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ORIGINAL CONTRIBUTION

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Dextran stabilized poly(methyl methacrylate) latex particles and their potential application for affinity purification of lectins

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Abstract Stable poly(methyl methacrylate) latex particles (220–360 nm in diameter) using dextran as the protective colloid were prepared and characterized in this study. Such an emulsion polymerization system follows Smith–Ewart Case III kinetics (i.e., the average number of free radicals per particle is greater than 0.5) due to the relatively large latex particle size. The carbohydrate content of these dextran modified emulsion polymers shows a maximum around 5% dextran based on total monomer weight. The latex stability during polymerization is closely related to the carbohydrate content of these latex particles, and it is controlled by the ratio of the thickness of the dextran adsorption layer to that of the electric double layer of the latex particles. The critical flocculation concentration (CFC) of the latex products correlates well with the latex stability during polymerization. The greater the total scrap produced during polymerization (i.e., the stronger the bridging flocculation), the lower is the CFC of the latex products. The affinity precipitation of concanavalin A (a model lectin used in this study) by the dextran modified PMMA latex particles is also illustrated in this study. The specifically adsorbed concanavalin A increases with the carbohydrate content of the dextran modified latex particles.

Key words Emulsion polymerization – poly(methyl methacrylate) – dextran stabilizer – concanavalin A – affinity purification – colloidal stability

Introduction

Emulsion polymer particles (ca. 0.05–0.5 μm in diameter) are produced by dispersion of hydrophobic monomers in water with the aid of surfactants, followed by addition of a persulfate initiator to start the reaction. These submicron particles are stabilized by surfactants adsorbed on their particle surfaces. Conventional mixed surfactants used in emulsion polymerization are amphiphilic compounds such as sodium dodecyl sulfate and nonylphenol-40 mole ethylene oxide adduct, which impart electrostatic [1, 2] and entropic [3, 4] repulsion forces, respectively, among the interactive latex particles. These small and mobile surfactant species may easily diffuse toward the surface layer of the polymeric film. The application properties of the latex products (such as adhesion and water resistance) may thus be greatly reduced. The water-soluble polymer, dextran, may become surface-active by grafting the hydrophobic oligomeric radicals onto dextran via the hydrogen abstraction mechanism during emulsion polymerization. The amphiphilic graft copolymer formed in situ is just like a nonionic surfactant (termed as protective colloid) and, therefore, it may help nucleate and stabilize the primary particles during polymerization. The application properties of such latex products should not be impaired because the grafted dextran have become an integral part of the emulsion polymer.

These submicron particles chemically incorporated with dextran on their particle surfaces may find potential application in affinity purification of carbohydrate-binding proteins (lectins) due to the extremely large particle surface area. Lectins are carbohydrate-binding proteins other than enzyme or antibody. It is well known that the lectin (e.g., concanavalin A (Con A) used as a model compound in this study) shows an equal or better specific affinity for carbohydrates (e.g., dextran) as compared to antibodies for antigens [5]. Con A has dimeric ($pH = 2.0$–5.8) or tetrameric ($pH > 5.8$) structure [6]. Con A may thus specifically bind to the surface dextran which are chemically coupled onto different latex particles. This will result in a crosslinked network structure and, thereby, induces selective precipitation of the resultant large flocs by gravitational or centrifugal force. The selective precipitation technique [7–12] involves binding of Con A onto the dextran modified latex particle surface, precipitation of the resultant complex out of the crude extract, and removal of the supernatant. This is followed by dissociation and recovery of the desired product from the precipitate.

To the best of our knowledge, the literature dealing with emulsion polymerization using dextran as the protective colloid had been nil. The objective of this work was to synthesize poly(methyl methacrylate) (PMMA) latices in the presence of dextran and then study their colloidal characteristics such as the particle size, redispersibility of these latex particles subjected to centrifugal force, extent of particle surface modification by dextran, zeta potential, and critical flocculation concentration. Another goal of this project was to demonstrate the affinity precipitation of Con A by the dextran modified PMMA latex particles.

Experimental

Materials

The chemicals used in this work include: methyl methacrylate (MMA) (Kaohsiung Monomer Co.); dextran with a molecular weight of approximate 5×10^5 g/mol (Fluka Biochemika); sodium persulfate (Riedel-de Haen); sodium chloride (Riedel-de Haen); sodium hydroxide (Riedel-de Haen); hydrochloric acid (Nacalai Tesque, Inc.); sodium phosphate, monobasic monohydrate (J.T. Baker); sulfuric acid (Shimakyu's Pure Chemicals); phenol (Nacalai Tesque Inc.); concanavalin A (Con A) (Type III, Sigma); nitrogen (Ching-Feng-Harng Co.); deionized water (Barnsted, Nanopure Ultrapure Water System, specific conductance < 0.057 μS/cm). The monomer MMA was distilled under reduced pressure before use. All other chemicals were used as received.

