Doxorubicin-Loaded Poly(butylcyanoacrylate) Nanoparticles Produced by Emulsifier-Free Emulsion Polymerization

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ABSTRACT: Doxorubicin-loaded poly(butylcyanoacrylate) (PBCA) nanoparticles (NPs) were prepared by an emulsifier-free emulsion polymerization technique. The pH values of the polymerization medium and the weight ratios of doxorubicin to butylcyanoacrylate had a significant effect on the mean particle size. The particle diameter determined by transmission electron microscopy showed that the nanoparticles were predominantly less than 50 nm. Drug loading and entrapment efficiency increased with increasing pH of the medium. The surface tension of the polymerization media increased with increasing polymerization time and reached a plateau after 4 h. Doxorubicin-loaded PBCA NPs carried a positive charge, and the zeta potential of drug-loaded nanoparticles increased with the increase of the polymerization pH. Molecular weight, analyzed by gel permeation chromatography, showed that the nanoparticles mainly consisted of oligomers of PBCA. The release rate of doxorubicin from nanoparticles in biological phosphate buffer was very slow, with a half-life of 111.43 h. The results indicate that drug-loaded nanoparticles can be prepared by an emulsifier-free emulsion polymerization technique and that the resulting nanoparticles might be suitable for targeting drug delivery vehicles for clinical application. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 517–526, 2000

Key words: doxorubicin; butylcyanoacrylate nanoparticles; emulsifier-free emulsion polymerization technique; targeting drug delivery system

INTRODUCTION

Approaches that kill tumor cells while leaving nontarget tissues unaffected are highly desirable for the treatment of cancer. Polymeric nanoparticles have been designed as drug carriers with the objective of delivering active molecules to the intended target and thus improving the therapeutic index. Numerous investigations have showed that the biological distribution of a drug can be modified, both at the cellular and at the organ level, using a nanoparticulate delivery system. After intravenous injection, drug-loaded nanoparticles similar to other colloidal drug carriers accumulate in the mononuclear phagocyte system, depending on the surface characteristics of the carrier system. This altered biodistribution profile of the encapsulated drugs could be used to target drugs to specific sites at cellular or subcellular levels for an optimized release of the drug.[1,2] The polymers from which the nanoparticles are prepared may be polymerized in advance or formed simultaneously with the nanoparticles by *in situ* polymerization. Poly(alkylcyanoacrylates) (PACA), which have been extensively used as surgical glues, are bioresorbable by a bioerosion

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mechanism.[3] PACA nanoparticles have been developed by anionic emulsion polymerization in water in the presence of a stabilizing agent and loaded with different pharmacological drugs.[4–6] Alkylcyanoacrylates may be polymerized by anions through an anionic mechanism[5–7] and by a covalent base through a zwitterionic mechanism.[7–9] The drug may be added during polymerization and be entrapped within the nanoparticles polymer network or covalently bound to the polymer.[5] The surfactants that are often added in the aqueous polymerization medium are polysorbates, poloxamers, dextran, and cyclodextrin. The hydroxyl group in the surfactant molecules may also act as a base to initiate polymerization of the alkylcyanoacrylates, competing with polymerization initiated by the basic group in the drug molecule and decreasing the drug-loading capacity and entrapment efficiency.[10,11] Surfactants are often limited in intravenous formulation because of their high toxicity and side effects. To reduce these toxic substances, further purification of the products sometimes is essential, which is accomplished by ultracentrifugation, ultrafiltration, gel chromatography, dialysis, or a combination of these methods.[1]

Drug-loaded nanoparticles prepared by emulsifier-free emulsion polymerization[12] might solve the problem caused by surfactants and improve drug loading and entrapment efficiency. Polymeric latexes, such as polystyrene and polyalkylacrylate and their copolymers, can be prepared with no emulsifier but an excess of initiator. Oligomers containing sulfonic acid groups and radical functions will then act effectively as emulsifiers in the polymerization and are subsequently incorporated into the polymer molecules. The polymer particles formed are sufficiently stable and do not contain any contaminants.[12–15] However, those polymers produced by emulsifier-free emulsion polymerization are not biodegradable. The risk of polymeric tissue or cellular overloading and side effects restricts their possible clinical use.

It has been reported that biodegradable poly(ethylbutylcyanoacrylate) nanoparticles were successfully prepared in double-distilled water in the absence of stabilizer.[16] Micelles may be able to form in the polymerization process because the surface tension of cyanoacrylate esters is below 37.5 mN m^{-1} and those monomers can be initiated by weak bases that may be present as end groups in the polymers,[17] forming water-soluble surface-active substances.[12] The molecular-weight determination made by gel permeation chromatography suggested that nanoparticles were built by an entanglement of numerous small oligomeric subunits rather than by coils consisting of one or several long polymeric chains.[6,7,11,18] When doxorubicin was present in the polymerization medium, the GPC profiles changed greatly —from unimodal distribution with a main peak of low molecular weight to a bimodal distribution with a new peak of high molecular weight and a main peak with slightly increasing molecular weight. These GPC results show that doxorubicin can act as an initiator (by its free amine function) in the anionic emulsion polymerization of a cyanoacrylic monomer and might be covalently linked to the beginning of the polymer chain.[6,19]

