Block Copolymer Nanoparticles of Ethylene Oxide and Isobutyl Cyanoacrylate

Young Kweon Choi, You Han Bae,† and Sung Wan Kim*

Center for Controlled Chemical Delivery, Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, 570 Biomedical Polymers Building, Room 205, Salt Lake City, Utah 84112

Received April 24, 1995; Revised Manuscript Received September 5, 1995®

ABSTRACT: Di- and triblock copolymers of poly(isobutyl cyanoacrylate) and poly(ethylene oxide) have been prepared and characterized. The synthesis involved capping of monohydroxyl- and dihydroxyl-terminated poly(ethylene oxide) with triphenylphosphine, followed by the zwitterionic polymerization of isobutyl cyanoacrylate with the triphenylphosphine-terminated poly(ethylene oxide) macroinitiator to yield the AB- or ABA-type block copolymers. The molecular weight and composition of the resulting block copolymers were close to theoretical values. Spherical nanospheres were prepared from the obtained block copolymers for potential biomedical applications.

Introduction

Recently, a new type of colloidal drug carrier was developed: biodegradable nanospheres with hydrophilic surface properties.[1,2] This approach to intravenously injected drug carriers may prolong the half life in blood and also alter their body distribution. The surface hydrophilicity of this biodegradable carrier was obtained by the adsorption of nonionic surfactants, such as Poloxamer, or from graft copolymers containing poly(ethylene oxide) (PEO). It was reported that the PEO surface of the nanoparticles reduced phagocytic uptake and thus prolonged blood circulating time (dysopsonic effect).

Lactide homo- or copolymers have been used to fabricate biodegradable particulate drug carriers due to their acceptable biocompatibility properties and nontoxic degradation products.[3,4] More recent studies demonstrated that nanoparticles (spheres or capsules) made of poly(alkyl cyanoacrylates) can be carriers for various drugs, including peptides and proteins.[5] Commercially, alkyl cyanoacrylates have been widely used as adhesives in industry and medicine due to their "instantaneous" polymerization properties. This class of monomer undergoes ionic polymerization in the presence of moisture or tertiary amino compounds.[6] Unlike other ionic polymerizations, however, controlling the polymerization process due to the higher propagation rate compared to the initiation rate and chain doubling process was difficult.[6,7] In addition, few articles have been found in the literature concerning copolymers of alkyl cyanoacrylate.[8,9]

This paper reports a zwitterionic synthesis of biodegradable AB- or ABA-type block copolymers comprising PEO (B block) and poly(isobutyl cyanoacylate) (PBCA; A block), utilizing triphenylphosphine end-capped mono- and dihydroxy PEO's. This paper also describes the preparation of nanoparticles with the obtained block copolymers for potential biomedical applications.

Experimental Section

Materials. PEO 3400 and monomethoxy PEO 2000 were purchased from Aldrich Co. (Milwaukee, WI) and were dehydrated by azeotropic distillation with benzene followed by further drying at 100 °C under vacuum. Isobutyl cyanoacrylate was obtained from Sigma Co. (St. Louis, MO) and used without further purification. (4-Bromophenyl)diphenylphosphine (Aldrich Co.) was recrystallized from ethanol and dried under vacuum at room temperature. Tetrahydrofuran (THF) was distilled over lithium aluminum hydride and sodium metal, successively, and a THF solution of potassium naphthalene was freshly prepared prior to use, as previously described.[10]

Preparation of the Macroinitiator. Reactions were carried out under nitrogen in a graduated glass apparatus, and chemicals were introduced through a rubber septum with a Gastight syringe. The dried PEO was dissolved in THF and titrated with 0.1 mol/L of potassium naphthalene/THF solution in a nitrogen atmosphere until a stable pale green solution color was observed. (4-Bromophenyl)diphenylphosphine in THF at a concentration equivalent to the hydroxyl content of PEO was added dropwise into the solution of potassium poly(ethylene glycol)ate. After it was stirred for 4 h at room temperature and diluted with dry THF to 0.01 M concentration, the initiator solution was decanted and the supernatant was used for polymerization.

