2. Condon, M. Z., Jensen, E. A., Watts, A. B., and Pope, C. W., J. Agr. Food Chem., *2*, 822–826 (1954).
3. Daniels, F., Mathews, J. H., and Williams, J. W., "Experimental Physical Chemistry," McGraw-Hill, New York (1934).
4. Haddon, R., Schwartz, A. K., Williams, P. A., Thurber, F. H., Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R. T., and Altschul, A. M., Cotton Gin and Oil Mill Press, *51*, (9), 18–20 (1950).
5. King, W. H., Wolford, L. T., Thurber, F. H., Altschul, A. M., Watts, A. B., Pope, C. W., and Conly, J., J. Am. Oil Chemists' Soc., *33*, 71–74 (1956).
6. King, W. H., Knoepfler, N. B., Hoffpauir, C. L., and McCourtney, E. J., J. Am. Oil Chemists' Soc., *34*, 27–30 (1957).
7. Lyman, C. M., Chang, W. Y., and Couch, J. R., J. Nutrition, *49*, 679–690 (1954).
8. Milligan, J. L., and Bird, H. R., Poultry Sci., *30*, 651–657 (1951).
9. Neurath, N., Greenstein, J. P., Putnam, F. W., and Erickson, J. O., Chem. Revs., *34*, 157–265 (1944).

[Received September 9, 1957]

The Behavior of Distilled Monoglycerides in the Presence of Water[1]

G. Y. BROKAW and W. C. LYMAN, Distillation Products Industries, Division of Eastman Kodak Company, Rochester, New York

THE EFFECT of purified monoglycerides on interfacial tension with water has been studied for triglycerides (1, 2) and for petrolatum and amyl acetate (2). Also the effect of monoglycerides on water absorption into cottonseed oil and petrolatum has been reported (1). Comparison with mono- and diglyceride mixtures indicated that the effects were not always proportional to monoglyceride content.

In the absence of fat or petrolatum some unusual qualitative differences became evident. This paper describes gels and other structures resulting from the combination of several distilled monoglycerides with water, and with water plus other materials.

Gels

When distilled monoglycerides (C_{14} or higher, see footnote, Table I) are melted and added to water at the same temperature, a firm "gel" is formed (Figure 1). The gel, usually containing about 15% to 25% by weight of water, is not dispersible in water and is difficultly soluble in oil. If the excess water is decanted, the gel can be worked mechanically into an optically clear solid with a consistency resembling very heavy stopcock grease.

Upon cooling a gel below the melting point of the monoglyceride, crystals begin to form. If crystallization proceeds appreciably, the gel structure deteriorates, and a white creamy paste is produced.

Thus the lower temperature limit of stability is related to melting point; 1-monostearin (m.p. 81°C.) gels are stable above about 80°C., and 1-monoolein (m.p. 35°C.) gels are stable above about 20°C. Mixed monoglycerides, such as those from natural fats and oils, yield mixtures of gel and paste which are not readily dispersed in water.

The upper temperature limit of stability is very close to 100°C. In most cases, if a piece of gel is heated on a spatula, the water begins to boil out before the solid structure deteriorates. Highly unsaturated monoglycerides (*e.g.*, from cottonseed oil or soybean oil) form gels which change at about 98°C. into water and oil layers.

Upon standing exposed to air, a gel loses water. The equilibrium status is not yet fully defined, but the water content at low relative humidity is probably well below 5%. Thus a gel can be kept only in a closed container or under extreme humidity conditions. One monoolein gel was kept in a stoppered bottle at room temperature for more than a year without appreciable change in appearance.

Water is partially soluble in a pure molten monoglyceride. As water is added, the apparent melting point drops somewhat, then the viscosity of the molten material begins to increase. The viscosity becomes high enough at about 10% and 15% by weight of water (monoolein at 25°C.) to result in a soft nonpourable transparent "solid." As the ratio of water is further increased, the solid becomes firmer until no more water can be incorporated.

A typical gel preparation contains 22% to 23% of water. But by severe mechanical manipulation at room temperature, it has been possible to prepare a monoolein gel containing up to about 45% of water without leaking. The gel may be of hydrogen bonded structure since preliminary measurements indicate a conductivity similar to that of the distilled water used ($K = 2.5 \times 10^{-6}$ mhos. per cm.) and appreciably higher than that of a typical water-in-oil emulsion ($K = <5 \times 10^{-8}$ mhos. per cm.).

