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# Some aggregation effects observed with an emulsion dispersed in saline containing cetrimide*

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Aggregation behaviour has been observed when an emulsion containing liquid paraffin and cetostearyl alcohol as disperse phase is diluted in saline containing cetrimide below the critical micelle concentration. This aggregation has been examined with the Coulter Counter. Under standardised conditions the apparent size of the aggregated particles is directly related to the logarithm of the molar concentration of the cetrimide present over three orders of magnitude. Above the critical micelle concentration the particles appear to be deaggregated.

EMULSIONS most frequently employed as pharmaceutical semi-solid vehicles are more strictly to be thought of as dispersions or solid-liquid suspensions. The optical size analysis of a sample of a typical pharmaceutical emulsion, cetrimide cream, B.Vet.C. 1953, exposed to ultrasonic irradiation, suggested that substantial numbers of sub-micron particles were present. This was confirmed by electron microscopy and a centrifugal method, as described by Freshwater, Groves & Scarlett (1965). Both these methods are likely to underestimate the numbers of particles in the top size range and another method was sought to confirm an optical microscope analysis. Although it is generally accepted that the Coulter Counter is of doubtful value in sizing particles below 0·5 $\mu$, the instrument has been used for emulsion systems (Higuchi, Okada & Lemberger, 1962; Wachtel & La Mer, 1962; Swift & Friedlander, 1964; Rowe, 1965) and appeared to be ideal for the system under examination.

The principle and operation of the Counter has been described by others (Coulter, 1956; Kubitschek, 1960) but any sample for analysis has first to be diluted with electrolyte. Visual examination of dilutions of the cetrimide cream formulation in a wide range of concentrations and types of electrolyte showed that much flocculation was occurring, the rate appearing to be related to the concentration of electrolyte. However, the addition of cetrimide to 0·154 M sodium chloride solution (physiological saline) gave the least flocculation. As the work proceeded it became clear that, under some conditions, aggregation could take place. The conditions enabling the Counter to be applied to the particle size analysis of other cetrimide emulsion systems have been investigated.

## Experimental

### MATERIALS

All materials were of commercial pharmaceutical quality. The cetrimide sample was a purified pharmaceutical grade consisting principally of tetradecyltrimethylammonium bromide. The critical micelle concentration (CMC) of this sample in 0·154 M sodium chloride solution was measured by a number of methods (Table 1) and taken as 0·003 M.

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TABLE 1. THE CMC OF CETRIMIDE IN 0·154 M SODIUM CHLORIDE MEASURED BY DIFFERENT METHODS (AT 25° C)

| Method | CMC (M) |
|---|---|
| Dropweight surface tension .. .. .. | 0·0015 |
| Titration Sky Blue FF[1] .. .. .. .. | 0·0025 |
| Adsorption spectrum Sky Blue FF[2] .. .. | 0·003 |

[1] Corrin & Harkins (1947).
[2] Corrin & Harkins (1946).

The emulsion under examination was a sample of cetrimide cream, B.Vet.C. 1953, which had been subjected to ultrasonic irradiation before cooling to room temperature. The resulting dispersion was allowed to age for six months before examination.

### INSTRUMENTATION

All particle counts were made on a Coulter Counter Model A (Industrial) fitted with 30 and 70 μ orifice tubes previously calibrated with polystyrene and polyvinyltoluene latices and pollens of known diameter.

### DILUTION MEDIA

Since dilution media for use with the Counter require to be substantially free of extraneous particulate contamination, a commercially available physiological saline solution for injection (Groves & Major, 1964; Groves, 1965) was employed. Quantitative dilutions of a filtered concentrated solution of cetrimide in saline were prepared as required.

### DILUTION PROCEDURE

To reduce the particle concentration of an emulsion to a suitable level for counting it is necessary to make the dilution in at least two stages, as noted by Higuchi & others (1962) and Rowe (1965). Since the CMC of cetrimide was known to be approximately 0·003 M in 0·154 M sodium chloride (Table 1) the primary dilutions were prepared in saline containing either 0·03 M or 0·0003 M cetrimide. A 50 mg sample of the cetrimide cream was accurately weighed on a watch glass and triturated with 100 mg glycerin using a camel hair brush. The dispersion was then slowly diluted with the required cetrimide-saline before making up to 50 ml. The primary dilutions were kept in plastic stoppered tubes on a slowly rocking table at ambient room temperature (24–28°) until required for analysis. This technique was found to be sufficient to prevent creaming of a diluted preparation without undue aeration.

