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Formation of positively charged poly(butyl cyanoacrylate) nanoparticles stabilized with chitosan

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Abstract To investigate the formation of positively charged nanoparticles (NP) stabilized with chitosan, positively charged poly(butyl cyanoacrylate) (PBCA) NP were prepared by emulsion polymerization in the presence of chitosan as a polymeric stabilizer at low pH. The effect of physicochemical factors such as the pH, the concentration and the volume of the chitosan solution, the chitosan molecular weight and the temperature on the mean particle size and the turbidity of PBCA-NP was investigated. Particle size was determined using a transmission electron microscope. The chemical interaction between chitosan and PBCA was identified by Fourier transform infrared (FT-IR) spectroscopy and the grafting percentage at various pH values was determined. The zeta potential of PBCA-NP coated with chitosan was determined from the electrophoretic mobility in 10 mM NaCl. The pH, the concentration and the volume of the chitosan solution and the molecular weight of chitosan were shown to be important factors in controlling the mean particle size of NP in the range 10–100 nm. FT-IR spectra indicated that chitosan was covalently linked to PBCA and the maximum grafting percentage reached about 120% w/w at pH 2.0. Nimodipine as a model drug was successfully incorporated into chitosan-stabilized PBCA-NP with a mean particle diameter of 31.6 nm. PBCA-NP coated with chitosan carried a positive charge. The results indicate that positively charged NP may be produced in the presence of cationic polysaccharide chitosan and might increase their potential use as a targeting drug delivery system.

Key words Chitosan · Positive charge · Nanoparticles · Poly(butyl cyanoacrylate) · Targeting drug delivery system

Introduction

The potential use of polymeric nanoparticles (NP) as drug carriers for site-specific drug targeting in vivo has led to the development of many different colloidal drug delivery vehicles [1, 2]. Of these, poly(alkyl cyanoacrylate) (PACA) NP have been investigated extensively for possibilities of clinical use due to their ease of preparation, low toxicity and biodegradability [1–3]. PACA-NP can be prepared by an anionic emulsion polymerization process, in which drops of water-insoluble monomers are emulsified in the aqueous phase. Polymerization takes place in micelles after diffusion of monomer molecules through the water phase and is initiated by anionic ions [1–3]. The influences of physicochemical factors and stabilizers on the resultant particle size and size distribution of PACA-NP were investigated [4–7]. Polymerization of cyanoacrylate monomers usually requires a pH below 3.5. Above this pH, the reaction rate is too high to allow the formation of small particles. The stabilizers often added to the aqueous polymerization medium are polysorbates [6, 8, 9], poloxamers [6,

10], dextran [6, 9–11], cyclodextrin [6], poly(ethylene glycol) (PEG) [12, 13] and poly(ethylene glycol *tert*-octyphenyl ether) [9]. The hydroxy group in these stabilizer molecules might initiate the polymerization of alkyl cyanoacrylates and the stabilizer might covalently bind to the PACA [6]. Molecular-weight determination made by gel permeation chromatography has suggested that the NP were built by an entanglement of numerous small oligomeric subunits rather than by the rolling up of one or several long polymeric chains [11, 14, 15]. The molecular-weight distribution for cyanoacrylate polymer produced in the presence of Dextran 70 or Poloxamer 188 showed a bimodal distribution [15]. The type and concentration of the stabilizer are very important factors for controlling the particle size [6, 11], the surface properties [6, 7, 11, 12], drug release [9, 14] and the uptake of NP by organisms [1–3]. The anionic polymerization of alkyl cyanoacrylates initiated by organic bases, tertiary amines and phosphines has been inferred to involve a zwitterionic mechanism. In these reactions the growing species, through propagating carbanionically, are believed to be zwitterions, which have their countercations, ammonium or phosphonium, ''built-in'' as the initial group in the chain, and are not independently mobile as in conventional ionic polymerization [16–18].

