THE EFFECT OF WATER ON THE PHYSICOCHEMICAL AND MECHANICAL PROPERTIES OF GELATIN

M.-F. Pinhas, J. M. V. Blanshard*, W. Derbyshire and J. R. Mitchell

Department Applied Biochemistry & Food Science School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, England

Abstract

Strips of gelatin have been prepared by extrusion at different water contents varying from 20 to 50% H_2O (dry weight basis, d.w.b.). The processes of subsequent hydration or dehydration of these strips were followed by dynamic mechanical thermal analysis (DMTA), wide-angle X-ray diffraction and NMR relaxation measurements. A comparison of the calculated dependence of the T_g of gelatin (T_g anhydrous, 200°C) on water content (using the Ten Brinke and Karasz equation) with experimental results derived from DMTA showed that freshly extruded material followed the theoretical plot below 25% H_2O (d.w.b.), but at higher water contents, the T_g deviated positively, probably due in part to the effect of delayed re-equilibration of water content after thawing of separated ice crystals. The experimental results determined after storage for one week fell on a different line, with a T_g of 145°C for anhydrous gelatin Possibly, the T_g is elevated by crystallization – a view supported by the WAXS spectra. The NMR relaxation results also showed a profound mobilization of the gelatin protons at water contents greater than 25% d.w.b.

Keywords: crystallization, gelatin, glass transition, mechanical properties, relaxation times

Introduction

In recent years, there has been substantial interest in the glass transition behaviour of biopolymers. The dependence of the glass transition temperature (T_g) of biopolymers on the content of plasticizers, in particular sugar and water, has been investigated [1, 2, 6]. Much of this work has been confined to water contents below 0.2 g H_2O/g dry solid, where the T_g is above the freezing point of water. This paper reports the behaviour of gelatin–water systems, including T_g and molecular mobility over the 9–50% H_2O d.w.b. range; another paper concerning the gelatin–sugar–water system at water contents in the range 20–50% (d.w.b.) is in preparation.

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* Author to whom all correspondence should be addressed.

Gelatin is extracted after partial hydrolysis of collagenous raw material from the skin, connective tissues and bones of animals. It is characterized by a unique composition and sequence of amino acids and possesses a number of distinctive functional properties that make it an important ingredient in many foods, including food confectionery products [3, 4].

The purpose of this investigation was to explore the relationships between molecular mobility, order, and the dynamic mechanical properties of gelatin. Information about changes in the dynamic mechanical modulus and loss factor as a function of temperature and moisture content was obtained by dynamic mechanical thermal analysis (DMTA). Order within the system was monitored by X-ray diffraction, and the order and mobility of molecules by NMR relaxation. Homogeneous samples were prepared by extrusion.

Materials and methods

Gelatin

The gelatin was provided by Sanofi Bio Industries (225 bloom limed gelatin, batch ABN 1084), in the form of granules dried to about 12% moisture content, and used as received.

Extrusion of gelatin

Gelatin granules were extruded using a Clextral BS-2I co-rotating, inter-meshing, twin-screw extruder. Extrusion conditions were a barrel of 400 mm length, a screw speed of 200 rpm, and four electrically heated zones at 40°C, 90, 110 and 80°C. A slit die was fitted with an aperture of 0.7 mm width and 30 mm height.

Through variation of the settings on the injection pump, it was possible to prepare samples with a range of water contents from 20 to 50% (d.w.b.). Some samples were used directly after extrusion. Those samples with the extreme levels of water were stored for one week in six relative humidity (RH) boxes (covering the RH range 59–100%), with the aim of following the hydration or dehydration of the gelatin and comparing the results obtained for such samples (moisture range, 9–50% d.w.b.) with those for samples studied immediately after extrusion. One particular concern was to determine whether the physical properties were dependent upon the hydration history of the sample.

DMTA

A dynamic mechanical thermal analyser, Mark I (Polymer Laboratories), was used in the single-cantilever bending mode, at a frequency of 1 Hz and a strain setting of ×1. This strain corresponded to a nominal peak-to-peak dis-

placement of 16 μm. Ribbons of extruded gelatin were cut into 8×20 mm strips. The samples were heated from –50 to 150°C at 5°C min⁻¹. The storage (E') or loss (E'') modulus and loss factor (tan δ) were recorded and plotted against temperature, for further evaluation of the change in the moduli with temperature.

