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Preparation of and Drug Release from W/O/W Type Double Emulsions Containing Anticancer Agents

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Water-in-oil-in-water (W/O/W) type double emulsions were prepared by two-step emulsification procedures. The structures of W/O/W type double emulsions and the release characteristics of cytarabine and 5-fluorouracil (5-FU) from W/O/W type double emulsions were studied.

W/O/W type double emulsions were ruptured within 24 h when sorbitan monooleate (SO-10) or sorbitan sesquioleate (SO-15) was used as a lipophilic emulsifier. On the other hand, W/O/W type double emulsions prepared in a hydrogenated castor oil (HCO-10) in peanut oil were still intact after 24 h. Therefore, the release characteristics of drugs were studied in HCO-10 in peanut oil.

The release of cytarabine from W/O/W type double emulsions was very prolonged. The release of cytarabine was enhanced with increase in the volume of inner aqueous phase or increase in the concentration of HCO-10. The concentration of cytarabine and the concentration of a hydrophilic emulsifier (HCO-60), however, had little effect on the release of cytarabine. Whereas the release of 5-FU from W/O/W type double emulsions was as fast as from a simple solution, the release was very prolonged when the pH value of the inner aqueous phase was adjusted to 10.0.

These results show the utility of W/O/W type double emulsions for sustained release preparations and raise the possibility of control of drug release from W/O/W type double emulsions.

Keywords——W/O/W type double emulsion; multiple emulsion; prolonged release; cytarabine; 5-fluorouracil; hydrogenated castor oil; physical stability

Water-in-oil-in-water (W/O/W) type double emulsions were observed as long ago as 1925.[1] They have been prepared recently in high yield by two-step emulsification procedures by Matsumoto *et al.*[2] Properties of W/O/W type double emulsions have subsequently been investigated.

The use of W/O/W type double emulsions in medicine has also been investigated to prolong drug release,[3] to immobilize enzymes,[4] to treat overdose,[5] and to obtain better immunologic adjuvants.[6] Moreover, W/O/W type double emulsions have been examined as a formulation for treatment of cancer. Hashida *et al.*[7,8] stabilized a W/O type emulsion and a W/O/W type double emulsion by gelling the inner aqueous phase and demonstrated the utility of a sphere-in-oil-in-water (S/O/W) type double emulsion as a drug carrier to lymphatics. Mimaki *et al.*[9] administered a W/O/W type double emulsion containing adriamycin to mice bearing Ehrlich solid tumor and observed an increase in survival time. In these applications, the stability of and release properties of drugs from W/O/W type double emulsions are very important.

In this work, we tried to prepare stable W/O/W type double emulsions by examining suitable combinations of oils and surfactants, and we examined the release patterns of anticancer agents.

Experimental

Materials——Sesame oil and peanut oil were purchased from Nakarai Chemicals Co., Kyoto. Nonionic surfactants, sorbitan monooleate (SO-10), sorbitan sesquioleate (SO-15) and hydrogenated castor oils (HCO-10 and HCO-60) were generously supplied by Nikko Chemicals Co., Tokyo. 5-Fluorouracil (5-FU) and cytarabine were gifts from Kyowa Hakko Kogyo Co., Tokyo, and Nippon Shinyaku Co., Kyoto, respectively. Water was distilled and treated with a Milli-Q reagent-grade water system (Millipore Corp., Massachusetts). All other chemicals were of reagent grade.

Preparation of W/O/W Type Double Emulsions——An improved version of the two-step emulsification procedure was employed (Chart 1). An aqueous solution of a drug (an inner aqueous phase) was introduced into an

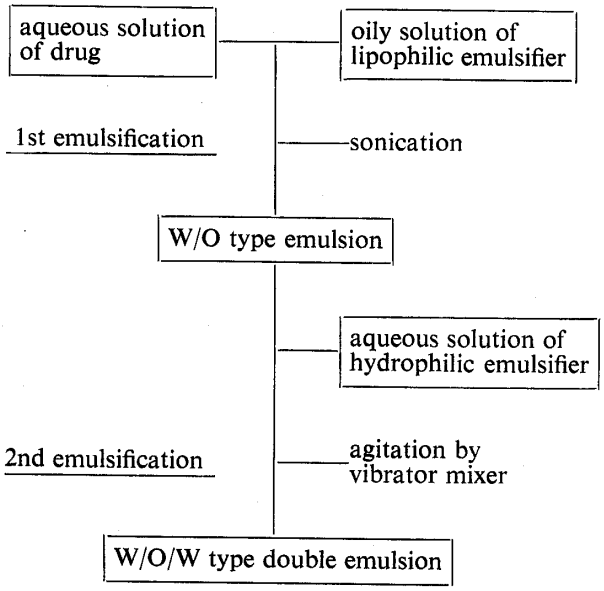

Chart 1. Preparation of W/O/W Type Double Emulsion by a Two-Step Procedure of Emulsification

