TABLE I

Effect of Prolonged Storage of Cottonseed Pigment Glands and Pure Gossypol on Their Acute Oral Toxicity and Gossypol Content

Sample No.	Description	Before storage		After storage		
		Gossypol content	Oral LD50 value	Time stored	Gossypol content	Oral LD50 value
		%	mg./kg.	yrs.-mos.	%	mg./kg.
1.	Untreated cottonseed pigment glands	....[a]	1060	9–7	36.8[b]	1100
2.	Sample 1 heated dry for 1 hr. at 105°C	....[a]	1110	8–3	35.1[b]	1310
3.	Untreated cottonseed pigment glands	....[a]	1350	9–0	29.7[b]	1480
4.	Sample 3 heated dry for 1 hr. at 103°C	....[a]	1520	8–5	27.3[b]	1710
5.	Untreated cottonseed pigment glands	32.5[b]	1430	5–1	30.2[b]	1410
6.	Untreated cottonseed pigment glands	28.6[b]	2170	4–9	27.0[b]	1965
7.	Pure gossypol	ca. 100.0[b]	2480	5–8	....	2200[c]
8.	Pure gossypol	ca. 100.0[b]	2600	7–4	....	2315[c]
9.	Untreated cottonseed pigment glands	37.8[b]	1140	....	....	....
10.	Untreated cottonseed pigment glands	34.3[b]	1345	....	....	....
11.	Untreated cottonseed pigment glands	30.3[b]	1635	....	....	....
12.	Untreated cottonseed pigment glands	34.1[b]	1845	....	....	....

[a] Analyzed by the then current antimony trichloride method of Boatner *et al.* (10, 11).
[b] Analyzed by the Official A.O.C.S. method of Pons and Guthrie (9).
[c] Administered in soybean oil instead of distilled water.

Experimental

The acute oral toxicity was determined on male rats (150–220 g.) of the Holtzman strain, which had been fasting for 18 hours with water *ad libitum*. Each rat was individually caged in an air-conditioned animal room maintained at 78 ± 1°F. and *ca.* 45% relative humidity. All of the samples had been stored in sealed containers in coolers held at 2 to 10°C. The pigment glands (or gossypol) were thoroughly mixed with distilled water and administered in a single dose. After intubation all animals were allowed free access to stock diet and water. Calculation of the median lethal dose (LD50) was made after one week by the method of Reed and Muench (8). Duplicate gossypol analyses on the stored samples were made by each of two independent laboratories, according to the method of Pons and Guthrie (9).

Results

A summary of the LD50 values and the gossypol analyses before and after storage is given in Table I. The total number of rats used was 1,148. It may be noted that there was no great change either in the LD50 values or in the analyzed gossypol content of the samples stored from 4¾ to 9½ years. As was also the case before storage, there is no apparent correlation between the acute oral toxicity of the various stored samples of cottonseed pigment glands and their analyzed gossypol content. In all cases the samples of cottonseed pigment glands containing only 27.0 to 37.8% gossypol were more toxic than pure gossypol.

Summary

Six samples of cottonseed pigment glands and two samples of pure gossypol stored for more than four to nine years were re-evaluated for their acute oral toxicity in the rat and re-analyzed for gossypol content. There was no appreciable effect on the acute oral toxicity or gossypol content after these long storage periods.

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An Electron Microscope Study of Certain Dispersions of Detergents in Oil[1]

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IN CONTRAST to the extensive investigations of soaps and detergents in aqueous solution, comparatively few studies have been reported on such compounds in hydrocarbon solvents. The studies which have been made show that the hydrocarbon systems are similar in many ways to aqueous systems (7, 9). Micelles of soaps and detergents in hydrocarbon solvents are generally assumed to be "inverted" (*i.e.*, with polar heads in the interior of the micelle). In liquid systems of this type, micelles have been postulated which are threadlike or rodlike (8), spherical (14), platelike (18), and lamellar (11). Arkin and Singleterry (1) and Singleterry and Weinberger (16) have presented evidence to show that the critical concentration for micelle formation in benzene solutions of calcium xenyl stearate is less than 10^{-6} moles/liter at room temperature and that the size and shape of soap micelles in benzene may be markedly altered by small amounts of water.

