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Polyelectrolyte complexation between cationic and anionic polyelectrolytes with complementary polymer-bound reactive groups of amine and acetoacetate: effect of mono- and divalent salts

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Abstract The copolymers of methacrylic acid with 0–50 % of 2-(methacryloyloxy)ethyl acetoacetate, and the copolymers of 2-(methacryloyloxy)ethyl trimethyl ammonium chloride with 0–50 % of 2-aminoethyl methacrylate were prepared by free radical polymerization in various solvents. The polyelectrolytes were characterized by potentiometric titration, NMR, viscometry and size exclusion chromatographic techniques. Polyelectrolyte complex formation between oppositely charged polyelectrolytes with polymer-bound (non)reactive polyelectrolytes was studied in the presence of NaCl and CaCl₂ as a function of ionic strength. The physical nature and efficiency of polyelectrolyte complexes that form gels, liquid coacervates, or soluble complexes depend on variables such as ionic strength and charge ratio. The highest complexation efficiency was obtained when a 1:1 charge ratio was used. Liquid coacervates were obtained with high ionic strength NaCl. The inclusion of small amounts of CaCl₂ led to drastic property changes in the polyelectrolyte complexes. The divalent calcium ion (Ca²⁺) displaced the poly[2-(methacryloyloxyethyl) trimethyl ammonium chloride] from the complexes and appeared as a new form of liquid coacervate in a range of ionic strength (50–200 mM CaCl₂). The reactivity of the polyelectrolyte system was demonstrated by studying the formation of coacervate complexes. Herein, the feasibility of cross-linking of the resulting coacervate via complementary poly(methacrylic acid-co-2-(methacryloyloxy)ethyl acetoacetate)-bound acetoacetate groups with poly(2-(methacryloyloxy)ethyl trimethyl ammonium chloride-co-2-aminoethyl methacrylate)-bound amine groups is discussed in detail.

Keywords Polyelectrolyte complexes · Coacervation · Microspheres · Ionic strength · Self-reactive polyelectrolyte

Introduction

Polyelectrolytes are macromolecules that have charged or chargeable groups when dissolved in polar solvents like water. Combining solutions of oppositely charged polyelectrolytes can lead to formation of layer-by-layer assemblies [1, 2], soluble [3], liquid coacervate [4], gel-like [5] and even solid [6, 7] polyelectrolyte complexes (PECs) that, in the latter three cases, phase separation takes place from solution. Polyelectrolyte complexes are a topic of considerable interest [1, 8–11] and have been used for a number of applications such as separation membranes [12, 13], immobilization of enzymes [14] or cells [15], coatings [16], drug delivery [17, 18], gene carriers [19, 20], and extraction of proteins [21].

The entropy of the complex increases with the release of small counter ions upon PEC formation. The presence of small amounts of salts or counter ions helps polyelectrolyte complexes rearrange and drive toward thermodynamic equilibrium [22]. Further addition of salt can induce a change in the conformation of the preformed polyelectrolyte complexes, depending on the nature of salts [23], counter ions and the ionic groups of the polyelectrolytes [24]. The resulting complex can be insoluble in water, organic or common solvents. In general, the stoichiometric composition of these polyelectrolyte complexes depends on the degree of dissociation of the two polyelectrolytes. The equilibrium water content in the PECs can vary from 30 to 90 % by weight [25]. In general, the nature and efficiency of polyelectrolyte complexes depend on number of variables such as charge ratio, charge density, ionic

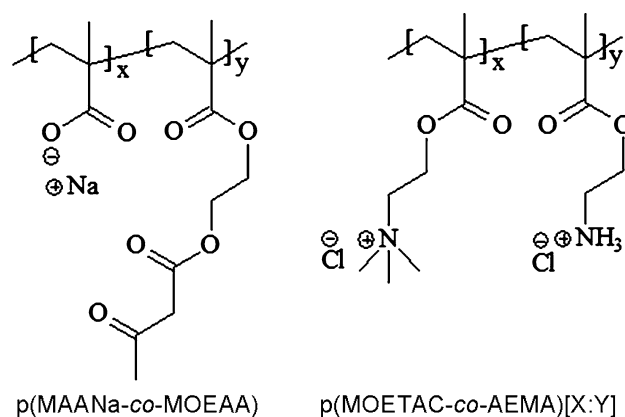
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strength, polymer concentration and structure, molecular weight of the polymer, pH and temperature [9, 26, 27]. The nature, efficiency and structural properties of the polyelectrolyte complexes have been extensively studied by the research groups of Kabanov et al. [11] and Tsuchida et al. [11, 28, 29].

When interaction between the polyelectrolytes is weak, the solution can separate into two immiscible liquid phases, in a process called “liquid coacervation” (from Latin, *coacere*: heaping together) with a polymer-rich coacervate and a polymer-lean supernatant phase [30]. When only a single polymeric species is present, the process of coacervation is referred to as “simple coacervation”.

Complex coacervation involves the interactions of two oppositely charged polyelectrolytes in aqueous media, where the opposite charges attract each other, but are not strong enough to cause precipitation [30]. One of the best-studied examples of complex coacervation is the gelatin–gum arabic system [31]. Wang et al. prepared microcapsules by an in situ coacervation method in which polyallylamine was adsorbed onto the polystyrene sulfonate-doped CaCO_3 microparticles, followed by cross-linking of the polyallylamine hydrochloride with glutaraldehyde and core removal in a solution of ethylenediaminetetraacetic acid (EDTA) with or without polyallylamine [32]. Cationic polyacrylamide copolymers (cPAM) above a certain molecular weight (MW) can form coacervate complexes with anionic sulfonated Kraft lignin (SKL). Coacervation efficiency increases with increasing MW of cPAM. Below the cPAM critical molar mass, colloidal complexes are formed instead [4]. Anionic polyphosphazenes can form microdroplets by NaCl-induced simple aqueous coacervation. These microdroplets can be stabilized by the addition of CaCl_2 to form hydrogel microspheres. This method is especially appealing for protein encapsulation [33]. Complex coacervation between polyelectrolytes with strong ionic groups and/or multivalent ionic salts, which are far from the thermodynamic equilibrium, is dependent on the MW, charge density, concentration, hydrophobic/hydrophilic content of the polymer, and the nature of the medium such as temperature, pH, ionic strength or the presence of non-solvents. Simple and complex coacervation processes have been extensively applied in the field of encapsulation, as well as for protein separations [34–37].

