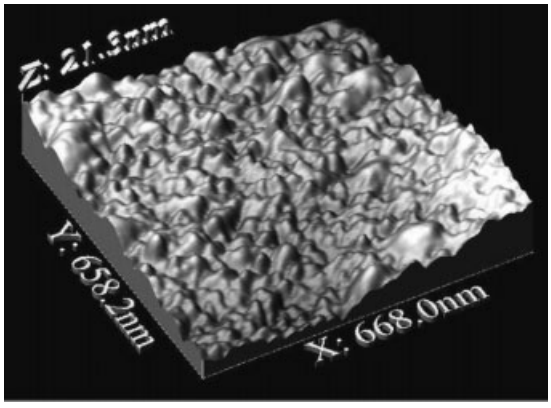
Stable Aqueous Dispersion of PEGylated Nanoparticles by Polyelectrolyte Complex Formation

Dilyana Paneva, Laetitia Mespouille, François Bougard, Nevena Manolova, Philippe Degée, Iliya Rashkov, Philippe Dubois*

Stable aqueous dispersions of nanoparticles were prepared by polyelectrolyte complex formation between well-defined poly(ethylene glycol)-*block*-poly(2-acrylamido-2-methyl-1-propane sodium sulfonate) and poly(ethylene glycol)-*block*-poly[2-(dimethylamino)ethyl methacrylate] diblock copolymers. Controlled synthesis of the copolymers was achieved by water-based atom transfer radical polymerization (ATRP). The nanoparticles were characterized by a quite narrow and monomodal size distribution as evidenced by dynamic light scattering (DLS) and confirmed by atomic force microscopy (AFM) after solution casting and freeze-drying.



Introduction

Depending on their composition and sequence length, amphiphilic block copolymers spontaneously form spherical, rod-like, lamellar, or vesicular aggregates in selective solvents. One typical form of block copolymer assembly is polymeric micelles with a hydrophobic core surrounded by a hydrophilic corona shell. They are of special interest as drug delivery systems with pharmacokinetics governed by their sizes and surface properties rather than by loaded drug properties.[1] When formed through electrostatic interactions between pairs of oppositely charged polymers, entropy gain resulting from low-molecular weight counterions release constitutes the driving force for nearly spherical polyelectrolyte complexes (PECs) in aqueous solutions.[2] As the effective charge ratio approaches unity, neutralization leads to water-insoluble complexes which may further aggregate up to macrophase segregation. However, the use of diblock copolymers composed of neutral poly(ethylene glycol) (PEG) segment and polyelectrolyte block opens the way for the preparation of stabilized aqueous colloids preventing the secondary aggregation of nanoparticles.[3] Moreover, the obtained nanoparticles are characterized by narrow size distribution and sizes centered around 50 nm.[4,5] In drug delivery systems,

D. Paneva, N. Manolova, I. Rashkov
Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
D. Paneva, L. Mespouille, F. Bougard, N. Manolova, P. Degée, P. Dubois
Laboratory of Polymeric and Composite Materials, University of Mons-Hainaut, Place du Parc 20, B-7000 Mons, Belgium
E-mail: philippe.dubois@umh.ac.be

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PEG segments also improve the biocompatibility of polymer materials suppressing non-specific interactions with blood proteins and leading to stealthy PEC nanoparticles.[6]

Recently, we reported a comprehensive study on the formation of PECs between poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) synthesized by atom transfer radical polymerization (ATRP) and poly(2-acrylamido-2-methyl-1-propane sodium sulfonate) (PAMPSNa) polymerized via redox-initiation reaction. It was shown how parameters such as pH, polycation-to-polyanion molar ratio, and polymer concentration in aqueous solutions could affect PECs' formation, i.e., yield, size, swelling degree, and stability of the PECs.[7] Next, we reported on the preparation of well-defined poly(ethylene glycol)-*block*-poly(2-acrylamido-2-methyl-1-propane sodium sulfonate) [PEG-*b*-PAMPS] block copolymers with predictable molar masses and narrow polydispersity indices (PDI $\approx$ 1.4 at 80% conversion) by water-based ATRP.[8]

Herein, we aim at describing the preparation of stabilized nanoparticle dispersions as potential drug delivery systems by mixing aqueous solutions of well-defined block copolymers PEG-*b*-PDMAEMA and PEG-*b*-PAMPS with similar molar masses of the polyelectrolyte block, both prepared by controlled ATRP (Scheme 1). Effect of PEG molar mass on the stability and sizes of the PEC particles was examined by dynamic light scattering (DLS) and atomic force microscopy (AFM). As far as the latter technique is concerned, a special two-step procedure involving solution casting, followed by freeze-drying was applied which leveled off the substrate effect and preserved nanoparticles' geometry from solution to freeze-dried state.

