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Core-shell type of nanoparticles composed of poly[(*n*-butyl cyanoacrylate)-*co*-(2-octyl cyanoacrylate)] copolymers for drug delivery application: Synthesis, characterization and *in vitro* degradation

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Abstract

Core-shell type of nanoparticles (NPs) with manipulated degradation rate and balanced hydrophilic/hydrophobic properties were designed and characterized. The NPs based on the copolymers of *n*-butyl cyanoacrylate (BCA) and 2-octyl cyanoacrylate (OCA) were prepared by anion emulsion polymerization in 0.01N HCl solution with pluronic F127 as the stabilizer. These NPs were spherical in shape and with size smaller than 100 nm in a narrow distribution. The particle size, zeta potential, molecular weight, hydrophobicity and degradation rate of the copolymer depended on its composition significantly. *In vitro* chemical hydrolytic studies indicated that the degradation rate of the NPs could be controlled over 200-fold by adjusting the BCA/OCA ratio. Differential scanning calorimetry (DSC) measurements verified the existence of copolymer with tapered structure which was induced by the reactivity difference of the monomers. A BCA/OCA core-shell structure is postulated that the OCA rich segments were mainly located in the core of the NPs. The cytotoxicity of poly(2-octyl cyanoactylate) (POCA) is quite lower than that of poly(*n*-butyl cyanoacrylate) (PBCA) and the toxicity of poly(BCA-*co*-OCA) NPs is similar to that of PBCA NPs.

Keywords: Core-shell type nanoparticles; Drug delivery carrier; Cyanoacrylate; Hydrolysis

1. Introduction

Biodegradable polymeric nanoparticles (NPs) have become important carriers for various drugs, peptides and gene delivery. The active agent encapsulated in the NPs tends to have higher biological stability, thereby, increasing the therapeutic efficiency and reducing the associated side effects. With appropriate design of carrier material (e.g. composition and surface properties), these NPs may possess higher capacity of active agent protecting from degradation and reduce premature elimination so as to reach the target in a controlled manner (Davis and Illum, 1983; Kreuter, 1991; Soppimath et al., 2001). Biodegradable polymers based on poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactide-*co*-glycolide) (PLGA) (Brannon-Peppas, 1995), poly(ε-caprolactone) (PCL) (Shenoy and Amiji, 2005) and poly(alkyl cyanoacrylate) (PACA) (Vauthier et al., 2003b) have been studied extensively as the carrier and have succeeded in development of the nanoparticulate formulations for intravenous administration.

PACA has been used as drug carrier for parenteral administation and tissue glue in surgery. The NPs composed of PACA (Couvreur et al., 1979b) were first prepared as the drug carrier by anion initiated emulsion polymerization in acidic water solution containing stabilizer (Couvreur et al., 1979a). Thus prepared NPs had advantages as easy preparation, high utility size ranges, stable and non-solvent residues. Moreover, PACA NPs have the ability to absorb or encapsulate a wide range of drugs, such as insulin (Damge et al., 1990; Behan and Birkinshaw, 2001b), pilocarpine (Wood et al., 1985), vaccines (Kreuter, 1988), oligonucleotide delivery (Nakada et al., 1996) and anti-tumor drugs (Vauthier et al., 2003a). Researches prove that PACA NPs are quite satisfied to be the drug delivery carriers and have already entered the developing stage of human body's clinical experiment for cancer therapy (Kattan et al., 1992; Vauthier et al.,

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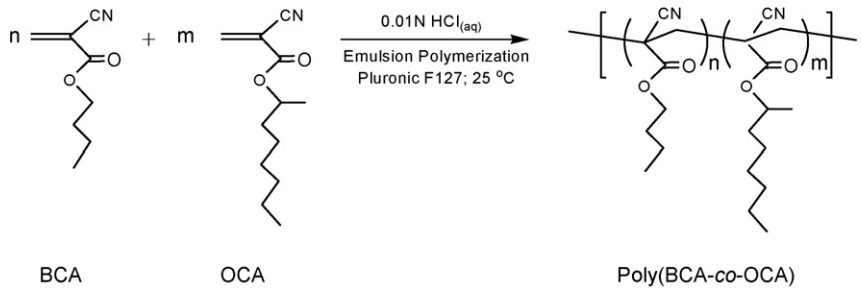

Scheme 1. Synthesis of poly(BCA-*co*-OCA) NPs.

2003b). The more stimulated and particular finding was that the drug-loaded PACA NPs coated with polysorbates with more than 20 polyoxyethylene units have overcome the barrier of blood–brain barrier to transport drug into the brain (Kreuter, 2001). However, the utility of PACA NPs is still limited because the controls of compatibility and release rate for various drugs are difficult with the structure of a homopolymer.

