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Soluble polyelectrolyte complexes composed of poly(ethylene oxide)-*block*-poly(sodium methacrylate) and poly(methacryloyloxyethyl trimethylammonium chloride)

Susanna Holappa, Toni Andersson, Lauri Kantonen, Patrik Plattner, Heikki Tenhu*

Laboratory of Polymer Chemistry, Department of Chemistry, University of Helsinki, PB 55, FIN-00014 Helsinki, Finland

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Abstract

Complexation between poly(methacryloyloxyethyl trimethylammonium chloride), PMOTAC, and poly(ethylene oxide)-*block*-poly(sodium methacrylate), PEO-*block*-PMANa with MANa blocks with two different molecular weights has been investigated by light scattering, LS, viscosimetry and conductivity measurements. Owing to the PEO blocks the polyelectrolyte complexes are water-soluble particles. Effects of the ratio of the oppositely charged monomer units, the ionic strength of the solution, and solution pH have been studied. With the 1:1 mixing ratio stable polyelectrolyte complexes, PECs, with spherical shape were formed. When either the cationic or anionic component was in excess, charged non-stoichiometric complexes were formed. The complexes were stable also in solutions with comparatively high ionic strength, though stronger secondary aggregation was observed. By changing the solution pH the degree of dissociation of PEO-*block*-PMANa could be adjusted. In the vicinity of the $pK_{a,\mathrm{average}}$ of PMAA, a minimum in the particle size and a maximum in the solution conductivity were observed. In solutions with lower pH, a typical self-complexation of PEO-*block*-PMAA was detected. Also, unexpected interactions between PMOTAC and the self-complexes of PEO-*block*-PMAA were observed.

Keywords: Polyelectrolyte complex; Poly(ethylene oxide)-*block*-poly(methacrylic acid); Light scattering

1. Introduction

Polyelectrolyte complexes (PEC) have an important role in nature. By mimicking the complex formation known to take place in living organisms, new methods for example in medicine, like in drug delivery and gene therapy, can be developed [1–6]. However, it is of great importance to know the mechanisms of the complexation and the effects of varying conditions to the formation as well as the solubility and stability of the complexes. Intensive research has been widely done on this field [7,8]. However, as the chemical and physical properties and environmental conditions can be varied almost limitlessly the field is still vastly studied.

Several variables affect the formation mechanisms and the stability of the PECs in water. Firstly, the strength of the PE as an acid or a base is of essential importance. Weak polyelectrolytes with unequal molecular weights can form thermodynamically stable PECs in the presence of low molecular weight salt [9,10]. Strong PEs and/or high molecular weight PEs form more aggregated PECs and the formation is strongly kinetically controlled [11,12]. The molecular weight of the PEs has its influence [13–15]. Typically, PECs with the anion to cation ratio close to unity are not soluble in water. The complex particles may, however, be stabilised as aqueous colloids. Whether the stabilisation of the particles is electrostatic or steric, influences on the applicable monomer unit ratio range, as in the complexes of homo-PEs or PEs with neutral segments, respectively. Electrostatically stabilised homo-PECs have a limited range of monomer unit mixing ratios. As the ratio approaches unity, very often secondary aggregation takes place, leading to precipitation. Also, the stability of homo-PECs is very sensitive to the ionic strength of the solution [12,16,17]. The colloidal stability of the PECs can be improved by utilising double-hydrophilic block copolymers. The steric stabilisation provided by the neutral segment enables the formation of equimolar PEC

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* Corresponding author. Tel.: +358-9-191-50334; fax: +358-9-191-50330.

E-mail address: heikki.tenhu@helsinki.fi (H. Tenhu).

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particles which are stable in much broader pH range in comparison to the complexes of homo-PEs [18–21].

In this work the complexation of a diblock copolymer comprising of an anionic block and an electroneutral one, poly(ethylene oxide-*block*-sodium methacrylate), PEO-*block*-PMANa, with a polycation, poly(methacryloyloxyethyl trimethylammonium chloride), PMOTAC, has been studied focusing on some of the abovementioned variables.

2. Experimental

2.1. Materials

For syntheses of polyanions: 4,4′-azobis(4-cyanopentanoic acid), ACPA, was purchased from Fluka and it was dried under vacuum for 48 h. Tri(methyl silyl)methacrylate (TMSMA), Aldrich, was distilled in vacuum. 3-Mercaptopropionic acid, 3-MPA, Fluka, was used as received. α-Amino-ω-methoxy-PEG, MW 5000, Shearwater Polymers, Inc., was dried under vacuum at 50 °C overnight. *N*-hydroxysuccinimide (HoSu), Fluka, and 1,3-dicyclohexylcarbodiimide (DCC), Fluka, were used as received. Dioxane, acetone, and acetonitrile were dried on molecular sieves.

For syntheses of polycation: methacryloyloxyethyl trimethylammonium chloride (MOTAC) as an 75% aqueous solution, Aldrich, ammonnium peroxydisulphate (APS), Merck, and *N*,*N*,*N*,*N*-tetramethyl ethylene diamine, Aldrich, were used as received. Phosphate buffer solution pH 7, Titrisol, was diluted with distilled and deionised water, Elgastat UHQPS.