Gel Preparation

Two techniques were used to prepare gels in order to demonstrate the effect of temperature and of the ratio of water on their physical properties. In the first case the monoglycerides listed in Table I (1 g. in each experiment) were added to water (100 g.) and stirred as the temperature was raised. Two transitions occurred in most cases, one in all cases. At about 10°–15°C. below the melting point the monoglyceride smoothed into a creamy dispersion. Then the monoglyceride coagulated into a non-dispersible lump which was translucent to transparent. This last conversion was called the "gel point."

Upon cooling this mixture of gel and water below the gel point, but above the first transition, and holding for a prolonged period of time with agitation, the

TABLE I

Gel Points of Monoglycerides

Monoglycerides [a]	m.p. °C.	"Gel point" °C.
Distilled monolaurin	54–57	No gel to 99°C.
Distilled monomyristin	62–65	85
Distilled monopalmitin	67–68	68
Distilled monostearin	70–72	70
1-Monomyristin	70.5	No gel to 99°C.
1-Monopalmitin	74–74.5	82
1-Monostearin	81–81.5	81
Distilled monoolein	35	<20

[a] Distilled products usually contain about 90–92% 1-monoglyceride, 5–8% of 2-monoglyceride, and 1–3% of glycerine, diglycerides, and free fatty acids (3).

---

[1] Communication No. 240 from the laboratories of Distillation Products Industries.

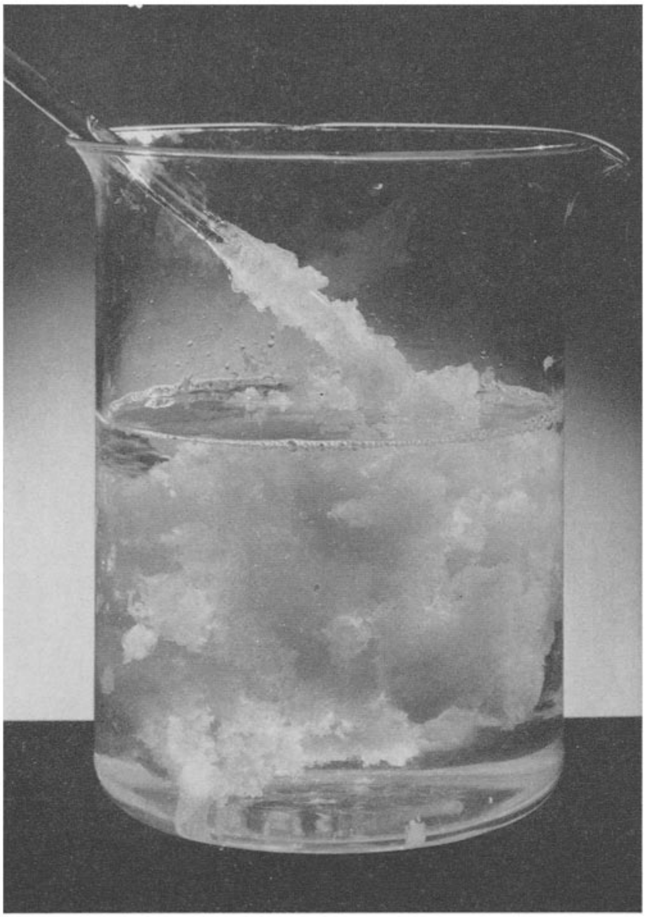

FIG. 1. Gel produced by adding monoolein to water.

smooth creamy dispersion could be regenerated. Upon cooling below the first transition point a marked viscosity increase occurred. This change in viscosity was reversible with temperature (see "Solutions").

By the second preparative technique the monoglyceride was heated to 96°–98°C., and vigorous agitation was begun. Boiling water was added very slowly, allowing time for any solid gel to be "dissolved." It was sometimes necessary to scrape the agitator shaft or container walls to release solid gel. After the desired amount of water was added, with adequate allowance for evaporation, the mixture was set aside to cool before weighing. If monoolein was used, the product could be examined at room temperature.

Using monoolein, it was found that additions of 1% to 5% water lowered the melting point and produced a viscous, creamy fluid at 25°C. At water contents of 10% the mixture was clearer optically, but the viscosity increased. At about 15% water the mixture was non-pourable. It was not possible to incorporate more than about 40% by weight (based upon the monoglyceride) of water by this technique. Cold manipulation of the resultant gel with additional water permitted a maximum incorporation of about 9 parts of water to 10 parts of monoglyceride.

"Solutions"

About 10 gal. of a mixture of 80% water and 20% distilled monoglycerides prepared from hydrogenated lard were held at 61.5°C. over-night. Beneath the surface crust there was a pool of clear liquid which solidified at 58°C. This solidification temperature was identical with the first transition temperature, where the viscosity change occurred.