PACA-NP are characterized by a negative surface charge, which might be attributed to adsorption of anions from the aqueous phase [7, 11, 12]. The in vitro negative charge may help to prevent the coalescence of NP; however, in vivo it promotes the adsorption of cationic substances, such as protein, sodium and calcium ions, which are present in the biological fluids, and this leads to the neutralization of the surface charge, the breakdown of the system and the leakage of the entrapped drug. Compared with negatively charged NP, positively charged NP may have improved stability in the presence of biological cations [19] and may be favourable for some drugs due to their interaction with negatively charged biological membranes and site-specific targeting in vivo [20, 21]. Thus, positively charged colloidal drug carriers [19, 22–25] are gaining increasing importance for drug delivery following intravenous, oral and ocular administration. In an attempt to provide a positive charge to a colloidal system, chitosan has been used in the preparation and stabilization of polyester nanocapsules [19, 26], NP [24], submicron-sized emulsions [19], microcapsules [25] and liposomes [27]. Chitosan is a cationic high-molecular-weight heteropolysaccharide composed mainly of β-(1,4)-2-deoxy-2-amino-D-glucopyranose units and partially of β-(1,4)-2-deoxy-2-acetamido-D-glucopyranose. Because of favourable biological properties such as biodegradability, biocompatibility and nontoxicity, chitosan has attracted great attention in pharmaceutical and biomedical fields [28, 29].

The aim of this research was to investigate the formation of positively charged poly(butyl cyanoacrylate) (PBCA)-NP in the presence of chitosan for the potential use as a targeting drug delivery system. We chose the polysaccharide chitosan as the stabilizer of PBCA-NP due to its cationic character and biocompatibility. Factors affecting particle size were investigated, the interaction of chitosan with PBCA was analysed using Fourier transform (FT)-IR spectroscopy and the zeta potential of PBCA-NP coated with chitosan was determined from the electrophoretic mobility. Finally, the potential of the positively charged PBCA-NP for entrapment of lipophilic compounds was assessed using nimodipine [30], a second-generation dihydropyridine with apparent selectivity for cerebral blood vessels, as a model drug.

Materials and methods

Materials

Chitosan (Nantong Shuanglin Biological Product, China) was obtained from lobster with 88% deacetylation. Three different molecular weights of chitosan (4.3×10^4, 14.5×10^4, 21.0×10^4) were determined by a viscometric method and were calculated using the Mark–Houwink equation, in which k and α depend on the degree of deacetylation of chitosan [31]. Nimodipine was purchased from Shangdong Xinhua Pharmaceutical Co. (Shangdong, China). Butyl cyanoacrylate monomer was synthesized by Xian Chemical Institute (Xian, China). All other reagents were of analytical grade.

Preparation of chitosan-stabilized NP

PBCA-NP were prepared by emulsion polymerization as described in detail elsewhere [1–6]. Briefly, the desired amount (500 μl) of monomer was dropped into an acidic solution of chitosan adjusted with hydrochloric acid under constant magnetic stirring (approximately 1000 rpm). Stirring was maintained for at least 6 h until the polymerization was complete.

The nimodipine-loaded NP were prepared by dropping a clear mixture of 50 mg nimodipine, 250 μl acetone and 500 μl monomer into 0.5% w/v chitosan (M_r 14.5×10^4) solution at pH 1.5 under constant magnetic stirring (approximately 1000 rpm). After polymerization was complete, acetone was removed under reduced pressure at 50 °C.

Particle size

The particle size and the shape of the chitosan-stabilized NP were observed with a JEOL JEM-100 transmission electron microscope (TEM). Each suspension was diluted to an appropriate solid content and a drop of it was placed onto a collodion support on copper grids, followed by negative staining with an aqueous solution of sodium phosphotungstate. The mean diameter (D_n) and the polydispersity index (D_w/D_n) were calculated by counting more than 200 NP.

Turbidity of the NP suspension

The PBCA-NP suspension was diluted to 1 g PBCA/l with distilled water of the same pH. The turbidity was measured at 590 nm using

a spectrophotometer (721C spectrophotometer, no.3, Analytical Instrumental Factory, Shanghai, China).

Extract of PBCA from chitosan-stabilized NP

After being dried to constant weight at 50 °C, the chitosan-stabilized PBCA-NP were extracted to constant weight with acetone using a Soxhlet extractor. The residue was dried under reduced pressure at 50 °C and accurately weighed. The grafting percentage was defined as follows: grafting percentage = (weight of PBCA branches introduced/weight of chitosan) × 100.

