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Sodium Caseinate Stabilized Zein Colloidal Particles

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The present work deals with the preparation and stabilization of zein colloidal particles using sodium caseinate as electrosteric stabilizer. Colloidal particles with well-defined size range (120−150 nm) and negative surface potential (−29 to −47 mV) were obtained using a simple antisolvent precipitation method. Due to the presence of caseinate, the stabilized colloidal particles showed a shift of isoelectric point (IEP) from 6.0 to around pH 5.0 and thus prevent the aggregation of zein near its native IEP (pH 6.2). The particles also showed good stability to varying ionic strength (15 mM−1.5 M NaCl). Furthermore, stabilized particles retained the property of redispersibility after drying. In vitro protein hydrolysis study confirmed that the presence of caseinate did not alter the digestibility of zein. Such colloidal particles could potentially serve as all-natural delivery systems for bioactive molecules in food, pharmaceutical, and agricultural formulations.

KEYWORDS: Zein; aggregation; colloid stability; sodium caseinate; electrosteric stabilization

INTRODUCTION

Zein, a proline-rich protein obtained from corn, has been studied as a potential biomaterial for delivery systems (*1*, *2*). The insoluble characteristic of zein makes it a good candidate for development of natural biopolymeric colloidal particles, which can be used for controlled delivery of drugs or other functional molecules such as nutraceuticals (*3*). However, the use of zein colloidal particles in foods and for oral drug delivery may not be optimal because zein as a biomacromolecule has an isoelectric point at around pH 6.2 (*4*). Thus, colloidal particles of zein close to neutral pH lose physical stability both at product conditions and at physiological pH in the intestine, leading to aggregation. Another major challenge that needs to be addressed is providing protection against aggregation during drying (lyophilization) for preparation of redispersible powders. A general way of preventing this aggregation is by providing steric stabilization using surfactants (*5*). However, use of surfactants in oral products is not preferred because (i) surfactants can be used only to a limited level due to cytotoxicity issues and (ii) surfactants often impart their own undesirable taste to the product (*6*). Hence, stabilization of colloidal particles using natural biopolymers has gained increased attention in recent years as it assures acceptable taste, biocompatibility, and biodegradability.

Sodium caseinate, a soluble mixture of several different caseins (αs1, αs2, β, and κ), is widely used as an ingredient in the food industry (*7*). The surface-active caseins contain hydrophilic and hydrophobic groups in various sequences and proportions. Sodium caseinate has been reported to act as an emulsifier/stabilizer due to the combination of electrostatic and steric stabilization (*7*). It is an edible protein and has a bland taste and thus is a suitable candidate for use as a stabilizer in our study.

In this paper, sodium caseinate is used to stabilize zein colloidal particles to make them more applicable for the development of an all-natural oral delivery system. Their stability as a function of pH and ionic strength, redispersibility after drying, and enzyme hydrolysis are investigated.

MATERIALS AND METHODS

Materials. Zein was purchased from Sigma Aldrich Inc., USA. Sodium caseinate (food grade) was purchased from Arla Foods, U.K. HCl (1 N) and NaOH (1 N) solutions were purchased from Merck Co., Germany. Absolute ethanol was obtained from VWR BDH Chemicals, U.K. Reagents and buffer salts for protein hydrolysis studies including *o*-phthaldialdehyde (OPA), dithiotreitol (DTT), disodium tetraborate, sodium doceyl sulfate, pepsin, pancreatin, NaCl, and $CaCl_2$ were purchased from Sigma Aldrich Inc., USA. Water purified by a Milli-Q system was used for all experiments.

Particle Synthesis. Colloidal particles were prepared using a modified method based on antisolvent precipitation (*3*). Precisely, zein (2.5 g) was dissolved in 100 mL of ethanol/water binary solvent (80:20 v/v) to form a stock solution, followed by the addition of these solutions to 250 mL of Milli-Q water without and with (0.1−2.0% w/v) sodium caseinate, under continuous stirring (1000 rpm) using a magnetic stirrer (model EM3300T, Labotech Inc., Germany). The dispersions thus formed were subjected to solvent removal under reduced pressure using a rotary evaporator (Rotavapor R-114, Buchi, Switzerland). These dispersions were then subjected to centrifugation at 4000 rpm for 10 min to separate out the minor part of zein that formed larger aggregates. The final dispersions were then stored at 4 °C until used for evaluation of particle size, shape, and ζ-potential. The pH of the resulting dispersion (without caseinate) was 4.0 ± 0.1, and dispersion with caseinate had pH in the range of 4.5−6.5. To obtain solid powder samples, dispersions were further concentrated by ultracentrifugation at 28000 rpm for 1 h (Optima L-70K ultracentrifuge, Beckman Instruments Inc., USA) and air-dried overnight (at 25 °C in the oven).

