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ORIGINAL CONTRIBUTION

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Colloidal stability of chitosan-modified poly(methyl methacrylate) latex particles

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Abstract Experiments of coagulation kinetics were used to study the influence of the electrolyte concentration on the colloidal stability of cationic poly(methyl methacrylate) latex particles with various degrees of chitosan modification. For the chitosan-free latex products prepared by various levels of 2,2′ azobis(2-amidinopropane) dihydrochloride (V-50) at constant pH, the critical coagulation concentration (ccc) increases with increasing V-50 concentration, due to the enhanced particle surface charge density. On the other hand, the chitosan-modified latex products at constant pH do not exhibit very different values of ccc. This result is attributed to the counterbalance between two opposite effects related to the grafted chitosan, that is, the increased particle surface charge density and the enhanced shift of the particle's shear plane toward the aqueous phase with the chitosan content. The ccc of the latex products with various degrees of chitosan modification decreases significantly when the pH increases from 3 to 7. This is because the degree of ionization of the surface amino groups (the particle surface charge density) decreases with increasing pH. As a result, the stability of the colloidal system decreases significantly with increasing pH. The apparent Hamaker constant and diffuse potential were obtained from the coagulation kinetics data. These two parameters along with the zeta potential and particle size data for the latex samples taken immediately after the end of the coagulation experiments were also used to study the effect of ionic strength on the colloidal stability of the latex particles.

Key words Colloidal stability – Chitosan-modified latex particles – Electrostatic/steric stabilization – Coagulation kinetics

Introduction

In our previous work, stable chitosan-modified poly(methyl methacrylate) (PMMA) latex particles were prepared by using 2,2′-azobis(2-amidinopropane) dihydrochloride (V-50) as the cationic initiator [1]. Chitosan can be chemically incorporated (or strongly absorbed) onto the PMMA particle surface. The water-soluble polymer, chitosan, may become surface-active by grafting the hydrophobic MMA oligomeric radicals onto chitosan, presumably via the hydrogen abstraction mechanism during emulsion polymerization. The amphiphilic graft copolymer formed in situ is just like an electrosteric stabilizer and, therefore, it may help nucleate and stabilize the primary particles during polymerization. As a result, the grafted chitosan molecules become an integral part of the emulsion polymer. The isoelectric point (IEP) of these latex products is 10.7. The pK_b of chitosan is 7.7 [2] and calculation based on this value of pK_b shows that about 80% of the -NH_2

groups of chitosan grafted onto the PMMA particle surface is protonated into the -NH_3^+ form at pH 7. Due to the extremely large particle surface area available for adsorption of proteins, the submicron latex particles have been used to purify proteins from a crude biological mixture [3–6]. Below pH 10.7, these positively charged chitosan-modified PMMA particles may thus find potential application in the recovery of the negatively charged proteins by the electrostatic interaction mechanism, as illustrated by adsorption of bovine serum albumin (BSA, IEP = 4.8) on these colloidal particles at pH 7 [1]. The surface chitosan species have a large number of amino groups available for ligand immobilization for protein adsorption via affinity interaction. In addition, chitosan can form a hydrophilic layer around the particles, thereby leading to adsorption of protein without denaturation.

It was also shown that the colloidal stability of these chitosan-modified PMMA latex particles has a significant influence on the BSA adsorption process [1]. First, the stability of these colloidal particles is very sensitive to changes in the aqueous electrolyte concentration. The presence of counterions will compress the electric double layer of the latex particles, decrease the electrostatic repulsion force between two approaching particles and, thereby, reduce the stability of these particles significantly. This colloidal phenomenon can be adequately described by the well-known DLVO theory [7, 8]. Secondly, adsorption of BSA on the particles takes place due to the electrostatic attraction force between the negatively charged BSA molecules and the positively charged particles. This kind of self-promoting adsorption process [9, 10] causes charge neutralization, reduces the particle surface charge density, and then destabilizes the colloidal particles. Both factors will contribute to the destabilization of the latex particles, thereby inducing the precipitation of the resultant flocs. The coagulation rate and the structure of the resultant flocs may have an important effect on the adsorption of BSA onto the chitosan-modified PMMA latex particles. For example, the number of effective binding sites on the particle surface may be greatly reduced owing to the steric hindrance effect provided by the formation of flocs. This may result in a decrease in the binding capacity of the polymeric support. On the other hand, formation of large enough flocs may facilitate their precipitation out of the crude extract by gravitational or centrifugal force. An optimal condition may thus exist in the protein purification process. A better understanding of the fundamental aspects of the colloidal stability of these chitosan-modified PMMA latex particles is essential for successful process development. In order to decouple the ionic strength effect and the charge neutralization effect, this paper presents the effect of the electrolyte (NaCl) concentration on the stability of the colloidal system in the absence of BSA. The results obtained from the isothermal equilibrium adsorption of BSA on the latex particles will appear in a forthcoming paper. The studies on the synthesis and characterization of the chitosan-modified PMMA latex particles will then form a solid basis to achieve the ultimate goal of a series of research projects. The final goal is twofold. The first aim is to chemically couple the ligand through the surface amino groups of chitosan for the affinity purification of the target protein. It is also interesting to note that the hydrophilic chitosan layer around the particle may serve as an effective spacer for the coupled ligand. Another major thrust for developing such a colloidal system is to immobilize an enzyme on the particle surface through the amino groups of chitosan. In this manner, the immobilized enzyme may retain its full activity due to the hydrophilic nature of chitosan.

