

Fat Emulsion. Effect of Heat on Solubility of Hydrophilic Emulsifiers¹

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EMULSIONS of the oil-in-water (O/W) type, which are prepared for use in intravenous alimentation, must be sterile. Sterilization is accomplished by autoclaving at 121° for 10 to 20 min. This heating imposes a severe physical strain on the emulsion, yet the majority of the particles of dispersed oil must have a diameter at or less than 0.5 μ .

It is generally agreed that the dispersed phase in stable O/W emulsions are spherical, small particles coated with a liquid close-packed film of the emulsifier (7). Of the natural emulsions found in the human intestine the average diameter of the oil particles is 0.5 μ . Although mineral oil is not emulsified in the intestines, it has been found experimentally that the intestinal walls can absorb particles of paraffin oil which are 0.5 μ in diameter (3).

The oil particles in stable emulsions are covered usually by emulsifying agents, used singly or in systems so as to provide both a lipophilic and a hydrophilic moiety. The former group is anchored in the oil droplet, and it is this group which forms the film on the oil particles. The lipophilic entity is usually a long-chain alkyl group, for example, the stearyl group. The hydrophilic part of the molecule of

emulsifying agent must have an affinity for water or a tendency toward some degree of water solubility. Further, hydrophilic emulsifying agents should maintain their solubility in water at those temperatures likely to be encountered in emulsion preparation and sterilization.

The hydrophilic characteristics of a large number of non-ionic emulsifying agents are imparted by their content of various amounts of polyoxyethylene groups. The bonds between the water molecules and such emulsifying agents, which in turn link the oil to the water, are hydrogen bonds. The energy of dissociation of all hydrogen bonds between two oxygen atoms for which data are available is of the order of magnitude of 7 kcal./mole (9, 10). This is a weak bond compared with energies of 25-100 kcal./mole that are characteristic of most single covalent bonds. Simply the kinetic energy of motion at the sterilization temperature (121°) is sufficient to break such bonds, even in the absence of other factors.

In the present investigation the solubility at elevated temperatures of some water-soluble or dispersible emulsifying agents of the polyoxyethylene type was studied. For comparison, some emulsifiers with hydrophilic groups of a different nature, and some with limited or no hydrophilicity, were investigated. An emulsifier of the non-ionic type exhibits inverse

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TABLE I
The Water-Solubility and Emulsification Characteristics of Various Emulsifiers

