

Effect of the Initial Concentration of Emulsifying Agents on the Ultracentrifugal Stability of Oil-in-Water Emulsions

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ABSTRACT

The rate of loss of oil from 50% Nujol-50% water emulsions stabilized with varying concentrations of Tween 20 or Triton X-100, and from 50% olive oil-50% water emulsions stabilized with sodium dodecyl sulfate (SDS), was determined by ultracentrifugation at 39,460 rpm. In all cases the ultracentrifugal stability increased with increasing initial concentration of emulsifier, although, unlike the behavior of Nujol-water-SDS emulsions, the rate of separation of oil decreased with time. The quantity of olive oil separated after a given time of centrifugation decreased linearly with increasing concentration of SDS, reaching zero at a concentration of SDS somewhat below the critical micelle concentration, and at adsorption of SDS corresponding to only about half the saturation capacity. The ultracentrifugal stability of Nujol emulsions with Tween 20 or Triton X-100 continued to increase slowly with increasing concentration of emulsifier, even at concentrations far greater than the critical micelle concentration.

INTRODUCTION

Since the early work of Garrett (1), Rehfeld (2), and Vold and Groot (3), there has been much interest in the use of the ultracentrifuge to investigate the stability of emulsions. Useful summaries include a review article by Garrett (4) and recent work reporting on the behavior of a broader assortment of oils and emulsifying agents (5,6).

The earlier work (2,3) had shown clearly that increasing the concentration of sodium dodecylsulfate (SDS) decreased the rate at which oil separated from Nujol or benzene emulsions up to the critical micelle concentration (CMC) of the surfactant, after which further increases had little effect. The conclusion was qualitatively the same whether equilibrium or initial concentrations of emulsifier were considered, and whether or not the drop size distribution was maintained constant in the series of emulsions being compared. Other studies (7,8) have shown that the average drop size decreases as the initial concentration of emulsifier present during emulsification is increased, so long as the equilibrium concentration present is below the CMC, and that the ultracentrifugal stability of Nujol-water-SDS emulsions increases with increasing equilibrium concentration of SDS up to the critical micelle concentration (9).

In the present investigation the dependence of the ultracentrifugal stability of Nujol-water emulsions was studied as a function of the initial concentration of Tween 20 and Triton X-100, and of olive oil-water emulsions of the equilibrium concentration of SDS. Since it is the equilibrium concentration of the surfactant in the aqueous phase that determines the extent of adsorption at the interface, this would have been the preferable parameter in all cases. Unfortunately all attempts to develop an adequate analytical method for determination of Triton X-100 or Tween 20 in the equilibrium aqueous phase of the emulsion were unsuccessful (6); so the results with these emulsions are reported in terms of initial concentrations. The drop size distribution was kept constant in all emulsions with a varying concentration of a given surfactant by preparing them by dilution from a single master batch (3), so as to permit unambiguous relation of observed changes in ultracentrifugal stability to changes in the concentration of emulsifier.

Despite its limitations, establishment of a direct relationship between the initial concentration of the emulsifier and the stability of the emulsions formed is of value in predicting optimum conditions of formulation. In interpreting the present results, however, it should be remembered that the state of the system in the ultracentrifuge differs greatly from that of a free-standing emulsion (9). The typical important nonionic surfactants employed behave similarly to pure SDS, in that the ultracentrifugal stability of the emulsions formed increases with increasing concentration of surfactant; but instead of leveling off to a limiting value at the CMC continues to increase slowly even at much higher concentrations.

It is also of interest that changing from a nonpolar (Nujol) to a polar (olive) oil results in separation of oil in the ultracentrifuge at a decreasing rather than constant rate as time of centrifugation increases, but causes no qualitative change in the dependence on emulsifier concentration.

