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Synthesis and characterization of original 2-(dimethylamino)ethyl methacrylate/poly(ethyleneglycol) star-copolymers

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ABSTRACT

Novel synthetic transfection vectors with linear triblock and star-shaped diblock copolymer architectures have been synthesized by atom transfer radical polymerization (ATRP). Based on 2-(dimethylamino)ethyl methacrylate (DMAEMA) and copolymerization with poly(ethyleneglycol) α-methoxy, ω-methacrylate (MAPEG), the synthesis was realized using CuBr ligated with 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as catalytic complex and either ethyl 2-bromoisobutyrate (EB^{i}B) or bis(α-bromoisobutyryl) *N*-methyl diethanolamine (DEA) or tris(α-bromoisobutyryl) triethanolamine (TEA) as (multifunctional)initiator. The polymers were characterized by GPC and NMR. The solution properties of these homopolymers and palm-tree-like copolymers were investigated by viscometry either in pure water or in buffered aqueous solutions. Interestingly, all the synthesized polymers show polyelectrolyte effect in Millipore water (25 °C) and in Hepes (20 mM) buffer solution (pH 7.4, NaCl 155 mM, 25 °C). Fitting of these viscometric data according to either Fuoss or Fedors equation allows for calculating the intrinsic viscosity of the polymers. These results are compared with dynamic light scattering (DLS) experiments to determine absolute masses. Finally, DEA based palm-tree-like copolymer is investigated to AFM measurement and micelles were observed at pH 8.

1. Introduction

Over the last decade, increased interest has focused on water-soluble cationic polymers, such as poly(L-lysine) [1–3], derivatized chitosan [4,5] and tertiary amino ethyl methacrylate-based (co)polymers [6–12]. These polymers or copolymers, characterized by a polyelectrolyte behavior, have been used as DNA binding agents either as pure compounds or as mixtures in nonviral systems, forming so-called polyplexes related to gene delivery systems of interest strategy of gene therapy [13]. Corresponding di- or triblock copolymers, built from a cationic segment and another hydrophilic sequence (such as poly(ethyleneglycol) (PEG)) with self-assembling properties in appropriate conditions, can exhibit specific thermo- and pH sensitivity [14], which changes the properties in solution and then affects the delivery system. Our group is interested especially in poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), which is a weak polybase ($pK_a \sim 7.0$) [12] and displays a well marked polyelectrolyte behavior in water [15,16]. Recently, we have reported that complexes obtained by self-assembly of the pCMVb plasmid with linear PDMAEMA polymers can be used to transfect Cos-7 cells [17]. That study also highlighted that the transfection was possible only in the absence of serum. However, this drawback could be overcome via PEGylation of the polycationic polymer, i.e. via poly(2-(dimethylamino)ethyl methacrylate)-*b*-poly(ethyleneglycol) α-methoxy, ω-methacrylate (PDMAEMA-*b*-PMAPEG) palm-tree-like

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copolymers. The hydrophilic PEG sequences proved to form a highly hydrated corona around the polyplex surface, therefore, improving the hemocompatibility of the transfecting system.

The aim of the investigation described herein is at first to synthesize novel macromolecular architectures to improve the efficiency of the cell transfection. In this context, we extended the strategy to introduce poly(ethylene glycol) sequences/grafts into 2-(dimethylamino)ethyl methacrylate (DMAEMA)-based polymers with two- and three-arm cores (Fig. 1) to be used as multifunctional initiators in atom transfer radical polymerization (ATRP), which is one of the most robust controlled/'living' radical polymerization methods since it can be applied to a wide variety of functional monomers such as DMAEMA [18,19] and provides well-defined polymers [20–22].

In order to assess the conformational transition of these polyelectrolytes in solution, the viscometric properties of linear, two-arm and three-arm star-shaped PDMAEMA-based polymers and PDMAEMA-*b*-PMAPEG copolymers were investigated in salt-free solution. The influence of the macromolecular parameters such as molar mass, composition and structure are described. In order to avoid limitations induced by the partial protonation of PDMAEMA chains in Millipore water, the viscometric behavior of PDMAEMA (co)polymers in Hepes buffer solution (pH 7.4) containing NaCl was also studied. Dilute solutions of copolymers were investigated by dynamic light scattering (DLS) to measure of the hydrodynamic radius (R_h) of the copolymer and to determine absolute masses. Knowing the R_h and the intrinsic viscosity, the viscosity Einstein's equation allows approximating the M_v value of the copolymer. Finally, freeze-drying deposition of triblock copolymer solution has been realized to investigate their self-assembly potential by AFM at pH 8.

2. Experimental

2.1. Materials

Ethyl 2-bromoisobutyrate (EB^{i}B), 2-bromo-2-isobutyrylbromide, *N*-methyl diethanolamine, triethanolamine, 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) and copper(I) bromide (CuBr) were purchased from Aldrich and used as received. Triethylamine (Fluka) and tetrahydrofuran (THF, Chem-Lab) were dried over calcium hydride for 24 h at room temperature, distilled under reduced pressure and stored over molecular sieves (4 Å). 2-(Dimethylamino)ethyl methacrylate (DMAEMA, Aldrich) and poly(ethylene glycol) α-methoxy, ω-methacrylate (MAPEG, Aldrich, M_n = 480 as determined by ^{1}H NMR spectroscopy, which corresponds to a PEG graft M_n of 395) were passed through a column of basic alumina to remove the stabilizing agents prior to use.

