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# Low-temperature scanning electron microscopy of the phase inversion process in a cream formulation

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## Summary

The microstructure of a modified cetomacrogol cream B.P. has been studied using a cryo-SEM technique. Etching of the aqueous phase was used to determine the nature of the continuous phase. A phase inversion from w/o to o/w occurs during the cream manufacture. Additionally, the technique has been used to monitor the structure of a cream formulation during long-term storage. A link between microstructure and emulsion stability was observed.

## Introduction

Within the pharmaceutical industry there is a continuing programme of development to improve the effectiveness of medicines, not only in the discovery and development of new active compounds but also in the improvement of the formulation vehicle stability. For one class of medicines — topicals – advancements have been focussed on investigating the complex microstructure of the ointment and emulsion bases. Many analytical methods have been used including differential scanning calorimetry, light microscopy, rheology and scanning electron microscopy (SEM) (see, for example, De Mann 1982; Junginger et al., 1981; Junginger 1984; Drzymala and Krajczyk 1985; Eccleston 1985; Eccleston and Beattie 1988; Rowe and McMahon, 1987, 1989). It is the application of this last technique to emulsion structure that this paper addresses.

Most recently, Rowe and McMahon (1989) have studied cream and gel structures using both SEM and transmission electron microscopy, the former with a specialised low-temperature technique (cryo-SEM) that enables the study of aqueous based systems which cannot be examined using conventional SEM methods. However, these and previous cryo-SEM studies have not considered the preparation of semi solids, this area having particular importance for bulk scale manufacture since minor changes in processing can alter the cosmetic and physical characteristics of the formulation base. In this paper we have examined the manufacture of a modified, commonly used

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TABLE 1

*Formulae of cetomacrogol creams and reference samples (expressed as % w/w)*

| Formulae | Sample A | Sample B | Modified cetomacrogol cream B.P. | Cetomacrogol cream B.P. |
|---|---|---|---|---|
| Cetomacrogol 1000 | 6.2 | 5.5 | 3.6 | 1.8 |
| Stearyl alcohol | 28.2 | 25.2 | 16.4 | 7.2 [a] |
| White soft paraffin | – | – | – | 15 |
| Liquid paraffin | – | 64.4 | 41.82 | 6 |
| Phenoxyethanol | 1.7 | 1.5 | 1 | 1 [b] |
| Water | 60.2 | – | 35 | 69 |
| Active drug | 3.7 | 3.4 | 2.18 | – |

[a] Cetostearyl alcohol.

[b] Chlorocresol used as preservative.

*NB*: Sample A and sample B components in same ratio as modified cetomacrogol cream B.P. formula – normalised to 100%.

pharmaceutical cream base — cetomacrogol cream B.P. Particular attention was paid to the cream formation process when the B.P. formula was modified by reducing the water content (by some 50%) and increasing the surfactant level from 1.8 to 3.6% w/w; the rationale behind these changes being the likely improved stability of water labile drugs within this base. In order to simplify the formula further, the white soft paraffin component of the B.P. formula was substituted with light grade liquid paraffin (see Table 1). In addition, a further extension of the applications of the cryo-SEM technique was sought when the ageing of cream samples was monitored over $1\frac{1}{2}$ years in order to assess the suitability of this method for long-term storage testing of emulsions.

## Materials and Methods

*Materials*

Cetomacrogol 1000 (Croda, Bx.11143.U.K.), stearyl alcohol (Henkel Chemicals, Bx.2925097, F.R.G.), liquid paraffin (Castrol Bx. 0839/3 U.K.), phenoxytol (Nippa Laboratories Bx. 56, U.K.) distilled water and active drug (Beecham Pharmaceuticals, Worthing, U.K.) were used.

*Sample composition*

Table 1 lists the formulae of the cetomacrogol creams and reference samples.

*Sample preparation*

The modified cetomacrogol cream and samples A and B were made using a small-scale (1 kg) cream mixer (Beecham Research Division Workshop, Worthing). Heating and cooling of the various components was by means of a water jacket around the vessel. The mixer had stirring, homogenising and vacuum facilities with temperature control to $\pm 0.5\,^{\circ}$C. All batches were approx. 625 g.

*Sample A and modified cetomacrogol cream.* For sample A and the modified cream the oily components (cetomacrogol 1000, stearyl alcohol and liquid paraffin) were heated to 65 °C, the active drug added and the mixture stirred and homogenised for 10 min. Phenoxyethanol (the preservative) was then added and the mixer contents stirred/homogenised for a further 5 min. The water (preheated to 65 °C) was then added and the resulting emulsion mixed for 10 min. Sample A was then allowed to cool whereas for the modified cream a sample was taken at 65 °C and examined using the cryo-SEM technique. The cream was then cooled and on cracking, appropriate solid and liquid fractions of the cream were sampled using a preheated pipette. On further cooling the cream reformed.

