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Improvement of the formation percentage of water-in-oil-in-water multiple emulsion by the addition of surfactants in the internal aqueous phase of the emulsion

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Summary

In order to improve the formation percentage of w/o/w emulsions, experiments were conducted in order to elucidate the influence of the addition of various kinds of surfactants in the internal aqueous phase of the emulsion, the formation percentage of w/o/w emulsion as compared with that without additives, and with sodium chloride or sorbitol. Several physico-chemical properties of intact w/o/w emulsion or each phase, such as the osmolarity, interfacial tension and viscosity, were investigated in order to determine the governing factor in improving the formation percentage of w/o/w emulsion. w/o/w emulsion was prepared via a two-step emulsification procedure. New coccine was employed as a marker to evaluate the formation percentage of w/o/w emulsion. Sodium alkylsulfonate, sodium alkylsulfate, sodium alkylcarbonate and polyoxyethylene (20) sorbitan monooleate were used as additives in the internal aqueous phase of w/o/w emulsion as well as sodium chloride and sorbitol. The formation percentage of w/o/w emulsion was improved by increasing the concentration of the additives, viz., various kinds of surfactants in the internal aqueous phase of the emulsion, as well as sodium chloride or sorbitol. This could be attributed to a decrease in interfacial tension between the internal aqueous phase of the w/o/w emulsion and the oily phase of the emulsion.

Introduction

Water-in-oil-in-water multiple emulsions (w/o/w emulsions) are complex systems having several potential applications in developing 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 led to several research efforts being devoted to overcoming this drawback, for example, via the addition of sodium chloride or bovine serum albumin (Kita et al., 1977), glucose (Matsumoto et al., 1980) and dextrose (Adeyeye et al., 1990).

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Although a considerable body of data concerning the efficacy or activity of surfactants as emulsifiers has been published (Matsumoto et al., 1987; Adeyeye et al., 1991), few studies have been directed at the use of surfactants as additives in the internal aqueous phase of w/o/w emulsion.

The objectives of the present study were to assess improving the formation percentage of the w/o/w emulsion by the addition of surfactants in the internal aqueous phase of the emulsion in comparison with the cases where no addition was made (control), and where the additives NaCl or sorbitol were present. Furthermore, we attempted to identify the governing factor(s) among the physico-chemical properties contributing to the improvement in formation percentage of the intact w/o/w emulsion, or each phase, for example, osmolarity, interfacial tension and viscosity.

Materials and Methods

Materials

Food dye Red No. 102 (new coccine) (2-Hydroxyazonaphthalene-4′,6,8-trisulfonic acid trisodium salt) used as a marker, entrapped in the internal aqueous phase to monitor the formation percentage of the 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 oily phase, sorbitan monooleate (SMO 80, equivalent to Span 80®) as a lipophilic surfactant, polyoxyethylene (20) sorbitan monolaurate (PSML 20, equivalent to Tween 20®) and polyoxyethylene (20) sorbitan monooleate (PSMO 80, equivalent to Tween 80®) as hydrophilic surfactant were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Sodium alkylsulfonate, sodium alkylsulfate and sodium alkylcarbonate were purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). The chemicals mentioned above were used without further purification. Other chemicals employed were of analytical or reagent grade.

A dialysis membrane tube was purchased from Spectrum Medical Industries, Inc. (U.S.A.).

Preparation of w / o / w emulsion

Preparation of the internal aqueous phase Each type of sodium alkylsulfonate and sodium alkylsulfate (listed in Table 1) and each type of sodium alkylcarbonate and PSMO 80 (shown in Table 2) were dissolved in an isotonic 0.236 M citric acid : 0.123 M disodium phosphate buffer (pH 6.33), to give concentrations both above and below the critical micelle concentration (CMC).

The CMC of each surfactant was determined at 20°C using a surface tensiometer (model CBVP-3A, Kyowa Kaimenkagaku Co., Ltd, Japan) operating on the principle of the glass plate hanging method. Sodium chloride was dissolved in the isotonic 0.236 M citric acid : 0.123 M disodium phosphate buffer (pH 6.33) at concentrations ranging from 0 to 1.8% (w/v), while sorbitol was dissolved in the same buffer at concentrations from 0 to 11.2% (w/v). New coccine at 0.2% (w/v) was dissolved in the above solutions to

TABLE 1

Sodium alkylsulfonate and sodium alkylsulfate employed as additives to the internal aqueous phase of w / o / w emulsion

