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# Preparation and characterization of water-soluble pH-sensitive nanocarriers for drug delivery

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**Abstract**

pH-sensitive drug delivery systems can be engineered to release their contents or change their physicochemical properties in response to variations in the acidity of the surroundings. The present work describes the preparation and characterization of novel polymeric micelles (PM) composed of amphiphilic pH-responsive poly(*N*-isopropylacrylamide) (PNIPAM) or poly(alkyl(meth)acrylate) derivatives. On one hand, acidification of the PNIPAM copolymers induces a coil-to-globule transition that can be exploited to destabilize the intracellular vesicle membranes. In this work, PNIPAM-based PM were loaded with either doxorubicin or aluminium chloride phthalocyanine and their cytotoxicity was assessed in murine tumoral models. On the other hand, poly(alkyl(meth)acrylate) copolymers can be designed to interact with either hydrophobic drugs or polyions and release their cargo upon an increase in pH.


*Keywords:* Block copolymers; pH-controlled release; Polymeric micelles; Anticancer agents; Poorly soluble drugs

## 1. Introduction

Over the last two decades, the field of drug delivery has witnessed the emergence of nanoscale systems such as polymeric micelles (PM) (Kataoka et al., 2001; Jones and Leroux, 1999), nanoparticles (Torchilin, 2000; Moghimi et al., 2001) and polymer conjugates (Duncan et al., 2001). In particular, PM were developed as a valuable alternative to "classical" low molecular weight surfactants (e.g. polysorbate and Cremophor® EL) owing to their relatively low toxicity and high drug loading capacity (Fig. 1A) (Torchilin, 1998). Furthermore, such core-shell assemblies obtained via the hydrophobic association of amphiphilic polymers generally exhibit low critical association concentrations (CAC), resulting in remarkable stability in solution. Recently, Kataoka et al. (1996) proposed to rely on the contribution of forces other than hydrophobic interactions to trigger the self-assembly of polymeric chains into micelles. Micellar formation was indeed successfully induced by electrostatic interactions between oppositely charged polymers and macromolecular drugs (e.g. oligonucleotides and plasmid DNA (Kakizawa and Kataoka, 2002)) (Fig. 1B) to yield so-called polyion complex micelles (PICM). This novel type of PM might overcome diverse problems met in gene and antisense therapy (Harada-Shiba et al., 2002). Efforts are now

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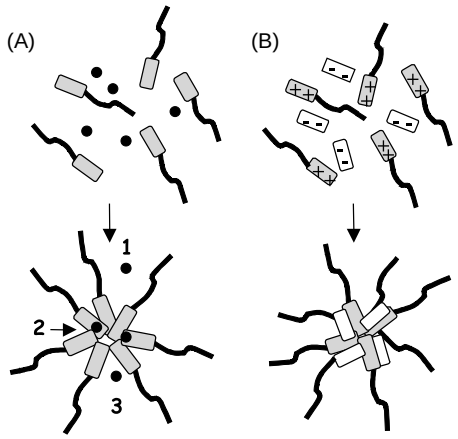


Fig. 1. Micelle formation: (A) core-shell assemblies capable of entrapping hydrophobic drugs and (B) formation of PICM from neutralization and condensation of oppositely charged polymer and drug.

being directed at solving the stability problems occurring with PICM in solution (Kataoka et al., 2001; Kakizawa et al., 1999; Kakizawa and Kataoka, 2002).

PM present numerous advantages, including their straightforward synthesis through self-association in aqueous milieu. The architectural features of PM further grant the system with valuable properties. The highly hydrated corona, composed of flexible and non-ionic polymer chains, can prevent the adsorption of opsonins on the carrier, thereby reducing its recognition by phagocytic cells and the ensuing rapid clearance. The stealth properties of PM can thus lead to increased circulation times in the bloodstream (Allen et al., 1999). Additionally, the core-shell structure provides a protective palisade against external stresses. As shown in Fig. 1A, incorporated poorly water-soluble drug molecules will partition in the outer shell (position 1), inner core (position 2), or at their interface (position 3), with core segregation being the most common case.