By analysis the clear liquid contained 21% monoglyceride. Some of it was separated and dried. The melting point of the recovered monoglyceride and the molecular weight of the fatty acids were the same as for the original monoglyceride, indicating no palmitin-stearin fractionation.

The clear liquid exists as much as 10° to 12°C. below the melting point of the monoglyceride (down to about 58°C.), and yet does not appear to be a true solution. One sample held at 61.5°C. slowly separated into a bottom layer of water (immiscible with this liquid), a middle layer of original liquid, and a top layer apparently of solid monoglyceride. Another sample gelled when heated to about 66° to 67°C. Because of this crystallization, or gelation, it was necessary in this preparation to keep the container wall between 58°C. and 66°C.

Diglyceride and Triglyceride Additives

It can be seen from Table I that the gel point varies with the chain length of the fatty acid in the monoglyceride and with its purity. This latter point is illustrated in that pure 1-monopalmitin has a lesser tendency to gel (or at least has a higher gel point) than distilled monopalmitin. On the other hand, the relatively impure mono- and diglyceride mixtures do not gel.

Triglycerides and distilled diglycerides were added to distilled monoglycerides (all preparations from lard), and the technique used for obtaining the data in Table I was then employed to find the amount of each of these additives necessary to prevent gelation. Table II shows the results. It can be seen that the addition of 20% triglycerides was as effective as the addition of 40% diglycerides in preventing gel formation under the experimental conditions. It is thus possible to use higher concentrations of monoglyceride in aqueous systems without gelation by using monoglyceride-triglyceride blends than by using mono- and diglyceride mixtures.

TABLE II

Prevention of Gelation by Other Glycerides

% ratio of monoglyceride to diluent	Effect	
	Diglyceride	Triglyceride
90:10	Gel	Gel
85:15	Gel	Partial gel
80:20	Partial gel	No gel
65:35	Partial gel	No gel
60:40	No gel	No gel

If the technique is employed of adding relatively small quantities of water to a blend of as little as 80% monoglyceride and as much as 20% triglyceride, an optically clear, non-pourable mixture can be obtained which appears to have a loose gel structure. This however will easily absorb additional water, changing into the customary white, opaque water-in-oil emulsion. Much higher ratios of triglyceride than 2 to 8 result in white, opaque emulsions as soon as the water solubility limit is reached. At ratios of 1 to 9 or below, the solid, clear gels will not absorb additional water or disperse readily into fat-water mixtures.

The solubility of water in blends of monoglyceride and triglyceride is usually about 10% to 25% of the monoglyceride content (2 to 5 mols. of water per mol.

of monoglyceride). The effect of adding water to a mixture of 50% monoolein and 50% of cottonseed oil is shown in Table III. The monoglyceride solubilizes the water in the cottonseed oil and also water-solubilizes the monoglyceride. Similar effects have been obtained with hydrocarbon solvents as well as with triglycerides at appreciably lower concentrations of monoglyceride.

TABLE III

Monoolein (1 pt.)—Cottonseed Oil (1 pt.)—Water Mixtures

% H_2O added—based on monoolein	Physical state at:			
	60°F.	80°F.	105°F.	115°F.
0	S	S	L	L
5	S	L	L	L
10	CL	L	L	L
15	CL	L	L	L
20	CL	L	L	LL
25	CL	L	CL	LL
30	CL	CL	CL	LL

S = Solid
L = Liquid
CL = Cloudy Liquid
LL = Two Liquid Layers

Many water-soluble materials can be dissolved in the water before putting it into the oil solution. Organic acids, such as ascorbic and nicotinic acid, are readily dissolved whereas inorganic salts, such as sodium chloride, are much less soluble. All of these results agree with micellar solubilization as described by Singleterry (4).

Solvent Additives

Gels can be dissolved in solvents, such as ethanol and petroleum ether. This is to be expected for ethanol since liquid distilled monoglycerides are soluble in 50% aqueous ethanol.

A study of mixtures of monoolein, ethanol, and water was carried out by pipetting small quantities of water into solutions of monoolein in ethanol. The mixtures were stirred thoroughly after each addition of water. At the solubility limit, cloudiness developed, then at a later stage for most of these mixtures an albuminous appearance developed, which quickly changed into a cloudy appearance again. The albuminous stage had essentially all of the physical characteristics of a Type A co-emulsion described under Surface-Active Additives. A peculiar, relatively low viscosity, clear region was then obtained at certain cencentrations. The system is quite temperature-dependent with gels forming from the cloudy mixtures when warmed. The results at 25°C. are summarized in Figure 2, with the boundary lines (except for the initial solubility limit) being more diffuse than shown. Extreme viscosities prevented satisfactory measurement of water additions to solutions with more than 8 parts of monoolein to 2 of ethanol.

