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Preparation and Characterization of β-Carotene Nanodispersions Prepared by Solvent Displacement Technique

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This work demonstrated the preparation of protein-stabilized β-carotene nanodispersions using the solvent displacement technique. The emulsifying performance of sodium caseinate (SC), whey protein concentrate (WPC), whey protein isolate (WPI), and a whey protein hydrolysate (WPH, 18% degree of hydrolysis) was compared in terms of particle size and ζ-potential of the nanodispersions. SC-stabilized nanodispersions exhibited a bimodal particle size distribution: large particles (stabilized by casein micelles) with a mean particle size of 171 nm and small particles (stabilized by casein submicelles) of 13 nm. This was confirmed with transmission electron microscopy analysis. Most of the β-carotene precipitated (87.6%) was stabilized in the small particles. On the other hand, the nanodispersions stabilized by the whey proteins were polydispersed with larger mean particle sizes. The mean particle size of WPC and WPI was 1730 and 201 nm, respectively. The SC-stabilized nanodispersion was expected to be more stable as indicated by its higher absolute ζ-potential value (−31 mV) compared to that of WPC (−15 mV) and WPI (−16 mV). Partially hydrolyzed whey protein possessed improved emulsifying properties as shown by WPH-stabilized samples. It was interesting to note that increasing the SC concentration from 0.05 to 0.5 wt % increased the particle size of β-carotene stabilized by casein micelles, while the reverse was true for those stabilized by SC submicelles. Microfluidization at 100 MPa of SC solution dissociated the casein micelles, resulting in a decrease in mean particle size of the casein micelle-stabilized particles when the SC solution was used to prepare nanodispersions. The results from this work showed that protein-stabilized β-carotene nanodispersions could be prepared using the solvent displacement technique.

KEYWORDS: β-Carotene; nanodispersion; sodium caseinate; solvent displacement

INTRODUCTION

Nanoparticles and nanodispersions have received much attention in the pharmaceutical industry over the past decade. This is mainly due to the ability of the nanoparticles and nanodispersions to control drug release and distribution as well as reduce the inherent limitations of slow and incomplete dissolution of water-insoluble drugs (*1*). In the food industry, nanodispersions of water-insoluble active compounds are attractive food ingredients as they enable more food formulations and improve the bioavailability of the active compounds (*2*). With a particle size in the nanometer range nanodispersions are physically more stable compared to the conventional dispersions which contain particles in micrometer sizes.

Solvent displacement, sometimes known as nanoprecipitation, is perhaps the simplest method for preparation of the nanodispersions of water-insoluble bioactive compounds. The method generally consists of mixing an aqueous phase containing an emulsifier with a water-miscible organic solvent such as ethanol or acetone. The organic solvent usually consists of the dissolved bioactive compound, a polymer, and optionally oil and a lipophilic emulsifier. Nanoparticles are formed instantaneously by rapid diffusion of the organic solvent in the aqueous phase, which induces interfacial nanodeposition of the bioactive compound at the interface between the organic and aqueous phases (*3*,*4*). The polymer, which diffuses together with the organic solvent, is stranded at the interface and incorporated with the bioactive compound. The emulsifier in the aqueous phase stabilizes the nanoparticles and prevents aggregation of the particles. The organic solvent is then evaporated from the nanodispersion under reduced pressure. Although the usefulness of the solvent displacement technique is limited to water-miscible organic solvents and it may be difficult to choose a suitable organic solvent for the process, the technique allows preparation of nanodispersions in one step at low energy input with a high yield of encapsulation (*1*, *3*, *5*).

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More often than not polymers such as poly(D,L-lactide) and poly(D,L-lactide-co-glycolide) are used to incorporate with the bioactive compound in the nanoparticles for controlled drug release purposes (*1*, *6*). The type and concentration of polymer used not only influence the drug release time but also have a significant effect on the particle size, surface charge, and polydispersity of the nanoparticles (*1*, *6*). For food applications in which controlled release of the bioactive compounds is not necessary, polymer can be omitted from the formulation. While many works have been done on production of drug nanoparticles using the solvent-displacement technique, information on preparation of bioactive compound nanodispersions for food applications without the presence of polymer is relatively scarce.

Particles in a nanodispersion are stabilized by an emulsifier or a combination of emulsifiers. There are a large variety of emulsifiers used in the food industry, and milk proteins are among the most important emulsifiers. The success of milk protein emulsifiers lies on their ability to adsorb on an oil−water interface rapidly, and they prevent droplet coalescence mainly by electrostatic repulsion (*7*). The surface activity and flexibility of the protein molecules, which allows them to unfold and spread at an interface, are important properties contributing to the emulsifying capacity of the protein. The order of surface activity for various milk protein components is β-casein > serum albumin > α-lactoalbumin > α_s-casein > κ-casein > β-lactoglobulin (*8*).

