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Solvent-free synthesis and purification of poly[2-(dimethylamino)ethyl methacrylate] by atom transfer radical polymerization

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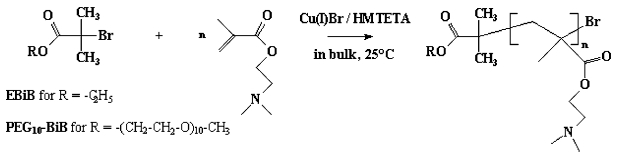
Solvent-free synthesis of well-defined poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) (co)polymers was performed by atom transfer radical polymerization conducted under very mild conditions (in bulk at 25 °C). The pH-dependence and the thermo-responsive behaviour of PDMAEMA in aqueous solution were operated to isolate and purify the (co)polymers without using any organic solvent or further catalyst extraction. The viscosity in aqueous solution of so-purified PDMAEMA homopolymers and their block copolymers with poly(ethylene glycol) (PEG) was studied as a function of molar mass and concentration and a typical polyelectrolyte behaviour was observed. these catalyst-deprived polycations are able to form stable and non toxic complexes with DNA, showing good transfection efficacies in gene therapy.

Recently, amino-functionalized methacrylate-based polymers such as poly[2-(dimethylamino)ethyl methacrylate] PDMAEMA have been reported to be efficient polycationic condensing agents for non-viral-DNA delivery owing to inherent amine protonation in physiological media.[1–3] To get a total lack of toxicity, such an application field however requires a high purity level of the polymeric vectors deprived from any residual monomer, solvent, and catalyst. On the other hand, it is also of key-interest to be able to finely control and tune up all the molecular parameters of such polycations. Our research aims at synthesizing well-defined PDMAEMA homopolymers and their related copolymers with poly(ethylene glycol) (PEG) (Scheme 1) for their potential to serve as vectors in gene therapy. In fact, in addition to their well known biocompatibility, the PEG sequences have proved to suppress non-specific interactions with blood proteins and prevent cellular uptake *via* the mononuclear phagocyte system *in vivo*.[4,5] Ideally, both (co)polymer synthesis, recovery and purification steps are to be performed in the absence of any organic solvent and under very mild experimental conditions.



Scheme 1 Copper(I) mediated living radical polymerization of DMAEMA using alkylbromide (macro)initiator.

Perfectly well-tailored (co)polymers in terms of architecture, molar mass and composition are thus highly desirable, which explains why research on controlled/'living' radical polymerization has grown so rapidly in recent years. One of the most successful systems is atom transfer radical polymerization (ATRP).[6] Controlled polymerization in ATRP is achieved by establishing a dynamic equilibrium between the propagating and dormant species with a transition metal complexes acting as a reversible halogen atom transfer reagent. As a result, the concentration of the propagating species is greatly lowered, and the contribution of termination side-reaction to the overall polymerization reaction is basically suppressed. ATRP has shown tolerance of a variety of functional groups including tertiary amines. Accordingly 2-(dimethylamino)ethyl methacrylate (DMAEMA) has been recently polymerized by ATRP.[7–9] However in all cases organic solvents were required for carrying out the polymerization or simply for recovering and purifying the polymers. Furthermore the transition metal-based complex used as ATRP catalyst is removed by passing the crude polymer in solution through a silica gel or basic alumina column.[9,10]

In this contribution, well-defined PDMAEMA have been synthesized by solvent-free ATRP using copper bromide complexed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) ligands as the catalyst, and 2-ethylbromoisobutyrate (EBiB) or 2-(monomethoxy-capped-PEG)bromoisobutyrate (PEG_{10}-BiB) as the (macro)initiator under mild conditions.† The bulk polymerisation of DMAEMA reached 80 and 90% conversion within 4 h at 25 °C using EBiB and PEG_{10}BiB, respectively (Table 1). For the sake of comparison, polymerisation reactions were conducted in THF solution with the same molar proportion of reactants. Polymer samples were characterized in terms of their molar masses and composition by Size Exclusion Chromatography (SEC) and by proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. For PEG_{10}-BiB-

Table 1 Results of the polymerisation of DMAEMA catalyzed by CuBr/HMTETA using alkylbromide (macro)initiator