Analysis of Particle Size and Surface Potential. The particle size and ζ-potential of dispersions were measured by dynamic light scattering (DLS) using a Zetasizer Nano (Malvern Instruments Ltd., UK) after appropriate dilution. For studying the effect of pH on ζ-potential, an MPT-2 multipurpose autotitrator (ZEN10001) was used in combination with the Zetasizer (0.1 N HCl and 0.1 N NaOH were used as titrants). All measurements were carried out at 25 °C, and the results reported are the average of three readings.

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Transmission Electron Microscopy (TEM). The shape of formed particles was analyzed by taking TEM photographs using a Technai transmission electron microscope (FEI Co., The Netherlands). The particles were dispersed in medium (i.e., Milli-Q water at appropriate pH), and one drop of the diluted dispersion was placed on a 200-mesh carbon-coated copper grid. The photographs were taken at various magnifications and 100 kV voltage.

Solid State Characterization. Differential scanning calorimetry (DSC) studies were performed on samples weighing 10 mg in flat-bottom aluminum pans using a Perkin-Elmer Pyris I DSC. The samples were heated from 30 to 230 °C at a heating rate of 10 °C/min. Inert atmosphere was maintained by nitrogen purging at a flow rate of 20 mL/min. Fourier transform infrared (FTIR) spectra of the samples were obtained in the range from 400 to 4000 cm^{-1} using a Bio-Rad FTS-6000 spectrometer. X-ray diffraction (XRD) patterns were recorded on a Bruker D8-Discover diffractometer with a copper target, voltage of 40 kV, current of 40 mA, and a scanning rate of 3°/min.

Stability Evaluation. Aggregation study was carried out by adding 250 μL of sample to 10 mL of phosphate buffer, pH 7.4, and noting visually the appearance of aggregated particles. The effect of ionic strength on colloidal stability was studied by adding 250 μL of sample to a series of solutions (volume of 10 mL) having various ionic strengths (from 15 mM to 1.5 M NaCl) and checking for aggregation. To rule out the possible effect of pH, it was maintained at 4.0 ± 0.1 for studies with plain zein colloidal particles. In the case of caseinate stabilized colloidal particles, the study was carried out at pH 7. To assess the stability against drying, the dispersions were subjected to freeze-drying (Labconco Freezone 6 plus, Labconco Corp., USA). The dried samples were then redispersed using Milli-Q water, and the time taken for the redispersion was noted. The particle size distribution was measured before and after drying. To study the long-term physical stability, the dispersions were stored at 4 °C and at ambient temperature for 3 months. Physical stability was assessed through visual inspection for the occurrence of aggregation or sedimentation at the end of the storage period.

Protein Hydrolysis Study. *OPA Method.* This spectrophotometric method (adapted from the literature, *8*) was developed to quantify the degree of hydrolysis (DH) of proteins via a rapid (96-well microplates) and simple procedure. The method uses *o*-phthaldialdehyde (OPA) that reacts with primary amino groups and a SH compound (dithiotreitol, DTT) to form a compound that shows absorbance at 340 nm. A serine standard curve was used to quantify the DH of the proteins, because in reactions serine shows a response very close to the average response of amino acids. The results are either expressed as DH in (%) or as milliequivalents (mequiv) of serine-NH_2. The working reagent was prepared by mixing 400 mL of an OPA solution, prepared at 40 mg/mL in ethanol, and 20 mL of a 0.9 mg/mL DTT solution that was prepared in an aqueous buffer with 0.1 M disodium tetraborate and 0.1% w/v SDS. Concentrations of studied samples were as follows: zein colloidal particles, 2.5% w/v; caseinate solution, 5% w/v, corresponding to a 1:2 ratio of zein/caseinate used in preparation. For the mixture of zein/caseinate and stabilized zein colloid the total protein concentration was 7.5% (zein = 2.5% and caseinate = 5% w/v).

(*a*) *Gastric Step.* Five milliliters of sample was mixed with 0.335 mL of 10× (10 times diluted) saline (to have a 50 mM NaCl final concentration), 1 mL of 10 mg/mL pepsin in 0.1 N HCl, and demineralized water to have volume of 10 mL, and the pH was adjusted to pH 2 using small aliquots of 4 N HCl. The above mixture was incubated for 90 min at 37 °C. At the end of incubation, 20 μL of sample (diluted to 10× with the buffer) was mixed with 150 μL of working reagent and the optical density (OD) was read at 340 nm on a Spectramax 190 (Molecular Devices Ltd., Wokingham, U.K.).

(*b*) *Intestinal Step.* After the gastric step, intestinal conditions were achieved by mixing 1 mL of bile (prepared by adding 0.5 g to 9.5 mL of buffer containing 40 mM NaCl, 20 mM $CaCl_2$, and 5 mM Tris) and 5 mg of pancreatin in 1 mL of buffer containing 40 mM NaCl, 20 mM $CaCl_2$, and 5 mM Tris. The above mixture was incubated for 120 min at 37 °C. At the end of incubation, 20 μL of sample (diluted to 10× with the buffer) was mixed with 125 μL of working reagent and the OD was read at 340 nm on a Spectramax 190 (Molecular Devices Ltd.).

