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Synthesis of high loading and encapsulation efficient paclitaxel-loaded poly(*n*-butyl cyanoacrylate) nanoparticles via miniemulsion

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Abstract

The manufacture of stable paclitaxel-loaded poly(*n*-butyl cyanoacrylate) (PBCA) nanoparticles containing high loading and encapsulation efficiency simultaneously were achieved in the presence of pluronic F127 via miniemulsion. It was found that both drug loading and encapsulation efficiencies of PBCA nanoparticles prepared by miniemulsion were higher (approximately three times) than those obtained by emulsion with similar paclitaxel content in the feed monomer (1%, w/w). Furthermore, the loading and encapsulation efficiencies increased concurrently (to a maximum of 4 and 80%, respectively) with increasing paclitaxel content and these nanoparticles were spherical in shape and with size near 100 nm. XRD patterns revealed that paclitaxel in particles was distributed in the molecular or amorphous state or in the form of small crystals. The *in vitro* drug release profile of drug-loaded PBCA nanoparticles prepared from miniemulsion exhibited a gradual release; more than 80% (w/w) of the paclitaxel was released after 96 h.

Keywords: Paclitaxel; Cyanoacrylate; Miniemulsion; Encapsulation; Nanoparticle

1. Introduction

During the last decade, biodegradable poly(alkyl cyanoacrylates) (PACAs) nanoparticles (Lenaerts et al., 1984; O'Sullivan and Birkinshaw, 2002) have been studied as an effective drug delivery device for the sustained and localized administration of various pharmacologically active agents, such as cytotoxic drugs (Couvreur et al., 1980), antibiotics (Couvreur et al., 1992), peptide (Damgé et al., 1990; Tasset et al., 1995) and gene (Li et al., 2003). The PACAs nanoparticles are generated from emulsion or by dispersion polymerization in acidic aqueous solutions of surfactants (Couvreur et al., 1979) and their porous structure give them a high specific area on which various quantities of drugs, dissolved in the medium during or after polymerization, are adsorbed. Suitable surfactants are used to control the sizes of particles in the range of 20–770 nm, and surface characteristics such as zeta potential and the hydrophilic–hydrophobic property are modulated to meet the requisites of intravenous or oral administration, and the release of a drug in the selected tissue (Douglas et al., 1984, 1985; Vauthier et al., 2003b).

For therapeutic use, the drug loading efficiency of the nanoparticles must be maximized, to minimize the amount of carrier. Reducing the overall quantity of carrier to be administered can reduce the risk of damage caused by the hydrolyte of nanoparticles. Accordingly, the successful drug delivery by nanoparticles depends on a high loading efficiency. In various kinds of PACAs nanoparticles, PBCA was the most used drug carrier because it can interact with different kind of drugs (Salgueiro et al., 2002). The most common approach of preparing drug-loaded PBCA nanoparticles is either incorporation during the process of emulsion or adsorption by the surface of nanoparticles (Illum et al., 1986; Zhang et al., 1996). Adjusting the factors of production, such as the stabilizer, the pH of the medium and the amount and time of drug addition are useful in increasing the carrier capacity. The increase of compatibility between drug and nanoparticulate matrix via adjustable hydrophilic/hydrophobic properties of poly(BCA-co-OCA) might promote the drug loading and encapsulation efficiency (Huang and Lee, 2006). However, the maximum weight of the drugs that can be entrapped by PBCA nanoparti-

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cles is limited by the solubility of drugs in the reaction medium. An approach for increasing the loading effficiency of PBCA nanoparticles to hydrophobic drug is through interfacial polymerization (Damgé et al., 1987). Another alternative particularly efficient strategy is to use cyclodextrins to form complexes with the hydrophobic drug progesterone and then to perform polymerization in the medium (da Silveira et al., 1998). The progesterone content in the particles is 50 times higher than that in the absence of cyclodextrin.

Paclitaxel is a quite effective chemotherapeutic agent that is extracted from the bark of *Taxus brevifolia*. It has been clinically applied to cure a wide range of tumors, especially breast cancer and ovarian cancer (Singla et al., 2002). It cannot easily be clinically applied since it has a fairly low aqueous solubility and its sources are limited. The solubility of paclitaxel in water is less than 3 μg/ml (Liggins et al., 1997), so it is formulated in a 50:50 mixture of Cremophore EL (polyethoxylated castor oil) and ethanol, which is diluted by adding normal saline or dextrose solution (5%) before administration in the current clinical formulation (Waugh et al., 1991). However, Cremophore EL has various side effects, including hypersensitivity, neurotoxicity, nephrotoxicity and cardiotoxicity (Liebmann et al., 1993). One feasible mean of overcoming these shortcomings and increasing bioavailability and tumor accumulation is to encapsulate paclitaxel into biodegradable polymeric micro/nanoparticles (Ruan and Feng, 2003; Mu and Feng, 2003), liposomes (Sharma et al., 1997), core/shell nanoparticles (Oh et al., 2005), micelles (Liggins and Burt, 2002) or dendritic polymers (Ooya et al., 2003).

