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A systematic microscopical examination of gels and emulsions containing cetrimide and cetostearyl alcohol

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Summary

The structure of gels and emulsions containing the mixed emulsifier system of cetrimide and cetostearyl alcohol has been studied using differential interference contrast microscopy and freeze-etch electron microscopy. Evidence from these techniques suggest that in the ternary gel systems increasing the cetostearyl alcohol concentration results in a gradual change from a vesicular structure to one consisting of a liquid crystalline phase largely localized around particles of cetostearyl alcohol. The structure of the emulsion systems is very similar to that of the ternary gels with the oil droplets providing additional nuclei for the liquid crystalline phase. It appears that the structure of these systems is more complex and more ordered than was previously believed.

Introduction

The mixed emulsifier system of cetrimide and cetostearyl alcohol is frequently used in the formulation of antiseptic creams and much work has been done in an attempt to define the structure of these creams using both rheometry (Barry, 1971) and light microscopy (Barry and Saunders, 1970) as recently reviewed by Eccleston (1984). However, recent studies in the general area of colloid science have demon-

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strated that by the application of the relatively new techniques of differential interference contrast microscopy (Kacher et al., 1984) and freeze-etch electron microscopy (Friberg et al., 1976; James and Heathcock, 1979; Mummé-Young et al., 1983) it has been possible to observe structures in systems hitherto difficult to observe. Although the latter technique has been used to examine similar systems to the one used in this study (Gstirner et al., 1969; Junginger et al., 1981; Junginger and Heering, 1983) it has not been applied to study of gels and emulsions containing cetrimide and cetostearyl alcohol.

Materials and Methods

Preparation of the gels and emulsions

All the materials used were of Pharmacopoeial grade. The ternary systems T_1–T_8 were prepared according to the formulae in Table 1. Cetostearyl alcohol at 80°C was dispersed in aqueous cetrimide solution at the same temperature and stirred gently with a paddle stirrer for a period of 1 h before being allowed to cool to approximately 60°C. The mixture was then homogenized using a Silverson multipurpose high speed mixer until the setting point of the gel was reached or for a period of not more than 15 min. The gel was then allowed to cool to room temperature. The emulsions E_1–E_3 were prepared in a similar fashion except that the cetostearyl alcohol was dissolved in the liquid paraffin at 80°C before being added to the aqueous cetrimide solution at the same temperature. All systems were allowed to stand for at least 2 weeks before being tested.

Differential interference contrast microscopy

In this form of microscopy special birefringent crystal prisms (Wollastan prisms) are used to split the beam such that the shear between the interfering beams is less than the resolution of the microscope objective in use. As a result a single image is still seen but, by adjusting the prism on the objective side, very small changes in refractive index and thickness within the sample can be highlighted in colour contrast. Suitable adjustment gives a detail of relief-like appearance, exceptional brightness and resolution almost twice that obtainable in, for example, phase contrast microscopy. Furthermore the depth of focus is significantly reduced, eliminating 'out-of-focus' detail as well as permitting examination of the sample layer by layer. The technique is relatively simple involving the use of a standard microscope fitted with a differential interference contrast condenser and special objectives. The microscope used in this study was a Leitz-Wetzlar Dialux (Wetzlar, F.R.G.).

Freeze-etch electron microscopy

In essence this technique is a means of preparing 'wet' materials for examination in the electron microscope without involving drying, dilution or other gross perturbation. It was originally introduced to enable ultrastructure to be investigated in biological specimens (Steere, 1957; Moor et al., 1961), an area in which it is still employed extensively, but more recently it has been used for more general morpho-

TABLE 1

FORMULATIONS USED IN THIS STUDY (ALL CONCENTRATIONS % w/w)

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	E_1	E_2	E_3
Cetrimide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cetostearyl alcohol	0.25	0.5	1.0	2.5	4.0	6.0	8.0	10.0	10.0	10.0	10.0
Liquid paraffin	–	–	–	–	–	–	–	–	5.0	10.0	20.0
Purified water to	100	100	100	100	100	100	100	100	100	100	100

