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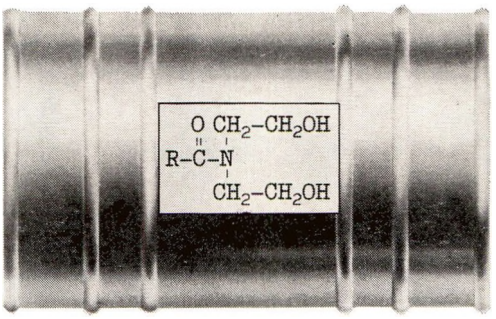

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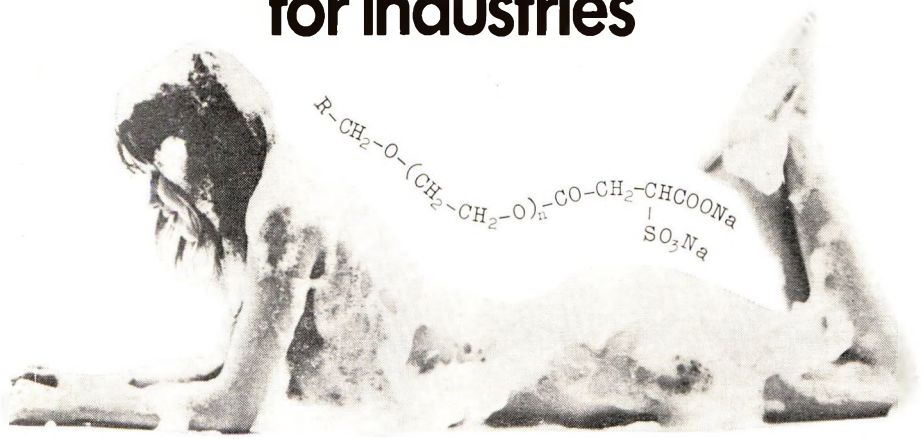

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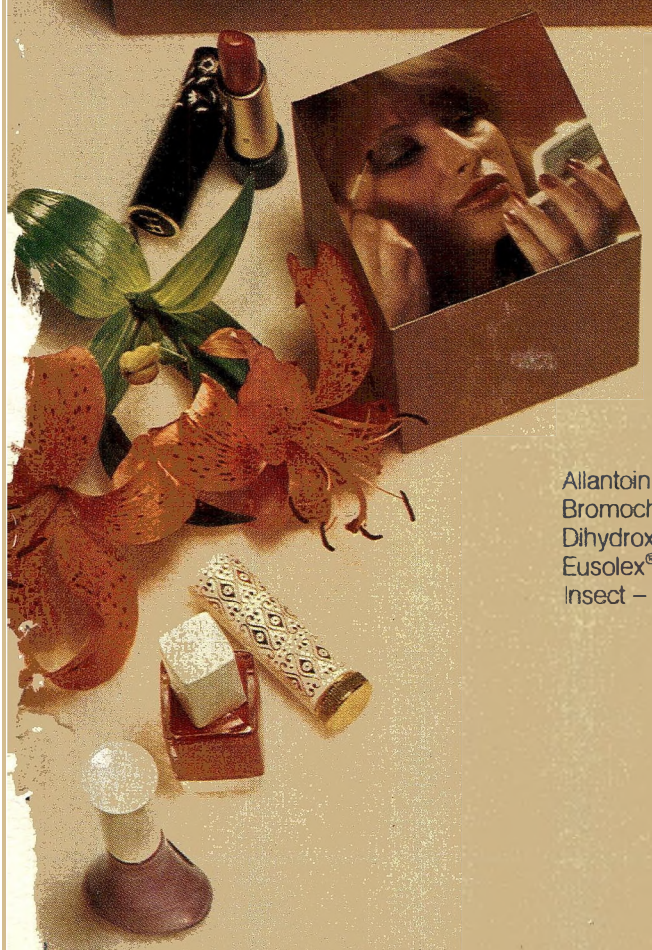

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for referenc without mutilating the pages of the Journal.

Measurement of biochemical parameters in the stratum corneum: MICHEL KERMICI, CATHERINE BODEREAU and GUY AUBIN. *Journal of the Society of Cosmetic Chemists* **28** 151–154 (1977)

Synopsis—The paper reports an original method to evaluate biochemical variations of the skin. From samplings of the stratum corneum upper layers, by stripping, enzymatic activities (lytic enzymes, deshydrogenases) have been quantitatively measured. The values determined by this method reflect the metabolic work present in the Malpighian layer. These enzymatic measurements, associated with the determination of other biochemical parameters (lipids, proteins, aminoacids) constitute an objective evaluation of the skin quality.

A critical study of the method is given and some experimental results, as a function of different human skin areas and skin types, are presented. Results of experiments, on swine and human skin, will show the interest of the technique.

A new approach to the theory of adsorption and permeability of surfactants on keratinic proteins: the specific behaviour of certain hydrophobic chains: J. GARCIA DOMINGUEZ, J. L. PARRA, MA R. INFANTE, CARLOS M. PELEJERO, FRANCISCO BALAGUER and T. SASTRE. *Journal of the Society of Cosmetic Chemists* **28** 165–182 (1977)

Synopsis—The sequence: detergency-adsorption-interaction-denaturation-permeability-irritation, caused by ionic surfactants on keratinic proteins such as stratum corneum has been studied. Adsorption and permeability of the protein or lipoprotein structures have been investigated, and a five step mechanism is proposed to explain the way in which ionic surfactants can be transferred from the interface protein-water to the inner cells of the dermis, resulting in irritation.

Two new theoretical approaches are proposed to justify the odd behaviour of the C_{12} anionic surfactants: solubility on hydrophobic sites, or a molecular conformation of the normal linear structure of the surfactant molecules in a more stable form, under conditions which give a minimum size for C_{12} surfactants.

Evaluation of O/W emulsion stability through zeta potential—1: DEVRAJ RAMBHAU, D. S. PHADKE and A. K. DORLE. *Journal of the Society of Cosmetic Chemists* **28** 183–196 (1977)

Synopsis—Ageing of emulsions prepared with sodium palmitate or cetyltrimethylammonium bromide under accelerated conditions such as elevated temperatures or higher centrifugal speeds resulted in an increase in mean globule diameter and decrease in viscosity and Zeta potential. A fair inverse correlation was observed between Zeta potential and ageing. The correlation between Zeta potential and mean globule diameter in sodium palmitate emulsions was not, however, as fair as

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in the case of emulsions prepared with cetyltrimethylammonium bromide. Changes in Zeta potential and viscosity on ageing showed a linear relation between them at all temperatures studied, in both the emulsions. Decay in Zeta potential with time at higher centrifugal speeds was linear and was dependent on speed of centrifugation. Mean globule diameter was linearly increased as Zeta potential was decreased at all centrifugal speeds studied. Emulsifier concentration studies revealed that at a critical concentration emulsions possess maximum Zeta potential. Maximum Zeta potential was associated with maximum emulsion stability. Possible mechanisms of Zeta potential decay at accelerated conditions were explained.

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Measurement of biochemical parameters in the stratum corneum

Presented at the ninth IFSCC Congress, June 1976, Boston, U.S.A.

MICHEL KERMICI, CATHERINE BODEREAU, and GUY AUBIN*
L'Oreal, France

Synopsis

The paper reports an original method to evaluate **biochemical** variations of the **skin**. From samplings of the **stratum corneum** upper layers, by **stripping**, enzymatic activities (lytic **enzymes**, deshydrogenases) have been quantitatively measured. The values determined by this method reflect the metabolic work present in the Malpighian layer. These enzymatic measurements, associated with the determination of other biochemical parameters (lipids, proteins, aminoacids) constitute an objective evaluation of the skin quality.

A critical study of the method is given and some experimental results, as a function of different human skin areas and skin types, are presented. Results of experiments, on swine and human skin, will show the interest of the technique.

Introduction

The study of cutaneous biochemistry has developed considerably during recent years, thanks to improvements in biochemical and histochemical methods. Apart from rare exceptions, these studies have been performed on man through biopsies. However, this method of obtaining samples causes stress and sometimes leaves unsightly scars.

This method is therefore not suitable for systematic studies, in particular those intended to evaluate the effects of various products, whether to assess their activity or their potential dermal toxicity.

The development of objective tests on human skin in vivo is, however, desirable to further research in cosmetics.

Several methods have been published with this aim. Often, however, the measurements are qualitative (1, 2, 3) or too complex to be used in routine tests (4, 5), though these methods are very interesting for fundamental studies.

Based on observations made in particular by the German school of dermatology (1, 2), which demonstrate the presence of enzymatic activity in the upper layers of the stratum/corneum, we have developed a method which makes it possible to make a quantitative evaluation of the different biochemical activities in the stratum corneum. The determinations are performed using samples obtained by strippings.

Various studies performed in our laboratories in support of this method showed that the biochemical variations observed in the upper layers of the stratum corneum were a reflection of the metabolic activity in the deep layers of the epidermis. It is therefore hoped that this method may prove useful in the study of cosmetic products.

The paper gives a critical study of the method. Examples of results will be given to illustrate the value of the method in the fields of dermatology and cosmetology.

* L'Oreal, Laboratoires de Recherche, 93601 Aulnay sur Bois.

PRINCIPLE

The principle of the method is the measurement of different biochemical activities directly from samples of the stratum corneum obtained by strippings, using adhesive strips. This method can be used in man, on all areas which are hairless or which have only a small amount of hair, and can also be used on pigs and laboratory animals provided that the density of the hairs is low, for example hairless animals.

METHOD OF OBTAINING SAMPLES

As far as is possible, the subjects providing the samples should apply nothing to the skin, at least during the 6 h preceding the test.

The samples should be taken in a room with controlled temperature and relative humidity ($\theta = 20°C \pm 2$; $RH = 50\% \pm 10$) from subjects who have been resting for 30 min. This ensures good homeostasis of the skin in relation to the ambient atmosphere.

Biochemical exploration of a given anatomical locality is performed in the following manner:

Epicutaneous lipids are first removed using the method of Shaefer and Kuhn-Bussius (7) by applying a 1·8 cm^2 plate of unpolished glass at a pressure of 1000 *g* for 30 sec. This pressure is obtained by using a dynamometric system which makes it possible to apply forces of 50–1,500 *g* on cutaneous surfaces ranging from 1 to 25 cm^2.

Immediately after obtaining this sample, a 4 cm^2 adhesive strip, either '471' or 'type clear' strips from Minnesota Mining and Manufacturing Co.®, is applied to the same place. This strip is applied for 15 sec. under a pressure of 250 g/cm^2, using the system mentioned above. The strip is then removed using tweezers. This stripping operation can be repeated several times on the same place.

In the context of the work described here, we have generally performed five consecutive strippings, keeping the first two for measurement of the proteins, the third for measurement of the acid phosphatases, the fourth for measurement of the activity of D glucuronidases and the fifth for determination of the activity of glucose 6 phosphate β dehydrogenases (G6PDH). 30 min. after the fifth stripping, it is possible to take another sample of lipids under the same conditions as previously.

As we shall see later on, this last measurement makes it possible to evaluate the speed of secretion of sebum at a given area.

After removal, the samples are stored at 0°C under nitrogen in hermetically sealed tubes.

METHODS FOR MEASUREMENTS

Lipids

The method of Cottet and Etienne (6) using the sulphophosphovanillin colorimetric method is applied directly to the sintered glass from the sampling, placed in a flat-bottomed tube.

The intensity of colour obtained is directly proportional to the amount of lipids, expressed as Trioleine, for quantities ranging from 20–150 μg. Absorptions are read at 525 nm.

This colorimetric method seems to us preferable since it is more accurate than the determination of the transmission of light through the sintered glass, which is the method proposed by Shaefer and Kuhn-Bussius (7).

Proteins

The protein samples obtained using the adhesive strips are measured by the method of Bramhall and his colleagues (8) which we have adapted to our needs.

The measurement consists of the adsorption of *Xylene Cyanin G*® on to the proteins. After removing the excess colourant by washing, the colourant fixed on the proteins is eluted and quantified by spectrophotometry at 610 nm. Coloration is proportional to the amount of proteins. The standard curve is set up using serum albumin, precipitated by trichloroacetic acid (7%) and fixed on Whatman No. 1 paper.

ENZYMATIC DETERMINATIONS

These are performed by direct incubation of the sample in the buffer substrate medium which is specific to the appropriate enzyme. The enzymatic reactions are observed using fluorimetric methods. These very sensitive determinations make it possible to detect faint enzymatic activity. In this way, acid phosphatases are determined by hydrolysis of α-naphthyl sodium phosphate and βD-glucuronidases by hydrolysis of α-naphthyl βD-sodium glucuronide. In these two cases, the liberation of the fluorescent naphthol group is observed. For these determinations we used the method published by Campbell and Moss (9) and Im and Hoopes (10), slightly modified. The activity of G6PDH is observed by reduction of the co-enzyme of the reaction, NADP*, by fluorimetry, according to Lowry (11).

WHY THESE PARAMETERS?

In the context of the development and control of the reliability of the method, the use of the parameters selected is easily explained.

The measure of the amount of proteins in the sample shows the state of desquamation of the skin, thus making it possible to evaluate the cutaneous reactions and to characterize the various types of skin.

The determination of acid phosphatases and βD glucuronidases is justified by the presence of these enzymes in the stratum corneum; these were demonstrated by Steigleder (1) in particular, whose work partly forms the basis of the beginning of our work. These two enzymes, localized in the cell lysosomes, are involved in the process of cellular dedifferentiation and keratinization (12) and because of this, are connected with the formation of the stratum corneum.

As for G6PDH, it plays an important role in the metabolism of the cell by virtue of its key position in the cycle of hexose monophosphates and it thus forms an excellent marker of cellular activity.

By histochemical studies on skin biopsies, Ohkawara and Halprin (13, 14) have shown that the activity of this enzyme increased significantly in cases of pathological disturbance and cutaneous stress.

* NADP = Nicotinamide adenine dinucleotide phosphate.

It was therefore of value to see whether we were able to detect this enzymatic activity in the upper layers of the epidermis, even though this enzyme had been considered exclusively endocellular. On this point, experience has shown us to be right, and the work of Shaefer and his colleagues (4, 15) has confirmed our initial results, demonstrating the existence of this enzyme in the upper layers of the epidermis, and showing that its presence at such a level was due to a limited extra-cellular flux.

CRITICAL STUDY OF THE METHOD

Adhesive strips

Several types of adhesive strips were used. The only ones which gave satisfaction were strips of '471' or type 'clear'. The other materials used, when in an aqueous medium, liberated substances which interfered with the determinations. The adhesive strips preferred have the added advantage of not curling up over themselves and of ensuring perfect contact with the substrates. These strips also make it possible to take samples of stratum corneum, layer by layer, without causing any stress for the subject. It must be emphasized, however, that there may exist differences in adhesion among rolls of adhesive of the same type. It is therefore appropriate to classify the different rolls, one compared with another, by testing them on adjacent areas of a forearm, for example.

The value of the sample of stratum corneum depends on the nature of the adhesive used in manufacturing the strip. We have performed different studies using the strip, type 'clear', to verify the extent of the sample of stratum corneum obtained by stripping. In this way, we determined that, on the skin of the forearm, it was necessary to do 20–25 strippings to remove the stratum corneum completely. These values are in agreement with the number of layers of stratum corneum found by Anderson and Cassidy (16) among others.

In another series of experiments, we performed a study of the transepidermal water loss, by the method of De Rigal and Leveque (17), as a function of the number of strippings. The surfaces sampled were the forearms of eight persons.

The mean values of our experimental data are presented graphically on *Fig. 1*. The hyperbolic shape of the curve suggests that the transepidermal water loss is a function of the thickness of the stratum corneum. Besides it has been demonstrated that the transepidermal water loss obeyed the Fick's law of diffusion (18).

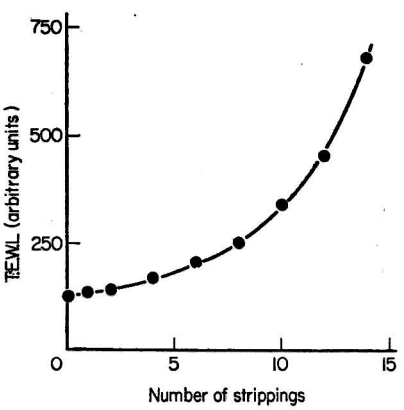

Figure 1. Variations of the transepidermal water loss plotted against the number of strippings.

On the other hand, the shape of the curve also shows that five strippings do not appear to alter fundamentally the barrier nature of the stratum corneum.

