Polyelectrolyte Complexation Between Poly(methacrylic acid, sodium salt) and Poly(diallyldimethylammonium chloride) or Poly[2-(methacryloyloxyethyl) trimethylammonium chloride]

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ABSTRACT: Polyelectrolyte complexes between poly(methacrylic acid, sodium salt) and poly(diallyldimethylammonium chloride) (PDADMAC) or poly[2-(methacryloyloxyethyl)trimethylammonium chloride] (PMOETAC) form gels, liquid phases, or soluble complexes depending on charge ratio, total polymer loading, polymer molecular weight, and ionic strength. Increasing the ionic strength of the medium led most polyelectrolyte pairs to transition from gel through liquid complexes (complex coacervate) to soluble complexes. These transitions shift to higher ionic strengths for higher molecular weight polymers, as well as for PMOETAC compared to PDADMAC. The complex phases swelled with increasing polymer loading, ultimately merging with the supernatant phase at a critical polymer loading. The isolated liquid complex phases below and above this critical loading were temperature-sensitive, showing cloud points followed by macroscopic phase separation upon heating. Incorporating 5 mol % lauryl methacrylate into the polyanion led to increased complex yield with PDADMAC, and increased resistance to ionic strength. In contrast, incorporating 30 mol % of oligo(ethylene glycol) methacrylate into the polyanion led to decreased complex yield, and to lower resistance to ionic strength. Two polyelectrolyte systems that produced liquid complexes were used to encapsulate hydrophobic oils, and in one case were used to demonstrate the feasibility of crosslinking the resulting capsule walls. © 2007 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 45: 4129–4143, 2007
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INTRODUCTION

The interaction of oppositely charged polyelectrolytes to form polyelectrolyte complexes (PECs) is a topic of considerable interest[1–5] stemming in part from the potential to use PECs in a wide variety of applications including; separation membranes,[6,7] immobilization of enzymes[8] or cells,[9] drug carriers,[10] gene delivery tools,[11,12] and protein purification.[13] PECs can take the form of layer-by-layer (LBL) assemblies,[5,14–21] soluble complexes,[1,2,22,23] solid precipitates,[24–26] and liquid coacervates.[27–29] Both the nature of the interactions and the properties of the PECs formed depend on variables such as charge ratio, ionic strength, pH, temperature, polymer concentra-

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tion, charge density, molecular weight, and polymer structure.[1–5,27,28]

The deposition of PECs onto preformed particles or droplets often results in capsules where the polyelectrolyte coating may modify colloidal behavior, protect the fill and control its release. Encapsulation with PECs is typically achieved by either LBL assembly or complex coacervation. LBL assembly, introduced by Decher,[14] involves sequential deposition of polyanions and polycations, and allows excellent control of the thickness and composition of the layers.[5,21] Complex coacervation is a liquid–liquid phase separation brought about by charge neutralization between oppositely charged polyelectrolytes that yields two immiscible aqueous liquid phases. The denser, polymer-rich coacervate phase can deposit onto dispersed oils, solids or cells in a single step.[30,31] The thickness of the resulting capsule walls depends on the ratio of coacervate to available surface area rather than on the number of layers deposited, and can reach several microns. A well-known example is the liquid complex coacervate formed upon mixing aqueous solutions of gum acacia, a negatively charged polysaccharide derived from the acacia tree, with pig-skin derived gelatin, at pH = 4–5 and above the gel point of gelatin at 50 °C.[32,33]

In some instances, especially for liquid coacervates, it is necessary to further harden the PECs to withstand chemical or mechanical challenges in subsequent use. In the case of gelatin/acacia, the liquid complex formed at 50 °C is initially gelled by cooling below 35 °C, and often hardened by chemical crosslinking with glutaraldehyde or formaldehyde. Other PECs have been crosslinked by the addition of small-molecule crosslinkers,[10,32,34] although this often links only one of the two polyelectrolytes. Reaction of polymer-bound functional groups promoted by heating[35] or reagents such as carbodiimides,[36–38] or by photochemical approaches[39–42] have also been used. In some cases, it is desirable to employ two polyelectrolytes bearing complementary functional groups for crosslinking, as this would lead to covalent incorporation of both polyelectrolytes, avoids exposure of the capsule fill to small-molecule additives, heat or light, and might reduce environmental risks associated with the use of small-molecule crosslinkers such as formaldehyde and glutaraldehyde.
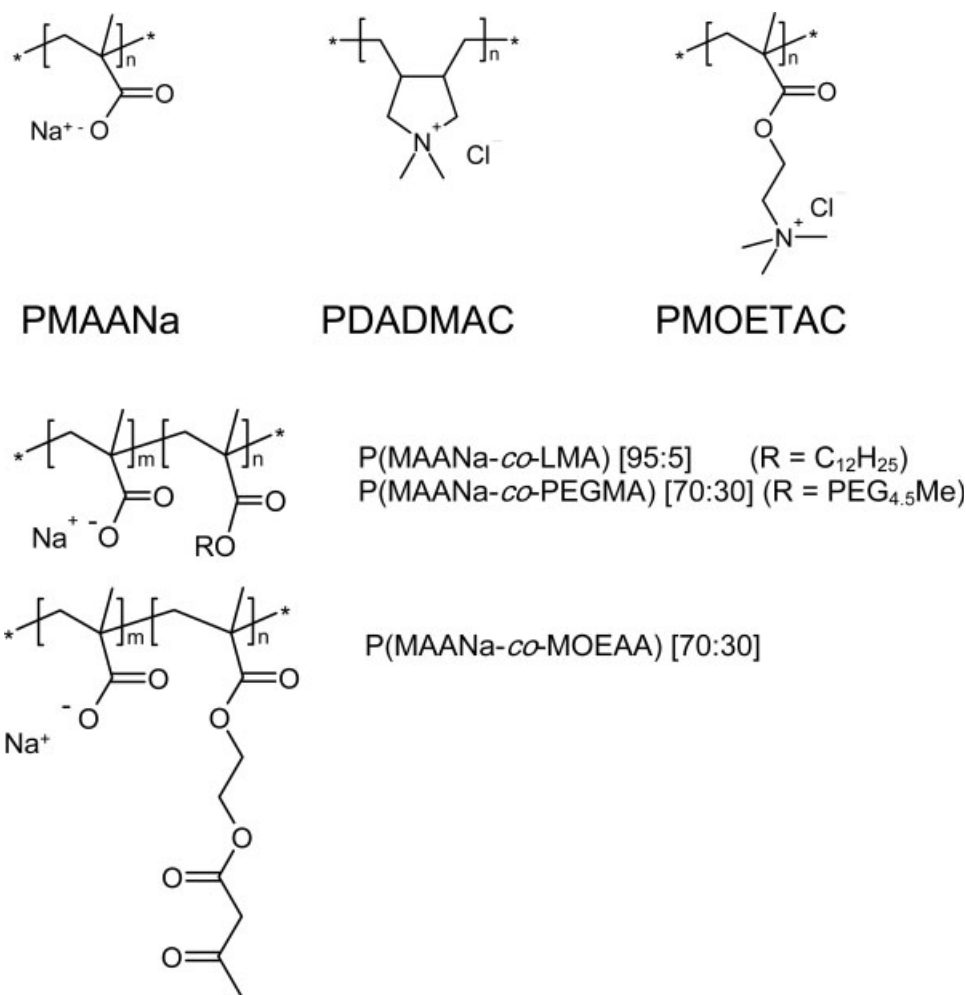
Complex coacervates are most often based on natural polymers including gum acacia and gelatin, with source-dependant variation in their properties. We here chose synthetic polyelectrolytes based on methacrylates and DADMAC because of their ease of synthesis, stability, and the large body of existing knowledge regarding possible comonomers, molecular weight control, and biomedical uses. Synthetic polyelectrolytes offer the ability to fine-tune key properties such as charge density, hydrophobic/hydrophilic balance and molecular weights, and can introduce additional functionality through appropriate comonomers. We have previously investigated synthetic polymers including polyelectrolytes that undergo coacervation.[43] This article describes our initial studies of such complexation, using three model polyelectrolytes, shown in Scheme 1, based on methacrylic acid (MAA), diallyldimethylammonium chloride (DADMAC) and 2-(methacryloyloxyethyl)trimethylammonium chloride (MOETAC). We examine how the physical nature of the complexes and the efficiency of complexation are affected by ionic strength, charge ratio, polymer loading, polymer molecular weight, and hydrophobic or hydrophilic comonomers such as lauryl methacrylate (LMA) and poly[(ethylene glycol)methyl ether methacrylate (PEGMA)]. Conditions under which liquid complex coacervates are formed are identified and some of the resulting complex coacervates are used to encapsulate hydrophobic oils. As well, the feasibility of crosslinking the resulting coacervate capsule walls via complementary polymer-bound reactive groups is investigated.[43] In the present work, polymer-bound acetoacetate groups, introduced via copolymerization of MAA with 2-(methacryloyloxy)ethyl acetoacetate (MOEAA), are used to react with polymer-bound amine groups in polyethyleneimine (PEI).