The amount of amino groups present in the hydrolysates (in mequiv) was calculated from the serine calibration curve using the following equation: $[\text{serine-NH}_2] = ((\text{OD}_{340\text{ nm}} - b_{\text{std}}\text{ curve})/a_{\text{std}}\text{ curve} \times d)/(P/X)$, where [serine-$NH_2$] is mequiv of serine-$NH_2$ per gram of protein, $OD_{340\text{ nm}}$ is raw data measurement at $\lambda = 340$ nm, b_{std} curve is the intercept of the linear regression equation of the serine standard curve, a_{std} curve is the slope of the linear regression equation of the serine standard curve, d is sample dilution, P is % protein concentration in the sample, and X is grams of sample per liter present in the stock solution. h (mequiv/g of protein in hydrolysate) and DH (%) were calculated as follows: $h = [\text{serine-NH}_2 - \beta]/\alpha$ and DH (%) $= (h/h_{\text{total}}) \times 100$, where β is taken from the intercept of a dose response curve of each particular protein substrate, α is taken from the slope of a dose response curve of each particular protein substrate, and h_{total} is total mequiv/g of protein substrate.

pH Stat Method. The assessment of protein hydrolysis using pH stat can only be carried out at neutral or alkaline conditions (*9*). Therefore, only the intestinal condition with pancreatin was studied by using this method. A Mettler DL 21 Titrator (Mettler Toledo Ltd., Leicester, U.K.) was used in the study, and conditions were as follows: In a total volume of 30 mL, 5 mL of sample was mixed with 225 mg of bile in 4.5 mL of buffer containing 40 mM NaCl, 20 mM $CaCl_2$, 5 mM Tris, 1.5 mL of saline, and 1.5 mL of 4 N NaOH (to adjust the pH to 7.3 prior to pretitration), and the rest of the volume was made up with Milli-Q water. Reaction was started with the addition of 30 mg of pancreatin dispersed in 3 mL of buffer. Similarly, conditions were set up for samples in the absence of pancreatin to serve as a control. The base used for maintaining constant pH was 0.1 M NaOH. The amount of base titrated is equivalent to the amount of amino acid released during protein hydrolysis. Thus, the amount of base consumed during the titration can be used as an indirect means of comparing the digestibilities of protein samples.

RESULTS AND DISCUSSION

Particle Synthesis and Characterization. The mechanism of formation of zein colloidal particles is well established (*3*). Zein particles can be made by controlled precipitation in ethanol/water solutions of zein by changing the quality of the solvent using water as antisolvent. The mean particle size of plain zein colloidal dispersion was found to be 147 ± 20 nm with positive surface ζ-potential = +34.5 mV measured at pH 4.0. Addition of sodium caseinate in the antisolvent aqueous phase did not show any major effect on the final particle size, but it caused the surface charge to shift from positive to negative. The precipitation of zein particles in aqueous solution of sodium caseinate could in principle be a competition of two processes: precipitation of zein, which is positively charged, followed by absorption of caseinate, which is negatively charged, and/or direct precipitation and aggregation of zein−caseinate coacervates formed due to direct electrostatic interaction. Providing that a sufficient amount of stabilizer is present, the first mechanism assures good stability and possible reduction of the resulting particle size owing to the arrest of the aggregation process. For the second mechanism, the casein molecules should molecularly interact directly with the soluble zein molecules requiring considerable solubility of casein in the water/ethanol mixture, which is not the case, and hence the second process can be neglected. Although a detailed understanding of the mechanism of particle formation is outside the scope of this paper, on the basis of the above consideration, absorption of caseinate on the zein particle surface, rather than direct coacervation, during the precipitation is the most likely mechanism. The mean particle size and surface potential of zein colloid stabilized using 2% w/v sodium caseinate (corresponding to a zein/caseinate ratio of 1:2) were observed to be 124 ± 32 nm and −41.5 mV (measured at pH 6.4), respectively. The spherical shape of the particles was confirmed by TEM (**Figure 1**). XRD diffractograms (Figure A in the Supporting Information) showed no sharp peaks, confirming the amorphous nature of particles. Figure B in the Supporting Information shows the FT-IR spectra of zein colloidal particles and caseinate stabilized zein colloidal particles. There were no major shifts of peaks or appearance of new peaks in the FT-IR spectra of the caseinate stabilized colloidal particles, indicating the absence of any chemical interaction