In this study, β-carotene nanodispersions were prepared without the presence of polymer. β-Carotene was chosen as a model for a water-insoluble bioactive compound since it is a common food ingredient and widely used in food formulation. The main focus of this work was to correlate the changes in the particle size and ζ-potential of the nanodispersions with their composition. Transmission electron microscopy (TEM) analysis was also undertaken for a selected formulation. The information on the physicochemical properties would contribute to development of active compound nanodispersions prepared by the solvent displacement technique.

MATERIALS AND METHODS

Materials. Sodium caseinate (SC), β-carotene, and sodium azide were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Commercial-grade whey protein concentrate (WPC, 34% protein purity) and whey protein isolate (WPI, 92−95% protein purity) were provided by Behn Meyer Foodtech Sdn. Bhd., Kuala Lumpur, Malaysia, while whey protein hydrolysate (WPH, degree of hydrolysis 18.1%, 96% protein purity) was contributed by Fonterra (NZ) Ltd., Auckland, New Zealand. Deionized water purified by a Milli-Q Organex system (Millipore, Bedford, MA) was used for preparation of the aqueous phase. All other chemicals used were of analytical grade.

Preparation of the β-Carotene Nanodispersions. Nanodispersions of β-carotene were prepared by modifying the method described by Fessi et al. (*9*), based on interfacial nanodeposition of β-carotene at the interface of acetone and aqueous phase after solvent displacement. The organic phase, which was acetone containing 0.015 wt % β-carotene, was added drop-by-drop to the aqueous phase consisting of 1 wt % protein and 0.02 wt % sodium azide in 0.05 M phosphate buffer, pH 7, under magnetic stirring. The ratio of organic phase to aqueous phase was set at 1:9 by volume. Acetone was evaporated off, and the nanodispersion was concentrated 5 times using a rotary evaporator (Eyela NE-1101, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Another series of experiments was carried out to study the effect of protein concentration by varying SC concentration from 0.001 to 5 wt %. Nanodispersions were also prepared with high-pressure homogenized SC solutions by subjecting the SC solutions to a microfluidizer (model M-110EH Microfluidizer Processor, Microfluidic Corp., Newton) operated at 100 MPa for 1−3 passes prior to the solvent displacement process. Nanodispersions of various β-carotene concentrations (0.006−0.015 wt % in acetone) were also produced to study the influence of β-carotene concentration.

Particle Size Analysis. Measurement of the mean particle diameter of the nanodispersions was done using a dynamic light scattering particle size analyzer which has a measuring range from 0.6 nm to 6 μm (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, U.K.). The refractive index for β-carotene of 1.47 (*10*) and water of 1.33 was used to calculate the particle size. The particle concentration in the sample was diluted to about 0.005 wt % with 0.05 M phosphate buffer (pH 7) prior to analysis to avoid multiple-scattering effect during measurement. The absorbance of nanodispersion particles was set at 0.3 (colored particle at 633 nm), and the temperature was 25 °C. The final particle diameter was calculated from the average of at least three measurements. The coefficient of variation, CV, of the particle size distribution was calculated from the standard deviation and mean value of the particle size. Measurement of the particle size was also carried out on the supernatant of the nanodispersions after centrifuging the samples at 10 000*g* for 30 min.

Zeta-Potential Measurement. The ζ-potential values of the nanodispersions were measured using the Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, U.K.), which measures the distribution of the electrophoretic mobility of particles with a size range from 3 nm to 10 μm using the laser doppler velocity technique. Since the ζ-potential is related to the electrophoretic mobility of the particles, the analyzer calculates the ζ-potential from the measured velocity using the Smoluchowski equation. The temperature during measurement was set at 25 °C, while the pH of the nanodispersions during the measurement was pH 7.