Polymerization process

Batch emulsion polymerization was carried out in a 250-ml glass reactor equipped with a 45° pitched 4-bladed agitator, a thermometer and a condenser. The polymerization procedure is described as follows. The reaction mixture including, e.g., 440 g H_2O, 0.975 g dextran (2% based on total monomer), and 48.850 g MMA was purged with nitrogen for 10 min to remove the dissolved oxygen while heated to 75 °C, followed by addition of the initiator solution comprising 10 g H_2O and 0.149 g $Na_2S_2O_8$ (0.305% based on total monomer weight) to start the reaction. The polymerization temperature was kept at 75 °C for 24 h, followed by a second shot of initiator solution (10 g H_2O and 0.149 g $Na_2S_2O_8$) and an increase in the temperature to 85 °C. Subsequently, the reaction system was kept at 85 °C for another 47 h to eliminate the residual monomer. The total solid content of the latex product is 9.83% for complete conversion. The agitation speed was kept constant at 400 rpm throughout this work. The concentration of dextran ([Dextran]) was varied from 0% to 10% based on total monomer.

The latex product was filtered through a 40-mesh (0.42 mm) screen and a 200-mesh (0.074 mm) screen in series to collect the filterable solids. Scraps adhering to the agitator, thermometer, and reactor wall were also collected. The total scrap data reported in this work include the 40-mesh scrap, 200-mesh filterable solids, and that adhering to the agitator, thermometer and reactor wall. Total solid content was determined by the gravimetric method.

Characterization of latex products

Particle size (d_p) data were obtained from the dynamic light scattering (DLS) method (Otsuka, Photal LPA-3000/3100). If necessary, the turbid latex sample was further diluted with deionized water to adjust the CPS value to the range of 8000–12000. The parameter 'accumulation times' was kept constant at 50. The d_p data reported in this work represent an average of at least three measurements and most of these data show an error of 10% or less. For comparison, transmission electron microscopy (TEM) (JEOL JSM-1200EXII) was also used to determine the number-average and weight-average particle sizes (abbreviated as d_n and d_w, respectively) for the latex products. At least 300 latex particles were measured for each latex sample.

Before the critical flocculation concentration (CFC) and zeta potential (ζ) measurements, the latex product was separated into the supernatant phase and the precipitate phase by centrifugation at 11 000 rpm for 15 min (Beckman, J2-21). The clear supernatant was withdrawn, followed by redispersion of the precipitate in fresh deionized water using a mini ultrasonic cleaner (Delta DG-1). This procedure was repeated at least three times to remove the free dextran in water. The transmittance of the resultant latex product (total solid content = 0.05%) was then measured by a spectrophotometer (Metertek SP-810) at 540 nm for determination of the critical flocculation concentration (CFC). The pH and ionic strength of the latex sample were adjusted by 0.5 N NaOH, 1 N HCl, and NaCl. The sample was mixed thoroughly for 30 min and it was then allowed to stand at room temperature for 1 h before the transmittance measurements. The ζ of these particles was determined by Zetamaster (Malvern). Five measurements were made for each latex sample and the average of these five measurements was reported as the ζ of the sample.

The carbohydrate content of the dextran modified emulsion polymers was determined by measuring the absorbance at 485 nm (Shimadzu, UV-160 A) after color development with the phenol-sulfuric acid reaction [13]. First, 1 ml of the dextran modified latex (solid content = 0.5%) was mixed with 0.5 ml phenol (5%) and 3 ml pure sulfuric acid. The reaction mixture was stirred for 10 min at 25 °C, followed by measuring the absorbance at 485 nm. The experiment using the latex sample with [Dextran] = 0% was taken as the reference (zero point).

Affinity precipitation of Con A

A protein solution was prepared by dissolving 0.2 g Con A (15% active) in 50 ml of pH 5 phosphate solution, followed by filtration through a 1.2 μm membrane to remove the insoluble species. The protein solution with a volume of 3 ml was then mixed with an equal volume of the dextran modified latex (solid content = 0.5%) to initiate the affinity precipitation of Con A. The reaction mixture was stirred for 1 h at 25 °C. The supernatant phase was collected by repeated centrifugation at 13 000 rpm for 10 min. This step was followed by determination of the concentration of Con A in the supernatant by UV absorbance at 280 nm (Shimadzu, UV-160 A).

