

## **DSC studies on the nature of freezing and nonfreezing water in polymer-water mixtures**

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### **Introduction**

The interaction of water with natural and synthetic polymers has been the subject of continued interest because hydration phenomena of polymers can modulate the physicochemical properties of such systems. Indeed, hydration of biomolecules such as proteins and enzymes is particularly important for the stability of the structure as well as proper functionality. On the other hand, hydrogels based on synthetic or natural polymers have become especially attractive not only for their use in biomedical applications but also for applications in other diverse fields, such as ion-conducting membranes, water retention in agriculture and sensors and actuators [1]. A detailed description of the water–macromolecule interactions and of their role in determining the structural, dynamic, and functional properties is a necessary prerequisite to optimize hydrogel characteristics for specific applications. It is known that water molecules in close proximity to biomolecules exhibit properties different than bulk water molecules and in order to describe these peculiar properties of water next to biomolecules the concept of a hydration shell has been employed. In a qualitative picture, the hydration shell consists of the first water layer or sometimes the first few water layers surrounding the biomolecule and interacting with it or at least noticeably influenced by it [2]. A variety of complex macromolecular structures can be formed depending on hydration and, therefore, on the degree of water – macromolecule association. Water may act also as a “plasticizer” affecting, thus, not only the conformation but also the dynamical properties at a molecular level [1].

Different conceptual frameworks have been used to describe the water–polymer associations in a range of hydrophilic polymers [1]. Terms such as “free, loosely bound, and tightly bound” or “freezable, bound freezable and non-freezable” help to describe the distribution of physical and chemical environments that water experiences in a hydrophilic polymer network leading thus to a classification of absorbed water into different states (classes). Alternatively, hydration properties have been discussed in terms of phase diagrams and of kinetic factors that inhibit the water crystallization. One method that is often used to distinguish between “free” or “bulk-like” and “bound” or “hydration” water is based on monitoring the thermodynamics of dehydration of the polymers. The desiccating force commonly used to distinguish between types of water is the freezing of water by employing differential scanning calorimetry (DSC) at subzero temperatures. In calorimetric experiments, the enthalpy of ice melting is monitored and recalculated to the mass of water that undergoes the melting transition. If this mass is lower than the total mass of water in the system, the difference in the masses constitutes the amount of nonfreezing (or non-freezable) water. Many experimental and theoretical works have focused on the investigation of the nature of nonfreezing water. Different states of water, physically binding to polymer chains, have been proposed, among them hydrogen-bond bound water, water

trapped in “nanocavities” in the polymer or simply water molecules that are not able to diffuse due to the vitrification of the amorphous polymer matrix.

The main objective of this work is to study the thermodynamics of water phases in polymer-water mixtures and to reveal the role of hydrogen bond networks existed in the biomolecules, as a function of added water molecules. To that end, we employ DSC method for monitoring the plasticization of the matrix and for studying the thermodynamics of the separate water phase (ice) formed at subzero temperatures, by gradual tuning the hydration level (from almost dry polymer up to highly concentrated solutions).

## Material and methods

In the present work we focus on the hydration properties of synthetic hydrogels based on poly(hydroxyethyl acrylate) (PHEA). In particular, we focus mostly on using PHEA copolymers, as well as PHEA/silica and copolymer/silica nanocomposites, as hydrogel matrices (xerogels) [1]. In addition, we investigate the hydration properties of a family of injectable natural hydrogels that mimic the composition of the extracellular matrix, with a homogeneous distribution of gelatin (Gel) and hyaluronic acid (HA) chains by enzymatic gelation of aqueous solutions of different mixtures of both polymers. Protein (Gel) and polysaccharide (HA) have been combined in an injectable crosslinked hydrogel with controlled Gel–HA ratio [3].

The hydration of the hydrogels was achieved by equilibration of the samples over certain salt solutions, i.e. within environments with varied relative humidity (RH). The hydration level is expressed in terms of water fraction,  $h_w$  ( $=m_{\text{water}}/m_{\text{total}}$ ). Thermal transitions of the materials were investigated in high purity helium (99.9995 %) atmosphere in the temperature range from  $-150$  to  $150$  °C using a TA Q200 series DSC instrument, calibrated with indium (for temperature and enthalpy) and sapphire (for heat capacity).