Experimental

The cationic latex products used in this work are the PMMA particles with various levels of chitosan modification described in Ref. [1]. For comparison, three chitosan-free latex products were also included in this study. Some physical properties of these latex products are summarized in Table 1, in which [V-50] and [C] represent the weight percentage (based on total monomer) of V-50 and chitosan, respectively, used in the synthesis work. These latex products were prepared by the batch surfactant-free emulsion polymerization technique. The weight percentages of chitosan (C_c) and V-50 ($C_{V\text{-}50}$) ultimately incorporated into the latex particles (based on PMMA weight) were determined by elemental analysis (see Table 1). Other chemicals include sodium chloride (Acros), hydrochloric acid (Wako), sodium hydroxide (Shimakyu), and deionized water (Barnsted, Nanopure Ultrapure Water System, specific conductance $< 0.057\ \mu$S/cm).

Experiments of coagulation kinetics [11–14] were used to determine the colloidal stability of the chitosan-modified PMMA latex products toward added NaCl. Before the experiments were conducted, the latex product was separated into the supernatant phase and the precipitate phase by centrifugation at 10 000 rpm for 30 min (Beckman J2-21). The clear supernatant was withdrawn, followed by redispersion of the precipitate in fresh deionized water by ultrasonification (Delta DG-1). This procedure was repeated 5 times to remove the free chitosan molecules and other impurities in the aqueous phase. The latex products are stable toward at least 5 cycles of centrifugation-redispersion, as shown by the particle-size data determined by the dynamic light-scattering technique

Table 1 Some physical properties of the poly(methyl methacrylate) (PMMA) latex particles with various levels of chitosan modification taken from Ref. [1]

Latex ID	V5	V10	V50	C1	C10	C20
[V-50] (%)	0.05	0.1	0.5	0.5	0.5	0.5
[C] (%)	–	–	–	0.1	1	2
d_w (nm)[a]	309	279	262	245	250	232
$C_{V\text{-}50}$ (%)[b]	0.8	1.7	8.3	8.3	8.3	8.3
C_c (%)[b]	–	–	–	0.08	0.9	1.7

[a] Weight-average particle size (monodisperse)

[b] Weight percentage of V-50 or chitosan (based on PMMA) incorporated into latex particles

(Otsuka Photal LPA-3000/3100). A latex sample (total solid content = 0.02%) with a volume of 0.6 ml was mixed rapidly with an equal volume of an aqueous solution comprising a prescribed quantity of NaCl to initiate the experiment of coagulation kinetics. The pH of the latex sample was adjusted by HCl and NaOH. The absorbance of the sample was monitored by a spectrophotometer (Shimadzu UV-1601) at 540 nm for determination of the coagulation kinetics. The zeta potential (ζ) and particle size (d_p) of the latex sample taken immediately after the end of the experiment were determined by Zetamaster (Malvern) and Photal LPA-3000/3100 (Otsuka), respectively. The reported ζ and d_p data represent an average of five and three measurements respectively. The dilution water used for the measurements of ζ and d_p had the same ionic strength and pH as the latex sample.

