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2015

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The version of record can be found at <https://www.doi.org/10.1002/adma.201500176>.

Saloplastics: Processing Compact Polyelectrolyte Complexes

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Abstract

Polyelectrolyte complexes, PECs, are prepared by mixing solutions of oppositely-charged polyelectrolytes. These diffuse, amorphous precipitates may be compacted into dense materials, CoPECs, by ultracentrifugation (ucPECs) or extrusion (exPECs). The presence of salt water is essential in plasticizing PECs to allow them to be reformed and fused. When hydrated, CoPECs are versatile, rugged, biocompatible, elastic materials with applications including bioinspired materials, supports for enzymes and (nano)composites. In this review, various methods for making CoPECs are described, as well as fundamental responses of CoPEC mechanical properties to salt concentration. Possible applications as synthetic cartilage, enzymatically active biocomposites, self-healing materials, and magnetic nanocomposites are presented.

Introduction

Structural polymers are often processed by softening or melting them with heat then reshaping them by extrusion, or molding. Such “thermoplastics,” which currently occupy major commercial markets, rarely contain ionic groups. In fact, the presence of a small amount of charge impacts the mechanical properties of thermoplastics significantly and can help make blends of polymers compatible.¹

Polyelectrolytes carry substantial amounts of charge on the polymer backbone. This high charge density endows polyelectrolytes with additional intriguing/confounding properties.² These charges tend to make polyelectrolytes water soluble and therefore useful for modifying aqueous rheological and associative properties. Most commercial applications of polyelectrolytes are centered on their use for water treatment (e.g. flocculation or descaling), rheology modification (e.g. shampoos, concrete plasticizers) or gels, especially superabsorbent gels (e.g. polyacrylic acid (co)polymers for diapers).² Polyelectrolyte rheology and interactions with other components, especially those that are charged, in complex mixtures are highly tunable by traditional macromolecular parameters (molecular weight, solvent quality, molecular architecture) as well as variables relating to their charge, such as the salt concentration.

Mixtures of polyelectrolytes with opposite charges spontaneously aggregate into hydrated complexes.³⁻⁵ Polyelectrolyte complexes, PECs, are held together by ion pairing interactions, Pol^+Pol^- , between oppositely-charged units on (bio)macromolecules.⁶ Because almost no enthalpy change is observed, it is concluded that the driving force for these polyvalent interactions is mainly the entropic release of counterions⁷⁻¹⁰ and water molecules¹¹ according to equation [1]

PECs exist in a range of morphologies, largely differentiated by water content and the way charge is balanced within the complex. The counterion content of PECs also controls their physical and mechanical properties.¹² Charges on polyelectrolytes are balanced either by the complementary polyelectrolyte (intrinsic charge compensation) or by counterions (extrinsic charge compensation).¹³ Non-stoichiometric PECs require extrinsic charge, whereas stoichiometric PECs may or may not contain ions.

Figure 1. Polyelectrolyte repeat unit charges for “extrinsic” sites are balanced by counterions whereas for “intrinsic” sites oppositely-charged repeat units are paired. This example is illustrated with poly(styrene sulfonate), PSS, and poly(diallyldimethylammonium), PDADMA, as polyanion and polycation, respectively.

Weak, fluid-like complexes between polyelectrolytes, often containing biomacromolecules such as proteins, are known as coacervates, described by Bungenberg de Jong around 1930³ and exploited in the food processing industry. When the first synthetic polyelectrolytes were complexed by Fuoss and Sadek in 1949, dense precipitates or particles resulted.¹⁴ There followed a brief period of intense property evaluation of PECs in the 1960's, mainly by Alan Michaels and coworkers.^{4, 6, 15-16} The initial set of properties were largely qualitative: insolubility in common solvents, infusibility, amorphous structure, virtually no salt counterions within the complex, high dielectric constant when wet, permeable to water and electrolytes, impermeable to macrosolutes, and swelling and plasticizing by aqueous electrolytes. But these properties were enough to project uses as membranes for dialysis, ultrafiltration, battery separators, fuel cell membranes, electrically conductive coatings, medical devices including contact lenses, and chemical sensors.⁶⁻⁷ Enthusiasm for dense PECs tapered away quickly because they were deemed “impossible” to process, as they were infusible and brittle when dry.^{6, 17} Interest in PECs remained low, even after

Michaels showed a route around the processing problem by dissolving PECs in aggressive ternary salts and casting them as films.^{4, 7}

Two morphologies of PEC evolved to address the processing challenges. Under certain conditions, such as an excess of one polyelectrolyte, dilute solution, and a strong mismatch of molecular weight, a stable colloidal dispersion of PEC nanoparticles, (quasisoluble PECs, q-PECs) may be obtained.¹⁸⁻²⁰ q-PECs rely on a shell of extrinsic charge to keep them dispersed.²¹ The Moscow State group²² elegantly demonstrated place-exchange reactions between certain types of polyelectrolytes in q-PECs. This labile behavior was contrasted with non-labile complexation between other pairings of polymers, illustrating the intricate kinetics of polyelectrolyte association and dissociation. q-PECs of a polycation, such as polyethyleneimine, and DNA are widely used for "gene delivery."²³

