Fabrication, Characterization and Pharmacokinetic Evaluation of Doxorubicin-Loaded Water-in-Oil-in-Water Microemulsions Using a Membrane Emulsification Technique

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Doxorubicin (DOX)-loaded water-in-oil-in-water (W/O/W) microemulsions were produced using a shirasu–porous–glass (SPG) membrane emulsification technique. Soybean oil was used as the oil phase; polyglycerol polyricinoleate (PGPR) or tetraglycerol polyricinoleate (TGPR) was used as the surfactant to stabilize the feed W/O emulsions, while Tween 20 was used in the external water phase to stabilize oil droplets containing water droplets. Increasing the feed pressure from 50 to 90 kPa increased the particle size of W/O/W emulsions, whereas it was decreased by increasing the agitator speed. The smallest particle sizes of multiple emulsions were obtained at the feed pressure of 50 kPa and agitator speed of 350 rpm. Under this set of conditions, the increase in the concentration of PGPR or TGPR showed a decrease in the particle size of DOX-loaded W/O/W emulsions. The optimized formulation comprising of 5% w/v PGPR and 3% w/v Tween 20 in the oil phase and external water phase, respectively, with 0.5% w/v of DOX had a particle size of 0.440±0.007 μm and polydispersity index of 0.220±0.087, which was supported by the transmission electron microscopy image. The formulations showed a sustained release profile in phosphate buffer solution (pH 7.4). The plasma concentrations of DOX after intravenous administration to rats were prolonged and gave approximately 17-fold higher area under the drug concentration–time curve (AUC) compared to free DOX solution. Thus, these results demonstrated that the SPG membrane emulsification technique could be used as a promising technique to prepare W/O/W microemulsions for delivering DOX with sustained release characteristics and better bioavailability.

Key words microemulsion; shirasu–porous–glass; doxorubicin; sustained release; pharmacokinetics

Over the past two decades, an increasing interest has been devoted to multiple emulsions due to their multiple compartment structure and an immense capability of encapsulating hydrophilic drug substances for sustained released characteristics.1–4) The potential application of water-in-oil-in-water (W/O/W) multiple emulsion is not only limited to pharmaceuticals but also in the field of cosmetics and food industry. In cosmetics and pharmaceuticals, W/O/W emulsions have been used for controlled release and targeted delivery of drugs.5–8) Food applications include the encapsulation of vitamin/minerals,9,10) and the production of low-calorie foods.11)

W/O/W emulsions are usually created using conventional homogenization technology in a two-step procedure.12) First, a primary water-in-oil (W/O) emulsion is prepared by homogenizing an oil phase and an aqueous phase together in the presence of a suitable oil-soluble emulsifier (low hydrophilic to lipophilic balance (HLB)<10). Second, W/O/W emulsion is prepared by homogenizing W/O emulsion with another aqueous phase in the presence of a suitable water-soluble emulsifier (high HLB>10).13) However, the second step often results in high polydispersity or low encapsulation efficiency. Therefore, the conventional homogenization techniques for preparing emulsions have been gradually replaced because of their high energy consumption, intricate technology and polydispersity of emulsions,13) and their intrinsic thermodynamic instability derived from the relatively larger droplets size.14)

Membrane emulsification is a relatively new method for the production of emulsions. The technique is attractive due to the low energy consumption, the better control of droplet size and droplet size distribution, and especially the scale-up possibility of the process. In particular, a special type of glass membrane, called shirasu–porous–glass (SPG) membranes, is potentially suitable for membrane emulsification due to their uniformly sized pores and wide range of available mean pore diameters.15) The multiple emulsions are produced by forcing a primary emulsion (W/O) through a microporous membrane into a continuous aqueous phase.16–19) This results in much less shear than in conventional emulsification processes so that the droplets are intact and both a high entrapment efficiency and monodispersity could be achieved.20,21)

