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^{1}H NMR study of amylose films plasticised by glycerol and water

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Abstract

Amylose–glycerol films prepared by casting from solution were hydrated to different water contents. Wide-angle X-ray diffraction analysis suggested that the films were partially crystalline and contained the B polymorph. At a given hydration level, the presence of glycerol did not affect the extent of crystallinity or polymorphism of amylose. Proton NMR relaxometry was used to study the effects of water and glycerol on the molecular mobility of amylose. Spin–spin and spin-lattice relaxation parameters suggested a step change in the mobility of amylose when sufficient plasticiser, water and glycerol, was added to decrease the glass transition temperature (T_g) such that it became equal to or smaller than the observation temperature (T), i.e. $(T - T_g) \geq 0$. The mobility of amylose probed through the properties of the rapidly decaying component of the spin–spin relaxation varied with temperature in an identical fashion for films in the glassy state regardless of their composition. In the rubbery state, the mobility of amylose increased with increasing glycerol and water contents, i.e. with increasing $(T - T_g)$. The mobility of amylose in the glassy state was best assessed through the spin-lattice relaxation times which suggested that at a given $(T - T_g)$, the addition of glycerol leads to a decreased mobility probably through its well documented antiplasticising role. The findings were discussed in relation to documented changes in important physical properties of amylose films such as elongation and gas permeability.

Keywords: Amylose; Molecular mobility; NMR relaxation; Glass transition

1. Introduction

In recent years there has been a considerable amount of research carried out on starch based films with most studies focusing on the behaviour of starch, and particularly amylose, in the presence of water and polyols (Gaudin, Lourdin, Le Botlan, Ilari, & Colonna, 1999; Lourdin, Ring, & Colonna, 1998; Moates, Noel, Parker, & Ring, 2001). The molecular relaxation processes studied using dielectric thermal analysis (DETA) in amylose–water–glycerol mixtures were found to be greatly dependent on glycerol content at concentrations below 20% (Lourdin et al., 1998). These authors suggested that in these systems, the dependence of the β-relaxation on glycerol content indicated that the measured low temperature relaxation was composed of β-motion of amylose and α-motion of glycerol. Above 20% glycerol, phase separation was suggested to occur. Furthermore, binary amylose–glycerol systems were examined by analysing the effect of glycerol on the properties of dry amylose films (Moates et al., 2001). Two separate relaxations were observed on the dynamic thermal mechanical analysis (DMTA), differential scanning calorimetry (DSC) and DETA thermograms of these amylose–glycerol systems. The lower relaxation was assigned to glycerol, based on the value of its activation energy, suggesting that amylose and glycerol are only partially miscible.

In another study, wheat starch was plasticised with water and sorbitol, and the molecular mechanism behind the macroscopic mechanical behaviour was investigated using DMTA and NMR relaxometry (Gaudin et al., 1999). As in the earlier work concerning amylose (Lourdin et al., 1998) the behaviour of wheat starch depended greatly on plasticiser content: below 27% sorbitol (dry basis), the films were brittle while a plastic behaviour was observed at higher concentrations. The authors proposed that their NMR results suggested that increasing sorbitol content up to 33% reduced the overall mobility of the liquid phase in the system. Above 33%, the mobility of the liquid phase

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increased, but remained less than the corresponding starch–water system. The authors concluded that ‘the results show the existence of two competitive effects: on the other hand sorbitol molecules bound to the most mobile parts of starch and were associated with a loss of system mobility, and on the other clusters of sorbitol associated with greater system mobility’.

In previously studies on amylose and amylopectin films in the presence of water and glycerol, the mechanical properties were found to change dramatically when glycerol content exceeded 20% (Myllärinen, Partanen, Seppälä, & Forssell, 2002) while their efficient oxygen barrier properties were lost above 20% water (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002).

Despite the high research activity on starch-glycerol films described above, the mechanism of glycerol plasticisation of hydrated starch films remains relatively unclear. In the present work, ^{1}H relaxometry was used to investigate the effects of water and glycerol on the molecular mobility of amylose with the aim of achieving a better understanding of the plasticisation of amylose by low molecular weight components. This is a process of great importance in future exploitation of starchy materials as described in a comprehensive recent review on starch by Parker and Ring (2001).

2. Materials and methods

2.1. Materials

Amylose was obtained as commercial potato starch isolate (A-05212) from Sigma-Aldrich Finland (Helsinki, Finland). Glycerol (1.04093.1000) was purchased from Merck Eurolab (Espoo, Finland).

2.2. Film preparation

Amylose was solubilised with glycerol (0, 10, 20 and 30% of dry weight) by heating in a pressure chamber to 140 °C. After cooling to 90 °C, the 2% amylose solutions were cast in pre-heated Teflon moulds and dried at 70 °C and RH 50% until films were formed. This was typically achieved within 4 h of drying. The films were ground with liquid nitrogen to avoid heat damage. The powders were then equilibrated for 7 days at 20 °C in RH chambers containing salt solutions: LiCl (RH 12%), $MgCl_2$ (RH 33%), $Mg(NO_3)_2$ (RH 54%), NaCl (RH 75%) and KNO_3 (RH 94%). The water contents of the films were approximated from the water vapour sorption isotherms (20 °C) been obtained previously (Myllärinen et al., 2002).

