Absolute characteristics and conformation of cationic polymers by hydrodynamic approaches: Poly(AEMA-*co*-MAEMA-*co*-DMAEMA)$_{stat}$ copolymers

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Cationic poly((2-aminoethyl)-methacrylate–*co*-*N*-methyl-(2-aminoethyl)-methacrylate-*co*-*N,N*-dimethyl-(2-aminoethyl)-methacrylate) copolymers were synthesized by reversible addition-fragmentation chain transfer polymerization in a wide range of molar masses $16,000 < M$,g mol^{-1} < 130,000 and narrow dispersities. The copolymers were investigated by a wide range of characterization techniques such as sedimentation velocity, intrinsic viscosity, translation diffusion, asymmetric flow field-flow fractionation, as well as standard SEC and NMR analysis. As a result a matrix of self-consistent parametric macromolecular characteristics and the absolute molar masses were obtained. Consistent Kuhn-Mark-Houwink-Sakurada scaling relationships were found for 0.2 M $NaCl$ water ($[\eta] = 0.002 \times M^{0.85}$, $s_0 = 0.0231 \times M^{0.41}$, $D_0 = 2,349 \times M^{-0.59}$) and 0.2 M $NaOH$ water ($[\eta] = 0.022 \times M^{0.50}$) solvent system. The corresponding conformational characteristics of the macromolecules, that define the polymer behavior in solution – equilibrium rigidity and diameter of the polymer chain, were determined to be A = 4.3 nm and A = 1.1 nm for 0.2 M $NaCl$ and 0.2 M $NaOH$ respectively. Additionally, the anisotropic optical properties were studied by the flow birefringence revealing information about optical anisotropy of the monomer unit ($\Delta a = -1.5 \times 10^{-25}$ cm^3). The presented study exemplifies the in-depth analytical characterization of cationic/charge polymers by the fundamental hydrodynamic approaches resolving the essential macromolecular characteristics and solution behavior.

1. Introduction

In the last decades, the field of macromolecular chemistry has expanded significantly resulting in the appearance of diverse polymeric systems for a broad range of applications. A special attention has always been reserved for charged polymeric species – polyelectrolytes, that are extensively used by both nature and industry [1,2]. Electrostatic interactions lead to a behavior of polyelectrolytes qualitatively completely different from those of uncharged polymers extending an area of potential applications [3,4]. Despite the continuing interest in polyelectrolytes, they remain one of the least understood states of soft matter. The fundamental aspect is to establish the influence of the molecular structure as well as position and type of charged moieties on the subsequent polymer properties. Methacrylate copolymers containing protonated amino groups within side chains represent very

promising and attractive polymer systems for both – potential applications and investigation of fundamental aspects regarding polyelectrolyte behavior and its properties. In order to overcome challenges faced by cationic polymers a variety of new methacrylate copolymers employing different amino functionalities were synthesized and investigated [5–8]. However, in spite of the advances made in this area there is still a lack in synthesis of new well-defined and chemically stable polymers with different amine functionalities that precludes further developments in this area [9,10].

Thereby, we have synthesized novel, pharmaceutically potential poly((2-aminoethyl)-methacrylate–*co*-*N*-methyl-(2-aminoethyl)-methacrylate-*co*-*N,N*-dimethyl-(2-aminoethyl)-methacrylate) copolymers employing different amine functionalities. The copolymers were fabricated using the reversible addition-fragmentation chain transfer polymerization technique resulting in a wide range of molar masses and relatively

low dispersities. Information concerning molecular, structural and conformational characteristics of the synthesized macromolecules can be extracted by a variety of different characterization techniques; the most used ones are represented by the family of mass spectrometry methods (MALDI-ToF-MS, ESI-MS), different variations of size exclusion chromatography (SEC) and nuclear magnetic resonance (NMR). However, the analysis and determination of the essential macromolecular parameters of charged polymeric species by the common analytical methods represent a non-trivial task and may fall in its accuracy; the possible solution can be then found in a family of specifically informative solution characterization methods – sedimentation velocity, translation diffusion and intrinsic viscosity. The methods were already developed at the early beginnings of the last century and have served as a base for today's polymer science [11–13]. In spite of the great foundation, these methods have receded from its position, in particular in the area of synthetic polymer investigations. We have therefore studied the synthesized p(AEMA-co-MAEMA-co-DMAEMA)$_{stat}$ copolymers by the fundamental methods of molecular hydrodynamics (viscometry, analytical ultracentrifugation, isothermal diffusion), optics (flow birefringence) as well as by the comparatively alternative and less used asymmetric flow field-flow fractionation (AF4) and common analytical methods such as SEC, NMR. The efforts were focused on the thorough polymer characterization to reveal basic structure-property relationships that define polymer behavior in solution.

2. Experimental

2.1. Materials

Unless otherwise stated, the chemical, were used without further purification. N,N-Dimethyl-(2-aminoethyl) methacrylate (DMAEMA), methacryloyl chloride, anhydrous triethylamine, 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPDB-COOH), neutral aluminium oxide and 2-(methylamino)ethanol were purchased from Sigma Aldrich. The inhibitor removal for DMAEMA was performed using the inhibitor remover from Sigma Aldrich. Di-tert-butyldicarbonate for the protection was from Alfa Aesar and 2-aminoethanol was purchased by TCI. 2-Bisazobutyronitile was purchased from Acros and recrystallized from methanol prior to use. Hydrochloric acid, dimethylformamide and tetrahydrofurane were purchased from VWR Chemicals, all other solvents used were obtained from standard suppliers.