2.2. Syntheses

To synthesise PEO-*block*-PMAA copolymers, TMSMA (10 mass%) was first radical polymerised using ACPA as an initiator and 3-MPA as a chain transfer agent. The molar ratios, monomer:initiator:chain transfer agent, in two TMSMA polymerisations were 100:1.3:1.7 and 100:0.25:0.3. Reactions were carried out in dioxane at 60 °C under nitrogen for 18 h. After the reaction, solvent was evaporated and the polymer dried under vacuum at room temperature overnight. The product was purified by precipitation from acetone to acetonitrile. It was filtered and dried under vacuum overnight. The second step of the synthesis was the activation of the carboxylic acid end groups using an excess amount of *N*-hydroxysuccinimide [22,23]. This was carried out in dioxane in the presence of DCC for two days. The reaction mixture was precipitated in acetonitrile and the product was dried under vacuum. In the final step the activated PTMSMA and the amino terminated PEO were attached to each other in a condensation reaction. The reaction was carried out in dioxane under nitrogen at room temperature in dark for two weeks. The product was precipitated in acetonitrile. All the reaction steps were done in dry conditions in order to prevent the hydrolysis of the protecting trimethylsilyl group. The protecting silyl groups were removed by washing the product first with 0.1 M NaOH and subsequently with water, after which the block copolymer was in its sodium methacrylate form, PEO-*block*-PMANa.

To synthesise the polycation, MOTAC (15 mass%) was radical polymerised using APS as an initiator (0.36 mole%). The reaction was carried out in an aqueous phosphate buffer, pH 7, at 65 °C under nitrogen for 19.5 h. Then, the reaction mixture was concentrated and the polymer was precipitated in acetone. The product was purified by dialysis in water–acetone mixture (10 vol% acetone) for five days [24].

2.3. Solution preparation

All samples were prepared by mixing 1 g/l stock solutions of polyanions and polycations in desired ratios. The mixing ratios are presented as the molar ratios of the charged units, $X = [\mathrm{MOTAC}]/[\mathrm{MANa}]$. The order of mixing was always the same, a polyanion solution was added to a polycation solution. In all the experiments the stock solutions were filtered through 0.45 μm filters. Magnetic stirring was used during the sample preparation and was continued overnight. The experiments to study the effect of the ratio of charged monomer units were conducted in 20 and 80 mM aqueous NaCl. The dilute NaCl solution was selected as a solvent in order to decrease the polyelectrolyte effect of the free polyelectrolytes, and also to obtain thermodynamically stable complexes [9,15]. The solution pH was controlled by phosphate buffer where the concentration of phosphate salts was 50 mM.

2.4. Methods

NMR spectroscopy. ^{1}H NMR (Varian Gemini 2000) was used to determine the chemical compositions of the polymers.

Size exclusion chromatography. The molecular weights were determined by size exclusion chromatography, SEC, (Waters) using refractive index detection. For the PTMSMA precursors, Styragel HR2, HR4 and HR6 columns were used with THF as an eluent. The columns were calibrated with PMMA standards (PSS Polymer Standards Service GmbH) in the range from 2400 to 850,000 g/mol. For the PEO-*block*-PMANa, Ultrahydrogel 250 and 2000 columns were used and 0.1 M aqueous $\mathrm{NaNO_3} + 3\%$ acetonitrile was used as an eluent. Molecular weight of PMOTAC was measured in 0.8 M aqueous $\mathrm{NaNO_3} + 3\%$ acetonitrile. The columns were calibrated with poly(ethylene oxide) standards (Polymer Laboratories), 4120–965,000 g/mol.

Laser light scattering. Static light scattering (SLS) and dynamic light scattering (DLS) measurements were conducted with a Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator, Ar-laser $\lambda = 514.5$ nm. The SLS data were analysed by using

Zimm's double extrapolation method. The specific refractive index increments, dn/dc, of the polymers and the polyelectrolyte complexes in 20 mM NaCl were determined with WYATT/OPTILAB 903 interferometric refractometer at 514.5 nm. The effect of the released counter ions to the mass concentration of PECs was taken into account via equation

$$C_{\mathrm{PEC}} = \frac{C_{\mathrm{PMOTAC}}V_{\mathrm{PMOTAC}}(1 - M_{\mathrm{Cl}^-}/M_{\mathrm{MOTAC}}f_{\mathrm{MOTAC}}) + C_{\mathrm{BC}}V_{\mathrm{BC}}w_{\mathrm{MAA}}(1 - M_{\mathrm{Na}^+}/M_{\mathrm{MAA}}f_{\mathrm{MAA}}) + C_{\mathrm{BC}}V_{\mathrm{BC}}w_{\mathrm{EO}}}{V_{\mathrm{PMOTAC}} + V_{\mathrm{BC}}} \tag{1}$$

where C_{PMOTAC}, C_{BC} and V_{PMOTAC}, V_{BC} are the concentrations and volumes of PMOTAC and block copolymer solutions; M_{Cl}^-, M_{Na}^+, M_{MOTAC} and M_{MAA} are the molar masses of the counter ions and the charged units of the PMOTAC and the block copolymers; f_{MOTAC} and f_{MAA} are the molar excess coefficients of the charged units; w_{MAA} and w_{EO} are the weight fractions of MAA and EO units in the block copolymers [modified from Ref. [25]].

The time correlation functions measured by DLS were analysed with a Laplace inversion program, CONTIN.

Conductometry. The conductivities were measured with Consort C833 multi-parameter analyser.

Viscosimetry. Reduced viscosity was measured by Micro-Ubbelohde viscometer with Lauda S5 detector, which was connected to Lauda PVS1 Processor Viscosity System with a PVS 2.49e analysis program.

3. Results and discussion

3.1. Syntheses

Linear block copolymers, PEO-*block*-PMANa, were obtained through preparing poly(trimethylsilyl methacrylate), PTMSMA, with a carboxyl end group originating from the initiator or the chain transfer agent, and by a subsequent connection of PTMSMA to an amino-terminated PEO. Molecular weights of the PEO-*block*-PMANa and PMOTAC used in the experiments are shown in Table 1. The molecular weight distributions of the polymers are broad as is typical for free radical polymerisation. The MW of the PEs is known to have an effect on the PEC formation [13–15]. In salt-free and very dilute salt solutions the effect is minimal due to the dominating kinetic control in the PEC formation. In more saline solutions the higher MW PEs are favoured in complexation in comparison to the low MW species. Whether the polydispersity of the PMOTAC and PEO-*block*-PMANa block copolymers affects the forming PECs will be considered later in the text.

