Shear-Induced Association and Gelation of Aqueous Solutions of Pectin

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We report effects of oscillatory and steady shear flows on intermolecular associations and gelation in a semidilute aqueous solution of pectin in the absence and presence of a hydrogen bond breaking agent (urea). The effect of urea on the growth of intermolecular complexes stabilized through hydrogen bonds is analyzed. It is shown that when the systems are exposed to high shear rates, prominent intermolecular associative junctions emerge upon return to low shear rates.

Introduction

Biomacromolecules such as polysaccharides may associate in aqueous solution to form supramolecular structures via intermolecular noncovalent interactions.[1] The considerable interest in these systems is due to their numerous applications, such as rheology modifiers, coatings, biomedical implants, and suspending agents for pharmaceutical delivery systems. The forces that usually hold together these supramolecular systems are hydrogen bonds and hydrophobic and electrostatic interactions. In the semidilute concentration regime, a situation may be attained where interchain association yields a connected network that spans through the whole sample volume (a physical gel is formed). In solutions of certain associating polymers it has been observed[2−7] that it is possible to induce and influence gelation by exposing them to an external stimulus such as a shear force. There are some theoretical studies[8−12] that address this phenomenon. Gelation under shear conditions may provide us with important information that can be crucial in explaining the mechanisms and factors governing the building up of supramolecular structures of association complexes and physical gels.

In this paper, we report the first observation of shear-induced gelation in an aqueous semidilute solution of pectin by probing oscillatory and steady shear flows. Our rheological measurements reveal a number of novel findings for this system, both with and without denaturing agent. In particular, we have provided information about the type and intensity of the intermolecular interactions operating in this system and also obtained some insight about the properties of the incipient gel.

Pectin is an 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.[13] Pectin consists primarily of (1→4)-linked α-D-galacturonyl units occasionally interrupted by (1→2)-linked α-L-rhamnopyranosyl residues (the latter may carry oligosaccharides).[13] Depending on the degree of methoxylation (DM), pectins are classified into high-methoxyl pectin (DM > 50%) and low-methoxyl pectin (DM < 50%). Light scattering results[14] from aqueous solutions of low-methoxyl citrus pectin fractions suggest that pectin is a semirigid polymer. Previous studies[13,15,16] have demonstrated that the degree of methoxylation governs the type of interactions operating in semidilute and gelling solutions these systems. In the case of high-methoxyl pectin, intermolecular associations are governed by both hydrogen bonds and hydrophobic interactions, while for low-methoxyl pectin, hydrogen-bonded intermolecular complexes are expected to play a dominant role. In the present work a low-methoxyl pectin (few hydrophobic groups) is employed; hence it is reasonable to assume that intermolecular hydrogen bonds play a major role in the shear-induced association or gelation process. To test this hypothesis, rheological measurements have also been executed in the presence of the hydrogen bond breaking agent urea.[17] This agent is a known denaturant and structure breaker in many biological systems. It has been observed that urea can disrupt the intermolecular hydrogen bonding between polysaccharide chains.[18]

Experimental Section

Materials and Solution Preparation. A low-methoxyl pectin sample, Pectin Classic CU701 (lot no. 0903185), was purchased from Herbstreith & Fox KG, Germany. According to the specifications from the manufacturer, this sample has a DM of 35% and the galacturonic acid content is 88%. To remove impurities, the sample was centrifuged for 12 h at 3800 rpm, dialyzed against water for 7 days, and freeze-dried prior to use. From capillary viscometry[19] on dilute solutions of pectin in the presence of 1 wt % sodium hexametaphosphate $[(NaPO_3)_6]$ (suppressing the tendency of forming aggregates), the molecular weight was estimated from intrinsic viscosity data ($[\eta] = 231$ mL/g) to be about 5×10^4 (using $[\eta] = (9.55 \times 10^{-2})M^{0.73}$ mL/g[20]).

The freeze-dried polymer was dissolved in water in the presence or absence of the denaturant urea; the pH of these solutions is close to 2.8. Solutions with a polymer concentration of 1.5 wt % (in the semidilute regime) were prepared and the concentration of urea was 4 M (4 M is the highest concentration that can be used without having problems to dissolve the polymer). Great care was exercised to ensure that the samples were homogeneous and freshly prepared. All the solutions were allowed to equilibrate for 12 h prior to measurement. All solutions were transferred to the measuring cell by very slow pouring.