Nonliquid soap-hydrocarbon systems have been fairly extensively investigated (3, 8, 10). Such sys-

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tems are microcrystalline pastes or gels. The electron microscope has been successfully used in the study of commercial greases by Farrington and Birdsall (5) and by others.

This paper presents some results obtained in an electron microscopic investigation of liquid dispersions of detergents and soaps in oil. This investigation was undertaken in connection with research on the dispersion of detergent additives in lubricating oils.

Experimental Methods

The detergents and soaps used in this investigation were experimental oil additives of commercial purity (with the exception of the lead oleate). The calcium cetyl phosphate is a mixture of mono- and dicetyl substituted phosphates (12). The sulfurized calcium alkyl phenate is made by neutralization of an alkyl phenol of approximately 300 molecular weight (4). The product is then reacted with slightly less than a stoichiometric amount of sulfur. These additives were available in the form of oil concentrates containing 30% to 40% by weight of the additive and were used without further purification in the preparation of oil solutions studied. Lead oleate was prepared by dissolving C.P. lead monoxide in a 10% excess of C.P. oleic acid (Eimer and Amend) at 90°C. in a vacuum oven. The product was washed three times with absolute ethanol and dried at 90°C. under vacuum. The oils used as solvents were a naphthenic SAE 40 petroleum oil (A) and a medicinal white oil (B), characteristics of which are given in Table I. These oils appear relatively particle-free under the electron microscope.

TABLE I

Characteristics of Oil Solvents

Oil	Naphthenic oil (A)	Medicinal white oil (B)
Density, d_4^{20}	0.907	0.896
Viscosity at 38°C., cs.	235	74
Viscosity at 99°C., cs.	15.0	7.6
Viscosity index	59	60
Principal hydrocarbon types	Naphthenic + some aromatic	Naphthenic (no aromatic)
Flash point, COC, °C.	230	180

The electron microscope used in this work was an R.C.A. Model EMU-2D.

The technique of specimen preparation follows. A very small amount of oil (<.1 mg.) was placed on a Parlodion film supported by a 200-mesh woven wire screen ⅛ in. in diameter. This oil was then spread thinly with one or two strokes of a thin glass rod. The thin oil film thus obtained (probably 1–3 μ thick) would not in its original condition be expected to permit effective observation of very small particles. The success of the method appears to depend upon the behavior of such a film in the electron microscope. When screens prepared in this way are examined in the electron microscope, the oil appears to have collected under the wires of the supporting screen. This is usually evidenced by the appearance of residual oil or of nonvolatile components of the oil at the corners of the screen openings. The Parlodion over the openings ordinarily appears to be free of oil. In some cases residual oil can be seen near the screen wires, completely ringing the oil-free area in the screen opening. The exact cause of this redistribution of the oil film is not clear, but it appears to occur very quickly when the specimen is struck by the electron beam. An effect which may be analogous can be observed by placing a suspension of carbon black in oil in a thin layer on a thin glass plate supported by two parallel metal rods an inch or two apart and heating from above with an infrared lamp. The oil is observed to collect over the metal rods, leaving the glass plate between the rods essentially free of oil and covered with particles of the suspended carbon black. In this case the redistribution of the oil apparently results from the temperature differential existing between the portion of the glass plate immediately over the the cool metal rods and that between the rods. A similar effect may explain the behavior of a thin oil film in the electron microscope. Alternatively it is possible that charging of the oil by the electron beam results in migration of the oil to the screen wires.