Doxorubicin is a well-established and widely accepted antineoplastic agent used in the treatment of many types of cancer. The duration of its use, hence its ultimate effectiveness as an anticancer agent, is limited by potentially irreversible, dose-dependent cardiotoxicity.[20] Thus, there is considerable interest in the development of agents that ameliorate or prevent this toxicity. Colloidal systems, such as liposomes[19,21] or polymeric nanoparticles,[1–2,5,19] have been designed as drug carriers with the objective of a selective delivery of the active molecule to the targeted organ. Doxorubicin-loaded PACA NPs have been successfully prepared by anionic polymerization in the presence of surfactants.[3,6,19,22–26] An *in vitro* study has shown that doxorubicin-loaded PACA NPs allows multidrug resistance to be overcome.[22] The *in vivo* investigation indicated that clearance of PACA NPs was very slow, with the highest proportions of the dose found in the liver, spleen, and lung.[5,19,27]

This article reports on the preparation of doxorubicin-loaded PBCA NPs by a novel method— emulsifier-free anionic emulsion polymerization—in order to avoid the side effects of surfactants. The formation of nanoparticles was also investigated. Drug loading and entrapment efficiency were determined using a spectrophotometer, the molecular weight was determined by GPC, and the zeta potential of nanoparticles was measured from electrophoretic mobility. *In vitro* drug release from doxorubicin-loaded PBCA NPs was evaluated using a dialysis bag diffusion technique.

EXPERIMENTAL

Materials

Butylcyanoacrylate was synthesized by Xian Chemical Institute (Xian, China). Doxorubicin

hydrochloride was purchased from Haimen Pharmaceutical Plant (Zhejiang, China). All other reagents were analytical grade.

Nanoparticle Preparation

PBCA nanoparticles were prepared by an emulsifier-free emulsion polymerization technique. Briefly, doxorubicin (2, 5, 10, or 20 mg) was dissolved into an aqueous solution (10 mL) of HCl with a pH value at 1.5, 2.0, or 2.5 or with citric acid with a concentration of 0.1% (w/v) (pH~2.75). Then, butylcyanoacrylate (100 mg) was added dropwise in the aqueous solution under magnetic stirring (approximately 1000 rpm). After completion of the polymerization, which lasted at least 4 h, 5 mL of the suspensions was adjusted to pH 6.0 with 0.1*N* NaOH to meet the pH values necessary to be classified as pharmaceutical dosages for parenteral injection.

Particle Size Determination

A sample was diluted with distilled water, and a drop of this dispersion was placed onto a collodion support on a copper grid. The latter was examined using a transmission electron microscope (TEM; JEOL TEM-100, Japan) after negative staining with an aqueous solution of sodium phosphotungstate. Nanoparticles larger than 50 nm were measured using a MASTERSIZE (Malvern Instruments Ltd., Malvern, UK).

Surface Tension

The surface tension of the polymerization medium was determined using the pendant drop method at a temperature of 25°C.

Determination of Drug-Entrapment Efficiency

The UV spectra were measured on a UV-3100 (Shimadzu, Japan). Doxorubicin, poly(butylcyanoacrylate), and nanoparticles were dissolved into tetrahydrofuran, and the UV spectra were determined over a wavelength range of 230–700 nm. Then a 10-mL doxorubicin-loaded nanoparticle suspension was diluted to 30 mL with distilled water and centrifuged at 50,000 rpm for 1 h (ultra Pro™ 80, Du Pont Company, Newtown, USA). The content of doxorubicin in the supernatant and sediment dissolved with tetrahydrofuran was measured using a Model 721 Spectrophotometer (No. 3 Analytical Instrumental Factory, Shanghai, China) at a maximum wavelength of 490 nm.

Molecular Weight Analysis

Centrifuged nanoparticles were dissolved in tetrahydrofuran and analyzed using polystyrene-calibrated gel permeation chromatography (GPC) (Waters Company, USA) with tetrahydrofuran as eluant at 25°C. Data from a refractive index detector (Waters Company, USA) were recorded and analyzed with a computer program.

Zeta Potential

The zeta potential of doxorubicin-loaded nanoparticles was determined from electrophoretic mobility measurements using a Model DPM-1 (experimental plant of Shanghai Bureau of Weights and Measures, China) to carry out electrophoresis in distilled water at 25°C.

In Vitro Drug-Release Studies

The dialysis bag diffusion technique was used to study the diffusion of doxorubicin from a nanoparticle suspension produced at pH 2.5 with a doxorubicin:monomer ratio of 1:5. An initial colloidal suspension (10 mL) containing doxorubicin was centrifuged to eliminate any unentrapped fraction, and the nanoparticles were suspended in 5 mL of biological phosphate buffer. A portion (2 mL) of this suspension was placed into a dialysis bag (M_w 10,000), and the dialysis bag was immersed in 20 mL of pH 7.4 biological phosphate buffer solution at 37 ± 0.5°C. At fixed time intervals, samples (2 ml) of the released solution were drawn and the concentration of doxorubicin was analyzed at maximum wavelength 490 nm. After determination, 2-mL samples of the released solution were returned, and each time this was done a little fresh buffer was added by weighing to maintain the release medium volume constant.