If Fick's law is accepted for the transepidermal water loss, that is to say, an inverse proportionality between the diffusion of water and the thickness of the membrane, it is possible to define the thickness of stratum corneum removed by stripping. Thus, it can be seen that (*Fig. 2*) four samples remove about $\frac{1}{4}$ of the stratum corneum, that it would require about ten to remove half and, by extrapolation, it can be seen that it would require about twenty-five to remove it completely.

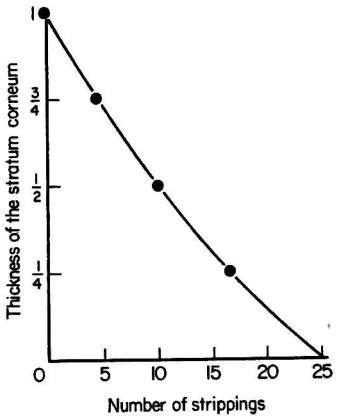

Figure 2. Variations of the thickness of stratum corneum against the number of strippings.

Fig. 2 shows that, as far as the tenth stripping the relation is linear between the thickness of the stratum corneum and the number of samplings; this suggests that the tissue removed is practically constant at each stripping.

Beyond the tenth stripping, the curve departs from linearity, i.e. the strips removing less material. It is probable that this is due to the stronger adhesion between cells in the lower layers of the stratum corneum.

Effect of the pressure exerted on the adhesive strip

Different determinations performed on the third and fifth stripping, related to the pressure exerted on the adhesive strip applied to the skin, showed that above a pressure of 100 g/cm², the activities measured did not vary (*Fig. 3*). For the purpose of standardization, we used pressures of 250 g/cm².

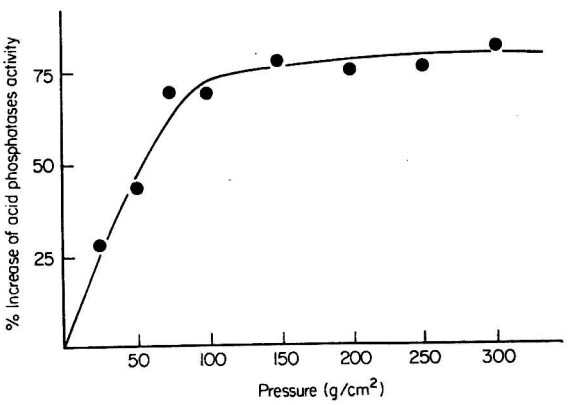

Figure 3. Acid phosphatase activity plotted against the pressure exerted on strips, during the sampling.

Effect of the period of application

The period of application of the dynamometric system to the skin has no effect on the cutaneous sample. A standardized time of 15 s. for each stripping makes it possible to obtain a suitable, homogeneous pressure.

Effect of the epicutaneous lipids on samples obtained by stripping

Does the presence of lipids on the surface of the skin upset the samples? One may believe that oily skins would show lower parametric values than they actually do.

In fact, experience has shown that, in individuals with normal or seborrheic skins, the first two strippings remove the surface lipids almost completely (*Fig. 4*).

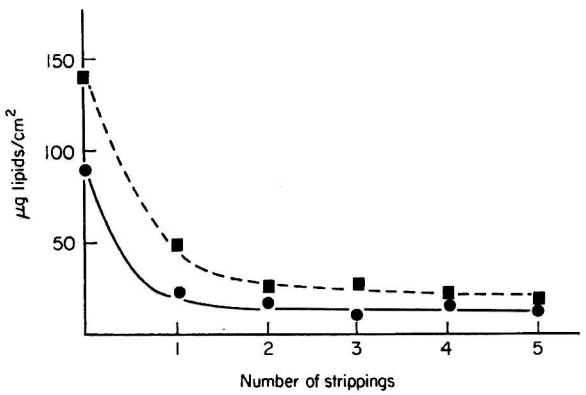

Figure 4. Elimination of external lipids plotted against the number of strippings.

Effect of the cutaneous bacteria on the measurements

The enzymatic activity detected in the upper layers of the stratum corneum have not a bacterial origin.

In fact, the number of bacteria present on 1 cm^2 of healthy skin varies from 10 to 10,000, of which more than 90% are not pathogenic. They only show weak acid phosphatase and glucuronidase activity (19, 20).

We have verified this fact by performing enzymatic determinations on the cutaneous flora (micrococci and corynebacteria) using samples obtained by the method of Reibel *et al.* (21). The quantities obtained all proved extremely small.

The activity of acid phosphatases in a suspension of cutaneous bacteria ($15 \cdot 10^8$/ml), measured by the Campbell and Moss's method (9) was $1 \cdot 10^{-4}$ mU/ml. The acid phosphatase values in the human skin (third stripping) are around $5 \cdot 10^{-2}$ mU/cm^2 (see Results section); therefore the enzymic activity, found in the upper layers of stratum corneum cannot be referred to bacteria.

Effect of time on the storage of the samples

The samples destined for enzymatic determinations may be stored dry at −20°C. After 24 h storage, it was established that the activities had not changed. Further, a gradual decrease in the initial activities was observed. There are no storage problems for lipids and proteins provided the samples are kept at 0°C under nitrogen.

RESULTS

The field of investigation opened by this method is immense. We have studied a number of problems, and in order to illustrate the various possibilities of the method, some results will be given, obtained in animal species as well as in man.

Reproducibility of the method

In order to verify the reproducibility of the method, we selected twenty subjects of varying age and sex with healthy skins of different types: some thicker, some more oily. On the forehead of each subject we marked five areas of 4 cm^2. We took a series of samples from each of these areas, and this was done four times at intervals of one week. Taking into account the different nature of the skin of the subjects, the values of the parameters monitored will also vary from one individual to another; we have therefore taken the average of the values obtained for each individual and each parameter and calculated the deviation in the types.

Table I shows the percentage of variations for type deviations compared to the mean. This table shows that, apart from the G6PDH where the type deviation was about 50% of the mean, all the other parameters measured show deviations representing about 25% of the mean value.

Table I. Variations of the type deviation compared to the mean (study on the forehead

	Epicutaneous lipids	Sebum excretion rate	Proteins (I+II)	Acid phosphatases (III)	G6PDH (V)
% ~/m	17–27	18–30	23–30	20–28	41–60

Number of subjects: 20; number of samples per subject and per parameter: 20; I, II, III, IV: number of order of stripping.

Although such variations may appear important, a priori, our studies have shown that in the case of affected skin, the values recorded are very different from those observed for healthy skins.

We found these variations to be identical in other experiments, conducted in different animal species, on the dorsal skin, as is shown in *Table II.*

Table II. Variations in the biochemical parameters in the stratum corneum according to the species of animal (study on dorsal skin)

Animal species	$n \times P$*	Proteins $\mu g/cm^2$	Acid phosph. $mU/cm^2 \times 10^{-2}$	βD glucuronidases $mU/cm^2 \times 10^{-3}$	G6PDH $mU/cm^2 \times 10^{-3}$
Rat Wistar hairless	40 × 1	5·3 ± 1·6	7·5 ± 2·2	8·8 ± 3·0	3·4 ± 1·6
Pig L.W. (4 months)	8 × 4	7·3 ± 1·9	15·4 ± 3·0	3·0 ± 0·8	5·1 ± 2·0
Man (30–40 yrs)	4 × 6	1·7 ± 0·5	4·8 ± 1·1	1·8 ± 0·3	2·3 ± 1·0

* n = number of subjects. P = number of samples.

Biochemical variations on the dorsal skin between different species of animals

Table II shows the mean values for 40 values in the rat, 32 values in the pig and 24 values in man, with the corresponding type deviations for each parameter.

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It was observed that the values were very different according to the species of animal under consideration.

In the pig, proteins and phosphatase activity were very clearly higher than in the other two species. Although the G6PDH were also higher, the difference was not significant.

Man, on the other hand, showed much lower values, whatever the parameter. The rat gave intermediate values except for βD glucuronidases, which were higher than in the other two species.

A comparison between the activities of acid phosphatases and βD glucuronidases is of interest insofar as there does not appear to be a correlation between these two types of activity in the stratum corneum, even though these two enzymes are described as being of lysosomal origin.

Biochemistry of stratum corneum depending on anatomical locality

Fig. 5 represents the values of proteins, acid phosphatases, βD glucuronidases and G6PDH determined on ten women aged from twenty to thirty-five years, having healthy skins and showing no signs of dermatoses. Thirteen anatomical localities were used.

It was observed immediately that the values for proteins, phosphatases and G6PDH did not vary in a significant manner between the various zones examined, with the exception of the forehead, for which much higher values were found. The backs of the hands also gave much higher protein values.

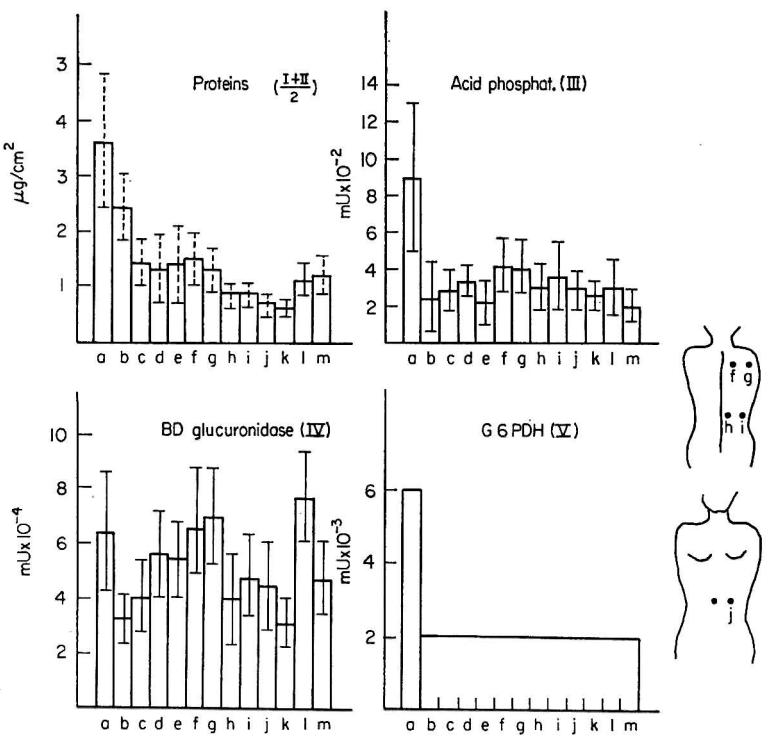

Figure 5. Biochemical variations in the upper layers of stratum corneum depending on anatomical locality. (a) Forehead; (b) hand; (c) forearm; (d) humerus; (e) armpit; (f) (g) (h) (i) back; (j) abdomen; (k) groin; (l) thigh; (m) calf.

The face and hands are subjected to external pollution to a more constant degree than all the other parts of the body studied. It is, therefore, perhaps not surprising that the results recorded were higher for these areas. Other factors may also intervene.

On this figure we have not given the mean values with the type deviation for G6PDH since we recorded a number of nil values. The curve given only shows that the values varied from 0 to $6 \cdot 10^{-3}$ mU/cm² for the forehead, and from 0 to $2 \cdot 10^{-3}$ mU/cm² for the other areas of the body.

As for the βD glucuronidases, we were not able to discern any differences between the different parts of the body.

Here again, there was no correlation observed between acid phosphatases and glucuronidases.

Biochemical variations on strippings depending on the degree of oiliness of the skin

Fig. 6 shows the variations of three biochemical activities determined on strippings of stratum corneum as a function of the degree of oiliness of the skin. The samples were taken from the foreheads of the subjects.

Determination of the epicutaneous lipids by the method of Shaefer made it possible to characterize the three main categories of skins: dry, normal and greasy.

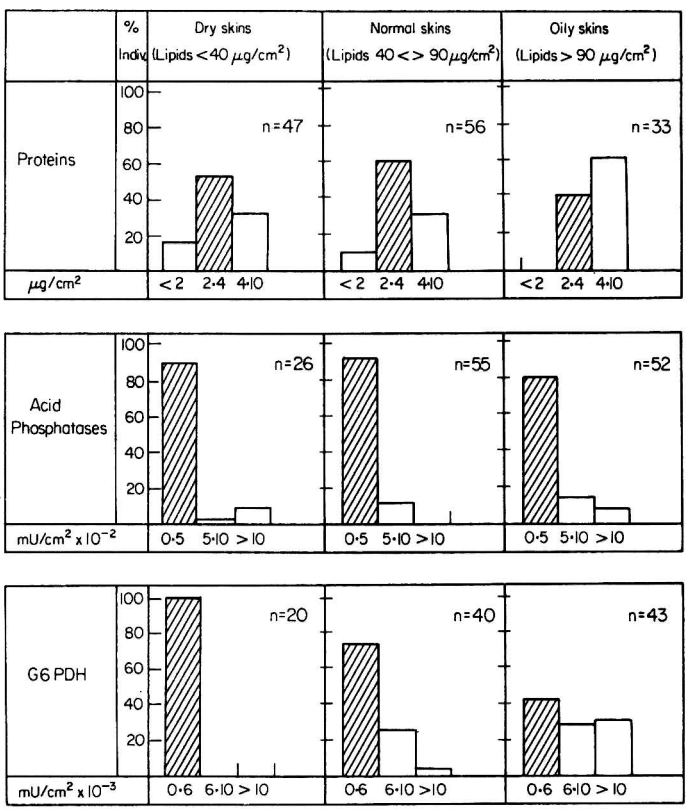

Figure 6. Biochemical variations on strippings depending on the degree of oiliness of the skin (forehead samplings) (Individual distribution plotted against measured values).

It must be emphasized here that this method of determination makes it possible to give a valuable, objective clinical assessment of the skin. The lipid values which make it possible to define the three classes of skin were established after examining more than three hundred people with skins showing varying degrees of oiliness. In addition, these values are close to those found by others using the same method, Agache for example (22).

This determination may be completed by a measurement of the 'sebum excretion rate' (S.E.R.) performed by taking a sample 30 min. after the last stripping. Our studies showed a correlation between the variations in epicutaneous lipids and S.E.R. in 80% of cases.

Examination of *Fig. 6* does not show immediately any significant variation in the acid phosphatases as a function of skin type. On the other hand, values for proteins and G6PDH, which were higher the greater the degree of oiliness, were detected in a certain number of people.

It would seem, in addition, that individuals with oily or seborrheic skins must be considered in two groups: those who simply show increased level of lipids (shaded part) and those where the excess of sebum is associated with greater than normal desquamation, characterized by the level of proteins, and a greater metabolic activity in the deep epidermal layers, characterized by the G6PDH measurement.

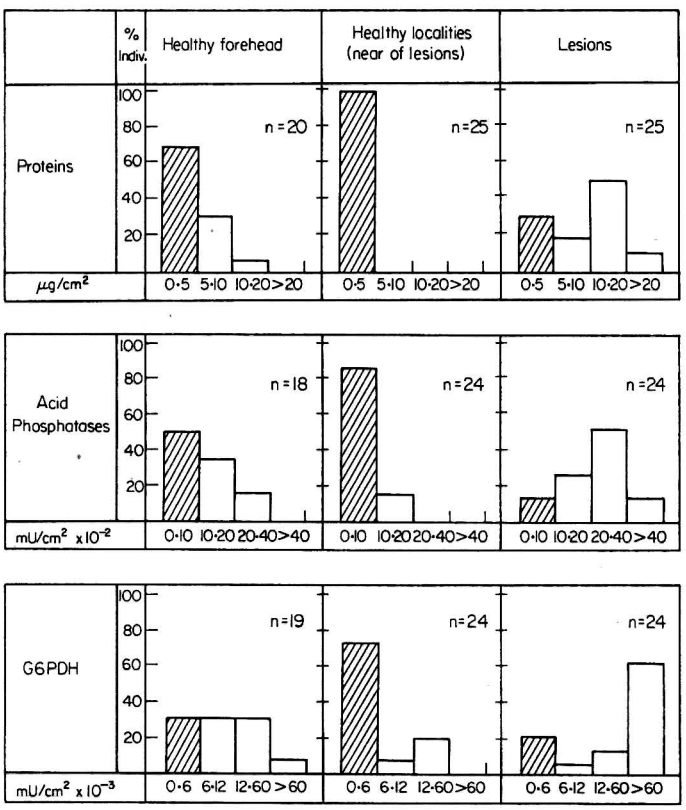

Figure 7. Biochemical activities in people affected by dermatoses (individual distribution plotted against measured values). Shaded part values from healthy persons.

Variation of the biochemical activities in people affected by dermatoses

Fig. 7 shows the biological variations observed in the upper layers of the stratum corneum in people affected by dermatoses of the keratosis type. This figure shows that with lesions, values for the three parameters studied were clearly higher than for healthy skin in the same anatomical localities. This result was expected. Nevertheless, it shows that the biochemical study using strippings of skin gives identical results to those found from biopsies by histochemistry or biochemical studies (14) and confirms the work of Shaefer and Zesch (15) on the biochemistry of the stratum corneum of people suffering from psoriasis.

