The Nanostructure of Polyelectrolyte Complexes of QPDMAEMA-b-POEGMA copolymers and Oppositely Charged Polyelectrolytes, and their Stability in the Presence of Serum Albumin

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Supporting Information

Synthesis & Characterization of Block Copolymers

1. Synthesis of block copolymers

The preparation of the quaternized poly(2-(dimethylamino ethyl methacrylate)-b-poly(oligo(ethylene glycol) methyl ether methacrylate) (QPDMAEMA-b-POEGMA) copolymer was achieved by synthesizing first the precursor poly(2-(dimethylamino ethyl methacrylate)-b-poly(oligo(ethylene glycol) methyl ether methacrylate) (PDMAEMA-b-POEGMA), employing reversible addition-fragmentation chain transfer (RAFT) polymerization, and following a two-step polymerization procedure. In particular, the PDMAEMA homopolymer was synthesized first, and was further utilized as a macro-chain transfer agent (macro-CTA) for the synthesis of the OEGMA second block (Scheme S1). The PDMAEMA-b-POEGMA copolymer was characterized by means of size exclusion chromatography (SEC), proton nuclear magnetic resonance (^{1}H-NMR) and Fourier-transform infrared spectroscopy (FTIR), to verify the successful synthesis and determine its molecular characteristics. Afterward, following a similar procedure as the one described in our previous work[1], the PDMAEMA-b-POEGMA copolymer was modified by the quaternization of the tertiary amine group of PDMAEMA block to the quaternary ammonium salt. Methyl iodide (CH_3I) was utilized as the quaternization agent, and was added in excess to a molar ratio CH_3I to amines 2:1, to achieve 100% conversion of the tertiary amine group to quaternary ammonium salt (Figure 1 in the main manuscript). The conversion of the PDMAEMA-b-POEGMA to quaternized

QPDMAEMA-b-POEGMA copolymer with permanent positive charge was examined by ^{1}H-NMR and FTIR.
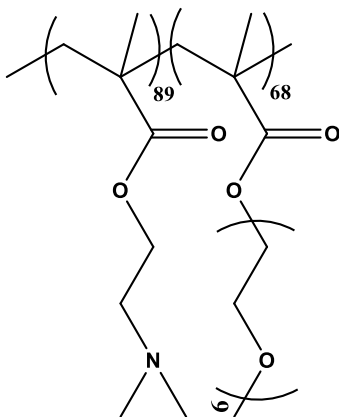
Scheme S1: Chemical structure of PDMAEMA-b-POEGMA copolymer precursor.

2. Molecular characterization of block copolymers

Molecular mass (M_w), molecular mass distribution and polydispersity index (M_w/M_n) of the synthesized PDMAEMA-b-POEGMA copolymer were determined by a SEC Waters System instrument (Waters Corporation, Milford, MA, USA). The calibration curve was set using linear polystyrene standards with narrow molecular mass distributions and average molecular masses in the range of 1,200-152,000 g·mol^{-1}. Tetrahydrofuran containing 5% *v/v* triethylamine was the mobile phase, at a flow rate 1 mL/min and temperature set at 30 °C. The determined molecular mass and polydispersity index (M_w/M_n) are presented in Table S1. The obtained molecular mass

is close to the stoichiometric one and the M_w/M_n is in a satisfactory range for RAFT polymerization procedures. Furthermore, the SEC chromatogram of PDMAEMA-b-POEGMA in Figure S1 has shifted to smaller elution volume, compared to the precursor PDMAEMA block, implying the increase of the molecular mass. Moreover, the relatively narrow, nearly symmetric and monomodal distribution of the chromatogram indicates a controlled synthetic route via the RAFT technique.

Table S1: Molecular characteristics of the synthesized copolymers.

Sample	M_w (g/mol) x 10^4 [a]	M_w/M_n [a]	(%) wt. (Q)PDMAEMA	(%) wt. POEGMA	n (units of (Q)PDMAEMA) [d]	m (units of POEGMA) [d]
PDMAEMA-b-POEGMA	4.64	1.17	30 [b]	70 [b]	89	68
QPDMAEMA-b-POEGMA	5.90	1.17	45 [c]	55 [c]	89	68

