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An investigation of the structural changes occurring in a cetostearyl alcohol/cetrimide/water gel after prolonged low temperature (4 °C) storage

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Structural changes in a ternary gel prepared using the mixed emulsifier system of cetrimide and cetostearyl alcohol after prolonged low temperature (4 °C) storage have been studied using freeze-etch transmission electron microscopy and other techniques. The system changed from an opaque smooth gel of high viscosity, low conductivity and low free water, to a pearlescent milky lotion of low viscosity, high conductivity and high free water. Subsequent equilibration of the thinned system to room temperature (25 °C) over 48 h produced an opaque granular gel of similar consistency, but slightly higher conductivity and higher free water than the initial sample. Microscopical examination by both differential interference contrast and freeze-etch electron microscopy showed the system changed from one consisting of a liquid crystalline network localized around cetostearyl alcohol particles, to a system consisting of large waxy plates coexisting with some residual liquid crystalline network. A supportive mechanism for the thinning of the ternary gel at prolonged low temperature storage has been inferred by comparing data with that produced by other workers studying the fusion of phospholipid membranes considered to be morphologically similar to the liquid crystalline network observed in this ternary gel.

The mixed emulsifier system of cetostearyl alcohol and cetrimide is used extensively in the preparation of pharmaceutical creams. The stability of these creams especially when stored at low temperatures for prolonged periods is of particular importance and much work has been done in this area specifically in determining the optimum properties of the cetyl and stearyl alcohols (the two most common fatty alcohols in the cetostearyl alcohol) necessary for good stability (Eccleston 1976; Fukushima & Yamaguchi 1983). However, apart from subjective reports that the creams become fluid and that platey crystals are formed leading to a pearlescent sheen, little work has been done on the actual structural changes that occur when these systems become unstable (Mapstone 1972; Fukushima et al 1977).

We have studied the structural changes that occur in a system consisting of a commercially available synthetic blend of cetostearyl alcohol, cetrimide and water when stored for prolonged periods at low temperature and subsequently equilibrated to room temperature in an attempt to define the mechanism for the instability.

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Materials and methods

All the materials used were of Pharmacopoeial grade. The cetostearyl alcohol consisted chiefly of cetyl alcohol (52·2%) and stearyl alcohol (40·6%) with minor amounts of C_{12} (0·21%), C_{14} (0·3%), C_{17} (0·2%) and C_{20} (0·1%) long chain alcohols (determined by gas chromatography as described by Patel et al 1985a).

A ternary gel containing 0·5% w/w cetrimide, 0·08% w/w propyl hydroxybenzoate and 10% w/w cetostearyl alcohol was prepared by the following method: Cetostearyl alcohol at 80 °C was dispersed in an aqueous solution of cetrimide and propyl hydroxybenzoate at the same temperature and stirred gently with a paddle stirrer for 1 h before being allowed to cool to approximately 60 °C. The dispersion was then homogenized using a Silverson multipurpose high speed mixer until the setting point of the gel was reached. The gel was then allowed to cool to room temperature and samples stored at both 25 and 4 °C.

Visual assessment of the gel was carried out using differential interference contrast microscopy and freeze-etch electron microscopy (Patel et al 1985b). The conductivities of the samples of gel stored under different conditions were determined at their respective storage temperature using a simple conductivity cell in the form of a probe coupled to a Universal Autobalance Bridge (Wayne Kerr, Type B642). Viscosity data were generated using a vibrational reed type rheometer (Bendix Ultraviscoson, Model 1800) at temperatures similar to those above. This is a non-destructive method of measuring the consistency of such systems relying on a special magnetorestrictive alloy blade vibrating at

28 kHz with an amplitude of 0·5 μm. The output from the instrument is given in terms of the product of the viscosity and density of the system, i.e. mPas gcm^{-3} (Roth & Rich 1953).

