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Improvement of the stability of water-in-oil-in-water multiple emulsions by the addition of surfactants in the internal aqueous phase of the emulsions

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Summary

In order to improve the stability of w/o/w emulsions, an attempt was made to elucidate the influence of addition of hydrophilic surfactants into the internal aqueous compartment of the emulsion, on the breakdown of the suspending vesicular globules of w/o/w emulsion, the changes in diameter of the internal aqueous compartments and of the suspending vesicular globules of w/o/w emulsion, as compared with emulsions without additives and with addition of sodium chloride. It was also attempted to measure the effect of addition of hydrophilic surfactant to the three different phases on the stability of w/o/w emulsions. The stability of diluted w/o/w emulsions with a large difference in osmolarity between the internal aqueous compartments and the external aqueous phase was also tested. w/o/w emulsions were prepared by the two-step emulsification procedure. New coccine was employed as a marker to check the yield of formation and the breakdown of the w/o/w emulsions. Sodium octanesulfonate was used as an additive in the internal aqueous compartment of the w/o/w emulsions as well as sodium chloride. Polyoxyethylene (20) sorbitan monooleate was used as an additive to the three different phases of the emulsions. The stability of w/o/w emulsions, as determined by coalescence of internal aqueous compartments and their increase in size by the influx of water to the internal aqueous compartment from the external aqueous phase, was improved by the addition of hydrophilic surfactant in the internal aqueous compartments of the emulsions. This was observed even under the condition of a large difference in osmolarity between the internal aqueous compartments and the external aqueous phase, and was affected by the concentration of hydrophilic surfactant. The addition of sodium chloride to the internal aqueous compartments and of hydrophilic surfactant to the external aqueous phase did not improve the stability of w/o/w emulsions. Only the addition of hydrophilic surfactant into the internal aqueous compartments was demonstrated to inhibit both coalescence of internal aqueous compartments and influx of water to the internal aqueous compartment from the external aqueous phase. On the other hand, the addition of hydrophilic surfactant into the oil phase was observed not to inhibit coalescence but water influx.

Introduction

Water-in-oil-in-water (w/o/w) multiple emulsions are complex systems that have many poten-

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tial applications for prolonged drug delivery systems (Yoshioka et al., 1982; Fukushima et al., 1983), drug overdose treatment (Chiang et al., 1978), cosmetics (Lin et al., 1975), and foods (Takahashi, 1986).

However, the inherent instability associated with these systems has stimulated many researchers to overcome this problem; for example, with the addition of sodium chloride or bovine serum albumin (Kita et al., 1977), glucose (Matsumoto et al., 1980), and dextrose (Adeyeye et al., 1990) to the internal aqueous compartment of w/o/w emulsions. There have been many studies related to the effects of lipophilic surfactants in the oil phase components (Adeyeye et al., 1991) and hydrophilic surfactants in the external aqueous phase (Matsumoto et al., 1987), and the volume ratio of each phase (Matsumoto et al., 1976), in order to improve the stability of w/o/w emulsions.

There are few investigations related to enhancing the stability of w/o/w emulsions by the addition of hydrophilic surfactant to the internal aqueous compartment of the emulsion.

The objectives of this study were to assess the improvement of the stability of w/o/w emulsions by the addition of hydrophilic surfactants to the internal aqueous compartment of the emulsion, as compared to emulsions without additives (control), and emulsions containing sodium chloride. Furthermore, it was attempted to elucidate the effects of addition of hydrophilic surfactant to internal, oil and external phases, on the stability of w/o/w emulsions. Those experiments described above were also carried out on the diluted w/o/w emulsions.

It is necessary to determine the change in diameter of the internal aqueous compartments when the stability of a w/o/w emulsion is examined (Matsumoto et al., 1980). In this study, percent release, and the change in size of the internal aqueous compartments and of the suspending vesicular globules of w/o/w emulsion, were employed as indices of stability.

Materials and Methods

Materials

Food dye Red No. 102 (new coccine), 2-hydroxyazonaphthalene-4′,6,8-trisulfonic acid trisodium salt, to act as a marker entrapped in the

TABLE 1

Formulations of w / o / w emulsions tested

Formulation		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
Internal aqueous phase	(ml)	50	50	50	50	50	50	50	50
(pH 6.33 isotonic buffer solution)									
New coccine	(mg)	100	100	100	100	100	100	100	100
Sodium chloride	(mg)	–	180	–	–	–	–	–	–
Na 1-octanesulfonate	(mg)	–	–	500	250	100	–	–	–
PSMO 80	(mg)	–	–	–	–	–	250	–	–
(Osmolarity) (mOsm/kg H_2O) [a]		297	404.5	389	339	314	299.5	297	297
Oil phase	(ml)	30	30	30	30	30	30	30	30
Liquid paraffin	(g)	19.25	19.25	19.25	19.25	19.25	19.25	19.25	19.25
SMO 80	(g)	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25
PSMO 80	(g)	–	–	–	–	–	–	150	–
Volume fraction	(ϕ w/o)	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
External aqueous phase	(ml)	80	80	80	80	80	80	80	80
(pH 6.33 isotonic buffer solution)									
PSML 20 (mg)	400	400	400	400	400	400	400	400	
PSMO 80	(mg)	–	–	–	–	–	–	–	400
(Osmolarity) (mOsm/kg H_2O)		299	299	299	299	299	299	299	299.5
Volume fraction	(ϕ w/o/w)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[a] Data are expressed as means obtained from duplicate samples.

internal aqueous phase to monitor the formation percentage and the breakdown of w/o/w emulsion, was purchased from San-ei Chemical Industries, Ltd, (Osaka, Japan). Liquid paraffin (specified grade in the Japanese official formulary of food additives) as an oil phase, sorbitan monooleate (SMO 80, equal to Span 80®) as a lipophilic surfactant, and polyoxyethylene (20) sorbitan monolaurate (PSML 20, equal to Tween 20®) and polyoxyethylene (20) sorbitan monooleate (PSMO 80, equal to Tween 80®) as nonionic hydrophilic surfactants were purchased from Wako Pure Chemical Industries, Ltd, (Osaka, Japan). Sodium octanesulfonate, as an anionic hydrophilic surfactant, was purchased from Tokyo Kasei Kogyo Co., Ltd, (Tokyo, Japan). These chemicals were used without further purification. Other chemicals employed were of analytical or reagent grade.