Wide-angle X-ray scattering (WAXS)

X-ray diffraction data were collected on a Philips (APD-15 system) diffractometer, using CuKα radiation (λ=1.5418 Ĺ) over the range, 2θ=4°–38°.

Relaxation time measurements

[1]H free-induction decays (FID) and relaxation-time measurements (spin-spin and spin lattice relaxation times) were performed on a Bruker Minispec PC 120 (20 MHz) operating at 40°C. The FID was obtained by recording the transient signal generated by a single excitation pulse. The spin-lattice relaxation was recorded using the inversion recovery procedure, and the spin-spin relaxation by a Carr-Purcell-Meiboom-Gill procedure [5], with a τ spacing of 262 ms between the 90° and 180° pulses.

Results and discussion

Extrusion

Homogeneous, continuous and clear strips of extrudate were produced. With the exception of the sample with the lowest moisture content, no bubbles were produced, presumably because the temperature of the sample at the die exit was below 100°C. No measurements were made on the sample containing bubbles.

DMTA

The glass transition was studied by observing the storage modulus, E', and tanδ as functions of temperature. In synthetic polymers, the position of the tanδ peak (measured at 1 Hz) is used as a convenient definition of T_g [1]; tanδ normally falls at a slightly higher temperature than that of the midpoint of the decrease in E'. Figure 1 shows tanδ as a function of temperature for directly extruded gelatin samples of different moisture contents. An identical form of the dependence of the shape of the curve on moisture content was found for samples that had been dehydrated or hydrated to the desired moisture content following extrusion. The tanδ curve shows a single and quite sharp peak (at low moisture contents). The peak decreases in intensity, shifts to lower temperature and broadens with increasing water content. A shoulder appears, which at the highest water content, can be clearly resolved into two different peaks. It is suggested that the lower temperature peak corresponds to the melting of ice,
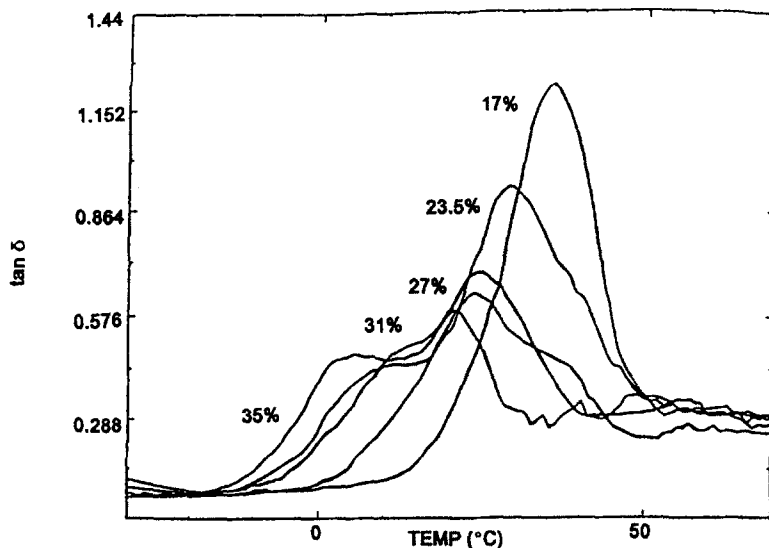
Fig. 1 DMTA loss factor, tanδ, as a function of temperature for extruded gelatin samples of different moisture contents (numbers represent water contents, % d.w.b.)

while the higher temperature peak is related to the glass transition of gelatin. When a high-water-content system is cooled to –50°C at the beginning of the DMTA experiment, ice is formed. When the temperature is increased, any ice crystals that have formed will melt (~0°C, first peak). The dependence of the position of the second tanδ peak on water content suggests that the water released as a result of ice melting does not, within the time scale of the DMTA experiment, diffuse and fully equilibrate through the sample to uniformly plasticize the gelatin [6]. In consequence, the amount of water actually interacting with the gelatin and playing the role of plasticizer [7] is somewhat less than expected theoretically. This provides a plausible explanation of why the T_g of the system, as measured by DMTA, deviates positively from the theoretical line at water contents≥27% (w/w d.w.b.).