It was also possible to dissolve 15% by weight of water into 30% to 50% solutions of monoolein in petroleum ether (Skellysolve B). Cloudiness developed in all cases before 25% water addition had been completed.

Surface-Active Additives

Mono- and diglyceride mixtures have been marketed in a "self-emulsified" or soap-containing form for many years. The relatively weak, water-in-oil emulsifying properties of mono- and diglyceride mixtures permit oil-in-water emulsions to be formed by

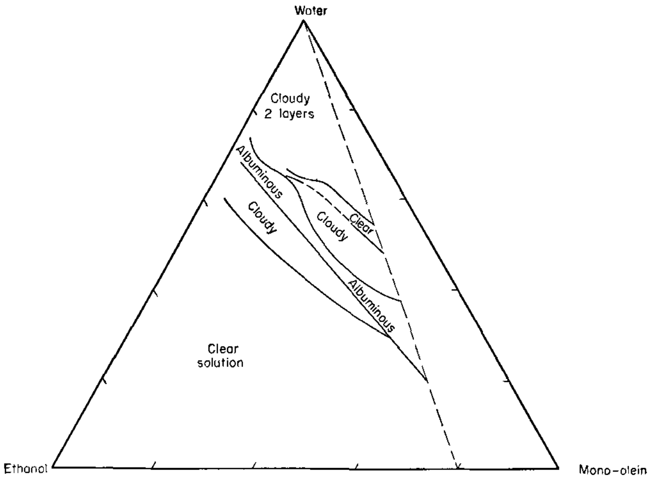

FIG. 2. The system monoolein-water-ethanol at 25°C. (MYVEROL type 18-71).

this technique. It is also possible to disperse distilled monoglycerides in water, using soap; the appearance of the emulsion is different.

Preparation of such a co-emulsified blend can be effected by mixing water, powdered soap, and molten monoglyceride and applying vigorous agitation. The temperature of agitation must be above the gel point. Extreme agitation, such as that in a Waring Blendor or even in a high-pressure dairy homogenizer, is necessary to provide the greatest degree of homogeneity and the greatest stability of the resultant emulsions.

Three types of monoglyceride co-emulsified blends exist. The first (Type A) exists at or above the normal gel temperature of the monoglyceride. It is amorphous by X-ray diffraction (as is the gel), is quite translucent, in some cases transparent, and has a consistency resembling egg white or a fresh dispersion of starch in hot water. It is thixotropic (Table IV). A dilute Type A co-emulsion, when whirled in a container, has been observed to back up as much as 180°, demonstrating an extremely elastic nature.

Type B emulsions result when Type A emulsions are cooled appreciably below the gel temperature of the monoglyceride. Type B is a crystalline dispersion having more of a translucent pudding appearance, not quite as viscous but still thixotropic (Table IV). It resembles a cooled or aged dispersion of starch in water. It has lost most of the elasticity and filamentous appearance. The crystal structure is *alpha*, as determined by comparison of X-ray diffraction patterns with those of Lutton (5).

TABLE IV

Thixotropy of Type A and B Emulsions [a]

Brookfield, viscometer speed, r.p.m.	Measured viscosity, cp.	
	Type A, 65°C.	Type B, 35°C.
20	430	280
10	700	400
4	1,600	600
2	2,300	1,000

[a] Ten g. distilled monoglycerides of hydrogenated lard, 200 g. water, and 0.5 g. sodium oleate.

Upon standing, most Type B emulsions change or "revert" to Type C. This is a white, opaque dispersion in water which resembles a normal dispersion of hard fat; the crystal structure is in the *beta* form. While some of these emulsions are undoubtedly complex structures, all three forms disperse in water.

As long as the temperature is maintained above the gel point, Type A emulsions appear to be stable indefinitely. The stability of Type B emulsions varies depending upon the composition, method of preparation, and temperature of storage but may be between minutes and months. Type C emulsions appear to be stable indefinitely, and the water can be evaporated to yield a very fine, free-flowing powder. Type A emulsions can be regenerated from either Type B or Type C by heating above the gel point. This cycle is shown in Figure 3.

