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(Received: January 29, 1998).

Ageing of Starch Based Systems as Observed with FT-IR and Solid State NMR Spectroscopy

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The retrogradation and physical ageing of model starch systems with respect to their glass transition temperatures T_g have been investigated by Fourier transform infrared spectroscopy and solid state NMR spectroscopy. Diffuse reflectance Fourier transform infrared (DRIFT) spectra demonstrate the commencing retrogradation of starch materials stored above their T_g by changes in peak lineshapes and intensities in the characteristic area between 995 cm^{-1} and 1020 cm^{-1} . Solid state NMR proton relaxation times in the rotating frame (proton $T_{1\rho}$) show a

characteristic course in relation to the storage conditions (time, humidity), for which a distinction is made between physical ageing which occurs below the T_g , and recrystallisation (retrogradation) which takes place above T_g . The proton $T_{1\rho}$'s of materials stored below T_g increase asymptotically in time due to physical ageing, whereas the proton $T_{1\rho}$'s of materials stored above T_g increase until a moisture content is reached that rises them above T_g , decrease due to further water absorption and then increase due to recrystallisation (retrogradation).

Introduction

Starch is one of the main energy providers in the human diet and it is being used for many years as a natural food-thickener [1]. In the food industry plasticisers like water and sugars are used to improve the food quality and to delay the loss of moisture and the staling of food products, which limit the shelf-life of bakery products.

Lately there is much interest in biodegradable plastics to replace synthetic short-lifecycle products. Starch, for

example, is a cheap biopolymer that is totally biodegradable forming carbon dioxide and water. Granular starch is mixed with suitable plasticisers to enable melting below the decomposition temperature, resulting in thermoplastic starch (TPS) [2, 3].

The time dependent behaviour of starch based materials during and after processing is of great importance in the food industry and for the development of bioplastics. Especially rheological and physico-chemical changes during storage (ageing) are important. Because the predominantly amor-

phous products are not at thermodynamic equilibrium, the systems will approach this equilibrium in time, which involves starch molecular rearrangement and starch recrystallisation. In this matter, one should distinguish between physical ageing below the glass transition (sub T_g effects) [4] and ageing above T_g , i.e. retrogradation [5, 6].

Recent research has demonstrated the applicability of advanced non-invasive solid state NMR [7–9] and infrared spectroscopic techniques [10–14], which have moved forward the understanding of molecular phenomena in starch materials. With these techniques the molecular mobility and organisation in starch based systems can be directly determined and in combination with other analytical techniques, it is possible to obtain more information about the ageing of such systems.

The shelf-life of fresh bakery products like cakes, bread and frozen products such as frozen dough is determined by ageing, causing staling [15, 16]. For biodegradable starch plastics ageing causes their embrittlement [17]. For both types of products ageing phenomena are caused by physical ageing and recrystallisation (retrogradation).

By analogy with these products, model systems were prepared by gelatinising and freeze drying native potato starch. After preparation, the products were aged at specific conditions (humidity, temperature, time) with respect to their glass-transitions (T_g) [4, 18]. We have monitored the physical ageing and retrogradation processes by solid state NMR spectroscopy, FT-IR spectroscopy and X-ray diffraction (XRD). To visualise both processes, we have correlated the proton relaxation times in the rotating frame (proton $T_{1\rho}$) with the storage time and the water content.

2 Experimental

2.1 Materials

Native potato starch, PN, (moisture content 17%) was supplied by AVEBE. Sodium azide was provided by Merck. Deionised water was used.

2.2 Sample preparation

Gelatinisation was performed in a Brabender viscometer. 42 g PN was mixed with 309 g water to gain a 10% dry weight starch dispersion. 0.1% wt sodium azide (NaN_3) was added as a preservative against fungi growth. The mixture was poured into the Brabender and stirred at 75 rpm. The mixture was heated from room temperature to 90°C with 2°C and held at 90°C for 55 min, until the viscosity had reached a nearly constant value. Then the mixture was frozen with liquid nitrogen and freeze dried over two days. The dried mixtures were ground and stored at -22°C before conditioning. Samples were at 20°C conditioned and relative humidities (RH) of 30%, 60% and 90%, respectively. At 30% RH as the water content is about 11% no retrogradation is expected because the systems are below their glass transition temperature [19]. Sub T_g phenomena, however, can be investigated on these samples [18]. At 90% RH the systems are above their T_g and retrogradation can be investigated on these samples [18]. Samples conditioned at 60% RH are stored at about their T_g and could exhibit both sub T_g and retrogradation phenomena.