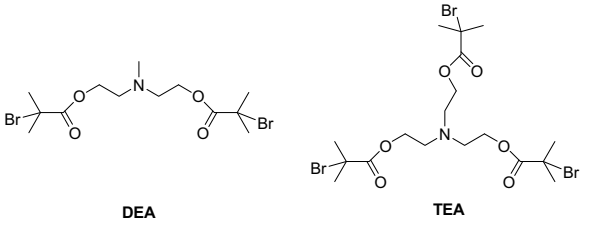

Fig. 1. Two-arm (bis(α-bromoisobutyryl) *N*-methyl diethanolamine, DEA) and three-arm (tris(α-bromoisobutyryl) triethanolamine, TEA) initiators for ATRP of DMAEMA.

2.2. Synthesis of bis(α-bromoisobutyryl) N-methyl diethanolamine (DEA)

N-Methyl diethanolamine (1 g, 8.39 mmol) and triethylamine (4.7 mL, 33.6 mmol) were added to 20 mL dry THF. 2-Bromo-2-isobutyrylbromide (7.72 g, 33.6 mmol) was added dropwise to this mixture over 1 h at 0 °C. After vigorous stirring for a further 2 h, the salt formed was filtered and the resulting clear solution was concentrated under vacuum at 30 °C before washing with 0.1 M Na_2CO_3. The product was extracted three times with dichloromethane and dried with $MgSO_4$. After filtration, the organic layer was evaporated under vacuum and purified by flash chromatography using heptane/ethyl acetate (10/90, R_f = 0.17) that afforded a dark-reddish brown oil. (2.64 g, yield: 75%) ^{1}H NMR: δ ppm = 4.24 (t, 4H), 2.76 (t, 4H), 2.37 (s, 3H), 1.91 (s, 12H).

2.3. Synthesis of tris(α-bromoisobutyryl) triethanolamine (TEA)

Triethanolamine (1 g, 6.70 mmol) and triethylamine (6.0 mL, 39.6 mmol) were added to 17 mL dry THF. 2-Bromo-2-isobutyrylbromide (9.11 g, 39.6 mmol) was added dropwise to this mixture over 1 h at 0 °C. It was treated as DEA and finally a dark-reddish brown oil was also obtained as reported elsewhere [23]. (2.84 g, yield: 71%) ^{1}H NMR: δ ppm = 4.18 (t, 6H), 2.89 (t, 6H), 1.86 (s, 18H).

2.4. Synthesis of PDMAEMA, DEA-PDMAEMA and TEA-PDMAEMA homopolymers and copolymers with PMAPEG

In a typical experimental run, a dried glass-tube was charged with CuBr (34 mg, 0.24 mmol) and a magnetic stir bar. The tube was closed with a three-way stopcock capped by a rubber septum and purged by three repeated vacuum/nitrogen cycles. Separately, in a dried flask, HMTETA (0.109 g, 0.47 mmol), DMAEMA (4 mL, 23.7 mmol), and when needed, 10 mL of THF or toluene were introduced and oxygen-degassed by bubbling with nitrogen for 5 min before transferring the mixture into the glass-tube placed in a oil bath maintained at a given temperature. Finally, degassed initiator (EB^{i}B, DEA and TEA) was added to the tube with a previously degassed syringe. The polymerization was stopped by immersing the tube into a liquid nitrogen bath and the polymer was selectively recovered by precipitation in sevenfold volume excess of cold heptane and dried until constant weight. Monomer conversion was determined by ^{1}H NMR or gravimetry. The catalyst was removed by passing through a column of basic alumina via THF or toluene solution. For the copolymerization with MAPEG,

after appropriate DMAEMA homopolymerization reaction time, previously nitrogen-bubbled MAPEG/THF (1/1 = v/v) solution (for example 1 mL/1 mL (0.97 M) for the entry 2a from Table 1) was added with a degassed syringe into the reaction glass-tube. Finally, the copolymerization was stopped and the copolymers isolated by following the aforementioned purification procedure.

2.5. Characterization

The molar fraction of MAPEG of the copolymers was determined by ^{1}H NMR by the method described in our previous work [15] with a BRUKER AMX-300 MHz spectrometer at room temperature in $CDCl_3$ added with 0.03% TMS. Gel permeation chromatography (GPC) was performed in THF + 2 wt% NEt_3 at 35 °C using a Polymer Laboratories liquid chromatograph equipped with a PL-DG802 degasser, an isocratic HPLC pump LC 1120 (flow rate = 1 mL/min), a Marathon autosampler (loop volume = 200 μL, solution conc. = 1 mg/mL), a PL-DRI refractive index detector and three columns: a PL gel 10 μm guard column and two PL gel Mixed-B 10 μm columns. Poly(methyl methacrylate) standards were used for calibration. Viscometry measurements were carried out at 25 °C using an Ubbelhode viscometer (inner diameter ϕ = 0.46 mm). The (co)polymer solutions were prepared 24 h before measurements by dissolution in Millipore water (pH = 6.5) or in an Hepes (20 mM) buffer solution (NaCl 155 mM, pH 7.4) and then maintained at 25 °C for at least 30 min to reach equilibrium temperature.