TEM Analysis. The noncentrifuged nanodispersion prepared with 1 wt % SC and 0.015 wt % β-carotene was used in TEM analysis for observing the microstructure and particle size distribution of the β-carotene particles. The sample was prepared using both freeze−fracture replica and resin embedding methods. For the freeze−fracture method the surface of the fractured sample was coated with a platinum layer followed by a carbon layer in vacuum. The metal atoms were applied at 45° to the fractured surface. For the resin embedding method sample preparation involved mixing the sample with 2% molten agar at a 1:1 (v/v) ratio and hardening the agar at room temperature. The agar was then cut into 1 × 1×1 mm cubes before fixing in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7) for 1 h at 4 °C. The sample was washed using 0.1 M phosphate buffer (pH 7), postfixed in 2% osmium tetraoxide for 2 h, and rewashed before it was dehydrated using a series of ethanol solutions (50%, 60%, 70%, 80%, 90%, 95%, and 100%). The sample was then embedded in Spurr's epoxy resin and incubated at 70 °C for 12 h. The harden resin block was cut into ultrathin sections (ca. 80 nm) using an ultramicrotome (LKB Ultratome V, LKB, Bromma, Sweden). The ultrathin sections were doubled stained with 2% uranyl acetate and Sato's lead solution. Morphologic examination of β-carotene particles was performed using a JEOL-1010 TEM (JEOL, Tokyo, Japan) working at an accelerating voltage of 80 kV.

Statistical Analysis. Statistical analysis was carried out for the experimental data by a one-way analysis of variance procedure using the SAS software package release 6.1 (*11*). The significant difference ($P < 0.05$) between means was further determined by Duncan's multiple range test. All of the reported values were the means of at least four measurements from two experiment replications.

RESULTS AND DISCUSSION

When the organic phase was added to the aqueous phase during preparation of β-carotene nanodispersions by the solvent displacement technique, a rapid interfacial spreading/turbulence was observed as a result of the mutual diffusion between the two phases. Davies and Rideal (*12*) suggested that the interfacial turbulence was caused by localized lowering of interfacial tension where the organic phase underwent rapid and erratic pulsations. The energy necessary for these jerky movements came from the free energy released as the solvent was redistributed to its equilibrium state (*3*). During the interfacial

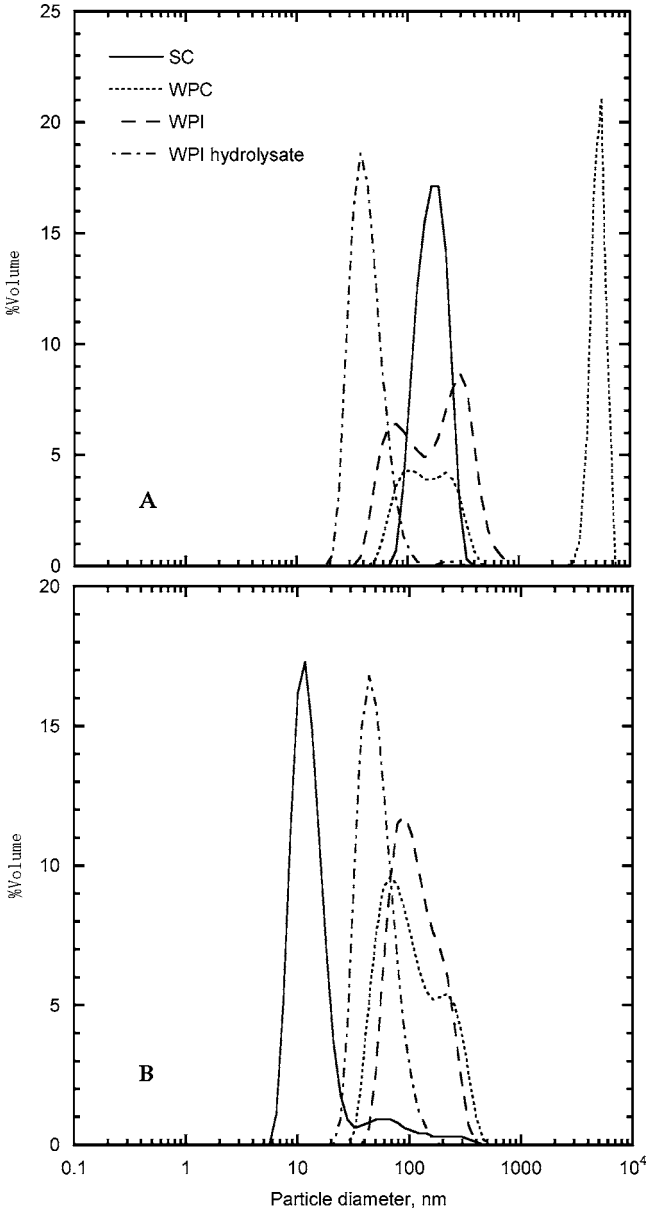

Figure 1. Particle size distribution of β-carotene nanodispersion prepared with sodium caseinate (SC), whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein hydrolysate (A) before and (B) after centrifugation at 10 000*g* for 30 min. The nanodispersions were prepared with 1 wt % protein solution.

turbulence acetone, which had a lower surface tension, moved to the aqueous phase. When the acetone and aqueous phase were mixed, two liquid flows were created instantly. The two flows enveloped each other and the boundary layers became thinner and thinner during the mixing process. The boundary layers of acetone were the sites of maximum β-carotene supersaturation, and nucleation and crystal growth took place. The solid β-carotene particles precipitated were then stabilized by SC through hydrophobic interactions. The affinity of the protein toward β-carotene was crucial for formation of the nanoparticles. In light of this proposed mechanism, it was expected that the characteristics of the protein had a profound effect on the particle size of β-carotene nanodispersions.

