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Effect of surfactant on fabrication and characterization of paclitaxel-loaded polybutylcyanoacrylate nanoparticulate delivery systems

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Abstract

The feasibility of applying biodegradable polybutylcyanoacrylate (PBCA) nanoparticulate delivery systems (NDSs) for the controlled release of paclitaxel was investigated. Paclitaxel-loaded and unloaded PBCA-NDSs containing various surfactants (dextran 70, cholesterol, polyvinyl alcohol and lecithin) were prepared by anionic polymerization. The effects of surfactant (1% w/v), surfactant combination (1% w/v each), and surfactant concentration (0.05, 1.0 and 2.5% w/v) on PBCA-NDSs were evaluated and characterized by particle size, zeta potential, entrapment efficiency, and in-vitro paclitaxel release kinetics. The physicochemical characteristics of PBCA-NDSs incorporated with various surfactants were significantly improved compared with PBCA-NDS without any surfactant, by decreasing particle size at least 3-fold as well as by increasing the zeta potential up to 18-fold to minimize the agglomeration of nanoparticles. Moreover, PBCA-NDSs incorporated with various surfactants demonstrated higher entrapment efficiency of paclitaxel. Results from the in-vitro release kinetic studies indicated that a more controlled biphasic zero-order release pattern of paclitaxel was observed for PBCA-NDSs incorporated with various surfactants. Compared with dextran 70 and polyvinyl alcohol, the naturally occurring lipids, lecithin and cholesterol, indicated greater advantages in improving the physicochemical properties of PBCA-NDSs, in terms of smaller particle size, higher zeta potential and better drug entrapment efficiency, and better controlled release of paclitaxel, in terms of lower release rate and prolonged action from PBCA-NDSs.

Introduction

Cancer is the second major cause of death in the USA, causing over 555 000 fatalities annually (American Cancer Society 2002). In recent years, the discovery of new agents, combination therapy and novel delivery of anticancer drugs have made it possible to achieve remission in patients with advanced stages of disease. Paclitaxel, discovered by the National Cancer Institute in 1967, is a diterpenoid pseudoalkaloid extracted from the bark of the Pacific yew or Western yew tree (Taxus brevifolia) (Singla et al 2002), and has a unique mechanism of action to inhibit cell growth (Horwitz 1992). However, there are currently two major limitations for the clinical application of paclitaxel: its limited availability and its high hydrophobicity (solubility ~0.6 mM in water). Adjuvants, such as Cremophor EL (polyoxyethylated castor oil) and ethanol, have been used in its clinical administration and have resulted in serious side-effects (e.g. hypersensitivity, nephrotoxicity and neurotoxicity), as well as physical instability and incompatibility with components of the infusion set. Because of these limitations, clinical administration of paclitaxel has been severely restricted and alternative dosage forms without Cremophor EL need to be developed (Singla et al 2002).

Approaches utilizing cosolvency (Bissery et al 1991), emulsification (Kan et al 1999), micellization (Miwa et al 1998), water-soluble prodrugs (Pendri et al 1998), cyclodextrin complexes (Lee et al 2001), albumin conjugates (Dosio et al 2001), HPMA conjugates (Duncan et al 2001), liposomes (Ceruti et al 2000), and microspheres (Mu & Feng 2001) have been reported, all with limited success. The use of biodegradable polymeric nanoparticles for controlled delivery of anticancer agents has the advantage of enhancing therapeutic efficacy and reducing systemic side-effects. Nanoparticulate

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carriers have been used for drug targeting and polyalkylcyanoacrylate nanoparticles are able to bypass the emergence of multidrug resistance, thereby increasing the efficacy of chemotherapy (Nemati et al 1996). These nanoparticulate carriers have also been found to concentrate in liver and avoid distribution in, for example, heart and kidney, where toxicity could be observed (Verdun et al 1990). Moreover, nanoparticles have the advantage of reduced particle size compared with microparticles, enabling intravenous as well as intramuscular and subcutaneous administration, and minimizing the risk of emboli and possible irritant reactions at the injection site. Among the various biodegradable nanoparticulate carriers, polylactide and polyalkylcyanoacrylate nanoparticles are frequently used to deliver various chemotherapeutic agents. Although the fabrication by the solvent evaporation technique of biodegradable particulate carriers by means of poly (D,L-lactide) (Liggins & Burt 2001) and poly (D,L-lactide-co-glycolic) acid coupled with various emulsifiers (Feng & Huang 2001) for the controlled release of paclitaxel has been reported, the feasibility of applying polyalkylcyanoacrylate in the fabrication of nanoparticle delivery systems by anionic polymerization has not been reported.