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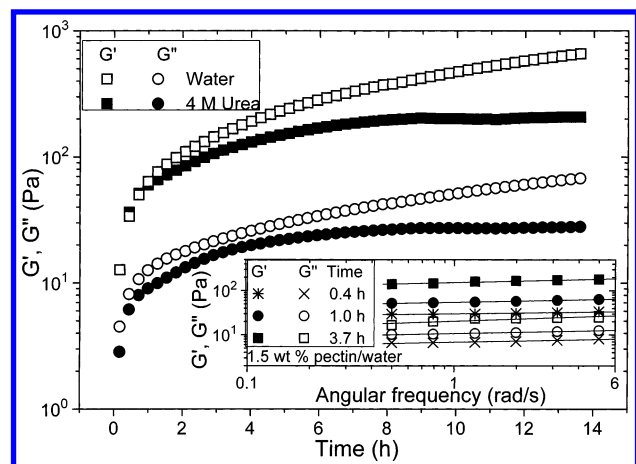‡ Department of Pharmaceutics.

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Figure 1. Time evolution of G' and G'' during sequential repeated measurements of the dynamic moduli in the angular frequency interval of $0.5-5$ rad/s at a fixed strain of 1% for 1.5 wt % pectin in aqueous solution. The inset plot shows the frequency dependencies of the dynamic moduli at different times for the pectin–water system.
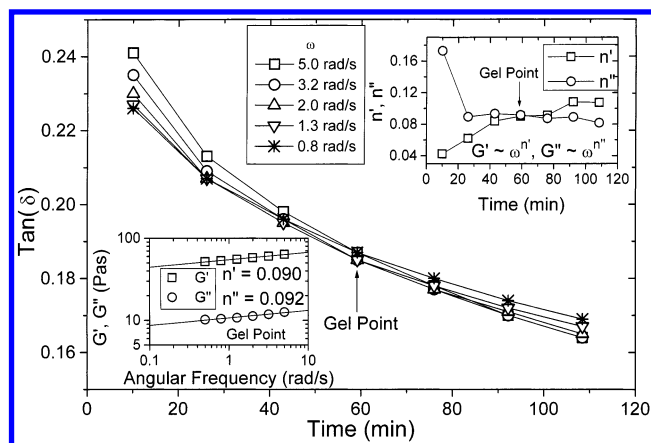

Figure 2. Illustration of methods for the determination of the gel point for the pectin (1.5 wt %)–water system. Viscoelastic loss tangent as a function of time for the angular frequencies is indicated. The upper inset plot shows changes of the apparent exponent n' for the storage and n'' for the loss moduli during the course of gelation. The lower inset plot shows the power law behavior of the dynamic moduli at the time of gelation.

Rheological Experiments. Oscillatory shear and viscosity experiments were conducted at 25 °C 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. To ensure the reproducibility of the measurements, great care was exercised when the sample was introduced into the rheometer to avoid shear effects on the solution. Measurements were repeated on fresh solutions and were found to be reproducible within 2%. The sample was applied on the plate in the solution state, and to prevent dehydration from the solution, the free surface of the sample was always covered with a thin layer of low-viscosity silicone oil (the viscoelastic response of the sample is virtually not affected by this layer). The measuring device is equipped with a temperature unit (Peltier plate) that gives very good temperature control over an extended time.

Results and Discussion

Oscillatory Shear Measurements. Time evolution of the storage (G') and loss (G'') modulus at a low angular frequency (5 rad/s) is depicted in Figure 1 for a pectin solution (1.5 wt %) without and with urea (4 M). The measurements were periodically repeated (every 16 min) in a narrow frequency interval ($0.5-5$ rad/s) at a small strain of 1% (see the inset of Figure 1) over an extended period of time. The most prominent feature is the marked increase of the dynamic moduli with time, especially for the system without added denaturant. Even at the start of the experiment, the elastic response is dominating ($G' > G''$ and nearly independent of frequency in the considered experimental window) and this trend is strengthened as time passes and the gel is developed. In the presence of denaturant, the dynamic moduli exhibit a considerably weaker increase as time elapses and no gel is formed. On the basis of the employed criteria for gelation (see the discussion below), we can argue that no gel is evolved during the time course in the presence of urea but only a viscosification of the sample is observed. The much lower values of the dynamic moduli in the presence of urea reflect that urea inhibits the formation of intermolecular hydrogen bonds.