RESULTS AND DISCUSSION

Influence of pH Value on Mean Diameter of Nanoparticles

Figure 1 shows the effect of the pH of the polymerization media on the number mean diameter determined by TEM. The nanoparticle size slightly decreased by increasing the pH value of a polymerization medium [Fig. 2(a,b)]. This result may be

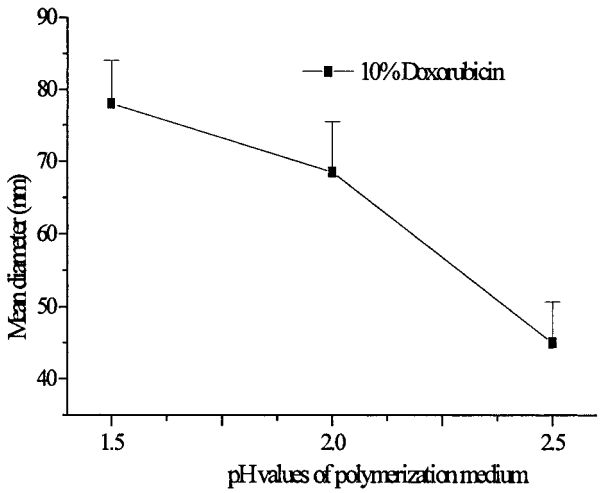


Figure 1 The effect of pH values of the polymerization medium on the mean diameter of nanoparticles.

explained by reference to the polymerization outline in Scheme 1.[7–9] Anionic and zwitterionic mechanisms should exist in the process of polymerization in the presence of doxorubicin. Doxorubicin may be covalently linked to the beginning of the polymer chain, acting as surface-active oligomers.[12] The activity of doxorubicin acting as an initiator may be a function of the pH values of the polymerization medium, and activity may increase with increasing pH values. When doxorubicin was present during the polymerization of nanoparticles, a major fraction of the drug was entrapped inside the nanoparticles.[23] The oligomer initiated by OH^- might also act as an emulsifier in the formation of nanoparticles because the hydroxyl is hydrophilic and PBCA is hydrophobic. At low pH, the polymerization time is significantly extended, and longer polymerization times result in longer chains and potentially larger particles. At high pH, the polymerization rate is rapid enough to form discrete nanoparticles and leads to direct polymerization of monomer droplets, producing amorphous polymeric particles.[28] It has been reported that doxorubicin may be both embedded into the polymeric matrix and adsorbed onto the surface of the nanoparticles when the drug is added during the nanoparticle formation process,[23] and the adsorption of doxorubicin on nanoparticles increases as the pH increases.[3] The adsorbed doxorubicin might decrease the coagulation of nanoparticles, which may further decrease the nanoparticle size. In the preparation of polystyrene lattices by the emulsifier-free technique, oligomers containing sulfonic acid groups might act effectively as surfactants.[12,13]

Influence of the Weight Ratio of Doxorubicin to Butylcyanoacrylate on the Mean Diameter of Nanoparticles

The effect of the ratio (w/w) of doxorubicin to the butylcyanoacrylate monomer on nanoparticle size is shown in Figure 3. With a constant monomer concentration, the number mean diameter of nanoparticles decreased with an increasing weight ratio of doxorubicin to monomer, from 0.02 to 0.2 (w/w) [Fig. 2(b,c)]. This result might also be attributed to something else: With more doxorubicin acting as initiator and becoming part of the polymer chain, the mean molecular weight of the prepared polymer will decrease and the doxorubicin adsorbed onto the nanoparticles will increase, leading to increasing numbers of nanoparticles with a decreasing mean size.

Influence of Acidifying Agents on Mean Diameter of Nanoparticles

The mean diameters, determined by TEM, of doxorubicin-loaded PBCA NPs produced in a hydrochloric acid solution (pH 2.0) and in 0.1% citric acid were 45.2 ± 5.6 (n = 3) and 46.3 ± 7.1 (n = 3), respectively. Figure 4 shows the acid effect on the number and volume diameter of doxorubicin-loaded PBCA NPs determined by photon correlation spectrometry (PCS). All reactions were performed in a 10-mL acidic aqueous solution containing 5 mg of doxorubicin and 50 mg of monomer. The results showed that hydrochloric and citric acids were found to be suitable acidifying agents for nanoparticle production with little difference in the result for particle diameter [Figs. 2(b,d)], as indicated by Douglas et al.[28] The volume mean diameter (D_v) and the number mean diameter (D_n) for particles larger than 50 nm were 325 nm and 106 nm, respectively, for the doxorubicin-loaded PBCA NPs produced in hydrochloric acid solution (pH 2.0), and 522 nm and 128 nm, respectively, for the 0.1% citric acid. Figure 4 also shows a more polydisperse particulate distribution for a pH above 2.0—for example, 0.1% citric acid with a pH of about 2.75. This is potentially more significant than the sizes themselves. The larger diameters for doxorubicin-loaded PBCA NPs produced in 0.1% citric acid might be attributed to the higher pH value of the polymerization medium. Figure 4(a) shows that some larger particles or coagulation of nanoparticles existed in suspension; however, there were few particles whose diameter was larger than 3 μm,