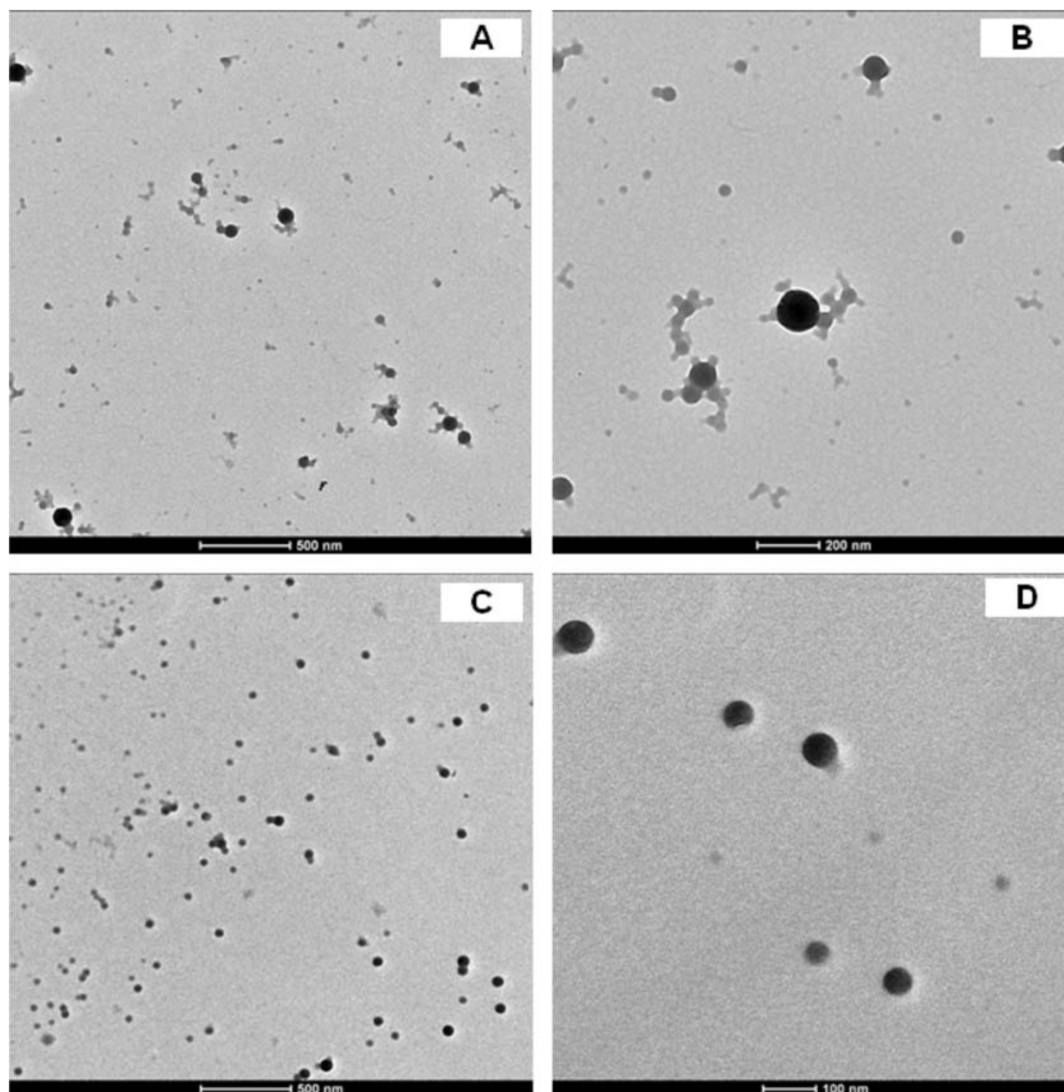

Figure 1. TEM images of zein colloidal particles (**A**, scale bar = 500 nm; and **B**, scale bar = 200 nm) and caseinate stabilized zein colloidal particles (**C**, scale bar = 500 nm; and **D**, scale bar = 100 nm).

between zein and caseinate. The DSC thermograms (Figure C in the Supporting Information) of zein colloidal particles and caseinate stabilized zein colloidal particles were almost superimposable and hence support our earlier assumption of the absence of any chemical interaction. Other transitions due to either adsorption/desorption were too minor to be detected by DSC.