FT-IR study

FT-IR spectra of chitosan, PBCA and the residue of chitosan-stabilized NP extracted with acetone were recorded using an IFS66V vacuum-type FT-IR spectrophotometer (Bruker, Germany). Potassium bromide tablets containing the samples were prepared prior to FT-IR analysis.

Zeta potential

The zeta potential of chitosan-coated PBCA-NP was determined from electrophoretic mobility measurements with a model DPM-1 electrophoresis meter (Experimental Plant of Shanghai Bureau of Weights and Measures, China) in 10 mM NaCl at 25 °C.

Results and discussion

Effect of pH on the size of the NP

The effect of the polymerization pH on the mean particle diameter and on the turbidity of PBCA-NP prepared from 500 μl butyl cyanoacrylate monomer dropped into 10 ml 0.5% w/v chitosan (M_r 14.5×10^4) solution is shown in Fig. 1. In the pH 1.0 medium, the particle size had a rather wide distribution; the size of most NP was 10–30 nm, but some larger particles (50–1500 nm) also appeared. The rather high turbidity might be due to the larger particles. At pH 1.5, the average diameter of PBCA-NP was 51 nm with a rather narrow distribution (Fig. 2A) and the turbidity was the lowest, indicating the NP were well dispersed in the polymerization medium. At pH 2.0 and higher, the size of the PBCA-NP decreased to 20–30 nm with a rather narrow distribution; however, the turbidity increased significantly, which may be the result of aggregates of NP. Under the TEM, many aggregates of NP were observed (Fig. 2B). As the pH was too low, the polymerization period was greatly extended. NP in this system became swollen with monomer and the molecular weight of PBCA was rather low because acids may act as transfer agents [4, 14]. Coagulation of these semifluid particles was not reversible and so a polydisperse system of larger particles was produced. At high pH and hence at a high initiator concentration of OH^-, the polymerization rate was too rapid to allow discrete particle formation, and this resulted in aggregates of NP and even the formation of a gel [4]. The optimum pH value observed in the pH profile was 1.5. At this pH polymerization was slow enough to give discrete particles but not so slow as to result in excessive particle coagulation.

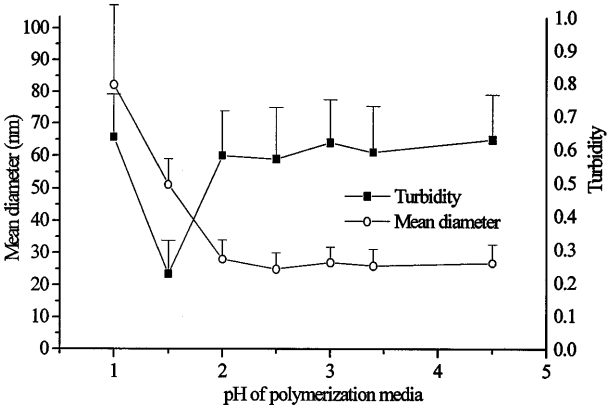


Fig. 1 Mean particle diameter and turbidity for Poly(butyl cyanoacrylate) nanoparticles (*PBCA-NP*) produced in 10 ml 0.5% w/v chitosan (M_r 14.5×10^4) at various pH values. Mean diameter (○), mean turbidity (■) ($N = 3$)

Effect of the concentration of chitosan on the size of the NP

The effect of the chitosan concentration on the mean particle diameter and on the turbidity of PBCA-NP prepared by dropping 500 μl monomer into 10 ml chitosan (M_r 14.5×10^4) solution at pH 1.5 is shown in Fig. 3. The results show that the mean particle diameter and the turbidity of PBCA-NP decrease with an increase in chitosan concentration. At low chitosan concentration the amount of stabilizer was insufficient to effectively cover the available surface, resulting in an unstable colloidal system. The smaller particles therefore agglomerated until the total surface area decreased to a point where the amount of chitosan available was sufficient to produce a stable suspension. When the chitosan concentration was lower than 0.2% w/v, the suspension was unstable. As the chitosan concentration increased, the agglomeration and the development of a narrowly dispersed NP population could be controlled; however, it was easy to form a gel in the polymerization process when the chitosan concentration was higher than 1.0% w/v and this might be due to the high concentration of chitosan amino and hydroxyl groups which may act as initiators in the polymerization medium.