EXPERIMENTAL

Materials

Methacrylic acid (MAA, 99%), 2-(methacryloyloxy)ethyltrimethylammonium chloride (MOETAC, 75 wt % solution), 2-(methacryloyloxy)ethyl acetoacetate (MOEAA, 95%), poly(ethylene glycol)monomethyl ether methacrylate (PEGMA, M_n = 300 Da), lauryl methacrylate (LMA, 96%), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (Vazo-56), methyl isobromobutyrate (Me*i*BrB, 99+%), cuprous chloride (99%), 2,2′-dipyridyl

PMAANa   **PDADMAC**   **PMOETAC**

P(MAANa-*co*-LMA) [95:5]   (R = $C_{12}H_{25}$)
P(MAANa-*co*-PEGMA) [70:30] (R = $PEG_{4.5}Me$)

P(MAANa-*co*-MOEAA) [70:30]

Scheme 1. Polyelectrolytes used in this study.

$(99^+\%)$, and polyethyleneimine (PEI, M_w = 2000 Da) were purchased from Sigma–Aldrich (Oakville, ON) and used as received. Azobis(isobutyronitrile) (AIBN) was received as a gift from Dupont and used as received. Poly(methacrylic acid, sodium salt) (PMAANa, M_n = 5400 Da; 30 wt % solution) and poly(diallyldimethylammonium chloride) (PDADMAC) with very low (40 wt % solution), low ($M_w \sim$ 100–200 kDa; 20 wt % solution), and high ($M_w \sim$ 400–500 kDa; 20 wt % solution) molecular weights were purchased from Aldrich and used as received. Ethyl ether, methanol, acetone, sodium chloride (Caledon Laboratories, Georgetown, ON), sodium sulfate (BDH, Toronto, ON), and ethanol (Commercial Alcohols, Brampton, ON) were used as received. Tetrahydrofuran (THF, Caledon Laboratories) was distilled over Na/K amalgam before use. HCl and NaOH solutions were pre-pared by diluting standard volumetric concentrates (Anachemia, Rouses Point, NY) with deionized water.

Low MW PMOETAC

Low MW PMOETAC was prepared by atom transfer radical polymerization using a method described in the literature.[44] CuCl (99 mg, 1.0 mmol) and 2,2′-bipyridine (390 mg, 2.5 mmol) were placed in a flask, which was sealed and flushed with nitrogen. MOETAC (8.3 g of 75 wt % solution, 6.22 g, 30 mmol), deionized water (2.1 mL), and isopropanol (3.7 mL) were mixed, bubbled with nitrogen for 20–30 min, and then transferred via a double-tipped needle to the flask containing CuCl and bipyridine. The mixture was stirred for 10 min to allow catalyst formation. Methyl isobromobutyrate (181 mg,

1.0 mmol) was dissolved in isopropanol (0.5 mL), and the mixture was bubbled with nitrogen for 5 min before being transferred via a double-tipped needle to the reaction flask. An exotherm was detected upon the addition of the initiator, and within 1 h, the reaction mixture had become a viscous slurry. The reaction was stopped after 2 h by exposing it to air. A small sample was removed, dried under a stream of air, dissolved in D_2O, and analyzed by ^1H NMR to determine monomer conversion. Water ($\sim$10 mL) was added to the remainder of the reaction mixture to produce a homogeneous solution. The reaction mixture was then dialyzed against deionized water using cellulose tubing with a 3.5 kDa MW cut-off and freeze-dried. Yield: 5.42 g (85%).

PMOETAC-770

MOETAC (10.00 g, 48.1 mmol) and Vazo-56 (0.131 g, 0.48 mmol) were dissolved in 90-mL water in a 125-mL HDPE bottle. The solution was bubbled with nitrogen for several minutes before the bottle was sealed. The bottle was placed in an oven and heated from 25 to 60 °C during 1 h, and then maintained at 60 °C for 23 h. The bottles were slowly rotated (4 rpm) in the oven to provide mixing. The polymer was isolated and purified by precipitation in acetone, or by dialysis (3.5 kDa MW cut-off) followed by freeze-drying. The purified polymer was then dried to a constant weight in a vacuum oven at 50 °C. Yield: 9.1 g (91%).

PMOETAC-300

PMOETAC-300 was prepared in a similar fashion except that 2 mol % of Vazo-56 was used.

P(MAA-co-LMA)

MAA (8.65 g, 100.5 mmol), LMA (1.35 g, 5.29 mmol), and AIBN (174 mg, 1.1 mmol, 1 mol %) were dissolved in THF/EtOH (1:1, 90 mL) in a 125-mL HDPE bottle. The solution was degassed and heated as described above for PMOETAC-770. The polymer was precipitated in ether (1 L), washed with ether, and then dried to a constant weight in a vacuum oven at 50 °C. P(MAA-co-LMA) [95:5] was obtained as a white powder in a yield of 9.63 g (96%).

P(MAA-co-PEGMA)

Polymerization and isolation were conducted in a similar fashion. MAA (4.01 g, 46.6 mmol), PEGMA (5.99 g, 20.0 mmol), and AIBN (109 mg, 0.67 mmol, 1 mol %) were heated in THF/ethanol (1:1, 90 mL), followed by precipitation in ether, to give 8.36 g (84%) P(MAA-co-PEGMA) [70:30].

P(MAA-co-MOEAA)

Polymerization and isolation were conducted in a similar fashion. MAA (4.84 g, 56.2 mmol), MOEAA (5.43 g, 24.1 mmol), and AIBN (132 mg, 0.80 mmol, 1 mol %) were heated in ethanol (100 mL), followed by precipitation in ether, to give 8.74 g (87%) P(MAA-co-MOEAA) [70:30].

Characterization

The composition of the P(MAA-co-MOEAA), P(MAA-co-PEGMA), and P(MAA-co-LMA) polymers was determined by ^1H nuclear magnetic resonance (NMR) spectroscopy using a Bruker AV 200 spectrometer for polymers dissolved in DMSO-d_6.

Size exclusion chromatography (SEC) was conducted using a system consisting of a Waters 515 HPLC pump, Waters 717 plus Autosampler, three columns (Waters Ultrahydrogel-120, -250, -500; 30 cm × 7.8 mm; 6-μm particles) and a Waters 2414 refractive index detector, and was calibrated with narrow molecular weight poly (ethylene glycol) standards (Waters). The eluent was 0.3 M Na_2SO_4 in 80:20 (v/v) water/methanol for PMOETAC. The P(MAA-co-MOEAA) and P(MAA-co-LMA) samples were neutralized with 1 M NaOH prior to SEC analysis, at pH 7, with 0.3 M $NaNO_3$ in 0.1 M phosphate buffer as the eluent.

Viscometry, conducted with an Ubbelohde viscometer (viscometer constant: 0.00314 cSt/s), was used to measure molecular weights of PMOETAC samples dissolved in 1 M NaCl at (20.0 ± 0.1) °C. All solutions were passed through 0.45-μm Acrodisc (Supor Membrane, Pall Corp.) filters before measurement. The intrinsic viscosity [η] was calculated by a double extrapolation of the Huggins plot (η_{sp}/c vs. c) to infinite dilution. The polymer molecular weights were calculated from the intrinsic viscosity using values for K and a found in the literature.[45]

Optical microscope images of complexes and capsules were taken using an Olympus BX51 optical microscope fitted with a Q-Imaging Retiga EXi digital camera and ImagePro software, or an Olympus BH-2 optical microscope fitted with Kodak DC120 digital camera and software.

Coacervation Efficiency

Solutions (typically 15.00 g) of PMAANa, PDADMAC, or PMOETAC at the appropriate concentration and ionic strength were mixed in a preweighed 50-mL polyethylene centrifuge tube. The solutions were mixed several times on a vortex mixer and allowed to stand. The mixture was centrifuged and the supernatant decanted or transferred by pipette to a preweighed, polystyrene weighing-dish. The centrifuge tube was weighed to determine the wet complex yield, and then both the complex and supernatant were dried to constant weight by heating at 65 °C. The dried complex and dried supernatant were weighed to determine the polymer content in the two phases.

Encapsulation

An oil-in-water emulsion was prepared by dispersing methyl benzoate (0.5 mL) in 10 mL of 1.31 % w/v PMOETAC-300 containing 325 mM NaCl with an overhead stirrer (800 rpm) for 30 min at room temperature (20 °C). To this mixture, an aqueous solution of PMAANa (10 mL, 0.69% w/v, 325 mM NaCl) was added dropwise, and the mixture was stirred for an additional 30 min before a sample was removed for examination by optical microscopy.