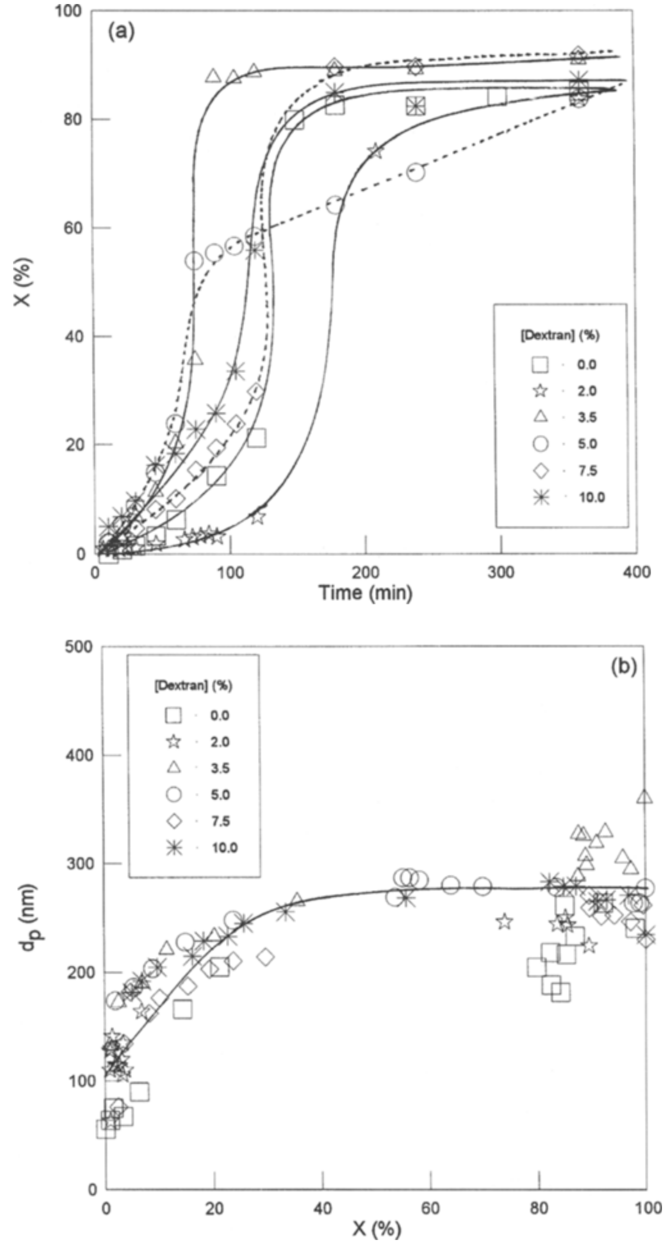


Fig. 1 Kinetic data: (a) monomer conversion as a function of time for the emulsion polymerizations and (b) latex particle size as a function of time for the emulsion polymerizations stabilized by various levels of dextran: [Dextran] = (□) 0.0%; (☆) 2.0%; (△) 3.5%; (○) 5.0%; (◇) 7.5%; (✳) 10.0%

Results and discussion

Emulsion polymerization

Figure 1 shows some kinetic data: (a) monomer conversion (X) versus time (t) and (b) d_p versus X data with [Dextran] = 0–10%. All the dextran stabilized polymerizations show a strong gel effect (i.e., a rapid increase in the polymerization rate with time due to the diffusion-controlled termination reaction), which is typical of the MMA emulsion polymerization at a temperature well below its glass transition temperature (115 °C) [14]. The rate of

Table 1 Results obtained from the emulsion polymerizations stabilized by various levels of dextran

[Dextran] [%]	0.0	2.0	3.5	5.0	7.5	10.0
d_{pf}[a] [nm]	304	224	360	296 ± 10[f]	230	240
$u/G^2 \times 10^{-1}$[b]	0.42	0.56	2.55	8.87 ± 0.17[f]	0.61	0.48
$d_{\mathrm{pf,rd}}$[c] [nm]	296 ± 14	237 ± 17	349 ± 19	286 ± 22	235 ± 8	265 ± 12
d_{w} [nm][d]	207	175	242	218	181	201
$d_{\mathrm{w}}/d_{\mathrm{n}}$[d]	1.007	1.016	1.013	1.424	1.016	1.022
$N_{\mathrm{f}} \times 10^{16}$ [1/L-H_2O]	0.633	1.550	0.368	0.597	1.359	1.169
$\mathrm{d}X/\mathrm{d}t \times 10^5$ [1/s]	4.075	0.956	7.131	7.186	4.850	4.800
n	1.21	0.11	3.51	2.15	0.62	0.70
Total scrap[e] [%]	4.5	1.2	6.3	9.9 ± 1.1[f]	0.7	1.8

[a] Particle size data of latex products based on DLS.
[b] A measure of particle size distribution of latex products based on DLS.
[c] An average d_{pf} in the redispersity test (at least 5 cycles).
[d] Particle size data of latex products based on TEM.
[e] Based on total monomer weight.
[f] An average of two identical experiments.

