

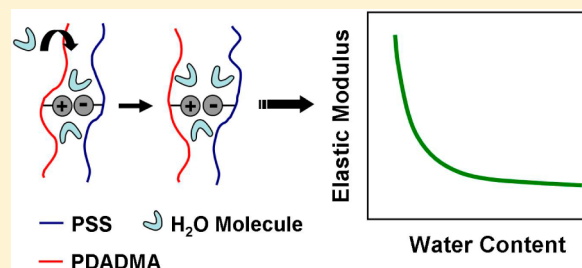
Mechanical Properties of Osmotically Stressed Polyelectrolyte Complexes and Multilayers: Water as a Plasticizer

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S Supporting Information

ABSTRACT: Compacted, macroporous complexes of poly(styrenesulfonate) and poly(diallyldimethylammonium) were dehydrated under defined osmotic stress using poly(ethylene glycol), PEG. A strong mechanical response to dehydration was observed. At the lowest osmotic stress applied, macropores within the complex were compacted, and the material became transparent. With additional osmotic stress, the decrease in water content with increasing stress slowed considerably, but the complex became much stiffer, the equilibrium modulus reaching several hundred MPa. Concurrently, the complexes became more brittle. Multilayers of the same polyelectrolytes reached equilibrium hydration levels much faster and also increased significantly in modulus. Using an empirical fit, the plasticizing efficiency of water was shown to be exceptionally strong.



INTRODUCTION

Polyelectrolyte complexes (PECs) are a versatile category of materials recognized for their ease of preparation and wide range of properties and applications.^{1,2} They are formed when oppositely charged polyelectrolytes interact in solution or at a surface, a process driven by ion-pairing and entropic release of water and counterions.^{1,3} PECs can be made with different morphologies, ranging from nanoparticles/films to macroscopic gels, depending on the method and conditions of preparation.⁴ PECs have been extensively studied as thin films prepared via the layer-by-layer technique.⁵ Complexes may also be prepared simply by mixing solutions of oppositely charged polyelectrolytes, yielding quasisoluble^{6,7} or solution-precipitated complexes⁸ or coacervates,^{9,10} where the final morphology depends on parameters such as relative polymer molecular weights, chemical composition, concentration, and ionic strength of the solutions.^{8,11,12} Other factors such as stoichiometry and order of mixing are also known to affect the form and composition of resulting precipitates.^{13,14}

The mechanical properties of PECs are strongly correlated with their composition and porosity. For example, poly(diallyldimethylammonium)/poly(styrenesulfonate) (PDADMA/PSS) complexes precipitated from high ionic strength solutions, then compacted by ultracentrifugation, were non-stoichiometric and porous with high water content, resulting in kPa stiffness and significant viscous response in tensile testing.¹⁵ In contrast, the same complexes prepared in low ionic strength and processed by extrusion were stoichiometric, homogeneous, compact, and less hydrated with MPa stiffness, similar to thin films of the same polyelectrolytes made by multilayering.¹⁶

Polyelectrolyte complexes and multilayers are viscoelastic in nature.^{17–19} In the earliest work on PECs it was observed that water is a critical component in plasticizing the mechanical

properties of PECs, which were reported to be brittle when dry and “tough and leathery” when wet or fully hydrated.^{1,2} Water has been studied as a potent plasticizer for many hydrophilic polymer systems, converting them from glassy to rubbery.^{20–22} Increasing the water content of a polymer was shown to decrease glass-transition temperatures, elastic modulus, and tensile strength and increase percent elongation—common characteristics of a plasticizer. Closer investigation of the mechanism of water plasticization showed that water plasticizes polymers not only by disrupting chain interactions (lubrication/gel theory) but also by creating free volume in the polymer network, enhancing chain mobility and softening the material, equivalent to increasing temperature.^{23–27}

In between the two extremes of “wet” and “dry” little is known concerning the control that water has over PEC modulus. It is possible to control the water content of a PEC by regulating the humidity of the atmosphere to which it is exposed. We measured the water content of PDADMA/PSS multilayers using such an approach²⁸ but did not track mechanical properties. Nolte et al.²⁹ followed the modulus of multilayers as a function of relative humidity but did not determine water content. Because of recent developments in producing large pieces of PEC by compaction^{15,30} or extrusion,¹⁶ we are now able to perform classical mechanical measurements on larger samples. In the present work, the chemical potential of the water was defined not by vapor pressure but by the addition of osmotic stressing agents to the solution in which a PEC was bathed. The osmotic pressure of

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the solution was precisely controlled by poly(ethylene glycol), which was excluded from the PEC.

The doping of PEC by salt weakens the physical network by breaking the ion pairing (cross-linking) between oppositely charged monomers.³¹ This effect was termed salt-induced plasticization and the complexes saloplastics.^{30,32} Saloplasticity permits the compaction of loose PEC precipitates into tough macroporous objects if sufficient salt is added. Because of the plasticity response of PECs immersed in solution, the salt concentration, or activity, was carefully controlled in the present work. Here, we show that the modulus of PEC is supremely sensitive to even small changes in the water content.

MATERIALS AND METHODS

Poly(4-styrenesulfonic acid) (PSS, molar mass $7.5 \times 10^4 \text{ g mol}^{-1}$, $M_w/M_n = 1.4$) and poly(diallyldimethylammonium chloride) (PDADMAC, molar mass 40×10^4 – $50 \times 10^4 \text{ g mol}^{-1}$, $M_w/M_n = 2.09$) were used as received from Sigma-Aldrich. Poly(ethylene glycol) (PEG, molar mass = 8000 g mol^{-1}) was purchased from Alfa Aesar. NaCl (ACS grade) from Aldrich was used to adjust the ionic strength of the solutions. All solutions were prepared with deionized water (Barnstead, E-pure).

Complexes were precipitated by mixing equimolar solutions of the oppositely charged polyelectrolytes (0.5 M based on the monomer unit). Both polyelectrolyte solutions were prepared in 2.5 M NaCl and the pH adjusted to 7 using 1 M NaOH. 20 mL of the PSS solution was added to 20 mL of PDADMAC solution while stirring for 10 min, forming a hydrated blob of complexed polyelectrolytes. The precipitates were centrifuged using a Beckman Coulter Optima XL-100 K ultracentrifuge with a 70Ti rotor (tube angle 23°) in polycarbonate thick wall centrifuge tubes at 190000g for 4 h at 25°C .

