CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 28, No. 4 April 1980

Regular Articles

[Chem. Pharm. Bull. 28(4)1009—1015(1980)]

Dosage Form Characteristics of Microsphere-in-oil Emulsions. I: Stability and Drug Release

Mitsuru Hashida, Toshio Yoshioka, Shozo Muranishi, and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University[1)]

(Received August 6, 1979)

The characteristics of microsphere-in-oil (S/O) emulsion relating to the stability and drug release properties were investigated in comparison with those of water-in-oil (W/O) emulsion. The mean globule sizes of the S/O emulsion and W/O emulsion were 1.6 μm and 1.9 μm, respectively. Visual observation of phase separation revealed that the S/O emulsion was more stable than the W/O emulsion, in good agreement with the results of microscopic observation of the globule coalescence. The S/O emulsion remained stable even after storage in a freezer for one month, whereas the W/O emulsion was completely destroyed by freezing. Concerning the rheological properties, both emulsions showed non-Newtonian plastic flow, but the S/O emulsion had a larger viscosity. Examination of the drug release characteristics showed that the S/O emulsion offered stable incorporation of a drug into the innermost aqueous phase even after redispersion into gelatin solution, in accord with the results of microscopic observation. These results suggest the superiority of the S/O emulsion as a drug delivery system. The reason for this is discussed in relation to several criteria having to do with physical properties.

Keywords——microsphere-in-oil emulsion; water-in-oil emulsion; drug delivery system; particle size; stability; rheological properties; drug release properties; multiple emulsion; microscopic observation

In pharmaceutical practice, various types of emulsion have been used as dosage forms for administering drugs.[2)] Today, however, emulsions are little used and are of only minor importance because of the inherent problem of stability. Therefore, the stabilization of thermodynamically unstable emulsified systems presents a challenging problem to pharmacists, since their utilization in drug delivery systems may offer novel and useful properties.

In our series of investigations on the utility of various types of emulsions as drug delivery systems, we have demonstrated an increased transport of anticancer agents to lymphatics by a water-in-oil(W/O) emulsion.[3)] Further, the best enhancement of drug delivery and successful prevention of lymphatic metastasis were obtained by employing a microsphere-in-oil(S/O) emulsion in which the W/O emulsion was improved through a replacement of its inner water

1) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*

2) P. Becher, "Emulsions: Theory and Practice," Reinhold Publishing Inc., New York, 1957; S.S. Davis, *J. Clin. Pharm.*, **1**, 11 (1976).

3) *a*) Y. Nakamoto, M. Hashida, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.*, **23**, 3125 (1975); *b*) M. Hashida, M. Egawa, S. Muranishi, and H. Sezaki, *J. Pharmacokin. Biopharm.*, **5**, 225 (1977).

droplets by gelled gelatin microspheres.[3b,4] Detailed examination of the delivery process of the emulsion and the drugs suggested that a stabilization of the drug incorporated into the emulsion droplets might be responsible for this superiority.[3b,5]

In the present investigation, we examine the dosage form characteristics of the S/O emulsion, such as the stability and drug release properties, and their relation to enhanced transport of the drug to the lymphatics, in comparison with those of the W/O emulsion.

Experimental

Materials——Sesame oil and gelatin were obtained from Nakarai Chemicals Co. Nonionic surfactants, the polyoxyethylene derivative of hydrogenated castor oil (HCO-60) and sorbitan sesquioleate (SO-15), were supplied by Nikko Chemicals Co. 5-Fluorouracil (5-FU) was supplied by Kyowa hakko Co. Radiolabeled 5-FU(6-^{3}H) was purchased from the Radiochemical Center, Amersham, England, with a specific radioactivity of 7.7 mCi/mg. All other chemicals were reagent grade products.

Preparation of the Emulsions——The compositions of two different types of emulsions are shown in Table I. Both emulsions were prepared by the sonic vibration method (Ohtake 150 sonicator). Sonification was carried out in a water bath maintained at 70° followed by rapid cooling to about 0°.