3.2. Complexation

3.2.1. Effect of the ratio of the charged monomers

The effect of the ratio of the charged repeating units on complex formation was studied with anionic block copolymers PEO-*block*-PMANa1 and PEO-*block*-PMANa2 and the cationic homopolymer PMOTAC in order to find out the effect of the length of the anionic block on the complexation. Also the effect of salt concentration was studied by comparing the complex formation in aqueous 20 and 80 mM NaCl solutions. As in many preceeding studies concerning the complexation of polyelectrolytes the most complete complexation was observed when the ratio of the charged repeating units was close to unity.

The particle size distributions for the complexes formed by PEO-*block*-PMANa1 and PMOTAC in the ionic strengths of 20 and 80 mM, and by PEO-*block*-PMANa2 and PMOTAC in the ionic strength of 20 mM are shown in Fig. 1. The effects of the length of the anionic block as well as that of the salt concentration are clearly seen from these figures. In each of the three cases the particles that are formed in the mixing ratios of 0.6–1.4 show narrow size distributions but different sizes, however. As the particle formation of PMOTAC with PEO-*block*-PMANa1 and PEO-*block*-PMANa2 is compared, it is seen that a longer anionic block increases the particle size, $D_{\mathrm{mean}} = 70$ and 120 nm, respectively. This may be explained by the smaller fraction of the solubilising PEO block in PEO-*block*-PMANa2 or by the larger dimensions of the PMANa

Table 1
Molecular weights of the polymers

	PTMSMA1	PTMSMA2	PMANa1	PMANa2	PEO-*block*-PMANa1	PEO-*block*-PMANa2	PMOTAC
$^{a}M_{w}$ (g/mol)					19,600	54,700	401,000
$^{b}M_{w}$ (g/mol)	44,900	77,600	20,100	64,800	22,800	74,800	299,000
$^{b}M_{n}$ (g/mol)	18,300	49,800	13,900	35,600	15,800	47,100	110,000
$^{c}M_{n}$ (g/mol)					18,200	36,700	
$^{d}M_{e}$ (g/mol)					$^{c}149$	$^{c}125$	208

[a] SLS.

[b] SEC.

[c] ^{1}H NMR, MW of PEO block 5000.

[d] M_e is mass per charge.

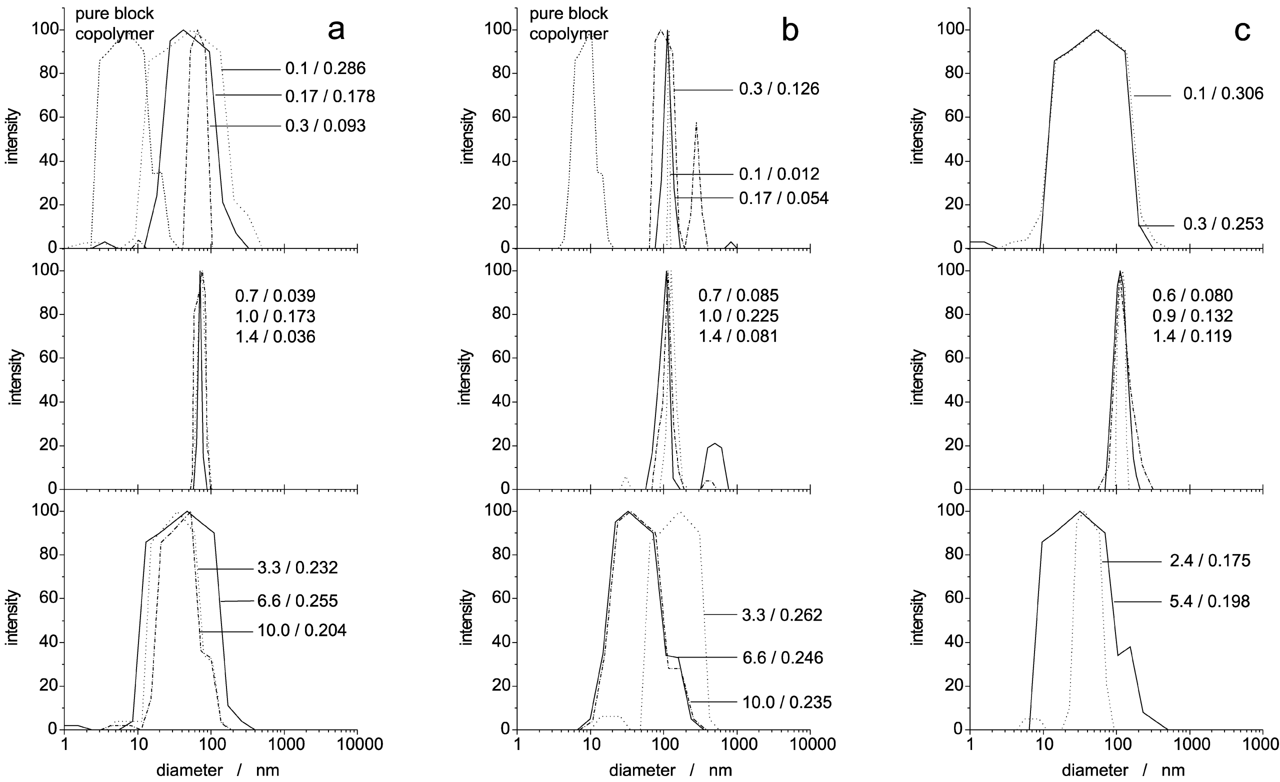


Fig. 1. The particle size distributions and polydispersities of the PEO-*block*-PMANa and PMOTAC complexes in different mixing ratios, [MOTAC]/[MANa], in aqueous NaCl solutions. (a) PMOTAC/PEO-*block*-PMANa1, 20 mM NaCl, (b) and 80 mM NaCl, and (c) PMOTAC/PEO-*block*-PMANa2, 20 mM NaCl. For every curve, mixing ratio/polydispersity is indicated.