Degradation is the process of chain scission, whereas erosion is the process of material loss and bioerodiable means a biological system is involved in the kinetic of the process. In the initial study of chemical degradation of PACA indicated that formaldehyde and cyanoacetate were produced from disconnecting the polymer chain during hydrolysis (Leonard et al., 1966; Vezin and Florence, 1980). Since the mechanism of producing formaldehyde in the degradation was hard to explain some results of the studies, more complicated mechanisms were proposed. For example, by analyzing the concentration of isobutanol and formaldehyde in the degradation of poly(isobutyl cyanoacrylate) NPs under alkaline medium or enzyme, Lenaerts et al. proved that the main degradation route consisted of hydrolyzing the ester groups and only few formaldehyde produced (Lenaerts et al., 1984).

The degradation rate of PACA NPs is fast in comparison with other biodegradable polymers such as PLA, PLGA, etc. In general, the degradation rate depends on several factors: such as particle size (Vezin and Florence, 1980), the ester chain length (Leonard et al., 1966; Müller et al., 1992), molecular weight of polymer (Vezin and Florence, 1980), pH of medium (Vezin and Florence, 1980; Lenaerts et al., 1984) and enzyme used (Lenaerts et al., 1984; Scherer et al., 1994; O'Sullivan and Birkinshaw, 2002). While the major mechanism of drug release from the PACA NPs is bioerosion, its drug release rate depends on the PACA hydrolytic rate (Lenaerts et al., 1984; Page-Clisson et al., 1998; O'Sullivan and Birkinshaw, 2004) as well.

Although different PACA NPs synthesized by anion emulsion polymerization have been studied, the degradation rate of PACA homopolymer still cannot be controlled in a wide range. Further, the kinds of drugs encapsulated and the loading/encapsulation efficiency may also be limited. In the present study, monomers of BCA and OCA were used for the synthesis of copolymers of poly(BCA-*co*-OCA) as the matrix materials for NPs. Such NPs tend to have the modulability for degradation rate while their controllable hydrophilic/hydrophobic properties may provide various compatibilities with different kinds of hydrophobic drugs. The NPs with different BCA/OCA ratios were prepared as shown in Scheme 1.

2. Materials and methods

2.1. Materials

The monomers of *n*-butyl cyanoacrylate and 2-octyl cyanoacrylate of 99.5% purity were obtained from Tongshen Enterprise Co., Ltd., Kaohsiung, Taiwan and were used as received. Pluronic F127 [poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer; MW 12,600 Da], 2-octanol, fetal bovine serum (FBS), penicillin, streptomycin, Dulbecco's modified Eagle medium (DMEM) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (USA). *N*-Butanol, *n*-hexanol were purchased from Fisher chemical (USA) and Tedia (USA) respectively and used as received. Hydrochloric acid (0.1N) and dimethylsufoxide were obtained from Merck (Germany). All solvents used were of analytical grade. The water used was purified by reverse osmosis (Milli-Q, Millipore).

2.2. Methods

2.2.1. Nanoparticle preparation

The NPs of PBCA, POCA and poly(BCA-*co*-OCA) with compositions of BCA/OCA: 75/25, 50/50 and 25/75% (w/w) were prepared individually by emulsion polymerization technique (Couvreur et al., 1979a). About 0.5 g of monomer or monomers mixture was added drop by drop to a 50 ml aqueous solution of 0.01N hydrochloric acid containing 0.3% (w/v) of pluronic F127 under stirring with a magnetic stirrer. The mixtures were stirred for 18 h in a 100 ml double-walled round-bottomed reactor surrounded by a thermostated water bath set at 25 °C. Then a solution of 1N sodium hydroxide solution was added to neutralize the suspension and allowed to stir for another 30 min. The NPs formed were separated by ultracentrifugation at 60,000 × *g* for 60 min (CP 100MX, Hitachi, Japan) and redispersed again in water and lyophilized.

Freeze-dried NPs were dissolved in acetone (20 mg/ml). Then water was added steadily until the color of the solution changed to milky white. The copolymer was collected by centrifugation at 10,000 × *g* for 10 min. The purified copolymer was obtained by repeating this procedure three times and collected for charac-

terization. The cytotoxicity studies were performed using stock NPs suspensions sterilized by filtration through sterile 0.22 μm membrane filters (Millipore®).

2.2.2. Scanning electron microscopy (SEM) studies

The morphology of NPs was observed by field emission scanning electron microscopy (FESEM; Hitachi S-4700). A drop of the diluted NPs suspension was placed on a 400 mesh carbon-coated copper grid. After drying, the samples were observed at 15 kV.

2.2.3. Particle size analysis

The particle size and the zeta potential of NPs were measured by photon correlation spectroscopy (PCS; Zetasizer 3000, Malvern Instruments, Malvern, UK) at 25 °C. The scattered light of wavelength 633 nm was detected at an angle of 90°. The NPs dispersion was diluted with water to an adequate concentration for better measurement. The mean hydrodynamic particle size was expressed as the value of *z*-average size. The width of the size distribution was indicated by polydispersity index (PI).

