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Interplay between Microscopic Structures and Macroscopic Viscoelastic Properties of Polyampholyte Gels

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Abstract Polyampholyte gels, which have hierarchical structures, exhibit excellent self-healing properties and have great promise for biomaterials and bioengineering. We investigated the relationship between microscopic structures and macroscopic viscoelastic properties of polyampholyte gels and found three factors influencing their viscoelastic properties, including the chemical crosslinking bonds, topological entanglements controlled by monomer concentration, and the ionic bonds. Ionic strength plays a major role on the strength of ionic bonds. A crossover point of elastic modulus and loss modulus was observed in the dynamic frequency sweeps at low monomer concentration or low chemical crosslinking density for gels with intermediate strength of ionic bonds. The solid-liquid transition signaled by the crossover point is a typical feature of dynamic associated gels, representing the dynamical association-dissociation of the ionic bonds and full relaxation of the topological entanglements in the gel network. While the crossover point disappears when the ionic bonds are too weak or too strong to form "permanent" bonds. Consistently, in the non-linear yielding measurement, gels with intermediate strength of the ionic bonds are ductile and yield at very large shear strain due to the self-healing properties and the dynamic association-dissociation of the ionic bonds. But the self-healing properties disappear when the ionic bond strength is too weak or too strong. Our work reveals the mechanism of how the dynamic association-dissociation of ionic bonds influences both the linear and non-linear viscoelastic properties of the polyampholyte gels.

Keywords Polyampholyte gel; Ionic bond; Topological entanglement; Self-healing; Hierarchical structure

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INTRODUCTION

Polyelectrolyte hydrogels exhibit various viscoelastic properties, which arise from non-covalent electrostatic interactions in covalent polymer networks, and can find wide applications in various fields such as wound dressings, contact lenses, drug delivery, and tissue engineering scaffolds.[1–5] However, the mechanical performance of traditional polyelectrolyte hydrogels is relatively weak due to their high-water content, limiting their application range.[6–9] Recently, several groups have proposed the concept of double-network hydrogels to enhance the mechanical performance.[10–16] In the double-network hydrogels, one network is formed by chemical bonds in the matrix, and the other network can be composed of non-covalent bonds, such as ionic bonds and hydrogen bonds, *etc*. Introducing a second network, serving as a sacrificial network allows the dissipation of energy through the fracture of this network during deformation, thereby enhancing the mechanical properties of the hydrogels. Specifically, there are several types of system for gels containing ionic bonds, including coacervates composed of polycations and polyions, polyelectrolytes complexed with multivalent ionic groups, polyion-complex hydrogels, polyampholyte gels, *etc*.[17–23] For example, Huang *et al.* proposed a simple two-step equilibrium method to prepare gels with synergistic ionic and metal-ligand bonds, which can significantly enhance the mechanical properties of the gels compared to the gels with only ionic bonds.[10] While Zou *et al.* studied a dual cross-linked hydrogel, where a physically crosslinked network by ionic coordination (Fe^{3+}) is imposed on a chemically crosslinked poly(acrylamide-*co*-acrylic acid) network by using rheology and proton multiple-quantum NMR spectroscopy. They found the heterogeneous structural evolution with increasing Fe^{3+} concentration and demonstrated the significant contribution of Fe^{3+} coordination to the moduli and mechanical properties of the gels.[24] Moreover, Toleutay *et al.* employed the hydrophobic monomer *n*-octadecyl acrylate for modification and prepared self-healing polyampholyte hydrogels through micellar polymerization in a sodium dodecyl sulfate aqueous solution. Their results show that the incorporation of hydrophobic *n*-octadecyl acrylate blocks into polyampholyte backbone *via* micellar polymerization increases the mechanical performance of the gels and can induce temperature-triggered self-healing behaviors.[25] Here we focused on polyampholyte gels, which consist of anionic and cationic repeating units randomly dispersed in the polymer

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gel network. Due to the randomness of the charge sequence, the opposite charges from inter-chain and intra-chain can complex and form multiple ionic bonds with a wide distribution of strengths.[17] Therefore, polyampholyte gels have rich viscoelastic properties and fatigue resistance, and they also exhibit excellent biological adhesive properties and anti-biofouling properties, thus make it a good candidate as structural biomaterials.[17,26–28]

Polyampholyte gels have hierarchical structures consisting of dynamic ionic bonds at ~0.1 nm scale, transient network at ~1 nm scale, permanent polymer network at the 10 nm scale, and bicontinuous phase network at ~100 nm scale.[29] Polyampholyte gel's hierarchical structures play a crucial role in determining the gel's performance. Li *et al.* developed tough and viscoelastic polyampholyte hydrogels and found the salt effect on mechanical properties, such as small strain moduli, large deformation energy dissipation, and fracture stretch ratio, can be effectively converted into frequency or strain rate dependences following the time-salt superposition principle.[30] Another study showed that during the process of dialysis against counterions, gel phase separation occurs due to the interplay of Coulombic forces and hydrophobic forces.[31] Furthermore, covalent network structure also shows great influence on the dynamic association of polyampholyte gels. Higher monomer concentration and crosslinking density enhanced the strength of the polymer network, restricting phase separation. Conversely, an increase in hydrophobic monomer content facilitated phase separation. However, how the hierarchical microscopic structures influence the viscoelastic properties of the polyampholyte gels is still unclear and needs to be further explored.

