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Studies on the Behavior of Natural Surfactant at the Oil-Water Interface. II. Behavior of Natural Surfactant at the Oil-Water Interface

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The behavior of natural surfactants was studied at an oil-water interface by measurement of the interfacial tension and the observation of the oil-water interface. Lecithin was used as an oil-soluble substance and sodium cholate was used as a water-soluble substance; *n*-paraffin, corn oil, unsaturated fatty acid and fatty alcohol were used as an oil phase. The reduction of interfacial tension was greatest in a dodecane–sodium cholate solution system, but the minimum interfacial tension was observed in an oleic acid–sodium cholate solution system. Spontaneous emulsification occurred in the oil phase when oleic acid was left in contact with sodium cholate solution. The most remarkable change was found in the system of oleic acid in contact with sodium cholate solution, in which crystal of cholic acid were formed at the interface.

At the oil-water inferface, various interfacial phenomena occur when the oil containing oil-soluble substance is allowed to contact the water containing water-soluble substance. Manabe and Okuyama[1)] have reported that there are many natural surfactants, especially in living body.

We have studied the molecular interactions at the interface of oil/water systems with various kinds of additives with respect to the tendency of spontaneous emulsification,[2–4)] and the behavior of natural surfactants, lecithin and cholesterol, at the oil-water interface.[5)]

In this work, lecithin and sodium cholate were used as oil-soluble and water-soluble natural surfactants, respectively. Lecithin is an important constituent of biological membranes and has been used widely in field such as the food industry as an excellent emulsifier.[6–8)] Sodium cholate to be used is one of the bile salts. Bile salts are necessary for absorption of oil-soluble vitamins such as Vitamin A, D, E, and K.[9–11)] Further, it is known that cholesterol is solubilized by a lecithin-bile salt micelle.[12)]

In the present investigation, the behavior of natural surfactants at the oil-water interface was studied by the measurements of interfacial tension; the interfacial products were observed by a polarizing microscope.

Experimental

Materials. *Natural Surfactants*: Lecithin from soybean, the so-called asolecithin (Associated Concentrates Inc.) of commercial grade containing 95—98% lipid, was used. Sodium cholate (Tokyo Kasei Ind. Ltd.) of reagent grade was used without further purification.

Oil Phase: Dodecane (Nikko petrochemical Co.), Corn oil (Ajinomoto Co.), *cis*-9-octadecenoic acid (oleic acid) and *cis*-9-octadecen-1-ol (oleyl alcohol) (Tokyo Kasei Co.) of a commercial grade were used without purification.

Water Phase: Double distilled and deionized water with an indicated resistivity of 18.0 megohm/cm was used.

Apparatus. The interfacial tension was measured by a Wilhelmy-type surface-tensiometer (Shimadzu Surface Tensiometer ST-1) and interfacial products were observed by a polarizing microscope with crossed nicols (Nippon Kogaku S-PO).

Procedure. Lecithin and sodium cholate were dissolved in oil and water, respectively, and the interfacial tension was measured at various concentrations of the solutes at 30 °C. These measurements were made 30, 50, and 15 min after the contact of oil and water phases in the case of dodecane and *cis*-9-octadecenoic acid, corn oil and *cis*-9-octadecen-1-ol, respectively. It was confirmed that the equilibria at these interfaces were reached after the times mentioned above.

Results and Discussion

The Lowering of Interfacial Tension. Figure 1 shows the effect of concentration of sodium cholate on the interfacial tension at the oil-water interface. As is shown, the interfacial tension decreased with increasing concentration of sodium cholate. In particular, the reduction of interfacial tension was the greatest in dodecane–sodium cholate solution system, but the minimum interfacial tension was observed in *cis*-9-octadecenoic acid–sodium cholate solution system, being about 1.7 dyn†/cm at higher concentration of sodium cholate. The interfacial tension of *cis*-9-octadecenoic acid–sodium cholate solution system was lower than *cis*-9-octadecen-1-ol. This may be due to a stronger

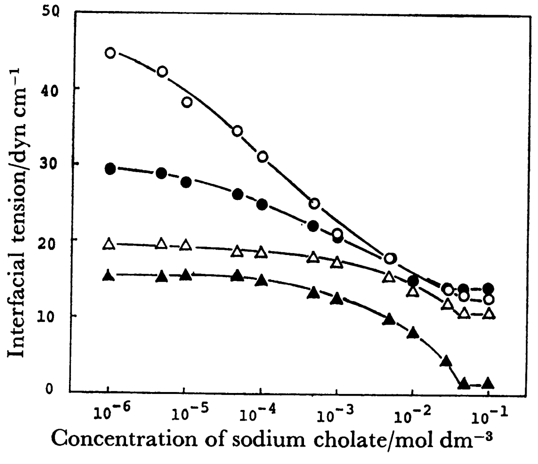

Fig. 1. The effect of sodium cholate on the interfacial tention of oil-water interface.
○: Dodecane, ●: corn oil, △: *cis*-9-octadecen-1-ol, ▲: *cis*-9-octadecenoic acid.