EXPERIMENTAL PROCEDURES

Materials

The sodium dodecylsulfate was the same Eastman sample used in a previous investigation (10) and contained less than 0.05% lauryl alcohol on the basis of its surface tension-concentration curve. Triton X-100 (Lot 2788) was used directly as furnished and is reported to be 100% polyoxyethylene *p-t* octyl phenyl ether with 9-10 ethylene

TABLE I

Dependence of Ultracentrifugal Stability and Fractional Saturation of the Oil-Water Interface of 50% Olive Oil-50% Water Emulsions on the Concentration of Sodium Dodecylsulfate (SDS)

Initial concentration of SDS, wt%	Equil. concentration of SDS, mmoles/liter	Fraction of saturation adsorption, θ	Induction period, min	Per cent oil separated after 75 min
0.20	2.79	0.264	0	37.0
0.25	3.59	0.332	3	24.0
0.30	4.98	0.388	1	19.5
0.35	5.67	0.422	20	8.5
0.40	6.75	0.465	65	2.0
0.45	7.72	0.515	96	0.0

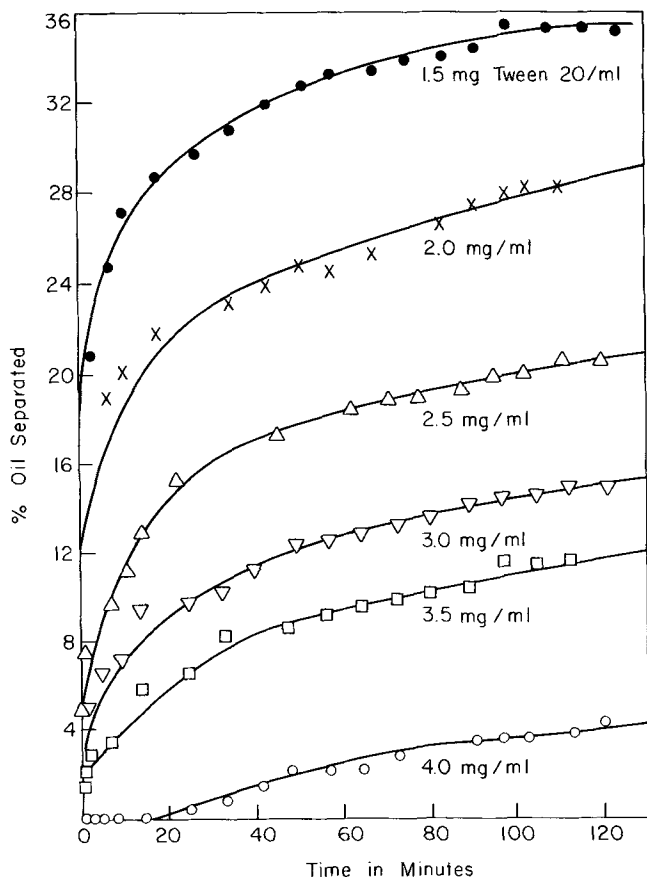


FIG. 1. Effect of the initial concentration of Tween 20 on the time dependence of the separation of oil from 50% Nujol-50% water emulsions.

oxide groups. The critical micelle concentration is reported to be in the vicinity of 0.19 mg/ml (11-13). Tween 20 was also used directly as furnished. It is reported to be polyoxyethylene (20) sorbitan monolaurate, HLB number 16.7, and to have a critical micelle concentration of 0.06 mg/ml (12). Nujol (Plough Inc., New York) and olive oil (Robinson Laboratories, San Francisco) were the same samples as in previous work (10) and were used without purification. Triple A Co. bottled distilled water was used directly.

Preparation and Characterization of Emulsions

Since differences in the conditions of preparation have a marked effect on the stability of two emulsions of the same final composition, it is necessary to specify the method of preparation of each set of emulsions in considerable detail, although in all cases the general procedure resembled that of Vold and Groot (3). Becher (16), for example, found that the extent of mechanical agitation during formation had a much greater effect on the drop size distribution of the resultant emulsion than did changes in the concentration of the emulsifying agent (7,8), although a different result might have been obtained had he worked at emulsifier concentrations below the critical micelle concentration.

In the following discussion emulsifier concentrations are expressed in terms of per cent, on the basis of the concentration initially present in the aqueous phase.

Emulsions with Tween 20

In the case of Nujol-water-SDS emulsions about half the total added SDS is adsorbed at the interface when the equilibrium concentration of SDS in the solution is at the critical micelle concentration. Since one of the original objectives of the present investigation had been to deter-

mine whether ultracentrifugal stability would also reach a limiting asymptotic value at the CMC of nonionic surfactants analogous to the behavior with SDS, it was hoped to study emulsions at equilibrium concentrations of nonionic surfactant both above and below the CMC. If the adsorption isotherm for Tween 20 at the Nujol-water interface were identical to that of SDS, this would predict that the CMC should be reached at an initial concentration of Tween in the aqueous phase of 0.012%. However 50% Nujol-50% water emulsions prepared at an initial concentration of Tween 20 of 0.012%, and even 0.05%, were so unstable as to be unusable in the ultracentrifuge.

