Thermodynamics of Water Stabilization of Carboxybetaine Hydrogels from Molecular Dynamics Simulations

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Ⓢ *Supporting Information*

ABSTRACT: Hydrogels are highly flexible network polymers being developed as scaffolds for tissue engineering and joint replacement. Their mechanical properties depend largely on their water content. To determine the associated mechanical and thermodynamic properties, we apply the new two-phase thermodynamics method (2PT) to short, molecular dynamics (MD) trajectories of solvated carboxybetaine methacrylate (CBMA) hydrogels. The calculated optimum water content agrees well with recent experiments. We find that the thermodynamics is dominated by a competition between the enthalpy of tightly bound water molecules (which enhance the population of low-energy states of the hydrogel) and the entropy-driven formation of a quasi-liquid water phase in the void volume. These new insights into the role of water in stabilizing hydrophilic motifs is expected to guide design strategies aimed at creating hydrogels with improved performance.

SECTION: Statistical Mechanics, Thermodynamics, Medium Effects
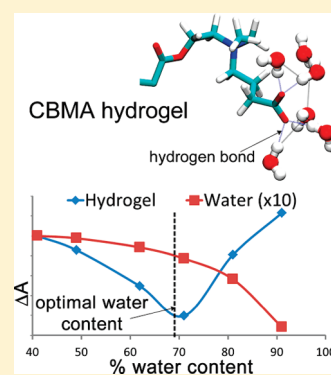
Because of their hydrophilic nature, biocompatibility, and high flexibility, hydrogels have attracted enormous interest for use as scaffolds in tissue engineering and joint replacement.[1,2] Most exciting are zwitterionic hydrogels made from biomaterials such as carboxybetaine methacrylate (CBMA) because they exhibit ultra-low-fouling;[3] that is, surfaces coated with these polymers allow little protein adsorption, greatly reducing degradation and thus enhancing the material stability.[4] Future progress in designing new materials with the specific mechanical responses required a detailed understanding of the molecular interactions that govern these systems. In particular, understanding of the role of water is critical because hydrogels are often composed of 90% water or more, and the transport of nutrients and cellular material through the hydrogel depends on the water content.[5]

It is known experimentally that hydrogels contain two kinds of waters, "bound" waters up until saturation and "free waters" afterward.[5−7] Here one imagines the water molecules coexisting in a bound−bulk-like equilibrium with the polymer matrix. This equilibrium determines the thermodynamics of the system and in turn the mechanical properties. Computer simulations are well-suited to testing these ideas, and indeed several studies have examined the structure and dynamics of water molecules in hydrogels, showing, for example, that the translational and rotational mobility of water molecules is significantly lower around polymer chains.[8,9] Recently, we used MD simulations to understand the three transition states of CBMA hydrogels under various swelling states (unpublished results). However, we did not consider the thermodynamics and, in particular, the role of water entropy.

As a step toward increased understating of the mechanism of water action in these hydrophilic motifs, we report here the free energy, entropy, and enthalpy of CBMA hydrogels[10] from 41 to 91% water using the two-phase thermodynamics (2PT) method to extract these properties from the MD. The details of the method have been presented elsewhere[11−13] and introduced in the Supporting Information. In brief, 2PT obtains the vibrational density of states (DoS) of the system from a Fourier transform of the atomic velocity autocorrelation function. This DoS is then partitioned into a diffusional part (which as a maximum at $\nu = 0$ and decreases rapidly with ν) and librational-vibrational part (that goes to 0 as $\nu \rightarrow 0$). Here the partition function for the librational−vibrational DoS uses the standard quantum Harmonic oscillator form, whereas the diffusional thermodynamics is based on a fit to hard sphere models.