2.2.4. Copolymer composition

^{1}H NMR (Varian Unity INOVA 500 MHz NMR, Darmstadt, Germany) spectra were obtained for purified neat polymers as well as copolymers in $CDCl_3$. The overall copolymer compositions were determined by comparing the integrated proton signal due to the two methylene protons of the BCA residues at δ4.3 with that of the one methane proton due to the OCA residues at δ5.0.

2.2.5. Molecular weight

The molecular weights of BCA-*co*-OCA copolymers were determined by gel permeation chromatography (GPC) equipped with a Waters 510 pump, 50, 10-3 and 10-4 Å Phenogel columns serially set (Phenomenex, USA) and a Waters 410 differential refractometer. The mobile phase was tetrahydrofuran (THF) at a flow rate of 1.0 ml/min. The purified copolymer was dissolved in THF to the concentration of 2% (w/v), then injected 50 μl to the system. Polystyrene standards of molecular weight between 1.3 and 377.4 kDa were used for the construction of calibration curve.

2.2.6. Differential scanning calorimeteric (DSC) analysis

The DSC measurements were performed on DSC2920, TA Instrument, USA. Polymer sample of 8–10 mg was sealed in hermetical aluminum pan. Heat flow was calibrated with the melting transition of indium. Temperature calibration was carried out with cyclohexane and dodecane. The thermograms were obtained from the second run after heating to melt, quenching with liquid nitrogen and reheating from −30 to 130 °C at a heating rate 5 °C/min under nitrogen (50 ml/min). The T_g was noted at the inflection point of the heat capacity jump.

2.2.7. Contact angle measurement

Contact angle measurements were done on contact angle meter (FACE CA-D, Kyowa Kaimenkagaku, Japan) at 25 °C. Purified polymers were dissolved in THF to the concentration of 2% (w/v) and spin-coated at 5000 rpm for 1 min on a cover glass and dried in air. One droplet (25 μl) of deionized water from a micrometric syringe was placed on the substrate and the contact angles on both sides of the drop image were measured at least for three liquid drops. The values were the means of these measurements within the angles of ±3°.

2.2.8. Chemical degradation of nanoparticles

Lyophilized NPs were redispersed in the phosphate buffer solution (PBS; pH 7.4) at a concentration of 2 mg/ml and placed in a shaking incubator (60 cycles/min) at 37 °C. At different time intervals, the NPs were separated from dispersion media by ultracentrifugation (CP 100MX, Hitachi, Japan) at 60,000 × *g* for 60 min. A 5 ml sample of supernatant was withdrawn from the dispersion, and mixed with 1 μl *n*-hexanol (internal standard). This solution was extracted by 2.5 ml of diethyl ether which was then injected into gas chromatography (HP 5890, USA) coupled with a mass spectrometer (HP 5972, USA). The GC column used was a DB-5MS (30 m × 0.25 mm i.d. and a film thickness 0.25 μm) capillary column. Carrier gas was helium at a flow rate of 1.5 ml/min. The oven temperature was ramped from 40 to 300 °C and held for 3 min. The temperature of the injector and detector were set at 220 and 280 °C, respectively.

The concentration of *n*-butanol and 2-octanol were determined from the calibration curves. Standard solutions of 0.003, 0.01, 0.05, 0.1 and 0.2 mg/ml of *n*-butanol and 2-octanol were prepared separately for calibration. The peak area ratios (*n*-butanol/*n*-hexanol and 2-octanol/*n*-hexanol) were analyzed from chromatographic patterns against the concentration of the respective calibration standards. The least-square linear regression was used to analyze data.

2.2.9. Cytotoxicity studies

The evaluation of the *in vitro* cytotoxicity of the NPs was carried out on human foreskin fibroblastic HS 68 cells viability for 3 days using the MTT assay (Mosmann, 1983). Sterile NPs suspensions were diluted with DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (10^4 IU/ml) to the concentration of 10 μg/ml. Fibroblastic cells were seeded at a density of 5×10^4 cells/well in a 24-well plate, and grew for 24 h. Thereafter, the cells were washed with PBS (pH 7.4) and incubated with diluted NPs suspension for 3 days at 37 °C and 5% (v/v) CO_2. After incubation, the upper medium was carefully removed and the cells were washed twice with PBS. Then, 1 ml MTT solution (0.5 mg/ml) was added to each well and allowed incubation for another 4 h. The intracellular blue formazon salt metabolizing the MTT by live cells was dissolved by adding 1 ml dimethylsufoxide and the absorbance values were measured by a multiwell microplate reader (SUNRISE TS, TECAN) at a wavelength of 570 nm. The relative cell viability (%) related to the control well containing cell culture medium without NPs was calculated by the following equation:

$$\text{relative cell viability (\%)} = \frac{[\text{absorbance}]_{\text{test}}}{[\text{absorbance}]_{\text{control}}} \times 100$$

Table 1
Results of particle size, polydispersity index (PI) and zeta potential of PBCA, POCA and poly(BCA-*co*-OCA) copolymeric NPs