*Sample B.* For this sample the four components were simply melted together, the drug added and the mixture stirred/homogenised until full dispersion was achieved; the cream was then cooled.

*Cryo-SEM details*

For each sample, approx. 2 μl was filled into a 1 mm hole in an aluminium stub mounted in a cryo specimen holder (Hexland CT1000, Oxford Instruments, Wantage, U.K.). A 1 mm hollow rivet was filled with a similar volume of cream and inverted onto the filled hole in the stub.

This assembly was frozen in subcooled liquid nitrogen (Robards and Sleytr, 1985) and then transferred under vacuum to the cryo-preparation chamber where a fracture face was produced by

knocking off the rivet against the cryo preparation stage.

In addition, an initial sample of the freshly prepared cream was taken at 65 °C into a pre-heated container. All specimen holder components were also preheated to 70 °C. Due to the lower viscosity of the hot sample a modification to the preparation method was required. The cream was transferred into a length of plastic cannula tubing, which was then inserted into the hole of an aluminium stub-specimen holder assembly, trimmed to a suitable length, and frozen and fractured as above. Etching of the specimen was performed on the cold stage of the SEM (Philips PSEM501, Pye Unicam, Cambridge, U.K.) at −80 °C as required. Prior to examination, the samples were sputter coated with gold for 2 min at 15 mA and 0.2 bar argon. Examination was normally carried out at 15 kV using a 12 mm working distance. Images were recorded on Kodak TMAX 120 roll film.

*Infrared spectroscopy*

A Perkin Elmer PE 782 infrared spectrophotometer was used to examine the composition of the liquid fraction of the cracked cream. Scanning range was 400–4000 $cm^{-1}$, with analysis on KBr discs.

## Results

*Cryo-SEM of creams*

During the preparation of this experimentally modified cetomacrogol cream an apparent phase inversion occurred, i.e. the cream cracked over the temperature range 50 °C to approx. 35 °C. Isolated samples of the two fractions of the cracked cream were examined using the cryo-SEM technique and a comparison was made with the two five-component reference samples A and B.

The oil-in-water emulsion structure of the finished cream at room temperature is shown in

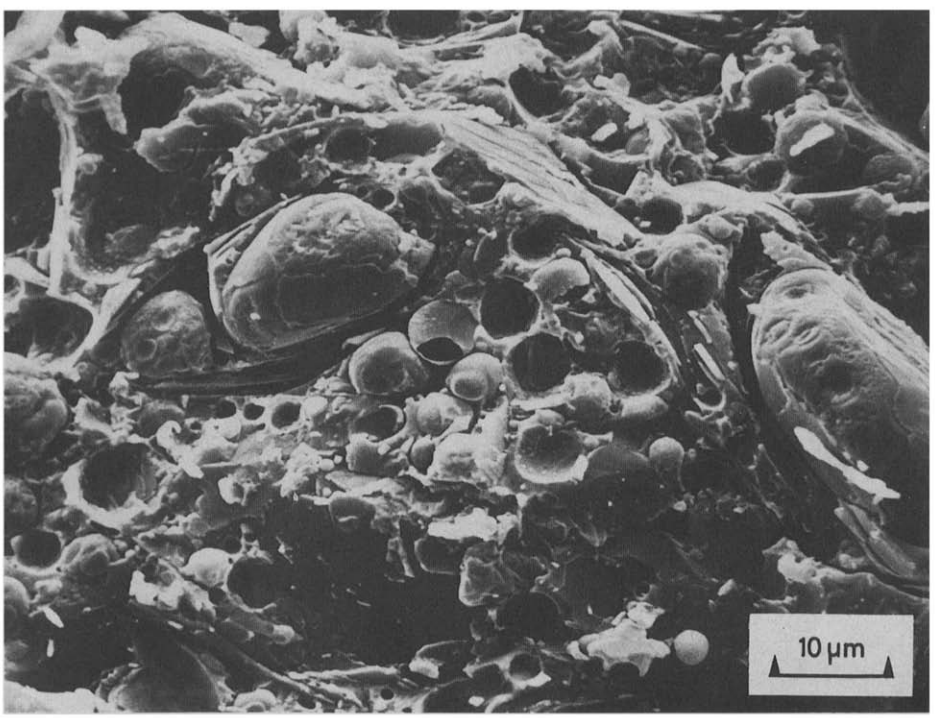


Fig. 1. Complete cream. Unetched.