polymerization (R_{p}) for emulsion polymerization can be written as

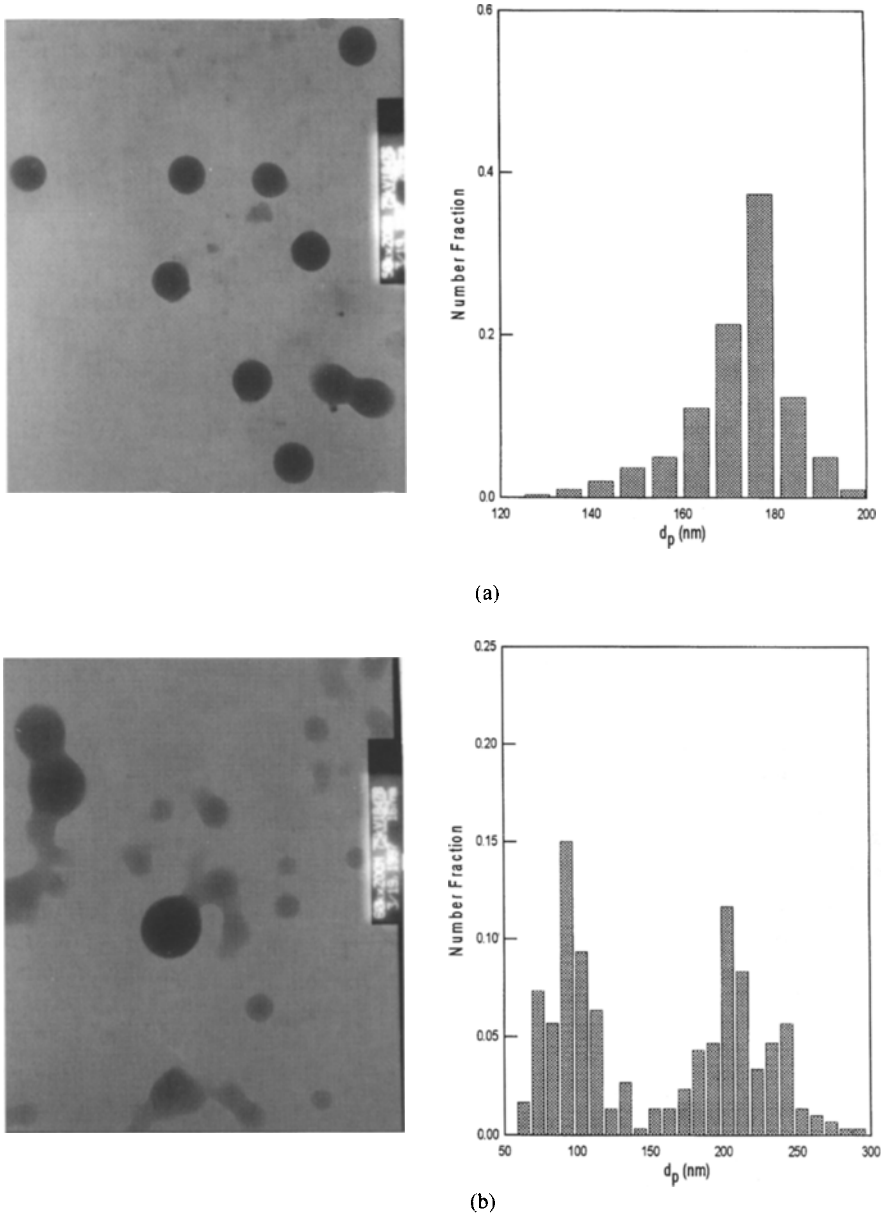
$$R_{\mathrm{p}} = [\mathrm{M}]_0 \mathrm{d}X/\mathrm{d}t = K_{\mathrm{p}}[\mathrm{M}]_{\mathrm{p}}(nN_{\mathrm{f}}/N_{\mathrm{a}}) , \qquad (1)$$

where K_{p} is the propagation rate constant, $[\mathrm{M}]_0$ is the initial concentration of monomer based on total water (e.g., 1.06 M in this work), $[\mathrm{M}]_{\mathrm{p}}$ is the concentration of monomer in the latex particles, N_{f} is the number of latex particles nucleated (see Table 1), n is the average number of free radicals per particle, and N_{a} is Avogadro's number. This equation can be used to calculate n if the parameters $\mathrm{d}X/\mathrm{d}t$, K_{p}, $[\mathrm{M}]_{\mathrm{p}}$, and N_{f} are known. The $\mathrm{d}X/\mathrm{d}t$ data were estimated by the slope of the least-squares best-fitted X vs. t straight line before the occurrence of the gel effect. The parameter K_{p} was estimated to be 602 1/M s at 75 °C [14]. Figure 1(b) shows that the latex particles stop growing at $X \sim 40\%$. This conversion represents the point at which the latex particles are saturated with monomer. Thus, $[\mathrm{M}]_{\mathrm{p}}$ was estimated to be 5.9 M. The data of $\mathrm{d}X/\mathrm{d}t$ and n as a function of [Dextran] are listed in Table 1. All the experiments result in a value of n greater than 0.5 (Smith–Ewart Case III kinetics [15]) except for the run with [Dextran] = 2.0%. This is probably caused by the relatively large latex particles stabilized by dextran ($d_{\mathrm{f}} > 230$ nm as shown in Table 1). As a consequence, the volume of the monomer-swollen polymer particles is large enough to allow more than one radical to coexist in the reaction loci. The run with [Dextran] = 2.0% follows Smith–Ewart Case I kinetics (i.e., $n < 0.5$) [15]. For the polymerization system with [Dextran] = 2.0%, the reason for the retarded reaction rate is not clear at this time. The rate of polymerization (proportional to $\mathrm{d}X/\mathrm{d}t$) or n seems to increase first to a maximum and then decrease with increasing [Dextran] besides the run with [Dextran] = 2.0% (see Table 1).

