Kinetics and thermal behaviour of the structure formation process in HMP/sucrose gelation

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The concentration and temperature dependence of the gelation kinetics of high-methoxyl pectin (HMP; 60% sucrose, pH 3) was investigated using measurements of small-amplitude oscillatory shear. The rate of gelation close to the gel point can be described as a second-order rate process using the kinetic model of Ross-Murphy (*Carbohydr. Polym.* 1991, **14**, 281) and a critical exponent close to that predicted by the percolation approach. The modulus after a long ageing time showed a power concentration dependence with an exponent around 3.1, higher than the classical square of concentration dependence, which was probably either due to the non-equilibrium state of the HMP gels even after long ageing times, or due to the proximity of the concentration range studied to the critical gelling concentration. The gelation rate of HMP/sucrose systems is strongly dependent on the temperature. An Arrhenius relationship was applied to describe this dependence. Two different processes are proposed to explain the discontinuity observed, each one having rates with different temperature dependence. The applicable kinetics at longer times are quite different, with a lower dependence on polymer concentration and ageing temperature. A non-isothermal kinetic model was used to describe the gelation process of the HMP/sucrose system during cooling.

Keywords: high-methoxyl pectins; gelation kinetics; gelation time

Gelation of polysaccharides is critical to formation of the desired texture in many food products. In biopolymer gels, the polymer chains form extended junction zones by means of side-by-side associations of a physical nature, in contrast to the typical single covalent bonds found in chemical cross-linking networks. Consequently, in physical gelation, the formation and breakdown of the junction zones is typically reversible, the crosslink functionality is very high, and the junction zones have a finite lifetime. The formation of this kind of transient network is determined by the chemical nature of the gelling system, temperature and time.

Pectins are a complex group of structural polysaccharides with an important role as primary constituents in the cell walls of many plants. They are also of significant importance in food technology, both from the point of view of ripening and processing of fruits and vegetables and their important functional properties, the most important of which is the ability to form gels. Pectins are anionic polysaccharides consisting of a linear backbone of $\alpha(1\rightarrow4)$-D-galacturonic acid residues partially esterified with methanol, with periodic interruptions by L-rhamnose residues 1,2-linked that make the backbone irregular, and with some other neutral sugars present as side chains.

Pectins with a degree of methylation higher than 50% (high-methoxyl pectins; HMPs) can gel at pH values lower than about 3.5, partially suppressing ionization of the carboxyl groups, and when the water activity is reduced by addition of a co-solute, typically sucrose at a concentration higher than 55% by weight.

HMP gelation is quite a complex process, in which several kinds of intermolecular interactions are involved.

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The junction zones in HMP gels are formed predominantly through aggregates involving a large and variable number of pectin chains, whose bonding stabilization consists of a combination of hydrogen bonds and hydrophobic interactions[1,2]. The different temperature dependencies of these intermolecular interactions in turn lead to the complex effect of temperature on the HMP gelation process[2,3]. In contrast to most polysaccharide gels, HMP gels are considered to be heat-irreversible[4].

In the present work, we studied structure development during the network formation of HMP/sucrose systems. We analysed the kinetic and thermal behaviour near the sol/gel transition and during ageing of the gels. The rheological parameter of interest was the storage modulus measured under dynamic shear at low strain amplitude; this parameter was assumed to be a measure of the number of elastically active chains in the pectin network. Changes in structure were followed as a function of time under isothermal conditions (cure curves) or non-isothermal conditions (temperature sweeps). The latter were achieved by decreasing the temperature at a constant rate. The study of structure formation in polysaccharide systems used as gelling agents in food formulations could be useful in understanding the kinetic and thermal behaviour of the food products in which they are incorporated as well as providing additional insights into the nature of the gelation process of these biopolymers.

Experimental

Materials

The pectin sample used was a commercial citrus pectin obtained from Bulmer (Hereford, UK). Its degree of methylation has been estimated by gas chromatography[5] to be 64%. The galacturonic acid content was estimated by colorimetry[6] to be 82%. The intrinsic viscosity was 4.8 dl g^{-1} determined in 0.1 mol l^{-1} NaCl at 25°C by capillary viscometry[7].

Preparation of the gels

The pectin was previously dispersed overnight in 0.1 mol l^{-1} citrate buffer (pH 3.0) at room temperature, and then centrifuged for 1 h at 28 000*g*. The dispersion was heated at 105°C in a paraffin bath and the required amount of sucrose was added under stirring. The total heating period was 10 min. The lost water was replaced and the sample was transferred to the instrument plate at the desired test temperature.

Rheological experiments

Dynamic rheological measurements were performed using a Carri-Med CS-50 controlled-stress rheometer (Carri-Med Ltd, Dorking, UK). Two different systems of measurement were used: (i) cone and plate geometry (radius = 25 mm, angle = 4°), which was used for time and frequency sweep experiments in isothermal conditions, and (ii) parallel plate geometry (gap = 2 mm, plate radius = 30 mm, with radial grooves in order to avoid gel slip), which was used for temperature sweeps in order to minimize the effect of temperature on the gap. After preparation, the sample was transferred to the instrument plate at the desired temperature, and the exposed surface was covered with a thin layer of low-viscosity paraffin oil to avoid solvent evaporation. The process of gel forming (cure experiments) was analysed by measuring the viscoelastic shear modulus $G^*(\omega)$ in the form of its storage (G') and loss (G'') components as a function of time at a frequency of 0.5 Hz. Mechanical spectra, $G'(\omega)$ and $G''(\omega)$, were obtained over the frequency range 0.005–5 Hz. Temperature sweeps were also performed at 0.5 Hz between 90°C and 20°C. Before the cooling scan during gel formation, the pectin/sucrose dispersion was kept in the rheometer for 5 min at 90°C. All experiments were performed in a constant strain mode with a low strain amplitude of 0.03.