Results and discussion

Representative absorbance at 540 nm versus time profiles for the latex product with 0.5 wt% V-50 and 2 wt% chitosan (designated as C20) are shown in Fig. 1. The coagulation kinetics data for other latex products also show similar absorbance versus time profiles. A general feature of the coagulation kinetics data is that, at constant NaCl concentration, the absorbance of the latex sample increases as the experiment proceeds. This trend indicates the scenario that the dimension of the flocculated latex particles continues to build up with the progress of the experiment. Moreover, at a certain point in time, the absorbance increases with increasing NaCl concentration, but the difference diminishes as the NaCl concentration approaches 200 mM. According to Fuchs [15], the stability ration (W) is defined as K_r/K_s, in which K_r and K_s are the rapid and slow flocculation rate constants, respectively. The value of W (e.g., see the run at [NaCl] = 15 mM in Fig. 1) can be estimated as the ratio of the asymptotic slope of the absorbance versus time curve at zero time (e.g., the initial slope at [NaCl] $\rightarrow$ 200 mM) to the slope of the absorbance versus time curve at zero time (e.g., the initial slope at [NaCl] = 15 mM) because K_r or K_s are proportional to the initial slope of the absorbance versus time curve and these two proportional constants are equal. The corresponding log W versus log [NaCl] data for the latex sample C20 are shown in Fig. 2. The discontinuous point in the log W versus log [NaCl] profile was then identified as the critical coagulation concentration (ccc) for C20. Furthermore, the diffuse potential (ψ_δ) and Hamaker constant (A) of the latex particles can be calculated according to the following equations [16]:

$$-\mathrm{d}\ \log W/\mathrm{d}\ \log[\mathrm{NaCl}] = 2.15\times10^{7} r\gamma^{2}/z^{2} \tag{1}$$

$$A = [1.73\times10^{-36}(\mathrm{d}\ \log W/\mathrm{d}\ \log[\mathrm{NaCl}])^{2}/r^{2}z^{2}\mathrm{ccc}]^{1/2} \tag{2}$$

where r is the particle radius, $\gamma = [\exp(ze\psi_\delta/2kT)-1]/[\exp(ze\psi_\delta/2kT)+1]$, z is the valence of the counterions, e is the electron charge, k is the Boltzmann constant, and T is the absolute temperature. The parameters ccc, A, and ψ_δ thus obtained are summarized in Table 2. The ζ and d_p data for the latex samples taken immediately after the end of the experiments of coagulation kinetics are also shown in Figs. 3 and 4, respectively.

The coagulation rate, as reflected by the slope of the absorbance versus time curve in Fig. 1 or the magnitude

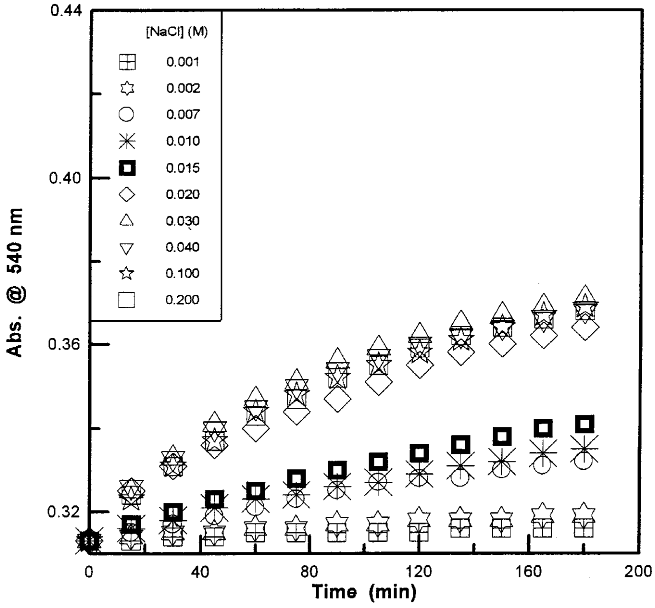

Fig. 1 Representative absorbance versus time profiles for the latex with 0.5 wt% 2,2′-azobis(2-amidinopropane) dihydrochloride (V-50) and 2 wt% chitosan (C)