More recently, thin films of PECs have been deposited on substrates by alternating exposure to polyelectrolytes.²⁴⁻²⁷ These polyelectrolyte multilayers, PEMUs, are dense, conformal coatings of the same material as a PEC.²⁸ The polyelectrolytes are combined in a stoichiometric proportion, or they are nonstoichiometric, depending on the polymers, the deposition conditions and even the thickness of the film.²⁹⁻³¹ PEC films of tunable composition were also obtained by deposition of PEC nanoparticles.³²

A dry PEC is like a polymeric salt with multiple interaction points. For over 50 years PECs have been claimed to be unprocessable as they are "infusible and insoluble in common solvents."^{6, 17} The polyelectrolyte multilayering method was therefore considered a breakthrough in providing dense, defect free coatings of a PEC.

Water turns out to be a major actor in PEC properties. From the earliest research, water has been known to plasticize PECs.^{6-7, 33} Unlike individual polyelectrolytes, which simply dissolve with the addition of water, PECs take up an equilibrium (i.e. defined) amount of water,³⁴ sufficient to hydrate the ion pairs.

In 2009 it was reported that PECs heavily plasticized (doped) with salt water could be compacted into tough, macroscopic articles under ultracentrifugal fields (Figure 2).³⁵ This property was termed "saloplasticity" to emphasize the role of salt in softening these compacted PECs (CoPECs). These large articles permit the use of standard polymer characterization equipment such as rheometry, tensile testing, optical microscopy of cross-sections and solid state NMR. A processing milestone was reached when a salt-plasticized PEC was extruded into fibers, rods, tape and tubes using a laboratory extruder.³⁶

Figure 2. Transformation of an as-precipitated (0.5M PSS and 0.5 M PDADMA, based on the monomer repeat unit in 2.5 M NaCl) blob of PDADMA/PSS complex into a tough, transparent PEC by saloplastic compaction under a high centrifugal field. The salt is required. The compacted PEC is actually porous but does not scatter light because of refractive index matching by the salt solution. The grid is 5 mm.

Saloplasticity is reminiscent of the intriguing “baroplastic” behavior demonstrated a few years ago by the Mayes group:³⁷⁻³⁹ certain block copolymers showed pressure-induced transitions from soft solid to melt-like behavior at temperatures far in excess of the glass transition temperature, T_g . These baroplastic systems contain nanophase dimensions of one high T_g and one low T_g component, the latter promoting flow under pressure. Potential advantages in energy savings during processing, as well as recycling, were highlighted. These same advantages may be realized by saloplastic materials and processing.

Plasticizing with salt water: Doping

Recent studies on the formation of polyelectrolyte multilayers have provided a great deal of insight on the relation between the fundamental properties of hydrated PEMUs and PECs and their counterion concentration. These studies demonstrated a slate of materials properties that were reversibly controlled by swelling or doping by salt. The concept of transforming a Pol^+Pol^- ion pair into counterion-compensated units, Pol^+Cl^- and Pol^-Na^+ , partially reversing Equation 1, is illustrated by Equation 2.

$$K_{dop} = \frac{y^2}{(1-y)a_{NaCl}^2} \quad [3]$$

The doping level, y , is the fraction of Pol^+Pol^- converted to extrinsic sites.⁴⁰ Thus, $1-y$, is the fraction of intrinsic sites or polyelectrolyte ion pairs. The counterion population is under reversible thermodynamic equilibrium, the doping constant given by Equation 3.⁴¹ If y reaches 1.0 the polymers are no longer associated and they dissolve. This is the condition reached by the ternary solvents (e.g. water, acetone and salt) developed by Michaels to dissolve complexes.⁷ The effectiveness of doping was found to follow a Hofmeister series,⁴² where SCN^- and ClO_4^- doped a complex most efficiently.⁴³ The rate of diffusion of ions

through stoichiometric, cylindrical samples of PDADMA/PSS complex was measured by electrical conductivity.

Figure 3. Doping level, y , in PDADMA/PSS exPEC versus salt activity for NaF (●); NaCH₃COO (◇); NaClO₃ (▲); NaCl (■); NaNO₃ (Δ); NaBr (○); NaI (◆); NaClO₄ (x); and NaSCN (□). Room temperature. Reproduced with permission from ref⁴³

X: Anion ^(a)	K_{dop}	$D_{\text{NaX}, y=0.5} (\times 10^{-7} \text{ cm}^2 \text{ s}^{-1})$
SCN ⁻	3.20	6.0
ClO ₄ ⁻	2.40	4.6
I ⁻	1.00	5.9
Br ⁻	0.42	8.0
NO ₃ ⁻	0.41	7.5
Cl ⁻	0.30	10.8
ClO ₃ ⁻	0.18	13.0
CH ₃ COO ⁻	0.02	15.9
F ⁻	0.008	4.0

^(a)cation is Na⁺

Table 1. Doping constants (K_{dop}) and diffusion coefficient ($D_{\text{NaX}, y=0.5}$) in PDADMA/PSS exPEC at $y = 0.5$ doping level. X⁻ is the anion with Na⁺ as a common cation. K_{dop} are from slopes in Figure 3.

