Development of a Novel Spontaneous Emulsification Method for Peptide Delivery Using Porous Silica Particles

Eiichi Toorisaka* and Yumi Nonaka

Department of Environment Science and Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube-shi, Yamaguchi 755-8611, JAPAN

Abstract: A new emulsification technique using porous silica particles was studied as a facile and instantaneous formation method for thermodynamically unstable emulsions. In this study, oil encapsulated in silica particles was released instantly upon the addition of a phosphate buffer, forming an oil-in-water (O/W) emulsion. Emulsion formation was inhibited or promoted using lipophilic or hydrophilic surfactant additives, respectively. We concluded that this phenomenon is affected by the wettability of the soybean oil on the silica surface, which is controlled by the surfactant. We prepared submicron size emulsions in a simple method involving the addition of the silica particles to an aqueous solution. This spontaneous emulsification technique could be applied to the formation of solid-in-oil-in-water (S/O/W) emulsions for oral delivery of hydrophilic peptide medicine.

Key words: spontaneous emulsification, silica particle, lipophilic surfactant, hydrophilic surfactant, peptide delivery

*Correspondence to: Eiichi Toorisaka, Department of Environment Science and Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube-shi, Yamaguchi 755-8611, JAPAN
E-mail: torisaka@yamaguchi-u.ac.jp

Accepted October 13, 2017 (received for review August 31, 2017)

NOTE
was introduced to the surface of the dried silica particles (100 ng) and allowed to adsorb onto the pores. A phosphate buffer (pH 6.8, 9.9 mL) was poured into a glass tube containing the silica particles with the oil phase. The size of the released oil droplets was measured using a laser-diffraction particle-size analyzer (Shimadzu, SALD-7100, Japan). The emulsification properties were estimated using O/W emulsions (without the peptide). Moreover, the peptide-releasing property of the S/O/W emulsion prepared using this method was analyzed. An FITC-labeled insulin (FITC-INS) was used as a model peptide. Insulin (from bovine pancreas) was obtained from Sigma–Aldrich (Japan). The silica-encapsulated oil phase (S/O suspension containing 1 mg/mL FITC-INS) was prepared using a previously reported procedure. The silica particles were added to the stomach-fluid (pH 1.2) or small-intestine fluid (pH 6.8) model. These fluids were stirred (100 rpm) at 37.0°C, and an aliquot was periodically withdrawn. The aqueous phase was separated by filtering the aliquot. The fluorescence due to the FITC-INS in the filtrate was detected using a fluorometer (exc. 488 nm; em. 530 nm, JASCO, FP-8300, Japan).

The oil phase was encapsulated into the silica particles using capillary action. The maximum encapsulation volume was estimated by analyzing the slide angle to entrap a sufficient amount of the suspension (data not shown). The slide angle rapidly increased when the suspension content was greater than 48%, indicating that the particles failed to entrap the additional volume of the suspension. When the added oil exceeded the volume of the pore space, the extra oil adhered to the surface of the silica particles. Based on this result, silica particles containing a 48% suspension were chosen for further examination of the emulsifying properties.

In this study, the porous silica particles were used to form the oil-in-water emulsion. The advantage of this emulsification technique is that the emulsion can be prepared simply by adding the silica particles into an aqueous solution. Figure 1 shows the PBS solution after adding the silica particles containing the soybean oil, oil with the lipophilic surfactant ER-290, and oil with the hydrophilic surfactant Tween 20 in addition to ER-290. When the silica particles containing only the oil were used as the emulsifying agent, the PBS solution gradually became turbid (Fig. 1a). By observing the silica particles under a microscope, we found that the oil droplets had been released from the surface of the silica (Fig. 1d). In contrast, when the lipophilic surfactant was contained in the oil, the oil droplets did not completely release (Fig. 1b). Moreover, as the hydrophilic surfactant was added into the oil, the oil droplets released (Fig. 1c). This result suggests that because the wettability of the water to the silica surface was higher than that of oil, the oil was stirred and forced out by the permeation of water into the pores of the silica particles.

We also believe that the lipophilic surfactant additive, ER-290, promoted the binding of the soybean oil with the silica surface (hydroxyl groups). In contrast, we believe that the hydrophilic surfactant, Tween 80, promoted the release of the soybean oil and enhanced the formation of oil droplets. We conclude that, when the oil phase contains a hydrophilic surfactant, the emulsification is spontaneous in the pores of the silica particles.

