

Characterization of Gelation of Aqueous Pectin via the Ugi Multicomponent Condensation Reaction

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Summary

Turbidity and rheological features during gelation of semidilute solutions of pectin via the Ugi multicomponent condensation reaction have been investigated at different polymer and cross-linker concentrations at ambient temperature. The gelation time of the system decreased with increasing polymer and cross-linker concentrations. At the gel point, a power law frequency dependence of the dynamic storage modulus ($G' \sim \omega^n$) and loss modulus ($G'' \sim \omega^n$) was observed with $n' = n'' = n$. The value of the power law exponent is about 0.6–0.7 for all the gelling systems, which is close to that predicted (0.7) from the percolation model. The elastic properties of the gels continue to grow over a long time in the post-gel region, and a solidlike response is observed at later stages during the gelation process. The turbidity of the gelling system is higher as the polymer and cross-linker concentrations rise, but the turbidity is virtually unaffected during an extended time after the formation of the incipient gel. It was demonstrated that polymer and cross-linker concentrations could be utilized to tune the physical properties, such as transparency and viscoelasticity, of the Ugi hydrogels.

Keywords

Biopolymers, Ugi reaction, rheology, turbidity, drug delivery systems, hydrogels

Introduction

Pectin is a biocompatible anionic polysaccharide, extracted from cell walls in most plants and is widely used as gelling agent, stabilizer, and emulsifier in many food products, and in the pharmaceutical industry [1]. Like most other polysaccharides, pectins are heterogeneous with respect to both chemical structure and molecular weight. Their composition varies with the source of pectin. Pectins (see Figure 1) consist primarily of (1→4) linked α -D-galacturonid units occasionally interrupted by (1→2) linked α -L-rhamnopyranosyl residues. Methyl groups esterify some of the galacturonic acid monomer units, and the degree of methoxylation (DM) is expressed

conventionally as a percentage of total galacturonic acid content. According to DM, pectins are frequently classified in two categories, namely the “high-methoxy” pectins with DM > 50% and the “low-methoxy” pectins with DM typically in the range ~ 30-40 %. For “high-methoxy” pectins, a combination of hydrogen bonding and hydrophobic interactions are usually responsible for chain association and gelation. While in the aqueous solutions of low-methoxy pectins, hydrogen-bonded intermolecular complexes are expected to play a dominant role in the chain association process [2,3].

Physical gels of pectins have been proposed as matrix for a colon drug delivery system [1]. Compared with a physical gel, the chemical gel has the advantages of high mechanical strength, versatile properties, and easy-control. Chemical hydrogels are also of interest in the development of drug delivery systems. In a previous investigation [4], the Ugi multicomponent reaction [5] was employed to produce hydrogels of alginate. As can be seen from the structure, the α -D-galacturonyl sequences of pectin are almost the exact mirror image of the α -L-guluronic acid regions in alginate. As might therefore be expected, the two materials will show some direct parallels in the reaction. However, in spite of this similarity in structure, it will be shown that the present gelling systems exhibit different characteristic physical features than the alginate hydrogels.

In this work, the gelling process of semidilute pectin solutions via the Ugi reaction has been studied by means of rheology and turbidimetry. The effects of pectin concentration and cross-linker density have been examined. The aim of this study is to understand the rheological and structural features of the systems during the gelation process. The results will show that it is possible to modulate the features of the gel network by altering the polymer concentration and the cross-linker density.

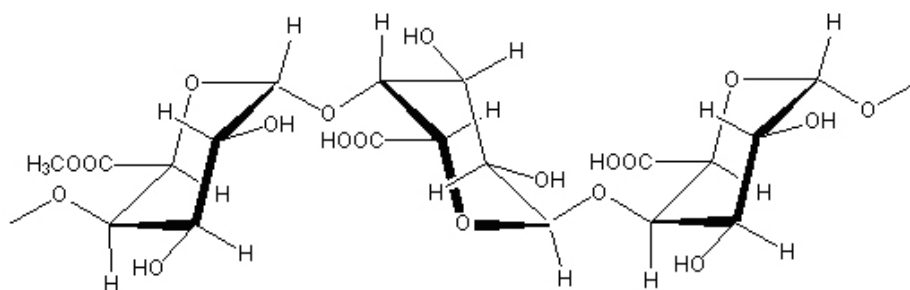


Figure 1. A schematic illustration of the principal structure of pectin.