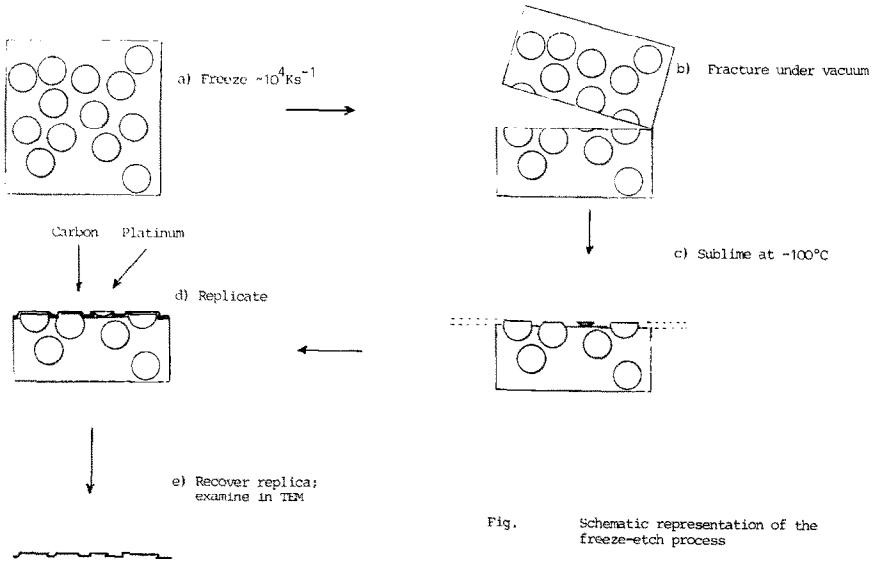

Fig. 1. A schematic representation for the preparation stages of the freeze-etch technique.

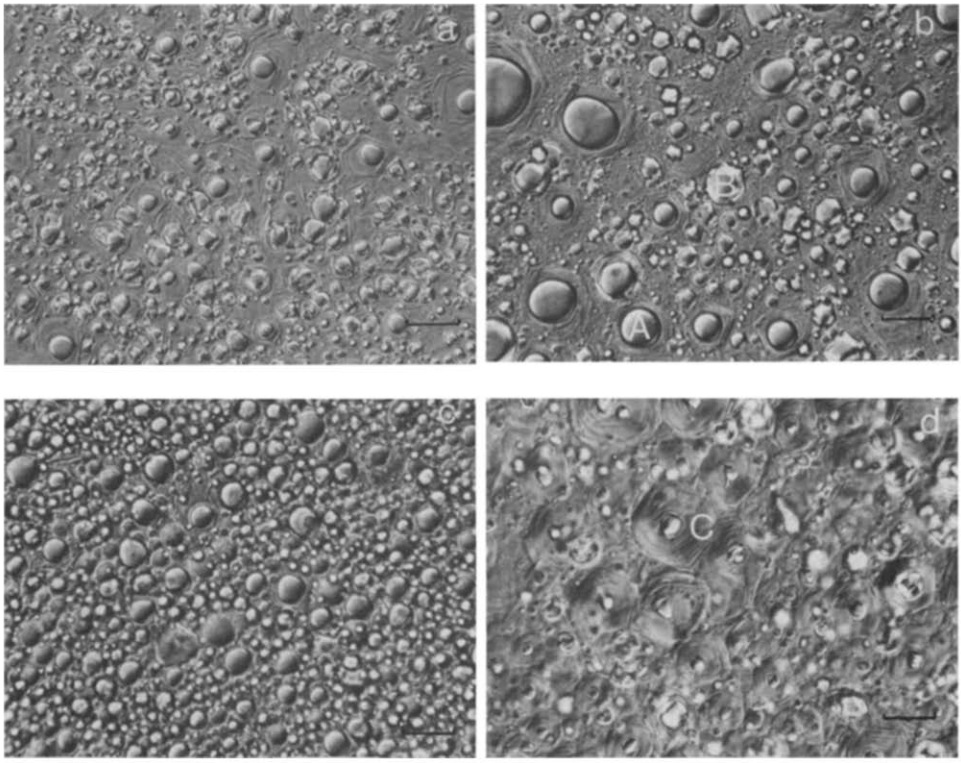

Fig. 2. Photomicrographs of emulsions E_1 (a), E_2 (b), E_3 (c), and T_8 (d). Feature A is a near spherical oil droplet of liquid paraffin, Feature B is a polyhedral-shaped particle of cetostearyl alcohol and Feature C is the lyotropic liquid crystalline network enveloping the oil and fatty alcohol. Bar = 25 μm.