2.3. ^{1}H NMR measurements

The samples were sealed in 10-mm NMR tubes. The measurements were performed at 20 °C using a Maran NMR spectrometer (Resonance Instruments, UK) operating at a resonance frequency of 23 MHz.

Spin–spin relaxation parameters were obtained from free induction decays (FID) acquired after a 90° pulse (typically 2–3.2 μs). The overall dead time of the spectrometer was ≤9 μs; 8192 data points with a dwell time of 1 μs were acquired. A relaxation delay of 5 s was inserted after each of the 32 scans. The magnitude data were fitted to the following equation (van den Dries, van Dusschoten, & Hemminga, 1998) using MS Excel© Solver:

$$S(t) = A \exp\left[\frac{-a^2t^2}{2}\right]\frac{\sin bt}{bt} + B\exp\left[\frac{-t}{T_{2m}}\right] + \varepsilon \qquad (1)$$

In this equation, the parameters A and B represent the contributions of the immobile and mobile protons, while T_{2m} represents the spin-spin relaxation time of the mobile component and ε is a baseline offset factor. The NMR spectrum of the rigid protons is assumed to be a rectangular line shape with a total width $2b$, convoluted with a Gaussian line shape with a standard deviation given by the parameter a (van den Dries et al., 1998; McBrierty & Packer, 1995).

The second moment, M_2, of the rigid component, which is a measure of the strength of the dipolar interactions, is given by:

$$M_2 = a^2 + \frac{1}{3}b^2 \qquad (2)$$

The spin-lattice relaxation times (T_1) were measured using the inversion recovery pulse sequence. FIDs were acquired at 18 values of 180–90° pulse spacing (τ) ranging from 8–800 ms with the same spectrometer parameters as above. The magnitude of the FID at 10 and 70 μs were selected to monitor the T_1 of the mobile ($S(70\ \mu s)$) and rigid ($S(10\ \mu s)-S(70\ \mu s)$) components. The results of each component were fitted to a single exponential using the following equation:

$$y(\tau) = A - B\exp(-\tau/T_1)) \qquad (3)$$

The rate of cross-relaxation between the rigid (amylose) and the mobile (plasticisers) components were measured using the Goldman-Shen pulse sequence (Goldman & Shen, 1966) modified to cancel T_1 effects (Kenwright & Packer, 1990). A value of 70 μs was used for the pulse spacing (τ_1) between the first and second 90° pulses in order to allow the solid component to fully decay. The spacing between the second and third 90° pulses (τ_2) was varied between 100 and 300 ms. In the original sequence, without the T_1 cancellation, the mobile component was observed to decrease before increasing according to T1 recovery. The initial decay was single exponential.

2.4. Glass transition temperatures

The glass transition temperature (T_g) values used in the ($T - T_g$) plots were calculated using the equation of ten

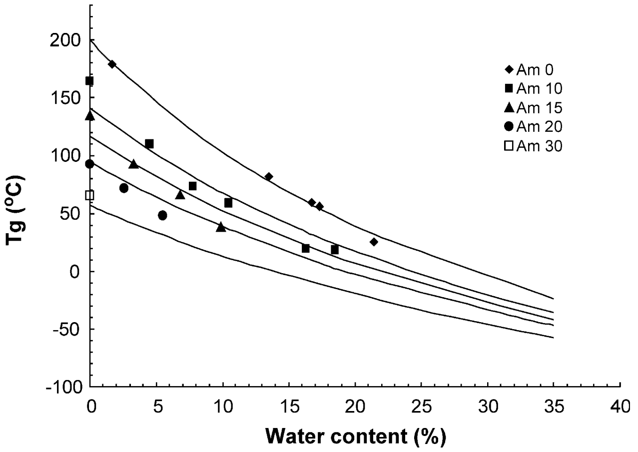

Fig. 1. The effects of water content and glycerol on the DSC measured T_g (adapted from Myllärinen et al., 2002). The lines were calculated using Eq. (4) as described in the text.