Secondary dilutions were prepared by pipetting 1·5 ml of the primary dilution into 100 ml of the appropriate cetrimide-saline solution contained in a 150 ml beaker on the Counter orifice stand.

### COUNTING PROCEDURE

Reproducible results could not be obtained in the system under examination unless the secondary dilution was kept stirred, presumably owing to creaming and flocculation. Since the stirrer fitted to the Counter was difficult to adjust to the slow constant speed needed to avoid stirring air bubbles into a solution otherwise prone to foaming, a constant speed stirrer rotating at 187·5 rpm was used. The stirrer was started immediately after preparation of the secondary dilution and counting begun a known time after dilution, starting at the highest instrument threshold and reducing this by 0·5 $\mu$ intervals until the complete size range had been covered. Background counts were taken using the same procedure.

## Results

### PRIMARY DILUTION IN 0·03 M CETRIMIDE

Using the 30 $\mu$ orifice tube the size distribution obtained with both primary and secondary dilutions in 0·03 M cetrimide is superimposed on the results obtained by other particle sizing methods (Freshwater & others, 1965) (Fig. 1). Reasonable agreement with the optical size analysis is apparent. From number size distribution the number volume mean diameter, $d_{vn}$, can be calculated as:

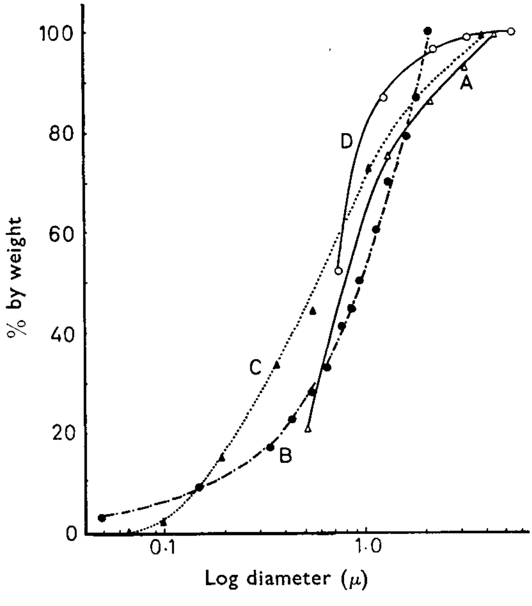

FIG. 1. The particle size distribution of a sample of cetrimide cream B.Vet.C. (1953) by different methods. A, optical microscope. B. electron microscope. C, centrifugal photosedimentometer. D, Coulter Counter.

$$d_{vn} = \left[\frac{\Sigma nd^3}{\Sigma n}\right]^{1/3} \qquad \text{(Heywood, 1963)}$$

As indicated in Fig. 1, $d_{vn}$ is 0·88 $\mu$. Making the identical analysis, but using a 70 $\mu$ orifice, the lowest limit of particle diameter detected was

1·5 μ and this gave a $d_{vn}$ value of 2·45 μ. Analyses with the 70 μ orifice tube, and using secondary dilutions of 0·003, 0·0003, and 0·00003 M cetrimide stirred for 1 hr, gave a $d_{vn}$ value of 2·4 μ in all instances. This indicates that under these conditions no significant change in the state of the dispersion has occurred.

### PRIMARY DILUTION IN 0·0003 M CETRIMIDE

It was found experimentally that aggregation was occurring in the secondary dilutions prepared from a 0·0003 M cetrimide-saline primary dispersion. A series of size distributions were obtained using the 70 μ orifice tube after 1 hr stirring in the presence of different concentrations of cetrimide. Some of these distributions are compared in Fig. 2 with the result obtained using the 30 μ orifice and 0·03 M cetrimide-saline. The apparent particle size is appreciably larger than the size indicated in Fig. 1. In addition, it will be seen that the apparent size increases as the concentration of the cetrimide present in the secondary dilution is reduced. It seemed likely that the size of aggregates was being measured by the instrument. For the purpose of direct comparison, each size distribution was expressed as a number volume mean diameter and plotted as a function of the logarithm of the molar concentration of cetrimide present in the secondary dilution, Fig. 3. The inflexion point shown in Fig. 3 corresponds to a concentration of 0·0003 M cetrimide, the concentration of the primary dilution. On a logarithmic scale there is a linear correlation between the apparent mean "particle" (or aggregate) size and concentration over three orders of magnitude. There is no evidence of an inflexion at the CMC (0·003 M).