Structure development rates

The influence of temperature on structure development rates (SDR), defined as dG'/dt, was evaluated by lowering the temperature at a constant rate (non-isothermal conditions), or by quenching the HMP/sucrose dispersions at fixed temperatures (isothermal conditions).

The evaluation of dG'/dt at each time was performed either by a simple forward-difference numerical method or after a polynomial fit or cubic spline interpolation, using TRATADATA software (Department of Chemical Engineering, University of Porto, Portugal), to smooth the data points. These treatments described the overall behaviour in a similar way.

As previously observed[8], SDRs calculated from G'–time data without previous fit and using the simple forward-difference numerical method showed much more scatter. This was also the case when using cubic spline interpolation, especially for the G'–time data obtained from the time sweep experiments, due to the high number of data points and the relative variation of G' obtained from point to point. For this reason, SDRs under isothermal conditions were calculated using the simple forward-difference numerical method, with subsequent smoothing of the dG'/dt data using a 'moving average' procedure, in which five data points were averaged around each given point to determine the output data. We believe that these treatments retain the character of the overall behaviour, will predict the correct curvatures, and that the parameters obtained will be reliable, while avoiding the scatter of data that has no physical significance.

Results and discussion

Effect of pectin concentration

Figure 1 shows the storage modulus as a function of time for different pectin concentrations during the ageing of HMP gels (pH 3, 60% w/w sucrose) at 20°C. Generally, the cure behaviour is typical of biopolymer gelation[9], with G' increasing rapidly at first and then more slowly, and where $G'' < G'$. With increasing pectin concentration, the largest changes occurred at an earlier stage. Thus the initial rise in the storage modulus was faster and the pseudo-plateau region was reached after a shorter time. When the pectin concentration was decreased, the sharp increase in G' was preceded by a period of time during which G' increased steadily and slowly. This period of time was also concentration-dependent, being shorter when the concentration was higher.

The initial period of gelation is plotted in expanded form in *Figure 2*. The time corresponding to the G'–G'' crossover clearly decreases with the increase in pectin

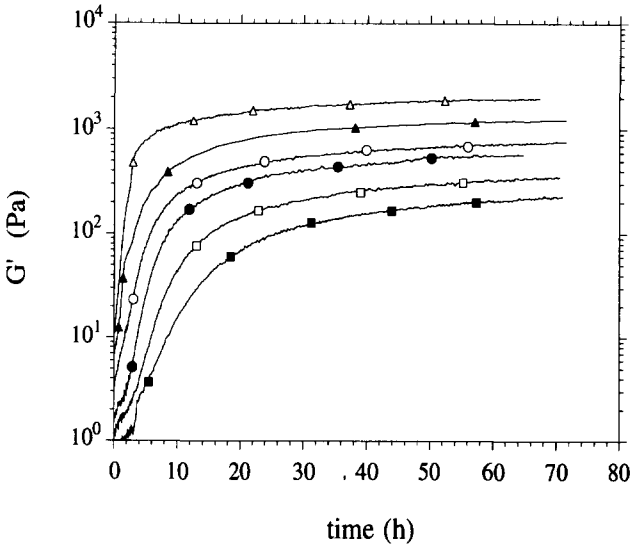

Figure 1 Cure curves on a semi-log scale recorded at 20°C and 0.5 Hz for various concentrations of HMP (60% w/w sucrose, pH 3): (■) 0.53%, (□) 0.60%, (●) 0.70%, (○) 0.80%, (▲) 0.91%, (△) 1.06%. Data were obtained every 3 min during the first 3 h, and then every 10 min. For clarity, only some of the experimental points are shown

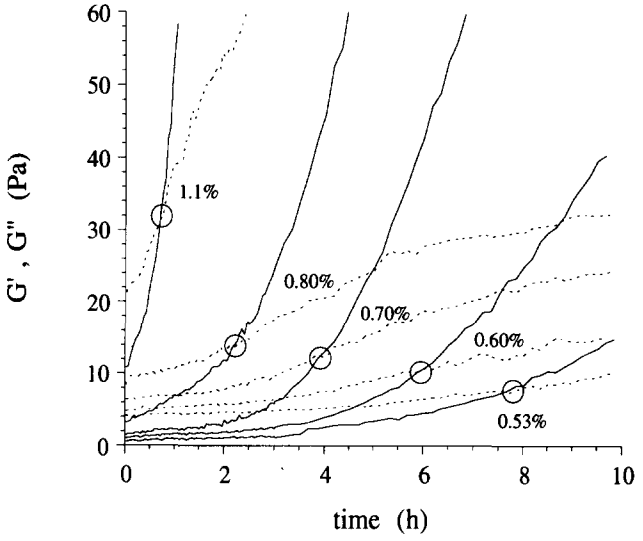

Figure 2 Initial increase of the dynamic modulus at 20°C and 0.5 Hz for various pectin concentrations (60% w/w sucrose, pH 3). Continuous lines denote the storage modulus (G') and dotted lines denote the loss modulus (G''). The circles show the time of G'–G'' crossover

concentration. Taking the crossover point as an apparent gelation time, we can observe that it was highly concentration-dependent, and ranged from 461 min to 38 min over the concentration range 0.53% to 1.06% (w/w). A power law relationship was obtained for the gelation rate (inverse of the gelation time) as a function of pectin concentration, with a slope of 3.6 (*Figure 3*). This value is higher than that previously reported by Oakenfull and Scott[10], which was around 2. The discrepancy may be due to the different samples used and particularly the different method of gel preparation. We note that these authors invariably obtained higher gelation rates even though they used a lower sucrose content (55%) and a higher pH (3.5).

