monly employed and provide a means of maximizing the information derived from minimum data.

Existing stability testing programs are based on the use of prescribed temperatures which are not altered from product to product. While the stability chart described in this report has been designed for a recommended stability testing program, it may be viewed generally as a prototype for other possible designs. Charts can be prepared for any combination of temperatures desired, whatever the value of α. The methods described can be applied to fit existing stability testing facilities. The use of a nomographic technique with conventional stability calculations has made the chart practical for routine application.

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Some Interfacial Properties of a Nonaqueous Emulsion

By RICHARD D. HAMILL*, FERRON A. OLSON, and ROBERT V. PETERSEN

Emulsions of glycerin and olive oil were formed using anionic surface-active agents. The two surfactants used were condensation products of ammonia and of 2-amino-2-methyl-1,3-propanediol (AMP) with the fatty acids available in olive oil. The minimum concentration of ammonia needed for emulsification was found to be 0.6 mg./100 ml. and 0.84 mg./100 ml. at phase volumes of 0.40 and 0.58, respectively. The minimum concentration of AMP was 2 mg./100 ml. and 2.7 mg./100 ml. for phase volumes of 0.40 and 0.58, respectively. Total interfacial area was calculated from photomicrographs of the emulsions and correlated with interfacial tensions. The effective mean molecular areas of the surfactants, in the low concentrations employed, were sufficient to form 89 to 98 per cent interfacial coverage.

PREVIOUS PUBLICATIONS (1, 2) have reported on the preparation of nonaqueous emulsions of olive oil and glycerin with the use of anionic, cationic, and nonionic surface-active agents. McMahon and co-workers (1) have demonstrated that oil can be emulsified with as little as 0.01% of amine employed. These workers also suggested that still lower concentrations might be effective in emulsifying this system. Further work with much lower concentrations of two anionic surfactants [condensation products of ammonia and of 2-amino-2-methyl-1,3-propanediol[1] (AMP) with the fatty acids available in olive oil] confirmed this hypothesis. Attention was also focused on the interfacial film available at these lower concentrations. Since oleic acid is the predominant fatty acid found in olive oil (3), it was assumed that the surfactant at the interface

could be represented simply as an ammonium or an AMP oleate. Therefore, if the dimensions of the oleate molecule and the total interfacial area in the emulsion were known, it would be a relatively simple matter to calculate the total interfacial coverage.

The dimensions reported for various oleate salts vary greatly. Kremnev and Kagan (4) reported values for the molecular areas of different oleate salts ranging from 50 Å^2 for sodium oleate to 258 Å^2 for cesium oleate. Calculation of the total interfacial coverage, based on a model with a dimension of 50 Å^2 showed a greatly insufficient monomolecular layer in the present system; whereas if the calculation is based on a 258 Å^2 model, an almost continuous monomolecular layer is shown. Thus, a knowledge of molecular dimensions in the glycerin–oil system is essential to the accurate calculation of interfacial coverage.

This study was designed to determine the minimum concentration of surfactant needed for emulsification and to obtain the interfacial tension data needed to confirm the effective area of the surfactant molecule.
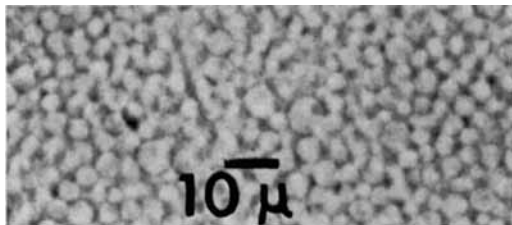
Fig. 1.—Photomicrograph of emulsion containing 0.6 mg. ammonia/100 ml. at a phase volume of 0.40.
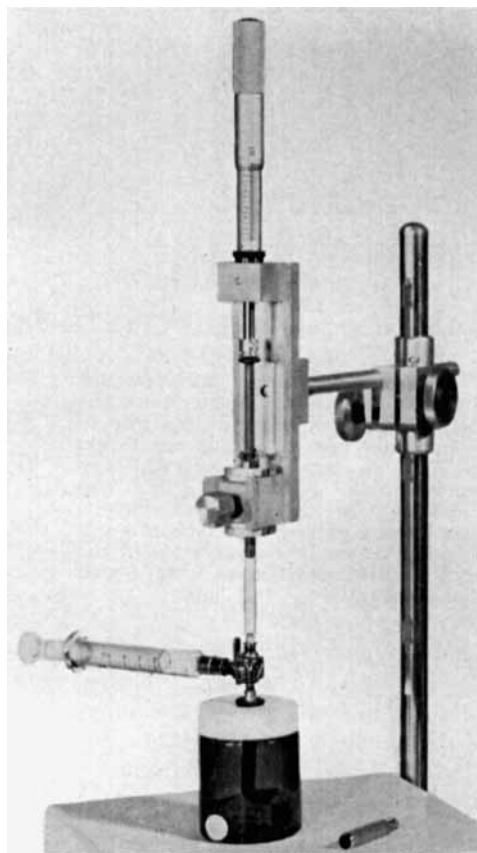

Fig. 2.—Drop-volume instrument.