Effect of the volume of chitosan on the size of the NP

The effect of the chitosan volume on the mean particle diameter and the turbidity of PBCA-NP which was

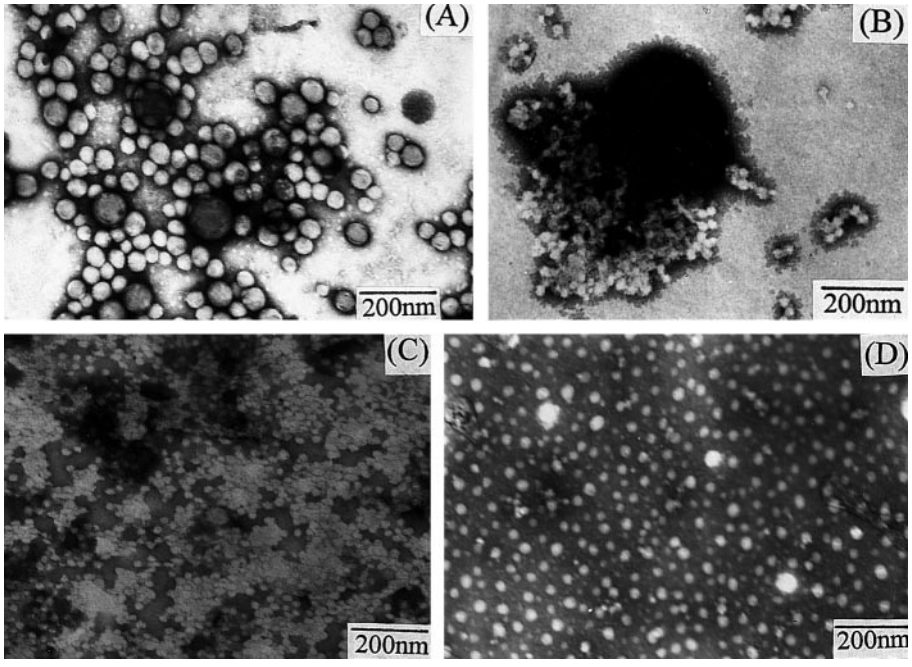


Fig. 2 Transmission electron microscope photographs of PBCA-NP produced **A** at pH 1.5 in 10 ml 0.5% w/v chitosan, **B** at pH 2.5 in 10 ml 0.5% w/v chitosan and **C** at pH 1.5 in 40 ml 0.5% w/v chitosan. **D** Nimodipine-loaded PBCA-NP produced in 10 ml 0.5% w/v chitosan at pH 1.5. The molecular weight of chitosan was 14.5×10^4. (Magnification 50 000×)

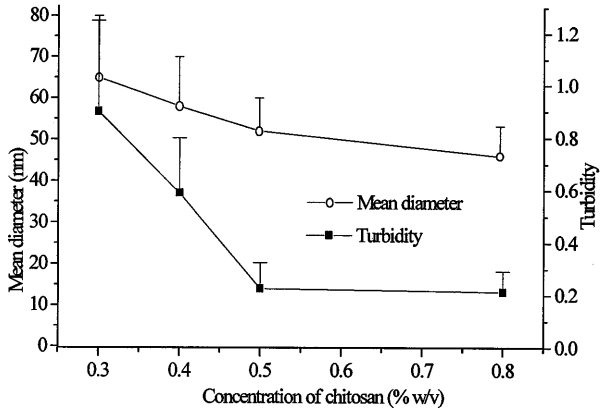


Fig. 3 Mean particle diameter and turbidity for PBCA-NP produced in various concentrations of chitosan (M_r 14.5×10^4) at pH 1.5. Mean diameter (○), mean turbidity (■) ($N = 3$)