2.6. Dynamic light scattering study

The mean hydrodynamic diameter and the size distribution of PDMAEMA-*b*-PMAPEG polymeric micelles were measured at a 90° angle with a Zeta-sizer apparatus (Malvern) equipped with a He–Ne laser (λ = 633 nm). After being filtered through a 1.2 μm Acrodisk® filter, the sample solutions (0.5 mg/mL) were measured at natural pH and 25 °C. Data were processed using multimodal size distribution (MSD) analysis based on non-negatively constrained least squares fitting (NNLS). The errors in the measurements of micellar sizes from DLS diagrams were within 10% around the mean value over 10 measurements for each sample, corresponding to a cumulate time of 1 min [24,25].

2.7. Atomic force microscopy

Thin deposits of micelles were prepared by applying 30 μL of aqueous solution (0.1 mg/mL, 25 °C, natural pH) onto freshly cleaved mica and the deposited solution was freeze-dried during 2 h [26]. Mica is a hydrophilic substrate (water contact angle < 10° for freshly cleaved mica) and negatively charged at neutral pH. The microscopic morphology of those deposits was analyzed with Atomic Force Microscopy (AFM). Height and phase AFM images were obtained with a Nanoscope IIIA MultiMode microscope (Veeco Inc., CA) operating in intermittent contact (Tapping mode) in air, using standard Si probes. The micelles diameter was measured at half-height; the standard deviation on these data is around 10 nm.

Table 1
Conditions for the synthesis of PDMAEMA-based (co)polymers by ATRP using the CuBr/HMTETA catalytic complex and alkylbromide initiators and its characterizations

Entry	Initiator	$[DMAEMA]_0/[initiator]_0$	Solvent	T (°C)	t (h)	M_n (calcd.)[a]	M_n (GPC)[b]	M_w/M_n[b]	Mol % MAPEG[c]
1a	EB^iB	200	THF	25	24	11,000[d]	11,200	1.24	
1b	EB^iB	100	Bulk	30	2	14,805[d]	22,900	1.31	
1c	EB^iB	300	Bulk	30	3	36,881[d]	45,400	1.25	
1d	EB^iB	514	THF	60	24	53,570[d]	64,600	1.30	
2a	EB^iB	95	THF	25	24	–	24,900	1.50	15
2b	EB^iB	110	THF	25	24	–	40,300	1.72	26
3a	DEA	47	THF	25	6	7000[d]	10,100	1.52	
3b	DEA	95	THF	25	6	12,700[d]	15,900	1.52	
3c	DEA	190	THF	60	24	28,900	30,300	1.48	
4a	DEA	48	THF	25	24	–	16,900	1.80	14
4b	DEA	95	THF	25	24	–	32,600	1.74	10
4c	DEA	190	THF	25	24	–	54,100	1.46	12
5a	TEA	100	Toluene	25	24	16,400	17,600	1.34	
5b	TEA	283	THF	25	4	23,500	24,800	1.38	
5c	TEA	283	THF	25	24	39,800	31,600	1.44	
5d	TEA	282	THF	25	24	42,200	44,000	1.59	
6a	TEA	71	THF	25	20	–	27,400	1.80	12
6b	TEA	141	THF	25	20	–	41,700	1.72	12
6c	TEA	286	THF	25	24	–	61,500	1.52	14

All the polymerizations were carried out with the ratio of $[initiator]_0/[CuBr]_0/[HMTETA]_0$ = 1/1/2; entry 1 corresponds to PDMAEMA, 2 to PDMAEMA-*b*-PMAPEG, 3 to DEA-PDAMEMA, 4 to DEA-PDMAEMA-*b*-PMAPEG, 5 to TEA-PDAMEMA, 6 to TEA-PDMAEMA-*b*-PMAPEG.

[a] As calculated from the following equation: M_n (calcd.) = $MW_{initiator}$ + [($[DMAEMA]_0/[initiator]_0$) × conversion by ^{1}H NMR spectroscopy × MW_{DMAEMA}].

[b] As determined by GPC with reference to PMMA standards.

[c] As determined by ^{1}H NMR spectroscopy from the relative intensities of the α-amino methylene protons of the DMAEMA derived repeating units at δ = 2.6 ppm and the methylene protons of the PEG sequences at δ = 3.65 ppm ($CDCl_3$).

[d] As determined by gravimetry (from the yield obtained after purification).