Colloidal Stability. Most applications of colloidal dispersion in oral products require stability at various pHvalues as well as in the presence of various electrolyte concentrations. It is known that the mechanism involved in stabilization of (polymer) colloids could be either electrostatic repulsion, steric stabilization, or a combination of both effects known as electrosteric stabilization (*10*−*12*). Electrosteric stabilization achieved via adsorption of a charged surface active (bio)polymer is one of the viable ways of stabilizing colloidal dispersions. The stabilization of hydrophobic colloids results from adsorption of an amphiphilic stabilizer on the surface due to hydrophobic interactions. Sodium caseinate, like many other proteins, tends to localize and adsorb on the surface of dispersed phase due to their amphiphilic and charged nature, providing an electrostatic and steric repulsion against aggregation (*7*). In the present case, there is strong electrostatic attraction due to the opposite charges on zein and casein during the precipitation process. However, the concentration of sodium caseinate used during the precipitation process affected the colloidal stabilization also due to the steric effects. It was observed that a minimum concentration of 0.30% sodium caseinate (corresponding to a zein/caseinate ratio of 1:0.3) was required for the formation of stable colloidal dispersion (**Figure 2**). At concentrations of 0.10 and 0.15% the dispersion formed had large aggregates, whereas at concentrations of 0.20 and 0.25% there was complete flocculation of particles, leaving the medium almost clear. This can be attributed to complete neutralization of the particle surface charge with insufficient steric stabilization. **Table 1** gives the pH of formed dispersions and ζ-potential values at various concentrations of sodium caseinate. To get further insight, the ζ-potential of colloidal dispersion was measured as a function of pH. Zein and caseins are known to have isoelectric points at 6.2 and 4.5, respectively (*4*, *13*). **Figure 3** shows ζ-potential curves as a function of pH for colloidal zein, sodium caseinate solution, and caseinate stabilized zein colloidal particles. As seen from **Figure 3**, zein colloidal particles showed an isoelectric point at pH 5.9 and sodium caseinate at pH 4.6, whereas the caseinate stabilized colloidal dispersion showed an isoelectric point (IEP) at around 5.0.

To summarize the results, at lower concentrations, that is, 0.10 and 0.15%, the surface covering is not enough, resulting in weaker steric stabilization and almost negligible electrostatic stabilization (due to the positive value of the ζ-potential). As the concentration is increased to 0.20 and 0.25%, the pH reaches close to 5.0 (which

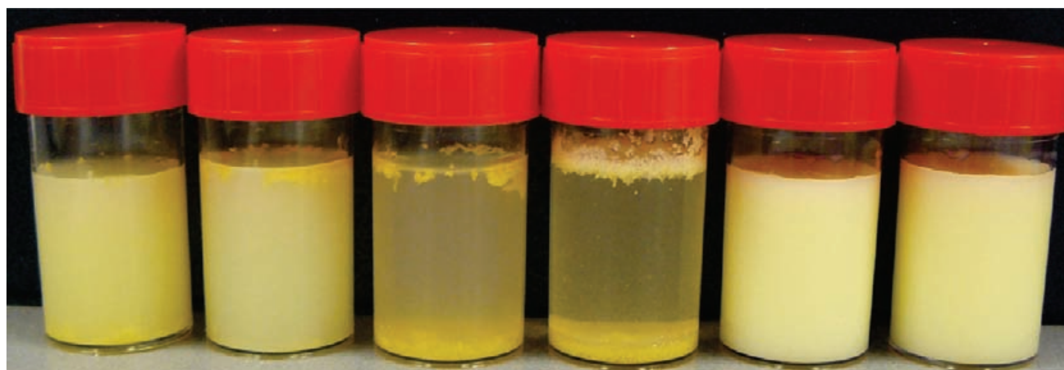

Figure 2. Zein dispersions prepared using different amounts of sodium caseinate. Starting from the left, zein colloids were prepared using 0.10, 0.15, 0.20, 0.25 0.30, and 0.35% sodium caseinate as stabilizer (corresponding to zein/caseinate ratios of 1:0.10−1:0.35). The concentration of zein was kept constant at 2.5% w/v.

Table 1. pH and ζ-Potential Values of Formed Colloidal Dispersions at Various Concentrations of Sodium Caseinate

concn of stabilizer (w/v)	pH of colloidal dispersion	ζ-potential (mV)
none	4.0	34.5 ± 1.3
0.10	4.2	11.4 ± 0.7
0.15	4.6	1.71 ± 0.6
0.20	5.0	unstable
0.25	5.1	unstable
0.30	5.7	−32.7 ± 0.8
0.35	5.9	−32.9 ± 0.4
0.40	6.0	−34.2 ± 0.6
0.45	6.1	−29.6 ± 1.5
0.50	5.7	−43.3 ± 1.2
0.75	5.9	−38.8 ± 2.4
1.00	6.1	−45.4 ± 2.5
1.25	6.3	−37.0 ± 3.4
1.50	6.4	−40.2 ± 0.9
1.75	6.5	−46.4 ± 4.4
2.00	6.4	−41.5 ± 3.6

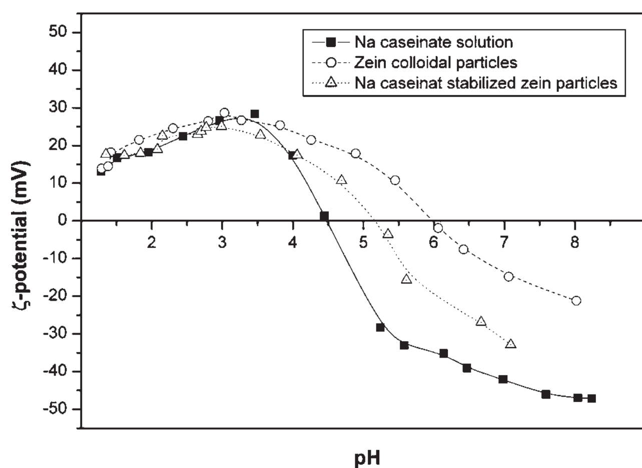

Figure 3. ζ-potential as a function of pH of plain zein colloidal particles, sodium caseinate, and zein colloids stabilized using 0.3% sodium caseinate (corresponding to a zein/caseinate ratio of 1:0.3).

