

PREPARATION OF POLYALKYLCYANOACRYLATE NANOPARTICLES WITH VARIOUS MORPHOLOGIES*

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Abstract The effects of various reaction conditions on the preparation of polyalkylcyanoacrylate (PACA) nanoparticles are studied. The PACA nanoparticles with different crosslinking degrees and morphology are prepared. Addition of crosslinkers can not only adjust the particle size, but also change the morphology of PACA nanoparticles. Moreover, the loose network structure of the PACA nanoparticles with “core/shell-like” morphology is investigated by AFM and TEM in detail.

Keywords: Nanoparticles; Biodegradability; PACA; Network structure; Drug carriers.

INTRODUCTION

Polyalkylcyanoacrylate nanoparticles (PACA-NPs), due to their low toxicity^[1], biocompatibility and biodegradability^[2], have been employed as carriers of drug delivery system (DDS) for a large variety of drugs since they were first introduced by Couvreur *et al.*^[3–5] in 1979. Especially, PACA-NPs are one of a few carriers that can penetrate the blood-brain barrier (BBB)^[6], and the exclusive carrier used potentially for delivery of multi-drug resistant (MDR) drugs^[7]. Surface-modified PACA-NPs^[8,9] can be employed as carriers for targeting drugs to specific sites of the organism^[10–12]. PACA-NPs can be prepared by polymerization of ACA monomers^[13] through three approaches: emulsion polymerization^[3–5, 14, 15], interfacial polymerization^[16–18] and emulsifier-free emulsion polymerization^[19]. Emulsion polymerization and interfacial polymerization have been extensively used to prepare PACA-NPs, while emulsifier-free emulsion polymerization is limited to some special conditions^[19]. However, the three methods entail severe restrictions due to: (1) lack of reproducibility for various batches of monomers^[20]; (2) low molar masses (400–3500 g/mol) of PACAs which are unsuitable for long-time release of drug^[21]; (3) contamination of drugs by additives^[16–17, 20]; (4) low drug-loading capacity, often less than 15 wt%^[7, 19, 22, 23].

In this paper we adopt free radical polymerization to prepare PACA-NPs. The products are found to be in various nano-morphologies, of which the size and structure are strongly dependent on the reaction conditions. The prepared PACA nanoparticles can be controlled not only to have monodisperse size distribution and regular spherical shape, but also high swelling performance. The PACA nanoparticles with different crosslinking degrees and morphology are prepared for the first time. Moreover, the loose network structure of the PACA nanoparticles with “core/shell-like” morphology has been investigated in detail.

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EXPERIMENTAL

Reagents

Ethyl-cyanoacrylate (ECA) and butyl-cyanoacrylate (BCA) were supplied as analytical grade and used without any further purification. Ethylene glycol dimethacrylate (EGDMA) was supplied by Aldrich. Benzoyl peroxide (BPO) was recrystallized from chloroform/methanol. Acetone and alcohol were dried and purified by distillation before use. Water was doubly distilled and deionized. The other reagents were also of analytical grade and used without any further purification.

PACA Nanoparticle Preparation

A typical procedure used to prepare the nanoparticles is as follows: A mixture of 0.25 mL ECA monomers, crosslinker EGDMA (2 wt% relative to the ECA monomers), initiator BPO and DMA (2 wt% and 1 wt% of the total monomers, respectively) was dissolved in 10 mL acetone. This reactant solution was slowly added dropwise within 1 h under mechanical stirring (650 r/min) into the acetone/alcohol (2/7 *V/V*, 90 mL) polymerization medium in a clean, dry 250 mL three-necked flask kept in an 18°C (defined as dispersing temperature in this paper) water bath. Then the reaction mixture was transferred into a 100 mL conical flask and heated to 45°C (defined as polymerization temperature in this paper) to react for 24 h. The preparation procedures for the other PACA nanoparticles were similar but with altered reaction conditions listed in Table 1.

Table 1. Reaction conditions for preparation of the PACA nanoparticles

Sample	Reactant composition		Polymerization medium			Note
	ECA (mL)	BCA (mL)	Acetone (mL)	Alcohol (mL)	Water (mL)	
I ^a	0.25	0	20	70	0	Fig. 1
II ^a	0.25	0.25	20	70	0	Fig. 2(a)
III ^{a, b}	0	0.25	20	70	8.2	Fig. 2(c)

^a For sample I, the crosslinker EGDMA dosage is in the range of 0–20 wt%, for sample II and III the crosslinker EGDMA dosage is fixed at 2 wt% (relative to the ECA monomers); ^b For sample III, appropriate amount of doubly distilled water (8.2 mL) must be added to polymerization medium beforehand.