Brinke et al. (1983), an extension of Couchman & Karasz, 1978 equation which describes the composition dependence of the T_g of the mixed systems:

$$T_g = \frac{\sum_i W_i \, \Delta Cp_i Tg_i}{\sum_i W_i \, \Delta Cp_i} \qquad (4)$$

where W_i, Tg_i and ΔCp_i refer to the weight fraction, the glass transition temperature and the difference in specific heat capacity between the liquid and the glassy states of each of the components of the mixed system at T_g. For water, the values $T_g = 134$ K and $\Delta C_p = 1.94\ \mathrm{J\,g^{-1}\,K^{-1}}$ (Sugisaki, Suga, & Seki, 1968) were used. The T_g and ΔC_p values for amylose were obtained by fitting Eq. (4) to the T_g versus water content data reported by Myllärinen et al. (2002) for amylose films and were found to be 473 and $0.54\ \mathrm{J\,g^{-1}\,K^{-1}}$, respectively (Fig. 1) while for glycerol, a ΔC_p value of $1.24\ \mathrm{J\,g^{-1}\,K^{-1}}$ was obtained when all the data obtained by the same authors for amylose systems containing 0, 10, 15, 20, 30 and 40% glycerol were fitted simultaneously to Eq. (4) using a T_g value of 185 K for glycerol (Simon, 1922) (Fig. 1). The values of $T_g = 473$ K and $\Delta C_p = 0.54\ \mathrm{J\,g^{-1}\,K^{-1}}$ calculated for amylose are in agreement with those reported by Orford, Parker, Ring, and Smith (1989), where a $T_g = 500 \pm 10$ K and $\Delta C_p = 0.49\ \mathrm{J\,g^{-1}\,K^{-1}}$ were found when analysing the DSC results of a series of saccharides ranging in DP from D-glucose up to amylose and amylopectin.

3. Results

Throughout this paper, amylose films with 0, 10, 20 and 30% glycerol are referred to as Am0, Am10, Am20 and Am30, respectively.

As reported previously, amylose tends to crystallise during the film casting procedure (Myllärinen et al., 2002; Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998). The X-ray diffractograms acquired on the amylose and amylose–glycerol films showed highly crystalline B-polymorph patterns (Fig. 2). Despite this crystallisation phenomenon, the films prepared by casting from an aqueous solution were translucent. For similar water content, the diffractograms showed little dependence on the glycerol concentration. As expected, the diffractograms became more resolved as the moisture content of the films increased by storing at higher relative humidity. This phenomenon was utilised by Hartley, Chevance, Hill, Mitchell, and

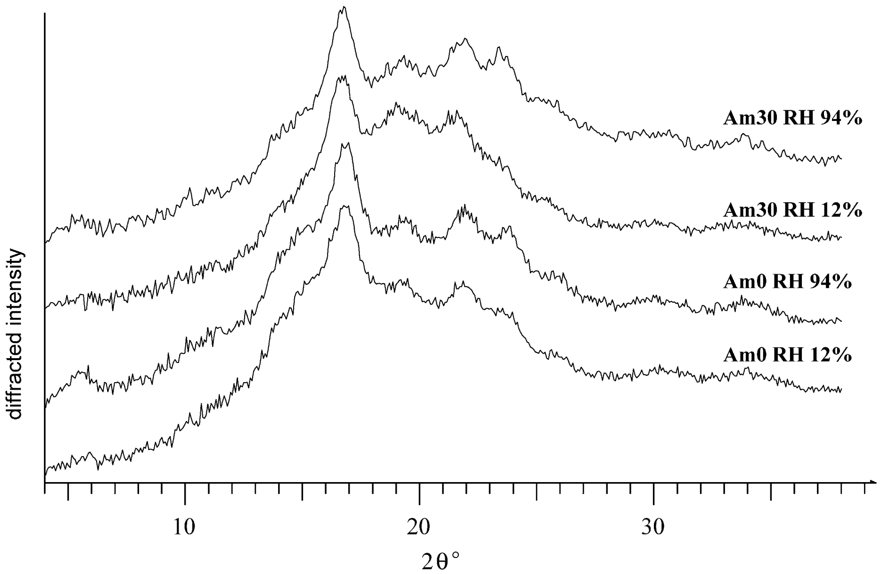

Fig. 2. X-ray powder diffraction patterns of amylose films containing 0 and 30% glycerol equilibrated at 12 and 94% relative humidity. The corresponding water contents for 0% glycerol were 8 and 20%, and for 30% glycerol 2 and 40%.

Blanshard (1995) to monitor the partitioning of water in starch-biopolymer mixtures.

The free induction decays of amylose samples with a range of water and glycerol contents were successfully fitted to Eq. (1). Typical results are shown in Fig. 3. The fast decaying component of the signal is caused by the rigid protons of the binary/ternary systems, whereas the slow decay is due to the mobile protons. The rigid component of the FIDs acquired on all the Am0 samples at 20 °C exhibited a strong beat pattern centred at approximately 50 μs reflecting strong dipolar interactions. Such interactions are very significant in solids and solid-like materials and have been described in many glassy carbohydrate systems e.g. maltose-water (van den Dries et al., 1998), carrageenan-water and carrageenan-glucose syrup-water (Kumagai, MacNaughton, Farhat & Mitchell, 2002), etc. The beat became less pronounced as the water and glycerol contents increased. This reflects the increased molecular mobility of amylose through plasticisation by water. The fact that the beat was observable even at the highest plasticisation level (30% glycerol, RH = 94%) indicated that the mobility of a significant fraction of the amylose protons, most probably those involved in ordered regions, is not significantly increased by water and glycerol plasticisation.