oily solution of a lipophilic emulsifier and emulsified with a sonicator (UR-20P, Tomy Seiko Co., Tokyo) in an ice-water bath to prepare a W/O type emulsion (1st emulsification). An aqueous solution of a hydrophilic emulsifier (an outer aqueous phase) was then introduced into the W/O type emulsion and the whole was agitated with a vibrator-mixer (MM-2, Kayagaki Rika Co., Tokyo) to prepare W/O/W type double emulsion (2nd emulsification). When not otherwise stated, experiments were carried out under the following conditions; the volume of the inner aqueous phase incorporating approximately 1.0% (w/v) drug was 0.4 ml, the weight of oily phase incorporating HCO-10 at various concentrations was 2 g, and the volume of the outer aqueous phase incorporating 0.5% (w/v) HCO-60 was 5 ml.

Examination of Physical Stability of W/O Type Emulsions Prepared at 1st Emulsification——Freshly prepared W/O type emulsions were introduced into glass cylinders and kept at 4 or 25 °C. The occurrence of phase separation was examined.

Microscopic Observation——Prepared emulsions were observed with an optical microscope (BH2, Olympus

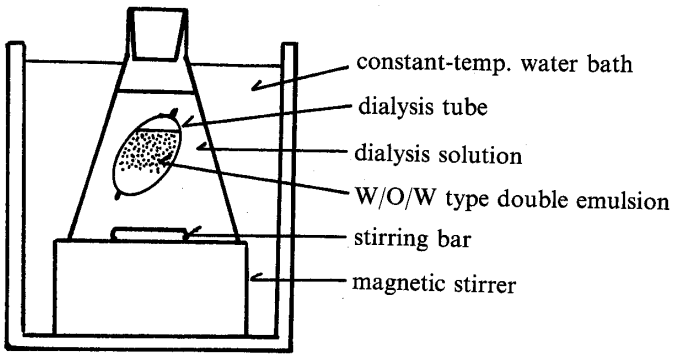

Fig. 1. Apparatus for Measurement of the Amount of Drug Released

Kogaku Co., Tokyo) and photomicrographs were taken.

Release Study——Release patterns of drugs from W/O/W type double emulsions were investigated by a dialysis method employing cellulose tubing (Spectrapore 2, Spectrum Medical Industries Inc., Los Angeles). The W/O/W type double emulsions were introduced into the dialysis tube with outer aqueous phase and dialyzed in a volume of 200 ml at 37.0 °C (Fig. 1); cytarabine in water and 5-FU in isotonic phosphate buffer solution, pH 7.4. The dialysis solution was vigorously agitated by a magnetic stirring bar to move the dialysis tube in the dialysis solution. At appropriate intervals, 5 ml of the dialysis solution was withdrawn and 5 ml of a fresh dialysis solution was added to maintain the original volume. The drug concentrations were analyzed spectrophotometrically (UV 240 spectrophotometer, Shimadzu Seisakusho Co., Kyoto); cytarabine at 271.0 nm and 5-FU at 266.0 nm. Percentages of drug released were calculated from Eq. (1).

$$\text{percent of drug released} = \frac{C_n + \dfrac{V_s \times (C_1 + C_2 + \cdots + C_{n-1})}{V_i + V_o + V_d}}{\dfrac{V_i \times C_i}{V_i + V_o + V_d}} \times 100 \qquad (1)$$

where

C_n; drug concentration at n-th sampling (mg/ml)
V_i; inner aqueous phase volume (ml)
V_o; outer aqueous phase volume (ml)
V_d; dialysis solution volume (ml)
C_i; initial drug concentration of inner aqueous phase (mg/ml)
V_s; sampling volume (ml)

