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A NEW METHOD FOR AUTOMATIC RECORDING THE STATES OF WATER IN GEL AND SOLUTION BY BROAD-LINE PULSED NMR

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An auto-recording system was developed for the measurement of the unfrozen water content and the spin-spin relaxation time as a continuous function of temperature by a broad-line pulsed nuclear magnetic resonance. The freezing-thawing hysteresis of gels, and the mobilities of solute and hydrated water molecules in frozen solutions of saccharides and alcohols were investigated.

The states of water in gel and solution systems have been extensively studied in cryobiology, cell biology, and muscle researches. The measurement of the unfrozen water (UFW) content and the relaxation times using the nuclear magnetic resonance methods is useful for these studies.[1)-7)] This method can be applied to food science and technology.[8)]

Quite recently, a system was made for the automatic measurement of the UFW content and the spin-spin relaxation time as a continuous function of temperature in our laboratory. The block diagram of the system is shown in Figure 1. Each sample of about 250 mg in a 7 mm outer diameter glass tube was cooled or warmed at the usual rate of about three degrees per minute, using nitrogen gas whose temperature was controled by a variable temperature unit (Bruker, B-ST 100/700). The freezing-thawing processes were measured by a broad-line pulsed NMR spectrometer (Bruker, minispec p 20) with the proton Larmor frequency of 20 MHz and a chromel-constantan thermocouple which was immersed in a sample and connected with a DC amp. The signal values at intervals of two μs of the free induction decay (FID) curve after 90° pulse

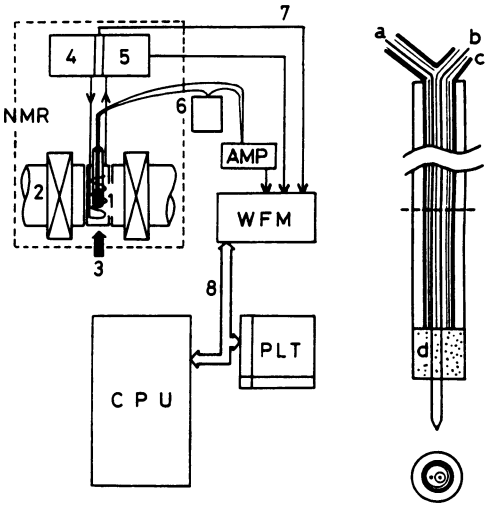

Figure 1. Block diagram of the system (left) and design of the thermocouple (right). NMR, AMP, WFM, PLT, CPU: see the text. 1: sample, 2: magnet, 3: nitrogen gas, 4: transmitter, 5: receiver, 6: thermocouple and cold junction of ice, 7: sampling trigger, 8: HP-IB interface bus. a,b: chromel-constantan, c: aluminum, d: thermal insulater, and all tubes are made of teflon.

and the sample temperature were A/D converted and memorized at every few seconds by a two channel wave form memory (WFM) apparatus (Kikusui, 8702S). The data were transferred to a minicomputer (CPU) system (YHP, HP 1000) by an HP-IB interface bus.

The right side of Figure 1 shows the design of the thermocouple. The WFM apparatus and the NMR spectrometer generates the electric clock noise, which makes it impossible to detect the FID curve because the thermocouple works as an antenna. For shielding this noise, aluminum metal which was grounded was used. A thermal insulater was necessary to reduced the heat conduction to the sample through the aluminum metal.

The following calculations were carried out in FORTRAN language. The real FID curves were obtained after calibrating the sensitivity of NMR using fifteen standards of cupric sulfate solutions in water-heavy water mixture with various proton concentrations and the same sample volume. This calibration was required as there was not linear relationship between proton content and the signal intensity. On the measurement of the FID curves, it generally occurs that a sample exhibits at least two different spin-spin relaxation times T_{2s} and $T_{2\ell}$, which correspond to solid (short T_2) and liquid (long T_2) state protons, respectively. The shape of the FID curve of liquid state protons is exponential, but that of solid state protons is Gaussian. As the population of liquid state protons, which was obtained by extrapolating the FID curve from 40 to 100 μs back to zero time,[3] was approximately equal to the liquid water content, it was reduced to UFW content after calibrating the Boltzmann effect. For practical sake, the UFW content was converted to a dry basis, i.e. gram water per gram dry matter ($gH_2O/gD.M.$). The UFW content was plotted as a function of temperature on semilogarithmic graph by an HP 9872A graphic plotter (PLT). This function was named the freezing curve (or the liquid content curve). At the same time, other temperature dependent curves, namely the solid content, T_{2s}, and $T_{2\ell}$ curves, can be obtained in the similar way.

Figure 2 shows the freezing curves of gelatin and waxy corn starch gels. There are remarkable hysteresis loops, or the large differences between freezing and thawing processes in the temperature range above -30°C. In the freezing process, water molecules are removed from gel networks and reduced to ice crystal, which does not melt below 0°C. For this reason, freezing-thawing hysteresis occurs. In many cases, $T_{2\ell}$ and solid content curves also have hysteresis loops. On the contrary, in the aqueous solutions of methanol, ethanol, glycerol, and oligosaccharides, hysteresis loops were not observed. This is due to the smoothness of rehydration or the limitless solubility in water. And the freezing curve of glycogen gel, whose hydrophilicity is much larger than that of starch, has no significant hysteresis. From these results, it can be said that the phase separation results in the freezing hysteresis.[6] The freezing-thawing procedure accelerrated the degradation or retrogradation of gels and the hysteresis loops become smaller.

