Stability and Flocculation of Oil Droplets in Dilute Emulsions

By HIROYUKI MIMA and NOBUYUKI KITAMORI

By using a Coulter counter, it was found that flocculation of oil droplets stabilized with acacia occurred even in dilute emulsions (8×10^{-3} to 10^{-4} vol. per cent oil). This phenomenon was reversible and the equilibrium in floc size distribution was attained rapidly, and the flocculation depended upon the concentration of oil droplets, the size distribution of primary droplets, and the amount of salts added. Since the rate of creaming depends upon the size of particles, the stability of emulsions is affected by the flocculation of emulsion droplets. The stability of emulsions in the presence of 0.1 per cent sodium chloride was measured quantitatively by measuring the fluorescence of anthracene dissolved in oil phase, and it is concluded from the experimental results that there is a distinct relationship between the floc-size and the stability of emulsions.

MANY STUDIES have been made on the stability of o/w emulsions. However, the previous methods to determine the stability, especially the rate of creaming, including measurement of the time elapsed to the beginning of creaming or the volume of the cream separated after a definite time, were not quantitative (1–5).

According to Stokes' law, it is known that the rate of creaming is affected by the size of particles, the difference between the gravities of oil phase and water phase, and the viscosity of water phase. The size of particles in emulsion depends

$$u = \frac{(P_1 - P_2)\, gd^2}{18\eta}$$

not only upon the size of primary droplets, but also upon the degree of flocculation of the primary droplets. As for suspensions, a study of the effect of suspensoid concentration on the degree of flocculation has been reported (6). However, no papers have yet dealt with the relationship between flocculation and concentration of oil in emulsions.

During the authors' studies on the applications of emulsions in the fields of pharmacy and foodstuff, it was observed that the creaming occurred rapidly in the original emulsions, which contained a high concentration of oil phase, while in the diluted emulsion, the rate of creaming was not so rapid. To clarify these phenomena, a Coulter counter was used to measure particle-size distribution of flocculated droplets (7, 8). From these experimental results, it was found that the more the emulsion was diluted, the more the modal diameter of the dispersed particles became smaller, and the ratio of the number of droplets per concentration of oil phase increased.

These facts indicate that the deflocculation depends upon the concentration of oil in the emulsion, but all the emulsion droplets were deflocculated to primary ones in very diluted concentration, where there was no relation between flocculation degree and oil concentration.

In order to elucidate the relationship between flocculation of oil droplets and emulsion stability, the rate of change in concentration of oil at a fixed depth was also determined quantitatively as a function of time by measuring the fluorescence of anthracene which had been dissolved in the oil phase of emulsion.

From these results, it was shown that there was a distinct relationship between the floc-size measured with the Coulter counter and the stability of emulsions.

EXPERIMENTAL

Material.—Liquid paraffin, olive oil, and orange oil were all reagent grade materials. Ethyl dihydroabietate was synthesized from abietic acid by esterification and hydrogenation; b.p. 160–195°/0.2 mm. Hg, d_{20}^{20} 1.0269, n_D^{20} 1.5230, acid value 3.0.

Gum arabic was used as an emulsion stabilizer. Sodium chloride and anthracene were of special grade. Ether used for extraction of anthracene was freed from fluorescent impurities by passage through an alumina column.

Preparation of Emulsions.—Anthracene was dissolved in oils to give 0.2% (w/v) solutions. Ten grams of gum arabic was dissolved in 1 L. of distilled water under stirring with a homo-mixer (Type Tokusyu Kikai Kogyo Co.). Twenty milliliters of oil (containing anthracene) was then added and pre-emulsified for 2 min. This relatively coarse emulsification was then followed by passing through a Monton Gaulin homogenizer under about 4500 p.s.i. Size distribution of primary droplets of emulsions depended upon the pressure under which the emulsion was forced through the valve of homogenizer. In addition, viscosity of oils and number of times of homogenization had effects on the droplet size distribution of the resultant emulsions. The emulsions containing 2.0% oil phase by volume were used as the original emulsions.