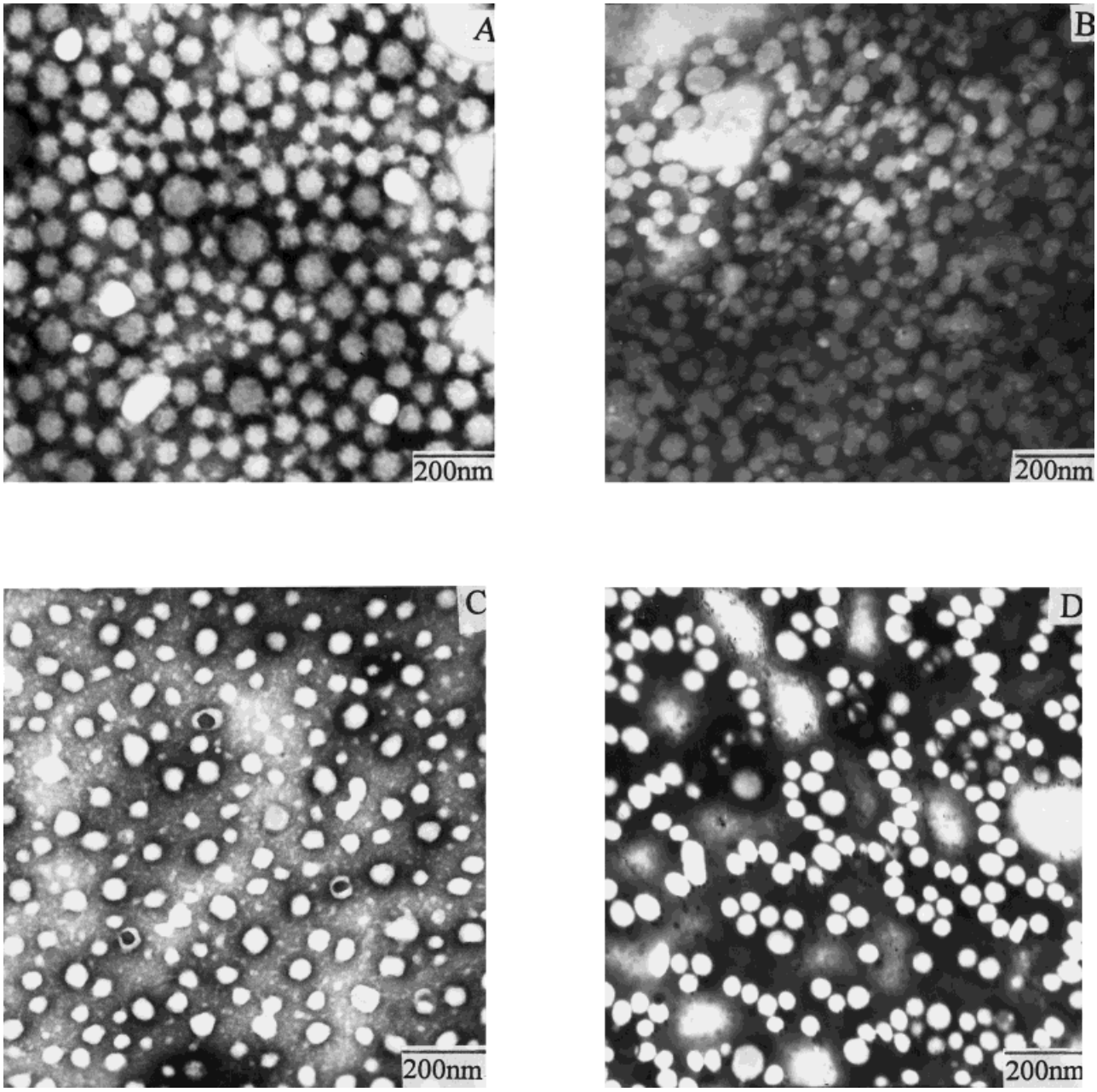
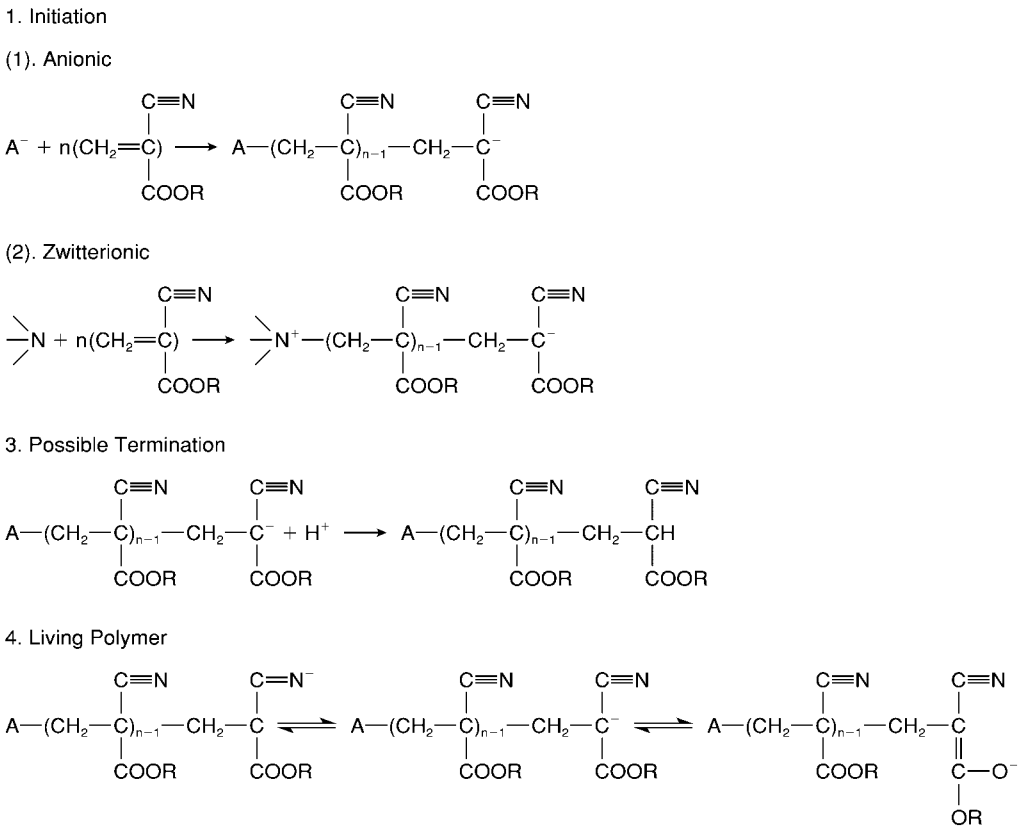