Table I. Composition of the W/O Emulsion and S/O Emulsion

W/O Emulsion		S/O Emulsion	
Water phase	0.35 ml	Water Phase	0.35 ml
(Anticancer agent)		(Anticancer agent)	
Distilled water	q.s.	Gelatin	70 mg
Oil phase	2.0 ml	Distilled water	q.s.
Sesame oil	1.84 ml	Oil Phase	2.0 ml
SO-15	0.13 ml	Sesame oil	1.84 ml
HCO-60	0.03 ml	SO-15	0.13 ml
		HCO-60	0.03 ml
Total volume	2.35 ml	Total volume	2.35 ml

Determination of the Particle Size of the Emulsions——The mean globule size of the internal (aqueous) phase was determined by the photomicrographic method. One drop of emulsion was diluted with a few drops of sesame oil and mixed well. One drop of this dilution was placed on a microscope slide and microphotographs of randomly chosen fields were taken. The magnification of the final photographs was 10000×. A total of about 1800 globules was recorded for both emulsions.

Determination of Emulsion Stability——Emulsion stability was evaluated by visual observation of the creaming process and microphotographic observation of the coalescence of globules. The emulsion sample was poured into a glass cylinder (inner diameter; 6 mm) to a height of 80 mm immediately after preparation and stoppered. The volume ratio of the separated phases was ascertained as a function of time at room temperature. The stability of both emulsions under freezing was examined by storing them in a freezer (−20°) for 1 month, followed by thawing at room temperature.

The size distribution change of the emulsion as a function of time was determined by the micrographic method described above.

Measurement of Viscosity of Emulsions——Emulsion viscosities were measured using a cone and plate viscosimeter (Haake Rotovisko, Germany) at shear rates from 668 to 12025 sec^{-1}. The measurements were made at 25°.

Measurement of Drug Release Rate from Emulsions——The apparatus used to determine drug release rate from emulsions is shown in Fig. 1; it was modified from that of Koizumi and Higuchi.[6] It consists of a small compartment with the emulsion above a larger stirred compartment acting as a sink (215 ml of distilled water). The small emulsion compartment was separated from the sink by a cellulose membrane (Visking, 36/32). The whole apparatus was submerged in a thermostated water bath at 37°. When aqueous

4) M. Hashida, Y. Takahashi, S. Muranishi, and H. Sezaki, *J. Pharmacokin. Biopharm.*, **5**, 241 (1977); M. Hashida, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.*, **25**, 2410 (1977).
5) M. Hashida, S. Muranishi, H. Sezaki, N. Tanigawa, K. Satomura, and Y. Hikasa, *Int. J. Pharm.*, **2**, 245 (1979).
6) T. Koizumi and W.I. Higuchi, *J. Pharm. Sci.*, **57**, 87 (1968).

solutions were studied, glass wool was used to avoid convective transport of the solute in the small compartment.

The concentration of solute, 5-FU, in the sink was determined by radioactivity measurement in a liquid scintillation system (Beckman LS-232).