Encapsulation and Crosslinking

An oil-in-water emulsion was prepared by dispersing paraffin oil (0.5 mL) in 10 mL of 1.31% w/v PMOETAC-300 containing 375 mM NaCl (pH 7) with an overhead stirrer (800 rpm) for 30 min at room temperature (20 °C). To this mixture, 10 mL of a 1.32% w/v solution of P(MAANa-*co*-MOEAA) [70:30] (1.05 equiv of anions) containing 375 mM NaCl (pH 7) was added dropwise. The mixture was stirred for an additional 30 min before 1.4 g of a 2 wt % PEI solution (pH 10.5) was added, and stirring was continued for 3 h at room temperature.

RESULTS AND DISCUSSION

Complexation studies were focused on interactions between pairs of homopolymers, PMAANa/PDADMAC and PMAANa/PMOETAC. The PMAANa employed in this work had a molecular weight (M_n) of 5.4 kDa. Three types of commercial PDADMAC obtained from Aldrich were used. Two of these have molecular weights described by Aldrich as low ($M_w \sim 150$ kDa) and high ($M_w \sim 450$ kDa), and are denoted as PDADMAC-150 and -450, respectively, in this article. The molecular weight of the third PDADMAC sample (PDADMAC-20) has been determined to be 20 kDa by viscometry.[43]

Two PMOETAC samples, PMOETAC-770 and PMOETAC-300, were prepared by free radical polymerization in water initiated with 1 and 2 mol % Vazo-56. Their molecular weights were measured by viscometry, using 1 M NaCl to suppress coil-expansion with dilution,[46] and were found to be $M_\eta = 770$ and 300 kDa, respectively. A third sample, PMOETAC-13, was prepared by atom transfer radical polymerization (ATRP) initiated with methyl isobromobutyrate (Me*i*BrB) in isopropanol/water (1:1 v/v).[44] After 2 h polymerization time, the conversion was determined to be 98% by ^{1}H NMR. Both ^{1}H NMR and viscometry were used to determine the molecular weight of the purified polymer. Comparison of the peak area of the signal due to the methoxy group, derived from the Me*i*BrB initiator, with those due to MOETAC in the ^{1}H NMR spectrum, gave a number-average molecular weight of $M_n = 6$ kDa. Viscometry in 1 M NaCl gave a viscosity-average molecular weight of $M_\eta = 13$ kDa.

Copolymers of MAA with MOEAA, PEGMA, or LMA were prepared by AIBN-initiated free radical polymerization in ethanol or THF/ethanol mixtures. P(MAA-*co*-MOEAA) [70:30], P(MAA-*co*-PEGMA) [70:30], P(MAA-*co*-LMA) [95:5] were determined to have compositions of [67:33], [68:32], and [93:7], respectively, by ^{1}H NMR. Thus, the compositions closely mirrored the comonomer feed ratios. This included samples removed at lower conversions (~30%), indicating that there is no drift in copolymer composition during polymerization. P(MAA-*co*-MOEAA) and P(MAA-*co*-LMA) were found to have molecular weights (M_n) of 42 and 7.1 kDa, respectively, by aqueous SEC. Polyanion solutions were prepared from the MAA copolymers by the addition of stoichiometric amounts of a 1.000 M NaOH solution.
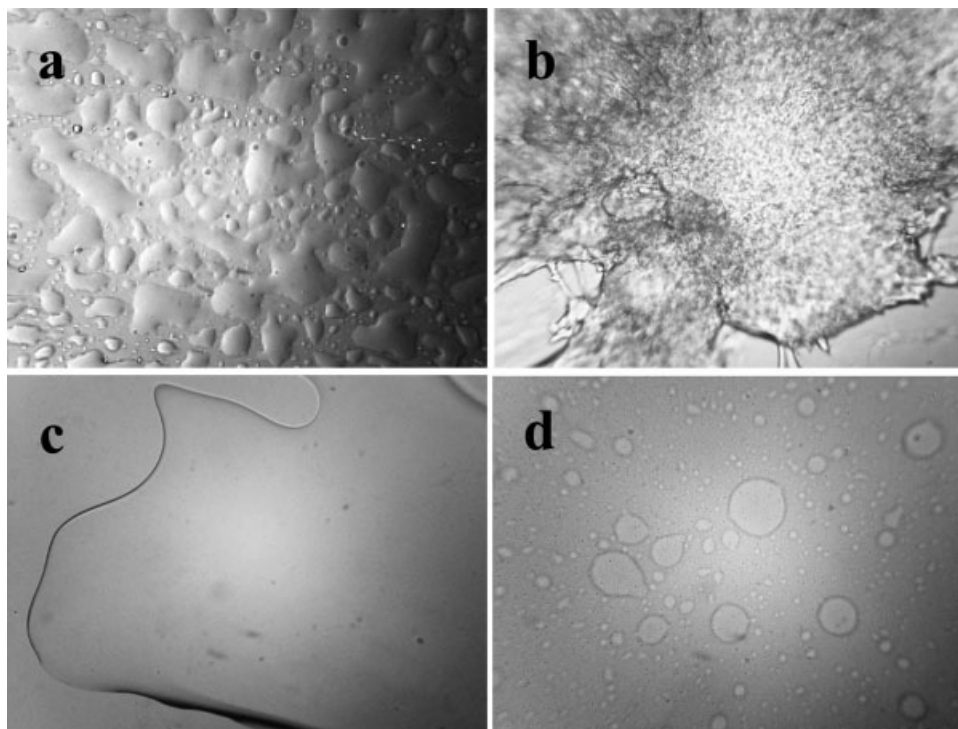
Figure 1. Optical microscope images of polyelectrolyte complexes: (a) liquid complex coacervate droplets PMAANa/PDADMAC-20 (no added NaCl). (a–d) PMAANa/PMOETAC-300, in presence of (b) 200 mM, (c) 300 mM, and (d) 400 mM added NaCl. All complexes were formed by combining the polyelectrolytes in ratios that result in 1 wt % total polymer loading with 1:1 charge ratios. Width of each image 5 mm.

Nature of the Complexes of PMAANa with PDADMAC-20, PDADMAC-450, and PMOETAC-300

Aqueous solutions of PMAANa were combined with solutions of PDADMAC-20, PDADMAC-450, or PMOETAC-300 to obtain mixtures with a 1:1 charge ratio and a total polymer concentration of 1 wt %. In each case, phase separation occurred immediately to form polyelectrolyte complexes (PECs). The PMAANa/PDADMAC-20 complex formed a separate liquid complex coacervate layer, which could be easily redispersed by shaking [Fig. 1(a)].

The analogous PMAANa complex with the higher molecular weight PDADMAC-450 formed a stiff gel, which turned into a liquid coacervate in presence of 300 mM NaCl due to partial shielding of the electrostatic interactions. Similar behavior is seen for the PMAANa/PMOETAC-300 complex [Fig. 1(b–d)], which is transformed from an opaque gel to a liquid complex coacervate as the salt concentration was increased from 200 mM [Fig. 1(b)] to 300 mM [Fig. 1(c)] to 400 mM NaCl [Fig. 1(d)]. The coacervate phase disappears entirely in the presence of 500 mM NaCl.

Factors Affecting Complexation Efficiency

As the above results illustrate, polyelectrolyte complexation may be effected by polymer properties and complexation conditions.[22,27,28,47–49] Therefore, a quantitative study was undertaken to examine the role of polymer concentration, charge ratio, ionic strength, and polymer molecular weight on the complexation of PMAANa with either PDADMAC or PMOETAC. Polyanion and polycation solutions were combined to give polyelectrolyte complexes over a range of polymer concentration, charge ratio and ionic strength. The phase-separated complexes were isolated from the supernatant and immediately weighed to determine the yield of the hydrated complex. Both the supernatant and the complex were then

dried to determine the amount of polymer (and NaCl) present in each phase. The relative amounts of polymer and water within the complex were obtained by comparison of the weights before and after drying. The expressions used to describe complex formation are shown below:

$$\text{Wt \% complex phase} = \frac{\text{wt. of complex} \times 100\%}{\text{total wt. of solution}}$$

Complexation efficiency

$$= \frac{\text{wt. of dry complex} \times 100\%}{\text{total polymer loading}}$$

Polymer fraction in complex

$$= \frac{\text{wt. of dry complex} \times 100\%}{\text{wt. of complex}}$$

The recovery of material was quantitative in all experiments: $(103 \pm 3)\%$ for PMAANa/PDADMAC-20 samples and $(98 \pm 3)\%$ for PMAANa/PMOETAC-300 samples, for example. For samples containing added NaCl, the values measured for complexation efficiency and polymer fraction have been corrected for the presence of the added NaCl in the complex, with the assumption that the NaCl is equally distributed between the complex and supernatant phases.