To cover the range from 0.15 to 0.40% Tween (emulsion set A091069), 150 ml Nujol and 120 ml 0.15% Tween (0.15 g/100 ml water) were stirred 5 min with the Brookfield counter-rotating mixer at 5000 rpm and passed eight times through a motorized Cenco hand homogenizer. Three more batches were prepared similarly, and all combined to yield 1080 ml of emulsion. After standing 24 hr, 135 ml aliquots were withdrawn from the stock emulsion and blended by gentle hand swirling with 15 ml solutions of Tween of varying concentration, to give a series of emulsions all of the same drop size distribution (3) and 50:50 oil-water volume ratio but with differing concentrations of Tween. To cover the range from 0.08 to 0.20% Tween (emulsion set M111969 TW), the same procedure was followed except that 120 ml of 0.1% instead of 0.2% aqueous Tween was used during the emulsification process; two batches were combined to yield 540 ml stock emulsion, from which 81 ml aliquots were withdrawn after 2 hr and 9 ml water or appropriate concentrations of Tween added as before to prepare the final set of emulsions of 50:50 oil-water volume ratio and varying concentrations of Tween.

Emulsions with Triton X-100

Emulsions containing 50% Nujol, 50% water and varying concentrations of Triton X-100 from 0.15 to 0.40% (emulsion set A090969) were prepared by emulsification at 0.15% Triton and subsequent addition of a more concentrated Triton solution as in the case of the Tween 20 emulsion set A091069. To cover the range from 0.08 to 0.18% (emulsion set M111969TR), the initial emulsification was carried out using 0.10% Triton solution following the same procedure as in the case of the Tween emulsion set M111969TW.

Olive Oil-Water-SDS Emulsions

These emulsions were prepared by exactly the same technique as was used in an earlier study of the effect of the concentration of SDS on the stability of Nujol-water emulsions (3) with an initial concentration of 0.2% SDS in the aqueous phase during emulsification, except that the emulsion was passed through the hand homogenizer eight instead of four times. Three batches were combined to yield 810 ml stock emulsion from which the final emulsions were prepared by blending in additional SDS solution.

The equilibrium concentration of SDS in the aqueous phase of these emulsions was determined by titration with cetyl pyridinium bromide solution (3). The adsorption data were well represented by the linear form of the Langmuir equation, thus permitting calculation of the specific interfacial area of the emulsion and the fraction of saturation of the interface with adsorbed SDS. Taking the area of an adsorbed SDS molecule at saturation adsorption to be 54\AA^2 (14), the specific interfacial area of the olive oil emulsion is $4.61 \times 10^4 \text{ cm}^2/\text{ml oil}$, a value two to four times larger than that of Nujol-water emulsions of the same composition prepared similarly. The data obtained with the olive-oil-water-SDS emulsions are summarized in Table I.