Surfactant	Molecular formula	Mol. Wt [a]	CMC [b] (% (w/v))
Sodium 1-butanesulfonate	$CH_3(CH_2)_3SO_3Na$	160.16	1.000–3.000
Sodium 1-pentanesulfonate	$CH_3(CH_2)_4SO_3Na$	174.20	1.001–5.007
Sodium 1-hexanesulfonate	$CH_3(CH_2)_5SO_3Na$	188.22	0.104–1.035
Sodium 1-heptanesulfonate	$CH_3(CH_2)_6SO_3Na$	202.25	0.501–2.005
Sodium 1-octanesulfonate	$CH_3(CH_2)_7SO_3Na$	216.28	0.302–1.008
Sodium 1-decanesulfonate	$CH_3(CH_2)_9SO_3Na$	244.33	0.050–0.500
Sodium 1-tetradecanesulfonate	$CH_3(CH_2)_{13}SO_3Na$	300.12	0.005–0.101
Sodium dodecylsulfate	$C_{12}H_{25}OSO_3Na$	288.38	0.004–0.040

[a] Molecular weight.

[b] Critical micelle concentration.

monitor the formation percentage of w/o/w emulsion. In order to assess the possibility of using new coccine as a marker for monitoring the formation percentage of w/o/w emulsion, advance determinations of the apparent partition coefficients of new coccine between each type of internal aqueous phase formula mentioned above, and the oily phase formula referred to in the following section, were performed. Consequently, it was confirmed that new coccine was a suitable marker to monitor the formation percentage of w/o/w emulsion, since partitioning from the internal aqueous phase formula to the oily phase formula was not observed. The osmolarity of each internal aqueous phase of w/o/w emulsions was measured with an osmometer (model 3W II, Advanced Instruments, Inc., MA) operating on the principle of freezing-depression, and was determined as a mean value from duplicate samples.

Preparation of the oily phase The formula of the oily phase used in these experiments was that described by Kawashima et al. (1991). SMO 80 was dissolved in liquid paraffin to a concentration of 30% (w/v).

Preparation of the external aqueous phase PSML 20 was dissolved in the isotonic 0.236 M citric acid : 0.123 M disodium phosphate buffer (pH 6.33) to a concentration of 0.5% (w/v). For examining the additional effect of surfactants on the formation percentage of w/o/w emulsion, each type of surfactant was dissolved in this solution to give its corresponding concentration.

Preparation procedure The w/o/w emulsion was prepared according to the two-step emulsification procedure described by Matsumoto et al. (1976). 50 ml of an internal aqueous solution was added dropwise to 30 ml of oily phase at a rate of 5.4 ml/min with the aid of a peristaltic pump (Atto Co., Ltd, Japan). During this stage, the system was agitated at 2700 rpm using a Polytron homogenizer (type X 1020, Ystral GmbH, Germany) equipped with a U-shaped blade (7.3 mm diameter, 12.0 mm height) at the bottom of the cylinder (7.8 mm internal diameter, 10.2 mm external diameter) having several small lattice windows. Next, the solution was poured into the external aqueous phase at a stretch, and the mixture was then immediately agitated at 3000 rpm for 1 min to yield the w/o/w emulsion using an Ultra-Disperser (model LK-21, Yamato Kagaku Co., Ltd, Japan), equipped with the same type of agitation parts, U-shaped blade (12.4 mm diameter, 10.0 mm height) and cylinder (13.0 mm internal diameter, 17.8 mm external diameter), as the Polytron homogenizer mentioned above.

The resultant w/o/w emulsions were stable for 1 month at 25°C after preparation. Scanning electron micrographs of the freeze-fractured w/o/w emulsion were taken on a T330A (Jeol Ltd, Japan).

Apparatus for dialysis test

Dialysis tests were conducted to determine the formation percentage of w/o/w emulsion. The dialysis apparatus shown in Fig. 1 was used in these experiments.