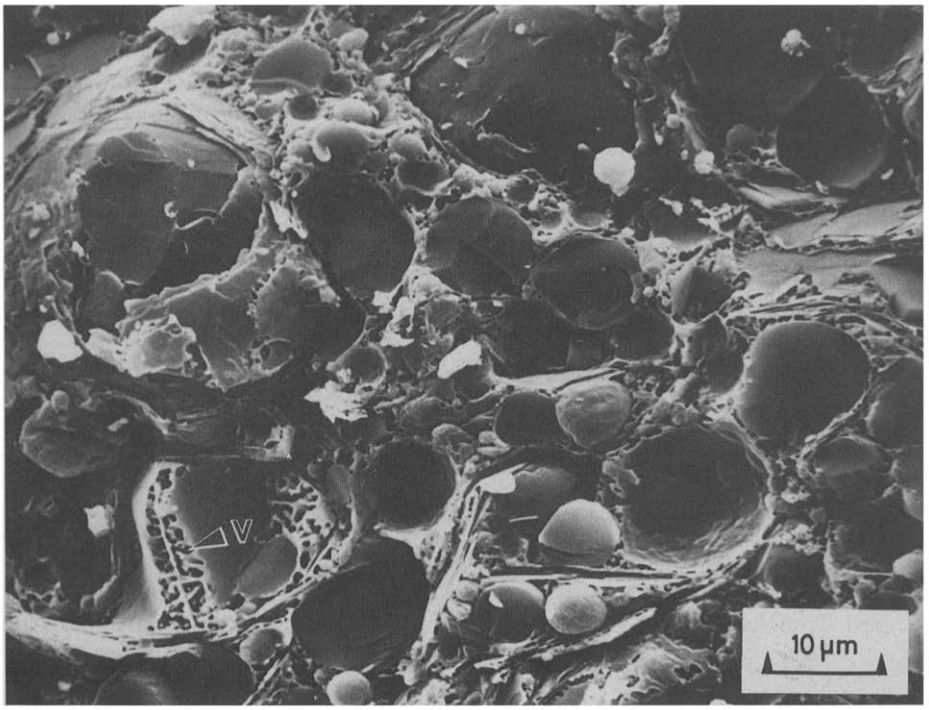


Fig. 2. Complete cream. Etched. Voids (v) reveal location of aqueous phase.

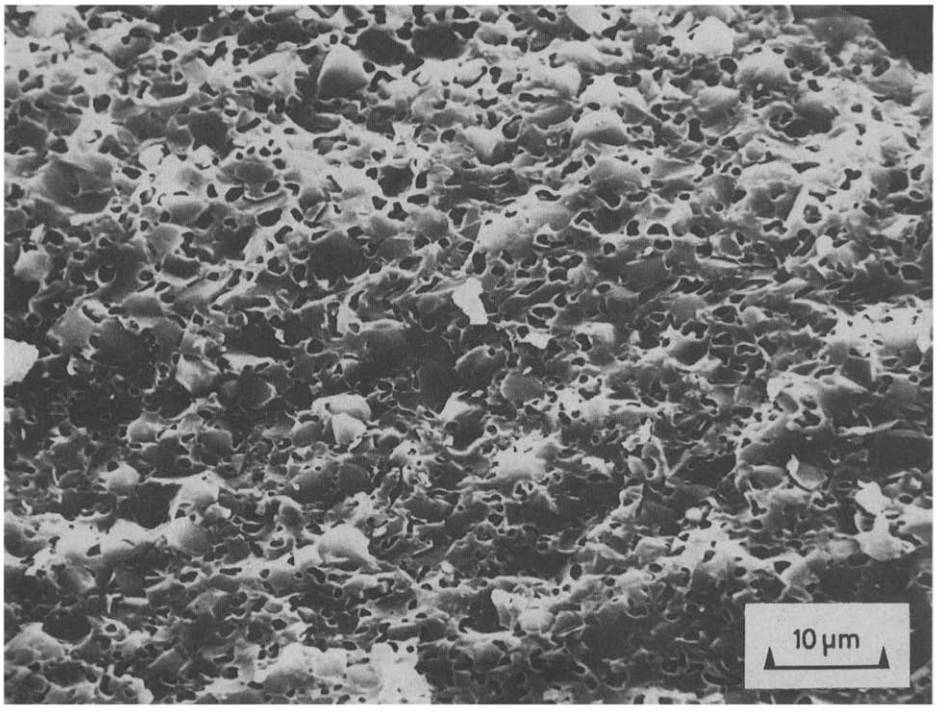


Fig. 3. Cream above cracking temperature.