In order to 'quantify' the effects of water content and glycerol on the mobility of amylose, responsible for the rigid component of the FID, the spin-spin relaxation second moment M_2 was calculated by fitting Eq. (1) to the FIDs acquired at different water and glycerol contents. M_2 reflects the strength of dipolar interactions. An increase in

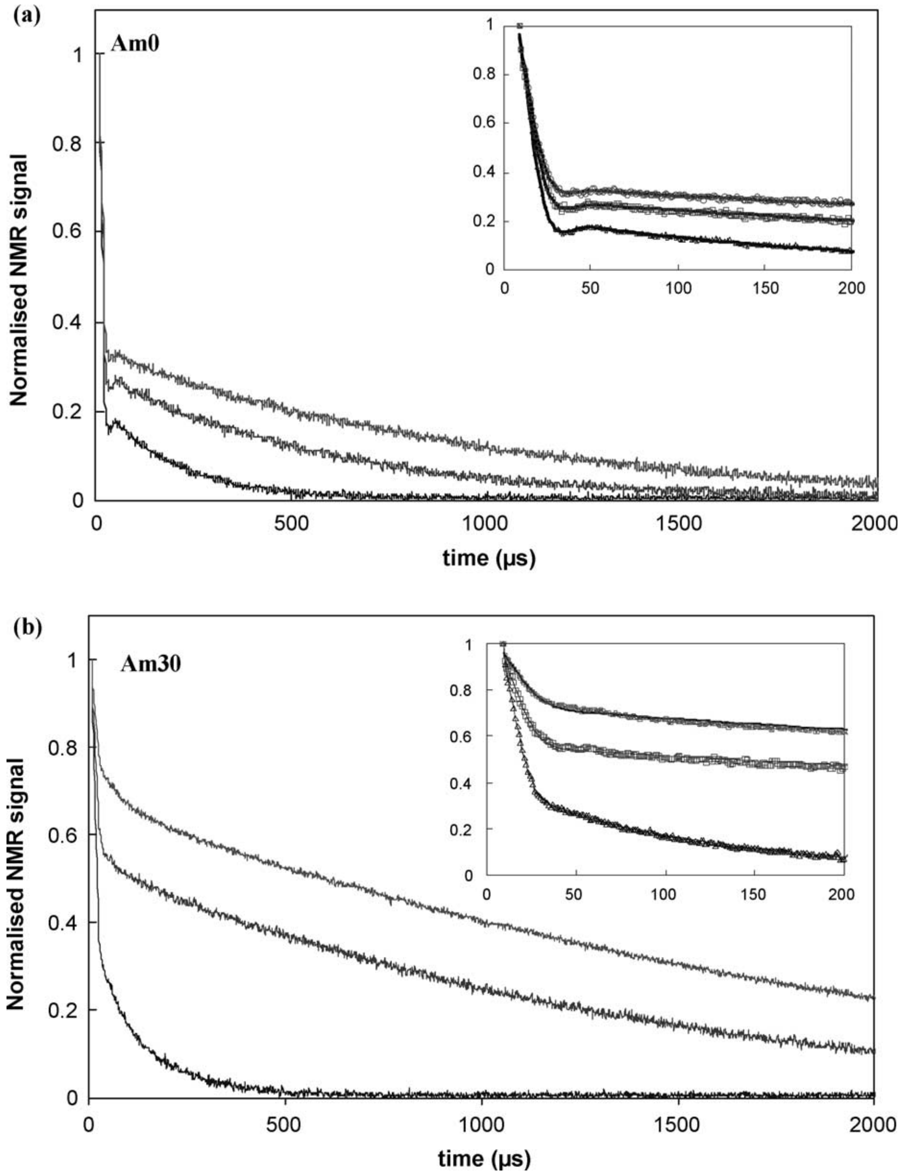

Fig. 3. Typical FIDs of Am0 (a) and Am30 (b) films equilibrated at, from bottom to top, 12, 54 and 94% RH. The signals where normalised to the signal at 9 μs. Both the experimental data and the fitted line shape (Eq. (1)) are included. The insets represent the first 200 μs of the FID.

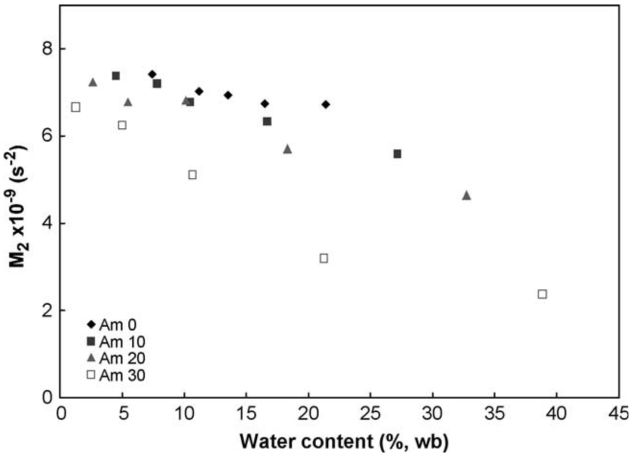

Fig. 4. Effect of water content and glycerol on the second moment of amylose samples.