3. Results and discussion

3.1. Synthesis and characterization

The preparation of the multifunctional initiators DEA and TEA has been carried out in the presence of a large excess amount of 2-bromo-2-isobutyrylbromide with triethylamine. After purification we can observe on the NMR spectra the presence of isobutyryl moiety at 1.91 ppm corresponding to 12 hydrogens for DEA and at 1.86 ppm corresponding to 18 hydrogens for TEA (Fig. 2). The synthesis of poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA)-based (co)polymers with various compositions has been carried out by atom transfer radical polymerization (ATRP) using copper(I) bromide (CuBr) ligated with 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as catalytic complex ($[Cu]_0/[HMTETA]_0 = 1/2$) and ethyl 2-bromoisobutyrate (EB^iB), bis(α-bromoisobutyryl) *N*-methyl diethanolamine (DEA) or tris(α-bromoisobutyryl) triethanolamine (TEA) as the initiator (Fig. 3). Table 1 shows the experimental conditions for the synthesis of the (co)polymers to be used for the viscosity and morphology studies, along with the results of the polymers analysis of composition as well as their apparent molecular characteristic. The homopolymerization of DMAEMA with EB^iB and absolute molar mass (M_n) determination by vapor pressure osmometry has been described in our previous study [15], showing that the M_n obtained was very close to theoretical one calculated with conversion. The homopolymerizations from DEA or TEA were carried out under the same conditions or at lower temperature when necessary to preserve the control over the polymerization then the copolymerization with MAPEG was carried out in the same condition. Fig. 4 shows the ^{1}H NMR spectra of DEA-PDMAEMA-*b*-PMAPEG and TEA-PDMAEMA-*b*-PMAPEG after purification. The peaks at 3.3–3.8 ppm corresponding to PEG segments were present in all copolymers, which were observed in our earlier study [15]. These spectra were also similar with linear copolymers of PDMAEMA-b-PMAPEG (entry 2 from Table 1). For all copolymers, the homopolymer obtained at the first step was analyzed with GPC before addition of MAPEG. Fig. 5 is GPC spectra of DEA-PDMAEMA-*b*-PMAPEG (4c from Table 1) showing successful copolymerization with the monomodal molecular weight distribution before (dotted line) and after (solid line) addition of MAPEG.

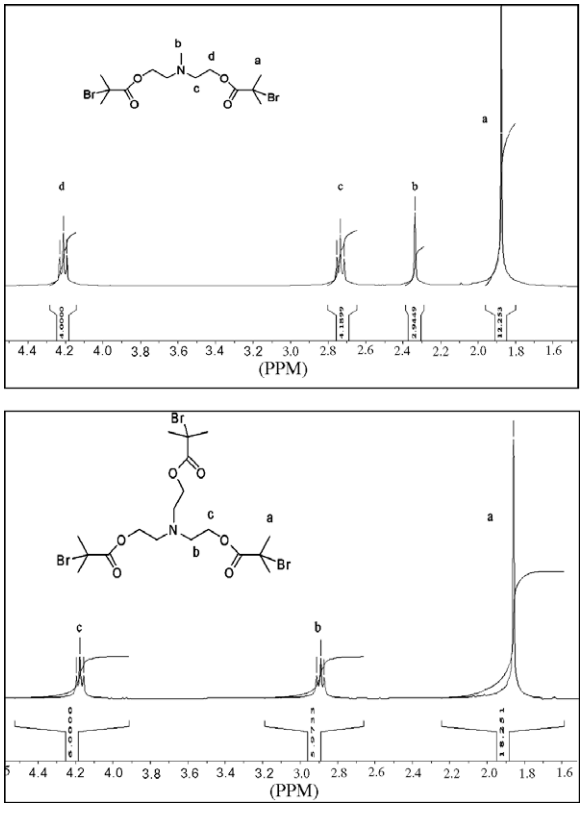

Fig. 2. ^{1}H NMR (300 MHz) spectra of multifunctional initiators DEA (top) and TEA (bottom) in $CDCl_3$.

3.2. Reduced viscosity measurements in millipore water

First, the effect of apparent molar mass (M_n (GPC)) on the reduced viscosity (η_{sp}/C) in pure water has been investigated for the series of PDMAEMA, DEA-PDMAEMA and TEA-PDMAEMA homopolymers. Fig. 6 shows some representative samples of the reduced viscosity of various homopolymers as a function of their concentration, from 10 down to 0.5 or 0.02 g/L. As expected for cationic polymer solutions, the reduced viscosity profile of all PDMAEMA-based homopolymers shows a polyelectrolyte behavior characterized by a sharp increase of the reduced viscosity in the low concentration range and the reduced viscosity slightly increases with the molar mass of the polymers too [29]. As widely reported, the polyelectrolyte effect is due to an expansion of the polyionic chain, which is caused by the enhanced dissociation of ionizable groups as the concentration decreases. Therefore, the intramolecular repulsive interactions between ionized groups (in our case the protonated amino groups) are stronger and spread all along the chain. Since the pK_a value of PDMAEMA in water is around 7.0, dissolution of PDMAEMA in pure water ($pH \approx 6$) leads to the protonation of a sizable part of the amino groups pending along the polymethacrylate backbone, so that the polyelectrolyte behavior is more effective at higher charge density per macromolecule. Among these series of (co)polymers this effect is clearly observed by the lower concentration at which the reduced viscosity starts to increase (solid symbols in Fig. 6). Within the series of PDMAEMA-*b*-PMAPEG, DEA-PDMAEMA-*b*-PMAPEG and TEA-PDMAEMA-*b*-PMAPEG copolymers having PEG segments giving palm-tree form, the same trends are observed (Fig. 6). Comparing the homopolymers with the corresponding copolymers of similar apparent molar mass, one can observe that the copolymers are characterized by a polyelectrolyte effect starting at higher concentration, i.e. the concentration at which the reduced viscosity starts to increase is higher than in case of the corresponding homopolymers. This is more likely explained by the presence of the hydrophilic PEG segments grafted like branches of a palm-tree, the steric effect of which is

