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Preparation and properties of chitosan microspheres based on polyglycerol polyricinoleate stabilized emulsions

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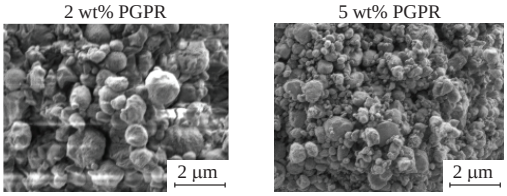
Chitosan microspheres were prepared by an emulsification–crosslinking technique with polyglycerol polyricinoleate (PGPR) as a surfactant to stabilize the pre-emulsions. The effect of a ratio between the components of the 2 wt% solution of acetic acid–PGPR–paraffin oil system on the properties of the pre-emulsion and microspheres was estimated.



Drug delivery systems for oral administration of therapeutic proteins and peptides, such as biodegradable nano- and microparticles, microcapsules and microspheres attract great attention because they can protect the active substance from degradation in the gastrointestinal tract. A desired release profile of protein drugs *in vivo* could be obtained.[1–5] Chitosan-based carriers, in particular, chitosan microspheres are of considerable interest due to their biocompatibility, biodegradability and mucoadhesive properties.[6–10] Conventionally, a simple water-in-oil (W/O) emulsification-crosslinking technique was used to obtain chitosan microspheres.[11–16]

The required properties of the drug carriers such as the efficiency of encapsulation and the profile of drug release in the small intestine can be regulated by changing the size, polydispersity and surface area of the microspheres particles.[17,18] These microsphere characteristics depend on the dispersity and aggregative stability of pre-emulsions. However, the relationships between the physicochemical properties of pre-emulsions and the final characteristics of particles as possible carriers for the delivery of therapeutic agents are scantily known.[19,20]

In this work, the chitosan microspheres were prepared by emulsion crosslinking with polyglycerol-6-polyricinoleate-15 (PG-6-PR) as a surfactant to stabilize the pre-emulsions. Polyglycerol polyricinoleates are nonionic surfactants with a low hydrophilic–lipophilic balance (HLB), which are used in the food industry to produce W/O emulsions. The relationship between the structure, stability and dispersity of the emulsions,† and their influence on the characteristics of chitosan microspheres were investigated.

† Emulsions were prepared by the addition of PG-6-PR (Hexaglyn PR-15, HLB 3.2, Nikko Chemicals) to paraffin oil (Pionier, Hansen & Rosenthal) followed by the incorporation of a 2% acetic acid solution with magnetic stirring (200 rpm) at 60 °C. Both phases were separately preheated up to the same temperature. The systems were homogenized (Ace Homogenizer model AM-11, Nihonseiki Kaisha) at 1500 rpm and room temperature for 5 min. Thereafter, the produced emulsions were magnetically stirred at 60 °C for 1 h.

Figure 1 shows the stability diagram of the 2 wt% solution of acetic acid–PG-6-PR–paraffin oil three-component system. Four different regions were observed: kinetically stable; unstable, in which a continuous oil layer was formed on top of W/O emulsion; creaming; and unstable, in which the oil and water phases were separated. The series of emulsion formulations (Figure 1, solid and dashed lines) were selected to examine the emulsion properties and to prepare the chitosan microspheres.

The effect of surfactant concentration (Figure 1, solid line) on the stability and dispersity of the emulsions and the characteristics of microspheres was studied. The surfactant concentration was varied from 2.0 to 5.0 wt% at a water-to-oil ratio of 8 : 2. The

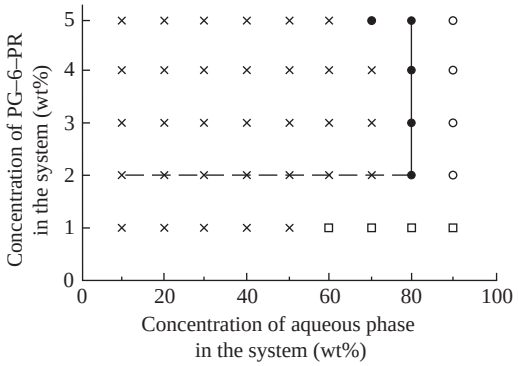


Figure 1 Stability diagram of the 2% solution of acetic acid–PG-6-PR–paraffin oil system. To construct the diagram, the freshly prepared emulsion samples were filled in glass tubes and stored at 60 °C for 24 h. Their stability (●) and the creaming (○) or separation of an excess oil phase (×) or both oil and water phases (□) in the emulsion were estimated visually.