Measurement of Size Distributions.—The method for measurement of droplet-size distribution of emulsions with the Coulter counter has been reported previously in detail (9). Our experiment followed this method. The aperture of 30 or 50 μ was used in accordance with the size of droplets. The threshold scale of the instrument was calibrated with polystyrene latex,[1] 0.769 and 1.305 μ in diameters. One-tenth per cent of saline was used as suspending medium. Since the floc-size distribution varied slightly with stirring, the measurement was carried out without stirring in this experiment. As the reproducible results were obtained with the same sample in the repeated measurements, it was thought that creaming did not occur during the measurement. The size distribution of emulsion was measured at various concentrations by diluting with 0.1% saline solution. Deflocculation was facilitated by dilution of emulsion, and at last the further dilution of emulsion resulted no longer in any change of the characteristic shape of the distribution curve. The size distribution of primary droplets was obtained at a diluted concentration under which the modal diameter of the distribution curve did not become smaller. The droplet size was also determined photomicroscopically. The floc-size distributions of emulsions were corrected according to the table of coincident passages supplied by Coulter Electronics, Inc. The modal diameters obtained from the floc-size distribution curves were used as the criterion of the degree of flocculation of droplets in emulsions.

With olive oil emulsion, the size distribution was measured at constant emulsion concentration (2 × 10^{-3}% oil) by diluting with varying salt concentration. The threshold scale of instrument was calibrated on every occasion of measurement.

Stability Measurement of Dilute Emulsions.—In these experiments, the concentration of oil phase of emulsion was 1 × 10^{-3} to 4 × 10^{-3}% and for this low concentration, a special method was necessary to determine the concentration of the oil in a small amount of samples collected from these emulsions. For example, when 1 ml. of a sample was collected, the amount of oil in the sample was only about 10^{-5} ml., which was too small to determine quantitatively with gas chromatography. For this reason, fluorescent analysis was used in order to determine the small amount of oil. The method is as follows:

The previously prepared stock emulsion containing anthracene was diluted to 1 × 10^{-3} to 8 × 10^{-3}% oil phase by volume with 0.1% saline solution. The diluted emulsions were allowed to stand in large glass cylinders (A) equipped with a small side orifice (B) closed by a rubber stopper (C) at the lower part of the cylinder. The vessel was about 9 cm. in diameter and about 60 cm. in height. The small orifice was fitted at the height of about 2 cm. from the bottom of the vessel. A long needle (D) was put into the vessel through a rubber stopper, and the tip of the needle was located at the center of the cylinder and just 2 cm. in height from the bottom (Fig. 1). Samples were easily collected through the needle at certain time intervals at the constant depth. Two milliliters of a sample emulsion was taken for one test, and the oil composition and the anthracene, of course, were extracted from

the sample with 5 ml. of refined ether, and the fluorescent strength of this ethereal solution was measured with Aminco's spectrophotofluorometer at 404 mμ by activating at 252 mμ. The volume of the emulsion in the cylinder was 2.1 L., and the total volume of the collected samples was 20–25 ml., which is only about 1% of the whole volume of emulsion, and is negligibly small. It was found that the fluorescence of the extract remained constant within an error with the same sample, in which the concentration of oil remained constant at a certain depth, and also there was a linear relationship between anthracene concentration (parallel to oil concentration) and strength of fluorescence. However, the strength of fluorescence of anthracene in orange oil decreased with time. Therefore, in the case of orange oil, it was necessary to use controls that were equal in concentration to that of test emulsion and were shaken at certain intervals in order to prevent creaming. The difference in fluorescence between the test emulsion and the control was considered as the decrease of anthracene due to creaming.

RESULTS AND DISCUSSION

Floc-Size Distribution.—The floc-size distribution of olive oil, liquid paraffin, and orange oil emulsions at various concentrations measured with a Coulter counter are given in Figs. 2 and 3 and Table I. The droplet-size distributions are included in these figures. These figures clearly show that the peak of the floc-size distribution curve migrates to larger droplet size with increase in emulsion concentration, and to the contrary, with decrease in emulsion concentration, they shift to smaller size. Table I shows the total number particles counted by the Coulter counter at various oil concentrations. According to these data, particle *number is not only parallel to oil concentration,* but less than the number calculated from the numbers of primary droplets. The measured number
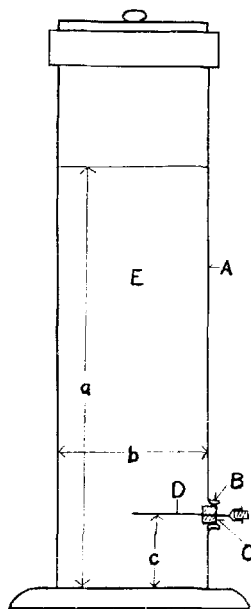

Fig. 1.—Apparatus for the determination of emulsion stability. Key: A, cylinder; B, side orifice; C, rubber stopper; D, needle; E, test emulsion; a, about 50 cm.; b, about 9 cm.; c, 2 cm.

