Comparative Study of Two Preparation Methods of w/o/w Emulsions: Stirring and Membrane Emulsification

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Since a membrane method which employs a porous glass membrane for preparation of water-in-oil-in-water (w/o/w) emulsion from water-in-oil emulsions has been developed recently, a comparative study was made of properties of w/o/w emulsions prepared by a two-stage stirring method and this membrane method. w/o/w emulsions encapsulating cytarabine, doxorubicin and vancomycin, were prepared by the two techniques using the same formulation. Particles of the w/o/w emulsion prepared by the stirring method were less homogeneous, whereas particle distribution of the emulsion prepared by the membrane method was sharp and close to monodispersion. The mean particle size depended on pore size of the membrane. The membrane method proved the more useful of the two for encapsulation of low molecular weight drugs. Drug release from the emulsion prepared by this means was slower than that from the emulsion prepared by the stirring method. The membrane method was therefore found useful for preparation of a homogeneous w/o/w emulsion, effective encapsulation of small drugs and control of drug release.

Key words w/o/w emulsion; membrane emulsification technique; two-stage stirring method; cytarabine; doxorubicin; vancomycin

The potential for water-in-oil-in-water type multiple emulsions (w/o/w emulsion) in drug delivery has been studied widely.[1–3] An ideal w/o/w emulsion which shows good stability or good drug entrapment efficiency, however, has not yet been developed. The two-stage stirring emulsification method (stirring method), which employs a sonicator for the 1st emulsification and a high-speed homogenizer for the 2nd, has long been used to prepare w/o/w emulsions.[4–6] However, the wide particle size distribution and low drug entrapment efficiency are problems of the stirring method.[7] Furthermore, this wide particle size distribution of the emulsion causes the system to be unstable.

Recently, a new emulsification method, the membrane emulsification technique (membrane method), has been developed and studied.[8–10] A controlled-pore glass membrane (Shirasu porous glass membrane; SPG membrane) was used to prepare w/o/w emulsions. Particle size of the w/o/w emulsion prepared by the membrane method was reportedly controlled by the pore size of the SPG membrane and the particle size distribution of the emulsion was monodispersion.[9] Higashi *et al.*[10] prepared the w/o/w emulsion entrapping epirubicin by the membrane method to use it as an arterial-injection therapy for patients with hepatocellular carcinoma, evaluated the method and reported its clinical usefulness.

The aim of this study was to compare pharmaceutical properties of w/o/w emulsions prepared by the stirring and the membrane methods. In the previous study,[11] we used a Lipiodol Ultra-Fluid (LPD) and isopropyl myristate (IPM) oil mixture for an oily phase of the w/o/w emulsion to minimize the difference in specific gravity between oily phase and aqueous phase, and were able to prepare a stable w/o/w emulsion. In this study, w/o/w emulsions were prepared by the two techniques with the same formulation and their pharmaceutical properties were compared. For entrapped water-soluble drugs, cytarabine, doxorubicin hydrochloride and vancomycin hydrochloride were used. Cytarabine and doxorubicin were used for treatment of cancer and vancomycin was used as a model of peptide drugs. All three drugs are water-soluble but are barely soluble in the oil used here. w/o/w emulsions which entrapped these water-soluble drugs were prepared by the two methods and the drug entrapment efficiency, particle size distribution, and drug releasing behavior were measured and characteristics of the techniques were evaluated.

Materials and Methods

Materials Cytarabine (M.W. = 243.22), doxorubicin (M.W. = 579.98) and vancomycin (M.W. = 1485.73) were generously supplied by Nihon Shinyaku Co. (Kyoto, Japan), Kyowa Hakko Kogyo Co. (Tokyo, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. Nonionic surfactants, HCO-40 and Pluronic F-88 (F-88) were gifts from Nikko Chemicals Co. (Tokyo) and Asahidenka Kogyo Co. (Tokyo), respectively. LPD (specific gravity, 1.28 at 20 °C) was purhased from Marion Merrell Dow Co. (Tokyo) and IPM (specific gravity, 0.89 at 20 °C) was a gift from Nippon Oil and Fats Co. (Tokyo).