block to be able to complex higher number of polycations [26]. In the stoichiometric complexes the number of anionic block copolymers that the polycations can occupy were calculated from the NMR data of the block copolymers and the SEC data of PMOTAC. One PMOTAC can bind 4.5 PEO-*block*-PMANa1 chains and 2.0 PEO-*block*-PMANa2 chains. So, if the particle cores in both cases would consist of a same number of PMOTAC chains with a stoichiometric amount of the anionic block copolymers, the number of the solubilising PEO chains on the particle surface would be more than twice less in the case of PEO-*block*-PMANa2 than in the case of PEO-*block*-PMANa1. On the other hand, the lower molecular weight of the anionic block in the PEO-*block*-PMANa1 reduces the probability to interact with many polycations at the same time, which also could cause the formation of smaller particles.

Comparison of the complexes formed by PEO-*block*-PMANa1 with PMOTAC in 20 and 80 mM NaCl solutions supports the first conclusion above. Increasing the salt concentration increases the particle size. This is due to the salting out phenomenon. As the particle size increases, the growth is stronger in volume than in surface area. Thus, the relative number of PEO chains on the particle surface increases.

Zintchenko et al. have reported that the length of the PEO chain is a more dominant factor in the PEC stabilisation compared to its proportion [26]. It appears that the molecular weight 5000 of PEO is just in the limit to be sufficient for stabilising the stoichiometric complexes. Although the size distributions measured at a scattering angle 90° were narrow and monomodal (Fig. 1), the size distributions detected in other angles were often bimodal. The occasionally observed low intensity peak indicates the appearance of a temporal aggregate formation of the PECs [16]. Most clearly this was observed in the complexes of PEO-*block*-PMANa1 and PMOTAC in 80 mM NaCl solution. In the PECs formed with the mixing ratios of 0.7 (0.6 in the case of PEO-*block*-PMANa2) and 1.4, the equilibrium between the steric and electrostatic stabilisation is optimal for stabilising the PECs. Size distributions are equal regardless of the scattering angle, aggregation of PECs was not observed.

The polydispersity index (PDI) of the particles was determined from the μ_2/Γ^2 ratio from the cumultant fit of the second order, indicated in Fig. 1 next to the mixing ratios. The optimal stabilisation of the particles with the mixing ratios of 0.7 and 1.4 becomes very apparent here as well. In those ratios PDIs have their minimum values. Comparing these values to the PDI value of 0.06 obtained by Gohy et al. [20] for the PEC system consisting of poly(2-vinylpyridine)-*block*-poly(ethylene oxide), M_w/M_n 1.05, and sodium poly(4-styrenesulfonate), M_w/M_n 1.1, implies that the polydisperse nature of the polymers used in the present study has a negligible effect on the size distribution of the PECs. This is true especially in the case of the complexes formed by the shorter PEO-*block*-PMANa1, where the PDI is <0.04 in the mixing ratios of 0.7 and 1.4. The higher PDI value of the stoichiometric complexes rises from the temporal aggregate formation as discussed above.

For the PECs formed with a further excess of either anionic or cationic components the size distributions are substancially different from those of the PECs formed with the mixing ratios of 0.6–1.4, with an exception of the PEO-*block*-PMANa1 complexes in 80 mM NaCl solution. In general, the deviations from the stoichiometric mixing ratios broaden the size distributions and decrease the mean diameter of the particles. There are several possible reasons for the broadening of the size distributions. The solutions may have heterogeneous compositions, comprising of stoichiometric complexes and uncomplexed polymers. On the other hand, the solutions may contain strongly charged PECs, which have bound all the excess polymers. The broadness of the distributions would then arise from the polydispersity of the particles plus the polyelectrolyte effect. Also, both stoichiometric and strongly charged PECs may coexist [9,11,27–29].

The diffusion coefficients of the complexes of PEO-*block*-PMANa1 and PMOTAC in 20 mM NaCl solution, determined from the first cumulant of the cumulant fit of the third order, $D = \Gamma/q^2$, are plotted against the square of the scattering vector, q^2, in Fig. 2. With the mixing ratios 0.3–1.4, where the size distributions are narrow, the diffusion coefficients are angular independent. When the size distributions are broader, clear angular dependence is observed. The particles whose diffusion coefficient does not show any angular dependence are typically spherical in shape. In the case of elongated structures the rotational and translational contributions to the diffusion coefficient can be detected separately [20,21,30,31]. However, the composition of the complex solutions in non-stoichiometric mixing ratios is very often polydisperse, which property also induces angular dependence of the diffusion coefficients [11].