Complex coacervation formed by the addition of salts, which is quite different from the soluble PECs, could turn into complete dissolution by negligible changes that depend on the characteristic properties of polyelectrolytes and salts [10, 38]. Trinh et al. [39] determined critical salt concentrations using various combinations of polyanions and polycations. Stover et al. [40, 41] used different polymers including polyelectrolytes to visualize their



Scheme 1 Synthetic polyelectrolytes investigated in this study

polyelectrolytic properties in the formation of liquid coacervates.

The present article describes the formation of polyelectrolyte complexes based on methacrylic acid (MAA) and 2-(methacryloyloxyethyl)trimethyl ammonium hydrochloride (MOETAC), that is shown in Scheme 1. The author examines how the physical nature of the complexes and the efficiency of complexation are affected by NaCl and CaCl_2 salts, as a function of ionic strength and charge ratio. Conditions are examined under which liquid complex coacervates formations are identified and their cross-linking properties are investigated in detail. In the present work, polymer-bound acetoacetate groups, introduced via copolymerization of MAA with 2-(methacryloyloxy) ethyl acetoacetate, are used to react with polymer-bound amine groups, introduced via copolymerization of MOETAC with 2-aminoethyl methacrylate.

Experimental

Materials

Unless otherwise stated, all monomers and salts were purchased from Sigma Aldrich, and solvents were purchased from Caledon Laboratories (Caledon, ON, Canada) and were used as received. The 2,2'-azobis(2-methylpropanimidine) dihydrochloride (Vazo-56) and azobisisobutyronitrile (AIBN) initiators were obtained from Sigma and recrystallized in methanol. De-ionized water was used as a solvent in the preparation of polyelectrolyte complexes.

Synthesis of poly(MOETAC) (pMOETAC)

Poly(MOETAC) was prepared by free radical polymerization, as described previously [41]. In brief, MOETAC

(5.10 g, 24.5 mmol) and Vazo-56 (0.134 g, 0.49 mmol) were dissolved in de-ionized water (50 mL). The dry nitrogen was bubbled through the reaction mixture for 15 min. The polymerization was continued in an oven for 24 h at 60 °C, while the reaction container was rotated at 10 rpm. The polymer product was precipitated and isolated in excess acetone (2.5 L). The white polymer product was dried in a vacuum oven at 50 °C to obtain 3.9 g p(MOE-TAC) (yield 78 %).

Synthesis of poly(MOETAC-*co*-AEMA), [p(MO90-A10), p(MO80-A20), p(MO70-A30), p(MO50-A50)]

Poly(MO90-A10) was prepared by free radical polymerization following procedure described above for pMOE-TAC. In general, MOETAC (7.24 g, 34.8 mmol), 2-aminoethyl methacrylate hydrochloride salt (AEMA, 2.76 g, 16.6 mmol) and Vazo-56 (0.271 g, 0.98 mmol) were dissolved in de-ionized water (100 mL). The reaction mixture was bubbled with dry nitrogen for 15 min and then placed in an HB-1000 hybridizer. The polymerization was continued in an oven at 60 °C for 21 h, while the reaction mixture was rotated at 10 rpm. After the elapsed time, the polymer product was precipitated and isolated in excess acetone (2 L). The solid polymer product was then dried in a vacuum oven at 50 °C to give 7.88 g p(MO90-*co*-A10) (yield 79 %). p(MO80-*co*-A20) (yield 81 %), p(MO70-*co*-A30) (yield 80 %), and p(MO50-*co*-A50) (yield 78 %) were prepared in a similar fashion.

Synthesis of poly(MAA) (pMAA)

Poly(MAA) was synthesized according to a procedure described elsewhere in the literature [42]. In brief, methacrylic acid (10.00 g, 116 mmol) and AIBN (0.19 g, 1.16 mmol) were dissolved in ethyl alcohol (45 mL). The solution was gently bubbled with dry nitrogen for 10 min and then placed in an HB-1000 hybridizer. The polymerization was continued in an HB-1000 hybridizer for 24 h at 60 °C, while the reaction solution was rotated at 10 rpm to provide uniform mixing. The polymer product was precipitated and isolated in excess diethyl ether (2 L). The white polymer product was dried to a constant weight in a vacuum oven at 50 °C to give 9.22 g pMAA (yield 92 %). The anionic form of pMAA, poly(methacrylic acid sodium salt) pMAANa was prepared by the addition of equimolar amount of 1 M NaOH to the pMAA.

Synthesis of poly(MAANa-*co*-MOEAA), [p(MA90-M10), p(MA80-M20), p(MA70-M30), p(MA50-M50)]

p(MA90-M10) was prepared by free radical polymerization, as previously described [42]. In brief, MAA (7.84 g,

91 mmol), 2-(methacryloyloxy) ethyl acetoacetate (MO-EAA, 95 %, 2.16 g, 10.1 mmol) and AIBN (166 mg, 1.01 mmol) were dissolved in ethyl alcohol (90 mL). The reaction mixture was bubbled with dry nitrogen for 15 min. The polymerization reaction was continued for 24 h at 60 °C, while the reaction mixture was rotated at 10 rpm to give uniform mixing. After the reaction time, the p(MA90-M10) was precipitated in excess diethyl ether (2 L). The precipitated product was then dried to constant weight at 50 °C in a vacuum oven to give 9.38 g p(MA90-M10) (yield 94 %). p(MA80-M20) (polymerized in ethanol, yield 85 %), p(MA70-M30) (polymerized in ethyl alcohol, yield 87 %), and p(MA50-M50) (polymerized in 50:50 mixture of THF and ethyl alcohol, yield 86 %) were prepared in a similar fashion. Poly(MAANa-*co*-MOEAA) was prepared by the addition of equimolar amount of 1 M NaOH with respect to MAA polymers.