In this study, the relationship between the microscopic structures and their macroscopically viscoelastic properties of the polyampholyte gels was studied by tuning various parameters, including the total monomer concentration of anionic and cationic monomers C_m, the molar charge ratio of anionic monomers to total monomers R, the chemical crosslinking density XL, and the ionic strength C_s inside the gels by using different swelling methods. This work may reveal the physical mechanisms of the microscopic relaxation of the dynamical ionic bonds and the macroscopic viscoelastic properties of the polyampholyte gels. It can also provide fundamental guidance for quantitively tuning the viscoelastic properties of polyampholyte gels in applications of biomaterials and bioengineering, especially in saline environments.

EXPERIMENTAL

Materials

Anionic monomer sodium *p*-styrene sulfonate (NaSS, ≥90%, Sigma-Aldrich), cationic monomer 3-(methacryloylamino) propyl-trimethylammonium chloride (MPTC, 50 wt% in water, Sigma-Aldrich), crosslinker *N*,*N*′-methylenebis (acrylamide) (MBAA, 2 wt% in water, Macklin), UV initiator *α*-ketoglutaric acid (99.0%–101.0%, Sigma-Aldrich), sodium chloride (NaCl, 99.8%, Macklin) were used as received . Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Synthesis of Polyampholyte Gels

Polyampholyte hydrogels were synthesized using free radical polymerization. Four parameters were tuned here, they are the total monomer concentration of anionic and cationic monomers C_m, the molar charge ratio of anionic monomer to total monomer R, the chemical crosslinking density XL, and salt concentration C_s. The salt concentration was changed by different swelling methods. Take the as-prepared gel with C_m=2 mol/L, R=0.525, XL=0.5% as an example. 6.495 g of NaSS, 12.582 mL of MPTC (50 wt%), 2.314 mL of MBAA (2 wt%) and 43.8 mg of *α*-ketoglutaric acid are mixed with certain amount of concentrated pregel solutions, then deionized water is added until the total volume is 30 mL. The pregel solution is transferred into a petri dish and purged with nitrogen for 30 min to remove oxygen. The reaction is initiated by irradiating the solution by the UV light with wavelength of 365 nm, and the reaction was continued for 8 h at room temperature.

Rheology Measurements

All rheological measurements were performed on a stress-controlled rheometer (Anton Paar MCR 502) with a 25 mm roughened parallel-plate geometry. Silicon oil was coated on the edge of the parallel plate to prevent water evaporation. Dynamic frequency sweep measurements were conducted in the linear viscoelastic region (shear strain γ=1%). Dynamic strain sweep measurements were conducted at a fixed frequency. All the measurements were carried out at room temperature.

RESULTS AND DISCUSSION

Polyampholyte gels have hierarchical structures at different length scales. They have reversible ionic bond at ~0.1 nm scale, a transient network made of ionic clusters at ~1 nm scale, a permanent polymer network made of chemical crosslinking bonds and trapped topological entanglements at ~10 nm scale, and a microphase separated network consists of bicontinuous dense/sparse polymer phases in water at ~100 nm scale, and such a bicontinuous phase network at ~100 nm scale depends on the strength of ionic bonds and molecular weight between the effective crosslinking points.[32,33] To study the relationship between the microscopic structures and their macroscopically viscoelastic properties of the polyampholyte gels, four key experimental parameters were tuned here, they are the total monomer concentration of anionic and cationic monomers C_m, the molar charge ratio of anionic monomers to total monomers R, the chemical crosslinking density XL, and the ionic strength C_s inside the gels.