Figure 3 shows the thawing processes of aqueous solutions of several saccharides. For the purpose of observing only the solute protons, the freezing curves of the deuterium oxide solutions were measured at the same concentration, and it was suggested that the solute protons appear at Y point (in the case of glucose; and at similar points for other saccharides). Hence it is thought that only the

UFW protons contribute to the freezing curve between X and Y points. At Y point, the $T_{2\ell}$ value begins to decrease due to the interaction between the UFW and solute protons. And at Z point, the solid content begins to decrease rapidly. From these facts, it was suggested that the molecular motions are activated in the following order; at first the hydrated or tightly-bound water molecules (X point), and secondly the solute molecules (Y point), and at last the ice-like or loosely-bound water molecules (Z point).

Figure 3 shows that each curve has a shoulder (glucose) or a plateau (other saccharides) between X and Y points. If each hydroxyl group has one hydrated or tightly-bound water molecule, the UFW content expected to be between 0.33 (polysaccharides) and 0.50 $gH_2O/gD.M.$ (glucose). It is recognized from the figure that these materials have lower hydrated water content than 0.33 $gH_2O/gD.M.$, except for waxy corn starch. But near Z point, each oligosaccharide curve has a shoulder, and the real content of UFW, which is calculated by subtracting the contribution of the solute protons, is at least 0.50 $gH_2O/gD.M.$

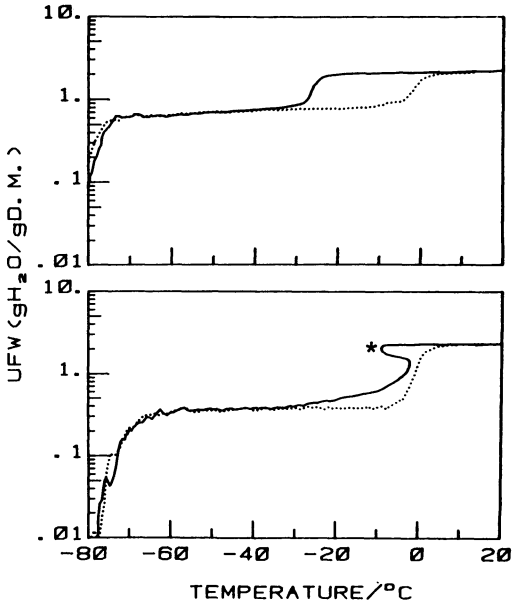

Figure 2. The freezing (———) and thawing (.....) processes of gels after heating at 90°C for three minutes; 35% gelatin (upper), and 37% waxy corn starch (lower). The symbol * denotes the supercooling.

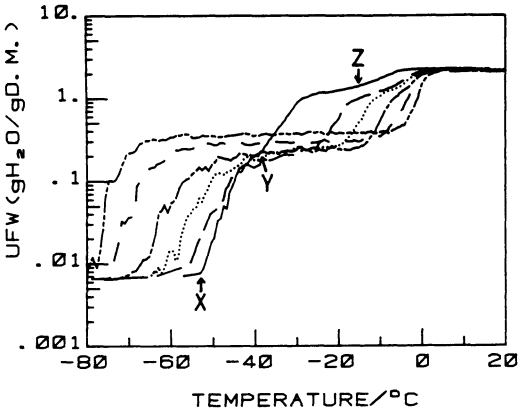

Figure 3. The thawing processes of 40% saccharide aqueous solutions; glucose (———), maltose (—— ——), maltotriose (.....), maltopentaose (— - —), glycogen (— —), and gelatinized waxy corn starch (— - - —). X,Y,Z: see the text.

The following mechanism of the cluster formation in the freezing process is suggested from these results. Solute and hydrated water molecules are clustered by freezing of loosely-bound water (Z point). After reversible dehydration of hydrated water molecules near the surfaces of clusters, or an increase of concentration, solute molecules form hydrogen bonds with each other and hold hydrated water in common (Y point). Each cluster is solidified as a whole in the ice matrix (X point). The inverse process is the thawing. This suggestion is confirmed by the measurements at other cooling rates and concentrations. It can be said that the molecular size has an apparent relationship to the temperatures of X, Y, and Z points.

Figure 4 shows the freezing curve of mannitol aqueous solution. Refreezing takes place at about -22°C in the thawing process after cooling at the rate of six

degrees per minute. By measuring the deuterium oxide solution at the same concentration, it was shown that such a peak was negligible. This suggests that the peak comes from the refreezing of hydrated water molecules after beginning of molecular motions, and at that time the separation into two phases of solute and ice crystal takes place. But in the cooling process at the rate of one degree per minute or more slowly, the solute crystalized out at about -20°C, and such a peak was not observed as the phase separation had been completed in the cooling process. Immersing the tube of sample directly in liquid nitrogen, the sample was frozen rapidly and the height of the peak was reduced to one third of that of Figure 4. It is thought that solutes are in the state similar to that of a matrix. Freeze-drying after rapid freezing gave high solubility powder of this alcohol.

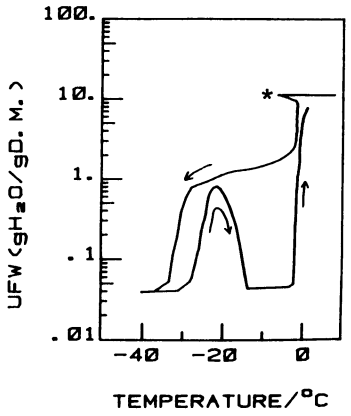

Figure 4. The freezing curve of 9% mannitol aqueous solution. Arrows indicate the process of change, and the symbol * denotes the supercooling.

As shown above, the freezing curve method is a powerful tool for studying the states of water in frozen solutions. One of the advantages of the freezing curve is its close relation to the properties of foods such as water activity and water holding capacity, so this method can be used for characterization and classification of foods and food stuffs, and clarification of cooking processes, and storage and drying techniques. This method will be also applied to studies of intermolecular interactions in biological systems and pharmaceutical science.

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