Unlike other latex products, the latex stabilized by 5.0% dextran shows a bimodal particle size distribution based on DLS measurements, as shown by the very high u/G^2 in Table 1. The parameter u/G^2 is a measure of the breadth of the particle size distribution. The greater the value of u/G^2, the broader is the particle size distribution. To test the reproducibility, the experiment with [Dextran] = 5.0% was carried out twice. As shown in Table 1, the resultant d_{pf}, u/G^2 and total scrap are 287 ± 18 nm, 8.87 ± 0.17 and 9.9 ± 1.1%, respectively. The reproducibility is reasonably good, considering that the latex stability is quite poor during polymerization as reflected in the very high total scrap data. The $d_{\mathrm{w}}/d_{\mathrm{n}}$ (polydispersity index) data (see Table 1) and representative particle size distribution profiles with [Dextran] = 2.0 and 5.0% (see Fig. 2) determined by TEM also show that all latices are relatively monodisperse except the latex stabilized by 5% dextran (bimodal). The particle size distribution profiles with [Dextran] = 0.0, 3.5, 7.5 and 10.0% are similar to that stabilized by 2.0% dextran shown in Fig. 2.

Figure 3 shows the carbohydrate content of the dextran modified emulsion polymers as a function of [Dextran]. The greater the absorbance at 485 nm, the greater is the amount of dextran grafted onto the particle surface. The carbohydrate content increases gradually and then jumps up to a maximum when [Dextran] increases from 0.0% to 5.0%. After the maximal point is reached, the carbohydrate content then starts to decrease with increasing [Dextran] (5.0% → 10.0%). The decreased carbohydrate content with increasing [Dextran] is probably related to the degree of chain transfer of the water-soluble oligomeric radicals to dextran in the aqueous phase. This chain transfer reaction results in a free radical on the dextran chain and, then, initiates the propagation reaction with MMA dissolved in water. The PMMA-modified

Fig. 2 Representative TEM photographs and particle size distribution profiles: [Dextran] = (a) 2.0%; (b) 5.0%



dextran may become surface-active and chemically adsorbed onto the latex particle surface in the subsequent emulsion polymerization. When [Dextran] is high enough the level of PMMA grafted onto dextran may not be sufficient to render the PMMA grafted dextran chains amphiphilic. Thus, only a small proportion of dextran initially charged to the reactor can be chemically incorporated into the latex particles.

Figure 4 shows the ζ versus [Dextran] data at 0.01 M NaCl and various pH values. The negative sign of the ordinate simply indicates that the latex particles carry negative charges. At constant pH, the ζ of these particles first decreases to a minimum when [Dextran] increases from 0 to 5%. After the minimal point is reached, the ζ then starts to increase with increasing [Dextran]. This result can be explained by the fact that these particles are covered by a layer of dextran. This hydrophilic polymer layer will shift the shear plane toward the aqueous solution and, consequently, reduce the ζ. Thus, the greater the carbohydrate content, the lower is the ζ of the dextran modified latex particles. Indeed, the ζ data correlate reasonably well with the carbohydrate content of the emulsion polymers (see Figs. 3 and 4). At constant [Dextran], the ζ increases with pH (see Fig. 4). The

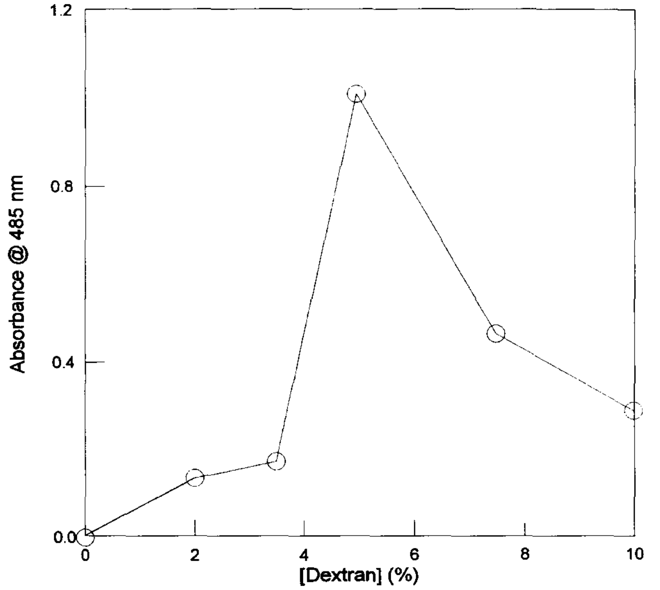


Fig. 3 Absorbance at 485 nm as a function of [Dextran] after color development with the phenol–sulfuric acid reaction

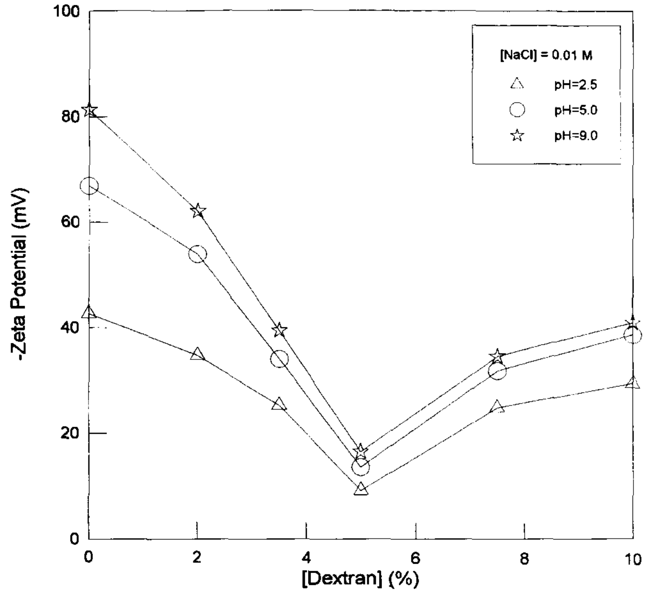


Fig. 4 Zeta potential as a function of [Dextran] at 0.01 M NaCl: (△) pH 2.5; (○) pH 5.0; (☆) pH 9.0

