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Chitosan-Gelatin Nanoemulsions Prepared by SPG (Shirasu Porous Glass) Membrane Process and Their Characteristics

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Abstract

In the present study, chitosan-gelatin nanoemulsions (CGN) were prepared by Shirasu Porous Glass (SPG) membrane process. The continuous phase (oil phase) consisted of soybean oil and Tween 80, the dispersed phase (water phase) consisted of 0.1% chitosan and 0.1 % gelatin solution. Microscopic observations indicated that the emulsions with mean diameter (Dm) of 300 and 500 nm were reasonably formed. The mean diameter of CGN basically depended upon the SPG membrane pore diameter, meanwhile it was linearly correlated to the amount of Tween 80 with a correlation constant of 0.93-0.99. The CGN prepared with pore diameter (D_p) of 300 and 500 nm, there were great differences in emulsion viscosity when compared to those of control, especially, the CGN prepared with D_p=500 nm was maintained the same level of emulsion viscosity during storage of 9 days.

Key words: chitosan-gelatin, nanoemulsion, SPG membrane, droplet size, stability

Introduction

Protein and polysaccharides play a key role in the structuration and stabilization of food systems through their gelling, thickening, and surface-stabilizing functional properties. Proteins possess generally good emulsifying and foaming properties due to their amphoteric structure and their ability to interact with each other. Chitosan, a polycationic polysaccharide derived from the natural polymer chitin, forms polyelectrolyte complexes with polyanionic polymers such as alginate. Chitosan is used widely especially for pharmaceuticals, food products, cosmetics and biomedicals because of its biological properties including biocompatibility (e.g. non-toxic, biodegradable, natural), bioactivity, reduced blood cholesterol levels and immune system stimulant effect (Knorr, 1982; Knapczyk, Krowczynski, Krzek, Brezeski, Nurnberg, Schenk and Struszcyk, 1989; Miyazaki, Yamaguchi and Takada, 1990). During recent years, there has been an increasing interest in the use of such chitosan microspheres as mucoadhesive drug delivery system, especially for nasal and peroral delivery of peptide drugs, in order to improve the drug absorption (Lueben, Leeuw, Langemeÿer, Boer, Verhoef and Junginger, 1996). Chitosan micro- or nanospheres can be prepared by a modified emulsification/ separation method and loaded such as alginate, gelatin and polyethylene oxide (Chithambra, Sunny and Jayakrishnan, 2002). Chitosan-coated alginate microspheres containing a lipophilic marker dissolved in an edible oil, were prepared by emulsification/internal gelation and the potential use as oral controlled release system investigated (Ribeiro, Neufeld, Arnaud and Chaumeil, 1999). Chitosan microcapsules were prepared by a water/oil emulsion techniques as controlled release systems for insulin (Aiedeh, Gianasi, Orienti and Orman, 1997). Large (>100 μm) and small (<10 μm) chitosan which were prepared by an emulsion method were used to deliver via different routes the anti-inflammatory drug (Acikgoz, Kas, Orman and Hincal, 1996), the antineoplastic agent (Jameela and Jayaktishnan, 1995), the anticancer agents (Akbuga and Durmaz, 1994) and other active materials (Hassan, Parish and Gallo, 1992). Nanoemulsions are colloidal system that the droplet size is nano-meter order, and are transparent or semitransparent

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solution. However, most emulsification process such as microfludizers, homogenizers, and stirred vessels are consumed a high energy and high shear. Recently, low shear and energy saving membrane emulsification process have emerged as a handy and size-controllable technique for treating shear-sensitive ingredients or surfactants. One of those, SPG (Shirasu Porous Glass) membrane process is known which possesses a uniform pore size distribution with a free choice of the nominal pore size ranging from 100~1,800 nm (Omi, Ma, and Nagai, 2000). In the present study, chitosan-gelatin nanoemulsions were prepared by SPG membrane process, using different size of SPG tubes and various amounts of Tween 80. In addition, the physicochemical properties of these nanoemulsions were determined and evaluated in order to apply to food and nutraceutical ingredient as a stabilizer.

Materials and Methods

Materials

Chitosan was prepared by the following steps such as demineralization, deproteinization and deacetylation from crab shell. After that, for the preparation of the water-soluble chitosan, the chitosan (degree of deacetylation 93%, molecular weight 330,000) was dissolved in the mixed aqueous solution of 20% hydrochloride solution and distilled water, and after the mixture was adjusted pH to 5.6, it was stirred for 3hrs. And then, the reaction mixture subjected to a continuous membrane filtration process, and the permeate freeze- dried (Yu, Park and Hong, 1999). Casein and gluten were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soy protein isolate (SPI 500E) was purchased from Protein Technologies International (St. Louis, MO, USA), and gelatin (bloom 380) was purchased from Kyonggi Gelatin (Yongin, Korea). Soybean oil was purchased from Dongbang Oil Co. (Incheon, Korea) and Tween 80 was purchased from Sigma Chemical Co. (St. Louis, MO, USA), were used as the dispersion (oil) phase.