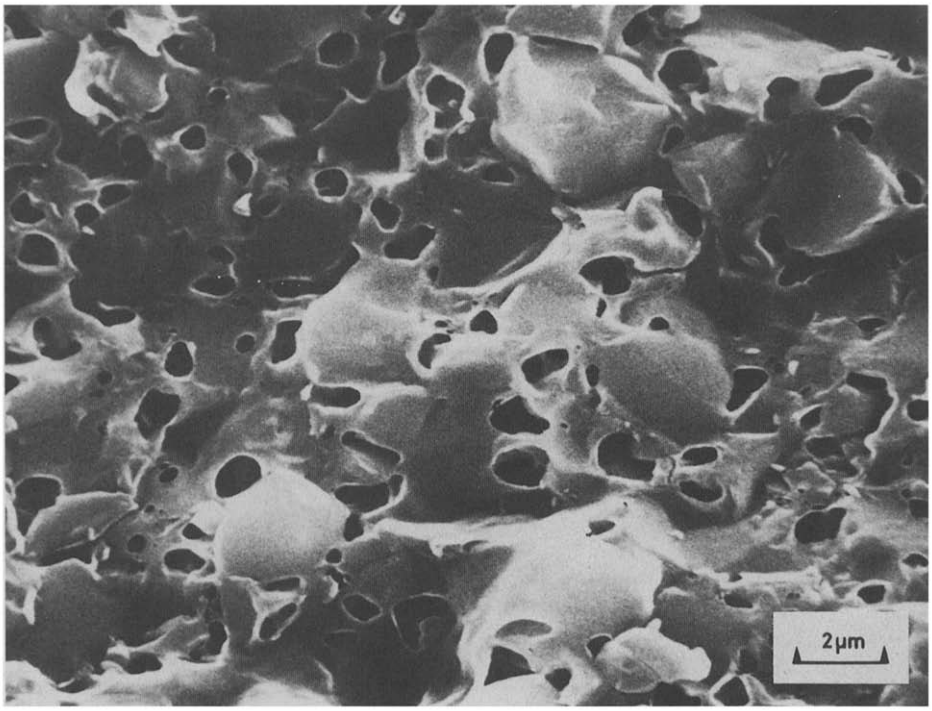


Fig. 4. Cream above cracking temperature.

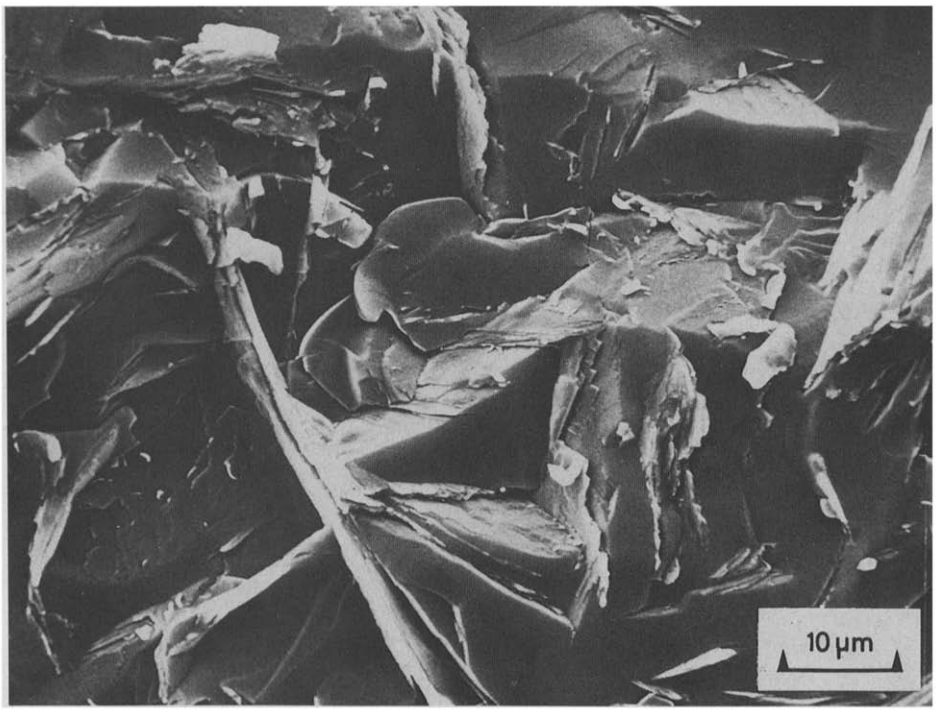


Fig. 5. Liquid phase of cracked cream.

