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^{1}H NMR and Rheological Studies of the Calcium Induced Gelation Process in Aqueous Low Methoxyl Pectin Solutions

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The ^{1}H NMR relaxometry in combination with water proton spin–spin relaxation time measurements and rheometry have been applied to study the ionic gelation of 1% w/w aqueous low methoxyl pectin solution induced by divalent Ca^{2+} cations from a calcium chloride solution. The model-free approach to the analysis of ^{1}H NMR relaxometry data has been used to separate the information on the static (β) and dynamic ($\langle\tau_c\rangle$) behaviour of the systems tested. The ^{1}H NMR results confirm that the average mobility of both water and the pectin molecules is largely dependent on the concentration of the cross-linking agent. The character of this dependency (β, $\langle\tau_c\rangle$ and T_2 vs. $CaCl_2$ concentration) is consistent with the two-stage gelation process of low methoxyl pectin, in which the formation of strongly linked dimer associations (in the range of 0–2.5 mM $CaCl_2$) is followed by the appearance of weak inter-dimer aggregations (for $CaCl_2 \geq 3.5$ mM). The presence of the weak gel structure for the sample with 3.5 mM $CaCl_2$ has been confirmed by rheological measurements. Apart from that, the T_1 and T_2 relaxation times have been found to be highly sensitive to the syneresis phenomenon, which can be useful to monitor the low methoxyl pectin gel network stability.

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1. Introduction

Pectins are crucial structural polysaccharide components of the primary cell walls of most higher plants. Apart from the important structural and functional

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role they play in plant tissues being natural ion-exchangers, pectins are widely used as textural ingredients in food industry because of their thickening and gel-forming properties [1, 2]. The primary structure of pectin molecules consists of α(1-4) linked D-galacturonic acid residues (SR — "smooth region") interrupted by (1,2) linked L-rhamnose residues (HR — "hairy region") carrying some neutral sugars as side-chains (essentially galactose and arabinose residues). Various carboxyl groups in the pectin backbone (SR) may be esterified with methyl groups. The degree of esterification (DE) divides pectins into: high methoxyl pectins (HMP) (DE 50–80%) and low methoxyl pectins (LMP) (DE 25–50%) types with respect to their different mechanisms of gelation [3].

Low methoxyl (LM) pectins can form gels by two different types of intermolecular associations: (i) ionic associations in the presence of divalent cations (Ca^{2+}) governed by the egg-box mechanism [4, 5], and (ii) non-ionic associations in the acidic medium and in the absence of Ca^{2+} (based on hydrophobic interactions and hydrogen bond formation) [6]. A wide range of different methods have been applied to study both the dynamic and structural aspects of these gelation processes. Calcium induced gelation of LM pectins have been already studied by rheological methods [3, 7–13], circular dichroism [4], NMR [2, 14–18], dynamic light scattering (DLS) [19], and molecular modelling methods [5]. The results of the studies have confirmed a strong binding of calcium to pectin molecules and suggested a two-stage process of the ionic LM pectin gelation, in which the formation of strongly linked dimmer associations is followed by the appearance of weak inter-dimmer associations, mainly governed by electrostatic interactions. Furthermore, it has been proved that the ionic mechanism of LM pectin gelation depends on the chemical structure of the macromolecule (degree of esterification, molecular weight, distribution of rhamnose) and conditions of the environment (pH, ionic strength, concentration of pectin and calcium cations, temperature) [1, 3]. The microstructure of pectin gels has been investigated by transmission electron microscopy, light microscopy, and confocal laser scanning microscopy and the results have revealed that the network of pure LM pectin gels have the open character. The aggregated network of LM pectin gels with large pores of about 500 nm has been observed. Furthermore, the LM pectin gels have shown a more inhomogeneous structure, more flexible and branched network strands than purely HM pectin gels [20].