Another feature of PM is their small size (generally inferior to 100 nm) that can induce substantial changes in drug biodistribution. For instance, solid tumors present leaky vasculatures from which small micelles can spontaneously extravasate. PM loaded with anticancer drugs can thus be used to passively target the tumor via this enhanced permeation and retention effect (Jones and Leroux, 1999; Kabanov et al., 1989; Maeda et al., 2000; Yokoyama, 1998). Active targeting is also feasible by modifying the micelle so as to allow chemical recognition by a specific cell receptor (Kabanov et al., 1989; Yamamoto et al., 2001) or to induce a response to external stimuli. For instance, ligands such as sugar derivatives (Nagasaki et al., 2001) and antibodies (Kabanov et al., 1989; Rammohan et al., 2001) can be grafted at the micellar surface. The lock-and-key binding between the ligand and the specific receptor can then trigger PM endocytosis. Alternatively, drug carriers composed of stimuli-sensitive polymers can be designed to respond to variations in temperature (Cammas et al., 1997; Kim et al., 2000) and pH (Leroux et al., 2001) or to ultrasounds (Rapoport et al., 1999; Marin et al., 2001).

Recently, our group has succeeded in synthesizing a series of pH-responsive PM composed of randomly or terminally alkylated *N*-isopropylacrylamide (NIPAM) copolymers (Taillefer et al., 2000). The in vitro $IC_{50}$ of the photosensitizer aluminium chloride phthalocyanine (AlClPc) against EMT-6 murine mammary tumoral cells increased 2–10-fold when loaded into PNIPAM-based micelles versus control AlClPc/Cremophor® EL formulation (Taillefer et al., 2001). Control assays with empty micelles further revealed that the PNIPAM copolymers were practically not cytotoxic at the doses demonstrating in vitro activity (Le Garrec et al., 2002). In this work, we expound in details our current developments on pH-sensitive copolymers consisting of either PNIPAM derivatives or various poly(alkyl(meth)acrylate) diblock copolymers. These carriers should most likely improve existing drug delivery systems in terms of safety, drug targeting, bioavailability, and drug efficacy.

## 2. Materials and methods

### 2.1. Materials

All reagents were purchased from Aldrich. Copper(I) bromide (99.99% grade), anhydrous triethylamine, *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA), and *N*,*N*-dimethylformamide (DMF) were used without further purification. All vinyl monomers were distilled (or recrystallized) before polymerization. Tetrahydrofuran (THF) was distilled over sodium, with benzophenone as the drying indicator.

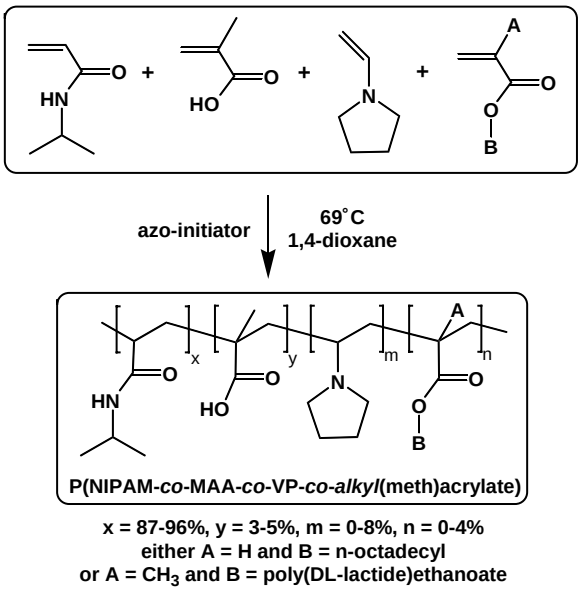


Fig. 2. Synthesis of pH-sensitive PNIPAM derivatives.

### 2.2. Preparation of pH-sensitive PNIPAM derivatives

Randomly alkylated PNIPAM copolymers were synthesized by free radical polymerization, as shown in Fig. 2 (Le Garrec et al., 2001, 2002). NIPAM, methacrylic acid (MAA, pH-sensitive moiety), *n*-octadecylacrylate (ODA, hydrophobic anchor), *N*-vinyl-2-pyrrolidone (VP, at different molar ratios as indicated by the monomers' subscripts) and 1,1′-azobis (cyclohexanecarbonitrile) (1.3 mol.%) were dissolved in distilled 1,4-dioxane (Le Garrec et al., 2002). For some experiments, the alkyl chain anchor was replaced by a hydrophobic macromolecular monomer, i.e. (poly(D,L-lactide)-ethanoate)methacrylate (HEMA-PDLLA) (Le Garrec et al., 2001). The reaction mixture was degassed under nitrogen for 15 min and polymerization carried out at 69 °C for 15 h. Polymers were recovered by precipitation in diethyl ether, resolubilized in THF, reprecipitated and washed extensively with diethyl ether. The polymers were finally dialyzed against water over 2 days (Spectra/Por No.1, molecular weight cut-off: 6000–8000), and isolated by freeze-drying.