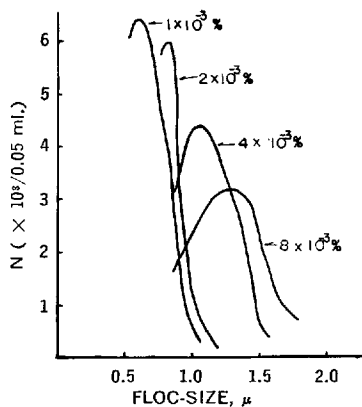[1] Supplied by J. W. Vanderhoff, Dow Chemical Co.

Fig. 2.—Change of floc-size distributions with concentration of emulsion (orange-oil emulsion).
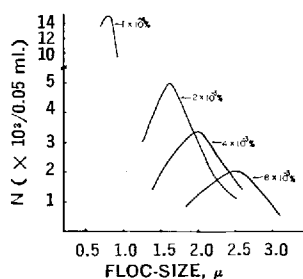

Fig. 3.—Change of floc-size distributions with concentration of emulsion (olive-oil emulsion).

TABLE I.—TOTAL NUMBERS OF PARTICLES IN ORANGE OIL EMULSION AT VARIOUS CONCENTRATIONS MEASURED WITH A COULTER COUNTER (IN 0.1% SODIUM CHLORIDE SOLUTION)

Emulsion Concn. ($\times 10^{-3}$%)	No. of Particles in 0.05 ml.	Relative No.	Relative No./Emulsion Concn.
8.0	61,500	1.000	1.00
4.0	58,000	0.943	1.89
2.0	48,500	0.789	3.16
1.0	49,000	0.797	6.38

of particles approached to the calculated number with dilution. At a very dilute concentration, for example, in 1×10^{-3}% orange oil emulsion, the characteristic shape of the distribution curve did not change, and only the peak height of curve decreased according to the concentration after dilution. At this concentration, the size distribution curve was regarded as that of primary droplets in emulsions. These size distributions were in good agreement with the photomicroscopic observation. It may be reasonable to explain these phenomena as the deflocculation of flocculated emulsion by dilution. The scheme of these phenomena is illustrated in Fig. 4.; *i.e.*, in concentrated emulsion, which represents 4×10^{-3} to 8×10^{-3}% of orange oil emulsion in Fig. 2, the flocculation occurred highly; in medium concentrated emulsion, which represents 2×10^{-3}% in Fig. 2, the flocculation is not so high, and in diluted emulsion which has

1×10^{-3}% of orange oil, the particles were separated to primary ones. Thus, determination of size distribution with a Coulter counter confirmed the presence of flocs in emulsion. In the previous studies of flocculation and deflocculation of oil droplets in soap-stabilized emulsions (2, 7), the rate of creaming of emulsion or cream volume after a definite time period has been taken as the criterion of flocculation. In addition, microscopic observation and turbidity measurement have been made for studies of flocculation. In comparison with these methods, a Coulter counter method is excellent for the determination of floc-size, as it makes it possible to measure more exact floc-size distribution than that obtained by the previous methods. However, this method is not suitable for the investigation of the effect of surfactant only on flocculation, as an electrolyte solution must be employed as a medium in the measurement.

In the present work, the flocculation of emulsion droplets stabilized with acacia (which was one of the strong anticoalescent agents) in 0.1% saline was investigated. Here only the effect of oil phase concentration on flocculation of droplets in the same kind of emulsion and the same salt concentration was studied.

Because the rate of flocculation seems to be rapid and 20–30 min. were necessary for a determination of the distribution curve, the rate of flocculation could not be determined with this method. Therefore, the determination of the distribution curve was carried out after 5–10 min. of dilution of emulsion.

According to Smoluckowski's theory of coagulation kinetics (10, 11), the time required for the droplets to collide by Brownian motion is a function of the Boltzman constant, the temperature, the viscosity of the continuous phase, and the number of primary droplets. The time required for the flocculation of these emulsion droplets (about 0.6–0.7 μ, 8×10^{-3} to 10^{-4}%) was not more than 10 min. The numbers measured at various concentrations with the Coulter counter (N) were regarded as the total numbers of flocs in emulsion and the numbers
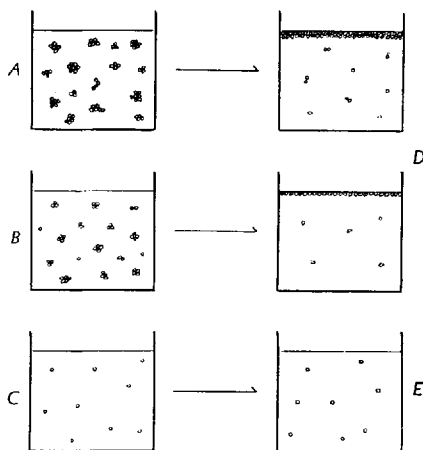

