre-coated plates (0.25 mm thick silica gel 60 F_{254}, Merck, Darmstadt, G.F.R.) used as received.

Preparation of derivatives

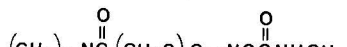
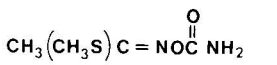
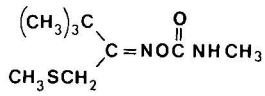
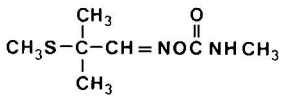
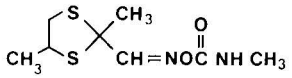
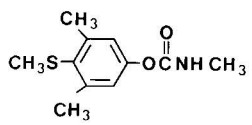
Preparation of oxamyl methoxime. Oxamyl (I) (1.0 g) was dissolved in 10 ml ethanol and 50 ml 1 *N* aqueous sodium hydroxide was added. After leaving this solution for 2 h at room temperature to ensure complete hydrolysis of oxamyl to its oxime, 2.0 ml dimethyl sulphate were added and the mixture stirred for a further 2 h. The product was extracted with dichloromethane (2 × 50 ml) and the pooled extracts, dried over anhydrous sodium sulphate, were rotary evaporated to a pale yellow oil. This material was purified by chromatography on a 100 mm × 30 mm I.D. silica gel column using diethyl ether–acetone mixtures as eluent to give oxamyl methoxime (II) (287 mg, 36%) as a colourless oil. Nuclear magnetic resonance (NMR) ($CDCl_3$): $\tau = 6.02$ ($-OCH_3$); $\tau = 6.91, 6.94$ [$(CH_3)_2N-$]; $\tau = 7.73$ ($-SCH_3$).

The methoximes and anisoles (substituted-phenyl methyl ethers) listed in Table I were prepared similarly from their respective carbamates, with the exception of the anisoles from methiocarb sulphoxide and methiocarb sulphone which were prepared by dilute peracetic acid oxidation of methiocarb anisole. The structures of these derivatives were confirmed by NMR. The methoxime of DS-15647 could not be satisfactorily prepared by the above methylation procedure and was not available for this study. Table I gives the TLC properties of the carbamates and their derivatives together with the chemical structures of the carbamates. The compounds were located on the TLC plates by quenching of the fluorescent indicator in the silica gel at 254 nm, or by spraying the plates with 0.5% 2,6-dibromo-*p*-benzoquinone-4-chlorimine in cyclohexane followed by heating to 110° for 15 min when the compounds appeared as brown spots on a pale background.

TABLE I

STRUCTURES AND TLC PROPERTIES OF CARBAMATES AND DERIVATIVES

Solvent systems: (A) hexane–acetone (2:1); (B) diethyl ether–acetone (4:1).

Compound	*Structure*	*R_F value*					
		Carbamates		*Corresponding phenol or oxime*		*Corresponding methoxime or anisole*	
		A	*B*	*A*	*B*	*A*	*B*
Oxamyl (DuPont 1410)		0.094	0.20	0.13	0.37	0.31	0.44
Methomyl		0.19	0.35	0.29	0.54	0.51	0.62
DuPont 1642		0.11	0.28				
DS-15647 (thiofanox)		0.40	0.59	0.53	0.75	—*	—*
DS-15647 sulphoxide**	–	0.09	0.11	—*	—*	—*	—*
DS-15647 sulphone**	–	0.23	0.37	—*	—*	—*	—*
Aldicarb		0.35	0.53	0.50	0.71	0.69	0.76
Aldicarb sulphoxide	–	0.03	0.07	0.07	0.16	0.19	0.25
Aldicarb sulphone	–	0.11	0.23	0.24	0.55	0.42	0.60
Tirpate		0.34	0.53	0.48	0.67	0.63	0.70
Methiocarb		0.47	0.69	0.50	0.73	0.65	0.77
Methiocarb sulphoxide	–	0.08	0.16	0.08	0.18	0.17	0.24
Methiocarb sulphone	–	0.21	0.49	0.24	0.56	0.39	0.62

* Compounds were not available for this study.

** The sulphide $-SCH_3$ is replaced by $-SOCH_3$ and $-SO_2CH_3$ in the sulphoxide and sulphone analogues, respectively.

Gas–liquid chromatography

Apparatus. The gas chromatograph used was a Pye 104 series oven fitted with a venting valve[21] and a United Analysts flame photometric detector operated in the sulphur mode (394-nm filter) according to the manufacturer's instructions. 0.9 m ×

3 mm O.D. stainless-steel columns were employed, packed with 80–100 mesh Chromosorb W coated with 0.5% Carbowax 20M + 5% SE-30 or with 5% QF-1. The nitrogen carrier gas flow-rate was maintained at 60 ml/min in the isothermal studies. The glass injection port, 90 mm × 3 mm I.D., was lightly packed with non-silanised glass wool and maintained at 210° (except where otherwise stated).

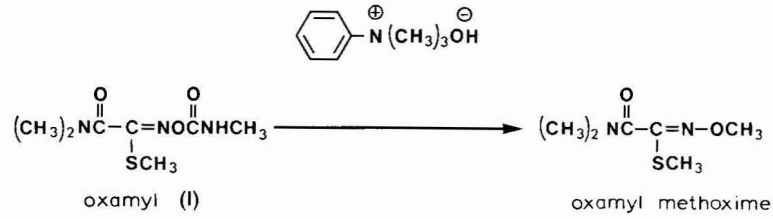
Injection procedure. Chemicals were usually taken into a 10-μl syringe in the following order: solvent (0.2 μl), sample solution (2.0 μl) and, finally, 0.1 *M* TMAH in methanol (0.5 μl). (The initial 0.2 μl of solvent may be omitted if so desired to simplify the injection procedure). Preformed derivatives were injected in the same volume of solvent (2.7 μl) without TMAH. The column effluent was vented to the atmosphere for 30–60 sec after injection.

Identification of the derivatives. The derivative formed by injection of oxamyl with TMAH was trapped in a cooled glass capillary from the column effluent. It was shown to be identical with authentic oxamyl methoxime (II) by co-chromatography on GLC and TLC, and by NMR. Derivatives formed similarly from other carbamates had the same GLC retention times as the corresponding methoximes or anisoles where these were available by independent synthesis.

Estimation of the yield of the derivatives. Yields of derivatives obtained from injection of carbamates (about 20 ng) with TMAH were estimated from the peak heights by interpolation from log–log calibration curves constructed for each of the authentic derivatives.

RESULTS AND DISCUSSION

Carbamates react with TMAH in the gas chromatograph to give derivatives with improved GLC stability and volatility. Phenyl N-methylcarbamates yield anisole derivatives by this procedure, and oximecarbamates yield methoximes, as illustrated by the reaction of oxamyl (I):



oxamyl (I) oxamyl methoxime (II)

The derivative formed thus from oxamyl was shown to be identical to independently synthesised oxamyl methoxime by GLC, TLC and NMR.

The yield of derivative from oxamyl (23 ng) injected with TMAH was examined using a range of conditions, and found to be unaffected by: varying the injection port temperature from 180°–300°; varying the volume of TMAH solution from 0.2–0.7 μl; reversing the order of the TMAH and the sample solution in the syringe. Thus, use of the procedure is straightforward and gives consistent results over a wide range of conditions.

Yields and GLC retention times of derivatives formed by this procedure from

TABLE II

YIELDS AND RETENTION TIMES OF THE DERIVATIVES FORMED BY THE ON-COLUMN REACTION OF TMAH WITH SOME CARBAMATES

Column, 0.5% Carbowax 20M + 5% SE-30.

Compound	*Column temperature (°C)*	*Retention time (min)*	*Yield (%)*
DuPont 1642	80	2.5	99
Methomyl	80	2.5	93
DS-15647	110	2.0	—*
DS-15647 sulphoxide	150	2.35	—
DS-15647 sulphone	150	2.60	—
Oxamyl	150	2.60	97
Methiocarb	150	3.6	90
Methiocarb sulphoxide	200	3.6	97
Methiocarb sulphone	200	3.9	100

* Yields were not measured for DS-15647 and its oxidation products as the authentic methoximes were not available.

some oximecarbamates (other than those from aldoximes) and substituted-phenyl N-methylcarbamates are given in Table II. The measured yields (for six compounds) were 90% or higher. Single peaks were obtained from each compound.

Yields of methoximes resulting from injection of aldoximecarbamates with TMAH varied from 0–91% (Table III), one cause being the competing thermal degradation of aldoximecarbamates to the corresponding nitriles[9]. Thus the fate of aldicarb by this procedure could be accounted for entirely by methoxime and nitrile formation (91% and 9%, respectively). However, Tirpate yielded only 38% methoxime, together with a smaller amount of another material presumed to be the nitrile. As there is also a 60–70% loss of preformed Tirpate methoxime on co-injection with

TABLE III

YIELDS AND RETENTION TIMES OF THE METHOXIME DERIVATIVES AND NITRILES FORMED BY THE ON-COLUMN REACTION OF TMAH WITH SOME ALDOXIMECARBAMATES

Column, 0.5% Carbowax 20M + 5% SE-30.

Compound	*Column temperature (°C)*	*Methoxime*		*Nitrile*	
		Retention time (min)	*Yield (%)*	*Retention time (min)*	*Yield (%)*
Aldicarb	80	2.3	91	1.45	9
Tirpate	130	3.4	38	2.7*	—**
Aldicarb sulphoxide	130	2.9	0	2.1*	—**
Aldicarb sulphone	150	1.5	0	1.25	8

* Retention time of the peak produced by injection of the carbamate alone and presumed to be the corresponding nitrile formed by thermal degradation. The more extensive degradation product methacrylonitrile has been reported previously to be formed during GLC of aldicarb sulphoxide[9], but this would not have been detected in our GLC system.

** The yields were small but could not be measured accurately in the absence of the authentic nitriles.

TMAH, it is likely that the methoxime is formed in high yield and subsequently partially decomposed to apparently involatile products, no other GLC peaks being observed.

Aldicarb sulphoxide and aldicarb sulphone injected with TMAH gave only low yields of the corresponding nitriles and no detectable amounts of the methoximes. In an attempt to elucidate the reactions occurring, some possible products from aldicarb sulphone were themselves injected with TMAH: aldicarb sulphone methoxime was largely decomposed, the only other peak observed representing variable but small amounts of the nitrile; aldicarb sulphone nitrile was about 90% decomposed, no other peaks being observed; aldicarb sulphone oxime yielded no peaks at all. This last observation suggests that the methoxime is not formed at all under these conditions (see below), but the alternative explanation, *viz.* that the methoxime is formed and then degraded perhaps via the nitrile, cannot be entirely ruled out. No attempt was made to increase yield of the methoxime derivatives by varying the GLC conditions or reaction procedure, because the aldoximecarbamates can be assayed adequately by thermal degradation in the gas chromatograph to the corresponding nitriles[9].

Fig. 1 shows the separation of carbamates in mixtures injected with TMAH using temperature programming. Because of the wide range of volatility of the derivatives, the carbamates were for convenience divided into two groups based upon their GLC retention times. Sulphoxides were not included as they were inadequately resolved from the corresponding sulphones, and in analytical procedures are likely to be oxidised to the sulphones prior to GLC estimation. The methoximes from oxamyl and DS-15647 sulphone were not resolved on the 0.5% Carbowax 20M + 5% SE-30 column, but could be separated on the 5% QF-1 column (retention times 2.75 and 2.28 min, respectively, at 180°).

The reaction of carbamates with TMAH appears to proceed in two steps: initial loss of the carbamate group to give the hydroxy moiety (oxime or phenol),

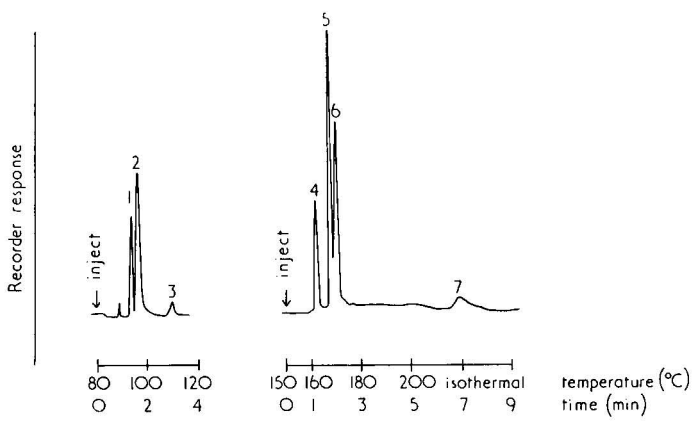


Fig. 1. Separation of carbamates in mixtures (20 ng of each compound) by on-column derivatisation with TMAH and temperature programming to 200°. Column, 0.5% Carbowax 20M + 5% SE-30; inlet pressure of nitrogen carrier gas, 10 p.s.i. 1 = Aldicarb; 2 = methomyl; 3 = DS-15647; 4 = Tirpate; 5 = oxamyl and DS-15647 sulphone, unresolved; 6 = methiocarb; 7 = methiocarb sulphone. The peak eluting at 89° is aldicarb nitrile.

which is then methylated. Carbamates having at least one unsubstituted position on the nitrogen are rapidly hydrolysed in aqueous alkali, and Ebing[22] observed that GLC injection of substituted-phenyl N-methylcarbamates with aqueous ammonia produced the corresponding phenols. Authentic oximes or phenols injected with TMAH gave derivatives of the same retention time as those produced similarly from the carbamates. Although the procedure gave similar yields of derivatives from both the carbamates and their hydrolysis products, this is perhaps fortuitous, especially for the aldoximecarbamates which can be degraded by competing reactions not readily available to their oximes.

The procedure gave reproducible results, six consecutive injections of oxamyl (23 ng) with TMAH giving a standard deviation of 4.9% about the mean peak height. GLC peak heights for oxamyl injected with TMAH and oxamyl methoxime fell on the same calibration curve (Fig. 2) over the range 10–250 picomoles.

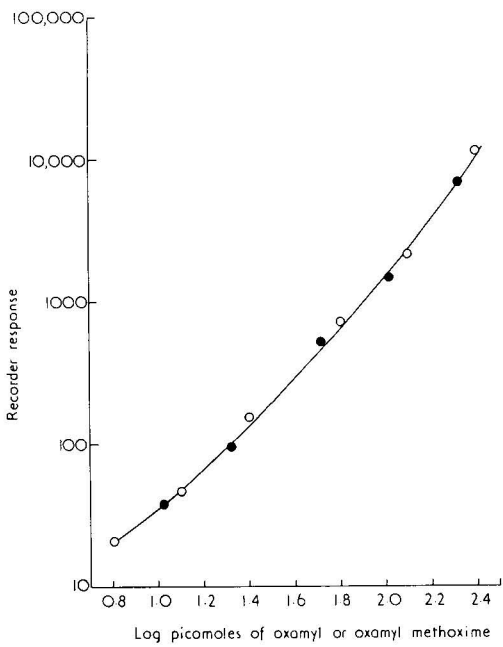


Fig. 2. Log–log calibration curve using the flame photometric detector in the sulphur mode. Column, 0.5% Carbowax 20M + 5% SE-30, maintained at 150°. ○ = Oxamyl methoxime; ● = oxamyl plus TMAH.

Few problems were encountered with the procedure although apparent yields of oxamyl methoxime could be reduced to about 80% if the injection port became contaminated with rubber fragments from the septum or carbonised involatiles from crop extracts, etc., and, at oven temperatures of less than 110°, injections made within 10 min of a previous TMAH-containing injection gave slightly reduced peak heights. Injections of the preformed derivatives with TMAH also gave similarly reduced responses under these conditions, probably caused by volatiles generated by the thermal decomposition of the TMAH[23] bleeding slowly into the detector and quenching the

sulphur emission[24]. Some accumulation of TMAH does occur in the GLC injection port, as injection of oxamyl alone following a series of injections with TMAH produced an appreciable peak of the methoxime. Conditioning the chromatograph overnight at the normal operating conditions removed this "memory effect".

Oxamyl has been estimated routinely in a wide range of soil and crop samples using on-column derivatisation with TMAH. This method does not distinguish between carbamates and their hydrolysis products, and the latter must be removed by "clean-up" procedures if estimation of the carbamates alone is required. Although this study has been confined to the assay of sulphur-containing carbamates using the highly sensitive and selective flame photometric detector, the derivatisation procedure should be applicable to the GLC analysis of other carbamates using different detectors.