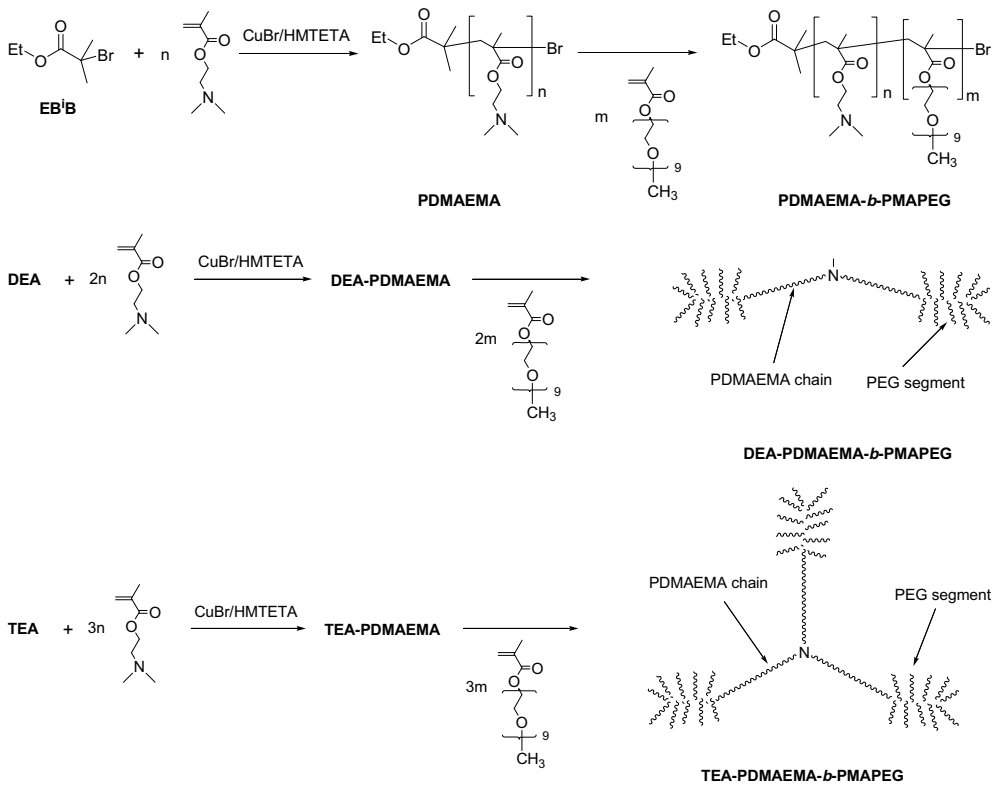

Fig. 3. Copper(I) mediated controlled/living radical (co)polymerization of DMAEMA using alkylbromide initiators.

expected to increase the overall hydrodynamic volume (Fig. 3). It is also worth pointing out that when comparing among the different structures as linear (co)polymers, two-arm (co)polymers and three-arm star-shaped (co)polymer of similar apparent molar mass, it was star-shaped (co)polymers which shows a polyelectrolyte effect at higher concentration then two others (Fig. 6). As the three-arm star-shaped (co)polymers have bulkier structure at the core site, important intramolecular repulsive interaction could be expected at higher concentration.

3.3. Intrinsic viscosity

Data shown in Fig. 6 can be linearized applying the Fuoss [27,28] Eq. (1) and Fedors [29] Eq. (2) equations:

$$\eta_{sp}/C = [\eta]/(1 + kC^{0.5}) \quad (1)$$

$$1/[2(\eta_r^{0.5} - 1)] = 1/[\eta]C - 1/[\eta]C_m \quad (2)$$

where η_{sp}, η_r, C, $[\eta]$, k and C_m are the specific viscosity, the relative viscosity, the concentration of the polymer, the intrinsic viscosity, the Fuoss constant and a polymer constant parameter, respectively. Fig. 7 shows some representative examples of the straight lines obtained using Fedors and Fuoss equations, respectively, allowing calculating intrinsic viscosity values (Table 2). Therefore, Fedors and Fuoss equations were applicable to dilute to moderately concentrated polymer solution, only corresponding dilute viscometric data were used to obtain intrinsic viscosity. As it could be expected, within each series of polymers the intrinsic viscosity increases with the apparent M_n values as determined by GPC. Comparing the intrinsic viscosity obtained with the two equations (Table 2), all values appear close to each other, showing that the two equations are well adapted for PDMAEMA and copolymers with PMAPEG for the viscometric characterization. The intrinsic viscosity values also confirm that the PDMAEMA-*b*-PMAPEG copolymers are characterized by a stronger polyelectrolyte effect compared with the PDMAEMA homopolymers of corresponding apparent molar mass, which also appears in the reduced viscosity values.

3.4. Reduced viscosity in pH 7.4 buffer solution

As the PDMAEMA is partially protonated in Millipore water, this effect could limit the solution properties when the pH changes and also influence the transfection experiments. In this context, the measurements of the reduced viscosity were also carried out in a buffer solution at pH 7.4 (Hepes 20 mM, NaCl 155 mM), which was used for transfection tests with cells [17]. The presence of 155 mM of NaCl in the Hepes buffer solution does not affect the polyelectrolyte behavior as shown in Fig. 8.