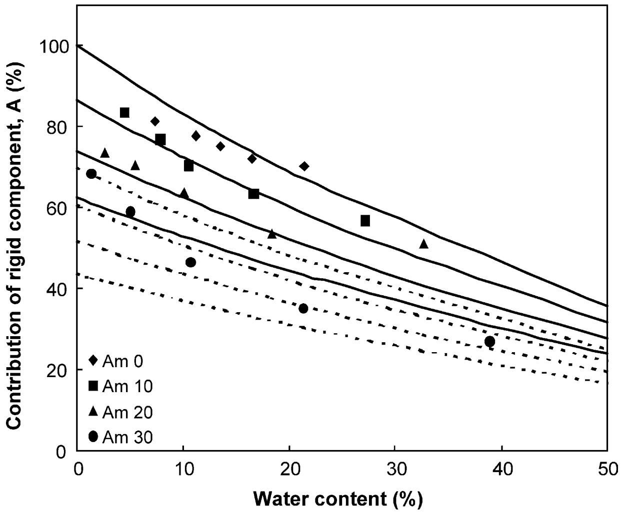

Fig. 5. Effect of water content and glycerol on the contribution of the rigid component (A) to the total signal estimated by fitting Eq. (2) to the FID data. From top to bottom: the lines represent the theoretical amylose contribution assuming slow (solid lines) and fast (dashed lines) proton exchange conditions.

molecular mobility leads to decrease of M_2 as dipolar interactions are increasingly averaged out by isotropic and anisotropic rotations. M_2 is also affected by proton density as dipolar interactions decrease with the sixth power of the distance between protons (van den Dries et al., 1998). Fig. 4 shows the effect of water and glycerol on the second moment of the rigid component of the FID reporting on the behaviour of amylose films. M_2 decreased as the water and glycerol contents increased. Water and glycerol have higher proton densities (ρ_{1H} mol ^{1}H per g) than amylose, namely 2/18 and 8/92, respectively, compared to 10/162. Therefore, increasing water and glycerol contents yield increased overall proton density. Thus the decrease in the second moment is indicative of the plasticisation of amylose and the resulting increase of its molecular mobility.

The effects of water and glycerol were also monitored by comparing the measured contribution of the rigid component (A%) obtained by fitting Eq. (2) to the FIDs acquired at different water contents to theoretical values calculated assuming that this component can be assigned to amylose and taking into account the proton densities of amylose, glycerol and water (Fig. 5). Except for the sample containing 30% glycerol, the measured A values agreed reasonably well with those predicted for slow proton exchange conditions considering the significant uncertainties in fitting the rigid component of the FID measured using a single pulse experiment and an overall dead time of 9 μs (Fig. 5, solid lines). At 30% glycerol, this correlation was far less convincing with the suggestion that in the highly plasticised films, a significant fraction of the amylose protons contribute to the liquid-like component of the FID. The contribution could be the result of either proton exchange (three exchangeable OH per glucosyl) or increased mobility of entire monomers (both OH and CH). It is not expected that all exchangeable protons are able to rapidly exchange (on the NMR experiment timescale, i.e. μs), as a large proportion of these would be involved in intra- and inter-molecular hydrogen bonds within the crystalline structure of amylose. The results in Fig. 5 show at low water contents, except for the control (0% amylose), a larger rigid component than anticipated based on the amylose contribution. This could be interpreted as indicative of a contribution from glycerol protons and linked to the widely documented (Lourdin, Bizot, & Colonna, 1997) antiplasticisation process, where higher than expected brittleness and density are observed.

In order to evaluate the correlation between glass-rubber behaviour and the changes in molecular mobility, the M_2 and the A% results were plotted against the scaled temperature ($T - T_g$) where $T = 20\,°C$ is the measurement temperature and T_g is the glass transition temperature calculated as described above (Figs. 6 and 7).

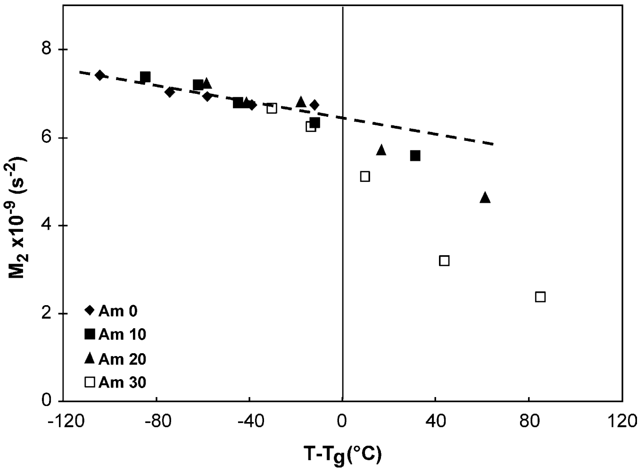

Fig. 6. The M_2 data of Fig. 4 plotted as a function of ($T - T_g$). The line was added a guide to the eye.