Figure 2 shows logE' as a function of temperature for extruded gelatin samples of different moisture contents. All the curves show a decrease over the same temperature range as that of the tanδ peak, and all show an increase at temperatures above those corresponding to tanδ. Such an increase has previously been described [8]. The gelatin ribbons clearly became stiffer, but the origin of the effect is uncertain. A possible explanation could involve some loss of moisture while the sample passed through the glass-rubber transition, though previous studies with starch strips have shown minimal loss of water on passage through T_g below 60°C. The T_g values corresponding to the change in the slope of E' and to the tanδ peak are plotted versus moisture content in Fig. 3. It might
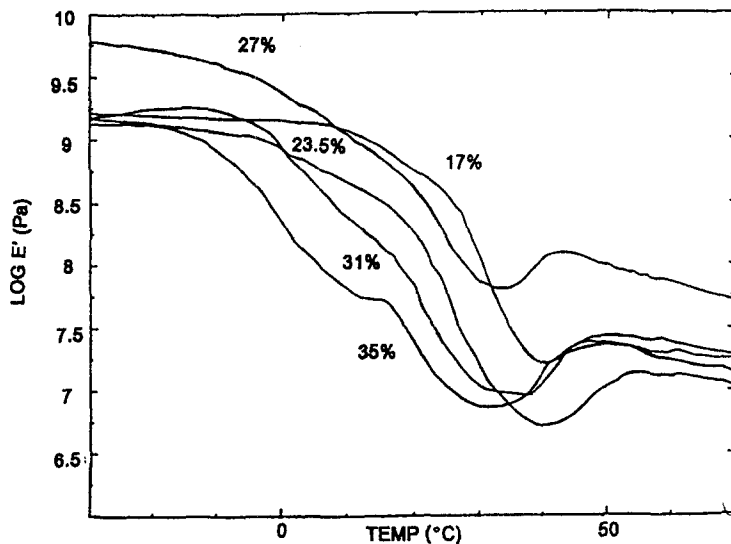
Fig. 2 Storage modulus (logE') as a function of temperature for extruded gelatin samples of different moisture contents (numbers represent water contents, % d.w.b.)
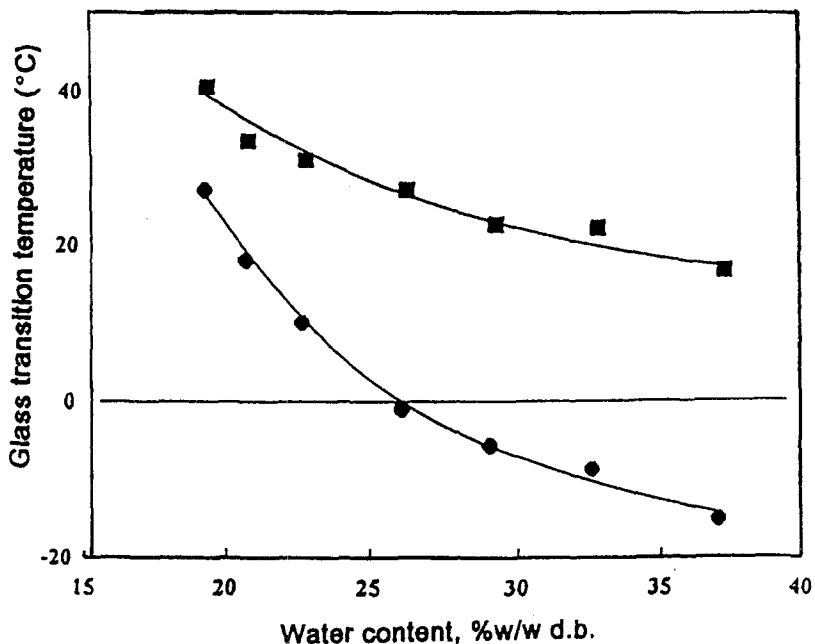

Fig. 3 T_g values corresponding to tanδ (■) and the drop in logE' (●), plotted as a function of water content

be expected that the temperature difference between the two mechanical phenomena would increase with increasing moisture content. There is some evidence (Fig. 2) that at high water contents, the change in E' starts at a temperature below that expected for ice melting. There could, therefore, be a component of this change in E' that is associated with a T_g' transition within the freeze-concentrated gelatin matrix [7]. If we assume that the T_g' of gelatin–water systems fals in the region of –5 to –15°C [9], then we would expect that some of the water released from the ice phase might diffuse into the gelatin matrix [10].