The pectin molecules as hydrocolloids affect the water behaviour. Near their surface pectins can bind, immobilise, and interact with water molecules. Thus, the analysis of the NMR water proton longitudinal (spin–lattice) T_1 and the transverse (spin–spin) T_2 relaxation processes can indirectly provide valuable information about the dynamics and structure of biomolecules in the water medium. In a recent publication [18], we have demonstrated that the water proton nuclear magnetic resonance dispersion of spin–lattice relaxation rates (^{1}H NMR dispersion (NMRD) or NMR relaxometry) [21] can be helpful in the analysis of ionic gelation

of LM pectin solution in the presence of sodium citrate buffer. ^{1}H NMR relaxometry has been widely used mainly to investigate proteins in solution [22–26] but it has also been applied to study more complex biological systems like gels or tissues [27–30]. NMR proton spin–spin relaxation time measurements have been found useful for monitoring the mobility and state of aggregation of biomolecules (proteins and polysaccharides) in solution and gelled systems [15, 30–38]. Therefore, in this paper we used ^{1}H NMRD method in combination with spin–spin relaxation measurements to study calcium induced gelation of LM pectin solution in the absence of the buffering salt (only in water medium). In addition, the rheological methods are used in order to check the existence of the gel network within the range of the visual sol–gel transition.

2. Materials and methods

2.1. Sample preparation

The low methoxyl pectin (potassium salts) from citrus fruits was obtained as dried powder from Sigma Chemicals (P-9311) and was used without further purification. The total galacturonic acid content in the sample was 60% and the average degree of its carboxyl groups esterification was 28%. The pectin was dispersed in distilled water, and then rapidly mixed on a magnetic stir plate at 70°C for 20–30 minutes. Afterwards, a cross-linking agent (calcium chloride solution) was added. All mixtures were stirred for another 5 minutes (homogenization process), and then carefully heated (to evaporate water) until a desired final weight of the sample. Homogeneous samples to study ionic gelation of LMP were prepared from 1% w/w aqueous pectin solution (pH: 5.20 ± 0.02) by adding appropriate amounts of the cross-linking agent (0.1 M $CaCl_2$). The calcium chloride concentration was varied from 0 mM to10 mM. All samples were stored in a refrigerator at 4°C for 24 hours, and were re-equilibrated to the room temperature prior to the NMR measurements.

The visual inspection of these samples was made, following the criteria of Garnier et al. [3]. For various concentrations of the calcium chloride different physical stages were observed: solution (0 mM $CaCl_2$), sol (1.5 mM, 2.5 mM, and 3.5 mM), pregel (5 mM $CaCl_2$), homogeneous gel (6.5 mM, 7.5 mM $CaCl_2$) and gel with syneresis (10 mM $CaCl_2$). All experiments involving the ionic gelation process of LM pectin were performed at 21°C.

2.2. ^1H NMR measurements

After the preparation procedure, the samples were placed in standard NMR tubes. No attempt was made to remove dissolved oxygen, as it was present in all samples.

Dispersions of proton spin–lattice relaxation rates R_1 were recorded with the fast field cycling relaxometer (Stelar Spin Master). The Larmor frequency was

changed between 0.01 MHz and 9 MHz. A typical switching time between low and high fields was 3 ms. A 90° pulse length of 5 μs was used, and the deadtime following the 90° pulse was 12 μs. For all experiments the data were averaged over four acquisitions. The temperature was controlled up to ± 0.1°C by the gas flow system of a conventional variable temperature control unit.

Measurements of transverse proton relaxation times T_2 were carried out using a Bruker DSX 400 NMR spectrometer operating at 400 Mz for protons. The Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence 90°_x-(τ-180 $^\circ_y$-τ-echo)$_n$ was applied, and the pulse spacing τ_{90-180° was 1 ms. Data were collected with a recycle delay time of 15 s and averaged over eight scans. The spin–spin relaxation times T_2 were determined from the CPMG echo decay envelope. The high-power solenoid coil was used. The 90° pulse length was about 4 μs.

2.3. Rheological measurements

Rheological measurements were performed by a RheoStress RS 150 Haake rheometer equipped with the double cone sensor system (DC 60: radius 32 mm, cone angle 1°). After preparation procedure, the samples were transferred onto a plate, preheated to 65°C and then quenched to 21°C. This temperature was kept for another 60 minutes, and then the mechanical spectra were recorded. Mechanical spectra showing the frequency-dependence of the storage modulus G', the loss modulus G'', and dynamical viscosity η^* were measured between 0.16 and 25 rad/s, at a low deformation of 0.03.