A 200 ml conical flask was used as a dialysis cell. A membrane dialysis tube was cut off in order to adjust the effective dialysis surface area to 18 cm^2, and both end terminals were locked

TABLE 2

Sodium alkylcarbonate and PSMO 80 [a] employed as additives to the internal aqueous phase of w/o/w emulsion

Surfactant	Molecular formula	Mol. Wt [b]	CMC (% (w/v)) [c]
Sodium propionate	CH_3CH_2COONa	96.06	2.005–8.001
Sodium *n*-caproate	$CH_3(CH_2)_4COONa$	138.14	0.500–1.001
Sodium *n*-caprylate	$CH_3(CH_2)_6COONa$	166.20	0.200–1.001
Sodium caprate	$CH_3(CH_2)_8COONa$	194.25	0.020–0.505
PSMO 80	–	–	0.005–0.051

[a] Polyoxyethylene (20) sorbitan monooleate.
[b] Molecular weight.
[c] Critical micelle concentration.

over by closures. One of the closures was combined with a magnetic stirrer to allow agitation of the test solution in the cell. The dialysis cell system was immersed in a water bath and maintained at 37°C.

Dialysis test

Procedure for dialysis test 200 ml of dialysis external solution, which was the same solution as the external aqueous phase, was heated in advance to 37°C and poured into the dialysis cell prior to the start of the test. Thereafter, 4 ml of w/o/w emulsion was placed in a dialysis membrane tube (1000 Da cutoff) that had one end locked over by a closure in advance. Subsequently, the other end was locked over by a closure and immersed in the external dialysis solution. About 1 ml of dialysis external solution was withdrawn periodically from the cell, and the concentration of new coccine was determined by spectrophotometry at 508 nm (model UV240, quartz cell with 1 cm length, Shimadzu, Japan). Immediately after measuring, the dialysis external solution sample was replaced in the dialysis cell and dialyzed for 6 h. In this study, experiments were conducted in duplicate.

Selection of pore size of dialysis membrane tube Experiments were conducted according to the procedure mentioned above with dialysis membrane pore sizes of 1000, 3500 and 8000 Da cutoff in order to select the respective pore size for the dialysis test. For the dialysis internal solution, about 50 mg of new coccine as a marker was dissolved in the isotonic 0.236 M citric acid : 0.123 M disodium phosphate buffer (pH 6.33), this buffer solution also being employed as an external dialysis solution. The dialysis experiments were carried out for 8–10 h, and were conducted in duplicate.

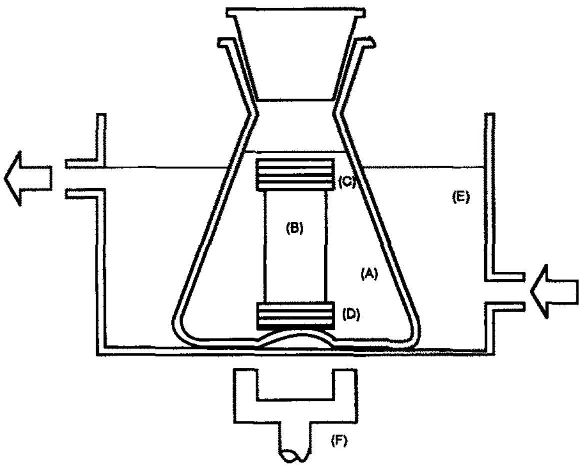

Fig. 1. Apparatus for dialysis test. (A) Dialysis cell (200 ml Erlenmeyer flask), (B) dialysis tube, (C) closure, (D) closure (attached to a stirrer bar), (E) water bath, (F) magnetic stirrer.

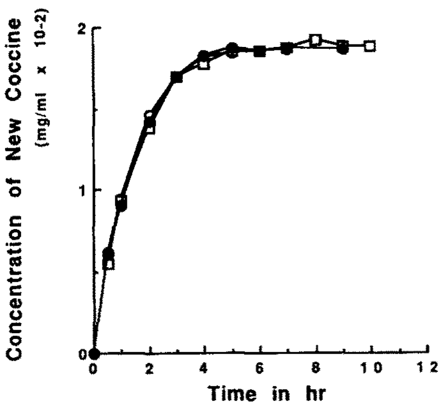

Fig. 2. Effect of pore size of dialysis membrane tube on the concentration of new coccine in the external dialysis solution. Cut off sites: (○) 1000 Da, (●) 3500 Da, (□) 8000 Da. Data are expressed as means ($n = 2$).

As indicated in Fig. 2, the time required for equilibration of the marker between the internal and external dialysis solutions was larger than 5 h in duration and was almost identical for each dialysis membrane tube. Accordingly, a pore size of 1000 Da cutoff was chosen for the dialysis tests.

Calculation of percentage of w/o/w emulsion formed

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

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

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

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_0 represent the volumes of the internal aqueous, external aqueous and oily phases, respectively, in the formulation for preparing w/o/w emulsion. V_d and V_s denote the volumes of the dialysis external solution and dialyzed emulsion, respectively.