Terminally alkylated PNIPAM copolymers were prepared following the same general procedure. More specifically, ODA and the alkylacrylate monomers were omitted ($n = 0$, Fig. 2) and the azo initiator replaced by 4,4′-azobis(4-cyano-*N*,*N*-dioctadecyl) pentanamide (DODA, 1.3 mol.%) in order to get two hydrophobic anchors per chain end (Le Garrec et al., 2002).

### 2.3. Preparation of (meth)acrylate-based diblock copolymers

Atom transfer radical polymerization (ATRP) of monomers was carried out in solution, using α-(2-bromoisobutyrylate)-ω-methoxypoly(ethylene glycol) as the ATRP macroinitiator ($M_n$: 2100) (Fig. 3; Ranger et al., 2001). The poly(ethylene glycol) (PEG) ATRP macroinitiator (1 eq.) was added to a solution containing PMDETA (1.1 eq.), copper (I) bromide (CuBr, 1.1 eq.) and monomers (5–50 eq., concentration of ∼0.8 M) in THF. The mixture was degassed with argon for 15–20 min at room temperature and the reaction carried out at 60 °C for 16 h. The polymerization reaction was stopped by pouring the mixture on a 10% ethanol/THF solution and by removing the copper complex by filtration on silica gel, with THF as eluent. Finally, the polymers were dialyzed against water over 2 days and recovered by freeze-drying. Ethylacrylate (EA) served as a hydrophobic monomer while *tert*-butylmethacrylate (*t*BMA) provided ionizable carboxylic acid units upon hydrolysis of the ester groups. Alternatively, amine-based methacrylate derivatives were used to introduce basic units.

### 2.4. Characterization of copolymers

Number ($M_n$) and weight-average ($M_w$) molecular weights were determined by size-exclusion chromatography (SEC) on a system equipped with a differential refractive index detector. SEC analyses were performed in THF, using monodispersed PEG standards for calibration. The polymer composition was determined by $^1$H NMR (600 MHz) in $CDCl_3$ with a relaxation time of 10 s. The $M_n$ of (meth)acrylate-based diblock copolymers was also obtained by $^1$H NMR via integration of the PEG terminal methoxy group.

### 2.5. Characterization of pH-sensitive PM

Particle size and distribution in aqueous media were obtained by dynamic light scattering (DLS) at 20 °C as

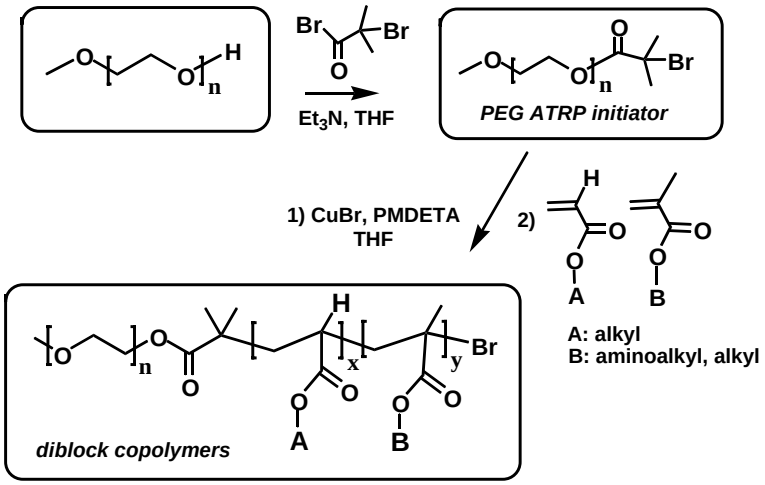


Fig. 3. Synthesis of (meth)acrylate-based diblock copolymers.

reported earlier (Ranger et al., 2001). Apparent CAC were determined by fluorescence spectroscopy using pyrene as a probe (Jones and Leroux, 1999; Astafevia et al., 1993). The pH of polymer phase transition was determined by static light-scattering at 480 nm in phosphate-buffered saline (PBS) at 37 °C (Le Garrec et al., 2002).