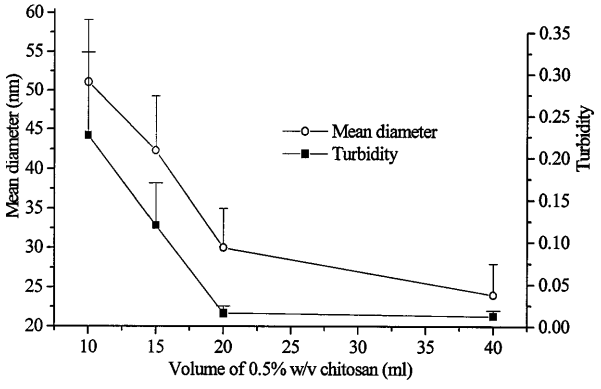


Fig. 4 Mean particle diameter and turbidity of PBCA-NP produced in different volumes of 0.5% w/v chitosan (M_r 14.5×10^4) at pH 1.5. Mean diameter (○), mean turbidity (■) ($N = 3$)

prepared by dropping 500 μl monomer into different volumes of 0.5% w/v chitosan (M_r 14.5×10^4) solution at pH 1.5 is shown in Fig. 4. The mean size and turbidity of PBCA-NP decreased as the volume increased. When the volume of chitosan was 10 ml, the mean particle diameter was about 50 nm; however, the mean particle diameter decreased to about 25 nm and the resultant NP showed near monodispersity as the volume increased to 40 ml (Fig. 2C). The results indicate that the volume of the chitosan solution has a significant effect on the NP size. An increase in the chitosan volume may not only produce smaller and more narrowly distributed nanoparticles, but may also simultaneously avoid the formation of a gel caused by the increasing chitosan concentration. At monomer concentrations below 1% w/v, similar results have been obtained for PBCA-NP produced in the presence of dextran [4].

Effect of the molecular weight of chitosan on the size of the NP

The effect of the chitosan molecular weight on the mean particle diameter and the turbidity of PBCA-NP is shown in Fig. 5. The results indicate, for the same chitosan concentration (0.5% w/v) and pH (1.5), that the mean particle diameter and the turbidity of PBCA-NP decrease with increasing chitosan molecular weight.

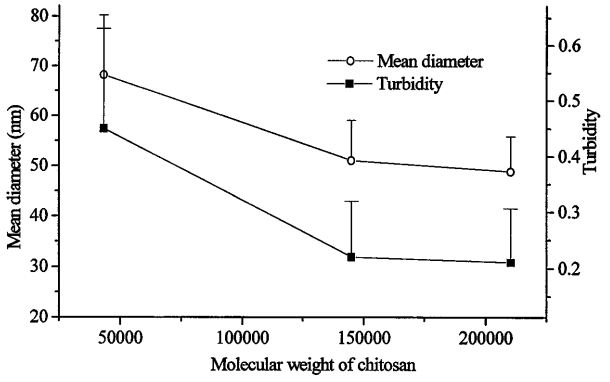


Fig. 5 Mean particle diameter and turbidity of PBCA-NP produced in 10 ml 0.5% w/v chitosan with different molecular weights at pH 1.5. Mean diameter (○), mean turbidity (■) ($N = 3$)

The results could be explained by considering particle formation and theoretical aspects of electrostatic and steric stabilization [32, 33]. The formation of an emulsion, which involves an increase in the interfacial area between the two phases, is accompanied by an increase in the free energy. Particle nucleation begins in the aqueous phase with the generation of oligomers containing the initiators. These oligomers agglomerate and nucleate a particle when polymerization has extended their size to the point where they become water-insoluble. The nucleate particles maintain their stability via the hydrophilic shell provided by the stabilizer which may concentrate on the particle–water interface to lower the interfacial tension [4, 6, 32]. A polymeric stabilizer should provide a combination of steric and electrostatic stabilization for colloidal particles [32, 33]. Chitosan is a polycationic polymer at acidic pH and could attach to the PBCA-NP surface to stabilize the NP through the electrosteric stabilization mechanism. The thickness of the chitosan layer attached to the NP may increase with increasing molecular weight, which might result in an increase in the steric repulsive energy and the electrostatic repulsive energy and finally in an increase in the total potential energy between the two particles. Thus, with increasing molecular weight, the stabilizing efficiency and effectiveness of chitosan should increase, leading to a decrease in the mean particle diameter and the turbidity in the formation of PBCA-NP. Similar results and explanations have been given for dextran [6] and many other polymeric surfactants [32].