Ultracentrifugal Studies

Ultracentrifugation of these emulsions was carried out at

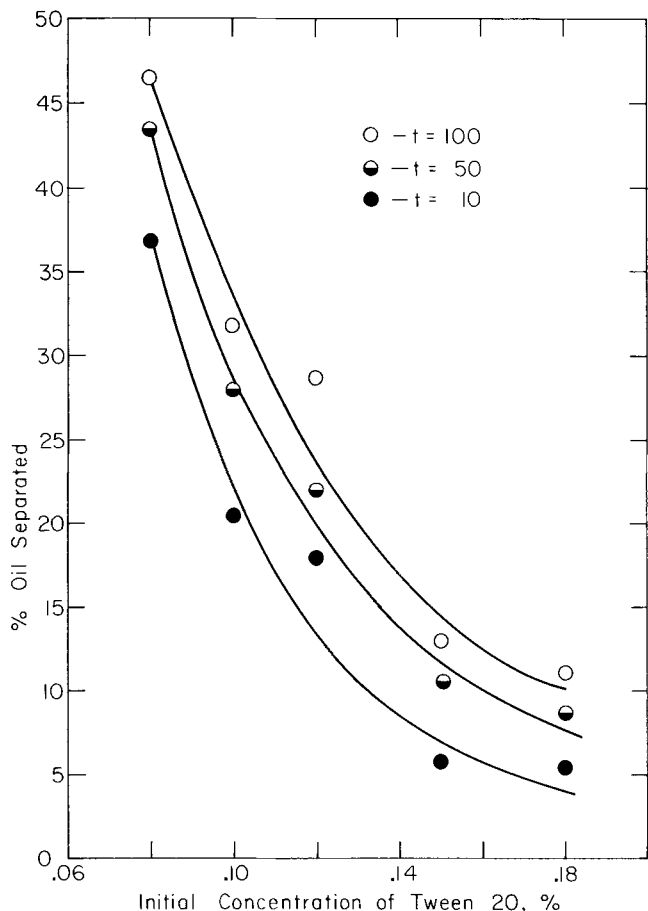


FIG. 2. Effect of the initial concentration of Tween 20 on the amount of oil separated from 50% Nujol-50% water emulsions M111969TW after varying times. ●, per cent separated after 10 min; ●, after 50 min; ○, after 100 min.

39,460 rpm and 25 C as in previous studies (3,10), and the desired quantities were calculated from the observational data by the same methods.

RESULTS

Nujol-Water-Tween 20 Emulsions

As is evident from Figure 1, plots of the per cent oil separated from those emulsions as a function of the time of ultracentrifugation yield smooth curves of continuously decreasing slope. The data conform well to the empirical equation (6),

$$t / \% \text{ oil}_{\text{sep}} = \frac{1}{b \% \text{ oil}_{\text{max}}} + \frac{t}{\% \text{ oil}_{\text{max}}}$$

where $\% \text{ oil}_{\text{sep}}$ is the fraction of the initial quantity of emulsified oil which has separated after time, t , $\% \text{ oil}_{\text{max}}$ is the extrapolated limiting value at infinite time, and b is a constant. This equation may be used with the data themselves, to calculate all of five possible quantitative parameters of emulsion stability: (a) the per cent oil separated at any give time; (b) the rate of separation of oil after time, t , (the slope of the curve of per cent oil separated vs. t); (c) the rate of separation of oil after any given constant fraction of the oil originally present has separated; (d) the extrapolated maximum amount of oil separable ($\% \text{ oil}_{\text{max}}$); and (e) the value of the empirical constant, b .

Figures 2 and 3 show the dependence of the amount of oil separated after 10, 50 and 100 min of centrifugation as a function of the concentration of Tween initially present in the aqueous phase of the emulsion over the two concentration ranges studied. Rates of separation of oil

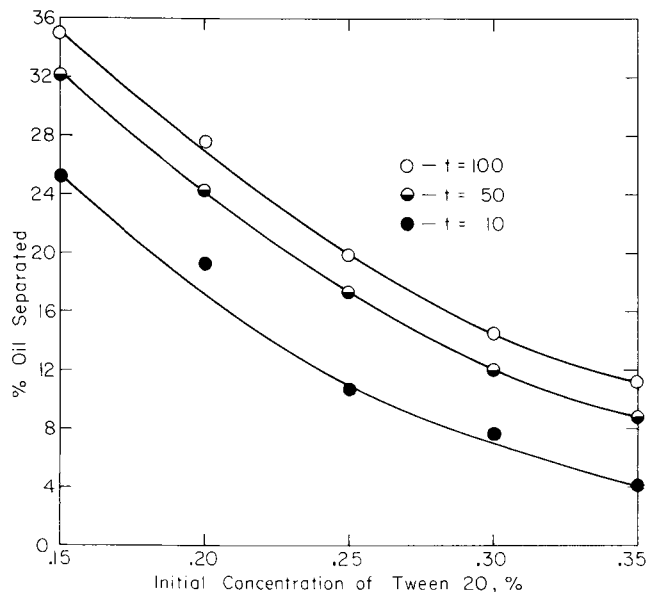


FIG. 3. Effect of the initial concentration of Tween 20 on the amount of oil separated from 50% Nujol-50% water emulsions A091069 after varying times. ●, per cent separated after 10 min; ●, after 50 min; ○, after 100 min.

after various times of centrifugation, calculated from the derivative of the empirical equation fitting the data, are assembled in Table II. Figure 4 shows the dependence of $\% \text{ oil}_{\text{max}}$ and b on the initial concentration of Tween in the aqueous phase of the emulsion. All these criteria are in qualitative agreement in showing that, for a given set of emulsions of the same drop size distribution over the concentration range studied, the stability increased continuously with increasing concentration of emulsifier with no abrupt change of slope.