The emulsification method using porous silica particles was compared with our general method. In addition, the effect of the oil-phase composition of the resulting emulsions was analyzed. Table 1 lists the mean diameters of the emulsified particles prepared using silica emulsification, self-emulsification, and homogenization. Figure 2 shows the particle-size distribution of the emulsions. The homogenization was conducted at 6500 rpm for 2 min. After adding the oil to the buffer, the self-emulsification was conducted by stirring the mixture at 100 rpm. When the self-emulsification and homogenization techniques were applied to the oil without the hydrophilic surfactant, an...
emulsion did not form. Furthermore, the self-emulsification technique required a higher concentration of the hydrophilic surfactant compared with that of the silica emulsification. Interestingly, in the addition of 20% Tween 80, the oil droplets were significantly finer in the silica emulsification than in the homogenizer emulsification. Moreover, finer oil droplets were formed as the concentration of the hydrophilic surfactant increased. This finding demonstrates that the addition of the hydrophilic surfactant to the oil phase promoted spontaneous emulsification in the pores of the silica particles.

Finally, the insulin-release behavior of the S/O/W emulsion formed using the silica particles was analyzed in the simulated-stomach fluid or small-intestine fluid (Fig. 3). The oil of composition C shown in Table 1 was used to prepare the S/O/W emulsion. In the simulated stomach fluid, the insulin (FITC-INS) was slightly removed from the emulsion. However, in the simulated intestinal fluid, the insulin was barely released. Moreover, a rapid release via the degradation of emulsion oil phase was observed in the sample containing intestinal fluid with lipase. This result demonstrates that the S/O/W emulsion retained its property in the case of the emulsification using the silica particles. We anticipate that the pores within the silica particles are useful for the formation of fine S/O/W emulsions as shown in Fig. 4.

In the present study, a novel emulsification method was
developed using porous silica particles. Using this method, a facile and instantaneous preparation of emulsions without mechanical aid was possible. The new method results in the formation of fine emulsions which are advantageous in applications in which stability is a concern. In particular, this emulsification method has great potential in the preparation of S/O/W emulsions for oral peptide delivery because the resultant products can promote the adsorption of peptides in the small intestine.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number JP 26630431.

References

1) Jafari, S.M.; Assadpoor, E.; He, Y.; Bhandari, B. Re-coalescence of emulsion droplets during high-energy emulsification. Food Hydrocoll. 22, 1191-1202 (2008).
2) Gaikwad, S.G.; Pandit, A.B. Ultrasound emulsification: effect of ultrasonic and physicochemical properties on dispersed phase volume and droplet size. Ultrason. Sonochem. 15, 554-563 (2008).
3) Santana, R.C.; Perrechil, F.A.; Cunha, R.L. High- and low-energy emulsifications for food applications: a focus on process parameters. Food Eng. Rev. 5, 107-122 (2013).
4) Prasert, W.; Gohtani, S. Effect of sucrose on phase/polyoxyethylene sorbitan fatty acid ester (Tween xx)/vegetable oil systems and food nano-emulsification using low-energy methods. J. Food Eng. 168, 119-128 (2016).
5) Anton, N.; Vandamme, TF. The universality of low-energy nano-emulsification. Int. J. Pharm. 377, 142-147 (2009).
6) Wakisaka, S.; Nishimura, T.; Gohtani, S. O/W nano-emulsion formation using an isothermal low-energy emulsification method in a mixture of polyglycerol polyricinoleate and hexaglycerol monolaurate with glycerol system. J. Ole Sci. 64, 405-413 (2015).
7) Komaiko, J.; McClements, D.J. Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. J. Food Eng. 146, 122-128 (2015).
8) Gursoy, R.N.; Benita, S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharma. 58, 173-182 (2004).
9) Leonaviciute, G.; Bernkop-Schnürch, A. Self-emulsifying drug delivery systems in oral (poly)peptide drug delivery. Expert Opin. Drug Deliv. 12, 1703-1716 (2015).
10) Toorisaka, E.; Ono, H.; Arimori, K.; Kamiya, N.; Goto, M. Hypoglycemic effect of surfactant–coated insulin solubilized in a novel solid-in-oil-in-water (S/O/W) emulsion. Int. J. Pharm. 252, 271-274 (2003).
11) Toorisaka, E.; Hashida, M.; Kamiya, N.; Ono, H.; Kokazu, Y.; Goto, M. An enteric-coated dry emulsion formulation for oral insulin delivery. J. Controlled Release 107, 91-96 (2005).