In the present work, we prepared the doxorubicin (DOX)-loaded W/O/W microemulsions using membrane emulsification by pressurizing the premixed W/O emulsions into the external water phase through SPG membrane. Soybean oil was used as the oil phase, polyglycerol polyricinoleate (PGPR) or tetraglycerol polyricinoleate (TGPR) as the hydrophobic emulsifiers (HLB value of 4), and Tween 20 (HLB value of 16.7) was chosen as the hydrophilic surfactant in the external water phase. Doxorubicin hydrochloride (DOX) was used as a hydrophilic model drug. DOX is an antineoplastic agent widely used in the treatment of acute leukemia, endometrial, ovarian and breast cancers.22) Despite good efficacy, its clinical utility may be hampered by cumulative, dose-limiting cardiotoxicity, myelosuppression, and drug resistance.23,24) Thus, W/O/W emulsion systems can be suitable drug carriers because of the encapsulation of the drug in the internal water phase and the low viscosity due to the external water phase. Moreover, in the form of an emulsion, it is possible to control release rates...
Experimental

Materials DOX was supplied by Dong-A chemical (Suwon, South Korea). Soybean oil was purchased from Sigma Chemical Co., Ltd. (Germany). Polyglycerol polyrincinoleate (SY-GLYSTER CRS-75) and tetracylglycerol polyrincinoleate (SY-GLYSTER CR-310) were supplied from Sakamoto Yakuhin Kogyo (Osaka, Japan). Tween 20 was purchased from DC Chemical Co., Ltd. (China). Semi-permeable membrane tubing (MEMBRA-CEL® Dialysis Tubing) was purchased from SERVA Electrophoresis (Heidelberg, Germany). A miniature kit for emulsification with an MPG module (microporous glass, a brand name of SPG) was purchased from Kiyomoto Works Co. (Miyazaki, Japan). All other chemical reagents were of analytical grade and were used without further purification.

Preparation of W/O/W Emulsions The W/O/W emulsions were prepared by a two-step process. The first-step emulsification consisted of preparing premixed W/O emulsions. For preparation of premixed W/O emulsions, various concentrations of the hydrophobic emulsifier PGPR or TGPR were initially mixed with soybean oil. The oil phase containing PGPR or TGPR was mixed with the water phase (pure water or drug solution). The premixed W/O emulsions were homogenized using a homogenizer (Ultra-Turrax® Model T25, IKA Works, Inc., Germany) at 9000 rpm for 5 min.

Membrane emulsification was used for the second step. A schematic diagram of the SPG membrane emulsification technique is shown in Fig. 1. The SPG membrane system consisted of a tube-shaped hydrophilic SPG membrane with an outer diameter of 10 mm, a thickness of 0.75 mm and a pore size of 1.4 µm, pressurized N2 gas, a dispersed phase container, a collecting vessel, and a stirrer. The premixed W/O emulsion as the dispersed phase was poured in a pressure-tight vessel that was connected to a nitrogen gas inlet attached to a PG-200-163GP-S pressure gauge (COPAL Electronics, Japan). The continuous phase, 100 mL of water containing 3% w/v Tween 20 was continuously stirred to prevent creaming of the droplets. Before starting the emulsification process, SPG membrane was dipped into the continuous phase and subjected to ultrasonic treatment for 30 min in order to wet the membrane thoroughly with the continuous phase.7) The dispersed phase was pumped under gas pressure through the pores of the membrane into the continuous phase, which circulated through the membrane module to form the W/O/W micro-emulsion droplets. The experiments were carried out over a wide range of feed pressures (50–90 kPa) and agitator speeds (150–450 rpm), at 25°C to optimize the process parameters.

Determination of Particle Size and Size Distribution NanoZS light-scattering particle size analyser (Malvern, U.K.) with non-invasive back scatter (NIBS®) technology was used to measure the droplet size and size distribution of the samples. It allowed detection of droplets in the range of 0.6–6000 nm. The data on particle size distribution were collected using Dispersion Technology Software (DTS) (nano) software (version 5.0) which was provided with the instrument. All the experiments were repeated three times and the values of the z-average diameters were used. The z-average diameter of the emulsion was derived from cumulative analysis by the Automeasure software (Malvern Instruments, Malvern, U.K.). The data were expressed as the mean±standard deviation (S.D.). A two-tailed unpaired Student’s t-test was performed with a significance level of p<0.05.