ACKNOWLEDGEMENTS

The authors thank the Union Carbide Corporation, E.I. du Pont de Nemours & Co., Farbenfabriken Bayer A.G., Diamond Shamrock Chemical Co., and the Minnesota Mining and Manufacturing Co. for gifts of chemicals, and D. Middleton and Fathima Jabbar for technical assistance.

REFERENCES

1 J. H. A. Ruzicka, *Proc. Soc. Anal. Chem.*, 10 (1973) 32.
2 W. Thornburg, *Anal. Chem.*, 47 (1975) 157R.
3 H. W. Dorough and J. H. Thorstenson, *J. Chromatogr. Sci.*, 13 (1975) 212.
4 W. P. Cochrane, *J. Chromatogr. Sci.*, 13 (1975) 246.
5 I. H. Williams, *Pesticide Sci.*, 3 (1972) 179.
6 L. Wheeler and A. Strother, *J. Chromatogr.*, 45 (1969) 362.
7 J. M. Peck and K. J. Harkiss, *J. Chromatogr. Sci.*, 9 (1971) 370.
8 E. J. Lorah and D. D. Hemphill, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 570.
9 J. B. Knaak, M. J. Tallant and L. J. Sullivan, *J. Agr. Food Chem.*, 14 (1966) 573.
10 H. L. Pease and J. J. Kirkland, *J. Agr. Food Chem.*, 16 (1968) 554.
11 M. C. Bowman and M. Beroza, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 1054.
12 H. A. Moye, *J. Agr. Food Chem.*, 19 (1971) 452.
13 R. Greenhalgh and J. Kovacicova, *J. Agr. Food Chem.*, 23 (1975) 325.
14 E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, 58 (1969) 370.
15 H. V. Street, *Clin. Chim. Acta*, 34 (1971) 357.
16 M. Widman, I. M. Nilsson, J. L. G. Nilsson, S. Agurell and K. Leander, *Life Sci.*, 10, Part II (1971) 157.
17 H. J. Kupferberg, *Clin. Chim. Acta*, 29 (1970) 283.
18 H. L. Davis, K. J. Falk and D. G. Bailey, *J. Chromatogr.*, 107 (1975) 61.
19 W. P. Cochrane and R. Purkayastha, *Toxicol. Environ. Chem. Rev.*, 1 (1973) 137.
20 F. S. Tanaka and R. G. Wien, *J. Chromatogr.*, 87 (1973) 85.
21 R. R. Claeys and T. Farr, *Anal. Chem.*, 40 (1968) 847.
22 W. Ebing, *Chimia*, 19 (1965) 501.
23 K. M. Williams and B. Halpern, *J. Chromatogr.*, 97 (1974) 267.
24 T. Sugiyama, Y. Suzuki and T. Takeuchi, *J. Chromatogr.*, 80 (1973) 61.

Journal of Chromatography, 125 (1976) 503–510

CHROM. 9221

SIMULTANEOUS DETERMINATION OF MEPERIDINE AND NORMEPERIDINE IN BIOFLUIDS

HAZEL H. SZETO* and CHARLES E. INTURRISI**

Department of Pharmacology, Cornell University Medical College, New York, N.Y. 10021 (U.S.A.)

(Received March 5th, 1976)

SUMMARY

A method employing solvent extraction and gas–liquid chromatography has been developed for the simultaneous determination of meperidine and its N-demethylated metabolite, normeperidine, in biofluids. Normeperidine is analyzed as the heptafluorobutyryl derivative. Using a flame ionization detector, the lower limit of sensitivity of the method is 0.02 μg/ml of biofluid for both compounds.

Samples of plasma obtained from obstetrical patients, following a single therapeutic dose, were found to contain higher levels of meperidine than concurrent samples of amniotic fluid. Normeperidine could not be detected in either biofluid after a single dose. There is, however, a gradual accumulation of normeperidine in plasma after repeated doses as determined in samples from cancer patients.

The method can also be used to determine the disposition of meperidine and the accumulation of normeperidine in the cat.

INTRODUCTION

Meperidine is a narcotic analgesic that is widely used for the relief of pain. In man, the major metabolic pathways of meperidine involve hydrolysis to form meperidinic acid, and N-demethylation to normeperidine, which is followed by hydrolysis to normeperidinic acid[1–4]. Normeperidine is the only metabolite that has been shown to possess significant pharmacologic activity[5] (Fig. 1). Animal studies have shown that the N-demethylated metabolite is half as potent as meperidine as an analgesic, but twice as potent as a convulsant[6,7].

Normeperidine has not been reported in human plasma after a single dose of meperidine. The purpose of this report is to describe a specific and sensitive method for the simultaneous determination of meperidine and normeperidine in biofluids.

* Predoctoral trainee supported by a Hoffmann-La Roche Fellowship.
** Andrew W. Mellon Teacher-Scientist, 1975–1976.

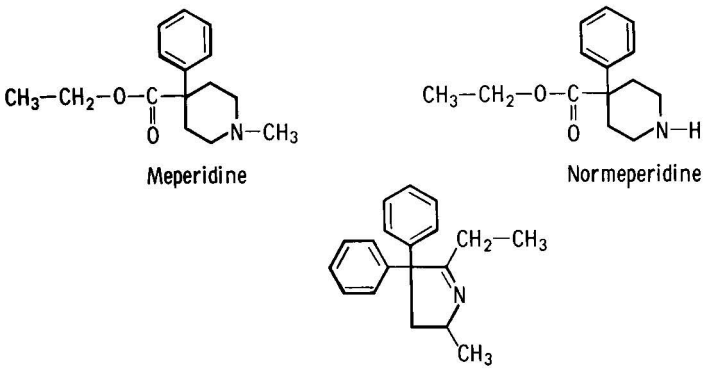


Fig. 1. Structural formulae of meperidine, normeperidine and the internal standard.

MATERIALS AND METHODS

Chemicals and reagents

Meperidine hydrochloride and normeperidine hydrochloride were generously supplied by Dr. F. Nachod of the Sterling-Winthrop Research Institute (Rensselaer, N.Y., U.S.A.). The internal standard, 2-ethyl-5-methyl-3,3 diphenyl-1-pyrolline hydrochloride, was provided by Dr. H. R. Sullivan of the Lilly Research Labs. (Indianapolis, Ind., U.S.A.) (Fig. 1).

The *n*-hexane and ethyl acetate are spectral grade, all other solvents are reagent grade. Anhydrous ethyl ether is obtained from Fisher (Pittsburgh, Pa., U.S.A.).

Stock solutions

Aqueous solutions of meperidine, normeperidine and the internal standard, each at a concentration of 4 μg/ml, are prepared and kept refrigerated.

Sample extraction from plasma

The method for extraction is adapted from that described by Inturrisi and Verebely[8] for the extraction of methadone from plasma.

To plasma (0.5—2.0 ml) in a 15-ml siliconized centrifuge tube with a PTFE-lined screw cap is added 0.15 ml of the solution of internal standard, 0.25 ml of 2.5 *N* sodium hydroxide and 2 drops of octyl alcohol. The sample is extracted with 5 ml of anhydrous ethyl ether by shaking for 5 min in an automatic shaker and centrifuged for 5 min at 500 *g*. The ethyl ether layer (upper) is carefully removed and saved. The extraction is repeated again with 5 ml of ethyl ether. The ether layers are combined and extracted with 5 ml of 0.2 *N* hydrochloric acid by shaking for 10 min and followed by centrifugation for 5 min. The ether phase (upper) is aspirated and discarded. The acid phase is then washed with 5 ml of hexane by shaking for 5 min and centrifuged for 3 min. The hexane wash (upper phase) is discarded. The washed acid phase is made alkaline by addition of 3 drops of 50% sodium hydroxide (pH >11), and extracted by shaking with 7 ml of ether for 10 min. After centrifugation for 5 min, the ether phase is carefully transferred to a 12-ml siliconized centrifuge tube and evaporated to dryness in a waterbath at 42°.

Sample extraction from amniotic fluid

Same procedure as described above for the extraction from plasma.

Preparation of the heptafluorobutyryl (HFB) derivative of normeperidine

A 3% solution (40 μl) of heptafluorobutyrylimidazole (HFBI) in ethyl acetate is added to the final dry extract, mixed and allowed to sit at room temperature in the dark for 30 min. The mixture is then dried by a gentle stream of nitrogen. The sample is reconstituted with 30 μl of cyclohexane by warming at 40°, and 1–2 μl is injected into the gas chromatograph.

Gas–liquid chromatography (GLC)

The GLC analysis is performed on a Varian Aerograph Model 2700 equipped with a flame ionization detector. The column is a 6-ft. long glass spiral with a 2-mm I.D. The packing material consists of 3% OV-17 on 80–100 mesh Gas-Chrom Q. The temperatures of the detector and the injection port are 275° and 250°, respectively. The carrier gas is helium at a flow-rate of 34 ml/min. Hydrogen and air flow are adjusted to give maximal detector response. Hydrogen flow-rate is 30–33 ml/min, and air flow-rate is 300–350 ml/min. The oven temperature is 175°. Detector sensitivity is varied from 4×10^{-11} to 16×10^{-11} A/mV at full scale as required.

Quantitation and calibration curves

Quantitation is performed by drawing in a baseline and measuring the peak height from the midpoint of the baseline. Calibration curves are constructed by plotting the ratio of the peak height of the compound to that of the internal standard against the concentration added. Standard calibration curves are obtained by adding meperidine and normeperidine in selected amounts from 0.04–1.0 μg to 2.0 ml of plasma or amniotic fluid and extracting according to the above procedure. The amount of each compound in a sample is determined by converting the peak height ratio into the absolute amount of compound present. Linearity of the standard curves in the range from 0.04–1.0 μg for both compounds allows for such conversion. The lower limit of sensitivity is defined as the lowest point on the calibration curve which gives at least a 5 unit deflection above baseline at attenuation 4×10^{-11}. This was found to be 0.02 μg/ml for both compounds in plasma and amniotic fluid.

RESULTS AND DISCUSSION

Many of the older methods for the determination of meperidine and normeperidine in human biofluids are based on spectrophotometric measurements of methyl-orange complexes[1–4,7]. These methods have been shown to be non-specific[9] and limited in sensitivity. Both of these considerations are critical in any method to be employed in pharmacokinetic studies. A fluorometric method for meperidine as described by Dal Cortivo *et al.*[10] has a lower limit of sensitivity of approx. 0.3 μg/ml. Recently several GLC methods have been reported for the quantitation of meperidine. Goehl and Davison[11] reported a GLC method for meperidine with a lower limit of sensitivity of 0.1 μg/ml. In 1974, Stambaugh and Wainer[12] published a method for quantitating 0.01 μg/ml of meperidine. These methods, however, do not also measure normeperidine levels. Klotz *et al.*[13] developed a method for both meperidine and nor-

meperidine by analyzing normeperidine as the trifluoroacetyl derivative, with a lower limit of sensitivity of 0.1 μg/ml. Chan *et al.*[14] reported a method for the simultaneous determination of meperidine and normeperidine using Carbowax as the stationary phase, which claimed to be capable of detecting as little as 0.02 μg/ml of both compounds in a 5-ml sample. However, we were not able to reproduce their method in our laboratory. The method we have developed supersedes most available methods both in sensitivity and by providing simultaneous quantitation of meperidine and normeperidine.

The conditions for the extraction of meperidine and normeperidine from biofluids were modified from the standard procedure for basic amines[8] to favor the recovery of both compounds. For example, we found that while *n*-butyl chloride would provide a good recovery of meperidine, a more polar solvent such as ethyl ether was necessary for optimal recovery of normeperidine.

Due to the close similarity in the chemical structure of meperidine and normeperidine, a commonly used non-polar phase such as SE-30 was not sufficient to separate the two compounds over a wide range of temperature conditions. The relatively more polar phases such as QF-1 and OV-17 allowed baseline separation, but did cause a moderate amount of tailing of the normeperidine. It was also found that although the linearity of the calibration curve for meperidine did extend down to 0.02 μg/ml, the linear range for normeperidine could be extended only to 0.08 μg/ml.

In order to overcome the problem of adsorption of normeperidine onto the packing material, it was necessary to form a derivative. A HFB derivative was chosen because it can be formed readily from normeperidine and results in a derivative with much improved column characteristics under the GLC conditions as described. Also, although not utilized at this time, the HFB derivative could be analyzed by an electron capture detector with a further increase in sensitivity.

Heptafluorobutyryl derivatives may be prepared from heptafluorobutyrylimidazole (HFBI) or heptafluorobutyrylanhydride (HFBA). HFBI is preferred over HFBA because it does not release acid into the reaction mixture, an inert base imidazole is produced instead. The derivative procedure is adapted from a general method described by Franken and Trijbels[15] for aliphatic amines. Conversion of normeperidine to the HFB derivative was found to be complete in 30 min at room temperature. And in agreement with the above paper, it was essential that the reaction be carried out in the dark, otherwise a greenish yellow color results and the conversion is not reproducible. It was found that most of the excess reagent could be removed by drying with nitrogen. Cyclohexane was chosen as the solvent to reconstitute the compounds for GLC because it did not dissolve any of the more polar by-products, and thus produced chromatograms without any interfering peaks. With the preparation of the HFB derivative, it was possible to extend the linearity of the calibration curve of normeperidine down to 0.02 μg/ml. The method as described can routinely be used to measure as little as 0.02 μg/ml of meperidine and normeperidine with an initial sample volume of 1 ml of biofluid. This high degree of sensitivity enables us to collect relatively small biofluid samples for pharmacokinetic studies.

Examples of chromatograms obtained under the conditions described above are given in Fig. 2. The multi-step extraction procedure results in an extract that is free of interfering peaks. In most cases it was possible to introduce samples into the gas chromatograph every 8–10 min.

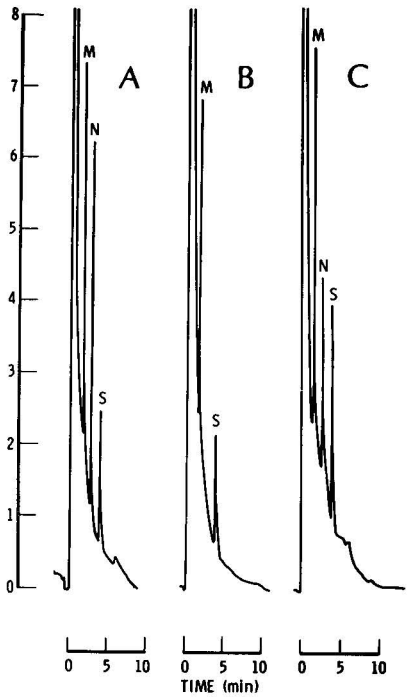


Fig. 2. Chromatograms of human plasma extracts. Retention times: meperidine (M), 1.8 min; normeperidine (N), 2.8 min; internal standard (S), 4.2 min. (A), Extract of control plasma to which was added 0.40 μg of meperidine (M), 0.40 μg of normeperidine (N), and 0.40 μg of internal standard. (B), Extract of plasma from an obstetric patient who received a single dose of meperidine, 100 mg i.m. (C), Extract of plasma from a cancer patient who received 10 doses of meperidine, 75 mg i.m. Detector sensitivity was 8×10^{-11} A/mV at full scale.

Fig. 2A shows the chromatogram obtained from an extract of control plasma to which was added 0.40 μg of meperidine, 0.40 μg of normeperidine, and 0.40 μg of internal standard. The retention times are: 1.8, 2.8, and 4.2 min, respectively.

Fig. 2B shows the chromatogram obtained from an extract of the plasma from an obstetrical patient who had received a single dose of meperidine, 100 mg intramuscular (i.m.). The sample was collected 30 min after the administration of the dose. The internal standard was added to 1.0 ml of sample plasma and the extract prepared as described in Materials and Methods. As can be seen in Fig. 2B the extract contained meperidine, but no normeperidine was detected.

Fig. 2C shows the chromatogram of an extract of a 0.5-ml plasma sample from a cancer patient who had received a total of 10 doses of meperidine, 75 mg i.m. The sample was collected 1 h after the last dose. It can be seen that the sample contained both meperidine and normeperidine.