Compacted complexes were cut into rectangular pieces and soaked in either 0.1 or 0.5 M NaCl, left to equilibrate for 1 week, and then transferred to PEG solutions. The activity of NaCl in PEG solutions was maintained between 0.01 and 0.5. Since PEG is known to change the activity of water and solutes in solution,^{33,34} the activity of NaCl in each PEG solution was determined with vapor pressure osmometry (VPO) and the amount of NaCl required to maintain activities of either 0.1 or 0.5 determined³⁵ (Supporting Information describes the procedure in more detail). The following PEG solutions were prepared by weight percentage (wt %) in water: PEG 0, 2, 5, 10, 15, 20, 25, 30, 35, and 40 wt %. To convert from concentration to activity, each PEG solution was prepared with the following NaCl concentrations: 0.05, 0.1, 0.15, 0.2, 0.25, and 0.5 M. Osmolalities of these solutions were measured using a Vapro 5520 VPO (Wescor, Inc.) at room temperature.

For water content measurements, cubes of salt-treated complexes about 0.7 cm on a side were weighed in the swollen state, then immersed in PEG–salt solutions, and left until they became completely transparent, which indicated their maximum dehydration under PEG osmotic stress. Samples were then weighed, dried in a vacuum oven at 100°C for 4 days, cooled under vacuum, and then weighed again. All measurements were done in duplicate, and the weight percentage of water in the complex was determined at each PEG–salt concentration.

To study the kinetics of dehydration of complexes in different PEG wt %, PEC pieces of equal mass ($\sim 0.5 \text{ g}$) were put in PEG 20, 30, and 50% (with NaCl activity 0.1), and the change in mass was recorded versus time to constant mass.

For tensile testing, centrifuged complexes were cut into slabs of dimension about $10 \times 4 \times 2 \text{ mm}$ and equilibrated in 0.1 or 0.5 NaCl. The samples were then put in a PEG 25% solution with NaCl activity of either 0.1 or 0.5 and left to dehydrate. For the single-sample experiment, the samples were tested in PEG 25% and then transferred to PEG 30%, where they were allowed to dehydrate for 3 days before they were tested in the higher PEG%. The same samples were allowed to dehydrate sequentially in the following PEG solutions: PEG 25, 30, 35, 40, 45, and 50%, all containing salt of activity either 0.1 or 0.5. For the multiple-sample experiment, all samples were first allowed to

dehydrate in PEG 25% and then transferred to one of the PEG solutions—30, 35, 40, 45, and 50%—and allowed to dehydrate for 1 week before they were mechanically tested.

The tensile testing machine (Thümler GmbH, Model: TH 2730) was equipped with a 100 N load cell for stress relaxation studies. Samples mounted on two plastic grips were stretched at a rate of 10 mm min^{-1} to a strain of less than 1%, and the decrease in stress was monitored for 150 s. Samples were maintained wet by the solutions during testing. In all experiments the temperature was $23 \pm 1^\circ\text{C}$. The equilibrium modulus was determined as the ratio of stress at equilibrium relaxation to the applied strain.

To check for crack formation, PEG-treated samples were imaged before and after mechanical testing using a Nikon SMZ 100 microscope equipped with DS-Ri 1 camera and NIS-Elements BR 3.1 imaging software. To monitor the porosity, $20 \mu\text{m}$ thick slices of complexes treated in PEG 25% were cut using a cryostat microtome (Leica CM 1850) and imaged at 100 \times magnification in epifluorescence mode using a Nikon Eclipse Ti inverted microscope equipped with Photometrics CoolSNAP HQ² camera and NIS-Elements AR 3.0 imaging software.

FTIR data on complexes in PEG were collected using a nitrogen-purged FTIR (Nicolet Nexus 470 with a DTGS detector) spectrometer. The resolution was 4 cm^{-1} , and 256 scans were averaged. PECs in 0.1 M NaCl and in PEG 50% with 0.1 NaCl activity were dried under vacuum at 100°C for 24 h. The dried complexes were crushed into fine powder, mixed with KBr, and pressed into pellets for FTIR.

For the studies on multilayers, PDADMA/PSS PEMUs were built using a robotic platform (StratoSequence V, nanoStrata Inc.) on 1 in. diameter, single side polished silicon wafers. The wafers were first cleaned in “piranha” (70% H_2SO_4 and 30% H_2O_2) for 20 min, then washed in deionized water, and dried with N_2 . *Caution: piranha is a strong oxidizer and should not be stored in closed containers.* The concentrations of PSS and PDADMAC solutions used for the buildup were 10 mM in 1.0 M NaCl concentration. A silicon wafer mounted on a shaft rotating at 300 rpm was alternated between polyelectrolyte solutions for 5 min with three 1 min water rinsing steps in between, until 30 layers (15 bilayers) were built. The PEMUs are denoted by (PDADMA/PSS)₁₅.

All multilayers were equilibrated in 10 mM NaCl for 18 h and tested for wet thickness and mechanical properties in salt using an atomic force microscope (AFM) and then transferred to PEG–salt solutions that varied between 5 and 50 in PEG wt % with salt activity of 0.01. The multilayers were left in PEG solutions for at least 24 h before they were tested again. (In PEG < 20%, samples were left for at least 3 days, measured, then left for extra 2 days, and measured again to make sure they had reached an equilibrium hydration state.)

The percentage of water remaining in the PEMUs after PEG dehydration was determined from the difference in film thickness. Film thickness in the wet state was measured using an MFP-3D AFM unit equipped with an ARC2 controller (Asylum Research Inc., Santa Barbara, CA) and Igor Pro software. Multi75AI (Budget Sensors) tips with a spring constant of $\sim 2 \text{ N m}^{-1}$ were used in ac mode for dry imaging and in contact mode for wet imaging. The cantilever was tuned to a frequency 10% lower than its resonance frequency. The films were scratched gently with tweezers, and a $90 \times 90 \mu\text{m}^2$ area around the scratch was scanned at a rate of 0.5 Hz to determine thickness. The percent change in thickness in wet and dry conditions reflects the water content (as percent volume) of the PEMUs in the different PEG solutions. Under ambient conditions (30% RH and rt), PDADMA/PSS PEMUs were found to contain 2.4 water molecules per ion pair.²⁸ Therefore, for accurate determination of water content in PEMUs, this amount of water was subtracted from the thickness at ambient conditions, t_a , to obtain the thickness of dry matrix (details on this calculation are provided in the Supporting Information).