In cancer therapy, numerous investigations have demonstrated that PACAs nanoparticles are excellent carrier colloids for use in chemotherapeutic agents, and can overcome multidrug resistance phenomena at both the cellular and the non-cellular levels (Vauthier et al., 2003a). However, in the conventional emulsion polymerization, drugs were dissolved in the polymerization medium before introducing monomer or added after the polymerization so that drugs were encapsulated during polymerization or adsorbed in the particle (Couvreur et al., 1990). In both cases, the solubility of drug in the reaction medium is important. The solubility of highly hydrophobic paclitaxel in water is poor, thus it seems impracticable to obtain paclitaxel-loaded PACAs nanoparticles with high loading efficiency by the conventional emulsion polymerization.

Miniemulsion is a relatively stable dispersion of oil droplets in water in the form of particles with sizes between 30 and 500 nm, prepared by shearing a system of oil, water, surfactant and a highly water-insoluble compound, a so-called hydrophobe whose function is to restrict degradation of droplets (Ostwald ripening) (Antonietti and Landfester, 2002). Ostwald ripening of the droplets is a phenomenon where large particles grow larger at the expense of smaller particles dissolving in the polymerization medium (Antonietti and Landfester, 2002). The smaller droplets will disappear if they are not stabilized to resist the diffusion degradation. The rate of this disappearance is fast, especially for the small droplets. The water-insoluble hydrophobe dissolved in the monomer droplets is incapable of transport between droplets, while it increases the osmotic pressure inside the droplets to suppress Ostwald ripening. In the suppression of coalescence and Ostwald ripening among droplets during polymerization, each droplet can be regarded as a nanoreactor, and the droplets are copied one-to-one to particles (Landfester et al., 1999). Since the loci of particle nucleation and subsequent propagation reaction are mainly in the submicron monomer droplets, the inorganic or organic materials that can be suspended or dissolved in the monomer are able to be incorporated in the polymeric particles (Erdem et al., 2000; Takasu et al., 2003).

This work utilizes miniemulsion polymerization to prepare high paclitaxel-loaded PBCA nanoparticles by ultrasonication for the purpose of cancer therapeutic application. This investigation reports the effects of surfactant concentration, paclitaxel content in the monomer and the method of synthesis on the loading capacity, the encapsulation efficiency and the properties of the paticles. The morphology of drugs in particles and *in vitro* drug release are also discussed.

2. Materials and methods

2.1. Materials

The monomer of *n*-butyl cyanoacrylate (BCA) with 99.5% purity was obtained from Tongshen Enterprise Co., Ltd., Kaohsiung, Taiwan and was used without further purification. Paclitaxel was purchased from DongTai Kangning Plant Co., Ltd., Jiangsu, China. Pluronic F127 [poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer; MW 12,600 Da] was obtained from Sigma (USA). HPLC grade tetrahydrofuran (THF), acetonitrile and dichloromethane (DCM) were purchased from Tedia (USA) and used as received. Hydrochloric acid (0.1 N) was obtained from Merck (Germany). Water, purified with the Millipore® (USA) Milli-Q system, was used throughout.

2.2. Methods

2.2.1. Preparation of nanoparticles

Paclitaxel-loaded PBCA nanoparticles were prepared by the miniemulsion and emulsion methods, respectively. Since BCA is an extremely active monomer, even the presence of a weak basic substance is capable of initiating the anionic polymerization. For obtaining a stable solution of monomer containing paclitaxel, it is required for BCA having high purity and containing little inhibitor of SO_2. Paclitaxel was dissolved in BCA with the aid of mild heating (60 °C) and sonication. For all experiments, 0.5 g of the paclitaxel containing or non-containing BCA monomer was added at once to a 50 ml aqueous solution of 0.01 N hydrochloric acid containing surfactant F127.

In miniemulsion, the aqueous solution and monomer were mixed using a magnetic stirrer at high speed (∼1000 rpm) for 5 min at room temperature (∼20 °C) to yield a pre-emulsion. The pre-emulsion was sonicated for 60 s in an ice bath with an energy output of 110 W using a Branson Sonifier (Model 450) and then gently stirred at 250 rpm for a further 3 h. To study the influence of pluronic F127 on drug loading and encapsulation efficiencies of paclitaxel-loaded PBCA nanoparticles, the

concentration of pluronic F127 in aqueous solution was varied from 0.03 to 0.5% (w/v) and the concentration of paclitaxel in feed monomer was 1% (w/w). Meanwhile, paclitaxel containing monomers with various paclitaxel concentrations (0, 1, 3, and 5%, w/w) were used to produce drug-loaded nanoparticles with constant 0.5% (w/v) of pluronic F127 in order to investigate the effects of drug concentrations on drug loading and encapsulation efficiencies.

While in the emulsion process, the paclitaxel containing monomer of 1% (w/w) was dispersed in the aqueous solution containing 0.5% (w/v) of pluronic F127 and polymerized with high speed stirring for 4 h at room temperature.

The resulting dispersions were filtered through a 1.0 μm filter to eliminate non-incorporated drugs and aggregated particles. Formed drug-loaded nanoparticles were separated by ultracentrifugation at 100,000 × *g* for 60 min (CP 100MX, Hitachi, Japan) at 4 °C and dispersed again in water and lyophilized for 3 days.