The method was employed in the determination of the plasma and amniotic fluid concentration of meperidine and normeperidine in obstetrical patients after a single dose of meperidine. Amniotic fluid was obtained by amniocentesis at various times after the administration of the dose, and a blood sample was collected at the same time. The results in Table I show that meperidine is present in amniotic fluid

TABLE I

PLASMA AND AMNIOTIC FLUID LEVELS OF MEPERIDINE IN OBSTETRICAL PATIENTS AFTER A SINGLE 100-mg I.M. DOSE

Subject	*Weeks of gestation*	*Time after dose (min)*	*Meperidine level (μg/ml)*	
			Plasma	*Amniotic fluid*
1	20	15	0.34	0.00
2	20	30	0.32	0.02
3	19	60	0.37	0.04
4	20	120	0.12	0.07

within 30 min after intramuscular administration, and the concentration remains lower than that in plasma up to 3 h after the dose. Normeperidine did not attain detectable levels in plasma or amniotic fluid during that time period.

The method has been used to compare the plasma levels of meperidine and normeperidine in samples from obstetrical patients given a single dose of meperidine with the plasma levels from cancer patients given multiple doses. Table II shows the data obtained from some of these patients. All samples were collected 1 h after the dose.

TABLE II

PLASMA LEVELS OF MEPERIDINE AND NORMEPERIDINE IN PATIENTS RECEIVING SINGLE AND MULTIPLE I.M. DOSES OF MEPERIDINE

Subjects 1–4 are obstetrical patients and subjects 5–8 are cancer patients.

Subject	*Dose (mg)*	*Number of doses*	*Time after dose (h)*	*Plasma level (μg/ml)*	
				Meperidine	*Normeperidine*
1	100	1	1	0.29	0.00
2	100	1	1	0.37	0.00
3	100	1	3	0.07	0.00
4	100	1	3.5	0.11	0.00
5	100	9	1	0.54	0.28
6	75	10	1	0.38	0.18
7	75	5	1	0.36	0.13
8	100	70	4	0.16	0.48

The data indicate that the peak plasma level of meperidine at 1 h after dosing was, on the average, somewhat higher in those patients given multiple doses compared to those given a single dose. The half-life of meperidine in normal volunteers after a single intravenous dose has been reported by Klotz *et al.*[13] and Mather *et al.*[16] to be 3.2 h and 3.7 h, respectively. Therefore, since the dosing interval in the cancer patients was 4–6 h, some accumulation of plasma meperidine could be expected. In agreement with the results of Klotz *et al.*[13] no normeperidine was detected in plasma up to 3.5 h after a single dose. However, we do find relatively high levels of normeperidine in the plasma of patients who have received multiple doses of the drug. The half-life of normeperidine in man is not known. It appears that there is an accumulation of the N-demethylated metabolite in plasma after repetitive doses, suggesting that the half-

life of the metabolite is longer than that of the parent drug. This finding may have considerable clinical significance since normeperidine has been shown in animal studies to be 2–3 times as toxic as meperidine[6]. Meperidine and normeperidine produce both excitatory and depressant effects with the excitatory effects predominant for normeperidine. In animals toxic doses of normeperidine led to convulsions with varying degrees of respiratory depression, and eventually death[6,7]. Although the depressant effects can be completely antagonized by a narcotic antagonist such as naloxone, Gilbert and Martin[17] have shown, in mice, that naloxone can only partially antagonize the convulsions produced by normeperidine.

This method was also used to study the disposition of meperidine and normeperidine in the plasma of a cat who received 4 doses of meperidine. Meperidine hydrochloride (5 mg/kg, i.m.) was injected at 4-h intervals and blood samples were collected at various times from a chronic indwelling catheter. The results are shown in Table III.

It can be seen that meperidine is rapidly absorbed after i.m. administration, and the levels fall rapidly during the first hour. Normeperidine was not detected in plasma until 3 h after the first dose. With each succeeding dose, the meperidine levels decline rapidly between the 1/4- and the 4-h samples. In contrast there is a gradual accumulation of normeperidine in the plasma following each dose of meperidine. Thus the ratio of normeperidine/meperidine at 4 h after dosing is increased from 0.26 after the first dose to 0.76 after the fourth dose.

TABLE III

PLASMA LEVELS OF MEPERIDINE AND NORMEPERIDINE IN A CAT FOLLOWING EACH OF 4 DOSES OF MEPERIDINE (5 mg/kg I.M.)

The dose was repeated at 4-h intervals.

Dose	*Time after dose (h)*	*Plasma level (μg/ml)*	
		Meperidine	*Normeperidine*
1st	1/12	6.02	0.00
	1/4	2.40	0.00
	1/2	2.00	0.00
	1	0.95	0.00
	2	0.74	0.00
	3	0.37	0.06
	4	0.39	0.10
2nd	1/4	3.87	0.13
	4	0.51	0.32
3rd	1/4	4.39	0.25
	4	0.78	0.36
4th	1/4	5.08	0.49
	4	0.93	0.71

These results from the cat are similar to those that we have found in preliminary studies in human subjects. The data indicate that N-demethylation is a major pathway of biotransformation of meperidine in both cat and man. Also both cat and man show an accumulation of normeperidine when given meperidine in repeated doses at the usual therapeutic dosing interval.

The development of a specific and sensitive method for the quantitation of meperidine and normeperidine will now permit us to study the pharmacokinetics of meperidine and normeperidine in man and in an animal model, the cat.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. Houde and A. Rogers, R. N. for their assistance in the cancer patient studies, and Dr. I. Zervoudakis for his assistance in the obstetrical patient studies. This research was supported in part by NIMH Grants No. DA-00297 and DA-4PG016.

REFERENCES

1 E. L. Way, A. I. Gimble, W. P. McKelway, H. Ross and C. Y. Sung, *J. Pharmacol. Exp. Ther.*, 96 (1949) 477.
2 J. J. Burns, B. L. Berger, P. A. Lief, A. Wollack, E. M. Papper and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 114 (1955) 289.
3 N. P. Plotnikoff, H. W. Elliott and E. L. Way, *J. Pharmacol. Exp. Ther.*, 104 (1952) 377.
4 N. P. Plotnikoff, E. L. Way and H. W. Elliott, *J. Pharmacol. Exp. Ther.*, 117 (1956) 414.
5 A. D. MacDonald, G. Woolfe, F. Bergel, A. L. Morrison and H. Rinderknecht, *Brit. J. Pharmacol.*, 1 (1946) 4.
6 J. W. Miller and H. H. Anderson, *J. Pharmacol. Exp. Ther.*, 112 (1954) 2190.
7 G. A. Deneau and K. Nakai, *Bulletin, Drug Addiction and Narcotics*, app. 6 (1961) 2460.
8 C. E. Inturrisi and K. Verebely, *J. Chromatogr.*, 65 (1972) 361.
9 A. H. Beckett, M. Rowland and E. J. Triggs, *Nature (London)*, 207 (1965) 200.
10 L. A. Dal Cortivo, M. M. De Mayo and S. B. Weinberg, *Anal. Chem.*, 42 (1970) 941.
11 T. J. Goehl and C. Davison, *J. Pharm. Sci.*, 62 (1973) 907.
12 J. E. Stambaugh and I. W. Wainer, *J. Clin. Pharmacol.*, 14 (1974) 552.
13 U. Klotz, T. S. McHorse, G. R. Wilkinson and S. Schenker, *Clin. Pharmacol. Ther.*, 16 (1974) 667.
14 K. Chan, M. J. Kendall and M. Mitchard, *J. Chromatogr.*, 89 (1974) 169.
15 J. J. Franken and M. M. F. Trijbels, *J. Chromatogr.*, 91 (1974) 425.
16 L. E. Mather, G. T. Tucker, A. E. Pflug, M. J. Lindop and C. Wilkerson, *Clin. Pharmacol. Ther.*, 17 (1975) 21.
17 P. E. Gilbert and W. R. Martin, *J. Pharmacol. Exp. Ther.*, 192 (1975) 538.

Journal of Chromatography, 125 (1976) 511–516

CHROM. 9155

AUSTAUSCHER MIT MONOBENZOKRYPTAND K[2_B.2.2.] ALS ANKERGRUPPE

E. BLASIUS und P. G. MAURER

Fachrichtung Anorganische Analytik und Radiochemie der Universität des Saarlandes, D-6600 Saarbrücken (B.R.D.)

(Eingegangen am 2. März 1976)

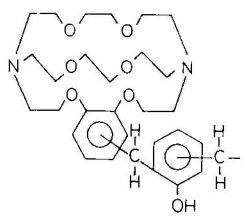
SUMMARY

Exchangers with monobenzocryptate K[2_B.2.2.] as anchor group

The capacity of the thermally and chemically very resistent exchanger with monobenzocryptate K[2_B.2.2.] as anchor group is 1.2 mmol/g of air-dried resin. This exchanger shows different selectivities for several alkali metal salts. Separations of alkali chlorides are described.

EINLEITUNG

Über den Einbau von Kronenverbindungen[1,2] und Kryptanden[2,3] als Ankergruppen in Kondensations- bzw. Polymerisationsharze wurde schon berichtet. In der vorliegenden Arbeit werden die Eigenschaften und Anwendungen des Austauschers mit K[2_B.2.2.] als Ankergruppe näher untersucht.



EXPERIMENTELLES

Bestimmungsverfahren

Zur Bestimmung der Verteilungskoeffizienten werden 250 mg lufttrockener Austauscher in ein Kunststoffgefäss von *ca.* 20 ml Inhalt eingewogen. Hinzu pipettiert man 5 ml einer Lösung, die 0.05 Mol/l der betrachteten Verbindung enthält. Nach dem Verschliessen des Gefässes wird auf einer Eigenbauschüttelmaschine mit 15 rpm bei Zimmertemperatur 24 h geschüttelt. Anschliessend ermittelt man die

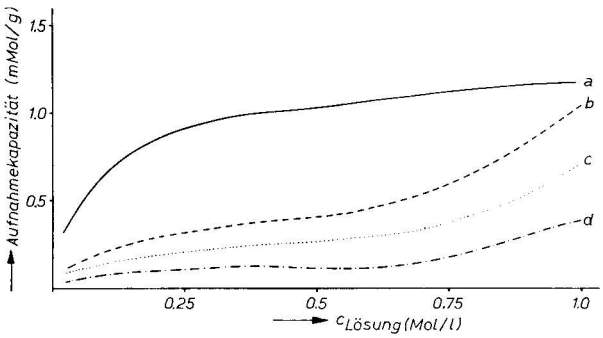


Fig. 1. Beladungskurven. (a) KSCN in reinem Methanol; (b) KSCN in wässriger Lösung; (c) KCl in wässriger Lösung; (d) NaCl in wässriger Lösung.

Konzentration des Kations bzw. des dazugehörigen Anions in der Lösung. Hierzu giesst man den Inhalt des Gefässes über ein trockenes Faltenfilter und entnimmt dem Filtrat sofort dreimal je 1 ml, die analysiert werden.

Alkaliionen werden flammenphotometrisch bzw. über die Halogenide nach Volhard titrimetrisch bestimmt. Die Beladungskapazität wird sinngemäss mit 500 mg Austauscher und 10 ml der entsprechenden Salzlösung, die zeitliche Einstellung der Gleichgewichte bei gleichen Bedingungen mit einer Konzentration von 1 Mol/l des Salzes ermittelt.

Trennungen

Eine Kolbenpumpe M 80 (Serva, Heidelberg, B.R.D.) mit verschiedenen Pumpleistungsstufen drückt das Elutionsmittel durch die Glasdrucksäule (Serva). Sie ist mit dem Austauscher gefüllt, den man vorher 2 h in einer Achatkugelmühle pulverisiert hat. Als Detektor dient das Differentialrefraktometer Multiref 901 (Optilab, Vällingby, Schweden) gekoppelt mit einem Schreiber Kompensograph III (Serva). Als Bezugslösung wird das reine Lösungsmittel verwendet.

Die Säulenbedingungen waren: Säulenhöhe, 30 cm; Säuleninnendurchmesser,

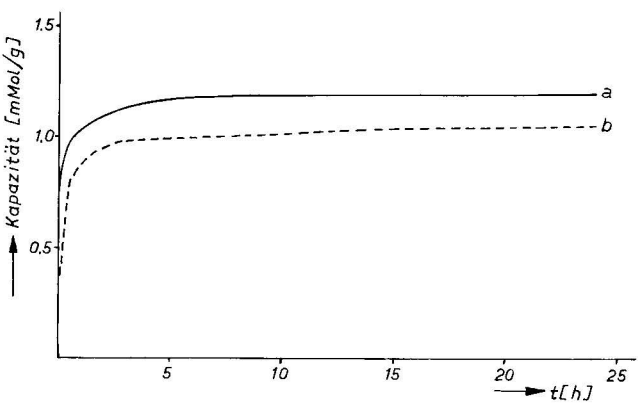


Fig. 2. Zeitliche Gleichgewichtseinstellung von KSCN (1 *M*). (a) In Methanol; (b) in Wasser.

TABELLE I

VERTEILUNGSKOEFFIZIENTEN α DER HALOGENIDE UND THIOCYANATE DER ELEMENTE DER ERSTEN HAUPTGRUPPE DES PSE IN METHANOL UND WASSER

Kation	*Methanol*				*Wasser*			
	Cl^-	Br^-	J^-	SCN^-	Cl^-	Br^-	J^-	SCN^-
Li^+	0.03	0.08	0.12	0.08	0.01	0.04	0.11	0.10
Na^+	0.25	0.39	0.50	0.47	0.07	0.10	0.16	0.16
K^+	0.57	0.68	0.69	0.70	0.11	0.14	0.18	0.18
Rb^+	0.41	0.42	0.53	–	0.07	0.08	0.15	–
Cs^+	0.28	0.34	0.47	–	0.03	0.06	0.10	–

0.32 cm; Harzbettvolumen, 2.0 ml; Arbeitsdruck, *ca.* 3 atü; Fördervolumen, 20–40 ml/h; Probevolumen, 10–100 μl.

ERGEBNISSE

Beladungskurven und zeitliche Einstellung der Gleichgewichte

In methanolischer KSCN-Lösung erreicht der Austauscher seine maximale Kapazität von 1.2 mMol/g lufttrockenem Austauscher. In Wasser ist die Aufnahme von Salzen geringer (Fig. 1). Ursache für diese Erscheinung ist die langsamere Gleichgewichtseinstellung und grössere Abhängigkeit von der Konzentration des aufzunehmenden Salzes.

Fig. 2 zeigt die zeitliche Einstellung der Gleichgewichte in Methanol und Wasser für KSCN. In methanolischer Lösung wird innerhalb 10 min über 80% der Kapazität des Austauschers ausgenutzt. In Wasser benötigt man hierzu bei gleicher Konzentration des aufzunehmenden Salzes die doppelte Zeit.

Verteilungskoeffizienten und Trennfaktoren

Untersuchungen über die Lage der Gleichgewichte werden mit Salzen der Alkalielemente durchgeführt. Tabelle I gibt die Ergebnisse der Untersuchungen in Wasser und Methanol in Form der Verteilungskoeffizienten α und Tabelle II der Trennfaktoren β wieder (Mittelwerte von je drei Bestimmungen). Die Verteilungskoeffizienten sind in Wasser wesentlich geringer als in Methanol. Die Auswertung

TABELLE II

TRENNFAKTOREN β DER HALOGENIDE UND THIOCYANATE DER ELEMENTE DER ERSTEN HAUPTGRUPPE DES PSE IN METHANOL UND WASSER*

Kation	*Methanol*				*Wasser*			
	Cl^-	Br^-	J^-	SCN^-	Cl^-	Br^-	J^-	SCN^-
Li^+	19.0	8.5	6.0	9.0	11.0	3.6	1.6	1.8
Na^+	2.3	1.8	1.3	1.5	1.6	1.4	1.2	1.2
K^+	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Rb^+	1.4	1.6	1.1	–	1.6	1.8	1.2	–
Cs^+	2.0	2.0	1.5	–	3.6	2.3	1.8	–

* Bezogen auf K^+.

