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Rheological evaluation of deacetylated gellan gum (Gelrite) for pharmaceutical use

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Summary

The general rheological properties of deacetylated gellan gum (Gelrite) were examined using a purpose-built air turbine viscometer, whose independent variable was shear stress. Continuous shear experiments on 1% w/w Gelrite dispersions in water prepared by autoclaving or an elevated heat treatment showed that such systems had similar pseudoplastic flow properties. The heat-treated dispersions were examined in greater detail and showed evidence of limited irreversible shear breakdown and little thixotropy. The dispersions decreased progressively in apparent viscosity with rise in temperature over the range 10–50°C but the activation energy of flow, derived from an Arrhenius-type plot, was low indicating good thermal stability. At low pH Gelrite dispersions were much less viscous. The apparent yield value and viscosity of dispersions increased disproportionately with increasing concentration of the polymer used. Dispersions of Gelrite were found to be sensitive to gelation at much lower concentration of divalent rather than monovalent cations. Creep testing confirmed that a 1% w/w Gelrite dispersion behaved as a pseudoplastic liquid, which was devoid of a static yield value.

Introduction

Deacetylated gellan gum, which is available commercially under the tradename of Gelrite, is a very interesting polymer with many potential applications in the pharmaceutical and food industries, aided by its lack of toxicity. It has the unique property that when dispersed in low concentration (< 1%) in water it forms a slightly viscous solution, which can subsequently increase markedly in apparent viscosity when introduced into the presence of physiological levels of cations. A useful pharmaceutical application of this property was disclosed in a recent patent (Irish Patent, 1986) whereby the polymer was used as a gelling agent to prolong the residence time of timolol eye-drops, being activated only on instillation of the preparation into the conjunctival sac of patients for treatment of glaucoma.

Gellan gum is an anionic extracellular polysaccharide material secreted by *Pseudomonas elodea*. The fermentation conditions necessary to produce a high yield, and details of its isolation from the fermentation liquor including a deacetylation step, together with its subsequent purification have been described (Moorhouse et al., 1981). Studies on the molecular characterization of gel-

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lan gum have been reported (Carroll et al., 1982; Brownsey et al., 1984; Gunning and Morris, 1990; Milas et al., 1990) confirming it to be a linear polymer with a tetrasaccharide repeating unit of L-rhamnose, D-glucose and D-glucuronate. The crystalline structure determined by X-ray diffraction analysis shows that the gellan molecule forms a parallel half-staggered double-helix, similar to other gel-forming polysaccharides. The likely gelling mechanism of the deacetylated form is based on the formation of double-helical junction zones followed by aggregation of the double-helical segments, which leads to a three dimensional network, and which is induced to form by cation and heat treatment (Grasdalen and Smidsroed, 1987; Chandrasekaran et al., 1988a,b; Chandrasekaran and Thailambal, 1990). Divalent cations, in particular, can coordinate to carboxylate groups to stabilize the gel formed.

There has been very little work reported on the rheological properties of the polymer (Moorhouse et al., 1981), despite the importance of this information in relation to potential applications. This reported study involves the use of continuous shear and creep testing to rheologically examine dilute aqueous dispersions of Gelrite in relation to parameters such as plasticity, thixotropy and stability to temperature and pH variation, which are likely to be of significance in its subsequent pharmaceutical uses.

Materials and Methods

Materials and their preparation

Dispersions of Gelrite (deacetylated gellan gum, Scott Laboratories Inc.) in deionized water were prepared either by autoclaving at 15 psi for 15 min (method A) or by heating while stirring at 90 ° C for 20 min (method B), prior to cooling. To study the effect of addition of monovalent cations, 0.5% w/w dispersions of Gelrite were prepared by method B in the presence of 0.5 M concentrations of sodium chloride or potassium chloride. Gelrite dispersions of 0.05% w/w were prepared by method B in the presence of 0.5 M concentrations of the divalent cations, calcium chloride and magnesium chloride. To study the effect of pH variation, the dispersion medium was altered to 0.1 N HCl, citrate-phosphate buffer (McIlvaine) or phosphate buffer (Sorensen) to yield pH values of 1.3, 5.0 or 7.0, respectively, for 1% w/w dispersions of Gelrite at 20 ° C. BDH Analar grade reagents and deionized water were used.

Rheological assessment

A modification of the concentric cylinder air turbine viscometer described by Davis et al. (1968), having a series of interchangeable cups and bobbins, was built and calibrated. An advantage of the instrument in comparison to conventional viscometers is that because its independent variable is shear stress, this permits apparent yield values to be determined directly rather than by extrapolation techniques. Also the instrument may be easily modified for creep testing, where samples are examined under conditions approaching their rheological ground state. Flow curves were based on the mean of five determinations and were performed at 20°C unless otherwise indicated.

