Ultrasonic Velocity and the Gel/Sol Transition for Differently Irradiated Gelatins

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Dedicated to Jannik Bjerrum on the occasion of his 70th birthday

The ultrasonic technique has been extended in a way that permits melting points and melting intervals for the gel/sol phase transition in diluted gelatin gels to be investigated. The method has been used in the investigation of two different gelatins with the same mechanical properties. The effect of γ-irradiation and accelerator irradiation on the two gelatins has furthermore been studied and a physico-chemical treatment of the data has been carried out with special emphasis on the melting enthalpies for the systems in question.

The effect of high energy radiation on gelatin has been intensively investigated mainly due to the importance of the material in food, film, and the pharmaceutical industry.[1] It is well known that the characteristics of the gelatin and the radiation, the dosage and the experimental conditions, have a vast influence on the obtained results but the mode of this influence is not clearly resolved. This situation is probably responsible for the fact that in the literature the effect of high energy radiation on gelatin appears to be contradictory, some works suggesting cross-linking[2] and other works suggesting degradation.[3] It seems likely, however, that the radiation effect is caused by a highly complex process which involves free radicals that may cause intermolecular cross-linking and peptide bond hydrolysis simultaneously. γ-Irradiation of dry gelatin using a ^{60}Co source does not change the viscosity or the melting point of the gelatin[4] whereas γ-irradiation on gelatin/water mixtures causes a change in those parameters[5] indicating that water molecules play a significant role.

A decrease in the viscosity and in the gel melting point is generally attributed to a decrease in the average molecular weight of the protein molecules. It is important to notice, however, that the melting point of the gel depends strongly on its thermal history. For instance, the melting point of a gel formed by slow cooling of a sol is different from that of a gel formed by quick chilling of the same sol. Consequently, the experimental conditions for the entire working procedure must be carefully specified in order for the obtained melting points to be comparable. Furthermore, it is necessary empirically to define the phase transition temperature. For instance, the melting point may be defined as the temperature at which a specified Neoprene ball inserted under the gel surface reaches the bottom of a tube of a gel that is heated by a certain number of degrees per hour,[6] or as the temperature at which the gel softens sufficiently to allow a drop of coloured Carbon Tetrachloride to sink through.[7] Although the gel melting pont, determined by either of these methods is arbitrary, it provides a useful guide to the changes in the structure that may arise in the gel. Of course, a measurement that gives a single value for the melting point, although a melting interval exists, only provides information about the strength of the last bonds that maintain the network throughout the system. Weaker links are presumably broken at lower temperature and probably even stronger links remain in the aggregates which persist at temperatures slightly above the quoted melting point. An experimental method that makes it possible to monitor very small changes in the gel structure over the entire melting

interval might provide progress in the understanding of the sol/gel transition in gelatin. Although the ultrasonic velocity has proved to be a useful tool for investigating polymer structure[10] it has not yet been used for investigations of the gel/sol transition temperature. This paper presents, for the first time, such an experiment and it is based on the ultrasound velocity at 4 MHz.

A serious problem concerning work on gelatin deals with the characterization of the sample. Modern work is often based on a gelatin specifically prepared in a way that makes the material well defined to the extent possible as far as the molecular weight distribution and composition are concerned. This work deals with two different commercially available gelatins, A and B, with the only basic specifications that they have identical rigidity or mechanical properties (Bloom factors). The work is based on changes in the unknown molecular weight distribution produced by means of controlled irradiation. Different doses of γ-irradiation from a ^{60}Co source are used for this purpose. Furthermore accelerator irradiation is used in order to evaluate possible differences between uncharged and charged irradiation.

The powdered gelatin was obtained from Nordisk Gelatine, Slangerup, Denmark with the specifications that gelatin A is prepared from pig skin and bone by acid extraction and gelatin B is prepared from calf skin and bone by basic extraction. The Bloom strength for both gelatins is 225.