Influence of Protein Type. Figure 1 compares the particle size distribution profiles of β-carotene nanodispersions prepared with SC, WPC, WPI, and WPH. In this study, at first the dynamic light scattering particle size analyzer showed that the SC-stabilized β-carotene nanodispersion exhibited a monomodal particle size distribution with a mean value of 171 nm (Figure 1A). However, after centrifuging the nanodispersion at 10 000*g* for 30 min, the supernatant was found to contain mostly particles with a mean diameter of 13 nm (Figure 1B). These observations indicated that, in fact, SC solution possessed a bimodal particle size distribution: the small particles were the SC submicelles, and the larger ones would be mainly the casein micelles that were not dissociated. The β-carotene precipitated during solvent displacement was stabilized by both the SC submicelles and the casein micelles. By measurement of the β-carotene concentration 87.6% of the β-carotene was stabilized by the submicelles while the remaining was by the casein micelles. The results were in agreement with the findings that a SC solution showed coexistence of predominantly submicellar species with a mean hydrodynamic diameter of about 10 nm and a small amount of supramolecular assemblies or casein micelles of >100 nm (*13*, *14*). SC is generally prepared from milk casein by either dialysis or precipitation at its isoelectric point (pH 4.6). This is followed by washing and adjusting the pH to neutral using sodium hydroxide. In these processes, most of the calcium and phosphate ions, which bind the casein submicelles together in casein micelles, are removed. As a result, the caseins exist mainly as submicelles in solution. Some of the casein micelles were not fully dissociated into the submicelles due to the residual calcium phosphate ions. The submicelles remained held together by colloidal calcium phosphate, which corresponded to the existence of large particles (mean particle size 171 nm) in the nanodispersion. During solvent displacement both the SC submicelles and casein micelles underwent conformational changes to maximize favorable interactions with the β-carotene precipitates and stabilized the nanoparticles. Since the aggregation process of the SC submicelles was similar to micelle formation in solutions of small molecule surfactants (*15*), the β-carotene particles were most likely being dynamically stabilized by the submicelles through hydrophobic interactions. As for the casein micelles, the β-carotene precipitates might be incorporated in the structure of the casein micelles during the dynamic micellization of the caseins.

Meanwhile, as shown in Figure 1A, WPC-stabilized nanodispersion exhibited a wide particle size distribution with a mean particle size of 1730 nm and CV 141%, partly due to the low protein content in WPC (34% protein purity). The β-carotene particles aggregated since there was insufficient protein available for stabilizing the β-carotene particles. The presence of extraneous especially the much bigger fat globules also caused the nanodispersion to become polydispersed as a whole. WPI had higher protein purity (>90%), and the nanodispersion prepared with WPI had a significantly ($P < 0.05$) smaller mean particle size (201 nm) and narrower size distribution (CV 66%) compared to that of WPC (Figure 1A). WPI-stabilized nanodispersions showed a bimodal particle size distribution, probably due to the heterogeneity of WPI in terms of molecular weight and lower structural flexibility compared to that of caseins. The molecular weights of the major whey protein fractions α-lactoalbumin, β-lactoglobulin, immunoglobulins, and serum albumin are 14, 18, 15−1000, and 69 kDa, respectively (*16*). Proteins with larger molecular weight were expected to be more rigid and form larger β-carotene particles. Being partially hydrolyzed the protein resulted in smaller molecular weight and improved protein flexibility. The β-carotene nanodispersion stabilized by WPH (18% degree of hydrolysis) in which 97% of the protein composition had a molecular weight <5 kDa

exhibited a monomodal particle size distribution and a mean particle size of 45 nm (Figure 1A). Centrifugation of the WPC- and WPI-stabilized nanodispersions removed the large particles, particles with a mean size of 126 and 127 nm remaining, respectively (Figure 1B). Their respective particle size distribution became narrower, but no new particle population was observed as in the case of SC-stabilized nanodispersion. As for the nanodispersion stabilized by WPH, centrifugation did not change its particle size profile and mean particle size much.