The amount of free water (as opposed to that bound up in the lyotropic liquid crystalline phase) expressed as a percentage of the total weight was evaluated using ultracentrifugation. 4·5 g of a sample of gel stored under different conditions was centrifuged at 150 000*g* for 3 h using an ultracentrifuge (Beckman, Model L5—65B) and the amount of free water (the bottom layer) determined by weighing. The concentrated sample of gel (the top layer) obtained by ultracentrifugation was analysed using differential scanning calorimetry (Perkin Elmer DSC 4) by heating 8–12 mg of sample at a rate of 1 °C min^{-1}.

Results

Changes in the properties of the ternary gel on storage are shown in Table 1. It can be seen that the system changed from an opaque smooth gel of high viscosity, low conductivity and low free water to a pearlescent milky lotion of low viscosity, high conductivity and high free water. Subsequent equilibration of the thinned system to room temperature over 48 h yielded changes in the conductivity and viscosity as shown in Table 1 and Fig. 1. The conductivity changes were similar to that seen on temperature cycling (Rowe & Patel 1985).

Table 1. Changes in the properties of the ternary gel on storage.

	Storage condition		
Property	A Initial	B 10 months 4 °C	C B equilibrated to 25 °C
Appearance	Opaque smooth gel	Pearlescent milky lotion	Opaque granular gel
Specific conductivity (μmhos cm^{-1})	15·3	46·5	27·8
Viscosity × density (mPas × gcm^{-3})	260	28	283
Free water content (% w/w)—determined by ultracentrifugation	23·8	78·4	42·2

Examination of the ternary gel by differential interference contrast microscopy showed a change from a system consisting of a liquid crystalline network localized around cetostearyl alcohol particles (Fig. 2A) to a system showing little evidence of a liquid crystalline network (Fig. 2B, 4 °C for 10 months) though a few cetostearyl alcohol particles surrounded by the ringed network were still visible. However, examination of the thinned system by freeze-etch electron microscopy (Figs 2C–E) showed that the system consisted of large waxy plates with some evidence of a more ordered network in places (Fig. 2E). Subsequent equilibration of the thinned ternary gel to room temperature resulted in some reforming of the original structure (Fig. 2F) resulting in an opaque granular gel of similar consistency but slightly higher conductivity and higher free water than the initial samples.

DSC curves of the top layers of the ultracentrifuged samples of the three gels showed a change from a system (room temperature sample) with a single endothermic peak at 54·7 °C (range 33·8–59·8 °C) to one (thinned sample) with two peaks at 54·3 °C (range 43·6–59·1 °C) and at 28·6 °C (range 24·4–31·9 °C) back to one (equilibrated sample) with a single peak at 53·7 °C (range 45·0–56·8 °C). Ultracentrifugation does not appear to alter the structure of the gel network.

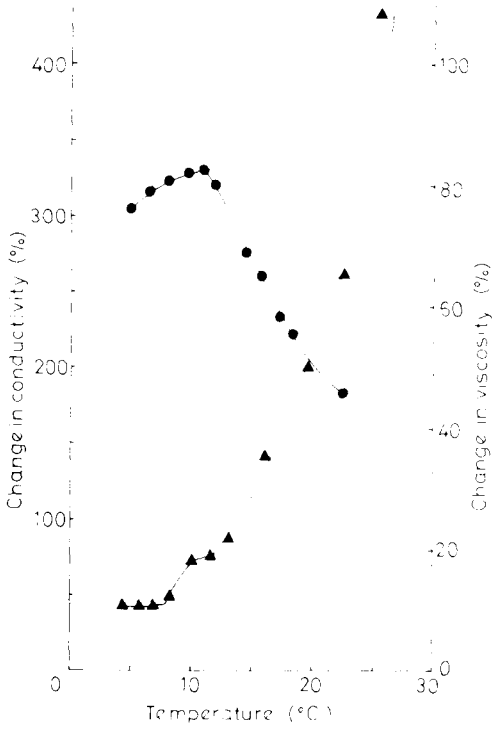

FIG. 1. The effect of re-equilibrating the thinned gel to room temperature on (●) the conductivity and (▲) the viscosity. Both expressed as a percentage of the initial sample.