[a] Determined by SEC, [b] Determined by ^{1}H-NMR, [c] Determined assuming 100% quaternization, [d] Calculated taking into consideration the molecular weight of DMAEMA monomer plus the molecular weight of the quaternization agent (CH_3I) and the molecular weight of the OEGMA monomer.
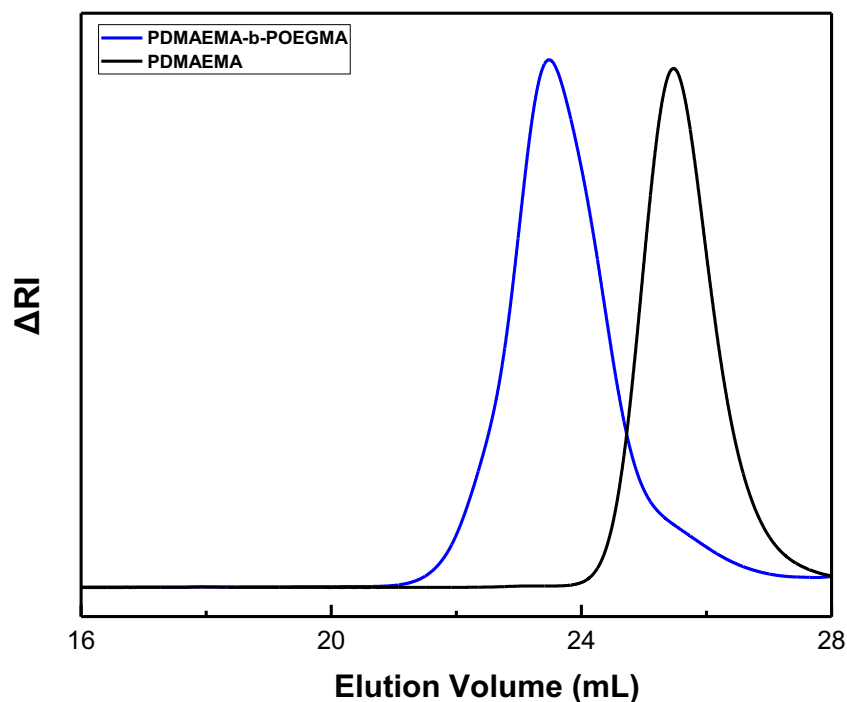
Figure S1: Chromatograms of the PDMAEMA precursor and PDMAEMA-b-POEGMA copolymer as obtained by Size Exclusion Chromatography (SEC) in THF/5% *v/v* Et3N.

^{1}H-NMR spectroscopy was utilized to confirm the chemical structure and to determine the mass composition (% wt.) of the synthesized block copolymers. The measurements were carried out on a Bruker AC 300 MHz FT-NMR spectrometer (Bruker, Billerica, MA, USA). The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. The ^{1}H-NMR spectrum of PDMAEMA-b-POEGMA (Figure S2) was recorded using deuterated chloroform (CDCl$_3$). The analysis of the ^{1}H-NMR spectrum (Figure S2) verified the expected chemical structure of the PDMAEMA-b-POEGMA copolymer by identifying the signals of the characteristic spectral peaks, which are listed below.

^{1}H-NMR spectral peaks of PDMAEMA-b-POEGMA copolymer (300MHz, CDCl$_3$, ppm): 4.06 (peak c$_1$: 2H, -OC$\textbf{\textit{H}}_2$CH$_2$N-), 3.87 (peak c$_2$: 2H, -(C=O)OC$\textbf{\textit{H}}_2$CH$_2$O), 3.64 (peak f: 36H, -(C$\textbf{\textit{H}}_2$C$\textbf{\textit{H}}_2$O)$_9$CH$_3$-), 3.36 (peak g: 3H, -(CH$_2$CH$_2$O)$_9$C$\textbf{\textit{H}}_3$-), 2.56 (peak d: 2H, -OCH$_2$C$\textbf{\textit{H}}_2$N-), 2.28 (peak e: 6H,-N(C$\textbf{\textit{H}}_3$)$_2$), 1.81 (peak b: 4H, -C$\textbf{\textit{H}}_2$C-), 0.87 (peak a: 6H, -CH$_2$CC$\textbf{\textit{H}}_3$-).