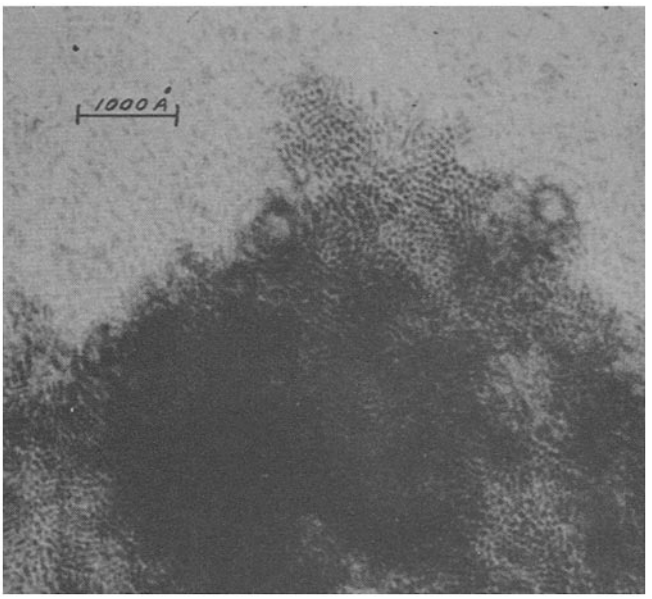

FIG. 1. Calcium cetyl phosphate in naphthenic oil (A). 130,000×.

Some evaporation of the oil undoubtedly occurs prior to and during the redistribution of the oil film, but this appears to be of secondary importance. Electron micrographs of carbon black suspensions in oil and of used lubricating oil specimens prepared by this technique indicate that no very marked concentration of particles occurs during this process. Some oils may occasionally collect in small patches on the Parlodion film as well as under the screen wires. Preparation of additional screens will however usually give a suitable film.

In most cases thinning of the oil film because of such redistribution together with some evaporation results in the mechanical grounding of even the smallest particles present in the oil. Electron micrographs are usually quite reproducible for successive screens, indicating that particles of a given size are deposited from an oil film of nearly constant thickness. Presumably the smallest particles are deposited when the film is thinnest.

Kodak lantern-slide, medium plates were exposed for about two seconds in taking micrographs. The electron micrographs presented were all taken at 16,000 × (except Figure 3, which was taken at 6,000 ×) and enlarged photographically.

Experimental Results

Electron micrographs at low magnifications of a 35% by weight suspension of calcium cetyl phosphate in naphthenic oil (A) show large numbers of apparently amorphous masses averaging several microns in diameter. At high magnifications however, wherever these particles are small enough or thin enough to permit observation of internal structure, they appear to be composed of agglomerated, approximately spherical micelles, apparently nearly uniform in size. A portion of such a particle is shown in Figure 1. Micelles are also found uniformly dispersed over the collodion film between the larger particles. These micelles appear rodlike in many instances although close inspection suggests that this may sometimes be caused by linear aggregation of "spherical" micelles.

Within the agglomerates the micelles form small colloid crystalline regions. The spacing between centers along the rows of micelles is variable. The closest, clearly measurable, average spacing between centers is about 67 Å although closer packing is indicated in other rows where the separate micelles are not clearly resolved. The individual micelles have apparent diameters averaging about 50 Å. A spherical micelle of this diameter could contain about 60 molecules.