Charge Ratio

The formation of PECs is normally maximized at a 1:1 ratio of anionic to cationic groups. Indeed, the yield and efficiency of complexation between PMAANa and either PDADMAC-20 or PMOETAC-300 were greatest at a 1:1 charge ratio (Figs. 2 and 3).
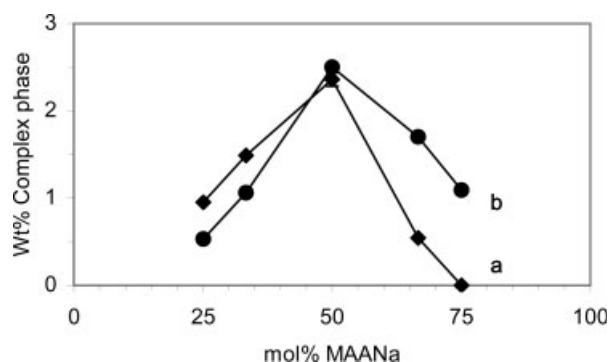

Figure 2. Complex yield as a function of the charge ratio, for complexes formed between PMAANa and (a) PDADMAC-20 or (b) PMOETAC-300. Total polymer loading is 1 wt %, with no added NaCl.
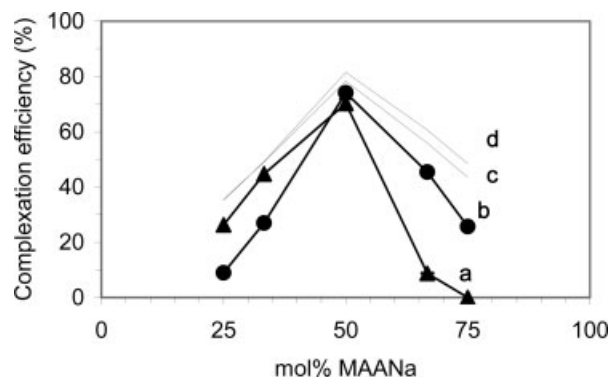
Figure 3. Complexation efficiency as a function of the charge ratio for complexes formed between PMAANa and (a) PDADMAC-20 or (b) PMOETAC-300. Total polymer loading was 1 wt %, with no added NaCl. Also shown are the two curves for efficiency, expected if there were quantitative formation of a 1:1 complex and complete loss of associated Na^+ and Cl^- ions—(c) PDADMAC-20 and (d) PMOETAC-300.

At 1:1 charge ratio, the observed complexation efficiencies of 70% for PDADMAC-20 and 74% for PMOETAC-300, correspond reasonably well to the values expected after accounting for the loss to the supernatant of up to 21.7 wt % (PDADMAC) and 18.5 wt % (PMOETAC) of the total polyelectrolyte weights as NaCl generated during complexation. Figure 3 shows that the complexation efficiency falls further below the theoretical at nonstoichiometric charge ratios, especially in presence of excess polyanion. This is likely due to the formation of some fraction of colloidally stable complex.

Ionic Strength

Ionic strength is known to strongly affect polyelectrolyte complexation.[22,27,28,47,48] For soluble complexes, typically formed at low polyelectrolyte concentrations and a nonstoichiometric mixing ratio, small increases in ionic strength often cause a reduction in hydrodynamic radius due to shielding of the charges of the excess polyelectrolyte within individual complex particles. This can also reduce repulsive interactions between colloidal particles, and hence facilitate aggregation and macroscopic phase separation. On the other hand, large increases in ionic strength can lead to shielding of opposite charges within the complexes, causing swelling and even dissolution of the complex. For example, the coacervation efficiency of gum acacia/gelatin complexes at a 1:1 charge ratio and 0.5%
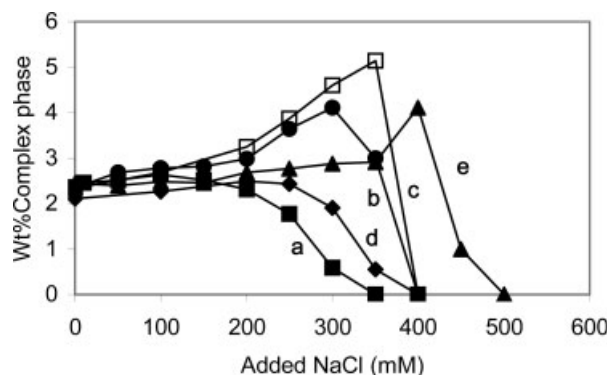
Figure 4. Complex yield as a function of ionic strength for PMAANa complexation with (a) PDAD-MAC-20 or (b) PDADMAC-150, (c) PDADMAC-450, (d) PMOETAC-13, or (e) PMOETAC-300. 1:1 Charge ratio, 1 wt % polymer loading.

(w/v) total polymer loading was zero in the absence of added salt, increased to >80% upon addition of ~1 mM NaCl, and then returned to zero as the ionic strength reached 10 mM.[28]

Thus, it is possible to control both the efficiency of complexation and the physical nature of the complex (solid, gel, liquid, soluble) by varying the ionic strength. The systems studied in this work lie at different points along the continuum between solid and soluble complexes, and the ability to use ionic strength to shift between solid and liquid complex may be of significant use in encapsulation. Indeed, as shown in Figure 1 above, raising the ionic strength from 0 to 400 mM caused the PMAANa/PMOE-TAC-300 complex to change from a gel to a liquid, while the PMAANa/PDADMAC-20 complex was converted from a liquid to a soluble complex. Below we explore the effect on complexation and complex properties, by varying the ionic strength through added salt and polymer loading.

The effect of added NaCl on the yield of complexes between PMAANa and PDADMAC-20, -150, -450 and PMOETAC-13 and -300 is displayed in Figure 4. The complex yield for 1 wt % total polymer loading in the absence of added NaCl is nearly identical [(2.40 ± 0.05)%] for four of the systems, while for PMAANa/PMOE-TAC-13 (curve d, Fig. 4) is slightly lower (2.1%). The complex yield increases as NaCl is added for all of the systems, and is most pronounced for the higher MW polycations. The complex yield for PDADMAC-450, the polycation with the highest MW, more than doubles in 350 mM NaCl. In each case, the yield reaches a maxi-

mum and then drops sharply to zero as the complex phase dissolves. The ionic strength required to dissolve the complexes is higher for polymers with higher MW (compare curves a and c, or d and e). For polymers with comparable MWs, it is higher for PMOETAC than PDADMAC (compare curves a and d, or c and e). It has been reported that PMAANa/PDADMAC complexes made from polymers with MWs of 114 and 250 kDa, respectively, underwent dissolution at about 500 mM NaCl consistent with the results in the present work for a PMAANa with a much lower MW.[47]

At low ionic strengths, all five of the PECs show complexation efficiencies (Fig. 5), i.e.: the fraction of the initial polyelectrolyte loading contained within the complex, near the values expected for complete polyanion/polycation complexation and loss of associated Na$^+$ and Cl$^-$ counter ions. Unlike the complex yield, the complexation efficiency does not rise appreciably with ionic strength, and even decreases at ionic strengths corresponding to maximum yields. The complexation efficiency results also show the greater resistance to ionic strength of complexes made from higher MW polymers, or from PMOETAC rather than PDADMAC.

The effect of ionic strength on the polymer weight fraction in the complex phases is shown in Figure 6. In the absence of added NaCl, four of the complex phases have polymer fractions close to 30%, while PMOETAC-13 [Fig. 6(d)] surprisingly shows the highest polymer fraction (38%). Increasing ionic strength causes a weakening of the electrostatic interactions between
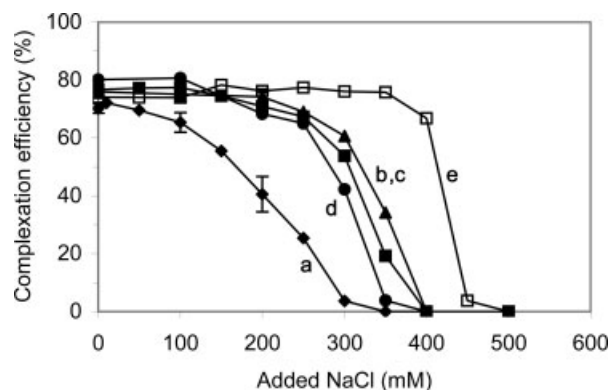