Ease of preparation, good encapsulation properties, low toxicity, biodegradability, and ability to cross the blood–brain barrier have made polyalkylcyanoacrylate nanoparticles ideal for cancer therapy, especially for brain tumours (Woods 1996; Kreuter et al 2002). Furthermore, clinical application of doxorubicin incorporated in polyisohexylcyanoacrylate has been studied (Kattan et al 1992). In the present investigation, the feasibility of applying polyalkylcyanoacrylate nanoparticles, such as polybutylcyanoacrylate (PBCA), as an alternative delivery system for paclitaxel was evaluated. PBCA has been used extensively as a surgical adhesive in the clinical setting. The modified emulsion polymerization, or anionic polymerization, was used to prepare the nanoparticulate delivery systems (NDSs) under various fabrication conditions (Kreuter 1994). The effect of various surfactants (dextran 70, cholesterol, l-α-lecithin and polyvinyl alcohol) on the fabrication of PBCA-NDSs was also investigated. The paclitaxel-loaded PBCA-NDSs were then evaluated and characterized by their particle size, zeta potential, entrapment efficiency, and in-vitro paclitaxel release kinetics in order to achieve smaller particle size, higher zeta potential, better entrapment efficiency, as well as desired release of paclitaxel. Thus, the risk of emboli as a result of the aggregation of nanoparticles could be minimized and the action of paclitaxel could be prolonged by controlled release dosage forms, after intravenous administration of PBCA-NDSs.

Materials and Methods

Materials

n-Butylcyanoacrylate, the monomer used for the polymerization of PBCA nanoparticles, was a gift from Permabond (Bridgewater, NJ, USA). Paclitaxel, dextran 70 (MW 68 800), cholesterol (5-cholesten-3β-ol, MW 386.7), anhydrous monobasic potassium phosphate and phosphate-buffered saline were purchased from Sigma Chemical Co. (St Louis, MO, USA). L-α-lecithin (soybean, MW 758.07) and polyvinyl alcohol (MW 30 000–70 000) were purchased from Fluka (Basel, Switzerland). All other reagents were of analytical grade.

Fabrication of paclitaxel-loaded PBCA-NDSs

The paclitaxel-loaded PBCA-NDSs were prepared using an acidic polymerization medium (0.01 M HCl, pH 2.33) containing surfactant (1% w/v) and 1 mL of paclitaxel solution (1 mg mL^{-1}) (Couvreur et al 1982). Butylcyanoacrylate (1% w/v) was then added dropwise under constant magnetic stirring (500–700 rev min^{-1}), in a stirrer (Corning PC420; Corning, Binghamton, NY, USA) with a glass coated stirring bar (VWR Scientific Products, S. Plainfield, NJ, USA). After 4 h of polymerization, the nanoparticle suspension was neutralized with sodium hydroxide (0.1 M) to a pH of around 6.0–7.0, then stirred for an additional 10 min to complete the polymerization. The resultant suspension was filtered through filter paper (8–12 μm, S and S no. 597), and ultracentrifuged (37 100 g) twice for 1 h each at 4 °C using an ultracentrifuge (Sorvall Ultracentrifuge, RC5C, rotor SA600; Kendro, Newton, CT, USA). After each centrifugation step, the supernatant was removed and the nanoparticles were resuspended in the same amount of deionized water using sonication (Laboratory Supplies Co., Inc, Hicksville, NY, USA). The nanoparticles were then lyophilized, in the presence of 4% mannitol as cryoprotector, under a pressure of 130×10^{-3} mbar overnight at −40 °C (Freezone 4.5; Labconco, Kansas City, MO, USA) and stored at 4 °C until further use. The PBCA-NDS without surfactant was prepared using identical procedures as described above, only in absence of any surfactant during the polymerization process.

The effect of surfactant on the fabrication of PBCA-NDSs was evaluated using 1% w/v dextran 70, lecithin and polyvinyl alcohol, and combinations (1% w/v each) of dextran 70, lecithin, polyvinyl alcohol and cholesterol. The effect of surfactant concentration on the fabrication of PBCA-NDSs containing dextran 70, lecithin and polyvinyl alcohol was evaluated at concentrations of 0.05, 1.0 and 2.5% w/v.