### 2.6. Drug loading method

Indomethacin, fenofibrate, and the anticancer drugs doxorubicin (DOX) and AlClPc were loaded into PM by a dialysis procedure as described elsewhere (Taillefer et al., 2000). Briefly, the polymer and the drug were dissolved in DMF and dialyzed for 24 h against water. Dialyzed solutions were filtered through 0.22 μm filters and recovered by lyophilization. Both DOX and AlClPc contents were assayed by spectrophotometry (Taillefer et al., 2000).

### 2.7. In vitro and in vivo assays

The cytotoxicity of drug-free and DOX-loaded PNIPAM-based micelles was assessed on C26 murine colon adenocarcinoma and EMT-6 murine mammary tumor cells (Fig. 4). In addition, PNIPAM micelles loaded with AlClPc were tested in vivo on female Balb/c mice (6–8 weeks) bearing i.d. implanted EMT-6 tumors (Le Garrec et al., 2002). Their in vivo antitumor activity was compared to AlClPc-Cremophor® EL micelles. Light at 650–700 nm (200 mW/cm$^2$ for a total light dose of 400 J/cm$^2$) was used for photoactivation of the drug (Le Garrec et al., 2002). Single-injection doses of 0.05 and 0.1 μmol/kg of AlClPc were tested for both PM and Cremophor® EL formulations.

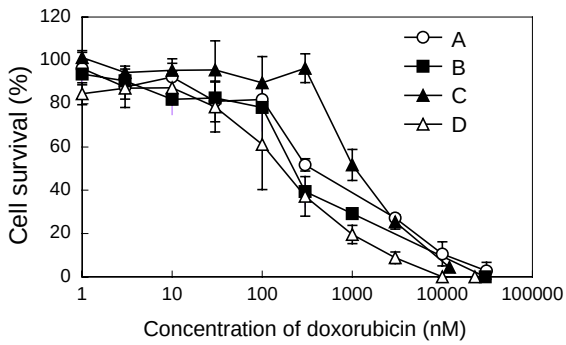


Fig. 4. In vitro cell survival after 72 h incubation with free DOX (A, open circle), DOX loaded in P(NIPAM$_{90}$-*co*-ODA$_{0.5}$-*co*-MAA$_{4.5}$-*co*-VP$_5$) (B, closed squares), in P(NIPAM$_{94}$-*co*-MAA$_{5.5}$-*co*-(HEMA-P(DLLA))$_{0.5}$) (C, closed triangles), and in P(NIPAM$_{94}$-*co*-MAA$_{5.8}$-*co*-(HEMA-P(DLLA))$_{0.2}$) (D, open triangles).

## 3. Results and discussion

### 3.1. Synthesis and characterization of pH-sensitive PNIPAM copolymers

Free radical polymerization using azo initiators led to a series of PNIPAM derivatives with alkyl chains located either at one chain end (DODA-based copolymers) or randomly distributed along the main chain (ODA-based copolymers). The ODA units could be replaced by the macromolecular monomer HEMA-PDLLA, which had an average polymerization degree of 12. The HEMA-PDLLA monomer was prepared from 2-hydroxyethylmethacrylate (HEMA) and D,L-lactide using aluminium catalyst, as previously reported (Barakat et al., 1994). In spite of the poor control over molecular weight, free radical polymerization yielded polymers small enough to be eliminated by renal filtration, with $M_n$ varying between 8000 and 14,000 (Table 1). $^1$H NMR analysis showed that there was a good correlation between the feed ratio and resulting polymer composition, except for VP. At most 50% of the VP input was incorporated into copolymer chains because of its low reactivity compared to (meth)acrylate monomer derivatives (de Queiro et al., 2000).

### 3.2. Characterization of PNIPAM-based micelles

PNIPAM copolymers containing hydrophobic moieties (i.e. DODA, ODA or PDLLA) and pH-sensitive units (i.e. MAA) self-assembled in water into micellar structures that could find applications as parenteral formulations. Size measurements usually revealed two distinct populations. Assemblies of about 18–40 nm were associated to single micelles, whereas the largest population (up to 400 nm) was attributed to secondary aggregates.

The incorporation of VP along the main chain did not significantly influence the size distribution and CAC measurements (Table 1). However, its presence slightly lowered the pH of phase transition from 6.05 to 5.81 for polymers displaying similar DODA and ODA contents. As expected, the pH of phase transition also decreased as the MAA content increased.