is the isoelectric point of stabilized colloid), eventually leading to the aggregation of the colloidal dispersion. Concentrations of 0.30% and above led to better coverage of surface particle, resulting in both steric and electrostatic stabilization (comparatively higher negative value of ζ-potential). The occurrence of a changed isoelectric point for the stabilized particles confirms that caseinate is absorbed on the zein surface, leading to the change in the surface charge. The corresponding change in the ζ-potential leads to a decrease in the effective IEP of the caseinate stabilized zein particles. The amount of caseinate needed to cover the particle surface to provide the most effective steric stabilization can be estimated from

$$C = \frac{6\Gamma\phi_p}{d}$$

where Γ is the surface coverage, ϕ_p is the volume fraction of particles, and d is the mean surface-averaged particle diameter. For Γ here, we approximated the value with the one known for emulsion systems, that is, 3 mg m^{-2} (*14*). Using these values, the concentration of adsorbed protein was estimated as 0.29% wt of caseinate per weight of zein colloidal particles, which is in good agreement with our experimental data as reported in **Table 1**, where colloid stability was obtained at a concentration of 0.30% wt. However, we used an excess of sodium caseinate to achieve easy redispersibility of dried powder.

Zein colloidal particles have a tendency to aggregate at neutral pH owing to their isoelectric pH of 6.2 (*15*). When plain zein colloid was added to buffer, an instantaneous aggregation was observed. On the other hand, it was seen that caseinate prevented aggregation of zein particles at a concentration of 1.25% w/v (corresponding to a zein/caseinate ratio of 1:1.25) and above (**Figure 4**).

The presence of electrosteric stabilizer further governs the sensitivity of the colloidal particles to the electrolytes (*16*). Accordingly, it was also found that a caseinate stabilized zein colloidal dispersion survived high ionic strength by showing no aggregation at studied concentrations (up to 1.5 M NaCl). Whereas the plain zein colloidal dispersion was stable only at lower ionic strength (30 mM NaCl and below), at higher ionic strength an instant aggregation was observed (**Figure 5**). The stability of the colloidal dispersion to high ionic strength in the current study suggests a strong steric stabilization due to effective coverage of stabilizer on the surface of colloidal particles and the role of electrostatic effect (as confirmed by the surface charge reversal).

For several industrial applications it is important to have easily dispersible powder formats. Colloidal particles prepared from hydrophobic polymers often tend to form aggregated lumps on drying, which are too difficult to redisperse to a stable colloidal dispersion. Sodium caseinate has been studied to have cryoprotectant activity owing to its capacity to bind water (*17*). Thus, the redispersibility of colloidal particles after drying was tested to evaluate the effect of the presence of caseinate on drying stability. After drying, the plain zein colloidal particles did not redisperse at all due to strong aggregation. Colloidal dispersions with 1.25% w/v caseinate and above showed complete redispersibility, whereas

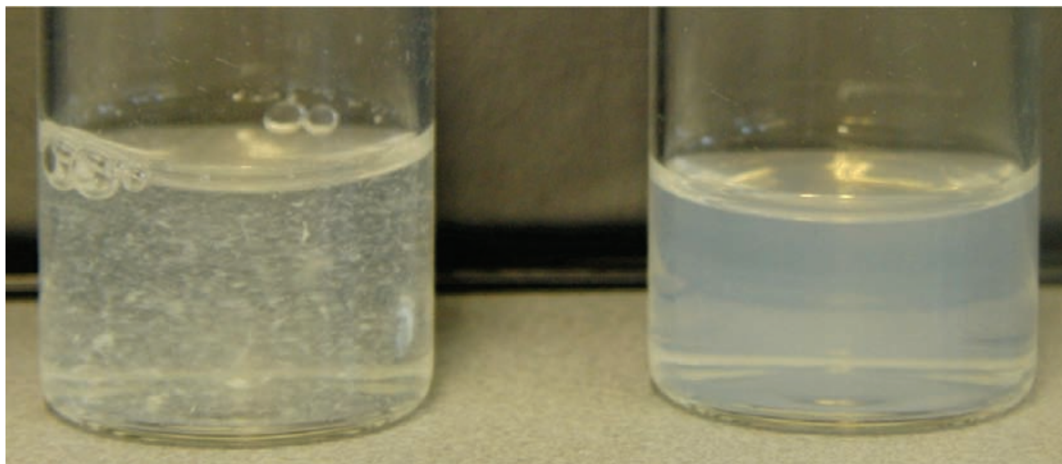

Figure 4. Photograph showing the aggregation of zein colloidal particles in phosphate buffer (7.4) on the left and stable colloidal dispersion of caseinate stabilized zein colloid on the right.