Block Copolymerization. Polymerization was carried out in a nitrogen atmosphere at room temperature. Isobutyl cyanoacrylate (0.05 M in THF) was added dropwise into the silanized glass apparatus containing the required amount of the macroinitiator. After stirring the reaction mixture for 1 h, polymerization was stopped by adding acidified methanol. The polymer was precipitated into an excess amount of distilled water, followed by washing with hexane and then drying under vacuum to a constant weight.

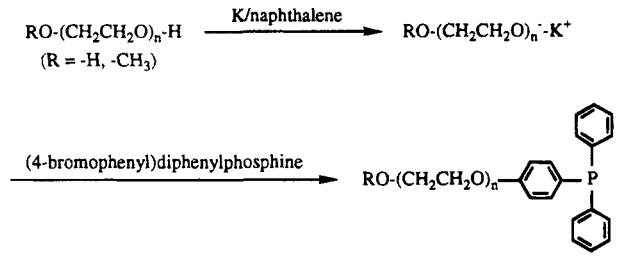
Preparation of Nanoparticles. Nanoparticles were prepared either by a solvent evaporation method[2] or the solvent exchange method. For the solvent evaporation method, a methylene chloride solution of PEO–PBCA block copolymers (0.5 g/2 mL) was emulsified in distilled water (10 mL) through the use of a probe sonicator (Branson, Danbury, CT) at 40 W and 70% duty cycle, pulsed mode. The methylene chloride was then removed by rotary evaporation, which was conducted at room temperature for 1 h under reduced pressure. Nanoparticles were also obtained by dialyzing the THF/copolymer solution (50 mg/10 mL) using Spectrapor dialysis tubing (molecular weight cutoff 3500) at 4 °C. In both cases, a white fine powder was obtained after freeze-drying the suspension of nanoparticles.

Measurements. ^{1}H-NMR spectra were run in $CDCl_3$ with tetramethylsilane as an internal standard on the Bruker AS 200 FT spectrometer. Gel permeation chromatography (GPC) was performed in THF with a Waters liquid chromatography equipped with Waters 501 pump, 712 WISP, 745 data module, R401 differential refractometer, and a set of columns (linear

* To whom correspondence should be addressed.
† Department of Materials Science and Engineering, Kwanju Institute of Science and Technology, 572 Sangam-dong, Kwangsan-ku, 506-303 Korea.
® Abstract published in *Advance ACS Abstracts,* October 15, 1995.

0024-9297/95/2228-8419$09.00/0

Scheme 1



and 500 Å). The molecular weight and molecular weight distribution were approximated from a calibration curve obtained from polystyrene standards (Polysciences, Inc.).

Quasi-elastic light scattering (QELS) measurements were performed at 25 °C and at a scattering angle of 90° by using a multiangle dynamic light scattering instrument (Brookhaven Instrument Corp.) with a Lexel Model 95 ion laser operated at a laser wavelength of 514.5 nm. A BI-2030AT 72-channel correlator was used to collect the autocorrelation function. The data were analyzed by the built-in Laplace-inversion algorithm.

The morphology of PEO–PBCA nanoparticles was studied on a scanning electron microscope (SEM; Cambridge Instruments, 250 Mk 3) using 3–10 kV. The samples were freeze dried, mounted on aluminum stubs with double-sided tape, and sputter coated with gold to a thickness of 200–500 Å.

Results and Discussion

A macrozwitterionic mechanism has been postulated for the polymerization of alkyl cyanoacrylate initiated by Lewis bases, such as phosphines and tertiary amines, while conventional initiators may provoke a classical anionic polymerization.[9,11] However, polymerization by tertiary amines yielded a polymer with higher molecular weight than the theoretical value, which is due to the comparable rate of propagation to initiation. In the case of graft copolymerization of alkyl cyanoacrylate onto poly(4-vinylpyridine), only one-third of the original pyridine groups were involved in the graft reaction, even in the presence of a chain killing agent.[8] In contrast, polymerization by phosphines demonstrated the classical (near ideal) living polymerization,[11] and the degree of polymerization (DP) was determined by the ratio of the moles of monomer consumed to the number of chains (i.e., DP = $[\text{monomer}]_0/[\text{initiator}]_0$).[12] Thus, polymerization of isobutyl cyanoacrylate with a triphenylphosphine-terminated macroinitiator was expected to yield PEO–PBCA block copolymers with the theoretical molecular weight and composition.