ODA-based copolymers exhibited lower CAC than terminally alkylated copolymers (33 mg/l versus 10 mg/l) while the substitution of ODA by PDLLA in-

Table 1
Characterization of pH-sensitive PNIPAM-based copolymers and micelles

| Polymer[a] | $M_n$[b] | PI[c] | Phase transition pH at 37 °C (±0.01) | CAC, in water at 20 °C (mg/l) | Micelle size[d] in PBS at 20 °C (nm) | | | Drug loading (% w/w) |
|---|---|---|---|---|---|---|---|---|
| P(NIPAM$_{87}$-*co*-MAA$_3$-*co*-ODA$_2$-*co*-VP$_8$) | 9,500 | 2.8 | 5.81 | 10 | 115 ± 33 (27%) | 27 ± 7 (62%) | <3 (11%) | AlClPc: 2.6 |
| DODA-P(NIPAM$_{96}$-*co*-MAA$_3$) | 8,000 | 2.2 | 6.05 | 34 | 98 ± 32 (36%) | 30 ± 8 (64%) | | AlClPc: 2.5 |
| DODA-P(NIPAM$_{88}$-*co*-MAA$_3$-*co*-VP$_8$) | 14,000 | 2.1 | 5.81 | 33 | 354 ± 98 (10%) | 30 ± 14 (80%) | <3 (10%) | AlClPc: 2.7 |
| P(NIPAM$_{90}$-*co*-MAA$_{4.5}$-*co*-ODA$_{0.5}$-*co*-VP$_5$) | 12,000 | 2.1 | 5.60 | 23 | 164 ± 32 (17%) | 40 ± 19 (83%) | | DOX: 2.1 |
| P(NIPAM$_{94}$-*co*-MAA$_{5.5}$-*co*-(HEMA-P(DLLA))$_{0.5}$) | 9,500 | 3.1 | 5.60 | 50 | 121 ± 38 (58%) | 28 ± 7 (10%) | <3 (33%) | DOX: 5.1 |
| P(NIPAM$_{94}$-*co*-MAA$_{5.8}$-*co*-(HEMA-P(DLLA))$_{0.2}$) | 12,000 | 3.0 | 5.45 | 100 | 195 ± 50 (48%) | 18 ± 3 (5%) | <3 (47%) | DOX: 4.5 |

[a] Numbers in subscript correspond to the concentration of monomer units in mol%.
[b] SEC analysis.
[c] Polydispersity index $M_w/M_n$ by SEC analysis.
[d] Mean ± S.D. of three measurements.

duced a major increase of the CAC. Up to 47% of the PNIPAM copolymers bearing PDLLA side chains existed as unimers (isolated polymer chains) in solution (Table 1). These results suggest that some of the polymer chains were free of PDLLA as a combined result of its very low content in the composition and the high polydispersity indices (PI) of polymers. Nevertheless, the highest drug loading levels were achieved with PNIPAM-based micelles containing PDLLA chains.

### 3.3. In vitro cytotoxicity of unloaded and DOX-loaded PNIPAM-based micelles

A previous in vitro study carried out in our laboratory demonstrated that AlClPc-loaded PNIPAM-based micelles presented enhanced activity on EMT-6 murine mammary tumor cells as compared to AlClPc-Cremophor® EL (Taillefer et al., 2001). Increased activity of pH-sensitive PM was attributed to endosomal membrane destabilization and possible cytoplasmic release of AlClPc following uptake by EMT-6 cells. It was also clearly shown that NIPAM copolymers (without drug) exhibited no significant cytotoxicity at the range of concentrations studied (Le Garrec et al., 2002).

Fig. 4 presents the cell survival of C26 murine colon adenocarcinoma cells following treatment with DOX. DOX-loaded micelles prepared with polymers B or D showed higher cytotoxic activity against C26 cells ($IC_{50}$: 347 and 243 nM, respectively) than free DOX (A, $IC_{50}$: 725 nM), while DOX-loaded micelles using polymer C were less cytotoxic ($IC_{50}$: 1164 nM). However, the increase in the cytotoxic activities of polymeric formulations B and D partially ensued from the intrinsic toxicity of the polymers themselves. Indeed, those NIPAM copolymers induced as much as 30% cell growth inhibition (data not shown) such that the net difference between the $IC_{50}$ of the polymeric formulations and free DOX vanishes. DOX is less hydrophobic than AlClPc and is thus probably released from PM prior to their endocytosis.