3.5. DLS characterization

As our major aim of this study was on the palm-tree-like copolymers, DLS analyses were investigated only for

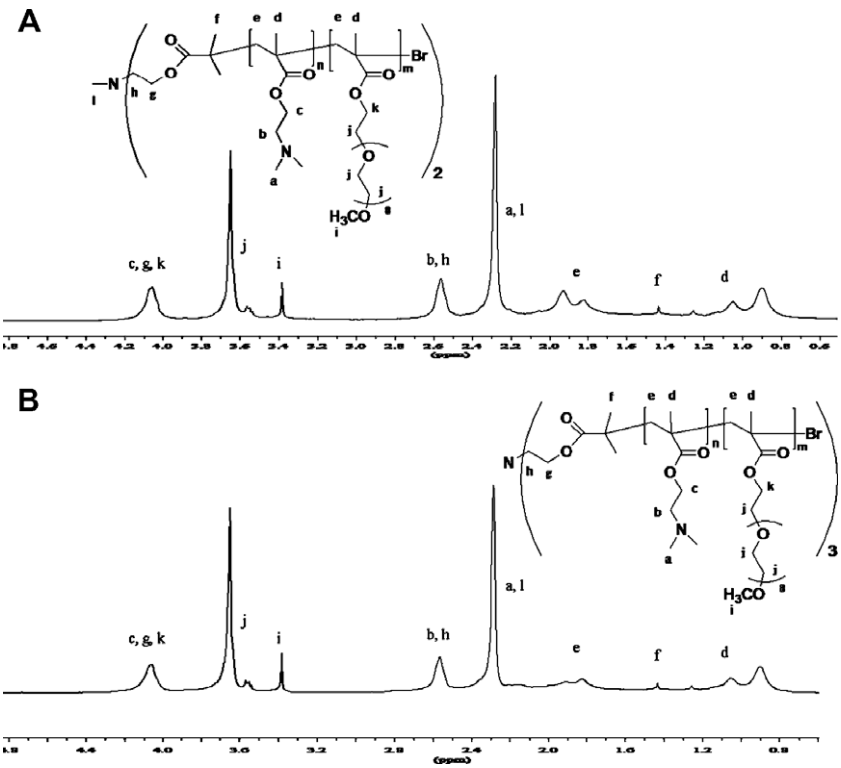

Fig. 4. ^{1}H NMR (300 MHz) spectra of DEA-PDMAEMA-*b*-PMAPEG (A, entry 4b from Table 1) and TEA-PDMAEMA-*b*-PMAPEG (B, entry 6a from Table 1) in $CDCl_3$.

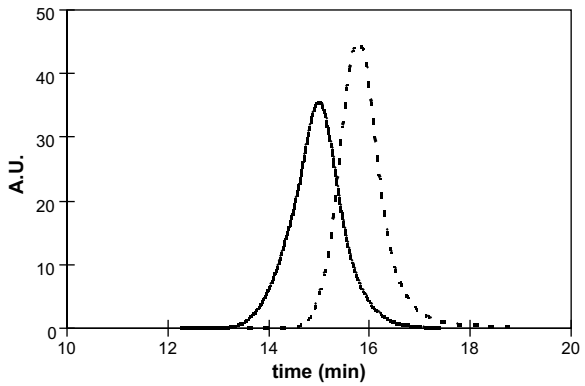

Fig. 5. GPC spectra of DEA-PDMAEMA-*b*-PMAPEG (solid line, entry 4c from Table 1) with its DEA-PDMAEMA homopolymer (dotted line, M_n = 21900, M_w/M_n = 1.38) analyzed before MAPEG addition.

the copolymers having PEG branches. The results obtained by DLS analyses indicate that all the copolymers independently of their architecture and their composition adopt a tri-modal apparent hydrodynamic distribution as shown in Fig. 9. This distribution type is commonly observed in micellar system solution. Moreover, the coexistence of different hydrodynamic radius (R_h) is a serious indication of a morphological transition within the sample. The lower population corresponds to the unimeric copolymer and present mean hydrodynamic radius between 8 and 15 nm. Second population corresponds to the self-associate structure like spherical or cylindrical micelles with hydrodynamic radius around 25 nm. Finally, the last population probably corresponds to secondary aggregates of several hundred nanometres or cylindrical objects. In the topic of this publication, the attention was focused on the first population. Indeed, the Einstein viscosity law [30] allows an approximation of the mean molecular weight (M_v), which could be written as:

$$[\eta] \cdot M_v = (10\pi N_a/3)R_h^3$$

where $[\eta]$, M_v and N_a are the intrinsic viscosity, the molecular weight and the Avogadro's number, respectively. According to this equation, some molecular weights were calculated from $[\eta]_{Fedors}$ and $[\eta]_{Fuoss}$ values (Table 3). Although there is a gap between the M_n determined by GPC and those calculated by the relationship of Einstein, these molecular weights are consistent with M_n obtained by GPC analyses. This difference should be due to the apparent measures of GPC calibrated on the basis of linear PMMA polymer, where the palm-tree-like copolymers have larger hydrodynamic volume than linear polymers.