Dialysis membrane tubes were purchased from Spectrum Medical Industries, Inc. (U.S.A.).

Preparation of w / o / w emulsion

Formulations of each w / o / w emulsion to be tested The formulations including the volume fractions of each phase, and the osmolarity of each aqueous phase of w/o/w emulsions are summarized in Table 1.

The basic formula of the oil phase used in these experiments was that described by Kawashima et al. (1991).

For the study of the effect of additives in the internal aqueous compartment of the w/o/w emulsion on the stability of the emulsion, w/o/w emulsions were prepared according to formulations (a)–(e) (Table 1). For the experiments to elucidate the effects of the addition of hydrophilic surfactant to each phase on the stability of the w/o/w emulsion, emulsions were prepared according to the formulations (f)–(h) (Table 1). Isotonic 0.236 M citric acid:0.123 M disodium phosphate buffer (pH 6.33) was employed as an aqueous phase.

The osmolarity of the aqueous phase of each type of w/o/w emulsion was measured with an osmometer (model 3W II, Advanced Instruments, Inc., MA) operating on the principle of depression of freezing point, and was determined as the mean value from duplicate samples.

Preparation procedure The w/o/w emulsions were prepared by the two-step emulsification procedure described in the previous study (Ohwaki et al., 1992).

The emulsification process for the first step, in which was prepared a single emulsion (w/o emulsion), employed a Polytron homogenizer (type X 1020, Ystral Gmbh, Germany), which had a vertical U-shaped blade (7.3 mm diameter, 12.0 mm height) that revolved at the bottom of cylinder (7.8 mm internal diameter, 10.2 mm external diameter) having several small lattice windows, to agitate the emulsion at 2700 rpm. Next, the resultant w/o emulsion was poured into the external aqueous phase at once, and instantly, the mixture was agitated at 3000 rpm for 1 min to yield the w/o/w emulsion with an Ultra-Disperser (model LK-21, Yamato Kagaku Co., Ltd, Japan), which had the same types of agitation parts, a vertical U-shaped blade (12.4 mm diameter, 10.0 mm height) and a cylinder (13.0 mm internal diameter, 17.8 mm external diameter), as the Polytron homoginizer.

After preparation of these w/o/w emulsions, they were stored at 25°C in order to investigate the stability of the emulsion by examining its characteristics, such as formation percentage, droplet diameter of internal aqueous compartments and the tendency for internal aqueous compartments in the emulsions to coalesce.

Determination of formation percentage and the breakdown of w / o / w emulsion

Dialysis studies were conducted to determine the formation percentage and the breakdown of the w/o/w emulsions. The procedure for the dialysis tests and the dialysis apparatus were as described in the previous study (Ohwaki et al., 1992).

In this study, experiments were conducted at 25°C in duplicate. From the data of the preliminary studies, a dialysis membrane pore size of M_r 1000 cutoff was chosen to be suitable for these dialysis experiments, and it was confirmed that the dialysis duration of 6 h was sufficient to determine the formation percentage or the breakdown of the w/o/w emulsion.

The percentage of w/o/w emulsion formed, $\alpha(\%)$, was calculated according to Eqn 1 (Kawashima et al., 1991):

$$\alpha(\%) = 100\{1 - n_1 V^* / (n_{10} - n_1) V_1\} \qquad (1)$$

$$V^* = V_2 + V_d (V_1 + V_2 + V_o) / V_s \qquad (2)$$

where n_{10} denotes the initial concentration of new coccine as a marker in the internal aqueous phase, and n_1 is the concentration of new coccine dialyzed in the external dialysis solution. V_1, V_2, and V_o represent the volume of the internal aqueous compartment, external aqueous phase, and oil phase, respectively, in the formulation to prepare the w/o/w emulsion. V_d and V_s denote the volumes of the dialysis external solution and dialyzed emulsion, respectively.

Percent release, $r(\%)$, at the initial time point or at each interval of the storage duration was calculated according to Eqn 3 in these experiments,

$$r(\%) = 100 - \alpha(\%) \qquad (3)$$

Percent release at the initial time point indicated the breakdown rate of the w/o/w emulsion on preparation, and that at each interval of the storage duration indicated the alteration from the initial percent release caused by the breakdown of the w/o/w emulsion.

Determination of droplet diameter of internal aqueous compartments of the original w/o/w emulsions

The droplet diameter of the internal aqueous compartments of the w/o/w emulsions, used in the stability test at 25°C, was determined using a Coulter® counter (model N4, Coulter Electronics, Inc., FL), operating on the principle of statistical analysis of measurements performed by Brownian motion and photon correlation spectroscopy. A sample of the w/o/w emulsion of about 5 g was introduced into a centrifugal glass tube and spun at 1000 rpm for 5 min. Then, precisely 0.1 g of the supernatant, which was a w/o emulsion, was withdrawn from the tube into a 30 ml beaker and diluted with 20 g of the oil phase of the w/o/w emulsion. It was then stirred gently, becoming transparent, and gas was extracted by a vacuum pump to obtain the test sample. As a result of a preliminary study, since the droplet diameter of the internal aqueous compartments of the w/o/w emulsion prepared for centrifugation at 1000, 2000, and 3000 rpm for 5 min was almost the same as that of the primary w/o emulsion, the centrifugation conditions of 1000 rpm for 5 min were adopted in this experiment. The mean value was obtained from triplicate samples.