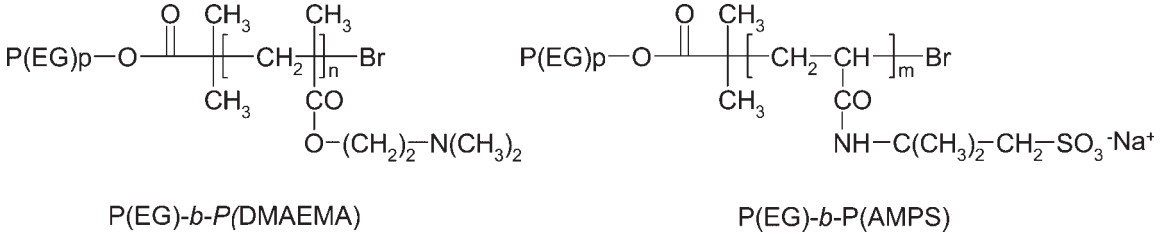
Experimental Part

Materials

2-Acrylamido-2-methyl-1-propane sulfonic acid (AMPS, 99%, MW = 207.25 g · mol^{-1}, Aldrich), 2-bromo-2-methylpropionylbromide (Br^{i}BBr, 98%, MW = 229.90 g · mol^{-1}, Aldrich), α-methoxy-ω-hydroxy poly(ethylene oxide) oligomers (MeO-P[EG]$_p$-OH; p = 12 ($\overline{M}_n$ = 550 g · mol^{-1}, PDI = 1.06, Aldrich), and p = 113 ($\overline{M}_n$ = 5 000 g · mol^{-1}, PDI = 1.06, Fluka)), 2,2′-bipyridyl (Bpy, >99%, MW = 156.18 g · mol^{-1}, Aldrich) and copper (I) bromide (CuBr, >99%, MW = 143.45 g · mol^{-1}, Aldrich) were used as received. Triethylamine (99%, MW = 101.19 g · mol^{-1}, Fluka) was dried over barium oxide for 48 h and distilled just before use. Tetrahydrofuran (THF, Labscan, 99%) and 2-(dimethylamino)ethyl methacrylate (DMAEMA, Aldrich) were passed through a basic alumina column to remove the stabilizing agents. The monomer was then stored under N_2 at −20 °C. All other reagents were of analytical grade, and used as received.

Synthesis and Characterization of PEG-*b*-PDMAEMA and PEG-*b*-PAMPS Block Copolymers

PEG-based diblock copolymers were synthesized by using α-methoxy-ω-(2-bromo-2-methylisobutyrate) poly(ethylene glycol) (MeO-P(EG)$_p$-Br^{i}B) with p = 12 or 113 as macroinitiators. As already reported,[8] quantitative esterification between MeO-P(EG)$_p$-OH and 2-bromo-2-methylpropionylbromide (Br^{i}BBr) was achieved after 24 h in THF at rt. using a slight molar excess of Br^{i}BBr and triethylamine. Poly(ethylene glycol)-*block*-poly[2-(dimethylamino) ethyl methacrylate] [P(EG)$_p$-*b*-P(DMAEMA)$_n$] diblock copolymers (Table 1, entries 1 and 2) were obtained by water-based ATRP, according to a procedure disclosed by Armes.[9] Briefly, the polymerization of DMAEMA ([DMAEMA]$_0$ = 1.5 mol · L^{-1}) was conducted in water/isopropyl alcohol mixtures (1:1 v/v) in the presence of NaCl using MeO-P(EG)$_p$-Br^{i}B with p of 12 or 113 as macroinitiators and CuBr · 2Bpy as the catalyst ([DMAEMA]$_0$/[MeO-P(EG)$_p$-Br^{i}B]$_0$/[CuBr]$_0$/[bpy]$_0$/[NaCl]$_0$ = 70:1:1:2:2). It comes out that P(EG)$_p$-*b*-P(DMAEMA)$_n$ diblock copolymers have compositions very close to theoretical values assuming a living process and quite narrow molar mass distributions as determined by ^{1}H NMR and SEC (in THF + 2 wt.-% NEt_3 at 35 °C according to a poly(methyl methacrylate) calibration), respectively (Table 1, entries 1 and 2). P(EG)$_p$-*b*-P(AMPS)$_m$ diblock copolymers were prepared according to our recently published procedure.[8] Controlled water-based AMPS copolymerization was initiated by MeO-P(EG)$_p$-Br^{i}B (with p = 12 or 113) in water/methanol mixtures (3:1 v/v) at 60 °C and at pH 10 for 3 h using CuBr · 2Bpy as the catalyst and NaCl as extra halide ions ([AMPS]$_0$ = 1.2 mol · L^{-1} and [AMPS]/[MeO-P(EG)$_p$-Br^{i}B]/[CuBr]/[Bpy]/[NaCl] = 70:1:1:2:2). Table 1 (entries 3 and 4) shows macromolecular characteristics of the diblock copolymers as determined by ^{1}H NMR and SEC as performed in 0.05 M CH_3COOH/CH_3COONa aqueous solution at 30 °C with respect to a poly(ethylene oxide) calibration. Whatever the copolymer, it was carefully purified from copper residues by filtration through alumina or silica gel column and dialysis so that the metal content lower than 1 ppm was determined by ICP-AES.[7,8,10]