Atomic absorption

The levels of the cations Mg^{2+}, Ca^{2+}, K^+ and Na^+ were determined by ashing 25 g samples of Gelrite in a furnace at a temperature not exceeding 500 ° C, followed by digestion with hot dilute hydrochloric acid and dilution with doubly distilled and deionized water. Samples were determined using an atomic absorption spectrophotometer (Pye Unicam, model SP9).

Results and Discussion

Effect of method of preparation of dispersions

Samples containing 1% w/w Gelrite were prepared by method A or method B and their flow curves are shown in Fig. 1. As can be seen from the graphs, both methods of preparation gave similar plots indicating an equivalent dispersion of the polymer and confirming that the polymer is stable to a high temperature autoclaving cycle. The flow curves indicate that at the concentration examined a pseudoplastic system with an apparent yield value is obtained. In all subsequent

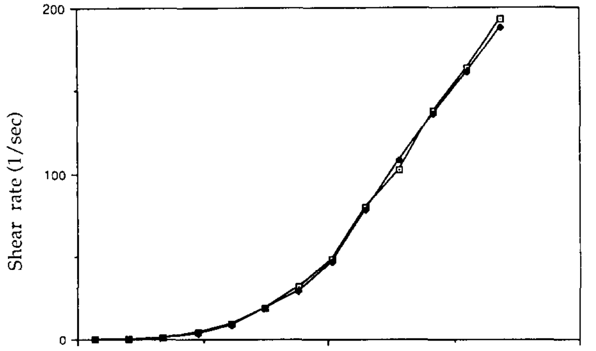

Fig. 1. Effect of method of preparation of 1% w/w Gelrite dispersion: autoclaving (□), heat at 90 °C (◆).

experiments method B was used for preparing polymer dispersions.

Reversible versus irreversible shear breakdown

Samples of 1% w/w Gelrite dispersions were prepared and used to check whether the material exhibited reversible or irreversible shear breakdown. To distinguish between thixotropy and irreversible shear breakdown, it is necessary to test for recovery of structure on resting. Having allowed samples to rest overnight in the viscometer, an initial flow curve was obtained at time 0 min over a range of shear stresses. Using the same sample, successive flow curves were obtained at time 30, 90 and 900 min after the commencement of the experiment. The resultant flow curves are shown in Fig. 2.

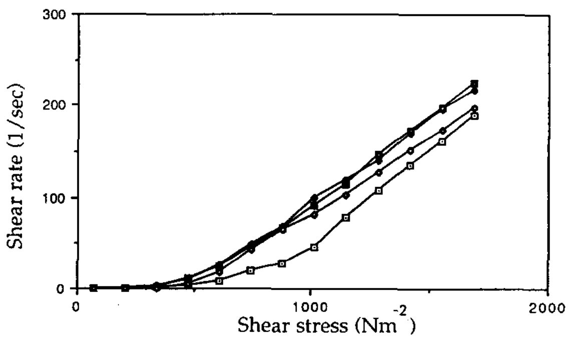

Fig. 2. Flow curves for 1% w/w Gelrite dispersions: time 0 min (□), 30 min (◆), 90 min (■), 900 min (◇).

As can be seen from the flow curves, after 900 min the structure does show some recovery, but not to the original resting state. There is some irreversible breakdown of the structure due to continuous shearing after 30 and 90 min. At the higher shear rates, the shear stress of the material at time 0 and 900 min is similar. The structure does show some degree of irreversible shear breakdown however, as it does not recover to its original level of structure within the time scale of the experiment. At low rates of shear there is a large increase in apparent viscosity consistent with the presence of weak intermolecular associations that are subsequently destroyed at higher rates of shear. The flow curves are similar at 30 and 90 min in that the sample recovers to the same level at both times. This finding indicates that the time dependent phenomenon is a combination of both reversible and irreversible effects which are not distinguishable from each other in conventional flow curves.

Effect of temperature

The rheological properties of many materials vary enormously with temperature. Gelrite has good thermal stability, being able to withstand an autoclaving cycle without loss of gel strength. Both up and down flow curves for 1% w/w Gelrite over the temperature range 10–50°C are plotted in Fig. 3. The up and down curves at each temperature do not differ significantly, indicating that the material shows little thixotropy. Also, the

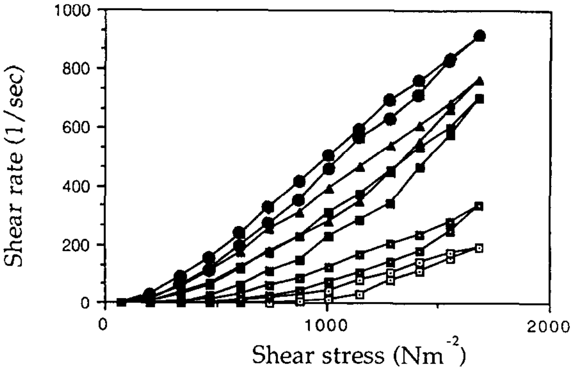

Fig. 3. Flow curves indicating the effect of temperature on 1% w/w Gelrite dispersions: 10°C (□), 20°C (■), 30°C (■), 40 °C (▲), 50°C (●).