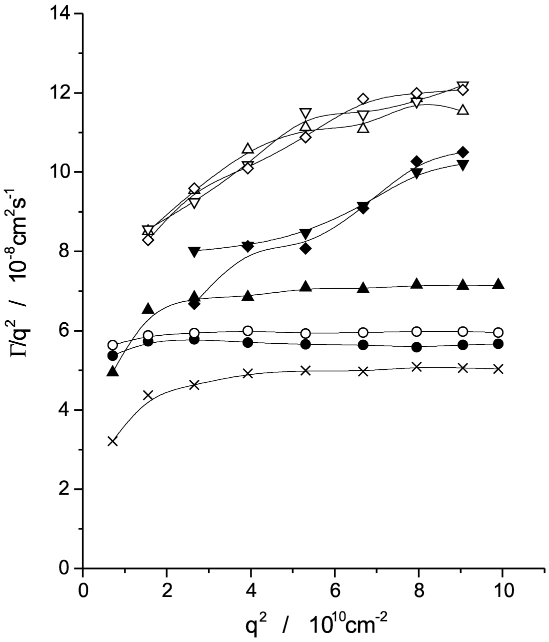


Fig. 2. The diffusion coefficients, $D = \Gamma/q^2$, of the complexes of PEO-*block*-PMANa1 and PMOTAC in different mixing ratios, $X =$ [MOTAC]/[MANa], plotted against the square of the scattering vector, q^2. Keys to symbols: cross $X = 1$; filled circle $X = 0.7$; filled upward triangle $X = 0.3$; filled downward triangle $X = 0.17$; filled diamond $X = 0.1$; open circle $X = 1.4$; open upward triangle $X = 3.3$; open downward triangle $X = 6.6$; open diamond $X = 10$.

The data collected by SLS for the PECs with cation/anion ratio 0.3, 1 and 3.3 is shown in Table 2. The R_g/R_h ratio gives further information about the architecture of the complex particles. The experimental R_g/R_h ratios of the PEO-*block*-PMANa1 complexes with mixing ratios of 0.3 and 1 equal 0.91 and 0.83, respectively, indicative of compact structures as compared to the theoretical value of 0.775 for hard spheres. In comparison the R_g/R_h ratio of the stoichiometric PEO-*block*-PMANa2 complexes equals 1.33 indicating a looser structure. The R_g/R_h ratio of 1.2 has been obtained for particles which were prepared by crosslinking bifunctional linear polystyrenes with tetrafunctional crosslinking agent at subcritical concentrations [32]. In comparison, R_g/R_h ratios of 1.27 and 0.75 were determined for a linear PS and for microemulsion polymerisate with the same branching density in similar conditions, respectively. These comparisons demonstrate the influence of many small loops, which do not significantly alter the chain dimensions, compared with linear chains. Thus, in spite of the stoichiometric ratio of the charged units it appears that in the complexes of the longer PEO-*block*-PMANa2 and PMOTAC, there still exists uncomplexed fractions of the PEs that form loops. The R_g/R_h ratios of 2.16–2.37 for the complexes of PEO-*block*-PMANa1 with mixing ratio 3.3 and the complexes of PEO-*block*-PMANa2 with mixing ratio 0.3 and 3.3 support the conclusion made from the angular dependency of the diffusion coefficients. The R_g/R_h ratio is expected to be >2.0 for rod-like particles.

The molecular weights allow the calculation of the number of polyelectrolyte chains per PEC, α. The equation used for stoichiometric complexes is:

$$\alpha_{\mathrm{PE}} = \frac{M_{\mathrm{w,PEC}}}{M_{\mathrm{w,PE}}} \frac{M_{\mathrm{e,PE}}}{M_{\mathrm{e,BC}} + M_{\mathrm{e,PMOTAC}} - M_{\mathrm{e,NaCl}}} \tag{2a}$$

The equations for the block copolymer and PMOTAC in cation/anion = 0.3 complexes are:

$$\alpha_{\mathrm{BC}} = \frac{M_{\mathrm{w,PEC}}}{M_{\mathrm{w,BC}}} \times \frac{0.3M_{\mathrm{e,BC}}}{3(M_{\mathrm{e,BC}} - 0.3M_{\mathrm{e,Na^+}}) + (M_{\mathrm{e,PMOTAC}} - M_{\mathrm{e,Cl^-}})} \tag{2b}$$

Table 2
SLS data for the polyelectrolyte complexes

PEC of PMOTAC and	Cation/anion	M_w (g/mol)	R_g (nm)	R_g/R_h	α_{PMOTAC}	α_{BC}
PEO-*block*-PMANa1						
	0.3	1.25×10^6	28.6	0.91	1.0	48
	1	3.84×10^7	36.2	0.83	67	976
	3.3	7.75×10^5	45.7	2.16	1.7	7.5
PEO-*block*-PMANa2						
	0.3	5.78×10^5	56.2	2.23	0.5	7.5
	1	5.14×10^8	92.5	1.33	969	4271
	3.3	7.48×10^5	47.1	2.37	1.7	2.2

$$\alpha_{\mathrm{PMOTAC}} = \frac{M_{\mathrm{w,PEC}}}{M_{\mathrm{w,PMOTAC}}} \times \frac{0.3M_{\mathrm{e,PMOTAC}}}{(M_{\mathrm{e,BC}} - 0.3M_{\mathrm{e,Na^+}}) + 0.3(M_{\mathrm{e,PMOTAC}} - M_{\mathrm{e,Cl^-}})} \tag{2c}$$

where the subscripts PE and BC refer to the polyelectrolyte in question and to the block copolymer in question; M_w is the molecular weight determined by SLS, M_e is the mass per charge; coefficients 3 and 0.3 refer to the molar fractions of the charged units. $0.3 \times M^+_{e,\mathrm{Na}}$ and $1 \times M_{e,Cl^-}$ are the fractions of released counterions. In the case of the cation/anion = 3.3 complexes the coefficients for molar fractions of the charged units are used vice versa as well as the coefficients for the released counterions. The effect of the length of the anionic block is different in each of the three mixing ratios. The requirement for sufficient number of solubilising PEO blocks in a PEC causes more than a tenfold difference in the molecular weights between the two different stoichiometric complexes. In the case of the cationic excess the complexes are similar in size and shape. When the anionic components are in excess there appears to be a difference in the number of block copolymers that a PMOTAC chain is able to bind depending on the molecular weight of the anionic component. In case of the shorter PEO-*block*-PMANa1, one complex contains one PMOTAC plus 48 block copolymers. However, in the case of the longer PEO-*block*-PMANa2 the corresponding values indicate that only half of the block copolymers are integrated into the complexes. The R_g/R_h ratio of the stoichiometric complex of PEO-*block*-PMANa2 already indicated that the structure of the complex is loose in comparison to the corresponding PEO-*block*-PMANa1 complex. Thus, the lower uptake of the block copolymers in the complexes of anionic excess is apparently also due to the imperfect organisation.