The gel point of the pectin (1.5 wt %)–water system can be determined by observation of a frequency-independent value[21] of tan δ ($=G''/G'$) obtained from a multifrequency plot of tan δ versus elapsed time (see Figure 2). An alternative method[22] 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 $n' = n'' = n$ (see the upper inset of Figure 2). Both methods yield the same gelation time (60 min) for this system that has been subjected to periodical oscillatory shear perturbations over an extended time. At the time of gelation, the $G'(\omega)$ and $G''(\omega)$ curves become parallel and power laws in frequency are observed (see the lower inset of Figure 2) with power law exponents of 0.09. The finding that the dynamic moduli for incipient gels are nearly frequency-independent has previously been reported for other polysaccharide systems.[23-26]

Muthukumar elaborated a theoretical model,[27] based on the assumption that variations in the strand length between cross-linking points of the gel network give rise to changes of the excluded volume interactions, to rationalize values of the relaxation exponent n in the whole physically accessible range ($0 < n < 1$). In this approach, a relationship between n and the fractal morphology of the incipient gel network was established:[27]

$$n = d(d + 2 - 2d_f)/2(d + 2 - d_f) \qquad (1)$$

where d ($d = 3$) is the spatial dimension and d_f is the fractal dimension, which relates the mass of a molecular cluster to its radius of gyration by $R^{d_f} \sim M$. In the framework of eq 1, all values of the relaxation exponent $0 < n < 1$ are possible for a fractal in the physically realizable domain $1 \leq d_f \leq 3$. The value of the fractal dimension obtained for the present incipient gel is 2.4, which suggests a rather compact homogeneous network structure.[23,28] Values of the fractal dimension around 2.5 have been reported[26,29-31] for various polysaccharide systems. However, in a recent light scattering study[32] on gelling semidilute aqueous solutions of high-methoxy pectin in the presence of sucrose, a fractal dimension of 1.6 was found, suggesting a network of swollen chains. This indicates that the structural morphology of this network, where gelation is induced through sucrose, is different from the shear-induced gel discussed above.

The oscillatory shear-induced gelation may be rationalized in the following way. The application of shear deformation to the pectin system may induce alignment of the semirigid chains and it is also possible that additional stretching of the chains occurs.[33] Both stretching and alignment of the chains may result
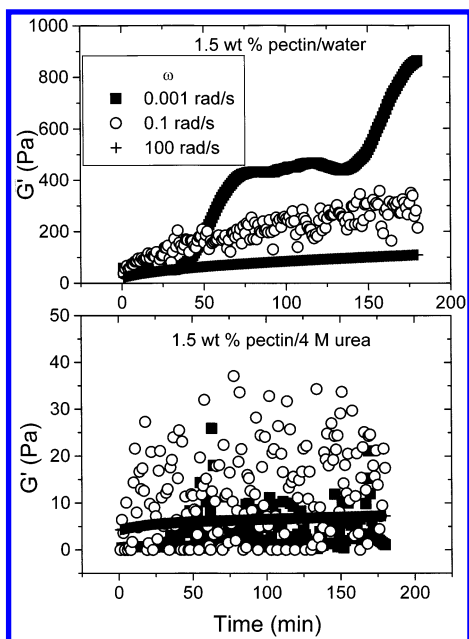
Figure 3. Time evolution of the storage modulus for the systems indicated during continuous measurements at the angular frequencies indicated and at a constant strain of 1%.

in more sites that become available for intermolecular junctions through hydrogen bonds. As a result, the chains hydrogen-bond to each other to form an interconnected three-dimensional gel network. It has been suggested that the network is in the form of staggered ladderlike structures.[12] The essential features of this process are cooperative mobility, orientational effects, and cooperative zipping of stretched chains through hydrogen bonds. Although the investigated pectin sample contains relatively few hydrophobic moieties, it is possible that because of large deformations of the network organization, the hydrophobic "stickers" may become more accessible to intermolecular associations and thereby contribute to the formation of the gel network. Because of the relatively low polymer concentration and the low molecular weight of the sample, the effect of entanglements can be ignored.