Results and Discussion

Physical Stability of W/O Type Emulsions

The physical stability of four systems was examined at 4 and 25 °C: 30% SO-10 in sesame oil, 30% SO-10 in peanut oil, 10% HCO-10 in sesame oil and 10% HCO-10 in peanut oil. When sesame oil was employed, phase separation started immediately and almost all the water separated after 6 h at both 4 and 25 °C. When peanut oil was employed, on the other hand, phase separation did not occur for a long time at 4 °C, possibly because peanut oil solidified at 4 °C. Sesame oil did not solidify at 4 °C. Since the contents of saturated fatty acids in peanut oil are greater than that in sesame oil,[10] peanut oil solidifies at a higher temperature than sesame oil. Moreover, water was not separated in the HCO-10 in peanut oil system for over six months at 4 °C because HCO-10 also solidified at 4 °C. At 25 °C, however, phase separation started immediately in the systems of HCO-10 in peanut oil and SO-10 in peanut oil. These results suggest that HCO-10 and peanut oil are suitable materials for a preparation to be stored under refrigeration.

Structure of W/O/W Type Double Emulsions

The structure of a W/O/W type double emulsion freshly prepared with SO-10 in peanut oil is shown in Fig. 2A. Although the W/O/W type double emulsion had some inner aqueous droplets immediately after preparation, coalescence among inner aqueous droplets soon started so that the inner aqueous droplets formed one larger droplet as shown in Fig. 2A. Figure 2B shows the structure of a ruptured W/O/W type double emulsion after a 24 h incubation in the apparatus shown in Fig. 1. It may be considered that in this system the W/O/W type double emulsion is unstable so that coalescence between the inner aqueous droplets and the outer aqueous phase occurred and no inner aqueous droplet remained within the oily phase. Figure 3 illustrates the process of rupture of a W/O/W type double emulsion in this system.

The other three systems, SO-10 in sesame oil, SO-15 in peanut oil and SO-15 in sesame oil, were similar to SO-10 in peanut oil in rupture behaviour.

Figure 4A shows the structure of the W/O/W type double emulsion freshly prepared with

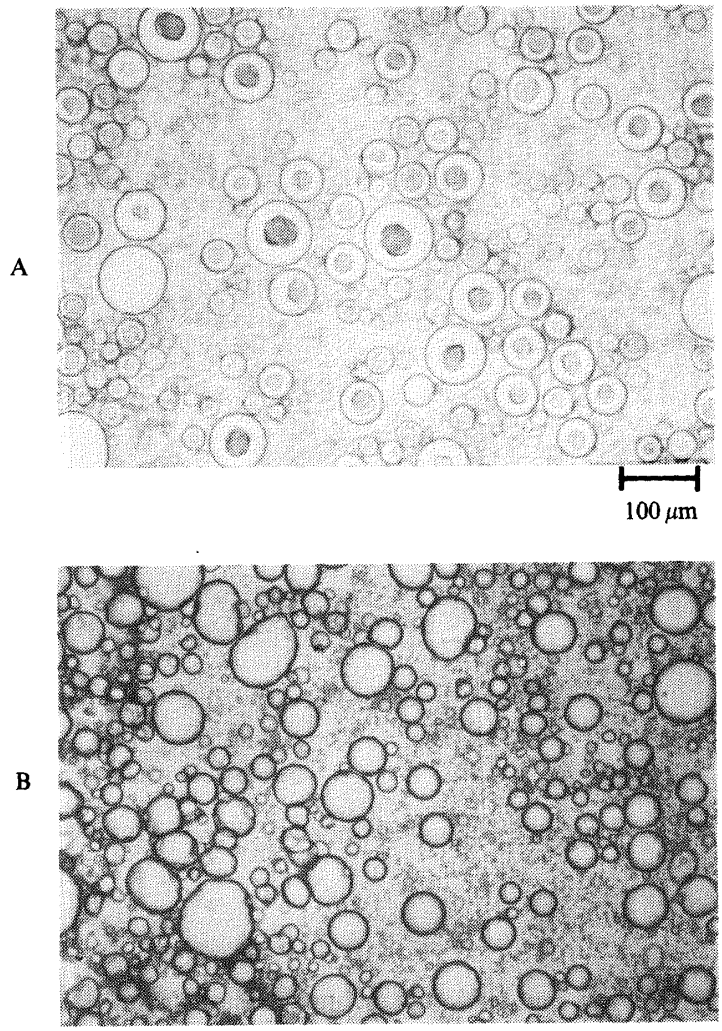

Fig. 2. Photomicrographs of W/O/W Type Double Emulsion of SO-10 in Peanut Oil Immediately after Preparation (A) and Ruptured Emulsion 24 h after Preparation (B)