This figure also shows that, in a number of the people affected by dermatoses, abnormally high values for proteins, acid phosphatases and G6PDH were observed on their healthy foreheads. Such observations suggest that either there is a change in cutaneous metabolism even in apparently healthy areas, or there are structural modifications in the stratum corneum which cause, in particular, a greater extra-cellular enzymatic flux.

In the case of this second theory, there would perhaps be good reason to be careful in the use of certain cosmetic products on such subjects.

Study of the effect of substances applied to the skin

Using the method of stripping, we have studied the action of different substances and cosmetic formulations. *Fig. 8* shows, by way of example, the effect of *Cetavlon®* (cetyl trimethyl ammonium bromide) on the skin.

The product was applied 5 days a week in quantities of 0·1–0·2 ml/cm^{-2} of a 5% wv^{-1} solution. After 15 min. contact, the application areas (100 cm^2 on the backs of pigs and humans, 15 cm^2 in the rat) were appropriately rinsed with lukewarm water.

The figure shows the biochemical variations observed, as a function of time, on the treated areas compared with the control areas.

First of all, this study revealed the completely different behaviour of the skin of the rat compared with human skin, whereas for the three parameters studied, there was quite a remarkable similarity in behaviour in the skin of the pig and of humans. From this figure it can be seen that we did not carry on the experiment in the rat for more than 10 days; the oedematous and in some cases severely cracked condition of the skin of the animals alone provided evidence of dermatoxicity without the need for supplementary analyses.

On the other hand, in humans and pigs, where only drying of the skin, in some cases hardly detectable, was observed clinically, the stripping method makes it possible to assess the variations in desquamation of the skin by measuring the level of proteins. It can be seen that under the influence of *Cetavlon*, the amount of proteins recovered on strippings was up to 3 times greater than the amount recovered from the control areas after 15 days of treatment.

A two-phase development of activity was observed for the two enzymes which are shown in the figure (βD glucuronidase and G6PDH). This variation is not accidental since it is also found here for glucuronidases and the G6PDH. It can also be observed for other enzymatic activity. This phenomenon was also found when sodium lauryl sulphate was used in the place of *Cetavlon* (23).

In spite of the physico-chemical action of detergents, a biological effect corresponding to an acceleration of cutaneous metabolism in the deep epidermal layers could be detected after the first application, as may be demonstrated histologically (areas of

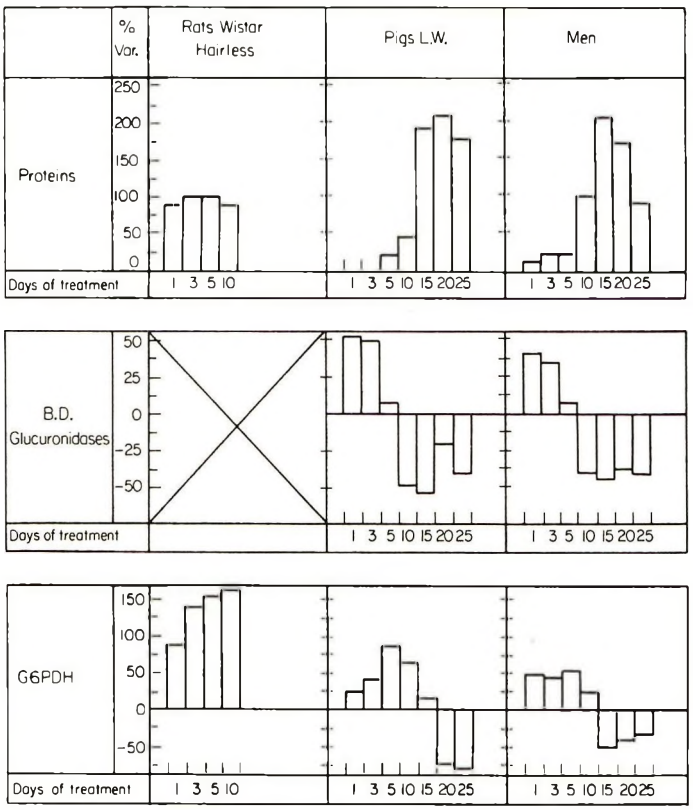

Figure 8. Effect of Cetavlon [R] on biochemical activities of stratum corneum. % variations plotted against the number of applications.

acanthosis, significant numbers of mitoses). In addition, one may suppose that the responses recorded in the first parts of the histogram would be lower than those actually produced, since what is being determined is the resulting effect of an increase induced by, and a decrease caused by, the physico-chemical action.

This physico-chemical action appears all the more clearly since beyond the fifth application there is the phenomenon of homeostasis which limits the biological response, and this explains the lower values recorded in the second part of the histograms.

We have chosen to cite this example, since the effects of *Cetavlon* and of Na lauryl sulphate have been very thoroughly studied (24, 25) and, as in the case of psoriasis previously mentioned, the results obtained by biochemical analysis of the surface corroborate those results from cutaneous biopsies.

CONCLUSION

These results merit further elaboration. We simply wanted to show, by these examples, that in different fields of research, the method proposed makes it possible to understand better the healthy human skin, its reactivity towards different substances administered, in particular on topical application, and the biochemical modifications inherent in different states, whether pathological or not. The results are in argeement with those

found at cutaneous biopsies. We therefore have available a simple and practical means of investigation.

In the context of this paper, the number of parameters studied has been limited intentionally. It is obvious that numerous other determinations could be carried out using samples obtained by stripping.

Using this method it would be easy to determine free amino acids, uronic acids, various enzymatic activities such as catalases and various dehydrogenases. Also, in this study we have only considered samples on the first five strippings, but it is also possible, and perhaps more valuable, to carry out determinations on deeper samples.

In conclusion, biochemical measures of this type may prove to be valuable supplements to the selection of methods at present available, by satisfying one of the major preoccupations of cosmetologists, which is to know the skin better in order to best ensure its hygiene, protection and beauty.

Acknowledgments

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A new approach to the theory of adsorption and permeability of surfactants on keratinic proteins: the specific behaviour of certain hydrophobic chains

Presented in June 1976 at the IFSCC IXth Congress, Boston, U.S.A.

J. GARCÍA DOMÍNGUEZ,* J. L. PARRA,* Mª R. INFANTE,* CARLOS M. PELEJERO,† FRANCISCO BALAGUER† *and* T. SASTRE† **Patronato 'Juan de la Cierva' Barcelona, Spain,*
†R. & D. Laboratory, Antonio Puig, S.A. Barcelona, Spain

Synopsis

The sequence: **detergency-adsorption-interaction-denaturation-permeability-irritation,** caused by ionic **surfactants** on keratinic **proteins** such as **stratum corneum** has been studied. Adsorption and permeability of the protein or lipoprotein structures have been investigated, and a five step mechanism is proposed to explain the way in which ionic surfactants can be transferred from the interface protein-water to the inner cells of the dermis, resulting in irritation.

Two new theoretical approaches are proposed to justify the odd behaviour of the C_{12} anionic surfactants: solubility on hydrophobic sites, or a molecular conformation of the normal linear structure of the surfactant molecules in a more stable form, under conditions which give a minimum size for C_{12} surfactants.

Introduction

The irritation phenomena on human skin have been intensively studied in the last few years by many authors (1–4). Irritation by itself is a complex process involving many factors which have been studied qualitatively and individually, but the specific importance of each of them on the whole phenomenon have not yet been well defined.

It has been well established that, both *in vitro* and *in vivo*, several ionic surfactants in aqueous solutions are able to increase cutaneous permeability. The ionic surfactants (R—COO—Na, R—OSO_3—Na, R—SO_3—Na, R—NH_3—Cl) having an alkyl chain of C_{12}–C_{14} are the ones showing higher values for permeability, denaturation, adsorption, etc., which finally result in a greater tendency to produce irritation on the human skin.

Before any anionic surfactant interacts with the protein or with other lipidic components of the human skin, the surfactant must pass through the interface water/stratum corneum. This penetration process is governed by the physicochemical laws of any solid/liquid interface and by the equilibrium micelle→individual molecules in the solution. Once this barrier is crossed by the surfactant, adsorption and real interaction between the surfactant and the proteinic support (and other components) occurs.

In our work we have considered: (a) The adsorption process of surfactants on both stratum corneum and a keratinic protein such as wool fibres, while considering the types of links involved, and the influence of the chain length of the C_8–C_{16} alkyl sulphates. (b) The interaction subsequent to adsorption which should result in a modification of the reactivity of the native protein. (c) Migration of surfactant molecules through the mass of the proteinic structure and especially the 'speed of specific migration', i.e. the ability of a molecule of surfactant to migrate through the support, compared with other members, belonging to the same family under the conditions of the treatment, which can play an important role on the biochemical effect referred to as 'irritation'. We can thus say according to the last assumption that 'irritation caused by surfactants depends on their migration and their characteristics'. In this paper a feasible mechanism to explain the migration of ionic surfactants on biological tissues containing proteins such as human skin, is proposed.

The higher rate of migration of certain surfactants (those having C_{12} in their hydrophobic chain) can be explained if a special conformation of the aliphatic chain takes place. In the discussion a theoretical approach to such a possibility is indicated.

This specific behaviour of C_{12} chains on adsorption (5), extraction of proteins (6), migration (7), conductivity (8), etc. was checked by studying the modification in the response of the cystine present in the proteinic structure of human hair to reductive cleavage with thioglycolic acid, in the presence of (C_6–C_{18}) alkylsulphates. The influence of the position of the —O—SO_3 group in the alkyl-sulphate chain, on the amount of protein extracted from human callus has also been investigated.

Experimental

MATERIALS

Human hair. Caucasian human hair was used, previously purified by Soxhlet extraction with ether and ethanol and finally, washed with distilled water and air dried.

Cuticle cells. The cuticle cells were isolated from human hair by using the Kassenbeck p-Toluene-sulphonic acid/ethyleneglycol method (9).

Human callus. Human callus obtained from a chiropodist was powdered and lipids eliminated by extraction with a mixture of chloroform : methanol 2 : 1 (v/v). (10). Finally, it was sieved at 200 mesh.

Wool fibres. Australian Merino 64's wool was used, extracted with diethyl ether, rinsed in distilled water and dried at room temperature.

Surfactants. Sodium alkyl sulphates in the range (C_6–C_{18}) were synthesized in our laboratories from the pure fatty alcohols by treatment with chlorosulphonic acid and final neutralization with sodium hydroxide (11).

Sodium alkyl sulphates with carbon numbers of C_8, C_{10} and C_{12} and having the sulphate group in position 2 and 5 respectively, were prepared in a similar way.

Thioglycolic acid. The thioglycolic acid used as a reducing agent was of reagent grade.

TREATMENTS

Treatments of human hair with thioglycolic acid solutions. Human hair (1 g) was wetted out with distilled water. The reaction of human hair fibres with thioglycolic acid was

carried out in a flask containing a 5% solution of the reductive agent. The pH was adjusted to the desired value for each treatment. When reduction of cystine disulphide bonds present in the keratin was performed with thioglycolic acid solutions in the presence of surfactants, 2% of the surface active agent was added to the reaction flask. The pH of the bath was maintained constant during the treatment by the addition of diluted NaOH or HCl and controlled by an Autotitrator (Radiometer TTT-11). The reaction bath was mechanically shaken (20 rpm) and temperature maintained at 37°C ±0·1°C during 60 min. The liquor : human hair ratio was 100 : 1. After treatment, the fibres were filtered, washed with distilled water (200 ml) and dried under a vacuum, over P_2O_5.

Treatments of cuticle cells isolated from human hair with thioglycolic acid solutions. For cuticle cells, a treatment procedure identical with the above was used, except that after treatment, concentrated sulphuric acid was added to the bath in such quantity as to give a 6N solution. No filtration was carried out before hydrolysis although some insoluble materials were present. Hydrolysis was carried out in a nitrogen atmosphere. The estimation of the cysteine residues formed during the treatment, due to the reduction of cystine disulphide bonds by the thioglycolic acid solution, was made using the Ellman method (12).

Treatments of human callus with anionic surfactants. Human callus was treated with a 0·015 M surfactant solution at 40°C for 60 min at pH 3·5. The reaction vessel was submitted to constant agitation at 500 rpm. After treatment the human callus was filtered and washed with distilled water at 40°C to remove all excess of surfactants and the washing liquids were added to the original treatment bath. The protein liquor ratio used was 1 : 300.

The amount of surfactant adsorbed on the human callus was analysed in the spent liquor by difference using the two phase titration method (13).

The content of aminoacids in the proteinic material extracted during the treatment was estimated with an aminoacid autoanalyzer (14).

Treatments of virgin wool fibres with anionic surfactants. Wool was treated with 0·01 N solutions of different sodium alkylsulphates at 40°C and pH 2,2 for 60 min using a similar procedure as described for human callus.

In the case of sodium dodecylsulphate, additional treatments were performed to study the ionic and/or hydrophobic interaction of the surfactant molecules on the wool fibres. The treatments were carried out in water and water-ethanol (1 : 1 v/v) solutions, at pH 2·2 and 50°C for different periods of time.

Results

THE INFLUENCE OF DIFFERENT ALKYLSULPHATES ON THE REACTIVITY OF CYSTINE IN HUMAN HAIR TO REDUCTIVE CLEAVAGE

Human hair and its cuticular fraction were treated with 5% thioglycolic acid solution at 37°C (±1°C) for 60 min at the pH range of 2·5–11·8. In *Fig. 1*, the amount of cysteine found in each case has been plotted against the pH of the treatment solution.

The results clearly show the presence of two maxima values of reduction at pH values of 3·25 and 4·40. The reduction curve up to pH 6 increases progressively with the pH of the treatment; after pH 11·5 solubilization of the human hair due to the combined effect of cystine cleavage plus the peptide hydrolysis will occur.

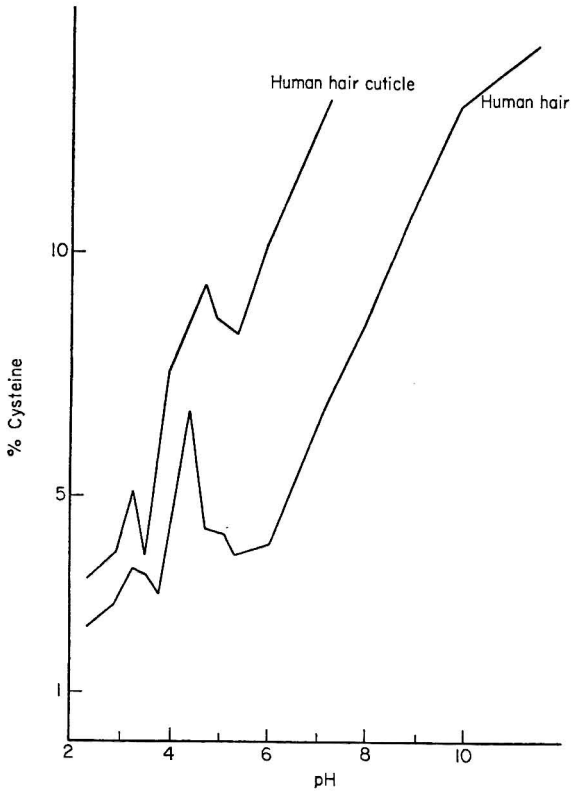

Figure 1. Reduction curve of human hair and its cuticular fraction with a 5% solution of thioglycolic acid.

When cuticle cells were isolated from human hair and submitted to a process identical to the above described, a curve showing a similar trend was obtained (see *Fig. 1*). It is interesting to note that the first maximum of the reduction occurs at pH 3·25 for both proteins, whilst the second shows a maximum for the cuticle at pH 4·75 as compared with pH 4·40 for human hair. Cystine present in cuticle, reacts in a greater amount at every pH value comparatively with human hair fibres.

In the literature, no indication of the presence of these two maxima values for reduction has been described as the treatment with thioglycolic acid versus pH has always been done in the presence of buffers (15). In our case, the pH was maintained constant during the treatment, without adding any buffer. We believe that as the presence of buffers modifies the behaviour of the ionic links present in the protein, especially those surrounding the cystine residues, the accessibility of thioglycolate ions to the disulphide bonds of cystine is altered. In fact, the influence of phthalate or borate ions during the treatment of human hair can play an important role on the stability of the ionic links. According to Kauzmann (16), the electrolytes tend to strengthen the hydrophobic bonds whereas they weaken salt linkages (by producing a stabilizing Debye Hückel atmosphere around the charged groups when they are dissociated). If so, in the pH range 3·80–4·20 i.e., within the isoelectric region of human hair, the presence of buffers reduces the strength of the ionic links, making the cystine residues more accessible to the thioglycolate ions. When no buffers are used to maintain a constant pH, these ionic links will

show a greater stability and the reduction at such pH values will be lowered. This area of least reduction (pH 3·80–4·20) corresponds to the minimum region observed between the two maxima in *Fig. 1.*

Another possible explanation could be that there may be different reactivities at the two pH values, with the hydrophobic or hydrophilic group surrounding the cystine residues incorporated into the protein. If so, the presence of ionic surfactants would show a great modification in the two maxima of reduction, i.e. the one belonging to the cystine located in a hydrophobic area.