3. The theory

The water proton spin–lattice relaxation rate R_1 for isotropic aqueous biopolymer systems is practically determined by homonuclear dipole–dipole interactions [39] and may be estimated from the following equation:

$$R_1 = \alpha + \beta[0.2J(\omega) + 0.8(2\omega)], \tag{1}$$

where α (includes the non-dispersive term) and β are constants, and $J(\omega)$ is the Lorentzian spectral density function given by

$$J(\omega) = \frac{\tau}{1 + (\omega\tau)^2}, \tag{2}$$

where $\omega = 2\pi\nu$ (ν — Larmor frequency), τ is the correlation time.

The ^{1}H NMRD study of water in concentrated protein solutions and semisolid biological systems like gels or tissues has shown the dispersion profiles of longitudinal relaxation rates R_1 extended over a frequency range wider than that for the Lorentzian dispersion form [18, 22–29]. Therefore, the dispersion profiles of such type cannot be correctly reproduced by the Lorentzian spectral density function (except relatively dilute protein solutions), however, they are successfully

described by means of the model-free analysis [18, 25–27]. The model-free approach [25] to the analysis of the stretched dispersion profiles assumes the multi--Lorentzian form of the spectral density function (SDF) (see (1) and (3)) given by

$$J(\omega) = \frac{\sum_{n=1}^{N} c_n \frac{\tau_n}{+(\omega\tau)^2}}{\sum_n^N c_n}, \tag{3}$$

where $\sum_n^N c_n = c(0)$ indicates the mean-square fluctuation.

This form of the spectral density function represents "any stationary Markov process obeying detailed balance, thus virtually covers all cases of complex dynamics" [25]. The number of terms in the multi-Lorentzian form SDF can be determined by any nonlinear method of parameter estimation (e.g. the Levenberg–Marquardt algorithm). Therefore the model-free analysis, gives N values of individual correlation times τ and N weight coefficients c, which do not necessarily have a defined physical meaning, in contrast to the quantities β and $\langle\tau_c\rangle$ which are well physically defined. The $\langle\tau_c\rangle$ values are related to the average correlation time by the following relationship:

$$\langle\tau_c\rangle = \frac{\sum_n c_n\tau_n}{\sum_n c_n}. \tag{4}$$

The β parameter is proportional to the mean-square fluctuations $c(0)$ (which are independent of the dynamics) and is defined in terms of the integral of the dispersion profile. These quantities are model-independent and allow a separation of the static (β parameter), and dynamical ($\langle\tau\rangle$ parameter) aspects of the information contained in the dispersion data [25].

4. Results and discussion

The dispersion of water proton spin–lattice relaxation rates R_1 of 1% w/w aqueous LM pectin solutions was measured as a function of calcium chloride concentration (0–10 mM $CaCl_2$) in order to observe the influence of calcium cations on binding LM pectin molecules in the gel network.

The experimental ^{1}H NMRD data with the model-free fits are shown in Fig. 1. The dispersion profiles were analysed in terms of Eq. (1) with the spectral density function containing, generally a sum of three Lorentzian terms according to Eq. (3). The model-free fit gave the β parameter (proportional to the mean-square fluctuations), three different correlation times, and the corresponding three weight coefficients, for each sample. The average correlation times $\langle\tau_c\rangle$ were calculated according to Eq. (4). The α values were estimated from measurements at higher frequency. The best fit parameters for the ^{1}H NMRD data, obtained by means of the model-free analysis and characteristic of samples, are collected in the Table.