Fig. 3 represents the specific viscosities of the PEC solutions in comparison to the specific viscosities of the pure uncomplexed species. The viscosities of the PEC solutions are plotted against the calculated mass concentration of the fraction exceeding the stoichiometric consumption of the component in excess. Thus, e.g. in the case of anionic excess, Fig. 3a,b, when the cation to anion ratio is zero, c in the abscissa has a value 1 g/l, and with the ratio equal to one, $c = 0$ g/l. Immediately it is clear that the solutions are not composed of stoichiometric complexes plus dissolved polymers. Since the contribution of the uncharged particles to the solution viscosity is of the order of nil, the viscosities of the PEC solutions should be similar to the viscosities of the pure polymer solutions due to the uncomplexed components. However, in each case different kinds of species build up the viscosity of the solutions. The effect of increasing ionic strength of the solutions due to the released counterions upon complex formation on the viscosities can be excluded because dilute NaCl solutions were chosen as solvents.

The PEC solutions with cationic excess, Fig. 3c, exhibit lower viscosities both in 20 and 80 mM NaCl solutions in comparison to the viscosities of the solutions of the pure component. In the ionic strength of 20 mM the viscosity curve of the PEO-*block*-PMANa2 complexes in cationic excess (data not shown) was identical to the PEO-*block*-PMANa1 complex solutions. This indicates that the PMANa block length has only a marginal effect on the resulting positively charged complexes. The particles consist of one or two (1.7 according to SLS, Table 2.) PMOTAC chains which have complexed the available anionic components. This way the complexes could be imagined to be necklaces with uncharged complexed segments as pearls and uncomplexed PMOTAC segments as strings. In the ionic strength of 80 mM the PECs consisting of only one PMOTAC as a host molecule are observed only at the mixing ratios of 6.6 and 10, Fig. 1. In the mixing ratio of 3.3 the ionic strength of the solution is still sufficient to screen the repulsive charges and to enable the formation of bigger complexes.

The excess of anionic component in the PECs induces the specific viscosities of the complex solutions to be higher in 20 mM NaCl solutions than the viscosities of the solutions of the pure component. Once again this confirms the formation of negatively charged particles. However, when anionic component is present in excess the molecular weight of PMANa block has a noticeable influence on the forming PECs. The specific viscosities of PEO-*block*-PMANa2 complex solutions are higher than the viscosities of the lower molecular weight PEO-*block*-PMANa1 complex

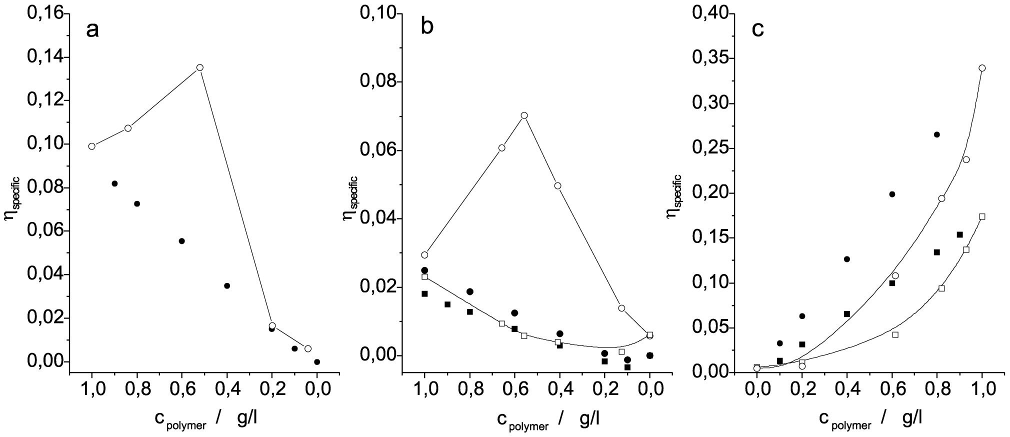


Fig. 3. Specific viscosities of PEO-*block*-PMANa and PMOTAC (filled symbols) and their complexes (open symbols) in aqueous NaCl solutions against polymer or excess polymer concentration, respectively. The component in excess is (a) PEO-*block*-PMANa2, 20 mM NaCl, (b) PEO-*block*-PMANa1, 20 mM NaCl (circles) and 80 mM NaCl (squares), (c) PMOTAC, 20 mM NaCl (circles) and 80 mM NaCl (squares). Notice different scaling on *y*-axes.

solutions. Also, the size distribution curves illustrate the difference in the mechanism of the particle formation. The size distribution of the PEO-*block*-PMANa1 complexes broadens as the mixing ratio is changed from 0.3 to 0.1, whereas such an effect is not seen in the size distributions of PEO-*block*-PMANa2 complexes. As already discussed earlier, the longer PMANa block in PEO-*block*-PMANa2 causes the formation of less regular structures in comparison to the lower molecular weight PEO-*block*-PMANa1. Thus, a polydisperse system builds up. Also, the particles formed have loose and presumably elongated structure, which induces higher viscosity. The reorganisation of lower molecular weight species is easier. Therefore, the particles formed by PEO-*block*-PMANa1 and PMOTAC are more monodisperse as well as more compact in nature. Consequently, the size distribution of the complexes with the mixing ratio of 0.3 is still relatively narrow. As the proportion of anionic components is increased the distributions broaden as a consequence of increasing polyelectrolyte effect. The typical slow mode in the CONTIN distributions is also observed in the case of anionic excess at the high and low detection angles (data not shown).