Chemical characterization

The chemical compositions and pK_a values of various copolymers of poly(MOETAC-*co*-AEMA) [x:y], pMAA and copolymers of poly(MAA-*co*-MOEAA) [x:y] were determined by potentiometric titration using PC titrate automatic titrator (Man Tech Associates). 1H Nuclear magnetic resonance (NMR) spectroscopy was also used to measure the chemical composition of various copolymers of poly(MAA-*co*-MOEAA) [x:y] in DMSO- d_6 solution on a Bruker AV 200 spectrometer (200 MHz). Optical microscope (OM) images of polyelectrolyte complexes were taken using an Olympus BX51 optical microscope fitted with a Q-Imaging Retiga EXi digital camera and ImagePro software.

Molecular weight determination

Size exclusion chromatography (SEC) (Waters, 515 HPLC pump, 717 plus Autosampler, Ultrahydrogel-120, -250, -500, 6 μ m particles, 30 cm \times 7.8 mm columns; 2414 refractive index detector) was used to determine the molecular weight and polydispersity index (molecular weight distribution) of pMAA and the copolymers of poly(MAA-*co*-MOEAA) with a flow rate of 0.8 mL/min of 0.3 M sodium nitrate in phosphate buffer (pH 7) as a mobile phase. The narrow dispersed polyethylene glycol standards were used to calibrate the SEC system. Solutions of 1 %w/v of pMAA or copolymers of poly(MAA-*co*-MOEAA) were prepared by addition of an equimolar amount of 1 M NaOH into the MAA precursor followed by dilution with the mobile phase, and was used for SEC.

The molecular weight of pMOETAC and the copolymers of poly(MOETAC-*co*-AEMA) were determined using a Ubbelohde viscometer (viscometer constant 0.00314 cSt/s).

The relative viscosity of the polymer solutions was measured using 1 M NaCl as a solvent at 20.0 ± 0.1 °C. Each data point was taken in triplicate to have an average measurement on the same mixture. The solution temperature was controlled by a thermostat and monitored by a thermometer. A stopwatch with an uncertainty of ± 0.1 s was used to measure the flow times. By extrapolation of the Huggins plot (η_{sp}/c vs. c) to infinite dilution and taking the intercept, the intrinsic viscosity $[\eta]$ was determined. Microsoft Excel 2007 (Microsoft Corporation, Seattle, USA) was used to plot viscosities against concentrations to obtain straight line relationship with large linear regression coefficients ($R^2 = >0.99$). The viscosity average molecular weight (M_η) of the polymer was calculated from the intrinsic viscosity using the relationship $[\eta] = KM^a$ with values for K and a found in the literature [43].

Preparation of polyelectrolyte complexes

Aqueous solutions (15 mL each) of pMOETAC (0.197 g) and pMAANa (0.103 g), or their copolymers of appropriate concentrations (1 % w/v) were combined in a pre-weighed 50 mL polypropylene conical tube at room temperature (20 °C). The mixtures were agitated for 5 min on a vortex mixer, and allowed to settle for 30 min. The polyelectrolyte mixture was then centrifuged (3,500 rpm, 10 min), and the upper phase (supernatant) was separated and transferred to a pre-weighed weighing dish using a pipette. The pre-weighed conical tube was re-weighed to determine the yield of wet polyelectrolyte complex. Both polyelectrolyte complex and supernatant were dried at 60 °C until constant weights were reached to determine the weight of solids (polymer plus salts) present in each individual phase.

The complexation efficiency and complex yield were calculated as follows

$$\text{Complexation efficiency} = \frac{\text{Wt of the polymer in dry complex}}{\text{Total wt of the polymers}} \times 100\%$$

$$\text{Complex yield} = \frac{\text{Wt of the wet complex}}{\text{Total wt of the polymer solution}} \times 100\%$$

Results and discussion

Synthesis of polyelectrolytes

Synthetic polyelectrolytes may have the ability to alter or optimize polyelectrolyte properties by introducing new functional groups/blocks in the existing polyelectrolyte through reactive comonomers. One of the research goals was to develop polyelectrolytes that could chemically

cross-link after deposition via complementary reactive functional groups on the polyelectrolyte pairs, thus, avoiding the use of small cross-linker molecules. The reactive anionic and cationic polyelectrolytes were synthesized by conventional free radical polymerization. The copolymers of 2-[(methacryloyloxy)ethyl] trimethyl ammonium chloride with 0–50 % of 2-aminoethylmethacrylate hydrochloride, poly(MOETAC-*co*-AEMA), were prepared by 2,2'-azobis(2-methyl propionamide) dihydrochloride initiated polymerization in water at 60 °C. The molecular weight of p(MOETAC-*co*-AEMA) (0–50 % AEMA) was determined using the Mark–Houwink equation. The intrinsic viscosity $[\eta]$ was calculated by extrapolation of the Huggins plot (η_{sp}/c vs. c) to zero concentration, which is intended to eliminate intermolecular interactions and should be independent of the fluid shear rate. The intrinsic viscosity of a given copolymer composition depends upon the hydrodynamic volume occupied per unit mass of the macromolecule (polymer chain) and its excluded free volume. It is also influenced by the hydrodynamic properties and solvents, but there will be an average configuration, which gives one insight into the general shape of polymer molecules in solution. The polymer viscosity average molecular weight was determined using the intrinsic viscosity obtained from the intercept of the Huggins plot, and the K and a values, which were found in the literature [43]. The viscometric measurements for ionic, water-soluble polymers are not possible in pure water as the polymers show electrostatically induced coil expansion with dilution [44]. In the presence of added low molecular weight electrolytes such as NaCl, this coil expansion is suppressed, and the viscosity curve becomes linear as expected for any polyelectrolyte.