Scheme 1. Sketch of PEGylated copolymers used for PEC nanoparticle formation (for details, see Table 1).

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Table 1. Composition and macromolecular characteristics of DMAEMA- and AMPSNa-based copolymers used for PEC preparation.

Entry	Copolymer	Conversion[a)]	Polyelectrolyte block			Copolymers
		%	$\overline{M}_{n,\,theor.}$[b)]	$\overline{M}_{n,\,exp.}$[c)]	f[d)]	PDI[e)]
1	$P(EG)_{12}$-*b*-$P(DMAEMA)_{67}$	89	10 400	10 500	0.99	1.34
2	$P(EG)_{113}$-*b*-$P(DMAEMA)_{65}$	80	8 800	10 200	0.86	1.38
3	$P(EG)_{12}$-*b*-$P(AMPS)_{60}$	60	7 800	13 700	0.56	1.39
4	$P(EG)_{113}$-*b*-$P(AMPS)_{70}$	81	13 000	16 000	0.80	1.40

[a)]Conversion as determined by gravimetry; [b)]Theoretical number-average molar mass as calculated by $\overline{M}_{n,theor.}$ = [monomer]/[initiator] × conv. × MW (monomer) assuming a living process; [c)]Experimental number-average molar mass as determined by ^{1}H NMR spectroscopy: $\overline{M}_{n,exp.} = \overline{DP}_{exp\ exp.} \times$ MW(monomer) with $\overline{DP}_{exp}$ calculated from the relative intensities of the methylene protons of PEG sequence at 3.65 ppm and either methylene protons of CH_2–SO_3Na in PAMPS at 3.32 ppm or α-methylene amino protons of PDMAEMA at 2.55 ppm; [d)]Initiation efficiency as calculated from $\overline{M}_{n,theor.}/\overline{M}_{n,exp.}$; [e)]Polydispersity index (PDI) as determined by SEC (the conditions for SEC analyses are given in the text).

Preparation and Characterization of Aqueous Dispersions of PEGylated Nanoparticles by Polyelectrolyte Complex Formation

Generally speaking, PECs were formed by mixing aqueous solutions of the diblock copolymers PEG-*b*-PDMAEMA and PEG-*b*-PAMPS for various initial concentrations (from 0.01 to 0.1 mg · L^{-1}) and polycation-to-polyanion molar ratios (from 1:9 to 9:1). Tertiary amino groups of PDMAEMA block ($pK_a \approx 7.0$)[11] were first converted into their ammonium salts by dropwise addition of glacial acetic acid into deionized water copolymer solutions to reach pH 4. As previously shown, such conditions are required to achieve the maximum yield in macroscopic PECs for a polycation-to-polyanion molar ratio of 1.[7] Each polyelectrolyte copolymers' solution was then filtrated through 1.2 μm Acrodisk® filter before mixing it vigorously with its counterpart for 2 h at rt. As a rule, the higher polyelectrolyte volume was added to the smaller one dropwise with a syringe. In order to form stable aqueous dispersions from $P(EG)_{12}$-*b*-$P(DMAEMA)_{70}$ and $P(EG)_{12}$-*b*-$P(AMPS)_{60}$, initial solutions' concentration had to be lowered down to 0.05 mg · mL^{-1} (Table 2, entry 1).