Compaction by ultracentrifugation

As-precipitated PECs are diffuse with much entrained water (see Figure 2). They are obtained simply by mixing virtually any combination of water-soluble polycation and polyanion. Those complexes which pair strongly may take some time to completely associate, especially if salt is not added to the mixture. This slow association, reflecting nonlabile ion pairing between polyelectrolytes,^{18, 22} may be seen by the initial formation of transparent blobs of complex with the consistency of egg white. Added salt⁴⁴ with stirring helps the complex to associate more fully and allowing the complex to stand for some time leads to densification.

Ultracentrifugation effects a couple of morphological changes. First, much of the entrained water is expressed from the complex under the typical gravitational forces

(>150,000 g). Second, loose particles of complex are forced together. Added salt helps to plasticize the complex, allowing these particles to fuse and form a tough, continuous matrix. Even with careful control of polyelectrolyte stoichiometry, CoPECs obtained by ultracentrifugation are usually nonstoichiometric, due to the known control of mixing order on PEC composition.⁴⁵⁻⁴⁷ CoPECs are porous with pores containing water, ions and polyelectrolyte (Figure 4).

The extent of porosity depends how the CoPECs are prepared. It was found that equimolar PAH/PAA CoPECs, formed and stored in 2.5M NaCl, show pores with a typical diameter on the order of 20 - 40 μm.⁴⁹ These are roughly the same size as PEMU microcapsules, which exhibit excess osmotic pressure inside the capsule due to excess polyelectrolyte⁵⁰ as is also thought to be the case with strongly swollen, hydrated polylysine/hyaluronic acid multilayers.⁵¹ When these PAH/PAA CoPECs were rinsed for several hours in aqueous solutions of lower NaCl concentration the pore size increased as a result of the osmotic pressure generated by excess polyelectrolyte within the pores. For salt concentrations lower than 1.0M a second population of much smaller pores, typically a few μm in diameter, appeared (Figure 5).

Figure 5. Influence of the salt concentration on the microstructure of ultracentrifuged complexes prepared at 2.5 M NaCl, pH 7.4 having a 0.1M PAA to 0.1M PAH ratio of 1:1. Shown are fluorescent microscopy images (λ_{ex} 488 nm) of slices of complexes conditioned in solutions at pH 7.4 having different concentrations of NaCl. Scale bars are 100 μm . Reproduced with permission from ref⁴⁹

When the centrifugation speed was reduced during CoPEC formation, the size of the large pores increased.⁴⁹ Before centrifugation, the solution of polyelectrolyte complexes contained large blobs of complexes but also very large voids with typical sizes up to 200 μm . It is reasonable to assume that the large pores reflect the polymer distribution in the initial, as-precipitated, polyelectrolyte complex. When the CoPEC is exposed to aqueous solutions of salt at concentrations other than those at which the complex was formed, the equilibrium between the ions in solution and the ions on the CoPEC changes. When the salt concentration of the medium surrounding the CoPEC is decreased, ions diffuse out of the CoPEC. If the CoPEC is equimolar, this leads to a shrinking of the complex matrix and water loss due to enhanced interaction between the charges of the two polyelectrolytes. For PAH/PSS CoPECs, this shrinkage can lead both to an increase of the large pores and the formation of small pores. In the case of PDADMA/PSS a similar behavior was observed, yet only large pores were present. When the CoPEC is initially not exactly equimolar as it was the case for the first studies on PDADMA/PSS where it contained an excess of PSS,³⁵ it is found that the pores also contained free PSS chains.

For CoPECs made from pH-sensitive polyelectrolytes, such as PAH/PAA, solution pH during, and after, compaction controls the positive to negative charge ratio. When these CoPECs are prepared at pH 7.4 in 2.5 M NaCl and conditioned at 0.15M NaCl at different pHs, one observes that the sizes (both the large and smaller pores) of the different pores shrink as the pH departs from 7.4 (increasing or decreasing).

For non-equimolar CoPECs obtained for example by slow addition of one polyelectrolyte into the other polyelectrolyte solution at 2.5 M NaCl and conditioned at 0.15M NaCl, the porosity also decreases when the PAH/PAA ratio decreases. The material appears almost homogeneous (absence of pores) for a PAH/PAA ratio of 0.6. This can be explained by the fact that in this case the polyelectrolyte complex is highly swollen and

contains large amounts of counterions with their hydration water in order to neutralize the charges originating from the polyelectrolyte in excess.

