Evaluation of the Formation Conditions of a Spontaneous Emulsification Using Porous Silica Particles

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Abstract: Emulsions are colloid dispersions which are attractive for use as drug carriers due to their simple structure and facile preparation. However, their low physicochemical stability has been problematic. In order to solve this problem, a spontaneous emulsification technique composed of porous silica particles has been developed. In this study, we investigated the conditions for effective formation of protein-encapsulated solid-in-oil-in-water (S/O/W) emulsions using this technique. Porous silica particles having a hydrophilic surface promoted the formation of a fine and uniform emulsion. It was found that the progression of emulsification was affected by electrolytes in aqueous solution. Moreover, it was confirmed that the S/O/W emulsion prepared using this method could successfully encapsulate protein.

Key words: spontaneous emulsification, porous silica particle, lipophilic surfactant, hydrophilic surfactant, protein

1 Introduction

Currently, a variety of new drugs are being developed, from low molecular weight drugs produced by chemical syntheses to biopharmaceuticals by biosynthesis. Therefore, the development of carriers for efficient drug delivery in the body has become important. Drug carriers serve roles in maintenance of drug activity, control of drug pharmacokinetics, and effective delivery to disease sites. Emulsions, which are dispersions composed of water and oil, are one of the most popular types of particles employed as drug carriers¹. They are generally formed via mechanical or physicochemical methods².

Mechanical methods, including high-speed, high-pressure, and ultrasonic homogenization, which generate high shear stress are generally employed³⁵⁷. In recent years, membrane emulsification⁶ and microchannel emulsification⁷ have also been used for the preparation of emulsions with narrow size distributions. Comparatively, phase-transfer, D-phase, and self-emulsification etc. are employed as physicochemical methods⁸¹⁳. When emulsions prepared by these techniques are employed as drug carriers, physicochemical stability is needed for high drug transport efficiency and effectiveness. In order to achieve this, techniques including: 1) reduction of interfacial tension through addition of a surfactant, 2) increased electrostatic repulsion between particles through increased zeta potential, 3) increased viscosity of the continuous phase, and 4) inhibition of Ostwald ripening through control of fine particle size have been frequently employed.

Alternatively, spontaneous emulsification has been used to solve emulsion instability, enabling emulsification in solution at the target site (e.g., the small intestine via oral administration)¹²⁷. Using this method, emulsions can be formed immediately before use. This method generates liquid emulsion formulations having stability and portability. Emulsions formed spontaneously, such as those acting in the small intestine via oral administration of an oil, are referred to as self-emulsifying drug delivery systems (SEDDSs)¹³¹⁷. When a mixture of an oil and hydrophilic surfactant containing lipophilic drugs is added to water, a fine emulsion is formed. Then, an increase in the emulsion surface area occurs, producing an increase in lipophilic drug solubility, resulting in the promotion of adsorption by intestinal mucosa. Another spontaneous emulsification method involves dry emulsions¹⁸²⁰. Dry emulsions are formed by spray or freeze drying fine oil-in-water (O/W) emulsions containing hydrophilic or hydrophobic substances in an outer aqueous phase. When the formed solid particles are added to water, oil droplets are immediately released and fine O/W emulsions are reconstructed. Dry
emulsions can contain liquid emulsions in solid form and have been employed as oral administration carriers for lipophilic and hydrophilic drugs.

Recently, we developed a unique, spontaneous emulsification technique using porous silica particles. This method results in facile, instantaneous formation of thermodynamically unstable emulsions. Previous studies have shown that this emulsification method has great potential for the preparation of solid-in-oil-in-water (S/O/W) emulsions for oral peptide delivery. S/O/W emulsions have been employed to improve the absorption of protein drugs in the gastrointestinal tract. Establishing emulsion formation conditions using this method is important for developing highly reproducible and versatile formulations. Therefore, in this study, the necessary conditions for efficient emulsion formation using porous silica particles were estimated.