### 3.4. In vivo antitumor activity of AlClPc-loaded PNIPAM-based micelles

Taillefer et al. (2001) reported complete regression of EMT-6 tumors after i.v. administration of either AlClPc-loaded PM or AlClPc-Cremophor® EL at a dose of 0.25 μmol/kg. Both formulations did not show any preferential accumulation in tumors (Le Garrec et al., 2002).

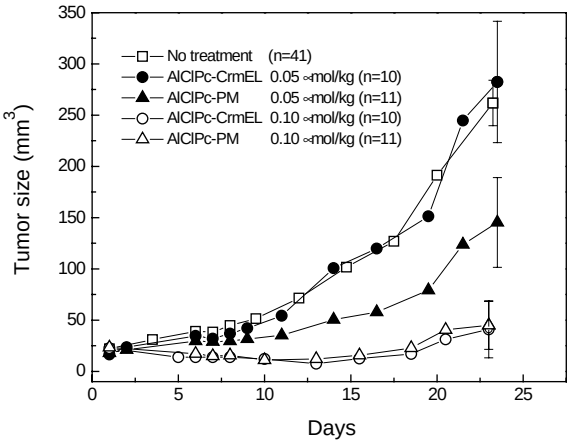


Fig. 5. In vivo antitumor activity of AlClPc incorporated into DODA-P(NIPAM$_{88}$-*co*-MAA$_3$-*co*-VP$_8$) PM (triangles) and Cremophor® EL (circles) after i.v. bolus injection of 0.05 and 0.10 μmol/kg in mice bearing EMT-6 tumor. Control mice (open squares) received no treatment. The SEM is shown on the last data point of each curve. Adapted from Le Garrec et al. (2002).

Since at 0.25 μmol/kg complete tumor regression was observed for PM and the control formulation, two different subtherapeutic AlClPc doses (0.05 and 0.1 μmol/kg) were administered to evaluate tumor growth after treatment. Fig. 5 shows that the antitumor activity of AlClPc incorporated into PM seemed to be improved in comparison with the AlClPc-Cremophor® EL formulation at 0.05 μmol/kg, although the differences in tumor volume were not statistically significant ($P > 0.05$ after 21 and 23 days). However, at that dose, photodynamic therapy with AlClPc-loaded PM yielded a higher incidence of cured mice (36%) compared to AlClPc-Cremophor® EL (10%) (data not shown).

### 3.5. (Meth)acrylate-based anionic diblock copolymers

Over the last decades, a wide variety of amphiphilic poly(methacrylate) and poly(acrylate) derivatives have been synthesized via “living”/controlled radical polymerization methods such as nitroxide-mediated polymerization (Benoit et al., 1999), reversible addition–fragmentation chain transfer (RAFT) poly-

Table 2
Characterization of PEG-*b*-(EA-*co*-MAA)

| Polymer[a] | $M_n$[b] | PI[c] | CAC (mg/l) in water at 20 °C | Size(nm)[d], in water at 20 °C | | |
|---|---|---|---|---|---|---|
| PEG-*b*-P($EA_7$-*co*-$MAA_7$) | 3,300 | 1.07 | 257 | 524 ± 69 (54%) | <3 (46%) | |
| PEG-*b*-P($EA_{17}$-*co*-$MAA_{17}$) | 5,100 | 1.09 | 108 | 347 ± 36 (56%) | 95 ± 5 (22%) | <3 (22%) |
| PEG-*b*-P($EA_{25}$-*co*-$MAA_{25}$) | 6,600 | 1.08 | 89 | 302 ± 16 (56%) | <3 (44%) | |

[a] Numbers in subscript correspond to the number of monomer units per chain.
[b] $^1$H NMR analysis.
[c] Polydispersity index, $M_w/M_n$ by SEC analysis.
[d] Mean ± S.D. of three measurements.

merization (Chiefari et al., 1998; Ray et al., 2003), and ATRP (Coessens et al., 2001). These synthetic pathways all have the common advantage of offering an accurate control over molecular weight and polydispersity.