Although, superficially, the form of the tanδ curve did not seem to depend on the hydration history of the samples, i.e. whether the samples were measured at the extrusion moisture content or dehydrated and then subsequently measured (the appearance of a second peak was observed in both curves at water contents of 27% and above), the temperature of the tanδ peak was higher for the dehydrated samples (Fig. 2). A possible interpretation is that the degree of ordering of the gelatin in the two systems was different. This possibility was investigated by X-ray diffraction (see below).

There are several equations relating the T_g of binary and ternary mixtures to the properties of the components. We analyzed the results in terms of the equation developed by Couchman and Karasz [11].

$$\ln T_g = \frac{\Sigma w_i \Delta C_{p_i} \ln T_{g_i}}{\Sigma w_i \Delta C_{p_i}} \qquad (1)$$

where ΔC_{p_i} is the value of the change in specific heat at T_{g_i} of component i and w_i is the weight-fraction of component i. The T_g and ΔC_p of amorphous water have been reported to be 136 K [12] and 1.94 J g^{-1}K^{-1} [13], respectively. (See addendum).

If it is assumed that ΔC_p is proportional to $1/T_{abs}$, the Couchman-Karasz equation may be simplified to the so-called Ten Brinke equation [14], which has the form:

$$T_g = \frac{\Sigma w_i \Delta C_{p_i} T_{g_i}}{\Sigma w_i \Delta C_{p_i}} \qquad (2)$$

This is related to the empirical Gordon-Taylor equation [15], used to account for the effect of water plasticization on T_g of water-plasticizable polymers:

$$T_g = \frac{w_i T_{g_i} + k w_2 T_{g_2}}{w_i + k w_2} \qquad (3)$$

where k is a constant. If $k=\Delta C_{p_2}/\Delta C_{p_1}$, Eq. (3) is equivalent to the Ten Brinke equation for a binary system.

In many cases, the T_g of pure biopolymers is difficult to measure, either due to the progressive unlocking of endothermic relaxation processes [16] or to degradation, and the intrinsic values of ΔC_p and T_g have to be estimated. The ΔC_p of amorphous gelatin has been reported [17] as $0.50 \pm 0.05 \ J \ g^{-1}(gK)^{-1}$.

Figure 4 compares the experimental T_g values obtained for the directly extruded samples with those for dehydrated or hydrated samples. Both types of samples behaved similarly, i.e. T_g decreases with increasing moisture content. The T_g values are different for lower moisture contents but become comparable at higher moisture contents. The T_g for the anhydrous polymer was determined from the best fit of the low moisture results, in the case of the dehydrated samples giving a value of $T_g = 147°C$ (solid curve, Fig. 4). This value is very different from the estimated value of 200°C. The dashed line, which is the fit of the experimental values for the directly extruded samples, gives for gelatin a T_g of 203°C and a ΔC_g of 0.45 $J/(gK)^{-1}$, but, as can be noticed, the fitted curve only matches the first few points, and it was not possible to obtain samples without bubbles at the lower moisture contents. Above 27% water, the T_g (or T_g') remains almost constant, even when the moisture content is increased; this point has already been discussed. In summary, the Ten Brinke equation [14] provides a good fit for the experimental DMTA values at low water contents. At higher
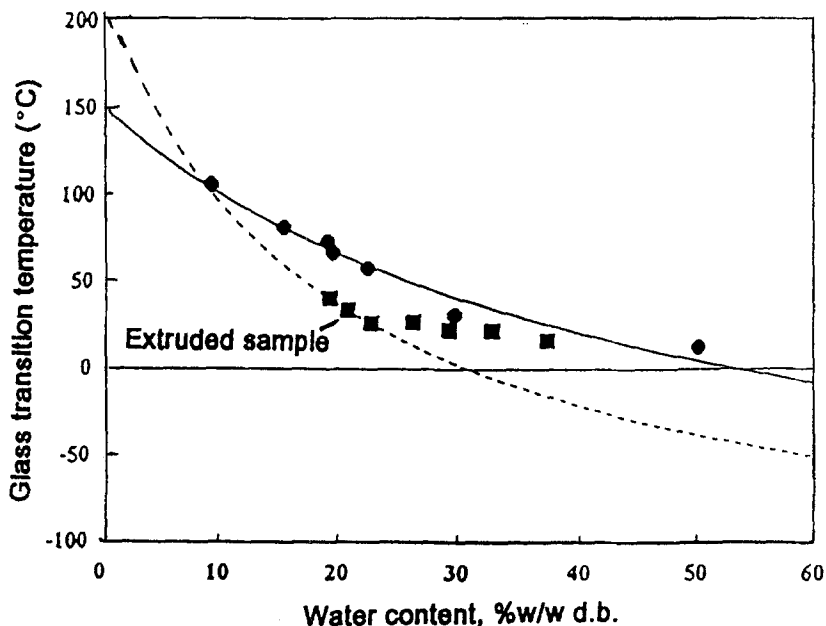