Figure 5. Complexation efficiency as a function of ionic strength for complexation of PMAANa with (a) PDADMAC-20 or (b) PDADMAC-150, (c) PDADMAC-450, (d) PMOETAC-13, or (e) PMOETAC-300. 1:1 Charge ratio, 1 wt % polymer loading.
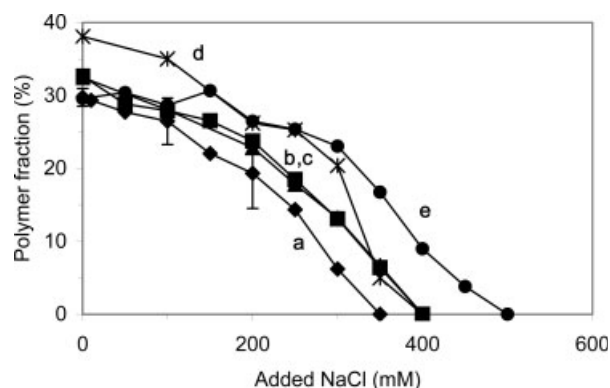
Figure 6. Polymer fraction in the complex phase as a function of ionic strength for complexation of PMAANa with (a) PDADMAC-20 or (b) PDADMAC-150, (c) PDADMAC-450, (d) PMOETAC-13, or (e) PMOETAC-300. 1:1 Charge ratio, 1 wt % polymer loading.

the chains and allows the complexes to swell. This swelling increases the complex yield, but decreases the polymer fraction in the complex phase. All the complexes show a steady decrease in polymer fraction as the ionic strength is increased. The complex made with PDADMAC-20 shows little change in complex yield between 0 and 150 mM NaCl, while the complexation efficiency and polymer fraction are steadily dropping in this range. This reveals that any swelling of this complex with increasing ionic strength is offset by the dissolution of some of the chains, plausibly those of lower molecular weight. The complexes made with polycations of higher MW have more electrostatic interactions per chain and thus higher ionic strength is needed to dissolve the complexes. The greater resistance to dissolution of the PMAANa/PMOE-TAC complexes may be a result of more efficient complexation due to the greater accessibility of the ammonium ions borne on the side chain in PMOETAC as compared to the main chain of PDADMAC.

The effect of ionic strength on complexes formed at nonstoichiometric charge ratios was also examined. The complexation efficiency for a PMAANa/PMOETAC-300 complex prepared at a 1:1 charge ratio (50 mol % MAANa) rose only slightly when the ionic strength was raised from 0 to 300 mM (curve e, Fig. 5). However, there is a more dramatic increase in efficiency for nonstoichiometric mixing ratios (Fig. 7). For example, the efficiency at a 3:1 ratio (75 mol% MAANa) is doubled from 23.8% at 0 mM to 49–51% in the presence of 100–300 mM NaCl.

For all nonstoichiometric ratios at $I = 300$ mM, the efficiency nears or exceeds the value expected for quantitative formation of a 1:1 complex and loss of the associated small ions (NaCl). As mentioned, increased ionic strength can allow rearrangement of nonstoichiometric soluble complexes and reduction of the colloidal stability of the initially formed complexes, both of which can lead to greater amounts of macroscopic phase separation. Complexation efficiencies slightly in excess of the "maximum" line in Figure 7 may result from the presence of small ions and/or more than 1 equiv of the excess polyelectrolyte within the complexes. The complex yield and complexation efficiency for nonstoichiometric PMAANa/PDADMAC-20 complexes were also found to increase in the presence of 100 mM NaCl (data not shown).

Polymer Loading

The total polyelectrolyte concentration also plays an important role in complexation. Low polyelectrolyte loadings often result in soluble complexes with little or no macroscopic phase separation. Phase separation becomes dominant at higher concentrations, but at still higher polymer concentrations, phase separation can disappear. The range of polymer concentrations in which phase separation is observed, depends on factors such as the nature and MW of the polyelectrolytes, temperature and the contribution to ionic strength, I, due to small ions. For
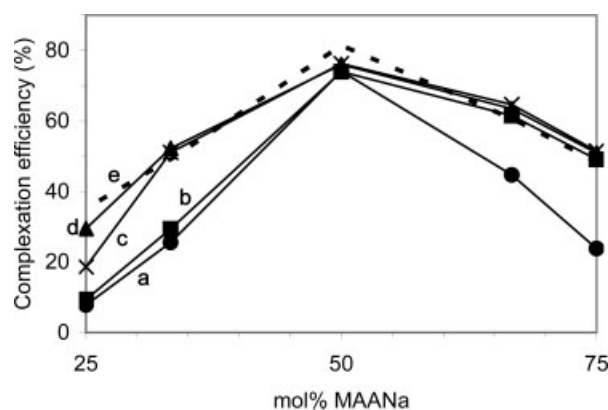

Figure 7. The effect of ionic strength on PMAANa/PMOETAC-300 complexation efficiency at various charge ratios for 1 wt % polymer loading. Added NaCl: (a) 0 mM, (b) 100 mM, (c) 200 mM, and (d) 300 mM. Also shown is the curve (e) for "maximum" efficiency if there were quantitative formation of 1:1 complex and loss of associated small ions (NaCl).

gelatin/acacia at a 1:1 charge ratio and $I = 0$, coacervation was observed only when the total polyelectrolyte concentration was between 0.5 and 14% (w/v).[28] In a study closely related to the present one, the complexation of PDADMAC and poly(acrylic acid, sodium salt) was examined as a function of mixing ratio, total polymer concentration, and polymer MW.[50,51] For most of the MWs studied, phase separation was observed at polymer concentrations up to about 13.2%, and then disappeared to give a macroscopically homogeneous one-phase system. The transition to a one-phase system was thought to be due to reaching a critical ionic strength for this polyelectrolyte pair.

The effect of polymer loading was explored here for complexes formed by PMAANa with PDADMAC or PMOETAC of different MW. At lower polymer loadings (<5% wt %), the complex yields increase with loading, and were independent of polycations (Fig. 8). Each of the systems exhibited upward curvature of the yield versus loading plot, without a corresponding increase in complexation efficiency (data not shown). For PDADMAC-20 and -450, an increase in loading from 1 to 8% causes 11- and 20-fold increases in complex yield, respectively, while the complexation efficiency either drops (from 70% to 57% for PDADMAC-20) or shows only a small increase (76% to 82% for PDADMAC-450). Thus, the dramatic increase in complex yield at higher loading is due to swelling of the complexes. The most pronounced increase in complex yield with loading is seen for the PMAANa/PDADMAC-450
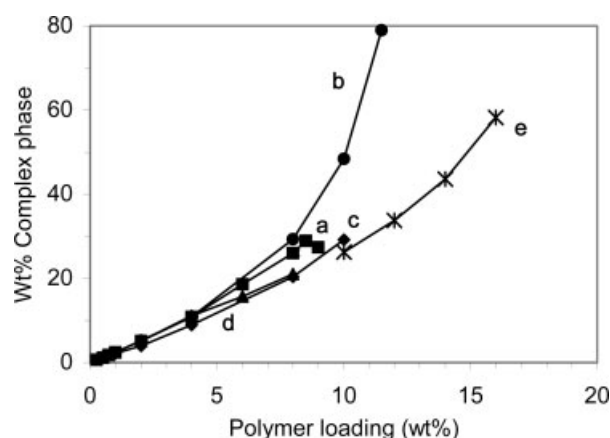

Figure 9. The polymer fraction in the complex (closed symbols) and the supernatant (open symbols) as a function of the total polymer loading for complexation of PMAANa with (a) PDADMAC-20, (b) PDADMAC-450, (c) PMOETAC-13, (d) PMOETAC-300, and (e) PMOETAC-770. Charge ratio = 1:1.

Figure 8. Complex yield as a function of total polymer loading for complexation of PMAANa with (a) PDADMAC-20, (b) PDADMAC-450, (c) PMOETAC-13, (d) PMOETAC-300, and (e) PMOETAC-770. Charge ratio = 1:1, no added NaCl.
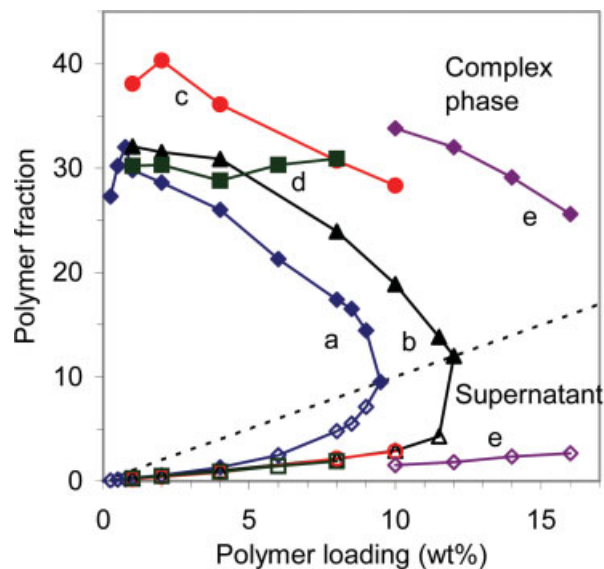
system, the system that had shown the greatest swelling in response to added NaCl (Fig. 4).