### 2 Experimental Procedures

Silica particles with a size distribution of 75–150 μm were provided by Fuji Silysia Chemical Ltd. (Kozoji-cho, Kasugai Aichi, Japan). The mean pore size of the two types of particles were 46.9 (Q-50) and 94.1 nm (Q-100). The silica particles were hydrophobized with KP-18C (Shin-Etsu Chemical Co., Tokyo, Japan). Emulsification using the silica particles was performed as follows: 1) a sample of soybean oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with or without surfactants was used as the oil phase; 2) sucrose erucic acid ester (ER-290), provided by Mitsubishi-Chemical Foods Co. (Tokyo, Japan), was used as a lipophilic surfactant, and polyoxyethylene sorbitan monooleate (Tween 80), from Tokyo Chemical Industry Co. (Tokyo, Japan), was used as a hydrophilic surfactant to promote the formation of dispersions of fine oil droplets; 3) the oil phase (100 μL) was introduced to the surface of dried silica particles (100 mg) and allowed to adsorb into the pores; and 4) phosphate buffer (pH 6.8, 9.9 mL) or distilled water (9.9 mL) was poured into a glass tube containing the silica particles and oil phase. The concentration of ER-290 was set to 20 wt%, which allowed the protein to be stable in the oil phase.

Emulsification properties were estimated using O/W emulsions without protein. The size of released oil droplets was measured using a laser diffraction particle size analyzer (SALD-7100; Shimadzu, Kyoto, Japan). Span values were calculated using the following equation:

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}}$$

where $D_{90}$, $D_{50}$, and $D_{10}$ are the highest droplet sizes containing 90, 50, and 10% of the volume of oil particles in the emulsion, respectively.

Emulsion release behavior of the silica particles was estimated through the turbidity of phosphate-buffered saline (PBS) after addition of the silica particles. Turbidity was measured at $\lambda = 600$ nm using a UV/vis spectrophotometer (ASV11D; AS ONE Corp., Osaka, Japan).

Protein encapsulation into the S/O/W emulsion prepared using this method was analyzed by confocal laser scanning microscopy. FITC-labeled albumin (FITC-ALB) was used as a model protein. Albumin (from bovine serum) was obtained from Sigma-Aldrich (Tokyo, Japan). Protein-encapsulated oil phase (S/O suspension, shown in Table 1, was used for preparation of this S/O suspension. Phosphate buffer (pH 6.8, 9.9 mL) was poured into a glass tube containing silica particles with this suspension. Then, S/O/W emulsion formation was observed using a confocal laser scanning microscope (LSM 5 Pascal; Zeiss Co., Tokyo, Japan).

### Table 1 Emulsification properties using hydrophilic versus hydrophobic silica particles.

| Oil phase composition [wt%] | Particle size [μm] (Span value) |
|-----------------------------|--------------------------------|
| Soybean oil | ER-290 | Tween 80 | Hydrophilic | Hydrophobic |
| a | 100 | 0 | 0 | 3.92 (2.34) | × |
| b | 90 | 0 | 10 | 0.58 (1.52) | × |
| c | 80 | 0 | 20 | 0.67 (1.92) | × |
| d | 60 | 0 | 40 | 0.72 (1.84) | 1.20 (2.56) |
| e | 80 | 20 | 0 | × | × |
| f | 70 | 20 | 10 | 5.41 (1.87) | 2.10 (5.08) |
| g | 60 | 20 | 20 | 0.43 (1.87) | 1.45 (4.50) |
| h | 40 | 20 | 40 | 0.36 (1.96) | 0.32 (1.85) |

× No emulsification occurred.
3 Results and Discussion

3.1 Effects of the surface properties of silica particles on spontaneous emulsification

In a previous article, hydrophilic silica particles with hydroxyl groups on their surface were used for emulsification. It has also been reported that emulsion formation using porous silica particles was inhibited or promoted using lipophilic or hydrophilic surfactant additives, respectively. In this study, the emulsification properties of hydrophilic and hydrophobic silica particles are compared. Table 1 shows the mean diameters and span values of emulsions formed in PBS using silica particles with each oil-phase composition. Lower span values represent narrower particle size distributions.