Effect of Total Monomer Concentration C_m

First the total monomer concentration C_m dependence was studied by dynamical frequency sweeps in the linear viscoelastic region. Here all the polyampholyte gels studied were in the as-prepared state without swelling, and with the fixed crosslinking density XL=0.5%, and the molar charge ratio of anionic monomers to total monomers R=0.52, which is at the charge balance point.[17] As shown in Fig. 1, the elastic modulus G' increases as C_m increases. For C_m=1 and 1.5 mol/L, a crossover point is observed for the elastic modulus G' and the loss modulus G'' at ω_c=0.59 rad/s for C_m=1 mol/L and ω_c=0.02 rad/s for C_m=1.5 mol/L, respectively. At high frequency they have solid-like behavior with $G'>G''$, while at low frequency they behave similarly to a weak gel with liquidlike behavior ($G''>G'$), implying that their response at high frequency is elastic dominated and at

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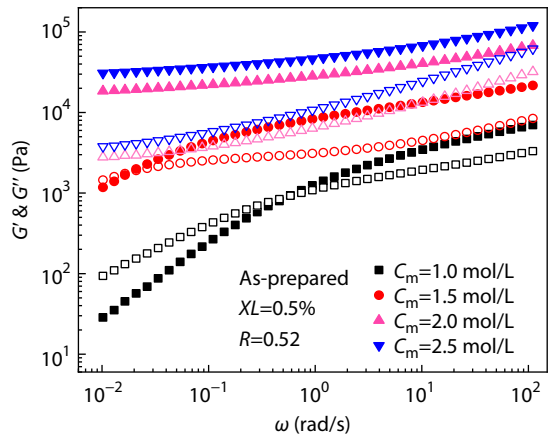

Fig. 1 Dynamic frequency sweeps of the polyampholyte gels in the as-prepared state with fixed crosslinking density XL=0.5%, molar charge ratio R=0.52, and different total monomer concentrations C_m. The shear strain is fixed at γ=1%. Closed (open) symbols represent storage (loss) modulus.

low frequency it is predominantly viscous.[34] Based on the hierarchical structures of the polyampholyte gels, the crossover points may be attributed to the dynamical association-dissociation of the ionic clusters and the full relaxation of the topological entanglements. The characteristic relaxation time ($2\pi/\omega_c$) increases from 10.6 s to 314 s as C_m increases from 1 mol/L to 1.5 mol/L. Because as the total monomer concentration C_m increases, the density of ionic clusters and topological entanglements becomes higher, so that the density of the dense phases in the bicontinuous dense/sparse polymer network increases, leading to a lower crossover point ω_c and longer relaxation time to make the dynamical ionic clusters and topological entanglements fully relax. As C_m further increases to 2 mol/L and above, the crossover point ω_c would go to even lower frequency, which is out of the measurement range here.

Effect of the Ionic Strength C_s Tuned by Using Different Swelling Methods

For polyampholyte hydrogels, the presence of salt accelerates the dissociation of ionic bonds.[10] Reducing the salt concentration allows the oppositely charged ions on the polyampholyte gel strands to form stronger ionic bonds, while increasing salt concentration favors ionic bonds dissociation. Here the ionic strength C_s inside the polyampholyte gels was changed by different swelling methods. As shown in Fig. 2(a), the gel sample with fixed crosslinking density XL=0.5%, molar charge ratio R=0.52 and total monomer concentration C_m=1.5 mol/L was measured in its three states: as-prepared state, swelled by deionized water, and swelled by 2 mol/L NaCl solutions. In the polyampholyte gels, the counterion of the anionic monomer NaSS is sodium, and the counterion of the cationic monomer MPTC is chloride, so that in the as-prepared gels the small ions are NaCl and some of these small counterions are binding with the backbone of the charged polymer gel strands, and other small ions are unbounded, depending on the degree of ionization α.[35] Therefore, for the as-prepared gel with C_m=1.5 mol/L, the ionic strength inside the polyampholyte gel at maximum would be 0.75 mol/L if the degree of ionization α is 1, otherwise the ionic strength C_s would be less than 0.75 mol/L if α<1. However, if the as-prepared gel with C_m=1.5 mol/L gels were swelled by 2 mol/L NaCl solution, whose NaCl concentration is higher than that inside the gel, the NaCl will diffuse into the gel network due to the osmotic pressure and Donnan equilibrium, thus the ionic strength C_s would increase. While if the gels were swelled by deionized water to remove the small ions, C_s would decrease significantly. Therefore, the ionic strength C_s inside the as-prepared gel is smaller than C_s of the gel swelled by 2 mol/L NaCl solution but is larger than gel swelled by deionized water. The ionic strength C_s can strongly influence the ionic clusters composed of physical ionic bonds in the polyampholyte gels. Figs. 2(a) and 2(b) show dynamic frequency sweeps for gels with C_m=1.5 and 2 mol/L, respectively, with the fixed crosslinking density XL=0.5% and molar charge ratio R=0.52. As the ionic strength C_s decreases, both the elastic modulus G' and loss modulus G'' increases due to the stronger physical ionic bonds at lower C_s. Interestingly, for gel with relatively low C_m of 1.5 mol/L (Fig. 2a), only the as-prepared gel with intermediate C_s has a crossover point ω_c for G' and G'', indicating that the association-dissociation dynamical process of ionic bonds may dominate the full relaxation of the gel network and the solid-liquid