## Results and Discussion

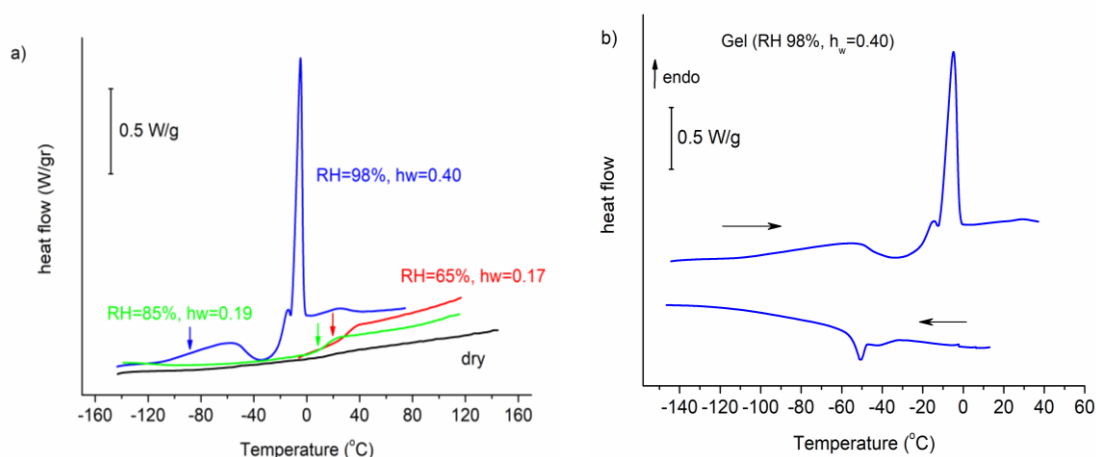


Figure 1. (a) DSC heating curves obtained on Gel hydrogels at various hydration levels indicated on the plot. The glass transition temperatures are marked by an arrow. (b) DSC cooling and heating curves of Gel hydrogel with  $h_w = 0.40$ . The heating rate was  $10$  °C/min.

Figure 1 show DSC heating curves obtained on Gel hydrogels at various hydration levels indicated in the plot. We observe the strong plasticization of the gelatin matrix. For the highest water fraction studied,  $h_w = 0.40$  for Gel, we observe in Figure 1b that during cooling the thermogram shows a weak exothermic peak that corresponds to

water crystallization (with strong super-cooling). During heating the thermogram shows strong cold crystallization process and subsequent (rather complex) melting of ice crystals formed during both, cooling and heating. The analysis of the spectrum reveals that the fraction of nonfreezing water in Gel is about 0.24. It is worth to notice that HA do not shows water crystallization at the hydration level of 0.40. Water crystallizes during cooling only for  $h_w \geq 0.60$ , whereas the analysis reveals that in that case the non-freezing water is again about 0.23. These results, on the one hand, highlight the, well known, property of HA to absorb large amount of water at elevated RH values, on the other, reveal that the nonfreezing fraction of absorbed water is similar in Gel and HA hydrogels, irrespectively of the total water fraction absorbed.

In Figure 2 we can see the strong plasticization action of water molecules being absorbed in neat PHEA hydrogels. In the range  $0 < h_w < 30\%$  (or 0.30) water does not phase separate at subzero temperatures, i.e. no ice crystals are formed, implying that water molecules are molecularly distributed, forming eventually water clusters, and affect strongly the molecular mobility and chain conformations of the PHEA macromolecules. For higher water fractions, ice is formed during cooling and the plasticization action of water stops. Interestingly, the  $T_g$  of PHEA is not affected by the formation of the ice phase.

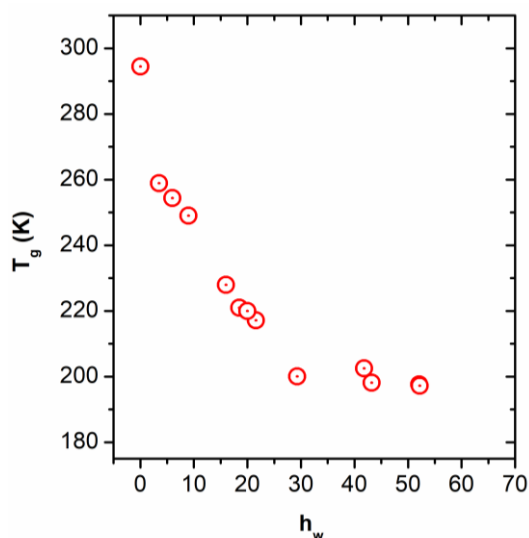


Figure 2. Glass transition temperature,  $T_g$ , measured by DSC, against water fraction  $h_w$  in neat PHEA hydrogels.

## Conclusions

DSC is a powerful tool for studying hydration properties of hydrogels. The method provide information regarding both, the molecular mobility of the hosting macromolecules and the thermodynamic properties of the separate ice phase formed at subzero temperatures.

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