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Evaluation of Emulsion Stability. The Effect of a Natural Emulsifier, Soya Sterol, on w/o Type Emulsions[1)]

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Using soya sterol (chemical composition by weight: β-sitosterol 56%; campesterol 28%; stigmasterol 4%; sterol hydrocarbons and cholesterol 6%; triterpene alcohols, keto-steroids and other steroid-like constituents 6%) and Span 85 as emulsifiers, with liquid paraffin as the dispersed phase in water, the effect of soya sterol and Span 85 on emulsion stability was evaluated in terms of the following five physicochemical parameters: (1) particle size, (2) degree of dispersion, (3) separation rate of dispersed droplets, (4) rheology of emulsion products, (5) sedimentation rate of dispersed droplets. An agitator and a homogenizer were used to produce the emulsions.

The following results were obtained. (1) The particle size of emulsions prepared with soya sterol was larger than that with Span 85, and consequently, soya sterol is a less effective emulsifier than Span 85. (2) Nevertheless, soya sterol has stronger stabilizing action on emulsions than Span 85, and this effect become especially marked when emulsification was carried out with a homogenizer at a high shear rate.

Keywords——agitator; emulsion stability; homogenizer; particle size; rheology; rheometer; sedimentation rate; soya sterol; Span 85; turbidimeter

Emulsion techniques are useful in diverse fields, such as the food, pharmaceutical, cosmetic, chemical and textile industries, where the safety and stability of emulsion products are important considerations. In pharmaceutical or cosmetic applications in particular, the safety of emulsion products, or in other words, the toxicity and medicinal effects of the emulsifier on the body, must be taken into consideration.[3)] From this point of view, natural emulsifiers are preferable to artificial emulsifiers and have been proved to be very useful in the preparation of drugs and cosmetics. However, there are many unsolved problems in connection with the stability of emulsions produced with natural emulsifiers.

In this study, we measured the stability of emulsions prepared with soya sterol, a plant sterol (chemical composition by weight: β-sitosterol 56%; campesterol 28%; stigmasterol 4%; sterol hydrocarbons and cholesterol 6%; triterpene alcohols, keto-steroids and other steroid-like constituents 6%) which was chosen as a natural emulsifier since it is not orally toxic, and is not irritating to the skin or eyes as determined by tests within the meaning of the Federal Hazardous Substances Labeling Act.[4)] Furthermore, as a surfactant soya sterol resembles cholesterol,[5)] which is used as an emulsifier for ointment bases, cold cream and other emulsion products. We chose Span 85 as an artificial emulsifier for comparison with soya sterol. The emulsion stabilities were evaluated in terms of the following five physicochemical parameters: (1) particle size distribution of droplets, (2) degree of dispersion, (3)

1) S. Noro, A. Takamura, T. Minowa, and M. Koishi, presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1979.
2) Location: *a) 1-22-1, Yato-cho, Tanashi-shi, Tokyo 188, Japan; b) 12, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.*
3) E.S. Lower, *Drug and Cosmet. Ind.*, **116**, 54 (1975).
4) General Mills Chemicals Inc., *Technical Bulletin*, 122, 3 (1978).
5) E.V. Truter, *J. Soc. Cosmet. Chem.*, 13, 180 (1962).

separation rate of dispersed droplets, (4) rheology of the emulsion product, (5) sedimentation rate of dispersed droplets.

Experimental

Apparatus——Sketches of the agitator and homogenizer[6] are shown in Fig. 1. The agitator was a stainless-steel agitation tank, 120 mm in diameter, with 4 baffles and a standard Rushton type with 6 blades.[7] The tank was placed in a water bath to maintain the desired liquid temperature. The homogenizer used was a type 15M-8TA machine from Gaulin Co., U.S.A. Dispersion of the material to be emulsified occurs not only while the materials pass through the opening between the valve and seat, but also when the resulting emulsion impinges against the retaining wall which surrounds the valve. Homogenizers were connected for multi-stage dispersion. Thus, the first stage of homogenization was usually done at the high pressure of 280 kg/cm^2, producing finely dispersed droplets, although they clumped. The second stage of homogenization, at the lower pressure of 60 kg/cm^2, broke up the clumps and produced emulsions of lower viscosity.

Materials——The continuous phase material used in this study was liquid paraffin (Taisei Pure Chemical Co., 58.3—60.5% by weight), and the dispersed phase material was distilled water (37.4—38.9% by weight). The natural and artificial emulsifiers used were soya sterol (Nisshin Oil Mills, Ltd., Tokyo) and Span 85, nonionic surfactant (Kao-Atlas Co., Tokyo), respectively. The emulsifier concentration in each emulsion was varied within the range of 0.6 to 4.3% by weight.