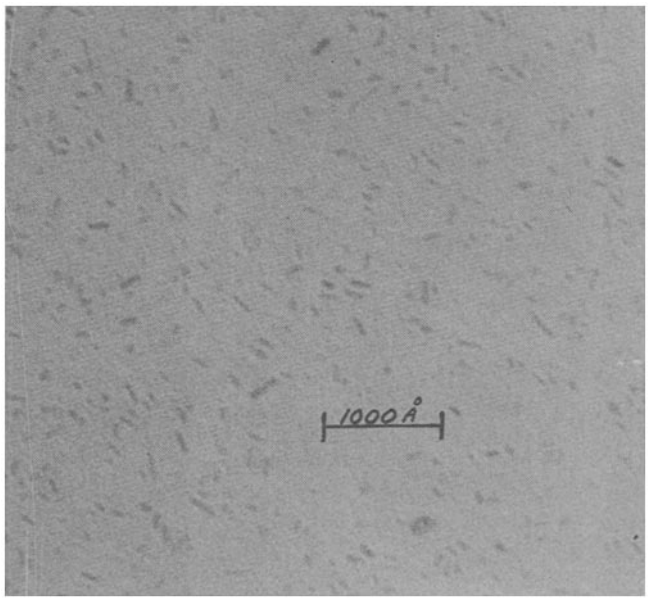

FIG. 2. Calcium cetyl phosphate + sulfurized calcium alkyl phenate in naphthenic oil (A). 150,000×.

Figure 2 illustrates the typical appearance of oil (A) originally containing a mixture of 8 x 10^{-3} moles of sulfurized calcium alkyl phenate and 5 x 10^{-3} moles of calcium cetyl phosphate per kilogram of solution. This solution is optically clear and apparently perfectly stable on prolonged standing. The small particles shown occur in such numbers and they are so uniformly distributed over the Parlodion film as to leave little doubt that they are detergent micelles or small linear aggregates of micelles. It is believed that these micelles contain a mixture of the two detergents. The micelles appear to be characteristically rodlike with apparent diameters between 40 Å and 70 Å. The most common diameter appears to be about 50 Å. The lengths are variable, ranging up to over 300 Å. Many of these micelles however appear to be roughly spherical in shape. These micelles appear to contain from about 25 molecules per micelle (for a 40-Å diameter sphere) upwards to several hundred for the longer rods. It can be seen that the rod-shaped micelles are quite frequently found in pairs or short rows. The spacing between centers of such paired or rowed micelles seems to be fairly uniform, averaging about 90 Å to 100 Å. This appears to correspond to a spacing between micelle surfaces of about 40 Å to 50 Å.

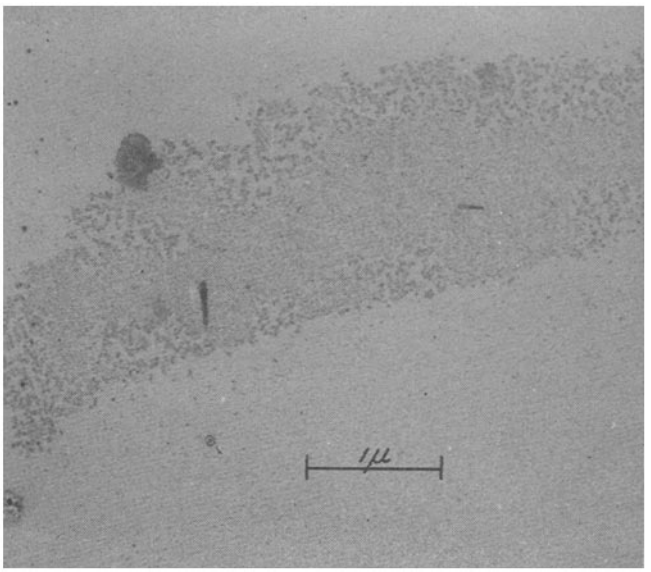

FIG. 3. A region in the same oil as shown in figure 2. 17,500×.