Fig. 4 T_g values corresponding to tanδ, plotted as a function of water content: experimental points for hydrated or dehydrated gelatin (●); experimental points for directly extruded gelatin (■)

water contents, due to phase separation within the system, it is not possible to obtain the T_g of the homogeneous mixture.

WAXS

Figure 5 illustrates the WAXS patterns of gelatin ribbons extruded at different water contents. All samples showed a broad amorphous peak between $2\theta = 16°$ and $28°$. At higher water contents, obtained either directly by extrusion or by hydration, a comparatively strong peak at $2\theta = 7°$ and a weaker one at $2\theta = 31°$ were observed. The peak at $2\theta = 7°$ is due to the repeat of the triple-helical protofibril in the direction perpendicular to the fibre axis of a junction zone [18, 19, 21]. According to Bragg's law ($n\lambda = 2d \sin\theta$), this peak corresponds to a repeat distance of 1.26 nm. The peak at $2\theta = 31°$ is probably derived from the amino-acid residues in the junction zones, at a repeat distance of 0.29 nm [18]. In contrast to our results, Itoh et al. [18] observed far more intense peaks at $7°$ and $31°$, possibly due to their samples being aged initially at a higher water content and only afterwards being dried to 10% moisture content.
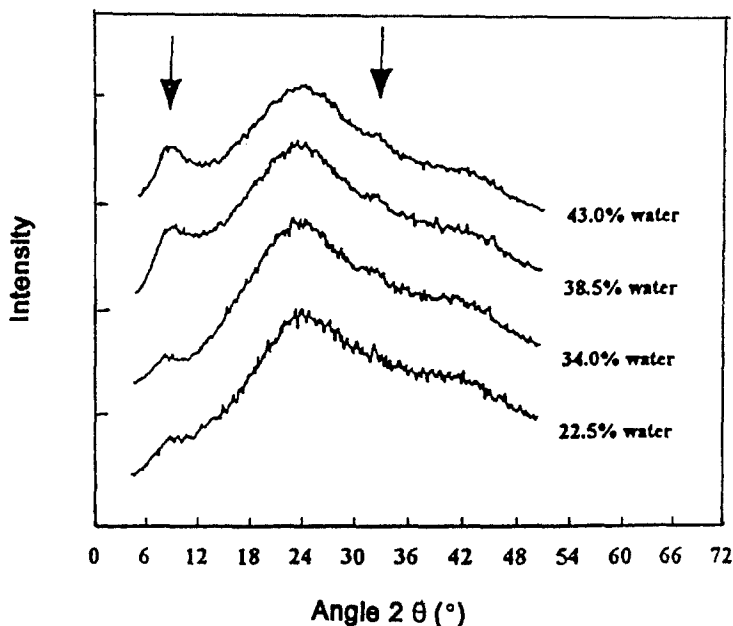