Experimental

Materials

A low-methoxyl pectin sample, Pectin Classic CU 701 (lot no.00306088), was kindly given to us by Herbstreith & Fox KG, Sweden. According to the specifications from the manufacturer, the galacturonic acid content is 88 mol % with a degree of methoxylation (DM) of 35 mol %. The molecular weight is estimated to be 50 000. The molecular weight distribution of the sample is not known, but it is probably broad. The overlap concentration, c^* , is approximately 0.6 wt % [6].

The raw pectin sample was dissolved in water under heavy stirring to reduce aggregation. Thereafter the solution was stirred overnight at room temperature to get a homogeneous solution, and the final concentration was 1.0 wt. %. The pectin solution was then centrifuged for 2.5 hours at 3800 rpm to remove large aggregates. The supernatant solution was dialyzed against water for 7 days to remove salt and other low-molecular weight impurities, and regenerated cellulose with a molecular weight cutoff of 8000 was used as dialyzing membrane. The dialyzed sample was then freeze-dried and the polymer was stored in a freezer to avoid degradation.

Formaldehyde, 1,5-diaminopentane (DAP), and cyclohexylisocyanide were purchased from Merck or Fluka and were of analytical grade. These chemicals were used without any further purification. Millipore water was utilized in the preparation of the solutions.

Solutions and gel formation

The freeze-dried polymer was dissolved in water and the solutions were homogenized by gentle stirring at room temperature overnight. The polymer concentrations are expressed in terms of weight percentage of polymer. All measurements were carried out on freshly prepared samples.

Formaldehyde, DAP, and cyclohexylisocyanide were added to the solution successively. The molar amount of bifunctional cross-linker DAP was calculated with respect to the molar amount of carbohydrate monomers thus determining the theoretical cross-linking density. The other components were added in an excess of about 40 %. For example, in the case of 2 wt. % pectin solution and 17 mol % cross-linking density, 0.2 g (1.1 mmol) pectin was dissolved in 10 g water, then 17.9 μl (0.26 mmol) formaldehyde, 22.1 μl (0.19 mmol) DAP and 32.3 μl (0.26 mmol) cyclohexylisocyanide were added successively. After the addition of each component, the solution was stirred vigorously to disperse the component homogeneously in the solution. After the last component cyclohexylisocyanide was added, the solution was stirred vigorously to ensure a homogeneously dispersed solution for the rheology and turbidity measurements. Cross-linker concentrations in the range 15-18 mol % were utilized and polymer concentrations (1-3 wt %) in the semidilute regime were studied. All measurements were carried out at 25 °C.

Turbidity Measurements

The transmittances of the gelling and nongelling pectin solutions were measured with a temperature controlled Helios Gamma (Thermo Spectronic, Cambridge, UK) spectrophotometer at a wavelength of 500 nm. We have chosen this wavelength because it is sensitive to turbidity changes, but the same trend in the turbidity will also be observed at other wavelengths. The apparatus is equipped with a temperature unit (Peltier plate) that gives an accurate temperature control over an extended time. The turbidity, τ , of the samples were calculated from:

$$\tau = (-1/L)\ln(I_t/I_0) \quad (1)$$

where L is the light path length in the cell (1 cm), I_t is the transmitted light intensity, and I_0 is the incident light intensity.