logical studies on colloid materials (Menold et al., 1976; Stewart and Sutton, 1984a). As the technique has been discussed in detail by these workers only an outline of the process is provided here. In our work the samples of ternary gels and emulsions were crash frozen in a chlorofluorohydrocarbon cryogen (Arcton 12, ICI Mond Division) to liquid nitrogen temperatures and then fractured to yield a suitable section plane. The exposed surface was etched by sublimation under moderately high vacuum conditions before being shadowed and replicated with evaporated platimum and carbon in a Cressington S30 freeze-etch module seated on a dedicated Edwards E306 vacuum coater. The replica was recovered, cleaned with methanol/acetone to remove all traces of adhering material, collected on copper netting, dried and then examined using a transmission electron microscope (EM300, Phillips).

A schematic representation of the method used is shown in Fig. 1

Results

Differential interference contrast microscopy

Typical photomicrographs of the ternary gel T_8 containing 10% w/w cetostearyl alcohol and the emulsions E_1, E_2, E_3 containing increasing concentrations of liquid

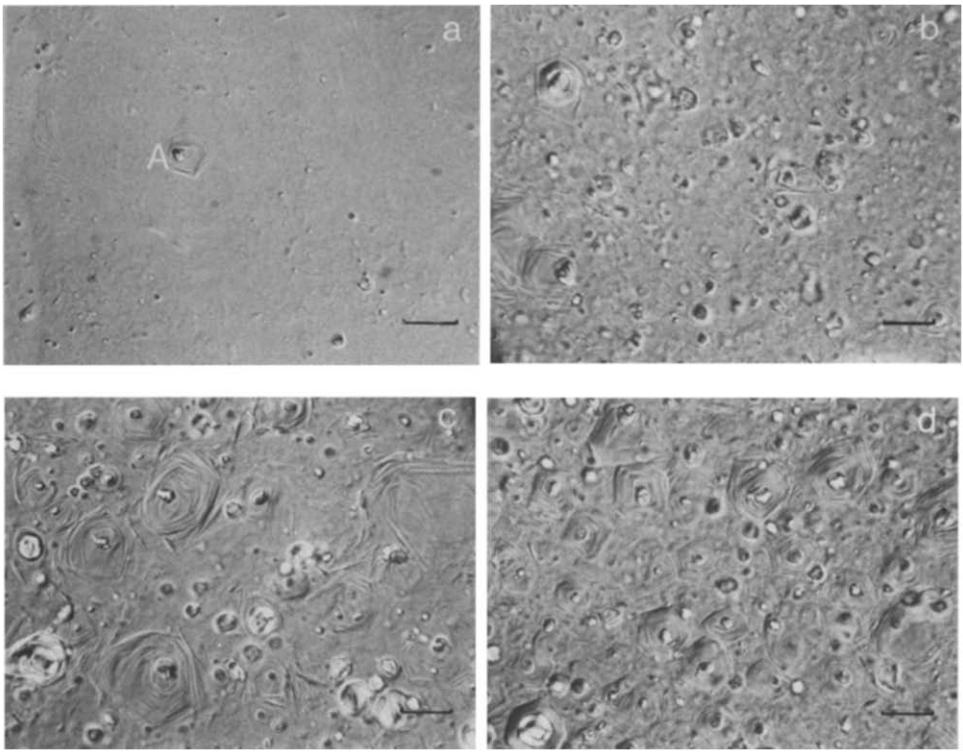

Fig. 3. Photomicrographs of the ternary gels T_3 (a), T_4 (b), T_5 (c) and T_6 (d). Feature A is believed to be a particle of cetostearyl alcohol embedded in a liquid crystalline network. Bar = 25 μm.

paraffin are shown in Fig. 2. The oil droplets can be easily recognized by their near spherical shape (Feature A) compared to the excess cetostearyl alcohol particles which tend to be polyhedral in shape (Feature B). Both the emulsions and gels contain a microstructure consisting of what can be best described as an 'onion-ring' lamellar phase, presumably the lyotropic liquid crystalline phase referred to by Barry and Saunders (1970). It is interesting to note that both the oil droplets and cetostearyl alcohol particles act as nuclei for this liquid crystalline phase. Fig. 3 shows the effect of increasing concentration of cetostearyl alcohol on the structure of the ternary gels. Fig. 3a is a photomicrograph of a 1.0% w/w cetostearyl alcohol formulation and is only beginning to show features (Feature A) normally seen in formulations with higher concentrations of cetostearyl alcohol. The liquid crystalline phase also tends to become more obvious at approximately 2.5% w/w cetostearyl