2.3 Polarised light microscopy

A Zeiss Axioplan MC 100 polarised light microscope was used with a blue colour filter. Magnifications of 100×, 200×

and 400× have been used. Samples were prepared by mixing a small amount of fine powder with a droplet of water.

2.4 X-ray diffraction

Wide Angle X-ray Scattering (WAXS) powder diffractograms were recorded on a Philips PC-APD diffractometer in the reflection geometry in the angular range 4–40° 2θ . The CuK radiation from the anode operating at 40 kV and 50 mA was monochromised using a 15 μm Ni foil. The diffractometer parameters were: divergence slit 1°, receiving slit 0.2 mm and scatter slit 2°. A proportional detector was used to detect the scattered radiation.

2.5 FT-IR spectroscopy

Diffuse reflectance Fourier transform infrared (DRIFT) spectra were recorded on a BioRad FTS-60A spectrometer with a liquid nitrogen cooled MCT detector using a Digilab diffuse reflectance accessory. DRIFT samples were prepared by mixing the fine powder starch sample with KBr (Uvasol®, Merck, <50 μm). The spectra obtained at resolution 4 cm^{-1} in the range 4000–550 cm^{-1} were averages of 256 scans. DRIFT spectra were recorded as *Kubelka-Munk* transformed spectra against a KBr background.

2.6 Solid state NMR spectroscopy

The ^{13}C NMR spectra were collected on a Bruker AMX 400 operating at 400 MHz for ^1H and 100.63 MHz for ^{13}C . Samples were spun at the magic angle (54.7°) with respect to the static magnetic field. Carbon and proton 90° pulse lengths were 5 μs in all experiments. Carbon chemical shifts relative to tetramethylsilane (TMS) were determined from the spectra, using solid glycine at room temperature as external reference. Samples were packed into 7-mm ceramic rotors and spun at 3–4 kHz. The ^{13}C spectra and proton $T_{1\rho}$ times were obtained with CP pulse sequence to enhance sensitivity [20]. Variable delay times up to 20 ms have been used for the proton $T_{1\rho}$ experiments. In ^{13}C CP/MAS experiments, the cross polarisation time was set to 2 ms with a recycle delay of 2 s.

3 Results and Discussion

3.1 X-ray diffraction and polarised light microscopy

X-ray diffraction has been used for a preliminary investigation of changes in crystallinity during processing and conditioning. The X-ray diffractograms of the freshly prepared freeze dried gelatinised PN show the typical unstructured pattern of a completely amorphous material. This is in agreement with polarised light microscopy, from which it was concluded that the granular structure of PN is totally disrupted, because no characteristic Maltese crosses have been found.

Storage up to 32 days at 20°C and at various humidities does not significantly increase the crystallinity of the prepared samples, which is seen from Figure 1, where the X-ray diffractograms of freeze dried gelatinised PN recorded after 7 and 32 days at 90% RH are presented. Powder XRD may be not sensitive enough to detect minor extents of recrystallisation or very small crystals.

3.2 Solid state NMR spectroscopy

The ^{13}C CP/MAS spectra of the freeze dried samples are typical of completely amorphous PN. This is in agreement with polarised light microscopy, where no granules have been detected, and in agreement with X-ray diffraction from