Characterization of paclitaxel-loaded PBCA-NDSs

The paclitaxel-loaded PBCA-NDSs were evaluated and characterized by their particle size, zeta potential and entrapment efficiency.

Lyophilized PBCA-NDSs (5 mg) were suspended in 20 mL of deionized water by sonication and filtered. The particle size and size distribution of PBCA-NDSs were measured by dynamic laser light scattering (Nicomp 380 DLS submicron particle sizer; Nicomp, Santa Barbara, CA, USA).

Lyophilized PBCA-NDSs (3 mg) were suspended in 10 mL phosphate buffer (0.02 M, potassium dihydrogen phosphate, pH 7.4) by sonication for 10 min. The zeta potential was analysed by a zeta potential analyser (Nicomp 380 ZLS) with a palladium electrode.

Lyophilized PBCA-NDSs (5 mg) containing paclitaxel were completely dissolved in 5 mL tetrahydrofuran solution. The solution was then filtered (0.22 μm; Pall Corporation, Germany) and the concentration of paclitaxel was assayed by high-performance liquid chromatography (HPLC). The entrapment efficiency was calculated as a ratio of the total entrapped paclitaxel to the total amount of paclitaxel used (i.e. 1 mg). The total entrapped paclitaxel was obtained as a ratio of assayed amount of paclitaxel in 5 mg of lyophilized PBCA-NDSs, determined by HPLC, to the total amount of lyophilized PBCA-NDSs, which was obtained from the fabrication of paclitaxel-loaded PBCA-NDSs.

In-vitro release kinetics of paclitaxel from PBCA-NDSs

To study the release kinetics of paclitaxel from PBCA-NDSs, a method utilizing a water shaker bath was used. In brief, 20 mg of the paclitaxel-loaded PBCA-NDSs was suspended in phosphate-buffered saline (100 mL) containing Tween 80 (0.1% v/v) as dissolution medium in screw-capped glass bottles. The bottles were placed in a water shaker bath (Forma Scientific, Thermo Forma, Marietta, OH, USA), which was maintained at 37 °C and shaken at 130 cycles min^{-1} throughout the duration of the study. Serial samples (1 mL each) were taken from the medium solution in each bottle and replaced with an equal volume of fresh medium at predetermined intervals. The samples collected were then centrifuged at 28 400 g for 5 min and the concentrations of paclitaxel in the supernatant were analysed by HPLC as outlined below.

Analytical analysis of paclitaxel

The HPLC system used for the analysis of paclitaxel was a HP series 1100 (HP, Palo Alto, CA, USA), equipped with a quaternary pump, an automatic sample processor, a UV detector, an integrator and a C18 column (5 μm, 250 × 4.6 mm) (Waters Spherisorb). The UV detector wavelength was set at 230 nm, and a combination of acetonitrile and water (starting from 38:62 to 70:30), at a flow rate of 1.5 mL min^{-1}, was used as the gradient mobile phase. Under these conditions, after injection of the sample (15 μL), a well-separated peak was detected at a retention time of 14 min with a sensitivity of 0.01 $\mu g\,mL^{-1}$.

Statistical analysis

The Student's t-test was used to determine statistically significant differences between PBCA-NDSs with and without the various surfactants for each parameter obtained from the analysis of particle size, zeta potential, entrapment efficiency, and in-vitro release kinetics of paclitaxel. One-way analysis of variance was used to determine the differences between the same parameters among PBCA-NDSs. If there was a difference among PBCA-NDSs, Student-Newman-Keuls method was used to make all pairwise comparisons. Both statistical analyses were carried using the StatMost program (version 3.0; Datamost Corporation, Sandy, UT, USA).

Results

Characterization of paclitaxel-loaded PBCA-NDSs

The effects of surfactant on the particle size, zeta potential and entrapment efficiency of PBCA-NDSs are summarized in Table 1.

Table 1 Comparison of physicochemical characteristics among various paclitaxel-loaded polybutylcyanoacrylate nanoparticulate delivery systems (PBCA-NDSs).