Fig. 4. Freeze-etch electrograph of the 1% w/w cetostearyl alcohol ternary gel (T_3). Note the marked vesicular structure and features such as A which are believed to be due to excess cetostearyl alcohol. Bar = 2 μm.

alcohol, while at concentrations in excess of 4.0% w/w, additional cetostearyl alcohol appears to act simply as nuclei for the liquid crystalline phase.

Freeze-etch electron microscopy

Typical electrographs of the ternary gel systems (Figs. 4–7) clearly parallel that seen in the optical micrographs but reveal more detail. At cetostearyl alcohol concentrations of 1.0% w/w and below, the system is predominantly vesicular in structure (Fig. 4) and as the concentration is increased the vesicles become more closely packed and begin to coexist with multi-layered (presumably liquid crystalline) structures (Figs. 5 and 6). At cetostearyl alcohol concentrations in excess of 2.5% w/w the multilayered structures are apparently nucleated by polyhedral shaped particles (Figs. 6 and 7) presumably the excess cetostearyl alcohol seen in the optical micrographs.

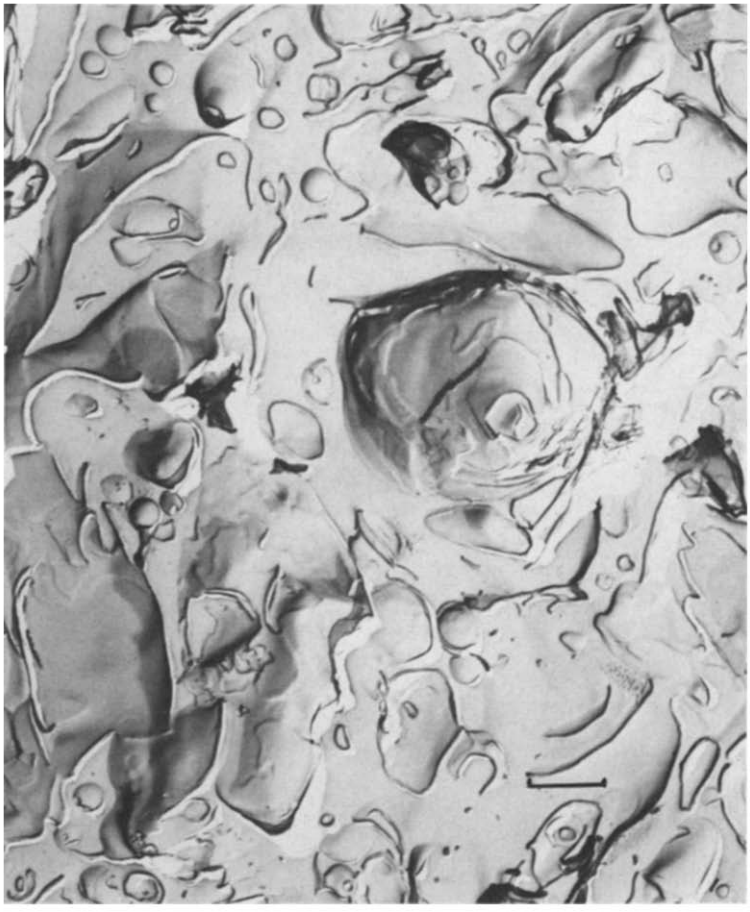

Fig. 5. Freeze-etch electrograph of the 2.5% w/w cetostearyl alcohol ternary gel (T_4). Bar = 2 μm.

Due to the likely importance of the volume fraction of the internal, i.e. the vesicular phase, in determining the physical properties of the ternary gels, this was calculated from the micrographs using standard stereological methods (Underwood, 1970; Stewart and Sutton, 1984b). It can be seen from Fig. 8 that the internal phase volume rises from a few percent at 0.25% cetostearyl alcohol to approximately 65% at 2.5% w/w cetostearyl alcohol where the system is becoming more close packed. For the same range of cetostearyl alcohol concentrations only a modest rise in mean vesicle diameter (0.5–1.0 μm) was observed.