Figure 2 TEM photographs of doxorubicin-loaded nanoparticles produced: (a) at pH 1.5 HCl with doxorubicin/monomer of 1/10 (w/w); (b) at pH 2.5 HCl with doxorubicin/monomer of 1/10 (w/w); (c) at pH 2.5 HCl with doxorubicin/monomer of 1/5 (w/w); (d) at 0.1% citric acid with doxorubicin/monomer of 1/10 (w/w).

indicating the suspension was suitable for intravenous administration. When the pH of the suspension was adjusted, using a sodium hydroxide solution, to be above 5.0, the nanoparticle suspension produced in the presence of hydrochloric acid was rather stable. The nanoparticle suspension produced with the citric acid, however, was unstable, which might result from the zeta potential decrease of doxorubicin-loaded nanoparticles caused by the formed trianion, trisodium citrate.

Surface Tension

The surface tension of the polymerization medium was measured during polymerization. The



Scheme 1 Possible polymerization mechanisms of alkylcyanoacrylates.

results are shown in Figure 5. Doxorubicin is not a surfactant and can't reduce the surface tension in neutral and acidic water solutions. After the monomer was added into the polymerization medium, however, the surface tension of the polymerizing emulsion dropped sharply, which might be because of the lower surface tension of the monomer (31.11 mN m^{-1})[17] and/or the newly formed surface-active oligomers initiated by doxorubicin and OH^-. And then the surface tension of the polymerization medium slowly rose to about 71.8 mN m^{-1} and did not change after 4 h of polymerization. The turbidity of the dispersion at 700 nm simultaneously increased with the increase of the surface tension. These results indicated that the polymerization process was completed when both the surface tension and the turbidity reached a plateau.

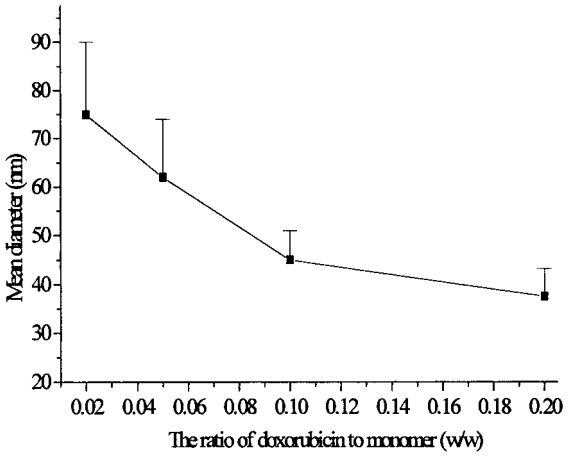


Figure 3 The effect of the weight ratio of doxorubicin to monomer on the mean diameter of nanoparticles.

Drug Loading and Entrapment Efficiency

The maximum wavelength of 490 nm was chosen as the detection wavelength because it is the maximum absorption wavelength for doxorubicin. Table I shows the drug loading and entrapment efficiency of doxorubicin-loaded nanoparticles produced in hydrochloric acid solution containing different amounts of doxorubicin. Drug loading and entrapment efficiency were affected greatly by the pH value of the suspension, and they in-

