Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Polymers 53 (2003) 459–468
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Rheology and gelation of water-insoluble dextran from *Leuconostoc mesenteroides* NRRL B-523

Prabhu Arcot Padmanabhan[a], Dong-Shik Kim[a,*], Daewon Pak[b], San Jun Sim[c]

[a]*Department of Chemical and Environmental Engineering, The University of Toledo, 3048 Nitschke Hall, Toledo, OH 43606, USA*
[b]*Water Environment and Remediation Research Center, KIST, Seoul, South Korea*
[c]*Department of Chemical Engineering, SungKyunKwan University, Suwon, South Korea*

Received 16 January 2003; received in revised form 21 April 2003; accepted 22 April 2003

Abstract

Dynamic oscillatory testing has been used to study the rheology of water-insoluble dextran. The rheological properties (storage and loss moduli) of dextran gel were measured and dextran was found to be neither a strong gel nor a weak gel, but an entanglement network at a concentration of 250 mg/ml. The extent of gelation, illustrated by the gel elastic modulus G', is found to decrease with increasing concentration of calcium ions. This was confirmed by shift of crossover frequencies towards higher values on the dynamic spectra and lower yield stress τ values obtained from stress ramp experiments. Finally, a comparison between gelation of dextran and alginate (a similar biopolymer) was made for clear understanding of effect of calcium ions on the dextran gelation.

Keywords: Rheology; Storage modulus; Loss modulus; Gelation; Insoluble dextran; *Leuconostoc mesenteroides*

1. Introduction

Extracellular microbial biopolymers (exopolymers, hereafter) are used extensively in many applications such as biofiltration for drinking water, hydraulic fracturing fluids in enhanced oil/gas production, immobilized biofilm reactors, biomedical materials, and food additives. These applications are attributed to the ability of exopolymers to modulate rheological properties of materials (Maier, Anderson, Karl, & Magnuson, 1992; Shamala & Prasad, 1995; Stoodley, Lewandowski, Boyle, & Lappin-Scott, 1999). This ability is determined by the physical properties of exopolymers. Therefore, physical properties such as surface charge, polarity and structure are considered important in controlling the behavior of exopolymers in applications.

For example, a better understanding of exopolymer rheology is crucial when we want to control the behavior of exopolymers accumulating on solid surfaces (i.e. biofilms). Biofilms mainly consist of exopolymers, cells, and other metabolic byproducts, and are usually resistant to shear stress in the flow systems. In many cases, biofilm formation is detrimental to the system, and usually difficult to prevent or remove once formed (Wingender, Neu, & Flemming, 1999). However, in some applications such as microbial enhanced oil recovery or biological filters, biofilms are used beneficially (Lappan & Fogler, 1994). Detrimental or beneficial, biofilm movement must be controlled carefully to minimize or promote the effect of biofilms. The other examples are adjusting the viscosity and flow behavior of artificial blood, bioreactors, or fracturing fluids (Christensen & Characklis, 1990; Costerton, Lewandowski, Caldwell, Korber, & Lappin-Scott, 1995). Here, a desired property of liquid solution can be adjusted by controlling the rheological properties of exopolymers added into the system.

Exopolymers are gelatinous matrices and become very stable when forming a gel, which results in complicated rheological behaviors. On the other hand, non-gel exopolymers are liquid-like and behave like a highly viscous liquid, less complex than gel exopolymers. Therefore, it is necessary to have a better understanding of the gelation of exopolymers. Some exopolymers such as alginate, gellan, and xanthan, have been studied, and the gelation mechanisms have been suggested (Flemming, Wingender, & Borchard, 2001; Morris, 1995; Rochefort & Middleman, 1987). These exopolymers commonly form multi-helical structures (Sutherland, 1994), and can aggregate through

* Corresponding author. Tel.: +1-419-530-8084; fax: +1-419-530-8086.
E-mail address: dong.kim@utoledo.edu (D.-S. Kim).

0144-8617/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0144-8617(03)00140-1

hydrogen bonding to form highly hydrated viscoelastic gels (Ross-Murphy, Shatwell, & Sutherland, 1996).

In this study, we have investigated dextran produced by *Leuconostoc mesenteroides* NRRL B523. This species is unique in producing both water-soluble and -insoluble dextrans when grown on sucrose (Padmanabhan & Kim, 2002). There have been numerous studies on the production of dextransucrase and water-soluble dextran from *L. mesenteroides* NRRL B-512F (Landon & Webb, 1990), but studies on the rheological properties of insoluble dextran are limited (Landon, Law, & Webb, 1993; Tecante & Munguia, 1986). The recent studies have shown that not only water-soluble dextrans, but also water-insoluble dextrans are of high industrial values (Stewart & Fogler, 2001).

One of the many factors involved in the gelation of exopolymers is the presence of cations, such as Ca^{2+} and Mg^{2+}. Influence of calcium ions on the mechanical properties of *Pseudomonas* biofilm was investigated by Flemming et al. There are certain critical concentration of calcium ions where the Young's modulus of the biofilm increases strongly and subsequently remains constant for higher calcium concentrations. This behavior is explained by the presence of calcium ions crosslinking alginate.

In this study, we used dynamic rheology to investigate the gelation of pure insoluble dextran produced under cell-free conditions. The effect of calcium ions on the gelation of the exopolymer was also studied. The relationship between the calcium ion concentration and the degree of gelation of the exopolysaccharide was examined.

2. Rheology background

Most biopolymers are viscoelastic, i.e. they exhibit solid (elastic)- and liquid (viscous)-like behavior. An elastic material will deform when subjected to an applied force and return to its original shape when that force is removed. In an ideally elastic system the amount of deformation (strain) is directly proportional to the force per unit area (stress). Because the rheological properties of viscoelastic materials are dependent on the rate of an applied shear, they are often tested using dynamic methods in which the shear is oscillated at various frequencies (ω).