To study the structure evolution of the network under a prolonged oscillatory shear perturbation (all data are collected at a fixed strain of 1%), a fixed angular frequency (three different values) was imposed on the systems over an extended period of time, and values of the storage modulus were collected continuously (see Figure 3). When the pectin−water system is subjected to a low angular frequency (0.001 rad/s), a gel structure is built up, with a plateau region of G' at intermediate times and a marked increase (at about 150 min) at longer times. This behavior suggests that, under this condition of deformation, the cooperative zipping of chains to form a stabilized gel network continues over a relatively long time. It has been argued[12] that at very weak deformations the rates of formation of energetic interactions (hydrogen bonds) should be very slow. At 0.1 rad/s, large fluctuations in the experimental data are a conspicuous feature and the overall effect is a moderate rise of G' in the course of time. This indicates that the rates of creation and destruction of intermolecular junctions are competing on the same time scale. At the highest value of ω (100 rad/s), the buildup of the structure of the network is suppressed and there is only a slight increase of G'.

In the presence of 4 M urea (the bottom panel of Figure 3), we cannot at the lower two values of ω trace a durable buildup of a network structure, but there are strong irregular variations in G' and this indicates a pronounced competition between forces
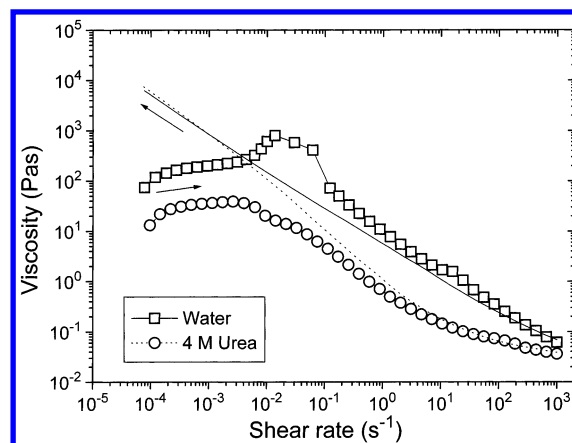

Figure 4. Shear rate dependence of the viscosity for 1.5 wt % pectin in aqueous solutions. Data obtained under increasing (→) and decreasing (←) shear rates have been collected.

that build up and break down the structure. At the highest value of ω, the perturbations are too strong to allow formation and stabilization of intermolecular junction zones.

Viscosity Measurements. The effect of shear rate on the measured viscosity for an aqueous solution (1.5 wt %) of pectin with and without urea is depicted in Figure 4. In the absence of urea, a shear-induced thickening occurs at low shear rates, probably due to orientation and stretching of the chains and thereby increasing the number of effective intermolecular bonds. At higher shear rates, the intermolecular junctions are disrupted at a rate faster than their rate of formation, resulting in a decrease in the junction density and hence a drop in the viscosity. The subsequent decline in shear rate (downramp curve) for the pectin−water system (solid curve) has also been monitored (see Figure 4). The frequently observed hysteresis phenomenon is established at intermediate shear rates by the fact that the downramp curve lies below the upramp one. However, a peculiar phenomenon appears at low shear rates, where the downramp curve lies considerably above the corresponding upramp curve. This suggests that the substantial perturbations applied to the system promote the accessibility of interchain bonds at low shear rates and the subsequent cooperative zipping of stretched chains (more evidence in this direction is given below).

In the presence of urea, the shear-induced thickening effect is negligible and the intermolecular associations are disrupted at high shear rates. The most startling feature is the restructuring of the network upon decreasing the shear rate (downramp curve). In this case, the downramp curve for the pectin system with urea is below the corresponding curve for the pectin−water system at high and intermediate shear rates, while at low shear rates the curves coincide with each other. The reason for this intriguing behavior is not clear at the present stage, but it seems that the effect of urea on the system is lost when the sample has been exposed to large shear perturbations prior to the decline toward the quiescent state (cf. discussion below).