3.6. Atomic force microscopy

AFM measurements were performed on the thin deposits prepared by freeze-drying of the copolymer solutions (0.5 mg/mL, pH 8, thermostatized at 25 °C) on mica. In agreement with DLS observations, spherical objects with

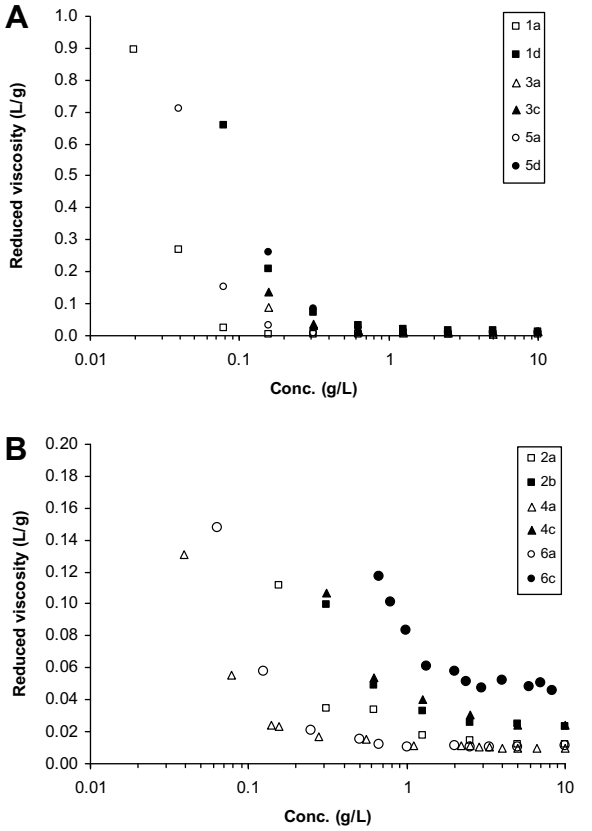

Fig. 6. Influence of the polymer molar mass and architecture on the reduced viscosities in Millipore water at 25 °C. For various PDMAEMA homopolymer architectures (A, entries 1, 3 and 5 from Table 1) and for various PDMAEMA-*b*-PMAPEG copolymer architectures (B, entries 2, 4 and 6 from Table 1) prepared with different initiators.

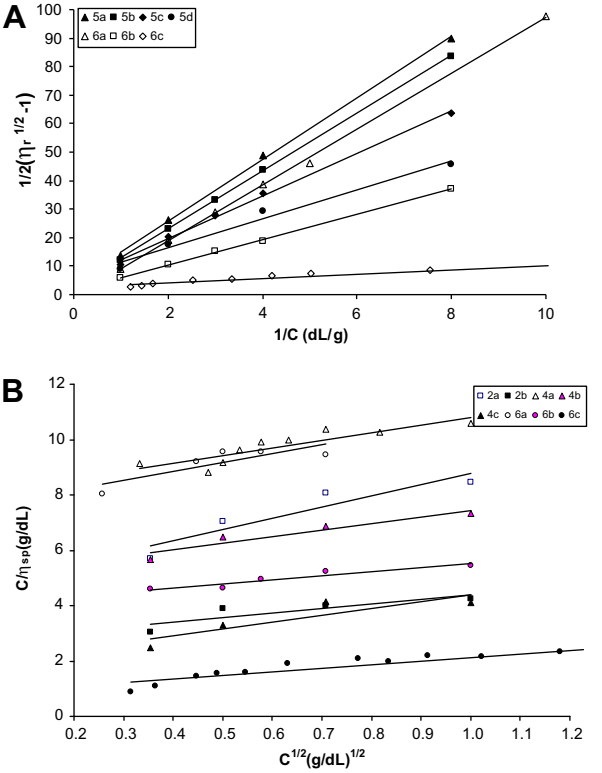

Fig. 7. Representation of the Fedors equation for TEA-PDMAEMA and TEA-PDMAEMA-*b*-PMAPEG (A, entries 5 and 6 from Table 1) and representation of the Fuoss equation for various PDMAEMA-*b*-PMAPEG copolymers prepared with different initiators (B, entries 2, 4 and 6 from Table 1).

a multimodal distribution around 40 nm were observed for the two-arm star-shaped copolymers (entry 4c from Table 2 and Fig. 10) but also cylindrical objects with a similar diameter while their length ranges from 100 to 250 nm. Self-assembly properties of copolymers seem to be particularly interesting and will be investigated in a further study.

4. Conclusions

A series of linear, two-arm and three-arm PDMAEMA homopolymers and their copolymers with PMAPEG 'branches' were synthesized by atom transfer radical polymerization (ATRP). The linear homopolymers form simple palm-tree-like structures when further copolymerized with PMAPEG. The second type also corresponds to linear homopolymers that are end-capped on both extremities after the second step copolymerization by two PMAPEG 'branches'. The third type consists of star-shaped PDMAEMA homopolymers and the extremity of each segment being thus covered by a PEG enriched brush after the copolymerization with PMAPEG. After characterization of the (co)polymer samples by GPC and NMR analyses, their aqueous solution properties were investigated by means of viscometric measurements. Whatever their molecular weight, composition and architecture, all

Table 2
Effect of apparent molar mass on the intrinsic viscosity [η] deduced from Fedors and Fuoss equations

Entry	$[\eta]_{Fedors}$ (dL/g)	$[\eta]_{Fuoss}$ (dL/g)
1a	0.077	0.077
1b	0.072	0.075
1c	0.159	0.181
1d	0.214	0.264
2a	0.192	0.212
2b	0.350	0.360
3a	0.114	0.141
3b	0.130	0.140
3c	0.132	0.139
4a	0.119	0.117
4b	0.185	0.197
4c	0.463	0.517
5a	0.092	0.098
5b	0.099	0.102
5c	0.149	0.159
5d	0.198	0.218
6a	0.123	0.120
6b	0.226	0.246
6c	1.02	1.36

Entry 1 corresponds to PDMAEMA, 2 to PDMAEMA-*b*-PMAPEG, 3 to DEA-PDAMEMA, 4 to DEA-PDMAEMA-*b*-PMAPEG, 5 to TEA-PDAMEMA, 6 to TEA-PDMAEMA-*b*-PMAPEG.