Sl. no.	Feed composition (wt.%)		Copolymer composition[a] (wt.%)		Particle size (nm)	PI	Zeta potential (mV)
	BCA	OCA	BCA	OCA			
1	100.0	0.0	100.0	0.0	74.3 ± 1.0	0.121	−21.6 ± 0.6
2	75.0	25.0	76.9	23.1	83.8 ± 3.1	0.104	−25.0 ± 0.1
3	50.0	50.0	51.1	48.9	89.6 ± 3.4	0.103	−24.9 ± 2.1
4	25.0	75.0	25.1	74.9	94.5 ± 0.6	0.082	−25.2 ± 1.3
5	0.0	100.0	0.0	100.0	98.1 ± 0.3	0.076	−27.7 ± 0.5

[a] Determined by ^{1}H NMR.

3. Results and discussion

3.1. Nanoparticle properties

In order to prepare the poly(BCA-*co*-OCA) NPs, respective monomers were mixed well and dispersed into polymerization media. During polymerization process, monomers reacted with each other to form copolymer as shown in Scheme 1. Feed compositions and characterization data of NPs are shown in Table 1. The weight ratio of BCA/OCA in the feed was varied from 100/0 to 0/100. The emulsion polymerization appeared stable and the levels of coagulation and precipitation were consistently very low with the help of surfactant pluronic F127.

The prepared NPs had spherical shape with narrow distribution. The SEM photograph of NPs is shown in Fig. 1 and it does not show any aggregation. The size of NPs increased from 74.3 to 98.1 nm and their PI decreased from 0.121 to 0.076 with increasing weight content of OCA in the feed as shown in Table 1. These results agree with the conclusion that the polarity of the polymer affects the stability and nucleation mechanism for latex synthesized in the presence of emulsifier (Aslamazova, 1995). The polarity of the polymer at the interface between solid and liquid causes the surface interactions. This interaction can decrease the surface energy and increase the stability of latexes. When the latexes have enough stability, the particle size is decreased. Therefore, the size of NPs increases as the content of OCA increases because the surface hydrophobicity of NPs increases.

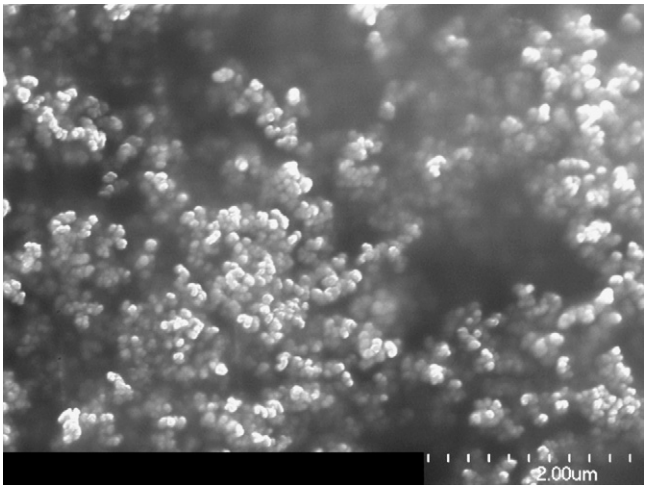

Fig. 1. SEM picture of POCA NPs.

If the monomer is slightly soluble in water, the initiation stage starts partially in the aqueous phase but not entirely in the emulsifier micelles (Aslamazova, 1995). The oligomeric radicals formatted from aqueous phase can react with other monomeric molecules dissolved in water and precipitate as a critical molecular length is reach or flocculate with other soluble oligomer radicals to format the primary particles. The mechanism of particle nucleation by the reaction mentioned above may increase the particle size distribution. The postulated particle formation mechanism of PBCA considered that the water-soluble oligomers generated in the droplets diffused out of the particles to water phase and then formed the primary particles (Behan et al., 2001a; Limouzin et al., 2003). Both the monomer and oligomer of OCA are difficult to be dissolved in aqueous phase, so the PI of the particles decreases with increasing weight content of OCA.

Zeta potential is the magnitude of the electrostatic potential at the plane of shear and is an important index to evaluate the stability of the particles. The zeta potential of all NPs exhibited a negative charge with value varied from −21.6 to −27.7 mV. The negative charges arise mainly from hydrolysis of the ester groups (Müller et al., 1992) and the carbanions at the ends of the polymeric chains (Chouinard et al., 1994). Furthermore, the negative charges increased slightly as the content of OCA increased. This phenomenon may be attributed to that the hydrophilic groups with negative charges connected on a more flexible polymeric chain are easier to migrate to the surface of the particles.

The overall copolymer compositions were estimated by NMR spectra and these data are also shown in Table 1. It was found that all compositions of copolymers calculated from NMR analysis agree with feed compositions well.

