Well-Defined Poly(butyl cyanoacrylate) Nanoparticles via Miniemulsion Polymerization

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Summary: Despite the high reactivity of the monomer, nanoparticles with a hydrophobic core based on poly(butyl cyanoacrylate) and a hydrophilic shell based on poly(ethylene oxide) PEO were synthesized in one step by miniemulsion polymerization. Colloidal properties of the nanoparticles were controlled by the structure and the amount of amphiphilic polymer in the aqueous phase, while the molecular weight of core depended on pH of the continuous phase and the polymerization mechanism (anionic or radical). The evolution of the molecular weight of the synthesized poly(butyl cyanoacrylate) was followed as a function of time at pH 7.4 by size exclusion chromatography. As expected, the degradation kinetics depended on the polymerization mechanism. Finally, a model compound, pyrene, was encapsulated in the synthesized nanoparticles. Its release was found to depend on the conditions of nanoparticles synthesis, especially on the polymerization mechanism.

Keywords: colloidal properties; drug delivery systems; miniemulsion; molecular weight distribution; nanoparticles

Introduction

Over the past decades, there has been considerable interest in developing biodegradable nanoparticles as effective drug delivery systems.[1,2] It is now well known that it is possible to modulate the interaction of these nanoparticles with the immune system by changing appropriately some of their physicochemical properties, such as size and surface characteristics.[3–7] In particular, it was suggested to modify the surface of the nanoparticles by highly hydrophilic polymers avoiding opsonins adsorption on particles that makes them recognizable for the macrophages of MPS.[8] Poly(ethylene oxide) (PEO) is one of the most studied hydrophilic polymer used for modification of surface properties of nanoparticulate carriers; leading to ''stealth nanoparticles''.[9,10] An additional and related research area focusses on the drug release rate, which seems to be mainly controlled by diffusion of drug through the matrix and (bio)degradation of nanoparticles.[11]

Poly(alkyl cyanoacrylate) nanoparticles have been studied in great details with a view to their use as materials for controlled drug release. PEO-coated poly(alkyl cyanoacrylate) nanoparticles are prepared essentially by two techniques. The first one involves anionic polymerization of alkyl cyanoacrylate in the presence of PEO.[12–14] The second one requires the synthesis of a PEO-poly(cyanoacrylate) copolymer for the formation of PEO-coated nanoparticles by nanoprecipitation.[15–17] In both cases, the final solid content of the suspensions is rather low. More recently, a few authors explored the possibilities offered by the miniemulsion polymerization technique. Taking advantage of the high stability of miniemulsions, they obtained poly(alkyl cyanoacrylate) nanoparticles dispersions with solid content

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up to 10%.[18–20] Furthermore, the molecular weight of the poly(alkyl cyanoacrylate) obtained could be controlled by the miniemulsion conditions.[20]

This paper aimed at a short but complete description of the synthesis and characterization of PEO-coated poly(butyl cyanoacrylate) (PBCA) nanoparticles obtained by miniemulsion polymerization. Despite the high reactivity of the monomer, nanoparticles of poly(butyl cyanoacrylate) were synthesized by radical and/or anionic miniemulsion polymerization stabilized with PEO-based surfactants of different PEO chain lengths: Brij®78, Brij®700, Tween®80 (Table 1). Particle size, surface coverage and hydrophilic layer thickness were measured as a function of the structure and the concentration of PEO derivatives in the aqueous phase. The molecular weight of the poly(butyl cyanoacrylate) was followed by size exclusion chromatography coupled with light scattering (SEC-MALLS). The influence of the polymerization mechanism on the final molecular weight and the degradability of the polymer were depicted. Finally, the encapsulation and release of a model compound were studied as a function of the conditions of nanoparticles preparation.

Materials and Methods

Materials

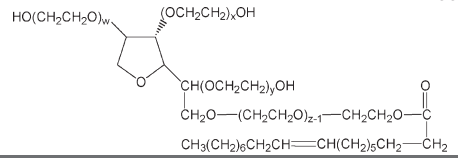
Butyl cyanoacrylate was obtained from Loctite (Ireland). Hydrochloric acid (1 M), dichloromethane (ChromasolV®, for HPLC, ≥99.9%) and dodecane (≥99%) were purchased from Aldrich. 2,2′-Azobis(2-methylpropanenitrile) (98%) (AIBN) and methanesulfonic acid (99%) were purchased from Acros. Brij®78, Brij®700, Tween®80 were purchased from Sigma. Aluminum potassium sulfate dodecahydrate (98%) ($KAl(SO_4)_2 \cdot 12H_2O$) was purchased from Lancaster. Tetrahydrofuran (THF, Chromanorm™ for HPLC), purchased from VWR Prolabo, was acidified with 5×10^{-4} M methanesulfonic acid. Pyrene (98%) from Aldrich was crystallized from hexadecane before use. MilliQ water was used for all the experiments.