PBCA-NDS	Particle size (nm)	Zeta potential (−mV)[a]	Entrapment efficiency (%)[b]
Polybutylcyanoacrylate	844.4 ± 105.8	1.21 ± 0.13	46.0 ± 3.3
Effect of surfactant (1%)[c]			
Dextran 70	260.5 ± 5.6	7.02 ± 0.21[d]	58.0 ± 0.5[d]
Lecithin	251.4 ± 17.9	10.05 ± 0.36[d]	71.0 ± 1.1[d]
Polyvinyl alcohol	260.8 ± 7.5	8.40 ± 0.45[d]	61.5 ± 1.7[d]
Effect of surfactant combination (1% each)[c]			
Lecithin/dextran 70	182.2 ± 10.5[e]	15.47 ± 0.55[e]	78.5 ± 1.1[e]
Lecithin/cholesterol	158.7 ± 5.5[e]	20.82 ± 2.00[e]	79.5 ± 0.4[e]
Polyvinyl alcohol/dextran 70	250.6 ± 24.2[e]	13.19 ± 0.68[e]	68.0 ± 1.1[e]

Data are presented as mean ± s.d., n = 3. [a]The negative sign was presented as negative charge of zeta potential. [b]Data were calculated from the ratio of entrapped amount in PBCA-NDSs to the total amount (i.e. 1 mg) of paclitaxel used in the fabrication of PBCA-NDSs. [c]The Student's t-test indicated a significant difference ($P < 0.001$) compared with PBCA-NDSs fabricated without any surfactant. [d]Analysis of variance indicated a significant difference ($P < 0.05$) among PBCA-NDSs incorporated with one surfactant. [e]Analysis of variance indicated a significant difference ($P < 0.05$) among PBCA-NDSs incorporated with a combination of two surfactants.

The PBCA-NDSs fabricated without surfactants had the largest mean particle size (844.1 ± 105.8 nm). The mean particle size of PBCA-NDSs incorporated with surfactant (dextran 70, lecithin and polyvinyl alcohol) was significantly reduced. The mean particle size of PBCA-NDSs incorporated with a combination of two surfactants was also significantly reduced for lecithin/dextran 70 and lecithin/cholesterol, but not for polyvinyl alcohol/dextran 70. Among the PBCA-NDSs studied, the mean particle size of PBCA-NDSs fabricated with a combination of lecithin and cholesterol was the smallest (158.7 ± 5.5 nm). The effect of surfactant concentration on the particle size of various PBCA-NDSs is shown in Figure 1. Results indicated that there was an almost linear decrease in the particle size with an increase in the surfactant concentration (range 0.05–1% w/v) for dextran 70 ($r^2 = 0.98$) and polyvinyl alcohol ($r^2 = 0.97$). In contrast, for lecithin, the mean particle size of PBCA-NDSs decreased (267.0 vs 251.4 nm) slightly with an increase in concentration of lecithin from 0.05 to 1.0% . However, as the concentration was further increased to 2.5% , the mean particle size increased dramatically to 345.5 ± 24 nm.

The PBCA-NDS fabricated without the use of any surfactants had the lowest mean zeta potential (1.21 ± 0.13 mV) (Table 1). The mean zeta potential of PBCA-NDSs incorporated with surfactant was significantly increased. The mean zeta potential of PBCA-NDSs incorporated with a combination of two surfactants (lecithin/dextran 70, lecithin/cholesterol and polyvinyl alcohol/dextran 70) was also significantly increased. Among all the PBCA-NDSs studied, the mean zeta potential of PBCA-NDSs fabricated using a combination of lecithin and cholesterol had the highest zeta potential (20.82 ± 2.00 mV).

PBCA-NDS fabricated without the use of any stabilizer had the lowest paclitaxel entrapment (46.0 ± 3.31%) (Table 1). The mean entrapment efficiency was significantly increased for PBCA-NDSs incorporated with dextran 70, lecithin and polyvinyl alcohol. The mean entrapment efficiency of PBCA-NDSs incorporated with a combination of two surfactants was also significantly increased. Among the PBCA-NDSs studied, the mean entrapment efficiency of PBCA-NDSs fabricated using a combination of lecithin and cholesterol had the highest entrapment efficiency (79.5 ± 0.4%). The effect of surfactant concentration on the entrapment efficiency of various PBCA-NDSs is shown in Figure 2. The mean values of entrapment efficiency of PBCA-NDSs decreased from 69.3 ± 2.2% to 45.2 ± 1.1% for dextran 70, from 76.3 ± 2.4% to 29.9 ± 2.1% for lecithin, and from 71.9 ± 1.3% to 20.2 ± 1.1% for polyvinyl alcohol, as the surfactant concentration was increased from 0.05 to 2.5% w/v.