Fig. 5 WAXS patterns of gelatin ribbons extruded at different water contents

Figure 6 shows the WAXS patterns for gelatin samples after storage for one week in RH boxes. Figure 7 shows a comparison of two samples with similar moisture contents (22.5% d.b.), one just after extrusion and the other after one week of storage in an RH box. The effect of hydration or dehydration of ex-
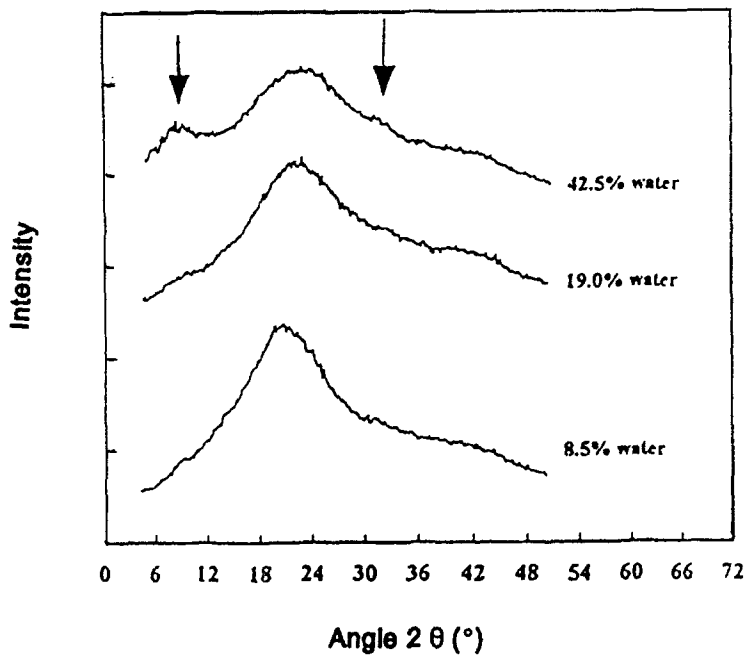
Fig. 6 WAXS patterns of gelatin after one week of storage in RH boxes, starting from a sample with 22.5% water d.w.b.
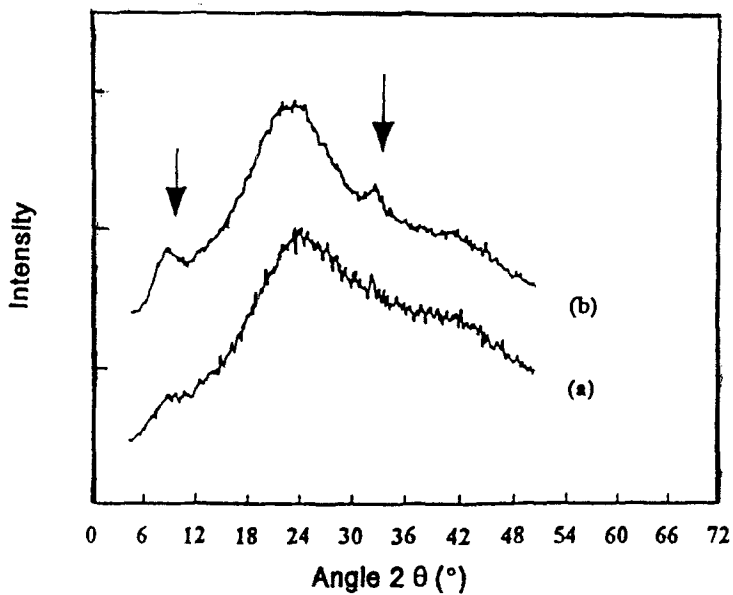

Fig. 7 Comparison of the WAXS patterns of two samples with similar water content (22.5% d.w.b.); (a) just after extrusion: no peak at $2\theta=31°$; (b) after extrusion and one week of storage in an RH box: one peak at $2\theta=31°$

truded samples was studied by placing samples in RH boxes. Hydration led to improved alignment of fibrils, due to expansion of the gel matrix and, consequently, greater molecular mobility, as found also by Pezron and Djabourov [20]. At higher water contents, some of the water can be frozen, i.e. it behaves as free water, as shown by the DMTA results.

When the gel is dried, the triple helices are compressed into groups or regions, probably resulting in their partial disorientation. In summary, it may be stated that at low water content, there is no order nor mobility of the chains within the system. At intermediate water content, there is some mobility and some order. At high moisture content, there is some order but no net orientation.

Relaxation-time measurements

NMR relaxation times are normally relatable to the mobilities of molecules residues containing the resonant nuclei. The amplitudes of the decay signals are related to their concentration. Information on the mobilisation of gelatin can be obtained from measurements of the spin-spin relaxation time (T_2) and spin-lattice relaxation time (T_1), which record different motional rates, slow (a few kHz) and rapid (at 20 MHz), respectively.