To check whether the second assumption is correct, and to know to what extent surfactants modify the reactivity of cystine present on the human hair, the treatment with thioglycolic acid at 5% was performed in the presence of sodium laurylsulphate (2%). In *Fig. 2* the amount of cysteine formed is plotted against the pH of the treatment bath. It can be seen that the amount of cystine reacting at pH 3·25 was 13·1% whilst in the absence of surfactant only 3·55% of cysteine was formed. Regarding the second maximum (pH 4·40) we can see that it nearly disappears; the slope of the curve in the pH range 6–11·5 is nearly the same.

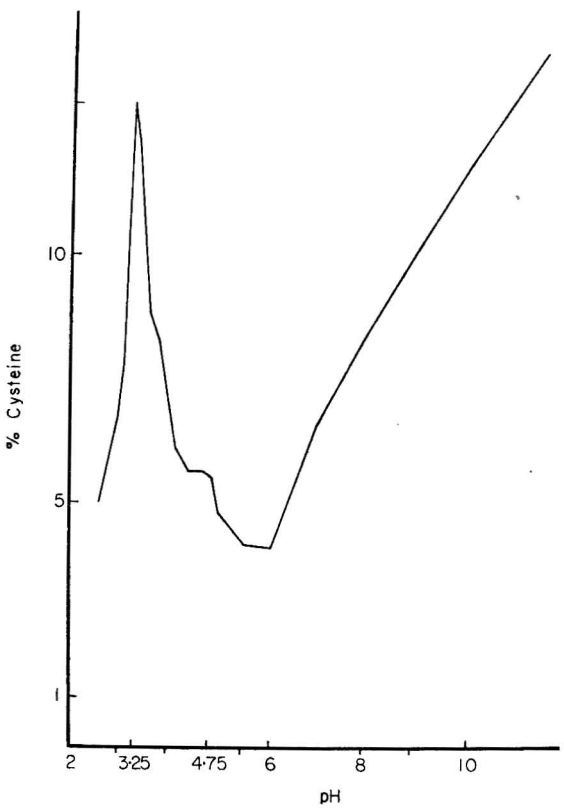

Figure 2. Reduction curve of human hair treated with a 5% solution of thioglycolic acid, in the presence of a 2% solution of sodium lauryl sulphate.

At this point of the investigation we were interested in knowing whether the hydrophobic or the hydrophilic part of the lauryl sulphate molecule was responsible for such behaviour. If the sulphate ion of the surfactant was the effective part of the

molecule, the same amount of cysteine would be obtained in the presence of any alkylsulphate independently of its hydrophobic chain. On the contrary, if the hydrophobic part of the surfactant were responsible for the phenomena and the influence related to the length of the aliphatic chain, a certain relationship between the chain-length and the amount of cysteine formed would occur. In *Fig. 3* we have plotted the amount of cysteine formed during the treatment of thioglycolic acid at pH 3·25 in the presence of different alkylsulphates as a function of the number of carbon atoms. It can be seen that the amount of cysteine is not directly related to the length of the aliphatic chain of the alkylsulphate; on the contrary, there are certain chain lengths for which the amount of cysteine formed is greater: C_6, C_{12} and C_{18}. The influence of the C_{12} chain is specially significant whereas the C_{16} chain has little effect on the original reactivity of the thioglycolic acid. It is notable that C_6, C_{12} and C_{18} are multiple values of C_6. In the last part of the paper some theoretical approaches to justify these findings are suggested.

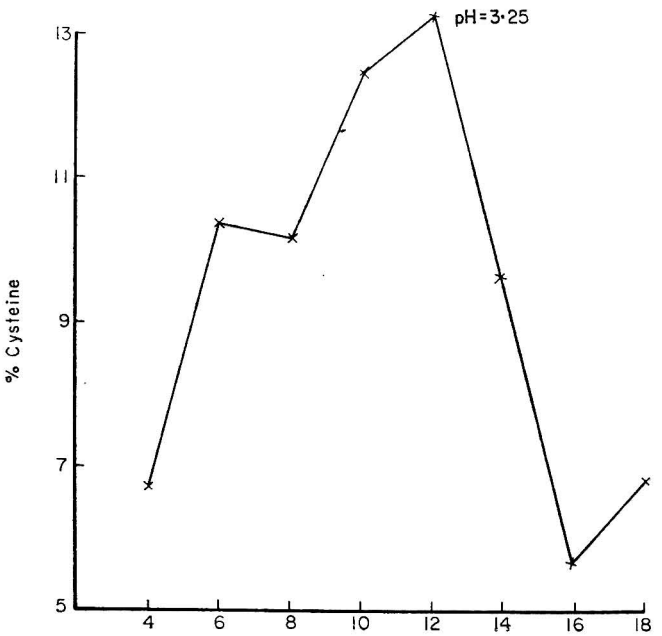

Figure 3. Influence of the number of carbons of the alkyl sulphate chain length on the reduction of human hair with thioglycollic acid at pH 3·25.

Nevertheless, a deeper study of the adsorption-interaction mechanism is necessary to understand the odd performance of the C_{12} alkylsulphates.

ADSORPTION OF DIFFERENT ALKYLSULPHATES ON HUMAN CALLUS AND WOOL FIBRES AS A FUNCTION OF THEIR CHAIN-LENGTH.

In *Fig. 4* the amount of different alkylsulphates adsorbed on human callus against the number of carbons of their hydrophobic chain length has been plotted. The results show that when each separate alkylsulphate is treated with the protein, the adsorption is a maximum for the C_{12} derivative under the same conditions. These results agree with the findings of other workers (5, 17, 18).

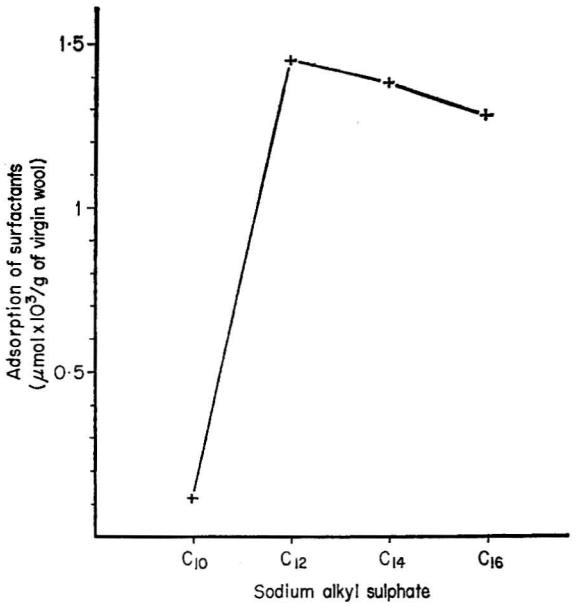

Figure 4. Human callus treated with a 0·015 M solution of different alkyl sulphates at pH 3·50, at 40°C, for 60 min.

Regarding the behaviour of wool fibres in relation to the adsorption of different alkylsulphates by this keratinic protein, wool fibres were treated at pH 2·2 at 40°C for 60 min. The amount of alkylsulphate adsorbed plotted against the number of carbons in the alkylsulphate is illustrated in *Fig. 5*.

It can be seen that sodium lauryl sulphate is adsorbed in greater amount than other members of the series.

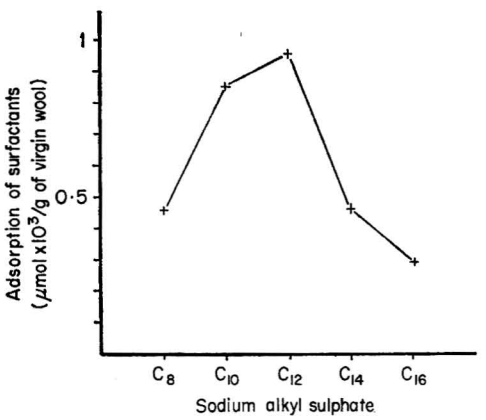

Figure 5. Virgin wool treated with sodium alkyl sulphates 0·01 M, pH = 2·2, 40°C, 60 min.

EXTRACTION OF PROTEINS FROM HUMAN CALLUS AS A FUNCTION OF THE CHAIN LENGTH OF DIFFERENT ALKYLSULPHATES.

Human callus free from lipids was treated with different alkylsulphates in the range C_{10}–C_{16} at pH 3·5 at 40°C for 60 min and the amount of protein extracted in each case estimated.

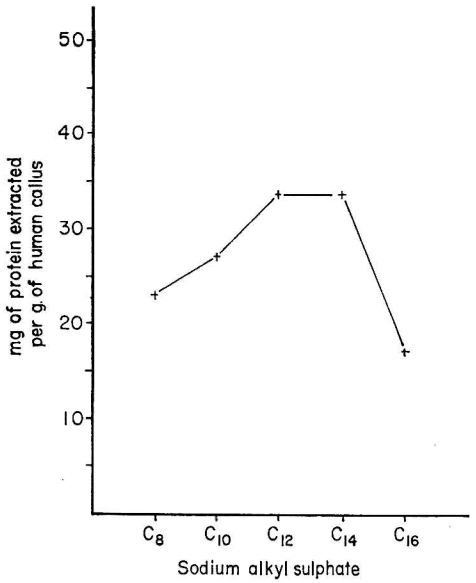

Figure 6. Human callus treated with a 0·015 M solution of different alkyl sulphates of pH 3·50, at 40°C for 60 min.

The results are given in *Fig. 6*. The C_{12} and C_{14} behave as very active protein extractors as compared with the remaining members of the series.

INFLUENCE OF THE POSITION OF THE $-OSO_3^-$ GROUP OF AN ALKYLSULPHATE ON THE EXTRACTION OF PROTEINS FROM HUMAN CALLUS.

Human callus free from lipids was treated at pH 3·5 at 40°C for 60 min with Sodium 1- or 2-Octyl-sulphate, Sodium 1- or 5-Decyl-sulphate, Sodium 1-Dodecyl-sulphate and Sodium 2-Dodecyl-sulphate.

The amount of protein extracted from the callus due to the surfactant interaction and the content in aminoacids on the protein extracted is given in Table 1. It can be seen that for C_8 and C_{10} the total amount of protein extracted differs significantly when the sulphate group is in position 1 or in positions 2 or 5.

The presence of the sulphate group in a position different from 1 can affect the conformation of the hydrophobic chain especially for position 5 (see discussion). In the case of laurylsulphate, the presence of the sulphate group in position 2 should not greatly modify the conformation of such a long hydrophobic chain and this accounts for the fact that the amounts of protein extracted are so similar (Table 1).

Tentative explanation of the theory of adsorption and permeability of surfactants on proteinic supports: Specific behaviour of certain hydrophobic chains. Many papers describe the specific behaviour of the C_{12} chain but no theoretical explanation of the phenomenon has been proposed, to our knowledge.

We believe that the adsorption and the interaction protein-ionic surfactant is firstly regulated by the solid/liquid interface before any surfactant molecule is bound to the protein. We propose the following tentative mechanism to explain the penetration (migration) of surfactants on protein supports:

Table 1. Aminoacid composition (μmol/l) of the protein extracted from human callus treated with alkylsulphates (C_8–C_{12}) having the sulphate group located in positions 1, 2 or 5.

Aminoacids	C_8		C_{10}		C_{12}	
	1–C_8	2–C_8	1–C_{10}	5–C_{10}	1–C_{12}	2–C_{12}
Aspartic acid	10	8	49	10	78	80
Threonine	22	21	30	28	12	29
Serine	60	48	123	110	167	92
Glutamic acid	11	8	105	66	72	107
Proline	7	5	13	6	21	16
Glycine	90	71	124	94	184	192
Alanine	35	30	49	35	69	65
Cystine	—	—	—	—	—	—
Valine	19	17	24	20	20	19
Methionine	3	2	8	4	4	7
Isoleucine	11	10	16	12	9	15
Leucine	18	15	32	20	24	63
Tyrosine	11	6	15	13	20	15
Phenylalanine	10	6	15	11	13	25
Lysine	70	64	73	65	100	62
Histidine	13	7	17	15	30	11
Arginine	3	4	8	5	10	42
Σ Aminoacids	393	322	701	514	863	840

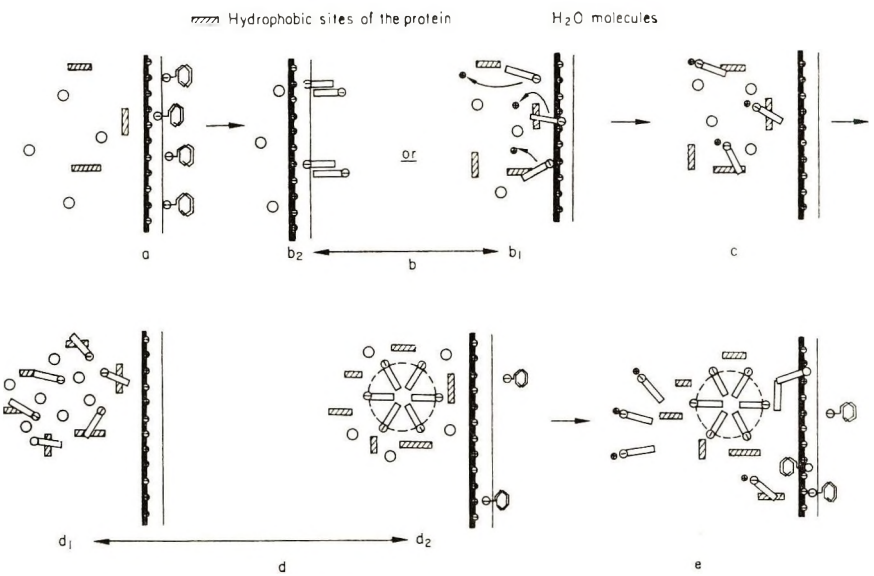

Figure 7. Feasible mechanisms of the migration process of surfactants through proteinic supports.

(a) Ionic attraction, (b) Hydrophobic interaction. b.1. surfactant-surfactant b.2 protein-surfactant, (c) Ionic exchange, (d) Formation of hydrophobic aggregation d.1. protein-surfactant d.2. surfactant-surfactant, (e) Migration of the hydrophobi aggregation.

In *Fig. 7* a possible schematic mechanism of the above stages is given.

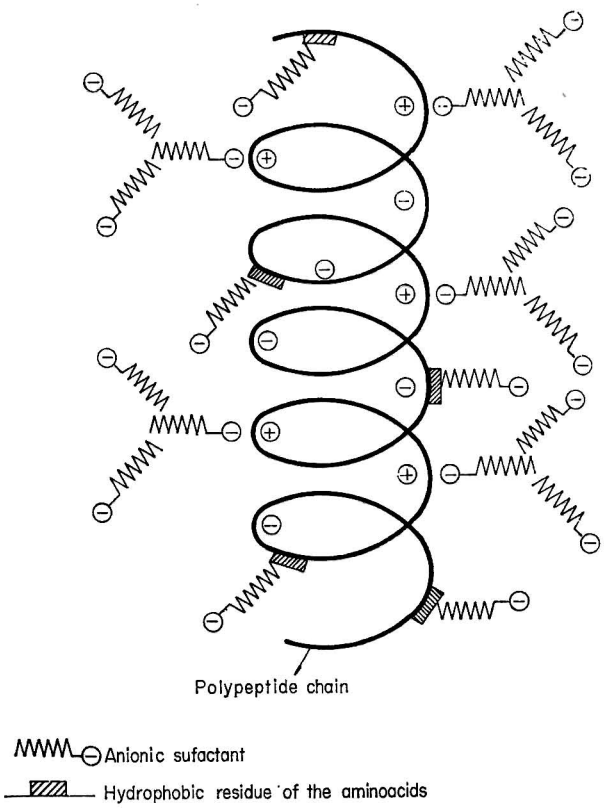

Figure 8.

The anionic surfactant will be linked to the NH_3^+ of the residual aminoacids of the protein (*see Fig. 8*) and the cationic surfactants will be linked to the corresponding COO^-. The number of molecules adsorbed will mainly depend on the pH of the bath. The ionic linkage is very important in the study of protein-surfactants interaction, due to the amphiphilic properties of the proteins.