Rheology experiments

Oscillatory shear sweep measurements were carried out in a Paar-Physica MCR 300 rheometer using a cone-and-plate-geometry, with a cone angle of 1° and a diameter of 75 mm. The samples were introduced onto the plate with great care to avoid shear effects on the solutions. To prevent evaporation of the solvent, the free surface of the sample was always covered with a thin layer of low-viscosity silicone oil (the viscoelastic response of the samples is virtually not affected by this layer). The measuring device is equipped with a temperature unit (Peltier element) that provides a good temperature control over an extended time. The values of strain amplitude were checked to ensure that all measurements were performed in the linear-viscoelastic regime, where the dynamic storage modulus (G') and loss modulus (G'') are independent of strain amplitude. The oscillating sweep experiments were carried over an extended angular frequency (ω) domain.

Analysis of Rheological Data

A simple power law can describe the rheological behavior of an incipient gel, where the dynamic moduli are related as [7]

$$G' = G''/\tan\delta = S\omega^n\Gamma(1-n)\cos\delta \quad (2)$$

where $\Gamma(1-n)$ is the gamma function, n is the relaxation exponent, and S is the gel strength parameter, which depends on the cross-linking density and the molecular chain flexibility [7]. This relationship has been utilized in this work to calculate the values of S . The phase angle (δ) between stress and strain is independent of frequency (ω) but proportional to the relaxation exponent:

$$\tan\delta = G''/G' = \tan(n\pi/2) \quad (3)$$

However, if the dynamic moduli exhibit very weak frequency dependence, it is convenient to follow the evolution of the rheological properties during gelation through the complex viscosity, with its absolute value $|\eta^*(\omega)|$ given by

$$|\eta^*(\omega)| = (G'^2 + G''^2)^{1/2}/\omega \quad (4)$$

In an analogous way as for the dynamic moduli, we may describe the frequency dependence of the absolute value of complex viscosity at the gel point (GP) in terms of a power law [8]

$$|\eta^*(\omega)| = aS\omega^m \quad (5)$$

with

$$m = n - 1 \quad (6)$$

and

$$a = \frac{\pi}{\Gamma(n)\sin(n\pi)} \quad (7)$$

where m is related to the relaxation exponent n . It is clear that if the frequency dependence of the dynamic moduli is weak (n is close to 0), a strong, easily detected

dependency of m (m approaches -1) is expected. Values of m close to zero indicate liquidlike behavior, whereas values of m approaching -1 suggest solidlike response. The values of S can also be calculated from the absolute value of complex viscosity (Equation (5)) and the results are consistent with those obtained from Equation (2). The gel point can be determined by observation of a frequency-independent value of $\tan \delta$ ($= G''/G'$) obtained from a multifrequency plot of $\tan \delta$ versus time [9]. An alternative method to determine the gel point is to plot against time the “apparent” viscoelastic exponents n' and n'' ($G' \sim \omega^{n'}$, $G'' \sim \omega^{n''}$) calculated from the frequency dependence of G' and G'' at each time of measurement and observing a crossover where $n' = n'' = n$ [10].