The dispersion profiles of the spin–lattice relaxation rate both in the aqueous LM pectin solution , sols and gels, show a strong stretching towards low frequencies

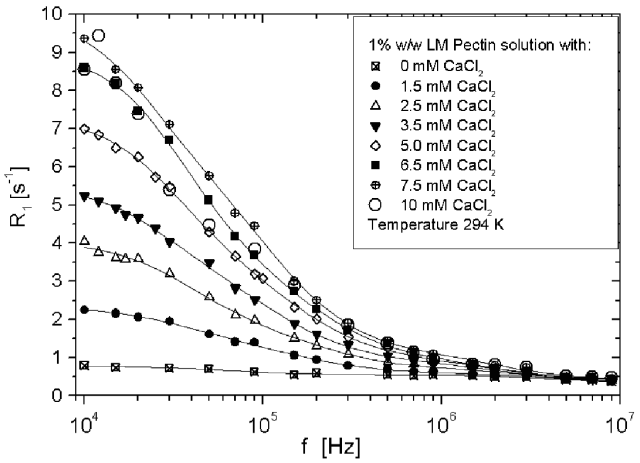

Fig. 1. Dispersion of the water ^{1}H spin–lattice relaxation rate in aqueous solution of 1% w/w LM pectin at pH 5.2 and 294 K without added calcium chloride solution and after addition of 1.5, 2.5, 3.5, 5, 6.5, 7.5, and 10 mM $CaCl_2$.

TABLE

Characteristic of samples and parameters of the ^{1}H NMR dispersion profiles obtained from the model-free analysis. 1LMPS = 1% w/w LM pectin solution.

Characteristic of samples			Model-free parameters		
Nr	Composition	Texture	α [1/s]	$\beta \times 10^7$ [s^{-2}]	$\langle\tau_c\rangle \times 10^{-7}$ [s]
1.	1LMPS pH 5.2	liquid-like	0.328	1.031	0.436
2.	1LMPS with 1.5 mM $CaCl_2$	liquid-like	0.349	1.218	1.603
3.	1LMPS with 2.5 mM $CaCl_2$	liquid-like	0.348	1.621	2.270
4.	1LMPS with 3.5 mM $CaCl_2$	liquid-like	0.398	1.453	3.513
3.	1LMPS with 5.0 mM $CaCl_2$	pregel	0.407	1.786	3.872
6.	1LMPS with 6.5 mM $CaCl_2$	gel	0.403	1.982	4.341
7.	1LMPS with 7.5 mM $CaCl_2$	gel	0.399	2.441	3.948

and have a much more stretched form than that of the Lorentzian dispersion. This extension is a consequence of a very complicated reorientational dynamics, distributions of proton exchange rates, and intermolecular dipole couplings in these systems. Similar results were observed in our earlier work for the ionic gelation of LM pectins in the presence of the sodium citrate buffer [18].

The slight frequency dependence of R_1 observed in the LM pectin solution becomes more significant after addition of the calcium chloride solution. With increasing $CaCl_2$ concentration the process of the calcium-induced associations of pectin molecules gets stronger. This effect, known as egg-box binding, has an

immediate impact on the dispersion profiles. With increasing concentration of the cross-linking agent (Ca^{2+}), the spin–lattice relaxation rates increase in the whole frequency range, but the character of this increase is different for different frequencies. The relaxation process is progressively enhanced with decreasing magnetic field strength. The dispersion data at the high field limit exhibit distinctly a lesser sensitivity to the presence of calcium cations than these at the low field range. The strong differences in the magnitude of the dispersion profiles in the low magnetic fields can be attributed to a decrease in the number of ionised sites on the pectin backbone surface as a result of the egg-box mechanism [4, 5].

Association process of LM pectin molecules induced by calcium cations is confirmed by the model-free parameters: $\langle\tau_c\rangle$ and β, presented in Fig. 2 as a function of the cross-linking agent concentration.

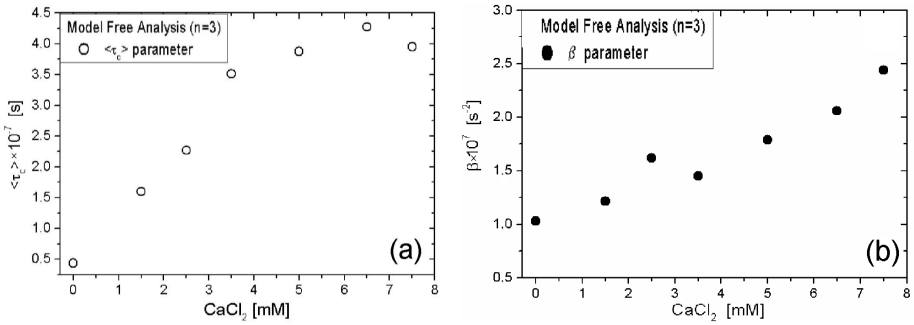

Fig. 2. Dynamic $\langle\tau_c\rangle$ (a) and static β (b) parameters for 1% w/w pectin solution versus the $CaCl_2$ concentration.