Figure 9 shows that polymer content in the complex and supernatant phases rapidly converges as the total polymer loading approaches the critical value of 9.5 wt % for PMAANa/PDADMAC-20, or 12 wt % for PMAANa/PDADMAC-450, where visible phase separation disappears (dashed line, Fig. 9). These critical polymer concentrations for transition to a macroscopic one-phase system are somewhat lower than the 13.2 wt % observed for the closely related poly(acrylic acid, sodium salt)/PDADMAC system,[50,51] likely because of the low MW of PMAANa (5.4 kDa). In approaching the critical loading, the PDADMAC-450 complex yield continues to increase while the complex yield for the PDADMAC-20 complex plateaus, as it more easily dissolves in the supernatant. At 9 and 11.5 wt % loading, the highest loadings to show phase separation for PDADMAC-20 and -450, respectively, both complex phases have polymer fractions of 14%. However, only 44% of the total polymer, most likely the higher MW fraction, is in the PDADMAC-20 complex compared to 92% for PDADMAC-450. Similar behavior was observed for these two systems in response to added salt (Figs. 4–6). The curves for PMOETAC

(Fig. 9, curves c, d, e) suggest the presence of a critical polymer loading closer to 20 wt %.

The decreasing polymer fraction in the complex leads to a corresponding decrease in the viscosity of the complex phase, similar to the effects seen for addition of NaCl. For example, the PMAANa/PDADMAC-450 complex gradually changes from a gel to a free flowing liquid coacervate as the loading is increased from 1 to 11.5 wt %.

Complex phases near their critical loading were also found to exhibit a dramatic temperature sensitivity. For instance, the PMAANa/PDADMAC-450 complex phase formed at 20 °C and 11.5 wt % total polymer loading was separated from the supernatant and then heated in a sealed tube. The clear liquid complex turned cloudy nearly immediately and when left overnight at 62 °C, separated into two layers with relative volumes of complex:supernatant ~60:40. The more concentrated complex phase was noticeably more viscous, even at 62 °C, than the initial complex at 20 °C. Similarly, the PMAANa/PDADMAC-20 solution formed at 9.5 wt % loading showed no phase separation at room temperature but separated into two phases with relative volumes of complex:supernatant of ~ 25:75 when heated to 62 °C. Figure 10 below illustrates the increasing phase separation upon heating of solutions containing 11.5 and 12 wt % PMAANa/PDADMAC-450, respectively.

Dissolution or hydration of the complexes exposes more hydrophobic portions of the polymer chains to water. The entropic penalty associated with hydrating these hydrophobic portions is greater at higher temperatures and the system responds by undergoing phase separation and dehydration, presumably to allow some of the hydrophobic groups to associate with each other. Temperature has been seen to affect complexation in other studies but the nature of this effect depends strongly on the system being studied. The yield of gelatin/albumin coacervation was increased at lower temperatures presumably because higher temperatures caused the loss of some electrostatic interactions.[28] Conversely, soluble complexes made from PDADMAC and PMAANa where the polyelectrolytes bore poly(ethylene glycol) grafts or blocks exhibited cloud points, and in some cases macroscopic phase separation, when heated.[52] The temperature-induced phase separation of the polyelectrolyte complexes examined in the present work might be useful in applications such as encapsulation, or purification via complexation.

The Effect of Comonomers

The nature of the complex and the efficiency of complexation are also influenced by the composition of the polyelectrolytes. Polymer properties such as charge density, hydrophobic/hydrophilic balance, and molecular weight can all be controlled through the selection of comonomers and polymerization conditions. In addition, it may be possible to adjust complex properties such as the colloidal stability or binding to surfaces through the introduction of appropriate chemical groups.

Two polyanions of differing hydrophobic/hydrophilic balance were prepared by radical copolymerization of methacrylic acid (MAA) with either 5 mol % lauryl methacrylate (LMA) or 30 mol % poly(ethylene glycol) methyl ether methacrylate (PEGMA). Following isolation and characterization of the copolymers, the MAA in the copolymers was neutralized by the addition of NaOH to give the two polyanions, P(MAANa-co-LMA) [95:5] and P(MAANa-co-PEGMA) [70:30]. In Figures 11 and 12, the complex yields and complexation efficiencies of these copolymers is compared with those of the parent polyanion, PMAANa. The polyanion containing 5 mol % of
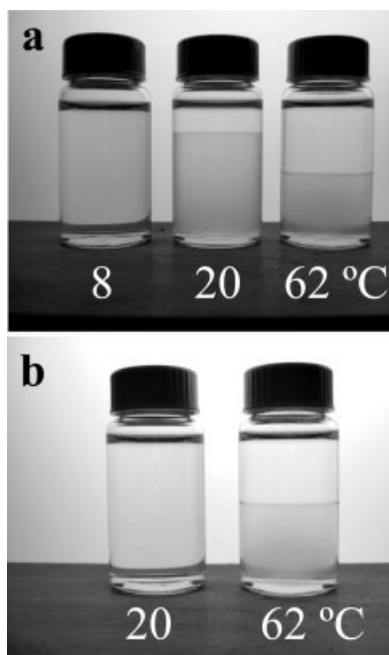

Figure 10. The effect of temperature on the PMAANa/PDADMAC-450 complexes formed at (a) 11.5 wt % and (b) 12 wt % total polymer loading.
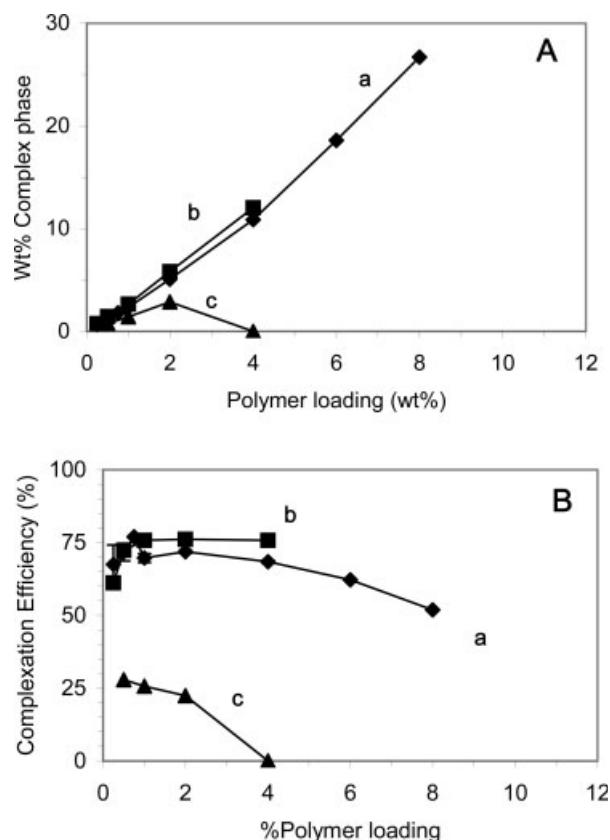
Figure 11. The effect of total polymer loading (wt %) on the (A) complex yield and (B) complexation efficiency for the complex formed between PDADMAC-20 and (a) PMAANa, (b) P(MAANa-LMA) [95:5], and (c) P(MAANa-PEGMA) [70:30]. Charge ratio = 1:1.

the hydrophobic LMA shows slightly higher yield and efficiency than the parent PMAANa over the range of ionic strengths and polymer loadings investigated. The polyanion that bears the uncharged, hydrophilic PEG groups shows much lower efficiency than the other two polyanions and no complex is formed when the ionic strength is increased by the addition of 100 mM NaCl or by raising the polymer loading to 4 wt %. The presence of PEG side chains has been shown previously to enhance the colloidal stability of soluble polyelectrolyte complexes and hinder macroscopic phase separation.[52–54]

As well as influencing the efficiency of complexation, these groups can alter the nature of the complex. Complexes made from the PEGMA-containing polymer had higher water content. At 1% polymer loading, the complex formed from P(MAANa-co-PEGMA) had a polymer fraction of 18%, while the complexes made from the other two polyanions had polymer frac-

tions of 29–30%. The introduction of a hydrophilic comonomer such as PEGMA might be used to obtain a coacervate under conditions where the parent polyelectrolytes would give a gel or solid complex.

Encapsulation of Oils with Complex Coacervates

Bungenberg De Jong[27] was the first to encapsulate hydrophobic liquid droplets with a coacervate, and complex coacervates have now been used to encapsulate oils or solids for a number of applications. The polar coacervate walls can protect volatile oils from evaporation. Consequently, model experiments were undertaken to examine whether coacervates of the type studied in this work could be used to coat the surface of hydrophobic oils.