The effect of temperature on the size of the NP

The effect of temperature on the mean particle diameter and the turbidity of PBCA-NP is shown in Fig 6. There was only a slight increase in the NP diameter with increasing temperature. The effect of temperature was obviously not so critical compared to the influence of pH. Temperature, therefore, did not constitute a suitable variable for the control of NP size and there is no advantage to be gained from production at other than ambient temperatures [4].

The formation mechanism of chitosan-stabilized PBCA-NP

The FT-IR spectra over the range 4000–400 cm^{-1} for chitosan, PBCA and chitosan-graft-PBCA are displayed in Fig. 7. After chitosan-stabilized NP had been extracted with acetone, the residue still possessed a –C≡N stretch (2250.2 cm^{-1}) and a carbonyl stretch (1750.8 cm^{-1}) corresponding to those of PBCA and a hydrogen-

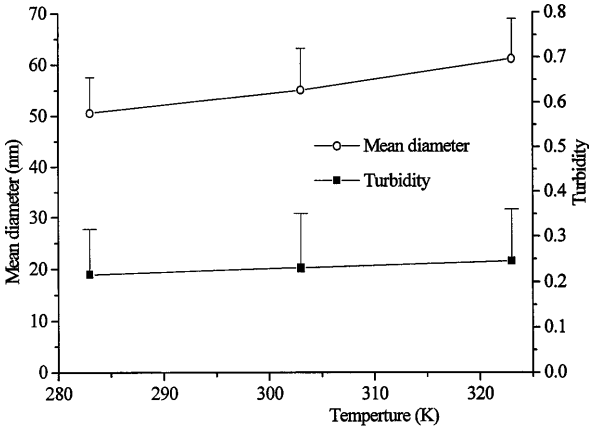


Fig. 6 Mean particle diameter and turbidity of PBCA-NP produced in 10 ml 0.5% w/v chitosan (M_r 14.5×10^4) at pH 1.5 at various temperatures. Mean diameter (○), mean turbidity (■) ($N = 3$)

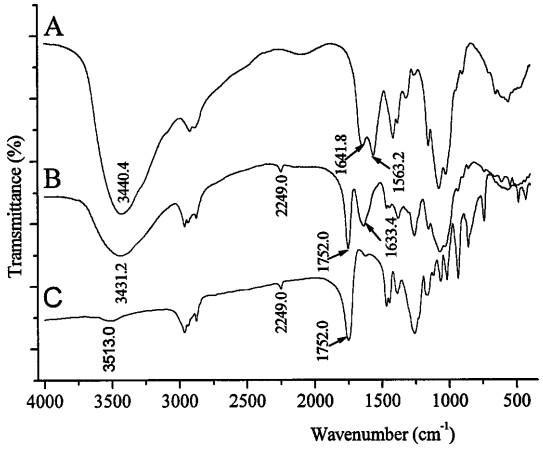


Fig. 7 Fourier transform IR spectra of chitosan (*A*), acetone-extracted residue of PBCA-NP produced in the presence of 0.5% w/v chitosan at pH 1.5 (*B*) and PBCA produced under aqueous conditions (*C*)

bonded OH stretch (3431.2 cm^{-1}) corresponding to that of chitosan. The amide II band at 1563.2 cm^{-1} observed in chitosan disappears or reduces dramatically, and the amide I band at 1641.8 cm^{-1} observed in chitosan shifts to 1633.4 cm^{-1}. These observations suggest that chitosan, especially the NH_2 group, may initiate the butyl cyanoacrylate monomer in the acidic polymerization medium, leading to chitosan chemically coupled to PBCA to form chitosan-stabilized NP. After chitosan-stabilized PBCA-NP had been extracted with acetone, hydroxyl-terminated PBCA homopolymers initiated by OH^- in PBCA-NP were removed due to the high dissolution of the homopolymers in the organic solvent, and the residue was chitosan and chitosan-graft-PBCA having short grafted PBCA chains because they did not dissolve in acetone. Douglas et al. [6] isolated PBCA from NP formed in the presence of Dextran 70. After removal of the cyanoacrylate polymer by dissolution in acetone, the derivatized dextran molecule still had a reduced intermolecular and intramolecular hydrogen-bonded OH stretch and a carbonyl stretch corresponding to that of PBCA [6]. According to the IR spectra of PACA, the presence of the base initiating species in the final polymer as an end group was demonstrated [34].