Composition of complexes

The properties (morphology and mechanical properties) of CoPECs formed by ultracentrifugation or extrusion of polyelectrolyte complexes depend strongly on the composition of these complexes. Although PECs are presumed to be equimolar at equilibrium (as many negative charges as positive ones),² equilibrium can be difficult to reach because the diffusion of the polyelectrolyte chains in complexes is much slower than in solution. Diffusion rates increase with the salt concentration and temperature. As a consequence of these kinetic limitations, the mixing order of the polyelectrolytes during complex formation influences their composition.⁴⁵⁻⁴⁷ For each polyanion/polycation system relevant experimental variables should be probed to establish their effect on stoichiometry.

Figure 6. Influence of the speed and order of addition of the polyelectrolyte solutions on the PAH/PAA ratio in PECs at 1 M (▲ PAA into PAH, ▼ PAH into PAA, ◆ simultaneous addition) and 2.5 M NaCl (□ PAA into PAH, ◇ PAH into PAA, ○ simultaneous addition). Reproduced with permission from ref⁴⁹

Extensive studies on mixing rate and order were performed on the PAH/PAA system.⁴⁹ As seen in Figure 6, the PAH/PAA ratio in the CoPEC depends on the mixing order and results in a deficit of polyelectrolyte poured into the reaction mixture. The departure from equimolarity decreases by increasing the speed of addition of the polyelectrolytes. Equimolarity is also approached by decreasing the concentration of the polyelectrolyte solutions. Equimolarity of the CoPEC is obtained when both polyelectrolytes are poured together in a beaker, as shown in Figure 6. In the case of PDADMA/PSS one

observes an excess of PSS when PSS is added into a solution of PDADMA.^{35, 48} These results show that it is difficult to predict the composition of a CoPEC of an unknown system.

Using PAA and PAH solutions of same concentration but at different pHs also changes the CoPEC composition.⁴⁹ An increase of the PAA content is observed below pH 4.5 when the charge of PAA decreases and an excess of PAH is measured above pH 8. Good agreement was obtained with the predicted ratio of PAA to PAH assuming intrinsic compensation and pK_a values of 4.0 and 11.0 respectively, which are close to pK_a values determined independently through potentiometric titration of PAH/PAA complexes.⁴⁹ Interestingly, it was found that the NaCl concentration had no major influence on the CoPEC composition (same composition at 1M and 2.5M NaCl), showing that the interactions between ion pairs of the two polyelectrolytes are stronger than the interactions between the polyelectrolyte charges and the salt ions.

Compaction by extrusion

Ultracentrifugation leads to macroporous, yet rugged, samples of compacted complex. These pores or voids are assumed to be derived from the original entrained water in the as-precipitated complex, which has a cottage cheese-like morphology, as seen in Figure 7A. The mechanism by which the volume within these large voids is redistributed into smaller ones, as shown in Figures 4 and 5, is not fully understood..

To eliminate porosity from PDADMA/PSS complexes extrusion through a small laboratory extruder was attempted.³⁶ As with the ultracentrifuging compaction techniques, it was found that plasticizing the starting complex with salt water helped to fuse the materials and render them flowable under the high shear forces in the extruder. These shear forces proved highly effective at removing almost all the pores in the complex. As seen in Figure 7, the diffuse, as-precipitated complex was transformed into a dense macroscopic CoPEC in one pass through the extruder. The region of the 1 mm diameter rod in Figure 7 nearest the surface, which experienced the greatest shear, was essentially free of pores. Using specific gravity measurements the pore volume was determined to be less than 1 vol%. For most extrusions, a combination of higher temperature (80-90 °C) and salt concentration (on the order of 1M) was found to be optimum, although it is possible to extrude PEC that is fully hydrated but has no salt.⁵²

The presence of water during extrusion is essential. PEC that was even slightly dehydrated below the equilibrium water content (about 10 water molecules per PDADMA/PSS ion pair³⁶) proved impossible to extrude. Dry PEC is brittle and impossible to process, as noted for many decades.^{4, 6, 53}

Figure 7. Optical autofluorescence microscopy images of 10 μm thick slices of PEC precipitated in 0.25 M NaCl, starting PEC (A) and extruded (B), and soaked in DI water. 450-490 nm excitation and 500-550 nm emission. All extruded PECs were initially precipitated from 0.125M PDADMAC and 0.125 M PSS in 0.25 M NaCl. Scale bar: 100 μm . Reproduced with permission from ref³⁶

The success of extrusion provided evidence that ion pair crosslinks slip, as permanently crosslinked materials are not good candidates for extrusion (if crosslinking is to be introduced into, say, a thermoplastic it is usually done during (reactive extrusion)⁵⁴⁻⁵⁶ or after the extrusion).

Figure 8. Stoichiometric complexes of PDADMA/PSS extruded, while doped with 1M NaCl solutions, into various shapes, (A) tape; (B) rod; (C&D) tube. The materials in the micrograph are dried. Scale bar 0.5 mm. Reproduced with permission from ref³⁶

The PEC could be extruded on a continuous basis to yield tape, wire, rods and tubes that adopt the dimensions of the extruder exit nozzle/head. The dense materials seen in Figure 8 were large enough for “standard” polymer mechanical characterization methods (rheology, tensile testing etc.).