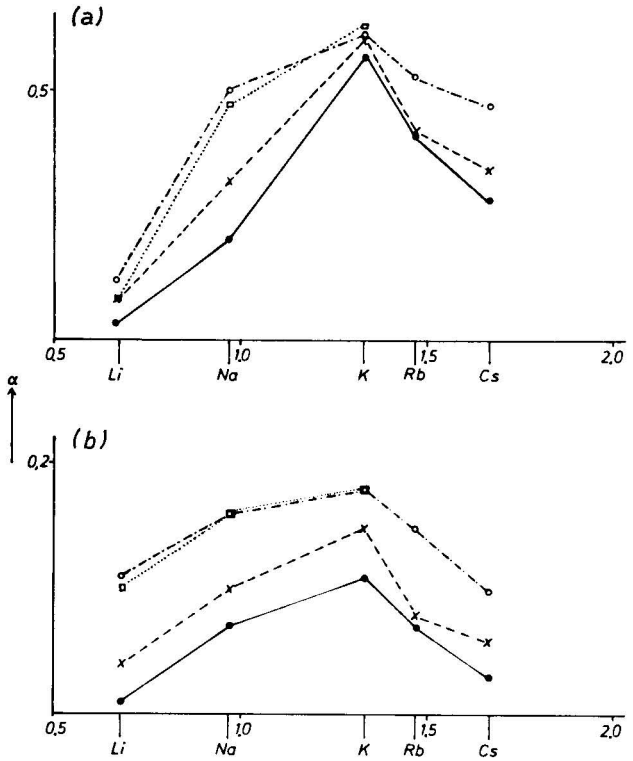


Fig. 3. Verteilungskoeffizienten der Alkalihalogenide und -thiocyanate in Methanol und Wasser in Abhängigkeit von den Ionenradien. (a) In Methanol; (b) in Wasser. ●—●, Cl^-; ×---×, Br^-; ○-.-○, J^-; □...□, SCN^-.

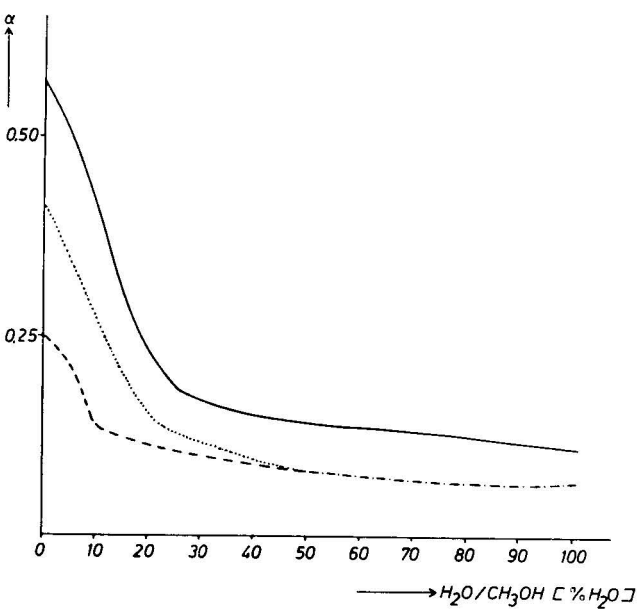


Fig. 4. Verteilungskoeffizienten α in Abhängigkeit vom Mischungsverhältnis Methanol–Wasser. ——, KCl; ·····, RbCl; — — —, NaCl.

der Trennfaktoren verdeutlicht jedoch, dass Trennungen der Alkalisalze mit Methanol und auch mit Wasser möglich sind.

Die Abhängigkeit der Verteilungskoeffizienten vom Ionenradius der Kationen gibt Fig. 3 wieder. Die Kurven zeigen gleichfalls die grosse Abhängigkeit der Salzaufnahme vom Anion. Leicht polarisierbare Anionen bewirken eine leichtere bzw. grössere Aufnahme des entsprechenden Kations. Die Maxima der Kurven in Fig. 3 liegen jeweils bei den K^+-Salzen, wie dies auch aus den Eigenschaften des monomeren Kryptanden[4] hervorgeht. Die grössten Verteilungskoeffizienten haben KSCN bzw. KJ sowohl in Methanol als auch in Wasser.

Die Abhängigkeit der Verteilungskoeffizienten α vom Mischungsverhältnis Wasser–Methanol ist erheblich (Fig. 4). Die Verteilungskoeffizienten von NaCl, KCl und RbCl werden mit zunehmendem Wassergehalt wesentlich kleiner, wobei zwischen 0–10% keine grossen Änderungen zu bemerken sind. Mit Salzen der Erdalkalielemente treten, obwohl auch hier die Eigenschaften des monomeren Kryptanden[4] gute

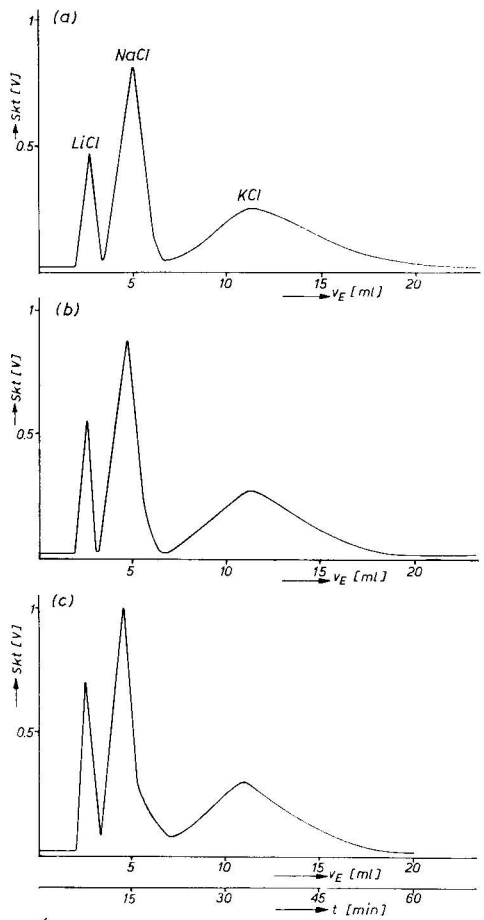


Fig. 5. Trennung von 0.06 mg LiCl, 0.35 mg NaCl und 0.80 mg KCl durch Elution mit Wasser bei verschiedenen Temperaturen und einer Durchflussgeschwindigkeit von 20 ml/h. (a) 25°; (b) 40°; (c) 50°. Skt[V] = Skalenteile Schreiberausschlag in Volt.

Trennergebnisse versprechen, nur kleine Verteilungskoeffizienten auf. Die Aufnahme an Erdalkalikationen ist in Methanol wie auch in Wasser zu gering. Messungen der zeitlichen Gleichgewichtseinstellung von Sr^{2+} bzw. Ba^{2+} weisen darauf hin, dass durch den Einfluss der zweifach positiven Ladung und der stabileren Solvathüllen die Einstellung der Gleichgewichte zu langsam ist.

Trennungen von Kationen der Alkalielemente

Der Austauscher kann zur Trennung von Alkalikationen untereinander durch Elution mit Wasser eingesetzt werden. Mit Methanol als Elutionsmittel erzielt man ebenfalls gute Ergebnisse, doch sind die Elutionsvolumina zu gross und daher auch die Trennzeiten zu lang.

Fig. 5 zeigt die Trennung von LiCl/NaCl/KCl bei einer Durchflussgeschwindigkeit von 20 ml/h bei 25°, 40° und 50°. Vollständige Trennung mit einem Totalelutionsvolumen von 20 ml Wasser wird innerhalb 1 h bei 40° erhalten. Bei 50° nimmt die Trennleistung des Austauschers wieder ab. Die Kationen erreichen zwar schneller ihre Gleichgewichtseinstellung, doch gleichzeitig wird die Elution durch die Temperaturerhöhung des Elutionsmittels beschleunigt. Ein analoges Ergebnis wird auch bei der Trennung von CsCl/RbCl/KCl erzielt.

Allgemein sollte eine schwach saure Reaktion des Elutionsmittels vermieden werden, denn nach längerer Zeit zeigt der Austauscher aufgrund der Protonierung des Stickstoffs an der Ankergruppe schlechtere Trennleistung. In diesem Fall muss der Austauscher mit einer wässrigen 0.1 *M* Triäthanolaminlösung regeneriert werden. Mit Hilfe von Eichmessungen kann auch eine quantitative Auswertung der Elutionskurven erfolgen. Weiterhin haben Vorversuche gezeigt, dass Halogenid- bzw. Pseudohalogenidionen bei gleichem Alkalikation getrennt und Salzkonvertierungen vorgenommen werden können.

ZUSAMMENFASSUNG

Der thermisch und chemisch stabile Austauscher mit Monobenzokryptand $K[2_B.2.2.]$ als Ankergruppe besitzt eine Kapazität von 1.2 mMol/g lufttrockenem Harz. Er weist unterschiedliche Selektivitäten für die verschiedenen Salze der Alkalielemente auf. Trennungen der Alkalichloride untereinander werden beschrieben.

DANK

Für finanzielle Unterstützung danken wir der Deutschen Forschungsgemeinschaft, Bonn-Bad Godesberg.

LITERATUR

1 E. Blasius, W. Adrian, K.-P. Janzen und G. Klautke, *J. Chromatogr.*, 96 (1974) 89.
2 E. Blasius und K.-P. Janzen, *Chem.-Ing.-Tech.*, 47 (1975) 594.
3 E. Blasius und P. G. Maurer, *Makromol. Chem.*, im Druck.
4 B. Dietrich, J. M. Lehn und J. P. Sauvage, *J.C.S. Chem. Comm.*, (1973) 15.

Journal of Chromatography, 125 (1976) 517–522

CHROM. 9181

DAS VERHALTEN VON RHENIUM(VII) AN SEPHADEX G-10

H. M. ORTNER und H. DALMONEGO
Metallwerk Plansee AG & Co. KG., A-6600 Reutte, Tirol (Österreich)
(Eingegangen am 4. März 1976)

SUMMARY

Behaviour of rhenium(VII) on Sephadex G-10

The elution behaviour of 10^{-1}–10^{-4} *M* solutions of ammonium perrhenate on Sephadex G-10 columns was investigated in a pH interval of 7–12. Rhenium(VII) was observed to be adsorbed to the dextran gel according to anti-Langmuir isotherms at pH 7–10, *i.e.*, the sorption is more pronounced at higher concentrations. Still stronger sorption was found at pH 12 due to the elevated hydroxyl group content of the gel at this pH value. Reproducibility of elution profiles was optimal at pH 10.

EINLEITUNG

In einer vorherigen Arbeit wurden erste Untersuchungen zum Verhalten von Ammoniumperrhenatlösungen an dem am engsten vernetzten Dextrangel Sephadex G-10 durchgeführt[1]. Dabei wurde ein von den Isopolysäurebildnern Vanadin(V)[2], Molybdän(VI)[3] und Wolfram(VI)[4,5] abweichendes Verhalten des Rhenium(VII) im schwach sauren bis alkalischen Bereich festgestellt.

Bereits bei pH 10 wird eine 0.1 *M* Ammoniumperrhenatlösung mit beträchtlicher Verzögerung und einem K_d-Wert von 4.53 eluiert. Dabei tritt schwache Bartbildung auf, was auf eine Sorptionsisotherme des Anti-Langmuir Typs schliessen lässt. Gestalt und Position der Elutionsbande bleiben bis in den schwach sauren Bereich unverändert. Bei pH 3 ist anstelle der Bartbildung schwache Schwanzbildung zu beobachten und bei pH 2 und darunter tritt eine noch stärkere Verzögerung auf. Auch die Ausbeuten sinken stark ab. Dieses Phänomen ist auf die von den Isopolysäurebildnern her bekannte Chelatkomplexbildung mit der Gelmatrix zurückzuführen, welche bei Rhenium erst im stärker sauren Medium einsetzt. In vorliegender Arbeit soll nun das Rhenium(VII)-Verhalten im neutralen und alkalischen Bereich näher untersucht werden.

EXPERIMENTELLES

Geräte und quantitative Bestimmungsmethoden

Zur Säulenchromatographie wurde eine Säule K 16/100 (Innendurchmesser 1.60 cm; Länge 100 cm) der Firma Pharmacia (Uppsala, Schweden) sowie eine

peristaltische Pumpe 4912A und ein Fraktionssammler LKB 7000 Ultro Rac, beide von LKB (Bromma, Schweden) verwendet.

Zur röntgenfluoreszenzspektrometrischen Rheniumbestimmung stand ein Philips Sequenzspektrometer PW 1220/C zur Verfügung. Die Messungen wurden unter folgenden Messparametern durchgeführt: Goldröhre, 50 kV, 40 mA; PVC-Probenbehälter mit Boden aus 6 μm Mylarfolie und mit Deckel; LiF(200)-Kristall; feiner Kollimator (Lamellenabstand 160 μm); Zählzeit 20 oder 40 sec; Messungen an der Rhenium $L\alpha_1$-Linie in 1. Ordnung mit Proportional- und Szintillationszähler in Serie; automatische Impulshöhendiskriminierung durch Kristallabschwächer für LiF(200) und festes Fenster. Rheniumkonzentrationen zwischen 10^{-3}–10^{-5} *M* wurden photometrisch mit α-Furildioxim bei 532 nm erfasst[6].

Rheniumkonzentrationen unter 10^{-5} *M* wurden durch γ-Aktivitätsmessungen der mit ^{186}Re markierten Lösungen bestimmt. Diese Messungen wurden mit einem 1.75 × 2 Zoll NaI(Tl)-Bohrlochkristall in Halterung Philips PW 4313 mit Photomultiplier und Bleiabschirmung PW 4356 und in Verbindung mit einem Philips PW 4620 Standard Analogue Unit sowie einem PW 4630 Standard Digital Unit durchgeführt. Messung im Bereich des 137 keV-Peaks bei 946.50 V, Abschwächung 2^1, Schwelle 30 Potentiometereinheiten und 17% Fenster.

Materialien

Sephadex G-10 (fine) wurde von Pharmacia (Uppsala, Schweden) bezogen, Ammoniumperrhenat von H. C. Starck (Goslar, B.R.D.). Alle anderen Reagenzien waren z.A. Reagenzien von Merck (Darmstadt, B.R.D.).

Herstellung der Perrhenataufgabelösungen

NH_4ReO_4 wurde in Wasser gelöst. Die pH-Einstellung für pH 8–10 erfolgte mit Ammoniak, für pH 11–12 mit Natronlauge.

ERGEBNISSE UND DISKUSSION

Einfluss der Durchflussgeschwindigkeit bei pH 10 (Tabelle I, Versuche Nr. 1–5)

Zunächst wurde der Einfluss der Durchflussgeschwindigkeit auf das Elutionsprofil 0.1 *M* bzw. 0.01 *M* Perrhenatlösungen bei pH 10 untersucht. Der Einfluss variierender Durchflussgeschwindigkeiten ist gering. Bei 0.01 *M* Lösungen ist die K_d-Wert Vorverschiebung von 3.80 auf 3.45 bei Erhöhung der Durchflussgeschwindigkeit von 25 auf 50 ml/h·cm^2 deutlicher als für 0.1 *M* Lösungen (Vorverschiebung von 4.84 auf 4.72). Es fällt auf, dass 0.01 *M* Lösungen mit wesentlich niedrigeren K_d-Werten und mit ausgeprägterer Bartbildung eluiert werden als 0.1 *M* Lösungen. Daher wurde als nächstes die Konzentrationsabhängigkeit des Elutionsverhaltens untersucht.

Konzentrationsabhängigkeit des Elutionsverhaltens bei pH 10 (Tabelle I, Versuche Nr. 2, 4, 6–9)

In Fig. 1 sind die Elutionsprofile der entsprechenden Versuche dargestellt. Das Elutionsprofil für 0.05 *M* Lösungen ist mit dem für 0.1 *M* Lösungen praktisch identisch. Lediglich die Peakbreite ist etwas geringer. Dieses Profil ist der Übersichtlichkeit halber nicht in Fig. 1 enthalten.