EXPERIMENTAL

The olive oil used was U.S.P. quality,[2] and the glycerin was 99+% purity,[2] confirmed by its refractive index. No further purification was performed. Appropriate concentrations of ammonia and AMP were prepared from stock solutions (by weight) of ammonia gas and AMP in glycerin.

The specific order of mixing the ingredients to be emulsified was the same as that reported previously (1). Briefly, the stock solution of amine was added to the requisite amount of glycerin contained in a Waring Blendor equipped with a Polytron Rotor-Stator[3] and mixed thoroughly. The appropriate amount of oil then was added as a slow continuous stream during emulsification. Mixing was continued for approximately 1 min. after the

complete addition of the oil. Deaeration of the emulsion was effected while stirring *in vacuo.*

The two phase volumes chosen were 0.40 and 0.58. A phase volume of 0.58 corresponds to the 50% weight combination used in previous work (1, 2).

Phase-contrast photomicrography[4] was utilized in the determination of the droplet diameters of freshly prepared emulsions. Conventional microscopic and staining techniques were found unsatisfactory.

Figure 1 shows one of the photomicrographs used to determine droplet size. Two or three 1-in. squares were arbitrarily selected from each photomicrograph, and the diameters of 100 to 200 droplets within these areas were measured. The average droplet diameter (D_m) was calculated statistically using

$$D_m = \sqrt[3]{\frac{\Sigma n D^3}{\Sigma n}} \qquad \text{(Eq. 1)}[5]$$

where n is the number of droplets with a diameter of D. With the mean droplet diameter known, total interfacial area was calculated.

An emulsion was considered stable if it did not display separation after standing at room temperature for 1 week.

Interfacial tension was measured by means of a drop volume instrument[6] (Fig. 2), which was constructed especially for this study. The 2-in. micrometer head reads to 1×10^{-4} in. and depresses a 1-ml. tuberculin syringe. The syringe was calibrated with the micrometer to give volumes measurable to 4×10^{-4} ml. The 5-ml. syringe attached horizontally to a three-way stopcock served to refill the 1-ml. syringe without disassembling the apparatus. The metal tip was constructed of seamless stainless steel tubing attached to a needle hub. The tip was tapered to give a sharp edge at the inside surface of the tubing (0.257-in. i.d.). It was siliconized with Desicote[7] to insure a lyophobic surface. The syringes, stopcock, and metal tip were washed in warm acetone for several hours before use, and extreme care was used to avoid contamination. An amber ointment jar was used to hold the oil and to receive the glycerin drops. An approximately 0.5-in. hole was made in the plastic cap of the jar to accommodate the metal tip. The metal tip was held in place with a tight-fitting seal. A hole was made near the bottom of the glass jar and an ampul-type rubber stopper inserted. Nitrogen, after passing through Drierite,[8] was bubbled through the oil for 1 hr. The nitrogen was introduced by way of a needle passed through the rubber stopper. The glass container was cleaned with cleaning solution before each use.

Olive oil was placed in the container, and glycerin with either ammonia or AMP in solution was added through the syringe and metal tip. The temperature in the room was controlled at 25 ± 1°.

Interfacial tensions were calculated from the data obtained by

$$\gamma = \frac{v(\rho - \rho_0)g}{f2\pi r} \qquad \text{(Eq. 2)}$$

In this equation, γ is interfacial tension, v is the volume of the drop, $\rho - \rho_0$ is the density difference between the two liquids, g is gravity, and f is a correction factor,[9] varying with the drop volume and the radius, r, of the metal tip $(r/v^{1/3})$.

Preliminary observations established that there was a decrease in drop volume, with an increase in time for drop formation. Therefore, the procedure employed was to form drops of various volumes and record the time needed for the drop to fall from the tip. To maintain a high degree of accuracy in determining interfacial tension by the drop volume method, the ratio $r/v^{1/3}$ must fall within a narrow range of values. Since r remains constant for a given metal tip, any change in v is reflected by a change in the ratio $r/v^{1/3}$. The procedure employed in this study imposed limitations on the range of drop volumes which could be recorded. These limitations did not allow measurement of volumes after interfacial tension equilibrium had been attained. Since the graph of interfacial tension *versus* time is a curve, extrapolation to some time longer than that possible to measure could not be accomplished with the accuracy desired. Therefore, the data were plotted as interfacial tension *versus* the reciprocal of time on log-log paper. A fairly linear line was obtained, thereby facilitating accurate extrapolation. It was assumed that at 1000 sec. equilibrium had been attained and that this point could be used as a reference between different surfactant concentrations.