When soybean oil was used as the oil phase, no oil droplets were released from the hydrophobic silica particles compared to the hydrophilic ones. When hydrophilic surfactant Tween 80 was added to the oil, emulsification of hydrophilic silica particles progressed well; however, emulsification did not proceed with hydrophilic silica particles unless a large amount of surfactant was added. When lipophilic surfactant was added to the oil phase, emulsification did not occur for either silica particle. However, emulsification did proceed upon the addition of hydrophilic surfactant. The emulsions formed using the hydrophilic silica particles tended to be finer and more uniform than those formed using hydrophobic particles (Fig. 1). The hydrophobic silica particles were disadvantageous for water penetration into pores due to their low surface wettability. Therefore, it was concluded that the water uptake ability of the surfactant greatly affects the emulsification of silica particles.

3.2 Emulsification conditions of the oil phase with lipophilic surfactant

When hydrophilic silica-particle encapsulated soybean oil and lipophilic surfactant ER-290 were added to PBS, emulsification did not proceed. However, emulsification did occur when the mixture was added to pure water.

In order to clarify this phenomenon, the effect of salt concentration in water on emulsification was evaluated. Silica particles containing soybean oil or soybean oil and ER-290 were added to PBS, and the effect of NaCl concentration on emulsification was evaluated. Emulsification behavior was confirmed by turbidity, and the progress of emulsification was evaluated as a relative ratio of the turbidity of the emulsion solution prepared without NaCl (Fig. 2). As the NaCl concentration increased, the relative turbidity decreased. This shows that emulsification was suppressed when the NaCl concentration was increased. Additionally, this emulsification-inhibiting effect by NaCl was noticeable when lipophilic surfactant was used. Next, emulsification behavior was compared in aqueous solutions containing electrolytes NaCl and MgCl₂, and non-electrolyte glucose (Fig. 3). It was found that emulsification was inhibited in aqueous solutions containing electrolytes having higher charges. From these results,

Fig. 1 Particle size distributions of emulsions formed using: (i) hydrophilic and (ii) hydrophobic silica particles; oil phase composition: (●) f, (□) g, and (▲) h (listed in Table 1).

Fig. 2 Emulsification behavior in aqueous solutions with different NaCl concentrations. Composition of oil phase: Oil/ER-290/Tween80 (wt%) = (●) 80/20/0, (■) 100/0/0.
it was expected that when a cationic substance is present in water, it becomes a binder between the hydrophilic group \(\text{sucrose}\) of ER-290 and hydroxyl groups on the silica surface, suppressing the approach of water into the silica pores.

3.3 Effects of the addition method of hydrophilic surfactant on emulsification

In aqueous solutions containing salts, such as physiological fluids, lipophilic surfactant-contained oil phase release from silica particles is suppressed. As lipophilic surfactants are always used in formulations of S/O/W emulsions, it becomes important to use a hydrophilic surfactant to promote emulsification and stable emulsion formation. Thus, the effect of hydrophilic surfactant concentration on the size of emulsion oil droplets within silica particles was evaluated (Fig. 4). In this evaluation, silica particles having mean pore sizes of 100 or 50 nm were used. It was shown that particle diameter decreased as the amount of hydrophilic surfactant added increased. The same trend appeared regardless of the pore size of the particles. Moreover, it was found that Tween 80 concentrations \(> 20\) wt\% promoted the formation of fine emulsions. For comparison, the same evaluation was performed using hydrophilic surfactant alone in aqueous phase. There was no significant difference in the particle size even when the concentration of hydrophilic surfactant in water was increased. Therefore, it was concluded that fine emulsion formation is promoted by adding hydrophilic surfactant to the oil phase.

3.4 Formation of S/O/W emulsions using silica particles

Finally, the formation of S/O/W emulsions was estimated using an oil phase containing complexed FITC-ALB and ER-290. It is important to form S/O/W emulsions of encapsulated proteins in oil droplets after release of the emulsion from silica particles for applications in oral protein delivery. Emulsions released in PBS were observed with a confocal laser scanning microscope (Fig. 5). It was observed that the interiors of the oil droplets strongly fluoresced. This shows that emulsions containing albumin in the oil phase were formed; in other words, formation of a S/O/W emulsion.

4 Conclusion

In this study, the formation conditions of fine S/O/W emulsions using porous silica particles were examined. Use of hydrophilic, porous silica particles promoted the intro-
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Conflict of Interest Declaration

The authors declare no conflicts of interest.

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