2.2.2. Analysis of particle size

The particle size and size distribution of drug-loaded PBCA nanoparticles were elucidated by photon correlation spectroscopy (PCS; Zetasizer 3000, Malvern Instruments, Malvern, UK) at 25 °C. Scattered light with a wavelength of 633 nm was detected at an angle of 90°. The dispersion was diluted with deionized water to a favorable concentration for better measurement. The average hydrodynamic particle size was expressed as the value of z-average size ±S.D. from three replicate samples. The width of the size distribution was indicated by the polydispersity index (P.I.).

2.2.3. Analysis of particle surface charge

Drug-loaded and unloaded PBCA nanoparticles suspensions were diluted with deionized water to ensure that the signal intensity is suitable for the instrument. The zeta potential was measured by laser Doppler velocimetry (Zetasizer 3000, Malvern Instruments, Malvern, UK) at 25 °C. Values are presented as mean ± S.D. from three replicate samples.

2.2.4. Molecular weight

The molecular weight of PBCA was determined by gel permeation chromatography (GPC) equipped with a Waters 510 pump, 50, 10^3 and 10^4 Å Phenogel columns arranged in series (Phenomenex, USA) and a Waters 410 differential refractometer. The mobile phase was THF at a flow rate of 1.0 ml/min. Freeze-dried drug-loaded nanoparticles were dissolved in acetone (10 mg/ml). Then, water was added steadily until the solution became milky white. The PBCA polymer was collected by centrifugation at 10,000 × *g* for 10 min and redispersed in deionized water. The purified polymer obtained by repeating this procedure three times was dissolved in THF to a concentration of 2% (w/v); 60 μl of this solution was then injected to the system. Polystyrene standards with molecular weights of between 1.3 and 377.4 kDa were used for the construction of calibration curve. Results were quoted as average number molecular weight ($\overline{M_n}$).

2.2.5. Particulate morphology

The morphology of the nanoparticles was observed by field emission scanning electron microscopy (FE-SEM; Hitachi S-4700, Japan). A drop of diluted nanoparticles suspension was placed on a 400 mesh carbon-coated copper grid. After drying, the samples were sputter-coated with a gold-palladium alloy and analyzed at an electron voltage of 5 kV.

2.2.6. Drug loading and encapsulation efficiencies

The method for determining the paclitaxel loading and encapsulation efficiency of PBCA nanoparticles was, according to the previous workers (Ruan and Feng, 2003). 6 mg of lyophilized nanoparticles were dissolved in 1 ml DCM and 6 ml acetonitrile/water (50/50, v/v) was then added and stirred under dry nitrogen stream to evaporate DCM at room temperature, and thus the paclitaxel payload in nanoparticles was determined. The resulting solution was filtered through 0.45 μm PTFE membrane filters. 20 μl of the filtered solution was injected into a high performance liquid chromatographic (HPLC) apparatus. The HPLC apparatus was equipped with a Waters 510 solvent delivery pump, a Luna C18 (2) column (5 μm, 250 mm × 4.6 mm, Phenomenex, USA) and a UV/VIS detector (Laballiance, USA), operated at a wavelength of 227 nm. The mobile phase was acetonitrile/water (50/50, v/v) and the flow rate was 1.0 ml/min. The concentration of drug in the solution was obtained from the calibration curve, which relates peak areas and concentrations. The curve is linear in the range of 50–50,000 ng/ml with a correlation coefficient of $R^2 = 1.0$. Results were expressed as the means of three measurements.

The recovery efficiency of this extraction procedure was examined using a known weight of paclitaxel: 0.03 to 0.3 mg, mixed with 6 mg of drug-free PBCA nanoparticles, and the procedure of extraction, described previously, was repeated. All the recoveries were approximately 95%, which reveal that approximate 95% of the original paclitaxel can be extracted by this procedure from the mixture of paclitaxel and PBCA nanoparticles. The loading efficiencies of paclitaxel in PBCA nanoparticles determined by this procedure of extraction were corrected accordingly.

The drug loading efficiency (L.E.) and drug encapsulation efficiency (E.E.) were defined as follows.

$$\text{Drug loading efficiency (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles}} \times 100$$

$$\text{Drug encapsulation efficiency (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of feed drug}} \times 100$$

2.2.7. X-ray powder (XRD) diffraction

XRD patterns of paclitaxel powder, PBCA nanoparticles and paclitaxel-loaded PBCA nanoparticles were obtained using a XDS 2000 diffractometer (Scintag, USA) with Seifert ID 3000 software. The scanning range of 2θ was from 5° to 40° and the

scanning rate was 1° 2θ/min with a step width of 0.02°. The X-ray source was CuKα radiation (40 kV, 35 mA).

2.2.8. In vitro release of paclitaxel

Two milligrams of lyophilized drug-loaded nanoparticles were redispersed in 10 ml of phosphate buffer solution (PBS, pH 7.4 containing 0.1%, w/v Tween 80) in a capped centrifuge tube. The tube was placed in a shaking incubator (120 cycles/min) at 37 °C. Tween 80 was used to increase the solubility of paclitaxel in the release medium and to reduce the association of the drug with the container surface. At predetermined times, the tube was centrifuged at 39,000 × *g* for 20 min. The collected particles were redispersed in 10 ml fresh PBS, containing Tween 80, for continuous release studies. The release of paclitaxel in the supernatant was extracted with 2 ml DCM and then 1 ml of acetonitrile/water (50/50, v/v) was added to the extract. After DCM was evaporated by a dry nitrogen stream, the drug concentration in the clear solution was analyzed by HPLC under the same analytic conditions as described above.

