Chem. Pharm. Bull. **40**(5) 1240—1246 (1992) Vol. 40, No. 5

Stabilization of Water/Oil/Water Multiple Emulsion with Hypertonic Inner Aqueous Phase

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Water/oil/water emulsion (w/o/w emulsion) was prepared with liquid paraffin, a hydrophobic surfactant (Span 80) and a hydrophilic surfactant (Tween 20) by a two-step emulsification procedure. The percentage of solute entrapped in the inner aqueous phase and the viscosity of the emulsion system increased with an increase in the concentration of solute (glucose or sodium chloride) initially added to the inner phase of the emulsion. The oil membrane of the w/o/w emulsion, which entrapped the hypertonic inner aqueous phase, was thick, and the release of the solute from the emulsion was slow. The viscosity of w/o/w emulsion entrapping a hypertonic inner aqueous phase was large, and consequently, separation of the aqueous phase was delayed. A decrease in the encapsulation efficiency of w/o/w emulsion during storage was considered to be the result of a rupture of the oil membrane. Destruction of the w/o/w emulsion followed first-order kinetics. The rate constant of destruction of w/o/w emulsion at room temperature could be predicted by measuring the destruction rate at a higher storage temperature.

Keywords w/o/w emulsion; stability; solute in inner aqueous phase; trapped percentage; droplet size; viscosity

Introduction

Water/oil/water emulsions (w/o/w emulsions) have many potential applications in the fields of cosmetics,[1,2] food[3] and medicine. The potential medical applications include acting as carriers for lymphatic drug delivery,[4,5] carriers for controlled drug release,[6-8] a substitute for red blood cells,[9,10] a reservoir to absorb drug overdose[11] and so on. However, w/o/w emulsion systems are thermodynamically unstable, which results in various problems such as instability during storage, including leakage of the content from the inner phase, flocculation of inner aqueous and w/o/w emulsion droplets, phase separation and so on, all of which must be overcome before the emulsions can be employed for practical purposes.

Florence and Whitehill[12] suggested three methods to stabilize w/o/w emulsions as follows: i) the use of a high-viscosity oil phase to prevent or decrease diffusion of individual surfactant molecules and water molecules, ii) the polymerization of interfacially adsorbed surfactant molecules, and iii) the gelation of the oily or aqueous phases of the emulsions. Nevertheless, the effective stabilization of w/o/w emulsions has not yet been accomplished.

Water-soluble drugs or water-soluble markers are generally added initially in the inner aqueous phase of w/o/w emulsions, and the percentage of the drugs or markers entrapped in the inner phase and the change in this value during storage are employed as indices of the formation and stability of w/o/w emulsions, respectively. However, the effects of the concentrations of drugs or markers in the inner phase on the encapsulation efficiency and on the stability of w/o/w emulsions[13] have not been fully established. It is generally considered that stable w/o/w emulsion can be obtained under an isotonic condition between the inner and outer aqueous phases.

In the present study, w/o/w emulsions were prepared with various concentrations of solutes in the inner and outer aqueous phases, and the effect of solute concentration on the stability of the w/o/w emulsions was investigated by measuring changes in the percentage of trapped solute, viscosity, droplet size and separation of the aqueous phase during storage. A method was found for stabilizing w/o/w emulsion by using a high solute concentration in the inner aqueous phase.

Experimental

Materials The lipophilic surfactant, sorbitan monooleate (Span 80, Kishida Chemical Co.), and the hydrophilic surfactant, polyoxyethylene sorbitan monolaurate (Tween 20, Kishida Chemical Co.), were used without further purification. The liquid paraffin specified in the Japanese official formulary of food additives was used. All other chemicals used were reagent-grade products.

Preparation of w/o/w Emulsion w/o/w emulsion was prepared by means of the two-step emulsification procedure described by Matsumoto *et al.*[14] In the first emulsification stage, 100 ml of inner aqueous phase was dropped into 60 ml of the oil phase, which consisted of 70% (w/w) liquid paraffin and 30% (w/w) Span 80, at the rate 1.7—2.0 ml/min with the aid of a feeding pump, CV-1 (Tokyo Kagaku Seiki Co.). The resultant w/o emulsion was dropped into 0.5% (w/v) Tween 20 aqueous solution (160 ml) at the rate 6.0—8.0 ml/min *via* the pump to produce a w/o/w emulsion (second emulsification stage). The agitating speeds of the system at the first and second emulsification stages were 390 and 630 rpm, respectively, using a Chemy B-150 stirrer (Tokyo Rikakikai Co.) with double four-bladed propellers.

Sodium chloride or glucose (0.01—0.10 mol/l) was added to the inner aqueous phase as a water-soluble marker. The outer aqueous phase contained 0—0.15 mol/l glucose.

Aliquots of prepared w/o/w emulsions were stored at 5, 30 and 40 °C.