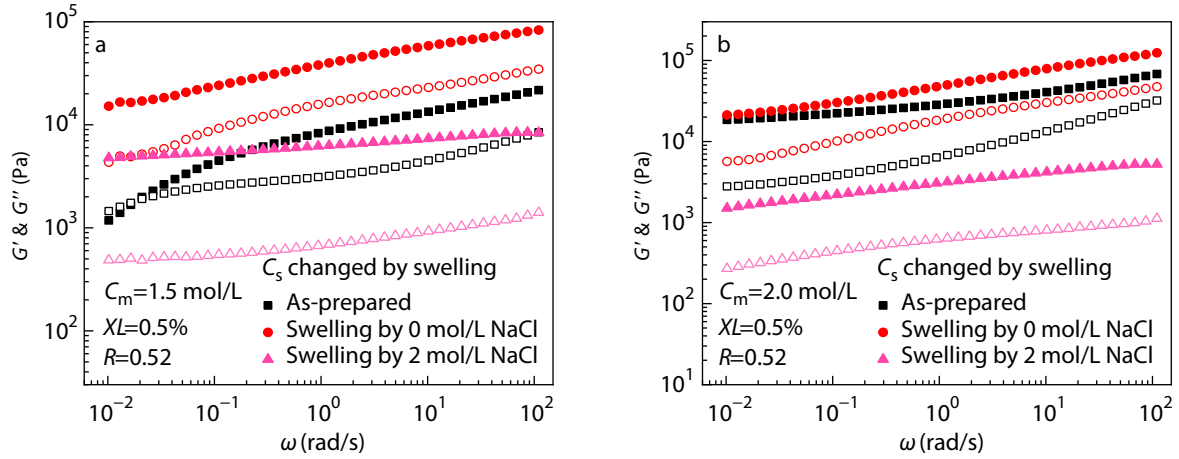

Fig. 2 Dynamic frequency sweeps for gels with fixed crosslinking density XL=0.5%, molar charge ratio R=0.52 and with different total monomer concentrations (a) C_m=1.5 mol/L and (b) C_m=2.0 mol/L. Each sample was measured in its three states: as-prepared state, swelled by deionized water, and swelled by 2 mol/L NaCl solutions. The shear strain is fixed at γ=1%. Closed (open) symbols represent storage (loss) modulus.

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transition here. While for gel swelled by deionized water, the physical ionic bonds are strong enough to form "permanent" bonds due to the low ionic strength C_s. Therefore, we cannot observe the crossover point of G' and G'' which represents the dynamic association-dissociation of the "permanent" bonds. On the other hand, for gel swelled by 2 mol/L NaCl solution, the physical ionic bonds almost disappear due to the Debye screening at high C_s, and the moduli are only correlated to the chemical crosslinkers and topological entanglements, so that there is no crossover point of G' and G''. Furthermore, the moduli of gels swelled by 2 mol/L NaCl solution show less frequency dependence, which is similar to the behavior of chemical crosslinked gels. When the total monomer concentration C_m is 2 mol/L (Fig. 2b), gels in all of its three states do not exhibit any crossover points of G' and G'' in the dynamic frequency sweeps. Because at high total monomer concentration C_m, the density of the topological entanglements increases, and they can prohibit the full relaxation of the whole gel network to induce a solid-liquid transition in the experimental time scale. Only at relatively low C_m with the intermediate strength of the ionic bonds, the crossover point ω_c of G' and G'' can be observed in the experimental time scale. The solid-liquid transition signaled by the crossover point of G' and G'' in the dynamic frequency sweep is a typical feature of dynamic associated gels, representing the characteristic relaxation time of the dynamic association-dissociation of the ionic bonds to make the gel network fully relax.

Besides, the swelling ratio of these gels were also studied. The gel volume was measured every day until its volume does not change anymore, indicating that the gel has reached the swelling equilibrium. The volume fraction Φ of the gel is defined as:

$$\Phi = \frac{V_d}{V} = \frac{V_d}{V_0} \times \frac{V_0}{V} = \Phi_0 \times \frac{V_0}{V} \quad (1)$$

where V_d is the volume of the gel in the dried state, V is the volume of the gel when it reaches swelling equilibrium, and V_0 is the volume of the gel in the as-prepared state. For the same gel sample, we can assume that the volume fraction of the gel in the as-prepared state $\Phi_0 = V_d/V_0$, so that we can get $\Phi/\Phi_0 = V_0/V$.