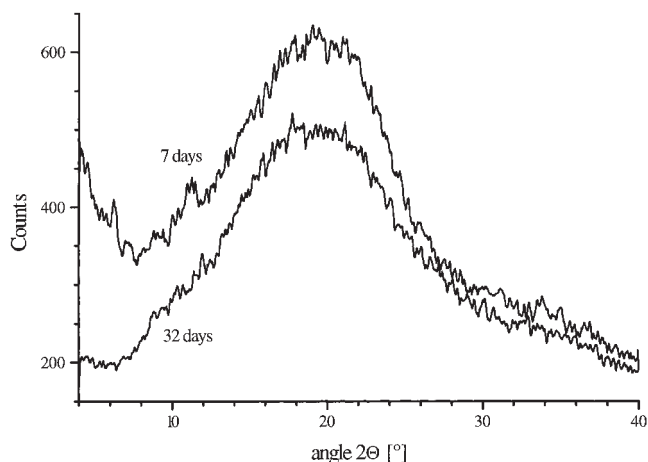


Fig. 1. XRD diffractogram of freeze dried gelatinised PN stored for 7 and 32 days at 20 °C and 90 % RH.

which only a broad, unstructured signal is detected. In the CP/MAS spectra, for carbon C-1 only one broad resonance without splitting is seen (Figure 5). The spectra of the conditioned samples show no significant changes in lineshapes or chemical shifts during storage at different humidities. Both CP/MAS and powder XRD experiments may be not sensitive enough to detect small variations in molecular structure, minor extents of recrystallisation or very small crystals.

3.3 FT-IR spectroscopy

Infrared spectroscopy has been used for investigating changes in starch structure on a short range molecular level. Especially interesting are the peaks at 1047 cm^{-1} characteristic of the more organised part of starch, at 1022 cm^{-1} characteristic of amorphous starch, and at 995 cm^{-1} which is sensitive to water [3, 21].

The infrared spectra of freeze dried gelatinised PN conditioned at 20 °C and 30 % and at 60 % relative humidity showed no major changes during 32 days (Figures 2 and 3).

At 90 % RH, depicted in Figure 4, the ratios of the peak intensities at 1047 cm^{-1} and 1022 cm^{-1} increased from 0.90 to 0.94, 0.98 and 0.99 after 7, 14, 21 and 32 days, respectively, implying a reducing amount of amorphous material, giving a more organised starch because retrogradation commenced. An increasing resemblance to native starch is observed during conditioning, for which this intensity ratio is 1.0.

3.4 Proton $T_{1\rho}$ measurements

The proton $T_{1\rho}$ relaxation times have been determined for the gelatinised and freeze dried PN samples conditioned at 30 %, 60 % and 90 % RH during storage for 32 days. In general, proton $T_{1\rho}$ relaxation times determine the rate of spin diffusion, which is governed by the strength of dipole-dipole interactions. Therefore, the rate of spin diffusion among the ^1H spins provides information about the domain sizes in polymer blends and about the molecular motion at the spin locking frequency of different phases in a homopolymer [20].

Figure 6 shows the results of the proton $T_{1\rho}$ measurements of the gelatinised PN samples conditioned at different humidities. The proton $T_{1\rho}$ values of the most intense signal at 72 ppm (glucose ring carbons 2, 3 and 5) have been used. Significant differences are observed between starch samples conditioned at 30 % relative humidity and the samples stored at 60 % and 90 % RH. After freeze drying the gelatinised samples have moisture contents of 11 %. This fresh gelatinised and freeze dried sub T_g material is expected to have a quite low proton $T_{1\rho}$ relaxation time, due to the low density (0.02 g/ml) of these materials after freeze drying and the completely amorphous character.

The initial moisture content of 11 % is about the equilibrium moisture content at 30 % relative humidity. For gelatinised freeze dried PN samples conditioned at 30 % RH, no retrogradation, but only sub T_g phenomena are expected to occur. During storage, at first an increase in proton $T_{1\rho}$ is detected and after one week the proton $T_{1\rho}$ value becomes constant. The increase in proton $T_{1\rho}$ relaxation time is caused