Percentage of Marker Entrapped in the Inner Aqueous Phase of w/o/w Emulsion Ten milliliters of w/o/w emulsion, prepared freshly or stored, was placed in seamless cellulose tubing (Viskase Sales Co., U.S.A.) and dialyzed in 90 ml of 0.5% (w/v) Tween 20 aqueous solution for 10 h. The concentration of sodium chloride in the dialysate was determined by measuring its electroconductivity with a DS-7M instrument (Horiba Co.), and that of glucose was determined spectrophotometrically at 490 nm by use of the phenol–sulfuric acid reaction.[15]

The percentage of the marker trapped in the inner aqueous phase of w/o/w emulsion, α(%), *i.e.*, the volume percentage of the inner aqueous phase entrapped in the oil membrane, was calculated according to Eq. 1 based on the assumption that markers detected in the dialysis medium originated only from leakage during the preparation of the w/o/w emulsion and from the expulsion of inner aqueous droplets by thermal motion or destruction of the oil membrane during storage:

$$n_{10}V_1(1-\alpha/100)=n_1[V_d+V_s\{V_1(1-\alpha/100)+V_2\}/(V_1+V_2+V_0)] \times (V_1+V_2+V_0)/V_s \qquad (1)$$

where n_{10} is the initial concentration of the marker in the inner aqueous phase; n_1 is the concentration of the marker in the dialysate; V_1, V_2, V_0 represent the volumes of inner aqueous, outer aqueous and oil phases in the formulation to prepare w/o/w emulsion, respectively; and V_d and V_s are the volumes of dialysis medium and dialyzed emulsion, respectively.

If V^* is defined by Eq. 2, α is given by Eq. 3.

$$V^*=V_2+V_d(V_1+V_2+V_0)/V_s \qquad (2)$$

$$\alpha=100\{1-n_1V^*/(n_{10}-n_1)V_1\} \qquad (3)$$

Measurement of Droplet Size and Electron Micrography of w/o/w

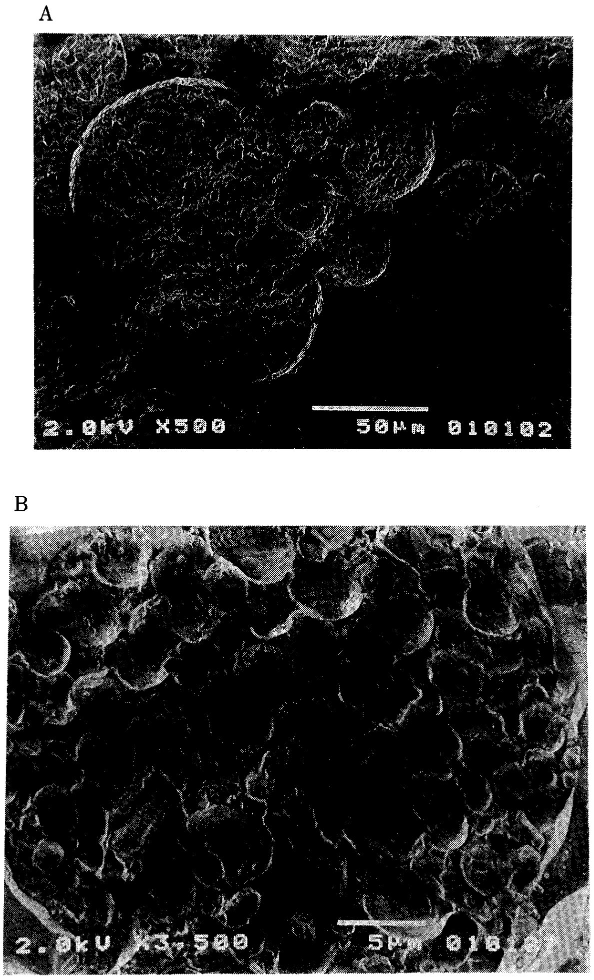

Fig. 1. Scanning Electron Micrograph of w/o/w Emulsion

Inner aqueous phase: 0.02 mol/l sodium chloride. Outer aqueous phase: water. (A) Micrograph of w/o/w emulsion system. (B) Magnified micrograph of w/o/w emulsion droplet. Mean diameter of inner aqueous phase: 1.87 µm.

Emulsion The scanning electron micrographs of w/o/w emulsion shown in Figs. 1A and B were taken with a T330A instrument (JEOL Ltd.). The inner aqueous droplet sizes were directly measured with a magnified micrograph (Fig. 1B) using a particle size analyzer, TGZ3 (Carl Zeiss Co., Germany).

Optical photomicrographs of w/o/w emulsions diluted five times with 0.5% (w/v) Tween 20 aqueous solution and those of w/o emulsions prepared at the first emulsification stage were taken. Heywood diameters of w/o/w emulsion droplets and those of the inner aqueous droplets in w/o emulsions were measured with a particle size analyzer, TGZ3 (Carl Zeiss Co., Germany).

Effective liquid membrane thickness, h, was calculated from the diluted w/o/w emulsion droplet size, D, and percentage of trapped marker by using Eqs. 4 and 5 as follows:

$$n\pi D^3/6=\alpha V_1/100+V_0 \qquad (4)$$

$$n\pi d^3/6=\alpha V_1/100 \qquad (5)$$

$$\begin{aligned} h&=(D-d)/2 \\ &=D[1-\{\alpha V_1/(\alpha V_1+100V_0)\}^{1/3}]/2 \qquad (6)\end{aligned}$$

where d is the diameter of an inner aqueous droplet and n is the number of w/o/w emulsion droplets in the whole multiple emulsion system.

The percentages of trapped solute, α, in Eqs. 4 and 5 were calculated with a diluted emulsion system by means of the way described in the previous paragraph.