Determination of the influx of water from the external aqueous phase of the diluted w/o/w emulsion to the internal aqueous compartments of the emulsion

In order to examine the influence of hydrophilic surfactants on the stability of the internal aqueous compartments of w/o/w emulsion under the condition of greatly different osmolarity between the internal aqueous compartment and the external dispersed phase, the following experiment was conducted.

A 0.1 g sample of the w/o/w emulsion was weighed accurately into a 20 ml beaker and diluted precisely with 10 g of deionized distilled water. The w/o/w emulsion containing 0.5% (w/v) PSMO 80 in the external aqueous phase of the emulsion was diluted precisely with 10 g of PSMO 80 aqueous solution (0.5% w/v). One drop of this was then poured onto a glass slide with a small depression in the center (0.05 cm in depth, 1.0 cm in diameter), and covered with a cover glass to prevent the evaporation of water, to be used as a sample for microscopic observation. After preparation, the sample was stored at 25°C and microscopic examination was carried out periodically.

The osmolarity of the external aqueous phase of these diluted w/o/w emulsions was the same value, 5 mOsm/kg H_2O. The particle size of the w/o/w emulsion was determined from a photomicrograph as the Feret diameter ($n = 100$).

TABLE 2

Changes of percent release of new coccine, droplet diameter of internal aqueous compartment and volume ratio of internal aqueous compartment compared to initial values against the storage duration for each type of w / o / w emulsion [a]

Storage duration (days)	Percent release					Droplet diameter (nm) [b]					Volume ratio [c]				
	Control	Sodium chloride 0.36% (w/v)	Na 1-octanesulfonate			Control	Sodium chloride 0.36% (w/v)	Na 1-octanesulfonate			Control	Sodium chloride 0.36% (w/v)	Na 1-octanesulfonate		
	–		0.2% (w/v)	0.5% (w/v)	1% (w/v)	–		0.2% (w/v)	0.5% (w/v)	1% (w/v)	–		0.2% (w/v)	0.5% (w/v)	1% (w/v)
Formation (%)	91.7	91.44	95.89	97.74	96.92	–	–	–	–	–	–	–	–	–	–
Initial	8.30	8.56	4.11	2.26	3.08	116.50	126.50	152.50	148.50	143.50	1	1	1	1	1
1	7.97	7.66	3.51	2.26	3.76	171.50	177.33	148.50	143.00	143.50	3.19	2.755	0.923	0.893	1
5	8.30	7.89	3.06	3.20	3.19	199.50	220.00	159.50	160.00	166.00	5.022	5.26	1.441	1.251	1.548
10	6.96	8.00	3.65	2.39	3.31	178.00	232.50	201.10	155.00	174.00	3.567	6.209	2.239	1.137	1.783
20	7.07	7.77	4.14	3.01	3.42	171.50	210.50	238.50	171.00	189.67	3.19	2.905	3.825	1.527	2.309
30	7.74	8.11	3.95	2.91	3.42	173.00	206.00	247.00	177.40	168.13	3.275	4.318	4.249	1.705	1.608

[a] w/o/w emulsions were prepared according to the formulations shown in Table 1 (a)–(e).

[b] Data are expressed as means obtained from triplicate samples.

[c] Volume ratio was calculated from the following equation:

volume ratio (–) = mean volume of internal aqueous compartment of w/o/w emulsion at each storage time/mean volume of internal aqueous compartment of initial emulsion.

Results and Discussion

Effect of the addition of sodium octanesulfonate in the internal aqueous compartment on the stability of w / o / w emulsion

The influences of sodium octanesulfonate, an anionic hydrophilic surfactant, added into the internal aqueous compartment on the stability of the w/o/w emulsion, such as breakage of w/o/w emulsion and coalescence of the internal aqueous compartment, were investigated in comparison with sodium chloride and without additives (control).

In advance, to check the feasibility of using new coccine as a marker for monitoring the yield of formation and the breakdown of the w/o/w emulsion, the apparent partition coefficients of new coccine between each type of aqueous phase formula and the oil phase formula were measured. Consequently, it was confirmed that new coccine was a suitable marker to monitor the yield of formation and the breakdown of the w/o/w emulsion, since there was no observed partitioning from the aqueous phase formula to the oil phase formula and no interaction between new coccine and additives, such as hydrophilic surfactant and sodium chloride, was considered in these w/o/w emulsion formulae.

Stability of original w / o / w emulsions

Table 2 shows the changes of percentage release of new coccine, the droplet diameter of the internal aqueous compartments, and the volume ratio of the internal aqueous compartment to the initial value with different storage durations for each type of w/o/w emulsion, whose formulations are given in Table 1 (a)–(e).

The increase in release of new coccine indicated the extent of breakdown of the w/o/w emulsion. For sodium chloride and control, the percentage release was almost the same with each storage duration, and for sodium octanesulfonate, it was less than that for sodium chloride or control at all added concentrations, and its percentage release was about one third. The percentage release at 0.5% (w/v) of sodium octanesulfonate was slightly smaller as compared with those at 0.2 or 1% (w/v). In our previous study, the concentrations of 0.2 and 1% (w/v) were demonstrated to be below and above the critical micelle concentration (CMC), respectively (Ohwaki et al., 1992). The released amount hardly increased with greater storage time for the five types of w/o/w emulsions, and the percentage release at each storage time point was almost the same as the initial value. This indicates that the suspending vesicular globules of the w/o/w emulsion did not break down with storage at 25°C.