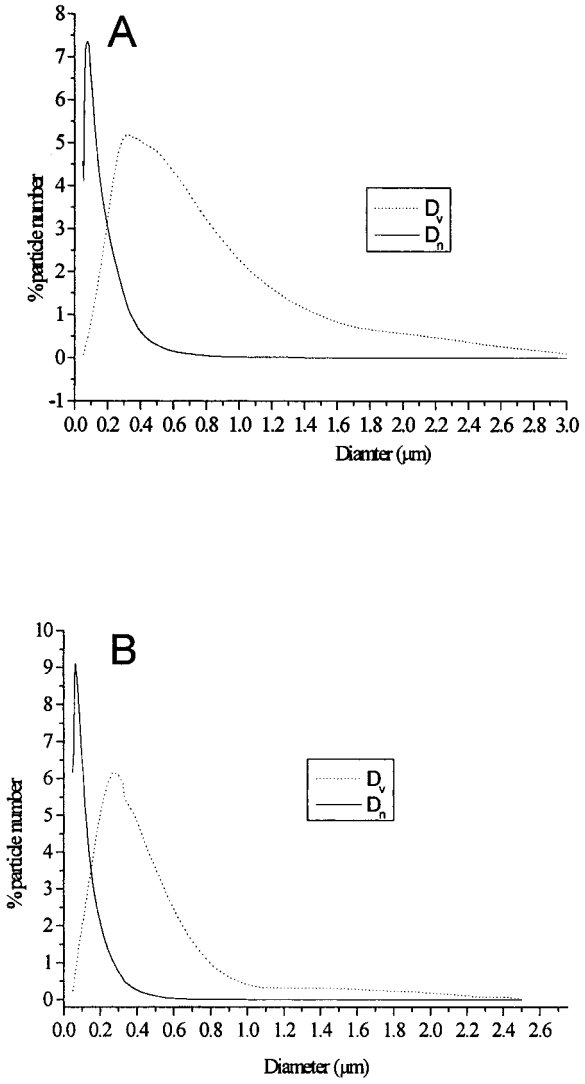


Figure 4 The PCS diameter of doxorubicin-loaded PBCA NPs produced (a) at 0.1% citric acid and (b) at pH 2.0 HCl solution.

creased significantly when the pH of the nanoparticle suspension was elevated to 6.0 by adjusting it with a 0.1*N* NaOH solution. Drug-adsorptive capacity depends on the surface properties of nanoparticles, the pH of the solution, the pKa of the adsorbate, and so on. The desorption zone corresponded to the pKa (8.2) of doxorubicin. The results were in agreement with the basic character of the molecule.[3]

Molecular Weight Analysis

Figure 6 shows GPC profiles of poly(butylcyanoacrylate) polymerized in different conditions, with peaks for polymers of two different molecular weights (peaks 1 and 2) and a peak for monomeric residue (peak 3). Table II shows the mass-average (M_w) and number-average (M_n) molecular weight and polydispersity (M_w/M_n) of peaks in the GPC profiles. The main peak with low molecular weight (peak 2) made up the bulk of the nanoparticles. When polymerized in 0.1% citric acid, the average molecular weight of PBCA produced in the presence of doxorubicin [Fig. 6)(a)] increased significantly as compared with those in the absence of doxorubicin [Fig. 6(b)], indicating that doxorubicin might act as an initiator in the polymerization.[6,19,21] In Figure 6(b) the very small peak with high molecular weight (peak 1) might be the result of continued polymerization within the PBCA latexes. PBCA polymerized in the presence of doxorubicin in a HCl solution with a pH of 1.5 shows a similar molecular-weight distribution [Fig. 6(c)], except for a lower molecular

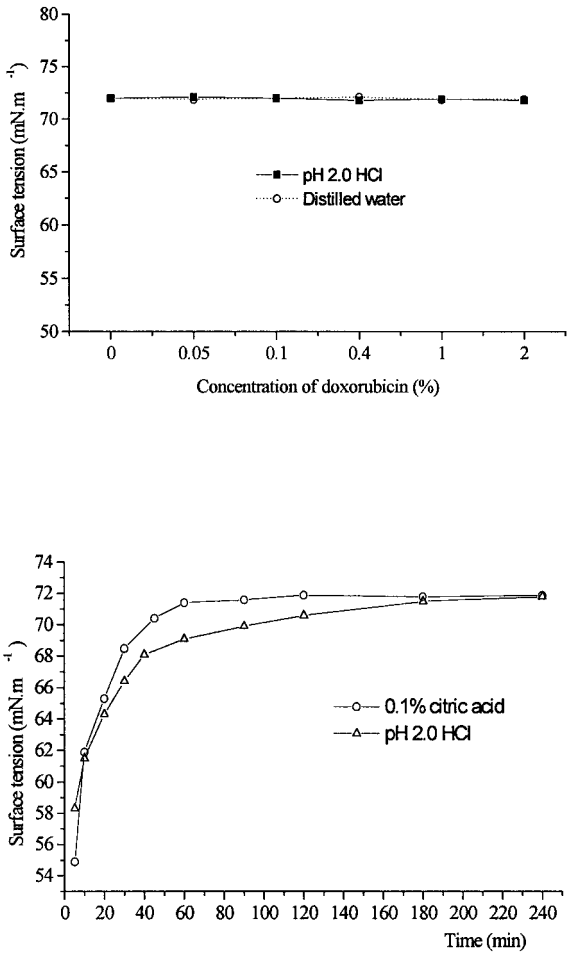


Figure 5 The surface tension of doxorubicin in (a) distilled water and pH 2.0 HCl solution and in (b) polymerization medium of doxorubicin-loaded PBCA NPs.