3. Results and discussion

3.1. Influence of synthetic method on paclitaxel-loaded nanoparticles

Particle sizes, polydispersities, zeta potentials and drug loading and encapsulation efficiencies of paclitaxel-loaded PBCA nanoparticles prepared from emulsion or miniemulsion, respectively, are present in Table 1. Nanoparticles prepared by the emulsion method were smaller and had a narrower size distribution, with lower drug loading and encapsulation efficiencies than those obtained by miniemulsion. These results revealed that miniemulsion polymerization was an effective method for encapsulating paclitaxel in PBCA nanoparticles. The paclitaxel-loaded PBCA nanoparticles prepared from the conventional emulsion process by encapsulating the pre-dissolved drug in the medium had encapsulation efficiency between 46 and 78.5% (w/w) with various kinds of surfactants (Mitra and Lin, 2003). However, low drug loading efficiency was expected because the amount of paclitaxel dissolved in the polymerization medium was too low for entrapment into the nanoparticles.

Fig. 1 presents the processes of miniemulsion (Antonietti and Landfester, 2002), emulsion and the conventional emulsion method (Guise et al., 1990) for preparing drug-loaded PBCA nanoparticles. In the oil-in-water miniemulsion technique, the drug-containing monomer was homogenized by high energy shear generated by ultrasonification, to obtain tiny monomer droplets with sizes of under 500 nm. Polymerization of these monomer droplets was initiated by the hydroxide ions captured from the medium, and then these monomer droplets were converted into drug-loaded particles. If Ostwald ripening and the coalescence between particles can be suppressed effectively, the drug dissolved in monomer can be completely encapsulated in PBCA nanoparticles by the miniemulsion polymerization method. In an emulsion polymerization system, the largest fraction of the monomer is dispersed in the form of monomer droplets and the principle reaction sites are located in the monomer-swollen micelles. As polymerization proceeds, the monomer molecules transport from the monomer droplets into the aqueous phase and subsequently into the reaction sites. The highly hydrophobic drug dissolved in the monomer droplets is almost insoluble in water; this makes drug molecules to diffuse out of monomer droplets with difficulty and therefore restricts the transport of drug from droplets to reaction sites. A large precipitate of the drug and appreciable aggregates are formed at the bottom of the reactor and on the stirrer. It is responsible for the low efficiency of the encapsulation of the drug in the PBCA nanoparticles obtained by the emulsion polymerization process. Also, the conventional emulsion method can not produce highly paclitaxel-loaded PBCA nanoparticles as well because only a limited amount of drug can be dissolved in the reaction medium (Vauthier et al., 2003a).

The zeta potential of PBCA nanoparticles prepared by emulsion polymerization was -0.3 ± 0.3 mV; the negative charges are significantly lower than the -19.3 ± 2.2 mV of the PBCA nanoparticles prepared by miniemulsion. The negative charges of PACA nanoparticles might originate mainly from the hydrolysis of the ester groups of PBCA (Müller et al., 1992) and the carbanions at the ends of the polymeric chains (Chouinard et al., 1994). Increase of the non-ionic surfactant on the particle surface would increase the distance between the shear surface and the particle surface, which resulted a decrease in the magnitude of the zeta potential (Duro et al., 1998). Thus, the lower magnitude of the zeta potential implied that greater amounts of non-ionic surfactant – pluronic F127 covered the particulate surface. Furthermore, non-ionic surfactants provide steric repulsion between the particles and reduce the surface tension of the particles. Therefore, the size of the PBCA nanoparticles prepared by the emulsion method is smaller than that of those prepared by the miniemulsion method. The drug-loaded PBCA nanoparticles prepared by miniemulsion polymerization had higher polydispersity index might be attributed to that Ostwald ripening was not suppressed effectively (Antonietti and Landfester, 2002). On the other hand, the higher zeta potential could increase the repulsion between particles and therefore decrease the particle size distribution. However, the influence of Ostwald ripening on particle size distribution might be larger than that of the zeta potential, which resulted an overall higher polydispersity. Similar phe-

Table 1
Effects of synthetic method on particle properties and drug loading efficiency

Method	Average diameter ± S.D. (nm)	Polydispersity	Zeta potential ± S.D. (mV)	$\overline{M_n}$ (g mol^{-1})	L.E. ± S.D. (%)	E.E. ± S.D. (%)
Emulsion[a]	56.2 ± 2.0	0.132	−0.3 ± 0.3	3488	0.18 ± 0.02	18.0 ± 2.0
Miniemulsion[a]	99.7 ± 4.4	0.248	−19.3 ± 2.2	3267	0.56 ± 0.04	56.6 ± 4.0

[a] Feed of monomer containing 1% (w/w) paclitaxel; surfactant concentration, 0.5 g/ml.