The sol was made up from the powdered gelatin by adding cold water and then heating slowly to 60 °C. The pH of all the samples was 5 ± 0.5. The solution was placed in the measuring cell and kept at 55 °C for 4 h. The cooling rate was 17.5 degrees per hour. The system was kept at 10 °C for 7 h and was then heated at the heating rate of 17.5 °C/h. The system was monitored during the heating period only. Experiments involving the cooling period as well show the characteristic hysteresis behaviour resulting in considerably less reproducible values of the gel setting point. Each gelatin probe was investigated in 3 concentrations (2.0, 3.5, and 5.0 % w/w) and each experiment was repeated twice giving rise to the uncertainties given in Table 1.

The γ-irradiation using a ^{60}Co source and the accelerator irradiation were carried out at RISØ, Roskilde, Denmark. Before irradiation the powdered gelatin was equilibrated over saturated $NaNO_2$ solution at 25 °C for 12 h corresponding to an atmosphere with 64 % water content.

EXPERIMENTAL

The sound velocity was measured by means of the sing around technique. In principle the sample is kept in a cylindrical cell, the ends of which are sealed with piezoelectrical quartz crystals. By means of an oscillator the sending crystal is excited by a radio frequency signal of 4 MHz for a duration time of 3 μs. The resulting sound wave produced in the system is detected by the other crystal and the created voltage triggers the oscillator to produce a second frequency signal across the sending crystal and so on. Thus the pulse repetition frequency in the circuit is related to the velocity of the sound wave that travels through the sample. The equipment is a modified NUS − Sonic Solution Monitor. The modification involves an attachment that makes it possible for the oscillator successively to operate two different cells and to print the pulse repetition frequency as well as the temperature inside the cells with convenient time intervals. The change in temperature is controlled by a synchronous motor that drives the contact thermometer of the thermostat. The following additional equipment is used: frequency counter (Philips PM 6630), digital printer (Philips PM 2466) and digital thermometer (Doric).

THEORY

The sound velocity, u, is given by eqn. (1)

$$u^2 = 1/(\rho\beta) \tag{1}$$

where ρ and β denote the density and the adiabatic compressibility of the system, respectively.

The inflection point of the plot of sound velocity vs. temperature may be regarded as the average melting temperature of the gel whereas the further curvature shows a melting interval, indicating structural changes at slightly lower as well as at slightly higher temperatures.

The enthalpy change for the gel/sol transition is related to the dependence of the average melting temperature, T_g, on the concentration of gelatin. The following equation[11] may be derived on the basis of several approximations that are only fulfilled if small concentration variations are involved below about 5 % overall concentration:

$$d(\ln c)/dT_g = \Delta H°/(RT_g^2) \tag{2}$$

c and T_g being the concentration of gelatin (w/w) and the temperature in Kelvin degrees, respectively.

The enthalpy change is that of the cross-linking process provided that the links holding the gel together result mainly from a binary association of polymer chains. Thus $\Delta H°$ represents the heat of reaction for the process:

1 mol cross-links → 2 mol cross-linking loci
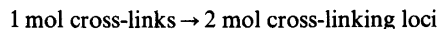
RESULTS

Fig. 1 shows a typical plot of the sound velocity vs. temperature when the matured gel is heated following the above described procedure. The curvature clearly indicates a melting interval. The data obtained from the different gelatins are given in Table 1. Table 2 gives the melting enthalpies for the different gelatins calculated on the basis of eqn. (2). All standard deviations are smaller than 0.1 %.