The Gibbs adsorption equation (Eq. 3) relating surface excess, Γ, to the dependence of interfacial tension on concentration was used to calculate the interfacial molecular area, σ.

$$\Gamma = -\frac{1}{RT}\left(\frac{d\gamma}{d\ln c}\right) = \frac{1}{N\sigma} \qquad \text{(Eq. 3)}$$

The terms R, T, and N are the molar gas constant, absolute temperature, and Avogadro's number, respectively. Concentration is used in Eq. 3 instead of activity because at the concentrations employed, activity should equal concentration. Rearranging Eq. 3

$$\sigma = -\frac{RT}{N}\left(\frac{d\ln c}{d\gamma}\right)$$

and substituting

$$\frac{R}{N} = k$$

gave the final equation used for the molecular area calculations (Eq. 4), in which $(d\ln c)/(d\gamma)$ was obtained from interfacial tension measurements at two known concentrations.

$$\sigma = -kT\left(\frac{d\ln c}{d\gamma}\right) \qquad \text{(Eq. 4)}$$

RESULTS

All emulsions formed were of the oil/glycerin type, verified by dilution of the external phase.

[9] Obtained from Table I-4, p. 25, Adamson, A. W., "Physical Chemistry of Surfaces," Interscience Publishers, Inc., New York, N. Y., 1960.

TABLE I.—MINIMUM AMINE CONCENTRATION AND SURFACTANT INTERFACIAL COVERAGE

Surfactant Precursor	Emulsion Phase Volume	Amine Concn., mg./100 ml.	Droplet Diameter, μ	Mean Effective Area, $\text{Å}.^2$/molecule	Monolayer, %
NH₃	0.40	0.6	2.98	354	94
	0.58	0.84	4.55	229	89
AMP	0.40	2.0	4.63	419	92
	0.58	2.7	5.91	375	98

The minimum amine concentration and the surfactant interfacial coverage is shown in Table I. With respect to ammonia, a minimum concentration of 0.6 mg./100 ml. in a phase volume of 0.40 produced a stable emulsion with a droplet diameter of 2.98 μ, whereas a minimum concentration of 0.84 mg./100 ml. in a phase volume of 0.58 produced a stable emulsion with a droplet diameter of 4.55 μ. When AMP was used as the surfactant precursor, a minimum concentration of 2 mg./100 ml. in a phase volume of 0.40 produced a stable emulsion with a droplet diameter of 4.63 μ, whereas a minimum concentration of 2.7 mg./100 ml. in a phase volume of 0.58 produced a stable emulsion with a droplet diameter of 5.91 μ.

The apparent effective area of the ammonium surfactant molecule was 354 Å.^{2} for a phase volume of 0.40 and 229 Å.^{2} for a phase volume of 0.58. These areas correspond to 94 and 89% interfacial coverage, respectively. (See Table I.)

The AMP surfactant molecule was found to be 419 Å.^{2} and 375 Å.^{2} for phase volumes of 0.40 and 0.58, corresponding to an interfacial coverage of 92 and 98%, respectively.

DISCUSSION

The data presented indicate that stable emulsions of glycerin and olive oil can be formed with exceedingly low concentrations of both surfactant precursors. For example, ammonia in a concentration of only 0.6 mg./100 ml. produced a stable emulsion with a phase volume of 0.40. A possible explanation for the low concentrations of surfactant precursors needed for stable emulsification may be found in the work reported by Valko (5) on comicellization. Comicellization is defined as the phenomenon whereby a surface-active agent in a concentration considerably below its own critical micelle concentration solubilizes an insoluble compound in comparatively large quantities. Valko found comicellization exhibited by the condensation products of fatty acids and fatty acid mixtures with an amine. Furthermore, McBain (6) has suggested that emulsification results when the saturation point of micelles is exceeded and further states ". . . . that when the micelle becomes saturated the addition of still more liquid results in a transition from solution to emulsions." If in the glycerin and oil system employed in the present studies micelles have not been formed, it seems entirely possible that comicellization may be involved.