Macroscopic properties

Polyelectrolyte complexes are amorphous. Neutron scattering of deuterated PSS within PDADMA/PSS complexes showed the polyelectrolyte chains to be rather compact Gaussian coils.⁵⁷ Unlike most mixtures of neutral polymers, ion pairing interactions between polyelectrolyte chains maintain complete mixing at a molecular level.⁵⁸⁻⁶¹ In addition, these ion pairs act like physical crosslinks/entanglements which appear to be static for rapid deformations (short times)¹² and labile for slow deformations (i.e. viscoelastic).⁶²

The bulk mechanical properties of CoPECs are strongly influenced by porosity, and even more strongly by hydration. Water is a highly efficient plasticizer for polyelectrolyte complexes.^{6, 63} Because dry PECs are brittle it is more convenient to measure mechanical and other properties of PECs when they are full hydrated by water, which is accomplished by simply maintaining the PEC in contact with aqueous solution. Characterizing PECs while they are in contact with aqueous solution is, in any case, appropriate, as many of the proposed bio-interface, biomaterials and membrane applications require the material to be immersed in water. Figure 9 shows how the modulus of a thin film of PEC (a PDADMA/PSS multilayer) is strongly influenced by water content. In this case, the equilibrium water content of the PEC was controlled by the addition of increasing concentrations of polyethylene glycol osmotic stressing agent. The exponent of the fit of modulus to water/polymer ratio, known as the “plasticizing efficiency,” is 117, which is high.⁶³

Figure 9. Equilibrium elastic modulus of (PDADMA/PSS)₁₅ PEMUs *versus* mole ratio of water to polymer matrix of the PEMU. The red line is a fit to $y = 4200 \times e^{-117x} + 17$. Reproduced with permission from ref⁶³

Due to their extensive porosity, CoPECs compacted by ultracentrifugation, ucPECs, are more deformable than those compacted by extrusion, exPECs. Both PEC morphologies depend on the salt concentration of the solution in which material is bathed. For example, 1:1 PAH/PAA ucPECs prepared from 0.1M PAH and 0.1M PAA in 2.5 M NaCl and then conditioned in 0.15 M NaCl show excellent stretchability with an ultimate tensile strain of about 500% (see Figure 10) and an ultimate tensile stress of 3.2 MPa.⁴⁹

Figure 10. Stretching of an ultracentrifuged PAH/PAA complex with a PAA to PAH ratio of 1:1 conditioned in 0.15 M NaCl, pH 7.4. From top to bottom: before stretching; stretching to 4.5 times the initial length; after 10 min the force is removed and the complex goes nearly entirely back to its original length (here, after letting it relax for 5 min). Original length of complex is 2 cm.

When brought back to the non-stretched state, the material recovers its initial dimensions after a few minutes. The Young's modulus of this ucPEC was around 300 kPa and its toughness about 1.7 MJ m^{-3} , a value about half that of PDADMA/PSSC³⁶ and comparable to that of poly(methyl methacrylate) which is about 2 MJ m^{-3} . Frequency dependent dynamic shear stress measurements show that at low frequency the PAH/PAA ucPECs possess a gel-like behavior associated with the physical crosslinks formed by ion pairs. At intermediate frequencies a marked viscoelastic behavior is observed.⁴⁹ This behavior is associated with the relaxation modes of the polymer chains constituting the gel whereas at high frequency a progressive freezing of intra-molecular relaxations is observed. As one shifts away from stoichiometry the PAH/PAA CoPECs become softer (decrease of G' and G'' at a given frequency when shifting away from the 1:1 PAA:PAH ratio, see Figure 11). At 1:1 stoichiometry the ionic crosslink density of CoPECs is highest and the water content lowest.

Figure 11. G and $\tan(\delta)$ at (a) 0.01 and (b) 1 Hz depending on the PAA to PAH ratio in the complex at 0.15 M NaCl, pH 7.4. Reproduced with permission from ref⁴⁹

For the same pair of polyelectrolytes, exPECS have a higher modulus than ucPECS. The water content in exPECs is also much lower because the pores have almost been eliminated. Figure 12 shows how water content in a PDADMA/PSS exPEC, increases with increasing salt concentration. Doping by salt, as depicted quantitatively in Figure 3, brings more water into the PEC. The water content does not depend on the number of times the PEC has been extruded. This doping and water content makes the PEC significantly softer (Figure 12). Also shown on Figure 12 are data for the modulus of a PDADMA/PSS multilayer (nominally the same material as PDADMA/PSS exPEC but only a few μm thick)) as a function of salt. The apparent modulus for the multilayer is higher because it was measured on a much faster timescale (70 ms).¹² The modulus in these saloplastic materials is under reversible control of salt concentration. The time required for pieces of solid PEC to reach doping equilibrium can be estimated from the diffusion data in Table 1. Porous PEC equilibrates faster.