Nujol-Water-Triton X-100 Emulsions

Both sets of emulsions, those prepared with 0.1% Triton and studied over the concentration range from 0.08 to 0.18% Triton, and those prepared with 0.15% Triton and studied over the concentration range from 0.15 to 0.40% Triton, gave curves of per cent oil separated vs. time of centrifugation of continuously decreasing slope. The data conformed to the empirical equation in the case of the emulsions prepared initially at 0.1% Triton, corresponding to region III-A of the master curve of Reference 6, whereas in the case of those prepared initially at 0.15% Triton this equation was no longer followed (region III-B of Reference 6). This is in accord with previous indications that the empirical equation tends not to apply in the case of emulsions which separate only a little oil very slowly. In the present instance emulsions with 0.20% Triton originally in the aqueous phase separated only 9.5% oil after 200 min of centrifugation, while those with higher concentrations underwent no oil separation over periods up to 2 hr, and so were too stable to be studied by this technique at the ultracentrifugal speed employed. Where the empirical equation is obeyed, rates of separation of oil were calculated from its derivative. Where it was not followed, the rates were obtained from the slope of the tangent to the curve at the specified time of centrifugation.

The separation of oil after three different periods of centrifugation at 39,460 rpm is shown as a function of the concentration of Triton initially present in the aqueous phase in Figure 5. With both sets of emulsions the stability increases with increasing concentration of Triton, although the set made at an initial concentration of 0.15% Triton is less stable than that made at an initial concentration of 0.10% Triton, when the two are compared at the same total initial concentration of Triton.

TABLE II

Dependence of the Rate of Oil Separation and Constants of Empirical Equation of 50% Nujol-50% Water Emulsions on Initial Concentration of Tween 20

Initial concentration of Tween 20, wt%	Rate of separation of oil, %/min				% oil _{max}	b, min ⁻¹ × 10 ²
	25 min	50 min	75 min	100 min		
Emulsions M111969TW						
0.08	0.26	0.08	0.04	0.02	48.5	20.6
0.10	0.26	0.09	0.04	0.03	34.5	11.2
0.12	0.24	0.08	0.04	0.02	31.2	10.8
0.15	0.15	0.07	0.04	0.02	15.4	4.6
0.18	0.14	0.05	0.03	0.02	13.7	4.3
Emulsions A091069						
0.15	0.25	0.078	0.038	0.022	37.8	14.8
0.20	0.21	0.068	0.033	0.020	29.5	13.0
0.25	0.19	0.066	0.033	0.027	22.4	9.0
0.30	0.18	0.078	0.043	0.025	17.9	4.3
0.35	0.14	0.067	0.038	0.025	14.3	3.4
0.40	—	0.053 ^a	—	0.020 ^a	—	—

^aUnlike the rates for the other emulsions, which were calculated from the differential of the empirical equation, these rates were determined from the slope of the tangent to the curve of per cent oil separated vs. time, since the data for this most concentrated emulsion did not obey the empirical equation.

Table III shows the rate of oil separation after various times of centrifugation of the emulsions for those made at the lower concentration of Triton, together with the calculated values of % oil_{max} and of *b*. It is interesting to note, by comparison with Figure 4, that even though these emulsions contain generally lower concentrations of emulsifier than those stabilized with Tween they are more stable, in that the values of *b* are smaller, and the extrapolated limiting separation of oil also less.

Olive Oil-Water-SDS Emulsions

Olive oil emulsions made with 0.2% SDS had the same behavior in the ultracentrifuge as Nujol-water-Tween 20 emulsions, the rate of separation of oil decreasing with time

and conforming to the empirical equation. Emulsions containing more than 0.2% SDS were very stable, having an induction period after attainment of full centrifugal speed before any oil separated, and not obeying the empirical equation but giving curves of per cent oil separated vs. time like those obtained with Triton X-100 at higher concentrations. Since emulsions containing more than 0.2% SDS were too stable to permit any meaningful comparison of the rate of separation of oil, relative stability was inferred from the differing amounts of oil separated after 75 min of centrifugation. These are given in Table I. This relatively long interval, chosen arbitrarily, was necessary because of the lengthy induction period with the emulsions containing the higher concentrations of SDS. Interestingly, plots of the amount of oil separated after 75 min vs. either initial or equilibrium concentration of SDS are essentially linear, the equilibrium plot extrapolating to zero oil separation in 75