Formulation of the w/o/w Emulsion The formulation of the w/o/w emulsion was based on our previous findings.[10] That is, the composition ratio of the internal aqueous phase : oily phase: external aqueous phase was fixed at 1 : 4 : 5 in this study. The internal aqueous phase was the normal saline solution of each drug. Each drug solution was prepared as an isotonic solution dissolved in a normal saline, and each drug concentration was as follows: cytarabine, 5 mg/ml; doxorubicin, 5 mg/ml; and vancomycin, 10 mg/ml. The oily phase was the oil mixture of LPD and IPM (4.5 : 5.5) containing 5% of HCO-40. The external aqueous phase was 5% F-88 in normal saline solution. The above formulation was used for preparation of both w/o/w emulsions.

Methods of Preparation of the w/o/w Emulsion The means of preparing a w/o/w emulsion by the stirring method (s-w/o/w) has been reported.[11] Rotation rate at 1st and 2nd emulsification stages was 20000 rpm and 6000 rpm, respectively. Preparation of a w/o/w emulsion by the membrane method (m-w/o/w) followed the method of Nakajima *et al.*[8] That is, a w/o emulsion was first prepared, this was injected into the emulsifying module of the membrane emulsification system (Microkit, Kiyomoto Ironworks Co., Miyazaki, Japan) attaching an SPG membrane (pore size being 4.07 μm) and was pressed through the membrane into the external phase by the pressure of N_2 gas (0.35 kgf/cm^2).

Measurement of the Particle Size and Viscosity of the w/o/w Emulsion Particle size of the w/o/w emulsion at 37 °C was measured by a particle distribution analyzer using dynamic light-scattering (Model

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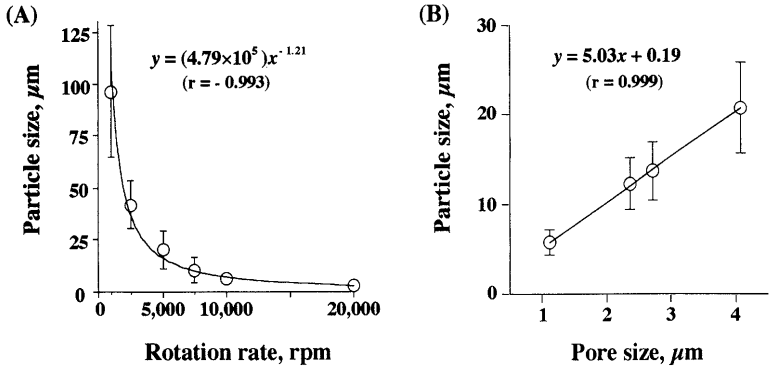

Fig. 1. Relationship between the Particle Size of w/o/w Emulsion Produced and the Rotation Rate of Homogenizer (A) or the Pore Size of SPG Membrane (B)

CIS-1, Galai, München, Germany). One-hundred microliters of w/o/w emulsion was diluted with 3 ml of normal saline and the volume mean particle size was measured. Viscosity of the emulsion was measured by a cone-plate type rotary viscometer (E-type, Tokyo Keiki, Tokyo, Japan); 1.2 ml of w/o/w emulsion was placed on the viscometer and its viscosity was measured at 37 °C under several shear stresses.

Drug Release Study Entrapment efficiency of each drug in the internal aqueous phase of the w/o/w emulsion and release pattern of each drug from the emulsion was determined as reported.[11] The amount of drug entrapped into the emulsion was first measured, then drug release tests were performed at 37 °C. Drug concentrations at appropriate intervals (1, 3, 6, 9, 12, 24, 48 and 72 h) were measured and the percent of drug released ascertained. When the release test was carried out directly at 37 °C, the total amount of drug released from w/o/w emulsion was nearly equal to the above method. It was thus considered that phase changes in the emulsion or film formulation inside the dialysis tube did not occur in this study.

Measurement of Drug Concentrations The concentration of cytarabine was analyzed spectrophotometrically (UV 240 spectrophotometer, Shimadzu Seisakusho Co., Kyoto) at 272 nm. Determination of doxorubicin was performed by the method of Masuike *et al.*,[12] and was analyzed by high performance liquid chromatography (HPLC) (LC-6A, R-500RF, CR-3A, Shimadzu) under the following conditions: column, LiChrospher 100 RP-18 (Merck, Darmstadt, Germany); mobile phase, methanol: 1 N formic acid buffer, pH 3.0=1:1; flow rate, 1 ml/min; temperature, 30 °C; Ex, 485 nm; Em, 575 nm; and injection volume, 10 μl. Determination of vancomycin was made by the method of Jehl *et al.*[13] using by HPLC (LC 6A, SPD-3A, CR-3A, Shimadzu) under the following conditions: column, LiChrospher 100 RP-18; mobile phase, acetonitrile: 0.02 M ammonium acetate buffer, pH 5.4=9:1; flow rate, 1 ml/min; temperature, 50 °C; absorbance, 280 nm; and injection volume, 50 μl.