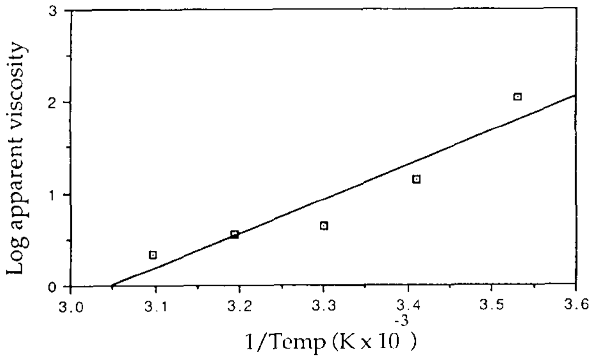

Fig. 4. Arrhenius-type plot.

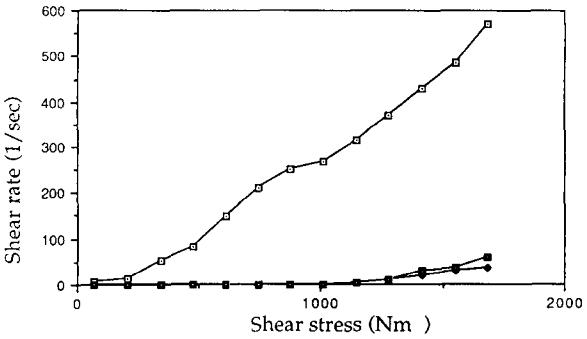

Fig. 5. Flow curves indicating the effect of variation in pH on 1% w/w Gelrite dispersions: pH 1.3 (□), pH 5 (◆), pH 7 (■).

apparent yield value and the apparent viscosity at the apex of the loop both decrease with increase in temperature as reported previously by Berney and Deasy (1979) for Carbopol 934, which is a carboxyvinyl polymer used in the formulation of pharmaceutical suspensions.

The apparent activation energy of flow, E, is a useful index of the thermal stability of polymeric dispersions. Low activation energies indicate that the apparent viscosity varies little with change in temperature. Fig. 4 shows a linear plot of log apparent viscosity at the apex against $1/T$ (absolute), from the slope of which the activation energy of flow was calculated to be 1.425 kcal/mol. This moderately low value obtained indicates reasonable rheological stability over normal use conditions, which would be of advantage in lessening sedimentation of dispersed solids in preparations such as suspensions or gels during expected temperature fluctuation in storage.

Effect of pH

Fig. 5 shows the flow curves obtained at the three different pH values examined. At the lower pH values the polymer dispersions are much less viscous, though the effect may be contributed to by the ionic content of the buffers used. The reduction in apparent viscosity is presumably due to less ionization of carboxyl groups resulting in decreased polymer extension solubility in the aqueous vehicle. The factor should be considered when using Gelrite in the formulation of pharmaceutical suspensions. It is also important in complex coacervation systems involving the polymer and gelatin for the microencapsulation of drugs, which have been reported to be dependent on the pH of the manufacturing vehicle (Deasy, 1984; Chilver and Morris, 1987).

Effect of concentration and batch

The effect of increasing the polymer concentration over the range 1–3% is shown in Fig. 6. All plots are non-Newtonian showing pseudoplastic flow. The shear thinning character is suggestive of the breakdown of a weak network or gel structure. The magnitude of the apparent yield value and the apparent viscosity at constant shear rate both tend to disproportionately increase with increasing polymer concentration. At the constant maximum shear stress examined as indicated at the apex of each plot, the apparent vicosities are 9.0, 65.8 and 247.8 N m^{-2} s^{-1} for the 1, 2 and 3% concentrations, respectively. This

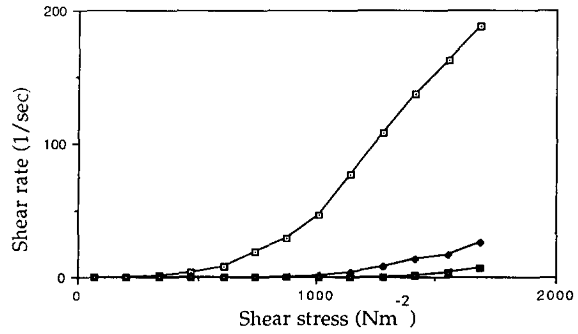

Fig. 6. Flow curves indicating the effect of variation in Gelrite concentration: 1% (□), 2% (◆), 3% (■).

non-linear increase in apparent viscosity is indicative of increasing polymer chain interaction with increase in polymer concentration, as observed generally with hydrophilic colloids.