gradually increased ζ with pH is probably caused by the carboxyl groups derived from hydrolysis of the sulfate end-groups and subsequent oxidation of the generated hydroxyl groups [16]. The ionized carboxyl groups may increase the particle surface charge density and, thereby, increase the ζ of the dextran modified latex particles.

The data of total scrap as a function of [Dextran] are shown as circular data points in Fig. 5. The total scrap produced during polymerization first decreases and then increases to a maximum when [Dextran] increases from 0.0% to 5.0%. After the maximal point is reached, the total scrap then starts to decrease with increasing [Dextran] (5% → 10%). The total scrap produced is presumably controlled by the ratio of the thickness of the dextran adsorption layer (δ) to that of the electric double layer of the latex particles ($1/\kappa$) (i.e., $\delta/(1/\kappa) = \kappa\delta$) [17–19]. The electric double layer around the latex particles originates from the sulfate group (SO_4^-) on the particle surface derived from the persulfate initiator. The ratio $\kappa\delta$ may play an important role in the bridging flocculation process. According to De Witt and van de Ven [18], at lower values of $\kappa\delta$ (i.e., $1/\kappa \gg \delta$) the electrostatic repulsion force predominates the interparticle interaction process, leading to a relatively stable colloid. The colloid becomes unstable due to the bridging flocculation mechanism when the ratio $\kappa\delta$ is equal to unity (i.e., $1/\kappa \sim \delta$).

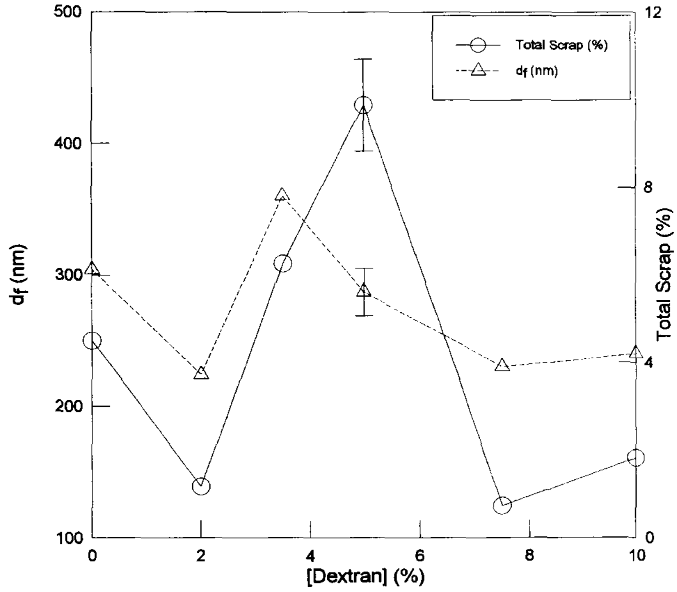


Fig. 5 Total scrap and final latex particle size as a function of [Dextran]: (○) total scrap; (△) final latex particle size

In this work, the concentration of the initiator was kept constant at 0.305% based on total monomer weight. Thus, the polymerization system with [Dextran] ranging from 0.0% to 10.0% might have comparable values of $1/\kappa$. Incorporation of 2.0% dextran into the emulsion polymerization reduces the total scrap from 4.5% to 1.2% in comparison with the surfactant-free emulsion polymerization ([Dextran] = 0.0%). Reduction in the formation of coagulum for the dextran stabilized latex is attributed to the synergetic effect provided by the electrostatic and steric repulsion forces. It is postulated that the ratio $\kappa\delta$ is relatively small (i.e., $1/\kappa \gg \delta$) for the run with [Dextran] = 2.0%. When [Dextran] increases from 2.0% to 5.0% the carbohydrate content of the dextran modified latex particles (or δ) increases (see Fig. 3). This will result in a rapid increase in total scrap because the ratio $\kappa\delta$ approaches unity (i.e., $1/\kappa \sim \delta$) and such a change may induce bridging flocculation among the interactive particles. Intensive flocculation may thus lead to formation of large flocs during polymerization. Further increasing [Dextran] from 5.0% to 10.0%, the polymerization system becomes stable again (i.e., $1/\kappa \gg \delta$) due to the decreased carbohydrate content (see Fig. 3). It is interesting to note that the particle size of latex products (d_f) versus [Dextran] profiles show a similar trend as the total scrap versus [Dextran] profiles (see the triangular data points in Fig. 5). The degree of bridging flocculation is more or less reflected in the d_f data. The greater the carbohydrate content (i.e., the stronger the bridging flocculation), the larger is the resultant latex particle size.