The effective areas of the surfactants at the interface were found to be 229 Å.^{2} and 354 Å.^{2} for the ammonium molecule and 375 Å.^{2} and 419 Å.^{2} for

the AMP molecule. The large areas exhibited by these molecules may be explained by the work of Kremnev and Kagan (4). They concluded that the size of the cation has an effect on the nature of the interfacial film and that soaps of quite large ions may stabilize emulsions with quite dilute interfacial films. These workers found that cesium oleate, the largest cation reported, displayed the largest molecular area (258 Å.²). According to Pauling (7), the crystal radius of the Cs⁺ ion is 1.69 Å., compared with 1.48 Å. for the NH₄⁺ ion. These dimensions do not take into consideration the effects of solvation in different solvents which can alter their effective size. The comparison is made here to emphasize the order of magnitude of the size of the cation and the area exhibited by their respective molecules. The radii of the Cs⁺ and NH₄⁺ ions are fairly close to one another. Observations of the effective area of the ammonium surfactant differ with the concentration employed (229 Å.² with 0.6 mg./100 ml. and 354 Å.² with 0.84 mg./100 ml.) but are comparable in magnitude to the cesium molecule. As would be expected, the AMP molecule is considerably larger and consequently exhibited a larger molecular area.

It became apparent during this study that the interfacial film was more complex than the simple model of the oleate initially conceived. This is explained readily on the basis of the composition of olive oil. Oleic acid provides over 85% of the approximately 91% unsaturated fatty acids. Saturated acids make up the remainder, with palmitic acid being predominant (3). It must be assumed that in these studies the fatty acids in the form of glyceryl esters also represent the amount and kind of *free* fatty acids available in olive oil to combine with the added ammonia or AMP. Thus, a mixed film or monolayer is thought to be present in the glycerin–olive oil emulsion since the concentrations of added amine were not sufficient to combine with all the fatty acids present. The mixed film is composed of the various fatty acids in both their amine salt form and in their free form.

Considerable evidence supports the interpretation that the monolayer observed here represents a mixed film and that emulsions are more stable because of the presence of this mixed film. Mixed monolayers on water have been found to exhibit molecular areas different from the molecular area of the pure components. Dervichian (8, 9) has discussed examples of this where changes in molecular areas were observed to occur at stoichiometric proportions of the constituents. Varying mixtures of myristic acid and trimyristin monolayers changed mean molecular areas in an almost precisely additive way. This evidence also offers more explanation for the fact that the surfactant molecules reported here were quite large. With regard to the effect of mixed films on emulsion stability, Osipow (10) states that, "qualitatively, it is known that combinations of water-insoluble long chain polar compounds and ionic surfactants form mixed surface films." He also states that more stable o/w emulsions are produced with these mixed films than with ionic surfactants alone. Schulman and Cockbain (11) stated that *optimum* emulsion stability was attained when the monomolecular film was electrically charged, stable, and in a condensed state. In this way, they continued, the interfacial tension is reduced to extremely small values. They concluded that these conditions could be satisfied when the film consists of a molecular complex of two suitable substances, one of which is an oil soluble substance and the other an ionizable water-soluble substance. Lawrence (12) reported that more stable o/w emulsions are obtained when the ionic surfactant and the water-insoluble polar compounds have the same hydrocarbon chain length. Although a condensed film was not present in the system employed, all other requirements for emulsion stability were satisfied.

Calculations of the molecular dimensions of the surfactants established that emulsification was accomplished with 89 to 98% continuous monomolecular film. This fact also demonstrated that an essentially complete film is needed to produce an emulsion complying with the stability criteria utilized in this study.

SUMMARY

The minimum concentration of surfactant needed to emulsify the nonaqueous system glycerin and olive oil was determined. At phase volumes of 0.40 and 0.58, 0.6 mg./100 ml. and 0.84 mg./100 ml. of ammonia, respectively, were needed. The minimum concentration of 2-amino-2-methyl-1,3-propanediol (AMP) was found to be 2 mg./100 ml. and 2.7 mg./100 ml. for phase volumes of 0.40 and 0.58, respectively.

The droplet diameters of the respective emulsions were determined by phase contrast photomicrography.

Interfacial tension studies were employed to determine the effective size of the surfactant molecules at the interface. Both the ammonium and the AMP molecules were shown to be very large in the low concentrations employed. The ammonium salt varied from 229 Å.² to 354 Å.², whereas the AMP salt varied from 375 Å.² to 419 Å.².

Correlation of the molecular sizes of the surfactant molecules with total interfacial areas calculated from the mean droplet diameters indicated that there existed from 89 to 94% interfacial coverage with the ammonia emulsions and from 92 to 98% interfacial coverage with the AMP emulsions.

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