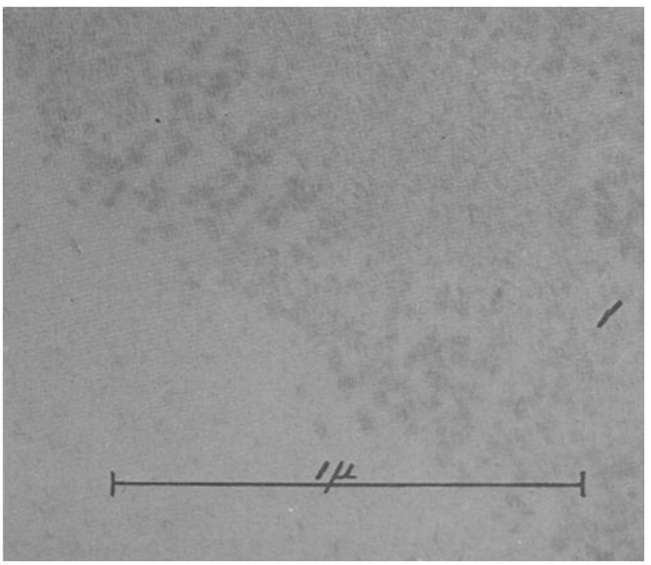

FIG. 4. Enlarged view of a portion of the region shown in figure 3. 62,000×.

Figures 3 and 4 show, at lower magnifications, an unusual area on a different specimen screen of the same oil solution shown in Figure 2, where the micelle concentration is considerably higher than elsewhere on the screen. The shape of this area is reminiscent of the characteristic spindle shape of tactoids. The rodlike micelles are seen in parallel alignment in rows containing up to 10 micelles with a nearly uniform spacing of 80 Å to 90 Å between centers. The individual micelles appear to be 40 Å to 50 Å in diameter.

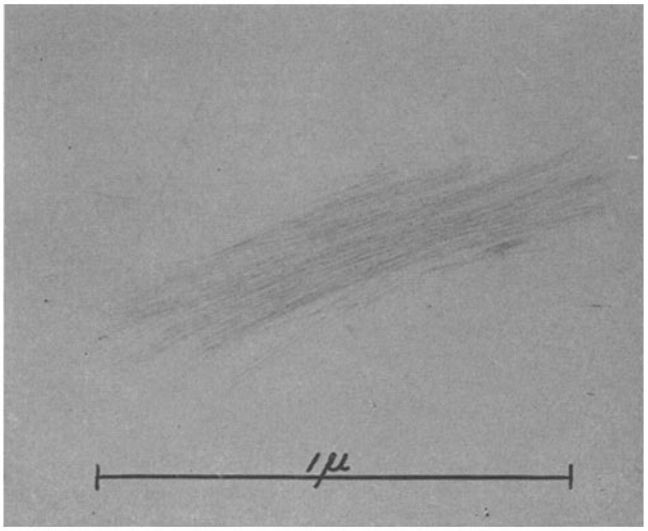

FIG. 5. Paracrystal found in a calcium cetyl phosphate + sulfurized calcium alkyl phenate dispersion in naphthenic oil (A). 62,000×.

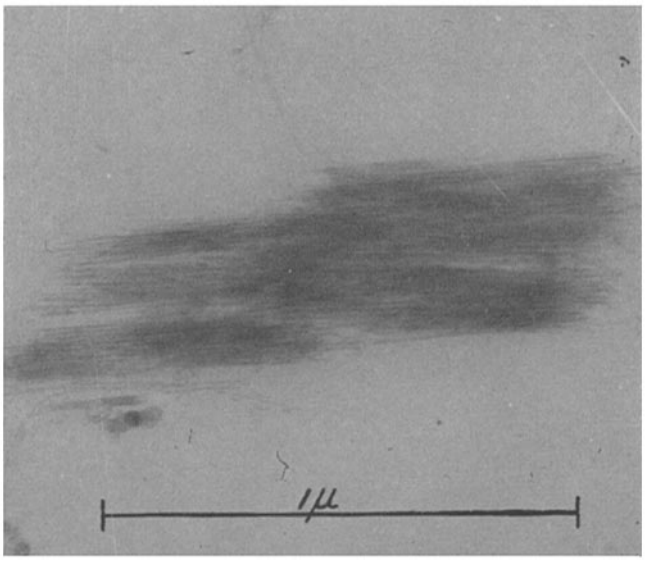

FIG. 6. Another example of the paracrystalline form shown in figure 5. 62,000×.