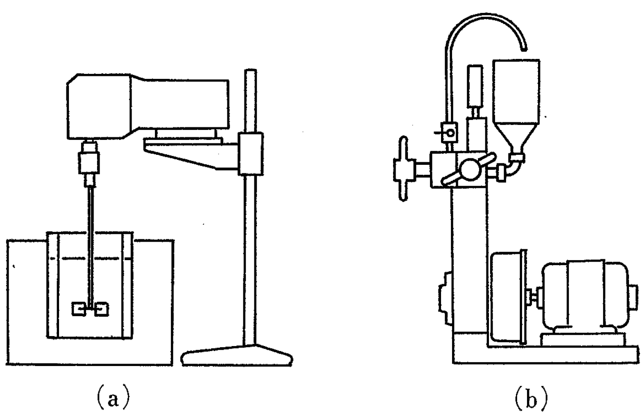

Fig. 1. Sketches of the Apparatus used

(a) agitator.
(b) homogenizer.

Procedures——Emulsifier was dissolved in liquid paraffin, kept at 85.0° in the agitation tank, and water, heated to 85.0°, was added to the solution. The temperature of the mixture was maintained at 85.0° for 30 minutes after starting emulsification in the agitator. The resulting water-in-oil emulsion was then gradually cooled to 20.0° over 30 minutes. The impeller speed was kept constant at 530 rpm throughout emulsification and cooling. After agitation for 1 hr, the first sample was taken to evaluate the emulsion stability.

The resulting coarse emulsion was quickly introduced into the homogenizer from the agitator, and was vigorously stirred again, being sheared at high velocity between the narrow valve and the seat of the homogenizer, so that each droplet of the dispersed phase was broken up or homogenized to a smaller size. The homogenized emulsion was also sampled to evaluated its stability.

To evaluate the stability of emulsions, the following four types of measurement[8] were carried out to examine the four parameters mentioned in the last paragraph of the introduction: particle size measurement of dispersed droplets by photomicrography (Nikon AFM, Nihon Kōgaku Co.); relative turbidity measurement with a PT-201 type turbidimeter (Nihon Seimitsu Kōgaku Co., Tokyo); measurement of the degree of separation or the height of the drainage phase after leaving the emulsion to stand in a glass tube at 20.0° for 7 days; measurement of the rheological properties with an RM-1 type rheometer (Shimadzu Seisakusho, Kyoto). Using the viscosity data and the Stokes equation, a fifth stability parameter, *i.e.* the sedimentation rate of dispersed droplets, was obtained.

Results and Discussion

The particle size distribution curves of emulsions prepared with the agitator only and stabilized with soya sterol or Span 85 are shown in Fig. 2. Changes of particle size distribution with changes of the emulsifier concentration showed similar tendencies in both emulsifiers. That is to say, the size distribution curves shifted to the low side of the diameter axis and became narrower and sharper with increase of the emulsifier concentration. These results

6) L.H. Rees, *Chem. Eng.*, **13**, 86 (1974).
7) S. Tsukiyama, A. Takamura, and N. Nakura, *Yakugaku Zasshi*, **94**, 490 (1974).
8) S. Noro, A. Takamura, and M. Koishi, *Chem. Pharm. Bull.*, **27**, 309 (1979).

suggest that the emulsifiers not only facilitate the subdivision of droplets, but also produce uniform droplets, probably due to the reduction of interfacial tension.

Since a broader size distribution and a larger standard deviation were observed for the emulsion prepared with soya sterol than for the emulsion with Span 85, the surface-activity of soya sterol is considered to be weaker than that of Span 85.