However, if we take into consideration the more realistic kinetic model of Ross-Murphy[11], higher exponents are expected from a log–log plot of gelation rate *versus* concentration, even for polymer concentrations well above the critical gelling concentration (c_0), without the need to assume high-order kinetics. According to this approach, the gelation time (t_g) is given by

$$t_g \approx \frac{K}{((c/c_0)^n - 1)^p} \quad (1)$$

where t_g is the gelation time, K is a proportionality constant, n is the kinetic order, generally taken to be 2, and p is a critical exponent, $0 < p < 3$.

Considering a second-order reaction ($n = 2$), and taking $p = 1.8$ (the critical value of the percolation exponent[12]), the limiting value of the slope of a log–log plot of $1/t_g$ *versus* c at high c/c_0 will be equal to np, in agreement with the slope of 3.6 obtained.

Kinetic effects on structure development close to the gelation threshold were quantified by measuring the rate of change in G' ($\omega = 0.5$ Hz), i.e. dG'/dt, near the G'–G'' crossover. Both dG'/dt and the gel strength at the gelation threshold were sensitive to changes in pectin concentration. The rate of change in G' near the gel point increased as the pectin concentration was increased (*Figure 4*). A high power dependence was obtained, $dG'/dt \propto c^{4.1}$, in satisfactory agreement with the dependence of the gelation rate on concentration previously found. Even more pronounced concentration dependence has been reported for a wide range of systems. For bovine serum albumin gelation[13], the slope of modulus growth near the gel point was found to be proportional to c^{27} for low concentrations, and dG'/dt was proportional to c^6 even at higher concentrations. For physical gels of poly(acrylonitrile) in dimethylformamide close to the critical gelling concentration, Bisschops[14] found $dG'/dt \propto c^{22}$. In contrast, Nolte *et al.*[15] found a much lower power dependence of dG'/dt on polymer concentration ($dG'/dt \propto c^{1.25}$) for the gelation of xanthan with chromium ions, even close to the critical gelling concentration. Close to the critical concentration, the interpretation of these exponents is complex. It is

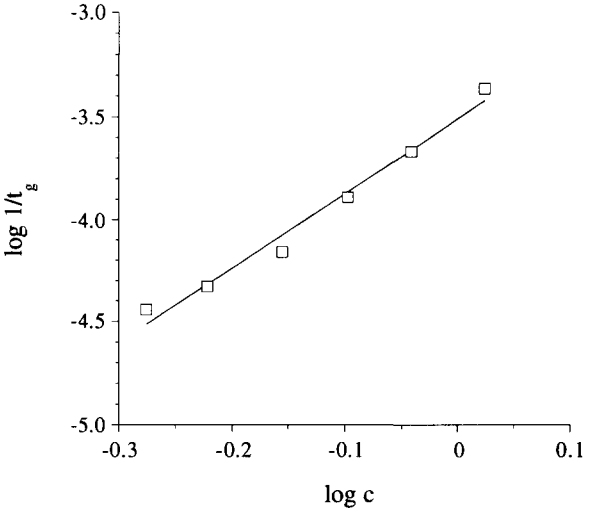

Figure 3 Effect of pectin concentration (%, w/w) on the gelation rate (inverse of the apparent gelation time, $1/t_g$ (s^{-1})). The best least squares fit has a slope of 3.6 and $R^2 = 0.981$

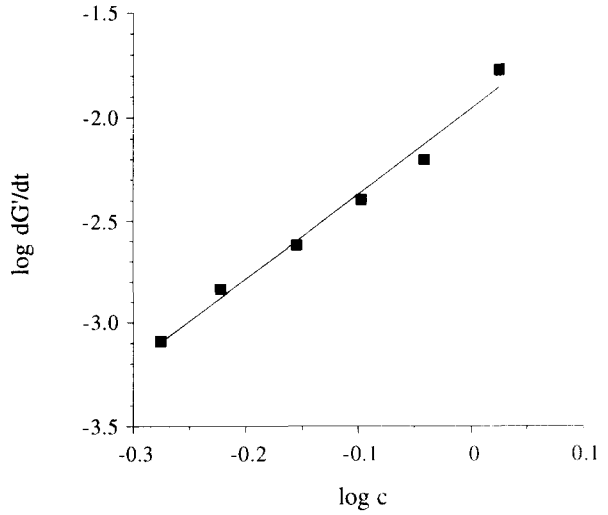

Figure 4 Effect of pectin concentration (%, w/w) on the rate of change of G' at the gelation threshold. The best least squares fit has a slope of 4.1 and $R^2 = 0.986$

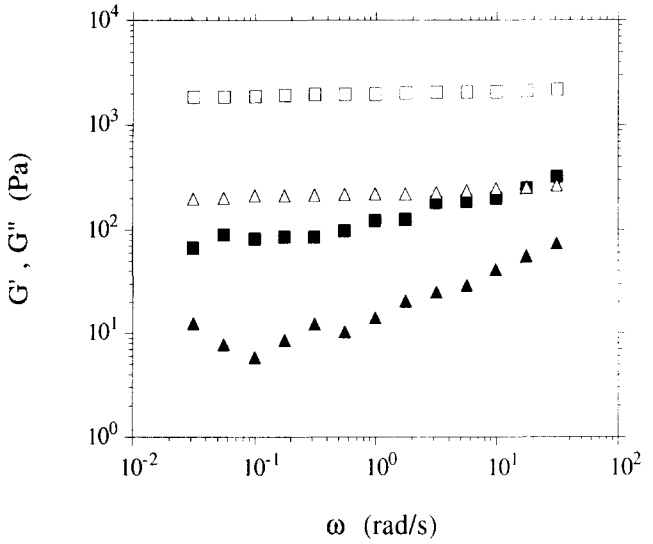

Figure 5 Mechanical spectra of gels aged for 72 h at 20°C, for two different pectin concentrations: 0.53% (△, G'; ▲, G'') and 1.06% (□, G'; ■, G'')

likely that the different mechanisms of macromolecular association involved are responsible for the wide range of values observed.