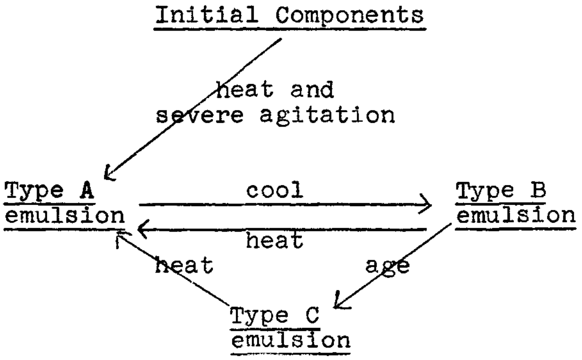

FIG. 3.

Effective co-emulsifiers are the strongly hydrophilic types, which are usually water-soluble, and may be anionic, nonionic, or cationic. Examples which have been used successfully include ordinary sodium soaps, sodium lauryl sulfate, polyoxy compounds (Tween 80), sucrose monopalmitate, quaternaries, and certain protein hydrolysates. The more hydrophilic materials are more effective, and less is required to accomplish the same result. Table V shows that an optimum quantity of co-emulsifier exists, at least when sodium oleate is the co-emulsifier.

TABLE V

Type B Emulsion [a] Stability of Distilled Monoglycerides from Hydrogenated Lard with Sodium Oleate

Sodium oleate g.	Time (days) to develop β-crystals at 25°C.
0.2	3
0.4	3
0.5	28
0.7	10
0.9	4
2.0	1

[a] 20 g. of monoglyceride and 200 g. of water.

Summary

When concentrated monoglycerides (such as distilled products) are heated to about their melting point with water, a gel is formed. The exact temperature of gelation is dependent upon molecular weight of the fatty acid (monolaurin does not gel, but monopalmitin does) and upon the the purity of the monoglyceride.

Additives can prevent gelation, with triglycerides (15% to 20% required) about twice as effective as diglycerides (30% to 40% required). Highly hydrophilic co-emulsifiers prevent gelation, resulting in one of three types of emulsions, at least two of which are thixotropic.

Water is partially soluble in monoglycerides and in monoglyceride containing blends. By this technique many water-soluble materials can be incorporated into an oil solution.

Acknowledgments

Appreciation is expressed to R. L. Griffith of the Eastman Kodak Research Laboratories for determining and interpreting X-ray diffraction patterns.

REFERENCES

1. Kuhrt, N. H., Welch, E. A., and Kovarik, F. J., J. Am. Oil Chemists' Soc., *27*, 310–313 (1950).
2. Gros, Audrey T., and Feuge, R. O., J. Am. Oil Chemists' Soc., *28*, 1–4 (1951).
3. Brokaw, G. Y., Perry, E. S., and Lyman, W. C., J. Am. Oil Chemists' Soc., *32*, 194–197 (1955).
4. Singleterry, C. R., J. Am. Oil Chemists' Soc., *32*, 446–452 (1955).
5. Lutton, E. S., and Jackson, F. L., J. Am. Chem. Soc., *70*, 2445–2449 (1948).

[Received June 12, 1957]

Oxidized Fatty Acid-Protein Complexes[1]

K. A. NARAYAN and F. A. KUMMEROW, Department of Food Technology,
University of Illinois, Urbana, Illinois

PROTEINS ARE KNOWN to form complexes with a wide variety of substances, such as alkyl benzene sulfonates (1), p-amino azo benzene (2), gossypol (3), phospholipides (4), sterols (5), and various other lipide materials. However most of these complexes are labile and could be easily ruptured to give back the constituent molecules in their original form by use of simple techniques, such as extraction with a suitable solvent system. Although considerable work has been done on lipoprotein complexes, very little is known regarding the structure of these conjugated proteins (6). Dervichian considered the association between the lipide and the protein to involve a weak lipide-lipide linkage, which could be split by ether when the water barrier was removed, and a somewhat stronger lipide-protein linkage that could be split by use of boiling alcohol (7). The presence of a strongly bound lipoprotein which is resistant to solvent action has been reported by Folch *et al.* as occurring in brain tissues (8).

Recently Tappel observed that unsaturated fatty acids and esters could react with proteins forming rather stable complexes which could not be ruptured by solvent treatments (9). He attributed the stability of these complexes as being caused by a chemical union between the aldehydes produced during autoxidation of the lipide and the reactive amino groups of the protein. Tappel obtained these stable lipide-pro-

---

[1] Portion of a thesis presented by K. A. Narayan as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology. Supported by a grant-in-aid from Swift and Company and by research grant No. C-1932 from the National Institute of Health, U. S. Public Health Service, Department of Health, Education and Welfare.