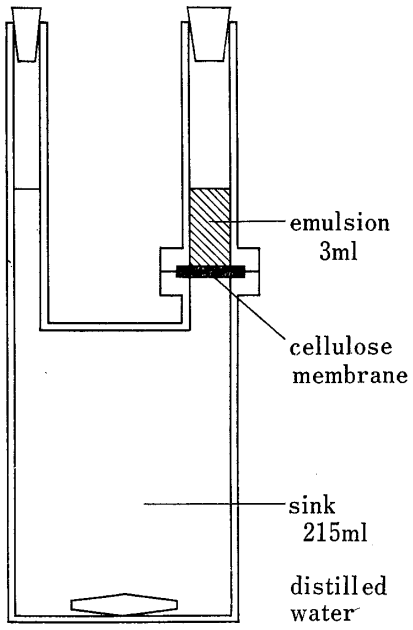

Fig. 1. Apparatus for Determining the Release Rate of a Drug from Emulsions

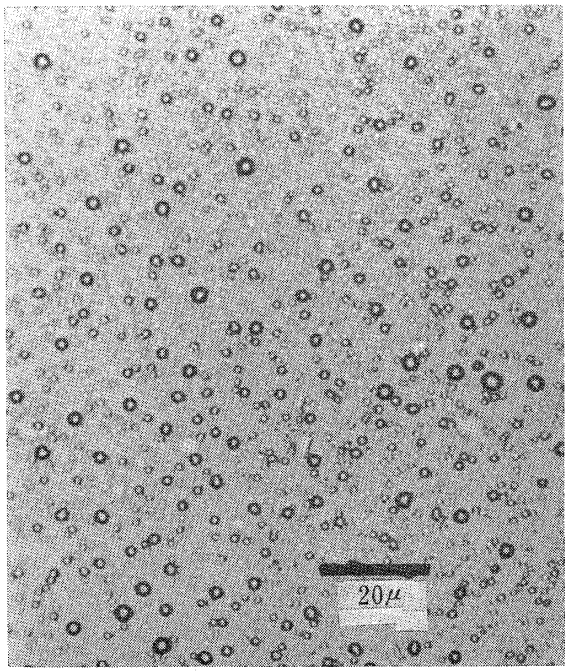

Fig. 2. Microphotograph of S/O Emulsion

Results

Particle-size Analysis

Fig. 2 is a microphotograph of S/O emulsion immediately after preparation. It can be seen that the particles formed by gelatin gel are uniformly dispersed in the oil phase. The W/O emulsion also gave similar microphotographs at the early stage after preparation. From these photographs, the sizes of about 1800 globules were measured for each emulsion.

Both emulsion types show rather similar size distribution patterns, though the size distribution of the S/O emulsion is slightly narrower than that of the W/O emulsion. The approximate arithmetic average particle sizes of S/O emulsion and W/O emulsion were 1.6 μm and 1.9 μm, respectively.

Stability

Fig. 3 shows the visually estimated phase-separation processes of both emulsions. As is obvious in this figure, W/O emulsion separated fairly rapidly into three phases, *i.e.* oil phase, cream layer, and water phase, from the top. On the other hand, the separation of S/O emulsion proceeded slowly and only a small amount of oil was separated from the cream layer at 72 hr after preparation.

Fig. 4 shows the results of size distribution analysis at 24 hr after preparation. S/O emulsion exhibited flocculation of the gelled globules but did not show coalescence nor any increase in particle diameter. The mean particle size was not significantly different from the initial value. The aqueous globules of W/O emulsion aggregated and combined to form larger droplets; *i.e.* coalescence occurred, and the mean diameter of droplets became greater.

The emulsion stability was also examined under freezing at −20° for one month. After thawing at room temperature, W/O emulsion showed almost complete separation into two