The storage modulus continued to increase slightly and continuously as a result of the slower formation and rearrangement of junction zones, reaching a pseudo-plateau region. Even after a relatively long period of ageing, G' continued to increase steadily in this region, as observed by plotting G' as a function of log ageing time (data not shown). This non-equilibrium behaviour may result from a continuous reorganization of the network. As expected for a network that has attained a state close to equilibrium, the kinetics operating after a longer period are quite different. The rate of change in G' after the pseudo-plateau was reached was much smaller, and practically independent of polymer concentration.

However, the magnitude of the modulus after ageing the gels was strongly dependent on concentration. *Figure 5* shows the frequency dependence of the magnitude of G' and G'', after 72 h of ageing, for the highest and lowest pectin concentrations studied. The general patterns are similar and characteristic of a 'true' gel. The storage modulus is almost independent of the experimental frequency range, and its value is about one order of magnitude higher than that of the loss modulus.

The concentration dependence of the storage modulus (G'), measured after ageing times of 48 h and 72 h for HMP gels in the concentration range 0.5–1.1% (w/w), is shown in *Figure 6*. The modulus increased with increasing pectin concentration, as a consequence of the increase in the number of junction zones resulting in an increase in the number of elastically active chains. In this concentration range, a power law dependence of G' on concentration (c) was observed, $G' \propto c^a$, with $a \approx 3$–3.2, depending on ageing time or frequency of measurement. Many reports have suggested that the elastic modulus of gels is proportional to the square of the concentration[9], i.e. a lower dependence than that observed for our HMP gels. This limiting behaviour was observed when the modulus was measured for much higher concentrations than the critical gelling concentration (c_0) and after long ageing times. Near the critical gel concentration, a large and variable power law dependence is observed.

The experimental conditions that have been used during the measurements performed on the HMP gels may be close enough to the critical gelling concentration to be the cause of the observed concentration dependence, and the modulus still increased monotonously with time even after 3 days of ageing, which may also explain the observed high concentration dependence of the gel modulus. From the results obtained by Comby *et al.*[16] from stress relaxation studies on HMP gels prepared under similar conditions, with a pectin concentration range of 1.4–1.8% (w/w), we obtained a high power concentration dependence of the elastic modulus in the range 2.8–2.9, in satisfactory agreement with our results for a slightly lower pectin concentration range.

Recently, it has been suggested that macromolecular

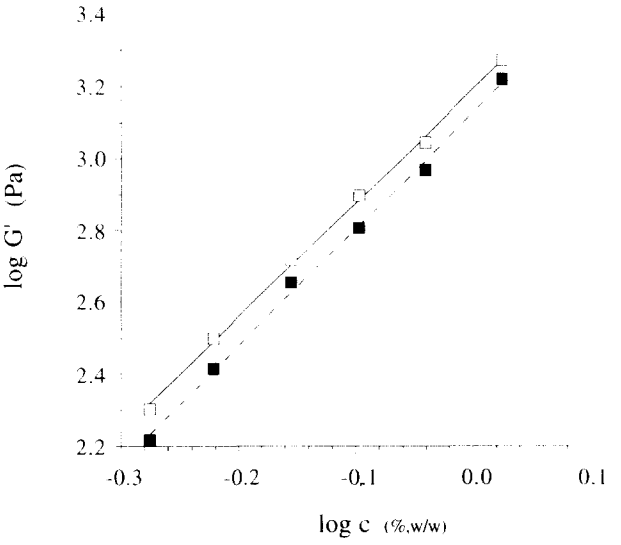

Figure 6 Concentration dependence of the storage modulus (G') of gels aged for 48 h (■) and 72 h (□), measured at 20°C and 0.056 rad s^{-1}, in the concentration range 0.5–1.1% w/w (pH 3, 60% w/w sucrose). A power law dependence of G' on c is observed, with $G' \propto c^{3.2}$ at 48 h and $G' \propto c^{3.1}$ at 72 h

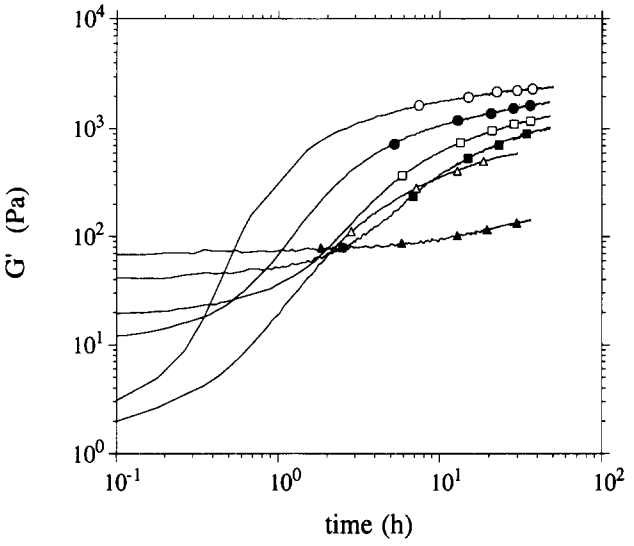

Figure 7 Storage modulus (G') as a function of the ageing time for 1% HMP (60% sucrose, pH 3), at several ageing temperatures: (▲) 5°C, (■) 10°C, (□) 15°C, (●) 20°C, (○) 30°C, (△) 50°C. Data were obtained every 3 min during the first 3 h, and then every 10 min. For clarity, only some of the experimental points are shown