Characterization of latex products

The redispersibility of the latex particles is important because centrifugation is often used to separate the precipitate from the crude extract for protein purification. The latex sample with a solid content of 0.05% was repeatedly centrifuged at 13 000 rpm for 10 min (Beckman, J2-21) and dispersed in a mini ultrasonic cleaner for at least five times. No significant changes in the particle size were observed (see the d_{pf} and $d_{pf,rd}$ data shown in Table 1). The redispersibility (mechanical stability) of these latex products is thus satisfactory for downstream bioprocessing.

For clarity, Fig. 6 only shows three typical sets of transmittance vs. [NaCl] data for the latex samples at pH 5.0 with [Dextran] = 0.0%, 5.0%, and 7.5%, respectively. Other transmittance vs. [NaCl] data for the samples at pH 5.0 ([Dextran] = 2.0%, 3.5%, and 10.0%) show similar trends. At pH 2.5 or pH 9.0, the samples with various levels of [Dextran] also show similar transmittance vs. [NaCl] profiles. The parameter [NaCl] represents the concentration of sodium chloride used in the transmittance measurements. The transmittance data for the sample with [Dextran] = 7.5%, for example, are maintained at a relatively constant level when [NaCl] increases from 0.01 to 0.88 M. Beyond 0.88 M NaCl, the transmittance starts to increase sharply with [NaCl] as larger flocs start to form and then precipitate under the influence of the gravitational force. The point at which [NaCl] = 0.88 M is thereby identified as the critical flocculation concentration (CFC).

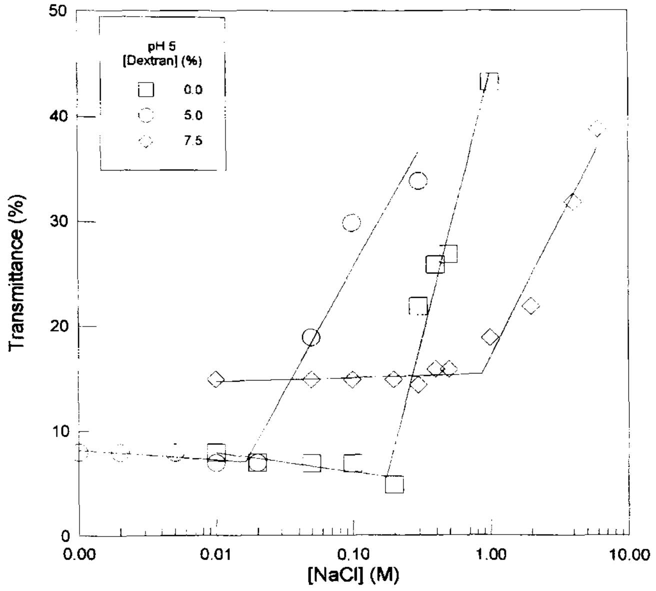


Fig. 6 Transmittance at 540 nm as a function of [NaCl] at pH 5: [Dextran] = (□) 0.0%; (○) 5.0%; (◇) 7.5%

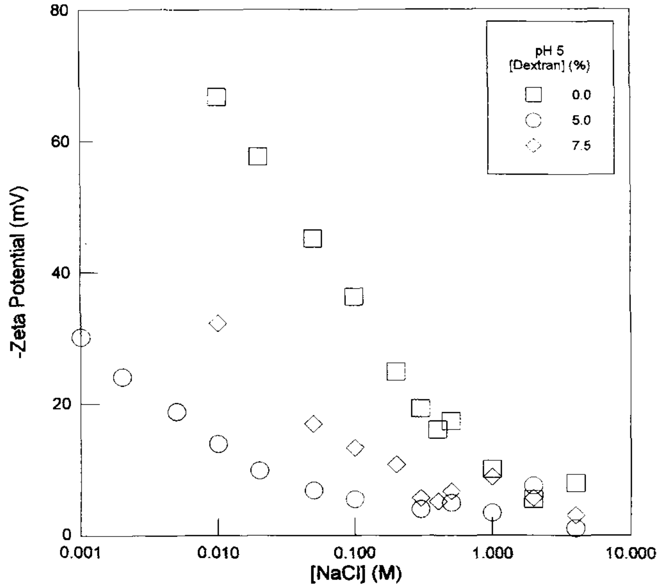


Fig. 7 Zeta potential as a function of [NaCl] at pH 5: [Dextran] = (□) 0.0%; (○) 5.0%; (◇) 7.5%

Figure 7 shows the corresponding data of ζ as a function of [NaCl] for the latex samples at pH 5.0 with [Dextran] = 0.0%, 5.0%, and 7.5%, respectively. It should be noted that the ζ of the latex particles stabilized by 5.0% dextran only represents an overall average because the sample shows a bimodal particle size distribution. At

Table 2 UV absorbance data at 280 nm obtained from the experiments of affinity adsorption of Con A by the dextran modified latex particles

[Dextran] [%]	0	0	2	2	2	7.5
Solid content[a] [%]	0	0.5	0.1	0.5	1.0	0.5
Absorbance	0.289[b]	0.217	0.197	0.173	0.130	0.135

[a] Total solid content of the latex sample.