Moreover, the signal at 2.28 (peak e, Figure S2) corresponding to the -CH$_3$ protons of the tertiary amino group of PDMAEMA block (peak e, 6H, -N(CH$_3$)$_2$) and the one at 3.64 ppm of the POEGMA ethylene glycol side chain (peak f, 36H, -(CH$_2$CH$_2$O)$_9$CH$_3$-) were utilized for the estimation of the copolymer composition.[1] The mass composition value was very close to the stoichiometric one and is also included in Table S1.
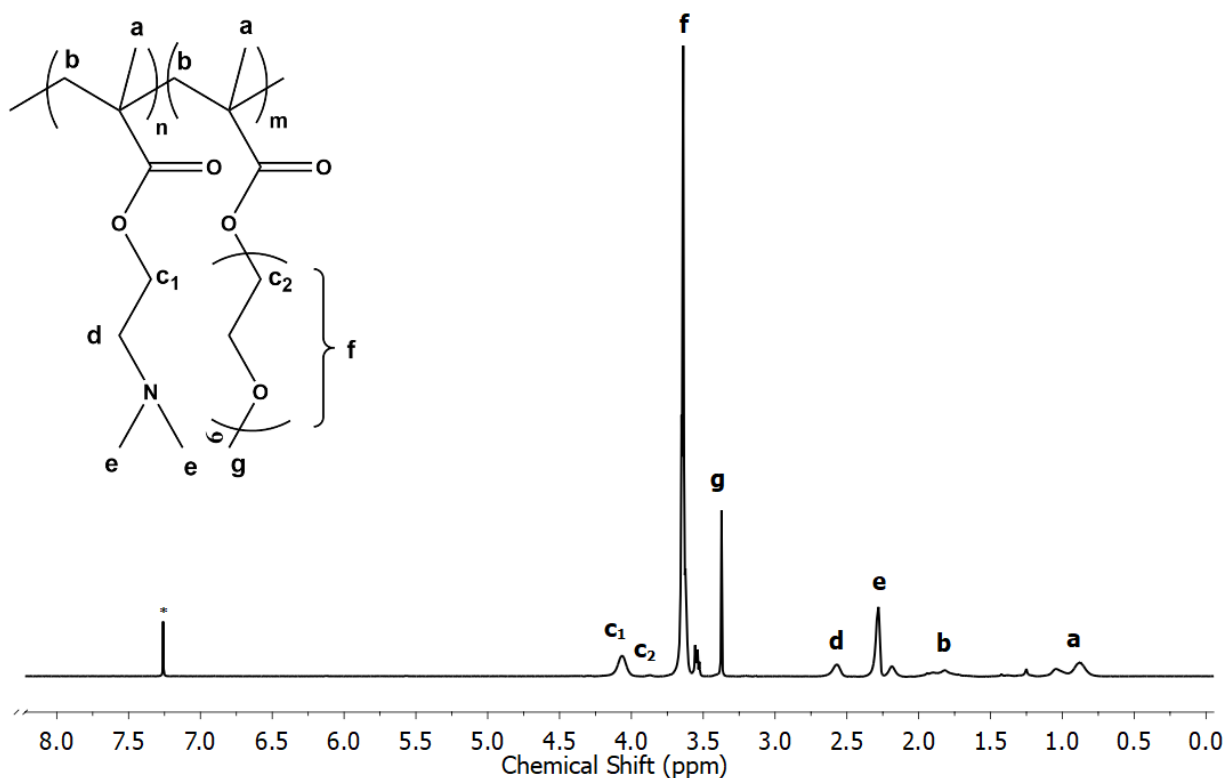
PDMAEMA-b-POEGMA

Figure S2: ^{1}H-NMR spectrum of PDMAEMA-b-POEGMA copolymer in CDCl$_3$. The asterisk (*) at 7.26 ppm denotes the peak of the solvent.

In the case of the quaternized QPDMAEMA-b-POEGMA copolymer, ^{1}H-NMR measurements were performed utilizing deuterium oxide (D$_2$O) as solvent and the recorded spectrum is presented in Figure S3. The ^{1}H-NMR spectral peaks of QPDMAEMA-b-POEGMA copolymer (300MHz, D$_2$O, ppm) are: 4.54 (peak c$_1$: 2H, -OC**H$_2$**CH$_2$N-), 4.20 (peak c$_2$: 2H, -(C=O)OC**H$_2$**CH$_2$O), 3.73 (peak f: 36H, -(C**H$_2$C H$_2$**O)$_9$CH$_3$-), 3.41 (peak g: 3H, -(CH$_2$CH$_2$O)$_9$C**H$_3$**-), 3.36 (peak e: 9H,-N(C**H$_3$**)$_3$), 3.07 (peak d: 2H, -OCH$_2$C**H$_2$**N-), 2.10 (peak b: 4H, -C**H$_2$**C-), 1.10 (peak a: 6H, -CH$_2$CC**H$_3$**-).