In the aqueous treatment of proteins with anionic surfactants we must take into account not only the ionic links but also the hydrophobic interaction. If in the treatment, a mixture of aqueous alidue is used, the hydrophobic interaction does not take place. In *Fig. 9* the number of surfactant molecules linked to the protein via ionic (alcohol-water) or ionic plus hydrophobic bonds (water only) is illustrated (19).

Nevertheless, if we are using a hydroalcoholic medium less surfactant is adsorbed, due to the inhibition of the hydrophobic interaction.

The interaction protein-surfactant in an aqueous medium modifies the isoelectric pH value of the protein so that a soluble fraction of the protein will be found in the treatment bath.

(a) *Ionic attraction.* In aqueous solution, surfactants form micelles of spherical shape allowing the hydrophobic chain to orientate away from the water. When a micelle gets near a protein surface, it will undergo a deformation and the monomeric molecules get ionically attached to the ionic residues of the protein (*Fig. 10*), leaving some surfactant molecules in the aqueous medium.

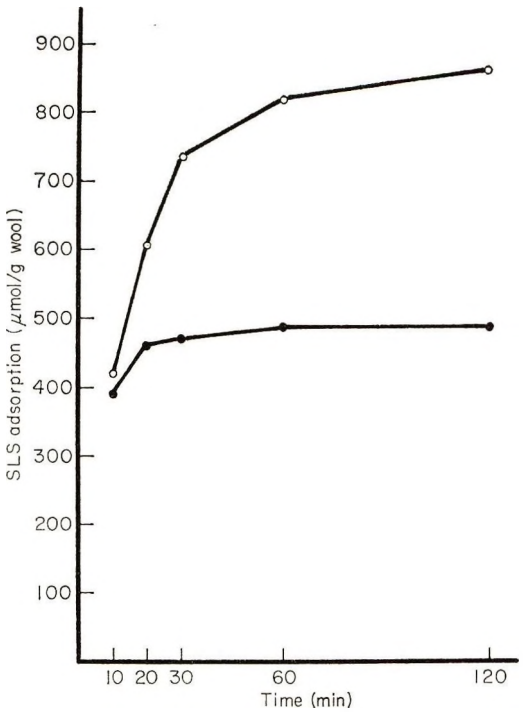

Figure 9. Adsorption of SLS solutions (pH 2·2), via ionic or hydrophobic link on wool fibres at 50°C, as a function of the time of treatment. ○ water; ● water-ethanol 1 : 1 (v/v).

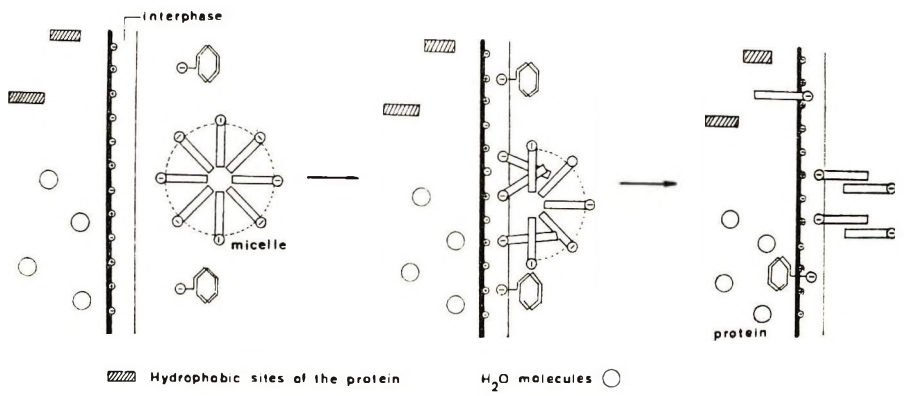

Figure 10. Feasible schematic mechanism of the interaction. Surfactant-Proteinic support (Stage a).

(b) *Hydrophobic interaction.* Once the monomolecular layer of surfactant is attached to the protein, new molecules can be attracted to the previous ones via hydrophobic interaction forming a second layer. The hydrophobic residues of the protein also allow the attachment of the surfactant molecules via the hydrophobic linkage. In other words, there are two possible ways of hydrophobic attachment: surfactant-surfactant and/or protein-surfactant (*see Fig. 7*).

(c) *Ionic exchange.* Proteins can be considered as amphoteric polyamides behaving like ionic exchangers. Below the isoelectric point of the protein, the cationic residues of the aminoacids can accept the anion of the alkyl-sulphate.

(d) *Formation of hydrophobic agregations.* The hydrophobic residues of the surfactants already fixed ionically to the α-helix of the protein can be the nucleous of attraction of new surfactant molecules forming what we can call 'pseudomicelles' (*Fig. 7, stage d*). Also, the hydrophobic sties of the protein can attract the hydrophobic residues of the surfactant. If so, it would be possible for the sulphate group of the surfactant to be attracted ionically in another site via the above ionic exchange mechanism. This is a possible explanation of the migration of the surfactant molecules into the protein.

(e) *Migration of the hydrophobic aggregation.* If the concentration of sodium alkylsulphate in the interface is great enough, the pseudomicelles can migrate into the inner part of the protein by the combined processes described. It must be taken into account that each ionic exchange process promotes changes in the internal pH of the morphological structure of the protein. The alkalinity produced is neutralized through a physiological mechanism; when this is not really possible, a certain irritation appears.

The answer of the living cells to the irritation promoted by the presence of the surfactant molecules can be either, to throw them back outside the dermis to the stratum corneum or to degrade them biologically into the skin structure.

Discussion

The relationship of the hydrophobic chain length of an alkylsulphate to its reactivity *in vitro* or *in vivo* results in the conclusion that alkylsulphates having a C_{12} chain always behave as the most effective agents for such purposes as adsorption, denaturation, epidermal electrical conductance, modification on the reactivity of a protein, solubilization of proteins, etc., among the series C_8–C_{16}.

The degree of hydrophobicity of ionic surfactants obviously increases with the number of carbon atoms in each series. On the contrary, solubility in aqueous solution follows an inverse tendency, whereas the solubility in fatty components is proportional to the degree of hydrophobicity. Adsorption and migration of surfactants greatly depend on the chemical composition of the support. In the case of human skin, the first layer interacting with the surfactant is the stratum corneum. This fraction is formed by keratinic proteins imbibed by those lipophilic compounds resulting from keratinization. The lipid fraction contains: hydrocarbons, cholesterol esters, waxes, mono, di, and tryglycerides, fatty acids, cholesterol, phospholipids, lipoproteins, etc. The degree of humidity at the stratum corneum level is regulated by the presence of urea, pyrrolidine carboxylic acid, aminoacids, mineral salts, lactates and water. Essentially, it is formed by fatty and proteinic components.

When an anionic surfactant is dissolved in water at a concentration below the C.M.C. the surfactant molecules tend to be adsorbed on the liquid/air, and human stratum corneum/water, interfaces.

The molecules adsorbed in the liquid/air interface are oriented in such a way that the ionic group of the surfactant remains in the aqueous phase, repelling the hydrophobic group outwards from the aqueous phase. Regarding the interface, stratum corneum/water, the ionic surfactant molecules are also oriented leaving the ionic group in the aqueous phase, whilst the hydrophobic residue is solubilized in the fatty components of

the stratum corneum. Then, according to Wepierre (20) the percutaneous penetration is regulated by the partition stratum corneum/water. Probably this partition is more favourable for the stratum corneum in those structures having C_{12} in the hydrophobic chain for a particular ionic group i.e. $—COO^-$, $—OSO_3^-$, $—SO_3^-$, etc. If so, this may be the reason for the odd behaviour of C_{12} anionic surfactant. We believe that this may not be the only reason, because when the solid phase contains no fatty components, as in the case of human hair washed with organic solvent, wool fibres, or the pure proteinic fractions of human skin (as callus cells to which lipids have been eliminated) we have found, as well as other authors (4, 5), that the adsorption of surfactant and extraction of proteins is also a maximum for the series of anionic surfactants having a C_{12} carbon chain. In this case, the partition coefficient cannot be the only answer to such a specific behaviour. In addition to this, we have shown in this paper (*Fig. 3*) that when a fatty free human hair is treated with a reducing reagent such as thioglycolic acid at pH 3·25 in the presence of a series of alkylsulphates in the range C_6–C_{18}, a maximum cystine reductive cleavage takes place when the surfactant contains a 12 carbon atoms hydrophobic chain.

This finding seems to indicate that other factors besides the partition coefficient, specific hydrophobic/hydrophilic balance, specific solubility, etc. should be taken into account to explain why the C_{12} anionic surfactant behaves in such a special and particular way.

Our work shows that there is another possible explanation for the behaviour of lauryl anionic surfactant from the point of view of adsorption and migration which in some ways would lead to consider this type of surfactant as a potential irritative agent. We believe that, when an anionic surfactant is dissolved in an aqueous medium below the C.M.C., the molecules as such, are first adsorbed in the interface liquid/air. Those molecules remaining in solution that are adsorbed in a fat free solid/liquid interface should adapt their structure in such a way that the area they expose to the aqueous media or to the water present in the solid support, contains the maximum of hydrophobic material in the minimum surface area. This tentative new approach to the understanding of the behaviour of anionic surfactants in aqueous media, leads to the assumption that the hydrophobic chain can never expose its stretched structure to the water phase. Ideally, the conformation in which the ratio volume/surface is a maximum is the spherical, as this is the conformation adopted when the micelles are formed. Nevertheless it appears reasonable to accept that the hydrophobic chain could adapt its structure to a coiled shape should this be possible (the α-helix conformation of proteins is an example of this). In the theoretical case that each of the turns of the coil is formed by six carbon atoms (like an open cyclohexane) we would obtain maximum volume/area ratios for those hydrophobic chains being multiples of six, i.e. C_6, C_{12}, C_{18}.

In *Figs. 11, 12 & 13*, the three theoretical possible conformations of C_{12} alkylsulphate can be observed.

This can only occur when the concentration of surfactant into the solid support or in the aqueous phase is below that C.M.C. because when this concentration is reached, the number of surfactant molecules in the medium is enough to allow the formation of micelles, via hydrophobic bonds, repelling water from the hydrophobic surroundings. Would it be possible for the first stage in the formation of micelles to start from the linkage of surfactant molecules already conformed in the coil structure above mentioned?

Regarding the particular behaviour of C_{12} alkylsulphates, several hypotheses could be postulated. Firstly, when a hydrocarbon residue is surrounded by water molecules, the

Figure 11. Conformation in zig-zag of a lauryl sulphate molecule.

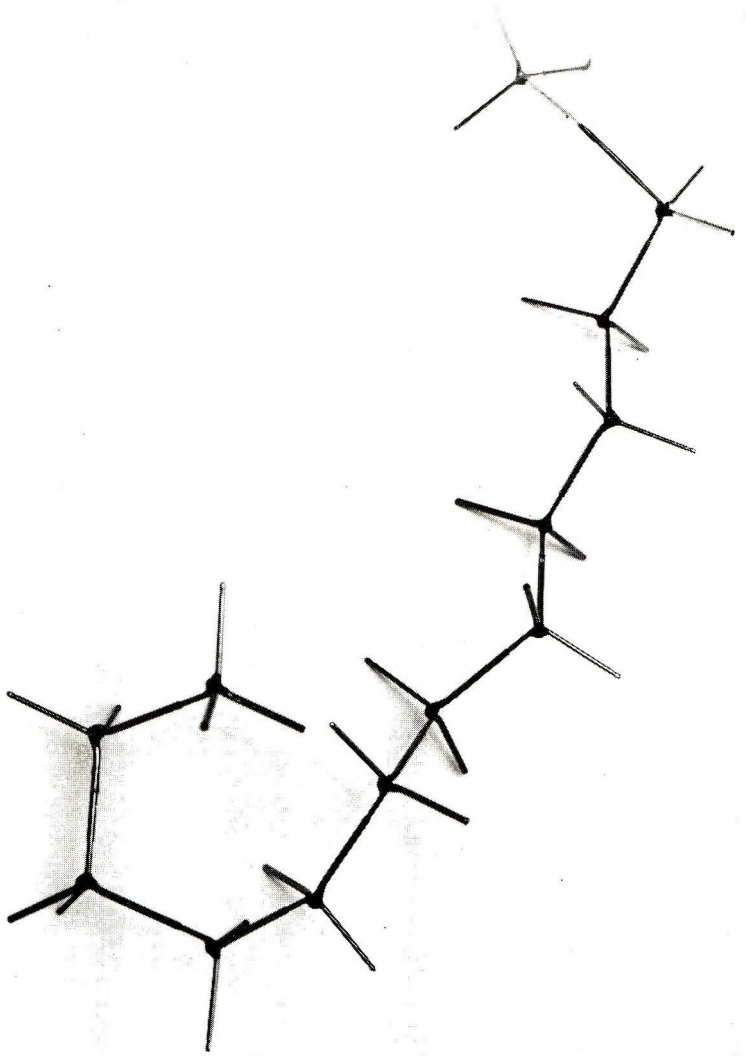

Figure 12. Conformation of a lauryl sulphate showing one open cyclohexane.

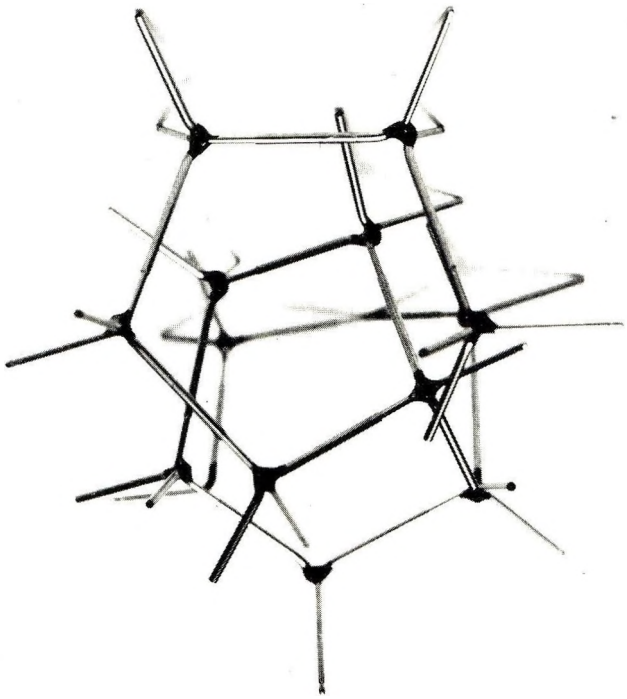

Figure 13. Structure of a lauryl sulphate with a double open cyclohexane conformation.

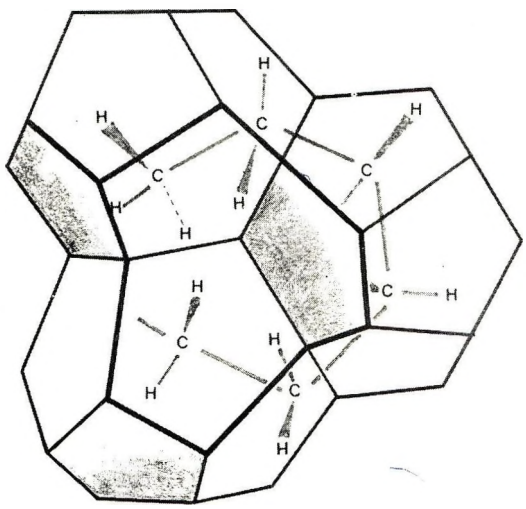

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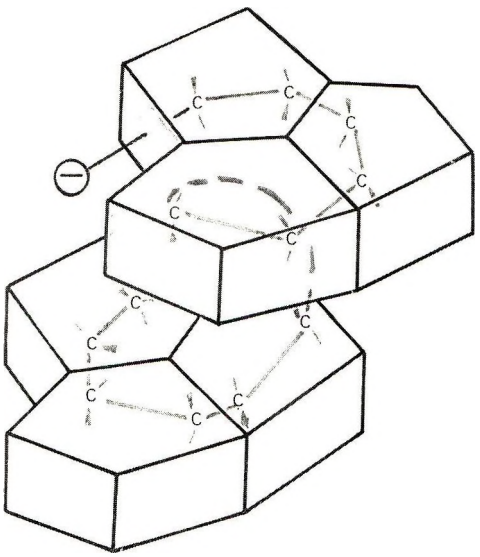
Figure 14. A non-polar chain inserted into a multiple water polyhedric clathrate in fixed conformation.



ure 15. A 12-carbon aliphatic chain inserted into two modulus of water polyhedric clathrates.

process brings about a large negative unitary entropy change which is intimately related to the unique structure of water itself. This great decrease in entropy shows that the water structure modifications should be considered as a very important factor. The earliest explanation originated as the 'iceberg' concept of Frank & Evans (21) in which water molecules in the vicinity of an apolar group become more ordered. An extension of this concept postulates 'Flickering clusters' in which by a cooperative effect, existing hydrogen bonds facilitate the formation of many more, resulting in a temporary cluster of water molecules in the vicinity (22).