microcrystallization plays an important role in the gelation mechanism of HMP[17]. The junction zones in HMP gels are predominantly formed through aggregates involving a large and variable number of pectin chains[1,4]. Some inhomogeneities in the pectin/sucrose network, with regions of some degree of crystallinity, are expected due to regularly arranged macromolecular segments formed by lateral packing of pectin chains. However, since the modulus continues to increase for long periods of time, it is very likely that there is a considerable degree of conformational flexibility in the junction zones in the HMP gels. Therefore, it is rather unlikely that the junction zones can be characterized by a high degree of crystallinity. However, as was reported for some gelled systems[18], the crystalline nature of the junction zones may also lead to a higher dependence of the pseudo-equilibrium modulus upon concentration, and this hypothesis should not be totally discarded. The rheological techniques employed are not suitable for determining whether the junction zones in our gelled system are predominantly formed by crystallites. To elucidate this point fully, other analytical techniques, such as small-angle neutron and X-ray scattering on gels, should be used.

Effect of temperature on gelation rate

Figure 7 shows the storage modulus (G') of an HMP/sucrose gel as a function of the ageing time at several ageing temperatures. The viscoelastic behaviour through the gel point and after ageing of the HMP gel is very sensitive to changes in temperature, and these aspects have been previously reported[3]. Here we are particularly interested in the effect of temperature on the kinetics of gelation. At 5°C, the gelation is slow and a weak gel was obtained, providing clear evidence of the very important role of hydrophobic interaction in the network structure of HMP gels. The pseudo-equilibrium storage modulus increased for temperatures up to 30°C and then decreased. At an intermediate temperature range, hydrogen bonds and hydrophobic interactions/van der Waals' forces can together contribute to the build-up of a network with the highest elasticity. This particular thermal behaviour of the HMP/sucrose system has been explained on the basis of the opposing effects of temperature on the types of interactions that stabilize this kind of gel[2,3].

The rate of gelation ($1/t_g$) of HMP/sucrose systems increased with increasing temperature in the range 15–60°C. Below 15°C, the gelation rate, based on the apparent gelation time, increased with a decrease in temperature. Macromolecular aggregation mediated by hydrogen bonding should predominate in this temperature range.

Between 15°C and 60°C, an Arrhenius treatment yielding activation energies for the process could be satisfactorily applied (*Figure 8*), and is useful to describe the thermal behaviour of the system at the gelation threshold. Two different temperature ranges could be identified, one between 15 and 30°C and other between 30 and 60°C, yielding apparent activation energies of 46.4 kJ mol^{-1} and 7.7 kJ mol^{-1}, respectively. Clearly the gelation rate is energetically favoured above 30°C. It is interesting that this inflexion temperature also corresponds to the ageing temperature at which the long-term modulus showed a maximum[4].

If a simple kinetic process were involved, a single linear relationship would be expected for the entire temperature range. A hypothesis to explain the observed behaviour can be advanced, based on two different processes involved in the initial gelation period of the pectin, each having rates with different temperature dependence. The first process is that of initial intermolecular local contacts, essentially by hydrogen bonding and involving short chain segments. This process will be the rate-determining process above 30°C. Hydrophobic interactions are well known to increase with increasing temperature and are also involved in HMP gelation. The second rate process can be idealized as corresponding to the subsequent lateral aggregation of chains and enlargement of junction zones, essentially governed by hydrophobic interactions, probably stabilized or strengthened by van der Waals' forces, and additionally

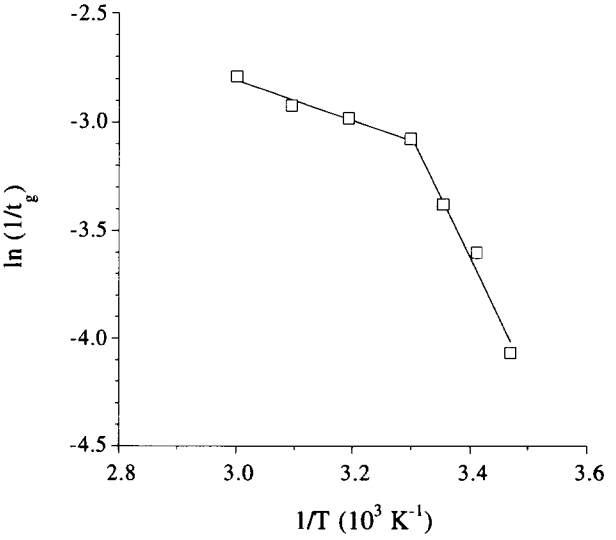

Figure 8 Arrhenius treatment for the effect of temperature on the gelation rate ($1/t_g$, s^{-1}) of 1% HMP dispersions (60% w/w sucrose, pH 3), at different ageing temperatures in the range 15–60°C