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† Throughout this paper 1 dyn$=10^{-5}$ N.

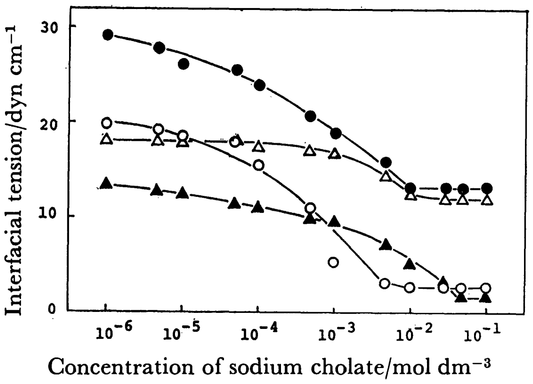

Fig. 2. The effect of sodium cholate on the interfacial tention of oil containing lecithin (2×10^{-3} wt%)-water interface.

○: Dodecane, ●: corn oil, △: *cis*-9-octadecen-1-ol, ▲: *cis*-9-octadecenoic acid.

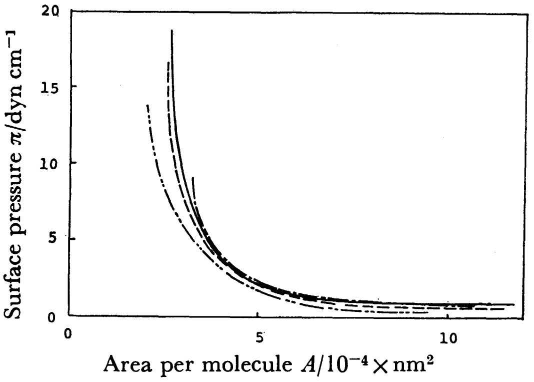

Fig. 3. π-A curves for various oils.

——: Dodecane, ------: corn oil, —·—: *cis*-9-octadecen-1-ol, —··—: *cis*-9-octadecenoic acid.

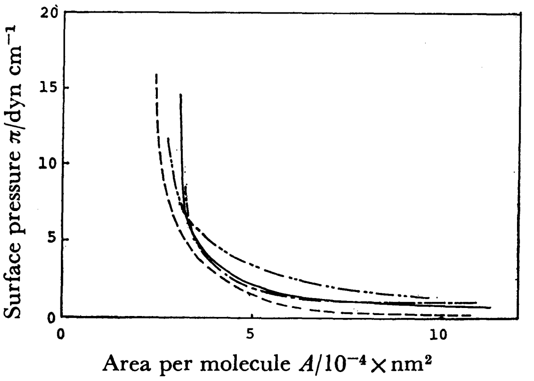

Fig. 4. π-A curves for various oils containing lecithin (2×10^{-3} wt%).

——: Dodecane, ------: corn oil, —·—: *cis*-9-octadecen-1-ol, —··—: *cis*-9-octadecenoic acid.

interaction between the carboxyl group of *cis*-9-octadecenoic acid and cholate ions than between *cis*-9-octadecen-1-ol and cholate ions.

Figure 2 shows the effect of the concentration of sodium cholate on the interfacial tension at the oil (containing 2×10^{-3} wt% lecithin)-water interface. As can be seen, the interfacial tension showed similar results to that of Fig. 1 except for dodecane, the interfacial tension of which was considerably lowered by the addition of a low concentration of lecithin. From the results, it was confirmed that lecithin affected most strongly the interfacial tension of dodecane–water.