Results and discussion

Effect of Polymer Concentration at a Fixed Cross-linker Level

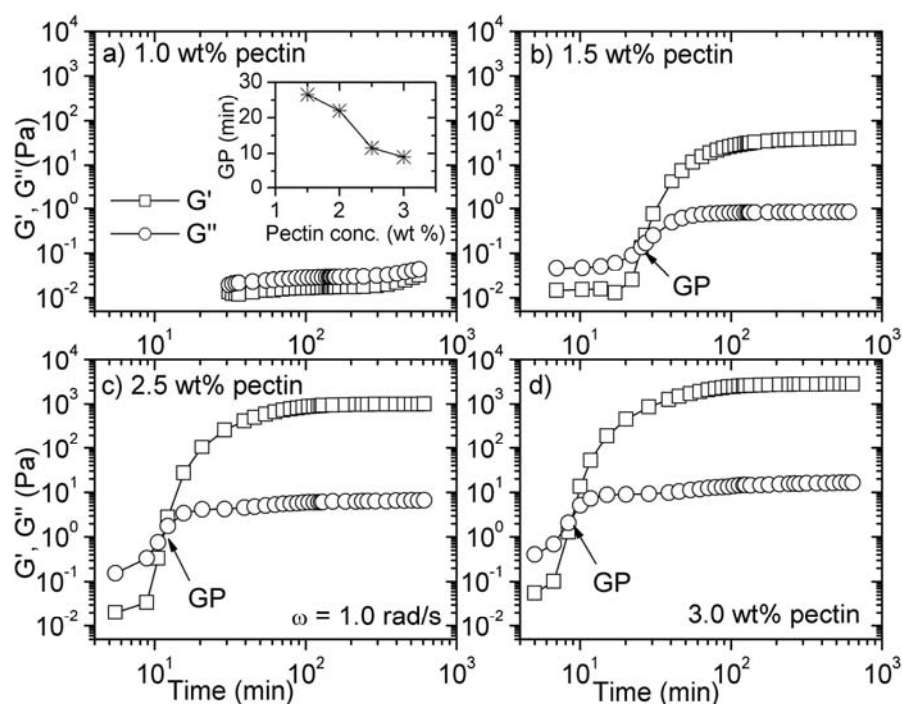


Figure 2. Time evolution of the dynamic moduli (G' , and G'') for the polymer concentrations of 1.0 wt. % (a), 1.5 wt. % (b), 2.5 wt. % (c) and 3.0 wt. % (d) at a fixed cross-linker concentration of 17 mol %. The gel point (GP) is plotted as a function of polymer concentration in the inset plot in (a).

Time evolution of the storage modulus G' and the loss modulus G'' (at a fixed angular frequency of 1.0 rad/s) is depicted in Figure 2 for systems with different polymer concentrations at a constant cross-linker level (17 mol %). Due to very short gelation times it is difficult to determine the gel point (GP) using the methods described above. Therefore, the crossover of G' and G'' was used to estimate the approximate gel point, as indicated by arrows in Figure 2. These gel points correspond approximately to

those found by the “tilting tube” method [11]. For the 1.0 wt. % solution, no gelation is detected but only a trivial viscosification of the solution occurs, whereas at higher polymer concentrations gels are formed and the gel point decreases with increasing polymer concentration (see the inset plot of Figure 2a). This trend signals that more sites are available for interpolymer cross-linking as the polymer concentration increases; hence the connectivity of the network is established earlier and a faster gelation occurs. Up to the gel point, G' is always smaller than G'' , indicating that the viscous response is the dominating rheological behavior of the systems. After the gel point, G' rises and becomes larger than G'' , which is a characteristic feature of the elastic response that is predominant in the postgel region. The values of G' and G'' at the gel point increase when the polymer concentration is raised. This is due to the enhancement of interpolymer interactions (caused mainly by hydrogen bonds) in the samples at higher polymer concentrations.

Time evolution of the complex viscosity (at a constant angular frequency of 1.0 rad/s) in the course of gelation (the gel points are indicated by arrows) is depicted in Figure 3a for systems with different polymer concentrations and a fixed cross-linking density (17 mol %). The values of η^* are higher as the polymer concentration rises, and the growth of η^* during the initial stage of the gelation process is more marked as the polymer concentration increases. We note that η^* levels off after a long time in the post-gel region, because most of the cross-linker zones have been established and the cross-linker reaction is completed. The findings can be rationalized in the following way. At a low polymer concentration, the necessary connectivity of the network is not established and no gel evolves. When the polymer concentration increases the number of “active sites” for cross-linking of the polymer network rises, and many intermolecular bridges are formed, favoring a faster gelation and stronger gel-networks are created (higher values of the plateau regions at long gelling times).