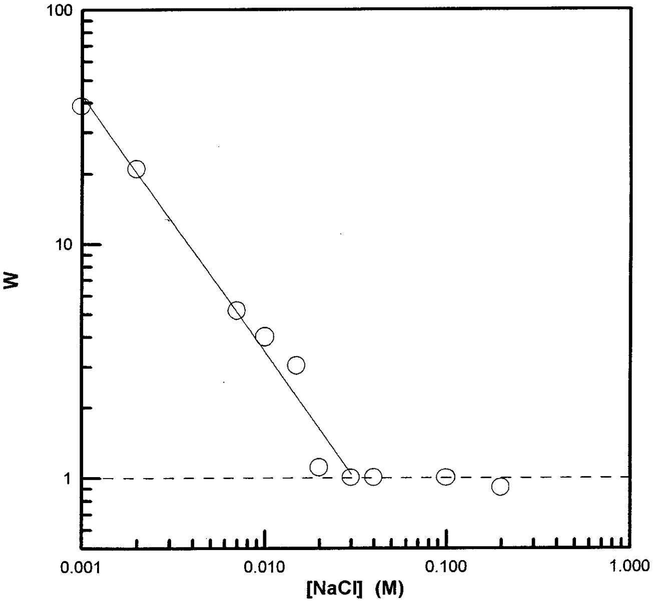

Fig. 2 Representative stability ratio versus NaCl concentration profiles for the latex with [V-50] = 0.5 wt% and [C] = 2 wt%

Table 2 Critical coagulation concentration, Hamaker constant, diffuse potential, and other parameters obtained from experiments of coagulation kinetics

Latex ID	V5	V10	V50	C1	C10	C20
			pH 3			
ccc (mM)	69	106	151	175	177	172
$A \times 10^{-21}$ (J)	0.97	1.67	2.83	3.56	2.25	1.62
ψ_δ (mM)	5.5	8.0	11.4	13.4	10.6	8.9
			pH 7			
ccc (mM)	50	55	60	37	39	33
$A \times 10^{-21}$ (J)	1.10	1.55	1.46	2.69	1.89	2.15
ψ_δ (mM)	5.4	6.5	6.5	7.8	6.7	6.8

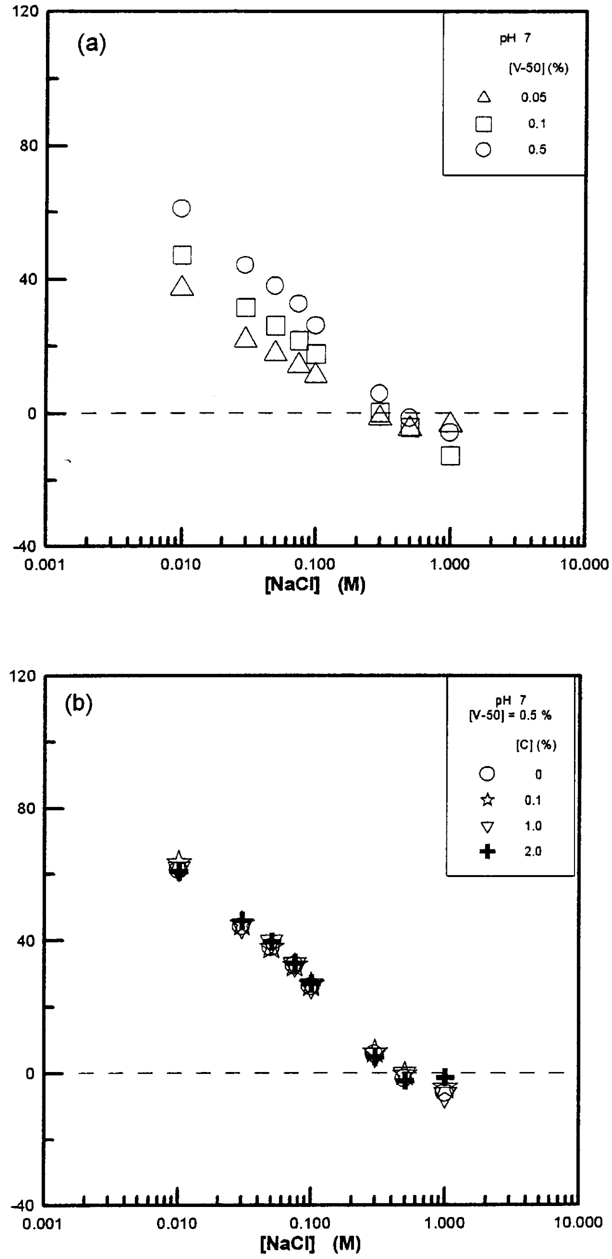

Fig. 3a, b Representative zeta potential versus NaCl concentration profiles for the latex samples at pH 7 taken immediately after the end of the experiments of coagulation kinetics. **a** [C] = 0 wt% and [V-50]: (△) 0.05 wt%; (□) 0.1 wt%; (○) 0.5 wt%. **b** [V-50] = 0.5 wt% and [C]: (○) 0 wt%; (☆) 0.1 wt%; (▽) 1 wt%; (+)2 wt%