The dynamic elastic modulus (G') is the in-phase component of stress with an oscillating strain and the viscous modulus (G'') is the out-of-phase component of stress and is a measure of energy lost through viscous flow. For a perfectly elastic material $G' = G$ (shear modulus) and $G'' = 0$, while for a Newtonian fluid $G' = 0$ and $G'' = \omega\eta$, where η is viscosity. Ideally, the elastic properties of a material should be measured instantaneously with an applied stress or strain, while viscous properties should be measured over long time periods.

Gelation is the phenomenon by which a crosslinked polymeric material undergoes a phase transition from the liquid to the solid state at a critical point of time, temperature, concentration, etc. (Winter & Chambon, 1986). Hence, the sol–gel transition, known as the gel point (Winter, 1989), occurs at some critical extent of the crosslinking reaction. Winter and Chambon proposed a general criterion that can be used to identify a gel point. They have shown that at the gel point, both the elastic modulus (G') and the viscous modulus (G'') exhibit a power-law dependence on the frequency of oscillation (ω). The corresponding expressions describing dynamic moduli at a gel point are as follows

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

$$G''(\omega) = S\Gamma(1-n)\sin(n\pi/2)\omega^n \quad (2)$$

where S is the gel strength parameter, Γ is the γ (Gamma) function, and n is the relaxation component.

The relaxation exponent n can have values in the range of $0 < n < 1$. Thus, at the gel point, the storage and viscous moduli depend on frequency in an identical manner, corresponding to parallel lines in a frequency spectrum:

$$G'(\omega) \propto G''(\omega) \propto \omega^n \quad (3)$$

The phase angle (δ) between stress and strain is independent of frequency but proportional to n, and the loss tangent ($\tan\delta$) is independent of frequency at the gel point.

$$\delta = n\pi/2 \qquad \text{or} \qquad \tan\delta = G''/G' = \tan(n\pi/2) \quad (4)$$

3. Experimental

3.1. Materials

L. mesenteroides NRRL-B523 (ATCC 14935) was used for the synthesis of cell-free insoluble dextran. It is a facultative anaerobe that grows under mesophilic conditions and placed in the freezer for long-term storage. Every 30 days an aliquot was transferred from the freezer, streaked onto slants prepared using Difco *Lactobacillus* MRS broth, grown for 24 h, and stored at 4 °C. The slants were discarded after 30 days of 4 °C storage.

3.1.1. Dextran synthesis

The details of growth medium and cell-free water-insoluble dextran synthesis can be found in Padmanabhan and Kim (2002). The cell-bound enzyme, dextransucrase, was used in the synthesis of cell-free insoluble dextran. The procedure can be briefly summarized as follows.

After 24 h of fermentation, the growth medium (phosphate-buffered saline medium) was centrifuged at 3500*g* for 30 min to obtain the insoluble biomass pellet (insoluble exopolymers and cells) at the bottom. The insoluble pellet was washed with deionized water and the biomass pellet was used for the production of insoluble cell-free dextran. A

small amount of the pellet (approximately 2 ml) was transferred into a 500 ml batch reactor containing 200 ml of sodium acetate buffer (pH 5.4) containing 130 μM $CaCl_2$ and 15% sucrose as the initiator and substrate for dextransucrase, respectively. After 22 h, the entire medium was centrifuged at 3500*g* for 20 min to collect the cell-free insoluble dextran as a pellet at the bottom. The pellet (insoluble dextran) was saved for concentration measurement.

3.2. Preparation of dextran samples

Water-insoluble cell-free dextran obtained was washed with deionized water and centrifuged again to remove any remaining sucrose. The pellet was stored in 50 ml Falcon (Fisher Scientific, Hanover Park, IL) centrifuge tube for freeze drying.

Before freeze drying, the tubes were put in liquid nitrogen (70 K) for 10 min to freeze the sample completely to form a solid. The tubes (with caps open) were then freeze-dried using Labconco lyophilizer at −47 °C at a vacuum pressure of 130×10^{-3} mBar for 48 h. The samples were stored at room temperature for rheological property measurements.

3.3. Preparation of gels

Gels of dextran (250 mg/ml total biopolymer concentration) were prepared by first mixing weighed freeze dried dextran and distilled water at 25 °C. All samples were allowed to stand for 12 h at room temperature before any further rheological testing. Various calcium chloride ($CaCl_2$) concentrations were added into each sample. The concentrations of $CaCl_2$ tested were 1.9, 3.8, 11.4, 26.6, 38, 76, and 114 mM.

3.4. Rheological characterization

Dynamic rheological measurements were conducted using a Rheometrics Scientifics' RDA III dynamic analyzer, provided with torsional tools, parallel plates and LN2 controller for low temperatures. The prepared dextran gels were placed on the parallel plate and then subjected to dynamic tests in the frequency range of 0.1–500 rad/s. The conditions of the test are given in Appendix A. The yield stress and the loss tangent (tan δ) calculations were performed with the software, RSI Orchestrator version V6.5.5, supplied by Rheometrics Scientific. These experiments allowed us to study the material microstructure through measurement of elastic (G') and viscous moduli (G'') as a function of frequency or stress. Dynamic stress sweeps were conducted before frequency sweeps so that the samples were not damaged by the sudden change in direction of motor. All experiments were repeated in duplicate.

4. Results and discussion

4.1. Rheological measurements

The dynamic frequency sweeps were conducted on all the samples, maintaining the concentration of dextran at 250 mg/ml. Fig. 1 depicts the frequency spectrum of dextran exopolymer only (no calcium ions). The motivation for using dynamic measurements to study the rheological behavior of dextran is to reinforce the concept that, for materials with 'gel-like' behavior, additional valuable information can be obtained from small-strain oscillatory experiments that are missed in the traditional steady shear experiments.