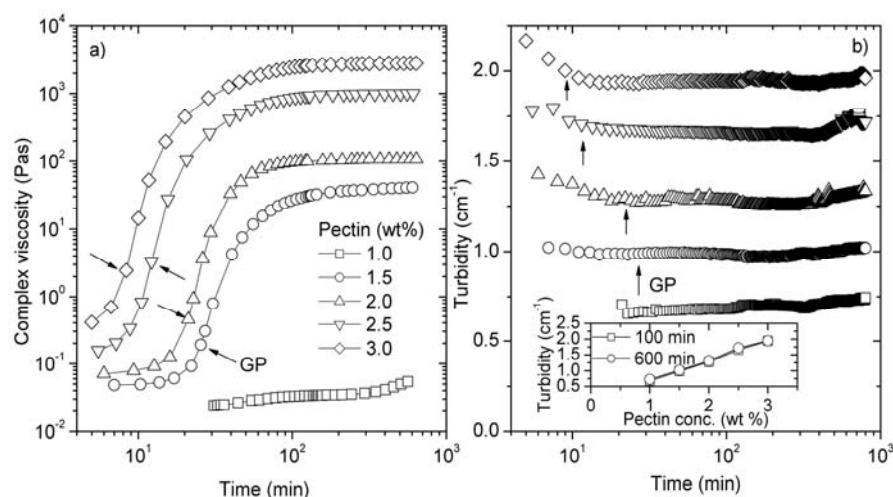


Figure 3. Time evolution of the complex viscosity (at constant angular frequency of 1.0 rad/s) (a) and turbidity (every second point is shown) (b) for the polymer concentration of 1.0 wt. %, 1.5 wt. %, 2.0 wt. %, 2.5 wt. % and 3.0 wt. % at a fixed cross-linker concentration of 17 mol %. Comparison of the values of turbidity at 100 and 600 minute for the gelling system of different pectin concentration is illustrated in the inset plot of (b).

Figure 3b illustrates the effect of increasing polymer concentration on the turbidity (τ) in the course of gelation (the GPs are indicated by arrows). We notice that the value of τ rises with increasing polymer concentration. This is partly ascribed to a trivial concentration effect according to the Lambert-Beers law, but enhanced association at higher polymer concentrations may also contribute to this behavior. The turbidity of the gelling systems decreases in the pre-gel region, and τ is practically constant in the post-gel regime as can be seen from Figure 3b. The initial decrease of τ may be associated with the break-up of the intermolecular hydrogen bonds in the pectin solution, caused by the cross-linker DAP. This substance may act as a hydrogen bond breaking agent. The inset plot of Figure 3b shows that the turbidities increase marginally when the reactions proceed from 100 to 600 minutes, suggesting that the heterogeneity of the gel-network is practically unaffected in the post-gel region. Studies on other gelling systems have revealed that heterogeneities are usually developed in the course of the curing process in the post-gel regime [4,12,13]. Some characteristic data for incipient gels obtained from different polymer concentration at a fixed cross-linking density of 17 mol % are presented in Figure 4. Because of very short gelation times it has been difficult to determine the gel point with great accuracy. An approximate value of the relaxation exponent n is around 0.6~0.7 for the systems with different polymer concentrations. The values are fairly close to that predicted from the percolation model [14,15]. A fractal dimension d_f of the incipient gel network is evaluated by using Muthukumars theory assuming fully screened excluded volume interactions (Rouse dynamics) [16,17]:

$$n = d(d + 2 - 2d_f)/2(d + 2 + - d_f) \quad (8)$$

where d ($d=3$) is the spatial dimension. The values of d_f for all the above systems are around 2. However, values of the fractal dimension around 2.5 have been reported for various polysaccharide systems forming physical hydrogels [7,13,18,19], which suggests a rather compact homogeneous network structure. The value of $d_f \sim 2.0$ in the present work indicates that the structure morphology of this network, where gelation is induced through chemical cross-linking, is different from the physical gel. It is evident from these results that the gel strength (S) increases with polymer concentration at a fixed level of cross-linker addition, which comes from the formation of more entanglements and effective intermolecular bridges at higher polymer concentration.