In-vitro release kinetics of paclitaxel from paclitaxel-loaded PBCA-NDSs

The effects of surfactant on the in-vitro release kinetics of paclitaxel from paclitaxel-loaded PBCA-NDSs are outlined in Table 2. To better compare the rate of controlled release of paclitaxel from various PBCA-NDSs, the release profiles of paclitaxel were plotted on the basis of matrix diffusion-controlled drug release kinetics (i.e. linear Q vs $t^{\frac{1}{2}}$ relationship) (Figure 3). Results in Figure 3 indicate that all paclitaxel-loaded PBCA-NDSs showed a characteristic biphasic zero-order release of paclitaxel with an initial fast release for a period of between 9 and

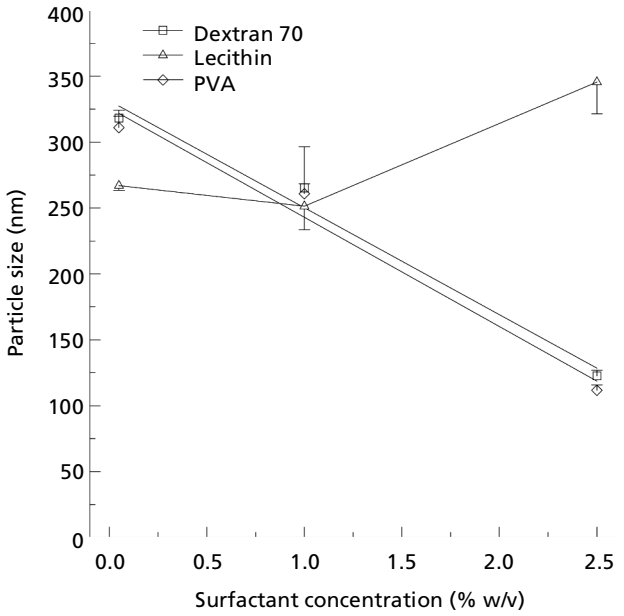

Figure 1 Effect of surfactant concentration on the particle size (mean ± s.d.) of paclitaxel-loaded polybutylcyanoacrylate nanoparticulate delivery systems. PVA, polyvinyl alcohol.

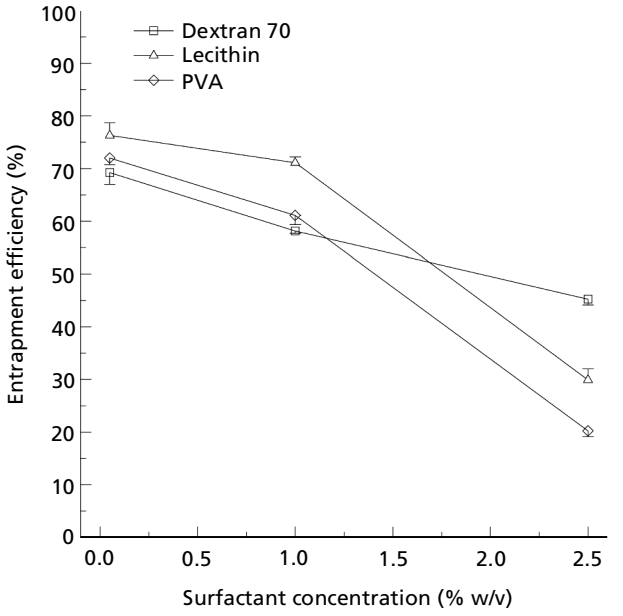

Figure 2 Effect of surfactant concentration on the entrapment efficiency (mean ± s.d.) of paclitaxel-loaded polybutylcyanoacrylate nanoparticulate delivery systems. PVA, polyvinyl alcohol.

Table 2 Comparison of release kinetics of paclitaxel from various paclitaxel-loaded polybutylcyanoacrylate nanoparticulate delivery systems (PBCA-NDSs).

PBCA-NDS	Release flux ($\mu g\,h^{-\frac{1}{2}}$)[a]		Amount released at 48 h (%)[b]
	Phase I	Phase II	
Polybutylcyanoacrylate	102.5 ± 16.0	12.8 ± 11.1	88.7 ± 3.3
Effect of surfactant (1%)			
Dextran 70	93.7 ± 22.2	17.5 ± 14.5	64.9 ± 4.7[c,d]
Lecithin	74.8 ± 9.5	15.0 ± 6.4	38.2 ± 3.1[c,d]
Polyvinyl alcohol	88.5 ± 8.3	19.8 ± 5.9	55.6 ± 3.8[c,d]
Effect of surfactant combination (1% each)			
Lecithin/dextran 70	60.4 ± 14.7[d]	7.8 ± 5.1	32.9 ± 5.7[c,e]
Lecithin/cholesterol	78.9 ± 7.7	3.4 ± 2.4	26.5 ± 3.7[c,e]
Polyvinyl alcohol/dextran 70	88.2 ± 12.3	9.2 ± 4.8	48.5 ± 4.7[c,e]