The interfacial membranes formed by proteins are electrically charged, and therefore, the major mechanism preventing particle aggregation in protein-stabilized dispersions is electrostatic repulsion (*7*). A minimum ζ-potential of ±30 mV is required for a nanodispersion to remain physically stable if the emulsifier is stabilizing the nanodispersion by electrostatic repulsion (*17*). The ζ-potential of β-carotene particles was −31 mV, suggesting that the SC-stabilized nanodispersion was stable against aggregation. The polar regions of the caseins are dominated by phosphoseryl residues and thus carry a large net negative charge (*8*). For example, α_{s1}- and β-caseins have a net negative charge of −22*e* and −14*e*, respectively, at neutral pH (*18*). Similar to the caseins, whey proteins also have net negative charge. However, the sequence distribution of polar and charged residues of whey proteins is rather uniform. Consequently, these proteins fold intramolecularly, thereby burying most of their hydrophobic residues (*8*). The net negative charge of α-lactalbumin and β-lactoglobulin is −10*e* and −2*e*, respectively (*8*). This was the reason why the whey proteins-stabilized β-carotene nanodispersions had significantly ($P < 0.05$) lower surface charge than the nanodispersions stabilized by SC. The ζ-potential of the WPC- and WPI-stabilized nanodispersions was −15 and −16 mV, respectively. Partially hydrolyzed the protein unfolded the protein structures and exposed some of the polar regions, as indicated in the slight increase in ζ-potential of the WPH-stabilized nanodispersion (−17 mV). Due to its potential in stabilizing the β-carotene nanodispersion, SC was used as the emulsifier to study other factors affecting the characteristics of the nanodispersion.

Influence of SC Concentration. Figure 2A shows the influence of SC concentration in the particle size distribution profile of the β-carotene nanodispersions without centrifugation. While the critical micelle concentrations (CMC) of pure β- and κ-caseins were reported to be 0.05% and 0.2−0.5%, respectively (*19*), the CMC of SC in this study determined by the pendant-drop interfacial tension method was 0.5 wt %. Below the CMC the casein micelles might dissociate and exist as monomeric structures or casein assemblies. A small portion of the β-carotene precipitated during solvent displacement was stabilized by these casein assemblies, which had a mean particle size of 56-63 nm (Figure 2A). As the SC concentration was raised to its CMC (0.5 wt %) the caseins self-associated to form micelles and the β-carotene particle size increased significantly ($P < 0.05$) to 137 nm. Increasing the SC concentration to 1 wt % resulted in the increase in β-carotene particle size to 171 nm. While a certain number of casein monomers (ca. 20−50) were needed to form a complete structure of a micelle, more caseins were available at 1 wt % SC to be adsorbed onto the surface the β-carotene particles and/or form complete micelle structure. Nanodipersions prepared with higher SC concentration (>1 wt %) did not significantly ($P > 0.05$) increase the β-carotene particle size since the surface of the particles was saturated with caseins. More micelles were formed, rather than growth in particle size of the individual micelles, when the CMC of SC was exceeded (*20*).