Type	POE ^a groups per molecule		Inversion temperature °C.	Appearance on cooling to 25°C.	Emulsion description (diameters of oil drops in microns)	
	No.	Wt. %			Before autoclaving ^b	After autoclaving
POE glycol monoesters						
Laurate (Ethofat C-15).....	5	52	—	Dispersion	2-2.5, many 2.5-7.0, clusters	Oil-water phases
Laurate (Ethofat C-20).....	10	68	32	Solution	2, many 7.0	Oil-water phases
Laurate (Ethofat C-25).....	15	76	66	Solution	2, many 7.0	Oil drops-emulsion
Laurate (Ethofat C-60).....	50	91	92	Solution	0.7-1.5, some 4.0	Oil drops-emulsion
Stearate (Ethofat 60-25).....	15	70	66	Solution	0.7-1.5, some 5.0	Oil-water phases
Stearate (Ethofat 60-60).....	50	89	98	Solution	0.7-1.5, few 5.0	Stable 0.7-1.4, few 5.0
Red oil (Ethofat 142-15).....	5	—	—	Dispersion-	—	—
POE amines						
Lauryl (Ethomeen C-15).....	5	54	37	Solution	0.7-1.5, many 2.0-4.0	5-mm. Oil layer-emulsion
Lauryl (Ethomeen C-20).....	10	71	98	Solution	0.7-1.5, many 3.5	Oil-water phases
Stearyl (Ethomeen 18-15).....	5	45	—	Gel	0.7-1.5, some 2-3, few 5.0	5-mm. Oil layer-emulsion
Stearyl (Ethomeen 18-20).....	10	62	74	Dispersion	—	—
POE amides						
Lauryl (Ethomid HT-15).....	5	51	—	Dispersion	1.0-1.5, many 4.0-5.0	Oil-water phases
Lauryl (Ethomid HT-25).....	15	75	95	Solution	1.0-1.5	5-mm. Oil layer-emulsion
POE sorbitan monoesters						
Laurate (Tween 20).....	—	—	95	Solution	0.7-1.5, many 2.0-3.5	Oil-drops-emulsion
Stearate (Tween 60).....	—	—	76	Solution	0.7-1.5, some 5.0	2-mm. Oil layer-emulsion
Oleate (Tween 80).....	—	—	93	Solution	0.7-1.5, some 4.0-5.0	Small oil layer-emulsion
POE sorbitan polyesters						
Dilaurate (G7076H).....	—	—	—	Dispersion	0.7-1.5	2-mm. Oil layer-emulsion
Tristearate (Tween 85).....	—	—	—	Dispersion	—	—
Mixed triglyceride (G931).....	—	—	—	Dispersion	1.0-	Stable 1.0
POE alcohols						
Lauryl (Brij 30).....	—	—	—	Dispersion	0.7-	1-mm. Oil layer
Lauryl (Brij 35).....	(< C50)	—	100	Solution	0.7-, some 2.0	Stable 0.7, some 2.0
Ethylene Propylene Oxide						
(Pluronic F-68).....	—	80	100	Solution	0.7-, some 2.0-3.0	Oil-water phases
Sorbitan esters						
Monolaurate (Span 20).....	—	—	—	Gel	0.7-1.5, few 2.0-3.0	Oil-water phases
Monopalmitate (Span 40).....	—	—	—	Gel	0.7-1.5	Oil-water phases
Monostearate (Span 60).....	—	—	—	Gel	0.7-1.5	Oil-water phases
Tristearate (Span 65).....	—	—	—	Dispersion	—	—
Trioleate (Span 85).....	—	—	—	Oil layer	—	—
Glycerol sorbitan laurate (G672).....	—	—	—	Dispersion	0.7-, some 4.0	Large oil drops-emulsion
Purified soybean phosphatides.....	—	—	—	Dispersion	0.7-, many 2.0	Oil drops-emulsion
Polyglycerol oleic ester (Demal 14).....	—	—	—	Dispersion	2.0-3.0	Oil-water phases
Polyethylene glycol stearate (PGS-400).....	—	—	—	Dispersion	0.7-, some 2.0	1-mm. Oil layer
Monoglyceride (Myvacet).....	—	—	—	Dispersion	Many 20-50	Oil-water phases
Di-acetyl tartaric ester of mono- and diglyceride (TEM).....	—	—	—	Dispersion	1.0-, many 3.0	Oil layer-emulsion

^a Polyoxyethylene.

^b Maximum temperature during homogenization 62°C.

solubility with temperature because it associates with water. At the point of inverse solubility or breaking of the hydrogen bonds with water, solutions of such emulsifiers become turbid. Those emulsifiers which maintain complete solubility at the highest temperatures should aid in forming the most heat-stable emulsions.

Emulsifiers

The cross-sectional areas of the lipophilic long-chain fatty acid groups have been determined by many investigators, using the Langmuir balance on monomolecular films (6, 5, 1). The cross-sectional area of each palmitoyl and stearoyl group was found to be approximately 22 \AA^2 whether it was measured as the acid, or as part of an ester, mono-, di-, or triglyceride molecule. Similarly the cross-sectional area of each oleoyl group was found to be twice as great or 46 \AA^2 . Therefore a calculation can be made as to the approximate amount of an emulsifier needed to cover completely the surface area of the oil droplets of a given particle size in an O/W emulsion. In the work reported here 1% of the various emulsifiers (based on the total weight of the solutions or dispersions) was used, providing a minimum of about 0.03 mole, which was 2 to 4 times the amount of emulsifier required to form a monolayer. The emulsifiers used are listed in Table I.³