The effect of the pH of the polymerization medium on the grafting percentages (w/w) is shown in Fig. 8. The results indicate that the pH of the polymerization medium has a significant influence on the weight percentage of PBCA covalently linked to chitosan. Each chitosan molecule might contain a great number of cyanoacrylate polymer moieties covalently linked via any of the available chitosan amino and hydroxyl groups. A lower-pH polymerization medium may result in a lower grafting percentage because acids may act as chain-transfer agents in the polymerization process and thus decrease the mean molecular weight of PBCA [4, 14, 35]. When the pH was higher than 2.5, the grafting percentage dropped, which might be due to competition between OH^- and chitosan for initiation of the polymerization. The highest amount of PBCA chemically associated to chitosan was obtained at pH 2.0 with a grafting percentage of about 120% w/w. The highest amount of PEG association with poly(isobutyl cyanoacrylate) (PIBCA) NP was obtained with monomethoxy-PEG at pH 1.5, and, in the case of PEG-PIBCA nanoparticles, the maximum amount of PEG associated was obtained at pH 1.0 [12]. A lot of monomers have been grafted onto chitosan by various initiator methods and the grafting percentage and grafting efficiency varies with monomer species, initiator methods, media, etc. [36].

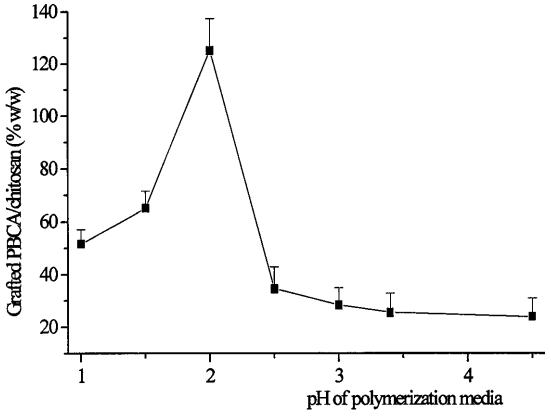
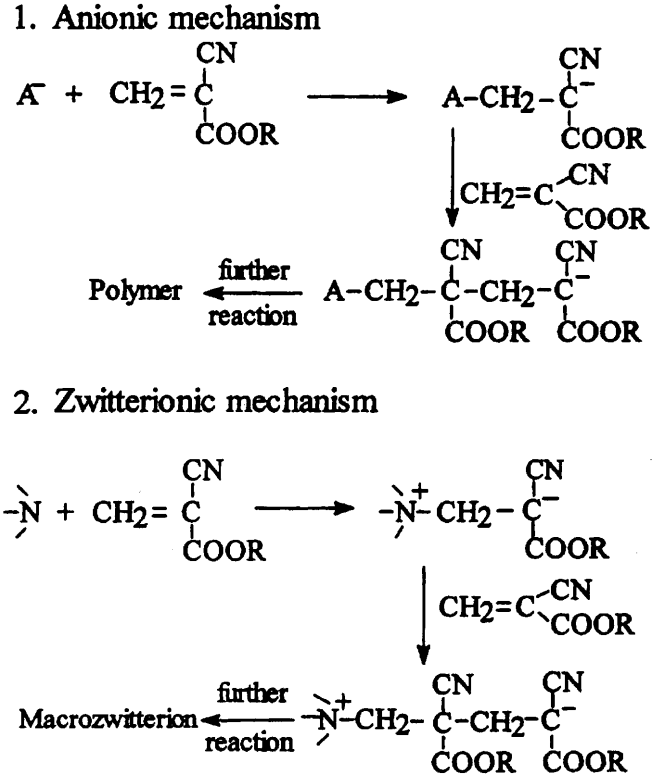