For the emulsion systems the behaviour is only marginally more complex than seen in the ternary systems (Fig. 9). By far the most important phenomenon is the tendency for the oil droplets to act as nuclei for the liquid crystalline phase, confirming the interpretation of the optical micrographs.

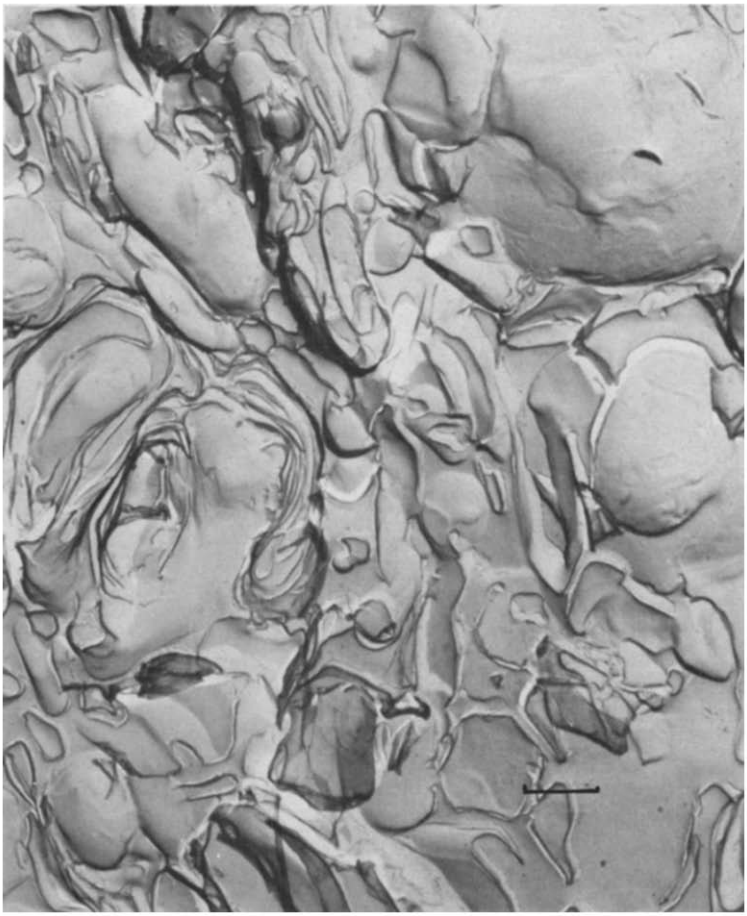

Fig. 6 Freeze-etch electrograph of the 4.0% w/w cetostearyl alcohol ternary gel (T_5). Note the vesicular structure coexisting with the excess cetostearyl alcohol and liquid crystalline network.