The apparent modulus of PEMU thin films was measured via nanoindentation by AFM as described by Moussallem et al.³⁶ The same tips, with a spring constant of $\sim 2 \text{ N m}^{-1}$, were used in the force measurements. The optical lever sensitivity (OLP) of the tip was calibrated on a glass slide and the spring constant of the tip was

calibrated in air using the thermal fluctuation technique. The tip was immersed in solution (salt or PEG + salt), and the OLP was recalibrated. Force maps of 4×5 were performed on bare silicon wafer where the tip was recalibrated and then on the PEMU with a scan size of $20 \times 20 \mu\text{m}$. All force maps were performed *in situ* with the sample immersed in solution. The distance from the surface, scan rate and trigger point of the tip were set to $1 \mu\text{m}$, 0.5 Hz , and 1 V , respectively, for most of the mechanical studies. For the studies at different strain rates, the distance, scan rate, and trigger point were tuned to attain different tip velocities. All force curves were analyzed using a punch model (Supporting Information).

RESULTS AND DISCUSSION

Polyelectrolyte Complexes. Solution-precipitated complexes of PSS and PDADMA were compacted using (ultra)-centrifugal fields. As previously reported, equimolar solutions at relatively high polymer (0.5 M) and NaCl (2.5 M) concentrations yielded compact macroporous pieces of PEC, CoPECs.^{15,30} Although the solutions were stoichiometric, kinetic limitations to complexation provided CoPECs with a slight (14%) excess of PSS, most of it as free chains, contributing to the osmotic swelling of the closed-shell pores. The equilibrium modulus of CoPECs varied between ~ 10 and 500 kPa depending on the ionic strength of the medium. CoPECs behave as viscoelastic solids under uniaxial¹⁵ and shear deformation.³⁰ Their viscoelastic response was modeled using a three-element generalized Maxwell model yielding two major relaxation mechanisms, thought to be a water flow dependent mechanism from frictional drag of fluid in the pores (long-term relaxation), and a water flow-independent mechanism from energy dissipating interactions within the solid matrix (short-term relaxation).¹⁵ Since both of these are influenced strongly by porosity, we employed a PEG osmotic stressing agent to remove water and collapse the material.

Osmotic stress is the controlled removal of water from a sample under osmotic pressure applied by a solution of inert polymer. The work required to extract the water is equivalent to work needed to bring the molecules in solution closer causing their crystallization or precipitation.^{37,38} Osmotic stress has been widely applied, for example, measuring intermolecular forces in stacks of lipid bilayers³⁹ and arrays of biopolymers (DNA,⁴⁰ proteins,⁴¹ and polysaccharides⁴²), quantifying conformational changes in enzymes,⁴³ and measuring the changes in hydration accompanying reactions such as oxygen binding to hemoglobin.⁴⁴ The method has been also extended to study interactions in solutions of synthetic polymers⁴⁵ and colloids.⁴⁶

PEG is a commonly used osmotic stressor: it is neutral, inert, and highly water-soluble with a high second virial coefficient ($A_2 \sim 10^{-3} \text{ mL mol g}^{-2}$)^{47,48} and large hydrodynamic radius ($R_g = 32 \text{ \AA}$ for PEG 8000 at 20°C) in aqueous solution.⁴⁹ Each PEG monomer unit can bind two to three water molecules by hydrogen bonding. At high molecular weights, more of the less tightly bound water molecules are trapped within the chains resulting in more hydration.^{50,51} The large chain size of PEG makes it efficient at excluding other molecules in solution, giving it advantages over other osmotic stressing agents (such as sucrose and NaCl) as it does not interact directly with the molecules of interest.^{52,53}

PEG with MW 8000 was employed here, which is large enough to be excluded from the PEC but still to exert sufficiently high osmotic pressure (Figure S3). The osmotic pressure of PEG 8000 at room temperature and different wt % was measured by vapor pressure osmometry (Figure S4). It

exerted almost 3 orders of magnitude higher osmotic pressure than the free PSS chains in the pores,¹⁵ which makes it efficient in osmotically compacting CoPECs.

Figure 1 shows time lapse pictures of a piece of CoPEC that was soaked in water (Figure 1a) and then in PEG 40%.

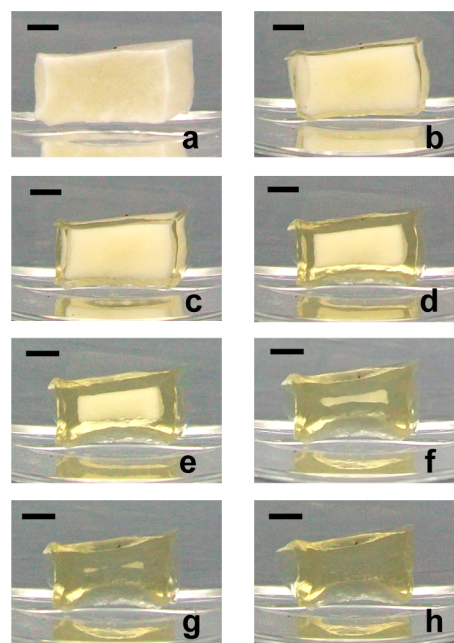


Figure 1. Pictures of PDADMA/PSS complexes soaked in PEG 40% for (a) 0 min, (b) 35 min, (c) 2 h, (d) 7 h, (e) 11 h, (f) 22 h 15 min, (g) 32 h 40 min, and (h) 50 h. Scale bar is 0.25 cm.