PEO derivatization with triphenylphosphine is shown in Scheme 1. First, the hydroxyl end groups of PEO were transformed into potassium alkoxides by titration with potassium naphthalene in THF at room temperature. The development of a stable green color may indicate that all hydroxyls reacted in the presence of a slight excess of potassium naphthalene.

Triphenylphosphine groups were then introduced through the reaction of potassium alkoxides with the halogenated phosphine compound. During the reaction between the PEO alkoxide and halgenated phosphine, precipitation was observed, most probably the formation of potassium bromide. The macroinitiator was isolated by precipitation in diethyl ether and characterized by ^{1}H-NMR. Figure 1 is the ^{1}H-NMR spectrum of the isolated macroinitiator, which clearly shows the presence of triphenyl phosphine groups (δ = 7.5–7.8 ppm) as well as the oxyethylene units of PEO (δ = 3.72 ppm). However, when the polymerization of isobutyl cyanoacrylate was initiated with the isolated macroinitiator, the

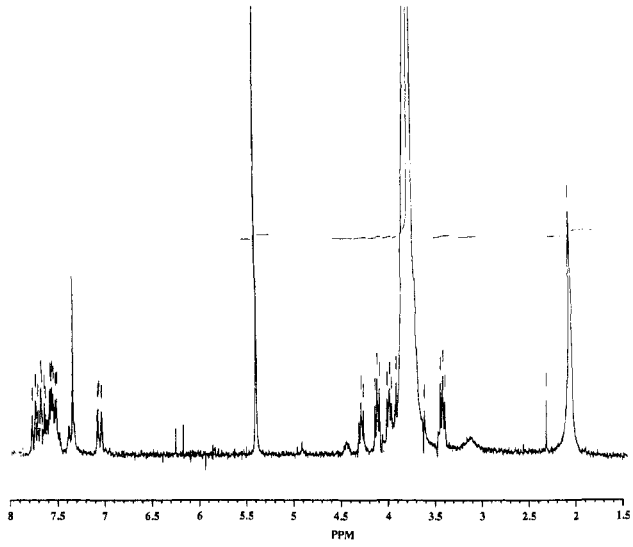


Figure 1. ^{1}H-NMR spectrum of a triphenylphosphine-terminated PEO macroinitiator.

GPC analysis of the resulting polymer showed a bimodal molecular weight distribution, indicating the presence of some decomposed macroinitiators which remained unreacted. The resonance signal of the triphenyl protons can be affected by oxidation of phosphine, due to the deshielding effect of oxygen. Thus, from the fact that the triphenylphosphine group of the isolated macroinitiator was differentiated from triphenylphosphine (δ = 7.35 ppm), it can be assumed that the phosphine group was oxidized during isolation. Oxidation of phosphines by air has been found under mild conditions.[12,13]

Polymerization of isobutyl cyanoacrylate was conducted with the nonisolated macroinitiator which was prepared under dry nitrogen. Polymerization occurred immediately and the monomer was almost entirely consumed. The sharp, unimodal GPC trace indicated that all phosphine macroinitiators were utilized. A small amount of PEO alkoxide salts may exist unreacted with (4-bromophenyl)diphenylphosphine. It is possible that they can initiate isobutyl cyanoacrylate monomer, resulting in classic anionic polymerization. Separate experiments, in which isobutyl cyanoacrylate was initiated by PEO alkoxide salts, revealed that the polymerization occurred immediately and was not controllable in terms of the molecular weight and composition. In contrast, polymerization initiated by phosphines followed the near ideal living polymerization. The polymerization rate initiated by phosphine initiator was very low compared to that with alkoxide initiator. Usually the polymerization took about 30 min, which is consistent with the result obtained by Miura et al.[9] Thus, the possibility of the initiation by PEO alkoxides may be ignored.