The copolymers of methacrylic acid with 0–30 % 2-(methacryloyloxy) ethyl acetoacetate, poly(MAA-*co*-MOEAA), were prepared using a free radical initiator, AIBN, in ethyl alcohol at 60 °C, and 40–50 % of 2-(methacryloyloxy) ethyl acetoacetate were polymerized in similar fashion in 1:1 THF/ethyl alcohol, followed by precipitation in diethyl ether. The compositions of anionic copolymers were determined by potentiometric titration using water or 1:1 methyl alcohol/water. The copolymer compositions were also determined by ^1H NMR and comparisons were made between the peak area of the methylene group derived from the MOEAA with the peak area of the methyl group derived from MAA/MOEAA. Not surprisingly for copolymerization of pairs of methacrylate monomers, the composition of the synthesized copolymers obtained from titration and/or ^1H NMR closely matched the feed ratio of the two monomers. The MW and the MW distribution (M_w/M_n) of the anionic polymers were determined using SEC with a phosphate buffer at pH 7. The

Table 1 Polyelectrolyte properties

Polyelectrolytes	Comonomers feed compositions (mol %)	Monomer to initiator ratio (mol %)	Polymer composition	Molecular weight (kDa)/(PDI)	pKa
pMOETAC	–	10:1	–	774 ^c	–
p(MO90-A10)	90:10	10:2	90:10 (± 1) ^a	290 ^c	6.9 ^f
p(MO80-A20)	80:20	10:2	80:20 (± 3) ^a	231 ^c	7.1 ^f
p(MO70-A30)	70:30	10:2	70:30 (± 4) ^a	178 ^c	7.3 ^f
p(MO50-A50)	50:50	10:2	50:50 (± 8) ^a	158 ^c	7.3 ^f
pMAA	–	10:1	–	40 ^d (1.59) ^e	6.3 ^f /7.2 ^g
p(MA90-M10)	90:10	10:1	90:10 (± 1) ^b	29 ^d (1.68) ^e	7.2 ^g
p(MA80-M20)	80:20	10:1	80:20 (± 1) ^b	41 ^d (2.01) ^e	7.1 ^g
p(MA70-M30)	70:30	10:1	70:30 (± 4) ^b	42 ^d (2.38) ^e	7.1 ^g
p(MA50-M50)	50:50	10:1	50:50 (± 6) ^b	31 ^d (2.44) ^e	7.0 ^g

^a % composition was determined by potentiometric titration

^b % composition was determined by ¹H NMR using a Bruker AV 200 spectrometer for samples dissolved in DMSO-*d*₆

^c Viscosity average molecular weight (*M*_n) measured by viscometry

^d Number average molecular weight (*M*_n) measured by SEC

^e Polydispersity index (PDI = *M*_w/*M*_n) measured by SEC

^f From titration in water

^g From titration in 50 % methanol

structure and properties of the synthetic polyelectrolytes used in this study are described in Scheme 1 and Table 1.

Effect of ionic strength on polyelectrolyte complexes

The nature of the polyelectrolyte complexes (PECs) formed by interaction between polycations and polyanions depends on a number of factors such as pH, charge density, charge ratio, ionic strength, temperature, solvent and the rigidity of the polymer chains [1, 23, 25, 45]. This research aimed to execute a quantitative survey to examine the effect of ionic strength (NaCl and CaCl₂) on the complexation of non-cross-linkable polyanions and polycations mentioned in Table 1, and realize their ability to form covalent cross-linkable complexes within their reactive domains.

The anionic copolymers and different salts (NaCl and CaCl₂) were used to realize the structural properties of anionic polyelectrolytes. As usual, NaCl and all polyanions (Table 1) did not show any macroscopic phase separation. However, the Ca/pMAA complex that was prepared by mixing a 1 % w/v solution of pMAA with 50–200 mM CaCl₂, formed liquid coacervates (Fig. 1a). The p(MA90-M10) and p(MA80-M20) gave similar but more viscous liquid complexes (images not shown). Ca/p(MA70-M30) and Ca/p(MA50-M50) did not show any visible macroscopic phase separation, likely due to the lower anionic (carboxylate) content of these polyelectrolytes. The absence of solid gels formed by the latter polyanions reflects their weaker calcium binding ability and suggests

that they should maintain some flexibility within the cross-linked polyelectrolyte complexes.

In the second stage, further experiments were designed to visualize the nature of the polyelectrolyte complexes and strength of the interactions between the non-reactive polycation, pMOETAC, and the polyanion, pMAA, in the presence of gelling divalent (Ca²⁺) and non-gelling monovalent (Na⁺) ions.

When the aqueous solutions of pMOETAC and pMAA in 1:1 charge ratio were mixed at room temperature (20 °C) in the absence of salts (NaCl, CaCl₂), phase separation occurred immediately. The solid pMOETAC/pMAA complex, in the presence of only 31.5 mM NaCl derived from the counter ions, settled and adhered to the walls of the centrifuge tube. This solid-like behavior was consistent with the relatively low water content of 70 wt% (30 % PEC). We know that the strength of the interactions in polyelectrolyte complexes can be tuned by changing the ionic strength. Addition of Na⁺ and Cl[−] can shield the electrostatic attraction between the oppositely charged polyelectrolytes. The OM images in Fig. 2 show the appearance of the complexes obtained by combining individual solutions of the two polyelectrolytes containing: (a) 0, (b) 200, and (c) 400 mM added NaCl. The images show increasingly swollen complexes, due to the increasing shielding of the polyelectrolyte interaction. Upon addition of salts, the appearance of the polyelectrolyte complexes gradually changed from gel to a liquid before dissolution. The complex shown in Fig. 2c is a liquid hydrogel, also known as a “complex coacervate”. Addition