Determination of interfacial tension

The interfacial tension between the internal aqueous and oily phases was measured at 25°C with a surface tensiometer (model CBVP-A3, Kyowa Kaimenkagaku Co., Ltd, Japan), operating on the principle of the glass plate hanging method. The measurement procedure followed the established method. The mean value of the interfacial tension was determined on duplicate samples.

Measurement of viscosity of w / o / w emulsion

The viscosity of fresh w/o/w emulsions was measured at 37°C using a viscometer (Tokyo Keiki Co., Ltd, Japan) equipped with a cone (radius, 4.8 cm; angle, 0.8°). The viscosity was evaluated at a time of 2 min and rate of rotation of 5 rpm. The mean value of the viscosity was determined on duplicate samples.

Results and Discussion

In order to improve the formation percentage of w/o/w emulsion, experiments were conducted for the evaluation of each of the effects resulting from the addition of various kinds of surfactants in the internal aqueous phase of w/o/w emulsion, as compared with those in the case where no addition was made and where sodium chloride or sorbitol was added. Furthermore, several of the physico-chemical properties of the w/o/w emulsion itself, or of each phase, such as the osmolarity, interfacial tension and viscosity, were determined in order to predict the efficacy of the additives in the internal aqueous phase at improving the formation percentage of w/o/w emulsion.

A scanning electron micrograph of w/o/w emulsion in the present system is shown in Fig. 3.

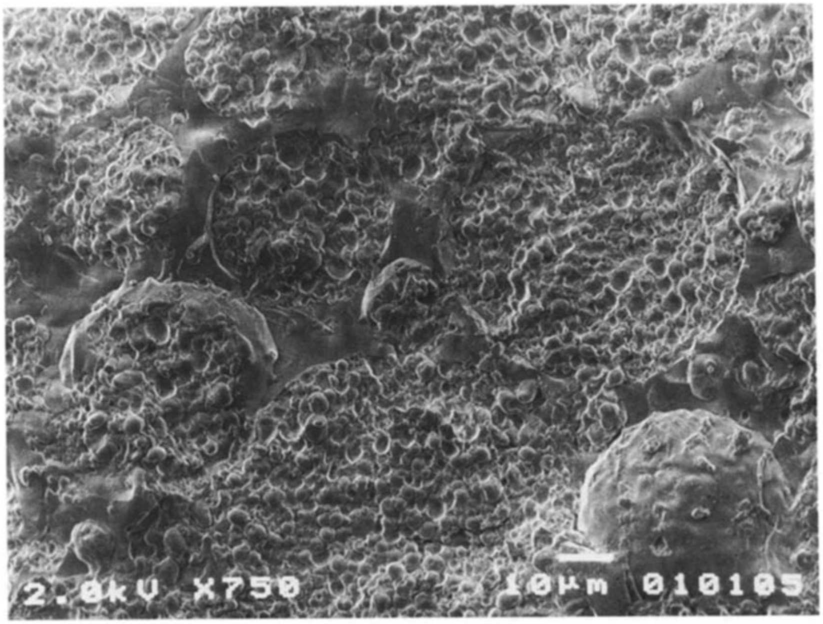

Fig. 3. Scanning electron micrograph of w/o/w emulsion.

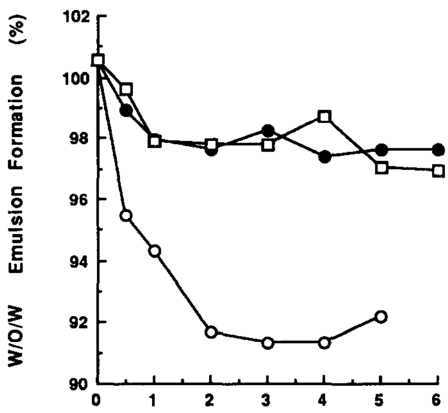

Fig. 4. Typical curves showing the dependence of the formation percentage of w/o/w emulsion on the duration of the dialysis test time. (○) Control, (●) sodium 1-octanesulfonate (2% w/v), (□) sodium *n*-caprylate (2% w/v). Data are expressed as means ($n = 2$).