It can be observed that dextran behaves like a typical macromolecular entangled biopolymer, with the elastic (storage) modulus G' dominating over the viscous (loss) modulus G'' in the high frequency range. A crossover point ($\omega_c \sim 11$ rad/s) was observed at intermediate frequencies and at lower frequencies or longer relaxation times, the viscous modulus predominates, with G' and G'' traces showing characteristic slopes of −2 and −1, respectively. These values are representative of the trends shown by Fig. 1 and not an exact numerical value. The crossover frequency suggests the beginning of the elastic plateau region and expected to reach higher values as the material becomes more dilute (as shown later).

The viscosity and loss tangent are plotted in Fig. 2. The figure clearly shows that the viscosity of dextran sample decreases with increase in shear rate. This is a typical characteristic of pseudoplastic fluid showing a shear-thinning behavior, normally expected in polymer solutions, where the fluid molecular weight is high. In these systems, as the shear rate increases, the stress also increases, but its dependence on shear rate is less than linear; therefore viscosity decreases.

4.2. Entanglement network

This dextran sample, showing a strong G' vs. frequency dependence and a $G'-G''$ crossover (Fig. 1), can be categorized as an entanglement network by distinguishing it from (1) crosslinked network gels (strong gels or chemical gels) which show very little G' vs. frequency dependence (Fig. 3a), and (2) physical gels which are intermediate between the two, showing G' vs. frequency dependence but no $G'-G''$ crossover (Fig. 3b). The impossibility for the chains to cross each other by cutting through their backbones creates an entanglement. Entanglements play the role of temporary crosslinks and constitute the physical junctions that are continuously disrupted and reformed among the chains.

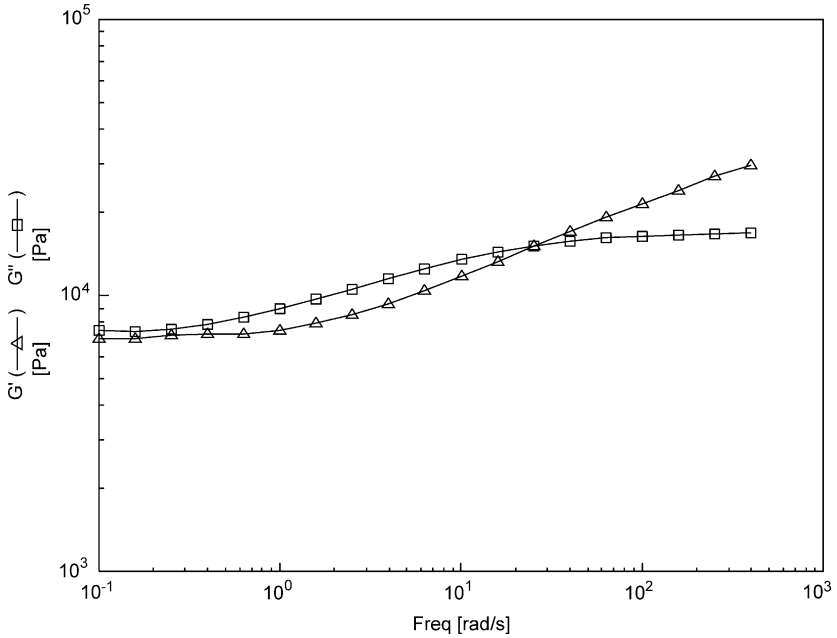

Fig. 1. Dynamic elastic (G') and viscous moduli (G'') of an insoluble dextran sample (0 mM $CaCl_2$) as a function of frequency.

4.3. Effect of calcium ions

The effect of calcium ions on the gelation of water-insoluble dextran was analyzed by conducting both dynamic and shear stress experiments. Figs. 4 and 5 show the dynamic spectra for the dextran–$CaCl_2$ samples (1.9, 3.8, 11.4, 26.6, 38, 76, and 114 mM) tested. Two trends that are common in all the figures are (1) there is a crossover point in the experimental frequency range used and (2) increased dependence of G' on frequency. This shows that all samples are entanglement networks and do not become strong gels.

When the crossover frequencies were pushed to higher values (ω_c(low) ~ 13 rad/s; ω_c(high) ~ 114 rad/s), there was a strong frequency dependence of the moduli over the entire range and the addition of calcium ions dropped the moduli by approximately 85%. The reduced $G'(\omega)/G''(\omega)$ ratio (Fig. 6) above ω_c indicates the structure entanglement is substantially decreased. The elastic modulus G', considered as the primary indicator of gel-like behavior,

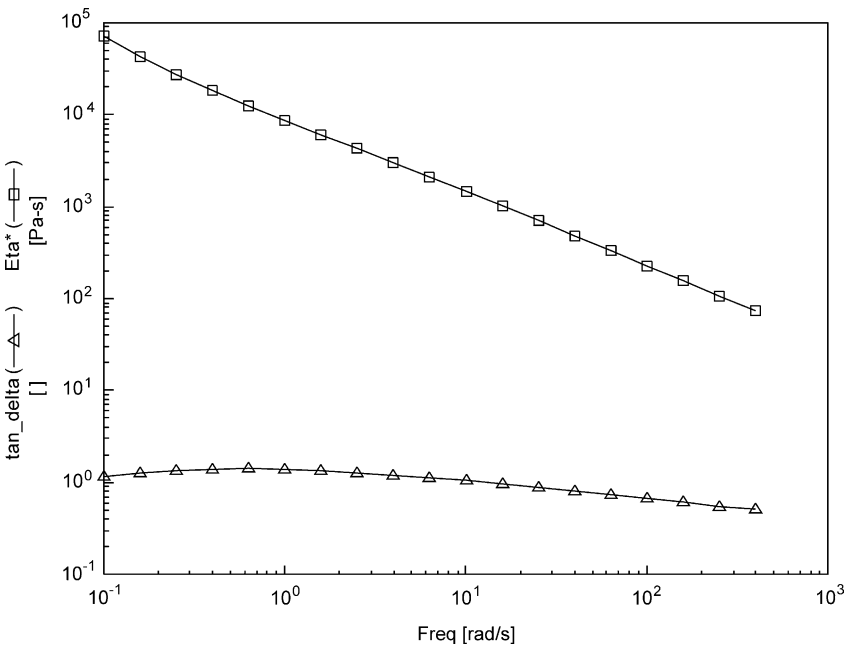

Fig. 2. Viscosity and loss tangent of dextran sample (no calcium ions added).