Methyl benzoate was dispersed with an overhead stirrer in a PMOETAC-300 solution before
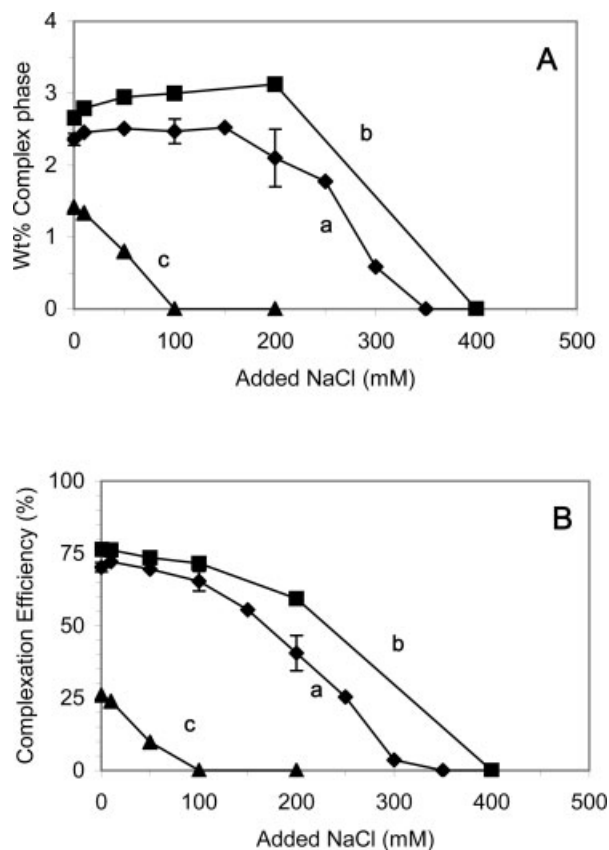

Figure 12. The effect of ionic strength on (A) complex yield and (B) complexation efficiency for the complex formed between PDADMAC-20 and (a) PMAANa, (b) P(MAANa-LMA) [95:5], and (c) P(MAANa-PEGMA) [70:30]. Charge ratio = 1:1.
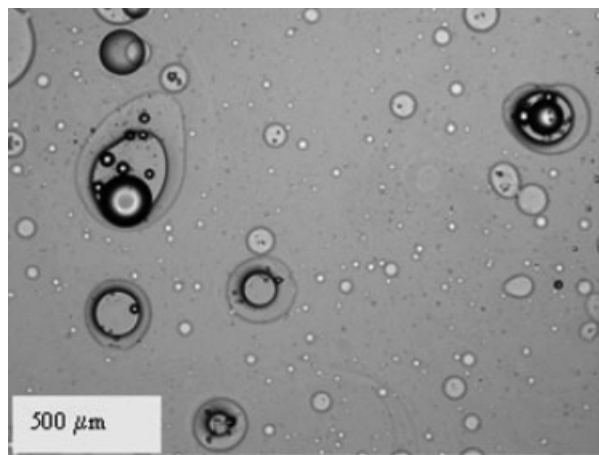
Figure 13. Optical microscopic image of methyl benzoate encapsulated with a complex made from PMAANa/PMOETAC-300 at a 1:1 charge ratio. Polymer loading: 1%, ionic strength: 325 mM.

PMAANa was added. The mixture contained 325 mM added NaCl at which ionic strength the PMAANa/PMOETAC complex exists as a liquid coacervate. Optical microscopy showed the presence of coacervate droplets and capsules where the coacervate coats both methyl benzoate droplets and air bubbles (Fig. 13). To be useful for encapsulation, the coacervate coating would need to be gelled, either physically or chemically. One means of gelling the PMAANa/PMOETAC capsule would be to reduce the ionic strength following encapsulation by diluting the solution. At reduced ionic strength (200 mM or lower, for example), the complex would become a gel [Fig. 1(b)].

Encapsulation and Crosslinking of the Capsule Walls

The use of synthetic polyelectrolytes allows the introduction of reactive groups that may be used to crosslink the polyelectrolyte complex during or after encapsulation.[43] Toward this end, a polyanion containing acetoacetate groups, P(MAANa-*co*-MOEAA) [70:30], was combined with PMOETAC-300 (1.05:1 mixing ratio) and used to encapsulate paraffin oil giving capsules similar to those shown in Figure 13. There was no appreciable change in the appearance of the capsules when a small amount of 2 wt % aqueous poly(ethyleneimine) (PEI) was added to the stirred capsule suspension [Fig. 14(a)]. PEI is a branched polyamine with a net cationic charge that would be attracted to the anionic complex. The free amine groups of PEI may react with acetoacetate groups on the polyanion chains to form covalent crosslinks as shown in Scheme 2. The capsules formed before PEI treatment disappeared when exposed to a 4 wt % NaCl solution, since the complex is held together by electrostatic interactions only. The PEI-treated capsule walls survived the NaCl challenge [Fig. 14(b)] showing that the walls were chemically crosslinked.

The polyelectrolyte complexes formed between PMAANa and PDADMAC or PMOETAC may prove useful for LBL assembly, encapsulation, protein purification, or other applications because of the ability to (a) vary the complex nature by changing polymer properties and/or complexation conditions, and (b) covalently crosslink the complexes. We are planning to use these
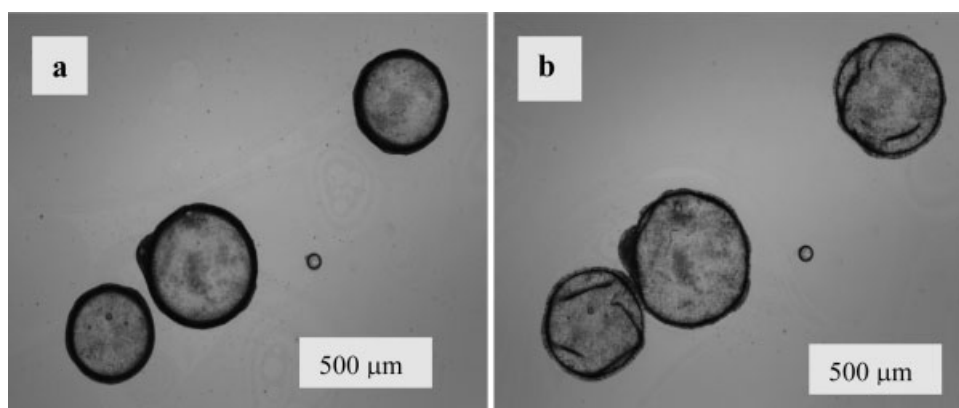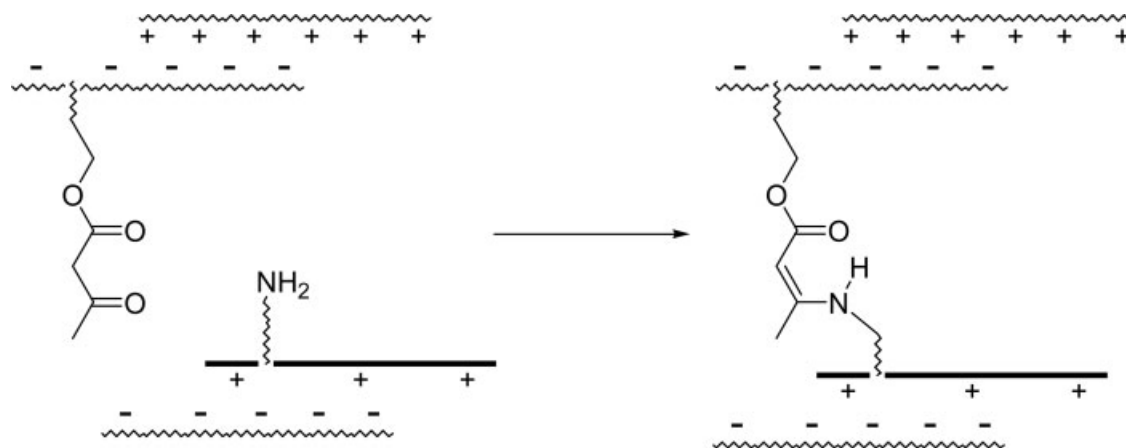

Figure 14. Optical microscopic image of paraffin oil encapsulated with a complex made from P(MAANa-*co*-MOEAA)/PMOETAC-300 at a 1.05:1 charge ratio and an ionic strength of 375 mM: (a) after crosslinking with PEI and (b) after addition of excess 4% NaCl (684 mM).

Scheme 2. Reaction between acetoacetate and amine groups to form crosslinked polyelectrolyte complex.

and similar approaches for the encapsulation of live cells used in enzyme-replacement therapy.

CONCLUSIONS

Polyelectrolyte complexes formed between poly (methacrylic acid, sodium salt) (PMAANa) and poly(diallyldimethylammonium chloride) (PDAD MAC) or poly[2-(methacryloyloxyethyl)trimethy-lammonium chloride] (PMOETAC) were shown to form gels, liquids, or soluble complexes, depending on the conditions used. Liquid coacervates were favored by higher ionic strength, whether from added NaCl or from higher polymer loading, and by using polyelectrolytes of lower molecular weight.