Dehydration and loss of porosity of the complex were evident from the formation of the thin transparent shell that grew radially inward with time, until the whole complex became transparent, indicating an equilibrium dehydration of the material. The change in optical properties of the complexes was related to the presence of pores.³⁰ The opacity observed at low salt concentrations resulted from scattering due to the difference in refractive index between bulk PEC and pore solution. The increasing transparency observed during PEG treatment was attributed to loss of pores as a result of PEC dehydration. Shrinkage of the entire sample was also evident with increasing time in PEG solution. The rate of CoPEC dehydration in PEG was faster with higher PEG wt % (kinetics are shown in Figures S5 and S6). Thus, by manipulating both concentration of, and time of immersion in, PEG solution, the shell-to-core dimensions in a piece of complex can be controlled, providing a means for controlling the properties of this composite material. It is worth mentioning that a core-shell morphology is a similar structure to the intervertebral disk, which is a composite of two cartilage domains that differ in their mechanical stiffness: a highly hydrated core (nucleus pulposus) of kPa range modulus, surrounded by stiff, less hydrated shell (MPa range) that helps maintaining the disk structure under deformation (annulus fibrosis).⁵⁴

For more quantitative studies, it was important to maintain a constant ionic strength (more precisely, ionic activity), as increasing ionic strength dopes PEC and plasticizes it. It was shown that, in PEG-salt mixtures, salt ions do not interact with PEG chains under aqueous conditions,⁴⁸ and it was concluded from a study on the molar conductance of NaCl in PEG-water

mixtures that ion–ion interactions of salt are not altered by PEG and that the decrease in molar conductance with increasing volume fraction of PEG could only be related to the “obstruction” of ion motion by the polymer chains.³³ Because PEG decreases water activity,³⁵ the activity of NaCl increases with the addition of PEG. To maintain constant NaCl activity, the activities of NaCl in different PEG wt % were estimated from the corresponding osmolalities determined by vapor pressure osmometry³⁵ and used to calculate the amount of salt required to create the desired ion activity in a given PEG wt % (Supporting Information, Figures S1 and S2).

The influence of NaCl doping in concert with PEG dehydration was verified by measuring the water content of CoPEC in increasing PEG wt % in NaCl of activity 0.1 or 0.5. Figure 2 shows decreasing water content with increasing osmotic stress.

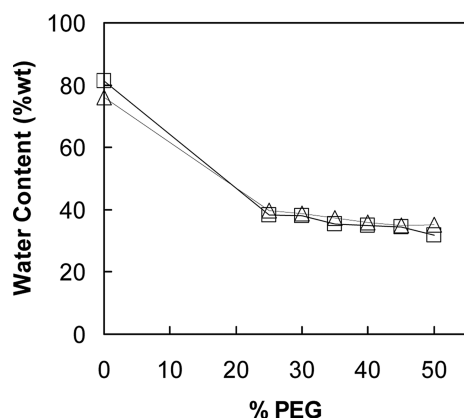


Figure 2. Weight percentage of water content in PEC samples with increasing PEG wt % solutions: (□) data for complexes pretreated in 0.1 M NaCl; (△) data for complexes pretreated in 0.5 M NaCl. PEG solutions contained 0.1 or 0.5 activity NaCl. The error bars are smaller than the dimensions of the symbols.

PDADMA/PSS complexes lost more than 50% of their water due to osmotic dehydration in PEG. There appear to be two regimes of water extraction. The lowest PEG concentration employed (25 wt %) accounts for most of the water loss. Because of the length of time needed for equilibration, the use of still lower PEG wt % was not possible. As PEG wt % increases water continues to be extracted, but at a much lower slope. In a recent study of PDADMA/PSS multilayers we observed this initial large weight loss and attributed it to dehydration of nanopores and additional water outside the hydration shell of polymer ion pairs.³

The initial decrease in the water content of CoPECs at PEG 25% was a result of macropore compaction. Since the amount of water in CoPEC pores is dictated by the osmotic pressure of the trapped PSS chains, the osmotic pressure of the external medium must be greater than in the pores. Microscopy of slices of complex before and after treatment in PEG 25% revealed no pores at this level of dehydration (Figure 3) consistent with the optical clarity of the material. To maintain optical clarity an upper limit of about 100 nm may be placed on pore size, if there are any left. It was possible to regenerate the pores by soaking the compacted complex in salt solution over a long period of time (Figures S7 and S8). Presumably, the slight excess of trapped PSS provides a “memory” of the porosity.

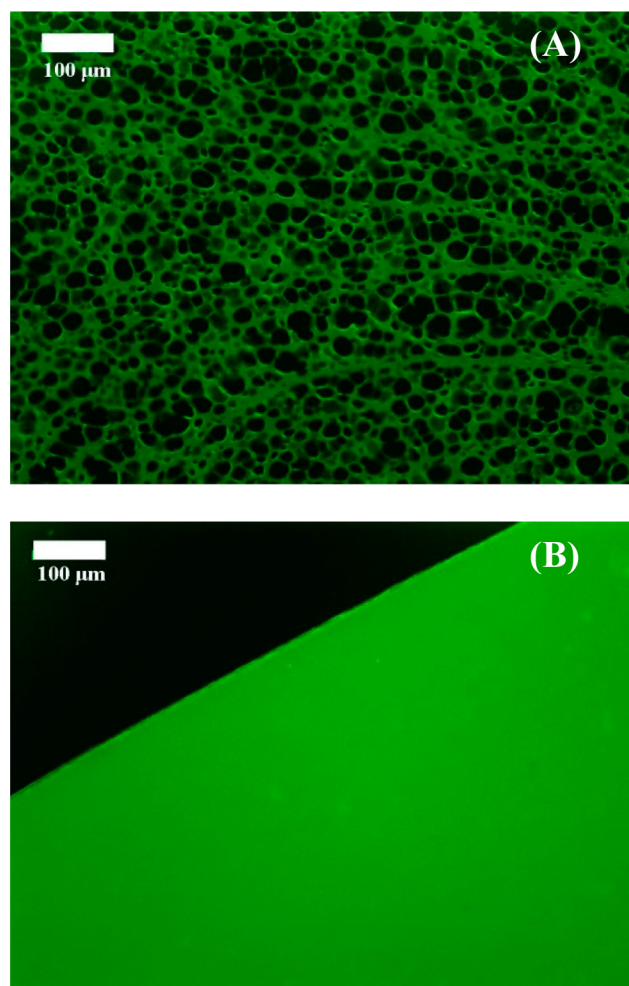


Figure 3. Fluorescence micrographs of thin PDADMA/PSS CoPEC sections in (A) 0.1 M NaCl (10 μ m thick section) and (B) 25% PEG with 0.1 NaCl activity (20 μ m thick section).

In a study on poly(vinyl alcohol) (PVA) hydrogels as possible bioimplants, PEG was used to simulate the *in vivo* osmotic environment in articular cartilage.⁵⁵ Porosity was shown to decrease by half after 4 weeks in a PEG solution with 0.95 atm osmotic pressure (equivalent to 7.6 wt % of PEG 8000) as the pores collapsed due to water expulsion. The decrease in pore volume and water content of the gels under osmotic stress resulted in stiffer gels. For PDADMA/PSS CoPECs, PEG 25%, with osmotic pressure of 11.5 atm, extracted the pore water.