In Vitro Release Study Each 2 mL of W/O/W emulsion and free drug solution (as a control) containing 0.5% DOX was put into the MEMBRA-CEL® dialysis membrane bag. Both the ends of the membrane bag were sealed with a Medi-cell clip to prevent leakage. Each bag was immersed in placed in 200 mL phosphate buffer solution (PBS, pH 7.4) contained in the jars of the dissolution tester (DST-600; Fine Chemical, Hwasung, South Korea). The temperature of the water-bath was maintained at 36.5°C and the stirring was done using the paddle at 50 rpm. At pre-determined intervals, 1 mL of the medium was sampled and filtered.25,26) The resulting solution was then analyzed by HPLC as described below.
**Pharmacokinetic Study** In Vivo Experiments Male Sprague-Dawley rats weighing 250±20 g were fasted for 12 h prior to the experiments, but allowed free access to water. Eight rats were divided into two groups. Under light ether anaesthesia, the femoral arteries and veins of rats were cannulated with PE-50 polyethylene tubing. After complete recovery from anaesthesia, DOX-loaded W/O/W emulsion or DOX solution were administered intravenously to the femoral vein through the catheter at a dose of 5 mg/kg as doxorubicin, respectively. Blood samples (0.2 mL) were collected via the femoral artery at designated time intervals and put into heparinized glass tubes and centrifuged at 12000×g for 10 min using a 5415C centrifuge (Eppendorf, Hauppauge, NY, U.S.A.).

All animal care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999, and amended in 2008 by the Society of Toxicology. Furthermore, the protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Yeungnam University.

Blood Sample Analysis Plasma (0.1 mL) was thoroughly mixed with 0.1 mL of acetonitrile and 25 μL of acetonitrile solution containing daunomycin (100 μg/mL) as an internal standard. It was centrifuged at 12000×g for 10 min to precipitate the proteins. The supernatant layer (0.2 mL) was evaporated under N2 (g) for 10 min using a 5415C centrifuge (Eppendorf, Hauppauge, NY, U.S.A.).

**Table 1. Compositions of Primary W/O Emulsion**

<table>
<thead>
<tr>
<th>W/O emulsion</th>
<th>Soybean oil (mL)</th>
<th>PGPR (g)</th>
<th>TGPR (g)</th>
<th>Distilled water (mL)</th>
<th>Phase separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>20</td>
<td>0.20</td>
<td>—</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>P2</td>
<td>20</td>
<td>0.40</td>
<td>—</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>P3</td>
<td>20</td>
<td>0.60</td>
<td>—</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>P4</td>
<td>20</td>
<td>0.80</td>
<td>—</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>P5</td>
<td>20</td>
<td>1.00</td>
<td>—</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>T1</td>
<td>20</td>
<td>—</td>
<td>0.20</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>—</td>
<td>0.40</td>
<td>5</td>
<td>+</td>
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<tr>
<td>T3</td>
<td>20</td>
<td>—</td>
<td>0.60</td>
<td>5</td>
<td>+</td>
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<tr>
<td>T4</td>
<td>20</td>
<td>—</td>
<td>0.80</td>
<td>5</td>
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<tr>
<td>T5</td>
<td>20</td>
<td>—</td>
<td>1.00</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>T6</td>
<td>20</td>
<td>—</td>
<td>1.20</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>T7</td>
<td>20</td>
<td>—</td>
<td>1.40</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>T8</td>
<td>20</td>
<td>—</td>
<td>1.60</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>T9</td>
<td>20</td>
<td>—</td>
<td>1.80</td>
<td>5</td>
<td>−</td>
</tr>
</tbody>
</table>

+: Phase separation for 24 h. −: No phase separation for 3 d.

Levels of statistical significance (p<0.05) were assessed using the Student’s t-test between two means for unpaired data. All data are expressed as mean±standard deviation (S.D.).

**Results and Discussion**

**Effect of Hydrophobic Emulsifiers on Phase Separation of Primary W/O Emulsions** In order to develop a stable DOX-loaded W/O/W emulsions using membrane emulsification technique, various process parameters were optimized. First, the volume fraction of water to oil was determined by adding different quantities of distilled water (10–50% v/v) to the soybean oil containing 2% (w/v) PGPR, prior to homogenization. Emulsions with 20–30% volume of water were stable without phase separation for seven days. The increase in water phase induced greater instability, which may be due to the increased incidence of coalescence and bridging at higher water volume, both of which led to reduction in total water droplet surface area. Similar results were demonstrated that 20–30% of dispersed phase to continuous phase was the optimum volume fraction for an emulsion to have good stability and high encapsulation efficiency. Therefore, the volume fraction of water and oil in primary W/O emulsion, were set at 0.25 in all the following experiments.