The molecular weights of BCA, OCA and copolymeric NPs were determined by GPC measurements. All samples showed a single and slightly broad distribution curve. The average number molecular weight ($\bar{M}_n$) increased with increasing OCA content as shown in Fig. 2 while the $\bar{M}_n$ of PBCA and POCA were 2913 and 6137 g/mol, respectively.

3.2. Thermal analysis

Fig. 3a shows the DSC curves of the copolymers with different compositions. The values of T_g shifted from 85.8 to 39.8 °C

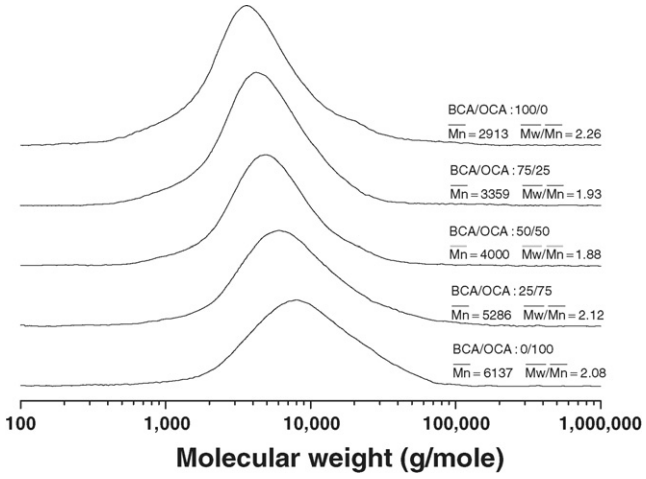

Fig. 2. GPC curves for PBCA, POCA and poly(BCA-*co*-OCA) at varying BCA/OCA weight composition.

as the weight content of OCA increased from 0 to 100%. The decrease of T_g with respect to increasing OCA content is due to the flexibility of the OCA segments. Only one T_g was observed in every copolymer. The variation of T_g with copolymer composition is linear as shown in Fig. 3b and obeys the Fox's law equation:

$$\frac{1}{T_g} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}} \quad (1)$$

where T_g is the glass transition temperature of the copolymer, T_{g1} and T_{g2} are glass transition temperatures of the homopolymers 1 and 2, respectively, and w is the weight fraction of the monomer in the copolymer. This relationship suggests a random structure for the copolymer. Nevertheless, the transition temperature intervals of these copolymers are broad indicating a possible gradient composition (Rivera et al., 2005). The reactivity of OCA is lower than that of BCA because of its longer alkyl chain which decreases the ability of electron-withdrawing (Henry, 1986). According to the theory of anionic copolymerization for the monomers with different reactivity (Odian, 2004), the composition of the copolymer chain is richer for the monomer BCA with higher reactivity in the initial stage of reaction and will gradually shift toward OCA richer composition with the macromolecules growing progressively, but the final composition is the same with the feed ratio if the conversion is completed.

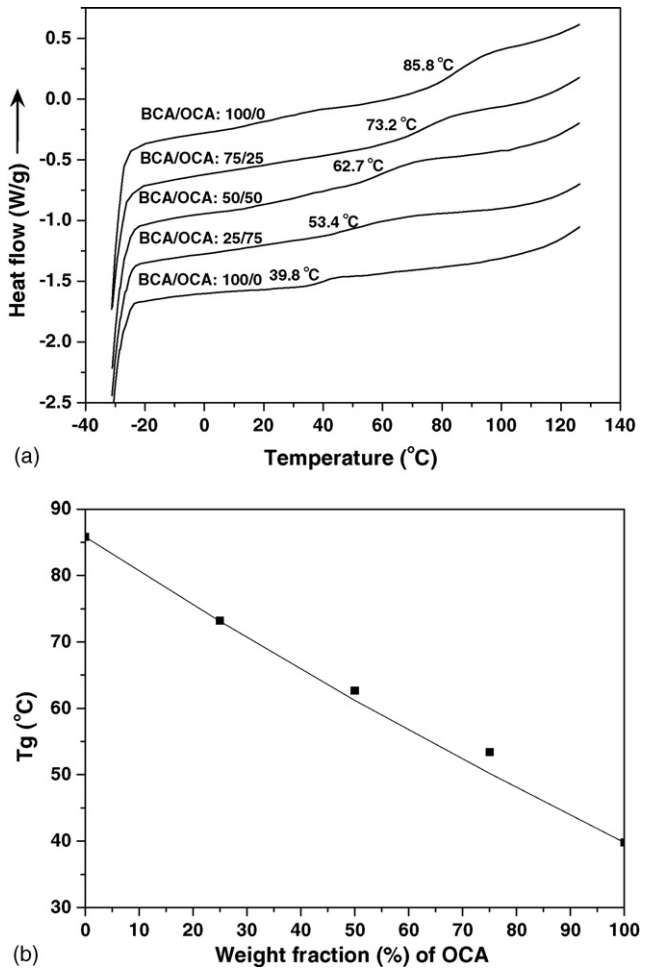

Fig. 3. (a) DSC thermographs for poly(BCA-*co*-OCA) at varying BCA/OCA weight composition. (b) Effect of OCA weight fraction on glass transition temperature (T_g) of poly(BCA-*co*-OCA). Block line: prediction from Fox's law equation; (■) experimental values.