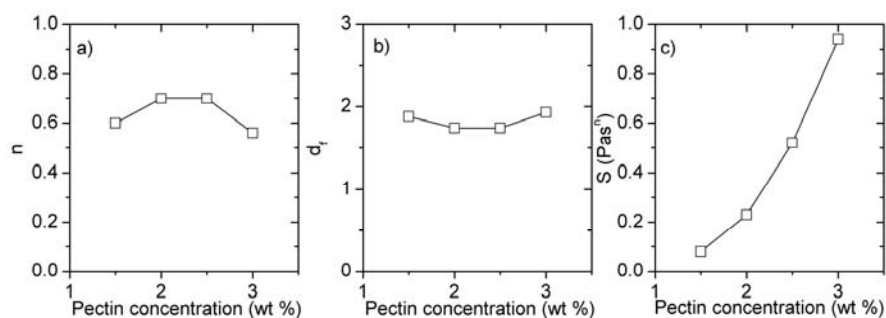


Figure 4. Effects of pectin concentration on the viscoelastic exponent n (a), on the fractal dimension d_f (b), and on the gel strength parameter S (c) of the incipient gels with a fixed cross-linker concentration of 17 mol %.

Effect of Cross-Linker Density at a Constant Polymer Concentration

Figure 5 shows the time evolution of the complex viscosity at different levels of cross-linker addition for pectin solutions of a constant polymer concentration (2.5 wt. %). Due to the short times of gelation, from 9 to 20 minutes (see Figure 6), Figure 5 mainly illustrates the behavior in the post-gel region. The profiles of the curves are reminiscent of the corresponding ones for the time evolution of complex viscosity at different polymer concentrations (see Figure 3). The growth of the complex viscosity is shifted to longer times as the cross-linker concentration decreases and the plateau-like region of the curve appears at a later stage with lower values of η^* . This is expected because at lower cross-linker levels, the cross-linker agent should be consumed at earlier times and a more fragile gel-network should be formed. The effect of cross-linker concentration on the complex viscosity at a long time (600 min) is depicted in the inset of Figure 5. A steady rise of η^* is observed as the cross-linker concentration increases. This suggests that “harder” or “stronger” gels are developed at higher cross-linker density. This may indicate that the number of trapped entanglements between network strands is favored by enhanced cross-linking density. Furthermore, the probability of forming more junction zones will rise as the cross-linker concentration increases.

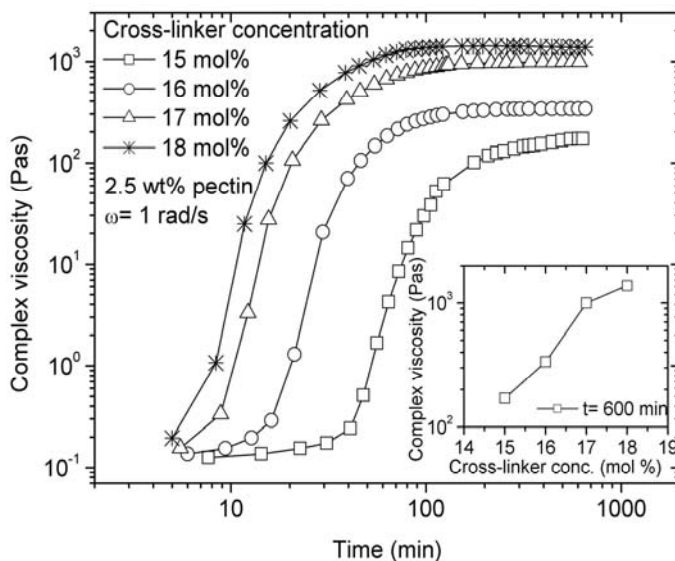


Figure 5. Time evolution of the complex viscosity for different levels of cross-linker additions at a fixed polymer concentration of 2.5 wt. %. The values of the complex viscosity at 600min (in the post-gel region for the gelling systems) as a function of cross-linker concentration are shown in the inset.