Two types of mechanism have been suggested[34] to come into play when a denaturing agent like urea is added to an aqueous solution with hydrogen-bonding sites on the polymer. The first is the specific binding of the denaturant to groups on the polymer chains, thereby weakening hydrogen bonding between polymer chains. The second possible mode of action of a denaturant is the breakdown of the hydrogen-bonded structure of the solvent ("structure breaking effect"). A strong shear deformation of the sample will probably cause a thorough restructuring or reorganization of the supramolecular system and perturbations of the
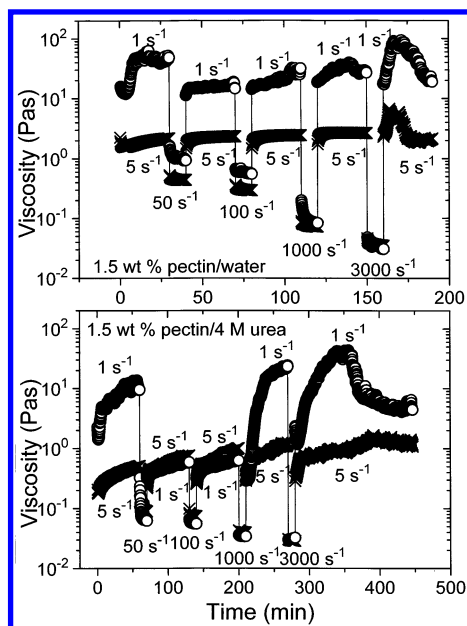
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Figure 5. Viscosity as a function of shear time under a continuous shear rate of 1 or 5 s^{-1}, interrupted periodically (duration of perturbation is 10 min) by augmented deformation shear rates (50, 100, 1000, and 3000 s^{-1}) for the systems.

local water structure around the polymer. It is possible that perturbations at high shear rates inhibit the effect of the denaturant so that intermolecular hydrogen bonds are formed on return to low shear rates. Under these conditions, urea may be able to hydrogen-bond with pectin chains and form a gel network. The effect of urea on the interaction between polysaccharide chains has recently been studied[35] by the osmotic stress method. In this paper it is argued that as the osmotic pressure increases, urea is able to hydrogen-bond with the polymer chains and form a new crystalline structure. However, there is also a possibility that enhanced hydrophobic associations are promoted between chains subjected to alignment and extra stretching. It has been reported[36] for polymer systems that the hydrophobic interactions are unaffected by the addition of urea.

A further elucidation of the buildup and breakdown of the structures evolved in these systems is depicted in Figure 5. In this case, a constant shear rate (1 or 5 s^{-1}) is applied to the system, and this process is periodically interrupted during a short time (10 min) by an enhanced perturbing shear rate of varying strength. Let us first discuss the results for the pectin−water system without urea (top panel of Figure 5). At the low steady shear rate (1 s^{-1}), the viscosity rises initially and an association structure is built up, followed by a subsequent breakdown of the structure at a shear rate of 50 s^{-1}. The main features can be summarized in the following way. (i) The breakdown of the associations is more pronounced as the magnitude of the deformation sequence is increased, but the times of restoration of the viscosity are rapid at all levels of shear perturbation. (ii) The general picture that emerges of the viscosity after recovering from the short augmented deformations is that the growth of the energetic associations is progressively more marked as the transitory shear rate perturbation is increased. This is a further indication that vigorous shear rate deformations are favorable for the succeeding creation of intermolecular associations at a low monitoring shear rate. The association structures formed after the highest shear rate perturbation break down as time passes, which is an indication that the interchain bonds are weak. (iii) When a higher (5 s^{-1}) steady shear rate is imposed upon the system, the values of the viscosity are lower, the times of

restoration of the viscosity are longer, and the growth of association complexes is only observed after the highest level (3000 s^{-1}) of shear rate deformation. However, these associations are gradually disrupted in the course of time.

In the presence of urea (the bottom panel of Figure 5), we observe an initial growth of the viscosity at the low steady shear rate (1 s^{-1}), but the most sensational finding is the dramatic rise of the viscosity (almost 2 orders of magnitude; the values of the viscosity are virtually the same as those for the system without urea) after being exposed to the larger two transitory shear rate perturbations (1000 and 3000 s^{-1}). This is further evidence that substantial shear rate deformations of the pectin system in the presence of urea can build up intermolecular associative junctions upon returning to a low (1 s^{-1}) steady shear rate. When a higher value of the steady shear rate (5 s^{-1}) is imposed, the level of continuous perturbation is too high to allow a pronounced growth of the weak intermolecular associative junctions.

Conclusion

In summary, we have shown the influence of oscillatory and steady shear flows on association and gelation in a semidilute aqueous solution of pectin with and without urea. The effect of urea on the growth of association complexes, stabilized through hydrogen bonds, has been scrutinized. Especially, it has been demonstrated that high shear rates can have a drastic impact on the buildup of association structures in systems that are conveyed back to low shear rates.

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