SPG membrane emulsification apparatus

The membrane emulsification apparatus was assembled as illustrated in Fig. 1. The continuous phase circulates outside of the SPG membrane tube in the vessel. The dispersion phase permeates from interior of the SPG tube under nitrogen gas pressure. The dimensions of the SPG tubes used were 10 mm outer diameter, 8 mm inner diameter and pore diameter, D_p=300 and 500 nm. The vessel volume for dispersion phase is 200 ml and the allowance of maximum pressure is 3.9 MPa. The circulating pump for continuous phase is a magnetic pump (MD-15FY-N, Iwaki Co., Tokyo, Japan), which has a maximum capacity of 10 *l*/min and total head of 3~4 m.

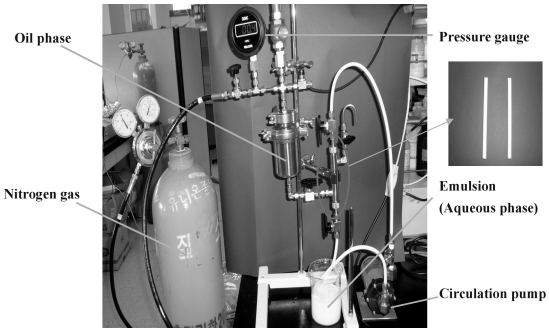

Fig. 1. Schematic outline of SPG membrane process.

Preparation of the chitosan-gelatin nanoemulsion (CGN)

Before emulsification, the SPG tube was pre-soaked in the continuous phase in an ultrasonic bath for 30 min. Then the SPG tube was set in the apparatus. The dispersion phase was delivered into the SPG tube and, after discharge of the air from the interior of the SPG tube, the continuous phase was circulated from the vessel to SPG tube. The permeant pressure of the dispersion phase was gradually increased and dispersion phase was emulsified into the continuous phase by passage through the membrane. For the continuous phase, 0.1% (w/w) chitosan was mixed into 0.1% (w/v) gelatin, and 3% (w/v) NaCl was added to the mixture to achieve a monodispersion. The mixture of soybean oil and Tween 80 (5~9%, w/v) was used as the dispersion phase.

Preparation of emulsion by the rotor-stator system

The continuous phase containing 0.1% (w/w) chitosan and 0.1% (w/v) gelatin, and the dispersion phase, containing soybean oil and Tween 80 (10%, w/v), were placed in a

cup of rotor-stator system (Ultra-Turrax T25, Janke & Kunkel, Germany), and the solutions were emulsified at 2,500 rpm for 3 min.

Observation of nanoemulsions by visible light microscope

The emulsions were observed using an DMRB light microscope (Leica Microsystems, Wetzlar, Germany) equipped with a image analysis system.

Analysis of droplet size

Emulsion droplet size distributions of CGN were analyzed using a laser diffraction instrument (Granulometer 1064, USA). 10ml of emulsion was diluted in deionized water and circulated through the measuring zone. Results are presented in terms of mean diameter and coefficient of dispersion (a). The a can be used as an index of monodispersion and defined as follows:

$$\alpha = S_d / S_p$$

where S_d is a standard deviation of the droplet diameter and S_p is the average droplet diameter of an emulsion.

Viscosity stability of emulsion

The viscosity of the emulsion was measured using a rotational viscometer (Brookfield Engineering Laboratories, INC, DVII+, MA, USA) during storage with a No. 2 spindle at 25°C for 9 days.

Results and Discussion

Preparation of CGN and droplet size

The CGN were prepared using SPG membrane process with D_p=300 and 500 nm, and the control emulsion were prepared by rotor-stator system. Physical properties of CGN prepared at different concentrations of Tween 80 and SPG membrane pore size in the rotor-stator system are shown in Table 1. The mean diameter of CGN were increased with increasing amounts of Tween 80 and that the CGN are dependent on the D_p of the membrane. It can be seen that the α values of CGN were 0.004~0.04, so it was evident that each emulsion was monodispersed. Generally, the smaller the α values, the more monodispersed emulsions, and one could defined monodispersed emulsion for a equal or smaller than 0.35 (Katoh, Asano, Furuya, Sotoyama and Tomita, 1996). Microscopic views of CGN and control emulsions prepared with three different amounts of Tween 80 (5, 7 and 9%), are shown in Fig. 2. Microscopic observations indicated that the emulsions with D_p=300 and 500 nm were reasonably formed. The droplet size of CGN was remarkably uniform and smaller than compared with that control (A) obtained by the rotor-stator system. Especially, the CGN (B) prepared with 9% addition of Tween 80 showed the highest concentration of the emulsion, as observed in Fig. 2. In membrane emulsification, the effect of the wall shear stress on reducing emulsion is appeared at the different membrane pore sizes, being