Entry	(Macro) initiator	Solvent	T/°C	t/h	Theor. DP	Exp. DP[c]	Conv. (%)	M_n[d]	M_n[d]	MWD[d]
[a]1	EBiB	THF	60	16	93	—	93	14 000	—	1.19
[b]2	EBiB	— (bulk)	25	1	17	—	17	7 700	—	1.12
[b]3	EBiB	— (bulk)	25	2	64	—	64	14 300	—	1.15
[b]4	EBiB	— (bulk)	25	4	80	—	80	19 700	—	1.15
[b]5	EBiB	— (bulk)	25	4	180	—	90	27 500	—	1.39
[a]6	PEG_{10}BiB	THF	60	4	97	116	93	13 500	18 800	1.28
[b]7	PEG_{10}BiB	— (bulk)	25	4	91	118	91	16 200	19 200	
[b]8	PEG_{10}BiB	— (bulk)	25	4	192	199	89	30 800	31 900	1.30
[b]9	PEG_{10}BiB	— (bulk)	25	4	370	ND	91	41 800	43 100	1.50

[a] Polymerization of DMAEMA, $[DMAEMA]_0$ = 2.95 M, $[(macro)initiator]_0:[CuBr]_0:[HMTETA]_0$ = 1:1:2. [b] Polymerization of DMAEMA, $[DMAEMA]_0$ = 5.93 M, $[(macro)initiator]_0:[CuBr]_0:[HMTETA]_0$ = 1:1:2. [c] Experimental degree of polymerization estimated by ^{1}H-NMR (see text). [d] Determined by SEC relative to a poly(methyl methacrylate) (PMMA) calibration (in THF + 2% triethylamine at 35 °C).

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block-PDMAEMA block copolymers, the experimental degree of polymerization (exp. DP) has been estimated by ^{1}H-NMR by comparing the relative intensity of protons of methylene groups of PEG_{10} sequence at 3.65 ppm (in $CDCl_3$) and α-amino methyl groups of PDMAEMA at 2.3 ppm (Table 1). Exp. DP's are in rather good agreement with the expected values (Theor. DP) calculated from the initial DMAEMA-to-(macro)initiator molar ratio. Further confirming the control over the polymerization, monomodal and narrow molecular weight distributions (MWD) have been recorded by SEC with polydispersity indices ranging between 1.1 and 1.5.

One of the main drawbacks in ATRP is the relative difficulty of removing the coloured catalyst residues from the (co)polymers. Usually the PDMAEMA chains are isolated by precipitating the crude reaction solution from petroleum ether (Fig. 1.-1-) and the catalyst removal is achieved by passing the recovered polymer in solution through a basic alumina column. It has been found that PDMAEMA polymers can be readily isolated from residual monomers and transition metal complexes without using any organic solvent or further catalyst extraction. Our organic solvent-free purification takes advantage of the pH-dependence and the thermo-responsive behaviour of PDMAEMA in aqueous media. After polymerization, acidic water at pH ~ 4 is added for solubilizing the crude PDMAEMA at rt (Fig. 1.-2-) while PDMAEMA chains selectively precipitate by increasing both the pH to *ca*. 12 and temperature to *ca*. 65 °C (Fig. 1.-3-). Colourless PDMAEMA is finally recovered by simple filtration (Fig. 1.-4-), without any trace of red precipitate that could be formed by the precipitation of reduced Cu(0).[11] This is further confirmed by the extremely low quantity of residual copper in the so-isolated (co)polymer, which has been measured by Inductively Coupled Plasma spectroscopy (ICP) and proved to be lower than 5 ppm attesting for the high efficiency of the proposed purification method. Interestingly, ^{1}H-NMR spectrometry of the so-recovered PDMAEMA chains does not reveal any contamination by residual HMTETA ligand and carboxylic acid functions, which attests for the absence of hydrolytic cleavage of the methacrylate ester bonds provoked by the pH variations.