We showed in an earlier study that porosity in CoPECs controls their mechanical properties.¹⁵ Pores decrease the equilibrium modulus and enhance the viscous response of the material, and because they contain most of the excess PSS, they contribute to the equilibrium modulus of the CoPECs as they exert osmotic resistance to loads.¹⁵ If water is extracted from CoPECs, they are expected to show a decrease in their damping or viscoelastic properties. The stress relaxation data in Figure 4 depicts increasing modulus with decreasing viscous behavior with increasing PEG wt %. With the loss of pore water, the hydrostatic (long-term relaxation) parameter is diminished. Comparing Figure 4A (PEG 0%) and Figure 4B (PEG 25%) shows that even after losing almost all pore water (Figure 3), samples still show considerable relaxation, which is attributed to slipping polymer entanglements from a material that has not

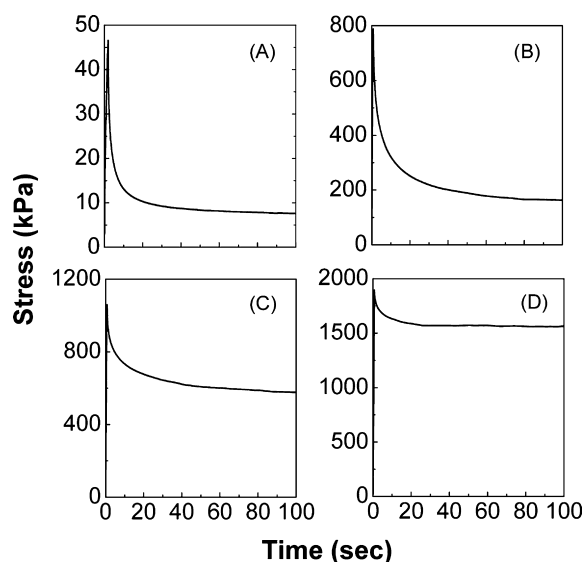


Figure 4. Stress relaxation of PDADMA/PSS CoPECs treated in (A) 0.1 M NaCl (0% PEG), (B) PEG 25%, (C) PEG 30%, and (D) PEG 40%. All PEG solutions prepared with 0.1 NaCl activity.

yet reached the glassy state. The extra few percent water content lost at higher PEG% resulted in major loss of viscous characteristic. The decrease in degree of relaxation seen in Figure 4C,D is an indication of restricted chain dynamics which implies loss in the intermolecular free volume available for chain relaxation,⁵⁶ consistent with approach to a glass.

Equilibrium elastic modulus values for PDADMA/PSS CoPECs treated in increasing PEG concentrations were determined from the stress relaxation plots at long times (i.e., steady-state stress at longer times in Figure 4). The moduli, shown in Figure 5, are almost 3 orders of magnitude larger than

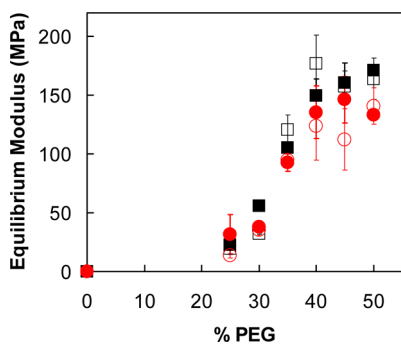


Figure 5. Equilibrium elastic modulus of PDADMA/PSS CoPECs dehydrated in different PEG% solutions. Squares represent data from samples that were pretreated in 0.1 M (■) or 0.5 M NaCl (□) and then soaked successively in increasing PEG% solutions and tested after few days in each PEG%. Circles represent data from samples pretreated in 0.1 M (●) or 0.5 M NaCl (○) and then left to dehydrate in PEG 25% before they were transferred to higher PEG solutions: 30, 35, 40, 45, or 50% to be tested after few days. All PEG solutions were prepared with NaCl activity of 0.1 or 0.5.

those of as-prepared CoPECs, suggesting that water provides fundamental control over both the elastic and damping characteristics. At equilibrium relaxation, viscoelastic materials behave as elastic solids.⁵⁷ Based on classical theories of elasticity for swollen polymer gels, the higher the degree of swelling, the

lower the elastic modulus as a result of the decrease in polymer volume fraction.⁵⁸

It is interesting that a small change in water content with increasing PEG wt % (Figure 2) resulted in almost 2 orders of magnitude increase in the elastic modulus between PEG 25% and 50%. There was more scatter in modulus data for the stiffer samples, which was of concern. Two approaches to increasing the modulus were taken. In the first approach, samples were soaked in PEG 25% until they became transparent, then mechanically tested, then left to further dehydrate in the next higher PEG wt % and tested few days after. This procedure was repeated on the same sample up to PEG 50%. The decrease in the slope of modulus at high PEG concentrations, and increasing scatter, was related to formation of cracks in the samples most clearly seen at PEG 45% and 50% after testing (Figure S9). To check whether crack formation was a result of extensive mechanical deformation on the same samples or to dehydration, the experiment was repeated on samples that were all treated in PEG 25%, and then each was transferred to different, higher PEG% solution. The samples were stretched to a lower strain than in the previous experiment (<0.5%) to control crack formation, and all samples were imaged before and after mechanical testing. Results from the two experiments were comparable, again with more scattering of data at the high PEG concentrations (Figure 5). Microscope imaging revealed cracks in samples treated in PEG \geq 40% before any mechanical deformation, especially in regions with defects. Spontaneous crack formation is an indication of stress release in a constrained environment.²⁴

Polyelectrolyte Multilayers. Osmotic stressing of PEMUs and CoPECs was compared, since the former morphology of PEC is studied far more widely. There are a couple of other potential advantages to studying PEMUs: first, because they are much thinner than the CoPECs, stress-induced cracking of the more glassy compositions should be alleviated. Second, also because they are much thinner than CoPECs, PEMUs should reach equilibrium dehydration much faster, making it possible to study materials properties at lower osmotic pressures. NMR^{59,60} and permeation⁶¹ studies on PEMUs revealed pores (or mesh sizes) of molecular dimensions. In comparison to CoPECs, PEMUs can be considered a nonporous form of PEC, since the pore volume in the former can be as high as 80%.³⁰ PEG dehydration studies were done on (PDADMA/PSS)₁₅ PEMUs built in 1 M NaCl and then undoped in 10 mM NaCl. Under these conditions, PDADMA/PSS PEMUs swell due to increased internal osmotic pressure from trapped counterions.³¹ Wet thicknesses of the PEMUs were determined before and after dehydration using AFM to estimate water content. Figure 6 shows that as PEG wt % increases, water content decreases. Eventually, at the highest PEG concentration (50 wt %), the water decreases to 26 vol % or 24 wt %.