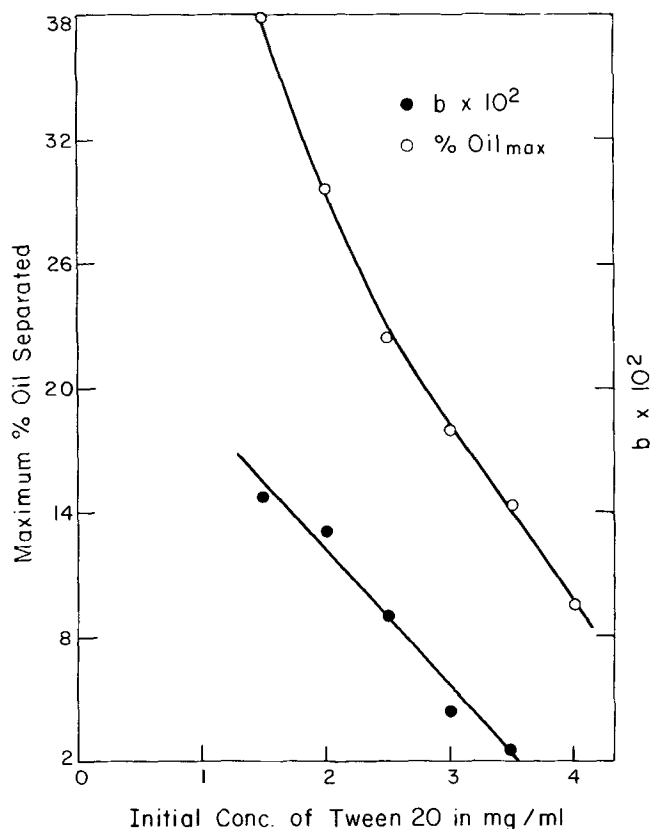


FIG. 4. Effect of the initial concentration of Tween 20 on the parameters of the empirical equation describing the separation of oil from 50% Nujol-50% water emulsions A091069.

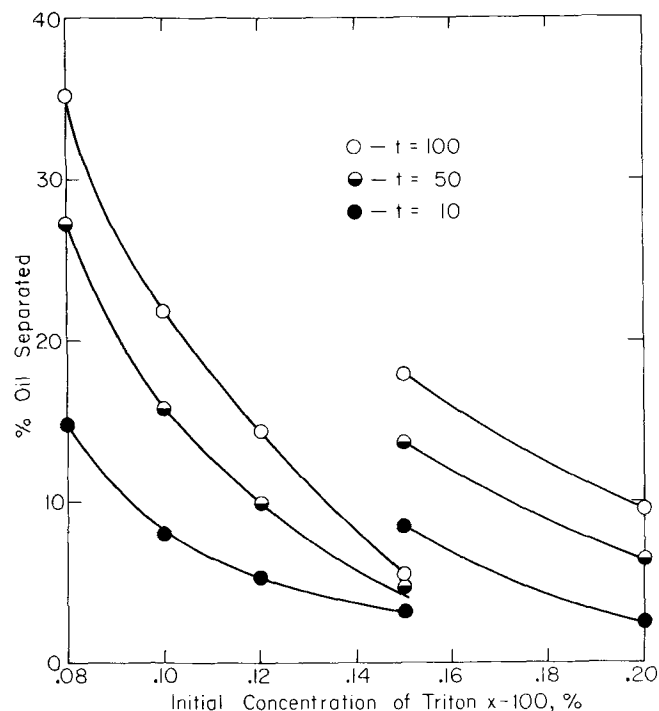


FIG. 5. Effect of the initial concentration of Triton X-100 on the amount of oil separated from 50% Nujol-50% water emulsions M111969TR and A090969 after varying times. ●, per cent separated after 10 min; ●, after 50 min; ○, after 100 min.

TABLE III^a

Dependence of the Rate of Oil Separation and Constants of Empirical Equation of 50% Nujol-50% Water Emulsions on Initial Concentration of Triton X-100

Initial concentration of Triton X-100, wt%	Rate of separation of oil, %/min				% oil _{max}	b, min ⁻¹ x 10 ²
	10 min	25 min	50 min	75 min		
Emulsions M111969TR						
0.08	—	0.45	0.21	0.12	45.1	3.4
0.10	—	0.29	0.16	0.10	31.6	2.2
0.12	—	0.18	0.11	0.07	24.0	1.6
Emulsions A090969						
0.15	0.21	0.11	0.09	0.08	—	—
0.20	0.12	0.09	0.07	0.06	—	—

^aRates of oil separation were determined from the differential of the empirical equation fitting the data for emulsions M111969TR, and from the slope of the tangent to the curve of per cent oil separated vs. time for emulsions A090969.

min at a concentration of 6.8×10^{-3} M SDS in the equilibrium aqueous phase.