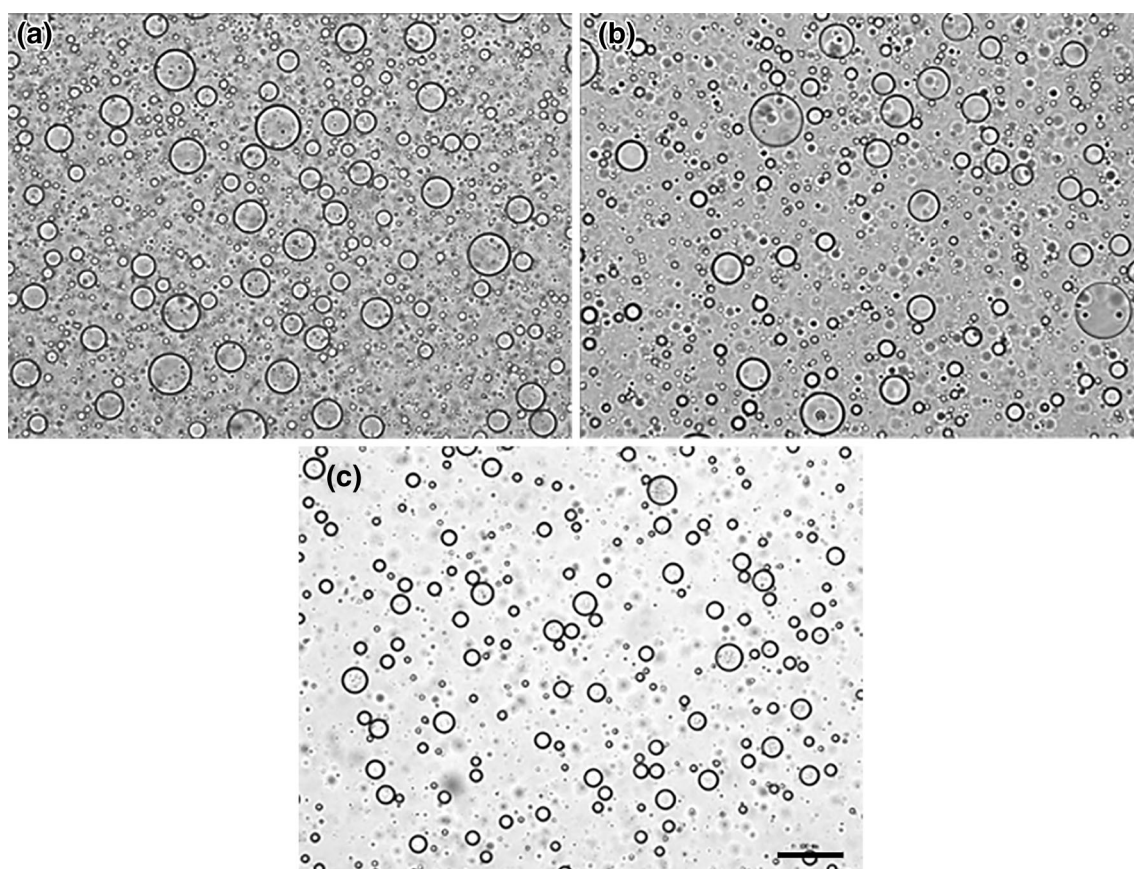


Fig. 1 OM images of 1 % w/v (pH 7) solutions of: **a** pMAANa in 200 mM CaCl_2 , and pMAANa and pMOETAC polyelectrolyte complexes prepared manually in **b** 100 mM and **c** 200 mM CaCl_2 . Scale bar 200 μm

of an excess of NaCl (600 mM NaCl) solution to a pMOETAC/pMAANa complex prepared in the absence of NaCl led to dissolution of the complex. These results indicated that the pMOETAC/pMAANa complex can exist as a gel or a liquid hydrogel (coacervate) depending on the ionic strength.

Upon addition of CaCl_2 , the pMOETAC/pMAANa gel complex prepared in absence of salts (Fig. 2a) changed to liquid coacervate. The OM images in Fig. 1 show the appearance of the complexes obtained by combining individual solutions of the two polyelectrolytes containing (b) 100, and (c) 200 mM added CaCl_2 .

The complexation efficiency, as measured by the weight fraction of dry polymer isolated from the complex formed by pMOETAC/pMAANa at 1:1 charge ratio in water, was $\sim 75\%$. This was consistent with the nearly quantitative polyanion/polycation complexation accompanied by the release of NaCl, which constitutes 18.5 wt% of pMOETAC/pMAANa at a 1:1 ratio. The fraction of polymer found in the complex remained fairly constant as concentration of NaCl was increased up to 400 mM, at a point which the complex started to dissolve (Fig. 3a).

In contrast, addition of CaCl_2 caused a rapid drop in the amount of polymer included in the complex, reaching a plateau for concentration of $\text{CaCl}_2 \geq 100$ mM (Fig. 3b). Calcium displaced the pMOETAC from the pMOETAC/pMAANa complex, to form a salt resistant, liquid coacervate composed almost entirely of Ca/pMAANa (pMOETAC:pMAANa = 3:97) as determined by ^1H NMR. At this stage, the weight of the complex was found to be very closely matched to what would be expected if only pMAANa was included in the complex (Fig. 4). The liquid nature of this coacervate reflected the weak calcium bridging between the polyanion chains and it indicated that these polyanions would likely exhibit some mobility within a complex unless physically entrapped or cross-linked.

Physical state of self-cross-linkable PECs

It has been hypothesized that the introduction of matched cross-linkable groups (primary amine and acetoacetate) into a pair of polycations and polyanions, respectively, would provide a route to in situ covalent cross-linking of this polyelectrolyte complex, without the use of low molecular weight cross-linking agents. To test this