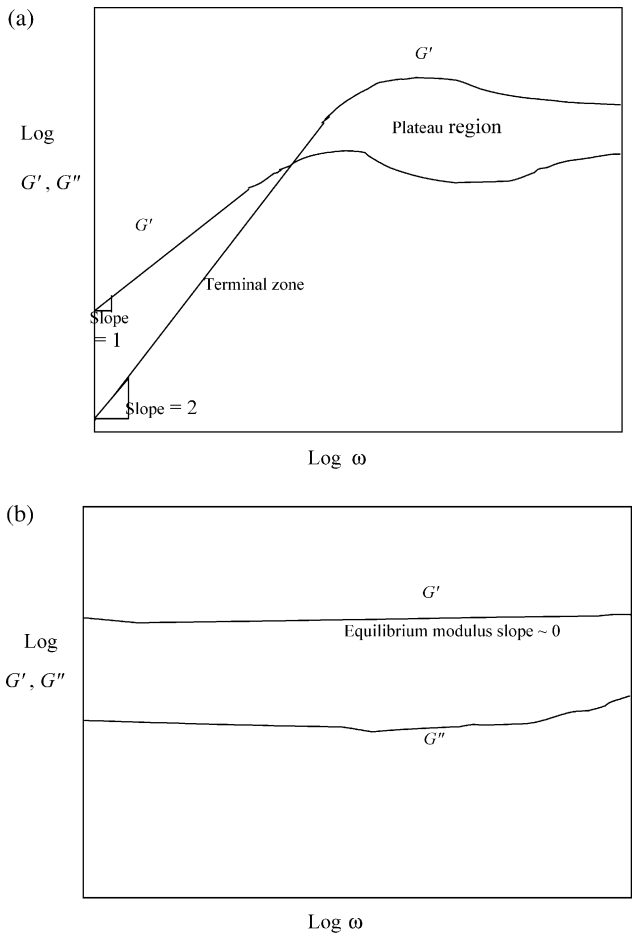

Fig. 3. Schematic of dynamic spectra expected (a) for entanglement network and (b) for a strong gel.

decreased with calcium concentration. Therefore, the addition of calcium ions decreased the gelation of water-insoluble cell-free dextran, thereby making the sample less elastic than the original dextran.

In order to better understand the mechanism involved in the action of calcium salt on the dextran exopolysaccharide, the gelation mechanism of alginate is compared with dextran. Alginates are naturally occurring polysaccharides extracted from the brown seaweeds. Bacteria, such as *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, also produce alginate-like polymers as exopolysaccharides (Sime, 1990).

Exopolymers such as dextrans and alginates are complex molecules, with both uncharged hydrophobic and charged/-polar hydrophilic regions, which can form multiple inter-molecular interactions in aqueous systems. These include attractive interactions between hydrophobic regions and between oppositely charged/polar regions, and repulsive interactions between similarly charged/polar regions. It is important to note that gelation is a process by which molecules bind to each other while *retaining or increasing* their bound water, while precipitation is the process by which molecules bind while *expelling* their bound water. The latter process, of course, eventually results in aggregated molecules shedding almost all their bound water and leaving the aqueous phase altogether. Hydrophilic interactions are water mediated ones facilitating gelation, while hydrophobic interactions expel water reducing gelation and facilitating precipitation (Tanford, 1980).

In the case of alginates, the molecules have an overall negative charge. In the absence of anions, this will result in the repulsive interactions dominating over the attractive ones, reducing gelation. The addition of divalent calcium

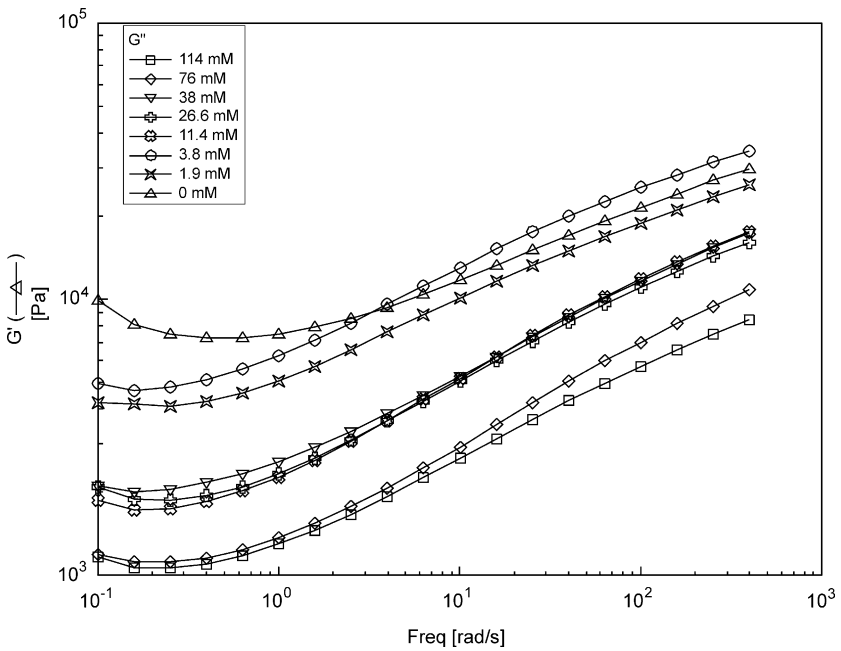

Fig. 4. Overlay of frequency sweeps (G') of dextran samples with different calcium chloride concentrations.