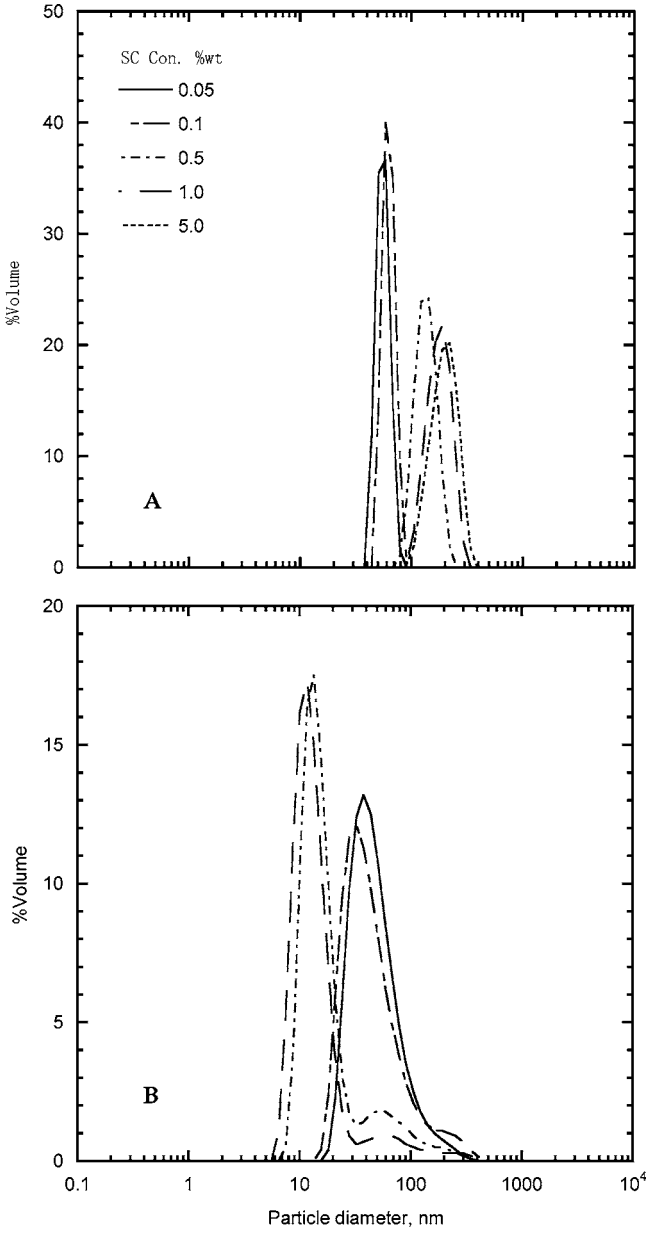

Figure 2. Effect of sodium caseinate concentration on the particle size distribution of β-carotene nanodispersion (A) before and (B) after centrifugation at 10 000*g* for 30 min.

Figure 2B depicts the particle size profile of β-carotene particles in the supernatant after centrifuging the nanodispersions at 10 000*g* for 30 min, which represents the fraction of the nanodispersions stabilized by the SC submicelles. At concentrations below the CMC of SC the mean particle size of β-carotene particles was 55−56 nm (Figure 2B). Due to the insufficiency of SC the β-carotene particles stabilized by SC submicelles aggregated to form larger particles. Increasing the SC concentration to 0.5 wt % significantly ($P < 0.05$) decreased the mean particle size to 15 nm, and this value remained almost unchanged with further increase in SC concentration. The results demonstrated that the SC submicelles behaved like small-molecule emulsifiers, in contrast to the casein micelles, when responding to changes in SC concentration.

Influence of Microfluidization. There was much evidence that high-pressure treatments (100−250 MPa) caused a more translucent appearance of milk and a reduction in the average casein micelle diameter, resulting from the casein micelle dissociation (*21*, *22*). Figure 3A depicts the β-carotene nano-

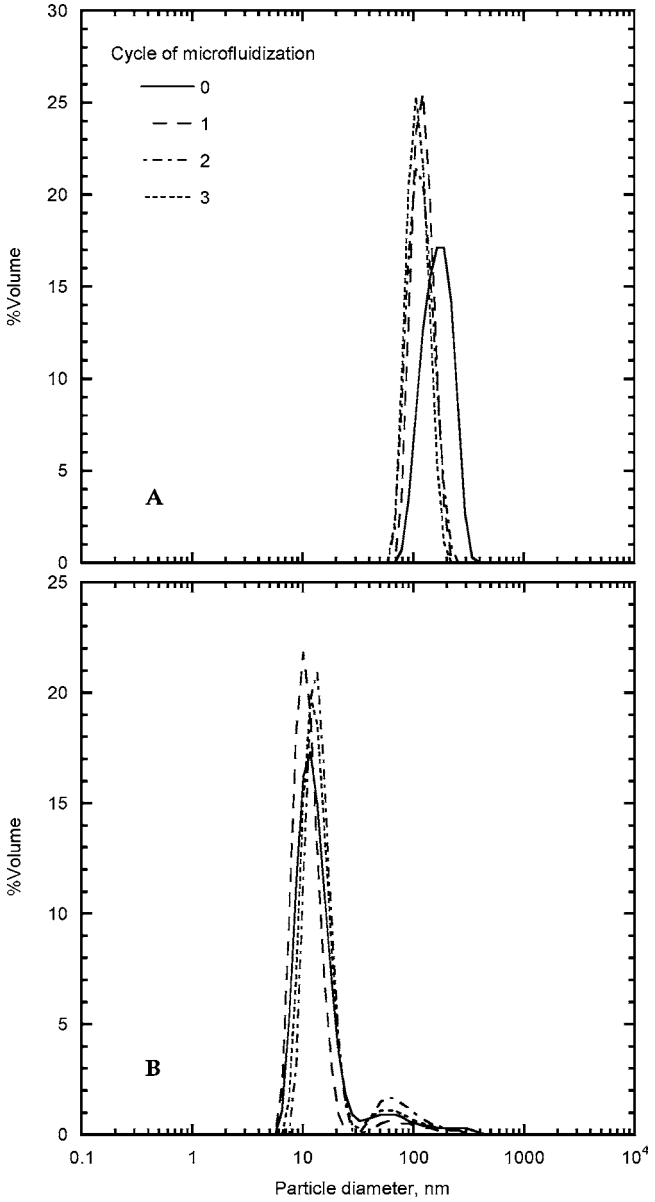

Figure 3. Effect of microfluidization on the particle size distribution of β-carotene nanodispersion prepared with 1 wt % sodium caseinate (A) before and (B) after centrifugation at 10 000*g* for 30 min. The microfluidization pressure was set at 100 MPa.