Discussion

The presence of large plate like crystals has long been associated with the instability and thinning of emulsions prepared using a mixed emulsifier consisting of a long chain fatty alcohol and surfactant (Barry 1968, 1970; Mapstone 1972). However, at that time, there were two schools of thought regarding the possible reasons for the instability. Mapstone (1972) postulated that crystallization resulted in there being insufficient alcohol left to stabilize the oil droplets, while Barry (1970) suggested that thinning was due to the destruction of the liquid crystalline network and that the main driving force for this was the crystallisation of the network either as free alcohol or as a mixed crystal of alcohol combined with the surfactant. A more plausible expla-

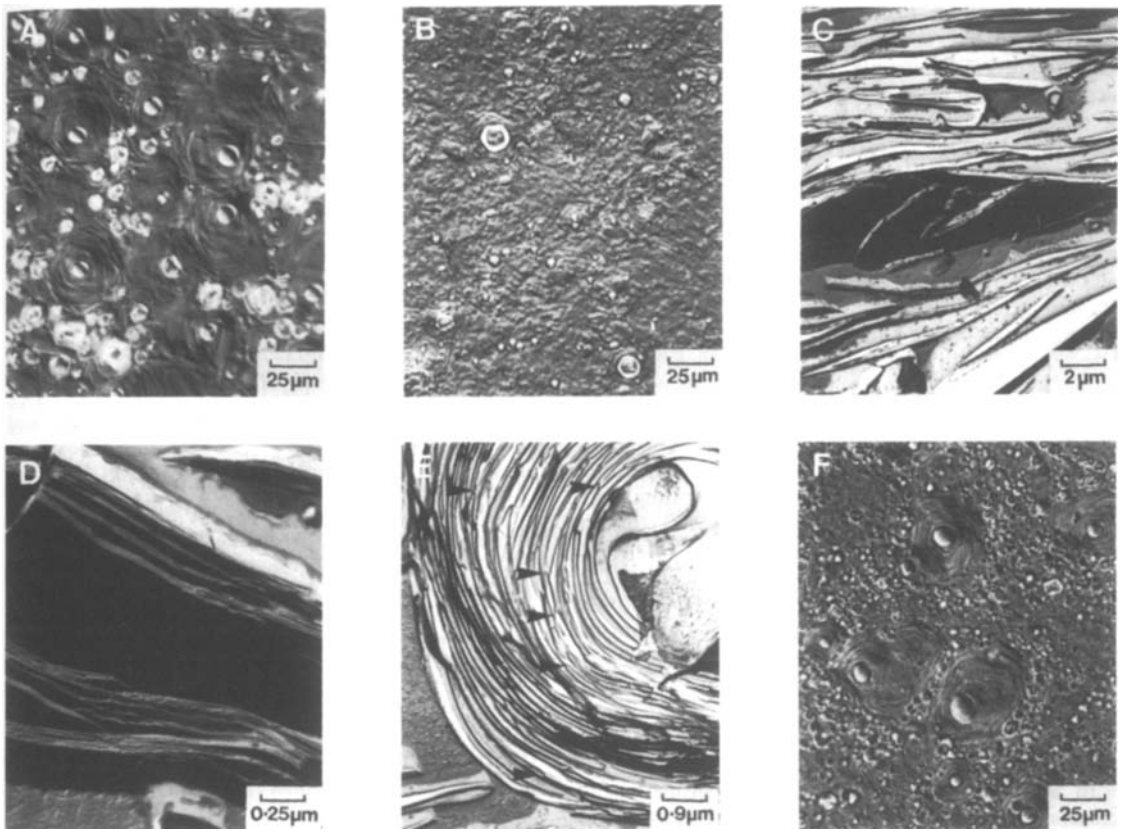

FIG. 2. A, differential interference contrast photomicrograph of the initial ternary gel; B, the sample stored at 4°C for 10 months; C, freeze-etch electron micrograph of the sample stored at 4 °C for 10 months; D, showing large platey crystals; E, showing evidence of residual liquid crystalline network and bilayer defects ▶; F, differential interference contrast photomicrograph of the thinned gel re-equilibrated to room temperature.