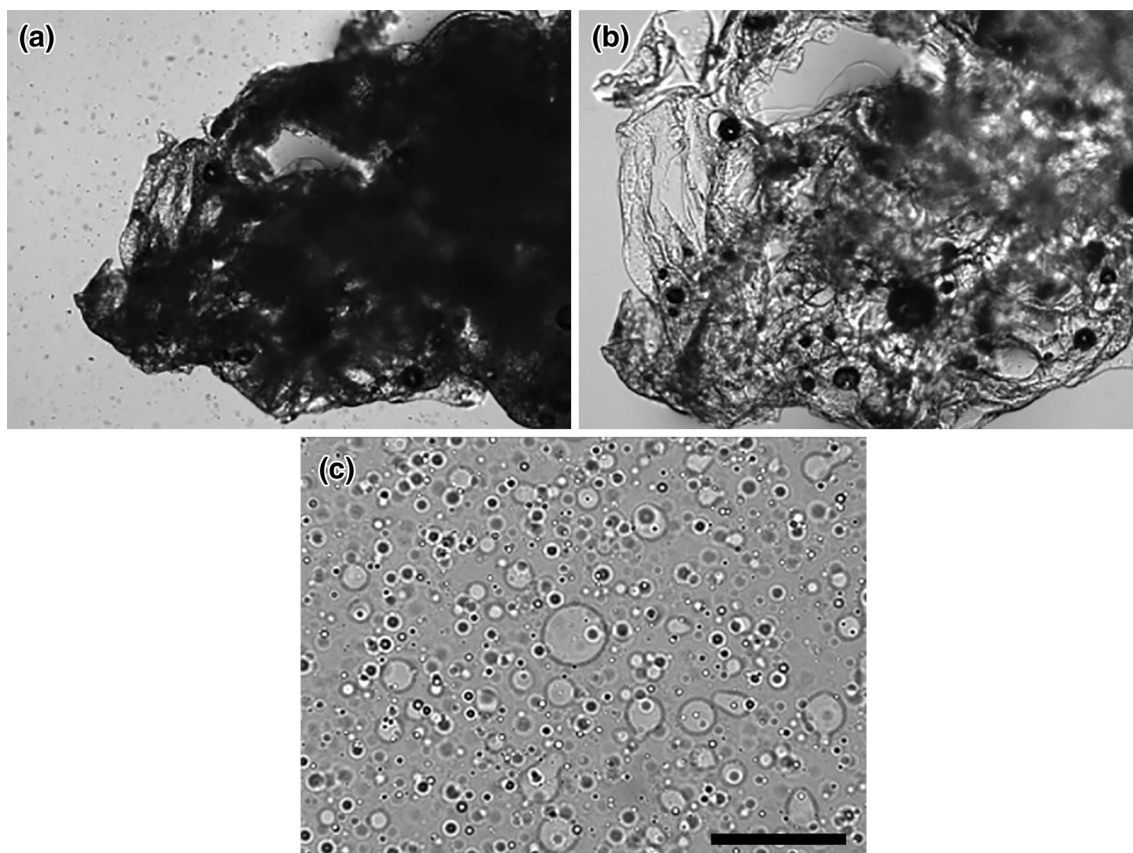


Fig. 2 OM images of pMAANa/pMOETAC polyelectrolyte complexes prepared from stock solutions on individual polyelectrolytes containing: **a** 0 mM, **b** 200 mM, and **c** 400 mM NaCl. All complexes formed with 1:1 charge ratio and 1 % w/v polymer loading. Scale bar 200 μ m

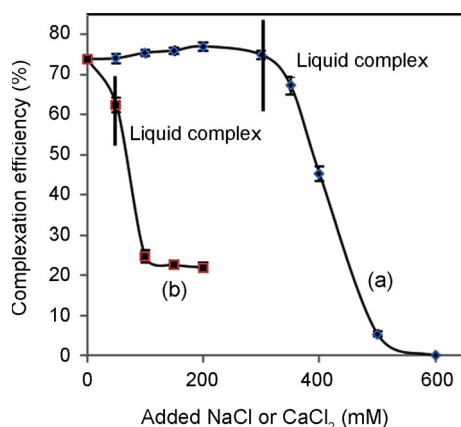


Fig. 3 Complexation efficiency of pMAANa with pMOETAC as a function of ionic strength of *a* NaCl and *b* CaCl₂. Total polymer loading is 1 % w/v, charge ratio is 1:1

hypothesis, a variety of self-cross-linkable polyelectrolytes has been synthesized, and some typical experiments were performed to study the feasibility for the preparation of well-defined/structured covalent cross-linked polyelectrolyte complexes. The main advantage of this approach was that the cross-linking reactions happened simultaneously in

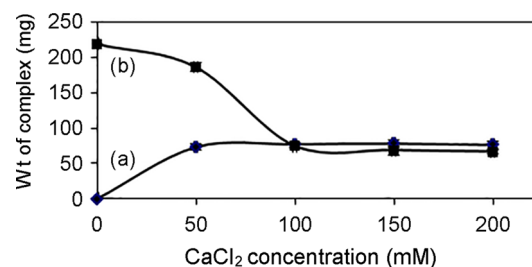


Fig. 4 Weight of the complex of pMAANa with pMOETAC as a function of ionic strength of CaCl₂: *a* pMAANa–CaCl₂ and *b* pMOETAC/pMAANa–CaCl₂. Total polymer loading is 1 % w/v (pMAANa-0.102 g, pMOETAC-0.197 g), charge ratio is 1:1

both phases. The ionic interactions occurred between the oppositely charged ions present in two different polyelectrolytes, which directed the polyelectrolytes together and facilitated the covalent cross-linking reactions between the two complementary reactive amino and acetoacetate groups present in polycation and polyanion, respectively.

Mixing p(MA90-M10) with p(MO90-A10) in a 1:1 charge ratio and at 1 % w/v total polymer loading at room temperature (20 °C) in the absence of NaCl, led to immediate phase separation and formation of a gel