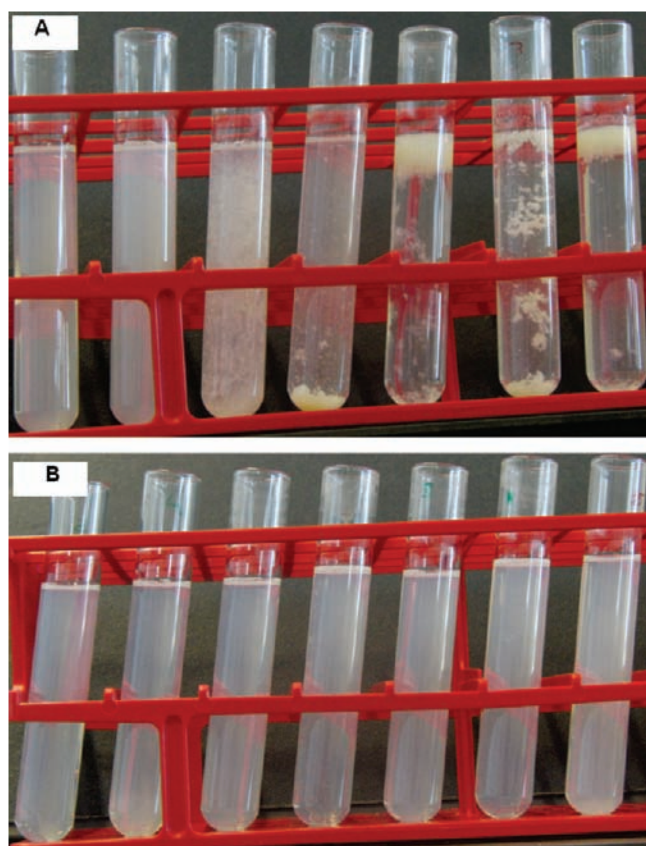

Figure 5. Photographs of (**A**) zein colloid (250 μL in 10 mL of medium with varying ionic strength, pH 4.0) and (**B**) caseinate stabilized zein colloid (250 mL in 10 mL of medium with varying ionic strength, pH 6.5). Ionic strength, from left to right: 15 mM, 30 mM, 75 mM, 150 mM, 300 mM, 750 mM, and 1.50 M.

samples with concentration of caseinate lower than 1.25% w/v showed incomplete redispersion, indicating partial aggregation of zein particles. The presence of an excess of sodium caseinate provided stabilization during drying and assured good redispersibility of the dried colloidal particles. The mean particle size and surface potential of the redispersed particles (ratio zein/caseinate of 1:2) were found to be 140 nm and −50.0 mV, respectively, suggesting no major changes in either particle size or surface potential (**Figure 6**). The dispersions stored at 4 °C and ambient temperatures were found to be stable for > 3 months, showing no signs of sedimentation or aggregation. In the case of plain zein dispersion, slow aggregation and sedimentation were observed after 2 weeks of storage at ambient temperature, probably suggesting the unstable nature of plain zein colloidal particles.

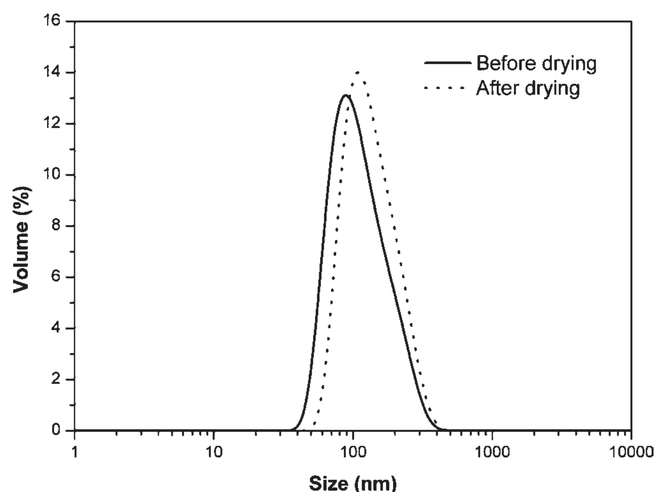

Figure 6. Volume-averaged particle size distribution graph of stabilized zein colloidal dispersion (zein/caseinate ratio of 1:2) before and after drying and redispersion.