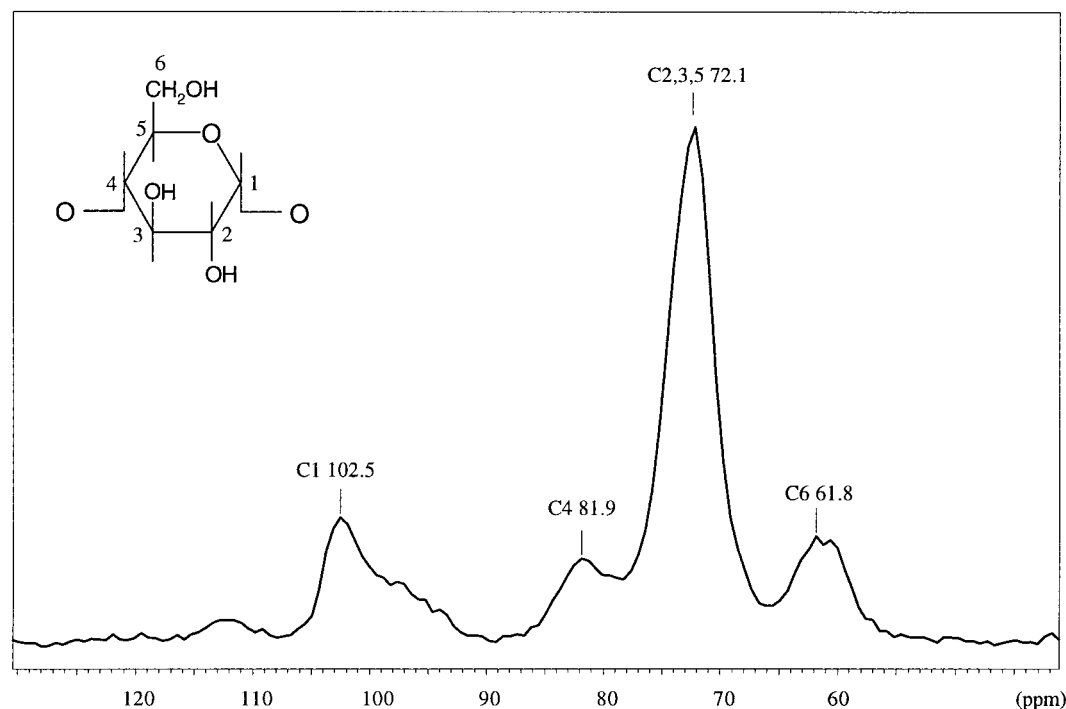


Fig. 5. ^{13}C CP/MAS NMR spectrum of fresh freeze dried gelatinised PN. Peak assignments with respect to the shown structure of the glucose ring.

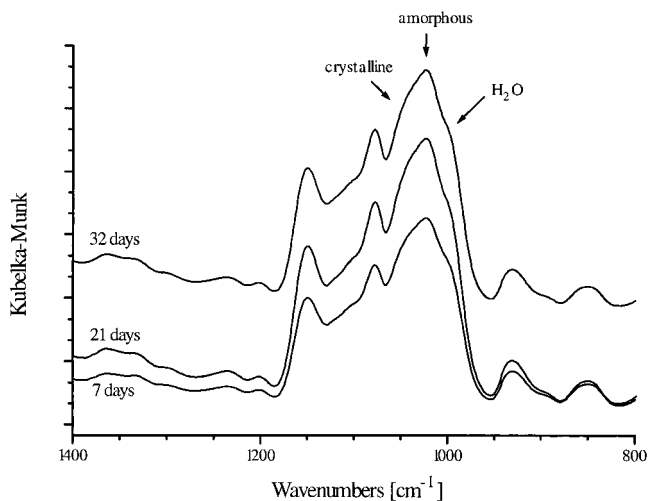


Fig. 2. FT-IR spectra of freeze dried gelatinised PN stored for 7, 21 and 32 days at 20 °C and 30 % RH.