The composition of the primary W/O emulsions (10 mL) and the effects of various PGPR concentrations (1–5% w/v) or TGPR concentrations (1–9%) on the phase separation with respect to the volume of soybean oil are shown in Table 1. The PGPR and TGPR were used as the hydrophobic emulsifiers due to their excellent water-binding capacity. For all the concentrations of PGPR tested, no phase separation into discrete oil and water phases was observed except formulation P1. In case of TGPR, lower concentrations showed phase separation (formulations T1–T5), while at higher concentrations (formulations T6–T9), no phase separation was observed. It suggested that the inadequate emulsifier would result in coalescence between water droplets which eventually would lead to phase separation. Moreover, due to the presence of the long hydrophilic polyglycerol chain in PGPR as compared to TGPR, the lower concentrations of PGPR were sufficient to stabilize the water droplets in primary W/O emulsions. The formulations which showed no phase separation were consid-
Determination of Concentration of Hydrophilic Emulsifier  

Tween 20, a non-ionic surfactant with HLB of 16.9 was selected as hydrophilic emulsifier because it is regarded as having a high level of stability and a low level of toxicity and is widely used in the preparation of emulsions. Preliminary W/O/W emulsions with 2% (w/v) PGPR in the oil phase and different concentrations of Tween 20 (1–3% w/v) in the external water phase were prepared by high speed homogenizer, considering 0.25 as the volume fraction of the dispersed phase to continuous phase. Although the droplet size was large (more than 50–300 µm), the decrease in the droplet size was clearly observed when the concentration of Tween 20 was increased from 1 to 3% (w/v). The results were in good agreement with those showing that the droplet size decreased when the concentration of hydrophilic emulsifier in the external water phase was increased. Therefore, the concentration of Tween 20 was set at 3% w/v for the preparation of narrowly sized W/O/W microemulsions by SPG membrane emulsification technique.

Effect of Process Parameters on Mean Droplet Size and Distribution

Effect of Feed Pressure on the Dispersion Phase  
Feed pressure is known to be one of the key factors that influence the formation of emulsion and particle size distribution. By controlling feed pressure in the emulsification process, the flux rate of the dispersed phase across the membrane channel, as well as the detachment of the droplets, can be modulated for the preparation of uniform emulsion droplets. The hydrophilic SPG membrane, with an average pore size of 1.4 µm was fitted in the SPG membrane equipment. The dispersed phase tank was filled with primary W/O emulsion and the continuous phase tank was filled with distilled water containing 3% w/v Tween 20. The system was run with constant agitator speed, but the applied feed pressure was varied. The results showed that increasing the feed pressure from 50 to 90 kPa increased the z-average particle size from ca. 0.678 to 0.854 µm, respectively, as shown in Fig. 2A. This was in general agreement with the literature. The increase in feed pressure causes the growth of droplets at the pores to occur faster, and the higher pressure of the fluid inside the growing droplets allows their shape to be held better, and so they become larger before detachment. In addition, as the rate of droplet formation increases with increasing pressure, the rate of interface formation increases. However, the rate of adsorption of emulsifier remains the same and interfacial tension is reduced more slowly relative to the formation rate. The droplet size distribution was increased at high feed pressure of 90 kPa than 50 kPa, as shown in Fig. 2B. These phenomena may be either due to the collision of the dispersed phase particles with the continuous phase components thereby breaking the emulsion or the delicate force balance against the interfacial tension was broken, thereby resulting in a nonuniform size distribution of the emulsion droplets. In this case, feed pressure of 50 kPa was chosen as an optimal condition for the preparation of DOX-loaded W/O/W emulsions in further studies.