In this work, ATRP was successfully used to prepare amphiphilic PEG-*b*-(EA-*co*-*t*BMA) with high synthetic yields (Davis and Matyjaszewski, 2000; Bednarek et al., 1999; Cheng et al., 2000). Polymer synthesis was initiated with a PEGylated macroinitiator (Ranger et al., 2001; Jankova et al., 1999). The *t*BMA units were then quantitatively hydrolyzed with HCl to introduce pH-responsive MAA units. PEG-*b*-(EA-*co*-MAA) had a $M_n$ between 3000 and 7000, with PI as low as 1.07 (Table 2). $^1$H NMR analysis revealed that the polymer compositions quantitatively correlated with the amount of monomers introduced.

PEG-*b*-(EA-*co*-MAA) self-associated in water to form nanometer-sized aggregates (Table 2). Such nanoaggregates could find practical applications for the oral or parenteral delivery of drugs. For example, PEGylated acidic copolymers could be complexed to cisplatin to reduce the drug toxicity after i.v. administration (Nushiyama et al., 1999; Nishiyama and Kataoka, 2001). These acidic copolymers present features that make them just as interesting for the oral delivery of poorly water-soluble drugs exhibiting low bioavailability. First, the PEG chains and the MAA units were both shown to interact with the intestinal mucosa (Bromberg et al., 1997; Bromberg and Ron, 1998; Bromberg, 2001). Additionally, the hydrophobic moieties along the polymer backbone can easily disperse and solubilize poorly water-soluble drugs (Leroux et al., 1996; Bromberg and Barr, 1999; Bromberg and Temchenko, 1999). Finally, drug release can be pH-triggered by a change in polarity of the core as the PM transit from the stomach to the small intestine. The MAA units of the polymer will indeed be protonated at the acidic pH of the stomach, thus allowing for the incorporation and protection of a hydrophobic drug in the core of the PM. The PM will subsequently dissociate and release their contents as they progress towards the intestinal environment where the MAA units get ionized.

As presented in Table 2, PEG-*b*-(EA-*co*-MAA) aggregate sizes ranged between 300 and 500 nm. The presence of such large aggregates may be explained by the formation hydrogen bonds between the oxygen atoms of the poly(ether) chains and the carboxylic acid groups of the MAA units (Lele and Hoffman, 2000; Mathur et al., 1998). An important proportion of copolymers existed as unimers (isolated polymer chain) as the MAA units can also interact with water molecules via hydrogen bonding. As expected, the CAC decreased as the hydrophobic segment length increased.

Fig. 6 illustrates the self-assembly of PEG-*b*-P($EA_7$-*co*-$MAA_7$) as a function of pH by spectrofluorimetry and light scattering. At low pH (<4.5), the MAA units are protonated and the polymer chains exist as aggregates (possibly aggregated micelles), as revealed by the high $I_{338}/I_{333}$ fluorescence ratio obtained from the pyrene excitation spectra. At higher pH, ionization of the MAA units induces dissociation of the micelles, resulting in a clear drop of light-scattering intensity and $I_{338}/I_{333}$ ratio.

A series of drug loading assays were performed with these (meth)acrylate-based diblock copolymers using indomethacin and fenofibrate as model drugs. Drug payloads reaching up to 6% (w/w) were achieved using a dialysis procedure. Drug-loaded nanoaggregates featured average sizes of 300 nm (data not shown).

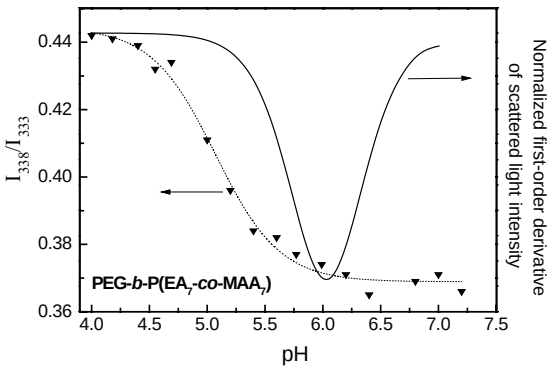


Fig. 6. Plots of the $I_{338}/I_{333}$ fluorescence ratio of pyrene in the presence of PEG-*b*-($EA_7$-*co*-$MAA_7$) at different pHs (triangles) and the normalized first-order derivative of light scattering of PEG-*b*-($EA_7$-*co*-$MAA_7$) (solid line). Adapted from Ranger et al.