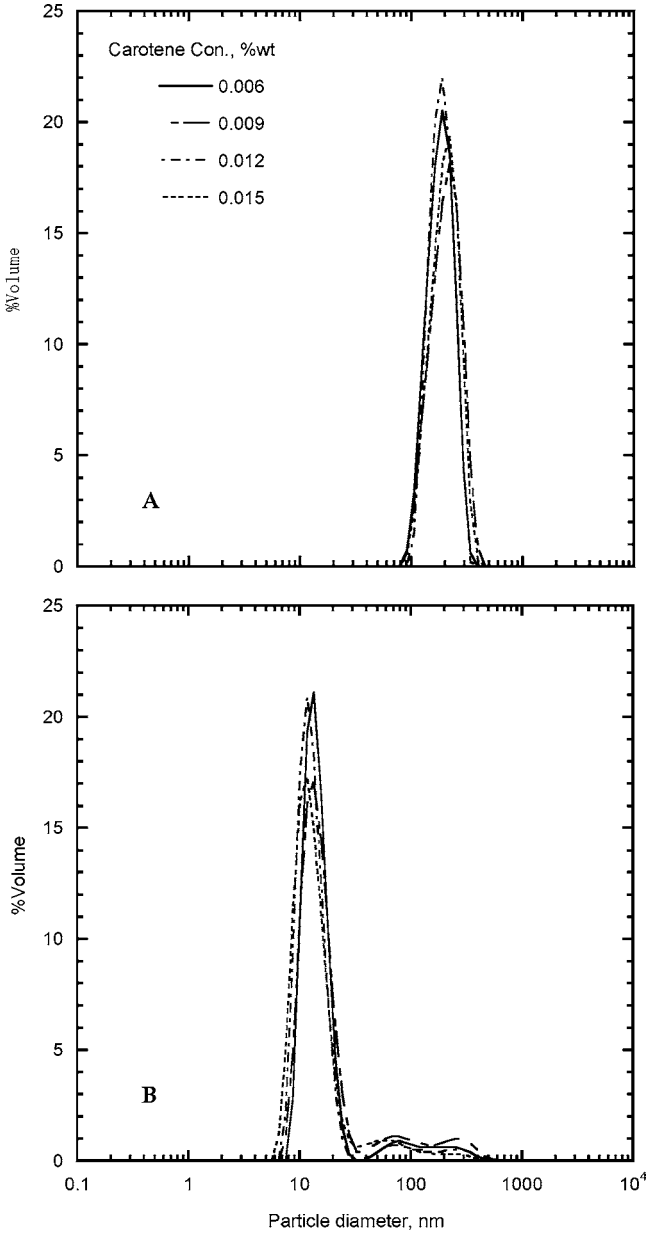

Figure 4. Effect of β-carotene concentration on the particle size distribution of β-carotene nanodispersion prepared with 1 wt % sodium caseinate (A) before and (B) after centrifugation at 10 000*g* for 30 min.

dispersions prepared from 1 wt % SC solutions with and without microfluidization at 100 MPa for 1−3 passes. The mean particle size of the β-carotene particles stabilized by these microfluidized casein micelles was 127 nm with a CV of 26%, which was significantly ($P < 0.05$) smaller than that prepared from untreated SC solution (Figure 3A). It was generally accepted that proteins could be dissociated due to inclusion of water into the structure (*23*), and such pressure-induced inclusion of water might explain the dissociation of casein micelles. It has been shown that high pressure induced solubilization of micellar calcium phosphate in casein micelles upon pressurization of milk (*22*). Dissociation of ion pairs was favored under pressure due to the negative volume change resulting from the more compact arrangement of water molecules around a charged ion than around an uncharged ion pair, leading to increased mineral solubility and ionization (*24*). The release of mineral ions caused the proteins to unfold their structure, and finally the micelles disintegrated. Other possible reason for casein micelle dissociation was the disruption of the hydrophobic interactions within the casein micelles. The partial loss of hydrophobic interaction caused the casein micelles to dissociate. Other electrostatic interactions were also readily disrupted under high pressure due to the electrostrictive effect of separate charges, which might further aid the micellar disruption.