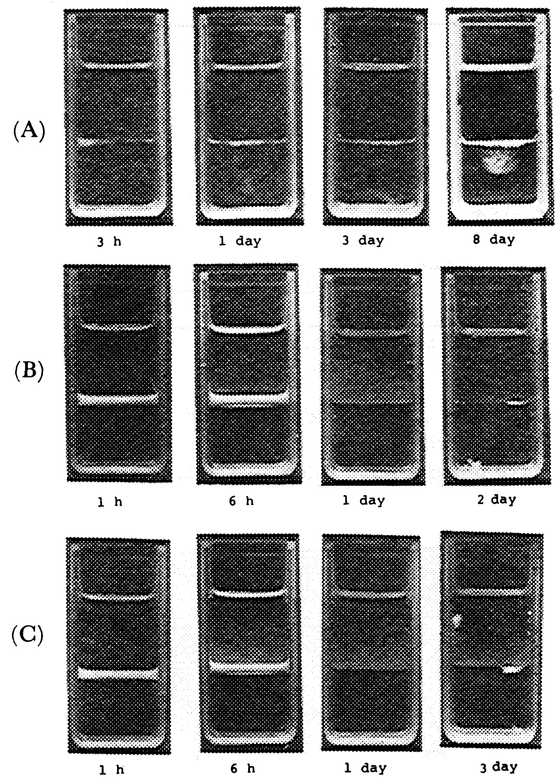

Fig. 5. The progress of spontaneous emulsification with time.

Water phase: sodium cholate 1×10^{-1} mol dm^{-3}.

Oil phase: (A) corn oil+lecithin 5×10^{-2} wt%. (B) *cis*-9-octadecenoic acid. (C) *cis*-9-octadecenoic acid+lecithin 1×10^{-2} wt%.

Figures 3 and 4 show the surface pressure (π)-area (A) curves calculated from Figs. 1 and 2 using the Gibbs equation. It is evident that the area is greatly decreased with increasing cholate ions at low surface pressure. This means that the molecules are tightly packed at higher surface pressure, the compressibility of the film being small.

Spontaneous Emulsification and Interfacial Product. Figure 5 shows the state when oils were left to stand in contact with sodium cholate solution. When corn oil containing lecithin was left in contact with aqueous solution, spontaneous emulsification occurred in the water phase.[5] However, no distinct change was recognized by the use of corn oil without lecithin. Further, the spontaneous emulsification was also observed when corn oil containing lecithin was left in contact with distilled water. From these results, it is found that lecithin affects the spontaneous emulsification when corn oil is used as the oil phase.

On the contrary, spontaneous emulsification occurred in the oil phase and the crystals were deposited at the oil-water interface when *cis*-9-octadecenoic acid was left in contact with sodium cholate solution. A similar change was also observed when *cis*-9-octadecenoic acid containing lecithin was used as the oil phase. However

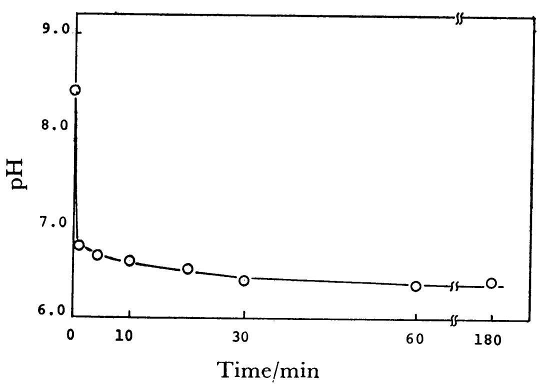

Fig. 6. The time dependence of pH of water phase in contact with oil phase.
Oil phase: *cis*-9-octadecenoic acid.
Water phase: sodium cholate 1×10^{-1} mol dm^{-3}.

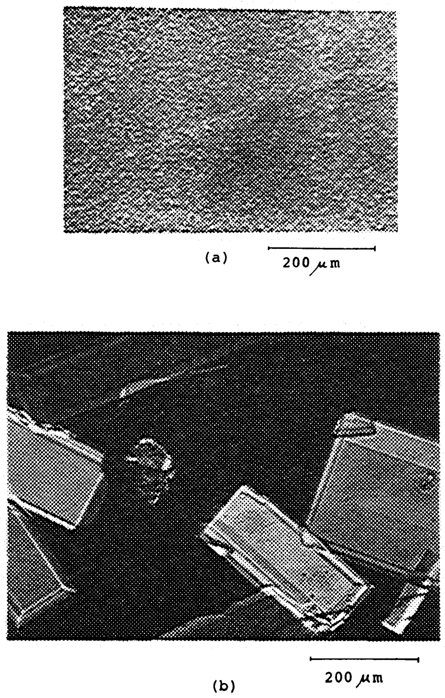

Fig. 7. The products formed at the oil-water interface.
(a) Thin milky film. Oil phase: dodecane+lecithin 1×10^{-2} wt%. Water phase: sodium cholate 1×10^{-1} mol dm^{-3}.
(b) Plate crystals. Oil phase: *cis*-9-octadecenoic acid +lecithin 2×10^{-2} wt%. Water phase: sodium cholate 1×10^{-1} mol dm^{-3}.

both spontaneous emulsification and crystal deposit were not observed when *cis*-9-octadecenoic acid containing lecithin was left in contact with water.[5] From these results, it is found that sodium cholate affects spontaneous emulsification when *cis*-9-octadecenoic acid is used as the oil phase.