According to the literature, the characteristic peak of the methyl protons of the quaternary amine is expected to be detected at approximately 3.32 ppm.[2] However, the CH$_3$ protons of POEGMA (peak g, 3H, -CH$_3$) are also detected in the same spectral region. Due to the overlap of the peaks e and g (Figure S3), the accurate estimation of the mass composition was difficult by ^{1}H-NMR spectroscopy. Nevertheless, the detection of the peak e (9H, -N(CH$_3$)$_3$) (Figure S3) at 3.36 ppm despite the overlap, along with the absence of signal at 2.28 ppm which would be attributed to methyl protons of tertiary amine (as in the case of the precursor PDMAEMA-b-POEGMA, Figure S2, peak e), indicate that the quaternization reaction was quantitative and the PDMAEMA-b-POEGMA precursor copolymer was successfully converted to the quaternized QPDMAEMA-b-POEGMA polyelectrolyte copolymer. Concerning the molecular characteristics of the quaternized copolymer, the mass composition and the molecular mass were estimated according to the molecular characteristics of the precursor copolymer and assuming that the conversion of the

tertiary to quaternary amine group is quantitative and occurs at 100%. The calculated values are included in Table S1.
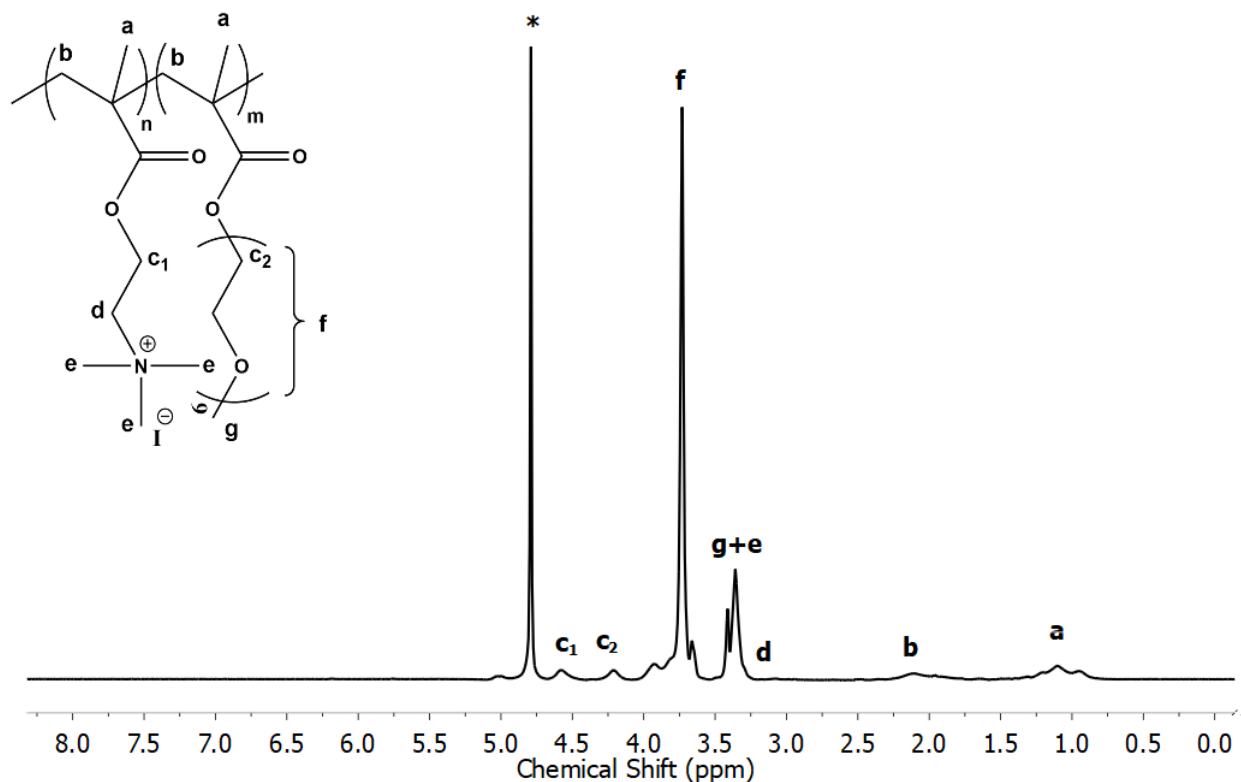

Figure S3: ^{1}H-NMR spectrum of QPDMAEMA-b-POEGMA copolymer in D$_2$O. The asterisk (*) at 4.79 ppm denotes the peak of the solvent.

Along with ^{1}H-NMR, FTIR spectroscopy in the form of ATR-FTIR was also implemented to identify the chemical structure of the synthesized copolymers and especially to verify the conversion of the tertiary amine to quaternary ammonium salt. The measurements were performed at room temperature in the range 5000–550 cm^{-1}, using a Fourier transform instrument (Bruker Equinox 55), equipped with a single bounce attenuated total reflectance (ATR) diamond accessory

(Dura-Samp1IR II by SensIR Technologies, Chapel Hill, NC, USA). The polymer samples were analyzed in the solid state and the spectra were recorded after 64 scans with resolution of 4 cm^{-1}.