In general, it is necessary to determine the change in droplet diameter of the internal aqueous compartments of a w/o/w emulsion when the stability of the emulsion is examined (Matsumoto et al., 1980). The droplet diameter of the internal aqueous compartment with sodium chloride as an additive in the internal aqueous compartment was greater at each storage time point than the initial value, and then increased until a storage duration of 10 days, thereafter decreasing gradually until 30 days. For the control, the droplet diameter increased until 5 days, and then decreased gradually until 20 days. An increase in droplet diameter was observed with control irrespective of the magnitude of the difference in osmolarity between the two aqueous compartments, the internal aqueous compartment and the external aqueous compartment (Table 2). This was thought to be caused not by the influx of water across the oil phase from the external aqueous phase to the internal aqueous compartment, but by the coalescence of the internal aqueous compartments with each other, since the existence of electrolytes in the aqueous compartments of the w/o/w emulsion tended to break down the oil films among the internal aqueous compartments of the emulsion (Matsumoto et al., 1981, 1985). In this experiment, since the buffer solutions employed as aqueous phases of an w/o/w emulsion contained electrolytes, the increase in droplet diameter of the internal aqueous compartment was attributed to coalescence.

For sodium octanesulfonate, the droplet diameter of the internal aqueous compartments at 0.2% (w/v) increased more markedly than those with other additive amounts, and was similar to that with sodium chloride. The droplet diameters at 0.5% (w/v) were almost equal to those at 1% (w/v) at each storage time point.

Moreover, in order to define the real volume increase of the internal aqueous compartment of the w/o/w emulsion, and to be able to compare values among these types of emulsions prepared, the volume ratio of the internal aqueous compartment of w/o/w emulsions for various storage durations at 25°C to that of the initial emulsion was obtained from the data on the mean droplet diameter. The volume ratio for sodium chloride at each storage time point was similar to that for control. For sodium octanesulfonate, the volume ratio at 0.2% (w/v) increased to a greater extent compared to those with other additive amounts, and was similar to that with control. The volume ratio at 0.5% (w/v) was the smallest of all at each storage time, and the stability of original w/o/w emulsion was found to be influenced by the concentration of sodium octanesulfonate added to the internal aqueous compartment.

In spite of almost the difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion being almost identical for sodium chloride and sodium octanesulfonate (1% w/v), the volume ratio for sodium chloride was larger than that for sodium octanesulfonate. It was suggested that the addition of sodium octanesulfonate to the internal aqueous compartment of the w/o/w emulsion was more effective at preventing the influx of water across the oil phase from the external aqueous phase to the internal aqueous compartment, or the coalescence of the internal aqueous compartments with each other, compared with sodium chloride or control.

Stability of diluted w / o / w emulsions

By diluting the w/o/w emulsions with deionized distilled water to magnify the difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion, transformation of the w/o/w emulsion, with respect to its features, diameter and volume, was investigated for the five original w/o/w emulsions listed in Table 1 (a)–(e).

It was reported that water influx across the oil phase of the w/o/w emulsion from the external

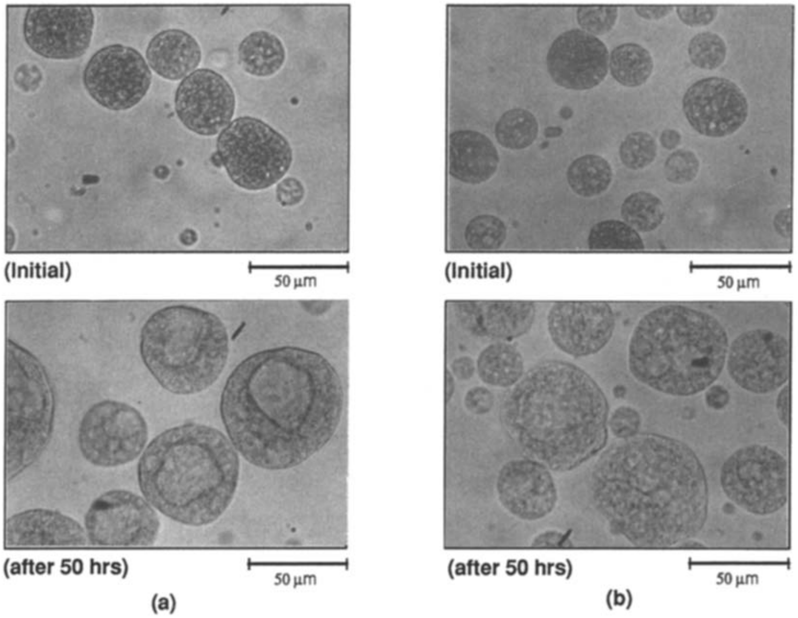

Fig. 1. Photomicrographs of the w/o/w emulsions diluted 100-fold with deionized distilled water. (a) Control (without additives). (b) Sodium chloride (0.36% w/v). Notations are the same as those in Table 1.

aqueous phase to the internal aqueous compartment occurred when the osmolarity of the internal aqueous compartment was greater than that of the external aqueous phase according to microscopic observation (Matsumoto et al., 1980). Figs. 1 and 2 show photomicrographs of the five types of w/o/w emulsions diluted 100-fold with deionized distilled water and stored at 25°C.

For control and sodium chloride as shown in Fig. 1, larger internal aqueous compartments were found in the diluted w/o/w emulsion after 50 h storage as compared with the initial emulsion, and the diameter of suspending vesicular globules of the w/o/w emulsion increased with storage time. In contrast, for sodium octanesulfonate in Fig. 2, the internal aqueous compartments of the w/o/w emulsion after 50 h were slightly larger than at the initial time point and were much smaller than those for control and sodium chloride. On comparison among three types of diluted w/o/w emulsions (Fig. 2 (c)–(e)), the internal aqueous compartments after 50 h were the largest of all at 0.2% (w/v) of sodium octanesulfonate.