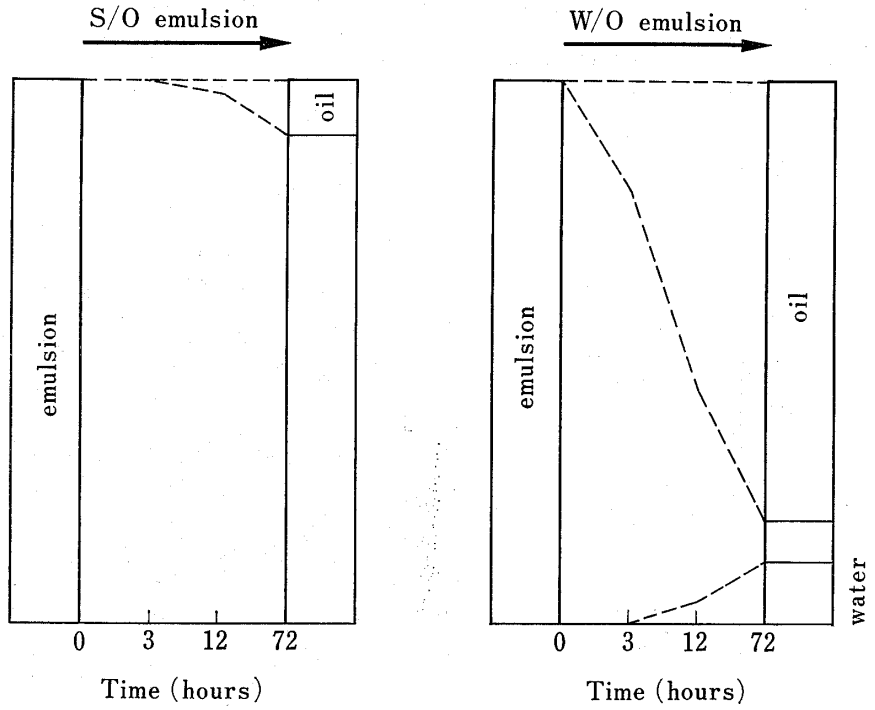

Fig. 3. Stability of S/O Emulsion and W/O Emulsion at Room Temperature

Results are expressed as the mean values of three experiments.

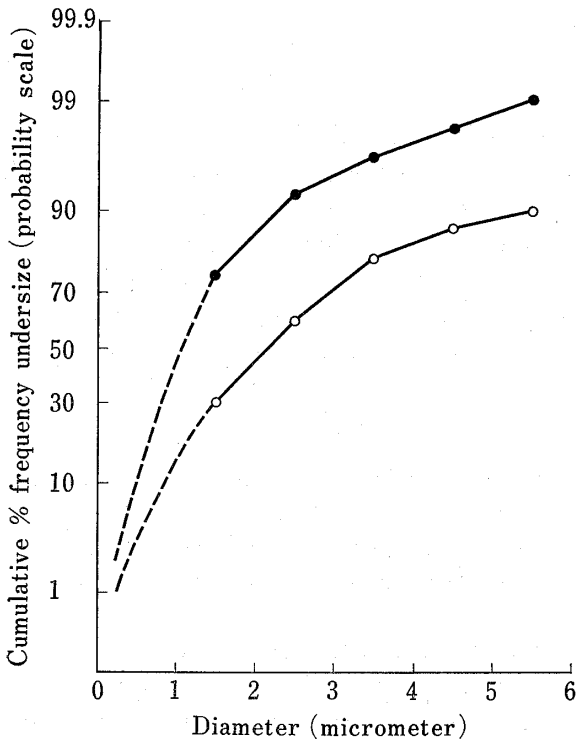

Fig. 4. Cumulative Particle Size Distribution of S/O Emulsion and W/O Emulsion at 24 hr after Preparation

●, S/O emulsion; ○, W/O emulsion.

phases *i.e.* the oil and aqueous layers. In contrast, thawed S/O emulsion exhibited slight creaming but no phase separation. This emulsion was easily reconstituted into a fine S/O emulsion by shaking vigorously.

Rheological Properties

Fig. 5(a) shows flow curves of both emulsions. Calculated viscosities are plotted against shear rate in Fig. 5(b). In this experiment, the rate of shear was changed downward from 12025 to 668 sec^{-1}. The results for sesame oil are also illustrated for comparison.

As is clear from Fig. 5(a), both emulsions exhibited non-Newtonian plastic flow, whereas sesame oil showed simple Newtonian flow. Both emulsions also showed thixotropy, although the up-curves are not illustrated in this figure. Comparing the two emulsion types, S/O emulsion showed a markedly larger viscosity, particularly in the range of slow shear rate. However, there was only a small difference at high shear rate, and at the shear rate of 12025 sec^{-1} the vis-