TABELLE I

SÄULENCHROMATOGRAPHISCHE VERSUCHE ZUM VERHALTEN VON RHENIUM(VII) AUF SEPHADEX G-10

Abkürzungen und Erläuterungen: Nr. = Versuchsnummer; Konz. = Aufgabekonzentration; pH = pH-Wert der Aufgabelösung und der Elutionslösung; Fig. = das Elutionsprofil ist in Fig. ... abgebildet; Gesamtausbeute = Angabe in % der Aufgabemenge, gemessen bis ... ml Elutionsvolumen; Peak, ml = Angabe des Peakelutionsvolumenbereiches; Durchflussgeschwindigkeit, Angabe in ml/h sowie in ml/h·cm². Sämtliche Versuche wurden bei Zimmertemperatur (20–24°) durchgeführt. Säulenbett: 1.60 × 90 cm (= 180 ml); Zwischenkornvolumen der Säule, v_0 = 68.4 ml; inneres Gelvolumen der Säule, v_i = 43.2 ml; Aufgabe- und Fraktionsvolumen jeweils 5.0 ml. Die pH-Einstellung des Elutionsmittels erfolgte mit Ammoniak für pH 10, mit Natronlauge für pH 11 und 12; für Versuche bei pH 7 wurde demineralisiertes Wasser als Elutionsmittel verwendet.

Nr.	*Konz. (M)*	*pH*	*Fig. Nr.*	*Gesamtausbeute*		*Peak*		*Durchflussgeschwindigkeit*		*Bemerkungen*
				%	*ml*	*ml*	*K_d-Wert*	*ml/h*	*ml/h cm²*	
Einfluss variierender Durchflussgeschwindigkeit für 0.1 M und 0.01 M Lösungen bei pH 10										
1	0.1	10.0	—	98	320	275–280	4.84	30	15	Bartbildung
2	0.1	10.0	1	99	320	275–280	4.84	50	25	Bartbildung
3	0.1	10.0	—	100	320	270–275	4.72	100	50	Bartbildung; Peak etwas vorverschoben
4	0.01	10.0	1	100	250	230–235	3.80	50	25	starke Bartbildung
5	0.01	10.0	—	98	250	215–220	3.45	100	50	starke Bartbildung; Peak etwas vorverschoben
Konzentrationsabhängigkeit des Elutionsverhaltens bei pH 10										
6	0.05	10.0	—	98	300	275–280	4.84	60	30	Elutionsprofil mit dem von Nr. 1 und 2 fast identisch
7	0.005	10.0	1	99	220	200–205	3.10	50	25	leichte Bartbildung
8	0.001	10.0	1	100	205	145–150	1.83	60	30	Peak symmetrisch
9	0.0001	10.0	1	100	170	110–115	1.02	60	30	Peak symmetrisch
Konzentrationsabhängigkeit des Elutionsverhaltens bei pH 7										
10	0.1	7.0	2	99	300	275–280	4.84	60	30	Bartbildung ab 90 ml
11	0.05	7.0	2	93	320	285–290	5.07	60	30	Bartbildung
12	0.05	7.0	—	95	320	290–295	5.19	60	30	
13	0.01	7.0	2	94	320	285–290	5.07	60	30	starke Bartbildung
14	0.01	7.0	—	92	350	290–295	5.19	60	30	starke Bartbildung
15	0.01	7.0	—	90	350	285–290	5.07	60	30	starke Bartbildung
16	0.005	7.0	2	99	300	275–285	4.84	60	30	starke Bartbildung
17	0.001	7.0	2	98	200	140–145	1.72	60	30	Peak weitgehend symmetrisch
18	0.0001	7.0	2	95	200	155–160	2.06	60	30	Peak symmetrisch
Elutionsverhalten im stärker basischen Bereich										
19	0.1	11.0	—	100	300	275–280	4.84	60	30	Bartbildung; Profil mit Nr. 2 und 10 praktisch identisch
20	0.05	11.0	—	98	350	267.5–272.5	4.67	60	30	keine Bartbildung, schwaches Tailing
21	0.05	11.0	—	96	350	280–285	4.96	60	30	schwaches Tailing
22	0.01	11.0	—	97	300	252.5–257.5	4.32	60	30	Peak symmetrisch
23	0.1	12.0	—	—	—	>350	>6.5	30	15	Elutionsschwierigkeiten durch Gelquellung; starke Elutionsverzögerung

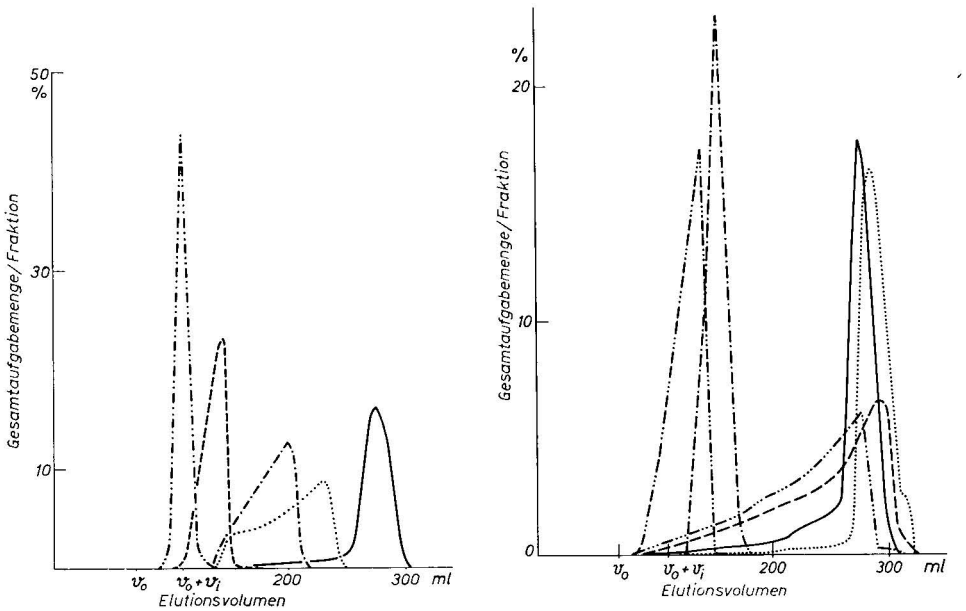


Fig. 1. Konzentrationsabhängigkeit des Elutionsverhaltens von Ammoniumperrhenatlösungen auf Sephadex G-10 bei pH 10.0. Durchflussgeschwindigkeit: 25–30 ml/h·cm²; Zimmertemperatur. ———, 0.1 *M* (Versuch Nr. 2, Tabelle I); · · · · · ·, 0.01 *M* (Versuch Nr. 4, Tabelle I); –·–·–·, 0.005 *M* (Versuch Nr. 7, Tabelle I); – – –, 0.001 *M* (Versuch Nr. 8, Tabelle I); –··–··, 0.0001 *M* (Versuch Nr. 9, Tabelle I). v_0 = Zwischenkornvolumen der Säule; $v_0 + v_i$ = Elutionsvolumen für eine Substanz mit völlig gleichmässiger Verteilung auf mobile und Gelphase.

Fig. 2. Konzentrationsabhängigkeit des Elutionsverhaltens von Ammoniumperrhenatlösungen auf Sephadex G-10 bei pH 7.0. Durchflussgeschwindigkeit: 30 ml/h cm²; Zimmertemperatur. ———, 0.1 *M* (Versuch Nr. 10, Tabelle I); · · · · · ·, 0.05 *M* (Versuch Nr. 11, Tabelle I); – – –, 0.01 *M* (Versuch Nr. 13, Tabelle I); –···–···, 0.005 *M* (Versuch Nr. 16, Tabelle I); – – · · · – –, 0.001 *M* (Versuch Nr. 17, Tabelle I); –·–·–·, 0.0001 *M* (Versuch Nr. 18, Tabelle I).

Die Sorptionstendenz nimmt mit sinkender Rheniumkonzentration ab. Für 10^{-4} *M* Lösungen entspricht das Peakelutionsvolumen schliesslich genau dem Wert $v_0 + v_i$ für völlig gleichmässige Verteilung auf innere Gelphase und äussere, mobile Phase. Ganz deutlich handelt es sich also um eine Sorption nach dem relativ selten auftretenden Anti-Langmuir Typ: bei hoher Aufgabekonzentration tritt deutliche Bartbildung bei hohem K_d-Wert auf, bei sinkender Aufgabekonzentration wandert das Peakelutionsvolumen nach vorn und die Bartbildung wird geringer. Eigentümlicherweise verläuft die Sorption des Perchloratanions, welches ähnlich gebaut ist wie das Perrhenatanion, nach dem Langmuir Typ[7]. Auch die Chelatkomplexbildung von Oxoionen an Dextrangelen verläuft wie die meisten Sorptionseffekte nach Langmuir-Isothermen. Eine befriedigende Erklärung für das abnormale Verhalten des Perrhenatanions konnte bislang noch nicht gefunden werden. Vielleicht handelt es sich um eine relativ schwache Komplexbildung, deren Dissoziationskonstante für die niedrigsten untersuchten Konzentrationen mit K_d-Werten um 1.0 bereits überschritten ist.

Konzentrationsabhängigkeit des Elutionsverhaltens bei pH 7 (Tabelle I, Versuche Nr. 10–18)

In Fig. 2 sind Elutionsprofile entsprechender Versuche dargestellt. Die Tendenz zur K_d-Wert Erniedrigung mit sinkender Aufgabekonzentration bleibt im Neutralbereich erhalten, die Sorption verläuft auch hier nach dem Anti-Langmuir-Typ. Die Reproduzierbarkeit der Elutionsprofile ist hier im absolut ungepufferten System (Elution mit demineralisiertem Wasser) erwartungsgemäss schlechter als bei pH 10 (vgl. die K_d-Wertschwankungen für Vers. Nr. 11 und 12, Vers. Nr. 12–14, sowie die K_d-Wertverschiebung 10^{-3}–10^{-4} *M* Lösungen von 1.72 auf 2.06). Die K_d-Werte für 0.1–0.005 *M* Lösungen liegen alle zwischen 4.84 und 5.19. Dies und die im Vergleich zu pH 10 deutlich höheren K_d-Werte für 10^{-3} und 10^{-4} *M* Lösungen deutet darauf hin, dass der Sorptionsverlauf nach einer Anti-Langmuir Isotherme bei pH 7 weniger ausgeprägt ist als bei pH 10. Die etwas schwankenden Ausbeuten für 0.05 und 0.01 *M* Lösungen sind auf messtechnische Probleme infolge der niedrigen Gehalte pro Eluatfraktion (starke Bartbildung) zurückzuführen (vgl. Fig. 2).

Elutionsverhalten im stärker basischen Bereich (Tabelle I, Versuche Nr. 19–23)

Das Elutionsprofil für 0.1 *M* Lösungen bei pH 11 entspricht vollkommen den Profilen bei pH 10 und pH 7. Bei hohen Aufgabekonzentrationen bleibt demnach im pH-Bereich 7–11 das Elutionsprofil konstant. Dies geht auch aus den in Tabelle II übersichtlich tabellierten K_d-Werten deutlich hervor. Bei pH 12 tritt hingegen bereits für 0.1 *M* Lösungen beträchtliche Elutionsverzögerung ein. Da Sephadex G-10 jedoch bei diesem pH-Wert bereits stark quillt, dies zu Elutionsschwierigkeiten führt, und überdies die Gefahr einer Schädigung der Gelmatrix besteht, wurde von weiteren Versuchen bei pH 12 abgesehen. Auch die Anzahl der Versuche bei pH 11 wurde aus dem letzteren Grund niedrig gehalten. Nachdem Hydroxylionen mit Kohlenhydraten Komplexe zu bilden imstande sind und NaOH bzw. KOH an Dextrangelen verzögert eluiert werden (z.B. mit K_d-Werten von 2.2 resp. 2.3 auf Sephadex G-25[8]), dürfte die ausgeprägte Elutionsverzögerung von Perrhenatlösungen bei pH 12 auf den erhöhten Hydroxylgruppengehalt von Dextrangelen bei diesem pH-Wert zurückzuführen sein. Dies deutet wieder darauf hin, dass die Rheniumsorption prinzipiell an den Gelhydroxylgruppen stattfindet. Im Gegensatz zu den Elutionsprofilen bei pH 10 und 7 tritt bei Elution 0.05 *M* Rheniumlösungen bei pH 11 keine Bartbildung, sondern leichtes Tailing auf, die K_d-Werte unterscheiden sich nicht von jenen für 0.05 *M* Lösungen bei pH 10 (vgl. Tabelle II).

Für 0.01 *M* Lösungen liegen wieder die K_d-Werte bei pH 7 höher als bei pH 10 und 11 (vgl. Tabelle II). Der niedrigste K_d-Wert für pH 10 deutet auf einen Elutionsverlauf nach einer besonders ausgeprägten Anti-Langmuir-Isotherme bei diesem pH-Wert hin. Dies geht auch aus einem K_d-Wert Vergleich bei pH 7 und 10 für 0.005–0.0001 *M* Lösungen hervor, wobei noch zu bedenken ist, dass bei pH 7 die K_d-Werte nicht so streng reproduzierbar sind wie bei pH 10.

ZUSAMMENFASSUNG

Es wurde das Elutionsverhalten 10^{-1}–10^{-4} *M* Ammoniumperrhenatlösungen auf Sephadex G-10 Säulen im pH-Bereich 7–12 untersucht. Dabei wurde festgestellt, dass die Rhenium(VII)-Sorption im pH-Bereich 7–10 nach Anti-Langmuir-Isothermen

TABELLE II

K_d-WERT ABHÄNGIGKEIT ÄQUIMOLARER PERRHENATLÖSUNGEN VOM pH-WERT
Bei einer Durchflussgeschwindigkeit von 25–30 ml/h cm².

M	*pH-Wert*	*K_d-Wert*	*Bemerkungen*
0.1	7.0	4.84	K_d-Wert zwischen pH 7 und 11 vom pH-Wert unabhängig
0.1	10.0	4.84	
0.1	11.0	4.84	
0.1	12.0	>6.5	
0.05	7.0	5.07	K_d-Werte bei pH 7 gegenüber pH 10 und 11 etwas erhöht
0.05	7.0	5.19	
0.05	10.0	4.84	
0.05	11.0	4.67	
0.05	11.0	4.96	
0.01	7.0	5.07	K_d-Werte bei pH 7 gegenüber pH 10 und 11 deutlich höher. Auch
0.01	7.0	5.19	bei pH 11 höher als bei pH 10
0.01	7.0	5.07	
0.01	10.0	3.80	
0.01	11.0	4.32	
0.005	7.0	4.84	Deutliche Diskrepanz; K_d-Wert bei pH 7 höher
0.005	10.0	3.10	
0.001	7.0	1.72	
0.001	10.0	1.83	
0.0001	7.0	2.06	Deutliche Diskrepanz
0.0001	10.0	1.02	

verläuft, d.h. die Sorption ist bei höheren Konzentrationen ausgeprägter als bei niedrigeren. Bei pH 12 tritt starke Sorption durch den bei diesem pH-Wert erhöhten Hydroxylgruppengehalt des Gels ein. Beste Reproduzierbarkeit der Elutionsprofile wurde bei pH 10 beobachtet.

LITERATUR

1 H. M. Ortner, *J. Chromatogr.*, 107 (1975) 335.
2 H. M. Ortner und H. Dalmonego, *J. Chromatogr.*, 89 (1974) 287.
3 H. M. Ortner und H. Dalmonego, *J. Chromatogr.*, 107 (1975) 341.
4 H. M. Ortner, H. Krainer und H. Dalmonego, *J. Chromatogr.*, 82 (1973) 249.
5 H. M. Ortner, *Anal. Chem.*, 47 (1975) 162.
6 O. G. Koch und G. A. Koch-Dedic, *Handbuch der Spurenanalsye*, Band 2, Springer, Berlin, 1974.
7 T. Ogata, N. Yoza und S. Ohashi, *J. Chromatogr.*, 58 (1971) 267.
8 B. Gelotte, *J. Chromatogr.*, 3 (1960) 330.

Journal of Chromatography, 125 (1976) 523–525

CHROM. 9329

Note

Separation of digoxin and dihydrodigoxin by thin-layer or paper chromatography

J. J. SABATKA, D. A. BRENT and J. MURPHY

Wellcome Research and Development Laboratories, Burroughs Wellcome Co., Research Triangle Park, N.C. 27709 (U.S.A.)

and

J. CHARLES, J. VANCE and M. H. GAULT

Department of Medicine, Memorial University, St. John's, Newfoundland (Canada)

(Received March 23rd, 1976)

Dihydrodigoxin is the major dihydro metabolite of digoxin in man[1]. Although the activity of this metabolite is unknown in man, experiments in animals show it to be approximately 1/10 as active as digoxin[2,3]. In addition, the production of the dihydro metabolite varies from patient to patient[1]. Quantitation of dihydrodigoxin and digoxin in plasma and urine is clinically important for the monitoring of patients on digoxin maintenance therapy and for the diagnosis of digoxin intoxication.