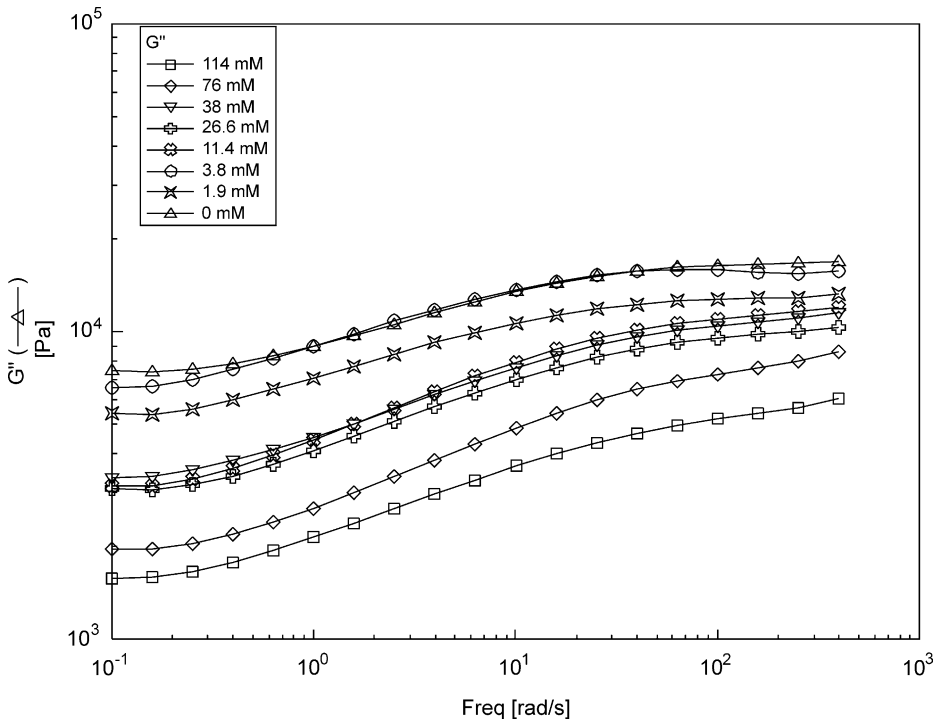

Fig. 5. Overlay of loss modulus (G'') of dextran samples with different calcium chloride concentrations.

ions can not only reduce this repulsion, but can also form intermolecular ionic bridges between negatively charged groups or zones. These bridges coupled with conformational changes can produce an expanded crosslinked network, referred to as egg boxes (Fig. 7), significantly enhancing gelation of the polymer (Clare, 1993; Powell, 1979; Smidsrød & Haug, 1971). This model of alginate network formation is referred to as the dimeric junction zone model. Other models, with varying degrees of similarity to the dimeric junction zone model, have also been proposed for these networks.

Unlike alginates, the dextrans are neutral overall. This means that even in the absence of any salt ions, attractive

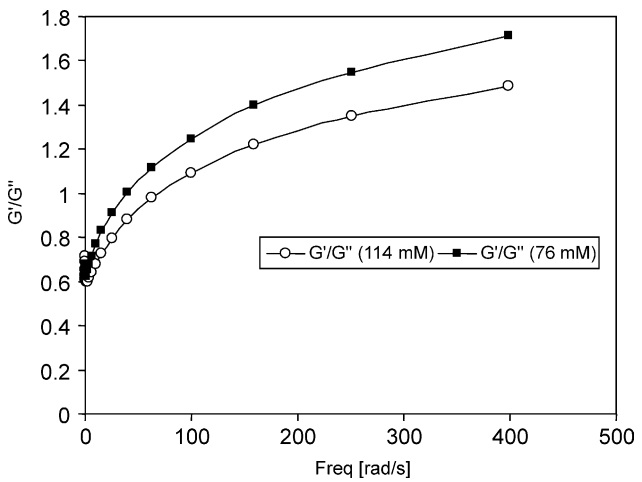

Fig. 6. Plot of $G'-G''$ vs. frequency for 76 and 114 mM $CaCl_2$ concentration samples.

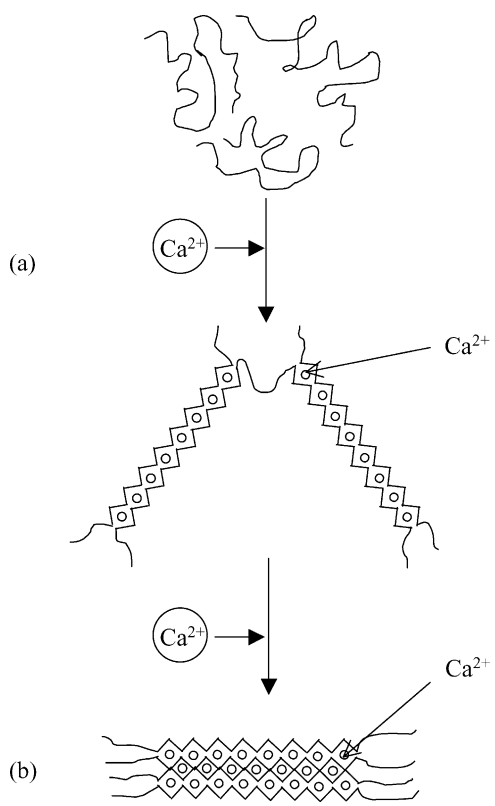

Fig. 7. Model for alginate network formation: (a) egg-box zones, (b) higher aggregates.