Nanoparticles Synthesis

Poly(ethylene oxide) derivatives were dissolved in 12.5 mL of solution of pH 1 or 2 on the day before. Weak heating is required to dissolve Brij®78. The organic phase was composed of 0.5 mL of BCA, 25 μL of dodecane and 5 mg of AIBN (in the case of radical polymerization). For the release studies, crystallized pyrene was added directly to the organic phase (10 g/L) before emulsification.

The organic phase was added dropwise to the aqueous solution. After stirring for 5 min at 1000 rpm and 4 °C, the formed emulsion was immediately sonicated (pulsed mode, 5 W, 1 min) using a Vibracell model 600 W instrument (Sonics & Material Inc., Danbury, CT). To avoid thermal polymerization, the emulsion was ice-cooled during sonication. Polymerization was then performed for 24 h at room temperature in the case of anionic polymerization or at 75 °C in the case of radical polymerization. Finally, the nanoparticles were dried by lyophilization. The dried products were analyzed by SEC-MALLS in acidified THF.

Table 1.
Structure and HLB values of Brij®78, Brij®700, Tween®80.

	HLB	Structure	
Brij®78	15	$CH_3(CH_2)_{17}-(OCH_2CH_2)_yOH$	Y = 20
Brij®700	18		Y = 100
Tween®80	15		

Characterization Techniques

Particle sizes were determined by dynamic light scattering at low concentrations using a HPPS-ET (Malvern Instruments) at 20 °C. The suspensions were diluted with aqueous solution of pH 1 and 2 depending on preparation conditions. d_H, *z*-average diameter and polydispersity index (PDI) were determined. PDI is an indicator of the particle size distribution ranging between 0 and 1. PDI values of less than 0.2 indicate a monodisperse emulsion, whereas large distributions show values above 0.5.

Electrophoretic mobility (μe) was studied in NaCl as a function of ionic strength. The experiments were carried out on a Zetasizer® Nano Series (Malvern Instruments, Malvern, UK). Miniemulsions were diluted before measurement. The zeta potential (ζ) was calculated from electrophoretic mobility using the modified Booth equation.[21] This equation allows the calculation of ζ values for any k and a values, where k^{-1} is the Debye length, related to the ionic strength, and a the radius of particles, whereas the classical Smoluchowski and Huckel equations are applicable only in two limiting cases, i.e., $ka > 100$ and $ka < 0.1$, respectively. ζ values were used to estimate the thickness of the adsorption layer using the Eversole and Boardman equation:[22,23]

$$\tanh(ze\xi/4k_bT) = \tanh(ze\xi_0/4k_bT)\exp(-k\Delta)$$

where z = 1 (electrolyte valence), ζ_0 is the surface potential, and Δ is the distance of the shear plane from the surface of the particle, corresponding approximately to the adsorbed layer thickness.

The amount of poly(ethylene oxide) derivatives at the particle surface (Γ) was directly determined by UV spectrophotometry using an I_2/KI solution (1% I_2, 2% KI w/v)[24] (direct method). 100 µl of I_2/KI solution is added to 4 mL of polymer solution. The mixture is stocked at room temperature; it is immune to light. Then, the absorbance of the solution is measured at 500 nm after exactly 15 min.

Size exclusion chromatography (SEC) was performed in acidified THF (sample concentration 1.5 wt.%) at room temperature using a Merck L-6200A HPLC pump equipped with a degaser, two columns PLgel Mixed 5 µm, 100 Å and 10E3 Å, 300 × 7.5 mm guard column (Polymer Laboratoires). Elution (0.7 mL/min) was dually monitored by multi-angle laser light scatting detection (MALLS) and differential refractometry (Merck RI-71).

Nanoparticles Degradation

Degradation of nanoparticles was carried out in phosphate buffer at pH 7.4. The nanoparticles obtained were tumbled at 37 °C in buffer solution (concentration 10 g/L). Samples were withdrawn during 48 h, and dried by lyophilization. The obtained products were analyzed by SEC-MALLS in acidified THF.