Current techniques for the assay of digoxin include inhibition of ^{86}Rb transport by the red blood cells[4], radioimmunoassay[5], and enzymatic isotope displacement[6]. None of these methods differentiate between digoxin and dihydrodigoxin. Cross reaction of dihydrodigoxin in digoxin assays may vary widely depending upon the nature of the assay, making determination of true digoxin levels difficult. Researchers have used gas chromatography–mass spectroscopy as well as combining mass spectrometry with other assays to determine the contribution of dihydrodigoxin[7,8]. Previous attempts to separate digoxin from its reduced metabolite, dihydrodigoxin, by solvent partitioning, column chromatography, paper chromatography and thin-layer chromatography (TLC) have proven unsuccessful[7,8]. We wish to report three chromatographic systems which give satisfactory separation of digoxin and dihydrodigoxin.

EXPERIMENTAL

Spray reagents

Reagent 1. 25% trichloroacetic acid solution in chloroform, containing 4 drops of 30% hydrogen peroxide in each 50 ml. Chromatograms are sprayed and heated at 90–100° for 2 min.

Reagent 2. Acetic anhydride–sulfuric acid–absolute ethanol (5:5:100).

Reagent 3. 0.05 ml *p*-anisaldehyde, 0.2 ml concentrated sulfuric acid, 10 ml glacial acetic acid. The plates are sprayed with freshly prepared reagent and heated at 110° for 4–6 min.

Reagent 4. 20 mg ascorbic acid, 19 ml methanol, 30 ml conc. hydrochloric

acid and 2.1 μl 30% hydrogen peroxide. The plates are sprayed and heated at 110° for 4 min.

Reagent 5. 10 ml of a freshly prepared 3% aqueous solution of chloramine T and 40 ml of 25% trichloroacetic acid in ethanol are mixed prior to spraying. The plates are heated at 110° for 8 min.

TLC on silica gel

Silica gel F_{254} plates (0.25 mm × 20 cm × 20 cm; E. Merck, Darmstadt, G.F.R.) were heat-activated at 100° for 1 h and stored in a desiccator. Plates were developed two times to 18 cm using chloroform–methanol–ammonia (9:1:1). The methanol used in this mixture had previously been saturated with silver nitrate. Chromatography tanks were fitted with saturation pads and allowed to equilibrate for 1 h. Plates were sprayed with the reagents described and visualized under long-wavelength UV light.

TLC on cellulose

Precoated cellulose plates (0.1 mm × 20 cm × 20 cm cellulose MN-300; Macherey, Nagel & Co., Düren, G.F.R.) were activated at 110° for 10 min and then dipped into a 25% formamide in acetone solution and air dried for 1 h. The plates were developed twice in a sandwich apparatus (Chromagram Developing apparatus No. 6071; Eastman-Kodak, Rochester, N.Y., U.S.A.) using chloroform saturated with formamide as the mobile phase. Plates were sprayed lightly with reagent 5 and visualized under long-wavelength UV light.

Paper chromatography

Paper chromatograms were run using Whatman No. 1 filter paper cut into 12 × 40 cm strips. The strips were impregnated with formamide by drawing them through a freshly prepared 30% solution of formamide in acetone contained in a chromatography trough. They were air dried for about 5 min. After spotting the sample, the strip was placed in a cylindrical chromatography tank (45 × 15 cm I.D.) containing a trough assembly 35–40 cm above the bottom of the tank. The tank was allowed to equilibrate for 30 min with 25 ml of the mobile phase (chloroform saturated with formamide) in the bottom. Then 20 ml of mobile phase were added to the trough. The chromatogram is developed to the bottom of the paper (about $1\frac{1}{2}$ h), and allowed to drip for about 4 h. The paper was removed and air dried (10 min) and in a 100° oven with free air circulation, until it was dry to the touch (15–20 min). Then it was sprayed heavily with reagent 1 and visualized under long-wavelength UV light.

RESULTS AND DISCUSSION

Chromatographic methods for the separation of digoxin and dihydrodigoxin were investigated. Digoxin and dihydrodigoxin appeared as symmetrical well separated spots with R_F values of 0.65 and 0.79 on cellulose plates and 0.33 and 0.41 on silica gel plates. Separation was also achieved on paper with the relative migrations of dihydrodigoxin to digoxin being 1.09 when the solvent flow is sufficient to bring dihydrodigoxin to the edge of the chromatogram.

To demonstrate the degree of separation achieved by the two TLC methods, the plates were scanned using a Schoeffel Spectrodensitometer (Model SD 3000) with

a reflectance mode attachment. Total fluorescence above 405 nm was measured using an excitation wavelength of 350 nm. The chromatograms which were sprayed with reagent 4 and the resulting scans are shown in Fig. 1. Chromatography using cellulose or paper gives greater separation of the two components than is obtained using silica gel. However using the former methods, R_F values are critically dependent on the percentage of formamide in the dipping solution and the drying times, whereas the silica gel method is simpler to carry out and more reproducible.

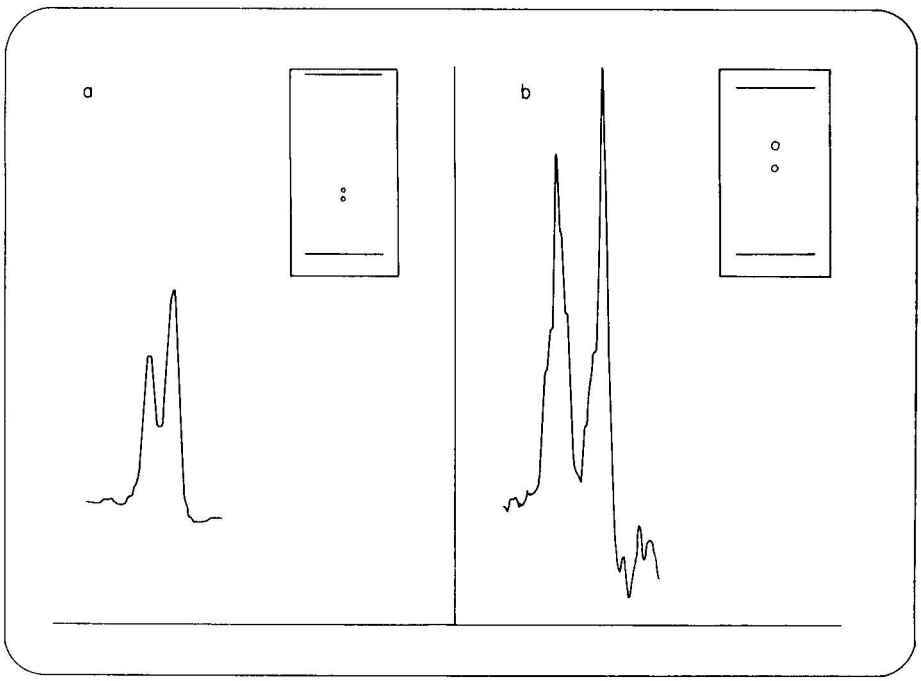


Fig. 1. Diagram of the TLC plate and fluorescence scan of digoxin–dihydrodigoxin separation using (a) silica gel plates and (b) cellulose plates.

These separations would be useful in conjunction with assays in which cross reaction is significant. It may be possible in the future to use these TLC methods for quantitation of digoxin and its metabolites using plate scanning methods. The limiting factor at present is a spray reagent that will allow detection of very low levels of digoxin and dihydrodigoxin. Using the Schoeffel spectrodensitometer reagent 4 was the most sensitive reagent tested with lower limits of 1 ng for digoxin and 10 ng for dihydrodigoxin.

REFERENCES

1 E. Watson, D. R. Clark and S. M. Kalman, *J. Pharmacol. Exp. Ther.*, 184 (1973) 424.
2 G. L. Lage and J. L. Spratt, *J. Pharmacol. Exp. Ther.*, 152 (1966) 501.
3 E. J. Bach and M. Reiter, *Arch. Exp. Pathol. Pharmacol.*, 248 (1964) 437.
4 J. W. Lowenstein and E. M. Corrill, *J. Lab. Clin. Med.*, 67 (1966) 1048.
5 G. C. Oliver, B. M. Parker, D. L. Brasfield and C. W. Parker, *J. Clin. Invest.*, 47 (1968) 1035.
6 G. Brooker and R. W. Jelliffe, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 28 (1968) 608.
7 D. R. Clark and S. M. Kalman, *Drug Metab. Dispos.*, 2 (1974) 148.
8 H. Greenwood, W. Snedden, R. P. Hayward and J. Landon, *Clin. Chim. Acta*, 62 (1975) 213.

Journal of Chromatography, 125 (1976) 526–529

CHROM. 9353

Note

Séparation par chromatographie en phase gazeuse de l'ε-hydroxynorleucine

CHRISTIAN PERIER, MARIE-CLAIRE RONZIÈRE, ALINE RATTNER et JACQUES FREY
Laboratoire de Biochimie, U.E.R. de Médecine, 30, rue Ferdinand Gambon, 42100 Saint-Étienne (France)
(Reçu le 2 février 1976)

Les fibres de collagène sont constituées par des chaînes polypeptidiques, de poids moléculaire 100,000, unies latéralement par des liaisons dites intramoléculaires pour former des tresses à trois brins, ces tresses sont également unies latéralement par des liaisons dites intermoléculaires et constituent ainsi la fibre elle-même. Les liaisons latérales sont des liaisons aldimines ou aldols formées entre une fonction aldéhyde d'un dérivé de la lysine (allysine ou acide α-amino-δ-semi-aldéhyde adipique) et une fonction aldéhyde d'une autre molécule d'allysine pour constituer une liaison aldol ou la fonction ε-aminée d'une molécule de lysine ou d'hydroxylysine pour constituer une liaison aldimine[1–4]. Si un échantillon de collagène est réduit par le borohydrure de sodium[5], les molécules d'allysine qui ne sont pas incluses dans des liaisons de réticulation seront réduites en ε-hydroxynorleucine; il en est de même des molécules d'allysine incluses dans des liaisons aldimines lorsqu'elles ont été libérées par une hydrolyse ménagée lors de la préparation du collagène acido-soluble.

Etant donné l'importance de l'ε-hydroxynorleucine pour apprécier le degré de réticulation d'un échantillon de collagène, mais aussi sa très faible concentration, nous avons cherché à la détecter par chromatographie en phase gazeuse.

MATÉRIEL ET MÉTHODE

Préparation de l'ε-hydroxynorleucine

Pour réaliser la synthèse de l'ε-hydroxynorleucine, nous avons suivi la technique proposée par Gaudry[6]. Le produit de départ de cette synthèse est le 2,3-dihydropyranne que l'on hydrolyse en δ-hydroxyvaléraldéhyde selon la technique de Schniepp et Geller[7].

Les étapes de la synthèse sont schématisées sur la Fig. 1. Par hydrolyse barytique de la 5-δ-hydroxybutylhydantoïne, nous obtenons un produit qui doit être l'ε-hydroxynorleucine.

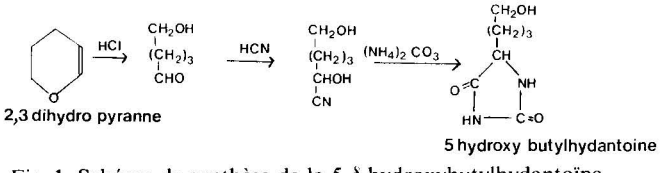


Fig. 1. Schéma de synthèse de la 5-δ-hydroxybutylhydantoïne.

Chromatographie en phase gazeuse

Afin d'obtenir des composés volatils, les acides aminés sont transformés en esters butyliques N-trifluoroacétylés en utilisant la méthode de Zumwalt *et al.*[8]. Les acides aminés en suspension dans 100 µl de dichlorométhane subissent successivement après évaporation du dichlorométhane, une estérification avec 2 ml de *n*-butanol anhydre saturé en acide chlorhydrique, pendant 2 h à 90°–100° en tubes bouchés. Les réactifs sont évaporés avant de procéder à l'acylation. Les dérivés remis en solution dans 500 µl de dichlorométhane sont alors traités par 50 µl d'anhydride trifluoroacétique pendant 2 h à 100° en tubes bouchés.

Les chromatographies sont effectuées sur 2 µl de cette solution. La séparation a lieu dans un chromatographe Fractovap GV (Erba Sciences) équipé d'une colonne en verre de 2 m × 3 mm (diamètre intérieur). La colonne est silylée et remplie de Gas-Chrom P, 100–120 mesh (Erba Sciences) imprégné de la phase fixe, SE-30 (méthyl siloxane) 1 %–QF-1 (méthyl fluoroalkylsilicone) 3 %. Une phase mobile est constituée par un flux d'azote d'un débit de 40 ml/min. Les acides aminés sont élués en fonction de la température qui est programmée de 100° à 250° à raison de 4°/min.

Les produits contenus dans l'effluent gazeux sont détectés par ionisation de flamme. Dans un précédent travail[9] nous avons utilisé cette méthode pour séparer les acides aminés contenus dans des hydrolysats d'échantillon de collagène.

RÉSULTATS ET DISCUSSION

Vérification de la structure des produits obtenus par synthèse

5-δ-Hydroxybutylhydantoïne. Le produit obtenu a un point de fusion de 160° mesuré par analyse thermique différentielle. Son spectre infrarouge permet de caractériser les groupements O–H, C–H, N–H, C=O qui se trouvent dans la molécule de la 5-δ-hydroxybutylhydantoïne. La courbe d'intégration du spectre de résonance magnétique nucléaire (RMN) précise l'existence de 12 protons: 8 protons aliphatiques, 1 proton de la fonction O–H, 2 protons des groupements N–H et 1 proton du carbone 5 qui correspondent à la structure théorique de la 5-δ-hydroxybutylhydantoïne.

ε-Hydroxynorleucine

ε-Hydroxynorleucine. Le point de fusion du composé se situe environ à 230°. Le produit obtenu est colorable par le ninhydrine alors que la 5-δ-hydroxybutylhydantoïne ne l'était pas. Son spectre infrarouge est caractéristique d'un acide aminé. Il permet l'identification des bandes d'absorption dues aux groupements: O–H, C–H, N–H, C=O et l'observation des déplacements de la bande C=0 vers 1600 cm^{-1}, déplacement que l'on remarque habituellement dans les spectres infrarouge des acides aminés. Le spectre de RMN dans l'eau lourde permet de confirmer la chaîne aliphatique de cet acide aminé qui comporte 13 protons dont 8 protons aliphatiques. Le spectre de masse de l'ester butylique di-O,N-trifluoroacétylé de l'ε-hydroxynorleucine montre un pic moléculaire $m/e = 395$ et les fragmentations caractéristiques de ce composé (Fig. 2).

Localisation sur les chromatogrammes de l'ε-hydroxynorleucine

On obtient une bonne séparation des acides aminés caractéristiques du colla-

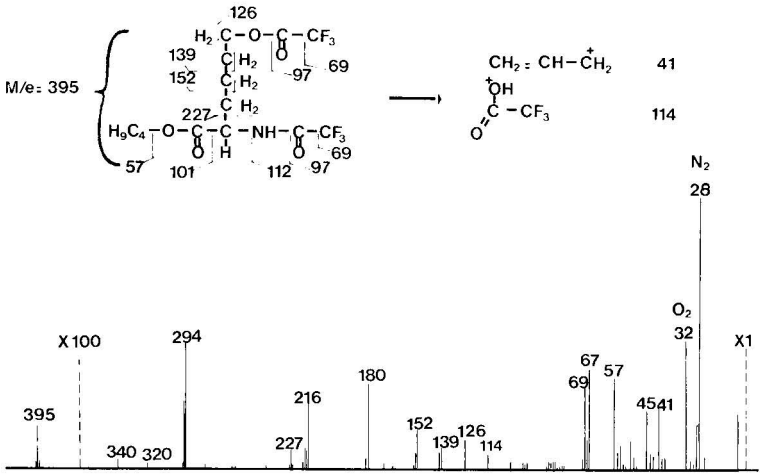


Fig. 2. Spectre de masse de l'ester butylique di-O,N-trifluoroacétylé de l'ε-hydroxynorleucine.