The characteristic data of gels from 2.5 wt. % pectin solutions with different cross-linking densities are presented in Figure 6. The result shows that the relaxation exponent n and the fractal dimension d_f are virtually independent of the level of cross-linker addition at this polymer concentration, whereas the gel strength parameter S is enhanced when the cross-linking density is increased from 16 mol % to 18 mol %. It

has been observed that S is sensitive to the changes of the strand length between the cross-linkers in the network [20,21]. When the cross-linking density is increased, the strand length is decreased, and higher values of S are expected.

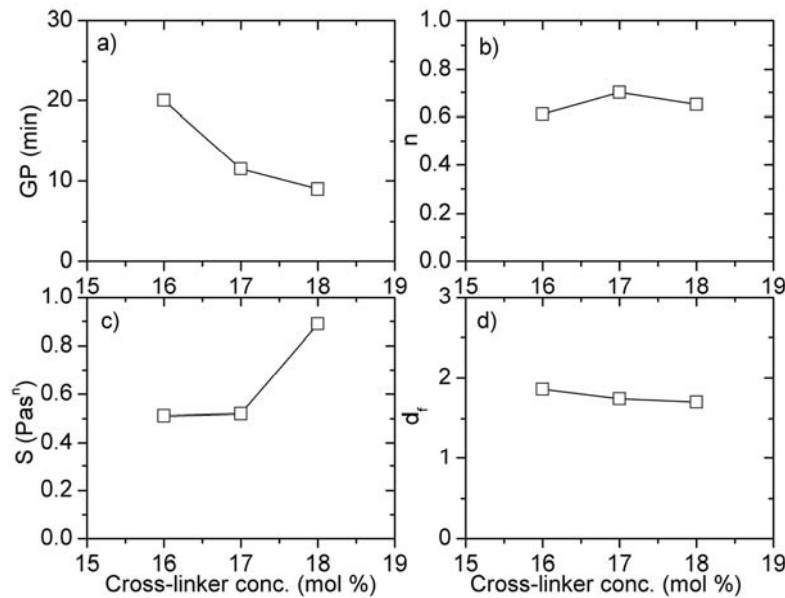


Figure 6. Effects of cross-linker concentration on the gel point (a), on the viscoelastic exponent n (b), on the gel strength parameter S (c), and on the fractal dimension d_f (d) of the gelation system at a pectin concentration of 2.5 wt. %.

The frequency dependences of the complex viscosity at different stages of the gelation process (where $\varepsilon = (t - t_g)/t_g$ is the relative distance from the gel point t_g) are illustrated for two gelling samples (with 16 mol % and 18 mol % cross-linker addition) in Figure 7. The values of the power law exponent m reveal that the properties of the samples change during the course of gelation from almost liquid-like ($m \approx 0$) in the pre-gel regime to virtually solid-like behavior ($m \approx -1$) in the post-gel zone. This finding illustrates that the gel-forming process, via the Ugi reaction, proceeds over an extended period. The results indicate that the gel network is strengthened during a long time after the formation of the incipient gel.

Figure 8 shows the time dependence of the turbidity for 2.5 wt. % pectin solutions with different levels of cross-linker addition. The general trend is that the turbidity decreases with increasing reaction time in the pre-gel region, and the change of turbidity becomes more pronounced as the concentration of the cross-linker agent increases. This change is attributed to the breakup of hydrogen bonds by the basic cross-linker DAP. The turbidity is practically constant during the whole gelation process for the gelling system with 16 mol % cross-linker addition, indicating no drastic growth of heterogeneities in the post-gel regime. Enhanced turbidity is observed for both systems with higher cross-linker concentration (17 and 18 mol %) at approximately 450 min and 350 min, respectively, and the increasing tendency is strengthened with increasing cross-linker concentration. This probably reflects that the

growth of the cross-linking zones (enhanced heterogeneity of the network), which comes from the unreacted components in the mixture and association complexes, is a function of both time and cross-linker concentration.