Effective total surface area of the liquid membrane, S, is calculated as

$$S=n\pi(D^2+d^2)/2 \qquad (7)$$

Effective membrane thickness and total surface area were calculated based on the assumption that one w/o/w emulsion droplet contained only one inner aqueous droplet, as shown in Fig. 2.

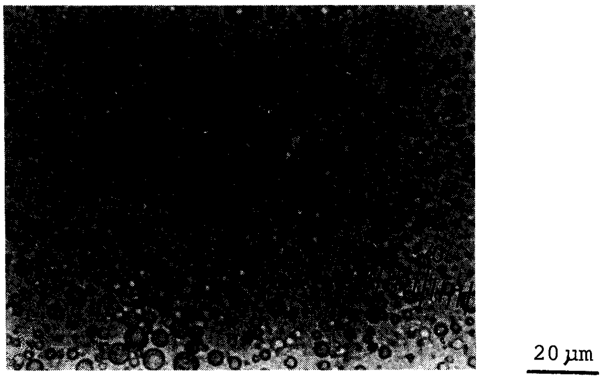

Fig. 2. Photomicrograph of Diluted w/o/w Emulsion

Prepared w/o/w emulsion was diluted five times with 0.5% (w/v) Tween 20 aqueous solution. Inner aqueous phase: 0.02 mol/l sodium chloride. Outer aqueous phase: water.

Measurement of Viscosity of w/o/w Emulsion Viscosities of w/o/w emulsions, both freshly prepared and stored, were measured with a cone-and-plate viscometer, Rheometer NRM-0 (Nippon Rheology Kiki Co.) at 20 °C. Cone radius and angle were 4.6 cm and 20′, respectively. Viscosity was measured at the shear rate of $120\,s^{-1}$, and this shear rate was reached in 40 s under conditions of uniformly accelerated shear rotation.

Percentage of Aqueous Phase Separated from w/o/w Emulsion System Twenty-five milliliters of prepared w/o/w emulsion was taken into a 25-ml glass graduated cylinder and allowed to stand. The volume of separated aqueous phase, V_{SEP}, was observed at intervals. The percentage of separated aqueous phase, β(%), was calculated as follows;

$$\beta=100(V_{SEP}/25)/\{(V_1+V_2)/(V_1+V_2+V_0)\} \qquad (8)$$

Determination of Interfacial Tension between Oil and Inner Aqueous Phases Interfacial tension between aqueous and oil phases was measured by the drop weight method at 20 °C. The aqueous phase was dropped into the oil phase and the interfacial tension was calculated by measuring the weight of an aqueous droplet. Aqueous phases employed were distilled water, 0.01, 0.03, 0.05, 0.075 and 0.1 mol/l glucose and sodium chloride aqueous solutions.

Results

Internal Structures of w/o/w Emulsions and Their Diluted Ones In the undiluted w/o/w emulsion system, multiple emulsion droplets contained many inner aqueous droplets and their sizes were approximately 8 to 90 µm as shown in Fig. 1. In contrast, a diluted w/o/w emulsion droplet had mostly single inner aqueous droplets and the size was relatively small, compared to undiluted w/o/w emulsion droplets, as shown in Fig. 2 and Table I.

Changes in Percentage of Solute Trapped in w/o/w Emulsion during Storage a) Effect of Temperature on Changes in Percentage of Trapped Solutes in Inner Aqueous Phase of w/o/w Emulsion during Storage A w/o/w emulsion was prepared with 0.03 mol/l glucose as the inner aqueous phase and without any solute in the outer aqueous phase. Aliquots of prepared emulsion were stored at 5, 30 and 40 °C. Figure 3 shows the changes in percentage of glucose entrapped in the inner aqueous phase during storage.

The percentage of trapped glucose in the emulsion stored at 5 °C showed little change, but the percentage in the emulsions stored at 30 and 40 °C remarkably decreased. Similar results were obtained with w/o/w emulsions prepared with other concentrations of glucose or sodium chloride in the inner aqueous phase.

b) Effect of Solutes in the Inner Aqueous Phase on Encapsulation Efficiency of w/o/w Emulsion w/o/w emulsions were prepared with various concentrations of solutes in the inner aqueous phase, and the changes in percentage of trapped solute during storage were investigated. The results are shown in Fig. 4. Entrapped solutes were glucose and sodium chloride in Figs. 4A and B, respectively.

Figures 4A and B show that w/o/w emulsion containing a high concentration of solute in the inner phase had large values of initially entrapped percentage and smaller decreases in the trapped percentage during storage. The emulsions in this figure were stored at 30 °C. Similar trends were observed at 5 and 40 °C.