Fig. 8 Percentage of grafted PBCA to chitosan by weight in the residue of chitosan-stabilized PBCA-NP produced at various pH values ($N = 3$)

Polymerization of alkyl cyanoacrylates can be initiated by nucleophilic agents due to the ease of polymerization of the double bond of the monomer caused by the highly electronegative nitrile and alkoxycarbonyl groups. It has been reported that anionic polymerization of alkyl cyanoacrylates might occur via an anionic mechanism, a zwitterionic mechanism or both of them, involving initiation by nucleophilic attack on the β-carbon of alkyl cyanoacrylate as outlined in Scheme 1



Scheme 1 Polymerization mechanisms of cyanoacrylic monomers

[4, 6, 16–18]. Hydroxyl and amino groups in chitosan and OH^- might act as initiators in the anionic emulsion polymerization process carried out in an aqueous phase.

Table 1 Zeta potential data of chitosan-coated poly(butyl cyanoacrylate) nanoparticles (*PBCA-NP*) in 10 mM NaCl

Sample	Zeta potential (mV)
Nimodipine loaded PBCA-NP coated with 0.5% w/v chitosan	+32.6 ± 2.4
PBCA-NP coated with 0.5% w/v chitosan	+39.5 ± 2.0
PBCA-NP coated with 0.25% w/v chitosan	+22.5 ± 3.3

According to the zwitterionic mechanism the basic initiating species may also be present in the final polymer as an end group. Chitosan-containing amino and hydroxyl groups might act as nucleophilic agents to initiate the butyl cyanoacrylate monomer and is therefore present in the final polymer as end group, resulting in an irreversible attachment between the chitosan and the NP through multipoint linkages.

Nimodipine-loaded PBCA-NP stabilized with chitosan

Figure 2D shows that nimodipine was successfully incorporated into the chitosan-stabilized PBCA-NP. No crystalline nimodipine was visible under the TEM for freshly produced NP. The NP exhibited monodispersity with a D_w/D_n of 1.02. The mean diameter of nimodipine-loaded NP was 31.6 nm and the size was a little smaller than that of the unloaded PBCA-NP, which might be due to the diffusion of acetone from the organic to the aqueous phase [37]. Polymeric NP prepared by a solvent diffusion method have been investigated elsewhere [2, 37].

Zeta potential

The zeta potentials of chitosan-coated PBCA-NP in 10 mM NaCl solution are shown in Table 1. The results indicate that PBCA-NP coated with chitosan carry positive charge. The zeta potential increases as the chitosan concentration increases, and nimodipine had a slight effect on the zeta potential. PACA-NP have a very negative zeta potential because of the adsorption of anions from the aqueous polymerization medium, but stabilizers, such as polysobates, poloxamers, dextrans and PEG, may increase the resulting zeta potential [7, 11, 12]. PBCA-NP produced in the presence of diethylaminoethyl dextran, with cationic tertiary and quaternary amino groups as stabilizer, were found to bear a net positive charge [7]. Because chitosan is a polycationic biopolymer [28, 29], positively charged polyester nanocapsules [19, 26], NP [24, 38], submicron-sized emulsions [19], microcapsules [25] and liposomes [27] have been produced by coating or interacting them with chitosan. Some investigators have succeeded in improving the stability of liposomes [27], emulsions and nanocapsules [19] using chitosan and its derivatives. It has been reported that the electrophoretic properties of colloids may affect their blood clearance properties and organ distribution in rats [21].

Conclusion

We have described the preparation of positively charged PBCA-NP stabilized with chitosan. The pH, the concentration, the volume and the molecular weight of chitosan have a great effect on the particle size and the turbidity of PBCA-NP. Chitosan can chemically couple to PBCA and the highest grafting percentage occurs at pH 2.0. Because of the polycationic character of chitosan, the NP coated with chitosan bear positive charge. Compared with negatively charged NP, positively charged NP might prevent destabilization by cation adsorption, give a better controlled release of drugs, favourably interact with negatively charged tissues, such as muscosa and elpithelia, and provide site-specific targeting for drugs in vivo. Therefore, positively charged targeting drug delivery vehicles may be considered as very promising drug carriers for various administration routes.

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