Effect of Agitator Speed  
The effect of the agitator speed was investigated using the same experimental setup. The feed pressure was set at 50 kPa, and the system was run with various agitator speeds. As shown in Figs. 3A and B, the increase in agitator speed from 150 to 450 rpm decreased the z-average particle size from ca. 0.856 to 0.648 µm, respectively, and also decreased the droplet size distribution. Although many mechanisms have been postulated for emulsion formation, the effects of agitator speed on the properties of an emulsion have not yet been clarified. However, in one of the study, it was reported that the particle size of emulsions decreased as the agitator speed increased, which was in close agreement with the present results. Thus, 350 rpm was selected as the optimal agitator speed for preparation of DOX-loaded W/O/W microemulsion, because it gave relatively uniform emulsion droplets with a narrow particle size distribution.

Based on these findings, the optimized conditions to produce an optimal uniform DOX-loaded W/O/W microemulsion using an SPG membrane were the feed pressure of 50 kPa and...
Effect of PGPR and TGPR on DOX-Loaded W/O/W Emulsion

The effect of various concentrations of PGPR and TGPR on droplet size and size distribution of DOX-loaded W/O/W emulsions were investigated. Initially, 625 mg of DOX was dissolved in 5 mL of internal water phase and various concentrations of PGPR (2–5% w/v) or TGPR (6–9% w/v) were added to 20 mL of the oil phase. Primary emulsification was carried out by using homogenization technique in order to obtain primary W/O emulsion. It was then filled into the dispersed phase tank, while the continuous phase tank was filled with 100 mL of distilled water containing 3% w/v Tween 20. Secondary emulsification was carried out under the optimized conditions using the SPG membrane emulsification technique at the feed pressure of 50 kPa and the agitator speed of 350 rpm. Figure 4 exhibited that the z-average particle size were decreased from ca. 0.690 to 0.440 µm as concentration of PGPR was increased from 2 to 5% w/v, and from ca. 0.765 to 0.610 µm as concentration of TGPR was increased from 6 to 9% w/v, respectively. Less emulsifier allows more coalescence of the droplets and this may explain the increase in droplet size.42) The lowest z-average particle size of 0.440±0.007 µm with polydispersity index of 0.220±0.087 were produced with the PGPR 5% w/v in the oil phase and 3% w/v Tween 20 in the external water phase. Moreover, the size and the spherical morphology of W/O/W microemulsion produced by SPG membrane emulsification technique were further supported by the TEM images as shown in Fig. 5. Thus, DOX-loaded W/O/W microemulsions had a narrowly sized, uniformly distributed spherical morphology.

**Encapsulation Efficiency**

The encapsulation efficiencies (EEs) of DOX-loaded W/O/W emulsions prepared with different concentrations of PGPR (between 2 and 5% w/v) or TGPR (between 6 and 9% w/v) in oil phase and Tween 20 in the external water phase are presented in Fig. 6. Increasing PGPR or TGPR concentrations resulted in higher EEs. In particular, at PGPR concentrations of 3 to 5% w/v and TGPR concentrations of 7 to 9% w/v, EEs of almost 100% were achieved. However, at PGPR concentration of 2% w/v and TGPR concentration of 6% w/v, the EEs of 76.2% and 69.0%, respectively was obtained indicating that almost 30% of the aqueous phase of the original primary emulsion was exposed to, and mixed with the external water phase during the SPG emulsification process. In addition, these multiple emulsions were unstable at low PGPR and TGPR concentrations, and resulted in a significant decrease in EE values compared to the initial EE values (data not shown). The decrease in the EE values was probably due to the rupture or expelling of the internal water droplets.43) In contrast, The EEs of W/O/W emulsions prepared with higher concentrations of PGPR (4 and 5% w/v) or TGPR (8 and 9% w/v) showed no significant change for at least three months at room temperature, indicating that DOX remained entrapped within the internal aqueous phase during the storage period.