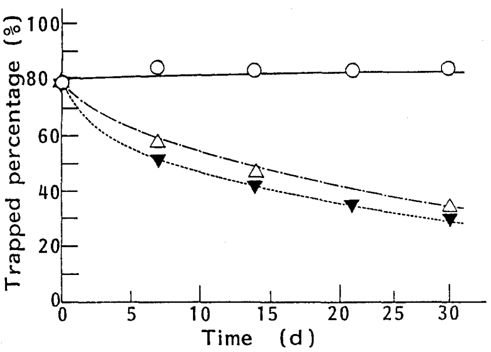

Fig. 3. Effect of Storage Temperature on the Stability of w/o/w Emulsion

Inner aqueous phase: 0.03 mol/l glucose. Outer aqueous phase: water. Storage temperature: ○, 5; △, 30; ▼, 40 °C.

c) Effect of Solutes in the Outer Aqueous Phase on Encapsulation Efficiency of w/o/w Emulsion w/o/w emulsions were prepared with 0.05 mol/l sodium chloride and with various concentrations of glucose in the inner and outer aqueous phases, respectively, and the changes in percentage of sodium chloride entrapped in the inner aqueous phase during storage were investigated. The results are shown in Fig. 5.

w/o/w emulsions with a high concentration of glucose in the outer phase had low encapsulation efficiency, and the decrease in trapped percentage during storage was large. The emulsions stored at 5 and 40 °C showed similar tendencies.

It became clear that w/o/w emulsion prepared with solutes at high and low concentrations in the inner and outer aqueous phases, respectively, *i.e.*, emulsion with a hypertonic inner phase, had a high encapsulation efficiency and was stable during storage.

Phase Separation of the System during Storage During storage of w/o/w emulsion in a glass cylinder, the transparent aqueous phase separated from the system. The separated percentages are shown in Fig. 6.

It was found that w/o/w emulsion prepared with a high concentration of solute in the inner phase and stored at a low temperature was stable with regard to phase separation as well as showing high entrapping efficiency.

Changes in Viscosity during Storage Figure 7 shows the changes in viscosity of w/o/w emulsions prepared with various concentrations of glucose in the inner phase during storage at 30 °C.

Table I. Effect of Solute Concentration in Inner Aqueous Phase on Percentage of Trapped Solute, Diameter of w/o/w Emulsion Droplets and Oil Membrane Thickness of w/o/w Emulsion

Inner aqueous phase	Outer aqueous phase	Percentage of entrapped solute (%)		Diameter of w/o/w emulsion droplet (μm)	Effective membrane thickness (h) (μm)	Effective total surface area (S) ($\times 10^{11}$ μm^2)	S/h ($\times 10^{11}$ μm)
		Immediately prepared emulsion	Five times diluted emulsion				
0.01 mol/l Sodium chloride	Water	78.2	a)	3.27	a)	a)	a)
0.03 mol/l Sodium chloride	Water	82.0	12.3	3.28	0.731	1.73	2.36
0.05 mol/l Sodium chloride	Water	83.6	16.0	4.03	0.816	1.53	1.88
0.075 mol/l Sodium chloride	Water	83.7	18.7	4.38	0.834	1.49	1.79

a) Percentage of entrapped solute in diluted w/o/w emulsion could not be detected.

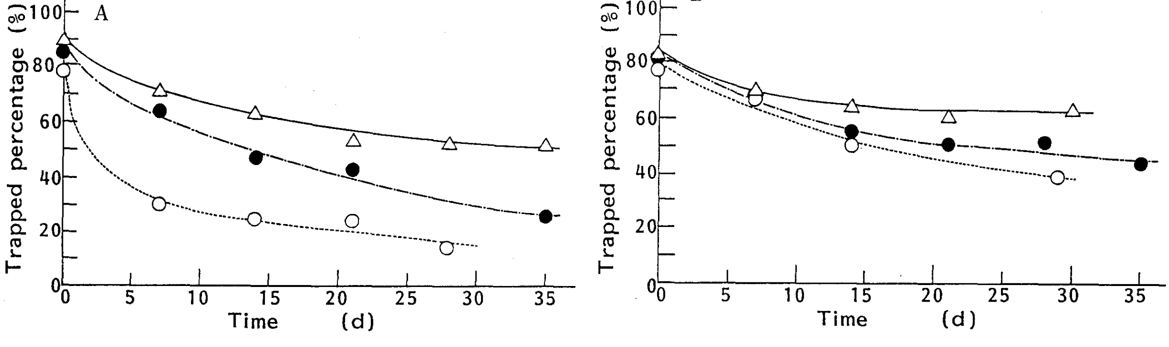

Fig. 4. Effect of Solute Concentration in Inner Aqueous Phase on the Stability of w/o/w Emulsion

Inner aqueous phase: A: ○, 0.01; ●, 0.05; △, 0.10 mol/l glucose. B: ○, 0.01; ●, 0.05; △, 0.10 mol/l sodium chloride. Outer aqueous phase: water. Stored at 30 °C.

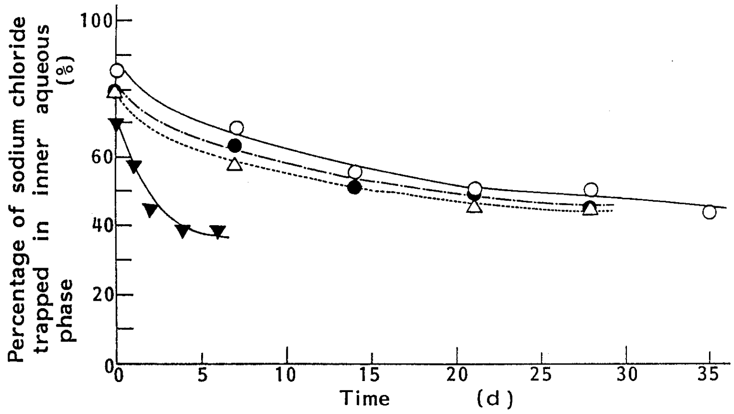

Fig. 5. Effect of Solute Concentration in Outer Aqueous Phase on the Stability of w/o/w Emulsion

Inner aqueous phase: 0.05 mol/l sodium chloride. Outer aqueous phase: ○, water; ●, 0.05; △, 0.10; ▼, 0.15 mol/l glucose. Stored at 30 °C.