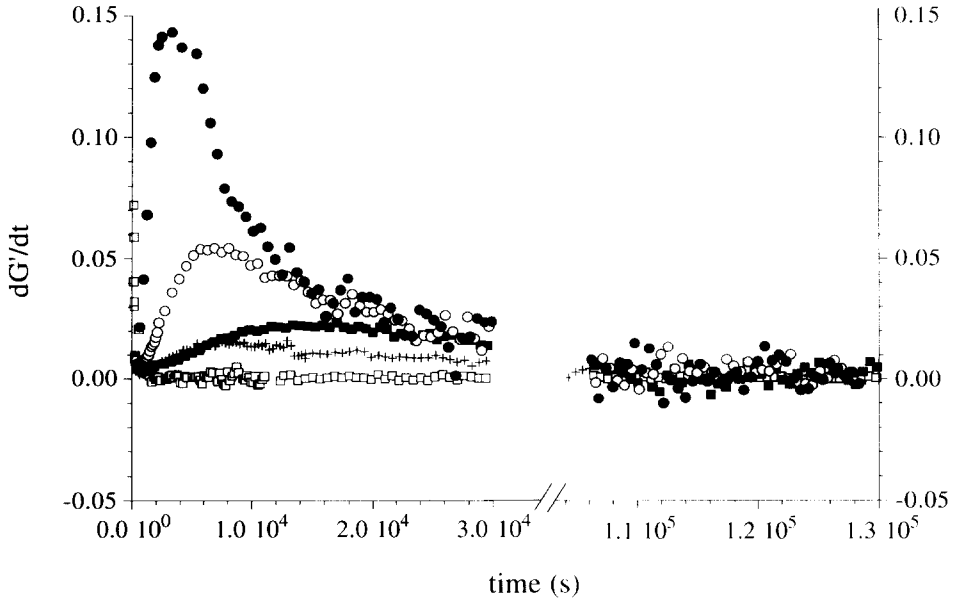

Figure 9 Structure development rates during ageing of a 1% HMP/60% sucrose dispersion (pH 3.0) under isothermal conditions at: (□) 5 C, (■) 15°C, (○) 20°C, (●) 30°C and (+) 50°C. SDRs were obtained by a numerical method followed by smoothing of the derivative values

by hydrogen bonds. This will be the rate-defining process below 30°C.

Besides their different temperature dependence, hydrogen bonds and hydrophobic interactions/van der Waals' forces also have different strengths and act at different distance scales. Therefore, it could be expected that, depending on temperature, different kinetics for the initial gelation period of the HMP/sucrose system would be applicable. Hydrogen bonds are less dependent on the separation of molecules and have enough energy to be stable even when developed in small numbers. Hydrogen bonds acting at larger intermolecular distances may be essential for the subsequent development of hydrophobic interactions and van der Waals' forces, through clustering of non-polar groups involving larger portions of the pectin chains.

Structure development rates for the ageing process

Gradual development of the network structure was reflected by a progressive increase in the storage modulus (G'). SDRs were measured under isothermal conditions at the different ageing temperatures previously studied and during the gelation process promoted by decreasing the temperature of the pectin dispersion (non-isothermal conditions).

Under isothermal conditions. As seen in *Figure 7*, the initial period of gelation is characterized by concave cure curves, after which they become convex and then almost linear. At 5°C, the convex region is not perceptible. This different behaviour, and the effect of the ageing temperature on the rate of ageing of the HMP gels, can be clearly seen if we analyse the SDRs at different ageing temperatures. *Figure 9* shows the SDRs as a function of the ageing time at different temperatures. Due to the rapid quenching of the sample to the desired ageing temperature, we can assume that all structural modifications during ageing occurred isothermally. Significant differences in SDRs were observed during the first 4 h of ageing. The general behaviour is an initial increase in SDR, followed by a decrease in the SDR, which continues to decrease steadily until the end of the ageing period. The sharpest initial increase in the ageing process was observed at 30°C, and the maximum SDR was reached after a shorter ageing time than at the other ageing temperatures. For ageing at 5°C, a different behaviour was observed, consisting of an initial rapid decrease during the first hour, followed by an almost constant SDR until the end of the ageing period. Between 15 and 50°C, the initial increase in SDR was due to the initially rapid increase in the storage modulus during the ageing of the pectin gel as previously observed. This exponential time dependence of the elasticity growth in the system corresponds to the rapid formation of junction zones between pectin chains. After this stage, the SDR decreases until it reaches a value that is always higher than zero but is almost independent of the ageing time or ageing temperature, which corresponds to a slow increase in the storage modulus and to the almost linear region of the cure curves. This stage corresponds to a slow reorganization of the network involving the creation of new junction zones, or an increase in the extension of the junctions between molecular chains.

The effect of ageing temperature on structure development rates has been quantified by calculating average structure development rates (SDR_{av}):

$$SDR_{av} = \frac{1}{t_1 - t_2} \int_{t_1}^{t_2} SDR \, dt \qquad (2)$$

The area under the curves of dG'/dt *versus* t was determined by calculating the sum of the trapezoids formed by the data points, between the lower limit ($t_1 = 720$ s) and the upper limit ($t_2 = 108\,000$ s) considered. The SDR_{av} values obtained are plotted in *Figure 10* as a function of the ageing temperature. As expected, the behaviour shown followed the bell-shaped dependence on temperature previously found for the elastic modulus of cured gels at different temperatures[4]. The maximum average rate of structure formation occurred around

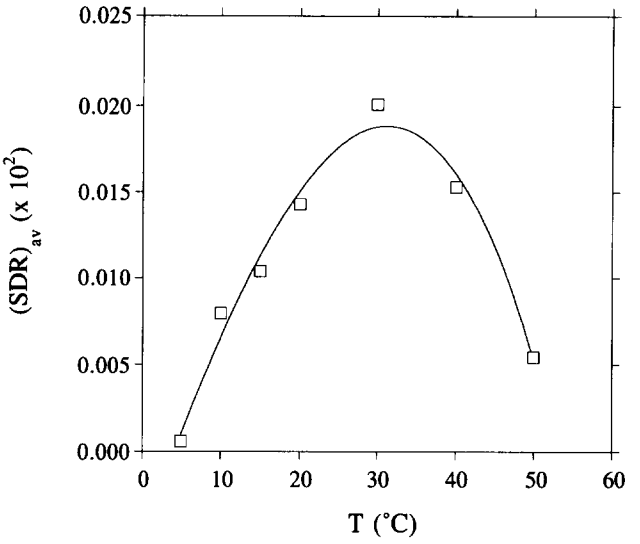

Figure 10 SDR_{av} at different ageing temperatures of a 1%HMP/60% sucrose dispersion (pH 3.0)

30°C, i.e. the same ageing temperature that resulted in a pectin gel with maximum elastic modulus (G').