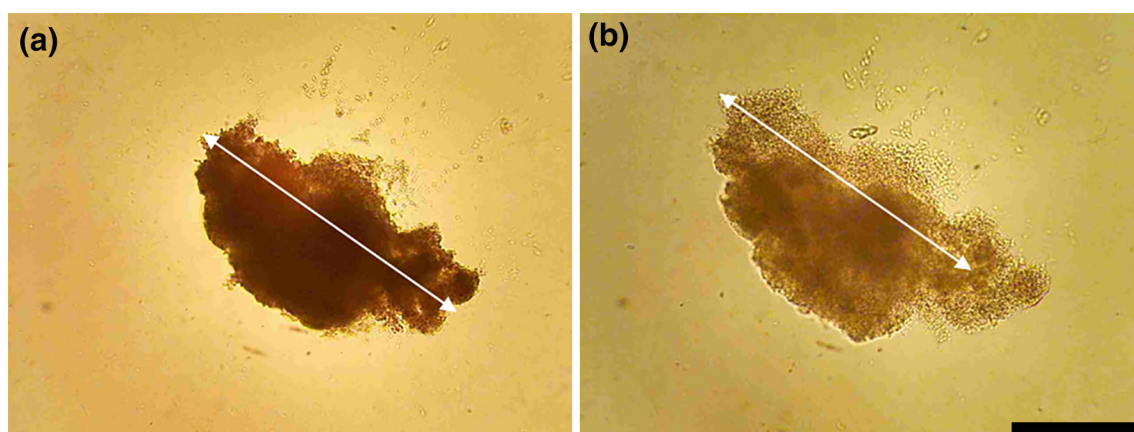
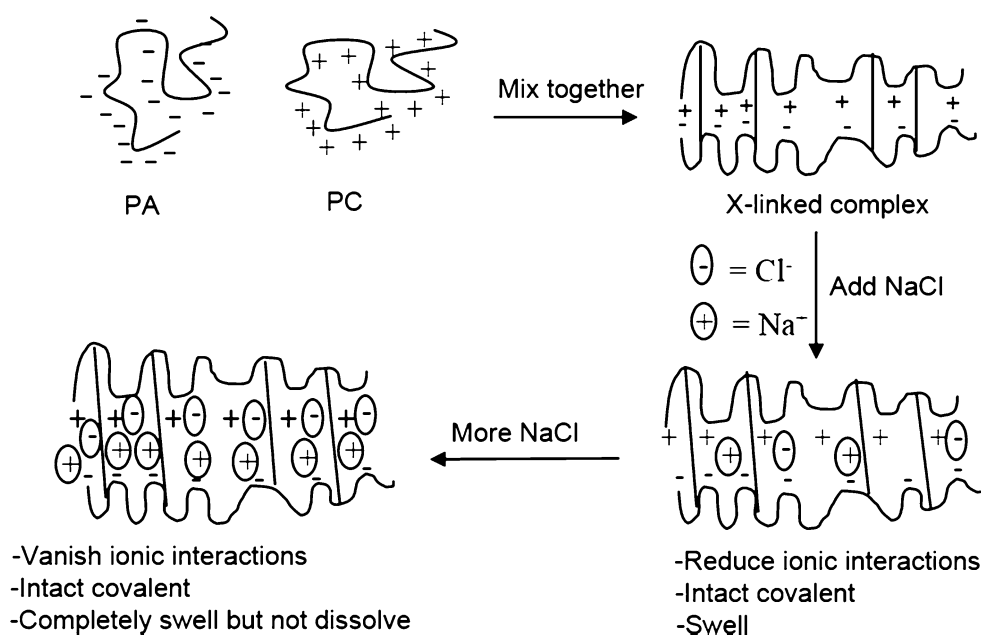


Fig. 5 OM images of p(MA90-M10) and p(MO90-A10) polyelectrolyte complex prepared in the absence of NaCl, before (a) and after (b) addition of 600 mM NaCl to the microscopic slide for 15 min.

Complex was formed at 1:1 charge ratio and 1 % w/v polymer loading. Scale bar 200 μ m

Scheme 2 The schematic representation of self-cross-linkable polyelectrolyte complexes in addition of excess NaCl. PA polyanion, PC polycation



complex (~ 30 wt% polymer) that stuck onto the wall of the polypropylene conical tube. The OM images in Fig. 5 show the appearance of a piece of solid complex formed by combining individual solutions of the two polyelectrolytes p(MA90-M10)/p(MO90-A10), before and after exposure to 600 mM NaCl. Increasing the ionic strength swelled the solid cross-linked complex slightly (Fig. 5b), due to the increasing shielding of the polyelectrolyte interaction. This indicated that the p(MA90-M10)/p(MO90-A10) complex formed a self-cross-linked complex, and therefore did not dissolve even at higher ionic strength. At high ionic strength, the electrostatic interactions between the polyelectrolytes were broken but the PECs survived due to the formation of the covalent cross-linked network within the complex. The schematic representation of self-cross-linkable polyelectrolyte complexes is shown in Scheme 2.

Upon exposure of CaCl_2 into p(MA90-M10)/p(MO90-A10), a similar swelling trend was observed. Likewise, all other cross-linkable analogous polycations and polyanions such as p(MA80-M20)/p(MO80-A20), p(MA70-M30)/p(MO70-A30), and p(MA50-M50)/p(MO50-A50) followed a similar cross-linking pattern (data not shown).

Effect of ionic strength on complexation efficiency of self-cross-linkable polyelectrolytes

The complexation yield and efficiency of p(MA90-M10) and p(MO90-A10) were determined using 1 % w/v total polymer loading, and was found to be greatest at 1:1 charge ratio, as shown in Figs. 6 and 7, which showed that they were in agreement with other PEC systems reported in the literature [10].

The complexation efficiency observed at 1:1 anionic to cationic ratio and 1 % w/v total polymer loading is ~ 80.0 %. The complexation efficiency decreased further from the maximum, when non-stoichiometric ratios were

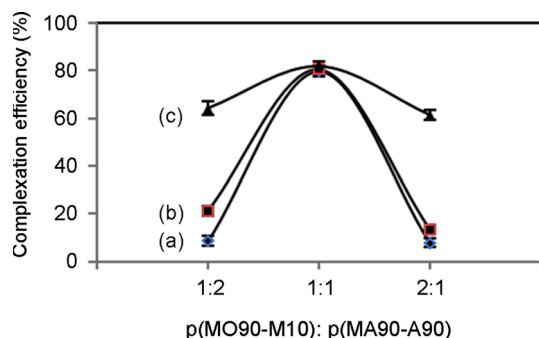


Fig. 6 The effect of ionic strength on p(MA90-M10) and p(MO90-A10) complexation efficiency at various charge ratios. 1 % w/v polymer loading. Added NaCl: *a* 0 mM, *b* 100 mM, and *c* 200 mM

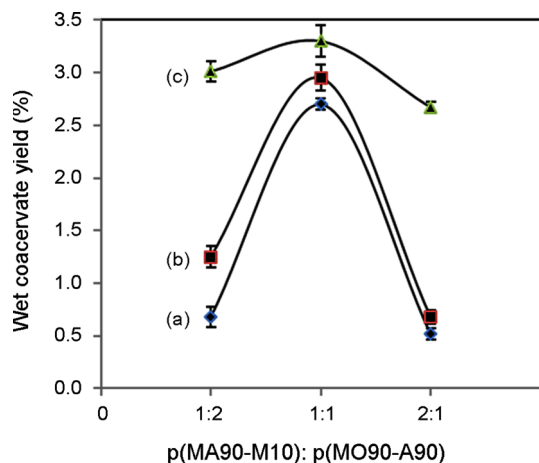


Fig. 7 Complex yields as a function of the charge ratio for complexes formed between p(MA90-M10) and p(MO90-A10). 1 % w/v polymer loading. Added NaCl: *a* 0 mM, *b* 100 mM, and *c* 200 mM

used (Fig. 6). However, with increasing ionic strength, the complexation efficiency increased dramatically. As mentioned previously, the addition of salt reduced the colloidal stability of the initially formed complexes, which allowed efficient aggregation. By the addition of CaCl_2 , coacervate complexes were formed with a similar trend of complexation efficiency and complex yield. Likewise, all other analogous reactive copolymer pairs, such as p(MA80-M20)/p(MO80-A20), p(MA70-M30)/p(MO70-A30), and p(MA50-M50)/p(MO50-A50), showed similar patterns (gel/coacervate) of complexation efficiency and complex yield following the addition of NaCl or CaCl_2 (data not shown).