The corresponding change in modulus of PEMUs at different PEG wt % was determined by nanoindentation. Force versus indentation curves, at different indentation rates, were collected for PEMUs in salt and PEG–salt solution and were fit up to 10% indentation to determine the elastic modulus. Dehydration of these nanometer thick films was fast as seen in a dehydration kinetics experiment on PEMU in PEG 30% (Figure S10); a jump in the modulus was seen after the first 7 min of adding PEG (which was the time needed to start the nanoindentation test) after which the value was almost stable. All multilayers were left for at least 12 h in PEG before testing; those in PEG lower than 20 wt % were tested after 3 and then 5 days to make

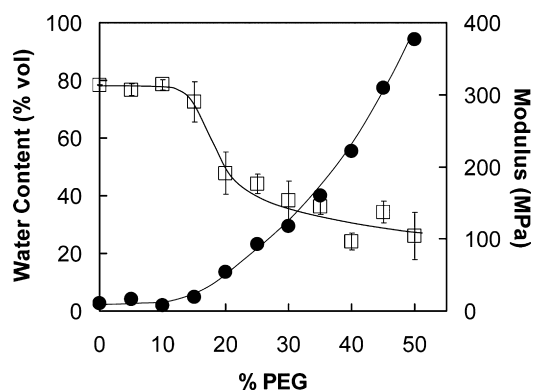


Figure 6. Change in volume percentage of water content in (PDADMA/PSS)₁₅ after soaking in PEG solutions (□) and the corresponding change in modulus (●). The multilayers were undoped in 10 mM NaCl and then transferred to PEG solutions with equivalent salt activity. Water content was calculated as percentage change in thickness of PEMUs which corresponds to change in volume.

sure they had reached equilibrium dehydration. Modulus values in Figure 6 were determined by extrapolating to zero strain rates (Figure 7 and Figure S11) to obtain the equilibrium

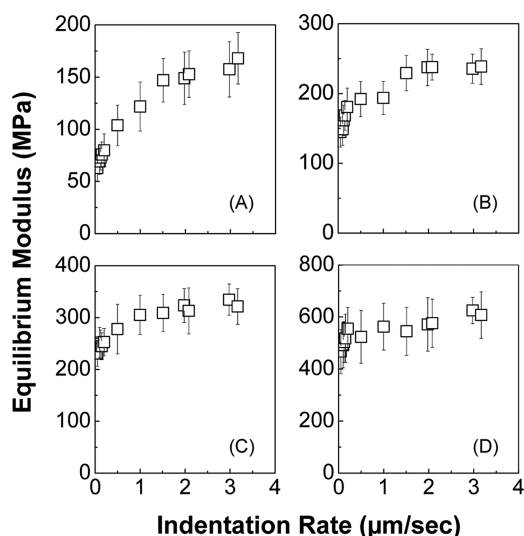


Figure 7. Change of elastic modulus of (PDADMA/PSS)₁₅ with increasing rate of indentation in PEG: (A) 20%, (B) 30%, (C) 40%, and (D) 50%. All PEMUs were first equilibrated in 10 mM NaCl and then soaked for 12 h in PEG solutions with 0.01 NaCl activity.

elastic modulus of the films, equivalent to the modulus of CoPECs determined by tensile stress relaxation experiments. The range of data points is wider with the PEMU samples, since PEG concentrations lower than 20% were possible. Up to about 10 wt % PEG (ca. 1.2 atm pressure) relatively little change was observed in water content and modulus. Again, there was a strong transition region in water content, but the PEMU modulus increased continuously even at the highest PEG%. There appears to be a threshold of about 1.2 atm, attributed to the osmotic pressure of trapped extrinsic sites, before water starts to be osmotically withdrawn from the PEMU.

In determining the modulus of thin films by nanoindentation, the film must be sufficiently thick to decouple the stiffness of the substrate from the measured modulus. For

our probe and conditions, it was shown that a film must be of thickness >200 nm in order to obtain a reliable value of the modulus by nanoindentation without interference from the substrate.⁶² Our PEMUs were at least 500 nm thick.

Viscous relaxation by nanoindentation is assessed by changing the indentation rate. Faster indentation rates are equivalent to short times or high-frequency deformation. We were able to detect viscoelasticity of PEMUs in salt solutions by nanoindentation at different strain rates.¹⁹ Figure 7 shows the change in modulus with indentation velocity for (PDADMA/PSS)₁₅ PEMUs in four of the PEG solutions (see Figure S11 for other PEG%). The percent increase in modulus with rate of deformation varied between 160% and 30% for PEG percentages between 5% and 50%, respectively, with increasing indentation velocity. Similar to CoPECs, a small incremental decrease in water content at the higher PEG concentrations resulted in noticeable transition toward a glassy response.

We have recently shown that the modulus of PDADMA/PSS multilayers ending in PSS is comparable to the bulk modulus,¹⁹ so the modulus values in Figures 6 and 7 should reflect bulk properties of the films under the conditions of the experiment. While nanoindentation provides good relative moduli (precision), assumptions in tip geometry often compromise accuracy. For a direct comparison of CoPEC and PEMU, nanoindentation was performed on a 50 μm thick section of PDADMA/PSS CoPEC that was dehydrated by PEG 25%. Figure 8 shows the change in modulus with indentation velocity

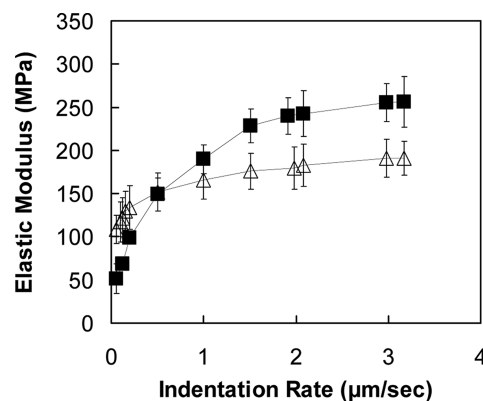
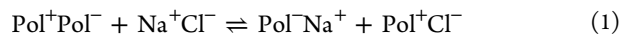


Figure 8. Nanoindentation at different strain rates on 50 μm thick slice of PDADMA/PSS CoPEC dehydrated in PEG 25% with 0.1 NaCl activity (■) and (PDADMA/PSS)₁₅ PEMU in PEG 25% with 0.01 NaCl activity (△).