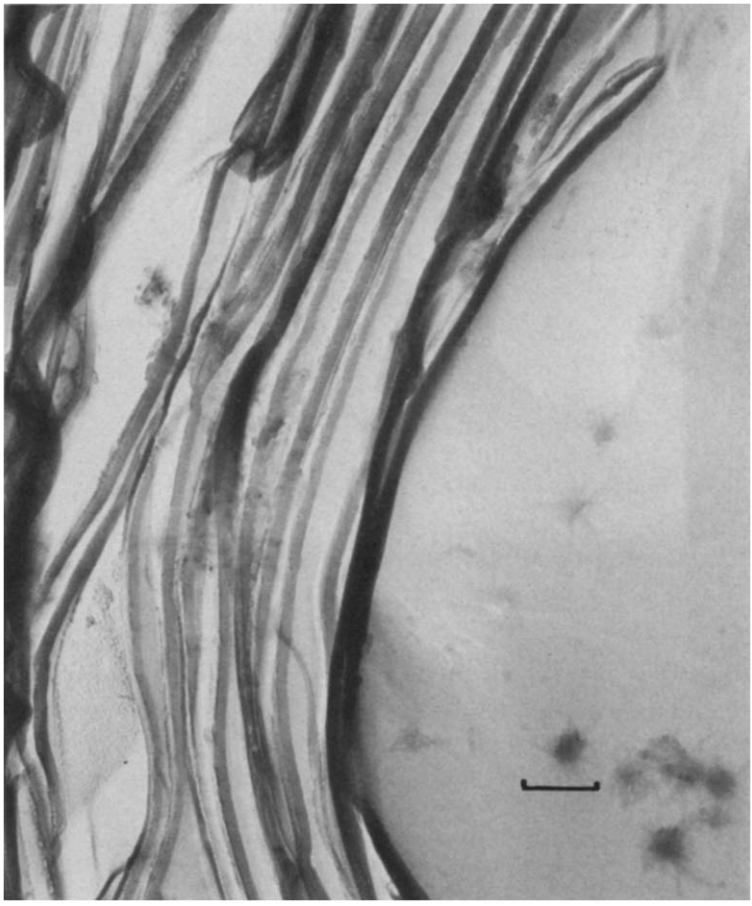

Fig. 7. High magnification electrograph of an apparent liquid crystalline gel region in the 4.0% w/w cetostearyl alcohol ternary gel (T_5). Bar = 0.3 μm.

Discussion

Evidence from the microscopical techniques suggests that in the ternary gel systems there is a gradual change from a vesicular structure to one consisting of a liquid crystalline phase largely localized around nuclei of excess cetostearyl alcohol particles. In all cases the volume included by the vesicles is many times that occupied by the nominal 'solids' content of the systems, close packing of the vesicles being reached at approximately 2.5% w/w cetostearyl alcohol. Presumably this phenomenon is responsible, at least in part, for the bodying effect in the system studied. The structure of the emulsion systems is very similar to that of the ternary gels with the oil droplets providing additional nuclei for the liquid crystalline phase.

The structures of the kind observed in these systems appear far from uncommon in other colloidal systems. Friberg (1979) for instance has attributed the exceptional

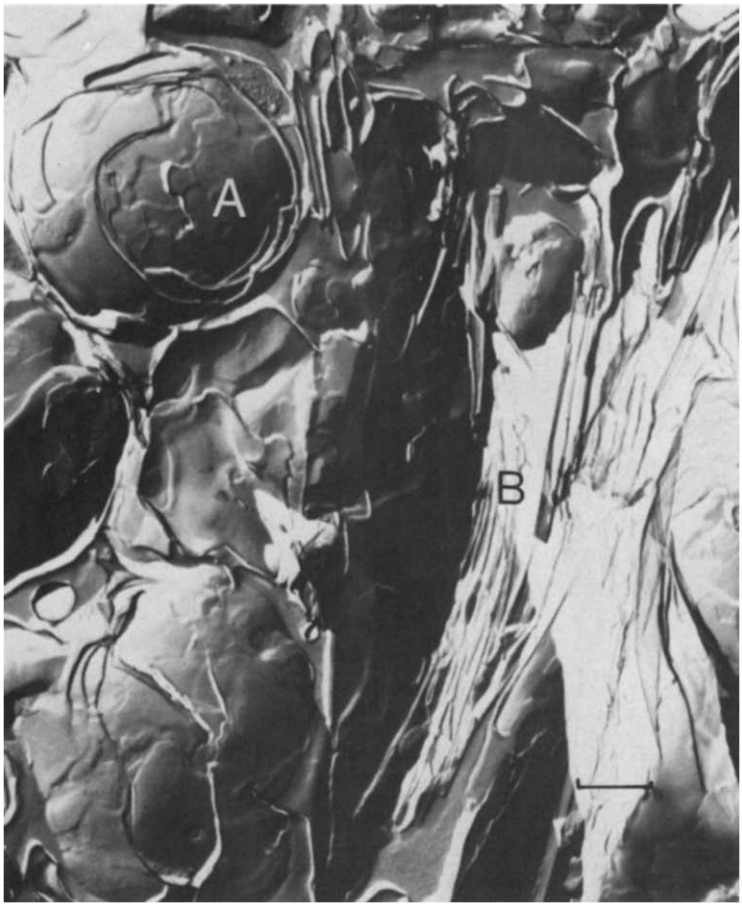

Fig. 8. Freeze-etch electrograph of an emulsion (E_3) containing 20% oil. Note the oil droplets (Feature A) and the lamellar liquid crystalline network (Feature B). Bar = 2 μm.

colloid stability of certain cosmetic creams to the formation of stabilising liquid crystalline layers around the droplets of the dispersed phase. Using freeze-etch electron microscopy Junginger et al. (1981) and Junginger and Heering (1983) have shown ordered gel like regions in pharmaceutical creams containing cetostearyl alcohol and a surfactant. However, there was no evidence for nucleation of the liquid crystalline phase around the excess fatty alcohol or oil droplets as observed in these systems. The vesicular structures are also not dissimilar to those observed by James and Heathcock (1979) on complex lyotropic systems.