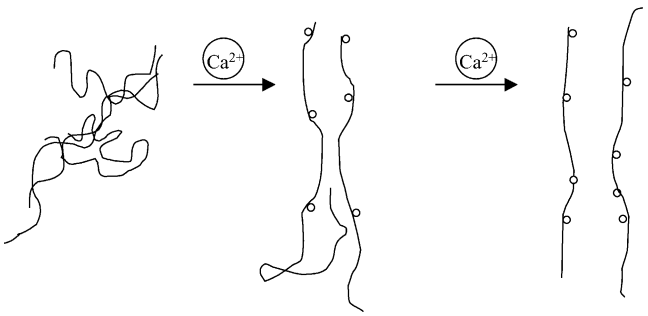

Fig. 8. Proposed model for insoluble dextran–calcium ion interaction.

interactions will be present in dextran solutions leading to increased gelation. As discussed above, it is only the attractive hydrophilic interactions that promote gelation and not the attractive hydrophobic interactions. The addition of salt ions to this system induces electrostatic shielding of the hydrophilic interactions, reducing their magnitude. Moreover, salt ions will enhance the hydrophobic interactions by an effect known as 'salting out' (Bergethon & Simons, 1990). This effect is the process by which the increasing concentration of salt ions results in more and more water molecules binding to them. The increased binding eventually results in the stripping of water molecules from the hydration layer surrounding hydrophobic groups, facilitating interactions between these groups. Thus, the addition of salt ions will decrease gelation by both decreasing the hydrophilic interactions and increasing the hydrophobic ones between dextran molecules. If the salt concentration is continuously increased, it will lead to the almost complete elimination of gelation, the precipitation of the dextran, and the viscosity of the solution returning to that of pure salt solutions. A schematic representation of the dextran gelation mechanism is given in Fig. 8.

It should be noted that, unlike in the dimeric junction zone model for the formation of alginate networks, the above explanation for the reduced gelation of dextrans with increasing salt concentration does not require the presence of divalent anions. This could provide one method of testing this hypothesis in future studies. Replacing the divalent calcium ion with the monovalent sodium ion should significantly reduce the gelation of alginates as the ability to form intermolecular ionic bridges will be greatly diminished. In dextran solutions, too, such a replacement will have an effect as the sodium ion will provide less electrostatic shielding and has less of a salting out effect, resulting in less interruption of gelation. However, this effect in dextran solutions is likely to be less pronounced than that in alginate solution.

4.4. Stress ramp tests

The stress-controlled experiments were conducted on all the dextran–$CaCl_2$ samples, to find the yield stress. A few sample plots are shown in Figs. 9–11. Yield stress is the finite value reached by the stress in stress vs. shear rate curve. Basically, there are two methods to estimate yield stress from τ vs. shear rate data. One is to plot $\tau-\gamma$ data in a bi-logarithmic plane and estimating the value of τ_y from the horizontal asymptotic branch of the curve (Method 1). Obviously, a reliable estimation of τ_y requires a data set extended towards very low values of γ. The second method is by drawing a tangent line to the point of inflexion and then fitting the corresponding data in various models (Method 2).

The yield stress values for the dextran samples, obtained by Methods 1 and 2 are tabulated in Table 1. The values obtained by Method 1 generally have less error range

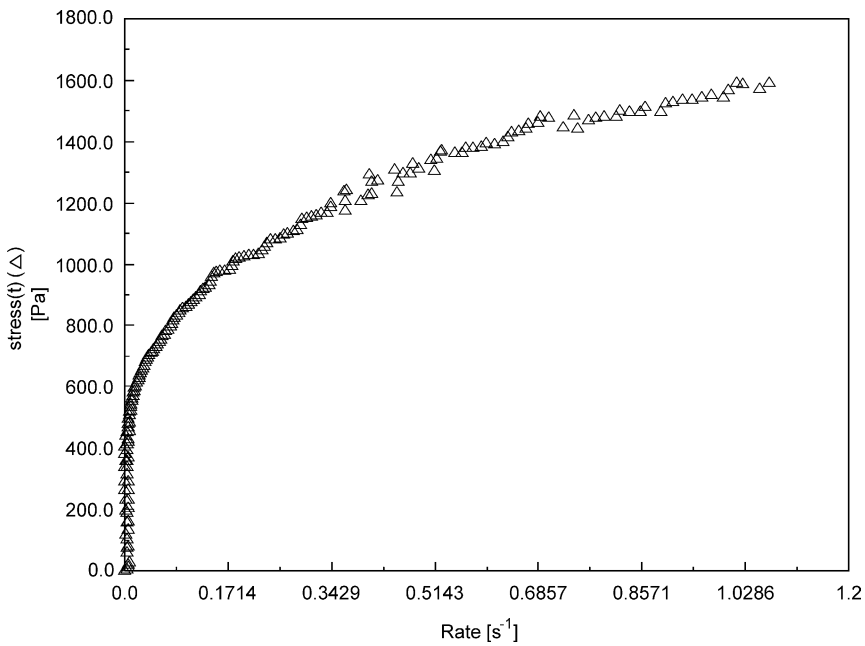

Fig. 9. Stress ramp curve for dextran sample (0 mM $CaCl_2$).

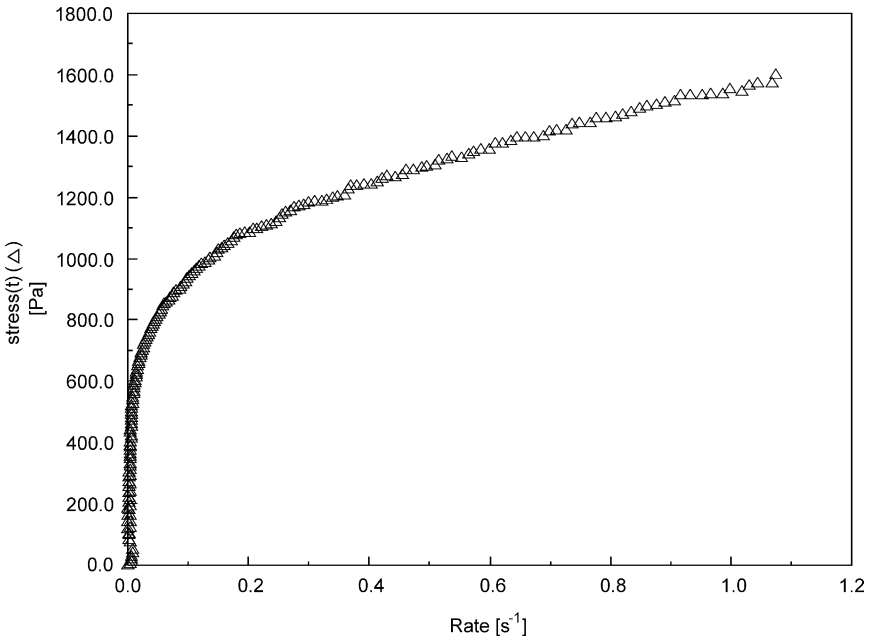

Fig. 10. Stress ramp curve for dextran–Ca^{2+} sample (1.9 mM $CaCl_2$).