[b] Absorbance of the mixture containing 3 ml Con A solution and 3 ml pH 5 phosphate solution.

constant [Dextran], the ζ of these latex particles decreases with increasing [NaCl]. Upon increasing [NaCl], the decreased ζ can be attributed to the increased ionic strength of the aqueous solution. The increased ionic strength will compress the electric double layer around these particles (i.e., decrease $1/\kappa$) and reduce ζ. This will cause the ratio $\kappa\delta$ to approach unity and, thereby, destabilize the dextran modified latex particles.

Figure 8 shows the CFC vs. [Dextran] data at various pH values. The CFC data correlate reasonably well with the latex stability during polymerization. The greater the total scrap (i.e., the stronger the bridging flocculation taking place during polymerization), the greater is the CFC of the latex products (see the total scrap data in Fig. 5). Furthermore, at constant [Dextran], the CFC increases rapidly and then levels off when pH increases from 2.5 to 9.0. This is again attributed to the carboxyl groups derived from hydrolysis of the sulfate end-groups and subsequent oxidation of the generated hydroxyl groups [16]. The ionized carboxyl groups may enhance the particle surface charge density and, thereby, increase the colloidal stability toward added salts.

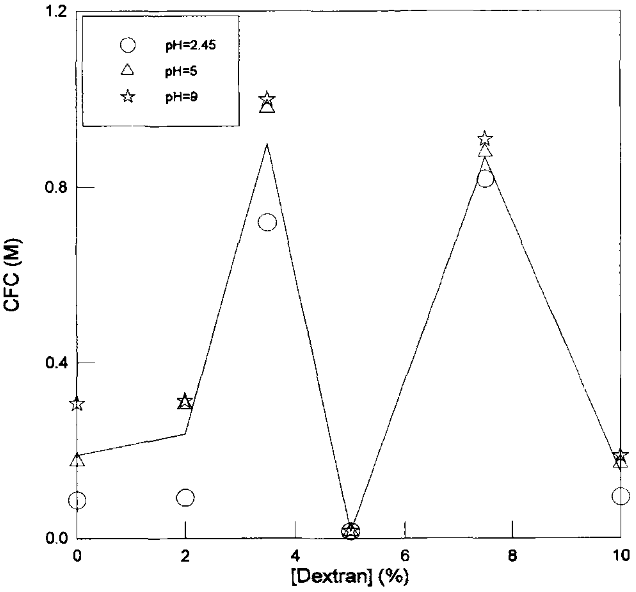


Fig. 8 Critical flocculation concentration as a function of [Dextran] at various pH values: (○) pH 2.5; (△) pH 5.0; (☆) pH 9.0

Affinity precipitation of Con A

The latex products prepared by 2.0% dextran (the lowest carbohydrate content) and 7.5% dextran (the second highest carbohydrate content) were chosen for demonstrating the affinity precipitation of Con A by the dextran modified latex particles. For reference, the latex product stabilized only by the anionic sulfate group ([Dextran] = 0.0%) was also included in this study. The experimental results are summarized in Table 2.

The total solid content of the latex samples was kept constant at 0.5% in the first series of experiments. The variable chosen for investigation was the carbohydrate content of these latex particles (i.e., [Dextran] = 0.0%, 2.0%, and 7.5%). The concentration of Con A in the supernatant phase is qualitatively related to the absorbance at 280 nm. The greater the absorbance (i.e., the higher the concentration of Con A in the supernatant phase), the less is the amount of Con A specifically adsorbed onto the latex particle surface. For reference, the absorbance of the mixture containing 3 ml Con A solution and 3 ml pH 5 phosphate solution was determined to be 0.289. Flocculation and precipitation of these dextran modified latex particles by Con A were observed in the course of experiments. The absorbance decreases from 0.217 to 0.135 with increasing carbohydrate content, as shown in Table 2. This result justifies the potential application of these dextran modified latex particles ([Dextran] = 2.0% and 7.5%) for affinity purification of Con A. The specifically adsorbed Con A may be dissociated and recovered from the precipitate phase by using glycine-HCl buffer solution (pH 2.0) [20]. After the affinity precipitation process was completed, some Con A were found to be adsorbed onto the particle surface without dextran ([Dextran] = 0.0%). The net charge of Con A at pH 5.0 should be positive because the isoelectric point (pI) of Con A is 7.8 [21]. Thus, the physically adsorbed Con A is probably due to the electrostatic interaction and it can be desorbed by using high salt concentration.

Finally, another series of experiments was carried out to study the effect of total solid content of the latex samples. In this case, [Dextran] was kept constant at 2.0% and the total solid content was varied from 0.1% to 1.0%. As shown in Table 2, the absorbance decreases from 0.197 to 0.130 with increasing total solid content. As expected, the larger the particle surface area, the more is the amount of Con A specifically adsorbed onto the dextran modified latex particle surface. This trend may thus serve as further evidence of affinity precipitation of Con A by the dextran modified latex particles.

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