HCO-10 in peanut oil. Coalescence among inner aqueous droplets was so slow that many small droplets remained observable as an inner aqueous phase. Figure 4B shows the structure of W/O/W type double emulsion after a 24 h incubation in the apparatus shown in Fig. 1. Most of the oil droplets have single inner aqueous droplets. Although several oil droplets do not have inner aqueous droplets, some of these oil droplets were probably produced by rupture of W/O/W type double emulsion during the process of taking the photomicrograph. From these results it is suggested that coalescence among inner aqueous droplets had occurred, but coalescence between inner aqueous droplets and outer aqueous phase had not occurred, so that each inner aqueous phase became a single large droplet after 24 h. However, coalescence between inner aqueous droplets and the outer aqueous phase occurred in the absence of agitation with a magnetic stirrer. Therefore, agitation seemed to stabilize the W/O/W type double emulsions against coalescence. Morimoto *et al.*[5] observed similar phenomena in SO-15 in liquid paraffin.

Release Patterns

Since HCO-10 in peanut oil was proved to provide a stable W/O/W type double emulsion, drug release was investigated in this system.

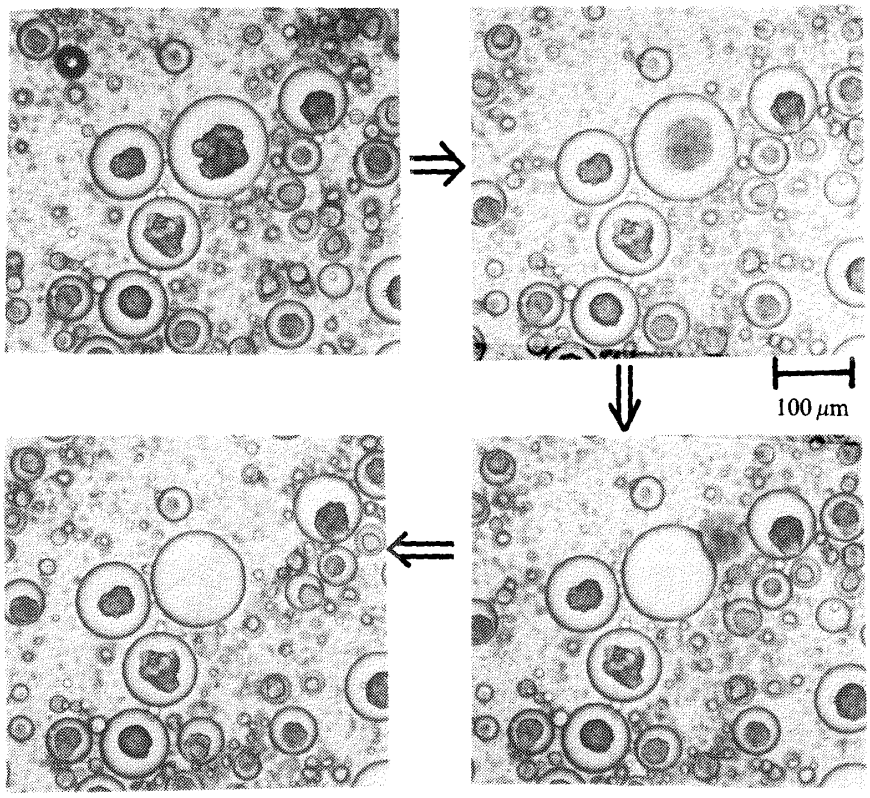

Fig. 3. Photomicrographs Illustrating Rupture of W/O/W Type Double Emulsion of SO-10 in Peanut Oil

Rupture proceeded within 5 s.

Koizumi and Higuchi[11] reported that a linear relationship was observed between the amount of drug released and the square root of time in drug release from a W/O type emulsion. Takehara *et al.*[12] also predicted a similar relationship based on a mathematical model. On the basis of these reports, Hashida *et al.*[8] examined the drug release from a W/O type emulsion and a W/O/W type double emulsion by plotting the amount of drug released against the square root of time. In this work also, the percentage of the drug released was plotted against the square root of time. Points and bars in each figure represent mean values+S.E.M. of three experiments. When the range of S.E.M. is smaller than the sizes of the symbols, only the mean values are plotted.