Results and Discussion

Figure 1 shows the relationship between the conditions at the second emulsification by the two methods and particle sizes of each w/o/w emulsion. The mean particle size was decreased with an increase in rotation rate at the second stage (Fig. 1 A). The relation between rotation rate and particle size of the w/o/w emulsion is described as follows:

$$y=(4.79\times10^5)x^{-1.21}$$

where x is rotation rate of the homogenizer and y is the mean particle size of s-w/o/w prepared by the stirring method (the correlation coefficient being −0.993). Particle size of s-w/o/w depended on the shear stress of the homogenizer, that is, the rotational energy. In general, the manufacturing of w/o/w emulsions depends not only on the rotational power but also on the formulation of the emulsion. Therefore, if the formulation is different, a different result could be expected.

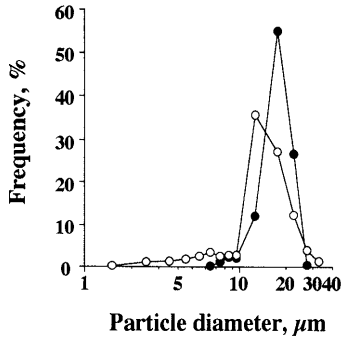

Fig. 2. Particle Size Distributions of s-w/o/w (○) and m-w/o/w (●)

On the other hand, the particle size of m-w/o/w depended on the pore size of the SPG membrane. The relation between membrane pore size and particle size of the w/o/w emulsion showed good correlation ($r=0.999$) and is described as:

$$y=5.03x+0.19$$

where x is a pore size of the SPG membrane and y is the mean particle size of m-w/o/w prepared by the membrane method (Fig. 1 B).

Distribution profiles of log-normal particle sizes of s-w/o/w and m-w/o/w are shown in Fig. 2. The particle size distribution of s-w/o/w was broader, that is, there were more small size particles than m-w/o/w. In contrast, the particle size distribution profile of m-w/o/w was sharp, in other words, more homogeneous particles were created by the membrane method. These are remarkable features of this method in which the w/o emulsion is pressed into an external aqueous phase through pores of the SPG membrane which are homogeneous in size.

Pharmaceutical properties of s-w/o/w and m-w/o/w are summarized in Table 1. Standard deviation (S.D.) of mean particle size of m-w/o/w emulsion was smaller than that of s-w/o/w. Viscosity of m-w/o/w was slightly lower than that of s-w/o/w; this was attributed to the many small particles existing in s-w/o/w. Both w/o/w emulsions tended to behave in a non-Newtonian manner and showed a plastic flow (yield value was 0.5 dyn/cm^2). Both techniques encapsulated vancomycin in each w/o/w emulsion very effectively, while the entrapment efficiency of cytarabine and doxorubicin into m-w/o/w was slightly higher than that of s-w/o/w. Relatively low molecular weight water-soluble drugs such as cytarabine could thus

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Table 1. Comparison of Pharmaceutical Parameters of s-w/o/w and m-w/o/w Entrapping Each of Three Drugs

	Cytarabine		Doxorubicin		Vancomycin	
	s-w/o/w	m-w/o/w	s-w/o/w	m-w/o/w	s-w/o/w	m-w/o/w
Mean diameter[a] (μm)	20.0±10.8	19.8±5.1	20.2±9.8	19.6±4.9	20.1±10.7	19.0±5.1
Viscosity[b] (cP)	19.2± 0.2	14.4±0.3	15.5±0.4	14.0±0.5	16.8± 0.3	15.9±0.5
Entrapment efficiency[b] (%)	15.0± 3.2	64.1±5.3	40.1±5.2	55.2±1.5	99.2± 0.3	99.5±0.2

a) Each value represents the mean±S.D. *b*) Each value represents the mean±S.E.M. of three experiments.