T_2 measurements

T_2 values were measured to assess the changes in biopolymer mobility with increased hydration. The T_2 for the solid component of the NMR signal, attributed to gelatin, increased as the water content was increased, probably because the plasticizing effect of water induced a greater mobility of the solid constituents [7]. Figure 8 shows the FID T_2 of the solid and liquid components as a function of water content. The liquid FID T_2 displays a sharp increase of 38% moisture. Figure 9 shows the normalised liquid/solid ratio compared to the expected curve, if the solid NMR signal refers to gelatin and the liquid to water. The solid amplitude (A_s) is proportional to $\rho_G G$, and the liquid amplitude (A_L) is proportional to $\rho_w W$, where ρ_i is the proton density of i, while G and W are the weights of gelatin and water, respectively. The values of ρ_i were 0.1111 for water and 0.0642 for gelatin; ρ for gelatin was calculated by taking into account the percentage of each amino acid in the gelatin [4, 21].

The predicted curve is a straight line with a slope equal to ρ_w/ρ_G. The ratio seems to follow the expected line up to 30% water content. Above 30%, a severe deviation occurs, consistent with a contribution by parts of the gelatin to the liquid signal. At high moisture contents, the spin-echo and liquid FID T_2 [5] signals probably contain contributions from the exchangeable protons of both water and the hydroxyl groups of gelatin. Mobilisation of gelatin occurs at a slightly higher water content than does the departure of the liquid/solid amplitude from the values expected if all the gelatin contributes to the solid signal.
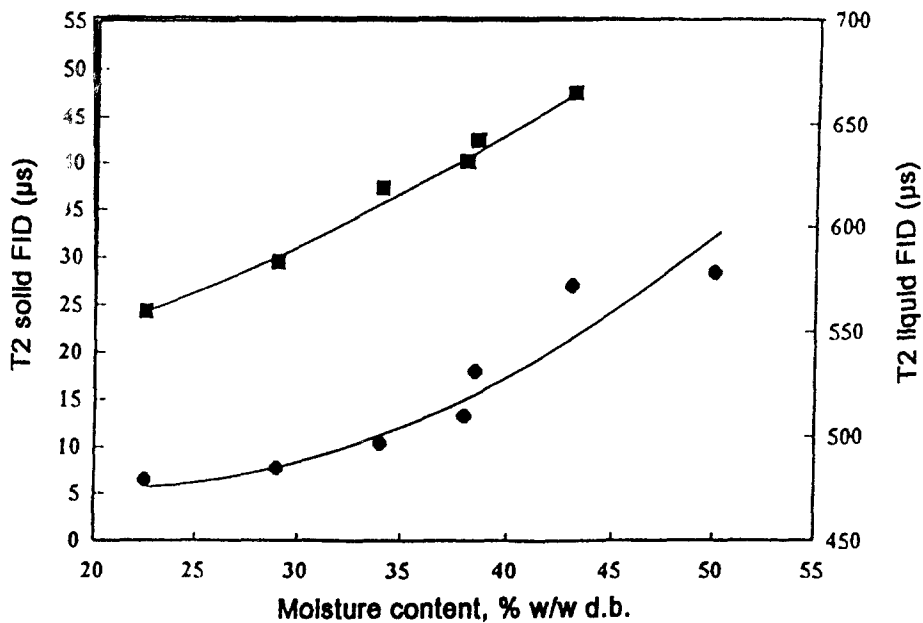
Fig. 8 Spin-spin relaxation times of the solid (■) and liquid (●) FID components, as a function of water content (20 MHz, 40°C)
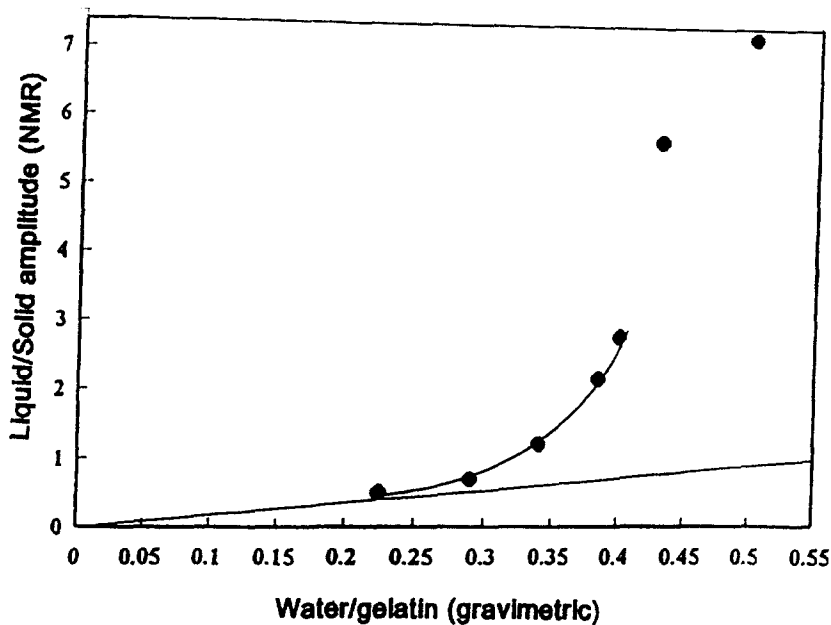