Under non-isothermal conditions. SDRs were also measured during the gelation process promoted by decreasing the temperature of the pectin dispersion (non-isothermal conditions) using temperature sweep experiments.

During gelation induced by cooling, the rate of structure development increased with decreasing temperature, showing a higher temperature dependence at the higher and lower temperature ranges (*Figure 11*). A temperature range where the rate of structure development increases with a decrease in temperature is characteristic of processes that involve a nucleation step. For example, the renaturation of DNA shows this effect at a temperature range below the melting temperature[19]. This behaviour can also be found during the gelation of biopolymers that undergo coil–helix transitions, such as gelatin[20] or agarose[21]. However, in these cases, the effect of temperature on the formation of ordered structures typically follows a sigmoidal shape, instead of the logarithmic shape observed (*Figure 11*), and, consequently, the effect of temperature on the rate of structure development shows a bell-shaped curve, with a maximum value at an intermediate temperature.

Non-isothermal kinetic data generation. Kinetic data were obtained for the structure development process during cooling of the pectin/sucrose dispersion. This kind of analysis is difficult due to the combined effects of time and temperature. Usually, kinetic data are obtained using time as an independent variable and changing the temperature and test sample for each run.

We have studied an approach that could be useful to take into consideration kinetic effects as well as thermal effects on biopolymer gelation. The method used was adapted from the 'linear increasing temperature method' proposed by Rhim *et al.*[22] to changes in structure followed under non-isothermal conditions (temperature sweeps) by linearly decreasing the temperature at a constant rate.

The non-isothermal kinetic model is based on combination of the classical rate equation, the Arrhenius equation, and the time–temperature relationship, yielding:

$$\ln\left(\frac{1}{G'^n}\frac{dG'}{dt}\right)=\ln k_0-\left(\frac{E_a}{RT}\right) \tag{3}$$

where G' is the storage modulus, taken as a measure of the number of junction zones in the network, n is the reaction rate order, t is the time, k_0 is the Arrhenius frequency factor, E_a is the activation energy of the process, R is the universal gas constant, and T is the absolute temperature.

To evaluate the reaction rate order of the process, we tried to use multiple linear regression (Statgraphics software version 5, STSC Inc., 1991), on the logarithmic form of Equation (3):

$$\ln\left(\frac{dG'}{dt}\right)=\ln k_0+n\ln G'-\left(\frac{E_a}{RT}\right) \tag{4}$$

However, a satisfactory linear relationship was not obtained whether data over the entire temperature range (90–20°C) or over shorter temperature ranges were used. The coefficients obtained from the model fitting had high standard errors, and their 95% confidence intervals were so large that the values obtained were not considered satisfactory.

One of the most important steps in using this method to describe the gelation process of biopolymers is evaluation of the reaction order, a parameter that may be complex and difficult to determine for this kind of process. At a first approximation, we can expect the collision of two macromolecules as the most probable first stage in intermolecular aggregation, giving rise to second-order kinetics. Therefore, we have assumed that, at least in the initial incipient gelation process of the HMP/sucrose aqueous system being analysed, macromolecular aggregation can be described by a bimolecular association equilibrium, i.e. $n=2$. This is also supported by the results obtained for the concentration dependence of the gelation rate. However, we cannot exclude a more complex mechanism due to the change

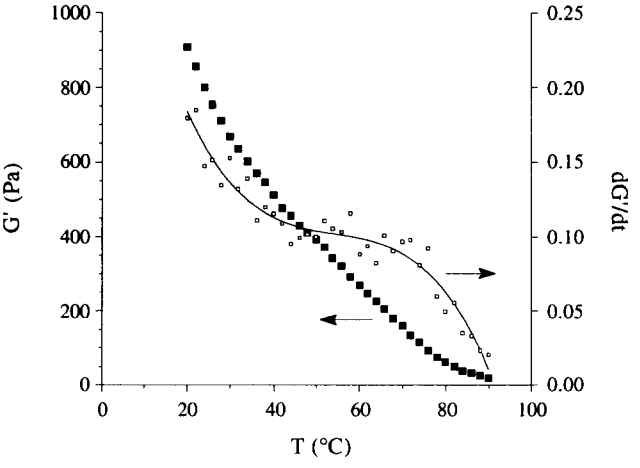

Figure 11 Influence of temperature on storage modulus (G') and dG'/dt (SDR) for a 1% HMP/60% sucrose dispersion under cooling from 90 to 20°C (0.5°C min^{-1}). (■) G'; (□) dG'/dt calculated after cubic splines fit; (——) dG'/dt calculated after polynomial equation fit

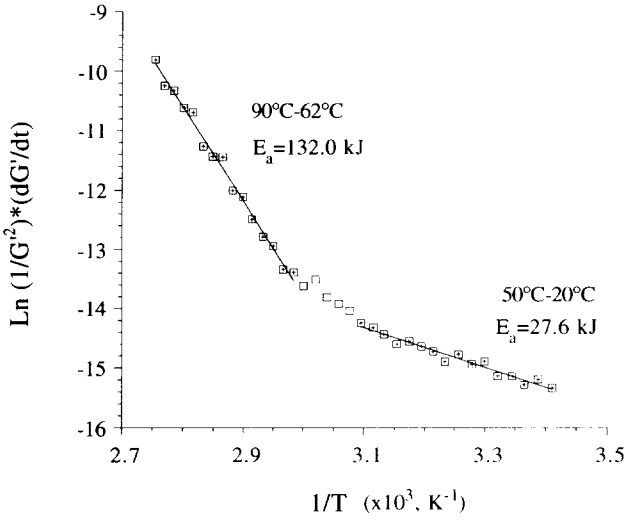

Figure 12 Arrhenius-type plot for the change in the storage modulus (G') during cooling of a 1% HMP/60% sucrose dispersion (pH 3.0), assuming second-order kinetics and a two-step process

in temperature or in the number of preformed junction zones during the cooling-induced gelation of our system.