The function of any emulsifier is to lower the interfacial tension between the oil and water phases. During the process of homogenization the interfacial area is increased tremendously. As an example, for every spherical droplet of 1-ml. volume (1.25 cm. in diameter) homogenized into spherical droplets 0.5μ in diameter, the interfacial area is increased from 4.9 cm.^2 to $1.2 \times 10^5 \text{ cm.}^2$ If the dispersed phase is a refined, bleached, and deodorized cottonseed oil, which is the oil used in many intravenous emulsions, the process of homogenization would result in a large increase in free energy. Since the interfacial tension of such cottonseed oil is $17.6 \text{ dyne cm.}^{-1}$, the free energy increase would be of the order of $2 \times 10^6 \text{ ergs}$ for each 1 ml. of oil emulsified into droplets 0.5μ in diameter (8). The resulting emulsion would be unstable in the absence of an emulsifying agent. To form easily a stable O/W emulsion having small particle size (approximately 0.5μ in diameter), the interfacial tension should approach zero in order to minimize the increase in free energy due to homogenization (2).

Experimental

One per cent solutions or dispersions of the various emulsifiers in water at room temperature were slowly heated until solubility inversion occurred. After inversion the samples were allowed to cool, and the temperature of clearing was observed. The warming procedure then was repeated. The inversion temperatures were found to be constant on repeated trials. The solubility characteristics are recorded in Table I. In addition to the polyoxyethylene type of emulsifiers other types are also included in Table I.³

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These latter emulsifiers have a limited solubility in water.

In order to determine whether the inversion temperature of emulsifiers in water-solution was related to their behavior in actual emulsions with oil, several emulsions containing 15% by weight of a refined, bleached, and deodorized sesame oil and 1% of an emulsifier (based on total weight) were made in a laboratory homogenizer. It had been experimentally determined previously that an emulsion had to be recycled through the homogenizer at a pressure of 3,500 p.s.i. in order to attain a particle size of about 0.5μ . The procedure used in all cases consisted of first passing the oil and emulsifier through the homogenizer and obtaining homogenization pressure of 3,500 p.s.i., then slowly adding half of the total amount of water. Next the mixture was recycled four times. The remaining water was added and the emulsion was recycled five times at the same pressure. The temperature resulting from homogenization was controlled by a heat exchanger and maintained between 60° – 63° . Particle size of the emulsion was determined by examination with a microscope having a calibrated micrometer scale and an oil immersion lens. A magnification power of 950 was employed. Particle-size determinations were made on undiluted emulsions and emulsions diluted with 4 parts of water. All emulsions were autoclaved in closed bottles with steam at 121° for 10 min. Results are given in Table I. An emulsion was considered to be stable to autoclaving if it maintained the particle size which it had before autoclaving. Each emulsion which was not obviously broken on autoclaving was reexamined microscopically to compare particle sizes before and after autoclaving.

In another series of emulsions of olive oil the temperature rise resulting from homogenization was not controlled, and the changes in particle size of each emulsion were followed as the pressure of homogenization and number of recyclings were increased. Data relating the increase in particle size with increase in temperature are given in Table II.³ In many cases the temperature of homogenization reached 85° – 90° on continuous recycling.

It is realized that the incorporation of a strongly lipophilic emulsifier in addition to a hydrophilic emulsifier would probably tend to prevent growth of