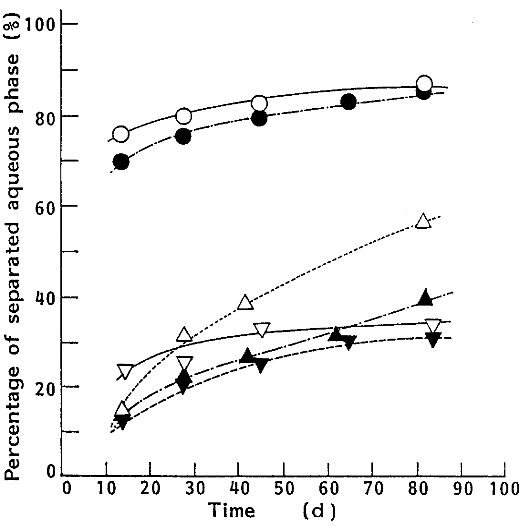

Fig. 6. Effect of Solute in Inner Aqueous Phase on the Separation of Aqueous Phase of w/o/w Emulsion

Inner aqueous phase: ○, ●, water; △, ▲, 0.03; ▽, ▼, 0.10 mol/l sodium chloride. Outer aqueous phase: water. Storage temperature: solid symbols, 25; open symbols, 35 °C.

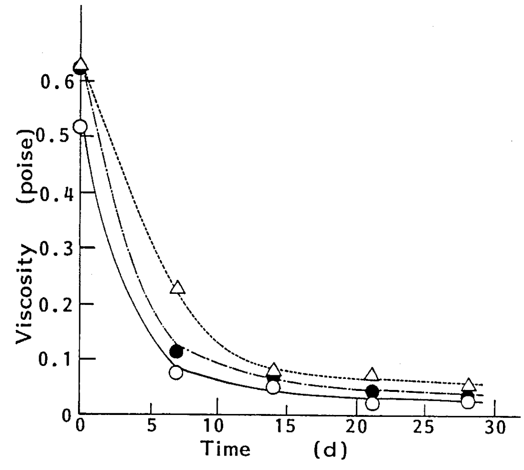

Fig. 7. Change in Viscosity of w/o/w Emulsion during Storage

Inner aqueous phase: ○, 0.03; ●, 0.075; △, 0.10 mol/l glucose. Outer aqueous phase: water. Stored at 30 °C.

It was found that the viscosity of the system decreased rapidly during storage. w/o/w emulsion containing a high concentration of solute in the inner phase initially had high viscosity.

Discussion

Morphological Change in w/o/w Emulsion with Dilution

During the dilution process, a multiple core w/o/w emulsion might be disintegrated into several or more single aqueous core w/o/w emulsion droplets.

During the dilution, the hydrophilic surfactant, Tween 20, in the diluting medium might adsorb preferentially at the interface between oil droplet and outer aqueous medium and thus reduce the interfacial tension. Therefore, the adsorbed surfactant could produce a new interface between the oil droplet and the outer aqueous medium, resulting in the disintegration of multiple core w/o/w emulsion droplets through a diluting process.

Droplet Size and Effective Membrane Thickness of w/o/w Emulsion and the Aqueous Droplet Size of Primary Emulsified w/o Emulsion Diluted w/o/w emulsion (=single core emulsion) droplet diameter and oil membrane thickness increased with an increase in the concentration of solute in the inner phase or with a decrease in the concentration in the outer phase as shown in Table I. This finding suggested that the diameter and the oil membrane thickness of the undiluted original emulsion with a hypertonic inner aqueous phase increased. The value of S/h for the diluted emulsion droplet in Table I can describe approximately that of original undiluted emulsion.

Figure 8 shows the aqueous droplet size of primary emulsified w/o emulsion.

Inner aqueous (Fig. 8) and diluted w/o/w emulsion (Table I) droplet sizes increased with an increase in the concentration of solute in the inner phase. Accordingly, the increase in diluted w/o/w emulsion droplet size with an increase in the concentration of solute in the inner phase was caused not only by permeation of water due to the osmotic gradient but also by an increase in the primary w/o emulsion droplet diameter.

It should be noted that the aqueous droplets in Fig. 8 are larger than the diluted w/o/w emulsion droplets in Table I. This discrepancy was caused by the difference of agitating speed between the first and second emulsification stages. The agitating speed at the second emulsification stage (630 rpm) was higher than that at the first stage (390 rpm), and primary w/o emulsion droplets were sheared and finely emulsified during the second emulsification procedure.

Stabilization of w/o/w emulsion with high concentrations of solute in the inner phase could be considered to be caused by thickening of the oil membrane as follows. Inner aqueous droplet diameters of the w/o/w emulsion containing high concentrations of solute in the inner phase increased initially during the preparation procedure. Accordingly, the total surface area decreased and the oil membrane became thicker owing to the decrease in specific surface area of the inner aqueous droplets. The w/o/w emulsion was stabilized by an increase in the sizes of inner aqueous droplets as a result of the use of a hypertonic inner aqueous phase, whether the decrease in encapsulation efficiency of w/o/w emulsion during storage was due to the destruction of the oil membrane or to the transport of solutes through the membrane.