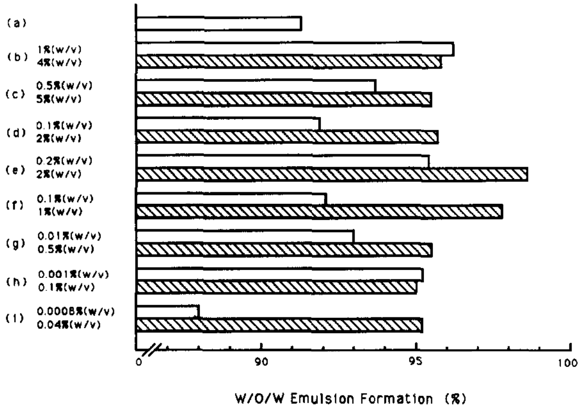

Fig. 5. Effect of the addition of various alkylsulfonates or alkylsulfates (sodium salts referred to in every case (b–i)) in the internal aqueous phase of w/o/w emulsion on the formation percentage of the emulsion. (a) Control; (b) 1-butanesulfonate, (c) 1-pentanesulfonate, (d) 1-hexanesulfonate, (e) 1-heptanesulfonate, (f) 1-octanesulfonate, (g) 1-decanesulfonate, (h) 1-tetradecanesulfonate, (i) dodecylsulfate Data are expressed as means ($n = 2$).

The formation of w/o/w emulsion could be confirmed and observed visually by electron microscopy. The emulsion droplets had diameters in the range 16–160 μm and contained numerous small aqueous droplets dispersed within them.

Fig. 4 depicts typical curves obtained for the formation percentage of w/o/w emulsion plotted as a function of the duration of the dialysis test time. For each curve, the time taken to reach the plateau was less than 2 h, and the plateau value indicated the yield of w/o/w emulsion. Consequently, we confirmed that a dialysis period of 6 h would suffice for evaluating the formation percentage of w/o/w emulsion.

Figs 5–7 demonstrate the effects of various types of additives in the internal aqueous phase of the w/o/w emulsion on the formation percentage of the emulsion. In Fig. 5, all of the alkylsulfonates and alkylsulfates at additive concentrations above the CMC increased the formation percentage of w/o/w emulsion as compared with the control (without additives). The highest value was attained with sodium 1-heptanesulfonate (2% w/v), whereas the formation percentages for the addition of sodium 1-butanesulfonate and sodium 1-tetradecanesulfonate at concentrations below the CMC were almost equal to those above the CMC. Also, the increasing effect of addition of the other sulfonates at less than the CMC was smaller in extent than that above the CMC, however, the formation percentage was greater than that of the control. For sodium dodecylsulfate, the formation percentage of w/o/w emulsion was found to be less than that of the control.

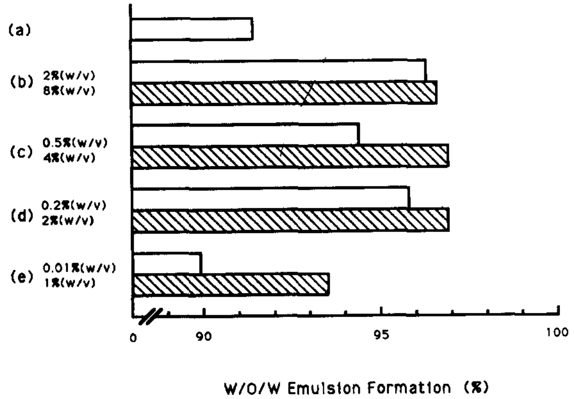

Fig. 6. Effect of the addition of various types of alkylsulfonate (Na salt used in every case (b–e)) in the internal aqueous phase of w/o/w emulsion on the formation percentage of the emulsion. (a) Control, (b) propionate, (c) *n*-caproate, (d) *n*-caprylate, (e) caprate. Data are expressed as means ($n = 2$).

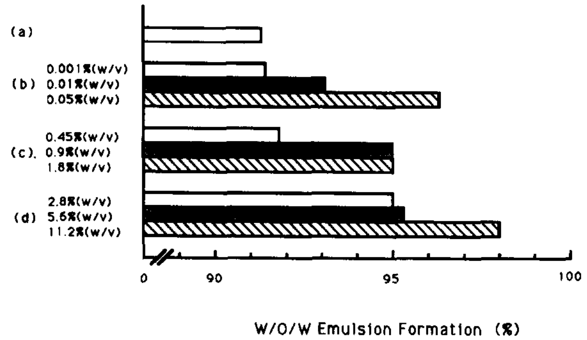

Fig. 7. Effect of the addition of several types of additives in the internal aqueous phase of w/o/w emulsion on the formation percentage of the emulsion. (a) Control, (b) PSMO 80, (c) NaCl; (d) sorbitol. Data are expressed as means ($n = 2$).