of *W* in Fig. 2, increases with increasing NaCl concentration due to the enhanced compression of the electric double layer around these chitosan-modified PMMA latex particles. This trend is consistent with the ζ and d_p data shown in Figs. 3b and 4b, respectively (see the cross data points for C20). The zeta potential of the colloidal particles decreases rapidly from about 60 to 45 mV as the NaCl concentration is increased from 10 to 30 mM. Furthermore, d_p remains relatively constant when the NaCl concentration increases from 1 to 30 mM. This is followed by a rapid increase in d_p due to the particle flocculation process upon further increasing the electrolyte concentration. These experimental data then suggest that the latex product C20 at pH 7 should possess a zeta potential of at least 45 mV in order to maintain adequate colloidal stability.

For the chitosan-free latex products prepared by various levels of V-50 (see V5, V10, and V50 in Table 2), at constant pH, ψ_δ increases with increasing V-50 content. The ζ data also show a similar trend (e.g., see the ζ data for the run at [NaCl] = 10 mM in Fig. 3a). This is attributed to the increased particle surface charge density (σ) with increasing V-50 content, as shown by the $C_{\text{V-50}}$ data in Table 1. As a consequence, the latex product prepared using a larger amount of V-50 should be more stable toward added NaCl and, indeed, it generally exhibits a higher value of the ccc (see Table 2). It is also interesting to note that *A* increases with increasing V-50 content, which is consistent with the work of Patey [17]. Considering two approaching colloidal particles possessing a relatively high value of σ, electrostatic attraction force can be induced between the NH_3^+ groups on one particle surface and the counterions (Cl^-) around the other particle. This can greatly increase the affinity between two interactive particles and, thereby enhance the apparent value of *A*. It should be noted that the influence of the V-50 content on the colloidal stability is less significant for the chitosan-free latex samples at pH 7 in comparison with the samples at pH 3. This result is closely related to the sensitivity of the surface properties of these colloidal particles to changes in pH [1]. It was shown that the ζ of the positively charged PMMA latex particles first remains relatively constant and then decreases rapidly when the pH of the latex sample increases from 2 to 10.7. This is because the fraction of the amino groups on the particle surface which has been ionized decreases with increasing pH. It is postulated that the pH effect may override the effect of the increased stability of the