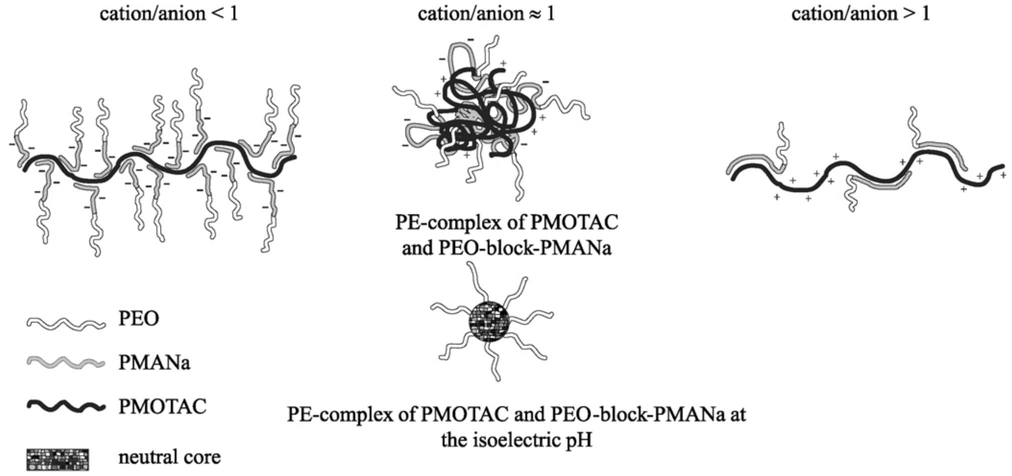
There is a maximum observed in the specific viscosity curves of the complex solutions of PMOTAC with PEO-*block*-PMANa1 and PEO-*block*-PMANa2 in the ionic strength of 20 mM. This indicates that there is a maximum in the number of the anionic species that the PMOTAC chain can bind [29,33]. As the mixing ratio is changed from 1 towards 0.1, the concentration of the host polycations is decreased since the total polymer concentration is kept constant in all the solutions. So, the increase in the solution viscosity is due to the increased anionic charge of the particles, which property exceeds the effect of decreasing particle concentration until the maximum in the binding number is reached. After the maximum point the solution viscosity decreases because of dilution. In Scheme 1 there is a schematic model of the PECs under varying mixing ratios.

In the ionic strength of 80 mM the specific viscosities of the complex solution and the pure PEO-*block*-PMANa1 solution are practically the same. However, according to the CONTIN size distribution there is no dissolved PEO-*block*-PMANa1 present. The repulsion caused by uncomplexed MANa units is not sufficient to break down the optimal sized particles but the excess charges act only as extra stabilisers in the particles.

3.2.2. *Effect of solution pH*

The effect of solution pH on the formation of stoichiometric complexes of PEO-*block*-PMANa2 and PMOTAC was studied. The polycation, PMOTAC, is a strong polyelectrolyte with quaternised ammonium groups. Thus the solution pH has a minimal effect on its degree of dissociation. In the following, it has been assumed that PMOTAC is fully dissociated through the whole pH range studied. On the other hand the polyanion block, PMAA, is a weak polyelectrolyte. By changing the solution pH the degree of dissociation is strongly affected. Also, in previous studies it has been proven that block copolymers of PEO and PMAA can go through self-complexation in aqueous solutions with low pH [21,34]. So, basically there are two variables affecting the complex formation upon varying the solution pH. The charge density of PEO-*block*-PMANa2 decreases as the solution pH is lowered. At the same time as a critical degree of protonation of PMANa block is reached, intra- and finally intermolecular hydrogen bonds between the PEO and PMAA blocks start to build up.

In Fig. 4 the mean diameters of PMOTAC, PEO-*block*-PMANa2 and their complex are plotted against the solution pH. At high pH the complex formation is very much similar to the complexation occurring in the NaCl solution. Both the anionic and the cationic species have high charge densities. The sufficient number of PEO chains on the particle surface for stabilisation is the factor that determines the number of aggregation and thus the size of the particles. The mean diameters of equimolar PEO-*block*-PMANa2/PMOTAC



Scheme 1. Schematic presentation of the PEC formation under different mixing ratios and solution pH.

complexes in 20 mM NaCl and 50 mM phosphate buffers, pH 7–9, are 120 nm and in the region of 200 nm, respectively. As the solution pH is lowered the size of the particles remains the same until around pH 6 it starts to decrease. At pH below 6 the intramolecular self-complexation of PEO-*block*-PMANa2 takes already place thus establishing additional hydrophobic domains. The minimum in the particle size is reached at pH 5.4. At this pH the degree of dissociation of PMANa block equals 0.77, based on the $pK_{a,average}$ of PMAA, which equals 4.88. Thus, the co-operation of Coulombic and hydrophobic interactions induces the formation of most densely packed PECs (Scheme 1). At even lower pH, the composition of the solution deviated from that expected, i.e. mixture of dissolved PMOTAC and self-complexes of PEO-*block*-PMANa2. An increased particle size was observed, which indicates attractive interactions between them.