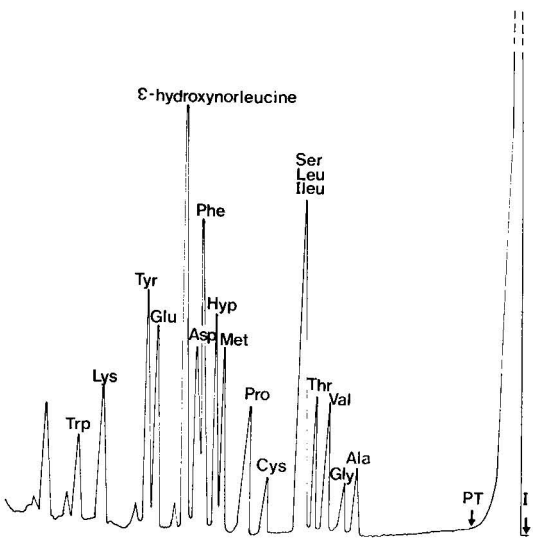


Fig. 3. Chromatographie en phase gazeuse d'un mélange d'acides aminés à raison de 5 nmoles de chacun d'entre eux en solution dans 2 μl de dichlorométhane. Phase stationnaire, QF-1 3%–SE-30 1% (60:40); phase mobile, azote, 40 ml/min. Programmation de température, de 100° à 250°, 4°/min.

gène (proline–hydroxyproline) en utilisant un mélange des phases QF-1 3%–SE-30 1% dans un rapport (60:40, p/p).

En comparant les chromatogrammes obtenus avec un mélange d'acides aminés à ceux obtenus après une surcharge en ε-hydroxynorleucine de synthèse, on observe que l'ε-hydroxynorleucine apparaît sur les chromatogrammes sous forme d'un pic nettement distinct de celui des autres acides aminés et se situant immédiatement après le pic de l'acide aspartique (Fig. 3).

REMERCIEMENT

Ce travail a bénéficié de l'aide de la D.G.R.S.T. par un contrat No. 74.7.0763 d'action complémentaire coordonnée de Chimie Analytique.

BIBLIOGRAPHIE

1 O. O. Blumenfeld et P. M. Gallop, *Proc. Nat. Acad. Sci. U.S.*, 56 (1966) 1260.
2 E. Schiffmann et G. R. Martin, *Arch. Biochem. Biophys.*, 138 (1970) 226.
3 A. Deshmukh, K. Deshmukh et M. E. Nimni, *Biochemistry*, 10 (1971) 2337.
4 R. C. Page, E. P. Benditt et C. R. Kirkwood, *Biochem. Biophys. Res. Commun.*, 33 (1968) 752.
5 A. J. Bailey et C. M. Peach, *Biochem. Biophys. Res. Commun.*, 33 (1968) 812.
6 R. Gaudry, *Canadian J. Res.*, 26B (1948) 387.
7 L. E. Schniepp et H. H. Geller, *J. Amer. Chem. Soc.*, 68 (1946) 1646.
8 R. W. Zumwalt, D. Roach et C. W. Gehrke, *J. Chromatogr.*, 53 (1970) 171.
9 C. Perier, J. Farjanel, M. Therme, M. Brunel et J. Frey, *Biochimie*, 56 (1974) 1003.

Journal of Chromatography, 125 (1976) 530–532

CHROM. 9198

Note

A simple screening method for urinary purines

I. R. F. BROWN

Department of Chemical Pathology, St. George's Hospital Medical School, University of London, Blackshaw Road, London, SW17 0QT (Great Britain)

(First received December 31st, 1975; revised manuscript received March 9th, 1976)

Although urinary screening methods are widely used in clinical chemistry as aids in the detection of abnormalities of amino acid and carbohydrate metabolism, relatively little attention has been paid to the use of such methods in the detection of disorders of purine metabolism[1]. Most of the methods available are cumbersome and can take several days to complete[2–6]. This paper describes a procedure that makes it possible in 1 day of purify, concentrate and separate the purines found in human urine.

MATERIALS AND METHODS

Urine (200 ml, containing approximately 200 mg of creatinine) was acidified by adding a few drops of concentrated hydrochloric acid. It was then applied to the top of a previously washed Dowex 250 × 15 mm 50W-X8 (H^+) column (20–50 mesh) and washed through with distilled water (200 ml). The adsorbed material, including the purines, was then removed from the column by washing with 5 *M* ammonia solution (200 ml). The eluate obtained was applied to the top of a 130 × 15 mm Dowex 2-X8-100 (OH^-) column (50–100 mesh), was then washed through with distilled water (200 ml) and the adsorbed material removed with 1 *M* hydrochloric acid (200 ml). The final acidic eluate, which contained the urinary purines, except for uric acid, was evaporated to dryness on a rotary evaporator. The dried material was dissolved in 6 *M* potassium hydroxide solution (1 ml), the solution neutralized by adding 6 *M* perchloric acid and the precipitated potassium perchlorate removed by centrifugation[7]. The final clear supernatant solution was spotted (1–10 μl) on thin layers of polyethyleneimine (PEI) cellulose pre-coated on plastic sheets (200 × 200 mm; Schleicher & Schüll, Dassel, G.F.R.) containing a fluorescent dye (maximum wavelength of absorption 254 nm). The plates were developed in the first direction with distilled water[8], dried and then in the second direction with 1.25 *M* sodium chloride solution[9]. Ultraviolet-absorbing spots were located by irradiating the plates with a short-wavelength (254-nm) lamp.

RESULTS AND DISCUSSION

The initial chromatographic steps on the Dowex 50W-X8 and Dowex 2-X8-100

resins are necessary in order to remove interfering substances from the urine. Tests showed that the following compounds were removed on washing the Dowex 50 column with water: uric acid, uracil, orotidine, orotic acid (partially), ψ-uridine and protein (albumin). The second column was shown to remove creatinine and urea. All of these interfering compounds are found in normal urine[4]. Purines were recovered quantitatively in the final acidic eluate from the second column.

The two-dimensional thin-layer chromatography was rapid and was completed in about 3 h. Purine extracts were prepared from both normal urine and pregnancy urine samples sent to the laboratory for routine oestrogen screening. No differences were observed between the two groups on chromatography and up to 19 spots could be located under ultraviolet light (Fig. 1). On the basis of their chromatographic mobilities, seven of these spots could be identified as xanthine, hypoxanthine, adenine, guanine, 1-methylhypoxanthine, 7-methylguanine and N^2-methylguanine. The most intense spots were those due to xanthine, hypoxanthine and 1-methylhypoxanthine. Other samples of purine bases were not available for comparison.

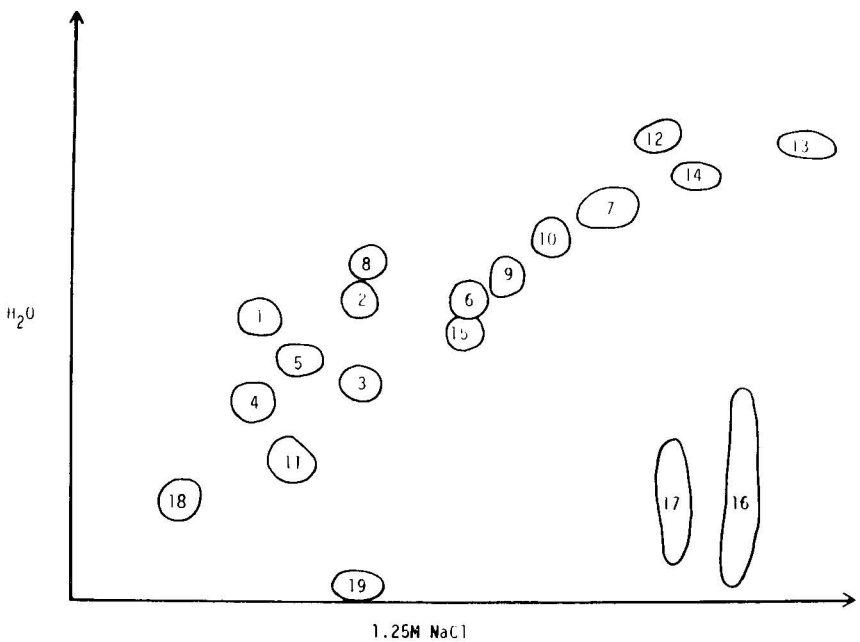


Fig. 1. Two-dimensional chromatogram of urinary purines. Spots: 1, adenine; 2, 7-methylguanine; 3, xanthine; 4, guanine; 5, N^2-methylguanine; 6, hypoxanthine; 7, 1-methylhypoxanthine; 8–19, unidentified. Spots 10, 15 and 17 were highly fluorescent.

The results obtained are comparable with those of other workers. Up to 22 spots have been noted in the paper chromatography of urinary purine preparations[5], although only about 15 purine bases have actually been identified in normal urine[3–5]. The present method should be useful in detecting such well documented inborn errors of purine metabolism as xanthinuria[10], in looking at the changes in purine urinary excretion patterns that occur in several types of cancer[11] and also in screening for

other as yet undiscovered metabolic disorders, particularly those relating to the pathogenesis of primary gout.

ACKNOWLEDGEMENT

Financial support was provided by the St. George's Hospital Medical Research Fund.

REFERENCES

1 C. E. Jones, in J. C. Touchstone (Editor), *Quantitative Thin Layer Chromatography*, Wiley, New York, 1973, p. 155.
2 B. Weissman, P. A. Bromberg and A. B. Gutman, *J. Biol. Chem.*, 224 (1957) 407.
3 H. A. Simmonds, *Clin. Chim. Acta*, 23 (1969) 319.
4 W. N. Kelley and J. B. Wyngaarden, *Clin. Chem.*, 16 (1970) 707.
5 D. H. Orrell, G. R. Haynes and D. N. Raine, *Ann. Clin. Biochem.*, 9 (1972) 53.
6 A. H. van Gennip, P. K. de Bree, C. van der Heiden, S. K. Wadman, J. Haverkamp and J. F. G. Vliegenthart, *Clin. Chim. Acta*, 45 (1973) 119.
7 R. Y. Thomson, in I. Smith (Editor), *Chromatographic and Electrophoretic Techniques*, Vol. I, Heinemann, London, 1967, p. 295.
8 P. Reyes, *Anal. Biochem.*, 50 (1972) 35.
9 C. E. Jones, E. E. Smith, W. Hicks and J. W. Crowell, *J. Lab. Clin. Med.*, 76 (1970) 163.
10 K. Engleman, R. W. E. Watts, J. R. Klinenberg, A. Sjoerdsma and J. E. Seegmiller, *Amer. J. Med.*, 37 (1964) 839.
11 S. S. Mirvish, J. Medalie, C. A. Linsell, E. Yousef and S. Reyad, *Cancer*, 27 (1971) 736

Journal of Chromatography, 125 (1976) 533

CHROM. 9559

Book Review

Comprehensive analytical chemistry, Vol. IID, Coulometric analysis, (by E. Bishop), edited by C. L. Wilson and D. W. Wilson, Elsevier, Amsterdam, Oxford, New York, 1975, XXVIII + 673 pp., price Dfl. 240.000, US$ 99.95, ISBN 0-444-41044-9.

The editors of *Comprehensive Analytical Chemistry* have been fortunate in being able to include this *magnum opus* in their series. It is a work of great scholarship, which will remain the source-book of coulometry for many years. So great was the amount of material that had to be digested, that it was decided to include only material published up to the end of 1969, except for a few essential papers by the author. The theoretical chapters, which form the first part of the book, and those on the practice of coulometry (Part II) also have general electrochemical implications, which will make the book invaluable to all electrochemists.

The theoretical section (Part I), after a simple introduction, gives a comprehensive, mathematical account of coulometric processes (electrode and other reactions), the effects of various parameters, kinetic factors, current and titration efficiencies, etc. Throughout this section, the 366, often complicated equations, are very clearly reproduced. Part II deals with the apparatus required for coulometry, including cells, power supplies, current measuring devices, and automatic and commercial instrumentation. The third section is devoted to procedures and applications in general. It discusses a wide variety of subjects, including the Faraday constant, coulometric preparation of standard solutions, current efficiency electrode surfaces, coulometry as an investigational tool, chronoelectrometry, and various special methods such as coulogravimetry, the determination of water, oxygen meters, voltammetry and stripping methods. The final part describes the applications of amperostatic and potentiostatic coulometry to a wide selection of elements and organic compounds. It describes techniques for end-point detection, the effect of titration conditions on titration curves, as well as detailed applications. In particular, the amperostatic generations of reductants and oxidants are considered at length. The book concludes with a list of 2444 references, a computer programme and a subject index.

There is no doubt that this book is one of the most significant analytical chemistry publications of this decade. It provides sound instruction in all aspects of coulometry and related subjects, and should be essential reading for all who are involved in electrochemistry. The price of the book is high, but the quality is guaranteed, and there will be no need to buy other books on this subject for many years to come.

Birmingham (Great Britain) A. TOWNSHEND

Journal of Chromatography, 125 (1976) 534–535

CHROM. 9479

Book Review

A dictionary of chromatography, by R. C. Denney, Macmillan, London, Basingstoke, 1976, XI + 191 pp., price £15.00, ISBN 0-333-17427-5.

There is no doubt that the idea of publishing a dictionary of chromatography was very timely as such a publication did not previously exist. Such a dictionary should reflect the *status quo* in chromatography and electrophoresis (which is also dealt with in this book) and have entries relating to the description of individual techniques, equipment, chromatographic materials and procedures, chromatogram evaluation and the theoretical background. I feel that it is clear which entries should be included in such a dictionary. Nomenclature, definitions and symbols will obviously be a source of problems because of the difficulty of obtaining a unifying view from the scattered literature.

With this book, the reviewer has to appreciate the materialization of the idea of compiling a dictionary, the logical arrangement of the individual entries and the figures that have been included to make the understanding of a particular term easier. Much attention is paid to terms related to the theory of chromatographic procedures, and to gas chromatography in particular. There are 374 entries in the dictionary, plus 321 references.

On the other hand, one cannot overlook the fact that there are numerous drawbacks to this book. The lack of balance among the individual techniques is probably its main disadvantage. Most attention has been paid to gas chromatography (about 40% of the entries), while thin-layer (TLC) and paper chromatography (PC) are almost neglected (5% of the entries). While there are almost no gaps in gas chromatography, the reader interested in TLC and PC will miss such fundamental entries as Paper, Layer, Twin layers, Foils, Sintered glass plates, Detection, Atomiser, Bioautography, Autoradiography, Diagonal chromatography, Over-run development, Horizontal development, Multiple development, Flying-spot densitometer, TAS procedure, Thermofractography, Fingerprints, Start, Front, Tailing, Martin's relationship, and many others. Moreover, in some other techniques some important entries are also missing, such as Isotachophoresis, Disc electrophoresis, Crossing electrophoresis, Deflection electrophoresis, Density gradient electrophoresis, Isoelectric focusing, Moving-boundary electrophoresis, Affinant, Reaction chromatography, Mass fragmentography, Chromatographic profiles, Peak, Ampholines, Argentation chromatography and Zone spreading. On the contrary, there are some terms that are obviously of secondary importance, such as Chabazite, Sedimentation potential and R_B. In numerous terms that are generally important in chromatography, only a single technique is emphasized. Thus, for instance, pellicular shape can be found not only in ion-exchange resins but also in sorbents for high-performance liquid chromatography (HPLC). Similarly, silica gel for HPLC is not mentioned.

Some terms are incorrectly defined, and a few may serve as illustrations here. Partition constant is today preferred to Partition coefficient; the sense of the word

Support is slightly different in TLC —the sorbents for liquid–liquid TLC are referred to as carriers; it appears that the explanation of Ascending development is more important than Ascending chromatogram; R_M (in the chapter Symbols) is incorrectly designated as Relative movement for related compounds; the term Chromatography is derived from the Greek Chroma and Grafein and not from Chromatos and Graphos; the term Desalting is used not only in gel permeation chromatography but equally for an operation used in sample preparation for TLC and PC; the use of abbreviations such as "l.c., p.c., g.c., t.l.c." is unusual, the capitalized versions LC, PC, GC and TLC being more common. In PC the milligram scale is not common. The term Spreader is not explained exactly. Also, some symbols and nomenclature could be subjected to discussion, but as mentioned above in this respect there is no uniformity in the literature.