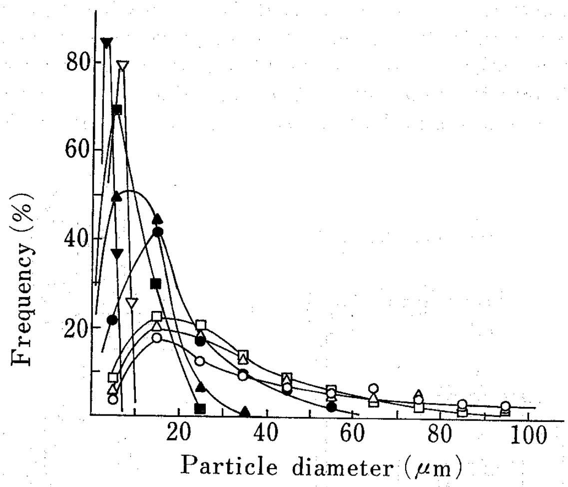

Fig. 2. Particle Size Distribution

○: soya sterol 0.6%, agitator,
△: soya sterol 1.1%, agitator,
□: soya sterol 2.1%, agitator,
●: Span 85 0.6%, agitator,
▲: Span 85 2.1%, agitator,
■: Span 85 4.3%, agitator,
▽: soya sterol 0.6—4.3%, agitator and homogenizer,
▼: Span 85 0.6—4.3%, agitator and homogenizer.

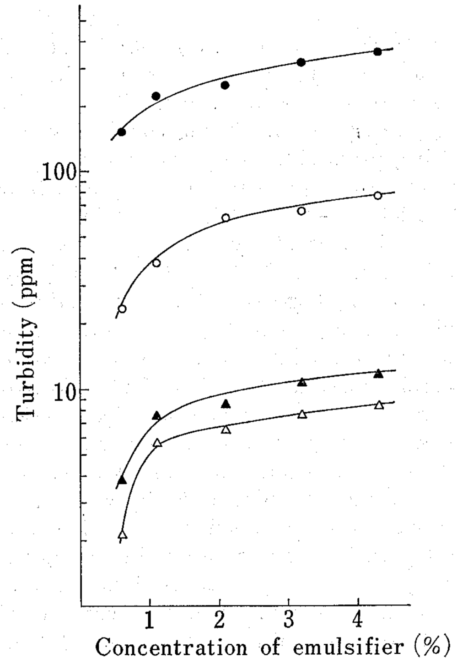

Fig. 3. Relation between Turbidity and the Concentration of Emulsifier

○: Span 85, agitator,
●: Span 85, agitator and homogenizer,
△: soya sterol, agitator,
▲: soya sterol, agitator and homogenizer.

Fig. 3 shows the effect of the concentrations of the two emulsifiers on the emulsion turbidity. The turbidity increased initially and levelled off later as the concentration of emulsifier increased. It was deduced that the average diameter of emulsion droplets became gradually smaller with increase of the emulsifier concentration.

At a given concentration, the turbidity of the emulsion prepared with Span 85 was much higher than that prepared with soya sterol. This may be interpreted as indicating that cohesion or coagulation is less marked in the emulsion formed with Span 85. Hence, the dispersing power of Span 85 in emulsion preparations was concluded to be better than that of soya sterol. Furthermore, it was deduced from Fig. 3 that the dispersing effect of emulsifier is more significant in an emulsion prepared by the successive use of the agitator and homogenizer than in an emulsion with the agitator alone.

The effect of emulsifier concentration on the height of the drainage phase is shown in Fig. 4. The drainage phase did not appear until several hundred minutes after the preparation of the emulsion. Initially, the drainage phase height increased linearly, and it gradually levelled off as the standing time increased. Generally speaking, the rate of drainage in the emulsion prepared with soya sterol was much slower than that in the emulsion with Span 85. Moreover, no drainage phase was found at all when the concentration of soya sterol exceeded 2.1% with the emulsion prepared using the agitator alone. Similar phenomena were observed in all the emulsion products prepared by the successive use of the agitator and homogenizer, regardless of soya sterol concentration.

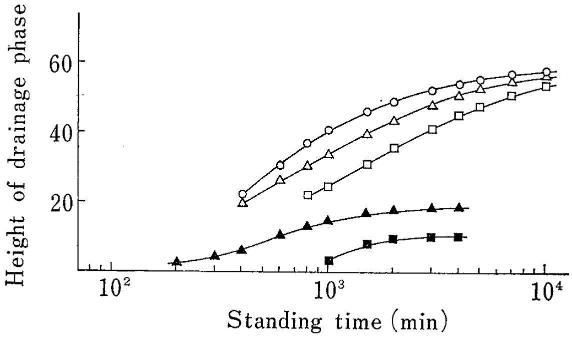

Fig. 4. Change of Height of the Drainage Phase with Standing Time

○: Span 85 0.6%, agitator,
△: Span 85 1.1%, agitator,
□: Span 85 2.1%, agitator,
▲: soya sterol 0.6%, agitator,
■: soya sterol 1.1%, agitator.