The freshly prepared emulsions were stored for five days at room temperature to separate an excess oil phase. Then, the ratio between the height of the emulsion layer and the total height of the liquid column was defined for each emulsion sample. Based on the obtained data, the concentrations of the dispersed phase in equilibrium emulsions were calculated. The excess oil phase was decanted and dispersion analysis of the samples was carried out.

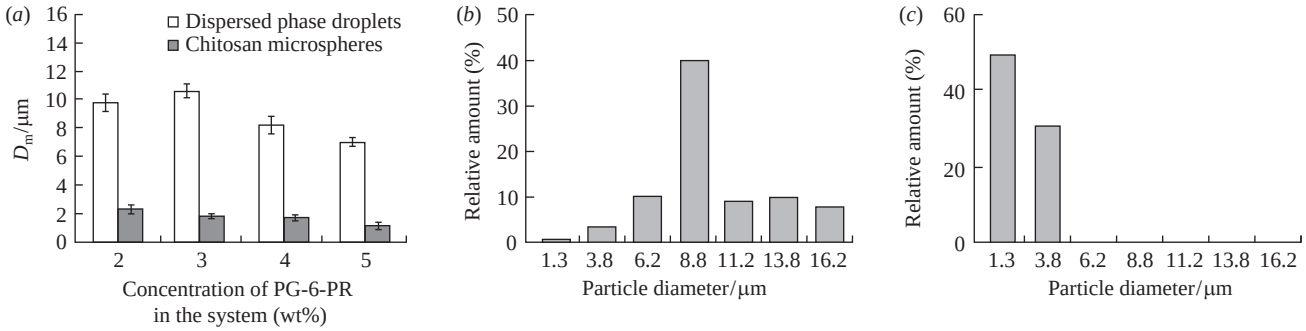


Figure 2 (*a*) Effect of surfactant concentration in the system (water-to-oil ratio 8 : 2) on the mass-average diameter of pre-emulsion droplets and chitosan microspheres; (*b*) the droplet size distribution of pre-emulsion (PG-6-PR 2.0 wt%) and (*c*) the size distribution of chitosan microspheres.

emulsions were kinetically stable and a dispersed phase concentration was 77.2 vol%. The droplet size in the equilibrium emulsions decreased with growing the concentration of PG-6-PR due to a decrease in the coalescence intensity [Figure 2(*a*)].‡ It was owing to a three-dimensional network structure formed by dispersed phase droplets covered with a durable layer of the adsorbed surfactant and the increased viscosity of a continuous phase caused by the formation of micelles and micellar aggregates (Figure S1, see Online Supplementary Materials).

Chitosan microspheres§ showed similar spherical shapes and a high degree of aggregation [Figure 3(*a*),(*b*)]. The increase in the surfactant concentration resulted in a decrease in the average diameter of particles and in the pre-emulsion droplet size [Figure 2(*a*)]. Average sizes of microspheres (1–2 μm) were much less than those of the pre-emulsion droplets (7–11 μm). This can be explained by the fact that the chitosan macromolecules adsorbed on the aqueous acetic acid solution/paraffin oil interface to create colloidal-adsorption layers with a gel structure that stabilizes the droplets of the dispersed phase.[17,21] This is also evidenced by a narrower particle size distribution of chitosan microspheres compared with that of the pre-emulsion drops [Figure 2(*b*),(*c*)].

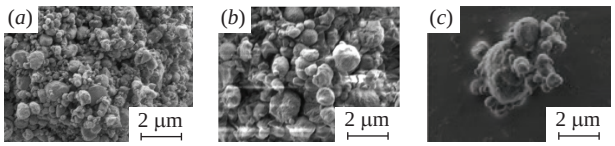


Figure 3 SEM micrographs of chitosan microspheres prepared at different water-to-oil ratios and PG-6-PR concentrations (wt%) in the system: (*a*) 8 : 2, 5.0, (*b*) 8 : 2, 2.0, (*c*) 2 : 8, 2.0, respectively.

‡ Particle size and size distribution of the emulsion droplets and microspheres were determined by a Biomed-2 optical microscope equipped with a Scopetek DCM 130 camera. Mass-average diameters of particles (D_m/μm) were calculated using the equation: $D_m = \Sigma n_i D_i^4 / \Sigma n_i D_i^3$, where n_i is the number of particles with diameter D_i.