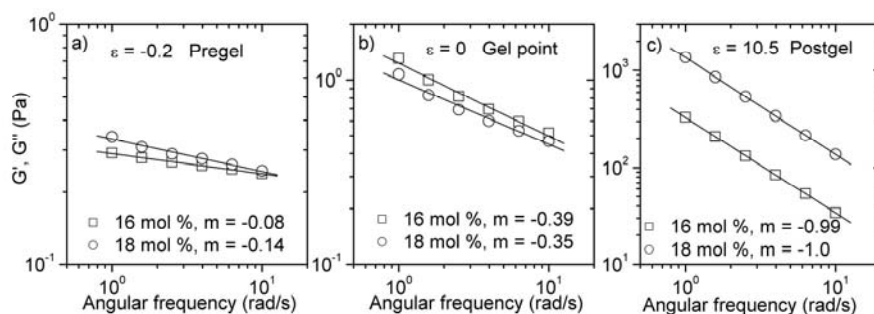


Figure 7. Frequency dependences of the complex viscosity for pectin solutions of 2.5 wt. % with two different levels of cross-linker addition. The values of the power law exponent m , expressing the frequency dependence of η^* , are indicated.

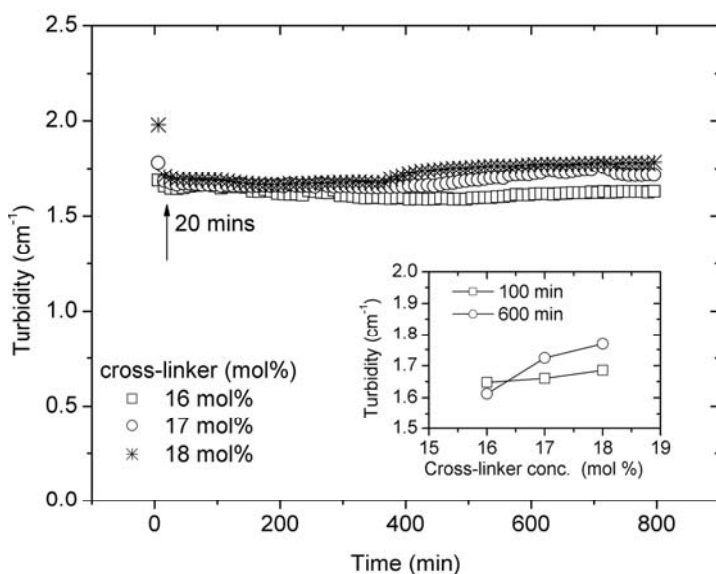


Figure 8. Time evolution of the turbidity in the course of gelation of a 2.5 wt. % pectin solution with different cross-linker concentrations (every tenth point is shown). The values of the turbidity at 100 and 600 minutes are plotted as a function of cross-linker concentration in the inset plot.

Conclusions

In this work, we have investigated turbidity and rheological features of gelling systems of pectin, prepared via the Ugi multicomponent condensation reaction. The effects of polymer and cross-linker concentrations have been studied. The main results can be summarized in the following way: (1) The Ugi condensation reaction is an

effective procedure for the synthesis of chemical hydrogels with tailor-made properties. (2) The rheological results reveal that increasing polymer and cross-linker concentrations promote faster gelation and give rise to stronger gels. (3) The incipient gels are weak, but they are strengthened as the cross-linker reaction proceeds in the post-gel region, as shown by the increasing value of the power law exponent m . (4) Though the gels are strengthened in the post-gel region, the turbidities for most gelling systems are virtually constant with the prolonging reaction time, indicating the heterogeneity of the networks exhibits no pronounced change. High cross-linker concentration causes an augmented turbidity in the late stage of the postgel region, which suggests the unreacted components in the mixture may play an essential role for the enhanced heterogeneity of the network. This study shows that by using the Ugi reaction, tailor-made hydrogels can be prepared for various pharmaceutical applications.

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