Complex yield was greatest when 1:1 charge ratios were used. In the absence of added salt, all the complexes formed at 1 wt % polymer loading had similar yields, efficiencies, and polymer fractions. As the ionic strength of the solutions was increased, the complexes underwent swelling and eventually dissolved. The ionic strength at which dissolution occurred was greater for polyelectrolytes of higher molecular weight, and for PMOETAC compared to PDAD-MAC. Likewise, when polymer loading was increased, the complexes became more swollen and above a critical loading, no macroscopic phase separation was observed for the complexes made with PDADMAC. The polyelectrolyte complexes formed near the critical loading were found to be temperature-sensitive, exhibiting cloud points and/or deswelling when heated.

When some of the carboxylate groups of PMAANa were replaced with hydrophobic lauryl (dodecyl) groups, the complex with PDADMAC was formed in a slightly higher yield and showed greater resistance to increasing ionic strength. When some of the carboxylates were replaced with hydrophilic but neutral poly(ethylene glycol) side-chains, the complex with PDADMAC was formed in much lower yield, and showed much lower resistance to ionic strength. Two polyelectrolyte systems that gave liquid complexes (coacervates) were used to encapsulate hydrophobic oils and, in one case, the crosslinking of the resulting capsule walls was demonstrated. The ability to vary the nature of the complex by varying ionic strength, polymer MW, polymer loading or temperature might be useful in encapsulation, protein purification, or other applications.

REFERENCES AND NOTES

1. Tsuchida, E.; Abe, K. Adv Polm Sci 1982, 45, 1–119.
2. Kabanov, V. A. In Macromolecular Complexes in Chemistry and Biology; Dubin, P.; Bock, J.; Davis, R.; Schulz, D. N.; Thies, C., Eds.; Springer-Verlag: Berlin, 1994; Chapter 10, pp 151–174.
3. Philipp, B.; Dautzenberg, H.; Linow, K.-J.; Kötz, J.; Dawydoff, W. Prog Polym Sci 1989, 14, 91–172.
4. Thünemann, A. F.; Müller, M.; Dautzenberg, H.; Joanny, J.-F.; Löwen, H. Adv Polym Sci 2004, 166, 113–171.

5. Sukhishvili, S. A.; Kharlampieva, E.; Izumrudov, V. Macromolecules 2006, 39, 8873–8881.

6. Iwatsubo, T.; Kusumocahyo, S. P.; Shinbo, T. J Appl Polym Sci 2002, 86, 265–271.

7. Kim, S.-G.; Lim, G.-T.; Jegal, J.; Lee, K. H. J Membr Sci 2000, 174, 1–15.

8. Dumitriu, S.; Chornet, E. Biotechnol Prog 1997, 13, 539–545.

9. Chu, C. H.; Kumagai, H.; Nakamura, K. J Appl Polym Sci 1996, 60, 1041–1047.

10. Mi, F. L.; Sung, H. W.; Shyu, S. S. Carbohydr Polym 2002, 48, 61–72.

11. Liu, W. G.; Yao, K. D.; Liu, Q. G. J Appl Polym Sci 2001, 82, 3391–3395.

12. Makita, N.; Yoshikawa, K. Biophys Chem 2002, 99, 43–53.

13. Wang, Y.; Gao, J. Y.; Dubin, P. L. Biotechnol Prog 1996, 12, 356–362.

14. Decher, G.; Hong, J. D. Makromol Chem Macromol Symp 1991, 46, 321–327.

15. Decher, G. Science 1997, 277, 1232–1237.

16. Sukhorukov, G. B.; Mohwald, H.; Decher, G.; Lvov, Y. M. Thin Solid Films 1996, 284/285, 220–223.

17. Schlenoff, J. B.; Dubas, S. T. Macromolecules 2001, 34, 592–598.

18. Mermut, O.; Barrett, C. J. J Phys Chem B 2003, 107, 2525–2530.

19. Choi, J.; Rubner, M. F. Macromolecules 2005, 38, 116–124.

20. Glinel, K.; Moussa, A.; Jonas, A. M.; Laschewsky, A. Langmuir 2002, 18, 1408–1412.

21. Sukhishvili, S. A. Curr Opin Colloid Interface Sci 2005, 10, 37–44.

22. Kabanov, V. A.; Zezin, A. B. Pure Appl Chem 1984, 56, 343–354.

23. Kabanov, V. A.; Zezin, A. B.; Rogacheva, V. B.; Gulyaeva, Z. G.; Zansochova, M. F.; Joosten, J. G. H.; Brackman, J. Macromolecules 1999, 32, 1904–1909.

24. Fredheim, G. E.; Christensen, B. E. Biomacromolecules 2003, 4, 232–239.

25. Paneva, D.; Mespouille, L.; Manolova, N.; Degee, P.; Rashkov, I.; Dubois, P. J Polym Sci Part A: Polym Chem 2006, 44, 5468–5479.

26. Michaels, A. S.; Miekka, R. G. J Phys Chem 1961, 65, 1765–1773.

27. Bungenberg De Jong, H. G. In Colloid Science; Kruyt, H., Ed.; Elsevier: New York, 1949; Vol. II, pp 335–432.

28. Burgess, D. J. J Colloid Interface Sci 1990, 140, 227–238.

29. Vanerek, A.; van de Ven, T. G. M. Colloids Surf Part A: Physicochem Eng Aspects 2006, 273, 55–62.

30. Arshady, R. Polym Eng Sci 1990, 30, 905–914.

31. Burgess, D. J. In Macromolecular Complexes in Chemistry and Biology; Dubin, P.; Bock, J.; Davis, R.; Schulz, D. N.; Thies, C., Eds.; Springer-Verlag: Berlin, 1994; Chapter 17, pp 285–300.

32. Fubao, X.; Cheng, G.; Bingxing, Y.; Ma, L. J Appl Polym Sci 2004, 91, 2669–2675.

33. Burgess, D. J.; Singh, O. N. J Pharm Pharmacol 1993, 45, 586–591.

34. Tong, W.; Gao, C.; Möhwald, H. Macromolecules 2006, 39, 335–340.

35. Stair, J. L.; Harris, J. J.; Bruening, M. L. Chem Mater 2001, 13, 2641–2648.

36. Schuetz, P.; Caruso, F. Adv Funct Mater 2003, 13, 929–937.

37. Schneider, A.; Vodouhê, C.; Richert, L.; Francius, G.; Guen, E. L.; Schaaf, P.; Voegel, J.-C.; Frisch, B.; Picart, C. Biomacromolecules 2006, 8, 139–145.

38. Mauser, T.; Déjugnat, C.; Sukhorukov, G. B. Macromol Rapid Commun 2004, 25, 1781–1785.

39. Pastoriza-Santos, I.; Scholer, B.; Caruso, F. Adv Funct Mater 2001, 11, 122–128.

40. Olugebefola, S. C.; Ryu, S.-W.; Nolte, A. J.; Rubner, M. F.; Mayes, A. M. Langmuir 2006, 22, 5958–5962.

41. Vuillaume, P. Y.; Jonas, A. M.; Laschewsky, A. Macromolecules 2002, 35, 5004–5012.

42. Wang, J.; Jia, X.; Zhong, H.; Luo, Y.; Zhao, X.; Cao, W.; Li, M.; Wei, Y. Chem Mater 2002, 14, 2854–2858.

43. Yin, X.; Stover, H. D. H. Macromolecules 2003, 36, 8773–8779.

44. Li, Y.; Armes, S. P; Jin, X.; Zhu, S. Macromolecules 2003, 36, 8268–8275.

45. Griebel, Th.; Kulicke, W.-M.; Hashemzadeh, A. Colloid Polym Sci 1991, 269, 113–120.

46. Mazumder, M. A. J.; Umar, Y.; Ali, S. A. Polymer 2004, 45, 125–132.

47. Dautzenberg, H.; Karibyants, N. Macromol Chem Phys 1999, 200, 118–125.

48. Dautzenberg, H.; Kriz, J. Langmuir 2003, 19, 5204–5211.

49. Dautzenberg, H. Macromolecules 1997, 30, 7810–7815.

50. Koetz, J.; Kosmella, S. Nuovo Cimento 1994, 16D, 865–871.

51. Kötz, J.; Beitz, T. Trends Polym Sci 1997, 5, 86–90.

52. Andersson, T.; Holappa, S.; Aseyev, V.; Tenhu, H. J Polym Sci Part A: Polym Chem 2003, 41, 1904–1914.

53. Dautzenberg, H. Macromol Chem Phys 2000, 201, 1765–1773.

54. Holappa, S.; Andersson, T.; Kantonen, L.; Plattner, P.; Tenhu, H. Polymer 2003, 44, 7907–7916.