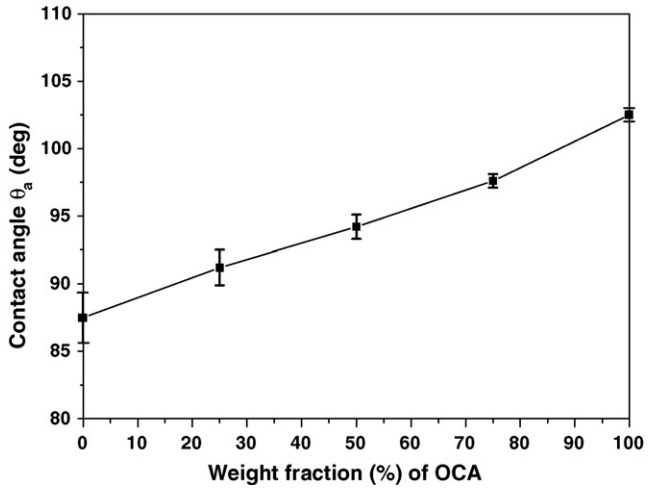

Fig. 4. Effect of weight fraction (%) of OCA on contact angles (mean ± S.D., $n = 5$).

3.3. Contact angle measurements

It is desirable to have polymer with balanced hydrophilic/hydrophobic properties so as to get better loading efficiency, storage stability and release characteristics as drug carrier. Such properties are closely related to the solid-state solubility of the drug in polymer. The hydrophobicity of polymer can usually be represented by contact angle and is shown in Fig. 4. The contact angle of water increases from 87.5° to 102.5° with the weight fraction of OCA increases from 0 to 100% in the copolymer. This tendency could be explained that the polar backbone was shielded by the alkyl side chain which diminished the surface tension therefore the hydrophobicity of the copolymer increased as the length and size of the alkyl chain increased (Grundke et al., 2001). Hence, the hydrophobicity of the copolymer can be controlled by selecting the composition properly.

3.4. Chemical degradation of nanoparticles

The rate of chemical degradation can be modulated by adjusting the monomer structure or monomer composition of the nanoparticles. For instance, the hydrolytic rate of PGA is faster than that of PLA, decreasing the PGA content in PLGA results in a prolonged degradation process (Asano et al., 1989). The alkyl cyanoacrylate analogue, OCA approved for skin closure is claimed to be less toxic and more flexible than BCA (Hollander and Singer, 1999). Furthermore, it can also be expected that POCA is a more hydrophobic polymer with slower degradation rate in comparison with PBCA because it has longer alkyl unit.

The degradation rates of NPs in PBS (pH 7.4) at 37 °C are significantly dependent on the content of OCA in the copolymer as present in Fig. 5a. About 8.5% of the ester groups hydrolyzed for PBCA NPs after 15 days and the degree of hydrolysis decreased with increasing OCA content in the copolymer. The value of degradation rate is 0.048% for POCA NPs after 15 days, it is much lower than that of PBCA. This result shows the presence of degradation pathway in the hydrolysis of the ester function (Lenaerts et al., 1984) for POCA NPs and agrees with the trend reported earlier for PACA homopolymer with different length of alkyl chain (Leonard et al., 1966). Since the longer alkyl side chains make them to shield effectively against the hydroxyl ions attack on the ester groups. Thus, the hydrolytic rate of poly(BCA-*co*-OCA) can be controlled in a wide range by varying the content of OCA in the copolymer.

The concentration of butanol and 2-octanol produced from hydrolyzed NPs are shown in Fig. 5b and c, respectively. It is significant that the total amounts of alkanol resulted from NPs degradation are contributed mainly from butanol. The relationships between the butanol produced and degradation time were nearly linear for NPs of PBCA and poly(BCA-*co*-OCA). Furthermore, 2-octanol was found only in copolymer with BCA/OCA composition of 25/75% (w/w) and pure POCA hydrolyzed after 15 days. Since BCA occupied most of shell when the BCA content is greater than 50% (w/w) and the mechanism of hydrolysis for PACA NPs is a surface degradation process (Müller et al., 1992; O'Sullivan and Birkinshaw, 2002). The hydrolysis of OCA was so slow (Fig. 5c) that the degradation product of OCA, 2-octanol, was only detectable for copolymer with higher OCA content. Data shown in Fig. 5 also verified a core-shell structure for the copolymer NP with BCA rich in the shell.