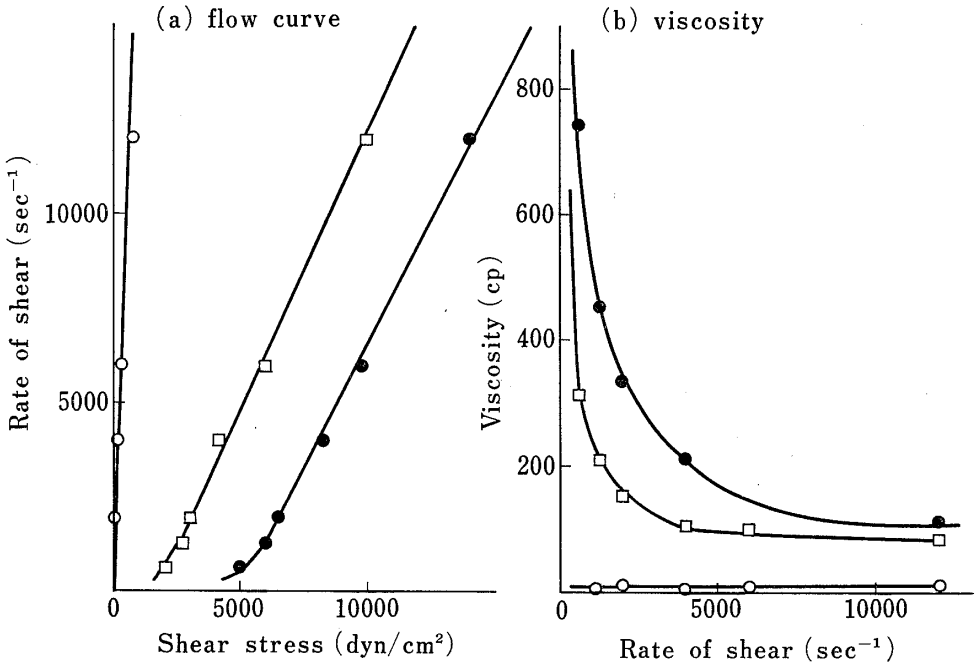

Fig. 5. Flow Curves and Viscosities of Various Oily Vzhicles at 25°

●, S/O emulsion; □, W/O emulsion; ○, sesame oil.
Results are expressed as the mean values of three experiments.

cosity of S/O emulsion was 116 cp and that of W/O emulsion was 83 cp. In contrast, the viscosity of sesame oil was about 8 cp.

Drug Release Characteristics

In order to clarify the drug release characteristics of both emulsions, the release rate of radiolabelled 5-FU incorporated into the inner phase of the emulsions was determined using the apparatus shown in Fig. 1.

Fig. 6. shows the release patterns of 5-FU from S/O emulsion and W/O emulsion. The amount of drug released was plotted against the square root of time. The mode of transfer of 5-FU through the membrane was also examined by applying aqueous 5-FU (Fig. 6). The plot for the aqueous solution was linear, suggesting that transport of 5-FU through the membrane occurred by a simple diffusion mechanism.[6] The rate of this transport was faster than that of release from emulsions, so that it can be considered that transmembrane movement does not play a significant role in the overall release of 5-FU from emulsions. Both emulsions showed linear release patterns in the earlier stage, up to 180 min after the start of the experiment. In this period, the release rates from both emulsions were slow and there was no significant difference between them, whereas enhanced release was observed in the subsequent period in the case of W/O emulsion. At 420 min after the start,

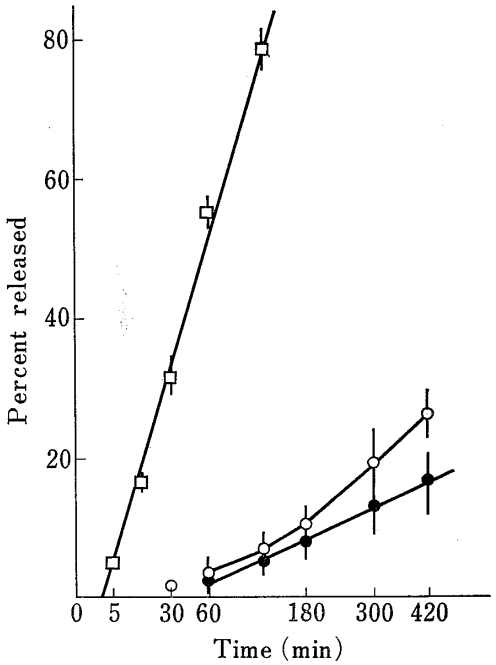

Fig. 6. *In Vitro* Release of 5-FU from S/O Emulsion and W/O Emulsion

●, S/O emulsion; ○, W/O emulsion; □, aqueous solution.
Results are expressed as the means±S.D. of four experiments.

a significant difference ($p<0.05$) was observed between the releases from W/O emulsion and S/O emulsion. This increased release rate was interpreted as reflecting the instability of W/O emulsion.