TABLE II
Effect of Homogenization Pressure and Recycling on Particle Size

Emulsifier	POE ^a groups per molecule		Number of recycles and pressure	Emulsion ^b appearance and particle size, microns
	No.	Wt. %		
POE glycol monoesters Lauryl	5	52	8; 2,500 p.s.i. 10; 3,500 p.s.i.	Poor, many 14.0 and up Worse, some 28.0, oil separation
Lauryl	15	69	10; 3,500 p.s.i. 20; 3,500 p.s.i.	General 0.7, some 2.0 Worse, many 3.0
Lauryl	50	92	7; 2,500 p.s.i. 7; 3,500 p.s.i.	General 0.7-1.5, few 3.0 Worse, general 1.5-2.0, some 3.0
Stearyl	50	88	7; 2,500 p.s.i. 1; 3,500 p.s.i. 8; 3,500 p.s.i.	Most 1.0-1.5 Many 3.0 Worse, general 3.0
Polyglycerol ester Oleic	—	—	7; 2,500 p.s.i. 1; 3,500 p.s.i. 15; 3,500 p.s.i. 20; 3,500 p.s.i.	General 0.7-1.5, few 3.0 General 0.7-1.5, few 3.0 Most 1.0-1.5, many 3.0-4.0 Worse, general 2.0-3.0

^a Polyoxyethylene.

^b Emulsion is 15% crude olive oil, 1% emulsifier, 5% dextrose.

particle size during homogenization and would also provide increased stability during autoclaving. Accordingly a limited number of emulsions containing 15% of sesame oil were prepared with various lipophilic emulsifiers in combination with some of the water-soluble emulsifiers containing in excess of 15 polyoxyethylene groups per alkyl group. Typical examples of such emulsions are given in Table III.³

TABLE III
Emulsions With Two Emulsifiers

Emulsifiers	Range of concentration (%) of emulsifiers studied	Stability to autoclaving
POE* lauryl alcohol.....	0.5-1.2	Unstable
Polyethylene-propylene oxide.....	0.3-0.75	
Diacyl tartaric acid ester of mono- and diglyceride.....	0.75-1.0	Unstable
Polyethylene-propylene oxide.....	0.5-0.75	
Glycerol sorbitan triglyceride.....	0.4-0.5	Unstable
Polyethylene-propylene oxide.....	0.5-0.6	
POE lauryl alcohol.....	0.45-0.75	Stable
Glycerol sorbitan triglyceride.....	0.45-0.75	
Polyglycol monester.....	0.3-1.2	Stable
Polyethylene-propylene oxide.....	0.5-0.75	
Diacyl tartaric acid ester of mono- and diglyceride.....	0.3-0.5	Stable
Polyethylene-propylene oxide.....	0.75	
Glycerol sorbitan laurate.....	0.2-0.8	Stable
Polyethylene-propylene oxide.....	0.3-1.2	

* Polyoxyethylene.

Results and Discussion

It is recognized, of course, that many good emulsifiers are not completely soluble in water but form dispersions. However, to provide increased emulsion stability at the temperature of sterilization, the emulsifier must be more hydrophilic than many oil-in-water emulsifiers that are satisfactory in ordinary use and must have an increased affinity for water at temperatures up to 120°C.

As seen from Table I, with a given type of emulsifier containing polyoxyethylene groups, the temperature at which solubility inversion occurs was found to increase as the weight percentage of polyoxyethylene groups increased. The variation in limit of solubility of the glycol lauryl ester type emulsifiers was from insoluble at room temperature to soluble up to about 92°C. as the weight percentage of polyoxyethylene groups increased from 52 to 91%. The glycol stearyl esters were more soluble than the lauryl esters at elevated temperatures. At identical inversion temperatures of 66° the weight percentage of polyoxyethylene groups were 76 and 70 for the lauryl and stearyl esters, respectively; at approximately the same weight percentage of polyoxyethylene groups, 70%, the inversion temperature of the lauryl ester was 32°, and of the stearyl ester, 66°. However a comparison of the types of emulsifiers of approximately the same weight percentage of polyoxyethylene groups shows widely different inversion temperatures. The amine type emulsifier was found to be more soluble than the amide, which in turn was more soluble than the ester type. In some of the polyoxyethylene emulsifiers the percentage of such groups was unknown. The results obtained are analogous to those reported by Griffin in his calculation of HLB values (4).