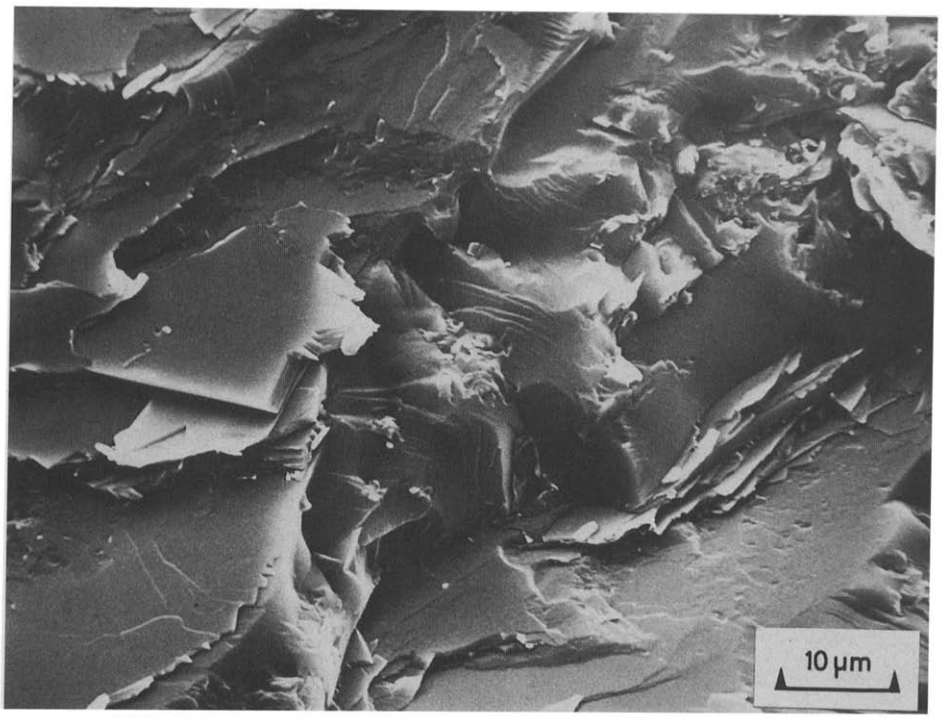


Fig. 6. Oil-based reference cream B.

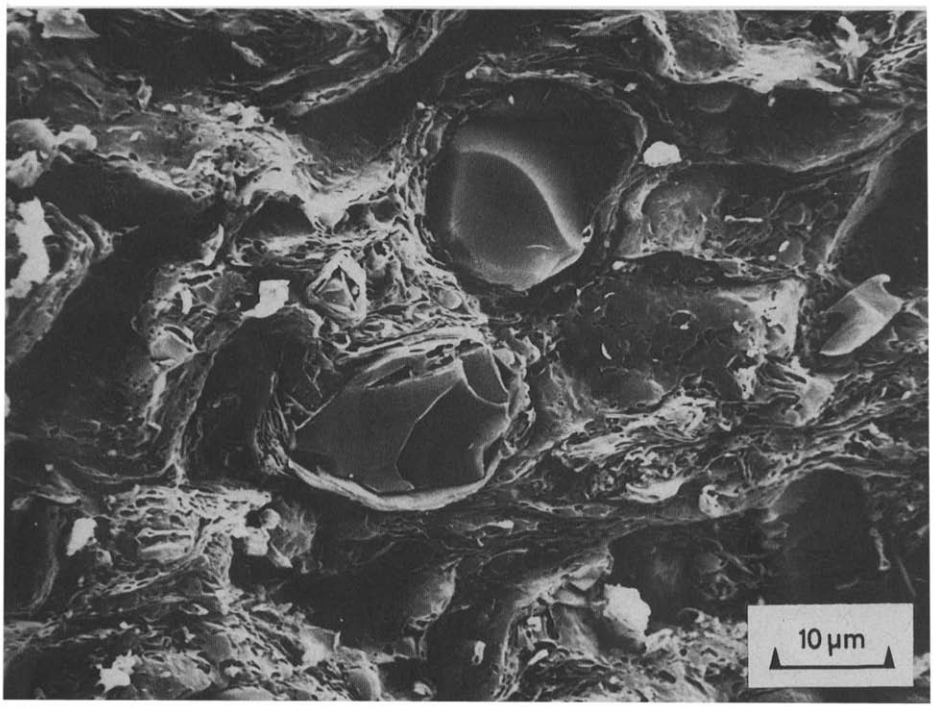


Fig. 7. Solid phase of cracked cream.