Fig. 3 shows the relationships between the diameter of suspending vesicular globules of the w/o/w emulsions diluted 100-fold with deionized distilled water and the storage duration at 25°C. The volume ratios of w/o/w emulsions at 25°C to that of the initial emulsion are plotted against storage durations in Fig. 4 based on the data shown in Fig. 3.

As shown in Fig. 3, plateaus were almost reached after 20 h for control and sodium chloride, and after 5 h for sodium octanesulfonate. The diameter in the plateau state for control was the largest, and was smallest for sodium octanesulfonate at the concentration of 0.5% (w/v).

Similar curves of the volume ratio with the same orders of magnitude of the plateau state in Fig. 3 were obtained in Fig. 4. Since we have confirmed that coalescence of diluted w/o/w emulsions with each other was not observed visually under the light microscope, the increase in

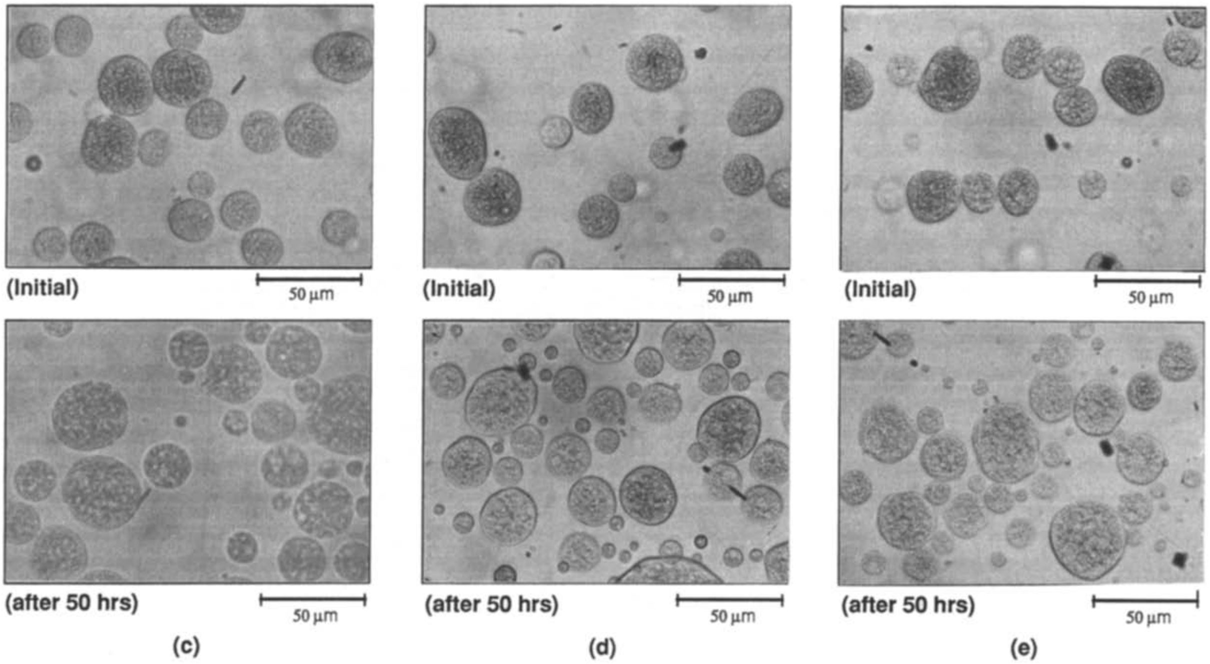

Fig. 2. Photomicrographs of the w/o/w emulsions diluted 100-fold with deionized distilled water. (c) Sodium octanesulfonate (1% w/v). (d) Sodium octanesulfonate (0.5% w/v). (e) Sodium octanesulfonate (0.2% w/v). Notations are the same as those in Table 1.

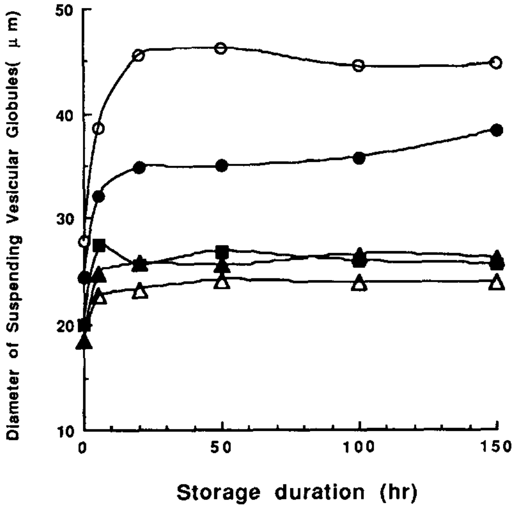

Fig. 3. Relationships between the diameter of suspending vesicular globules of the diluted w/o/w emulsion and the storage duration at 25°C. (○) Control, (●) sodium chloride (0.36% w/v), (■) sodium octanesulfonate (1% w/v), (△) sodium octanesulfonate (0.5% w/v), (▲) sodium octanesulfonate (0.2% w/v). Data are expressed as means ($n = 100$).

diameter or volume ratio could be caused by the influx of water across the oil phase from the external aqueous phase to the internal aqueous compartment, or by water uptake into the oil phase (Kita et al., 1978). In this experiment, since the formulations of the oil phases in these w/o/w emulsions whose formulations are shown in Table 1 (a)–(e) were the same, the amount of water uptake by the oil phase was considered to be equal. Therefore, the difference in volume ratio could indicate a difference in the amount of water influx across the oil phase from the external aqueous phase to the internal aqueous compartment of the diluted w/o/w emulsion.