Table I Drug Loading and Entrapment Efficiency of Doxorubicin-Loaded Nanoparticles in Different pH Media

Sample	pH 1.5	pH 2.5	pH 6.0
1. 5% doxorubicin			
Drug loading (%)	1.23 ± 0.10	2.05 ± 0.14	4.3 ± 0.13
Entrapment efficiency (%)	24.9 ± 1.7	40.0 ± 2.8	86.0 ± 2.6
2. 10% doxorubicin			
Drug loading (%)	1.81 ± 0.16	3.27 ± 0.30	6.8 ± 0.21
Entrapment efficiency (%)	18.1 ± 1.6	32.7 ± 3.0	67.9 ± 2.1
3. 20% doxorubicin			
Drug loading (%)	2.1 ± 0.24	4.26 ± 0.44	13.6 ± 0.38
Entrapment efficiency (%)	10.5 ± 1.2	21.3 ± 2.2	65.4 ± 1.9

weight, possibly caused by the lower pH of the polymerization medium. It has been reported that increasing the hydrogen ion concentration results in a reduction of molecular weight.[18] The GPC profiles show that a peak with a very low molecular weight corresponds to the monomeric residues.[6,21] Guise et al.[7] have shown that when vidarabine is presented in the polymerization medium, a small number of monomers might be initiated by zwitterionic mechanism, resulting in a dramatic increase in the polymer molecular weight.

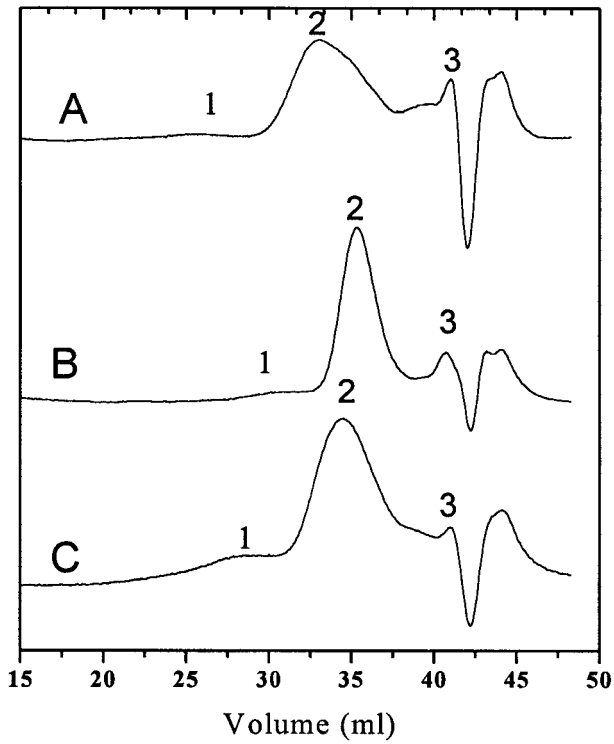


Figure 6 GPC chromatographs: (a) PBCA produced at 0.1% citric acid with doxorubicin/BCA (1/5 w/w); (b) PBCA produced at 0.1% citric acid without doxorubicin; (c) PBCA produced at pH 1.5 HCl with doxorubicin/BCA (1/5 w/w).

Zeta Potential

Figure 7 shows the zeta potential of doxorubicin-loaded PBCA NPs produced at different weight ratios of doxorubicin to monomer [Fig. 7(a)] and different pH values of polymerization medium [Fig. 7(b)]. The doxorubicin-loaded nanoparticles prepared in a polymerization medium with a pH of 1.5 carried a positive charge, and the weight ratios of drug to monomer had no significant effect on the zeta potential of nanoparticles, as the nanoparticles

Table II Molecular Weight and Polydispersity of Poly(butylcyanoacrylate) Polymerized at Different Conditions

Peak	M_w	M_n	M_w/M_n
Sample A			
Peak 1	77600	50800	1.53
Peak 2	3320	2050	1.62
Peak 3	214	156	1.37
Sample B			
Peak 1	11900	9300	1.27
Peak 2	1615	1160	1.39
Peak 3	214	156	1.37
Sample C			
Peak 1	76000	25000	3.02
Peak 2	2320	820	2.80
Peak 3	214	156	1.37