Fig. 4.—Schematic representation of the principal types of emulsion. Key: *A*, concentrated; *B*, medium; *C*, diluted; *D*, creaming occurs (unstable); *E*, no creaming occurs (stable).
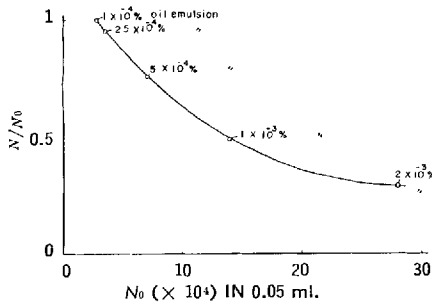
Fig. 5.—Relation between N/N_0 and N_0 obtained with ethyl dihydroabietate emulsion in 1% sodium chloride solution.
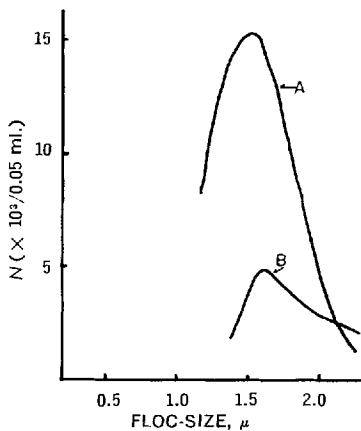

Fig. 6.—Floc-size distribution curves of 2 × 10^{-3}% olive oil emulsion in different salt concentrations. Key: A, in 1% NaCl solution; B, in 0.1% NaCl solution.

of primary droplets (N_0) which constituted the flocs were calculated from the total numbers of droplets measured in very diluted concentration, where the droplets did not form any flocs. The relation between N/N_0 and N_0 obtained with ethyl dihydroabietate emulsion in 1% saline is given in Fig. 5. The curve in Fig. 5 shows the relation similar to that of Smoluckowski's theory of coagulation. From these results, it is concluded that the flocculation is attributed to the collision of droplets, and the flocculation is reversible so that the equilibrium is attained rapidly after dilution.

In these cases, the flocculation of emulsion droplets was demonstrated in 0.1% saline solution. As pointed out earlier (12), it can be considered that the flocculation was affected by the concentration of salt. Actually, as shown in Fig. 6, the numbers of smaller particles in 1% NaCl solution were more than those in 0.1% NaCl solution at constant emulsion concentration.

Emulsion Stability.—Figures 7–10 illustrate the emulsion stabilities at various concentrations of olive oil, liquid paraffin, orange oil, and ethyl dihydroabietate emulsions. The changes in oil concentration of emulsion at fixed position upon standing indicate the floating or settling of oil droplets in emulsion at a certain rate.

The floating or settling rate depends mainly upon the size and the density difference between oils and media, and upon Brownian motion in small droplet size range. Actually, with ethyl dihydroabietate emulsions, oil concentration at the lower position of the test cylinder inclined to increase, because its density is slightly larger than that of water (Fig. 10), while with other emulsions oil concentration inclined to decrease. As shown in
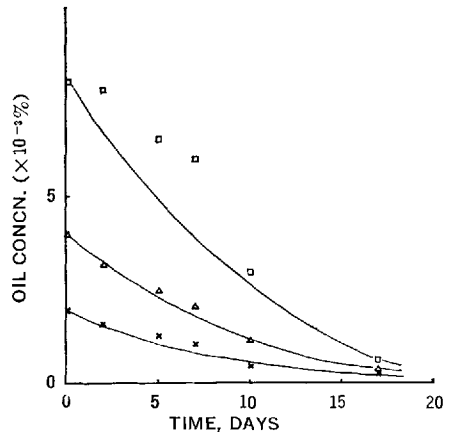

Fig. 7.—Stability of olive oil emulsion as reflected in changes with time of the concentration of the oil phase near the bottom of the test vessel. The 3 plots correspond to 3 different initial concentrations.
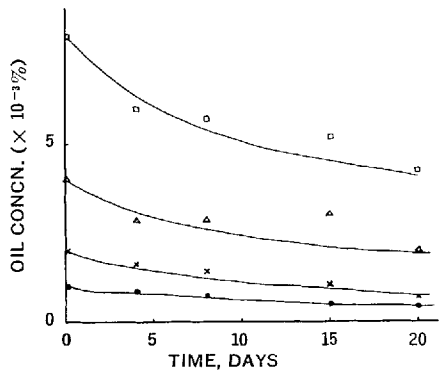

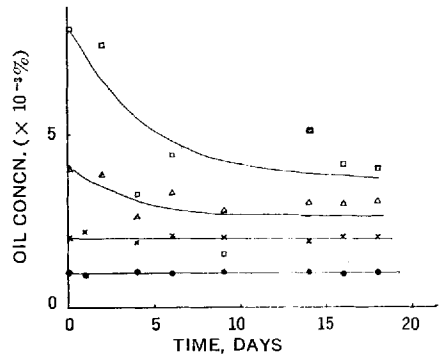Fig. 8.—Stability of liquid paraffin emulsion.