To study the thermodynamics of solvated CBMA systems, we first performed 25 ns of constant pressure (1 atm)−constant temperature (300 K) MD equilibration simulations on the various CBMA−water systems as detailed in the Supporting Information. During the last 5 ns, the system thermodynamics were evaluated every 0.5 ns by running short 20 ps trajectories using the 2PT method. The energies were then referenced to separate calculations of the dry CBMA polymer and bulk water. For example, at STP, the Helmholtz free-energy cost of transferring n water molecules
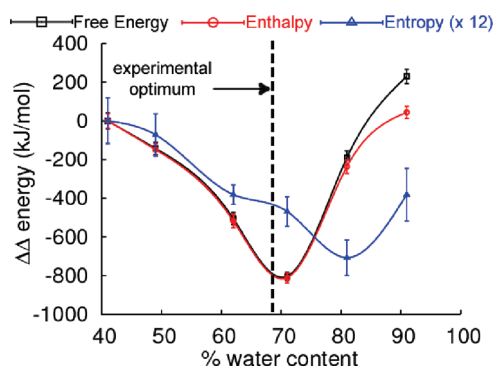
Figure 1. Calculated Helmholtz free energy (black squares), enthalpy (red circles), and total entropy (blue triangles) of hydrated CBMA hydrogels relative to the 41p (41% water) structure. The total entropy $[\Delta(T\Delta S)]$ is scaled $\times 12$ for presentation purposes. The experimental optimum water content is between 61p and 70p, as indicated. Data points are smooth by cubic spline for clarity. Error bars indicate the variance.
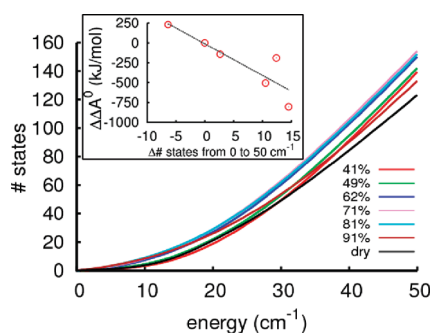

Figure 2. Population of the low energy states ($<50 \text{ cm}^{-1}$) of the CBMA hydrogel as a function of water content. The concerted breathing modes ($35-50 \text{ cm}^{-1}$) are enhanced because of the presence of the water molecules compared with the dry hydrogel (black). Inset: Correlation between the relative free energy and number of low-energy states of the hydrated hydrogel reference to the value at 41% water content. The best fit (solid line) gives $R^2 = 75\%$.

from the bulk into the CBMA matrix is

$$\Delta A^0 = A^{\text{complex}} - A^{\text{dry}}_{\text{CBMA}} - n\left(A^{\text{complex}}_{\text{water}} - A^{\text{bulk}}_{\text{water}}\right)$$

Our analysis allows us to separate the energetic change of the hydrogel and water. First, we focus on the thermodynamics of the hydrogel because we find this to dominate the total thermodynamics. As shown in Figure 1, we find a minimum in the relative free energy between the 62p (61%) and 71p structures. The minimum indicates the optimal water content and agrees with the experiment saturation point.[3] Below saturation, the free energy is dominated by enthalpy, but we find a slight decrease in entropy of the hydrogel in this regime. The entropic decrease is well approximated with a cubic function, and thus we interpret the decrease to be a thermodynamic manifestation of volumetric swelling into a vacuum.

The entropy change of the hydrogel plays is more significant for higher water contents. From 70 to 80% water content, the entropic decrease has twice the slope, indicating a fundamental reorganization of the polymer in response to the external stress. Here the hydrogel has reached it maximum swelling capacity; any
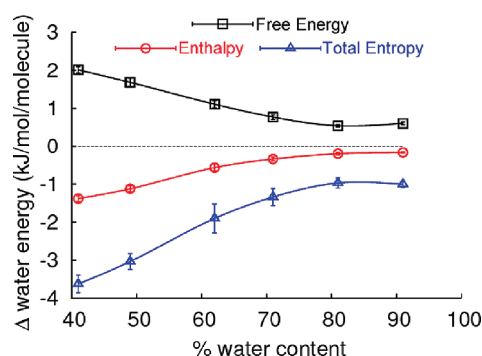

Figure 3. (a) Comparison of the per-water free energy (black squares), enthalpy (red circles), and total entropy (blue triangle) relative to the bulk.

further swelling necessarily involves unfavorable stretching of the bonds. This also causes a dramatic increase in the enthalpy (more positive—less favorable) as the additional strain energy in the hydrogel overwhelms favorable interactions with additional waters.

In examining the dominant enthalpic increase, we find that the potential energy does not fully account for enthalpic gain. As shown in Table S2 of the Supporting Information, the potential energy of the system does not lead to a minimum at the experimental equilibrium water content. Instead, we find that including zero-point energy (ZPE) and heat capacity corrections from the quantum modes are critical to capture the physics of these systems. Here the binding of water and subsequent swelling of the polymer forces the system to increase the population of higher energy vibrational states.

Evidence of a shift in vibrational state of the hydrogel with increasing water content is found by examining the populations of the $0-50 \text{ cm}^{-1}$ energy states from the DoS (Figure 2). These low-energy states arise primarily from librational motions of the hydrogel and thus are sensitive to the presence of guest molecules in the polymer matrix, such as water. We find that the 60p (increase of 22%) and 71p (increase of 25%) CMBA hydrogels has the highest population of low-energy states compared with the dry hydrogel, whereas the 91p (+8%) hydrogel has the lowest. For convenient comparison with the relative free-energy change, we reference the number of low-energy states to the 41p hydrogel. As shown in the inset of Figure 2, there is a 75% correlation between the two measures of thermodynamic stability and a 98% correlation if the largest outlier (81p) is eliminated. Indeed, we show below that waters in the 81p structure have special character.