Assuming a reaction order $n = 2$, Equation (3) was used to estimate the activation energy of the process, by using a linear least-squares routine (*Figure 12*). The process could be satisfactorily described by assuming a two-step process corresponding to two different temperature ranges. Between 90 and 62°C, the least-squares linear regression yielded $E_a = 132.0\ \text{kJ mol}^{-1}$ and $k_0 = 5.09 \times 10^{14}\ \text{Pa}^{-1}\,\text{s}^{-1}$, and $E_a = 27.6\ \text{kJ mol}^{-1}$ and $k_0 = 1.77 \times 10^{-2}\ \text{Pa}^{-1}\,\text{s}^{-1}$ between 50 and 20°C for the structure development process during cooling of the HMP/sucrose dispersion.

These values of apparent activation energies should not be compared directly with previously reported values of activation energies for cured HMP/sucrose gels, e.g. $E_a = 155\ \text{kJ mol}^{-1}$ and $130\ \text{kJ mol}^{-1}$ found for the gel relaxation processes by Plashchina *et al.*[23] and Kawabata[24], respectively, which were thought to be related to the formation/rupture of junction zones in preformed gels. In our data, every data point was estimated in a continuous manner during the initial period of gelation induced by cooling. Hence, the kinetic parameters were affected quantitatively by the previous thermal history of the material. The higher activation energy in the higher temperature range may reflect the higher energy barrier between the unaggregated and aggregated macromolecular states under temperature conditions disadvantageous to the development of intermolecular interactions that could promote formation of the pectin network. Under thermal conditions that favour interchain aggregation, as is the case below 50°C, the structure development process is energetically easier and the activation energy is lower.

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References

1 Walkinshaw, M.D. and Arnott, S. *J. Mol. Biol.* 1981, **153**, 1075
2 Oakenfull, D. and Scott, A. *J. Food Sci.* 1984, **49**, 1093
3 da Silva, J.A.L. and Gonçalves, M.P. in 'Cellulose Derivatives and Related Polysaccharides', Ellis Horwood, Chichester, in press
4 Morris, E.R., Gidley, M.J., Murray, E.J., Powell, D.A. and Rees, D.A. *Int. J. Biol. Macromol.* 1980, **2**, 327
5 McFeeters, R.F. and Armstrong, S.A. *Anal. Biochem.* 1984, **139**, 212
6 Scott, R.W. *Anal. Chem.* 1979, **51**(7), 936
7 da Silva, J.A.L., Gonçalves, M.P. and Rao, M.A. *J. Food Sci.* 1992, **57**, 443
8 Rao, M.A. and Cooley, H.J. *J. Food Sci.* 1993, **58**, 876
9 Clark, A.H. and Ross-Murphy, S.B. *Adv. Polym. Sci.* 1987, **83**, 57
10 Oakenfull, D. and Scott, A. in 'Gums and Stabilisers for the Food Industry 3' (Eds G.O. Phillips, D.J. Wedlock and P.A. Williams) Elsevier Applied Science Publishers, London, 1986, p 465
11 Ross-Murphy, S.B. *Carbohydr. Polym.* 1991, **14**, 281
12 Stauffer, D., Coniglio, A. and Adam, M. *Adv. Polym. Sci.* 1982, **44**, 103
13 Richardson, R.K. and Ross-Murphy, S.B. *Int. J. Biol. Macromol.* 1981, **3**, 315
14 Bisschops, J. *J. Polym. Sci.* 1955, **17**, 89
15 Nolte, H., John, S., Smidsrød, O. and Stokke, B.T. *Carbohydr. Polym.* 1992, **18**, 243
16 Comby, S., Doublier, J.L. and Lefebvre, J. in 'Gums and Stabilisers for the Food Industry 3' (Eds G.O. Phillips, D.J. Wedlock and P.A. Williams) Elsevier Applied Science Publishers, London, 1986, p 203
17 Sheyenson, V.A., Khomutov, L.I., Lashek, N.A. and Ptitchkina, N.M. in 'Gums and Stabilisers for the Food Industry 6' (Eds G.O. Phillips, P.A. Williams and D.J. Wedlock) Oxford University Press, London, 1992, p 311
18 Ferry, J.D. 'Viscoelastic Properties of Polymers', 3rd edition, Wiley, New York, 1980
19 Wetmur, J.G. and Davidson, N. *J. Mol. Biol.* 1968, **31**, 349
20 Djabourov, M., Leblond, J. and Papon, P. *J. Phys France* 1988, **49**, 319
21 Braudo, E.E., Muratalieva, I.R., Plaschina, I.G. and Tolstoguzov, V.B. *Carbohydr. Polym.* 1991, **15**, 317
22 Rhim, J.W., Nunes, R.V., Jones, V.A. and Swartzel, K.R. *J. Food Sci.* 1989, **54**, 446
23 Plaschina, I.G., Fomina, O.A., Braudo, E.E. and Tolstoguzov, V.B. *Colloid Polym. Sci.* 1979, **257**, 1180
24 Kawabata, A. *Mem. Tokyo Univ. Agric.* 1977, **19**, 115