DISCUSSION

In all cases studied the ultracentrifugal stability of any given set of emulsions all having the same drop size distribution increased with increasing initial concentration of emulsifier. This is true with all the possible quantitative criteria suggested: per cent oil separated after a fixed period, rate of oil separation after a fixed time, rate of oil separation after loss of a fixed fraction of the initially emulsified oil, and were available, the extrapolated maximum amount of oil separable under the given conditions and the value of the constant *b* in the empirical equation. It should be pointed out, however, as is clear from the data in Tables II and III and from the shape of the curves in Figure 1, that the rates of separation of oil tend toward a low limiting value independent of emulsifier concentration after longer periods of centrifugation; so comparisons of relative rate are more meaningful at shorter times.

It is not possible from the present data, in the absence of adsorption isotherms, to make any definitive conclusion as to whether the stability of Nujol-water emulsions prepared with Tween 20 or Triton X-100 would reach a constant value at the critical micelle concentration as in the case of emulsions with SDS (3). However it is significant that the amount of oil separating after a given time interval (Fig. 2, 3 and 5) continues to decrease with increasing concentration of surfactant, even though the initial concentrations become so high that the CMC must certainly have been far exceeded. Whether this continuing effect is due to a difference in the mechanism of stabilization by the non-ionic as contrasted with the anionic surfactant, to nonoccurrence of a sharp critical micelle concentration in the commercial surfactants, or to continuing adsorption of a specific more highly surface active fraction of the polymeric surfactant, can not be determined from the present data.

In the case of olive oil-water-SDS emulsions the ultracentrifugal stability increases with increasing concentration of SDS up to an equilibrium concentration of SDS of 0.068M, at and above which no oil has separated after 75 min of centrifugation. One might have expected this break point to have occurred at the CMC, 0.081M, as in the case of the Nujol-water emulsions. It is also significant that the limiting stability has already been reached, even though the interface contains only 0.52 of the maximum adsorption (calculated from the Langmuir equation fitting the data), presumably corresponding to a close-packed monolayer, as contrasted with the behavior in Nujol-water systems where the rate of oil separation did not become zero until the interface was completely covered with adsorbed SDS (15).

Possibly the lower limiting concentration of SDS in the present case may be due to natural surfactants already present in the olive oil used, since these could be adsorbed at the interface, and contribute to the stability in the same way as does lauryl alcohol added to Nujol-water emulsions stabilized with SDS (10).

The importance of the exact conditions existing during the emulsification process is brought out by comparison of the results with the two sets of Tween emulsions shown in Figures 2 and 3, and the two sets of Triton emulsions shown in Figure 5. In both cases, if comparison is made of emulsions of the same phase volume ratio and total initial concentration, the emulsion made at the higher concentration of surfactant is the less stable. Thus, after 50 min centrifugation a 50% Nujol-50% water emulsion with a total initial concentration of Tween 20 of 0.18% on the basis of the aqueous phase, made at a Tween concentration of 0.15%, had separated 27% of the oil (Fig. 3), whereas an otherwise identical emulsion, only made at a Tween concentration of 0.10%, had separated only 8.5% of the oil (Fig. 2).

This at first seems odd, since it has been shown (7,8) that increase in the concentration of surfactant present during emulsification results in a decrease in mean particle size (increase in specific interfacial area) and that this in turn results in an increase in ultracentrifugal stability (9). However the observed behavior may be rationalized in terms of the concept that the stability is strongly dependent on the fraction of the oil-water interface covered by adsorbed surfactant (10,15). Obviously, with the same total amount of emulsifier available, the emulsion having the smaller drop size (that prepared at the higher concentration of surfactant) would have a smaller fraction of the oil-water interface protected by adsorbed surfactant than would one of larger drop size and so, in accord with the results obtained, would be expected to be the less stable. This should not be the case if the two emulsions were compared at the same equilibrium concentration of emulsifier in the aqueous phase.

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