§ The microspheres were prepared by a modified method reported by Varshosaz *et al.*[16] Chitosan, (deacetylation, 82%; number-average molecular weight M_W = 200 kDa) was provided by Bioprogress. The chitosan solution (in 2% acetic acid) with a concentration of 10 g dm^{-3} was used as an aqueous phase. An aqueous solution of the crosslinking agent citric acid was added dropwise to emulsion samples with stirring at 60 °C. Then, the stirring was continued at the same temperature for 5.5 h. The chitosan-to-crosslinker mass ratio was 3 : 1. The as-prepared suspensions were stored at room temperature for five days to let the microspheres settle down. The top oil layer was decanted and microspheres were collected and washed with *n*-hexane four to six times followed by centrifugation at 6000 rpm for 10 min. Thereafter, the microspheres were dried in air. Finally, the product was washed with dichloromethane (3 × 50 ml) and stored in an oven at 60 °C for 1 h.

The shape and surface morphology of the chitosan microspheres were analyzed by scanning electron microscopy (Tescan MIRA 3 electron microscope) at an accelerating voltage of 1 kV. The samples of the microspheres were previously dried *in vacuo*.

The influence of the water-to-oil ratio (Figure 1, dashed line) on the properties of the pre-emulsions and the chitosan microspheres was evaluated. The concentrated emulsions were obtained at a water concentration of 30–80 wt% in the three-component system (Figure S2, see Online Supplementary Materials). At a high dispersed phase concentration, the emulsion droplets formed a three-dimensional particle network. At a low concentration, the sedimentation and the subsequent coalescence of water droplets occurred until the system reached the equilibrium. Therefore, the decrease in the water mass fraction of the system led to an increase in the droplet size during the storage of emulsions [Figure 4(*a*)].

The dependence of the average diameter of the chitosan microspheres [Figure 3(*b*),(*c*)] on the water-to-oil ratio in the system

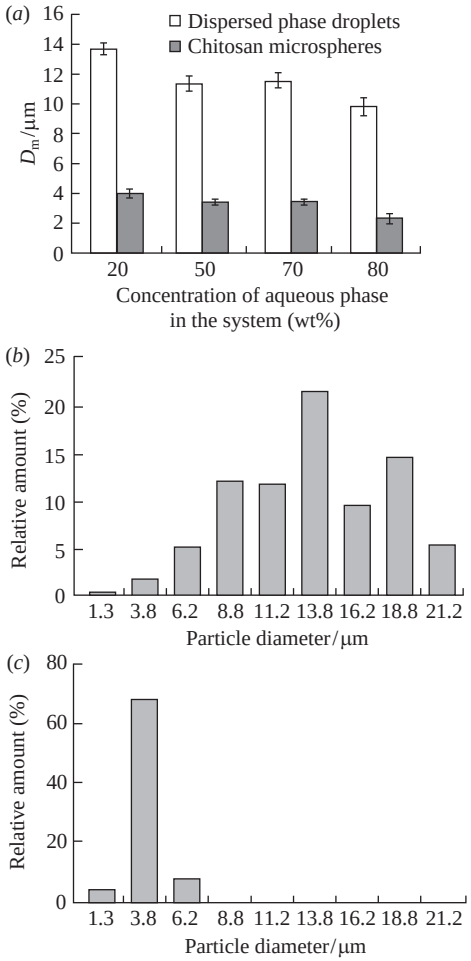


Figure 4 (*a*) Effect of the aqueous phase concentration (wt%) in the system (PG-6-PR 2.0 wt%) on the mass-average diameter of pre-emulsion droplets and chitosan microspheres; (*b*) the droplet size distribution of pre-emulsion (PG-6-PR 2.0 wt%, water-to-oil ratio 2 : 8) and (*c*) the chitosan microspheres size distribution.

correlated with that of average pre-emulsion droplet size on the same factor [Figure 4(*a*)]. Due to the formation of the colloidal-adsorption layers of chitosan at the water/oil interface that hindered the coagulation and coalescence of droplets, the size and polydispersity of the microspheres were significantly lower in comparison with those of the pre-emulsion drops (see Figure 4).

The formation of the network of droplets in the emulsions prepared at the high concentrations of water and PG-6-PR in the system led to the aggregation of chitosan microspheres during the separation and drying processes.

The particle surface area suitable for protein adsorption decreases due to the aggregation. Therefore, despite the fact that the high content of the aqueous phase and the surfactant in the system makes it possible to obtain particles with smaller sizes, we concluded that the use of lower water-to-oil ratios and PG-6-PR concentrations is preferable for the preparation of chitosan microspheres.

The study of the influence of the component ratio in the test system on the stability, dispersity and rheological properties of pre-emulsions allowed us to determine conditions for the preparation of chitosan microspheres with controllable sizes, polydispersity and specific surface areas. The regulation of these properties of microspheres is a factor responsible for the degree of sorption and the rate of protein release.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.01.023.

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