Characterization of PACA Nanoparticles

The prepared PACA nanoparticles were characterized by using an optical microscope (OM, Olympus IX-70 Japan), a transmission electron microscope (TEM, Philips EM-400, Philips TECNAI G²F-20) and an atomic force microscope (AFM, Digital Instruments Nanoscope IV (DI), USA). The tapping mode images were taken at room temperature with an image resolution of 256 × 256 pixels and a scan speed of 0.7–1.2 Hz in air).

RESULTS AND DISCUSSION

Preparation Conditions of PACA Nanoparticles

A series of experiments were designed to investigate the effects of the various reaction conditions on the preparation of the PACA nanoparticles. Reaction conditions and average diameters of the prepared PECA nanoparticles are listed in Table 2.

Polymerization temperature has strong effects on the mobility and self-assembly of PACA chains and the radical polymerization rate. As shown in Table 2, the uniform PACA nanoparticles can not be prepared under low polymerization temperature (35°C), as lots of small particles are produced during the long time of free radical polymerization process, particularly at 30°C precipitates are even formed. However, high polymerization temperature (70°C) is also adverse to nanoparticles preparation. The appropriate polymerization temperature is in the range of 40–60°C.

The ratio of acetone/alcohol, the distribution ratio of acetone in two parts, and the dispersing temperature also play important roles. As can be seen from Table 2, when the ratio of acetone/alcohol is lower than 20/80 or higher than 35/65, regular nanoparticles can not be prepared or even form precipitates. The appropriate ratio of acetone/alcohol is in the range of 25/75–30/70. For the distribution ratio of acetone, the appropriate value lies in the range of 10/20–15/15, beyond which regular nanoparticles can not be obtained. For the dispersing

temperature, when it is too low (10°C), regular nanoparticles can't be formed, and precipitates even occur at 6°C. However, high dispersing temperature (25°C) may lead to some large particle formation. This can be seen with the PACA product which displays a dual size distribution. The appropriate dispersing temperature is in the range of 16–22°C. Relatively, the stirring rate has minor effect on the preparation of PACA nanoparticles in the range of 450–1000 r/min, it may be due to the high miscibility of acetone and alcohol. However, when stirring rate is too low (250 r/min), some large particles are produced.

Table 2. Average diameters of the prepared PACA nanoparticles at different reaction conditions

Dispersing T (°C)	Nanoparticle size (nm)	Polymerization T (°C)	Nanoparticle size (nm)	ACT/ALH (V/V) ^c	Nanoparticle size (nm)	Distribution ratio (V/V) ^d	Nanoparticle size (nm)
6	Precipitate	30	Precipitate	15/85	Precipitate	5/25	— ^a
10	— ^a	35	— ^a	20/80	— ^a	10/20	130.7
13	127.1	40	121.1	25/75	143.6	15/15	128.8
18 ^e	130.7	45 ^e	130.7	30/70 ^e	130.7	20/10 ^e	— ^a
22	129.6	60	130.3	35/65	Precipitate	25/5	— ^a
25	124.5/600 ^b	70	— ^a	50/50	— ^a		

^a PECA nanoparticles with regular shape can't be prepared; ^b A few large particles are produced and the prepared PECA nanoparticles have dual size distribution; ^c The ratio of acetone/alcohol for polymerization medium; ^d Distribution ratio of acetone between reactant solution and polymerization medium; ^e Referring to the “fixed” conditions. In each condition-dependent measurement, only one condition is changed while the rest are fixed at these values.

To summarize, the most suitable preparation conditions are as follows: the dispersing temperature and polymerization temperature are 18°C and 45°C, respectively. The ratio of acetone/alcohol and the distribution ratio of acetone in two parts are 30/70 (V/V) and 10/20 (V/V), respectively. The stirring rate is in the range of 450–1000 r/min.

Preparation of PACA Nanoparticles with Different Morphology and Composition

Under optimized preparation conditions, PACA nanoparticles with different cross-linking degrees are prepared by adding different amounts of crosslinkers during preparation process. The TEM micrographs of the prepared PACA nanoparticles in Fig. 1 (sample I in Table 1) show that crosslinker dosage has strong effect on the size and morphology of PACA nanoparticles. The average diameter of PACA nanoparticles decreases from 138 nm to 40 nm as the crosslinker dosage increases from 0 to 20%. It is interesting that PACA nanoparticles with different morphology can be prepared by adjusting crosslinker dosage. Generally, the prepared PACA nanoparticles have “core/shell-like” morphology when the crosslinker dosage is in the range of 4%–14%.