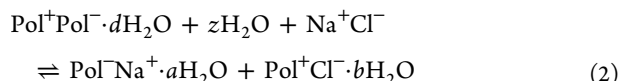
of the dehydrated CoPEC slice compared to a PEMU at the same PEG%. The modulus for PEC extrapolated to zero strain rate is comparable to the results for the macroscopic sample in Figure 5 (ca. 25 MPa). PEC and PEMU in Figure 8 show order-of-magnitude similarity, although the CoPEC has a stronger dependence on indentation rate, probably because of the nonstoichiometric composition (the excess of PSS decreases the ion pair density and increases the water content slightly).

Plasticization. The modulus of a PEC is known to depend on the external salt concentration, which dopes the PEC according to the following equilibrium:



where Pol^+Pol^- are paired polyelectrolyte repeat units and Pol^-Na^+ and Pol^+Cl^- are counterion-compensated repeat units

(all within the PEC). Thus, the result in Figure 2 that shows no significant effect of salt concentration on modulus was, at first, surprising. However, the full doping equation includes waters of hydration



where d , a , and b are the respective hydration levels of paired and counterion-compensated polyelectrolyte. z , the number of water molecules that enter the PEC along with each NaCl, is about 4 for PDADMA/PSS.³ Solutions of lower water activity (higher osmotic pressure) shift the equilibrium to the left in eq 2, implying no significant doping occurs in PEG solutions with NaCl activities of 0.1 or 0.5. Hence, the changes in modulus are related to water content, not changes in doping level.

In an initial attempt to explain the increase in modulus, it was assumed that the effective cross-linking density increased as water was removed. At high cross-link density, modulus is a stronger, nonlinear function of cross-linking.⁶³ Building on a recent analysis of PDADMA/PSS multilayers, which treated each ion pair as a cross-link,³² the shear modulus (G) was estimated from the number of moles of subchains per unit volume (ν), which is proportional to the cross-link density. At high cross-link density, segments between cross-links are shorter and stiffer with less degrees of freedom. Moduli were calculated using Smith's theory for a non-Gaussian distribution of chain lengths:⁶³

$$\frac{\nu RT}{G} = \frac{1}{\Phi_n} - \frac{6\bar{C}_n}{5q^2\bar{n}} \quad (3)$$

where \bar{n} is the geometric mean number of bonds in the network chain, \bar{C}_n is a characteristic ratio given by $\bar{C}_n = n^{0.57}$, and q , a dimensional factor determined by bond angles and length of the backbone chain, is equal to 0.83 for a network containing only carbon-carbon single bonds. A value of 0.55 was determined for Φ_n at 0 M NaCl previously.³² ν was calculated as follows:

$$\nu = 3c' \quad (4)$$

where c' is the effective cross-link density of the network, in our case assuming 0.3 network fraction.³²

Using the water content data determined by AFM, we estimated the effective cross-link density of the PEMUs at different PEG wt % (detailed calculation in Supporting Information).

Figure 9 shows the calculated change of equilibrium shear modulus (G_0) with effective cross-link density. G_0 was determined as $G_0 = E_0/3$, assuming an isotropic network with Poisson ratio of 0.5. The full line in this figure is a fit to eq 3. Clearly, the modulus of the PEG-dehydrated PEMUs is much higher than the predictions of the Smith model at the higher cross-linking densities. There is only agreement toward the nonosmotically stressed extreme.

Since the sharp increases in modulus could not be explained by an increase in effective cross-link density, we turn our attention to plasticizer arguments. A plasticizer is a small molecule that enhances the flexibility of a material, decreasing its glass-transition temperature.⁶⁴ Plasticizers can strongly influence the viscoelastic response of polymers. For example, shorter retardation times were seen with increasing plasticizer percentage in polymer-based coatings,⁶⁵ and both equilibrium and delayed elastic modulus values showed a decrease with

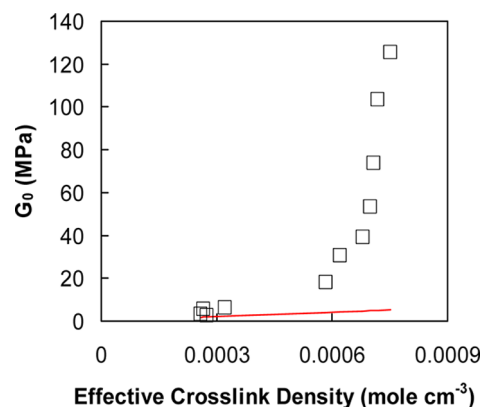


Figure 9. Change of equilibrium shear modulus (G_0) with effective cross-link density of (PDADMA/PSS)₁₅ PEMUs in different PEG solutions at constant salt activity (0.01 NaCl). The full line is fit to Smith theory for non-Gaussian chain distribution (eq 3).

plasticizer content and dependence on plasticizer properties in poly(vinyl chloride) (PVC).^{66–68}

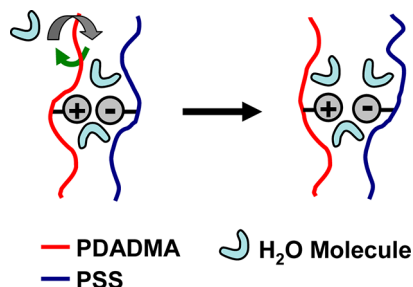
Three main theories attempt to explain the mechanism of plasticization: (1) lubricity theory treats the plasticizer as a lubricant that reduces intermolecular friction between polymer chains, (2) the gel theory extends lubricity theory considering a plasticizer to act by breaking and masking polymer–polymer interactions, thus reducing the rigidity of the material, and (3) the free volume theory suggests that a plasticizer increases the free volume between polymer chains allowing for more chain motion and viscous-like flow. Plasticization is often explained by the three theories combined,^{64,69} probably because of their conceptual overlap.