Table 1. Physical properties of chitosan-gelatin nanoemulsion and control emulsions depend on the concentration of Tween 80 by rotor-stator system and Shirasu porous glass membrane process

Process	Tween 80 (%)	Diameter at 10%(nm)	Diameter at 50%(nm)	Diameter at 90%(nm)	Mean diameter (nm)	α*	Specific surface(cm^2/g)
Rotor-stator	5	1,360	8,240	20,910	9,870	0.02	19479.4
	7	1,390	9,420	20,950	10,280	0.007	18426.8
	9	1,350	8,890	21,310	10,320	0.009	30207.2
SPG membrane 300 nm	5	590	2,420	6,330	3,240	0.004	47593.1
	7	590	2,670	10,230	4,330	0.005	39376.7
	9	1,040	2,690	18,500	4,700	0.08	44713.7
SPG membrane 500 nm	5	960	2,930	16,810	6,340	0.04	29821.1
	7	1,100	5,810	17,430	7,570	0.006	23562.5
	9	1,190	9,360	16,800	8,950	0.01	21772.6

*index of monodispersion

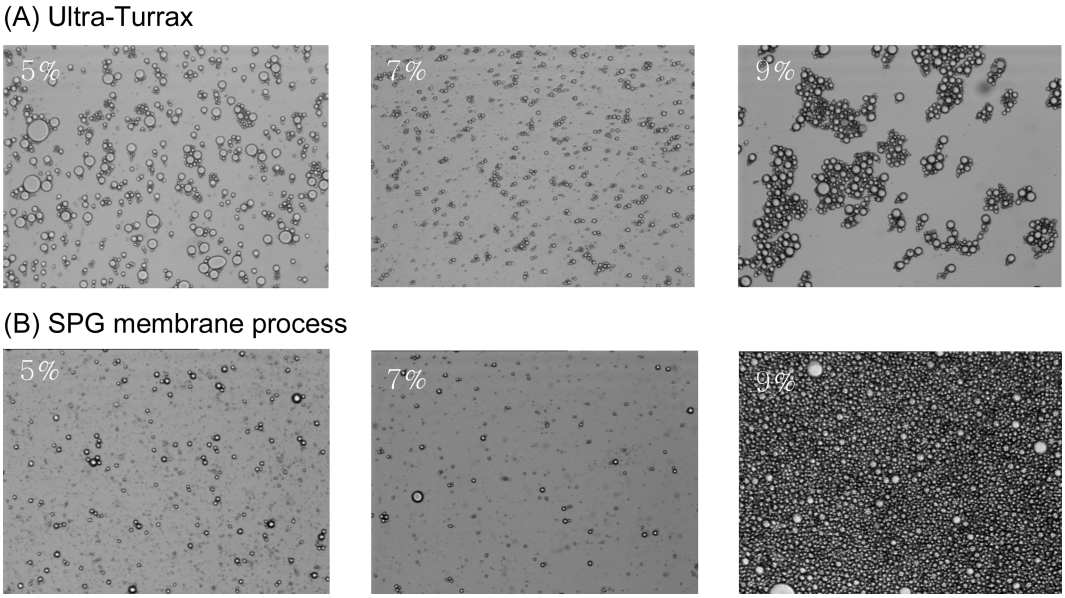

Fig. 2. Micrographs emulsions at the different concentration of Tween 80.

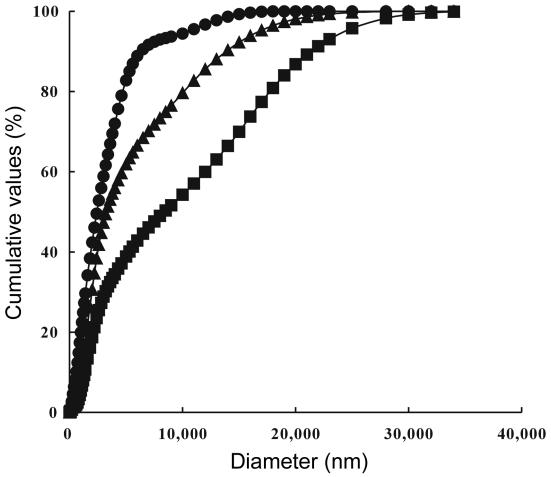

Fig. 3. The overlay curves for drop size distribution of CGN mixture prepared by rotor-stator system and SPG membrane process at 9% concentration of Tween 80: ■; rotor-stator, ●; D_p=300 nm, ▲; D_p=500 nm.

more effective for smaller membrane pore sizes (Schröder and Schubert, 1997). Fig. 3 shows the droplet size distributions of CGN and control prepared by the SPG membrane and the rotor-stator system. The mean diameter of the emulsions prepared by the SPG membrane were smaller than control within a narrow size distribution that they are depend on the pore diameter of the membrane.