Reduced viscosity of EBiB-PDMAEMA in aqueous solution has been measured at 25 °C (Fig. 2). As expected, the reduced viscosity profile of PDMAEMA increases as a function of polymer concentration. However high MW polymeric chains show a polyelectrolyte behaviour characterized by a sharp increase of the reduced viscosity at low concentration range. The polyelectrolyte effect can be interpreted in terms of the repulsive electrostatic interactions between the fixed charges on the polymer chain, *i.e*., the protonated amino groups pending all along the polycationic backbone. It means that a minimum chain length is necessary to display this polyelectrolyte effect. Similar behaviour (not shown here) is observed for PEG_{10}-BiB-*block*-PDMAEMA diblock copolymers. The pH-dependence of the reduced viscosity of PDMAEMA-based polymers will be examined in a forthcoming paper. The solvent-free ATRP and the new strategy for catalyst removal in water have allowed for the synthesis of well-defined PDMAEMA homopolymers and

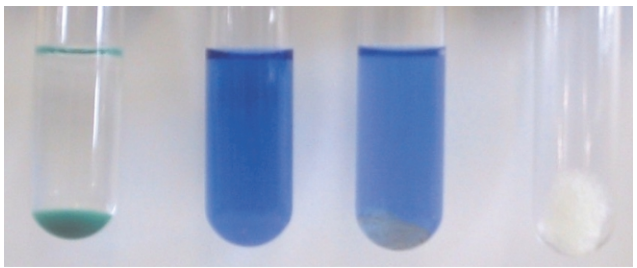

Fig. 1 Recovery of PDMAEMA: (from left to right) -1- by precipitation from petroleum ether, -2-soluble PDMAEMA in acidic water (pH ~ 4), -3-'free-organic-solvent' PDMAEMA precipitation from alkaline water (pH ~ 12), -4-colourless PDMAEMA isolated from 3 by filtration.

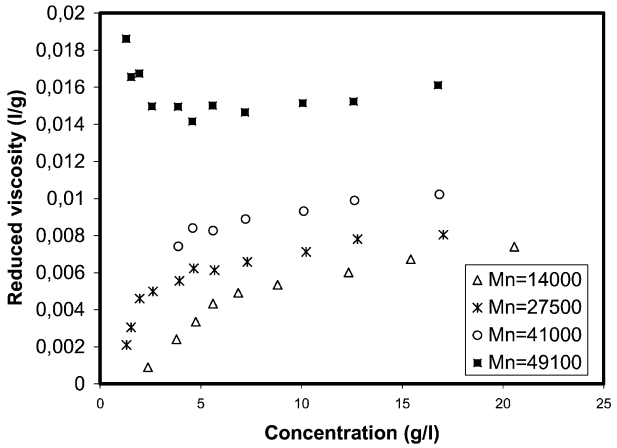

Fig. 2 Evolution of reduced viscosity of EBiB-PDMAEMA in aqueous solution as a function of concentration (pH = 6).

diblock copolymers with PEG. This polymerization method provides good control over molar masses and MWD with monomer conversions as high as 90%. Interestingly enough, these catalyst-deprived polycations are able to form stable and non-toxic complexes with DNA, showing good transfection efficacies in gene therapy. The formation of such DNA–polycations complexes, their lack of cytotoxicity and the gene delivery studies will be reported in a very near future.

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Notes and references

† Polymerization of DMAEMA was carried out in bulk (or in THF for the sake of comparison) using EBiB or PEG_{10}-BiB as (macro)initiator (Scheme 1). In a typical experiment run, a dry glass-tube was charged with CuBr (34 mg, 0.237 mmol) and a magnetic stir bar. The tube was fitted with a rubber septum and degassed by three freeze–pump–thaw cycles. Separately, a dry flask was charged with HMTETA (0.109 g, 0.474 mmol), DMAEMA (4 mL, 23.73 mmol), and when needed, 4 mL THF. The flask was fitted with a rubber septum and degassed for 5 min under nitrogen. This mixture was then transferred in a glass-tube containing CuBr, placed in a water bath maintained at 25 °C. Finally, degassed EBiB or PEG_{10}-BiB was added to the tube with a degassed syringe. The polymerization was terminated by immersing the tube into a liquid nitrogen bath. The catalyst was removed by solubilizing the crude polymer in water at pH ~ 4 and then by increasing the pH to *ca*. 12 and the temperature to *ca*. 65 °C for precipitating the PDMAEMA chains.

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