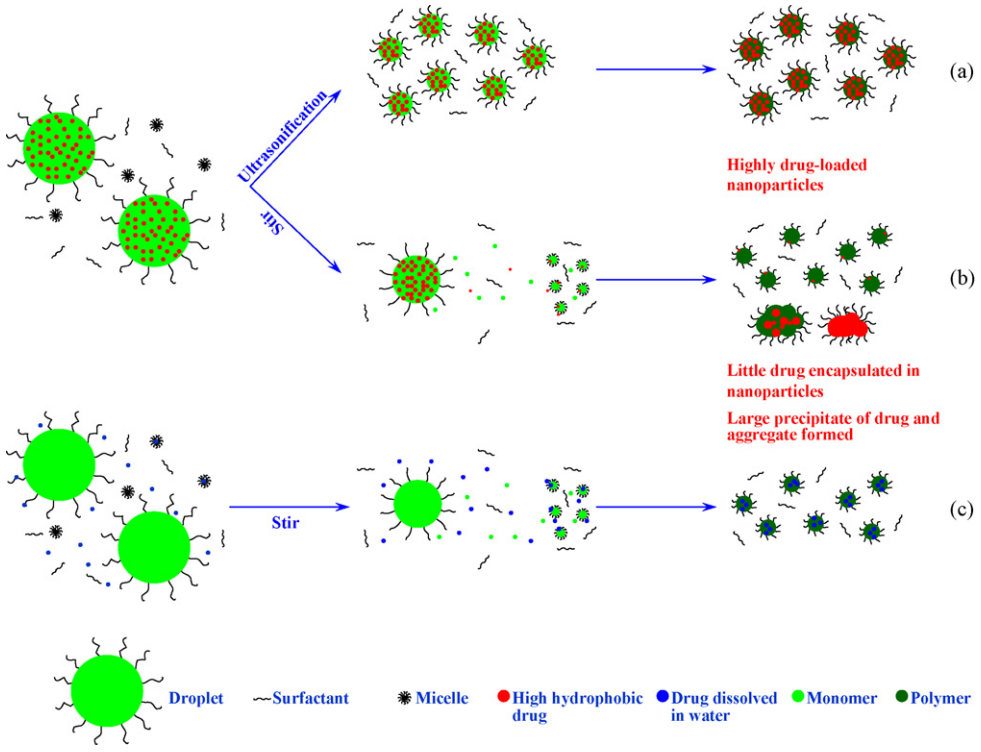

Fig. 1. Schematic illustration of process for encapsulating drug in PBCA nanoparticles by the methods of miniemulsion (a), emulsion polymerization (b) and the conventional emulsion polymerization (c).

nomenon was observed by Holzapfel (Holzapfel et al., 2005). He found that fluorescent carboxyl functionalized polystyrene particles with higher zeta potential prepared by miniemulsion also exhibit larger particle size distribution (PDI). It provides an evidence that the influence of Ostwald ripening on particle size distribution is stronger than that of electrostatics.

3.2. *Effect of the concentration of pluronic F127 on drug-loaded nanoparticles*

The effects of the concentration of pluronic F127 on particle size and polydispersity index of the paclitaxel-loaded PBCA nanoparticles prepared by miniemulsion are shown in Fig. 2. The particle size decreased from 183.4 to 99.7 nm and the size polydispersity index increased from 0.039 to 0.224 as the concentration of pluronic F127 increased from 0.03 to 0.5% (w/v). Similar results were reported for the miniemulsion of styrene using non-ionic surfactants of Lutensol AT50 and SE3030 (Bechthold et al., 2000). The stability of particle increases with increasing coverage of the surfaces of particles with surfactant. Therefore, the size of the particles decreases as the concentration of the surfactant increases. The width of the size distribution increases with the concentration of pluronic F127 perhaps because of excess surfactant (Kim et al., 2004) and the low efficiency of the suppressing of Ostwald ripening for paclitaxel.

Fig. 3 shows the effect of the concentration of pluronic F127 on paclitaxel loading and encapsulation efficiencies of PBCA nanoparticles prepared by miniemulsion at constant drug content in the monomer solution. The loading and encapsulation efficiencies increased with increasing surfactant concentration from 0.03 to 0.1% (w/v) and leveled off afterwards. At low surfactant concentration, the PBCA nanoparticles with porous surfaces would loss drugs from the surface into the suspension medium by diffusion (Kreuter, 1994). As the surfactant concentration increased, the porous surfaces of the PBCA nanoparticles gradually became smooth (Mitra and Lin, 2003), and therefore minimized the loss of drug during synthesis (Feng and Huang, 2001). Meanwhile, the particle size decreased as the surfactant concentration increased, resulting in an increase in the total particulate surface area, which promoted the total drug loss from the particles. These two opposing effects on drug entrapment compensated for each other and explained why the efficiency

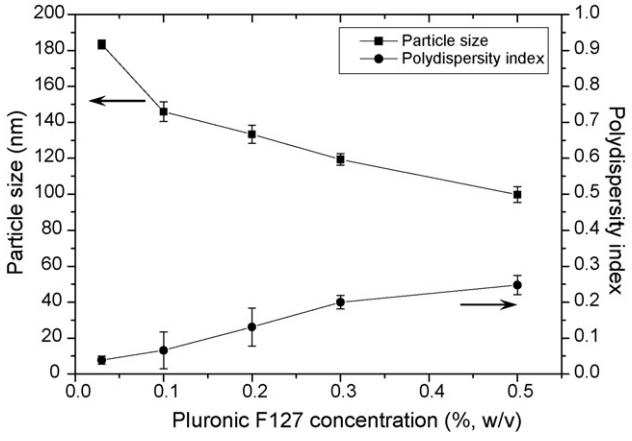

Fig. 2. Effect of pluronic F127 concentration on particle size and polydispersity index of paclitaxel-loaded PBCA nanoparticles.