Release of Cytarabine—Figure 5 shows the release patterns of cytarabine from the W/O/W type double emulsion and an aqueous solution. The release of cytarabine from the W/O/W type double emulsion was slower than that from the aqueous solution. When cytarabine was contained not in the inner aqueous phase but in the outer aqueous phase, the release pattern of cytarabine was similar to that from the simple aqueous solution (this result is not shown). These results suggest that the rate-limiting step was not transport through the dialysis tube but transport through the oily phase, and that the existence of W/O/W type double emulsion did not hinder the transport of cytarabine through the dialysis tube.

The W/O/W type double emulsion showed almost linear release in the early stage (up to 4 h after the start of the experiment). Reduced release was noted after 4 h, possibly due to creaming of the oily phase in the dialysis tube and incomplete sink condition in the dialysis solution.

Figure 6 shows the effect of the volume of the inner aqueous phase on the release pattern. As the volume was increased, the release of drug was enhanced. It was noted from

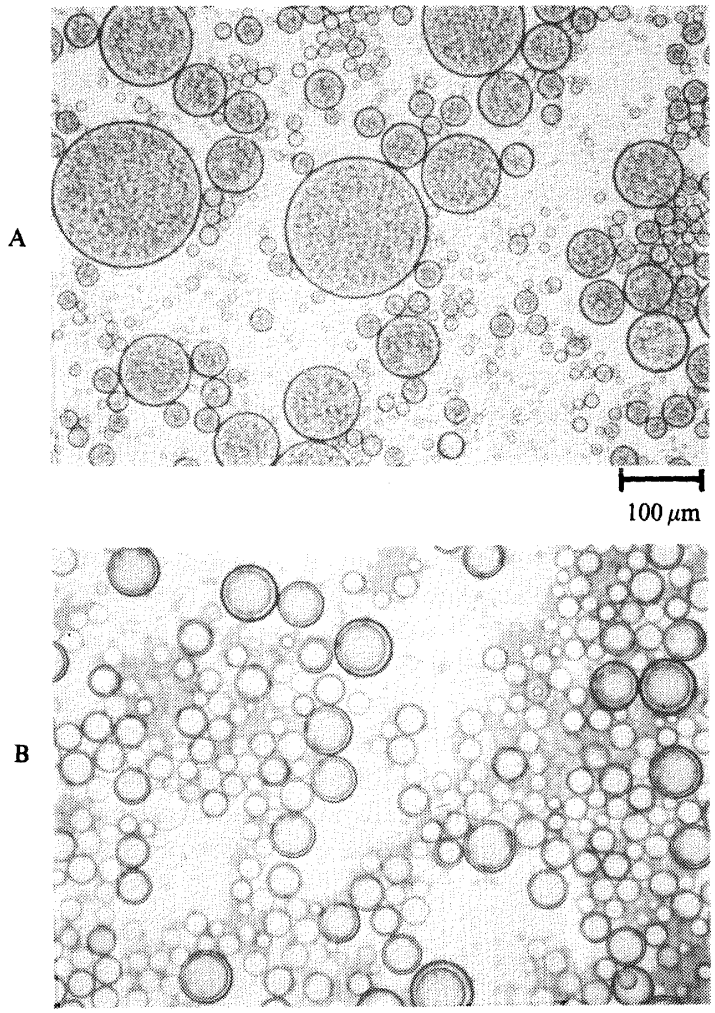

Fig. 4. Photomicrographs of W/O/W Type Double Emulsion of HCO-10 in Peanut Oil Immediately after Preparation (A) and 24 h after Preparation (B)

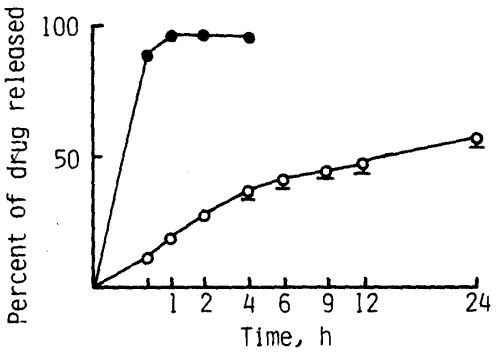

Fig. 5. Release Patterns of Cytarabine from W/O/W Type Double Emulsion (○) and Aqueous Solution (●)