The addition of all types of alkylcarbonates at concentrations above the CMC increased the formation percentage of w/o/w emulsion as compared with the control, as demonstrated in Fig. 6, and the formation percentages for sodium propionate, sodium *n*-caproate and sodium *n*-caprylate were almost identical. In contrast, increasing effect of the addition of alkylcarbonate at less than the CMC, except for sodium caprate, was of smaller extent than that above the CMC, however, the formation percentage exceeded the value for the control.

In Fig. 7, the formation percentage of w/o/w emulsion was observed to increase linearly with the concentration of PSMO 80, a nonionic surfactant, in the internal aqueous phase, however, the increasing effect at 0.001% (w/v) additive was not evident in comparison with the control. For sodium chloride, the formation percentages of w/o/w emulsion were almost identical at 0.9 and 1.8% (w/v) additive and were greater than that of the control. However, at 0.45% (w/v) additive, the formation percentage increased only slightly as compared with that of the control. Although each concentration of sorbitol was adjusted to the same osmolarity as that for the corresponding concentration of sodium chloride, the formation percentage of w/o/w emulsion at a sorbitol concentration of 2.8% (w/v) was almost equal to that at 5.6% (w/v), both values being greater than that of the control. Moreover, the increasing effect on the formation percentage at an additive concentration of 11.2% (w/v) was greater than those at both 2.8 and 5.6% (w/v). On comparison of the two additives, sodium chloride and sorbitol, the values determined for the formation percentage of w/o/w emulsion for sorbitol were greater than those for sodium chloride despite the almost identical values of the osmolarity at each concentration.

From the above results, it was demonstrated that the formation percentage of the w/o/w emulsion was increased by the addition of surfactants into the internal aqueous phase of the emulsion, as well as by sodium chloride or sorbitol.

We subsequently addressed the question of how the addition of surfactants in the internal aqueous phase increased the formation percentage of w/o/w emulsion as compared with that for sodium chloride or sorbitol by investigating several physico-chemical properties, for example, osmolarity of the internal aqueous phase, interfacial tension between the internal aqueous and oily phases and viscosity of w/o/w emulsion.

The relationship between the formation percentage of w/o/w emulsion and osmolarity of the internal aqueous phase of the emulsion at various concentrations of sodium chloride or sorbitol is illustrated in Fig. 8. A significant correlation (correlation coefficient, 0.8402; $p < 0.05$) was found between the two variables. It has been reported that the stability and formation percentage of w/o/w emulsion are enhanced by addition of sodium chloride or bovine serum albumin (Kita et al., 1977), glucose (Matsumoto et al., 1980), and dextrose (Adeyeye et al., 1990). These observations were found to be due to the increase in osmolarity of the internal aqueous phase or in the viscosity of the w/o/w emulsion. The results in Fig. 8 are consistent with the above studies. On the other hand, the increasing effect of surfactant addition in the internal aqueous phase of w/o/w emulsion on the formation percentage of the emulsion was not examined. Nevertheless, several studies on the enhancement of w/o/w emulsion formation have been performed, e.g., on the influence of lipophilic surfactants in the oily phase components (Adeyeye et al., 1991), hydrophilic surfactants (Matsumoto et al., 1987) and volume

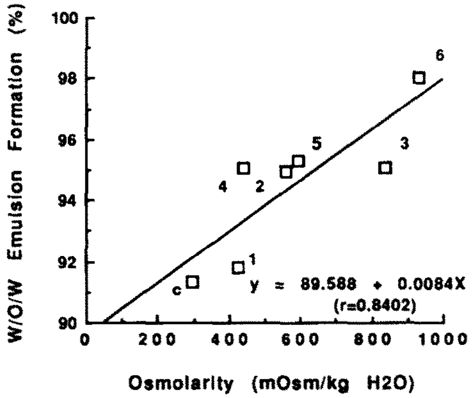

Fig. 8. Relationship between the formation percentage of w/o/w emulsion and osmolarity of the internal aqueous phase of the emulsion at various additive concentrations of sodium chloride or sorbitol in the internal aqueous phase. c: Control. Sodium chloride: (1) 0.45, (2) 0.9, (3) 1.8% (w/v); sorbitol: (4) 2.8, (5) 5.6, (6) 11.2% (w/v).