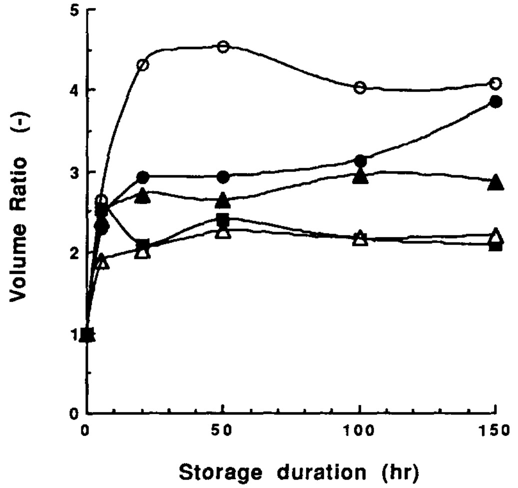

Fig. 4. Relationships between the volume ratio of the diluted w/o/w emulsion to that of the initial emulsion and the storage duration at 25°C. Symbols are the same as those in fig. 3.

As shown in Fig. 4, the volume ratio of diluted w/o/w emulsions increased with storage duration at 25°C compared to the initial value for control, despite the smallest difference in osmolarity occurring between the internal aqueous compartment and the external aqueous phase. For sodium octanesulfonate, the increase in volume ratio at the concentration of 0.2% (w/v) was the largest of all irrespective of the smaller difference in osmolarity between the two phases. The increase in volume ratio at 0.5% (w/v) was almost the equal to that at 1% (w/v). The mechanisms of water influx across the oil phase from the external aqueous phase to the internal aqueous compartment of the w/o/w emulsion, such as carrier transport of inversed micelles and water channels, have been reported (Kita et al., 1978). On the other hand, it was reported that a film like a liquid crystal was formed at the interfaces between the oil phase and the internal and external aqueous phases, on the formation of a w/o/w emulsion (Shinoda et al., 1968; Matsumoto, 1986, 1988). Moreover, the addition of hydrophilic surfactant in the internal aqueous compartment decreased the interfacial tension between the oil phase and the internal aqueous phase, and a film like a liquid crystal at the interface between the two phases would approach (Ohwaki et al., 1992). In this experiment, even below the additive concentration of sodium octanesulfonate at 0.2% w/v (less than the CMC at which micelles were not formed), the stability of the w/o/w emulsion was enhanced as compared with control. From these viewpoints, transport of water across the oil phase from the external aqueous phase to the internal aqueous compartment of the w/o/w emulsion would be prevented by the formation of closer film between the internal aqueous compartment and the oil phase, which is caused by the orientation of monomers of sodium octanesulfonate at the interface between the two phases. For sodium chloride, the increase in volume ratio was larger than those for sodium octanesulfonate, but smaller than that for control in spite of producing a greater difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion. Further studies are needed to elucidate how addition of sodium chloride to the internal aqueous compartment of a w/o/w emulsion would contribute to prevention of transport of water across the oil phase from the external aqueous phase to the internal aqueous compartments.

Effect of the addition of PSMO 80 to each phase on the stability of the w / o / w emulsion

In order to elucidate which additional phases of surfactant in the w/o/w emulsion (internal aqueous, oil or external aqueous phase) could contribute to the enhancement of stability of the emulsion, the effect of the addition of PSMO 80 to each phase on the stability of the w/o/w emulsion was studied. In this study, 0.5% (w/v) PSMO 80 was employed as a hydrophilic surfactant since its osmolarity was almost identical between the two aqueous phases of the w/o/w emulsions whose formulations are listed in Table 1 (f)–(h), and, in addition, 0.5% (w/v) PSMO 80 was able to dissolve in all phases of the w/o/w emulsion.

On the other hand, the stability of emulsions is known to be dependent on the HLB of the emulsifier. In this experiment, since the HLB number for a mixture of two surfactants, SMO 80 (HLB 4.3) and PSMO 80 (HLB 15), was found to be slightly increased according to calculation based on the law of additivity of HLB (Magdassi et al., 1984), it was supposed that the stability of w/o/w emulsion was not affected by the change in HLB number, as compared with that without PSMO 80 in the oil phase.

Stability of original w / o / w emulsions

Table 3 shows the effect of the addition of PSMO 80 to various phases on percent release of new coccine, droplet diameter of the internal aqueous compartment, and volume ratio of internal aqueous compartment to initial emulsion for each type of w/o/w emulsion whose formulations are shown in Table 1 (f)–(h).

The percent release of new coccine with addition to the oil phase was slightly greater than that for addition to the internal aqueous compartment at each storage time, and percent release with its

TABLE 3

Effect of the addition of polyoxyethylen (20) sorbitane monooleate (PSMO 80) to various phases on percent release of new coccine, droplet diameter of internal aqueous compartment, and volume ratio of internal aqueous compartment to initial emulsion for each type of w / o / w emulsion [a]

Storage duration (day)	Percent release			Droplet diameter (nm) [b]			Volume ratio [c]		
	Internal 0.5% (w/v)	Oil phase 0.5% (w/v)	External 0.5% (w/v)	Internal 0.5% (w/v)	Oil phase 0.5% (w/v)	External 0.5% (w/v)	Internal 0.5% (w/v)	Oil phase 0.5% (w/v)	External 0.5% (w/v)
Formation (%)	94.97	93.26	85.74	–	–	–	–	–	–
Initial	5.03	6.74	14.26	175.75	143.50	145.00	1	1	1
1	6.40	6.85	16.49	182.00	208.00	209.33	1.111	3.045	3.009
5	6.40	6.85	16.38	188.33	172.25	463.00	1.231	1.73	32.486
10	5.94	6.85	17.94	174.75	190.67	431.75	0.983	2.346	26.399
20	6.86	7.30	16.71	178.00	177.50	432.00	1.039	1.893	26.445
30	6.97	7.64	16.60	175.00	191.00	389.00	0.987	2.358	19.308

[a] w/o/w emulsions were prepared according to the formulations shown in Table 1 (f)–(h).

[b] Data are expressed as means obtained from triplicate samples.