Relationship between mean diameter and Tween 80

The relationship between the mean diameter of CGN and the amount of Tween 80 is shown in Fig. 4, a straight lines with a correlation coefficient of 0.93 and 0.99 for D_p=300 and 500 nm, were plotted respectively. It was found that this relationship is in agreement with the results reported by Asano and Sotoyama (Asano and Sotoyama, 1999). The diameter of emulsion droplet basically depends upon the membrane pore diameter.

Viscosity stability of the CGN and control emulsion

The viscosity stability of CGN and control emulsion for the 9 day storages are exhibited in Fig. 5. In the CGN prepared with D_p of 300 and 500 nm, there were a little differences in emulsion viscosity when compared to those of control, especially, the CGN prepared with D_p=500 nm was maintained the same level of emulsion viscosity during storage of 9 days. For control emulsion, 3 days after emulsification the viscosity decreased rapidly to below half level of stability with a D_p 500 nm. In the preparation of food emulsion using a membrane emulsification, a stable monodispersed emulsion could be prepared even with the droplet diameter of 500 nm or more (Asano and Sotoyama, 1999). Generally, the emulsion

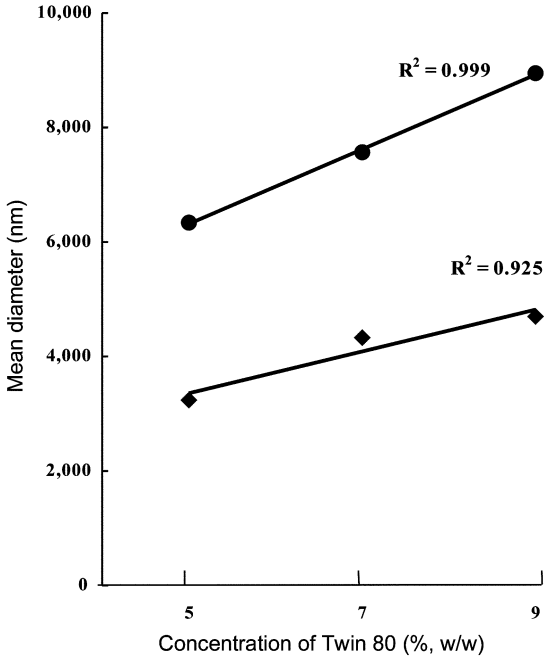

Fig. 4. The relationship between mean diameter of CGN and the concentrations of Tween 80 in the emulsions.◆; D_p=300 nm, ●; D_p=500 nm.

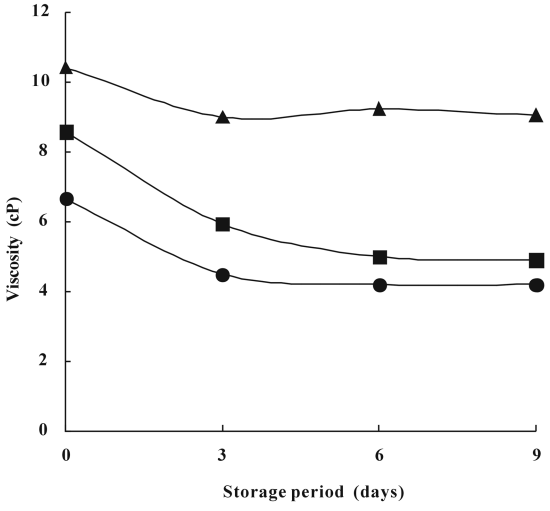

Fig. 5. Viscosity stability of CGN and control emulsions prepared by rotor-stator system and SPG membrane: ● ; CON, ■; D_p=300 nm, ▲; D_p=500 nm.

with a large D_p have inferior stability. Floury *et al.* (16) have suggested that the emulsion viscosity is strongly depend on the continuous phase viscosity but seem to be independent on the oil droplet sizes. As a result, we believe that the SPG membrane process will become a very useful means of obtaining stable and high quality nanoemulsion. At the same time, this technique seems to be applicable for design and development of functional nanospheres.

Conclusions

Nanoemulsions or microemulsions of chitosan-gelatin polymer can be prepared by the SPG membrane and the rotor-stator system. The size of the emulsions depend on the pore diameter of membrane as well as on the concentration of Tween 80. Especially, the emulsion formed uniformly without droplets collapsed as a stable monodispersed emulsion at 9% of Tween 80 concnetration. The viscosity stability of emulsion, sample prepared with D_p=500 nm showed a better stability than D_p=300 nm and control. In order to apply for food and biomaterial field as a functional nanospheres, the next step such as process optimization, encapsulation technique of emulsion and delivery characteristics should be investigated continuously.

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