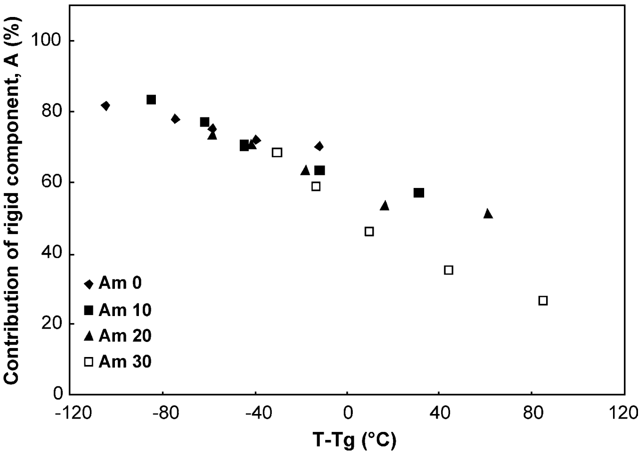

Fig. 7. The $A\%$ data of Fig. 5 plotted as a function of $(T - T_g)$. The line was added a guide to the eye.

Regardless of the level of plasticiser, both the M_2 and A results superimposed at negative values of $(T - T_g)$, i.e. in the glassy state, and decreased steadily as $(T - T_g)$ increased up to a value ~0 °C. The decrease of M_2 was steeper in the rubbery state, i.e. at $(T - T_g) > 0$ reflecting increased cooperative molecular motion of the amylose chains in the rubbery state. Except for the sample containing 30% glycerol, the contribution of the rigid component followed a monotonously decreasing pattern over the entire $(T - T_g)$ range studied. These results suggested that the effect of plasticisers on the mobility of amylose could be conveniently estimated using the scaled temperature $(T - T_g)$. While in the glassy state, i.e. at $(T - T_g) < 0$, the specific composition of the sample in terms of water and glycerol contents is irrelevant when estimating the molecular mobility of the biopolymer. In the rubbery state, i.e. at $(T - T_g) > 0$, the glycerol content affected the measured M_2 values; at a given $(T-T_g)$. The contribution of the rigid component (A) decreased and the mobility of this rigid component (monitored through M_2) increased with increasing glycerol content. This observation suggests that in addition to decreasing the glass transition temperature, glycerol afforded a greater mobility for amylose in the rubbery state (at a given $(T - T_g)$) than water. These results are in agreement with previously published results where glycerol was found to significantly affect the mechanical properties of amylose films (Myllärinen et al., 2002). The steeper increase of mobility in the rubbery state in glycerol containing systems could reflect the dramatic decrease in viscosity as $(T - T_g)$ increases above 0 °C and could be linked with the notion of strong glasses where the viscosity decreases in an Arrhenius manner and fragile glasses, where the viscosity decreases according to the much steeper Williams-Landel-Ferry equation as T increases above T_g and the system moves from a solid-like to a liquid-like behaviour (Angell, 1995).

Spin-lattice relaxation experiments were also used to study the plasticisation of amylose by water and glycerol. Spin-lattice relaxation is sensitive to magnetic fluctuations in the region of the Larmor frequency, which is a function of the static magnetic field (normally tens to hundreds of MHz for protons), or in the case of a T1 experiment, the strength of the radio frequency field. Spin–spin relaxation on the other hand is sensitive to magnetic fluctuations up to tens of kHz. As described above, the magnitude of the rigid component, approximated from $S(10\ \mu s)-S(70\ \mu s)$, and mobile component, approximated to $S(70\ \mu s)$, of the FIDs acquired after increasing recovery times (τ). The results were satisfactorily fitted to a single exponential using Eq. (3). A typical inversion recovery result is shown in Fig. 8.

At all glycerol contents, both the T_1 of the rigid and the mobile component of the FID decreased with increasing water content to reach a minimum before increasing at higher water contents (Fig. 9). This 'V-shape' mirrors the widely reported pattern of the dependence of T_1 on factors that increase molecular mobility, typically temperature. In the liquid state, a long spin-lattice relaxation time indicates high mobility which averages the magnetic field fluctuations to a low value causing inefficient relaxation. However, on the solid state side of the T_1 minimum the intensity of fluctuations are of too low a frequency to contribute significantly to relaxation (Ruan & Chen, 1998). The T_1 values of the rigid and mobile components of the FID were of the same order ranging between 48 and 105 ms over the entire range of water and glycerol contents investigated. The similarity in the T_1 s of the rigid and mobile components indicates efficient cross-relaxation between these two components. This could be via spin diffusion or actual chemical exchange between sites. Cross-relaxation times of 8 and 11 ms were measured using the Goldman-Shen pulse sequence (Kenwright & Packer, 1990) for the Am0 and Am30 samples equilibrated at 54% RH and containing 14