Fig. 9 Normalised liquid/solid amplitude of extruded gelatin as a function of water content: experimental values (●); predicted curve (–)

T_1 measurements

T_1 data were recorded by the inversion-recovery technique (180° pulse – delay (τ) – 90° pulse) [5]. Signals were acquired at 11 and 71 μs, the signal at 11 μs representing contributions from the solid and liquid, and the 71 μs signal corresponding to the liquid only. The solid signal was obtained by subtraction. Both the solid and liquid T_1 values are plotted in Fig. 10. At low water contents, the T_1 values obtained are similar for solid and liquid, strongly suggesting an effective spin-exchange mechanism. The extruded sample shows a minimum, probably corresponding to a broad distribution of molecular motions [16] and also to the presence of different amino acid sequences in the gelatin.
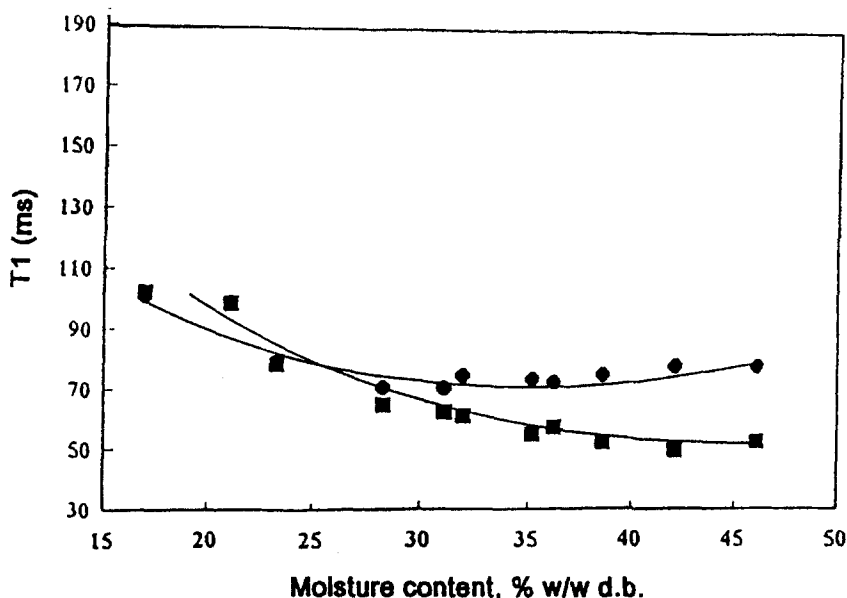

Fig. 10 Spin-lattice relaxation times of extruded gelatin as a function of water content: solid component (■) (recorded at 11 μs); liquid component (●) (recorded at 71 μs)

Conclusion

It is evident that the T_g of gelatin is very sensitive to water content, and water has a pronounced plasticizing effect on extruded samples. The T_g values for samples stored in RH boxes after extrusion were slightly different from the T_g values measured for samples of the same moisture content obtained by direct extrusion. There may be a molecular reordering during the hydration or dehydration process, which is indicated by the X-ray results. A future paper will present a study of the effect of sugars on the physicochemical properties (phase separation, solubility and partitioning of water) of gelatin/sugar/water systems.

Addendum

A referee kindly drew the attention of the authors to a paper by G. P. Johari (J. Chem. Phys., 98 (1993) 7324), which states that the ΔC_p associated with the glass-rubber transition of water is 0.089 J $K^{-1}g^{-1}$. However, the use of this figure in the Ten Brinke and Karasz equation, with the established values of $T_{g\ H_2O}$, $T_{g\ gelatin}$ and $\Delta C_{p\ gelatin}$, does not permit the fitting of our experimental data. This is an issue that requires further examination, not only for gelatin, but for other macromolecules.

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