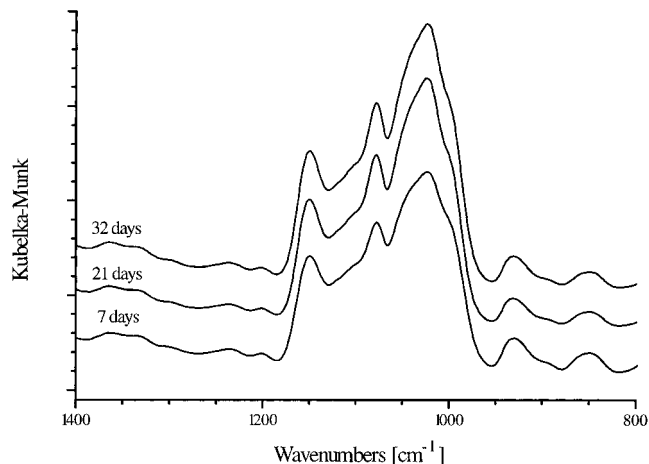


Fig. 3. FT-IR spectra of freeze dried gelatinised PN stored for 7, 21 and 32 days at 20 °C and 60 % RH.

by larger and better packed domains with stronger dipolar interactions of protons. This reorientation of the gelatinised PN is thought to be due to sub T_g physical ageing phenomena, which are related to a decrease in free volume or to an increase in water-starch (plasticiser-polymer) interactions, resulting in a stiffer material with a higher density [22, 23].

The gelatinised freeze dried PN samples, conditioned at 60 % and 90 % RH, show remarkable changes in the proton $T_{1\rho}$ after seven days of conditioning. To explain this behaviour, we have to consider again that two important facts are affecting the length of the proton $T_{1\rho}$. Firstly, changes in the domain size influence the relaxation behaviour resulting in longer relaxation times for larger domains and secondly, the mobility of protons can result in a more effective relaxation resulting in a shorter proton $T_{1\rho}$ for an increased mobility. These factors can lead to opposing effects.

The samples stored at 60 % and 90 % RH are initially equal to the samples stored at 30 % RH. They absorb water until their T_g is below room temperature. The interaction with this water causes a swelling of the starch network, resulting in bigger domains with a longer relaxation time $T_{1\rho}$, until the glass transition is reached. When enough water

is absorbed, the glass transition temperature will be below room temperature and the mobility of the starch chains increases significantly. Therefore we detect a decrease in relaxation time after seven days of storage. Reaching the equilibrium amount of water at these storage conditions, the T_g 's of the samples are below room temperature and we can detect the retrogradation resulting in an increase in proton $T_{1\rho}$, because of recrystallisation (less mobility) and a growth of domains of recrystallised material.

The gelatinised freeze dried PN samples conditioned at 60 % and 90 % RH, show a similar change in relaxation times during ageing. The changes are faster for the samples conditioned at 90 % than for the samples stored at 60 % RH due to the higher water contents of the former, as seen by the fact that the minimum in $T_{1\rho}$ is observed at an earlier stage during ageing. For the freeze dried gelatinised PN samples stored at 60 % RH, which are close to their T_g , one can expect both retrogradation and sub T_g phenomena. But one can conclude that retrogradation is the governing process because of the similar course of relaxation time compared to the gelatinised PN samples stored at 90 % RH (Figure 6).