Figure 2 is the ^{1}H-NMR spectrum of the PEO–poly(butyl cyanoacrylate) block copolymer, which was obtained from the polymerization with an unisolated macroinitiator. A chemical shift due to triphenylphosphine groups is still observed at δ = 7.4–7.7 ppm. Thus, it can be concluded that phosphine groups were successfully coupled at the end of the PEO and then initiated the isobutyl cyanoacrylate monomer, yielding a corresponding block copolymer. Signals at δ = 3.62 and δ = 2.42 ppm are assigned to methylene protons of PEO chains and PBCA backbone, respectively. Peaks at δ = 0.97, δ = 2.04, and δ = 4.03 ppm are attributed to the methyl, methine, and methylene protons in the pendant groups of PBCA chains, respectively.

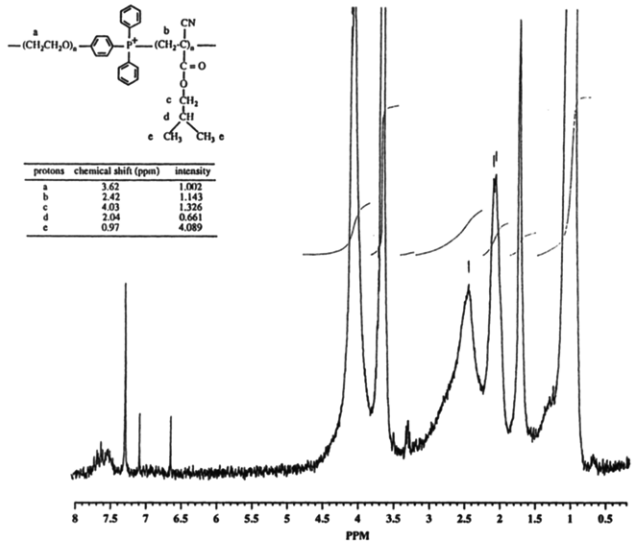


Figure 2. ^{1}H-NMR spectrum of a PEO–PBCA diblock copolymer prepared from momethoxy PEO 2000 (PEB-2).

Table 1. Results of the Anionic Polymerization of Isobutyl Cyanoacrylate (BCA) Initiated with Triphenylphosphine-Terminated PEO's in THF at Room Temperature

code	feed (mole ratio of EO to BCA)	copolymer comp[a] (mole ratio of EO to BCA)	number-average MW calcd	number-average MW NMR
PEB-1	20:30[b]	19:27	12 300	11 800
PEB-2	20:60[b]	25:66	22 700	20 400
PBEB-1	23:17[c]	22:13	11 900	10 200
PBEB-2	23:34[c]	26:33	20 500	18 100

[a] Calculated from ^{1}H-NMR spectra. [b] Diblock copolymer prepared from monomethoxy PEO 2000. [c] Triblock copolymer prepared from PEO 3400.

Table 1 summarizes the results for the block copolymerization. The experimental molecular weights determined by ^{1}H-NMR are close to the theoretical values calculated from the monomer to initiator ratio. These results may indicate that the polymerization of butyl cyanoacrylate by triphenylphosphine-terminated PEO yielded well-defined block copolymers.

Nanoparticles were prepared from diblock copolymers by either the solvent evaporation method or the solvent exchange method. Both methods yielded spherical nanoparticles. The particle size and distribution were measured by QELS (Figure 3), which shows unimodal size distribution. The mean particle size ranged from 100 to 700 nm, depending on the amount of organic solvent used and the composition of copolymers. The particle size and distribution were not changed, even after lyophilization. The lyophilized nanoparticles were easily redispersed in aqueous solutions without any change in size and shape.

A preliminary stability test established that the block copolymer nanoparticles dispersed in aqueous solutions did not aggregate over 1 week, presumably due to the decreased surface free energy of PEO-coated hydrophilic surface, while nanoparticles prepared from PBCA homopolymers began to aggregate after 1 day. Further investigations are needed to analyze the surface properties. The SEM photograph (Figure 4) shows the morphological feature of nanoparticles prepared by the solvent evaporation method; spherical shapes and smooth surfaces are observed.