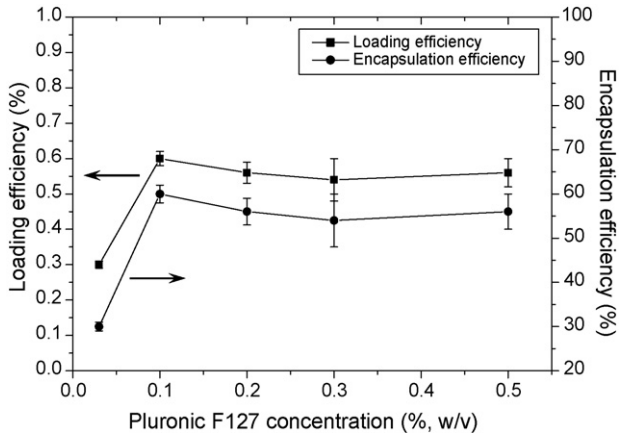

Fig. 3. Effect of pluronic F127 concentration on loading and encapsulation efficiency of paclitaxel-loaded PBCA nanoparticles (paclitaxel concent in feed monomer: 1%, w/w).

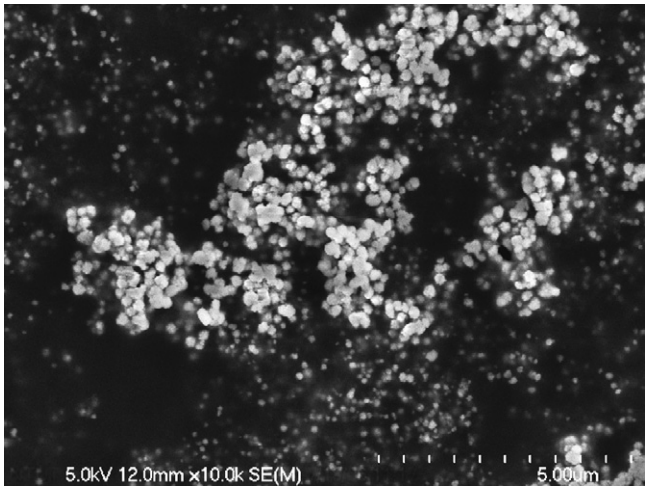

Fig. 4. FE-SEM picture of paclitaxel-loaded PBCA nanoparticles with 4.0% L.E. prepared by miniemulsion polymerization.

became almost constant as the concentration of the surfactant increased.

3.3. Content of paclitaxel in monomer

A stable, transparent monomer solution, containing 5% (w/w) paclitaxel, was obtained by heating it for several minutes at 60 °C. The FE-SEM photograph (Fig. 4) of the paclitaxel-loaded PBCA nanoparticles prepared by miniemulsion shows distinct spherical nanoparticles. As to miniemulsion, the content of paclitaxel in the monomer considerably affects the particle size, the size distribution, the zeta potential and the molecular weight

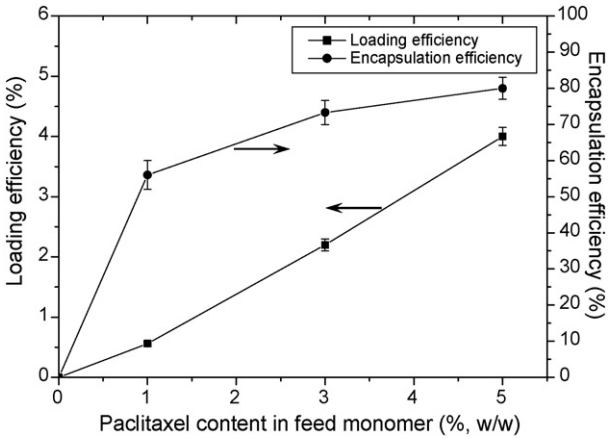

Fig. 5. The influence of paclitaxel content in feed monomer on loading and encapsulation efficiency of paclitaxel-loaded PBCA nanoparticles.

of PBCA as shown in Table 2. The value of negative charges on the particles were reduced from -33.9 ± 1.9 to -16.6 ± 2.4 mV as the paclitaxel content increased from 0 to 5% (w/w). The increase of paclitaxel in PBCA nanoparticles reduced the negative charges might be explained by the shielding effect of carboxylic groups by drugs molecules on the particle surface.