Figure 12. (Left) Water content vs. salt concentration for PDADMA/PSS exPECs after rehydration for 2 days in salt solution. The data is shown for PEC extruded (●), double extruded (◇), and triple extruded (▲). (Right) Equilibrium modulus in different salt solutions for PDADMA/PSS samples extruded (●), double extruded (◇), and triple extruded (▲). The points (×) are the modulus for a PEMU of PDADMA/PSS recorded by Jaber et al.¹² Reproduced with permission from ref³⁶

Access to the dense, pore-free exPEC morphology means that the properties of the polyelectrolyte complex itself may be explored. As with other measurements, the PEC remains in contact with aqueous solution of defined ionic strength to maintain an equilibrium content of water and counterions (doping level). Rheometry experiments of dense stacks of exPEC tapes revealed some interesting mechanical properties.⁶⁴

First, was the demonstration of a clear transition in the hydrated PECs that had all the hallmarks of a glass transition. While calorimetry showed only a weak signature for this

T_g , dynamic mechanical analysis clearly revealed the characteristic T_g signatures for storage (G') and loss (G'') modulus and $\tan\delta$ (Figure 13).⁶⁴

Figure 13. Variable (increasing and decreasing) temperature dynamic mechanical analysis for PDADMA/PSS exPEC in equilibrium with salt solutions at 0.1 Hz. Storage (G') (diamonds) and loss (G'') (squares) modulus, and $\tan\delta$ (triangles) show a clear glass transition for increasing (solid symbols) and decreasing (open symbols) temperature. Increasing [NaCl] from 0.1M (A) to 0.5M (B) to 1.0M (C) decreases T_g , as expected for a plasticizer. Reproduced with permission from ref⁶⁴

The transitions were reversible on the time scale of the experiment (Figure 13, see forward and reverse temperature scans).

Figure 13 also illustrates the plasticizing effect of salt addition by shifting the T_g to lower temperatures. This confirms the interplay between salt concentration and temperature suggested by Tan et al.⁶⁵ and time and salt concentration put forward by Spruijt et al.⁶² Thus, building on the well-known (but still not fully understood) time/temperature superposition principles for polymer mechanical properties,⁶⁶ there appears to be a time/temperature/salt doping equivalence for PECs. The data obtained for PDADMA/PSS exPECs suggested an empirical relationship between the three variables: $T_g = 38 + 2.3\ln f - 20[\text{NaCl}]^{6/5}$. Figure 14 shows the glass transition temperature as a function of frequency, f , for different salt concentrations and the fit to this equation. As with uncharged amorphous polymers, the time/temperature/salt relationship holds best at temperatures near and above T_g

Figure 14. (left) T_g vs frequency for ExPECs soaked in solutions of different [NaCl]: 0.1 M (◆), 0.5 M (●) and 1.0 M (▲). Solid lines are fits to $T_g = 38 + 2.3 \ln f - 20[\text{NaCl}]^{6/5}$ to illustrate time/temperature/salt superposition. (right) Equivalence of salt doping, time and temperature in saloplastics. Reproduced with permission from reference 64.

For extrusion of PDADMA/PSS, a lack of salt doping could be compensated by higher temperatures as long as the complex remained fully hydrated. Due to the high (maximum) density of ionic crosslinks, polymer chains were strained and highly aligned during the extrusion of undoped PEC.⁵² Aligned chains were quasi-persistent at room temperature but could be relaxed by exposure to either hot water or salt – another demonstration of temperature/salt equivalence. On a macroscopic scale, fiber samples shrunk in length by up to 80%. Samples could be restretched and dried, holding their new shape until they were relaxed again (shape memory). Chain orientation also led to PECs that were considerably tougher than anisotropic materials when dry.⁵² Figure 15 shows how fibers of oriented saloplastic can be tied in knots – previously unthinkable for dry PEC.

Figure 15. Photos of dry strained PEC fiber in a knot (b) and the maximum degree (~58°) the unstrained sample can be bent (c). The scale bars are 1 cm.

(Nano)composites

In principle, any material that can be mixed with the PEC before ultracentrifugation or extrusion will be dispersed into the compacted complex. The degree of dispersion will be determined, in part, by how well the material disperses in the starting PEC. Nanomaterials can be mixed with one, or both, of the polyelectrolytes prior to complexation. The first

demonstration of CoPEC nanocomposite formation was with iron oxide nanoparticles incorporated in PSS/PAH compacted by ultracentrifugation.⁴⁹ Because the resulting ucPECs were magnetic, chunks of them could be retrieved from a reaction mixture with a magnet.

Compacting PECs with added solids by ultracentrifugation is not ideal, since the high centrifugal fields will concentrate denser additives towards the bottom of the sample. Superparamagnetic iron oxide nanoparticles, IONs, 12 nm in diameter were coprecipitated with PDADMA/PSS PECs and extruded into fibers.⁶⁷ The bulk exPEC retained the magnetic properties of the ION additive, which permitted remote heating of the exPEC by radiofrequency fields. Figure 16 shows the superparamagnetic response from the IONs within PECs and the temperature increase of PEC wrapped around a thermometer and irradiated in the coils of an rf generator.