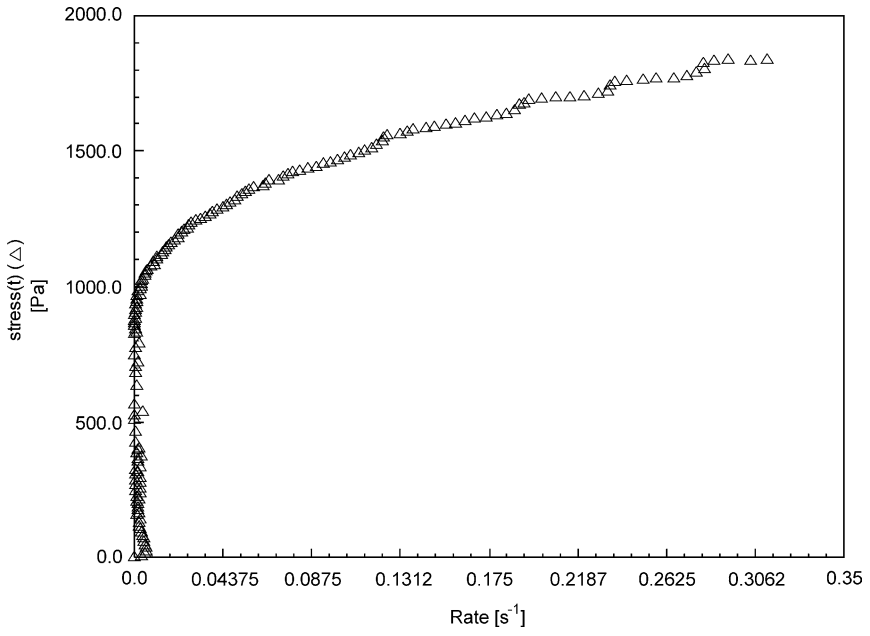

Fig. 11. Stress ramp curve for dextran–Ca^{2+} sample (3.8 mM $CaCl_2$).

compared to values from Method 2. The yield stress values decreased with increase in the concentration of calcium ions, which confirms that the samples became less elastic with addition of calcium salt.

4.5. Models

The model used in the calculation of yield stress was Bingham model, as it is the widely used model in the industrial field to correlate the viscosity data of pastes,

Table 1
Yield stress values obtained from Methods 1 and Method 2 (Bingham model)

Calcium chloride concentration (mM)	Yield stress by Method 1 (Pa)	Yield stress by Method 2 (Pa; Bingham model)
0	450 ± 10	540 ± 50
1.9	412 ± 10	535 ± 50
11.4	141 ± 10	280 ± 50
26.6	138 ± 10	226 ± 50
38	129 ± 10	188 ± 50
76	117 ± 10	142 ± 50
114	87 ± 10	97 ± 50

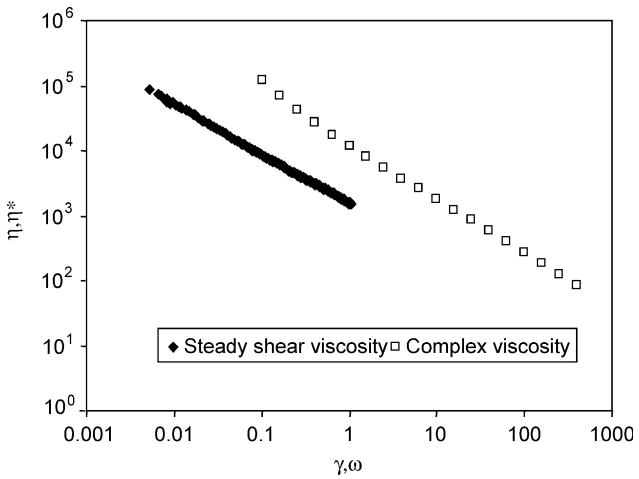

Fig. 12. Steady shear viscosity and complex viscosity plotted against shear rate and frequency for 0 mM Ca^{2+} dextran sample.

slurries and suspensions

$$\tau = \tau_y + \eta\gamma \quad (5)$$

where τ is shear stress, τ_y is yield stress, η is viscosity and γ is shear rate.

The yield stress values obtained with this model are higher than the values obtained by Method 1. This is possibly due to the error involved in drawing the tangent line and also in the selection of data points to be fitted.

4.6. Cox-Merz rule

This rule states that the magnitudes of the complex viscosity and the steady shear viscosity must be equal at equal values of frequency and shear rate. In other words, the values of complex and steady shear viscosity are the same for a given frequency and shear rate, at low values of γ and ω. This implies the convergence criteria that the complex and steady shear viscosities converge to one another as frequency and shear rate approach zero. An attempt (Fig. 12) is made for experimental verification of this condition. As shown in Fig. 12, the functional dependence of η and η^* are different and they do not show any tendency to converge. This is possible for certain structured systems, such as dextran.

5. Conclusions

The rheological behavior of water-insoluble dextran at a concentration of 250 mg/ml has been investigated using dynamic oscillatory shear testing. In particular, the effect of divalent cations (Ca^{2+}) on the gelation of dextran has been studied. The values of storage modulus suggest that the dextran sample (0 mM Ca^{2+}) is an entanglement network, and not a strong or weak gel. This is confirmed by a 'crossover' in G' and G'', at intermediate frequencies and $G'' > G'$ in the 'terminal zone', indicating a viscous liquid.