3.5. Proposed particles structure

The reactivity of BCA is larger than that of OCA, hence the chains of oligomer are expected to be rich in BCA at the initial stage while the copolymer chains are rich in OCA at the final stage of the polymerization. The consequence of a tapered structure for the copolymer of poly(BCA-*co*-OCA) (Rivera et al., 2005) and the linear plot of butanol concentration in NPs hydrolysis process indicate that BCA is rich in the shell layer of the particles while OCA is in the core. The proposed particle formation, structure and hydrolysis are shown in Fig. 6.

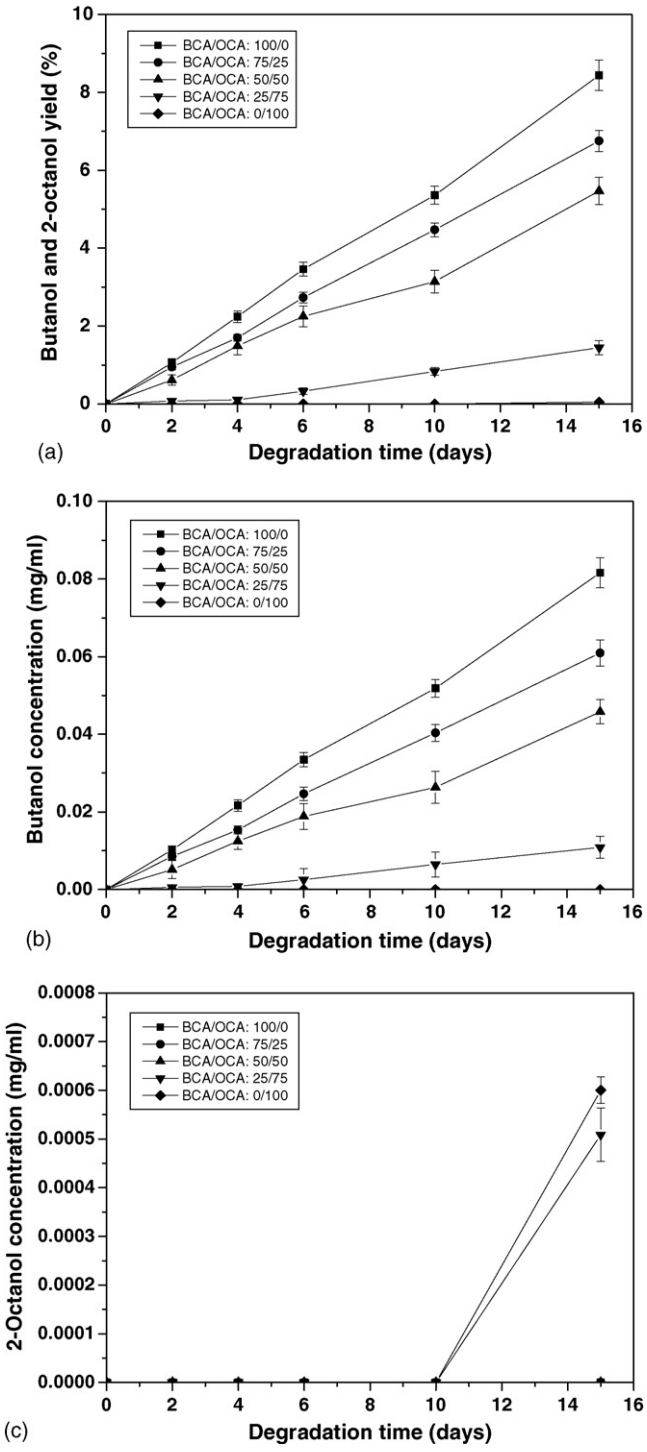

Fig. 5. Chemical degradation of poly(BCA-*co*-OCA) NPs in PBS (pH 7.4) at 37 °C: (a) butanol and 2-octanol yield (mean ± S.D., $n = 3$) presented as a molar percent of the total BCA and OCA units initially present in the NPs; (b) butanol concentration (mean ± S.D., $n = 3$); (c) 2-octanol concentration (mean ± S.D., $n = 3$).

Cyanoacrylate monomers are extremely reactive and the anionic initiation polymerization can take place even in acid aqueous (Behan et al., 2001a). Among the monomers used, BCA is highly reactive and has higher solubility in water in comparison to OCA. Thus OH^- anions react mostly with BCA in the

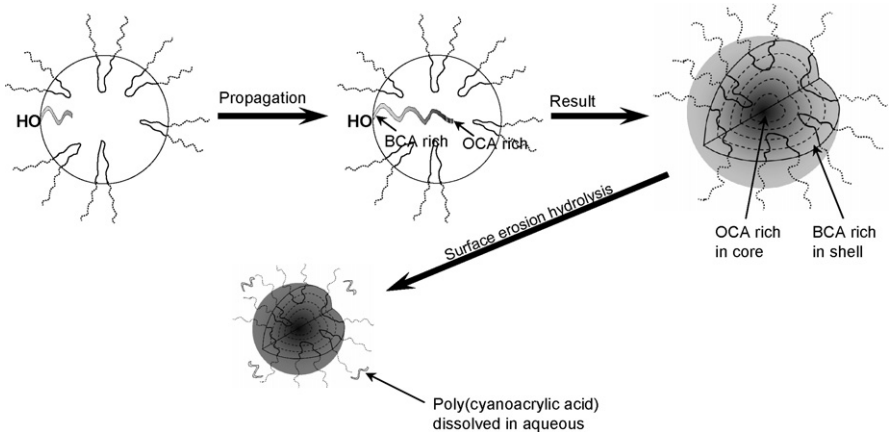

Fig. 6. Schematic representation of the proposed NPs formation, morphology and hydrolysis.