After addition of calcium chloride to the pectin solution the mean correlation times $\langle\tau_c\rangle$ and β parameter increase. The most significant dynamical changes were noted on varying the concentration of calcium chloride from 0 mM to 3.5 mM, for samples showing a typical liquid-like behaviour. The effect of calcium chloride addition on the $\langle\tau_c\rangle$ parameter is less pronounced for higher $CaCl_2$ concentrations (above 3.5 mM), but still shows the same tendency towards slowing down the dynamics. At the concentration of calcium chloride being equal to 6.5 mM, the gel state was observed. From this concentration of the salt the mean correlation time $\langle\tau_c\rangle$ remained almost unchanged. An addition of the cross-linking agent to the pectin solution had an important effect on the β parameter. With increasing concentration of calcium chloride the number of the junction zones on the pectin backbone increases, therefore the static β parameter increases, which confirms a substantial modification in the system's structure. These trends of $\langle\tau_c\rangle$ and β parameters can be explained by assuming a two-stage process of ionic LM pectin gelation in which the formation of strongly linked dimer associations (egg-box binding) is followed by the formation of weak inter-dimer aggregation [4, 5].

At the highest calcium chloride concentration value (10 mM) the shape of the dispersion profiles changes suggesting a change in the gel structure. This experimental result perfectly corresponds to the visual inspection of the syneresis phenomenon.

The visual inspection suggests that the sol–gel transition occurs between 5 mM (the texture is mushy — a pregel state) and 6.5 mM (the typical gel texture) concentration of the salts, but the model-free parameters do not show a drastic structural variation within this range. The rheological measurements were performed for these samples in order to probe the network formation. The viscoelastic profiles are presented in Fig. 3. Results in both cases show mechanical spectra typical of gelled systems, with the storage modulus (G') higher than the loss modulus G'' over most of the measuring frequency range and with the average $\log\eta^*$ vs. $\log\omega$ slope approaching the theoretical value of (–1), for a perfectly elastic (Hookean) network. Such results indicate that in both samples: with 5 mM $CaCl_2$ and 6.5 mM $CaCl_2$ the overall chain mobility of pectin molecules is very low [8, 11]. The solid-like response (G' modulus), as expected, is more evident for the sample with a higher concentration of calcium cations (6.5 mM $CaCl_2$ — a somewhat higher number of junction zones), but does not show sufficient differences compared to the frequency-dependence of G' modulus for the sample with a lower concentration of calcium chloride (5 mM $CaCl_2$). These mechanical spectra provide the experimental evidence that the visual sol–gel transition requires a higher concentration of the cross-linking agent than that following from the rheological criterion. The model-free parameters lead to the same conclusions and show that the gel starts to set earlier (below 5 mM $CaCl_2$) than for the sample whose texture has been classified as a mushy (pregel state) on visual observation.

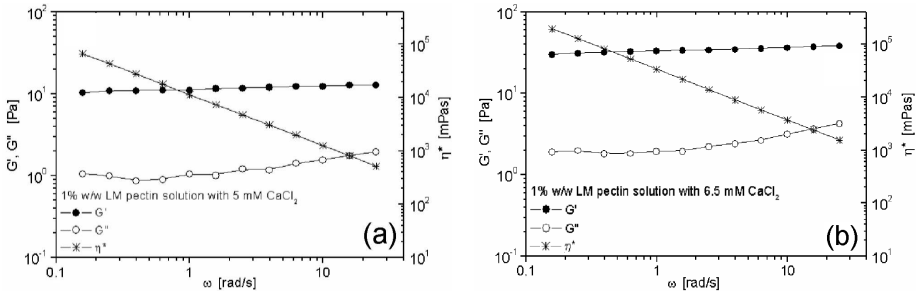

Fig. 3. Frequency dependences of storage modulus (G'), loss modulus (G''), and dynamical viscosity η^* for samples showing visual changes in the texture: (a) 1% w/w LM pectin solution in the presence of 5 mM $CaCl_2$ solution — the pregel state, (b) in the presence of 6.5 mM $CaCl_2$ solution — the gel state (3% strain, 294 K).