The recorded ATR-FTIR spectra of PDMAEMA-b-OEGMA copolymer as well as its quaternized derivative are depicted in Figure S4. The analysis of the ATR-FTIR spectral peaks below confirmed the expected chemical structure of the synthesized copolymers by identifying the major chemical groups of which the copolymers are comprised.[1,3]

ATR-FTIR spectral peaks of PDMAEMA-b-POEGMA), v (cm^{-1}), (s: stretching, b: bending): (CH$_2$): 2944(s), 2866(s) and 1454(b), (–N(CH$_3$)$_2$): 2821(s) and 2769(s), (C=O): 1726(s), (C-N): 1130(s), (C-O-C): 1100(s).

ATR-FTIR spectral peaks of QPDMAEMA-b-POEGMA), v (cm^{-1}), (s: stretching, b: bending): (O-H) (internal moisture) 3471(s), (C-N(CH$_3$)$_3^+$): 3005(s), 1474(s) and 950(s), (CH$_2$): 2870(s) and 1450(b), (C=O): 1726(s), (C-N): 1130(s), (C-O-C): 1100(s).

Furthermore, the disappearance of the characteristic bands of the tertiary amine groups N(CH$_3$)$_2$ at 2821 cm^{-1} and 2769 cm^{-1} in the spectrum of QPDMAEMA-b-POEGMA copolymer (in comparison with the spectrum of the precursor), as well as the presence of new absorption peaks at 3005 cm^{-1}, 1474 cm^{-1} and 950 cm^{-1} corresponding to quaternary amine groups, proved the successful quaternization modification.[1,3,4]

In conclusion, the overall results obtained by SEC, ^{1}H-NMR and FTIR demonstrated the synthesis of well-defined copolymers with the desirable molecular characteristics.
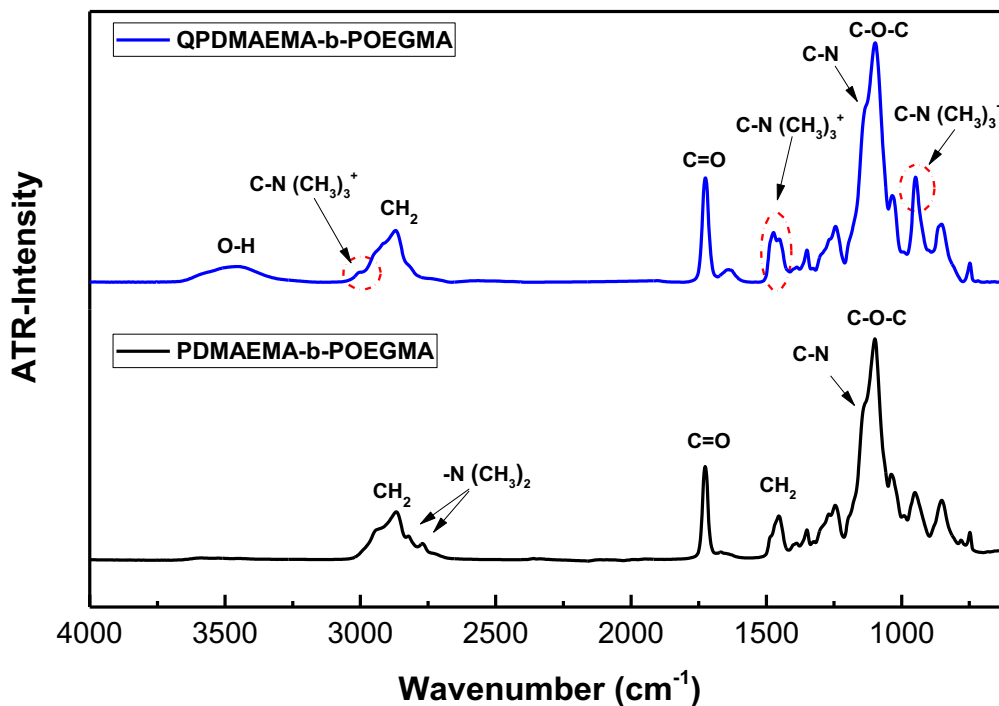
Figure 4: ATR-FTIR spectra of PDMAEMA-b-POEGMA and QPDMAEMA-b-POEGMA copolymers in solid state. The red circles denote the quaternary ammonium groups and therefore the successful quaternization of the PDMAEMA-b-POEGMA precursor copolymer.

References

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