Numerous studies investigated the state of water in polymer systems to explain the mechanism by which water affects mechanical response. An increase in free volume cavity size is associated with an increase in water content. There is also evidence of inter- and intramolecular disruption of polymer interactions by water molecules.^{23,26} Significant changes in the mechanical properties were observed with changes in water content.^{21,22,70} The elastic modulus of poly(*N*-isopropylacrylamide) gels showed a sharp exponential increase with decreasing water content.²¹ Stress–strain data at different water contents showed a failure envelope, which is characteristic of amorphous elastomer behavior at variable temperatures. This suggested water in hydrogels plays a role similar to temperature in elastomers, and the sharp transition in modulus (similar to the trend of our data in Figure 9) was analogous to glass-to-rubber transition normally seen for polymer networks with changes in temperature. A critical water content can be defined, at the inflection point of the transition similar to the glass-transition temperature, below which the complex behaves as a glassy material. Glass-to-rubber transition induced by temperature is also known to be associated with increased free volume.⁵⁷

For CoPECs it is clear that the removal of pores at lower osmotic pressure (Figure 2) accounts for an initial increase in modulus. The further strong increase in PEC modulus with dehydration of an apparently compact material (Figure 3) cannot be explained by removal of water from (nano)pores. Instead, it is believed that water hydrating polyelectrolyte ion pairs (ion pair “bound water”) is removed. It has been shown that bound water (“nonfreezing”) water provides the major contribution to plasticization of hydrated polymers.²³ Using a free volume framework, bound water provides volume for

polymer chain motion. An example of a polymer backbone chain motion is depicted in Scheme 1 in which a water molecule and a chain segment exchange positions. An equally valid argument could be made that water is “lubricating” the chain motion.

Scheme 1. Representation of How Water Provides Free Volume for Polyelectrolytes to Move in, Plasticizing a PEC^a



^aA water molecule and a polymer segment exchange place rapidly and reversibly.

Although the plasticizing effect of small molecules has been known for many decades, quantitative theories are incomplete. The effect of plasticizers is encapsulated in empirical relationships such as the following:

$$E_p = E_{p_0} e^{-k_m r_p} \quad (5)$$

where E_p is the elastic modulus of the plasticized material, E_{p_0} is the modulus at zero plasticizer content, and r_p is the mole ratio of the plasticizer. The exponent k_m is known as the plasticizer efficiency. Such a relationship has been employed, for example, in films of hydrophilic biopolymers (e.g., proteins).²⁰ Sothornvit et al.⁷¹ compared the effect of different plasticizers on the mechanical properties of β -lactoglobulin films.

A plot of the modulus versus mole ratio of water (relative to the polymer matrix) in PEG-treated PEMUs shows an exponential decrease in the modulus with increase in water content. The fit to eq 5 in Figure 10 gives a value of 4200 MPa for E_{p_0} and 117 for k_m . The value of E_{p_0} determined from the fit is close to that determined by AFM force curves on (PDADMA/PSS)₁₅, dried in a vacuum oven at 100 °C for 4 days (4.1 ± 0.8 GPa). Farhat et al. have shown that by heating

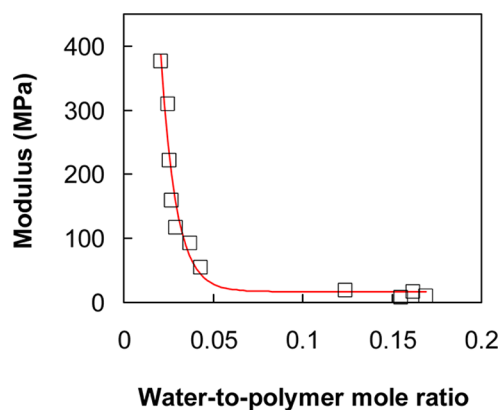


Figure 10. Change of equilibrium elastic modulus of (PDADMA/PSS)₁₅ PEMUs with mole fraction of water to polymer matrix of the PEMU. The red line is a fit to $y = 4200 \times e^{-117x} + 17$.

the multilayer to 100 °C, all water is removed.²⁸ Therefore, the modulus of “dry” PEC is in the range of 4 GPa. For comparison, the elastic modulus data of poly(vinyl chloride) (PVC) plasticized with different percentages of dioctyl phthalate (DOP) were fit to eq 5, and a k_m value for DOP of 51 was determined.⁶⁶ DOP is a common, efficient plasticizer for PVC, an important industrial thermoplastic that is normally brittle and difficult to process ($T_g = 90$ °C).⁶⁷ Water is about twice as efficient in plasticizing PDADMA/PSS PEMUs than is DOP in plasticizing PVC, consistent with the general finding that small molecular weight plasticizers show higher plasticizing efficiency.⁷¹

Normalizing modulus values for polymer volume fraction at each water content allows determining network properties irrespective of water contribution.^{21,22} For modulus values in Figure 10 it resulted in the same increasing trend at low water mole ratios. This indicates that interchain interactions are also involved in enhancing the stiffness of the complex with loss in water content, besides the contribution of interchain free volume, suggesting that water plasticizes PECs by enhancing both free volume and lubricity effects among the chains.

CONCLUSIONS

Osmotic stress is a powerful experimental variable for modifying the water content of the various morphologies of polyelectrolyte complex. Porosity may be eliminated with sufficient stress, and the remaining more strongly bound water may be systematically removed. The stages of dehydration will vary with morphology, but osmotic stressing is a useful method for homogenizing samples, such as multilayers, which may show different environments for molecules sorbed in them.⁷² Given the strong osmotic pressure of some physiological environments, mechanical properties of PECs proposed for biomedical applications should be tested under conditions of the appropriate osmotic stress. The strong, nonlinear changes in modulus that occur with water content point toward a highly efficient plasticizing efficiency of water. Partially dehydrated complexes are much stiffer than as-prepared PECs, suggesting exceptionally strong materials, including composites, may be made from them.

ASSOCIATED CONTENT

Supporting Information

Salt activity determination, force curve calculations, proof that PEG is excluded from PEC, osmotic pressure versus PEG wt %, PEC dehydration kinetics, rehydration kinetics, micrographs of crack formation, PEMU dehydration kinetics, calculations of effective cross-link densities, apparent modulus versus nano-indentation rate. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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