Data are presented as mean ± s.d., n = 3. [a]Phase I is the initial phase of the biphasic release profile; phase II is the second phase of the biphasic release profile. [b]Data were calculated from the ratio of the total amount of paclitaxel released at 48 h to the entrapped amount of paclitaxel in the PBCA-NDSs (Table 1). [c]The Student's t-test indicated a significant difference ($P < 0.001$) compared with PBCA-NDS fabricated without any surfactant. [d]Analysis of variance indicated a significant difference ($P < 0.05$) among PBCA-NDSs incorporated with one surfactant. [e]Analysis of variance indicated a significant difference ($P < 0.05$) among PBCA-NDSs incorporated with a combination of two surfactants.

16 h, followed by a second much slower release phase through to the end of the studies.

As outlined in Table 2, the mean values of initial release flux of paclitaxel from PBCA-NDSs fabricated without any surfactant ($102.5 \pm 16.0\,\mu g\,h^{-\frac{1}{2}}$) decreased with incorporation of surfactant. However, in the case of the second phase release flux, these values increased with surfactant incorporation. On the other hand, the mean values of release flux of paclitaxel from PBCA-NDSs incorporated with a combination of two surfactants decreased in the case of both initial and second phase release. Moreover, the mean values of cumulative amount of paclitaxel released at 48 h decreased significantly from PBCA-NDSs without any surfactant (88.7 ± 3.3%) to PBCA-NDSs fabricated with incorporation of a single surfactant and a combination of surfactants (Table 2).

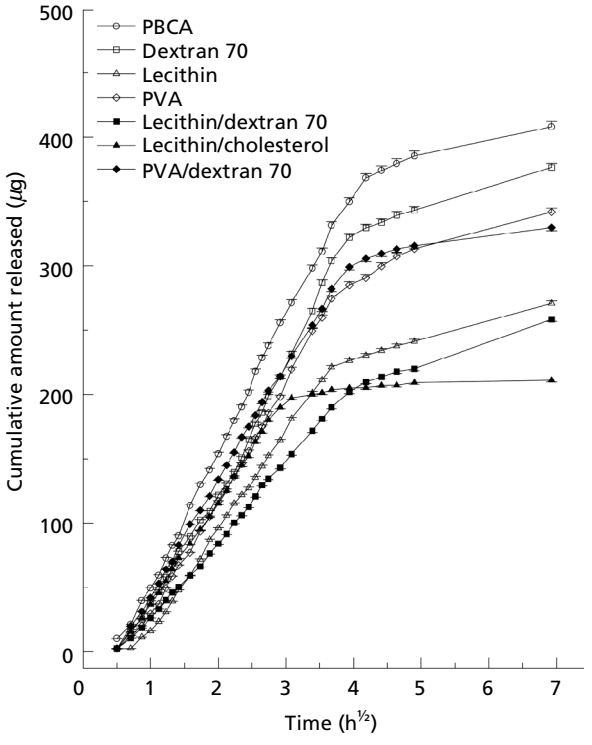

Figure 3 Comparative relationships of cumulative amount of paclitaxel released (mean ± s.d.) as a function of the square root of time from paclitaxel-loaded polybutylcyanoacrylate (PBCA) nanoparticulate delivery systems. PVA, polyvinyl alcohol.

Discussion

In the current study, PBCA-NDSs were successfully prepared by anionic polymerization in an aqueous dispersion medium of low pH at room temperature. Thus, the use of organic solvents and high temperatures, which might result in toxicity and instability of NDSs, was avoided. The fabrication of PBCA-NDSs without the use of organic solvents could be an advantage over the fabrication of particulate carriers by means of poly (D,L-lactide) (Liggins & Burt 2001) and poly (D,L-lactide-co-glycolide) (Feng & Huang 2001) using the solvent evaporation technique.