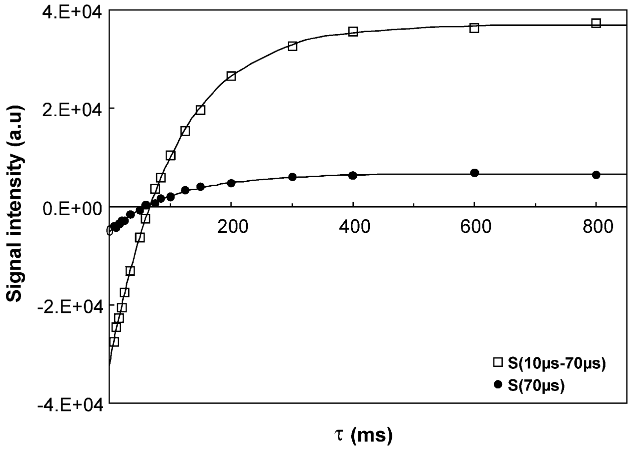

Fig. 8. Typical inversion recovery results for an Am0 sample equilibrated at RH = 12% (7.4% water) showing the behaviour of the rigid ($S(10\ \mu s)-S(S70\ \mu s)$) and liquid ($S(70\ \mu s)$) components of the FID and the best-fit lines (Eq. (3)).

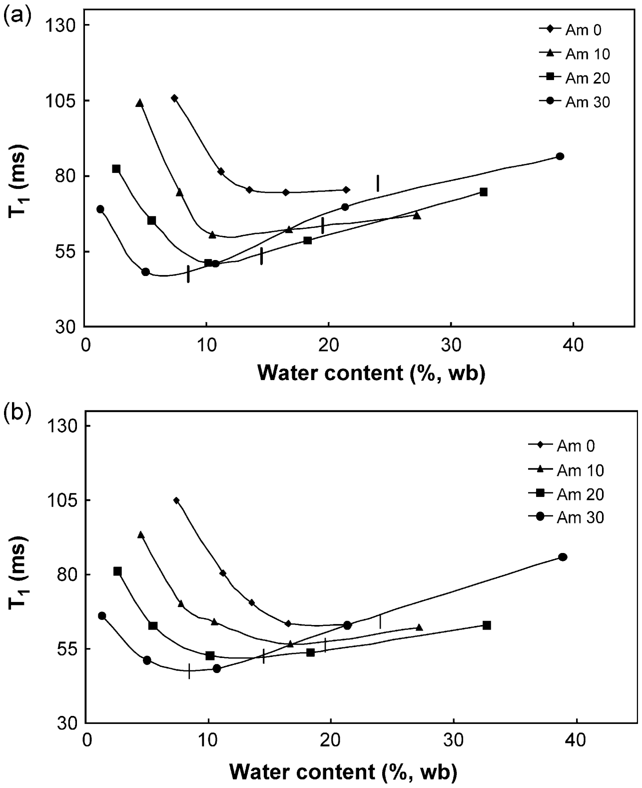

Fig. 9. T_1 relaxation times of the rigid (a) and mobile (b) components of the FID of the amylose films as a function of water content at 20 °C. The bars represent the approximate water content values corresponding to $T_g = 20$ °C.

and 11% water, respectively. Chemical exchange has been suggested to proceed more efficiently when a certain amount of water is present, whereas spin diffusion is dominant at low moisture contents. The values for the Goldman-Shen decay constants constrain the upper limit for exchange or spin diffusion. The spin-spin relaxation times, T_2^* (T_2 of the liquid component of the FID) were 3–50 times shorter than the cross-relaxation times, and so being substantially shorter, should not be affected by chemical exchange. The reported cross-relaxation times were of the same order as measured by Tanner et al. (1991) for native waxy maize starch containing 10% water at 30 °C (5 ms).

The incorporation of the glycerol into the samples affected the measured T_1 values by (i) decreasing T_1 at low water contents, (ii) by decreasing the water content at which the minimum of T_1 was observed, and (iii) by increasing the T_1 at high water and glycerol contents (Fig. 9). When the values of W_g, the water content at which T_g is equal to the measurement temperature (20 °C) were superimposed to the T_1 data, a reasonable correlation was found between W_g and the water contents at which the observed minimum T_1 occurred, particularly for the sample containing 30% glycerol (Fig. 9). The role of the relationship between the glass-rubber transition and the molecular mobility probed by the spin-lattice relaxation times was studied by superimposing the T_1 results on a plot as a function of $(T - T_g)$ (Fig. 10). For both the rigid and mobile components, there was a significant overlap in the results with an overall minimum of T_1 observed around $(T - T_g) = 0$. However, a more careful examination of the results revealed that while the T_1 values at $(T - T_g) > 0$ did not depend significantly on sample composition, both for the rigid and mobile components of the FID, the glycerol content had a measurable effect on T_1 at $(T - T_g) \leq 0$. The results suggest that when these samples are well into the glassy state, the value of T_1 at a given $(T - T_g)$ increases with increasing glycerol content. Since this part of the T_1 curve describes the solid state behaviour where T_1 is inversely correlated with mobility, the results suggest that in the glassy state, glycerol may decrease amylose mobility and act as an antiplasticiser.