Supporting evidence for the structural model of these systems is provided by conductivity measurements. Fig. 10 shows the effect of cetostearyl alcohol concentration on the specific conductivity of the ternary gel systems. The large decrease in conductivity over the cetostearyl alcohol concentration 0–1.0% w/w is consistent with the rapid increase in internal phase volume of the vesicles with the consequent

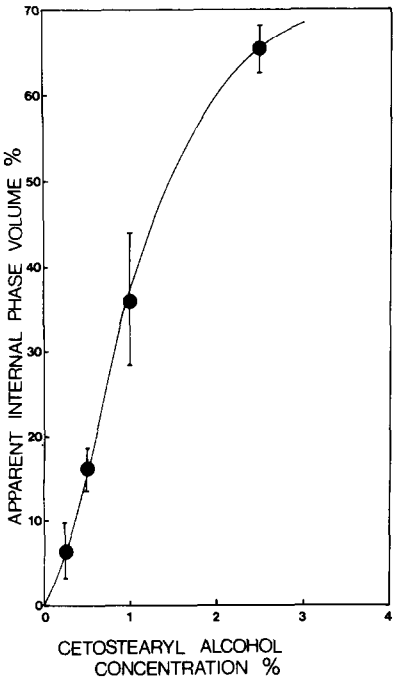

Fig. 9. The effect of cetostearyl alcohol concentration on the apparent internal phase volume (as % of total) for the ternary gel formulations.

occlusion of the continuous phase. The more gradual decrease in conductivities at higher concentrations is due to the increasing tortuosity of the conductive pathways as the liquid crystalline network becomes nucleated around the excess cetostearyl alcohol particles. A change in overall structure of the ternary gel systems of cetostearyl alcohol concentrations in excess of 2.5% w/w (ratio of 5 : 1 relative to the weight of cetrimide) has also been implicated from reflectance measurements (Rowe and Patel, 1985).

The overall conclusion of this work is that observed structure in our systems suggests a morphology that is more ordered than previously suggested (Barry and Saunders, 1970). This may be due, in part at least, to the specific conditions used to prepare the ternary gels and emulsions, viz. the prolonged heating at 80°C before cooling and homogenization, since this method is somewhat different from that used by Barry and Saunders (1970) and other workers who crash cool immediately on addition of the two phases. However, we would emphasize that the preparative conditions used in this study were chosen to provide an analogue of a large scale manufacturing process and hence are no more extraordinary than used by previous workers.

Currently we are using the approach described here to investigate all the various aspects of the physical chemistry of these systems especially the effect of preparative conditions and thermal stability.

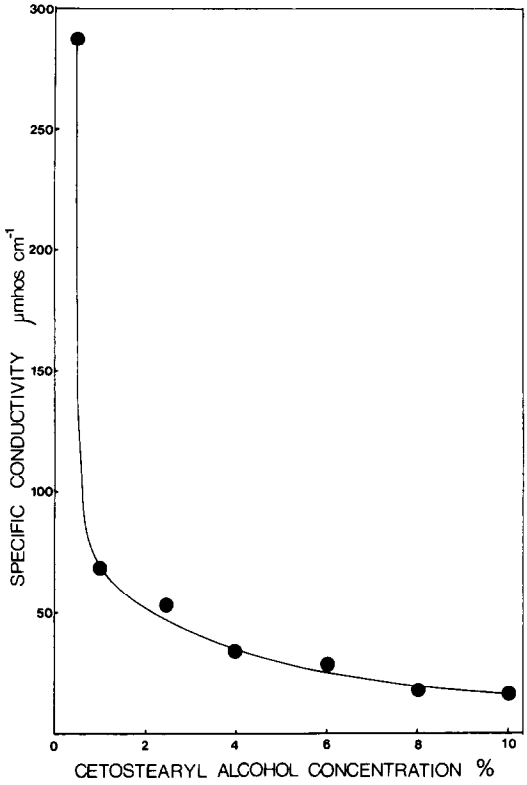

Fig. 10. The effect of cetostearyl alcohol concentration on the specific conductance for the ternary gel formulations.

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