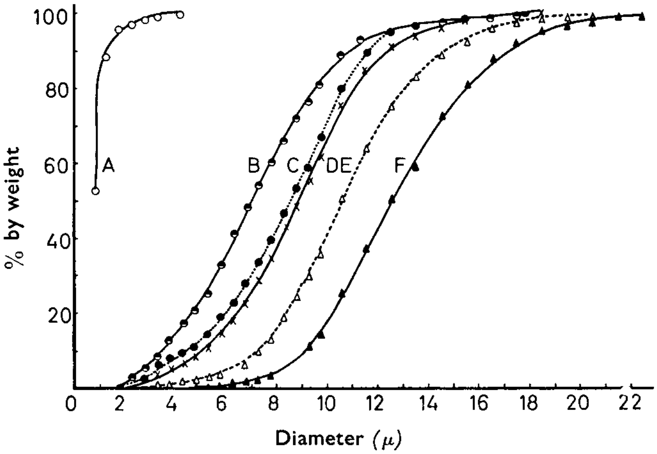

FIG. 2. The change in particle size distribution of the cetrimide cream sample with concentration of cetrimide present in the saline (Coulter Counter). A, 30 μ orifice tube, 1° and 2° dilutions 0·03 M cetrimide. B–F, 70 μ orifice tube, 1° dilution = 0·0003 M cetrimide. Cetrimide 2° dilution: B = 0·03 M; C = 0·003 M; D = 0·0015 M; E = 0·00015 M; F = 0·00003 M.

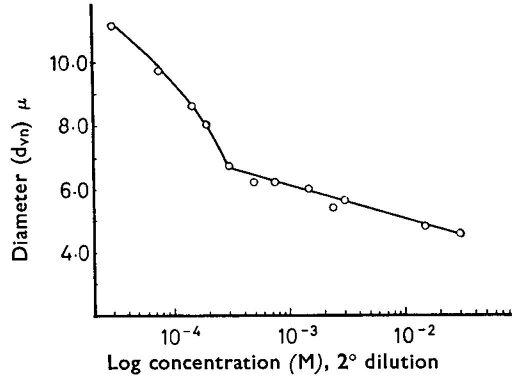

FIG. 3. The change of apparent mean diameter of emulsion particles with cetrimide concentration. 1° dilution = 0·0003 M cetrimide. 2° dilutions as shown.

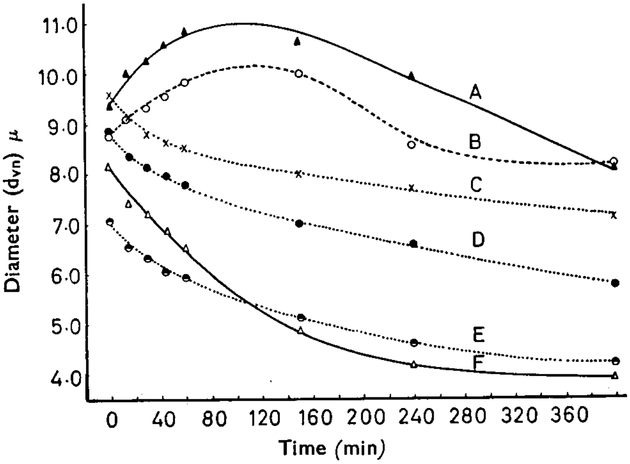

FIG. 4. The change of apparent particle size as a function of cetrimide concentration and time. 1° dilutions = 0·0003 M cetrimide. 2° dilutions: A = 0·00003 M; B = 0·000015 M; C = 0·0003 M; D = 0·00015 M; E = 0·003 M; F = 0·03 M.

The sizing technique described above required about 30 min for completion and was therefore not suited to a study of the rate at which the aggregation was proceeding. Accordingly, the procedure was abbreviated to enable an analysis to be made in 4–5 min by counting at wider size intervals. The results of this experiment are shown in Fig. 4 for dilutions maintained at constant temperature (25°) and constant rates of stirring for up to 400 min.

In all instances replicate experiments gave substantially identical results. Results shown are therefore those from one run under each set of conditions.