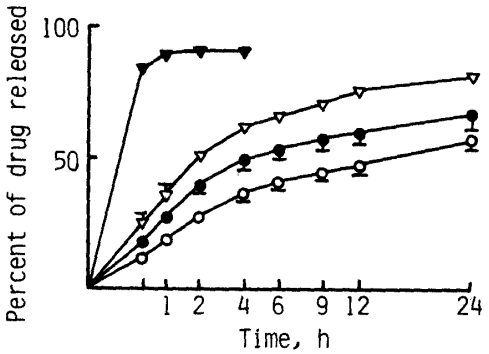

Fig. 6. Effect of Volume of the Inner Aqueous Phase on Release of Cytarabine from W/O/W Type Double Emulsion

Oily phase, 10% HCO-10 in peanut oil.
▼, 1.2 ml; ▽, 0.8 ml; ●, 0.6 ml; ○, 0.4 ml.

microscopic observations on freshly prepared W/O/W type double emulsions that coalescence between inner aqueous droplets and outer aqueous phase took place more frequently as the volume of inner aqueous phase was increased. Thus, it is suggested that the enhancement of

release may result from a decrease of the yield of W/O/W type double emulsion at the 2nd emulsification and the destabilization of the W/O/W type double emulsion. In addition, thinning of the oily phase may also be one of the factors producing the enhancement. When the volume of inner aqueous phase was 1.2 ml, the emulsion prepared at the 1st emulsification was not a W/O type emulsion but an O/W type emulsion. Therefore, a simple O/W type emulsion resulted at the 2nd emulsification. In this case, since the aqueous solution of the drug was not entrapped within the oily phase, the release of the drug was as fast as from the simple aqueous solution of the drug (shown in Fig. 5).

Figure 7 shows the effect of concentration of HCO-10 on the release pattern. As the concentration of HCO-10 was increased from 10 to 25%, the release of the drug in the early stage was increased. This enhancement may be explained by the change in particle sizes of the emulsion. Table I shows the effect of the concentration of HCO-10 on the particle sizes of the inner aqueous droplets which were measured in W/O type emulsions prepared at the 1st emulsification and oil droplets which contain the inner aqueous droplets. As the concentration of HCO-10 was increased, the sizes of the inner aqueous droplets decreased. Takehara *et al.*[12] observed that the release of drug from W/O type emulsion became faster as the droplet sizes of the aqueous phase became smaller. The release of drugs from W/O/W type double emulsions can be regarded as essentially similar to that from W/O type emulsions. Therefore, the decrease in size of the inner aqueous droplets resulted in an increase in the release rate of drugs from W/O/W type double emulsion. Further, the decrease in size of oil droplets containing the inner aqueous droplets by increasing the concentration of HCO-10 leads to an increase in the total surface area for drug release from oil droplets. This increase in the surface area for drug release resulted in an increase in the release rate of drugs. In addition, the increase in drug solubility in the oily phase with increasing concentration of

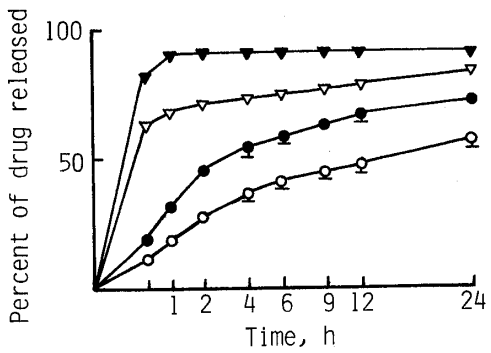

Fig. 7. Effect of Concentration of HCO-10 in the Oily Phase (Peanut Oil) on Release of Cytarabine from W/O/W Type Double Emulsion

▼, 25%; ▽, 20%; ●, 15%; ○, 10%.

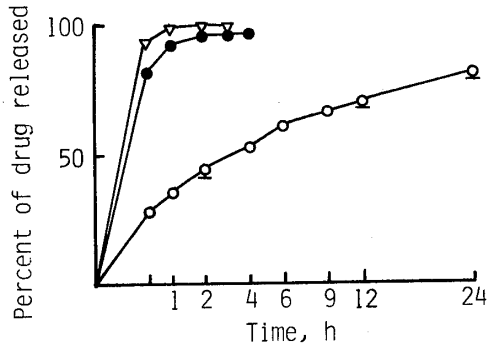

Fig. 8. Release Patterns of 5-FU from W/O/W Type Double Emulsions and Aqueous Solution

▽, aqueous solution; ●, double emulsion (inner aqueous phase, water); ○, double emulsion (inner aqueous phase, pH 10.0 carbonate buffer).