Calcium induced gelation of LM pectin can be also probed by the water proton spin–spin relaxation (T_2) process suitable for monitoring the mobility and state of aggregation of the polysaccharide chains.

Addition of LM pectin to water decreases the water proton transverse relaxation times. In general, two components of water proton T_2 relaxation were observed. The experimental results are presented in Figs. 4 and 5.

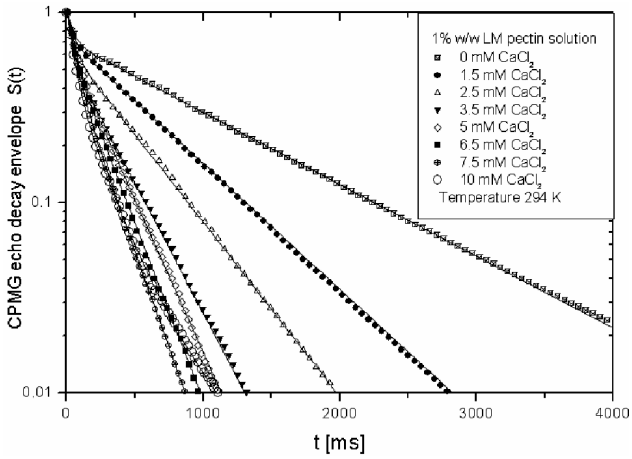

Fig. 4. Changes in the CPMG echo decay envelopes of 1% w/w LM pectin solutions observed without and with the cross-linking agent (Ca^{2+}) in different concentration, at 294 K.

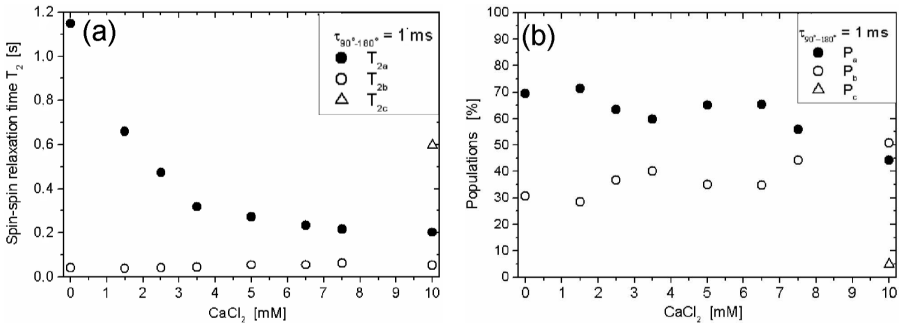

Fig. 5. Water proton spin–spin relaxation times (a) and their populations (b) versus calcium chloride concentration added to 1% w/w LM pectin solution in order to induce the gel network formation.

Bi-exponential decay suggests the presence of water protons in two different physical environments [15]. The slow components of spin–spin relaxation times (T_{2a}) can be associated with the fraction (P_a) of water molecules which are away from the pectin molecules. Since these water molecules have a relatively high and non-restricted mobility, the efficiency of the spin–spin relaxation processes is considerably limited. The fast components of the spin–spin relaxation (T_{2b}) times

may be ascribed to the fraction (P_b) of water molecules near the pectin surface. The Pb population of water may have restricted mobility due to interactions with the pectin molecules and/or formation of water clathrates around hydrophobic methyl ester groups [40] thus this fraction of water can more rapidly exchange spin energy with surrounding water molecules [15]. This explanation of the experimental results based on the "free" (or "bulk" — long T_2) and the "bound" (or "interfacial" — short T_2) water concept is a certain simplification as we are aware of the fact that the binding of water to the macromolecules sites is not the only factor determining the transverse relaxation behaviour.