In previous studies,[3b,5] it became clear that when S/O emulsion or W/O emulsion was injected into the interstitial space of the tissues, they were converted into the so-called multi-type emulsion, in which a large number of innermost globules are dispersed within the oil droplets. Under these conditions, the drug is considered to be contained in the innermost aqueous globules. To estimate the drug release rate from emulsions under such *in vivo* conditions, therefore, both emulsions were redispersed into 1% gelatin solution to form multiple emulsions and the release rates of 5-FU from them were determined in the same manner.

Fig. 7 shows microphotographs of the resulting S/O/W emulsion and W/O/W emulsion prepared as described above. As can be seen in these photographs, the S/O emulsion provided a uniform multiple emulsion retaining a large number of fine microspheres in the oil droplets. On the other hand, the W/O emulsion gave a coarse dispersion of relatively large aqueous globules in oil droplets, suggesting that some of the aqueous globules had coalesced and ruptured into the outside aqueous phase. Thus, some significant mixing of the two aqueous phases might have occurred.

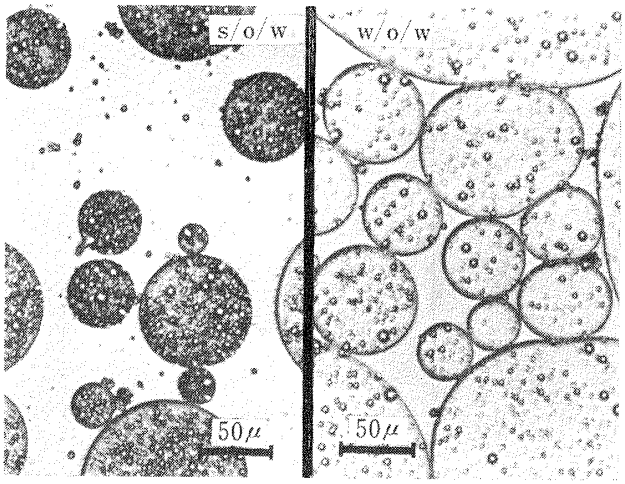

Fig. 7. Microphotographs of S/O/W Emulsion and W/O/W Emulsion Prepared by Dispersing S/O Emulsion or W/O Emulsion, Respectively, into 1% Gelatin Solution

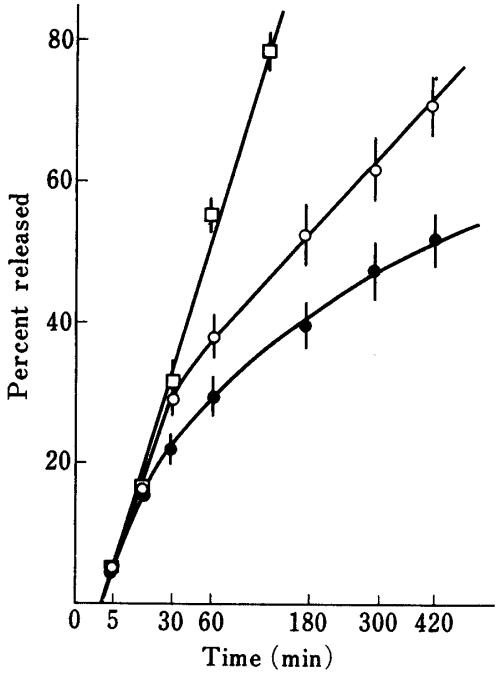

Fig. 8. *In Vitro* Release of 5-FU from S/O/W Emulsion and W/O/W Emulsion

●, S/O/W emulsion; ○, W/O/W emulsion; □, aqueous solution.
Results are expressed as the means±S.D. of four experiments.

