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Infrared spectroscopy used to study ice formation: the effect of trehalose, maltose and glucose on melting

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Abstract

We report the use of infrared (IR) spectroscopy to detect ice crystals in biological solutions. The method is based upon the temperature-dependence of the OH bending and stretch bands of water. By using mixtures of D₂O and H₂O, water's absorption bands can be made to be on scale in transmission mode. Water's stretch band moves to lower frequency and sharpens with freezing and the bending band goes to higher frequency becomes less sharp. The technique is demonstrated for the study of the hysteresis of freezing in the presence of glucosyl-sugars, namely glucose, maltose and trehalose.

Keywords

ice crystals; infrared; hysteresis

Liquid water is required for life, but ice can kill cells. It is desirable for storage of cells, tissue, pharmaceuticals and food-stuffs to have a means to study the conditions when ice forms [1]. In this Note we demonstrate the use of infrared spectroscopy to determine the presence of ice and to study melting. The method is based upon the temperature-dependence of the OH stretch band of water. The band moves to lower frequency with increasing H-bonding strength as temperature decreases [2], and with freezing, the stretch band becomes sharp [3]. We demonstrate the use IR absorption in altering the melting pattern of water by examining the effect of glucosyl carbohydrates. Our point is that by selecting a given water IR band, by using isotope dilution and by changing the pathlength, information on the presence of ice in an aqueous sample can be detected in IR transmission mode. We have previously used this technique to study water in cells [4], denaturants [5], ions [6] and cryosolvents [7]. However, we did not explore which absorption bands are suitable for the study of water's liquid/ice phase transition.

Infrared spectra of H₂O, D₂O, 5% D₂O in H₂O and 5% H₂O in D₂O in liquid and ice form are presented in Fig. 1A–D, respectively. The HOH bending mode, ν_2 , is at 1645 cm⁻¹ (Fig. 1A) and DOD the bending mode is at 1212 cm⁻¹ (Figure 1B) and both absorbance bands diminish in going from liquid to ice. Broad absorption, attributed to a combination of ν_2 and libration, has a maximum at 2150 cm⁻¹ for liquid and 2220 cm⁻¹ for ice H₂O at the temperatures given in Fig. 1A. These values are respectively 1470 cm⁻¹ and 1615 cm⁻¹ for D₂O (Fig. 1B). Using

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isotopically doped water the stretching frequency of OD (Figure 1C) and OH (Figure 1D) can be made to be on-scale. Freezing shifts the absorption of the stretch mode lower, the band narrows and becomes more intense. Therefore, all absorption bands of water in the mid-IR range can be used to detect the liquid/ice transition.

As temperature changes and melting occurs, there is a sharp transition in the absorbance. Large changes in the spectra are observed at the melting temperature and the melting is clearly defined. We also studied other mixtures of H₂O and D₂O. Mixtures show sharp, single transitions and the temperature of the transition is intermediate between 0 and 4, values for the respective melting of pure H₂O and D₂O, in accordance with the known melting point of mixtures [8]. Because ice formation requires a nucleation site, the observed freezing temperature is lower than the melting temperature. The effect of solutes on this hysteresis phenomenon is reported elsewhere [9].

The use of IR to measure melting of water is now demonstrated to show the effect of sugars. Glucose, trehalose and maltose are all glucosyl-based. In glucose, OH positions at 2, 3 and 4 are equatorial, which leads to the largest polar area of aldohexoses [10]. The major difference between the molecules lies in the number of glucose units and the linkage between units. Glucose is a monosaccharide, while trehalose and maltose are disaccharides with (1,1) and (1,4) linkages, respectively. Each of the glucosyl rings is able to distort water-water hydrogen bonds and interactions in the surrounding water layer. Sugars resemble water in that they are both OH rich, but at 0.5 M sugar, the absorbance in the OH stretch region is dominated by water. The hydrogen atoms on the hydroxyl positions of the sugar exchange with water, and so the effective amount of H in the solution has increased, but the OH stretch region is maintained on scale by reducing the pathlength and by diluting the sugar in D₂O rather than an H₂O/D₂O mixture.

The temperature dependence for OH stretch is plotted in Fig. 2 for 1% H₂O in D₂O (A) and solutions of 0.5 M trehalose (B), 0.5 M maltose (C), and 1 M glucose (D). The change in the DOD bending absorption is also shown in Fig. 2B, C and D. Trehalose and maltose act in the same way to broaden the melting temperature. Glucose at 1 M had a larger effect than 0.5 M trehalose and maltose. The gradual change in spectra for the concentrated sugar solution contrasts with the sharp phase transition of pure water. Our interpretation is as follows: as melting occurs, water molecules surrounding the sugars first show disorder. The concentration of sugar in the melted phase is high. As temperature further increases, the pool of melted water increases. As temperature is increased, by about 3 °C, all water is liquid for 0.5 M trehalose and maltose and about 1.5°C for 1 M glucose. The decrease in melting temperature for glucose relative to trehalose and maltose is in line with the idea that the sugar effect is colligative.

As noted, the concentration studied there was no detectable difference between the melting of trehalose and maltose solutions. This is in contradiction of molecular dynamics simulations which showed differing water distributions between trehalose and maltose [11]. However, in more concentrated solution regimes, the stronger interaction of trehalose with water could impact the effectiveness of this molecule relative to other carbohydrates under the water replacement hypothesis.

In conclusion, the IR absorption spectra of ice and liquid water are very different. By using a mixture of D₂O and H₂O the existence of ice in aqueous solution can be detected using IR spectroscopy in the transmission mode. The technique was used to examine the effect of glucosyl sugars. These sugars broaden and reduce the melting temperature of water.

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Fig. 1.

IR absorption spectra of H_2O and D_2O in isotopically mixed forms. Thick lines give liquid absorption, and thin lines give ice absorption. A) H_2O : liquid at 5°C , and ice at -10°C . B) D_2O : liquid at 5°C , and ice at -3°C . C) 5% D_2O in H_2O : liquid at 5°C and ice at -5°C . D) 5% H_2O in D_2O : liquid at 5°C , and ice at -5°C . No spacer was used (effectively 3 to 5 microns). IR spectra were obtained in the transmission mode using a Bruker IFS 66 Fourier transform infrared spectrometer (Bruker, Brookline, MA); the aperture of 2.0 mm and spectral resolution was 2 cm^{-1} . Approximately $20\text{ }\mu\text{L}$ of the sample was placed between CaF_2 windows. Temperature was maintained by circulating water through a cell holder made in the Johnson Research Foundation Instrumentation Shop (University of Pennsylvania). FisherBrand Type-

K thermocouple (Fisher Scientific, Pittsburgh, PA) measured the temperature at the sample with an accuracy of $\sim 0.1^{\circ}\text{C}$. Spectra were corrected for atmospheric contribution, smoothed with Savitsky-Golay algorithm, baseline corrected and converted to absorbance using OPUS v.5.0 software (Bruker).

Fig. 2.

IR absorption of 1% H₂O in D₂O as a function of temperature. A) D₂O doped with ~ 1% H₂O, (B) 0.5 M α - α trehalose in D₂O, (C) 0.5 M α - α maltose (Sigma Chem. Co) in D₂O (D) 1 M glucose in D₂O. Measurements were taken with increasing temperature at the wavenumber indicated. Conditions are given in legends of Fig. 1. Spacer for A: 25 micron; Spacer of B,C and D: none.