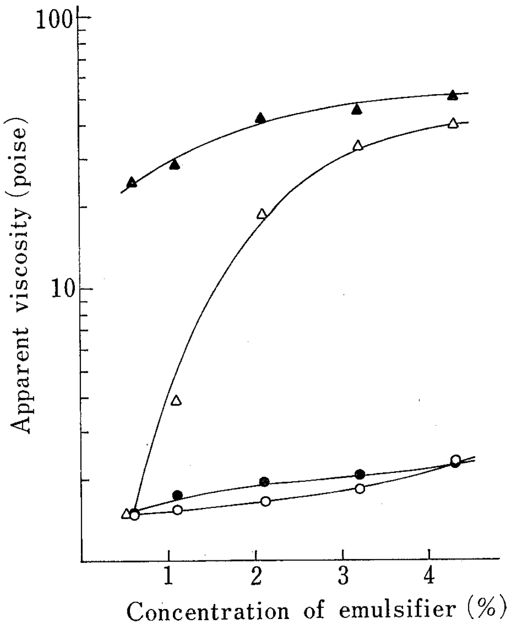

Fig. 5. Relation between the Apparent Viscosity and Concentration of Emulsifier

○: Span 85, agitator,
●: Span 85, agitator and homogenizer,
△: soya sterol, agitator,
▲: soya sterol, agitator and homogenizer.

The effect of emulsifier concentration on the viscosity of the emulsions is shown in Fig. 5. The viscosity values of emulsions prepared with the agitator alone did not depend upon the concentration of Span 85, and a similar tendency was found for emulsions prepared by the use of the agitator and homogenizer. Thus, the effect of Span 85 on emulsion formation seems to be insignificant from the standpoint of emulsion viscosity. On the other hand, in the case of emulsions prepared using soya sterol, different effects of emulsifier concentration on the emulsion viscosity were found with emulsions prepared by different procedures; that is to say, the viscosity of emulsions prepared with the agitator alone increased rapidly with the addition of emulsifier, while the viscosity of emulsions prepared by successive use of the agitator and homogenizer were relatively higher even at a lower concentration of soya sterol. These different viscosity tendencies with emulsions using Span 85 and soya sterol may be explained as follows. The addition of the relatively strongly surface-active Span 85 produces finely dispersed emulsions even when only a small amount is used, regardless of the preparative method used. Thus, an essentially constant viscosity value was obtained. The low value reflects the absence of cohesion or coagulation, as already mentioned in the discussion of turbidity. On the other hand, the addition of soya sterol seems to complicate the behavior of disperesed droplets, possibly involving cohesion or complex structure, as will be considered next in the discussion on the emulsion rheograms.

The two types of emulsion rheograms which were obtained by increasing the shear rate from 7.5 sec^{-1} to 75 sec^{-1} (up-curve) and decreasing from 75 sec^{-1} to 7.5 sec^{-1} (down-curve) are shown in Fig. 6.[9] The emulsions prepared with soya sterol had a higher value of viscosity than those prepared with Span 85. Furthermore, a difference was found in the relative position of hysteresis curves of soya sterol and Span 85. With soya sterol, the down-curve was displaced to the left of the up-curve, showing that the emulsion has a lower consistency at any rate of shear on the down-curve than it has on the up-curve. This result indicates that a breakdown

9) K. Umemura, *Yakuzaigaku*, 22, 28 (1962).

of structure occurred at the up-curve and that this was not reversed immediately on the down-curve, even though the stress was removed or reduced. In view of the tendency for lower viscosity at higher shear rate, it is possible that the gel structure of the emulsion begins to break down, probably resulting in the formation of aligned droplets. Accordingly, the emulsion at the up-curve undergoes a gel-to-sol transformation and exhibits shear-thinning, while at the down-curve a perfect sol-to-gel transformation cannot occur due to the very slow recovery of structure.

On the other hand, for the emulsion prepared with Span 85, little difference between the up-curve and the down-curve of the rheogram was observed. This suggests the absence of multiple phases and the coalescence and deformation of large globules in this w/o emulsion.

Other important factors affecting the emulsion stability remain to be discussed, *i.e.* the sedimentation velocity of individual droplets and the zeta potential of the electrical double layer surrounding each droplet. Using our viscosity data, the sedimentation velocity of droplets in emulsion products was calculated as follows. The velocity of sedimentation is expressed by the Stokes equation,

$$u = \frac{(\rho_c - \rho_d)g}{18\eta} d_p^2 \qquad (1)$$

where u is the terminal velocity in cm/sec, d_p is the diameter of the droplet in cm, ρ_c and ρ_d are the densities of the continuous and dispersed phases in g/cm^3, respectively, g is the acceleration due to gravity in cm/sec^2, and η is the viscosity of the medium or emulsion in g/cm·sec.