Figures 5 and 6 show examples of what appears to be a paracrystalline form of these paraffin chain salts present to a small but significant extent in other preparations of the oil described above. The calcium cetyl phosphate and the sulfurized calcium alkyl phenate were preparations different from those used in the oil shown in Figures 2, 3, and 4. The long fibers composing the paracrystal have approximately the same diameter (~50 Å) as the rodlike micelles. The spacing between fibers in Figure 5 appears to be about 80 Å while in Figure 6 spacings range from 80 Å to more than 100 Å. In all cases the fibers appear to be fairly stiff.

Figure 7 is an electron micrograph of a lead oleate suspension in medicinal white oil (B). The lead oleate was originally dissolved in the oil at 100°C. at a concentration of 3.5 x 10^{-2} moles per kilogram. On cooling, separation of the soap occurred to give a turbid suspension of gelatinous particles. When this suspension is examined under the electron microscope, the lead oleate precipitate is found to consist of large elongated particles, indistinct in outline, and frequently branched. These particles often appear to be quite flat on the Parlodion film and seem to be gelatinous in nature. When thin, the particles invariably give the impression of having internally fine structure in electron micrographs. Several slide preparations and "through-focus" exposures established the reality of this internal structure. Figure 7 shows a portion of one of the large particles of the soap precipitate. The precipitate is seen to contain numbers of small particles apparently nearly isodimensional and uniform in size. The average diameter of the particles appears to be about 40 Å. These particles are believed to be lead oleate micelles. The average micelle appears to contain about 35 molecules. No crystalline order can be seen, but a distinct tendency for the micelles to form short chains appears to exist. The particles usually appear to be separated by spacings of 20 Å to 40 Å. Oil is probably associated with the micelles in the agglomerate shown. These micelles may also be present in moderate numbers on the Parlodion film outside the agglomerate, but low contrast and the lack of a distinctive shape for the micelles preclude a definite decision on this point.

Discussion

At least two types of micelles, "spherical" and rodlike, appear in electron micrographs of oil dispersions of detergents and soaps. The exact shape of the "spherical" micelles cannot be determined with the electron microscope because of the extremely small size of these particles. It is reasonable to suppose however that such micelles are either compact "spheres" of the inverted Hartley type (with the polar heads of the molecules in the interior) or small disks or plates consisting of double layer platelets with polar heads together. Platelike micelles of roughly spherical shape have been assumed in aqueous solutions by Philippoff (13) and Dervichian (2) and more recently by Harkins (6). Van der Waarden (18) has recently assumed a platelike micelle form for metal naphtha sulfonates in hydrocarbons and mineral oils. On this assumption however the axial ratios calculated from viscosity data ranged from about 1/5 to 1/50. The micelles observed in the electron micrographs presented here do not appear to fit this description.

The rodlike micelles observed may correspond to the Lawrence or threadlike micelle (8), or they may be small linear aggregates of "spherical" micelles. In some instances, at least, these rods appear to be two molecular lengths in diameter and are of variable length.

Aggregation of both the "spherical" and the rodlike micelles to form colloid crystalline forms is indicated. The rodlike micelles occasionally appear to form paracrystals of long fibers. In these cases a definite fairly uniform spacing of from 80 Å to 100 Å appears to be maintained between centers of parallel rods or fibers. This apparently corresponds to a spacing of from 30 Å to 50 Å between particles.