Figure 16. (left) M-H curve for the ION-PEC fibers based on the weight of the composite. 0.5 wt% (—), 2.1% (---) and 4.5% (-·-), at 300K. (right) Temperature versus time for ION-PEC fibers containing (a) 0.0%; (b) 0.5%; (c) 2.1%; and (d) 4.5 wt% ION exposed to 1kW RF field. Reproduced with permission from ref⁶⁷

Biomaterials

The interface between natural systems and PECs in various formats, mainly in the ultrathin “multilayer” morphology, has been of intense interest. Hydrated PECs are rugged, supple, and offer the possibility of tailoring the surface via the composition of the “top” or last-added layer. A key question refers to the biocompatibility of PECs. This question has been partially but widely answered by many studies showing cells adhere, grow, and proliferate on multilayers, or dried films of PEC nanoparticles,³² showing these materials are not cytotoxic.⁶⁸⁻⁷³ There are understandable concerns regarding possible toxicity of a material that contains polycations, which are known to be individually cytotoxic.⁷⁴ In fact, pairing a polycation with a polyanion substantially reduces, or eliminates toxicity of the former,⁷⁵ as long as the positive surface charge density is not too great.⁷⁶

Much has been reported on the effect of substrate stiffness on cell migration and even stem cell differentiation.^{72, 77-79} Since PECs as multilayers are simple to deposit on almost any surface, many studies have looked into cell culture on multilayers. The fact that multilayers are ultrathin and relatively smooth facilitates optical microscopy on planar surfaces. Although studies of cell culture on compacted PECs are not yet available, it is reasonable to expect results similar to those on multilayers. The modulus range of hydrated PECs is in the 1kPa-10 MPa range, which is comparable to many living tissues.

The intervertebral disk (IVD), a fibrocartilaginous cushion between vertebrae, consists of an outer annulus fibrosus and an inner nucleus pulposus.⁸⁰ To illustrate a possible biomedical application of ucPECs as IVD-mimics, samples of ucPEC from poly(methacrylic acid) PMAA and PDADMA were prepared.³⁵ When in contact with pH 7 buffered saline (0.15M NaCl), the modulus of these porous CoPECs fell in the 1-10 kPa range. From 1 to 100 rad s⁻¹, the values of $|G^*|$ are close to those determined by Iatridis et al.⁸⁰ for the nucleus pulposus in the human intervertebral disc. $|G^*|$ spans the range 3 to 20 kPa. The loss angle is of the same magnitude as the IVD, but increases to higher values at lower frequencies for the PMAA/PDADMA ucPEC. The IVD is more structured than a PEC, but the materials share certain features, such as an excess of negative polyelectrolyte that maintains a high population of sodium ions, which ensures the material remains elastic by maintaining hydration.⁸¹

For “active” biomaterials applications, CoPECs can be functionalized with enzymes which remain viable despite the potential for multiple interaction points between natural (protein) and synthetic polyelectrolytes.⁸² As with nanomaterials, bioactive composites are made by including the enzyme in one of the polyelectrolyte solutions. The loading of enzyme into the final CoPEC depends on how it is processed. The optimal way of mixing the enzyme into the polyelectrolyte depends on the isoelectric point (IP) of the enzyme. When preparing a ucPEC of PAH/PAA containing alkaline phosphatase (ALP), for example, which has an IP of 4.5, it is advantageous to first mix the enzyme with the polycation (PAH) when working at pH 7 (above the enzyme’s IP) and then to add to this solution to the polyanion (PAA in this example).⁸³ Because ALP is net negatively charged under these conditions, it interacts with the polycation more strongly than it does with the polyanion.

In the case of ALP/PAH/PAA ucPECs, no enzyme leaked out of the ucPEC over time periods exceeding 5 days and the ucPEC showed strong enzymatic activity.⁸³ This CoPEC, in the form of a powder to increase the surface area/volume ratio, showed a higher enzymatic activity based on the mass of enzyme compared to homogeneous solutions of enzyme. This demonstrates that the enzyme activity is not only preserved but actually enhanced when it is embedded into the PAH/PAA ucPEC. The enhanced activity was explained by the strong interaction of phosphate groups, one product of the dephosphorylation reaction catalyzed by ALP, with PAH, reducing the inhibition of ALP by phosphate, which is sequestered by the PAH of the ucPEC as they are produced by the enzyme.

Another result with significant practical importance is that the host ucPEC enhances the thermal stability of the guest enzyme. Figure 17 compares the temperature response of ALP in buffered solution and in ucPEC.⁸³ At 60 °C, the activity of the enzyme in solution disappears within about 5 h but when immobilized in PAH/PAA CoPEC, made from 0.1M PAH and 0.1M PAA, it retains 80% of its initial activity over the same period. Higher temperatures induce higher activity, as seen in Figure 17, up to a point, but past about 55 °C the enzyme rapidly denatures if it is in solution, while it remains robust in the CoPEC. Such a result is consistent with previously reported behavior of proteins in polyelectrolyte multilayers.⁸⁴ It is assumed that the polyelectrolytes wrap the protein molecules which reduces the internal mobility and stabilizes their structure.