DISCUSSION

The irradiation of powdered gelatin A and B in doses from 2.5 to 10 Mrad causes a decrease in the melting point of the diluted gels prepared from the material after irradiation. The effect of the uncharged γ-irradiation differs slightly from that of the charged accelerator irradiation, the latter giving rise to smaller changes in the melting points. This may be related to different efficiencies as far as
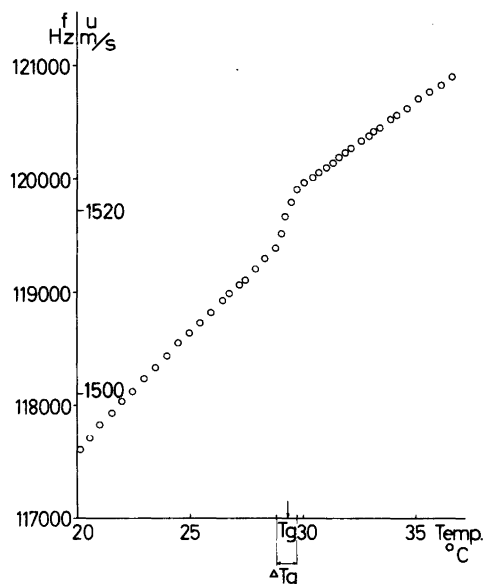

Fig. 1. A typical plot of the sound velocity, u, (and the pulse repetition frequency, f) vs. the temperature for 2.5 Mrad accelerator irradiated gelatin B-gel (3.5 % W/W).

penetration is concerned. The melting intervals, although these are difficult to obtain with satisfactory reproducibility, show a tendency to increase with increasing irradiation dosage indicating that

Table 1. Melting points (°C) and melting intervals for a typical gelatin gel (gelatin B, 3.5 % w/w). The irradiation has been carried out on the powdered gelatin.

	Non irradiated	γ-Irradiated			Accel. irradiated		
Mrad	—	2.5	5.0	10.0	2.5	5.0	10.0
T_g (±0.15)	30.26	28.77	27.96	26.76	29.61	28.89	27.05
ΔT_g (±0.09)	0.63	0.46	0.67	0.98	0.71	0.75	0.76

Table 2. Melting enthalpies for non irradiated and for differently irradiated gelatin A and gelatin B gels.

	$\Delta H°$ kJ per mol of cross-links Non irradiated	γ-Irradiated			Accel. irradiated		
Mrad	—	2.5	5.0	10.0	2.5	5.0	10.0
Gelatin A	309	309	293	207	256	215	287
Gelantin B	250	370	239	222	333	351	170

irradiation may cause a wider range of bond-strengths that maintain the gel network.

The enthalpy change reflecting the cross-link stability shows a vast variation with irradiation although the actual changes are difficult to correlate with the irradiation doses in a simple manner. A decrease in this enthalpy change and hence a decrease in the bond-strength that maintain the gel network may be attributed to a higher content of covalent links introduced in the dry bulk material by the irradiation. Thus further covalently cross-linked gelatin, when dissolved, may produce weaker gels on cooling because these additional links interfere with the freedom of the polypeptide chains to adopt the conformation necessary for the gel structure to be established. The fact that high irradiation doses cause decreasing enthalpy changes supports this point of view. Thus a decrease in the melting point for a diluted gel made up from irradiated material may also reflect that cross-linking has taken place in the bulk material. The general conclusion being that enthalpy changes rather than changes in melting points should be considered and that cross-linking is the dominating process when powdered gelatin is irradiated with high doses (5 – 10 Mrad).

It must be noticed that the two different gelatins, although they have the same Bloom strength, *i.e.* the same mechanical property, and about the same melting temperature show distinct differences as far as melting enthalpies are concerned. This clearly illustrates the need for further physico-chemical information in relation to the characterization of commercially available gelatins. The data furthermore indicate that the two gelatins respond differently on irradiation, and that may be important information, in particular if the gelatin is going to be involved in an irradiation procedure for sterilization or other preparation purposes.

Finally, the point may be stressed that although the melting temperatures and the melting intervals are affected only to a very small extent by irradiation, the values of the melting enthalpies of the diluted gels show a drastic dependence on gelatin type and on irradiation, giving preference to that parameter, if additional physico-chemical characterization of gelatin is desired. The ultrasonic method described in this paper may be a very useful tool for obtaining this information.

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