nation based on more recent investigations (Fukushima et al 1977; Junginger 1984) is that the double layers of the gel phase approach one another releasing water while at the same time changing their crystalline form (Eccleston 1984). The data generated in this study, i.e. the large platey crystals seen in Fig. 2D, E, accompanied by a decrease in the presence of a liquid crystalline network around the cetostearyl alcohol and a change in the DSC of the fatty alcohol itself, indicating the presence of a different mesomorphic form in the thinned gel, tend to confirm this hypothesis but unfortunately does not imply a mechanism as such.

A mechanism for thinning can be inferred by comparing the data with that produced by other workers studying the fusion of phospholipid membranes (Hui et al 1981). Phospholipid bilayer lamellae are morphologically similar to the liquid crystalline network in the ternary gel and would appear to behave in a similar fashion, i.e. they are unstable during freeze thaw cycles resulting in dehydration and fusion. The fusion mechanism proposed by those workers relies on the coincident effects of local dehydration and the formation of point defects or dislocations leading to bilayer disruption. The presence of dislocations in the lamellar liquid crystalline region in the thinned gel, Fig. 2F (these are classic examples of edge dislocations in smectic liquid crystals—de Gennes 1974) would appear to lend credence to the hypothesis that a similar mechanism can be applied to the thinning of the gel. Such a mechanism may be proposed as follows. In the ground state for a lamellar liquid crystal there is a constant layer thickness with no twisting or bending of the director (de Gennes 1974). If a strain is imposed on such a system, for example as a result of cooling, then the layers tend to contract and distortions and undulations occur. These systems are metastable and in time the layers tend to move through the formation of dislocations. Further strain imposition by further cooling or local dehydration will tend to produce even more dislocations leading to total fusion of some of the bilayers and the formation of large plates.

Although this mechanism is speculative, it is supported by further observations. Firstly, it can be seen that neither the surfactant nor any other ingredient plays a

primary role in the fusion mechanism [Laser Raman spectroscopy has confirmed that the liquid crystal bilayers are predominantly, if not totally, cetostearyl alcohol (Louden et al 1985)], and hence thinning will occur in any such system, a fact reported by other workers (Mapstone 1972; Fukushima & Yamaguchi 1983). (Polyethylene glycols promote membrane disruption and fusion in phospholipid bilayers (Knutton 1979; Boni et al 1981) and hence should be avoided in cream formulations.) Secondly, theoretically the process will be more rapid where there is a more extended random liquid crystalline network rather than the 'closed' more ordered network localized around the cetostearyl alcohol particles as in the gel prepared in this study. Evidence in our laboratories that the thinning process is more rapid in gels and emulsions that have first been cycled between −2 and +2 °C for four weeks before storage would support this since such cycling results in the production of a more extended randomly orientated gel network (Rowe & Patel 1985). Such a mechanism does not preclude the reformation of the gel network on equilibrating the thinned gel at 25 °C. The similarity of the conductivity curves (Fig. 1) with those reported for a single freeze/thaw cycle would support the mechanism of reformation as previously proposed (Rowe & Patel 1985). Such a mechanism would occur more rapidly than the fusion process hence the relatively rapid re-thickening seen in practice (Junginger 1984).

In a recent paper on the colloidal properties of creams, Junginger (1984) intimated that problems of their stability, including the ageing processes, could be resolved when the exact facts of the structure elements are known. Freeze fracture of freeze-etch transmission electron microscopy is an advanced method that allows the observation of the structural changes that can occur in such systems and this method combined with others such as laser Raman spectroscopy (Louden et al 1985), thermogravimetry (Junginger et al 1984) and low angle X-ray diffractometry (Fukushima et al 1977) should enable the mechanisms to be elucidated.

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