2.1.1. Synthesis of N-tert-butyloxycarbonyl-(2-aminoethyl)-methacrylate (BocAEMA)

N-Tert-butyloxycarbonyl-(2-aminoethyl)-methacrylate was synthesized according to a procedure of Kuroda et al. [14] The synthesis was performed according to the procedure which is described earlier. ^{1}H NMR (300 MHz, CDCl$_3$): δ [ppm] = 1.42 (s, 9H), 1.93 (s, 3H), 3.43 (m, 2H), 4.18 (t, 2H), 4.8 (br. s, 1H), 5.57 (s, 1H), 6.10 (s, 1H). ^{13}C NMR (300 MHz, CDCl$_3$): δ [ppm] = 18.2 (CH$_3$), 28.3 (CH$_3$), 39.6 (CH$_2$), 63.9 (CH$_2$), 79.5(CH$_2$), 125.8 (C$_{quart}$), 136.0 (C$_{quart}$), 155.7 (C$_{quart}$), 167.2 (C$_{quart}$).

2.1.2. Synthesis of N-methyl-N-tert-butyloxycarbonyl-(2-aminoethyl)-methacrylate (BocMAEMA)

N-Methyl-N-tert-butyloxycarbonyl-(2-aminoethyl)-methacrylate was synthesized according to a procedure of Sinclair et al. [7] 10 g 2-(Methylamino)ethanol was dissolved in 80 mL chloroform, cooled in an ice bath, 29 g di-tert-butylcarbonate in 80 mL was added dropwise and stirred at room temperature for one hour. The solvent was removed under reduced pressure and the mixture was purified by distillation (30 mbar, 180 °C). Under schlenk conditions 21.4 g of N-methyl-N-tert-butyloxycarbonyl-2-aminoethanol were diluted with 100 mL dry dichloromethane, 49.4 mL anhydrous triethylamine was added and the reaction mixture cooled in an ice bath. 17.7 mL Methacryloyl chloride in 100 mL dichloromethane was added dropwise and the reaction was

stirred at room temperature overnight. The mixture was washed with water as well as brine and dried over sodium sulfate. Further purification was done using a column chromatography with a mixture of cyclohexane and ethyl acetate (9:1–3:1).^{1}H NMR (300 MHz, CDCl$_3$): δ [ppm] = 1.96 (s, 9H), 2.76 (m, 3H), 3.30 (m, 2H), 4.56 (m, 2H), 5.65 (s, 1H), 6.30 (s, 1H). ^{13}C NMR (300 MHz, CDCl$_3$): δ [ppm] = 18.2 (CH$_3$), 28.3 (CH$_3$), 35.2 (CH$_2$), 47.9 (CH$_3$), 62.7 (CH$_2$), 79.7(CH$_2$), 126.0 (C$_{quart}$), 136.1 (C$_{quart}$), 155.8 (C$_{quart}$), 167.1 (C$_{quart}$).

2.2. Synthesis of the copolymers

Copolymers of BocAEMA, BocMAEMA and DMAEMA were prepared by the reversible addition-fragmentation chain transfer (RAFT) polymerization method (Fig. 1 SI). In a typical RAFT copolymerization reaction, 0.735 g of BocAEMA (3.18 10^{-3} mol), 0.773 g of BocMAEMA (3.18 10^{-3} mol), 0.98 mg of AIBN initiator (5.96 10^{-5} mol), 5.68 mg of CPDB-COOH RAFT agent (20.33 10^{-5} mol) and 5.03 mL DMF were mixed together with anisole as internal standard (0.347 mL) in a 25 mL reaction vial. The monomer concentration was kept at 1 mol L^{-1}. The reaction mixture was degassed with argon for at least 30 min and subsequently the reaction solution was placed in a preheated oil bath at 70 °C for 38 h. The copolymer was purified by two times precipitation from THF into a large volume of n-hexane and dried under reduced pressure. Conversion was measured by ^{1}H NMR spectroscopy (Figs. 2 and 3 SI) using anisole as internal standard.

2.3. Deprotection of the Boc-protected polymers

The Boc-protected copolymers were deprotected using diluted hydrochloric acid in methanol. In a typical procedure, 300 mg polymer was dissolved in 10 mL methanol and 1 mL of hydrochloric acid was added dropwise and stirred at room temperature overnight. The solvent was removed under reduced pressure, dissolved in water and freeze dried.

2.4. Viscosity measurements

Viscosity measurements were conducted using an AMVn viscometer (Anton Paar, Graz, Austria), with the capillary/ball combination of the measuring system. The respective flow times for the solvent and polymer solutions, η_0 and η, were measured at 25 °C, with relative viscosities $\eta_r = \eta/\eta_0$ in the range of 1.2–2.5, corresponding to dilute solutions. The extrapolation to zero concentration was performed by using both the Huggins (1) and the Kraemer (2) equations, and the average values were considered as the value of the intrinsic viscosity

$$\frac{\eta_r - 1}{c} = [\eta] + k_H [\eta]^2 c + \cdots \tag{1}$$

$$\frac{ln\eta_r}{c} = [\eta] + k_K [\eta]^2 c + \cdots \tag{2}$$

where η_r is the relative viscosity, c is polymer concentration, k_H and k_K are viscometric Huggins and Kraemer constants.

2.5. Analytical ultracentrifugation

Sedimentation velocity experiments were performed with a ProteomeLab XLI Protein Characterization System analytical ultracentrifuge (Beckman Coulter, Brea, CA), using conventional double-sector Epon centerpieces of 12 mm optical path length and a four-hole rotor (AN-60Ti). Rotor speed was 42,000 rpm. The cells were filled with 420 μL of a sample solution at an initial concentration and 440 μL of solvent (0.2 M NaCl water). Before the run, the rotor was equilibrated for approximately 1 h at 25 °C in the centrifuge. Sedimentation profiles were obtained by interference optics at the same temperature. Three concentrations of each sample were studied, covering a wide