Pyrene Release Kinetics

15 mg of pyrene-loaded nanoparticles were added to 1 L of a buffer solution containing 30% (v/v) ethanol at pH 7.4. 10 mL samples of the solution were withdrawn in regular over 48 h, then centrifuged at 30 000 g at 15 °C for 30 min. Clear supernatants were collected for determination of the free pyrene concentration by fluorescence spectroscopy. Fluorescence emission spectra were recorded on a Spexfluorolog-3 spectrometer equipped with a thermostatted cell compartment at 25 °C (slit width 0.5 mm, $\Delta\lambda$1/2–1.5 nm). Before each experiment, fluorescence emission spectra of reference solutions (fluorescein 0.04–0.12 mg/L in a pH 7.4 buffer solution containing 30% (v/v) ethanol) were recorded and their areas between 300 and 500 nm were measured by integration.

Results and Discussion

Synthesis of PEO-coated PBCA Nanoparticles

Butyl cyanoacrylate (BCA) is considered as one of the more reactive monomers in anionic or zwitterionic polymerization.[25] Drastic conditions are thus required in

order to emulsify the monomer in water while limiting its anionic polymerization. Therefore, different parameters such as pH,[8,26] temperature, sonication time[27] have to be carefully controlled during all the process. However, PEO-coated poly (butyl cyanoacrylate) nanoparticles were successfully synthesized by miniemulsion polymerization stabilized with Brij®78, Brij®700, Tween®80 (Table 1). No destabilization occurred during the polymerization and the amount of coagulum remained below 5 wt.%.

As expected,[28,29] particle size strongly depended on surfactant concentration in the aqueous phase; very small nanoparticles could be obtained at its high concentrations (Figure 1). On the contrary, temperature and polymerization mechanism had little influence on the particle size. In the case of Tween®80, particle size even decreased to values as low as 100 nm. These values are much smaller than those reported in the literature for BCA nanoparticles obtained by emulsion polymerization at pH 2 in the presence of Tween®80 (220–260 nm) [30]. Miniemulsion polymerization is thus a very efficient technique to optimize particle size for a given surfactant concentration.

At increasing concentrations of stabilizer in the aqueous phase, the amount of adsorbed surfactant increased to reach a value of 3.4 mg/m^2 for Tween®80, 2.8 mg/m^2 for Brij®78 and 1.6 mg/m^2 for Brij®700 (Figure 1). The maximum surface coverage thus decreased when the PEO chain length increased. Indeed, the already-adsorbed chains induce steric repulsion of the surfactant remaining in the aqueous phase, thereby reducing the final surface coverage.

Finally, the hydrophilic layer thickness was found to increase with increasing surfactant concentration for Brij®78 and Brij®700, while a constant value of about 8 nm was found for Tween®80 (Figure 2). Indeed, the conformation of the surfactant switched from a mushroom to a brush conformation because of steric hindrance induced by neighbouring chains at increasing surface coverage. The results are consistent with the structure of PEO derivatives: the hydrophilic layer thickness increased with increasing length of PEO chains.

Influence of Polymerization Mechanism

BCA is a highly reactive monomer. Its polymerization can theoretically proceed by three mechanisms: radical, anionic and

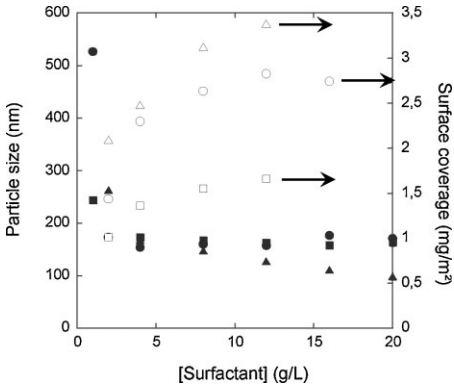


Figure 1.
Particle size (filled symbols) and surface coverage (empty symbols) as functions of the concentration of surfactant in the aqueous phase for poly(butyl cyanoacrylate) nanoparticles obtained by anionic miniemulsion polymerization at room temperature in the presence of Brij®78 (●), Brij®700 (■), and Tween®80 (▲). The oil volume fraction was 4%.