TABLE I. Size of Emulsions (μm)

Phase	Concentrations of HCO-10, %			
	10	15	20	25
Inner aqueous droplets	$11.6 \pm 0.6^{a)}$	$8.7 \pm 0.5^{a)}$	<5.0	Not visible
Oily droplets containing aqueous droplets	$55.3 \pm 3.1^{b)}$	$45.0 \pm 2.2^{b)}$	$9.0 \pm 0.7^{a)}$	<7.0

Results are expressed as the mean ± S.E.M. The numbers of droplets measured were 50 *a)* and 100 *b)*.

HCO-10 might also enhance the release rate of drugs. On the other hand, it is also suggested that with an increase in concentration of HCO-10, coalescence between inner aqueous droplets and outer aqueous phase becomes faster, so that the yield of W/O/W type double emulsion at the 2nd emulsification was decreased and/or W/O/W type double emulsion ruptured at an earlier stage, resulting in enhancement of the release rate at the early stage, especially under the conditions of 20% HCO-10 and 25% HCO-10. Further experiments are required to examine this enhancement.

Matsumoto *et al.*[2] showed that the yield of W/O/W type double emulsion decreased when the concentration of hydrophilic surfactants in the outer aqueous phase was increased. If the yield of W/O/W type double emulsions had ever decreased in the present study, the release rate of drug would have increased in the early stage. On the other hand, examination of the effect of concentration of HCO-60 in the outer aqueous phase (from 0 to 0.7%) on the release patterns of cytarabine indicated little effect. Therefore, it is suggested that the yield of W/O/W type double emulsions is not affected by the concentration of HCO-60 in the outer aqueous phase.

Examination of the effect of concentration of cytarabine in the inner aqueous phase (from 1 to 3%) on the release patterns showed that release patterns were not much affected by change in drug concentrations in spite of the expectation that osmotic pressure would increase as the concentration of cytarabine was increased. This result may indicate that the release rate of cytarabine can be easily controlled by adjusting the concentration of cytarabine initially introduced into the inner aqueous phase.

Release of 5-FU——Figure 8 shows the release patterns of 5-FU from an aqueous solution and W/O/W type double emulsions. When the inner aqueous phase was distilled water, the release of the drug from the W/O/W type double emulsion was as fast as from an aqueous solution. In this case, the W/O/W type double emulsion remained intact at 24 h. It is therefore considered that the permeability of 5-FU through the oily phase is very large. Hashida *et al.*[8] showed sustained release of 5-FU from a W/O/W type double emulsion. The difference in the release rates of 5-FU between the present experiment and Hashida's may be due to the difference in materials used.

When the inner aqueous phase was carbonate buffer at pH 10.0, on the other hand, the release of 5-FU from the W/O/W type double emulsion was sustained; only about 65% of the drug was released in 24 h. This result may be interpreted as follows. 5-FU was ionized at pH 10.0, because the pK_a of 5-FU is 8.0; thus, at pH 10.0, decreased partition of 5-FU from the inner aqueous phase to the oily phase resulted in the sustained release of 5-FU.

General Discussion

In the present study, we established a quick preparative method for W/O/W type double emulsions by improving Matsumoto's method,[2] and we also observed changes in emulsion structure with time and examined the release patterns of cytarabine and 5-FU. The stability of W/O/W type double emulsions prepared in the present study is still not sufficient, because even in the most stable system, HCO-10 in peanut oil, the W/O/W type double emulsion was ruptured in the absence of agitation.

The release of cytarabine from W/O/W type double emulsions was sustained, and was greatly affected by the concentration of HCO-10, a lypophilic emulsifier, and the volume of the inner aqueous phase, but was little affected by the concentrations of cytarabine and HCO-60, a hydrophilic surfactant. The release of 5-FU from W/O/W type double emulsions was prolonged when the drug was ionized by the employment of alkaline solution as the inner aqueous phase. These results suggest not only the utility of W/O/W type double emulsions for sustained release dosage forms but also the possibility of the control of drug release from

W/O/W type double emulsions.

Further studies on the preparation of more stable W/O/W type double emulsions and the release of other drugs, as well as application to *in vivo* systems, are now being undertaken.

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