Many authors have shown that in dilute aqueous carbohydrate systems enhancement of the spin–spin relaxation mainly depends on the rapid proton exchange mechanism between water and carbohydrate hydroxyl groups [30, 31, 34]. In the presence of aggregation or gelation processes another relaxation pathway can also be important, such as contributions from the exchange of water protons between the bulk and bound water phases by chemical exchange and/or molecular diffusion [30–36]. Unfortunately, it is difficult to distinguish exactly the contributions of these various relaxation mechanisms. Apart from the use of the water proton transverse relaxation measurements in combination with other techniques in the analysis of textural properties of many complex water/biopolymer systems, we perform these measurements in our study in order to probe the LM pectin network formation.

With increasing calcium chloride concentration the long component of the water proton spin–spin relaxation time T_{2a} decreases (from 1.15 s in the solution state to around 0.20 s in gelled systems). The most significant reduction of the transverse relaxation times, was observed for samples showing the visibly liquid form (from 0 mM to 3.5 mM $CaCl_2$). For higher $CaCl_2$ concentrations the enhancement of the spin–spin relaxation is considerably less pronounced or it is even almost unchanged. These results indicate that the polygalacturonate chains exhibit a high specificity for calcium binding, so initially mainly create strongly linked dimers, and next when enough calcium cations is available the chain dimer can aggregate into larger sheets. The egg-box dimer formation induced by calcium cations causes a gradual reduction of the mobility of pectin molecules because the cross-linked pectin molecules can more rapidly exchange spin energy with the surrounding water molecules than in the solution state. These observations are consistent with Wicker and Kerr findings [15] concerning ^{1}H NMR dispersion of water proton T_2 relaxation in LM pectin solution and gel systems. These authors noted two components of water proton transversal relaxation times in LM pectin solutions and substantial enhancement of the long component transversal relaxation (around five times) after addition to the LM pectin solution calcium cations at the concentration sufficient to the LM pectin gel formation. For the gelled system Wicker and Kerr also observed a flatter T_2 dispersion curve in comparison with the corresponding curve obtained for LM pectin solutions. These results

provided the experimental evidence of sufficient restriction of the chain mobility of pectin molecules after their cross-linking via calcium cations. The slow down of the relaxation process in aqueous LM pectin solution with increasing calcium chloride concentration has been observed also in DLS study [19], with a much greater contribution of the slow mode relaxation as the solution is driven from the sol to gel phase.

The shorter component of the water proton spin–spin relaxation T_{2b} shows a slight increase when the concentration of the cross-linking agent increases. The crucial rise of T_{2b} values was noted on varying the concentration of calcium chloride from 3.5 mM ($T_{2b} = 0.044$ s) to 7.5 mM ($T_{2b} = 0.062$ s). In this range of $CaCl_2$ concentration we also observed some changes in relative populations of transverse relaxation time components in comparison with less cross-linked samples (below 3.5 mM $CaCl_2$). The possible explanation is that the concentration of calcium chloride of 3.5 mM is sufficient to promote effective inter-dimer associations, essential in the gelling process of LM pectin. The existence of the weak gel structure in this sample confirms the rheological measurements. The mechanical spectrum for the sample of pectin solution in the presence of 3.5 mM $CaCl_2$ (Fig. 6) exhibits the weak gel-like character with G' values considerably lower than for samples with a higher degree of cross-linking (5 mM and 6.5 mM $CaCl_2$).

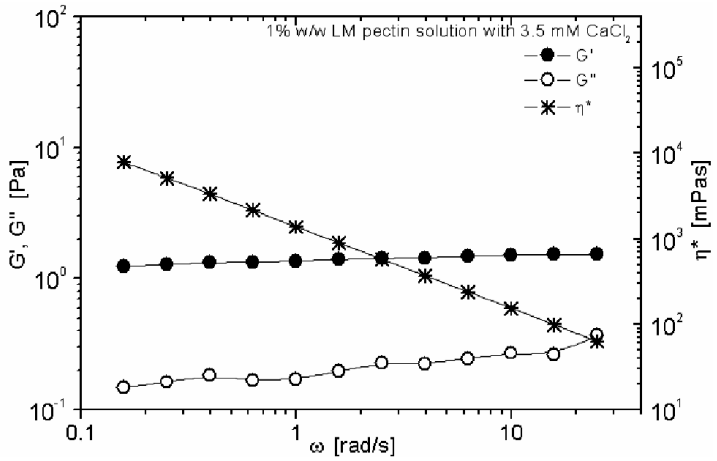

Fig. 6. Frequency dependences of storage modulus (G'), loss modulus (G'') and dynamical viscosity η^* for 1% w/w LM pectin solution in the presence of 3.5 mM $CaCl_2$ concentration (3% strain, 294 K).