The above equation, however, must be modified if the droplets do not settle freely or if the emulsion is not dilute. In most pharmaceutical emulsions, which contain dispersed droplets at a high concentration, the droplets exhibit hindered settling, *i.e.*, they interfere with one another as they fall. Thus, the Stokes equation may be rewritten in the following form,

$$u = k \frac{(\rho_c - \rho_d)}{\eta} d_p^2 \qquad (2)$$

where k is a constant which must be determined by experiment.

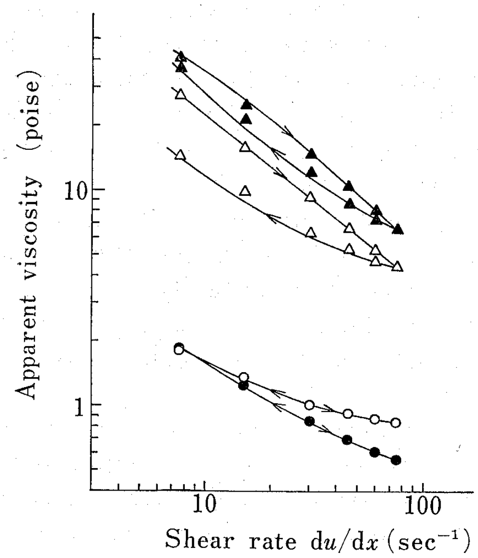

Fig. 6. Plot of Apparent Viscosity against Shear Rate

○: Span 85 2.1%, agitator,
●: Span 85 2.1%, agitator and homogenizer,
△: soya sterol 2.1%, agitator,
▲: soya sterol 2.1%, agitator and homogenizer.

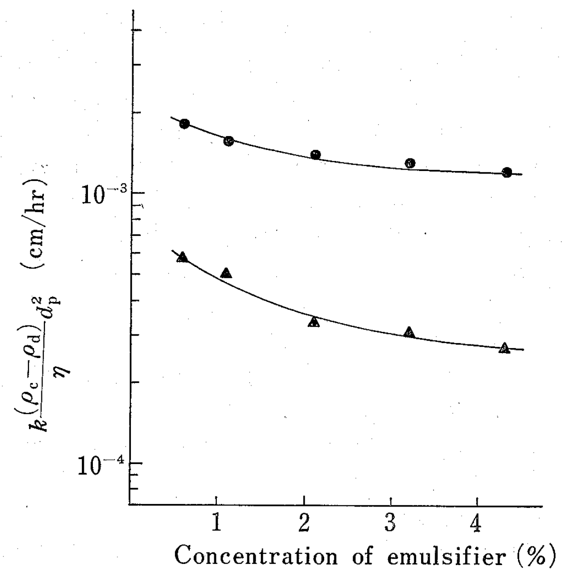

Fig. 7. Comparison of Emulsion Stability between Soya Sterol and Span 85

●: Span 85, agitator and homogenizer,
▲: soya sterol, agitator and homogenizer.

Fig. 7 shows the relation between the calculated velocity of sedimentation and the concentration of emulsifier. When the emulsion was prepared with soya sterol, the calculated value of sedimentation velocity became very small due to the high viscosity of the emulsion in spite of the large droplet size, as compared with the emulsion prepared with Span 85.

The results described above led us to the following conclusions. The particle size of the emulsion prepared with soya sterol was larger than that with Span 85, and consequently, soya sterol is a less effective emulsifier than Span 85. In the case of soya sterol, because of the strong affinity for oil exhibited by its oleophilic cyclopentano hydrophenanthrene ring, the molecules are oriented at the water-oil interface in such a way that only a part of the hydrophilic chain lies within the water phase, the other part being located in the oil phase on the other side of the interface. Within the floccules of droplets, the part of each droplet surface which faces another droplet flattens under the influence of various hydrodynamic factors. The emulsion prepared with soya sterol has a stable gel structure around the droplet surface, because the flattened globule surfaces are stabilized by the cyclopentano hydrophenanthrene ring of the soya sterol molecules. Accordingly, soya sterol is considered to stabilize an emulsion more effectively than Span 85, and this effect becomes particularly marked if emulsification is carried out in a homogenizer with a high shear rate.

The above-mentioned properties of emulsions prepared with soya sterol suggest that soya sterol may be suitable for use in pharmaceutical products such as ointments, emulsions, and suppositories.

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