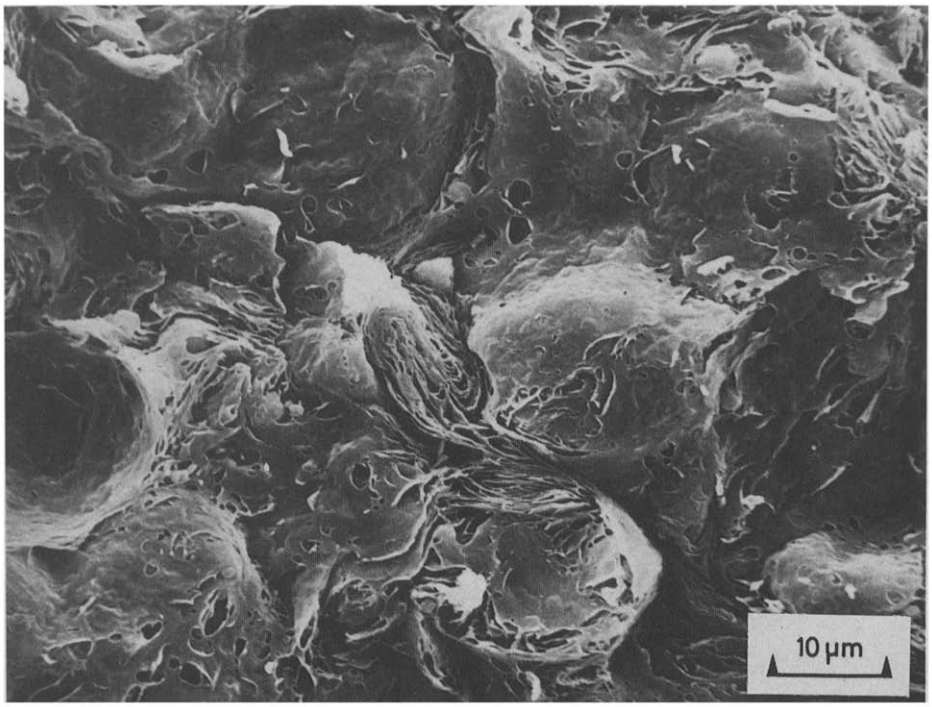


Fig. 8. Water-based reference cream A.

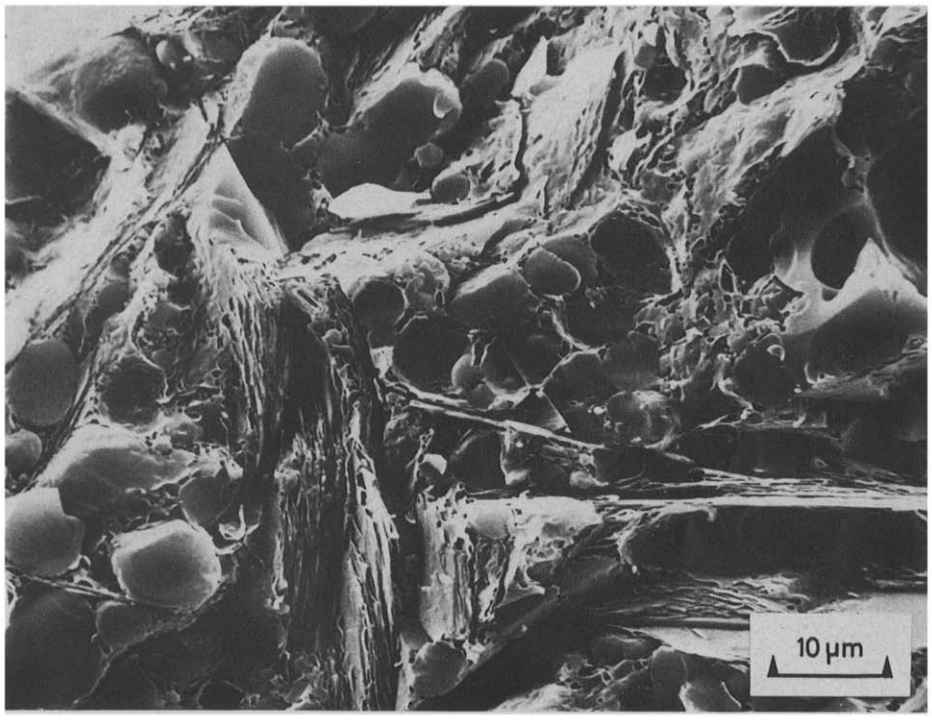


Fig. 9. Re-formed cream.