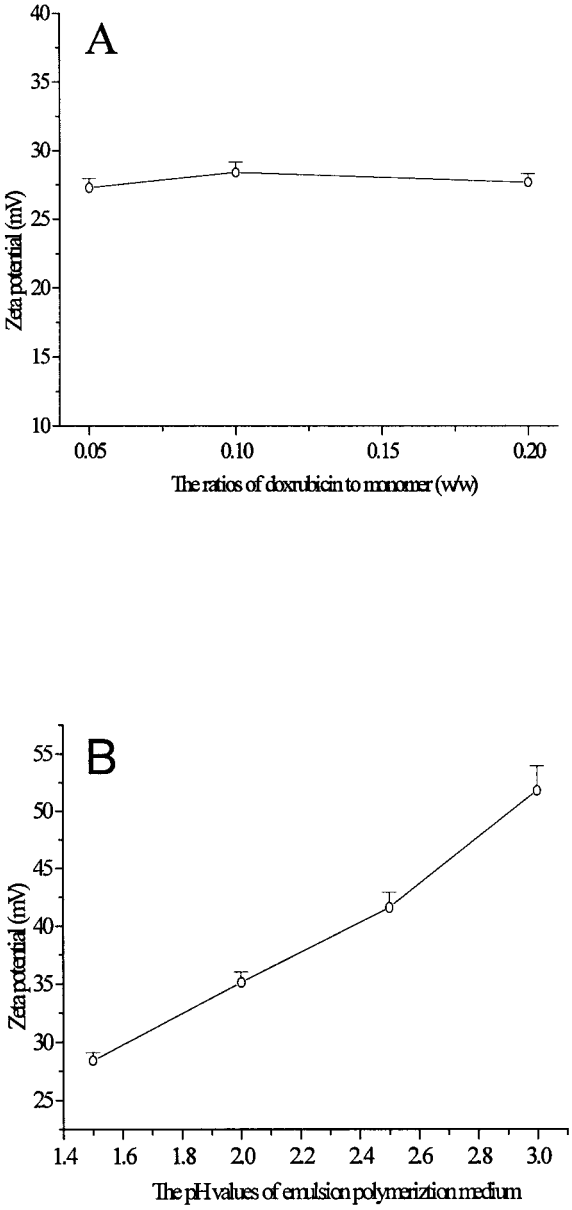


Figure 7 Zeta potential of doxorubicin-loaded PBCA NPs produced at (a) pH 1.5 HCl solution with various weight ratios of doxorubicin to monomer and at (b) various pH values of HCl solution with a fixed weight ratio of doxorubicin to monomer of 0.1.

were obtained at a doxorubicin to monomer ratio of 0.05 to 0.2 [Fig. 7(a)]. But the zeta potential went up as the pH of the polymerization medium increased [Fig. 7(b)], which might be from the increase of doxorubicin adsorption on the nanoparticles with the increasing pH[3] and the cationic character of doxorubicin in an acidic condition. The zeta potential of drug-loaded nanoparticles produced by emulsion polymerization in the presence of surfactants normally carries a negative charge.[6,30,31] The negative charge might be associated with the carbanion, which has been assumed to be present at the termination end of the polymeric chain[8,9,30] or/and the adsorption of anions from the aqueous phase.[6] When the amount of doxorubicin lowers the carrier capacity of nanoparticles, the zeta potential of nanoparticles might decrease, resulting in instability of the nanoparticle suspension.

In Vitro Release

Figure 8 shows the doxorubicin release from nanoparticles in the biological phosphate buffered saline at 37°C. The drug release data from nanoparticles were fitted to the Weibull distribution. The correlation coefficient of the equation was 0.9989, and the T_{30}, T_{50}, and T_{80} values were 23.41, 111.43, and 805.86 h, respectively. When the drug-loaded nanoparticles were added into the dialysis bag, two kinetic processes could control the appearance of doxorubicin, that is, release from the nanoparticles to the aqueous phase in the bag and diffusion of doxorubicin through the dialysis bag. According to the release profile of doxorubicin from nanoparticles, the release rate decreases with increasing time. The initial higher release rate might be mainly the result of the big surface area and desorption of doxorubicin from the nanoparticle surface. The lower release rate might be related to the polymer degradation rate, since doxorubicin might chemically couple to the

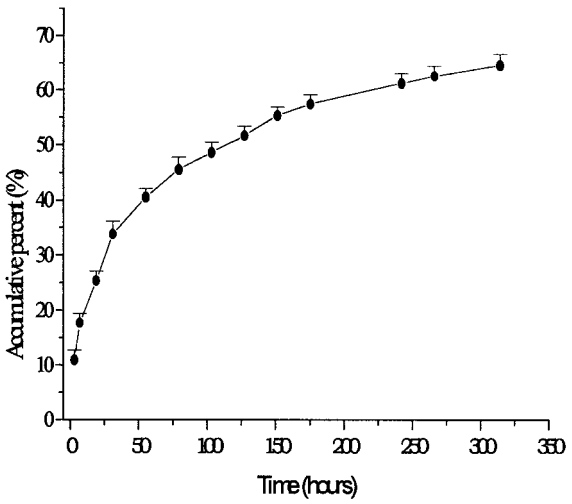


Figure 8 Release profile of doxorubicin from nanoparticles produced at pH 2.5 with a doxorubicin/monomer ratio of 1/5 in a biological phosphate buffer solution at 37°C.

PBCA and be entrapped into the nanoparticles in the polymerization process. About 1% of doxorubicin was released *in vitro* in 48 h in 2-morpholinoethansulfate buffer at pH 6.5.[32] The drug release could be a consequence of polymer degradation, which probably occurs through an enzymatic pathway consisting of ester hydrolysis of the side chain with production of water-soluble poly(cyanoacrylic acid). Th dactinomycin release rate from various cyanoacrylic polymers was found to correlate with the rate of polymer bioerosion.[4]

CONCLUSION

To avoid the side effect of nanoparticle suspension *in vivo* caused by surfactants, this study proposed a novel method—emulsifier-free emulsion polymerization technique—to produce doxorubicin-loaded PBCA NPs. The pH value of the polymerization medium and the weight ratio of doxorubicin to cyanoacrylic monomer had a significant effect on the particle size. Doxorubicin-loaded PBCA NPs prepared in a hydrochloric acid solution were rather stable when the pH was adjusted to be above 4.0, and the drug loading and entrapment efficiency increased as the pH increased. Investigation of the GPC showed that doxorubicin might act as initiator through a zwitterionic pathway, resulting in an increase in the polymer molecular weight. Doxorubicin-loaded nanoparticles achieved controlled release, with a half-life of 111.43 h. The zeta potential of drug-loaded nanoparticles carried a positive charge, which might be beneficial to the treatment of cancers. According to the described results, drug-loaded PACA NPs may be produced by the emulsifier-free emulsion polymerization technique, resulting in nanoparticles that might be suitable for intravenous administration; in addition, the targeting efficiency of the drug might be greatly improved.

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