For the polyampholyte gel with low monomer concentration of C_m=1.5 mol/L swelled by deionized water, the gel volume shrinks so that the volume fraction finally increases to Φ/Φ_0=1.42, because in the bicontinuous phase network, the dense phases composed of topological entanglements and ionic clusters would be more compact due to the lower ionic strength, thus the gel volume would shrink. While for gel with C_m=1.5 mol/L swelled by 2 mol/L NaCl solution, all the ionic bonds are screened so that the ionic clusters can open up. Therefore, the gel swells and the volume fraction finally decreases to Φ/Φ_0=0.54. For polymer gels, the elastic modulus G' is proportional to the gel volume fraction Φ following the scaling law of $G' \sim \Phi^a$ ($0<a<1$), and the a exponent value depends on the system.[7,36–38] Here the volume fraction change also contribute to the change of the elastic modulus G', making G' of the as-prepared gel higher than G' of the gel swelled by 2 mol/L NaCl solution, but is lower that G' of the gel swelled by deionized water (Fig. 2a). For the gel with C_m=2 mol/L swelled by deionized water, due to the higher density of the topological entanglements at higher C_m, the gel is resistant to collapse, resulting in minor volume fraction change after swelling by deionized water (Fig. 3b). However, when it is swelled by 2 mol/L NaCl solution, the gel swells due to the screening of the ionic bonds, and its volume finally decreases to Φ/Φ_0=0.5, leading to an order of magnitude decrease of G' after swelled by 2 mol/L NaCl solution (Fig. 2b).

The hierarchical structures of these polyampholyte gels are also expressed in the yielding measurements. The shear strain sweeps for gels with C_m=1.5 mol/L, and crosslinking density XL=0.5%, molar charge ratio R=0.52 in its three states are shown in Fig. 4(a). at low shear strain γ, both G' and G'' are nearly constant in the linear viscoelastic regime. As shear strain γ becomes larger, G' and G'' start to decrease, leading to the destruction and yielding of the gel structures. Here the yielding point γ_c is defined as the point where G'' is larger than or equal to G'.

For gel with C_m=1.5 mol/L swelled by deionized water, the yielding point γ_c=3.4%, which is dominated by the breakage of the ionic bonds. The ionic bonds become tighter and stronger and are more like "permanent" bonds at low C_s in the gel swelled by deionized water, so that they would break and yield at very low shear strain, make the gel sample very brittle. While for gel swelled by 2 mol/L NaCl solution, all the ionic bonds are screened and the yielding point γ_c=20.1%, in-

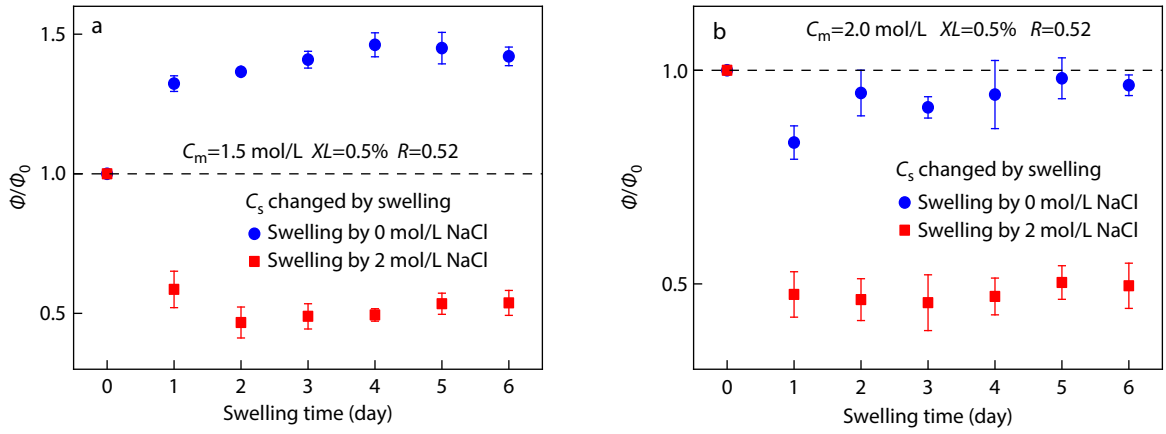

Fig. 3 Swelling kinetics of gels with fixed crosslinking density XL=0.5%, molar charge ratio R=0.52 and with different total monomer concentrations (a) C_m=1.5 mol/L and (b) C_m=2.0 mol/L. Gels are swelled by deionized water and 2 mol/L NaCl solutions. Φ is the volume fraction of the gel and Φ_0 is the volume fraction of the gel in the as-prepared state, Φ/Φ_0 is the normalized gel volume fraction.