The storage modulus and loss modulus of the dextran sample decreased with the addition of calcium ions. The two moduli of dextran sample with 114 mM Ca^{2+}, decreased approximately 85% compared to dextran with no calcium added. This indicates that the addition of calcium ions breaks the entanglement of the dextran making it less elastic unlike alginate, where addition of calcium ions enhances the gelation.

Acknowledgements

The authors would like to thank Dr Arunan Nadarajah in the Department of Chemical and Environmental Engineering at the University of Toledo for valuable comments and suggestions.

Appendix A

The conditions of the test for dynamic frequency sweeps on all samples are listed below:

1. Geometry type = parallel plates (ParaPlate).
2. Diameter = 50.0 mm.
3. Gap = 1.0 mm
4. Read test fixture = On.
5. Tool inertia = 0.0 g cm^2.
6. Change gap to match tool thermal expansion = Off.
7. Tool thermal expansion coefficient = 0.0 μm/°C.
8. Fluid density = 1.0 g/cm^3.
9. Test type = dynamic strain frequency sweep (DFreqSwp).
10. Strain = 15.0%.
11. Sweep mode = Log.
12. Initial frequency = 1 rad/s.
13. Final frequency = 500.0 rad/s.
14. Points per decade = 5.
15. Options:steady preshear = Off.
16. PreShear mode = Off.
17. Delay before test = Off.
18. Automatically start test when on temperature = Off.
19. Mode = apply constant static force.
20. Max autotension rate = 0.01 mm/s.
21. Measurement options: Cycles = 0.5, Time = 3 s.

References

Bergethon, P. R., & Simons, E. R. (1990). *Biophysical chemistry: Molecules to membranes*. New York: Springer, pp. 149–150, 171–180.

Christensen, B. E., & Characklis, W. G. (1990). Physical and chemical properties of biofilm. In W. G. Characklis, & K. Marshall (Eds.), *Biofilms* (pp. 93–130). New York: Wiley.

Clare, K. (1993). Alginate. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (3rd ed). San Diego: Academic Press.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annual Review of Microbiology*, *49*, 711–745.

Flemming, H. C., Wingender, J., & Borchard, W. (2001). Influence of calcium ions on the mechanical properties of a model biofilm of mucoid *Pseudomonas aeruginosa*. *Water Science and Technology*, *43*, 49–57.

Landon, R. S., Law, R. C. S., & Webb, C. (1993). Fermentation broth rheology during dextran production by *Leuconostoc mesenteroides* B-512F as a possible tool for control. *Applied Microbiology and Biotechnology*, *40*, 251–257.

Landon, R. S., & Webb, C. (1990). Separating enzyme production and product synthesis within a traditional fermentation process. *Process Biochemistry*, *34*, 19–23.

Lappan, R. E., & Fogler, H. S. (1994). *Leuconostoc mesenteroides* growth kinetics with application to bacterial profile modification. *Biotechnology and Bioengineering*, *43*, 865–873.

Maier, H., Anderson, M., Karl, C., & Magnuson, K. (1992). In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums: polysaccharides and their derivatives* (pp. 181–227). San Diego: Academic Press.

Morris, V. J. (1995). In M. S. Alistair (Ed.), *Food polysaccharides and their applications* (pp. 341–375). New York: Marcel Dekker.

Padmanabhan, P. A., & Kim, D.-S. (2002). Production of insoluble dextran using cell-bound dextransucrase of *Leuconostoc mesenteroides* NRRL B-523. *Carbohydrate Research*, *337*(17), 1529–1533.

Powell, D. A. (1979). Structure, solution properties and biological interactions of some microbial extracellular polysaccharides. In R. C. W. Berkeley, G. W. Gooday, & D. C. Ellwood (Eds.), *Microbial polysaccharides and polysaccharases* (pp. 117–160). New York: Academic Press.

Rochefort, W. E., & Middleman, S. (1987). Rheology of xanthan gum: salt, temperature, and strain effects in oscillatory and steady shear experiments. *Journal of Rheology*, *31*(4), 337–369.

Ross-Murphy, S. B., Shatwell, K. P., & Sutherland, I. W. (1996). Influence of acyl substituents on the interaction of xanthans with plant polysaccharides. *Food Hydrocolloids*, *10*, 117–122.

Shamala, T. R., & Prasad, M. S. (1995). Preliminary studies on the production of high and low viscosity dextran by *Leuconostoc* spp. *Process Biochemistry*, *30*, 237–241.

Sime, W. J. (1990). In P. Harris (Ed.), *Food gels* (pp. 53–78). Barking, UK: Elsevier Applied Science.

Smidsrød, I. W., & Haug, A. (1971). Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity at different ionic strengths. *Biopolymers*, *10*, 1213–1227.

Stewart, T. L., & Fogler, H. S. (2001). Biomass plug development and propagation in porous media. *Biotechnology and Bioengineering*, *72*, 353–363.

Stoodley, P., Lewandowski, Z., Boyle, J. D., & Lappin-Scott, H. M. (1999). Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: an in situ investigation of biofilm rheology. *Biotechnology and Bioengineering*, *65*, 83–92.

Sutherland, I. W. (1994). Structure–function relationships in microbial exopolysaccharides. *Biotechnology Advances*, *12*, 393–447.

Tanford, C. (1980). *The hydrophobic effect* (2nd ed). New York: Wiley.

Tecante, A., & Munguia, A. L. (1986). Rheological characterization of dextran-enzymatic synthesis media. *Journal of Applied Polymer Science*, *31*, 2337–2350.

Wingender, J., Neu, T. R., & Flemming, H. C. (1999). What are bacterial extracellular polymeric substances? In J. Wingender, T. R. Neu, & H. C. Flemming (Eds.), *Microbial extracellular polymeric substances: Characterization, structure and function* (pp. 1–15). New York: Springer.

Winter, H. H. (1989). Gel point. In H. H. Winter (Ed.), *Encyclopedia of polymer science and engineering* (pp. 37–43). New York: Wiley.

Winter, H. H., & Chambon, F. J. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology*, *30*, 367–382.