Complex coacervation of cross-linkable polyelectrolytes

An OM image of complex liquid coacervate of p(MA90-M10) and p(MO90-A10) at 100 mM CaCl_2 is shown in Fig. 8a. The size of liquid coacervate was found to be ~ 50 – 150 μm . p(MA80-M20) and p(MO80-A20) also provided similar cross-linked coacervate droplets at 50–100 mM CaCl_2 . However, reactive polyelectrolyte pairs, such as p(MA70-M30) and p(MO70-A30), and p(MA50-M50) and p(MO50-A50), did not provide cross-linked liquid coacervates; rather, it formed a swelled rigid gel which was in agreement with other PEC systems reported in the literature [46]. The rigid gel was formed due to faster and stronger interactions between the reactive functional groups, relative to their ionic counter parts.

pMOETAC/pMAANa at a 1:1 charge ratio at ~ 400 mM NaCl, the PECs appeared to be a liquid coacervate (Fig. 2c). In contrast, p(MO90-A10) and p(MA90-M10) in 0–2 M NaCl, the complex did not show any complex coacervates; rather, it showed a swelled polyelectrolyte complex gel that was comparable to Fig. 5. A

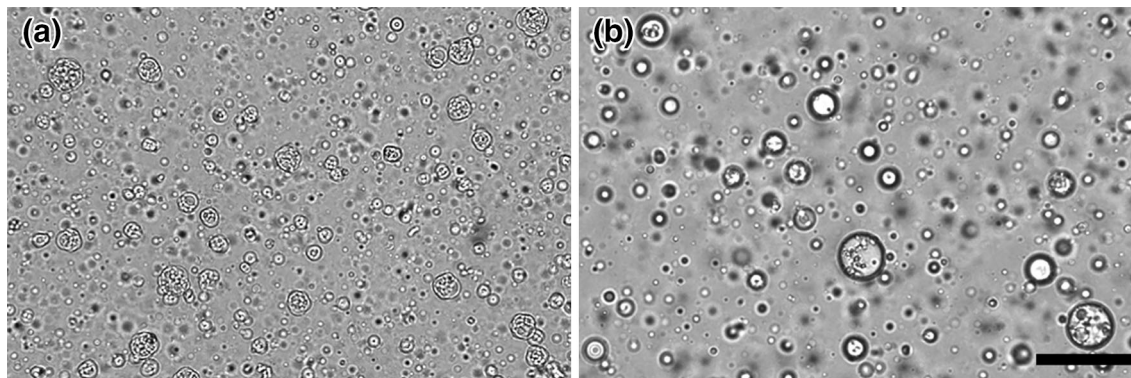


Fig. 8 OM image of microspheres formed by cross-linking between p(MA90-M10) and p(MO90-A10) cross-linked coacervate in 100 mM CaCl_2 solution. **a** Normal microsphere and **b** addition of excess 2 M NaCl, swelled microsphere. Scale bar 300 μm

colloid of an unstable complex coacervate of pMOETAC and p(MA70-M30) at 375 mM NaCl (image not shown) has been prepared. The coacervate droplets aggregated together within minutes without stirring. When 600 mM NaCl solution was added to the pMOETAC/p(MA70-M30) coacervate complex, it did dissolve. Using flexible PEI as a cross-linker, the pMOETAC/p(MA70-M30) liquid coacervate formed as covalently cross-linked microspheres, and became colloidally stable. Upon addition of excess salts (NaCl or CaCl_2), this cross-linked liquid coacervate swelled but did not dissolve. By increasing the ionic strength in the solution medium, the small counter ions (Na^+ and Cl^- , etc.) penetrated into the coacervate microspheres and the charge of polyelectrolytes was partially screened. After addition of excess NaCl, the polyelectrolytes were completely screened and the polymer chains expanded, leading to the swelling of coacervate microspheres. In this system, the swelling of the complex coacervate microspheres increased the microsphere diameter by $\sim 45\%$ of its original diameter. A similar swelling pattern was observed when p(MA90-M10)/p(MO90-A10) cross-linked coacervate droplets in 100 mM CaCl_2 (Fig. 8a) were exposed to excess 2 M NaCl (Fig. 8b).

Conclusion

The polyelectrolytes based on methacrylic acid, and 2-(methacryloyloxy)ethyl trimethyl ammonium chloride with complementary reactive functional groups of acetate and amine, respectively, were synthesized and characterized. The polyelectrolyte complexation between poly(methacrylic acid, sodium salt) and poly[2-(methacryloyloxyethyl) trimethyl ammonium chloride] and/or their reactive polymer-bound copolymers were found to form gels, liquid coacervates, and/or soluble complexes depending on variables such as ionic strength and charge ratio. Complex yield was greatest when a 1:1 charge ratio of polycations and polyanions was used. Liquid coacervates were favored by higher ionic strength, whether from added NaCl or CaCl_2 . In the absence of added salts, all complexes were formed at 1 % w/v total polymer loading with similar complexation efficiencies and yields. Increasing the ionic strength or changing the type of salts (NaCl or CaCl_2) resulted in changing nature of polyelectrolyte complexes from gels to soluble complexes through an intermediate phase of liquid coacervate. The ionic and covalent cross-linkable polyelectrolytes were used to prepare a stable cross-linked coacervate complex. The diverging nature of the polyelectrolyte complexes studied in this work lay at different points along the continuum between solid and soluble complexes, and the ability to use ionic strength and/or salt types to shift between solid and

liquid complex may be of significance for various applications such as flocculants, binders, coating, encapsulations and biotechnology.

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