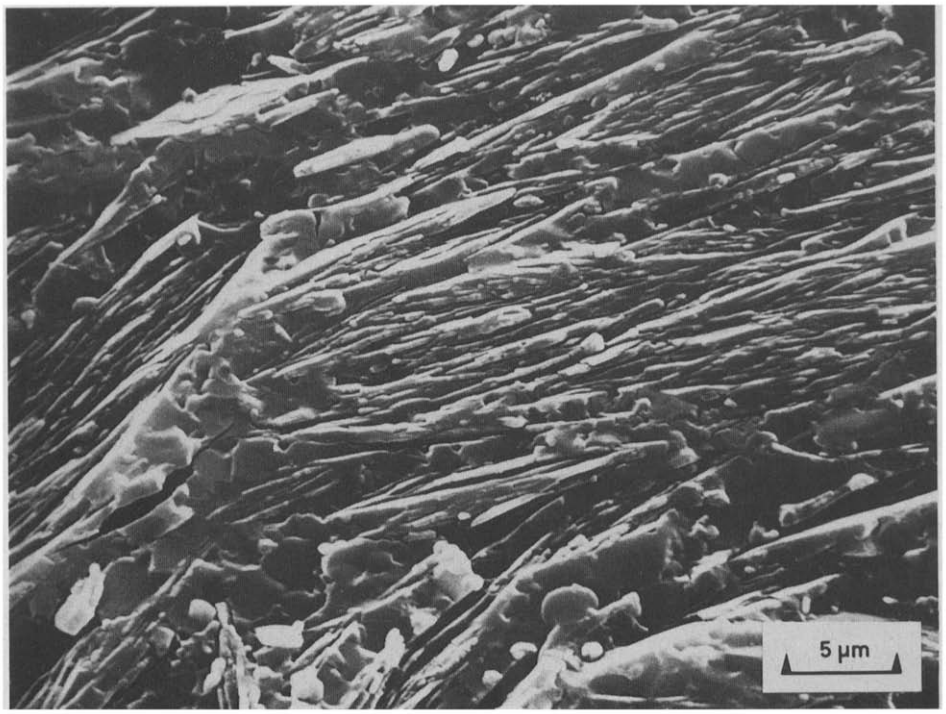


Fig. 10. Cream stored at 5 °C for 18 months.

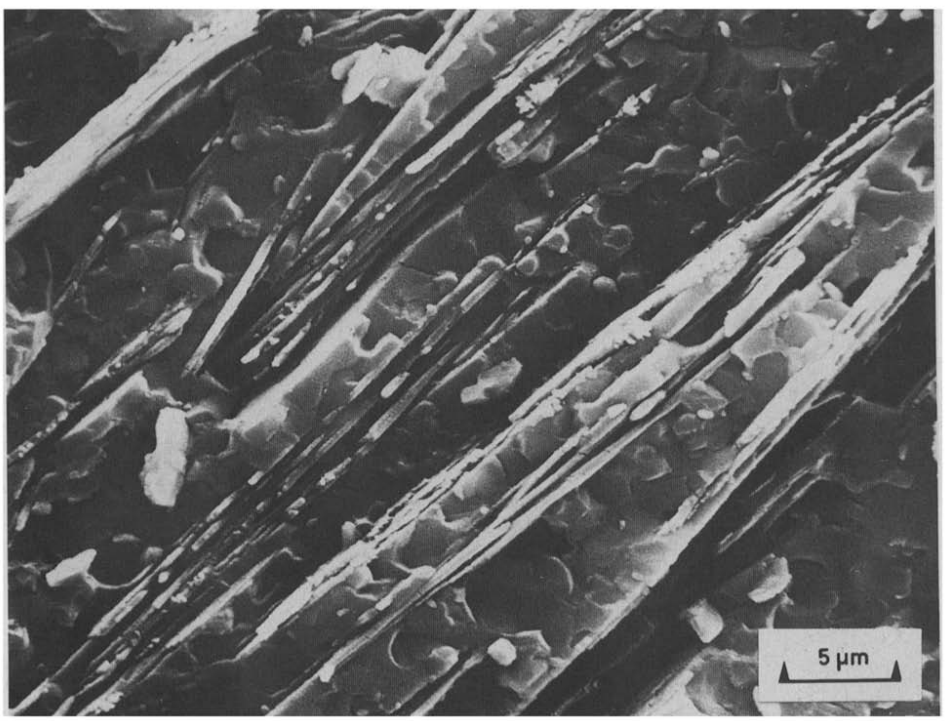


Fig. 11. Cream stored at 25 °C for 18 months.

Figs 1 and 2. Fig. 2 demonstrates the usefulness of etching the samples to reveal the location of the aqueous phase as indicated by the resultant voids (v). The aqueous phase in the finished cream can be seen to be located in the continuous phase liquid crystalline matrix.

All subsequent micrographs are of etched preparations.

The initial cream mixture sampled at 65 °C, i.e. above the cracking temperature, shows a quite different structure (Figs 3 and 4). The aqueous phase is seen to be dispersed as irregular droplets within a continuous oily matrix. This initial structure changes dramatically after cracking occurs during cooling, with two distinct components arising, viz. a solid and a liquid fraction.