The stability of a miniemulsion depends on the nature and concentration of the hydrophobe. There are many kinds of molecules can be used as hydrophobes in miniemulsion, such as hexadecane, dye, comonomer, silanes, polymer and so on. More hydrophobic agent (solubility in water is less than 0.1 ppm) can be used as an effective osmotic agent to suppress Ostwald ripening in miniemulsion (Landfester et al., 1999). Considering the application of drug-loaded nanoparticles in biomedicine, classical non-biocompatible or toxic hydrophobes were not permissible. The work on the encapsulation of organic pigment in polymeric particles by the miniemulsion method demonstrated that the hydrophobic pigment dissolved in monomer, contributing to the suppression of Ostwald ripening by increasing the osmotic pressure of the droplets (Chern et al., 1998; Lelu et al., 2003). In order to stabilize the miniemulsion system, hydrophobe is required to build up an osmotic pressure in the droplets for resisting Ostwald ripening. Table 2 shows that the polydispersity index slightly decreases as the paclitaxel content increases, indicating that highly hydrophobic paclitaxel (solubility in water: approximately 0.3 ppm) possessed the function of hydrophobe to inhibit Ostwald ripening in the miniemulsion process of BCA.

Fig. 5 shows the influence of paclitaxel content in the feed monomer on the loading and encapsulation efficiencies of paclitaxel-loaded PBCA nanoparticles prepared by miniemul-

Table 2
Effect of paclitaxel content in feed of monomer on properties of paclitaxel-loaded PBCA nanoparticles

Paclitaxel content (%, w/w)	Average diameter ± S.D. (nm)	Polydispersity	Zeta potential ± S.D. (mV)	$\overline{M_n}$ (g mol^{-1})
0	92.8 ± 3.8	0.263	−33.9 ± 1.9	2135
1	99.7 ± 4.4	0.248	−19.3 ± 2.2	3267
3	95.8 ± 5.1	0.253	−19.0 ± 2.1	3330
5	104.6 ± 4.7	0.241	−16.6 ± 2.4	3314

sion with pluronic F127 as the stabilizer. A loading efficiency of over 4% (w/w) and an encapsulation efficiency of 80% (w/w) can be achieved simultaneously when the drug content in the feed monomer is 5% (w/w). The trend of the increase of loading and encapsulation efficiencies simultaneously is contrary to that of the drug-loaded PBCA nanoparticles prepared by conventional emulsion method (Illum et al., 1986). In the conventional emulsion method (process (c) in Fig. 1), in order to have high loading efficiency, large amount of feed drug is required, however, large amount of feed drug results in low encapsulation efficiency (Illum et al., 1986). If the stability of paclitaxel containing monomer droplets can be maintained during polymerization in miniemulsion system, consequently these monomer droplets convert into drug-loaded particles. While for the unstable droplets, the smaller droplets will disappear and paclitaxel contained in these droplets will precipitate during polymerization as previous description for incorporating hydrophobic dye in polymeric particles by miniemulsion (Chern et al., 1998). Highly hydrophobic paclitaxel functions as a hydrophobe in BCA monomer, an increase of paclitaxel content in the monomer increases the stability of the droplets. Therefore, paclitaxel-loaded PBCA nanoparticles with high loading and encapsulation efficiencies could be obtained simultaneously by miniemulsion.

3.4. X-ray powder diffraction

The XRD spectra of paclitaxel, PBCA nanoparticles, paclitaxel-loaded PBCA nanoparticles prepared by emulsion and miniemulsion with various drug loading efficiencies from 0.56 to 4% (w/w) are shown in Fig. 6. These data are useful in analyzing the degree of crystallinity of the drug in PBCA nanoparticles. Paclitaxel exhibited several intense peaks at $2\theta = 5.6^\circ$, 9.9° and 12.7°. However, these peaks were not observed in the XRD patterns from samples of paclitaxel-loaded nanoparticles. The intensity of the XRD peak depends on the crystal size. Therefore, the diffractograms of the paclitaxel-loaded PBCA nanoparticles indicated that the drug would be either molecularly dispersed in the polymers or distributed in an amorphous state or crystalline with very small size. A similar result was observed for oil-soluble crystalline dyes distributed in particles prepared by miniemulsion (Takasu et al., 2003).

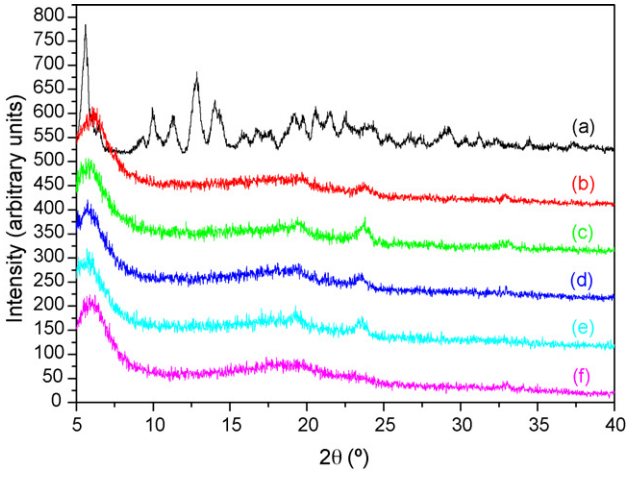

Fig. 6. X-ray powder diffraction patterns of: (a) paclitaxel, (b) PBCA nanoparticles, (c) paclitaxel-loaded PBCA nanoparticles prepared by emulsion method, (d) paclitaxel-loaded PBCA nanoparticles prepared by miniemulsion method, (e) paclitaxel-loaded PBCA nanoparticles with 2.2% L.E. prepared by miniemulsion method, and (f) paclitaxel-loaded PBCA nanoparticles with 4.0% L.E. prepared by miniemulsion method.