The release patterns of 5-FU from S/O/W emulsion and W/O/W emulsion are illustrated in Fig. 8. In contrast with the release patterns of primary emulsions, these multiple emulsions showed rapid transfer of 5-FU to the sink in the early period, which seemed similar to that obtained with an aqueous solution of 5-FU. S/O/W emulsion exhibited fast and slow release phase. These two phases can be interpreted as representing rapid release of the drug from the external aqueous phase and slow release from the primary emulsion, respectively, in accord

with the results of Brodin *et al.*[7] W/O emulsion showed relatively rapid release even at the later period, confirming rupture of the primary emulsion, as observed in the microphotograph shown in Fig. 7.

Discussion

In previous reports,[3,5] the mechanism for specific lymphatic delivery of anticancer agents by S/O emulsion and/or W/O emulsion was shown to involve oil droplets of multiplied emulsion formed by injection into the interstitial spaces acting as a carrier, incorporating the drug in the innermost aqueous globules. In order to improve the effectiveness of emulsions, it appears to be necessary to satisfy the following two conditions: (1) preferential transport of oil droplets of multiplied emulsion to the lymphatics, and (2) stable incorporation of the drug into the carrier droplets, though with release of the drug with a suitable rate at the target site.

In the present investigation, the microphotographs shown in Fig. 7 indicate that the S/O emulsion was converted to a more stable and better dispersed multiple emulsion than the W/O emulsion when they were redispersed into an aqueous solution of 1% gelatin. In accord with this finding, efficient incorporation of the drug into the multiplied S/O oil droplets was found with S/O emulsion (Fig. 6). These results seem to account for the superiority of the S/O emulsion as a lymphotropic delivery system, at least in connection with the second condition mentioned above.

On the other hand, from the viewpoint of supplying clinical formulations of good quality, it is desirable to develop a stable emulsion which can retain its form for a considerable time in storage. As shown in Figs. 3 and 4, the S/O emulsion is more stable than the W/O emulsion, and in particular, the excellent reconstitution of frozen S/O emulsion offers further support for the practical utility of the S/O emulsion.

As regards rheological properties, both S/O and W/O emulsions exhibited non-Newtonian plastic flow, and S/O emulsion showed the largest viscosity. This increased viscosity of the S/O emulsion at slow shear rate was considered to be advantageous in connection with stability on storage. Based on the calculations of Henderson *et al.*,[8] the flow rate of a pharmaceutical liquid is nearly 10000 sec^{-1} through a hypodermic needle with a radius of 0.05 cm at a flow of 2 ml per sec, so that both emulsions should show relatively small viscosity and good injectability.

The results presented in this report indicate that several physical characteristics of the S/O emulsion are fairly different from those of the W/O emulsion. Comparing these two emulsions, the major point of difference is that S/O emulsion has inner gel globules consisting of gelatin and water. Accordingly, these globules are considered to resemble a solid in that they may show resistance to coalescence. The formation of a network of chains of globules together with a partial inhibition of growth of large icecrystals in the gel may be responsible for the increased stability in the frozen state.

Kumano *et al.*[9] reported a method for preparing stable W/O emulsions stabilized by gels formed between surfactants and aqueous solutions of amino acids, and demonstrated the utility of this formulation for ointments or cosmetics. Our method is somewhat similar to this, but the preparation procedure seems to be easier, since we utilized a sol-gel alteration promoted by a decrease of temperature.

In conclusion, the present results demonstrate the superiority of the S/O emulsion as a drug delivery system for anticancer agents, having a predictable shelf-life and offering controlled drug release characteristics.

7) A. Brodin, D. Kavaliunas, and S. Frank, *Acta Pharm. Suetica*, **15**, 1 (1978).
8) N. Henderson, P. Meer, and H. Kostenbauder, *J. Pharm. Sci.*, **50**, 788 (1961).
9) Y. Kumano, S. Nakamura, S. Tahara, and S. Ohta, *J. Soc. Cosmet. Chem.*, **28**, 285 (1977).