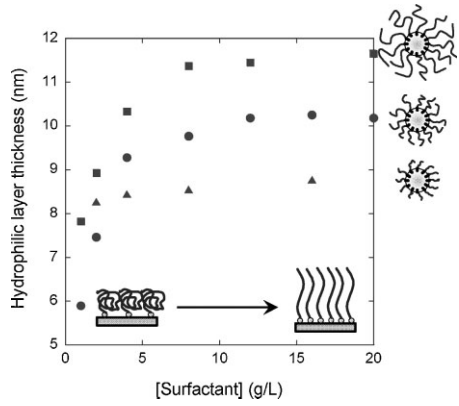


Figure 2.
Hydrophilic layer thickness as a function of the surfactant concentration in the aqueous phase for poly(butyl cyanoacrylate) nanoparticles obtained by anionic miniemulsion polymerization at room temperature in the presence of Brij®78 (●), Brij®700 (■), and Tween®80 (▲).

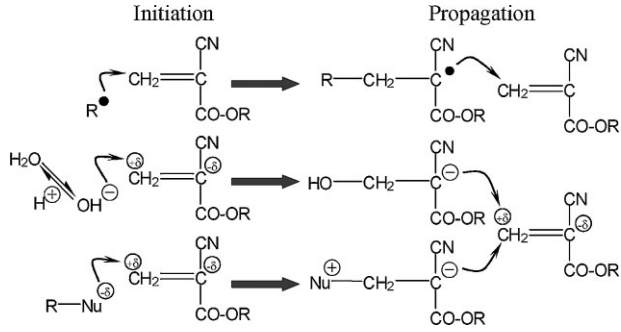


Figure 3.
Scheme of radical (A), anionic (B) and zwitterionic (C) polymerization of alkyl cyanoacrylates.

zwitterionic polymerization. In practice, the anionic and zwitterionic routes are favored because they are rapidly initiated at ambient temperature (Figure 3)[31].

However, the molecular weights of poly(butyl cyanoacrylate) obtained are generally low [18] (below 8 000) and strongly depend on pH of the polymerization medium.[31,32] To increase the molecular weight, higher pH value are required, which usually lead to a very fast, uncontrolled process. In order to synthesize high-molecular-weight PBCA, AIBN was added to the monomer prior to emulsification. Temperature, pH and polymerization mechanism were thus varied so as to adjust the final molecular weight of PBCA. Brij®78 was used to stabilize the miniemulsion ([Brij®78] = 4g/L). Table 2 collects the different conditions used to synthesize nanoparticles.

The average size of the obtained particles was close to 160 nm, whatever pH and polymerization method were used. The particle size is mainly controlled by surfactant concentration in continuous phase and not by the polymerization method. On the contrary, molecular weight of PBCA strongly depended on the synthesis conditions (Figure 4).

Two populations were observed in all cases. The first peak corresponds to molecular weight close to 4000 $g.mol^{-1}$ while the highest molecular weights reach values of 200000 $g.mol^{-1}$. As expected, lower pH favored low molar weight while higher temperature favored higher molecular weight in anionic polymerization. Furthermore, the presence of the AIBN initiator led to the highest molecular weight. Miniemulsion polymerization thus allowed controlling the final molecular weight of the nanoparticle cores.

PBCA can degrade *via* an unzipping process [8], leading to low-molecular-weight polymers. The evolution of the molecular weight of the synthesized PBCA was thus followed in a buffer solution at pH 7.4 (Figure 4). The population of higher molecular weight almost disappeared when the nanoparticles were obtained by anionic polymerization, while it remained constant when radical polymerization was used. Indeed, the polymer chain ends are different in the two cases and only chains terminated by a reaction with protons can depolymerize. Finally, a slight increase in the molecular weight of the second population was observed, due to a re-equilibrium of the propagation-depropagation steps.

Table 2.
Conditions of miniemulsion polymerization of BCA.

Run	pH	Polymerization	Pyrene
1	1	ionic at room temperature	No
2	2	ionic at room temperature	No
3	2	ionic at 75 °C	No
4	2	radical at 75 °C	No
5	1	ionic at room temperature	Yes
6	2	radical at 75 °C	Yes