The biodegradable, amphiphilic polymers, such as PEO–PBCA block copolymers, are known to be useful

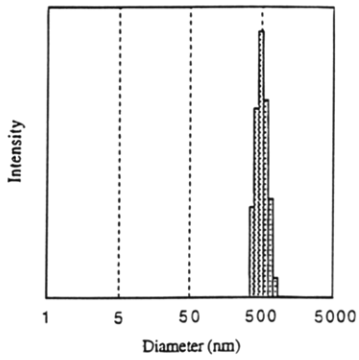


Figure 3. Quasi-elastic light scattering of nanoparticles prepared from a PEO–PBCA triblock copolymer (PBEB-1). The mean size was determined to be 431 nm.

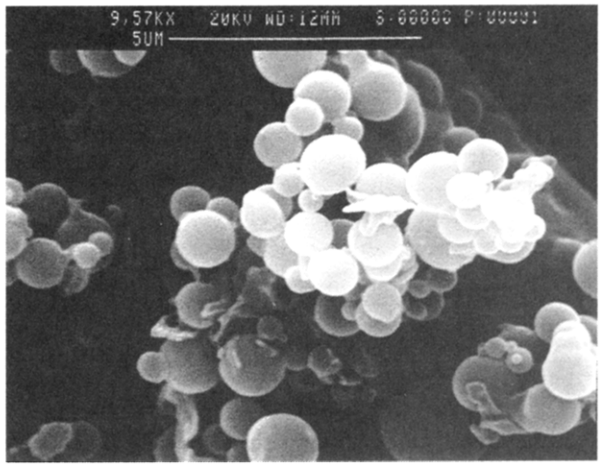


Figure 4. SEM photograph of the nanoparticles prepared from a PEO–PBCA triblock copolymer (PBEB-2).

due to their wide range of applications. For drug delivery they can be formulated into a variety of forms having diverse shape and function, i.e., polymeric micelles, surface-modified spheres, and hydrogel matrices. These polymers can also be used to modify the surface of biomedical devices. From a toxicological point of view, PEO–PBCA block copolymers may be acceptable since both PEO and PBCA are currently used in biomedical applications.

References and Notes

(1) Rudt, S.; Muller, R. H. *J. Controlled Release* **1993**, *25*, 51.
(2) Gref, R.; Minamitake, Y.; Peracchira, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600.
(3) Labarre, D.; Vittaz, M.; Spenlehauer, G.; Bazile, D.; Veillard, M. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **1994**, *21*, 91.
(4) Allemann, E.; Gurny, R.; Doelker, E. *Eur. J. Pharm. Biopharm.* **1993**, *39* (5), 173.
(5) Grangier, J. L.; Puygrenier, M.; Gautier, J. C.; Couvreur, P. *J. Controlled Release* **1991**, *15*, 3.
(6) Johnston, D. S.; Pepper, D. C. *Makromol. Chem.* **1981**, *182*, 421.
(7) Pepper, D. C. *J. Polym. Sci.: Polym. Symp.* **1978**, *62*, 65.
(8) Johnston, D. S.; Pepper, D. C. *Makromol. Chem.* **1981**, *182*, 407.
(9) Miura, M.; Akatsu, F.; Ito, H.; Nagakubo, K. *J. Polym. Sci.: Polym. Chem. Ed.* **1979**, *17*, 1565.
(10) Morton, M.; Milkovich, R. *J. Polym. Sci.: Part A* **1963**, *1*, 443.
(11) Johnston, D. S.; Pepper, D. C. *Makromol. Chem.* **1981**, *182*, 393.
(12) Pepper, D. C.; Ryan, B. *Makromol. Chem.* **1983**, *184*, 395.
(13) Rauhut, M. M.; Currier, H. A. *J. Org. Chem.* **1961**, *26*, 4626.

MA950552F