DLS was carried out after 48 h using a Brookhaven Instruments BI-160 goniometer equipped with a BI-9000AT digital correlator. A He-Ne laser operating at 633 nm wavelength and 17 mW power was used as a light source for the scattering angle of 90° while non-negatively constrained least squares: regularized protocol (CONTIN) was applied. The AFM images were obtained by using a Nanoscope IIIA Multimode microscope operating in intermittent contact (tapping mode) under air and silicium (Si) probes. For each sample, a drop of aqueous dispersion of PEC nanoparticles was deposited onto Si, followed by freeze-drying.

Results and Discussions

Recently, it has been shown that the molar mass of the PEG block in polyelectrolyte-containing copolymers has a significant effect on the aggregation level of the PECs, especially in terms of the stoichiometric ones.[3,5,12] As seen from Table 2, for PEG-*b*-PDMAEMA/PEG-*b*-PAMPS complexes at polycation-to-polyanion molar ratio of 1, the concentration for the preparation of stable aqueous dispersions depends strongly on PEG molar mass. The higher the PEG block molar mass the higher the initial solution concentration for the formation of stable aqueous PEC dispersions and the lower the particle size. The complex between $P(EG)_{12}$-*b*-$P(DMAEMA)_{70}$ and $P(EG)_{12}$-*b*-$P(AMPS)_{60}$ for a polycation-to-polyanion molar ratio of 1 and an initial solution concentration of 0.05 mg · mL^{-1} forms a stable but turbid dispersion of secondary aggregates with apparent mean average size of ca. 500 nm (Table 2, entry 1). This is consistent with the poor stabilizing effect of short PEG

Table 2. Mean apparent size of stoichiometric PECs aqueous dispersions as determined by DLS.

Entry	PECs composition[a)]	Mean apparent size
		nm
1	$P(EO)_{12}$-*b*-$P(DMAEMA)_{70}$/$P(EO)_{12}$-*b*-$P(AMPS)_{60}$ (0.05 mg · mL^{-1})	500 ± 0.080
2	$P(EO)_{113}$-*b*-$P(DMAEMA)_{65}$/$P(EO)_{113}$-*b*-$P(AMPS)_{70}$ (0.1 mg · mL^{-1})	50 ± 0.025

[a)]The initial concentration of the copolymers is given in brackets.

segments at least for the initial polycation-to-polyanion molar ratio close to 1.

In sharp contrast, the PECs' dispersions obtained from $P(EG)_{113}$-*b*-$P(DMAEMA)_{65}$ and $P(EG)_{113}$-*b*-$P(AMPS)_{70}$ diblock copolymers did not turn turbid, whatever the polycation-to-polyanion molar ratio (Table 2, entry 2), thus confirming the key role of PEG length and its spatial contribution on PECs stabilization, even for polyelectrolytes of similar polymerization degrees. Starting from initial copolymer solutions of 0.1 $mg \cdot mL^{-1}$ and polycation-to-polyanion ratio of 1, a quite narrow and monomodal PECs' size distribution was evidenced by DLS (Figure 1). There is neither obvious macroscopic precipitation nor secondary aggregate formation when PEG segments are long enough ($\overline{M}_{n,PEG} = 5\,000$). Such an observation is corroborated by AFM images of PEC nanoparticles (Figure 2). First, mica was used as a substrate but due to its preferred interactions with polycations, particles tend to merge and flatten out. Then, mica was substituted for Si which shows higher inertness. A layer of joined nanoparticles was obtained through the deposition of a drop of aqueous dispersion of PEC nanoparticles onto Si. In order to preserve the shape and the size of the nanoparticles close to those in the swollen state, thus enabling the comparison of the data from the DLS and AFM analyses, the AFM images were taken after freeze-drying of the dispersion deposited onto Si. Hemispherical particles with mean diameters from 25 to 60 nm are imaged, in good agreement with the mean apparent particle size determined by the DLS analysis (Table 2, entry 2; Figure 1). In other words, the applied two-step procedure allows preserving nanoparticles' geometry from solution to freeze-dried state. It should be noted that the dispersions were stable in the whole pH range, in which the nanoparticles existed. At pH values higher than 8, the PECs disintegrated and PEG-*b*-PDMAEMA and PEG-*b*-PAMPS dissolved. The nanoparticles did not aggregate either in acidic or in neutral medium and the obtained aqueous dispersions were stable against aggregation for more than one-month stay at ambient temperatures.