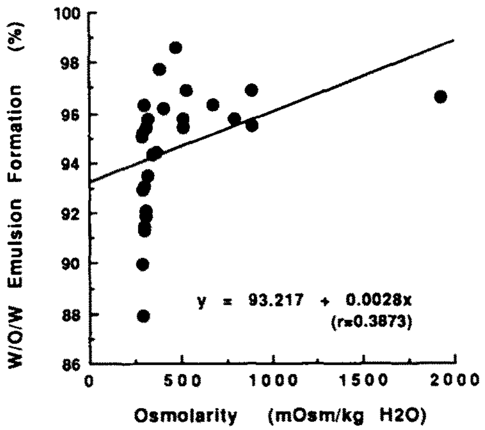

Fig. 9. Relationship between the formation percentage of w/o/w emulsion and osmolarity of the internal aqueous phase for various types of surfactants.

ratio of each phase (Matsumoto et al., 1976). The present paper demonstrates clearly that the formation percentage of w/o/w emulsion is improved by the addition of surfactants in the internal aqueous phase of the emulsion as well as by the presence of the additives sodium chloride or sorbitol.

Fig. 9 depicts the relationship between the formation percentage of w/o/w emulsion and osmolarity of the internal aqueous phase of the emulsion for various kinds of surfactants.

We found no significant correlation between the formation percentage of w/o/w emulsion and osmolarity of the internal aqueous phase (correlation coefficient: 0.3837). This indicates that another factor is the cause of the improvement in the formation percentage of w/o/w emulsion.

Fig. 10 shows the relationship between the formation percentage of w/o/w emulsion and the interfacial tension between the internal aqueous phase of w/o/w emulsion and the oily phase of the emulsion.

A significant correlation (correlation coefficient, 0.9434; $p < 0.01$) was observed between both variables for surfactants. It was possible to predict that leakage of the internal aqueous phase could be prevented to a greater extent with additives present during the stage of formation of w/o/w emulsion than without. This is due to the decrease in interfacial tension. Matsumoto (1986) reported that it was possible for smectic liquid crystals of a lipophilic surfactant to form in the case where the interfacial tension between the aqueous and oily phases decreased. Hence, the hypothesis was put forward that, with decreasing

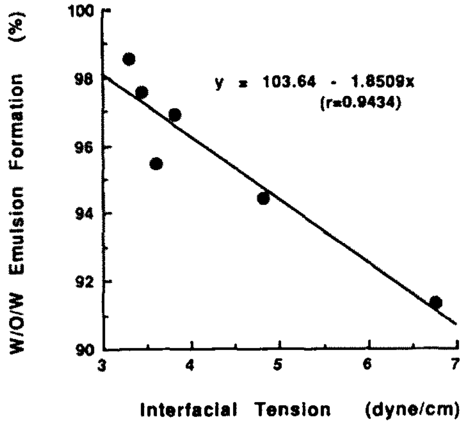

Fig. 10. Relationship between the formation percentage of w/o/w emulsion and the interfacial tension between the internal aqueous phase of w/o/w emulsion and the oily phase of the emulsion. (c) Control, (P) 1-pentanesulfonate (5% w/v), (H) 1-heptanesulfonate (2% w/v), (O) 1-octanesulfonate (1% w/v), (Co) *n*-caproate (4% w/v), (Cy) *n*-caprylate (2% w/v). (Na salt referred to in every case).

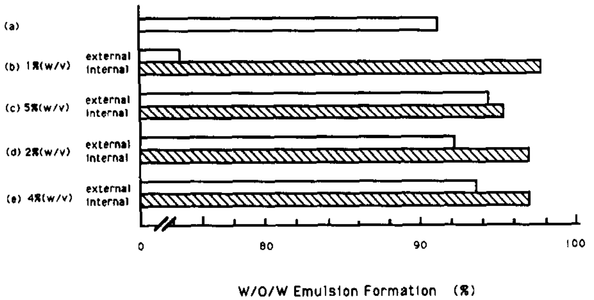

Fig. 11. Effect of added phase of surfactant on the formation percentage of w/o/w emulsion. (a) Control, (b) 1-octanesulfonate, (c) 1-pentanesulfonate, (d) *n*-caprylate, (e) *n*-caproate (Na salt referred to in every case (b–e)).

interfacial tension, the orientation of hydrophilic surfactants at the interface between the internal aqueous phase of w/o/w emulsion and the oily phase of the emulsion would lead to the formation of a liquid crystal closer to that of the smectic type.