The bound water molecules have decreased (favorable) enthalpy compared with the bulk due to electrostatic interactions with the CBMA hydrogel. However, these molecules have lower (unfavorable) entropy that dominates the free energy. The net result is an unfavorable free energy that increases monotonically and converges to the bulk value with increasing water content (Figure 3). Water molecules in the 41p hydrogel are the most thermodynamically unfavored compared with the bulk, losing 12.5 e.u. (e.u. = J/mol/K) of entropy or 20% of the total. Emphasizing the previous point, this entropic loss is somewhat compensated by a 1.4 kJ/mol or 4% increase in the enthalpy arising from increased potential energy (hydrogen bonding) and increased ZPE interactions with the hydrogel.

The almost linear increase in the entropy and enthalpy until the optimal water content suggests a transition from a 2D
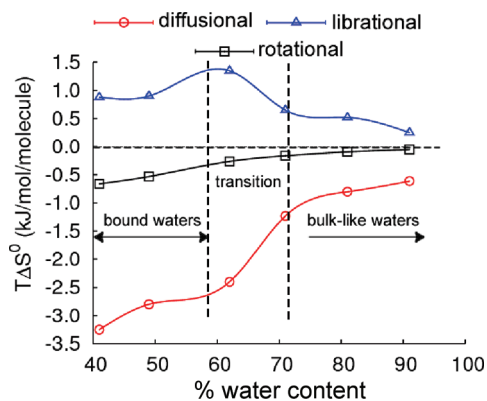
Figure 4. Diffusional (red circles), librational (blue triangles), and rotational (black squares) components of entropy of water molecules in the CBMA hydrogel relative to the bulk. By "bound waters", we mean those that are "confined spatially".

(absorbed on the hydrogel surface) to a 3D (bulk water) behavior. However, even at our highest water content structure (91p), a significant amount of water is still bound to the hydrogel. These interfacial water molecules have 4 e.u. less entropy than in a bulk water box, a 6% decrease that correlates well with the calculated solvent-accessible surface-area-to-volume ratio (Table S1 of the Supporting Information). Therefore, we conclude that water molecules in these hydrogels exist in a density-dependent solid (bound to hydrogel surface)—liquid (inside the void volume of the polymer matrix) equilibrium.

Further insights into the nature of the water molecules in the hydrogel structure are obtained by considering the two kinds of motions in rigid water molecules: translations and rotations, which are generally assumed to be uncorrelated. The translations can be further divided into diffusional (center of mass) and librational (low frequency rattling) motions. The 2PT analysis allows the thermodynamics arising from of each of these motions to be calculated straightforwardly (Table S4 of the Supporting Information).

As shown in Figure 4, the entropic decrease in the waters is primarily due to restricted translational motions. Restricted rotations account for between 18 (41p) and 8% (91p) of the decreased entropy and show smooth convergence to the bulk. We find that the surface water molecules loose an average of 1.0 ± 0.5 rotational degrees of freedom.

More significantly, there is a competition between the more dominant, restricted, diffusional motions of the bound water molecules and the enhanced librational motions associated with the coupling to the breathing motions of the hydrogel. The trends in these translational motions are not monotonic, however. Between 40 and 60% water content, the diffusional and librational entropies increase by ~0.5 kJ/mol. Near the optimum water content, the diffusional entropy of the water significantly increases, whereas the librational entropy decreases. This indicates a shift in the equilibrium between bound and bulk water molecules. That is, the thermodynamics of forming a low density liquid phase in the polymer matrix becomes equal to that of absorbing on the surface of the hydrogel at this point. Therefore, a significant portion of bound water molecules migrate into the liquid phase, enhancing their diffusional entropy at the expense of their librational entropy. Beyond the optimum water content, the bound—bulk water equilibrium shifts more toward the bulk.

In conclusion, we find that water molecules generally increase the population of the low-energy states of the hydrogel, suggesting that the main mechanism for thermodynamic stabilization is the coupling of the water librational motions to the hydrogel breath breathing modes. These results suggest a measurable thermodynamic isotope effect in these systems that would be dominated by ZPE for light molecules such as water. Experimental studies with heavy water can be used to test this theory.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Computational methods and tabulated energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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