stage of initiation reaction. Due to the hydrophilic nature, the OH functional groups stay at the water-particles interface. This induces the tapered structure of poly(BCA-*co*-OCA) chain thus BCA rich segment is outwards and OCA rich segment is inwards in the NPs as shown in Fig. 6. The core-shell type structure observed in this research also coincides with previous studies that particles prepared by emulsion copolymerization may form similar structure depending on the polymerization conditions, the hydrophilic/hydrophobic properties and the reactivity ratio of the monomers (Aymonier et al., 2001).

3.6. Cytotoxicity studies

The in vitro cytotoxicities of PBCA, POCA and poly(BCA-*co*-OCA) NPs were tested with human foreskin fibroblasts by the MTT assay at the concentration of 10 μg/ml. Fig. 7 shows that the cell viability is unchanged for POCA NPs but similar reductions are observed for PBCA and poly(BCA-*co*-OCA) NPs.

POCA NPs do not affect the viability and proliferation of human foreskin fibroblasts indicating their low cytotoxicity. On the other hand, the toxicities of these copolymer NPs are close to that of PBCA even though the OCA content in the copolymer is varied. The observed cytotoxicity for PACA composed of degradation products in the culture medium and the local concentration of toxicity product rising from particles adhering to cell membranes (Lherm et al., 1992). As reported in the literature (Zimmer and Kreuter, 1995), polycyanoacrylate NPs with longer alkyl chains exhibit better bioadhesion, which result higher local concentration of toxicity product. Therefore, poly(BCA-*co*-OCA) NPs with higher OCA content would have higher local concentration of toxicity product due to their better bioadhesion. On the other hand, as shown in Fig. 5, the degradation rate decreases with increasing OCA content in the poly(BCA-*co*-OCA) NPs. Since the apparent cytotoxicity contains both the degradation product and the toxicity product from NPs adhering to cell membranes, these two effects seem to compensate with each other and the apparent cytotoxicity remains the same for the copolymer NPs.

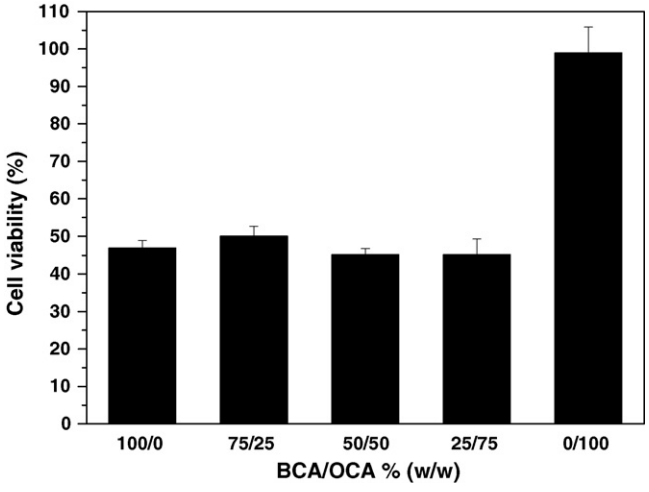

Fig. 7. *In vitro* cytotoxicity (mean ± S.D., $n = 3$) for poly(BCA-*co*-OCA) NPs at varying BCA/OCA (w/w) composition at the concentration of 10 μg/ml.

There are some disputes about the use of PACA as drug carriers, due to potential adverse tissue reactions and cytotoxicity effects (Ciapetti et al., 1994; Zange and Kissel, 1997). Studies suggest that PACA NPs would degrade slowly enough to prevent high local concentration of degradation products which could not be metabolized effectively (Lherm et al., 1992). Therefore, low toxicity of POCA NPs might be more suitable for drug carrier and chronic delivery of drugs.

4. Conclusion

This study has shown the feasibility of preparing the core-shell type of NPs for drug carrier. All the sizes of particles were less than 100 nm with narrow size distribution. The copolymers were taper-like structure as evidenced by DSC study. The hydrophobicity of NPs can be increased by increasing OCA content in the copolymer. Since the degradation rate may be manipulated in an extremely wide range through varying the monomer composition, the drug release rate may be controlled with some precision. Although the apparent toxicity of poly(BCA-*co*-OCA) NPs is similar to that of PBCA, their characterizations on physical–chemical properties show that poly(BCA-

co-OCA) NPs are more feasible to encapsulate hydrophobic drugs.

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