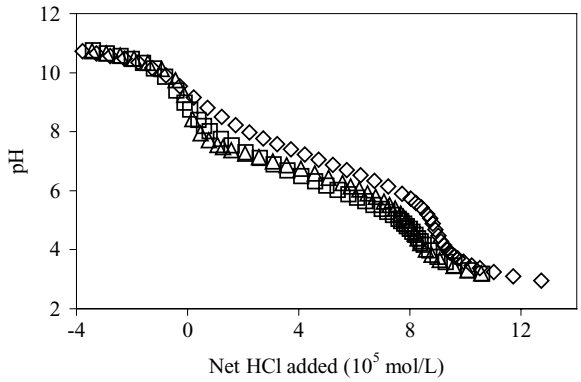


Fig. 7. Potentiometric titration curves for PEG-*b*-P(AEMA) (diamonds), PEG-*b*-P(DMAEMA) (squares) and PEG-*b*-P(DEAEMA) (triangles) copolymers at 1 mg/ml. Polymers presented $M_n$ of 4800, 4900 and 5300, respectively.

### *3.6. Methacrylate-based cationic diblock copolymers*

PICM ensue from the condensation and neutralization of oppositely charged macromolecules. As their self-assembly is driven by electrostatic interactions, their stability is predicted to depend on pH and ionic strength variations, i.e. conditions modifying the apparent ionization degree of the components. We therefore directed efforts at evaluating and relating the pH-responsiveness and stability of different cationic copolymer-based PICM to the nature of the ionizable units. Block copolymers composed of PEG and various amine-bearing monomers (namely, 2-aminoethyl methacrylate (AEMA), with a primary amine unit, 2-(*N*,*N*-dimethylamino)ethyl methacrylate (DMAEMA) and 2-(*N*,*N*-diethylamino)ethyl methacrylate, both with tertiary amine units of different hydrophobicity) were synthesized by ATRP (Zhang and Matyjaszewski, 1999). Potentiometric titration curves were generated by recording the changes in pH values following addition of increments of NaOH to fully protonated polymeric solutions. PEG-*b*-P(AEMA), PEG-*b*-P(DMAEMA), and PEG-*b*-P(DEAEMA) all behave as weak bases and presented buffering capacities, as evidenced by the presence of plateaus in Fig. 7. p$K_a$ of 7.1, 6.5 and 6.7 were obtained for the conjugate acids of PEG-*b*-P(AEMA), PEG-*b*-P(DMAEMA) and PEG-*b*-P(DEAEMA), respectively.

Clearly, the nature of the amine influences the electric properties of the polymers. PEG-*b*-P(AEMA), presenting primary amino groups, comes out as the most basic of all three polymers and is expected to form polyelectrolyte complexes over a broader pH range. Variations in the buffering capacity could further be associated with different intracellular behaviors given that the capture of protons by the amino groups is expected to play a role in the disruption of organelles (i.e. endosomes and lysosomes) upon internalization of the complexes (Boussif et al., 1995). The hydrophobicity of the monomers was also shown to alter the behavior of the polymers; the neutralized units of PEG-*b*-P(DEAEMA) are indeed hydrophobic enough to trigger their segregation into the core of traditional amphiphilic micelles (data not shown). Despite their different pH-sensitivities, all three copolymers were shown to condense heparin, a model macromolecular polyanionic drug, and trigger the formation of monodispersed PICM with diameters varying from 25 to 32 nm at acidic pH.

## 4. Conclusion

Several amphiphilic pH-sensitive copolymers were synthesized and their self-assembling properties in aqueous media characterized. These copolymers yielded micelles or nanoaggregates sensitive to pH variations. The PNIPAM derivatives were shown to be a potential safe alternative to Cremophor® EL for the solubilization of hydrophobic drugs. In addition, AlClPc-loaded PNIPAM-based micelles appeared to

be more efficient than their Cremophor® EL counterpart in an i.d. implanted mouse mammary tumor model. PEG-*b*-(EA-*co*-MAA) assemblies were shown to solubilize hydrophobic drugs and dissociate upon an increase of pH. Such a system could be used for the oral administration of poorly water soluble drugs presenting a low biovailability. Finally, the PEG-*b*-(aminoethylmethacrylate) derivatives might be key polymeric materials for the sequestration and protection of polyanionic drugs such as heparin and antisense oligonucleotides into PICM.

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