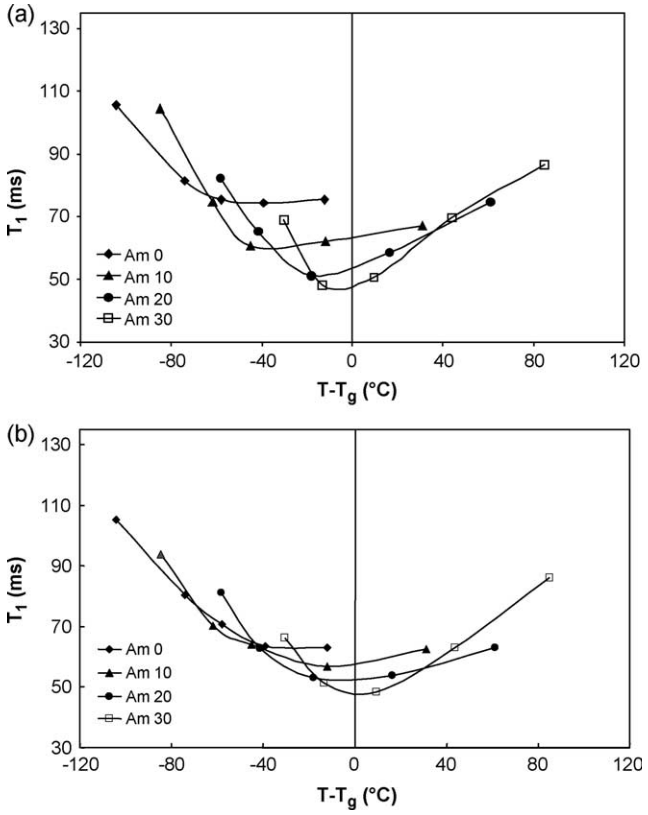

Fig. 10. The T_1 data of the previous figure plotted as a function of $(T - T_g)$.

4. Discussion

Monitoring effects of plasticisers on molecular mobility is only valuable if it can help to understand actual changes in technological properties of amylose films. Studies of amylose films in relation to their properties and potential are both well established (Langlois & Wagoner, 1967) and very active (see introduction). Being more resistant to enzymatic and acid hydrolysis (Myllärinen, Buleon, Lahtinen, & Forssell 2002), and also, mechanically stronger (Myllärinen et al., 2002) amylose offers much more functionality and potential to film applications than amylopectin, the other starch polymer. One important feature of the tensile properties of amylose film was, that elongation increased when sufficient amount of plasticiser was added e.g. 30% glycerol and 11% water (RH = 50%) (Myllärinen et al., 2002). This is in agreement with the findings of the present study where above *circa* 15% water, the sample containing 30% glycerol exhibits the highest molecular mobility probed by spin–spin relaxation. In contrast, the gradual loss of the excellent oxygen barrier properties of amylose films with increasing moisture content and the occurrence of dramatic changes at 20% water regardless of glycerol content (Forssell et al., 2002) cannot be directly linked to results of this study. It is not however unreasonable to suggest that O_2 transport through amylose films is not solely controlled by the molecular mobility of the polymer. It is for example possible that the partial phase separation often reported in starch–polyol films may enhance gas transport via a plasticiser-rich phase.

5. Conclusions

The roles of water and glycerol in plasticising partially crystalline amylose in ternary amylose–glycerol-water systems were investigated by monitoring proton mobility. Both spin-spin and spin-lattice relaxation parameters showed a significant change as the glass transition temperature (T_g) was decreased by the addition of plasticisers to reach the measurement temperature ($T = 20$ °C), i.e when $(T - T_g) \sim 0$ °C. The spin–spin relaxation data (M_2 and contribution of rigid component) reported successfully on the increased mobility of amylose in the rubbery state by the addition of water and glycerol, but were unable to distinguish between the amylose films with various water and glycerol contents in the glassy state. Data on spin-lattice relaxation times complemented the spin-relaxation results and showed that high frequency molecular motions were decreased in the glassy state by the addition of glycerol. This was attributed to the phenomenon of antiplasticisation, well documented for synthetic and natural polymers.

This study suggested that examining molecular mobility (and other physical properties) of amylose–glycerol–water systems is best achieved in the context of the scaled temperature ($T - T_g$), which explained most of the effects of water and glycerol. Both the spin–spin and spin-lattice relaxation results suggested that glycerol increases significantly amylose mobility at a given water content and particularly in the rubbery state at a given ($T - T_g$) (M_2), phenomenon which is practically observed as higher elongation.

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