# Discussion

These experiments suggest that a valid estimate of the size distribution of emulsion particles greater than 0·5 $\mu$ can be obtained with the Coulter Counter if the primary dilution is made in saline containing the emulsifier above its critical micelle concentration. The mean particle size increases if the emulsifier is below the CMC, indicating that aggregation is taking place. Rowe (1965) has independently described the use of electrolyte containing anionic or non-ionic emulsifiers. No reason was given for this but he does not appear to have encountered aggregation. On the other hand, Higuchi and his associates (loc. cit.) found that aggregation of hexadecane emulsions and polymer latices occurred in concentrations of sodium lauryl sulphate and dioctylsodiumsulphosuccinate above the CMC and not below. Using the creaming rate as an indication of aggregation, Cockbain (1952) reported that deaggregation took place below the CMC of soap-stabilised emulsions. Higuchi, Rhee & Flanagan (1965) also noted that a small amount of myristyl-$\gamma$-picolinium chloride increased the aggregation rate of polymer latex particles in the presence of dilute salt concentrations. Retardation was observed near, or above, the expected CMC for this cationic surface-active agent.

It would be anticipated that the presence of electrolyte decreases the thickness of the electrical double layer around each emulsion particle and hence affects the stability of the dispersion. Aggregation is therefore possible during a Coulter Counter size analysis which must be made in the presence of electrolyte. The fact that a cationic emulsion is readily susceptible whereas anionic systems are not, may be due to either the difference in thickness of the double layer or the strength of the van der Waals' forces involved at the interface.

Van den Tempel (1953) reviewed the flocculation and aggregation behaviour of oil-in-water dispersions. Higuchi & others (1962) noted that the Counter was suitable for investigating aggregation phenomena and this has been adequately confirmed (Higuchi, Okada, Stetter & Lemberger, 1963; Swift & Friedlander, 1964; Higuchi & others, 1965; Mourad & Lemberger, 1965 a, b). Following these authors, an attempt was made to treat the data in Fig. 4 according to Smoluchowski (1916), who predicted that a plot of the reciprocal of the total particle number with time should be linear. This was found to be valid for a short period of 60–100 min but was not applicable for the total period of the experiment. There are a number of important experimental differences between the present work and that of Higuchi and his colleagues, who have confined their attention to essentially monosize dispersions. As shown in Fig. 1, the present system has a wide size distribution and van den Tempel (1953) pointed out that such a system was likely to aggregate more rapidly. Friedlander (1961) showed that the decay in the total number of particles in a heterogeneous system should be of the form:

$$1/N_{\infty}^{2} \simeq t, \text{ where } N_{\infty} = \text{total number of particles and } t = \text{time.}$$

This decay law was also found to be inapplicable and, for the present, it appears that the data in Fig. 4 cannot be treated quantitatively. This may be because the data were based on counts above a threshold corresponding to 2·5 $\mu$ and it is known that the system contains particles down to at least 0·015 μ (Freshwater & others, 1965). As previously noted, the Counter is of limited value for counting particles below 0·5 μ diameter, and in its present state of development is unable to count the true total number of particles present in the dispersion. Any deductions which can be drawn must remain qualitative only.

Essentially, Fig. 4 shows the variation of a mean aggregate diameter with time and concentration of cetrimide. It will be seen that at 0·003 M (i.e. the same molarity as the primary concentration) there is a slow fall in aggregate size and this presumably represents a change in the size of the aggregate following a change of shear rate (Swift & Friedlander, 1964). Below the concentration of the primary dilution there is at first a relatively rapid increase in aggregate size followed after some 100 min by a slow deaggregation. The marked inflexion point at 0·0003 M noted in Fig. 3 is an indication of the same phenomenon.

This, then, suggests that an equilibrium aggregate size is formed slowly in the presence of the emulsifier. As the concentration of emulsifier is increased the aggregate size decreases in a regular fashion until, ultimately, the aggregates are reduced to the primary particles of the system under investigation. There are difficulties in establishing the time when equilibrium is reached although it is clear that it is in excess of the 400 min and appears, from other evidence, to be at least two or three days. It is of interest to note from Fig. 3 that the relationship is logarithmic, supporting the observations of Rehfeld (1962) and Rowe (1965) that the activity of surfactants increases as a log function of concentration.

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