A comparison of the liquid fraction with the oil-based reference cream B shows a striking similarity (Figs 5 and 6). In both cases the structure is homogeneous with no evidence of an aqueous phase after etching, suggesting the absence of water from the liquid phase of the cracked cream.

By contrast, the solid phase of the cracked cream shows a highly lamellar continuous aqueous matrix containing scattered large oil droplets (Fig. 7). This is also seen in the aqueous reference cream A which has a lamellar structure (Fig. 8), the lack of 'globules' reflecting the low oil content (35%) of this sample compared to the 60% oil composition of the modified formula.

Recombination of these two fractions occurs on continued mixing and cooling, with oil being incorporated as droplets from the liquid fraction into the solid fraction, resulting in an apparently stable complex oil-in-water emulsion (Fig. 9). However, examination of samples of such emulsions after storage for 18 months at 5 and 25 °C shows that structural changes do occur dependent upon storage temperature. The oil-in-water droplet and lamellar structure is maintained, but coalescence of oil droplets resulting in a coarser structure occurs at 25 °C which is not apparent after storage at 5 °C (Figs 10 and 11).

*Infrared spectroscopy*

The liquid fraction of the cracked cream was analysed using infrared spectroscopy. The resultant trace indicated an 88% 'paraffin' content and a 12% stearyl alcohol content. Also of note was the absence of the surfactant in representative samples of the liquid phase, implying the residence of the nonionic surfactant within the 'solid' (Fig. 7) phase of cracked cream.

## Discussion

The microstructural evidence points to the studied emulsion having inverted from being essentially a water-in-oil emulsion when formed at high temperatures (Figs 3 and 4) to being, on cooling, oil-in-water. Although Figs 3 and 4 do not show an even, homogeneous, continuous phase of oil, the sampling appears to have captured an intermediate stage of the inversion process from w/o to o/w whilst the aqueous phase is still dispersed. Careful preparation of the hot cream sample was made to ensure that the sample remained above the 'cracking' point until it was frozen in the subcooled nitrogen.

On cracking the excess oil is excluded from the non-ionic surfactant oil/water bilayers as water becomes the continuous phase. Indeed, the infrared analysis data on the excess liquid fractions indicate it to be wholly oil phase without surfactant. This excess if eventually incorporated back into the cream continuum to form the final o/w cream.

It is suggested that the apparent cracking and reformation of the modified cetomacrogol cream can be attributed to phase inversion of the emulsion as the initial cream emulsion is cooled. Shinoda and Kunieda (1983) have extensively studied this phase inversion process and have related the actual phase inversion temperature (PIT) to properties of the emulsion components such as the surfactant hydrophile/ lipophile balance (HLB) and the individual properties of the oils used in emulsion formation.

The HLB of the emulsifiers is approx. 6 (as calculated by reference to Shinoda and Kunieda (1983)), and for the emulsification of (paraffinic) mineral oil, Becher (1965) advises that the required HLB of the oil phase should be 4 for w/o emulsion or 10 for o/w, i.e. the model formula HLB lies in between and this could be an inherent

source of the instability. In addition, Shinoda and Kunieda (1983) have clearly stated that non-ionic surfactants tend to form w/o emulsions at high temperatures but revert to o/w at low temperatures, a process that has been graphically observed here.

One method of raising the observed PIT of 37 °C to an ideal value some 25–70 °C higher and hence form a more stable emulsion (Shinoda and Kunieda 1983), would be to increase the polyoxyethylene content of the non-ionic surfactant. For example, POE (23) lauryl alcohol ether (HLB 16.9) could be used. However, this surfactant is not as commonly used in pharmaceutical vehicles as POE (20) cetyl ether (i.e. cetomacrogol 1000), and may not be readily accepted.

It should be noted that a strict cooling rate of the cream was employed to limit the separation process (cf. Shinoda and Kunieda (1983)), and the improved reproducibility of the experimental cream manufacturing process has been monitored using rheology. In addition, alterations to the HLB of the surfactant components have been made.

The cryo-SEM technique has also been used to study storage temperature effects on the final emulsion stability. An increased bilayer spacing can be taken as a sign of emulsion breakdown as the temperature of storage is increased from 5 to 25 °C after an 18 month time period (Figs 10 and 11). This presumably is due to coalescence of the oil/water bilayers and dispersed oil droplets, and the fact that pure alcohols tend to form less stable emulsions than a combination of two alcohol homologues (Eccleston, 1984). It is therefore suggested that the cryo-SEM technique can help monitor long-term storage of semi-solids and is a useful extension to conventional analytical methods.

## Conclusion

This work has shown the usefulness of the cryo-SEM technique to improve understanding of the phase changes occurring in a modified version of a common pharmaceutical cream base. The micrographs appear to support the established phase inversion theories of non-ionic surfactant emulsions.

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