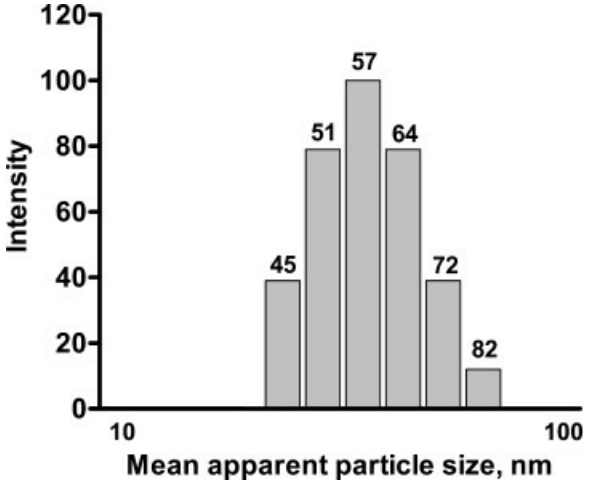

Figure 1. DLS of PECs as obtained by mixing 0.1 $mg \cdot mL^{-1}$ aqueous solutions of $P(EG)_{113}$-*b*-$P(DMAEMA)_{65}$ and $P(EG)_{113}$-*b*-$P(AMPS)_{70}$ for a polycation-to-polyanion molar ratio of 1.

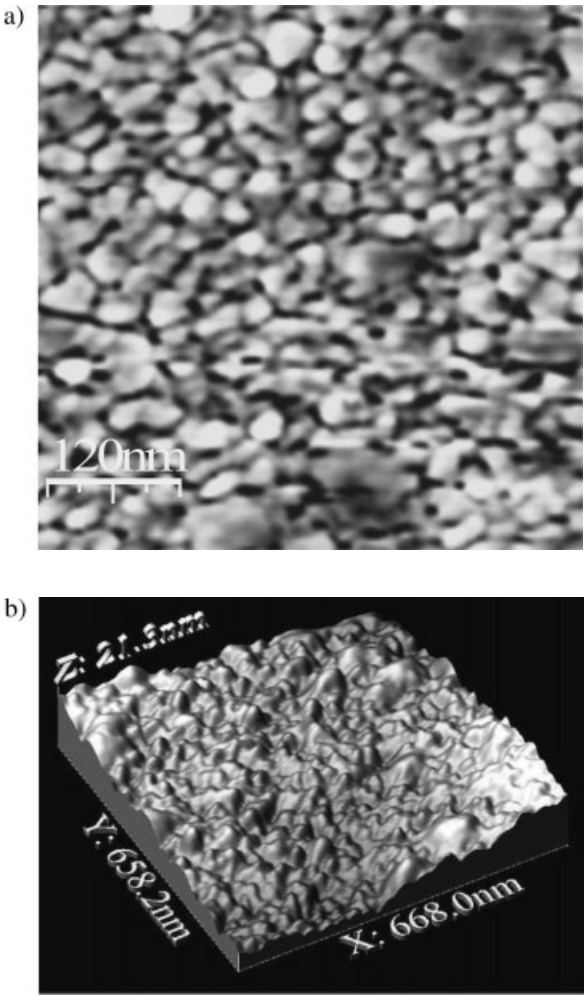

Figure 2. 2D phase (a) and 3D height (b) AFM micrograph of solution-cast and freeze-dried PECs made from $P(EG)_{113}$-*b*-$P(DMAEMA)_{65}$/$P(EG)_{113}$-*b*-$P(AMPS)_{70}$ for a polycation-to-polyanion ratio of 1 (on silicium substrate).

Conclusion

The preliminary studies on the PEC formation between PEG-*b*-PDMAEMA and PEG-*b*-PAMPS have revealed that the complex formation is a very convenient and straight approach towards the production of nanoparticle stabilized dispersion based on these polymer partners. Water-based ATRP of functional methacrylates and acrylamides allows synthesizing a large range of PEGylated diblock

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Macromolecular Journals

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copolymers with controlled molar masses and polydispersity indices. Combining PEG neutral segment to polyelectrolyte block allows preparing nanoparticle dispersions with a mean diameter of ca. 60 nm. Since the block copolymers reported herein have never been reported earlier at least via such a well-controlled copolymerization process, the formation of the PEGylated nanoparticles obtained from the resulting polyelectrolyte complex and the characterization of their stable aqueous dispersion appear to be original at least to the best of our knowledge. One could accordingly expect an attractive behavior of these as drug delivery nanoparticles. Such an application is currently under investigation and will be the subject of a forthcoming publication.

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