According to Pauling, in the pentagonal dodecahedron structure for liquid water it is assumed that the apolar group is inserted inside the dodecahedron as a clathrate structure. The presence of the apolar molecule inside the water tends to stabilize it, making it more 'crystalline' (23, 24).

Obviously, the water molecules integrated in such structures should give a conformation of the hydrophobic chain of the surfactant in order to keep it into a definitive number of clathrate units (*Fig. 14*).

Can the C_{12} alkylsulphate hydrophobic chain orient its structure in such a way that the whole length occupies completely a certain number of clathrates? If so, the water molecules surrounding the C_{12} chain should be so highly ordered that the volume occupied is kept to a minimum. This hypothesis could be justified by the results commented given in the paper which give for C_{12} a maximum adsorption and migration.

If the energy that the system can bring is enough to orient the C_{12} hydrophobic chain into a certain open type of double coil shape (6 carbon atoms in each winding), then the hydrophobic chain would show to the water phase a specific surface to allow a definite number of clathrates to envelop the hydrocarbon chain. As in the previous hypothesis, this structure could justify the odd behaviour of C_{12} alkylsulphates (*Fig. 15*).

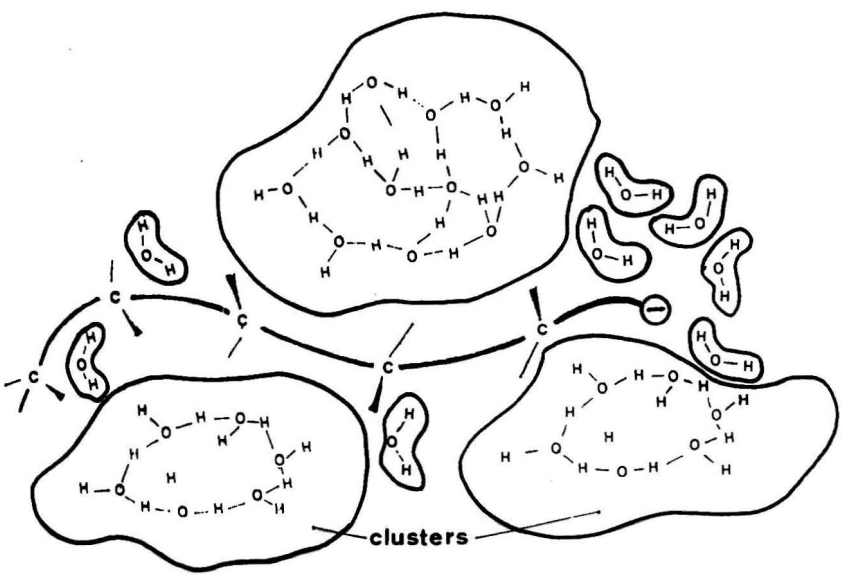


Figure 16. A non-polar chain of an alkylsulphate in conformation because of the surrounding water clusters.

Whatever hypothesis be the right one, we can say that the migration of surfactant molecules through the support is optimum when its hydrophobic chain has promoted a highly ordered water structure in its surroundings and its hydrophobic chain is organized in such a way as to allow such ordered disposition of water (*Fig. 16*). The mutual interaction hydrophobic chain-water and water-hydrophobic chain results in a molecule showing an optimum shape and size for the hydrophobic chain of 12 carbons.

In *Fig. 17* the length of the hydrophobic chain in Å for alkylsulphates against the number of carbon atoms is illustrated. We can see that if the hydrophobic chain of the surfactant does not conform (in other words, it remains linear) there is a direct relationship between the length and the number of carbon atoms. In our opinion, this means that if the molecule is in stretched form inside the protein, we cannot explain the specific behaviour of the C_{12} alkylsulphate. If we assume that the first conformation occurs in a 6 carbon open ring and that for the successive alkylsulphates the chain remains linear, then no specific size for any of them can be found. However, if for the C_{12} chain we consider that the second open-cyclohexane structure is formed, a minimum length for the C_{12} alkyl sulfate is observed.

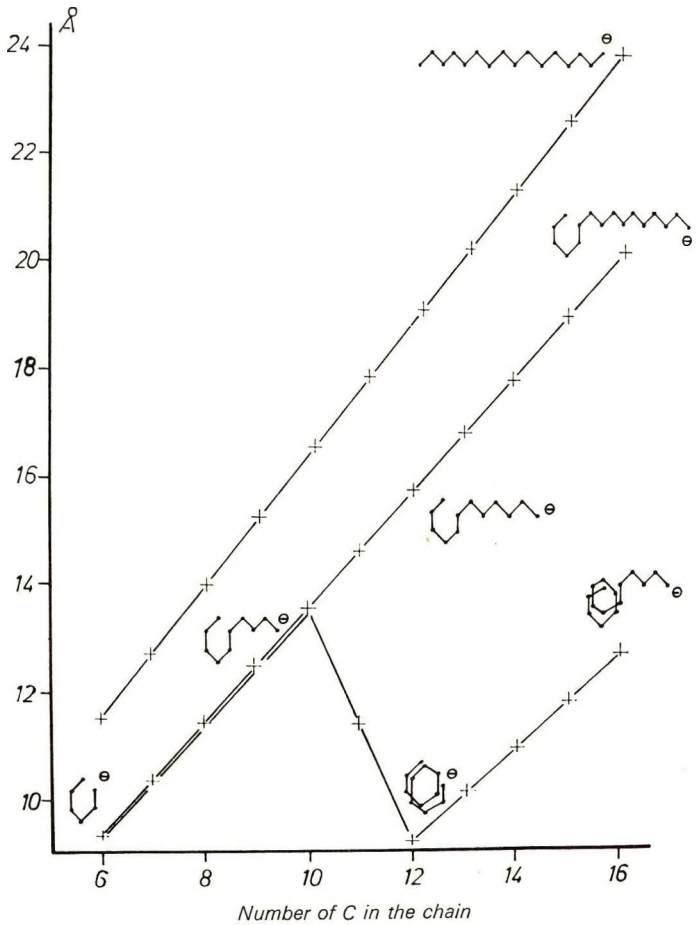


Figure 17. Length in Å for different conformations of alkyl sulphates.

In *Fig. 18* the size of the molecule has been studied by measuring the diagonal length. If we estimate the average value of the length of the aliphatic chain measured in the transverse and longitudinal directions, the relationship between the length in Å and the number of carbons shows also that the minimum size and obviously the optimum ratio area/volume, occurs for structures containing 12 carbon atoms.

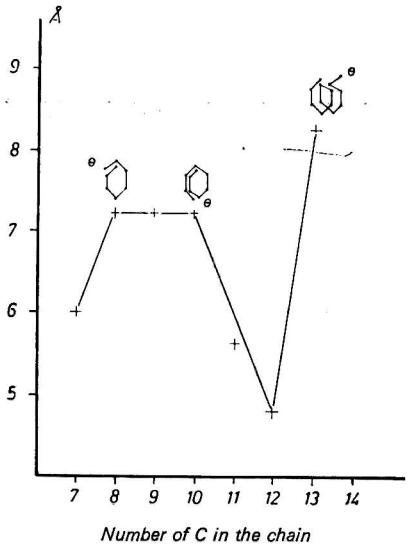


Figure 18. Diagonal length in A for different conformation of alkyl sulphates.

This theoretical assumption gives some justification to the specific behaviour of the C_{12} chain so far observed by many authors and for the results described in this paper. The smaller the cross-section of the molecule the smaller will be the steric hindrance that the polipeptide chain of the protein will oppose to its penetration.

Obviously, other properties like solubility, wetness and detergent power help to justify the quicker migration of the C_{12} surfactants, but these properties play their role when the surfactant is in solution. We believe that when the surfactant is adsorbed and migrates inside the protein, this cannot occur in the same way.

If an alkylsulphate, due to its spatial shape, migrates to the inner part of the skin structure, an ionic exchange can take place between the sodium ion and the protons at the physiological fluid level with the corresponding modification of the pH value of the skin. The organism will have to compensate for the consequent pH modification to maintain the pH constant at a value near neutrality. If this pH compensation does not take place, or it is more difficult in certain individuals, or if the quantity of surfactant that exchanges its sodium ion is very high in this particular area, it will appear a modification of the metabolism giving rise to a phenomenon known as irritation. The higher the number of adsorbed molecules the greater the possibility of modification not only of the ionic character but also of the hydrophobic character of the protein structure to which the surfactant is attached. Modification of the isoelectric point produces a solubilization of protein. The fact that solubilization is more suitable for the C_{12} alkylsulphates supports our postulate, we get a greater migration when we are dealing with structures containing such numbers of carbon atoms in their structure.

The fact that when we treat human hair with thioglycolic acid we get a maximum reactivity in the presence of C_{12} alkylsulphate shows that this molecule may have

migrated to protein zones where it has been possible for the thioglycolate ion to react with the cystine located in the structure.

It would be desirable from the cosmetic point of view that a given surfactant with identical detergent properties would show a minimum migration. Dealing with the C_{12} chain this can be accomplished if we prevent the double coil conformation mentioned previously. In fact, we have performed in our laboratory different experiments in which the anion sulphate is present in the position 2 or 5. After treatment with these molecules we have observed that the adsorption is lower than the one corresponding to the alkylsulphate having the anionic group in position 1 for an identical length in its hydrophobic chain. Another method of modificating the migration of surfactant and consequently the irritation it may produce, will be to straighten out the hydrophobic chain of the surfactant when interacting with the stratum corneum. The effect of a non-ionic surfactant on the decrease of the irritation produced by anionic surfactants is well known. Possibly, the non-ionic surfactants inhibit the conformation of the anionic surfactant.

Evidence of the conformation of hydrophobic chains of 8 or 10 carbon atoms of soaps or alkylsulphates in water have been obtained by Raman spectroscopy (25, 26). Optimum conformations can justify, as described in the paper, the fact that in any family of surfactants, a certain specificity in behaviour can be shown by some members of the series. These molecules will contain those chain lengths that can adopt a conformation able to allow the molecule to keep the contact surface hydrophobic chain-water to a minimum.

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Evaluation of O/W emulsion stability through zeta potential—I

Prediction of O/W emulsion stability through zeta potential by accelerated ageing tests

DEVRAJ RAMBHAU,* M.Pharm., D. S. PHADKE, M.Pharm. *and* A. K. DORLE, M.Pharm., Ph.D. **Department of Pharmaceutical Sciences, Nagpur University Campus, Amravati Road, Nagpur-10 (M.S.). Pin: 440010, India.*

Synopsis

Ageing of emulsions prepared with sodium palmitate or cetyltrimethylammonium bromide under **accelerated conditions** such as **elevated temperatures** or **higher centrifugal speeds** resulted in an increase in **mean globule diameter** and decrease in **viscosity** and **Zeta potential.** A fair inverse correlation was observed between Zeta potential and aging. The correlation between Zeta potential and mean globule diameter in sodium palmitate emulsions was not, however, as fair as in the case of emulsions prepared with cetyltrimethylammonium bromide. Changes in Zeta potential and viscosity on ageing showed a linear relation between them at all temperatures studied, in both the emulsions. Decay in Zeta potential with time at **higher centrifugal speeds** was linear and was dependent on speed of centrifugation. Mean globule diameter was linearly increased as Zeta potential was decreased at all centrifugal speeds studied. **Emulsifier concentration studies** revealed that at a **critical concentration** emulsions possess maximum Zeta potential. Maximum Zeta potential was associated with maximum emulsion stability. Possible mechanisms of Zeta potential decay at accelerated conditions were explained.

Introduction

Accelerated aging tests, which include storage at elevated temperatures or at higher centrifugal speeds are often used in prediction of shelf-life of an emulsion. Changes in size frequency, specific interfacial area and viscosity at elevated temperatures revealed that thermal effects are greater for some systems than for others and there is no real basis for comparison (1, 2, 3, 4, 5, 6). This shows the limitations of the applicability of the thermal methods towards the accelerated aging tests. As was felt by Wood and Catacalos, (5) even till today no clearly definable relation between temperature and rate of change of parameters used to evaluate emulsion stability, has been worked out Similarly the versatile use of analytical ultracentrifuge in the prediction of emulsion stability, has been questioned (7), although it was remarked as an excellent tool (9, 10 11, 12, 13) for the purpose.

The relevant literature on stress conditions clearly advocates a need for the investigation of any other parameter, which could be used to evaluate emulsion stability.

The possibility that electrical charge effects may be of significance in stabilizing emulsions was recognized at a fairly early date (15). However the surface charge which is reflected in the magnitude of Zeta potential was not worked out to any fruitful extent, a

a quantitative parameter, although its importance in the emulsion stability has been well recognized (18, 20, 19, 21, 8, 22, 16). Powis (24) observed, decrease in contact potential with time in his emulsions. Davies (25) derived an approximate equation relating surface potential with rate of coagulation. Our earlier report (26) has also indicated the correlation between Zeta potential and mean globule diameter in emulsions during ageing. Thomas, M. Riddick (21), showed that Zeta potential can be well correlated with the extent of adsorption and desorption of emulgents, at interface for most liquid/liquid systems. Jackson & Skauen (27) have indicated the utility of Zeta potential in the predication of emulsion stability.

Since the stability of an emulsion is a function of electrical potential at interface, measurement of the same in conjunction with other parameters was suggested by Becher (16). Therefore, the present study was undertaken with a view to investigate the probable utility of Zeta potential in the stability prediction of emulsions by means of accelerated ageing tests. Efforts were also made to correlate the changes in Zeta potential at accelerated conditions to that of the changes taking place simultaneously in mean globule diameter or viscosity.

Materials and methods

(i) Refined arachis oil (Postman Brand, India), (ii) Sodium palmitate (BDH), (iii) Cetyltrimethylammonium bromide (BDH), (iv) Distilled water.

Preparation of emulsions. Required amount of emulsifier, aqueous phase and oil was mixed and blended for required time as shown in Table 1. Emulsions thus formed were allowed to stand for an hour to suppress foam.

Table 1.

S. No.	Phase volume ratio oil : water v/v	Emulgent concentrations	Blending time in min
I	50 : 50	SP 1 g CTAB 0·5 g	5 min
II	50 : 100	SP 1 g CTAB 0·5 g	5 min
III	30 : 70	SP or CTAB 0·1 g, 0·2 g, 0·4 g, 0·6 g, 0·8 g and 1 g	2 min

(I) Thermal studies. (II) Centrifugal studies. (III) Emulsifier concentration studies. (SP = Sodium palmitate. CTAB = Cetyltrimethylammoniumbromide.)

THERMAL STUDIES

One hundred ml of emulsions were placed in each of the forty wide mouthed bottles and ten such bottles were stored in each of the four ovens maintained at 30°, 40°, 50° and 60°C ±1°C respectively. The determinations of Zeta potential (ZP), mean globule diameter (MGD) and apparent viscosity were carried out at an interval of 12 h for a period of 120 h. All measurements were performed when the samples attained room temperature.

Zeta potential measurement. Modified Abramson's flat cell microelectrophoresis apparatus was assembled in our laboratory for measuring electrophoretic migration (EM), from which Zeta potential (ZP) was calculated using Smoluchowski's equation (23). Platinum electrodes were used and readings were taken at a required e.m.f. ranging from 50–150 v maintaining current strength constant throughout the measurements. To obviate opacity problems, all emulsions were diluted to 1 in 250 by distilled water. The microelectrophoresis cell was rinsed with the diluted sample, prior to its use. Heat dissipation problems into cell were minimized by using the lamp only when it was essential. The polarity was reversed after every three readings. Depending on the degree of variation of EM value in first five readings 65–100 globules were tracked at predetermined stationary level with a stop watch having 0·1 second precision.

Globule size measurement. 1 in 200 dilution of emulsion was performed with a mixture of 75% v/v propylene glycol +25% v/v distilled water. Three hundred globules were measured for their size on a microscope equipped with a standardized eye-piece micrometer scale. From this data d_{ln} value was computed.

Viscosity measurement. Viscosity was calculated by treating the rheograms obtained on a multipoint instrument (Fischer MacMichael Viscosimeter).

CENTRIFUGAL STUDIES

Emulsions were centrifuged at 12000, 14000 and 16000 rpm for 15, 30, 45 and 60 min respectively, on a high speed centrifuge (UNIPAN, made in Poland). The determination of Zeta potential and mean globule diameter was carried out from the creamed emulsion layer for the controls and samples, following the methods described above.

EMULSIFIER CONCENTRATION STUDIES

In order to study the pattern of decay of Zeta potential in emulsions with varying stabilities, emulsions were prepared with six concentrations of each emulsifier as described in Table 1. They were screened for the magnitude of initial Zeta potential and changes therein after an interval of 24 h for a period of 120 h. All the emulsions were placed in wide mouthed bottles and were stored in an oven maintained at 50°C ±1°C.