The results of particle size analysis indicate that the surfactants played a significant role in decreasing the particle size because the polymerization medium generates the initiator OH^- ions as well as H^+ ions, which terminate the polymerization reaction. This causes the molecular weights after polymerization to be very low (Vansnick et al 1985). Furthermore, the glass transition temperature of polybutylcyanoacrylates produced by anionic polymerization was lower (56 °C) than that produced by the free

radical method (123 °C) (Kulkarni et al 1973). Because of the low molecular weights and the low glass transition temperature, the growing PBCA nanoparticles have been observed to be very soft and are thus prone to agglomeration, forming large particles (Table 1) (Kreuter 1994). Therefore, methodologies, such as incorporation of surfactant, to reduce the particle size of PBCA nanoparticles are necessary, which sterically stabilize the PBCA nanoparticles by forming a protective layer on the nanoparticle surface (Douglas et al 1985). The reduced particle size enables intravenous administration of PBCA-NDSs without adverse effects such as the risk of emboli and possible irritant reactions at the injection site.

Extensive X-ray photoelectron spectroscopy studies indicate that phospholipids (e.g. lecithin) have a greater tendency to migrate to the surface of their PLGA nanospheres than other surfactants (e.g. polyvinyl alcohol) (Feng & Huang 2001). Also, the more surfactant at the surface of the particle, the more effective stabilization and smaller particle size. In this study, lecithin produced PBCA-NDSs of smaller particle size than dextran 70 and polyvinyl alcohol (251.4 vs 260.5 and 260.8 nm), suggesting that a similar mechanism of lecithin exists between PLGA and PBCA nanospheres. Moreover, the combination of lecithin and cholesterol, both lipids, formed a more homogeneous and stable emulsion system and resulted in the smallest particle size of PBCA-NDSs.

As the surfactant concentration increased, the particle size of PBCA-NDSs decreased because, at lower concentrations, the insufficient amount of surfactant fails to cover all the surfaces of the PBCA nanoparticles. The uncovered PBCA nanoparticles then tend to aggregate until the total surface area of nanoparticles is decreased to a point such that the amount of surfactant available is sufficient to produce a stable system resulting in larger particle size of PBCA-NDSs. As the concentration was increased, it was possible to effectively stabilize all the particle surface area, resulting in the smaller particle size of PBCA-NDSs (Douglas et al 1985).

Lecithin showed a reverse trend compared with dextran 70 and polyvinyl alcohol because even at low concentrations, all the PBCA nanoparticles were completely covered with lecithin. Thus, the surplus lecithin within the formulation could form vesicles or incorporate into a multilayer arrangement surrounding the nanoparticles, resulting in an increased particle size of PBCA-NDSs. This happens because the excess lecithin in the system could no longer decrease the radius of curvature of the oil droplets and thus the phospholipids form other structures (Mosqueira et al 2000). Thus, from Figure 2, it could be concluded that the secondary structures significantly contaminate the nanoparticle formulation at concentrations greater than 1% lecithin and also at 1% lecithin the nanoparticle formulation might not be contaminated by secondary structures so as to significantly effect their physicochemical properties such as drug entrapment or release. Studies using more concentration levels should be carried out to confirm this finding.

The zeta potential analysis suggested that all PBCA-NDSs had a negative charge (i.e. negative zeta potential values) of nanoparticles at pH 7.4, which might be attributed to the ionization of groups on the side chain of polymer and surfactant, such as the cyano group (from PBCA), phosphatic group (from lecithin), and hydroxyl group (from cholesterol and polyvinyl alcohol) (Quellec et al 1998). The zeta potential values were consistent with those reported in the literature for polybutylcyanoacrylate (Douglas et al 1987), poly(isobutylcyanoacrylate) (Aboubakar et al 1999) and poly(lactic acid)-poly(ethylene oxide) nanoparticles (Govender et al 2000). The PBCA-NDSs produced were thus further stabilized due to steric repulsion between the nanoparticles. Lecithin, being an amphiphile, remains firmly attached to the surface of the particles by their anchoring moieties while maintaining an adsorbed layer thickness of sufficient magnitude to produce a strong steric repulsion, whereas dextran and polyvinyl alcohol, being totally hydrophilic polymers, form covalent linkages with the cyanoacrylate polymer moieties via hydroxyl groups and therefore remain attached to the particle surface by forming multipoint linkages (Douglas et al 1985). The nanoparticles of PBCA-NDSs produced with a combination of two surfactants showed even higher zeta potential due to the presence of a larger number of ionized groups on the surface nanoparticles. The larger values of zeta potential indicate higher interparticulate repulsion and thus decrease the chances of particle aggregation and embolism in-vivo.