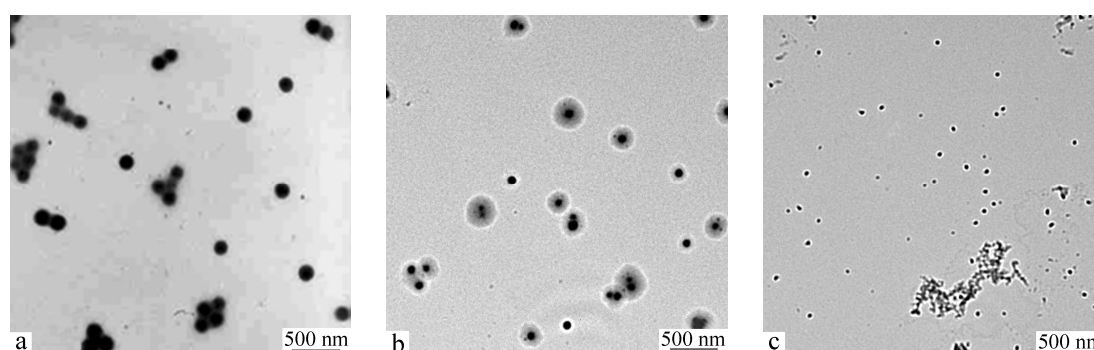


Fig. 1 TEM images of the nanoparticles with different cross-linking degrees: (a) 2%, (b) 6%, (c) 20%. The treatment process of all the samples in Fig. 1 is as follows. A drop of the sample is deposited onto a carbon-coated PVA-formal support on a copper grid, which is not stained. The grid is observed by TEM, operating at an acceleration voltage of 80 kV.

BCA monomer is usually used as raw materials to prepare PACA-NPs in view of its lower toxicity. Unfortunately, the PBCA nanoparticles can't be prepared successfully because the reactivity of BCA monomer is not as high as that of ECA monomer. One way is that ECA monomer can be used together with BCA monomer. The complex PACA nanoparticles (sample II in Table 1) with different monomer composition can be prepared by this way. Their high swelling performance has been observed by TEM and OM as shown in Figs. 2(a) and 2(b). The average diameter of the complex PACA nanoparticles is about 172 nm in TEM and 1.8 μm in OM, respectively. When pure BCA monomer is used with addition of appropriate amount of double-distilled water (8.2 mL), the average diameter of the PBCA nanoparticles obtained (sample III in Table 1) is about 120 nm as shown in Fig. 2(c). Additionally, Figure 2 shows that the morphology and particle size distribution of the PBCA nanoparticles are more heterogeneous as compared with PECA nanoparticles in Fig. 1(a). It may be the result of structure changing of PACA polymers (including their side ester groups and end groups) when ECA monomers are replaced by BCA monomers.

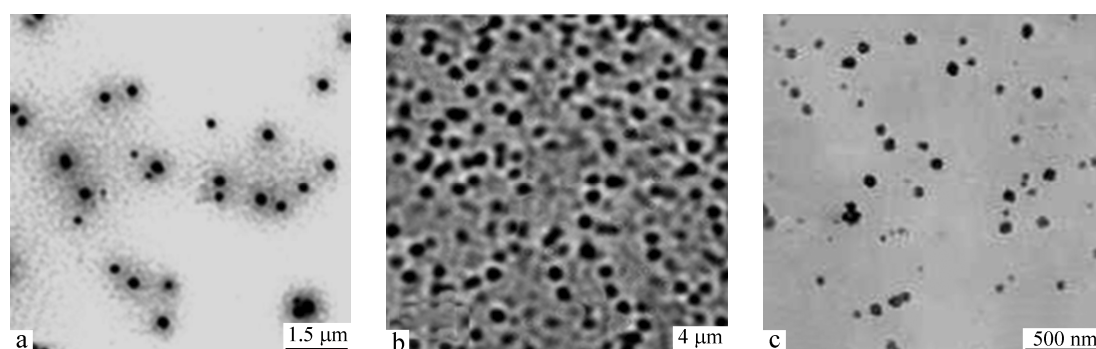


Fig. 2 Morphology of PACA nanoparticles: (a, b) TEM and OM images of complex PACA nanoparticles, respectively; (c) TEM image of PBCA nanoparticles

The treatment process of all the samples in Figs. 2(a) and 2(c) is as follows. A drop of the sample is deposited onto a carbon-coated PVA-formal support on a copper grid, which is not stained. The grid is observed by TEM, operating at an acceleration voltage of 80 kV. For the sample in Fig. 2(b), the sample solution is dropped onto a quartz wafer, followed by drying under atmosphere at room temperature, the sample is then observed by an optical microscope.