In the polyethylene-propylene oxide emulsifier, greatly different in chemical type from the glycols or amines, the limit of solubility was not reached at the boiling point of water at atmospheric pressure. Although its weight percentage of polyoxyethylene groups was exceeded by other materials with lower temperatures of inversion, this material exhibited the longest solubility range of all the emulsifiers tested.

Usually the initial particle size of the emulsion depends on the pressure of homogenization, under otherwise equal conditions. Data in Table I indicate that, in most cases, the general particle sizes immediately after the homogenization process, during which time a heat exchanger was used, were about the same. However the stabilities of the emulsions at the temperature of the autoclave varied greatly.

As the temperature rise due to homogenization increased on recycling without external cooling, the particle size in a given emulsion also tended to increase, as shown in Table II. In the case of the emulsifier with the low solubility inversion temperature of 32°, oil actually separated out on recycling as the temperature approached 90°. In the other cases oil was not visible on recycling, but the number of droplets larger than 3 μ per microscopic field increased. The initial temperature of homogenization was 25°, and the maximum temperature reached during continuous recycling was 85-90°.

Emulsions were made with some emulsifiers whose inversion temperatures were above 85°, which is the usual temperature reached during repeated recycling during homogenization at pressures as high as 3,500 p.s.i. With these emulsifiers the increase in particle size was not as great as with those emulsifiers which inverted under 85°. The polyethylene-propylene oxide was able to withstand the highest temperature reached by the homogenizer (85-90°C.).

The results obtained indicate that the combination of high temperature and extreme mechanical shear caused by homogenization at high pressure causes particle-size growth in emulsions containing only those emulsifiers which exhibit solubility inversion at temperatures lower than about 85°. It is apparent that the weight percentage of polyoxyethylene groups must reach a certain optimum for a given alkyl group for a given emulsifier which is in agreement with the report of Griffin (4). In general, emulsifiers require approximately 70% by weight of polyoxyethylene groups in order to withstand homogenization and sterilization in emulsions of the type investigated.

It is not the purpose of the present paper to enumerate those emulsions which are stable with respect to particle size and phase separation and those which are not. However it is evident from the data of Table III that a combination of hydrophilic-lipophilic emulsifiers results in a better emulsion than if either emulsifier is used alone. The results indicate that an emulsion can be improved with respect to particle size by the use of two emulsifiers rather than only one. For example, an emulsion containing a polyoxyethylene lauryl alcohol as the only emulsifier showed no separation of phases on autoclaving, but the particle size ranged up to 2 μ in diameter. By the addition of a more lipophilic emulsifier, polyoxyethylene sorbitol triglyceride, the particle size was uniformly about 0.7 μ . The assumption must be made that a more stable bond between the oil and water phases was obtained, and the change in free energy

of interfacial surface formation was less when two emulsifiers were used.

As indicated by the results, if two strongly hydrophilic emulsifiers are used, the resultant emulsion does not have good particle size, probably because of the limited number of oil-anchoring alkyl groups.

Summary

The bonds between some water-soluble emulsifiers and water are hydrogen bonds with low energies of dissociation, of the order of 7 kcal./mole. In the absence of other factors such bonds can be broken by the kinetic energy of motion at elevated temperatures.

The solubility of several emulsifiers was determined, and emulsions containing these emulsifiers at concentrations 2 to 4 times the amount required to make a monomolecular film of the oil droplets were made.

To provide emulsion stability at homogenization and sterilization temperatures the emulsifiers must be more hydrophilic than many oil-in-water emulsifiers that are satisfactory in ordinary use and must have an increased affinity for water in the temperature range of 5° to 120°. For a given type of emulsifier containing a given alkyl group, an optimum weight percentage of polyoxyethylene groups is required.

The solubility of an amine type emulsifier with the same alkyl group and approximately the same weight percentage of polyoxyethylene groups per molecule is greater than that of the corresponding

amide compound, which, in turn, is more soluble than the corresponding ester type of emulsifier, because of differences in chemical type. Polyethylene-propylene oxide had the longest solubility range of the emulsifiers tested.