Mechanism of Destruction of w/o/w Emulsion during Storage Several mechanisms of destruction of w/o/w emulsions have been considered, as described by Florence and Whitehill,[16] *i.e.*, coalescence of inner aqueous droplets in a w/o/w emulsion droplet, expulsion of the inner phase from a w/o/w emulsion droplet, coalescence of w/o/w emulsion droplets and so on.

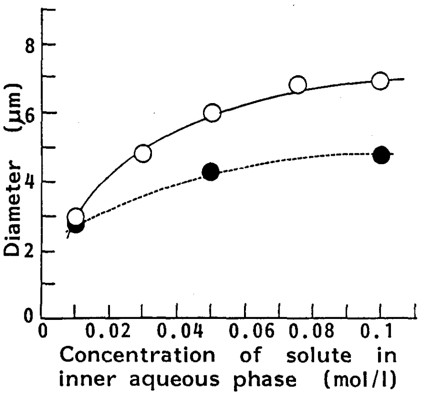

Fig. 8. Effect of Concentration of Solute in Inner Aqueous Phase on the Aqueous Droplet Size of w/o Emulsion Prepared at the First Emulsification Stage

Solute: ○, sodium chloride; ●, glucose.

We therefore investigated which process was dominant in causing the decrease in percentage of trapped solutes during storage, expulsion of the inner phase from w/o/w emulsion droplets by destruction of the oil membrane, or transport of water and solute molecules through the oil membrane owing to the osmotic and diffusional transports, respectively.

In the present study, the percentage of marker trapped in w/o/w emulsion was calculated by using Eq. 3. The permeations of water and marker through the oil membrane were neglected, although it was reported that water[17,18] as well as markers initially added to the inner aqueous phase[19,20] could migrate through the oil membrane. Such migration of the marker, if it occurs to a significant degree, will result in underestimation of the percentage of trapped marker with Eq. 3. If the migration of solutes through the oil membrane contributes markedly to the decrease in percentage of solute trapped in w/o/w emulsion during storage, the difference between observed and calculated percentages of trapped solutes will increase when S/h in Table I is large, *i.e.*, the concentration of solute in the inner phase is low. Therefore, the mechanism of decrease in trapped solute during storage was investigated by evaluating the validity of Eq. 3.

Mooney[21] represented the viscosity of an emulsion, η, as follows,

$$\eta=\eta_0\exp\{a\Phi/(1-k\Phi)\} \qquad (9)$$

where η_0 is the viscosity of the dispersing medium; Φ is the volume fraction of the dispersed phase; a and k are constants.

In the present study, Φ is represented as follows,

$$\Phi=(\alpha V_1/100+V_0)/(V_1+V_2+V_0) \qquad (10)$$

Therefore, the following relationship is obtained,

$$1/\ln(\eta/\eta_0)=1/a\Phi-k/a \qquad (11)$$

Figure 9 shows the relationship between $1/\Phi$ and $1/\ln(\eta/\eta_0)$ of w/o/w emulsions, immediately prepared and

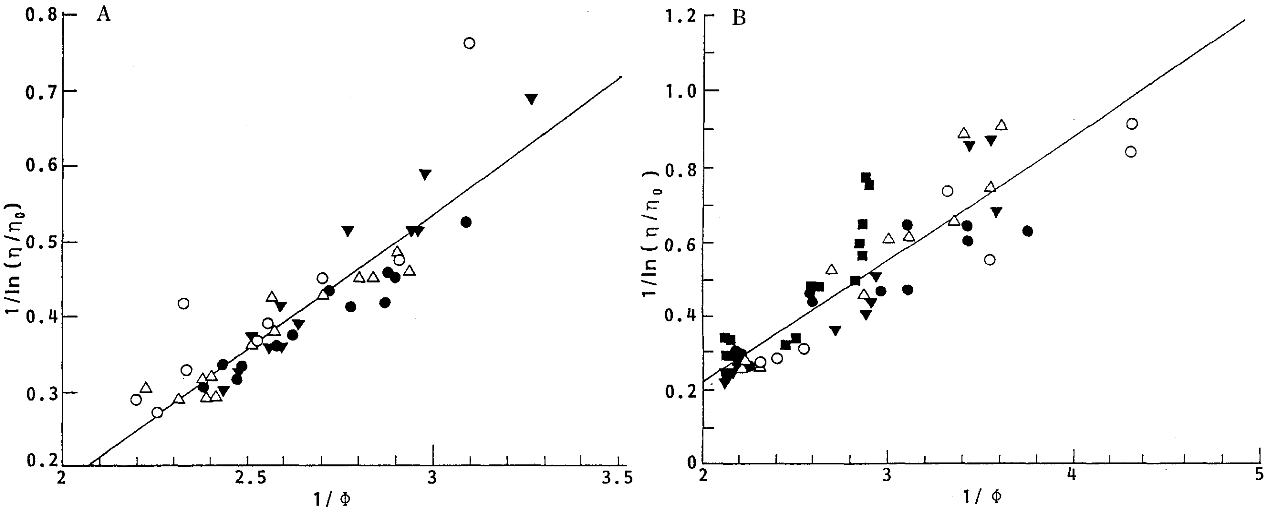

Fig. 9. Relationship between Volume Fraction of Dispersed Phase and Viscosity of w/o/w Emulsion