The PBCA-NDS fabricated without any surfactant showed low entrapment efficiency because of their porous surface (Kreuter 1994), which resulted in extensive drug loss from the nanoparticles as a result of diffusion into the suspending medium during the fabrication process. On the other hand, PBCA-NDSs fabricated with various surfactants had a smooth continuous surface with very few or no pores to minimize the drug loss during the fabrication process (Feng & Huang 2001). The incorporation of lipids (lecithin and/or cholesterol) resulted in a higher entrapment efficiency because the hydrophobic surfactants contributed to the further incorporation of the highly hydrophobic paclitaxel into the related formulation systems (Mu & Feng 2001).

The amount of surfactant plays a fundamental role in determining not only the size but also the entrapment efficiency of the nanoparticles. The results indicate that increasing the surfactant concentration decreases entrapment efficiency. This might be attributed to the fact that an increase in surfactant concentration results in a decrease in the particle size and an increase in surface area per unit volume, which consequently increases the possibility of drug loss by diffusion into the medium during the fabrication process (Gorner et al 1999). In addition, some drug molecules may bind to the excessive surfactant molecules and thus drug may be lost during the fabrication process.

The release profiles shown in Figure 3 indicated that the drug release from the PBCA nanoparticles was by a polymer matrix diffusion-controlled phenomenon. The biphasic release profile of paclitaxel from the PBCA-NDSs in this study was in accord with the in-vitro insulin release behaviour of PBCA nanoparticles (Zhang et al

2001). The initial fast release phase (9–12 h) might be due to the immediate diffusion and release of the portion of the drug located on and near the surface of the nanoparticles. Thereafter, the release profile shifted to a lower rate of release for the remaining time period, which might be due to diffusion of drug from the inner core of nanoparticles. The release from PBCA nanoparticles is proposed to be mainly by diffusion from the particles only partially augmented by polymer degradation. As long as the polymer surface exists, diffusion is the main contributor, so the drug release is only completed after the final degradation of the polymer particles (El-Samaligy & Rohdewald 1982).

It is interesting to note that PBCA-NDSs incorporated with hydrophobic surfactant, such as lecithin, had lower amounts of paclitaxel released at 48 h than PBCA-NDSs with hydrophilic surfactants, such as dextran 70 and polyvinyl alcohol (38.2% vs 64.9% and 55.6%, respectively) (Table 2). This could be attributed to the higher solubility of paclitaxel in hydrophobic surfactants incorporated in PBCA-NDSs than in aqueous dissolution medium. The amount released at 48 h decreased further to 26.5% for PBCA-NDSs fabricated using a combination of lecithin and cholesterol. Furthermore, the facts of higher solubility of paclitaxel in hydrophobic surfactants and the higher concentration of surfactant combination, which reduces surface porosity of the nanoparticles, have resulted in significantly slowing down the drug release and flattening of the release profiles.

The results of this study suggest that surfactants incorporated into the PBCA-NDSs can dramatically increase their effectiveness. However, the disadvantage with the use of surfactants in controlled drug delivery systems is their potential toxicity at higher concentrations. Surfactants are capable of causing disruption in biological membranes and display significant interaction with certain proteins. A high concentration of surfactants over a long period of time may disturb some bodily processes (Buckton 1995). Therefore, the optimal concentrations of surfactants need to be further evaluated in animal studies.

Conclusions

The physicochemical characteristics of PBCA-NDSs, fabricated by an anionic polymerization technique and incorporated with various surfactants, were significantly improved by decreasing particle size as well as by increasing the zeta potential to prevent agglomeration of nanoparticles. Moreover, PBCA-NDSs incorporated with various surfactants demonstrated higher entrapment efficiency of paclitaxel and allowed more controlled and/or prolonged release of paclitaxel. Based on the results of this study, the PBCA-NDSs with a combination of lecithin and dextran 70 could potentially be developed as a successful delivery system for paclitaxel as they show satisfactory physicochemical characteristics and controlled release of paclitaxel over 48 h. However, further investigations in animal studies have to be carried out. Finally, compared with dextran 70 and polyvinyl alcohol, the naturally occurring lipids, such as lecithin and cholesterol, had greater advantages in improving the physicochemical properties of PBCA-NDSs, in terms of smaller particle size, higher zeta potential and better drug entrapment efficiency, and better controlled release of paclitaxel, in terms of lower release rate and possible longer duration of treatment from PBCA-NDSs.

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