An increase in particle size or an appearance of two phases in emulsions prepared with emulsifiers which undergo solubility inversion below 85° was found. Emulsions prepared with emulsifiers whose inversion temperatures were above 85° maintained, generally, a low particle size on autoclaving, did not separate into a watery phase and an emulsion phase, and did not form a layer of oil.

Emulsions prepared with two emulsifiers, such that one had some lipophilic characteristics stronger than the other, were found to be stable and maintain a low particle size on autoclaving.

REFERENCES

1. Adam, N. K., Proc. Roy. Soc. (London), *99A*, 336 (1921).
2. Bull, H. B., "Biochemistry of the Lipids," p. 144, New York, John Wiley and Sons Inc., 1937.
3. Frazer, A. C., J. Physiol. (London), *103*, 306 (1944).
4. Griffin, William C., Am. Perfumer & Ess. Oil Rev., 26-29 (May 1955).
5. Harkins, W. D., Chem. Revs., *29*, 385 (1941).
6. Langmuir, I., J. Am. Chem. Soc., *39*, 1848 (1917).
7. Schulman, J. H., and Cockbain, E. G., Trans. Faraday Soc., *36*, 651 (1940).
8. Singleton, W. S., and Benerito, Ruth R., J. Am. Oil Chemists' Soc., *32*, 23 (1955).
9. Wheland, G., "Advanced Organic Chemistry," 2nd ed., p. 49, New York, John Wiley and Sons Inc., 1949.
10. Wheland, G., "The Theory of Resonance," p. 46, New York, John Wiley and Sons Inc., 1944.

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Dilatometric Properties of Some Butyropalmitins, Butyrostearins, and Acetopalmitins¹

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CERTAIN DI- AND TRIGLYCERIDES containing both short and long chain fatty acids normally solidify to waxy solids and as such possess a number of potential uses (3, 4). These glycerides are unique because of their ability to exist in waxy polymorphic forms which apparently are seldom thermodynamically stable but usually are stable for all practical purposes.

More than 20 articles and patents describe these unique glycerides, generally acetoglycerides, but only one of these articles is concerned with dilatometric behavior (6). It is a report of the dilatometric properties of 1-aceto-3-stearin and 1,2-diaceto-3-stearin. Two related articles are concerned primarily with other thermal properties and polymorphism of various glycerides (4, 7).

The present investigation was undertaken to obtain dilatometric and related data on six compounds which have heretofore not been described in the literature or for which information is meager. The six compounds, thought to have potential use in some practical applications, are: 1-butyro-3-palmitin, 1,2-dibutyro-3-palmitin, 1-butyro-3-stearin, 1,2-dibutyro-3-stearin, 1-aceto-3-palmitin, and 1,2-diaceto-3-palmitin.

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Materials and Procedures

Materials. Each of the aceto- and butyroglycerides was prepared by the direct esterification of 1-monopalmitin or 1-monostearin with either acetyl or butyryl chloride, using a procedure described previously (6). Briefly, the monoglyceride and acid chloride, each in chloroform solution, were mixed in the presence of pyridine and allowed to react at room temperature. After completion of the reaction the solution was washed successively with dilute acid, dilute alkali, and water. Then the chloroform was removed by stripping at a low temperature. The triglycerides were purified by several recrystallizations from acetone. The diglycerides were purified by cooling a hexane solution to remove the greater part of the uncombined monoglyceride, after which the partially purified diglycerides were recrystallized several times from acetone.

The monoglycerides used in the preparations possessed a purity of about 99%, estimated on the basis of melting points, hydroxyl values, and other analytical determinations. The acid chlorides were used as received from a manufacturer of reagent grade chemicals. The final aceto- and butyroglycerides possessed purities of 96% or better, with the triglycerides tending to be of higher purity than the diglycerides. The