Characterization of PACA Nanoparticles with “Core/Shell-like” Morphology

As shown in Fig. 1(b), the PACA nanoparticles with 6% crosslinker have special “core/shell-like” morphology. The same sample was also observed by TEM after it had been negatively stained with an aqueous solution of sodium phosphotungstate. The morphology of this sample has been well represented by Fig. 3(a). The loose network structure of the shell layer is stained and corresponds to the dark field in TEM image. Contrarily, the compact core of PACA nanoparticles is not stained and corresponds to the bright field in TEM image^[24]. The sample was also observed by AFM after having been dried under high vacuum. The morphology of the collapsed PACA nanoparticles is presented in Figs. 3(c) and 3(d), which show that PACA nanoparticles collapse and appear to be oblate. Their average diameter and height are about 400 nm and 20 nm, respectively. That is to say, the ratio of diameter/height is 20. PACA nanoparticles will further collapse to caky shape under the circumstance of high vacuum and electron beam when they are observed in high resolution TEM (Fig. 3b). The caky image shows an average diameter of 120 nm which is close to that of the “core” of PACA nanoparticles in Fig. 1(b). The morphology of PACA nanoparticles changes remarkably under different observation conditions, indicating that PACA nanoparticles have large flexibility and elasticity.

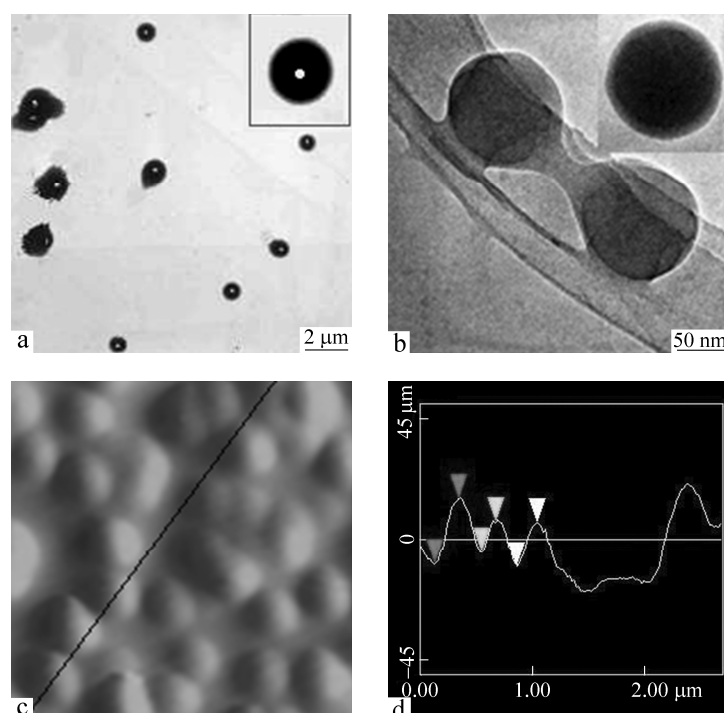


Fig. 3 PACA morphology at different observation conditions: (a) TEM image of the PACA nanoparticles stained with sodium phosphotungstate, (b) High resolution TEM image, (c) AFM image ($2\ \mu\text{m} \times 2\ \mu\text{m}$), (d) Height contour of (c)

The treatment process of the samples is as follows. For the sample in Fig. 3(a), 1 mL original PACA nanoparticle solution is dispersed in 10 mL double-distilled water, and a blue and transparent solution can be obtained after about 30 min. A drop of this solution is then deposited onto a carbon-coated PVA-formal support on a copper grid, which is further negatively stained with an aqueous solution of sodium phosphotungstate. The grid is observed by TEM, operating at an acceleration voltage of 80 kV. For the sample in Fig. 3(c), the sample solution is dropped onto a quartz wafer, followed by drying under high vacuum about 666.6 Pa at room temperature for 8 days, the sample is then observed by AFM

CONCLUSIONS

The preparation conditions of PACA nanoparticles have been studied systematically and the most suitable conditions are concluded. Under optimized preparation conditions, PACA nanoparticles with different crosslinking degrees can be prepared by adding different amounts of crosslinkers. Addition of crosslinkers can not only adjust the particle size, but also change the morphology of PACA nanoparticles. Moreover, the loosely crosslinked PACA nanoparticles exhibit “core/shell-like” morphology and high swelling performance, which will be very important for improving their drug-loading capacity. So we may anticipate that the prepared PACA nanoparticles will be very promising DDS with high drug-loading capacity.