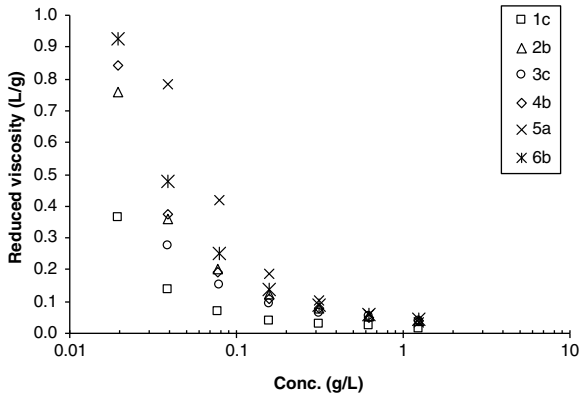

Fig. 8. Influence of the polymer molar mass and architecture on the reduced viscosities in Hepes buffer solution (pH 7.4) at 25 °C for various PDMAEMA homopolymers and PDMAEMA-*b*-PMAPEG copolymers prepared with different initiators (some representative samples were presented as the entries 1c, 2b, 3c, 4b, 5a and 6b from Table 1).

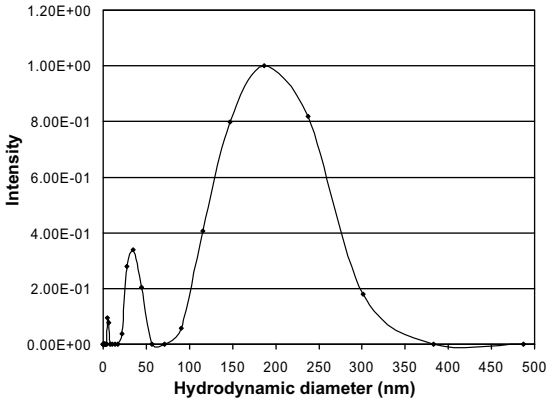

Fig. 9. DLS profile of 4c in the aqueous solution (0.5 mg/mL) at pH 8 and 25 °C.

Table 3
Hydrodynamic radius and molecular weight calculated from viscosity Einstein's law

Entry	R_h (nm)[a]	$M_{v\ Fedors}$[b] (g/mol)	$M_{v\ Fuoss}$[b] (g/mol)
2a	8	15,167	16,747
2b	13	38,325	39,420
4b	9	23,239	24,747
4c	15.5	45,234	50,510
6a	7.5	22,078	21,540
6b	11	33,978	36,985

[a] Hydrodynamic radius obtained by DLS analysis.
[b] Viscosimetric molecular weight calculated by viscosity Einstein's equation from Fedors' and Fuoss' intrinsic viscosity.

studied (co)polymers displayed characteristic polyelectrolyte behavior as evidenced by a sharp increase of the reduced viscosity at lower concentration. The intrinsic viscosity of the polymers was further approached by using the Fuoss and Fedors equations. The intrinsic viscosity is in good accordance with the apparent molar masses obtained by GPC, i.e. a higher molar mass leading to a higher intrinsic viscosity. The morphology of the copolymers was analyzed by DLS. All copolymers have same DLS profile forming self-associate object with hydrodynamic radius around 25 nm. AFM analysis of two-arm triblock copolymer synthesized from the difunctional initiator spontaneously shows the formation of spherical and cylindrical micelles in solution, which represent as a good candidates for encapsulating (bio)molecules in. The formation of spontaneous spherical and cylindrical micelles with ABA-type copolymers has been already reported but to the best of our knowledge it is the first time that they could be observed for pH-sensitive copolymers. Therefore, these compounds should allow tuning and controlling the gene release by a local decrease of pH as it occurs in direct vicinity of cancerous cells/tissues. This application for controlled gene delivery and their use in gene therapy is under current investigation.

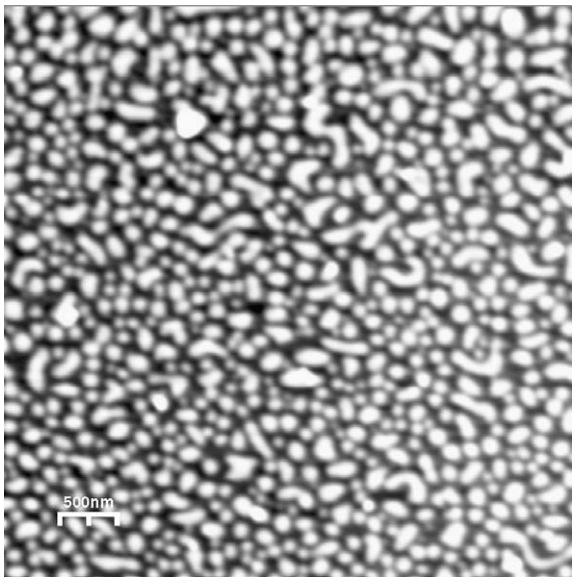

Fig. 10. AFM height image (5×5 μm) of spherical and cylindrical micelles obtained by freeze-drying on mica from a two-arms copolymer 4c solution at pH 8, for a concentration of 0.5 mg/mL and at 25 °C.

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