Rees (15) in a development of an earlier suggestion of Usher (17) has recently shown that, in colloidal suspensions of spherical particles in which there is an appreciable potential energy maximum for the interaction of two particles (a "stable" suspension), slow aggregation of the spheres can result in the formation of linear aggregates preferentially. If, in addi-

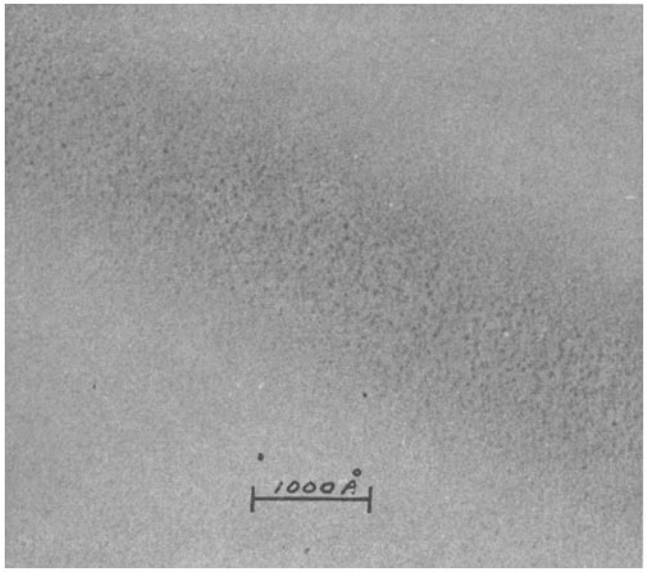

FIG. 7. Gelatinous precipitate of lead oleate in medicinal white oil (B). 150,000×.

tion, long-range attractive forces exist, the potential minimum thus arising is deeper parallel to the linear aggregate than at the ends. This would lead to a loose lateral association of the linear aggregates. Behavior of this type could apparently account for most of the phenomena observed in the electron micrographs presented in this paper if the rodlike micelles are assumed to be linear aggregates of spherical micelles.

Long-range attractive and short-range repulsive forces may exist in hydrocarbon solutions of detergents and soaps. It is difficult to believe however that such forces could arise from an electrokinetic potential in view of the extremely low electrical conductivity of such solutions. (The specific conductance of such oil solutions is of the order of 10^{-10} mho/cm. at 100°C.) The possible effects of electron bombardment in the electron microscope create some doubt however.

An obvious alternative explanation for the apparent short-range repulsion is that the micelles are solvated with oil. The layer of bound oil increases the effective particle radi by up to 25 Å. Such solvated micelles might be stabilized against aggregation in much the same manner as if an electrokinetic potential were present.

Further evidence is clearly necessary to permit a definite explanation of the spacings evident in the electron micrographs presented.

The limitations of the electron microscope are well known. The specimens viewed are always in vacuum and at a poorly defined temperature. The electron bombardment to which a specimen must be subjected introduces additional uncertainty. Surface-tension effects during the evaporation of the solvent may markedly alter the appearance of the specimen. Making due allowance for such complicating factors, it appears that the electron microscope is capable of yielding useful information about colloidal solutions of detergents in oil.

Summary

1. Electron micrographs of certain detergent and soap micelles presumably existing in oil solution are presented.

2. Both "spherical" and rodlike micelles appear to exist in oil. These micelles are apparently two molecular lengths in diameter. The rodlike micelles are of variable length, ranging up to over 300 Å. It seems likely that in some instances these rods are small linear aggregates of "spherical" micelles.

3. Association of rodlike micelles to form paracrystalline sheaves of long fibers is indicated. These structures apparently maintain fairly uniform spacings of from 80 Å to 100 Å between centers of the rods or fibers although the micelles appear to be only about 50 Å in diameter.

4. Aggregation of spherical micelles to form small colloid crystalline regions in large agglomerates is demonstrated.

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Direct Determination of Saturated Fatty Acids in Fats, Oils, and Methyl Esters[1]

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ALTHOUGH much has been published in recent years on the determination of polyunsaturated acids in fats and oils, there has been relatively little work done to improve or develop procedures for the determination of the saturated acids present. In 1925 Bertram described one of the first attempts to determine saturated acids directly (1). This method, which has become known as the Bertram Oxidation Method (4), involves the oxidation of the unsaturated linkages with permanganate, followed by a tedious pre-

[1] Presented at the spring meeting, American Oil Chemists' Society, New Orleans, La., April 28 to May 1, 1957