REFERENCES

- 1 Kante, B., Couvreur, P., Dubowas-Krack, G., Demeester, C., Guiot, P., Roland, M., Mercier, M. and Speiser, P., *J. Pharm. Sci.*, 1982, 71: 786
- 2 O’Sullivan, C. and Birkinshaw, C., *Polym. Degrad. Stab.*, 2002, 78: 7
- 3 Couvreur, P., Kante, B., Roland, M., Mercier, M. and Speiser, P., *J. Pharm. Pharmacol.*, 1979, 31: 331
- 4 Liu, T. and Shan, G.R., *Acta Polymerica Sinica*(in Chinese), 2009, (11): 1132

- 5 Cao, Z.H., Cui, Q.M. and Shan, G.R., *Acta Polymerica Sinica*(in Chinese), 2007, (12): 1116
- 6 Alyautdin, R.N., Petrov, V.E., Langer, K., Berthold, A., Kharkevich, D.A. and Kreuter, J., *Pharm. Res.*, 1997, 14: 325
- 7 Soma, C.E., Dubernet, C., Bentolila, D., Benita, S. and Couvreur, P., *Biomaterials*, 2000, 21: 1
- 8 Storm, G., Belliot, S., Daemen, T. and Lasic, D.D., *Adv. Drug. Deliv. Rev.*, 1995, 17: 31
- 9 Stolnik, S., Illum, L. and Davwas, S.S., *Adv. Drug. Deliv. Rev.*, 1995, 16: 195
- 10 Kreuter, J., *Adv. Drug. Deliv. Rev.*, 2001, 47: 65
- 11 Nwashioke, Y. and Yoshino, H., *Adv. Drug. Deliv. Rev.*, 2001, 47: 55
- 12 Gibaud, S., Rousseau, C., Weingarten, C., Favier, R., Douay, L., Andreux, J.P. and Couvreur, P., *J. Controll. Release*, 1998, 52: 131
- 13 Vauthier, C., Dubernet, C., Fattal, E., Pinto-Alphandary, H. and Couvreur, P., *Adv. Drug. Deliv. Rev.*, 2003, 55: 519
- 14 Lescure, F., Zimmer, C., Roy, D. and Couvreur, P., *J. Colloid. Interface Sci.*, 1992, 154: 77
- 15 Douglas, S.J., Illum, L. and Davwas, S.S., *J. Colloid. Interface Sci.*, 1985, 103: 154
- 16 Khoury-Fallouh, N. Al., Roblot-Treupel, L., Fessi, H., Devwassaguet, J.P. and Puwasieux, F., *Int. J. Pharm.*, 1986, 28: 125
- 17 Vranckx, H., Demoustier, M. and Deleers, M., *Eur. J. Pharm. Biopharm.*, 1996, 42: 345
- 18 Lambert, G., Fattal, E., Pinto-Alphandary, H., Gulik, A. and Couvreur, P., *Pharm. Res.*, 2000, 17: 707
- 19 Yang, S.H., *J. Appl. Polym. Sci.*, 2000, 78: 517
- 20 Behan, N., Birkinshaw, C. and Clarke, N., *Biomaterials*, 2001, 22: 1335
- 21 Limouzin, C., Caviggia, A., Ganachaud, F. and Hémerly, P., *Macromolecules*, 2003, 36: 667
- 22 Fontana, G., Pitarresi, G., Tomarchio, V., Carlwasi, B. and Biagio, P.L.S., *Biomaterials*, 1998, 19: 1009
- 23 Fresta, M., Cavallaro, G., Giammona, G., Wehrli, E. and Puglwasi, G., *Biomaterials*, 1996, 17: 751
- 24 Li, X., Chen, P., Qin, C. and Liao, S., *Chin. Polym. Mater. Sci. Eng.*, 1999, 15: 129