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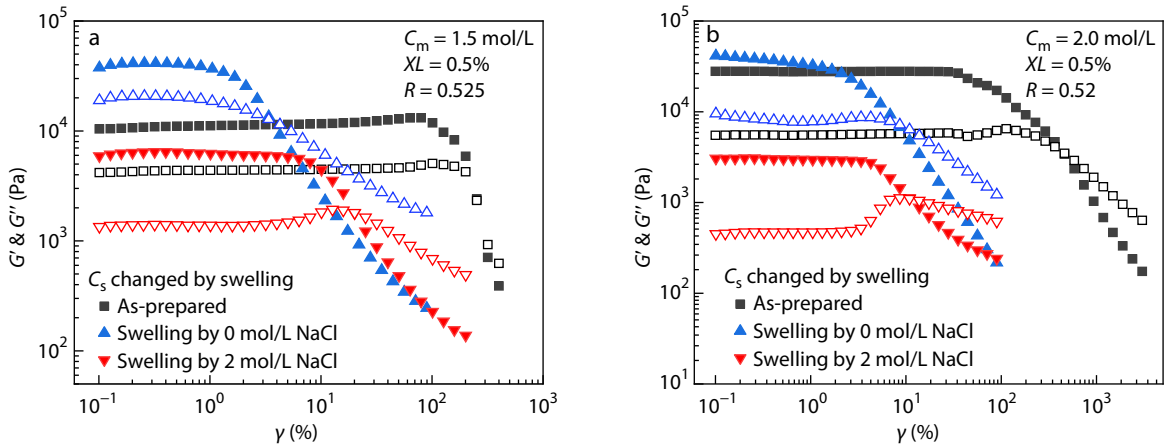

Fig. 4 Shear strain sweeps at ω=1 rad/s for gels with fixed crosslinking density XL=0.5%, molar charge ratio R=0.52 and with different total monomer concentrations (a) C_m=1.5 mol/L and (b) C_m=2.0 mol/L. Each sample was measured in its three states: as-prepared state, swelled by deionized water, and swelled by 2 mol/L NaCl solutions. Closed (open) symbols represent storage (loss) modulus.

dicating the breakage of chemical bonds dominates and makes the whole gel network yield. Interestingly, the as-prepared gel exhibits ductile properties with yielding point γ_c=253%. As mentioned before, the ionic bonds of the as-prepared gel have the intermediate strength, so that it can exhibit a crossover point ω_c of G' and G'' during the dynamic frequency sweep (Fig. 2a), indicating the dynamic dissociation-association of the ionic bonds with the intermediate strength. Here in the shear strain sweep, in the low shear strain regime, the ionic bonds will break up first, but the chemical bonds can still support the whole gel network and protect the gel from yielding. In the intermediate shear strain regime, the chemical bonds will break up, but the ionic bonds which broke up at the very beginning in the low shear strain regime can self-heal due to their dynamical association-dissociation properties. Although the chemical bonds already irreversible break up, but the self-healing ionic bonds and the dynamical association-dissociation process of these ionic bonds can support the whole gel network and prevent yielding until very large shear strain. And the self-healing ionic bonds in turn might also protect and delay the breakage of the chemical bonds at relatively larger shear strain. Please note that the self-healing properties and the association-dissociation process of the ionic bonds only show up at intermediate ionic strength, while for gel swelled by deionized water, the ionic bonds are strong enough to form the "permanent" bonds and thus will lose the self-healing properties. A summary of the yielding point γ_c values in Fig. 4 are listed in Table 1.

When the total monomer concentration C_m is 2 mol/L, the density of the topological entanglements increases, so that for the gel with C_m=2 mol/L swelled by deionized water, both the ionic bonds and the topological entanglements dominate the yielding points and make γ_c=8.6% (Fig. 4b), which is higher than γ_c for the gel with low C_m=1.5 mol/L swelled by deionized water. γ_c of the gels with C_m=2 mol/L swelled by 2 mol/L NaCl solution is similar to γ_c of the gels swelled by deionized water, whose yielding point is dominated by the chemical bonds and topological entanglements. The as-prepared gel with C_m=2 mol/L also exhibits very large yielding point at γ_c=462% due to the self-healing nature and the dynamical dissociation-association process of these ionic bonds with intermediate strength. Because the as-prepared gels with higher C_m have higher density of topological entanglements, so that the yielding point γ_c of the as-prepared gels with C_m=2 mol/L is much higher than γ_c of the as-prepared gels with C_m=1.5 mol/L, as shown in Table 1. Overall, the yielding point is determined by chemical bonds, topological entanglements and ionic bonds. The self-healing nature and the dynamical dissociation-association process of the ionic bonds with intermediate strength can make the whole gel network ductile with much larger γ_c. But the self-healing properties would disappear and the yielding point γ_c would shift to much lower value if the strength of the ionic bonds is so strong to form "permanent" bonds at low C_s or too weak due to Debye screening at high C_s.