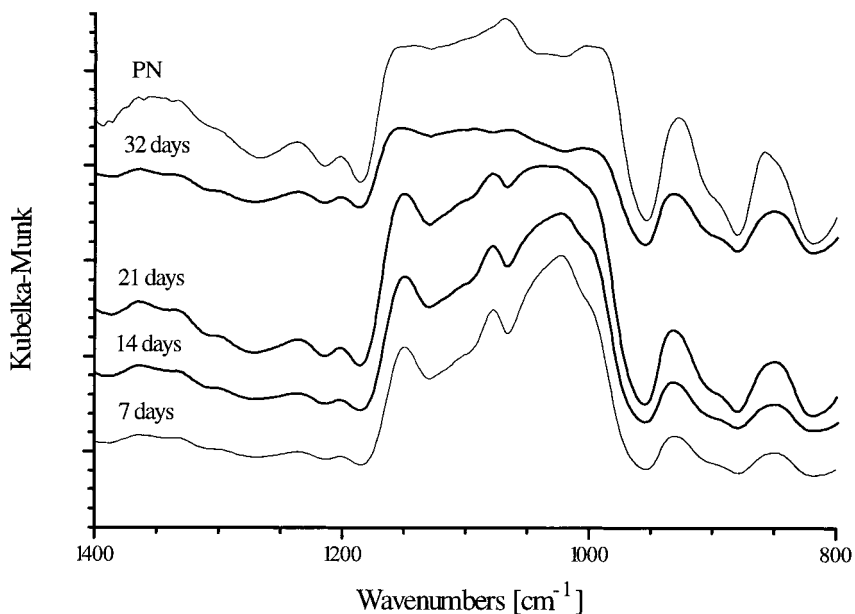


Fig. 4. FT-IR spectra of freeze dried gelatinised PN stored for 7, 14, 21 and 32 days at 20 °C and 90 % RH, and of native potato starch PN.

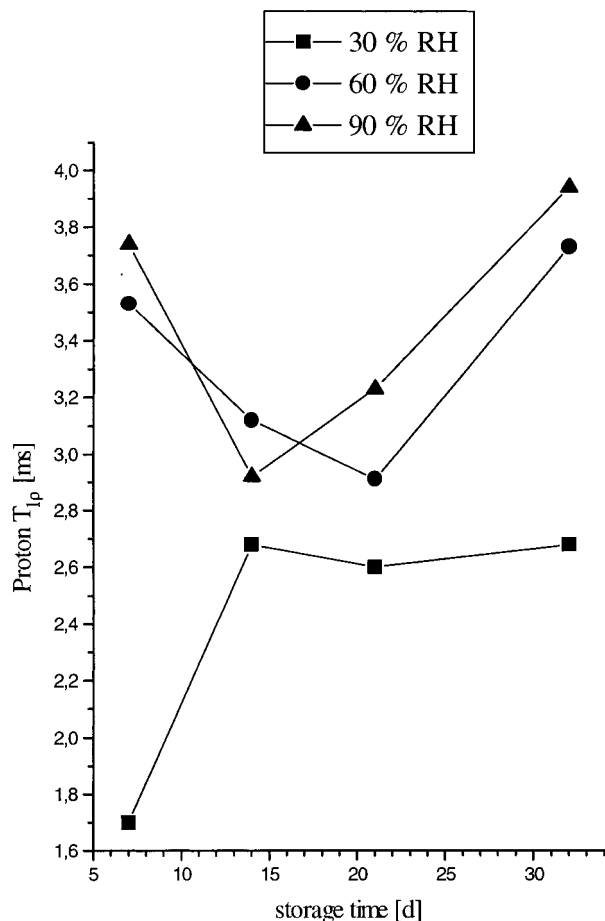


Fig. 6. Proton $T_{1\rho}$ relaxation times for freeze dried gelatinised PN conditioned at 30%, 60% and 90% RH.

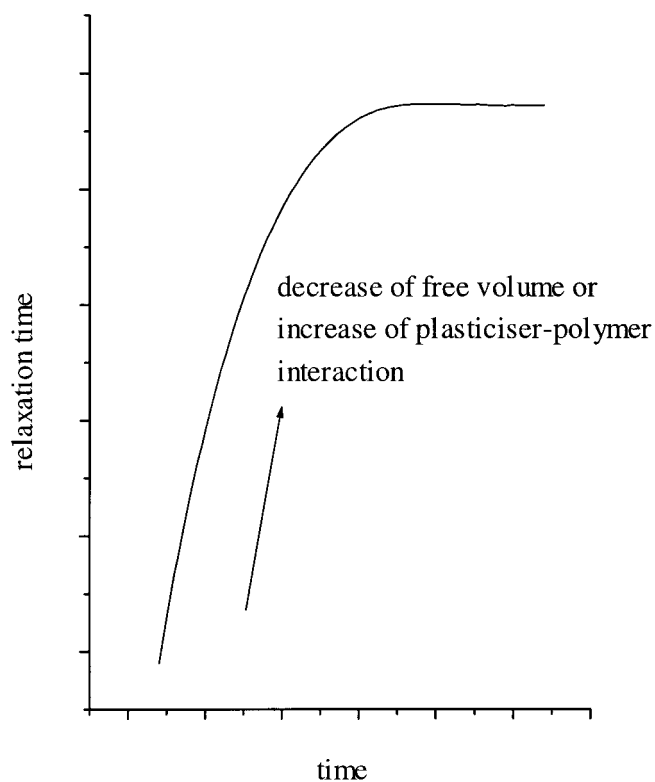


Fig. 7. Course of proton $T_{1\rho}$ relaxation times of dried gelatinized starch materials below T_g .