Fig. 5 presents the conductivities of the complex solutions of PEO-*block*-PMANa2 and PMOTAC in 20 mM NaCl and in 50 mM buffer solutions. In both figures both the measured conductivities and the calculated conductivities are plotted. Two calculated conductivities are presented, the first one shows the effect of the released counterions and the second one is the sum of the released counterions and the effect of the uncomplexed polyelectrolyte. The calculated conductivities of the excess polyelectrolytes were calculated by assuming a linear dependence between the polyelectrolyte concentration and the solution conductivity. In the solutions of varying pH, the excess fraction of uncomplexed MOTAC units was calculated based on the degree of dissociation of PMANa. The conductivity curve of the varying mixing ratios obeys well the calculated sum curve [28]. In the high and low ratios the measured and calculated values coincide, but as the ratio approaches unity the measured values lie below the calculated ones. Once again, this indicates that although the complexes may have overall electroneutrality there still exists uncomplexed loops. These charged loops attract ions from the solution, thus decreasing the conductivity.

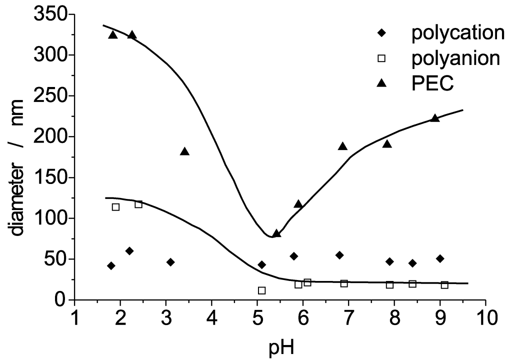


Fig. 4. The mean diameters of PMOTAC, PEO-*block*-PMANa2 and their complexes plotted against the solution pH. The lines are to help the eye.

The conductivity curve of the equimolar complex solutions against pH can be compared to the conductivities of the complex solutions with cationic excess of different mixing ratios. At high pH values, similar deviation of the calculated values from experimental ones is observed. Again, with decreasing pH the change begins around pH 6, the conductivity starts to increase and finally reaches the maximum at pH 5.4. Concurrently, the calculated and the measured curves overlap and they separate again below pH 3.5. The pH range 6–3.5 is the region where the intramolecular self-complexation of PEO-*block*-PMANa is observed, but not yet intermolecular. The reason why the experimental conductivity obeys the calculated one in the pH range 6–3.5 might be that the hydrophobic domains brought by the intramolecular complex segments makes the complex structure more dense, thus releasing a maximum amount of counterions into the solution. At low pH, if there were no interactions between the self-aggregates of PEO-*block*-PMANa2 and PMOTAC the experimental solution conductivity should be similar to the theoretical sum curve. However, as already seen from Fig. 4 the mean diameters detected from the mixed solutions of PEO-*block*-PMANa2 and PMOTAC at low pH are measurably higher than the mean diameters of the self-aggregates of

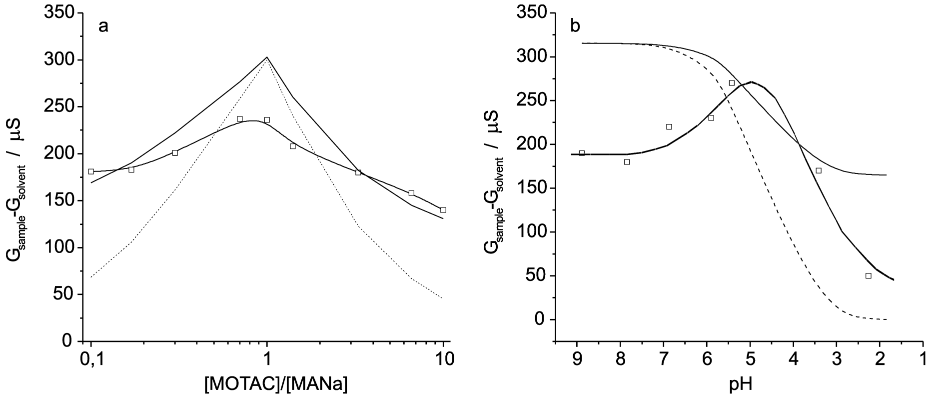


Fig. 5. The conductivities of the complex solutions of PEO-*block*-PMANa2 and PMOTAC in (a) 20 mM NaCl and in (b) 50 mM buffer solutions. Measured conductivities (squares, the lines are to help the eye), calculated conductivities from the released counterions (dotted lines) and the calculated sum conductivities of the released counterions and the uncomplexed polyelectrolyte (continuous lines without symbols).

PEO-*block*-PMANa2. Thus, it is not surprising that there is a decrease in the solution conductivity. The mechanism of the binding of PMOTAC to the self-aggregates of PEO-*block*-PMAA is not clear yet, but there are some proofs that PEO can bind into PMOTAC [35]. So, it is possible that PMOTAC is bound to the surface of the self-aggregates as loops, thus making the structure of the corona to correspond a structure of a PE-gel, which has a high affinity to counterions [36].

4. Conclusions

In the complexation studies of PEO-*block*-PMANa and PMOTAC the effect of the block length of the anionic part, the effect of the salt concentration of the solution, as well as the effect of solution pH were studied. The equimolar complexes are spherical in shape and monodisperse. Increasing the length of the anionic block increases the particle size, due to the smaller fraction of the solubilising PEO in the block copolymer. Also, in higher salt concentration larger number of stabilising PEOs are needed, and thus bigger particles are observed. In non-stoichiometric mixing ratios highly charged PECs are formed. A maximum in the number of the anionic species that the PMOTAC chain can bind was observed.

In high pH, the complexation of PEO-*block*-PMANa and PMOTAC is very similar to that observed in the aqueous NaCl solutions. In the pH range 3.5–6 the intramolecular complex segments of PEO-*block*-PMANa induce the formation of a more dense complex structure. At even lower pH, indications of binding of PMOTAC to the self-aggregates of PEO-*block*-PMANa were found.

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