At lower concentrations of the cross-linking agent (below 3.5 mM $CaCl_2$) and in the solution state the large effect of electrostatic repulsion decreases the probability of contact between pectin chains so that these samples show liquid--like behaviour without gel forming properties. When the concentration of the salt increases the effect of inter-dimer aggregations becomes more significant, leading to

the formation of the pregel and gel states with large pores and aggregated network strands. Such inhomogeneous LM pectin pregel and gel structures can promote an increase in the free volume [32] for molecular motions of water at macromolecular sites away from large multi-chain cross-links. This can explain a slight increase in T_{2b} values and also variation in relative populations P_a and P_b.

For the highest concentration of the cross-linking agent (10 mM $CaCl_2$) the tri-exponential decay of the CPMG echo envelope was noted and a drastic change in the distribution of populations in comparison to all other samples was observed. The short component of the water proton transverse relaxation time (T_{2b}) brings the greatest contribution to the CPMG echo decay envelope (50.8%), a little lower is the contribution of the long component T_{2a} (44.2%). The third and the longest component of the spin-spin relaxation time T_{2c}, makes only 5% of the overall echo decay magnitude, but clearly shows that the gel network forming in the presence of 10 mM concentration of calcium chloride is considerably defected. This conclusion is consistent with the results of T_1 dispersion measurements obtained for the same sample. These ^{1}H NMR results clearly show that the 10 mM concentration of $CaCl_2$ is too high to allow the formation of stable LM pectin gel, which can be visually observed as the syneresis phenomenon caused by the collapse of the network structure.

5. Conclusion

The ^{1}H NMR dispersion of spin–lattice relaxation rates ($1/T_1 = R_1$) and spin–spin relaxation time (T_2) measurements were applied to study calcium induced gelation of aqueous LM pectin solution. The model-free approach to the analysis of ^{1}H NMRD data was used to separate the information on the static (β parameter) and dynamic ($\langle\tau_c\rangle$ parameter) behaviour of the systems tested. Rheological measurements were applied as a complementary method to probe the network formation within the range of the concentration calcium chloride solution where the visual sol–gel transition was noted.

The ^{1}H NMR results suggest that the average mobility of both water and the pectin molecules is largely dependent on the concentration of the cross-linking agent (calcium cations) added to the aqueous LM pectin solution. The character of this dependency (β, $\langle\tau_c\rangle$, T_2 vs. $CaCl_2$ concentration) is consistent with the two-stage gelling process of LM pectin, in which the formation of strongly linked dimmer associations is followed by the formation of weak inter-dimer associations, mainly governed by electrostatic interactions [3, 4]. Water proton NMR findings for the concentration of calcium chloride between 0 and 2.5 mM are indicative of a decreasing mobility of pectin molecules mainly due to the cross-linking process. In this concentration range the large electrostatic repulsion effect between pectin chains restrains their associations, thereby the gel network cannot be formed (samples exhibit a typical liquid-like behaviour). Smaller dynamical changes were observed for higher $CaCl_2$ concentration (≥ 3.5 mM), which can be attributed to

the beginning of an effective aggregation process of pectin chains leading to the gel network formation.

The sample with 5 mM of calcium chloride concentration was considered a pregel because of its visual mushy texture, but the mechanical spectrum of this sample indicates a typical gel-like character. In rheological measurements the weak gel formation was observed for the pectin solution in the presence of 3.5 mM concentration of calcium chloride. This result confirms our NMR findings that the network is formed at a lower concentration of the cross-linking agent than that required according to the visual criterion.

The results of water proton NMR relaxation both spin–lattice T_1 and spin–spin T_2 revealed a high sensitivity to the syneresis phenomenon, which means that their measurements can be useful to monitor the LM pectin gel network stability.

Acknowledgments

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