Effect of Chemical Crosslinking Density *XL*

To investigate the influence of the chemical crosslinking density on the rheological properties of the gels, a series of as-prepared gels with different XL were studied with C_m of 1.5 and 2.0 mol/L, respectively. As shown in Fig. 5, as the chemical crosslinking density XL increases, both G' and G'' increase. For the as-prepared gels with fixed C_m=1.5 mol/L, R=0.52, the chemical crosslinking density XL is changed from 0% to 5.9% (Fig. 5a). At low crosslinking density XL in the range of 0%–0.5%, the crossover point ω_c of G' and G'' can be observed, indicating a solid-liquid transition. And the corresponding characteristic relaxation time ($2\pi/\omega_c$) increases from 6.7 s to 314 s as XL increases from 0% to 0.5%. The crossover points ω_c may arise from the

Table 1 Summary of the yielding point γ_c for gels in the shear strain sweeps.

C_m	1.5 mol/L, XL=0.5%, R=0.52			2.0 mol/L, XL=0.5%, R=0.52		
Swelling state	As-prepared	Swelling by 0 mol/L NaCl	Swelling by 2 mol/L NaCl	As-prepared	Swelling by 0 mol/L NaCl	Swelling by 2 mol/L NaCl
Yield points	253.0%	3.4%	20.1%	462.0%	8.6%	10.9%

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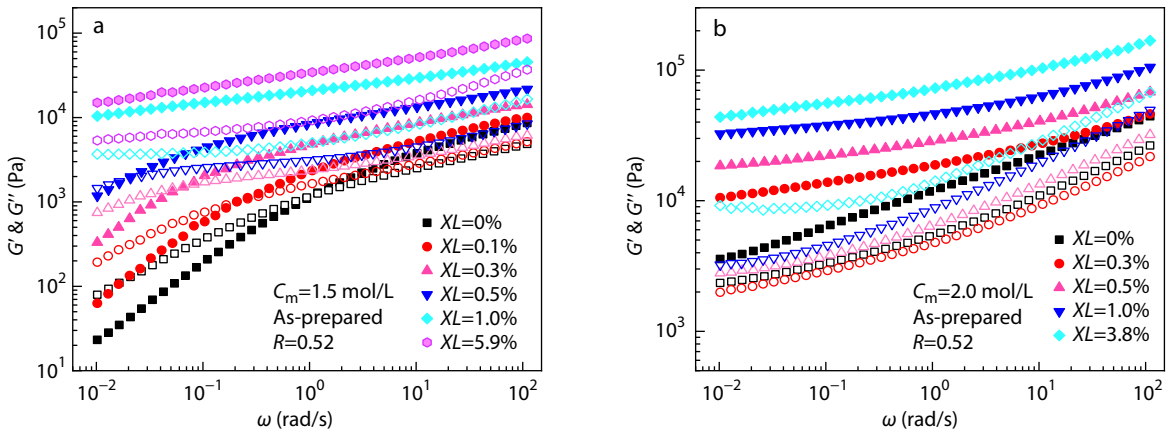

Fig. 5 Dynamic frequency sweeps of the polyampholyte gels in the as-prepared state with fixed molar charge ratio R=0.52, and fixed total monomer concentration of C_m=1.5 mol/L (a) and C_m=2 mol/L (b), the chemical crosslinking density XL is changed. The shear strain is fixed at γ=1%. Closed (open) symbols represent storage (loss) modulus.

dynamical association-dissociation of the ionic clusters and the full relaxation of the topological entanglements due to the low C_m and low XL. However, when the chemical crosslinking density XL further increases to 1% and 5.9%, the crossover point ω_c cannot be observed in the experimental time scale. Because when the chemical crosslinking density XL increases, the permanent chemical crosslinking bonds dominate and support the gel network and prevent the full relaxation of the gel network. While for the as-prepared gels with fixed C_m=2 mol/L, R=0.52, and the chemical crosslinking density XL is changed from 0% to 3.8% (Fig. 5b), no crossover point can be observed. Because now C_m become higher so that the density of the topological entanglements increases, and the topological entanglements dominate and prevent the full relaxation of the whole gel network to induce a solid-liquid transition in the experimental time scale. Besides, for chemical gels, the elastic modulus G' is independent of frequency and G'' is roughly an order of magnitude lower than G', indicating the elastic nature of the gels. While for physical gels with ionic bonds, they can be well described by the sticky Rouse model of associating polymer strands derived from ionic bonds with $G'\sim\omega^{0.5}$ relation.[39, 40] Here as the chemical crosslinking density XL increases, the elastic modulus G' has less frequency dependence, indicating they transfer from physical gels with ionic bonds to more like chemical gels dominated by chemical bonds.