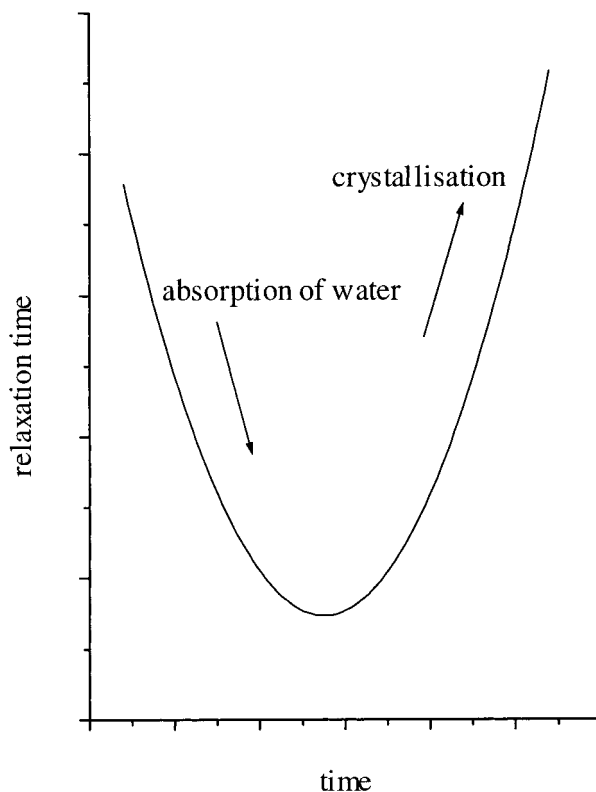


Fig. 8. Course of proton $T_{1\rho}$ relaxation times of dried gelatinized starch materials stored above T_g .

In general, the course of the proton $T_{1\rho}$ relaxation times during storage of potato starch can be determined in dependence of storage below or above T_g . Both situations are depicted in Figures 7 and 8. Below T_g the sub T_g physical ageing process causes a sort of asymptotic rising course of the proton $T_{1\rho}$ due to free volume relaxation or increased polymer-plasticiser interactions [22, 23]. The free volume decreases and the material becomes less flexible, resulting in slower movements and slower spin relaxation. Above T_g at first the absorption of water up to the equilibrium moisture content dominates the changes in starch chain mobility, resulting in the decrease of proton $T_{1\rho}$ because the material becomes more flexible. Above the glass rubber transition, the recrystallisation or retrogradation process starts. This process is the dominating factor after the equilibrium moisture content is reached, which is observed by an increase of proton $T_{1\rho}$ due to less flexibility.

4 Conclusions

FT-IR and solid state NMR spectroscopy are good techniques for observing physical ageing and retrogradation by means of spectral changes in lineshapes and linewidths and by the determination of relaxation times. In FT-IR spectroscopy changes in the area of 1050 cm^{-1} and 995 cm^{-1} imply the retrogradation of starch based materials stored above T_g . Proton $T_{1\rho}$ relaxation times are very sensitive to changes in the degree of crystallinity, domain size, molecular arrangement and the moisture content during retrogradation. The influence of the storage humidity in relation to the T_g can be monitored via the relaxation times. Below the T_g the decrease in free volume results in an asymptotic increase in relaxation times. During water absorption the relaxation

times are suggested to increase until the T_g is at about room temperature. Above the T_g further absorption of water increases the mobility of starch until the equilibrium moisture content has been reached. During and after this period retrogradation takes place and the relaxation times increase because of the development of crystallinity.

Acknowledgements

We would like to thank ATO-DLO for the use of the NMR, FT-IR and XRD facilities. Finally, we thank the Technology Foundation STW for financial support (Project UCH.4011).

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(Received: July 17, 1998).