[c] Volume ratio was calculated from the following equation:

volume ratio (–) = mean volume of internal aqueous compartment of w/o/w emulsion at each storage time/mean volume of internal aqueous compartment of initial emulsion addition to the external aqueous phase was about 2.4-times that for addition to the internal aqueous compartment at each storage time point. The increase in percent release of new coccine could have been caused by the addition of surfactant to the external aqueous phase of the emulsion. Slight breakdown of each type of w/o/w emulsion was observed based on the slight increase in percent release of new coccine from the emulsion with increase in storage duration.

The diameter of the internal aqueous compartment with addition of PSMO 80 to the oil phase was almost the same as that with addition of PSMO 80 to the internal aqueous compartment at each storage time, although the initial diameter was smaller with addition into the oil phase than into the internal aqueous compartment. With addition of PSMO 80 to the external aqueous phase, the diameter increased up to about 2.5-fold with storage duration, as compared with that for addition into the internal aqueous compartment.

The volume ratio of the internal aqueous compartment to the initial value, which was calculated from the mean diameters, was slightly larger with addition into the oil phase than that of addition into the internal aqueous compartment at each storage time point. On the other hand, the volume ratio with addition into the external aqueous phase increased about 20-fold with storage duration, as compared with that of addition into the internal aqueous compartment.

The results of this study indicate that with the addition of surfactant into the external aqueous phase, the internal aqueous compartments markedly increased in size with storage duration, which was presumably due to the coalescence of internal aqueous compartments with each other. The influx of water across the oil phase of the w/o/w emulsion from the external aqueous phase to the internal aqueous compartment was not considered to cause the increase in size of the internal aqueous compartments, because of the

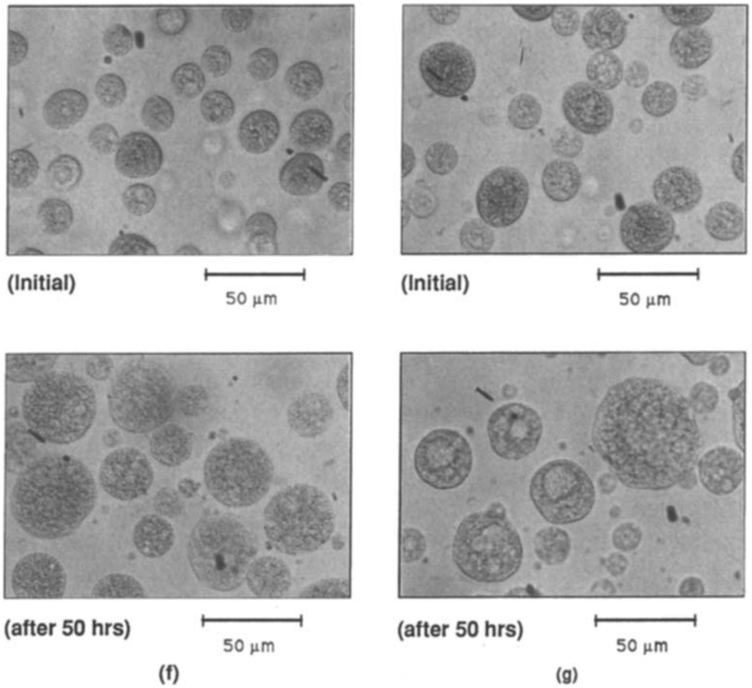

Fig. 5. Photomicrographs of the w/o/w emulsions diluted 100-fold with deionized distilled water. (f) Addition of PSMO 80 in the internal aqueous phase (0.5%w/v). (g) Addition of PSMO 80 in the oil phase (0.5% w/v). Notations are the same as those in Table 1.

almost equal osmolarity of the two aqueous phases.

Stability of diluted w / o / w emulsion

In order to investigate the effect of the-addition of surfactant to each phase on the transformation of w/o/w emulsions, such as features, diameter, and volume, under the condition of a large difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion, the original w/o/w emulsions, whose formulations are shown in Table 1 (f)–(h), were diluted with deionized distilled water. Figs. 5 and 6 show photomicrographs of the three types of w/o/w emulsions diluted 100-fold with deionized distilled water and stored at 25°C.

With the addition of 0.5% (w/v) PSMO 80 into the oil phase of the w/o/w emulsion in Fig. 5, larger internal aqueous compartments were found in the diluted w/o/w emulsion after 50 h storage, as compared with the initial emulsion, and the diameter of suspending vesicular globules of the diluted w/o/w emulsion increased with storage duration. With addition into the internal aqueous compartment, the droplet diameters of internal aqueous compartments after 50 h were slightly larger than at the initial time point, and the diameter of suspending vesicular globules was almost the same as with addition into the oil phase. In our preliminary study, the amount of water uptake into the oil phase in these three types of diluted w/o/w emulsions (Table 1 (f)–(h)) was found to be almost identical, from observation of the change in droplet diameter of a diluted single emulsion (o/w emulsion) prepared using the same formulations of oil phases as the three types of w/o/w emulsions (Table 1 (f)–(h)). Therefore, this indicates that coalescence of the internal aqueous compartments of diluted w/o/w emulsions readily occurred with the addition of surfactant into the oil phase in comparison to addition into the internal aqueous compartment, and the water uptake into the intenal aqueous compartment for both diluted w/o/w emulsions was almost equal under the condition of a large difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion.