Microfluidization at 100 MPa, however, had no effect on the particle size of SC submicelles. This was reflected on the SC submicelle-stabilized β-carotene particles in the nanodispersion (Figure 3B). Unlike the casein micelles, the structure of SC submicelles contained no colloidal calcium phosphate but hydrophobic and electrostatic interactions to maintain the structural integrity. In other words, there was no calcium phosphate cross-linkage between the caseins in SC submicelle structure for the mechanical pressure to break. Also, due to their relatively small structure/size, it was difficult, if not impossible, to disrupt the hydrophobic and electrostatic interactions within the SC submicelles, even with multiple passes of microfluidization.

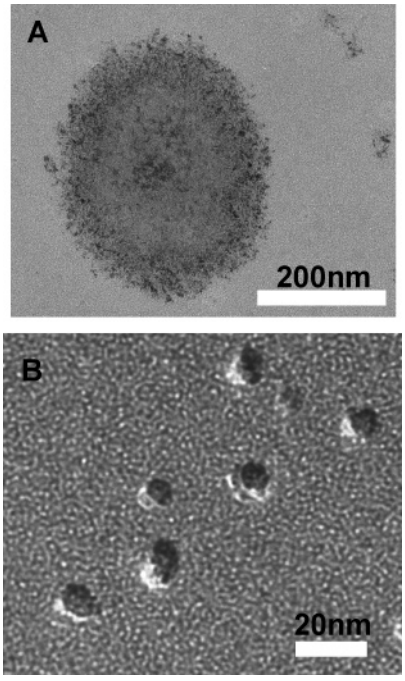

Figure 5. TEM images of β-carotene nanodispersion prepared with 1 wt % sodium caseinate. The samples were prepared by (A) resin embedding and (B) freeze–fracture replica methods.

Influences of β-Carotene Concentration. Figure 4A and B shows the particle size distribution of the β-carotene particles before and after centrifugation, respectively. The results showed that the mean particle size of β-carotene particles stabilized by both casein micelles and SC submicelles was independent from the β-carotene concentration in acetone under the experiment conditions. β-Carotene had a very low solubility in acetone (~0.015 wt %) at room temperature. At such a low concentration β-carotene precipitates formed during the solvent displacement process had a much smaller particle size compared to that of casein micelles and SC submicelles and did not have any significant influence on the nanodispersion particle size and particle size distribution.

TEM Analysis. Figure 5 shows the representative TEM images of the noncentrifuged β-carotene nanodispersion prepared with 1 wt % SC. The TEM sample was prepared using resin embedding (Figure 5A) and freeze−fracture replica methods (Figure 5B). The former method allows observation of the inner structure of the casein micelles containing β-carotene precipitates, while the latter provides high-magnification images and, therefore, is useful in observing fine particles. Figure 5A shows the TEM image of large particles (>200 nm), which corresponded to the casein micelle-stabilized β-carotene particles in the nanodispersion. It was apparent that the outer surface of the particles was quite diffuse, similar to the micelle periphery boundary of typical casein micelles in raw milk (*25*). The casein micelles were stained with uranyl acetate, which at neutral pH acted as a positive stain for proteins. The protein (casein)-rich regions were represented by dark regions on the image owing to the shadow cast by uranium atoms that scattered electrons in TEM electron beams (*25*). A gray/faint region in the center of the micelles was noted, indicating the existence of non-protein substances inside the casein micelles. The non-protein substances were probably the β-carotene precipitates, although more investigation was needed for conformation. Figure 5B shows that the particles in the sample exhibited spherical morphology with a diameter of 10−15 nm. These particles were most likely the β-carotene particles stabilized by the SC submicelles since the observation agreed well with the respective particle size analysis results. The particles look like solid objects with a well-defined boundary, in accordance with previous findings that SC submicelles formed a more uniform and smoother adsorption layer at the oil/water interface than the casein micelles (*26*).

Conclusion. This work demonstrated that protein-stabilized β-carotene nanodispersion could be prepared by the solvent displacement technique. The type of protein, owing to the nature of the protein in terms of molecular weight and structural flexibility, determined the β-carotene particle size and distribution. The β-carotene nanodispersions prepared with SC exhibited a bimodal particle size distribution. The β-carotene precipitates predominantly were stabilized by SC submicelles, while the remaining ones were by casein micelles. The presence of SC submicelle- and casein micelle-stabilized β-carotene particles has been confirmed by TEM analysis. Interestingly, these two types of β-carotene particles responded differently toward the effects of SC concentration and microfluidization in terms of particle size distribution. Although it is a great challenge to increase the β-carotene content in the nanodispersions, solvent displacement remains as an attractive technique to prepare the nanodispersions thanks to its simplicity and low-energy input.

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