3.5. In vitro release of paclitaxel

The *in vitro* release profiles of paclitaxel from paclitaxel-loaded PBCA nanoparticles prepared by emulsion and miniemulsion methods into PBS (pH 7.4) solution are presented in Fig. 7. Paclitaxel-loaded nanoparticles prepared by emulsion exhibit an initial rapid release to 85.6% (w/w) during the first 10 h, followed by a slow release to 93.2% (w/w) until 96 h. However, paclitaxel-loaded PBCA nanoparticles prepared by miniemulsion method show less burst effect and slower release profiles compared with emulsion method. The percentages of drug released after 96 h are 89.7 and 82.9% (w/w) for paclitaxel-loaded PBCA nanoparticles with loading efficiency of 0.56 and 4% (w/w), respectively.

The release profile of paclitaxel-loaded PBCA nanoparticles prepared by the emulsion method is similar to that of the drug-loaded nanoparticles prepared by conventional emulsion process (Mitra and Lin, 2003), which has a biphasic profile with an initial rapid release phase followed by a slower release phase. The initial rapid release phase indicates that some of the drug is on and near the surface of the particle, the second slow release phase might be caused by the release of the drug from the inner core of the particle. On the other hand, the release profile of paclitaxel-loaded PBCA nanoparticles prepared by miniemulsion show a less burst release which might indicate a larger fraction of paclitaxel distributed in the nanoparticles.

The slower release rate for the paclitaxel-loaded nanoparticles with larger amounts of drug loading shown in Fig. 7 is similar to the previous study on the release profile of rose

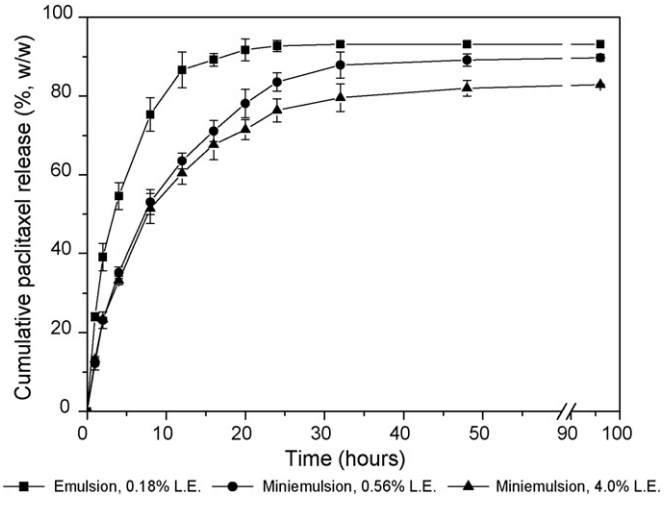

Fig. 7. *In vitro* cumulative paclitaxel release (mean ± S.D., $n = 3$) profile for paclitaxel-loaded PBCA nanoparticles prepared by emulsion and miniemulsion methods with drug loading efficiencies of 0.18, 0.56 and 4% (w/w).

bengal-loaded PBCA nanoparticles (Illum et al., 1986). The drug release rate for drug-loaded PBCA particles depends considerably on the hydrolytic rate of the polymeric matrix. It has been shown that the longer hydrophobic alkyl side chains would shield effectively against the hydroxyl ions to attack the ester groups of PACA and therefore decrease the hydrolysis rate of PACA (Huang and Lee, 2006). Accordingly, larger content of paclitaxel in PBCA nanoparticles would also increase the barrier for water and hydroxyl ions to penetrate and degrade PBCA polymer, which leads to the decrease of the hydrolytic rate of drug-loaded particles as well. Hence, the relative paclitaxel release rate decreases with increasing paclitaxel content in particles.

4. Conclusion

This study demonstrated the feasibility of encapsulating highly hydrophobic paclitaxel in PBCA nanoparticles by the miniemulsion process using pluronic F127 as a surfactant. Nanoparticles with high drug loading and encapsulation efficiencies could be obtained by miniemulsion, while emulsion polymerization provided nanoparticles with limited loading efficiency. The hydrophobic drug of paclitaxel can be used as a hydrophobe to suppress the effect of Ostwald ripening so to yield stable nanoparticles with a diameter of about 100 nm.

Data present also reveal that the surfactant concentration in miniemulsion has the effect on the particle size, the size distribution, the surface charge and the drug loading and encapsulation efficiency. XRD study indicates that paclitaxel encapsulated in PBCA nanoparticles is either molecularly dispersed in the polymers, distributed in an amorphous state or distributed in a crystalline state with crystal size too small to be detected. *In vitro* drug release reveals that the drug-loaded nanoparticles prepared by miniemulsion might contain more drug distributed in the polymeric matrix compared with those prepared by emulsion and the release rate decreases with increasing paclitaxel content in the particle.

The results of this study have demonstrated that the miniemulsion method can be feasibly employed to entrap highly hydrophobic drug of paclitaxel into PBCA nanoparticles with a high loading and encapsulation efficiency. This strategy can also be applied to encapsulate other hydrophobic drugs which cannot react with cyanoacrylate monomer, and to yield drug-loaded nanoparticles with high entrapment efficiency.

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