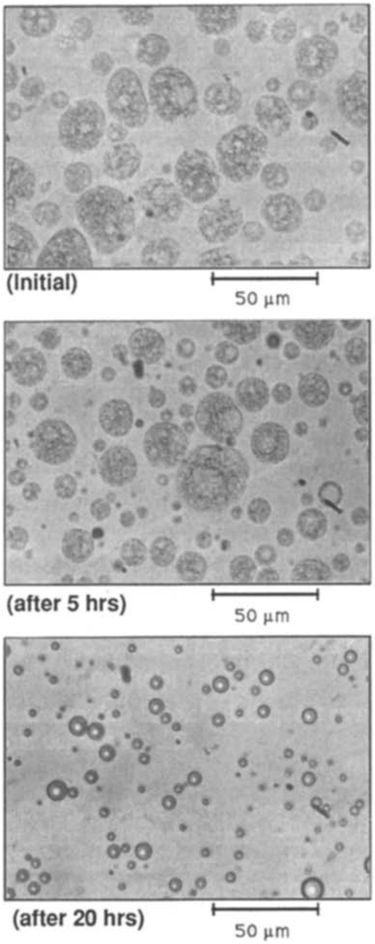

Fig. 6. Photomicrographs of w/o/w emulsions diluted 100-fold with 0.5% (w/v) of PSMO 80 aqueous solution. (h) Addition of PSMO 80 in the external aqueous phase (0.5% w/v). Notations is the same as that in Table 1.

As shown in Fig. 6, with the addition of 0.5% (w/v) PSMO 80 into the external aqueous phase of the w/o/w emulsion, the features of the diluted w/o/w emulsion after 5 h were similar to those of the initial emulsion. However, after 20 h, a single emulsion, which was an oil-in-water emulsion (o/w emulsion), was generated by transformation of the diluted w/o/w emulsion. This suggests that the addition of surfactant to the external aqueous phase could make the diluted w/o/w emulsion extremely unstable under the condition of a large difference in osmolarity between the internal aqueous compartment of the w/o/w emulsion and the external aqueous phase of the emulsion.

Table 4 shows the effect of addition of PSMO 80 to various phases on the diameter of suspending vesicular globules of the diluted w/o/w emulsion and the volume ratio of diluted w/o/w emulsions with various storage durations at 25°C to the initial emulsion for each type of original w/o/w emulsion shown in Table 1 (f)–(h).

The diameter of suspending vesicular globules with addition into the oil phase was almost the same as for addition into the internal aqueous compartment at each storage time point. With addition into the external aqueous phase, the diameter became smaller than that for other emulsions with increased storage duration, which could be caused by generation of the smaller o/w emulsion. With addition of PSMO 80 into the internal aqueous compartment and the oil phase, the increases in volume ratio were almost identical to those for addition of sodium octanesulfonate at 1% (w/v) into the internal aqueous compartment, shown in Fig. 4. On the other hand, on comparison at almost equal osmolarity in the inernal aqueous compartments of the w/o/w emulsions (Table 1 (e) and (f)), the increases in volume ratio with the addition of PSMO 80 into the internal aqueous compartment were smaller than that with the addition of sodium octansulfonate at 0.2% (w/v) into the internal aqueous compartment, which indicates that the addition of PSMO 80 enhanced the stability of w/o/w emulsion more than that of sodium octansulfonate at 0.2% (w/v).

Conclusion

The stability of w/o/w emulsions, as determined by breakdown and growth of internal aqueous compartments caused by coalescence or water influx across the oil phase, was enhanced by the addition of hydrophilic surfactant, sodium octanesulfonate or PSMO 80, to the internal aqueous compartment of the w/o/w emulsion. In particular, even under the condition of a large difference in osmolarity between both aqueous phases of the w/o/w emulsion, coalescence of the internal aqueous compartments or water influx into the compartments was inhibited by the addition of hydrophilic surfactant in the internal aqueous compartment. Moreover, the stability of w/o/w emulsions was affected by the concentration of hydrophilic surfactant added to the internal aqueous compartment, and was enhanced even at additive concentrations below the CMC

TABLE 4

Effect of the addition of Polyoxyethylene (20) sorbitane monooleate (PSMO 80) to various phases on diameter of Suspending vesicular globules of the diluted w / o / w emulsion and volume ratio of the diluted w / o / w emulsion a ed at various storage durations to the initial emulsion for each type of w / o / w, emulsion [a]

Storage duration (h)	Diameter of suspending vesicular globules [b] (μm)			Volume ratio (–) [c]		
	Internal 0.5% (w/v)	Oil phase 0.5% (w/v)	External 0.5% (w/v)	Internal 0.5% (w/v)	Oil phase 0.5% (w/v)	External 0.5% (w/v)
Initial	22.66	21.92	17.77	1	1	1
5	30.68	30.59	17.24	2.482	2.718	0.913
20	28.76	27.66	6.16	2.044	2.009	0.042
50	29.61	27.86	5.26	2.231	2.053	0.026
100	26.93	27.76	6.34	1.679	2.031	0.045
150	–	27.19	3.92	–	1.909	0.011

[a] w/o/w emulsions were prepared according to the formulations Shown in Table 1 (d)–(f).

[b] Data are expressed as means obtained from photomicrographs as the Feret diameter ($n = 100$).

[c] Volume ratio was calculated from the following equation:

volume ratio (–) mean volume of internal aqueous compartment of w/o/w emulsion at each storage time/mean volume of internal aqueous compartment of initial emulsion.

at which micelles were not formed, as compared with control. The closer film like a liquid crystal, which inhibited water influx into the internal aqueous compartment and coalescence of the internal aqueous compartments, was considered to be formed by the addition of hydrophilic surfactant in the internal aqueous compartment of the w/o/w emulsion. On the other hand, with addition of hydrophilic surfactant into the oil phase, coalescence of the internal aqueous compartments was observed in spite of the almost equal influx of water into the compartments as compared to that with addition into the internal aqueous compartment. With addition of hydrophilic surfactant in the oil phase, a different type of film, which allowed coalescence of the internal aqueous compartments but inhibited water influx into the internal aqueous compartment, from that with the addition in the internal aqueous compartment was suggested to be formed. Further studies are needed to elucidate the differences between these two types of films.

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