Inner aqueous phase: A: ○, 0.01; △, 0.03; ●, 0.05; ▼, 0.075 mol/l sodium chloride. B: ○, 0.01; △, 0.03; ●, 0.05; ▼, 0.075; ■, 0.10 mol/l glucose. Outer aqueous phase: water. Parameters of Eq. 10 calculated from regression lines: a and k of A are 2.77 and 1.51, respectively and those of B are 3.00 and 1.33, respectively. Correlation coefficient: A, 0.900 ($n=48$); B, 0.902 ($n=57$).

stored at 5, 30 and 40 °C. The inner phases of the emulsions contained various concentrations of sodium chloride and glucose in Figs. 9A and B, respectively.

If the migration of solutes through the oil membrane significantly contributes to a decrease in trapped percentage during storage, calculation of α with Eq. 3 will become imprecise. Therefore, in such an emulsion, changes in Φ during storage will be significantly dependent on S/h, and parameter a in Eq. 11 will change.

In both of Figs. 9A and B, a good linear relationship was observed with a correlation coefficient of more than 0.9. The values of parameter a calculated from the lines were 2.77 and 3.00 in Figs. 9A and B, respectively, which are close to Mooney's ideal value, 2.5. Independence of the parameter on the concentration of solutes, *i.e.*, S/h was found. Therefore, the estimation of trapped percentage with Eq. 3 was valid, and it was concluded that the contribution of the migration of solutes through the oil membrane to the decrease in the trapped percentage during storage was small. Consequently, the decrease in percentage of trapped solutes during storage was primarily due to the expulsion of the inner phase as a result of thermal motion or destruction of the oil membrane.

Kinetic Study of Breakdown of w/o/w Emulsion The amount of marker trapped in the oil droplet was plotted *versus* storage time on semi-logarithmic coordinates, since its decrease was determined by a rupture of the oil membrane of the droplet. The results are shown in Fig. 10.

Linear relationships were obtained for various inner aqueous phases at the initial stage of storage. The good accordance of the plots with first-order kinetics proved that the decrease in percentage of marker entrapped in the inner phase was caused predominantly by expulsion of the inner aqueous phase, presumably as a result of thermal motion and destruction of the oil membrane.

In each case, the data deviated somewhat from the regression line at the later stage, suggesting that the reduction of the decreasing rate of trapped solute might be caused by a thickening of the oil membrane, because oil molecules after expulsion of the inner aqueous droplets remained in the membrane of the w/o/w emulsion droplets.

Figure 11 shows the Arrhenius plots of the first-order kinetic rate constants and Table II shows the activation energies calculated from Fig. 11.

Activation energies ranged from 4.4 to 29.8 kcal/mol, which increased approximately with a decrease in S/h. This finding indicates that the dependence of the destruction rate on storage temperature was so large that the decrease in entrapping efficiency during storage could not have been caused by the transport of the marker through the oil membrane. This supports the view that the breakdown of the oil membrane should be the main cause of the decrease in the trapped percentage during storage.

The good accordance of breakdown of w/o/w emulsion

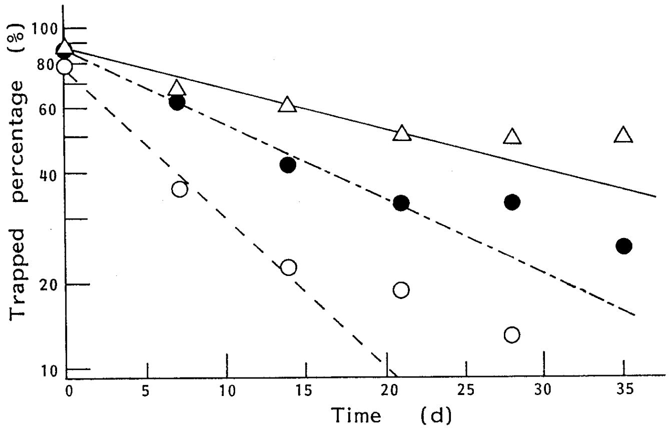

Fig. 10. Decrease in Percentage of Solute Trapped in w/o/w Emulsion during Storage

Lines are the least-squares regression lines corresponding to first-order kinetics. Inner aqueous phase: ○, 0.01; ●, 0.05; △, 0.10 mol/l glucose. Outer aqueous phase: water. Stored at 40 °C.