Results

Figs. 1 and *2* record the changes in Zp with time at various temperatures when plotted as log Zp against time in h. Zp decreases with ageing at all elevated temperatures studied in two emulsions made with SP or CTAB respectively. *Fig. 1* reveals that SP emulsions behave differently at elevated temperatures when compared to CTAB emulsions. Rate of decay of Zp was found to increase with increase in temperature in both the emulsions. But the change in rate of decay of Zp was sharp from 30–40°C in SP emulsions, whereas

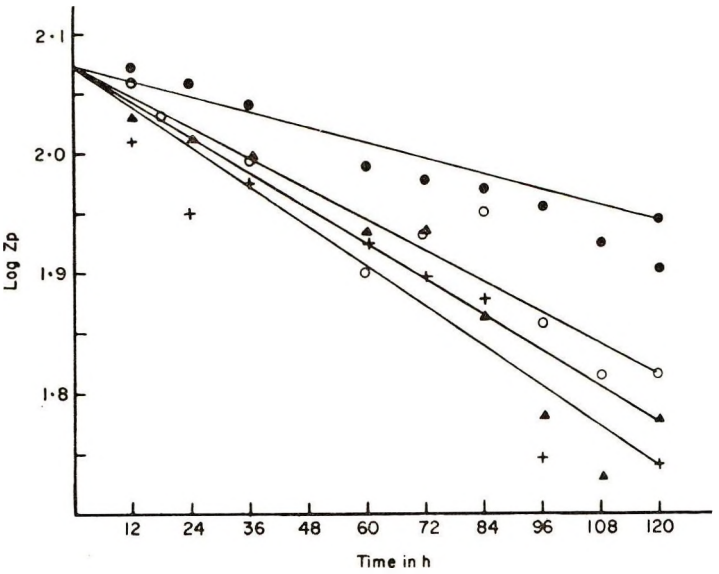

Figure 1. The change in the log of Zp as a function of time in emulsion prepared with SP, stored at different temperatures. ●, 30°C; ○, 40°C; ▲, 50°C; +, 60°C.

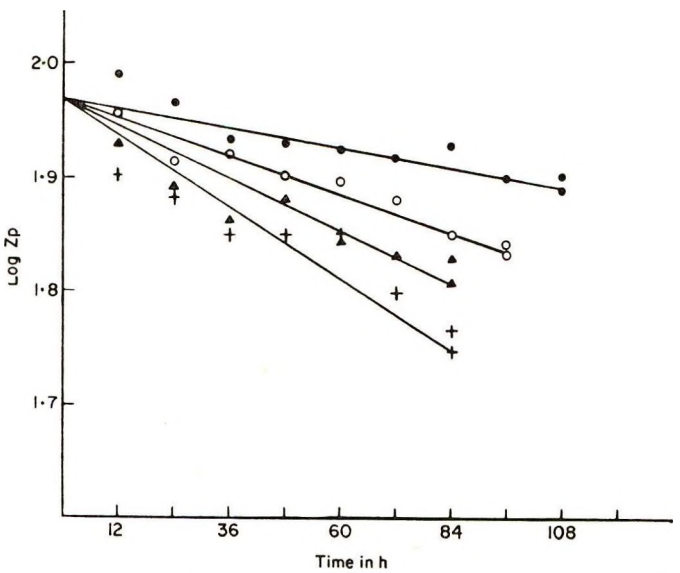

Figure 2. The change in the log of Zp as a function of time in emulsion prepared with CTAB, stored at different temperatures. ●, 30°C; ○, 40°C; ▲, 50°C; +, 60°C.

in CTAB emulsions it was not so sharp. Further changes at 40° to 50°C and 50° to 60°C were relatively gradual in both the emulsions. In SP emulsions it was observed that the changes in emulsion properties such as MGD or viscosity which are the symptoms of gross instability also changed sharply from 30° to 40°C, whereas the further changes from 40° to 50° and 60°C revealed a slow change. Gradual changes in MGD and viscosity were observed in CTAB emulsions.

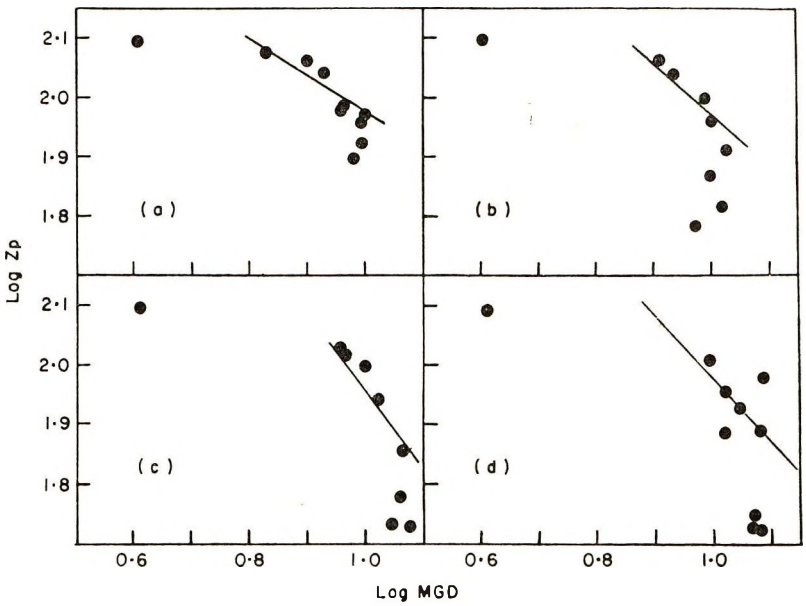

Figure 3. The relationship between log of Zp and log MGD in SP emulsions, stored at different temperatures. (a) 30°C; (b) 40°C; (c) 50°C; (d) 60°C.

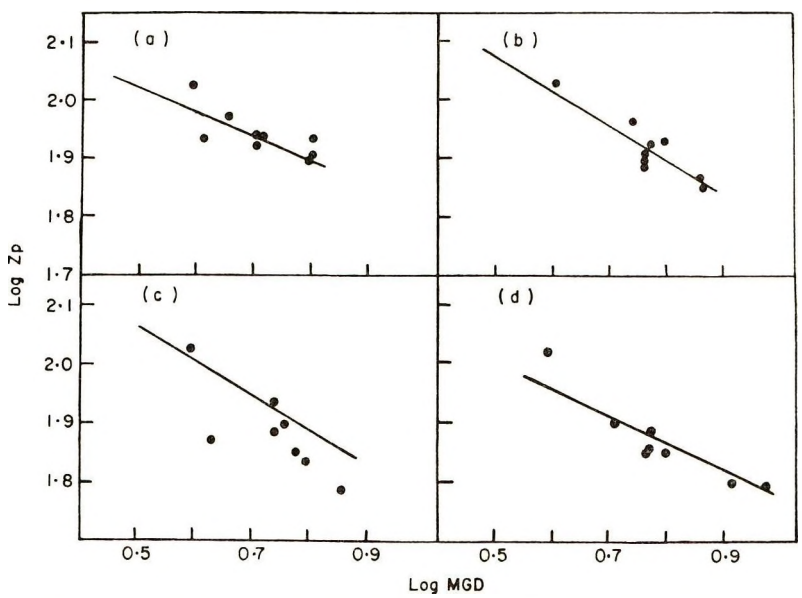

Figure 4. The relationship between log of Zp and log of MGD in CTAB emulsion, stored at different temperatures. (a) 30°C; (b) 40°C; (c) 50°C; (d) 60°C.

The *Figs. 3* and *4* show the changes in Zp with MGD during thermal ageing for SP and CTAB emulsions. Log of Zp and log of MGD bear an inverse relation. The linearity between these parameters is not marked in SP emulsions, but fairly good correlation was revealed in case of CTAB emulsions. In *Fig. 3* the initial Zp and MGD do not fall on the best fit lines drawn through the points. This may be due to the drastic effect of temperature on the coagulation rate of oil droplets.

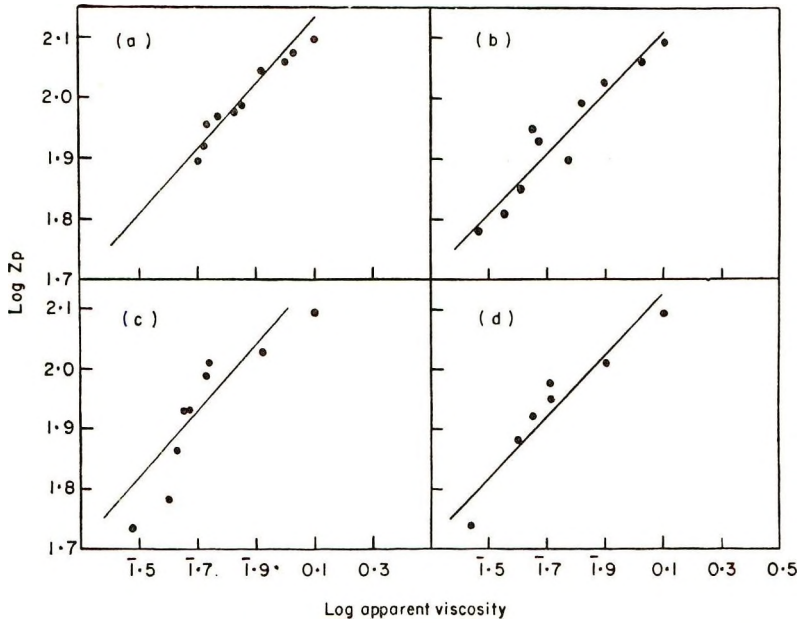

Figure 5. The relationship between log of Zp and log of apparent viscosity in SP emulsion, stored at different temperatures. (a) 30°C; (b) 40°C; (c) 50°C; (d) 60°C.

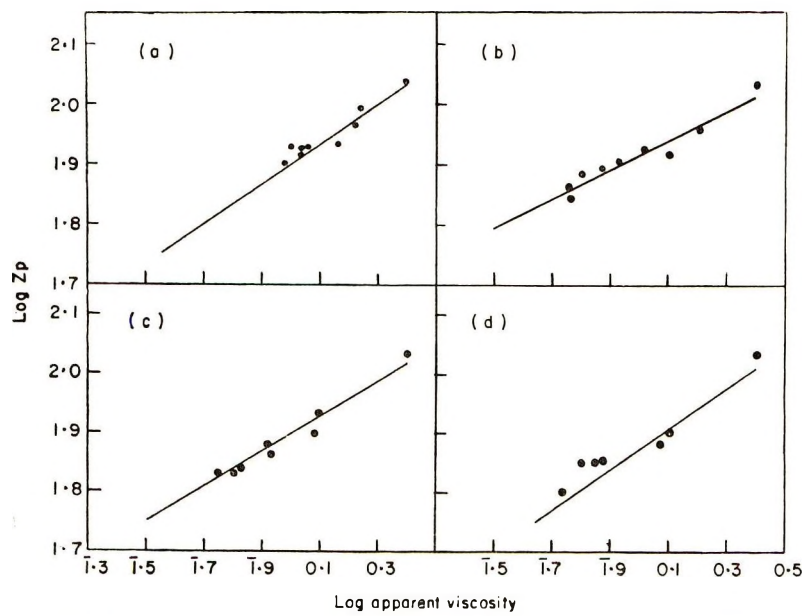

Figure 6. The relationship between log of Zp and log of apparent viscosity in CTAB emulsion, stored at different temperatures. (a) 30°C; (b) 40°C; (c) 50°C; (d) 60°C.

Figs. 5 and *6* reveal that apparent viscosity decreased at all temperatures studied with ageing. Log Zp vs log apparent viscosity plots for SP emulsion or CTAB emulsion revealed a significant correlation between them. The changes in apparent viscosity can be attributed to the changes in mean globule diameter and changes in viscoelastic properties of the interfacial film. Sherman's reports (28, 29) emphasize that the apparent

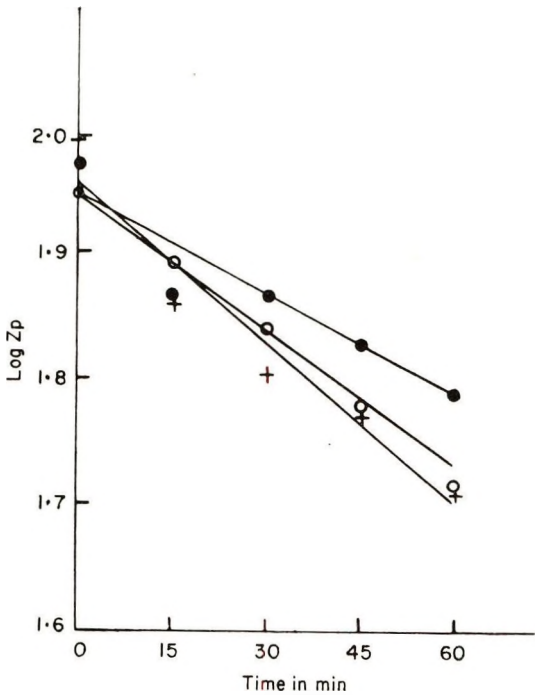

Figure 7. The change in log of Zp as a function of time of centrifugation at different centrifugal speeds for the SP emulsion. ●, 12000 rpm; ○, 14000 rpm; +, 16000 rpm.

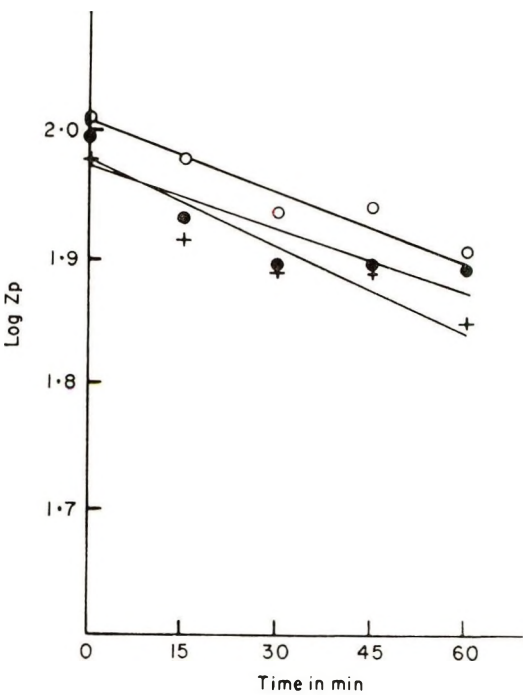

Figure 8. The change in log of Zp as a function of centrifugation time at different centrifugal speeds for CTAB emulsion. ●, 12000 rpm; ○, 14000 rpm; +, 16000 rpm.

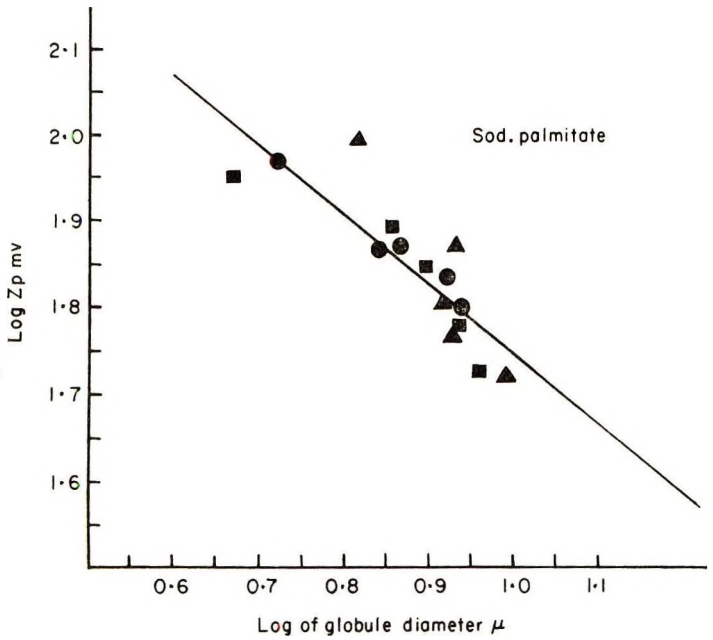

Figure 9. The relation between log ZP and log MGD, when SP emulsion was centrifuged for different time intervals at different speeds. ●, 12000 rpm; ■, 14000 rpm; ▲, 16000 rpm.