Figure 6 shows the change of pH of the aqueous phase with time when *cis*-9-octadecenoic acid was left in contact with sodium cholate solution. It is clear from this figure that the initial pH of the water phase decreased rapidly with time. It seems that sodium ions of the water phase reacted with *cis*-9-octadecenoic acid, hydrogen ions of the water phase increased and some of the cholate ions transferred to the oil phase with the hydrogen ions. The results indicate that spontaneous emulsification or crystal deposit occur in the oil phase. Davies *et al.* showed that the spontaneous emulsification was caused by one or more of the following possibilities:[13,14] i) The instability of the interface due to a non-uniform diffusion of the components of the phases across the interface, which disturbs the interface violently enough to form droplets of the adjacent bulk phase. ii) An accompanying diffusion of oil and an additive into water, which results in a dilution of the additive by the water, leaving oil droplets to form an emulsion. iii) The temporary appearance of a negative interfacial tension which causes an enlargement of the interface area.

Schulman *et al.*[15,16] have shown that spontaneous emulsification occurs when a complex is formed between an oil-soluble substance and a water-soluble surfactant at the oil-water interface. The spontaneous emulsification of oil containing lecithin in contact with sodium cholate solution is attributed to the formation on liquid crystal of lecithin or to the formation of mixed micelle consisting of lecithin and sodium cholate.

Figure 7 shows photographs of the interfacial products. The thin milky film formed at the dodecane-sodium cholate solution interface is shown in Fig. 7-a. The plate crystals deposited at the *cis*-9-octadecenoic acid–sodium cholate solution interface are shown in Fig. 7-b. Crystals were obtained a few days after the oil and water phase came into contact at sodium cholate concentrations above 5×10^{-2} mol dm^{-3}. This crystal was identified to be a single crystal of cholic acid by the measurement of the melting point, IR spectroscopy and X-ray diffraction.

Similar results were obtained when (9*Z*,12*Z*)-9,12-octadecadienoic acid (linoleic acid) was used as the oil phase and were not obtained when *cis*-9-octadecen-1-ol was used. As mentioned above, it seems that the hydrogen ions of *cis*-9-octadecenoic acid, (9*Z*,12*Z*)-9,12-octadecadienoic acid and cholate ion in water affect the formation of this single crystal.

References

1) T. Manabe and N. Okuyama, *Yukagaku*, **26**, 578 (1977).
2) K. Ogino and M. Ota, *Yukagaku*, **23**, 28 (1974).
3) K. Ogino and M. Ota, *Bull. Chem. Soc. Jpn.*, **49**, 1187 (1976).
4) K. Ogino and H. Umetsu, *Bull. Chem. Soc. Jpn.*, **51**, 1543 (1974).
5) K. Ogino and M. Onishi, *J. Colloid Interface Sci.*, **83**, 18 (1981).
6) J. Stanly, "Soybeans and Soybean Products," ed by K. S. Markley, Interscience Publishers, New York (1951), Vol. II, p. 593.
7) S. Ota, *Yukagaku*, **19**, 792 (1970).
8) S. Sato and A. Nishioka, *Yukagaku*, **28**, 773 (1979).
9) A. F. Hofmann, *Biochem. Biophys. Acta*, **70**, 306 (1960).
10) A. F. Hofmann, *Biochem. J.*, **89**, 57 (1963).
11) M. C. Carey and D. M. Small, *Arch. Int. Med.*, **130**, 506 (1972).
12) N. A. Mazer, R. F. Kwasnic, M. C. Carey, and G. B. Bendenk, "Micellization, Solubilization and Microemulsions,"

ed by K. L. Mittal, Plenum Press, New York (1977), Vol. 1, p. 383.

13) J. T. Davies and E. K. Rideal, "Interfacial Phenomena of Natural Surfactant," Academic Press, New York and London (1963), pp. 360—377.

14) J. T. Davies and D. A. Haydon, *Proc. 2nd Int. Congr. Surface Activety*, **1**, 417 (1957).

15) J. H. Schulman and E. G. Cockbain, *Trans. Faraday Soc.*, **36**, 651 (1940).

16) A. E. Alexander and J. H. Schulman, *Trans. Faraday Soc.*, **36**, 960 (1940).