The effect of the phase of surfactant addition on the formation percentage of w/o/w emulsion is shown in Fig. 11. The formation percentage for the addition of surfactants in the external aqueous phase was less than that in the internal aqueous phase, and was slightly greater than the control value, except for sodium 1-octanesulfonate. The lower value for the latter was believed to result from aggregation of the emulsion. From the results of this study, it was suggested that the addition of surfactant in the internal aqueous phase of w/o/w emulsion led to the formation of a more rigid interface.

It has been reported previously that a higher osmolarity of the internal aqueous phase of the w/o/w emulsion as compared to the external aqueous phase of the emulsion is the factor leading to the rise in viscosity of the w/o/w emulsion. This is caused by the transfer of water from the external aqueous phase to the internal aqueous phase (Kita et al., 1977), which also improves the formation percentage of w/o/w emulsion (Adeyeye et al., 1990). Furthermore, an equation expressing the relation between the logarithm of the relative viscosity of the emulsion and the volume ratio of the dispersed phase was proposed for the w/o/w emulsion system (Mooney, 1951).

Fig. 12 demonstrates this relationship as shown by the plots of the logarithm of the viscosity of the w/o/w emulsion vs osmolarity of the internal aqueous phase of the emulsion. A significant correlation (correlation coefficient, 0.8118; $p < 0.05$) was found to hold between these two physico-chemical properties for sodium chloride and sorbitol (Fig. 12a), as described previously, but not for surfactants (Fig. 12b). From these results, it was supposed that the transfer of water from the external aqueous phase of w/o/w emulsion to the internal aqueous phase of the emulsion was of smaller extent in the case of surfactants than that for sodium chloride or sorbitol, due to the formation of an interfacial barrier between the two phases.

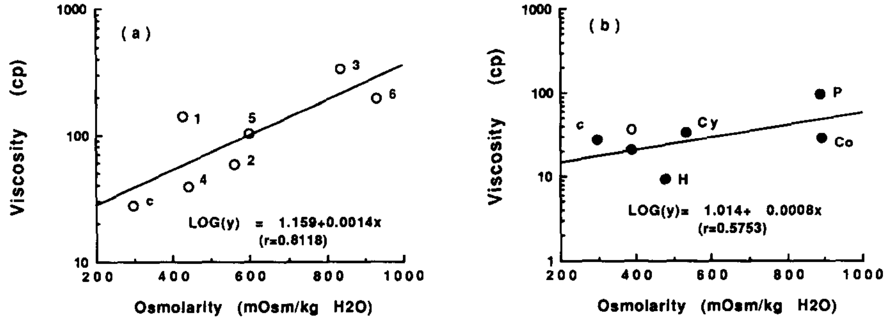

Fig. 12. Relationship between viscosity of the w/o/w emulsion and osmolarity of the internal aqueous phase of the emulsion (a) c: Control. Sodium chloride: (1) 0.45, (2) 0.9, (3) 1.8% (w/v); sorbitol: (4) 2.8, (5) 5.6, (6) 11.2% (w/v). (b) c: Control. (P) 1-pentanesulfonate (5% w/v), (H) 1-heptanesulfonate (2% w/v), (O) 1-octanesulfonate (1% w/v), (Co) 1-caproate (4% w/v), (Cy) 1-caprylate (2% w/v) (Na salt referred to in every case).

In conclusion, the formation percentage of the w/o/w emulsion was improved by increasing the additive concentration of various surfactants in the internal aqueous phase of the emulsion, as well as by sodium chloride or sorbitol. The increasing effect of surfactant addition on the formation percentage of w/o/w emulsion was observed to be linearly related to the change in interfacial tension between the internal aqueous phase of the w/o/w emulsion and the oily phase of the emulsion. Matsumoto (1988) reported that multiple emulsions, i.e., w/o/w and o/w/o emulsions, could be prepared in the neighborhood of a detergent phase like a liquid crystal. The detergent phase is where the hydrophilicity and lipophilicity of the surfactants are just balanced (Shinoda et al., 1968). As a result of this study, the decreasing interfacial tension between the internal aqueous phase of w/o/w emulsion and the oily phase of the emulsion is suggested to lead to the formation of a more closely smectic liquid crystal on the addition of a hydrophilic surfactant in the internal aqueous phase, hence providing the increasing effect on the formation percentage of w/o/w emulsion.

Further studies are necessary in order to elucidate the relationship between the formation percentage of w/o/w emulsion and the CMC for each surfactant.

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