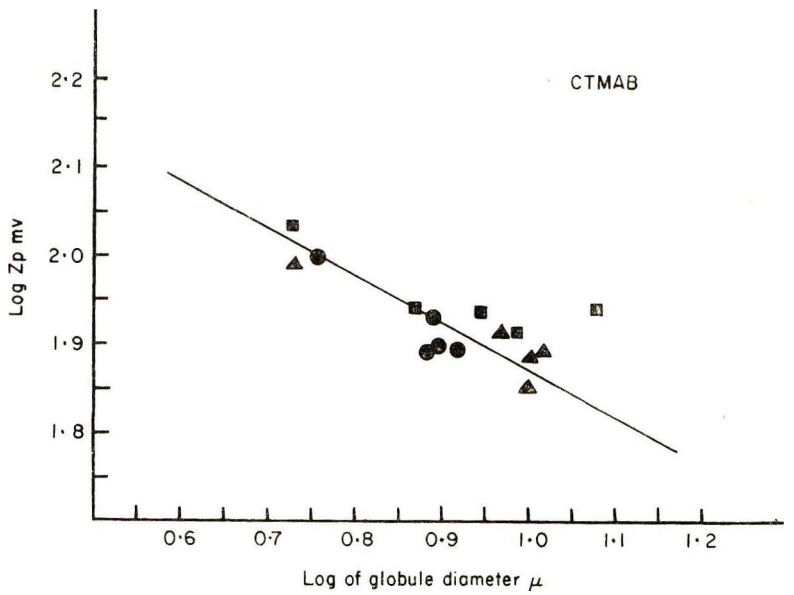

Figure 10. The relation between log Zp and log MGD when CTAB emulsion was centrifuged for different time intervals at different speeds. ●, 12000 rpm; ■, 14000 rpm; ▲, 16000 rpm.

viscosity changes in soap stabilized emulsions are dependent on the changes in mean globule diameter on ageing. Hence the linear relation between mean globule diameter and Zp which was not significant from the data points given in *Fig. 3* could be expected from the viscosity data given in *Fig.* 5.

Figs. 7 and *8* record the changes in Zp during centrifugal ageing for SP or CTAB emulsions. Determination of Zp after 15, 30, 45 and 60 min of centrifugation at 12000, 14000 and 16000 rpm resulted in a progressive fall of Zp with time of centrifugation in both the emulsions. The rate of decrease of Zp was found to be a function of rpm and time of centrifugation. With the increase in rpm rate of Zp decay was accelerated. Reduction in Zp value was associated with concomitant increase in MGD. *Figs. 9* and *10* show that the changes in Zp with respect to changes in MGD occurring because of centrifugal ageing at various speeds are fairly linear.

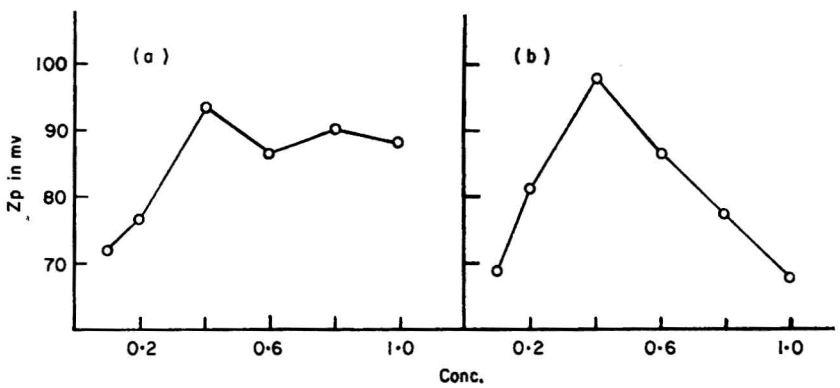

Figure 11. Effect of emulsifier concentration on the initial Zeta potential value of SP emulsion (a) and CTAB emulsion (b).

The emulsifier concentration studies on emulsions stabilized with various concentrations of SP or CTAB revealed that conditions of maximum stability with respect to Zp are satisfied at a concentration of 400 mg emulsifier/100 ml of emulsion for both the emulgents as shown in *Figs. 11a* and *11b*. In case of emulsions stabilized with SP, (*Fig. 11a*) Zp continuously increased up to the concentration of 400 mg after which there was a slow fall. The stability was also found to increase up to this concentration after which there was no further improvement. As may be seen from the *Fig. 12,* it was interesting to observe that the Zp of the broken emulsions (when emulsions showed distinct oil separation they were treated as broken) in all cases were near about 40–45 mv. The rate of fall of Zp markedly increased in emulsions stabilized with concentrations below the critical concentration, i.e. 400 mg and above it the rate of fall of Zp was slow as compared to low concentrations.

In the case of CTAB stabilized emulsions (*Fig. 11b*) the Zp continuously increased up to the concentration of 400 mg, after which there was a sudden drop in Zp which exhibited a direct impact on the stability of emulsions stabilized with 800 mg and 1 g of CTAB.

As shown in *Fig. 13* emulsions stabilized with 100 mg, 200 mg, 800 mg and 1 g have broken within 48–72 h, whereas only 400 mg and 600 mg stabilized emulsions withstood the thermal stress up to 96 h. Emulsions showed distinct oil separation when Zp was reduced to 60 mv.

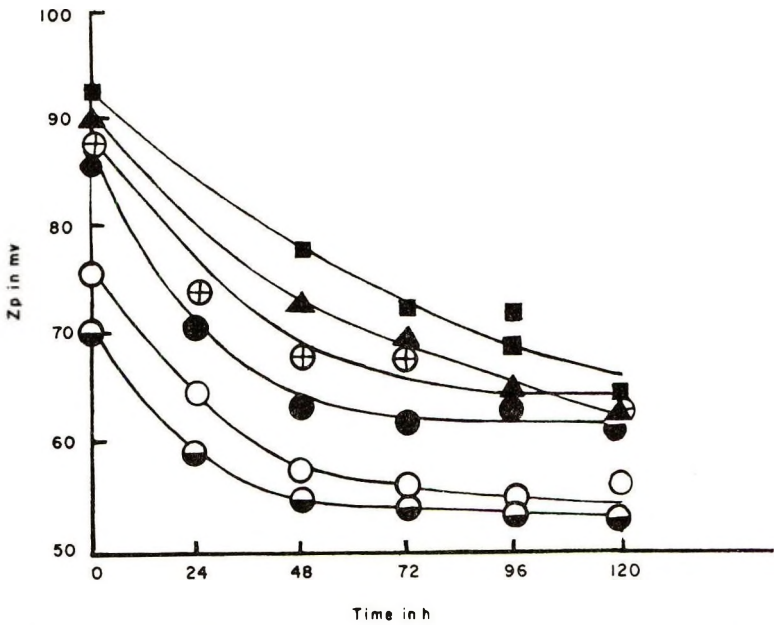

Figure 12. The change in Zp as a function of time in emulsions prepared with various concentrations of SP. ■———■, 0·4 $gml^{-1} \times 100$; ▲———▲, 0·6 $gml^{-1} \times 100$; ⊕———⊕, 0·8 $gml^{-1} \times 100$; ●———●, 1·0 $gml^{-1} \times 100$; ○———○, 0·2 $gml^{-1} \times 100$; ◒———◒, 0·1 $gml^{-1} \times 100$.

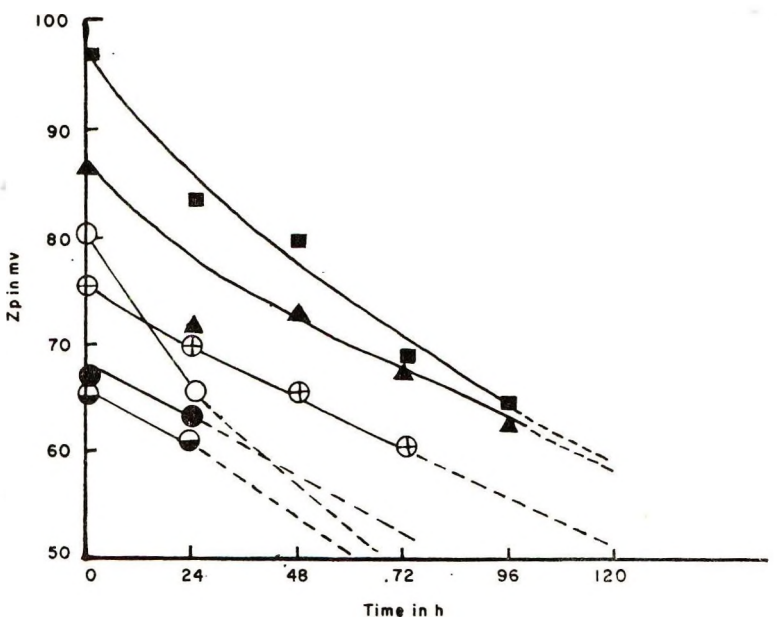

Figure 13. The change in Zp as a function of time in emulsions prepared with various concentrations of CTAB. Dotted line indicates oil separation. ■———■, 0·4 $gml^{-1} \times 100$; ▲———▲, 0·6 $gml^{-1} \times 100$; ⊕———⊕, 0·8 $gml^{-1} \times 100$; ●———●, 1·0 $gml^{-1} \times 100$; ○———○, 0·2 $gml^{-1} \times 100$; ◒———◒, 0·1 $gml^{-1} \times 100$.

Discussion

For the thermal studies the emulsions were prepared by employing 1g SP or 0·5 g CTAB. Microscopic examination of both the emulsions failed to indicate any appreciable occurence of aggregation of globules. The concentrations of the emulgents employed are higher than their cmc values, therefore the existence of micelles in the continuous phases of both the emulsions is obvious. Addition of emulsifiers in excess of cmc should only increase the number and size of micelles in the continuous phase without appreciable effect on density of packing of adsorbed emulgent (14). For ionized surfactants like SP or CTAB, increase in temperature should result in an increase of number of monomeric species and reduction in number and size of micelles (30). When this happens the escaping tendency of surface active molecules becomes quite high and emulgent molecules in effect 'bled off' (desorbed from) the interface to make up the micelles. Osipow (31) has postulated the mechanism of such desorption. According to him presence of micelles in juxtaposed interfacial films would increase electrical repulsion and favour the desorption of ionic emulgents from the oil/H_2O interface. Desorption will thus disturb the dynamic equilibrium between the surface active molecules oriented in the film and dissolved in the continuous phase. Davies (33) equation,

$$\text{Log}\left\{-\frac{1}{n}\frac{dn}{dt}\right\} \quad \frac{z.e.\Psi_0}{2{\cdot}303\ KT} + C$$

relating desorption rate dn/dt to that of surface potential is an evidence for the surface potential dependence on desorption rate. The surface potential Ψ_0 has been found empirically related to the Zp (ξ). The relation, $\xi = 0{\cdot}55\Psi_0$ has been claimed to hold reasonably well (36). Decrease in double layer thickness due to increased counter ions is another possibility which might decrease Zp. However above the cmc the increase in counter ions will be slower owing to the limited dissociation of counter ions from micelles (17). At higher temperatures such dissociation will be appreciable and consequently the thickness of the double layer should decrease at a rate approximately inversely proportional to the counter ion concentration. Decrease in double layer potential occurring because of desorption and increase in counter ion concentration will thus favour the process of coagulation. Brady *et al.* (34) have indicated such desorption of emulsifiers prior to coagulation in o/w emulsions. Lawrence and Mills (35) reported that in practice electrical factors provide energy of activation to the process of coagulation and derived equation

$$\left\{\frac{\text{Coalescence rate}}{A_1}\right\} = -\frac{B\Psi_0^2}{KT}$$

relating coalescence rate with surface potential. That before collision of two surfaces can occur an energy barrier proportional to Ψ_0^2 must be overcome was also recorded by Verwey and Overbeek (36). Lawrence and Mills (35) and Pethica and Few (37) tested the validity of the above equation at a constant temperature and observed an expected linear relation between Ψ_0 and coagulation rate, although the data points were too meagre to emphasize the relation with great confidence. The results of the present study reveal that, thermally induced desorption which resulted in the decreased double layer potential seems to be the basic factor initiating the coagulation. Once such coagulation is triggered, further changes in Zp with ageing can be attributed to the fall of surface coverage of oil droplets. Fall of surface coverage can only occur if there is a displacement of emulsifier molecule or degraded component of emulsifier into one of the phases. In the present

systems, due to steric reasons displacement of emulsifier molecules into discontinuous phase is possible. According to Boekenoogen (38) a dry neutralized peanut oil dissolved 0·1% sodium or potassium soap at 75°C. Although this solubility is rather low, the presence of small amounts of fatty acids was shown to increase the solubility of soap to a considerable degree (39). The soap also may dissolve colloidally in the oil. Newbay (40) found that 0·03% to 0·7% soap was dissolved in neutralized soyabean and cotton seed oils. Therefore solubility of SP or CTAB into arachis oil can be expected. Although we have not studied rate of hydrolysis of the present emulsifier, we are tempted to believe that elevated temperatures would bring about hydrolysis of such emulsifier. The fatty molecules of SP or CTAB may be favourably dissolved into the oil phase and thus further enhance the solubility of the emulsifier into the oil phase. As a result of this, fall of surface coverage and consequently fall in charge density should occur.

Thus changes in Zp with time could therefore be explained on the basis of its dependence on the rate processes such as desorption, coagulation and solubility of emulsifier and their chemical deterioration.

Centrifugal stress increases the rate of migration of dispersed globules to the superfacial emulsion layer. This is in accordance with Stoke's law. It is probable that due to increased concentration of the globules at superfacial emulsion layer, the aggregation tendency of the globules increases and drainage of continuous phase entrapped in between globules will take place, thus disrupting the emulsifier film, and leading to coagulation. The rate of globule size expansion followed first order kinetic pattern confirming earlier reports (41). The rate of globule size expansion and rate of Zp decay in both emulsions depended upon the magnitude and duration of centrifugation. T. Higuchi (42) has objected to the application of an over simplified Stoke's law for creaming of emulsions. Garrett (43) suggested a possible modification for Higuchis' hypothesis, to include adjustment of particles in packed bed with time to stimulate sweep out phenomenon which is characterized at higher centrifugal stresses (10). Powis (25) and Lewis (44) have attributed the decay in Zp to the Sweep out phenomenon. This was further confirmed by Addnik *et al.* (45), who observed the fall of the phase boundary potential with time when myristic acid was spread over water. During such fall of potential the water was being squeezed out from between chains themselves. He attributed this effect to the time effect, observed by Powis and suggested that similar phenomenon may occur in case of adherence of droplets in emulsions. That displacement of surfactant molecules from the interface because of desorption may also change Zp was explained recently by Vold (46). He recognized rate of desorption and electrostatic effects, as rate determining processes for demulsification caused by centrifugal stress.

Experiments on the effect of emulsifier concentration on the stability of the emulsions revealed that for maximum stability of emulsion a critical concentration of emulgent is essential. This is in accordance with an earlier report (31). However, in many cases stability appears to be maximized at cmc (13). There are reports that maximum stability of emulsion at the cmc is also associated with maximum Zp on globules (47). Although we have not considered cmc calculations in our studies, it was seen that the maximum initial Zp exists at a critical concentration (400 mg per 100 ml of emulsion) of emulsifier in case of both the emulsions. Increasing the concentration above the critical concentration caused decrease in Zp. The nature of the plots of concentration of emulsifier vs Zp, however, is not the same for the two emulsifiers. In case of SP emulsions there is a gradual fall in Zp by increasing the concentration above critical concentration, while in the case of CTAB emulsion there is a sudden fall. Such a difference in pattern is difficult to explain

at this stage, but one may consider the possibility of its being due to the differences between the stability behaviour of emulsions prepared with these two emulsifiers. Obviously there is need of further work to understand this aspect.

Conclusions

Zp is found to be a linear function of time at accelerated temperatures. Fall of Zp was always associated with decrease in emulsion stability as indicated by an increase in MGD, and a decrease in viscosity. There appears to be a rough correlation between Zp and MGD. A fair correlation was obtained between viscosity and Zp.

Decay in Zp was found to be a function of time at different centrifugal speeds. Reduction in Zp value during centrifugal ageing was associated with increase in mean globule diameter. Zp and MGD showed a linear relation between them.

The initial value of Zp was not a linear function of concentration of emulgent. At a critical concentration of 400 mg (SP or CTAB)/100 ml emulsion, the emulsions showed maximum stability, and were also associated with maximum initial Zp. The stability of CTAB emulsion was a critical function of emulsifier concentration below and above which emulsions were extremely unstable.

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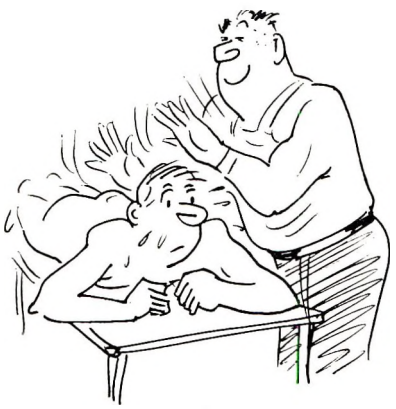

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