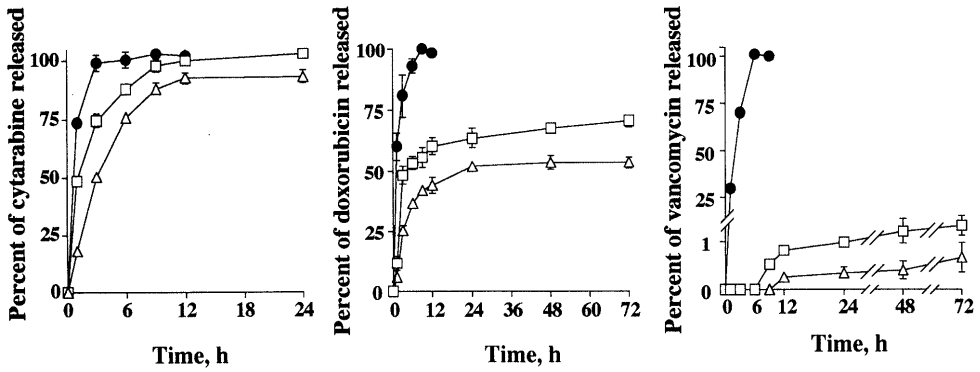

Fig. 3. Comparative Study on Release of Cytarabine, Doxorubicin and Vancomycin from s-w/o/w (squares), m-w/o/w (triangles) and Aqueous Solution (closed circles)

Each value and point represent the mean±S.E.M. of three experiments.

more effectively be entrapped into the emulsion by the membrane method than by the stirring method. It has been reported that the entrapment efficiency of a low molecular weight water-soluble drug into w/o/w emulsion was relatively low and the drug leaked from the emulsion easily.[7] The good entrapment efficiency into m-w/o/w may be hypothesized as due to the leakage of the drug by the indiscriminate shear by a high-speed homogenizer being relaxed in the membrane method in which the w/o emulsion is pressed into the external aqueous phase through the controlled-pore glass membrane.

Release profiles of three drugs from s-w/o/w and m-w/o/w are shown in Fig. 3. Drug release from m-w/o/w was more sustained than s-w/o/w in all three drugs. Cytarabine was released rapidly and the release was completed in 24 h. More than 30% of doxorubicin remained in the w/o/w emulsions 72 h after experiments were started, while little vancomycin was released from the emulsion during the 72 h experiment.

The property of drugs (molecular weight, substituent group, hydrophilicity and lipophilicity), the property of w/o/w emulsion or the interaction of drugs with the emulsion are believed related to drug entrapment efficiency and drug release property. The amount of drug released first depended on its molecular weight, and the release rate was reduced with increase in molecular weight. One rationale for prolonged drug release could be the effect of the interaction between a drug and the surface area (structure or surfactant) of w/o/w emulsion. Doxorubicin has a large chromophore and the structure of vancomycin is very complicated and bulky. The releases of these drugs from the surface of the w/o/w emulsion, which is covered with high molecular weight surfactants which form a complicated network, may be inhibited by the substituent group or the structure.

Next, the property of surface of the w/o/w emulsion is thought to be affected when a drug is released. Particles of m-w/o/w were more homogeneous and their size distribution was sharp (Fig. 2), while the particles of s-w/o/w were not homogeneous and there were many small particles (also Fig. 2). These differences are expected to come from their mechanisms of emulsification. Because there were many small particles in s-w/o/w, the surface area of the emulsion was expanded. Therefore, the drug release from s-w/o/w may be accelerated compared with m-w/o/w. Next, the effect of the surface structure property of the w/o/w emulsion on the release of drug from the emulsion is considered. It was reported that the orientation of the surfactant at the surface area of m-w/o/w was isotropic by an analysis of X-ray small angle scattering.[14] This report showed that the surfactant was adsorbed homogeneously on the surface of the emulsion, and therefore m-w/o/w could be prepared with a small amount of surfactant. The orientation in the surfactant of s-w/o/w was not always isotropic, however. When the drug is released from m-w/o/w, it may be prevented by the layer of the surfactant; because the surfactant of m-w/o/w is packed densely at the surface, the drug release rate is believed to be slower than that from s-w/o/w.

The present study demonstrates that the membrane emulsification system is a simple method of preparation of w/o/w emulsions; that is, complicated operations are not required. Although this technique can be used to prepare a homogeneous w/o/w emulsion, a great deal of time was necessary: about 12 h to prepare only 20 ml of m-w/o/w. In contrast, only about 30 min was required to prepare s-w/o/w. It is thus important to search for

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better formulation of the emulsion, such as kinds and concentrations of surfactants and compositions of the w/o/w emulsions, so that they can be made in a short time. Improvement in the mechanical system of the membrane method such as operation pressure is also required. It was shown here that the particles of m-w/o/w were nearly homogeneous; therefore, the detailed mechanism of drug release from the w/o/w emulsion and the detailed features of the surface and w/o/w emulsion itself have been clarified.

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