Figure 17. (left) Temperature resistance of alkaline phosphatase in a PAH/PAA CoPEC ($\blacktriangle$) and in solution ($\square$). Shown are the relative activities (activity at 37 °C; relative to a sample that was not heated) after different time intervals at 60 °C. (right) Influence of the temperature on the relative activity of alkaline phosphatase in the CoPEC ($\blacktriangle$) and in solution ($\square$). The activity at 35 °C was set to 1. Reproduced with permission from ref⁸³

Adhesion and self healing

As already discussed, when the solution salt concentration increases, salt penetrates into the CoPECs which increases the mobility of polyelectrolyte chains.^{22, 44, 85-86} This leads to interesting mending and self-healing properties.⁸⁷ For example, when an ucPEC is cut in two pieces and the two pieces are brought together they adhere right away to each other (mending) if the salt concentration is sufficiently high. If they are left together for a few hours at high salt concentration (for example, 3 hours in 2.5 M NaCl for a 1:1 PAH/PAA CoPEC), the material recovers its initial mechanical properties. These properties are recovered gradually - after a few minutes of contact up to 50% of the ultimate tensile strain is recovered (Figure 18).⁸⁷

Figure 18. Cutting, mending, and stretching of PAH/PAA CoPECs (all at room temperature): CoPECs were made compacting PAH/PAA precipitates by ultracentrifugation in the presence of salt. CoPECs were compressed into sheets and cut into shapes. The PAH in the left-hand piece of complex is labeled with rhodamine. For mending/adhesion they were contacted for a set time (here 15 min) in a specific salt concentration (here 2.5 M NaCl). Reprinted with permission from ref⁸⁷

Similarly, when a cut is made halfway through a PAH/PAA CoPEC, as shown in Figure 19, the material exhibits almost complete “self healing” after exposure to 1.0 M NaCl for several hours. The elasticity of the material keeps the cut faces in contact during healing

and no pressure or other manipulation is required. Both mending (adhesion) and healing are faster and more complete when the salt concentration of the solution into which they are immersed is higher.

Figure 19. Autonomous self-healing and elasticity of a strip of PAH/PAA ucPEC in 1 M NaCl. From top to bottom: the strip of CoPEC was conditioned in 1 M NaCl and cut halfway through while being stretched; when the force was removed the strip returned to its initial shape and after leaving the strip for 2 h in 1 M NaCl the cut had already partly disappeared; after a further 8 h the cut had healed nearly completely as shown by stretching. Reprinted with permission from ref⁸⁷

Future prospects

CoPECs represent a new class of materials with versatile properties. Due to the great variety of polyelectrolytes, both natural and synthetic, potential compositions are vast. Also, it is straightforward to add other particles and molecules to the PECs as they are being prepared and before they are compacted. Many of the most promising applications are at the bio-interface and include substrates and scaffolds for cell growth, biomechanical materials, (bio)catalysis supports and drug delivery vehicles. As a first direction it is clear that components for CoPECs must be broadened into biopolyelectrolytes, such as proteins (e.g. collagen and fibronectin), hyaluronic acid, chondroitin sulfate, cellulose and chitosan. "BioCoPECs" should, *a priori*, be ideal candidates to create scaffolds for tissue engineering. Indeed, they are porous with a porosity that can be tuned and they can be functionalized by the addition of bioactive macromolecules, growth factors or adhesion proteins. Their mechanical properties can be controlled and they can be degradable by the use of degradable biomacromolecules. These properties should allow the design of new materials

that can be processed on a large scale, whose interactions with cells can be tuned and, most importantly, whose vascularization can be encouraged.

CoPECs benefit from the large body of work and inspiration already invested in multilayers. They allow ideas originating in the field of ultrathin multilayers to be brought into three dimensions. CoPECs can be endowed with electrical conductivity using conducting polyelectrolytes or conducting polyelectrolyte complexes (e.g. PEDOT/PSS). Alternatively, conducting nanowires made from metal, semiconductors or carbon nanotubes could also be incorporated.

Much fundamental work is required, such as defining the construction rules for which polyelectrolytes under which conditions are best suited to CoPEC synthesis. For example, strongly-interacting polyelectrolytes may require higher salt concentrations or temperatures to process into CoPECs. The interplay of comonomers and blends of polyelectrolytes is not known. Transport properties of ions and neutral molecules must be determined to understand CoPECs used as membranes for dialysis, ultrafiltration, battery separators or fuel cell membranes. All these potential applications and fundamental questions regarding this intriguing form of soft matter are sure to stimulate further work in the area.

Acknowledgements

This work was supported by funding from the U.S. National Science Foundation, the Gutenberg Chair program of the Université de Strasbourg and the region d'Alsace, and icFRC (project PSC-005).

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