Table II. Activation Energy of Breakdown of w/o/w Emulsion

Inner aqueous phase	Outer aqueous phase	Activation energy (kcal/mol)
0.01 mol/l Sodium chloride	Water	4.74
0.05 mol/l Sodium chloride	Water	4.95
0.075 mol/l Sodium chloride	Water	5.33
0.10 mol/l Sodium chloride	Water	8.18
0.01 mol/l Glucose	Water	11.91
0.05 mol/l Glucose	Water	19.07
0.075 mol/l Glucose	Water	20.84
0.10 mol/l Glucose	Water	31.28

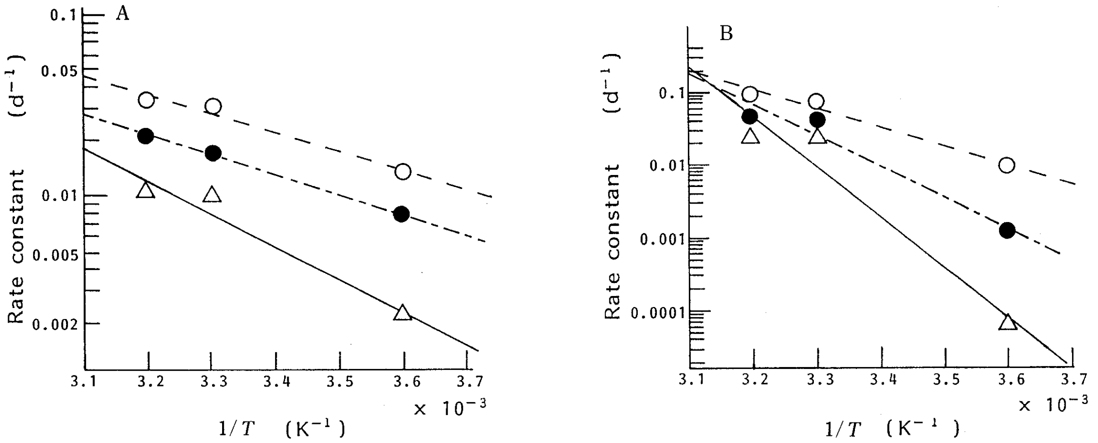

Fig. 11. Arrhenius Plots of First Order Rate Constants of the Decrease in Percentage of Solute Trapped in w/o/w Emulsion during Storage

Inner aqueous phase: A: ○, 0.01; ●, 0.05; △, 0.10 mol/l sodium chloride. B: ○, 0.01; ●, 0.05; △, 0.10 mol/l glucose. Other aqueous phase: water.

with a first-order kinetic model and the good linearity of the Arrhenius plots indicate that the stability of w/o/w emulsion at room temperature can be predicted from the result of accelerated tests at a higher temperature.

Mechanism of Stabilization of Oil Membrane with Hypertonic Inner Aqueous Phase w/o/w emulsion prepared with a high concentration of solute in the inner phase, *i.e.*, hypertonic inner aqueous phase, had large inner aqueous and w/o/w emulsion droplets and thick oil membranes. The thickening of the oil membrane was caused by the increase in the diameter of the inner aqueous droplet, because the volume of the oil phase was constant. To investigate the mechanism of increase in droplet size, interfacial tension between glucose or sodium chloride aqueous solution (0—0.1 mol/l) and the oil phase of w/o/w emulsion was measured. The determined values were 3.8—4.2 dyn/cm and no dependence of the value on the solute concentration could be detected. Therefore, the relationship between the concentration of solute in the inner phase and the droplet size could not be explained by changes in interfacial tension.

Another possible mechanism is changes in the hydrophilicity and lipophilicity balance of the surfactants and the hydration conditions of surfactants. Surfactant molecules are aligned at the oil–water interface and the hydrophilic groups of the molecules are hydrated. In the sodium chloride aqueous solution, sodium and chloride ions are hydrated with water molecules through an ion-dipole interaction. In the case of glucose, equatorial hydroxyl groups form hydrogen-bonds with water molecules and stabilize into a so-called iceberg structure. When glucose or sodium chloride is added to the emulsion system, these molecules or ions may deplete the hydration shell around the polar headgroups of surfactant molecules. Therefore, the lipophilicity of the surfactant would be increased. Accordingly, the curvature of the interface between oil and inner aqueous phases would decrease and the interaction of surfactant molecules would increase due to a hydrophobic interaction to form a relatively rigid surfactant molecule layer at the interface between the inner aqueous and oil phases as result of the decrease in effective bulkiness of the polar headgroups of the surfactant molecules. More activation energy would be required to destruct the oil membrane with such rigid surfactant layer, as indicated in Table II.

This speculation, that the hydrophilicity of a surfactant decreases with the addition of a water-soluble compound to the aqueous phase, is consistent with Shinoda's results,[22] showing that the phase inversion temperature of emulsion decreased with an increase in the concentration of salt in the aqueous phase. Consequently, the number of surfactant molecules aligned at the interface will increase and the inner aqueous and w/o/w emulsion droplets will become larger.

Conclusions

Inner aqueous and w/o/w emulsion droplets of w/o/w emulsion became larger with an increase in the concentration of solutes in the inner aqueous phase. This caused the oil membranes to become thicker. A decrease in entrapping capacity during storage is considered to be caused by the expulsion of the inner aqueous phase through a broken oil membrane. Since w/o/w emulsion containing a high concentration of solute in the inner phase had a thick oil membrane, leakage of the solute was delayed. Such an emulsion has high encapsulation efficiency and high viscosity. Consequently, the flocculation of droplets was inhibited and phase separation was delayed. Our results suggest that the addition of a water-soluble compound which does not interact with drugs to be encapsulated in the inner aqueous phase would improve the encapsulation efficiency and the stability of drug-carrying w/o/w emulsions.

Acknowledgment We thank Mr. Toshiaki Suzuki (JEOL Ltd., Tokyo, Japan) for taking the electron micrographs of the w/o/w emulsions. The experimental assistance of Messrs Tsutomu Nishide and Koji Suetake are sincerely acknowledged.

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