Pyrene Encapsulation and Release

Pyrene-loaded nanoparticles were prepared by anionic miniemulsion polymer-

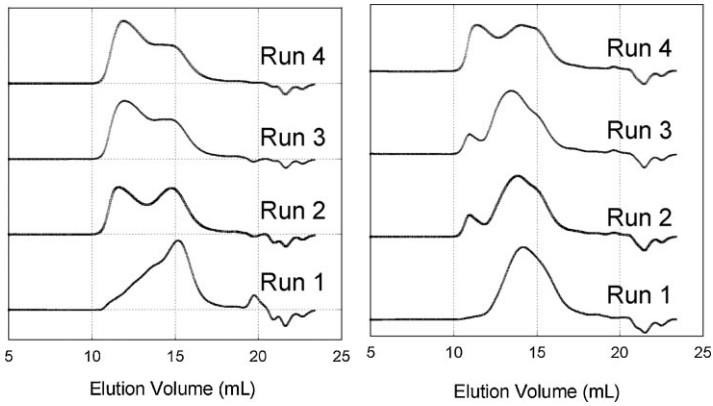


Figure 4.
SEC eluograms of PBCA obtained for different polymerization conditions (left) and after degradation in buffer solution at pH 7.4 for 8 h (right). Brij®78 was used to stabilize the miniemulsion ([Brij®78] = 4 g/L).

ization at pH 1 and room temperature (Run 5) and by radical polymerization at pH 2 and 75 °C (Run 6), in order to obtain different cores. Brij®78 was used to stabilize the miniemulsion ([Brij®78] = 4 g/L).

The size of nanoparticles obtained was close to 150 nm, which is consistent with the results obtained in the absence of pyrene. The pyrene encapsulation ratio was very high and close to 95%, as measured by UV/visible spectroscopy in THF.

To increase the solubility of pyrene in the continuous medium, the release test was carried out in pH 7.4 buffer solution containing 30% (v/v) ethanol at 37 °C (Figure 5).

The pyrene release kinetics strongly depended on the properties of the core of the nanoparticles. In Run 5 (anionic polymerization), 75% of pyrene was

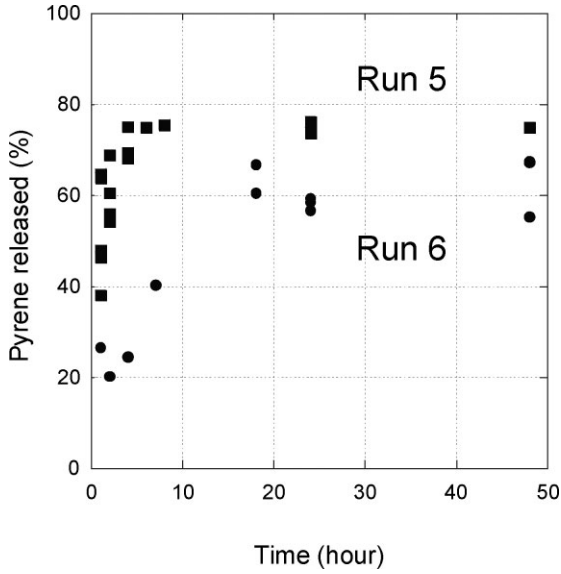


Figure 5.
The amount of released pyrene as a function of time; the continuous medium is a buffer solution of pH 7.4 containing 30% (v/v) ethanol.

released quickly in the course of the first 8 h. It was suggested that this partial release could be due to partition of the encapsulated compound between the matrix and buffer solution[33].

In Run 6, only 60% of pyrene was released in ca. 20 h. This slower release is ascribed to a higher molar mass of the core and probably to lower degradability of the PBCA obtained by radical polymerization.

Miniemulsion polymerization is thus a very convenient method to vary the release rate of an encapsulated compound because it allows varying the properties of the core of the nanoparticles while keeping other parameters, such as particle size and surface coverage, constant, simply by changing the polymerization mechanism.

Conclusions

Despite the high reactivity of the monomer, nanoparticles with a hydrophobic core based on poly(butyl cyanoacrylate) and a hydrophilic shell based on POE were synthesized in one step via miniemulsion polymerization. Particle size, surface coverage and hydrophilic layer thickness were controlled by the structure and the amount of amphiphilic polymer in the aqueous phase, while the molecular weight of the poly(butyl cyanoacrylate) depended on pH of the continuous phase and the polymerization mechanism (anionic or radical).

The evolution of molecular weight of the synthesized PBCA was followed as a function of time at pH 7.4 by size exclusion chromatography. As expected, the degradation kinetics depended on the polymerization mechanism (anionic or radical). Finally, a model compound, pyrene, was encapsulated in the synthesized nanoparticles. Its release depended on the conditions of nanoparticle synthesis, especially on the polymerization mechanism.

Miniemulsion polymerization is thus a very convenient method to control the final properties of the nanoparticles. Biological tests are now required to check the influence of these properties on the in vivo behavior of these nanoparticles.

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