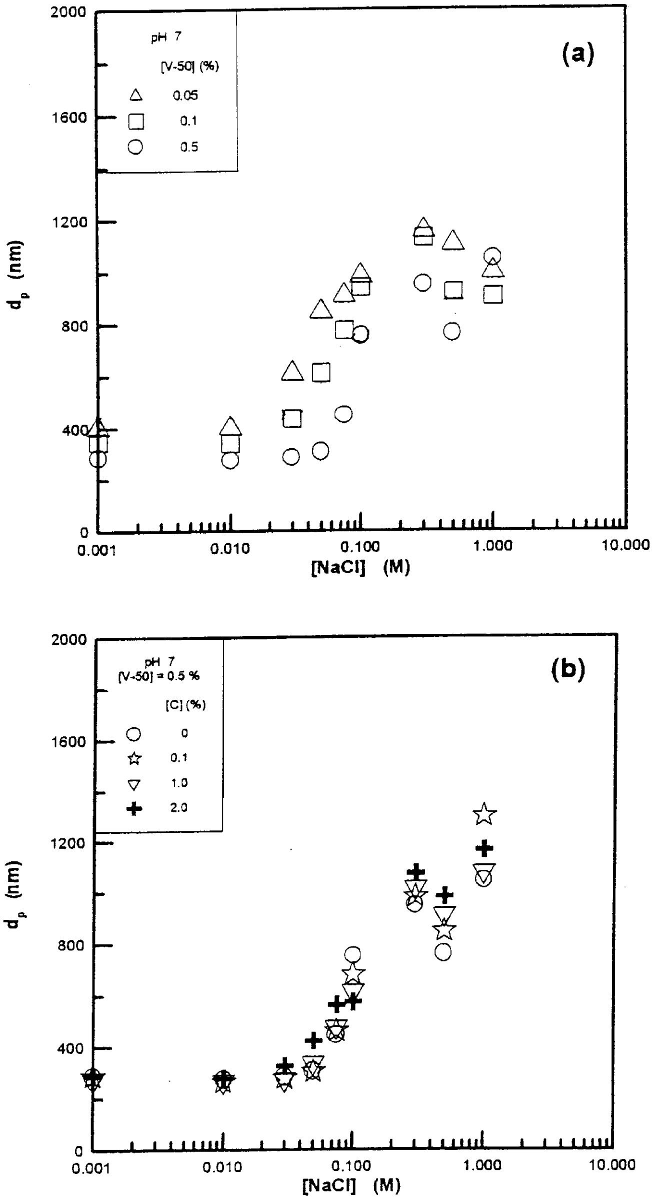

Fig. 4a, b Representative particle size versus NaCl concentration profiles for the latex samples at pH 7 taken immediately after the end of the experiments of coagulation kinetics. **a** [C] = 0 wt% and [V-50]: (△) 0.05 wt%; (□) 0.1 wt%; (○) 0.5 wt%. **b** [V-50] = 0.5 wt% and [C]: (○) 0 wt%; (☆) 0.1 wt%; (▽) 1 wt%; (+)2 wt%

colloidal particles with increasing V-50 content. As a result, the latex products V5, V10, and V50 at pH 7 do not show very different values of ccc, A, and ψ_δ. Table 2 also shows that the ccc, A, and ψ_δ decrease when the pH of the chitosan-free latex sample increases from 3 to 7. Thus for the latex sample at pH 7, the value of σ is not high enough to provide the interactive particles with adequate electrostatic repulsion force. This will then lead to a decrease in the stability (i.e., a reduction in ψ_δ and ccc) of the colloidal system. In addition, the decreased concentration of NH_3^+ groups on the particle surface may lower the induced electrostatic attraction force and, consequently, reduce the affinity between two approaching particles. This factor is responsible for the decreased apparent value of A as the pH of the latex sample is increased from 3 to7.

For the latex particles with 0.5 wt% V-50 and various degrees of chitosan modification ([C] = 0.1–2 wt%, C_c = 0.08–1.7 wt%), at constant pH, the ccc data seem to be relatively insensitive to changes in the chitosan content (C_c) (see C1, C10, and C20 in Table 2). This observation is further supported by the ζ and d_p data for the latex products toward added NaCl, as shown in Figs. 3b and 4b, respectively. For comparison, the ζ and d_p data for the latex product prepared with 0.5 wt% V-50 and 0 wt% chitosan (designated as V50) are also included in these plots. At constant NaCl concentration, the values of ζ and d_p for the chitosan-modified latex products do not vary significantly. This result may be due to the fact that these latex particles are covered by a layer of grafted chitosan. This hydrophilic polymer layer will shift the shear plane toward the aqueous solution and, thereby, reduce the ζ of the colloidal particles. Thus, the greater the degree of chitosan modification, the lower the ζ of the latex particles. On the other hand, the chemically incorporated chitosan will contribute to the particle surface charge density. In this case, the ζ of the latex particles is expected to increase with increasing C_c. It is therefore postulated that these two opposite effects related to the grafted chitosan are coincidentally counteracted by each other.