Fig. 9.—Stability of orange oil emulsion.

Figs. 8 and 9, the rates of decrease in oil concentration were initially rapid, gradually became slower, and attained to equilibria. The rate of decrease of
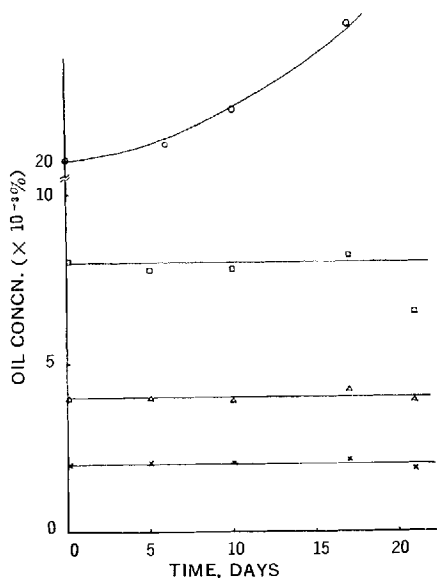

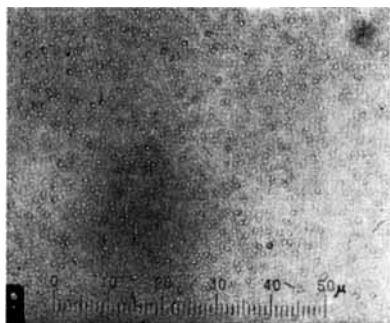Fig. 10.—Stability of ethyl dihydroabietate emulsion.

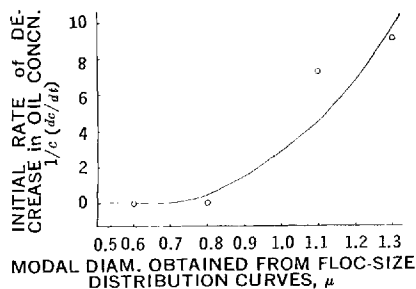Fig. 11.—Photomicrograph of orange oil emulsion.

Fig. 12.—Relation between stability and floc-size in orange oil emulsion.

concentrated emulsion is much more than that of diluted one, but the stability curve of the olive oil emulsion illustrated in Fig. 7 is slightly different from that of Figs. 8 and 9. In the very diluted emulsion of any oil, floating or settling of droplets does not occur, and therefore oil concentration remains constant. Even if droplets in emulsion float equally at any concentration, it follows that the rates of decrease in oil concentration of more concentrated emulsion are larger than those of more dilute emulsions. Therefore, the rate of creaming must be compared by using the specific rate of decrease in oil concentration, $1/c$ (dc/dt), where c is oil concentration at time t at a constant depth.

Relation between Flocculation and Stability of Emulsions.—Creaming of emulsions is the phenomenon that occurs in consequence of separation of oil droplets due to the density difference between the dispersed and continuous phases. Hence, the rate of creaming depends upon the density difference, the size of particles, and others according to Stokes' law. If the flocculation occurs in emulsion, a flocculated particle behaves as a larger droplet, although primary droplets were small. As shown in Figs. 2 and 3, the droplet size of the primary particles of emulsions prepared in this laboratory was about 0.6–0.7 μ and this was confirmed with photomicrograph (Fig. 11), in which none of the larger droplets were contained. From the data of the emulsion stability and the floc-size distribution measured with the Coulter counter, the relation between them may be concluded as follows.

For smaller droplets, a continuous random zig-zag motion called Brownian motion was expected to account for the stability against creaming; while, in the concentrated emulsion, Brownian motion of droplets affects the growth of flocs, and the larger flocs float according to Stokes' law. The relation between flocculation and emulsion stability of orange oil emulsion is given in Fig. 12. The modal diameter of floc-size measured with the Coulter counter was used as the size of flocs, and the specific rate of decrease in oil concentration was used as the measure of emulsion stability. The stability decreased rapidly with the increase of floc-size above a certain size. Below this size, creaming did not occur to any extent and emulsion was stable.

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