Effect of Molar Charge Ratio *R* in the Polyampholyte Gels

Lastly, the effect of molar charge ratio of anionic monomers to total monomers R is also investigated. Fig. 6 shows the elastic modulus G' obtained from dynamic frequency sweeps at ω=1 rad/s for polyampholyte gels in the as-prepared state with fixed total monomer concentration C_m=2 mol/L, chemical crosslinking density XL=0.5%, and with different molar charge ratio R. The gel with the molar charge ratio R=0.52, which is at the charge balance point,[17] has the highest elastic modulus G'. As long as R deviates further away from the charge balance point 0.52, no matter if R becomes larger or smaller than 0.52, their elastic modulus G' decreases. In polyampholyte gels, there are three factors influences their mechanical properties, they are the chemical crosslinking bonds determined by XL, topological entanglements controlled by total monomer concentration C_m,

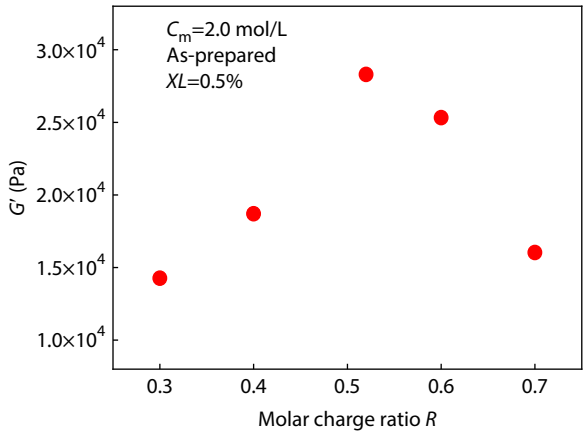

Fig. 6 Elastic modulus G' obtained from dynamic frequency sweeps at ω=1 rad/s for gels in the as-prepared state with fixed total monomer concentration C_m=2 mol/L, chemical crosslinking density XL=0.5%, and with different molar charge ratio R.

and the physical ionic bonds determined by R and the ionic strength C_s. Here C_m=2 mol/L and XL=0.5% are fixed so that the chemical crosslinking bonds and the topological entanglements are the same. Since the gels are all in their as-prepared state, so that the ionic strength inside the gels is also the same. Therefore, at the charge balance point R=0.52, the number of the ionic bonds can reach the maximum, thus the elastic modulus G' can reach the highest value. When R deviates from the charge balance point 0.52 from either side, the number of the ionic bonds would decrease, leading to the decrease of G'.

CONCLUSIONS

In summary, the relationship between microscopic structures and macroscopic rheological properties of the polyampholyte gels, which have hierarchical structures at different length scales was explored in this work. We systematically studied the effect of total monomer concentration C_m, the ionic strength C_s tuned by using different swelling methods, the chemical crosslinking density XL, and the molar charge ratio R on the viscoelastic and mechanical properties of the polyampholyte gels. We found that there are three factors that influence their mechanical

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properties, they are the chemical crosslinking bonds controlled by *XL*, topological entanglements controlled by total monomer concentration C_m, and the number and strength of the physical ionic bonds. In the dynamic frequency sweeps, a crossover point ω_c of *G*′ and *G*″ was observed at relatively low C_m or low *XL* for the as-prepared gels with intermediate strength of the ionic bonds. The solid-liquid transition signaled by the crossover point of *G*′ and *G*″ in the dynamic frequency sweep is a typical feature of dynamic associated gels, representing the dynamical association-dissociation of the ionic bonds to make the gel network fully relax. We also found that salt concentration played a major role on the strength of the ionic bonds, dialysis in high salt solution induced Debye screening of the ionic bonds, resulting in volume expansion and modulus reduction. Dialyzing out all salt ions with pure water can transfer the ionic bonds into "permanent" bonds, which can provide stronger association of gel network, resulting in volume shrinkage and modulus enhancement. Both situations result in the absence of crossover point ω_c. Moreover, in the shear strain sweeps, the self-healing properties and the dynamical association-dissociation process of the ionic bonds with intermediate ionic strength can support the gel network and prevent yielding until very large shear strain, and the self-healing ionic bonds in turn might also protect and delay the breakage of the chemical bonds at relatively larger shear strain, making the gel very ductile. But the self-healing properties would disappear if the strength of the ionic bonds is so strong to form “permanent” bonds, then the gel will become very brittle with very low yielding point. Besides, as the chemical crosslinking density *XL* increases, the elastic modulus G′ has less frequency dependence, indicating they transfer from physical gels with ionic bonds to more like chemical gels dominated by chemical bonds. Lastly, our results show that at the charge balance point *R*=0.52 the elastic modulus *G*′ approaches the highest value, where the number of the ionic bonds can reach the maximum. Our work reveals the mechanism of how the hierarchical microscopic structures, especially the dynamic association-dissociation of the reversible ionic bonds influence both the linear and non-linear viscoelastic properties of polyampholyte gels. This work may provide fundamental physics for their applications in biomaterials and bioengineering. A systematic and detailed investigation to compare the rheological properties of polyampholyte gels and polyelectrolyte gels will be carried out in our future work.

Conflict of Interests

The authors declare no interest conflict.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The author’s contact information: jiadi11@iccas.ac.cn.

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