At pH 3, the value of the ccc of the latex particles in the absence of chitosan (V50) is smaller than those for the chitosan-modified latex products C1, C10, and C20 (see Table 2). In contrast, at pH 7 the value of the ccc for V50 is much larger than those for C1, C10, and C20 (see Table 2). Note that these emulsion polymers contain exactly the same amount of V-50 ($C_{V\text{-}50}$ = 8.3 wt%) but various levels of grafted chitosan (C_c = 0–1.7 wt%). Such reverse behavior is probably caused by the bridging flocculation mechanism proposed by Pelssers et al. [18] and De Witt and van de Ven [19]. The colloidal stability of these chitosan-modified latex products is presumably controlled by the fraction of the particle surface covered by chitosan (θ) and the ratio of the thickness of the chitosan adsorption layer (δ) to the thickness of the electric double layer around the latex particle ($1/\kappa$). According to De Witt and van de Ven [19], at lower values of the ratio $\delta/(1/\kappa) = \kappa\delta$, the electrostatic repulsion force predominates the interparticle interaction process, leading to a relatively stable colloidal system. The colloidal system becomes unstable due to the bridging flocculation mechanism provided by adsorption of the chitosan chain on two neighboring particles when the ratio $\kappa\delta$ approaches unity. Under the condition of $\theta \rightarrow 1$ and $\kappa\delta > 1$, the colloidal system is stable as a result of steric stabilization. Based on simple mass balance, the particle surface concentration of chitosan (Γ) was estimated to be 3.9×10^{-9}, 4.5×10^{-8}, and 7.8×10^{-8} g/cm^2 for the latex products C1, C10, and C20, respectively. The magnitude of δ cannot be determined since information on the conformation of the surface chitosan is not available at this time. As Γ is increased, the particle surface becomes more crowded with the coupled chitosan molecules and, therefore, these hydrophilic species will extend to a greater extent toward the aqueous phase. Accordingly, δ is expected to increase with increasing Γ. The chitosan-modified latex products (C1, C10, and C20) at pH 3 possess a relatively high particle surface charge density (σ) and thus, these particles are surrounded by a rather thick electric double layer (i.e., $1/\kappa$ is very large), as shown by the ζ data (e.g., ζ = 77–87 mV at [NaCl] = 10 mM). It is postulated that, for these chitosan-modified latex products at pH 3, the effect of the increased $1/\kappa$ overrides the effect of the increased δ with Γ. This will then result in a relatively stable colloidal system due to the low value of $\kappa\delta$. Another possible contributing factor for the improved colloidal stability of the chitosan-modified latex particles is the synergistic electrostatic and steric stabilization mechanisms involved. Consequently, the chitosan-modified latex products exhibit larger values of the ccc than the chitosan-free latex product V50. By contrast, the ζ of the chitosan-modified latex products at pH 7 is lower (e.g., ζ = 67–69 mV at [NaCl] = 10 mM) because of the reduced degree of ionization of the surface amino groups. The decrease in ζ then causes a decrease in the electric double layer thickness (i.e., $1/\kappa$ is very small) and, thereby makes the ratio $\kappa\delta$ move close to unity. In this case, the bridging flocculation mechanism may play a more important role in the interparticle interaction process and, therefore, the chitosan-modified latex products exhibit smaller values of ccc than the chitosan-free latex product V50.

At constant pH, a maximum value of the ccc seems to exist around C_c = 0.9 wt% in the ccc versus C_c data for the chitosan-modified latex products (See C1, C10, and C20 in Tables 1, 2), although the experimental data are not solid enough to draw such a conclusion. This observation is probably related to the above-mentioned bridging flocculation mechanism. Increasing the chitosan content (C_c) from 0.08 to 0.9 wt% will result in an increase in Γ (3.9×10^{-9} to 4.5×10^{-8} g/cm^2). Accordingly, the synergistic electrostatic and steric stabilization effect provided by the particle surface NH_3^+ groups and chitosan is greatly enhanced. The value of the ccc for the chitosan-modified latex products may thus increase as a result of the enhanced colloidal stability with C_c. Upon further increasing C_c from 0.9 to 1.7 wt%, Γ increases from 4.5×10^{-8} to 7.8×10^{-8} g/cm^2 and, consequently, the surface chitosan molecules may protrude beyond the electric double layer (i.e., $\delta > 1/\kappa$). In this case, the probability of inducing the bridging flocculation process is increased significantly, leading to a decrease in the ccc. Tables 1 and 2 also show that both A and ψ_δ decrease with increasing C_c for the chitosan-modified latex products at pH 3. The ζ data for these colloidal particles at a NaCl concentration of 10 mM also show the same trend; that is, the ζ of the latex particles decreases from 87 to 77 mV when C_c increases from 0.9 to 1.7 wt%. This result is consistent with a mechanism involving the induced electrostatic attraction force between two approaching charged particles [17]; however, it is not clear why the ccc versus C_c data exhibit a maximum while A, ψ_δ and ζ ([NaCl] = 10 mM) all decrease monotonously with increasing C_c. In addition, there is no apparent correlation between C_c and the parameters ccc, A, and ψ_δ for the chitosan-modified latex products at pH 7. Further research is required to clarify these questions. Again, just like the chitosan-free latex products, the parameters ccc, A, ψ_δ, and ζ ([NaCl] = 10 mM) for the chitosan-modified latex samples at pH 3 are greater than those for the samples at pH 7 due to the higher particle surface charge density (see Table 2).

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