**In Vitro Drug Release**

The nature of the surfactants in the W/O/W emulsions can affect the DOX release profiles. As depicted in Figs. 7A and B, for both types of surfactants, the releases of DOX from W/O/W emulsions were decreased...
as surfactant concentrations increased. In addition, PGPR showed more release retarding capacity than TGPR at the lower concentration. The lower concentrations of surfactants resulted in a higher initial loss of DOX, indicating that it was released immediately after formation of the W/O/W emulsions. The internal water droplets in these emulsions appear to have an insufficient amount of PGPR or TGPR and would be more susceptible to damage by shear forces during the SPG membrane emulsification process. More elaborately, the release of DOX in W/O/W emulsions with lower concentration of PGPR (2% w/v), was very rapid and reached between 50–60% within first 6h, and up to 70% within 48h. Similarly, TGPR (6% w/v) showed the fast release profile in the first 6h and the release was up to 80% within 48h. At higher concentrations of PGPR and TGPR, the release of DOX was slower and in a sustained manner. The increased hydrophobic emulsifier concentration increase the viscosity in the oil phase, which might form the strong membrane, and slow down the water permeation coefficients, and the internal water droplets are more resistant to rupture or coalescence. On the contrary, the free DOX solution under similar experimental conditions released around 70% and 95% in 1 and 4h, respectively, which indicated immediate release of DOX as compared to the W/O/W emulsion. Since the dialysis membrane itself acted as the barrier for the drug release, the DOX released from the solution was extended upto 4h.

In order to assess the exact mechanism of drug release from the W/O/W emulsions, the rate of dissolution was calculated using Korsmeyer–Peppas equations, given below as follows:

\[
\frac{M_t}{M_i} = k t^n \\
\log \left( \frac{M_t}{M_i} \right) = \log k + n \log t
\]

where \(M_t/M_i\) is the fraction of drug dissolved at time \(t\), \(k\) is a kinetic constant and \(n\) is the release exponent which is an indication of the dissolution mechanism. The release exponent \(n\) value indicated that the mechanism of drug release was zero-order, non-Fickian, and Fickian (Higuchi model) whenever \(n=1\), \(0.5<n<1\) and \(n=0.5\), respectively. Our results showed that most \(n\) values were between 0.5 and 1, suggesting that the drug was released from the microemulsion system by non-Fickian, “anomalous” mechanism.

**Pharmacokinetic Evaluation** Figure 8 shows the mean plasma concentration–time profiles of DOX after intravenous administration of DOX solution and DOX-loaded W/O/W emulsions to rats at the dose of 5mg/kg DOX. It has been mentioned that the particle sizes of the W/O/W emulsions for the intravenous (i.v.) administration were recommended to be kept under 7µm in diameter in order to allow free flow of the particles in the blood vessels. Our formulation had the particle size of ca. 0.440µm which was considered suitable for the i.v. route of administration. The total plasma concentrations of DOX after administration of W/O/W emulsions were...
significantly higher than free DOX solution at \( p < 0.05 \), and maintained about 7 µg/mL up to 12 h. The corresponding non-compartmental pharmacokinetic parameters are listed in Table 2. The pharmacokinetic parameters of DOX-loaded W/O/W emulsions were significantly different from that in the DOX solution.46) The W/O/W emulsions gave a significantly higher \( AUC \) and hence ca. 17-fold better bioavailability compared to the DOX solution. The enhanced bioavailability of DOX might be due to the sustained release of DOX from the W/O/W microemulsion. Moreover, the MRT, \( t_{1/2\alpha} \), and \( K_{el} \) values of DOX from the W/O/W emulsions were significantly different from those of the DOX solution \( (p<0.05) \). Microemulsion formulations have distinct advantages when delivered parenterally because of the fine particle microemulsion is cleared more slowly and, therefore have a longer residence time in the body. Other advantages are that they exhibit a higher physical stability in plasma than liposomes and the internal oil phase is more resistant against drug leaching.48–50) Our results suggest that DOX-loaded W/O/W multiple emulsions would be useful for delivering DOX in a pattern that allows sustained release for a long time, leading to better bioavailability.

**Conclusion**

DOX-loaded W/O/W microemulsions were produced by Shirasu Porous Glass (SPG) membrane using W/O emulsions prepared by homogenization as feed emulsions. The optimized formulation was composed of 0.5% w/v DOX, 5% w/v PGPR and 3% w/v Tween 20 as sole hydrophobic and hydrophilic emulsifier with respect to the volume of oil phase of the primary W/O emulsion and the outer aqueous phase of the W/O/W microemulsion, respectively. The formulation showed the sustained release characteristic, prolonged the plasma drug concentration after intravenous administration and gave about 17-fold higher \( AUC \) compared to DOX solution. Thus, the SPG membrane emulsification technique could be used as the promising technique to prepare W/O/W microemulsions,
which could be effective for delivering anticancer agents with sustained release characteristics and better bioavailability.

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