From the printing point of view the book is well produced with few errors. It was a pleasure for the reviewer to see his own name correctly spelled in reference 123 whereas a mistake occurs in reference 204. In conclusion, one can say that the book will be useful in the area of gas chromatography, but with other chromatographic and electrophoretic techniques it unfortunately does not meet the aims specified by the author in the Introduction, namely "to provide a source of reference to the student, laboratory technician or general scientist seeking rapid information on chromatography".

Prague (Czechoslovakia) K. MACEK

Author Index

Erratum

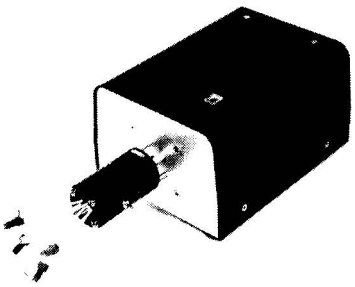
J. Chromatogr., 115 (1975) 250–255

The reported column temperatures should be increased by 15° to get the correct temperature.

Vol. 125, No. 3

journal of
chromatography news section

APPARATUS



N-923

6-PORT MOTORIZED VALVE

The standard model of the 6-port motorized valve from Altex Scientific permits automatic switching of one inlet flow stream to six different outlet lines or *vice versa*. Other flow switching patterns are also available, including a fixed sample loop injector. Motor driven indexing is electro-optical and takes less than 300 milliseconds using panel button or remote timer or programmer.

N-933

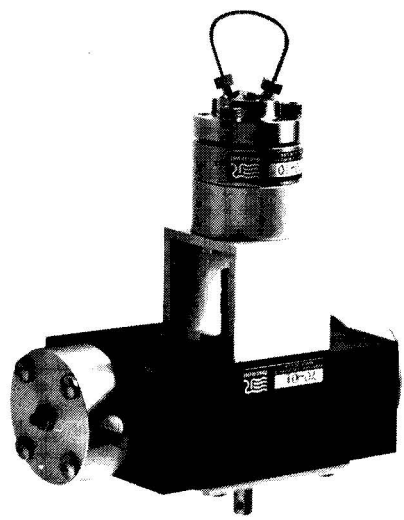
HARD-LAYER TLC PLATES

New England Nuclear is now manufacturing hard-layer TLC plates that feature a silica-gel coating and an inert, organic binder. The hard-layer surface can be written on without chipping. Suitable for a wide range of analyses, the pre-scored plates are additionally designed for auto-radiographic visualization.

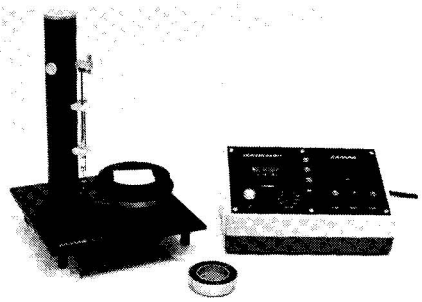
N-922

PNEUMATIC ACTUATOR FOR HPLC VALVES

Rheodyne's pneumatic actuator (Model 70-01) provides automatic operation of their highpressure liquid chromatographic sampling and switching valves. 60° rotary motion is provided by a double acting air cylinder. Operation range is between 40 and 125 p.s.i. air pressure. Available separately, the actuator can also be obtained as the automatic sample injection valve or as the automatic 3-way and 4-way valves.



For further information concerning any of the new items, apply to the publisher, using the reply cards provided, quoting the reference number printed at the beginning of the item.



N-927

U-CHAMBER SYSTEM FOR CIRCULAR TLC

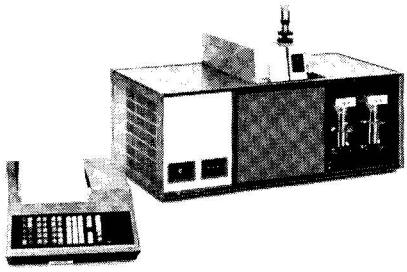
The Camag U-chamber system has been designed for circular chromatogram development using the new type high-performance TLC precoated plates. The system allows control of all phases (stationary, mobile, vapour of the chromatographic system). The mobile phase is fed from a syringe into the layer at a constant flow-rate. As sample injection into the flowing system is possible, the U-chamber offers experimentally correct conditions for direct transfer of information from TLC to column chromatography. A chromatogram is completed within 1–4 minutes and requires 0.07–0.2 ml of developing solvent.

N-921

HIGH-PERFORMANCE LIQUID CHROMATOGRAPH WITH BUILT-IN PROCESSOR

The Hewlett-Packard 1080 series high-performance liquid chromatograph is controlled by a built-in central processor, and gives users full control of separation parameters. All major components of the liquid chromatograph, including pumps, injector, columns and detector, are newly designed. The instrument consists of two modules: one containing the control terminal with a printer/plotter, the other comprising chromatographic system and central processor. The LC terminal provides interactive keyboard control over all operating parameters. The LC mainframe contains the injector, solvent containers, pumps, detector, column and the digital processor.

Once instructed the instrument automatically carries out the entire analysis. A final report contains the chromatogram and a complete report in the format of the analytical method chosen. The terminal can also plot the detector signal, flow,, percentage solvent composition and column-head pressure.



A single-wavelength UV detector is standard but the instrument is compatible with other detectors such as variable-wavelength UV–visible and refractive index detectors.

The HP 1084A model features gradient elution capability. Model HP 1082A features an isocratic system which can be upgraded to full gradient capability.

N-932

DATA SYSTEM FOR AUTOMATED CHROMATOGRAPHY

The new Varian data system (CDS-111) automatically integrates and calculates the data from gas and liquid chromatographs, automatically quantitating most chromatograms on its own. The areas of simple and complex peaks can be measured. Internal standard, external standard, calibration factor, relative response factor, area percent and normalized area percent methods, separately or in combination, are used to calculate the results in forms such as weight percent, mole percent or volume percent. The system interfaces with most gas and liquid chromatographs. In combination with the Varian GC 3700, the instrument can automate the whole chromatographic process from injection to final report. The latter shows the chromatographic results, as well as GC and AutoSampler parameters (e.g. carrier-gas flow, temperature, vial and rack number).

N-924

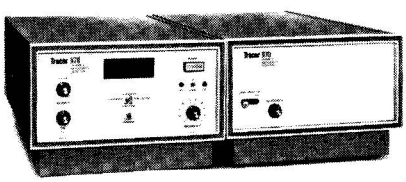
NEW RI DETECTOR

A new RI detector (the Multiref 901), for use in chromatography, is being marketed by the LC Instrument Co. (a subsidiary of Lachat Chemicals). Measurement is made using the interferometer principle in adjacent cells for the standard and the unknown. No temperature control is necessary, but thermostatic control is possible for measurements at temperatures other than ambient. Sensitivity is 10^{-8} RI units.

N-934

VARIABLE-WAVELENGTH DETECTOR

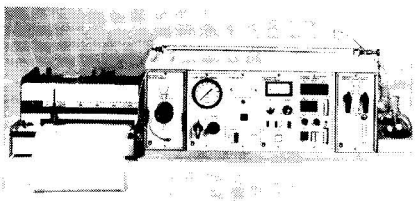
Tracor's Model 970 variable-wavelength detector has been developed specifically for use in high-pressure liquid chromatography, and covers the complete ultraviolet and visible spectral range. The detector is available separately or as part of a complete liquid chromatograph.



N-925

NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPH ON U.S. MARKET

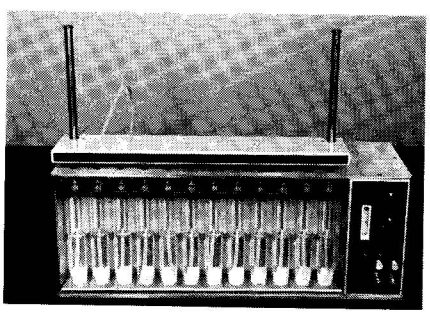
A modular high-performance liquid chromatograph has been introduced to the U.S. by the LC Instrument Co. (a subsidiary of Lachat Chemicals). UV, RI or fluorescence detectors are available. The unit is available with or without a gradient pump. The unit is available in two models: one with minimum components, the other with any desired combination of components.



N-928

CAMAG ELUQUICK

Eluquick is a new Camag instrument for the quantitative elution of sample zones from TLC sheets and is particularly useful in serial analysis where sample quantities are not extremely small. Sample areas are cut out, placed in test-tubes and elution solvent added. After a pre-set soaking time a stream of air is passed through the liquid for a time also pre-set, effecting a thorough but gentle mixing so that the adsorbent layer does not flake off the sheet. The clear eluate is removed by decanting, pipetting, aspirating, etc. 12 samples can be simultaneously eluted in the Eluquick.



CHEMICALS

N-915

SUPELCO CHROMATOGRAPHY CATALOGUE

Chromatography Catalogue No. 10 recently released by Supelco includes 100 pages of materials for gas, liquid, and thin-layer chromatography. A 15-page section is devoted to applications including acids, alcohols, amines, carbohydrates, drugs, fatty acids, hydrocarbons, pesticides, etc. The catalogue also includes a listing of high-purity lipid standards.

N-919

FICOLL-PAQUE™

Pharmacia Fine Chemicals has recently introduced Ficoll-Paque for the rapid isolation of viable lymphocytes. The solution is critically tested for density, sterility, reproducibility and function. It has a shelf life of 2 years when stored at room temperature and protected from direct light.

N-911

RADIOCHEMICALS CATALOGUE

Copies of New England Nuclear's 1976 catalogue are now available. Over 1000 labelled chemicals are listed, together with radionuclides, radioactive standards, and liquid scintillation chemicals, as well as details of customs services.

NEW BOOKS

A laboratory manual of qualitative organic analysis, by H.T. Openshaw, Cambridge Univ. Press, Cambridge, 3rd ed., 1976, 104 pp., price ca. £ 2.70, ISBN 0-521-29112-7.

Heteroaromatic nitrogen compounds: The azoles, by K. Schofield, M.R. Grimmett and B.R.T. Keene, Cambridge Univ. Press, Cambridge, 1976, ix + 437 pp., price £ 19.50, ISBN 0-521-20519-0.

Instrumental technology, Vol. 2, On-line analysis instruments, by E.B. Jones, Newnes-Butterworths, London, 1976, 282 pp., price £ 7.00, ISBN 0-408-00198-4.

MEETING

SAC CONFERENCE 1977

The Fourth SAC Conference will be held at the University of Birmingham from July 17th to 22nd, 1977. The Conference is organised by the Analytical Division of the Chemical Society and is sponsored by the International Union of Pure and Applied Chemistry. The scientific programme will cover all aspects of analytical chemistry, and will consist of plenary lectures, invited lectures, contributed papers (including poster presentations), workshops, and specialist sessions organized by the subject groups of the Analytical Division of the Chemical Society.

The invited and contributed papers will be arranged in lecture and poster sessions, the following aspects of analytical chemistry being included: insrrumentation in analysis, atomic spectroscopy, molecular spectroscopy, chromatography and other separation methods, nuclear and radiochemical analysis, microchemical techniques and analytical microscopy, thermal analysis, electroanalysis, including ion-selective electrodes, analysis in the life sciences, analysis in industry, pollution and environment control analysis, forensic analysis, newer techniques.

Contributions to the lecture and poster sessions should describe original, unpublished work that leads to the advancement of any of the general topics of analytical chemistry listed in the previous paragraph. Titles and abstracts in a form suitable for publication (up to 200 words) must be submitted before November 30th, 1976, to Dr. A. Townshend or Dr. A.M.G. Macdonald, Chemistry Department, The University, P.O. Box 363, Birmingham B15 2TT, Great Britain.

PUBLICATION SCHEDULE FOR 1976

Journal of Chromatography (incorporating *Chromatographic Reviews*)

MONTH	D 1975	J	F	M	A	M	J	J	A	S	O	N	D
JOURNAL	115/1 115/2	116/1 116/2	117/1 117/2	118/1 118/2	118/3 119	120/1 120/2	121/1 121/2	122 123/1	123/2	124/1 124/2 125/1	125/2 125/3	126 128/1	128/2 129
REVIEWS*					127/1				127/2			127/3	

* Volume 127 will consist of *Chromatographic Reviews*. The issues comprising this volume will not be published consecutively, but will appear at various times in the course of the year.

GENERAL INFORMATION

(A leaflet *Instructions to Authors* can be obtained by application to the publisher.)

Types of Contributions. (a) Original research work not previously published in a generally accessible language in other periodicals (Full-length papers). (b) Review articles. (c) Short communications and Notes. (d) Book reviews; News; Announcements. (e) Bibliography of Paper Chromatography, Thin-Layer Chromatography, Column Chromatography, Gas Chromatography and Electrophoretic Techniques. (f) Chromatographic Data.

Submission of Papers. Three copies of manuscripts in English, French or German should be sent to: Editorial office of the Journal of Chromatography, P.O. Box 681, Amsterdam, The Netherlands. For *Review articles*, an outline of the proposed article should first be forwarded to the Editorial office for preliminary discussion prior to preparation.

Manuscripts. The manuscript should be typed with double spacing on pages of uniform size and should be accompanied by a separate title page. The name and the complete address of the author to whom proofs are to be sent should be given on this page. Authors of papers in French or German are requested to supply an English translation of the title. A short running title of not more than 50 letters (including spaces between the words) is also required for Full-length papers and Review articles. All illustrations, photographs, tables, etc., should be on separate sheets.

Heading. The title of the paper should be concise and informative. The title should be followed by the authors' full names, academic or professional affiliations, and addresses.

Summary. Full-length papers and Review articles should have a summary of 50–100 words. In the case of French or German articles an additional summary in English, headed by an English translation of the title, should also be provided. (Short communications and Notes will be published without a summary.)

Illustrations. The figures should be submitted in a form suitable for reproduction, drawn in Indian ink on drawing or tracing paper. Particular attention should be paid to the size of the lettering to ensure that it does not become unreadable after reduction. Sharp, glossy photographs are required to obtain good halftones. Each illustration should have a legend, all the *legends* being typed together on a *separate sheet*. Coloured illustrations are reproduced at the author's expense.

References. References should be numbered in the order in which they are cited in the text and listed in numerical sequence on a separate sheet at the end of the article. The numbers should appear in the text at the appropriate places using superscript numerals. In the reference list, periodicals[1], books[2], and multi-author books[3] should be cited in accordance with the following examples:

1 A. T. James and A. J. P. Martin, *Biochem. J.*, 50 (1952) 679.
2 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 201.
3 R. D. Marshall and A. Neuberger, in A. Gottschalk (Editor), *Glycoproteins*, Vol. 5, Part A, Elsevier, Amsterdam, 2nd ed., 1972, Ch. 3, p. 251.

Abbreviations for the titles of journals should follow the system used by *Chemical Abstracts*.

Proofs. Two sets of proofs will be sent to the author to be carefully checked for printer's errors. Corrections must be restricted to instances in which the proof is at variance with the manuscript. "Extra corrections" will be inserted at the author's expense.

Reprints. Fifty reprints of Full-length papers, Short communications and Notes will be supplied free of charge. Additional reprints can be ordered by the authors. An order form containing price quotations will be sent to the authors together with the proofs of their article.

News. News releases of new products and developments, and information leaflets of meetings should be addressed to: The Editor of the News Section, Journal of Chromatography, Elsevier Scientific Publishing Company, P.O. Box 330, Amsterdam, The Netherlands.

Subscription orders. Subscription orders should be sent to: Elsevier Scientific Publishing Company, P.O. Box 211, Amsterdam, The Netherlands.

Publication. The *Journal of Chromatography* (including *Chromatographic Reviews*) appears fortnightly and has 14 volumes in 1976. The subscription price for 1976 [Vols. 115–128 and Supplementary Vol. 5 (Bibliography of Paper and Thin-Layer Chromatography 1970–1973)] is Dfl. 1470.00 plus Dfl. 180.00 (postage) (total US$ 660.00). Subscribers in the U.S.A., Canada and Japan receive their copies by air mail. Additional charges for air mail to other countries are available on request. Back volumes of the *Journal of Chromatography* (Vols. 1 through 114) are available at Dfl. 100.00 plus postage. Claims for issues not received should be made within three months of publication of the issue. If not, they cannot be honoured free of charge.

Advertisements. Advertisement rates are available from the publisher on request. The Editor of the journal accepts no responsibility for the content of the advertisements.