Protein Hydrolysis. Zein as a protein has a low digestibility due to its limited enzymatic degradation as known from the literature (*18*). The low digestibility of protein could be exploited for developing delivery systems in which the release of loaded bioactive molecules could be controlled by the slow digestion of zein (*1*). One of our major concerns was to check if there are any changes in the digestion characteristics of zein particles due to the presence of a relatively hydrophilic adsorbed layer of sodium caseinate. **Table 2** gives % DH values obtained from the OPA test. As seen from the result, zein colloidal dispersion showed minimal digestion with a % DH value of 2.39% at the end of the digestion study. In the case of caseinate stabilized zein colloidal dispersion, it was found that caseinate did not significantly alter the digestion of zein. The relatively higher value of % DH in the case of caseinate stabilized zein colloidal dispersion compared to plain zein colloidal dispersion could be attributed to the digestion of caseinate present on the surface of zein particles followed by digestion of a normal amount of zein. Results were further confirmed by results from

Table 2. Degree of Hydrolysis (% DH) of Zein Colloidal Particles, Caseinate Stabilized Zein Colloid, and Caseinate Solution Containing Equivalent Concentration of Caseinate Present in the Colloidal Dispersion

	zein colloid	caseinate stabilized zein colloid	caseinate solution
% DH in gastric conditions	2.06	5.01	3.33
%DH in intestinal conditions	0.33	5.14	4.97
total % DH	2.39	10.15	8.30

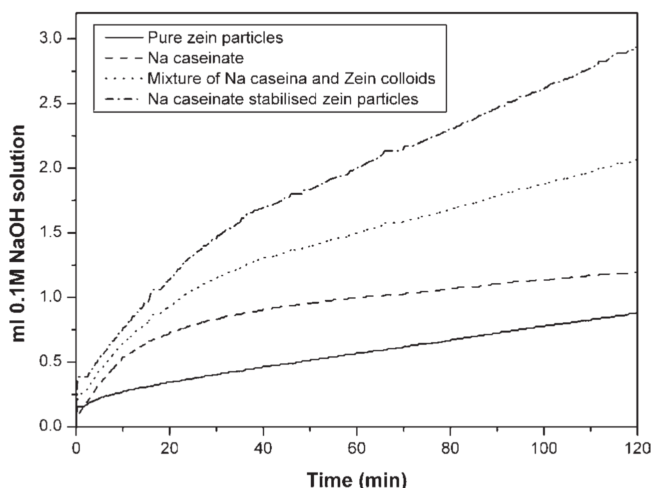

Figure 7. Relationship of amount of base consumption (mL of 0.1 M NaOH) against time of incubation during intestinal digestion for pure zein particles (2.5% w/v), sodium caseinate solution (5.0% w/v), a mixture of zein colloidal particles (2.5% w/v) and solution of sodium caseinate (5.0% w/v), and caseinate stabilized zein colloidal particles (2.5% w/v zein and 5.0% w/v sodium caseinate).

a pH stat study (**Figure 7**). The hydrolysis (reported as % base consumption) of caseinate stabilized zein colloidal dispersion followed the pattern of caseinate digestion with almost equal amounts of digestion in intestinal conditions. The relatively lower amount of base consumption in the case of caseinate zein mixture was due to some aggregation formed when the zein colloidal dispersion was mixed with caseinate solution. This interaction is due to direct interaction between the positively charged zein and caseinate, which is negatively charged. In addition, the final pH of the mixture is close to neutral, which will further promote aggregation of zein particles. Overall, from the protein hydrolysis studies it was found that stabilization with sodium caseinate did not change the digestion characteristics of the zein, which has low digestibility due to the higher proportion of nonpolar amino acids (*4*). The total protein concentration in the mixture of zein colloidal particles and sodium caseinate and in the casein-stabilized colloidal particles was higher than the caseinate solution (7.5% as compared to 5% w/v). This explains the higher % DH value for the mixture and stabilized colloidal particles.

In conclusion, zein colloidal particles stable at neutral pH were successfully prepared for the first time using sodium caseinate as electrosteric stabilizer. The particles displayed a concentration-dependent surface charge reversal due to the adsorption of caseinate. The resulting colloidal particles were also found to be stable at high ionic strength, which could be considered beneficial for stability in physiological conditions and products with high salt concentration. The dried powder sample could be redispersed easily, and thus it opens up new possibilities of using zein colloidal particles in dried form. It was also seen that the addition of caseinate resulted in enhanced physical stability of dispersion at ambient temperature. Because stabilization is merely due to the adsorption of protein on the particles, and the antisolvent used was water, this process is potentially easy to scale up. Such colloidal particles have the potential to be used as an all-natural biopolymer-based colloidal delivery system for encapsulating/embedding bioactive molecules in food (e.g., nutraceuticals), pharmaceutical (e.g., drug), and agricultural formulations. The applicability of these stabilized colloidal particles for delivery of bioactive compounds was successfully investigated in our recent work (*19*).

ABBREVIATIONS USED

DLS, dynamic light scattering; TEM, transmission electron microscopy; DSC, differential scanning calorimetry; XRD, X-ray diffraction; FTIR, Fourier transform infrared spectroscopy; OPA, *o*-phthaldialdehyde; DTT, dithiotreitol; DH, degree of hydrolysis; OD, optical density; mequiv, milliequivalent; IEP, isoelectric point.

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Supporting Information Available: DSC thermograms, XRD diffractograms, and FTIR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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