The 1% w/w flow curves obtained from Gelrite dispersions prepared from two different batches of the polymer were superimposable, indicating negligible batch to batch variation.

Effect of mono- and divalent cations

The 0.5 M concentrations of cations were prepared in the presence of 0.5% w/w Gelrite for the monovalent cations, Na^+ and K^+, and in the presence of 0.05% w/w Gelrite for the divalent cations, Ca^{2+} and Mg^{2+}. As shown in Fig. 7, gels of similar apparent viscosity can be formed with divalent cations at much lower concentrations of polymer than are required for gelation with monovalent cations. Of the cations examined at comparable molar strength, Ca^{2+} exerts the most profound effect on the gelation of the polymer. Gels prepared with Ca^{2+} do not remelt under normal conditions, but to increase the thermal reversibility of Gelrite, it is necessary to increase the polymer concentration and/or decrease the divalent ion concentration. Mg^{2+} produces a thermally reversible gel. K^+ exerts the more pronounced effect of the monovalent ions examined, as reflected in its greater apparent yield value and apparent viscosity at maximum shear stress, in comparison to Na^+.

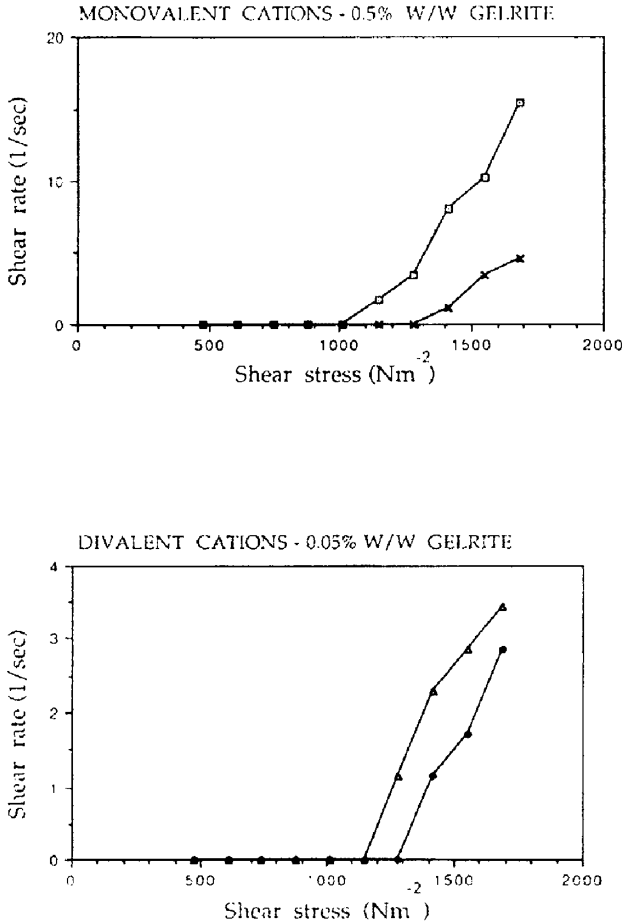

Fig. 7. Effect of various cations on flow curves of Gelrite dispersions: Na (□), K (×), Mg (△), Ca (◆).

TABLE 1

Level of cation in a Gelrite sample, determined by atomic absorption spectroscopy, compared to the reported literature value for a different sample

Cation	Level determined (% w/w)	Level reported (% w/w)
K^+	1.83	2.0
Na^+	0.54	0.1
Mg^{2+}	0.27	0.5
Ca^{2+}	0.87	0.1

The effect of cation addition to Gelrite dispersions is influenced by the levels of these cations already present in the dry polymer, arising from the extraction and purification steps used in its manufacture. Table 1 shows levels of cation determined by atomic absorption and also the reported values of Carroll et al. (1983) for a different sample, which are in poor agreement for Na^+ and Ca^{2+}. The major cation present by weight is K^+, but appreciable concentrations of the other cations of interest are also observed.

The reason why gels are formed with divalent cations at much reduced concentration than required with monovalent cations is due to the fact that the dispersed Gelrite molecules have uronic acid groups at junctional zones resulting from the parallel alignment of the helical polymers. It is believed that each divalent cation gives rise to much stronger double-helical interaction with these groups by displacing two monovalent cations

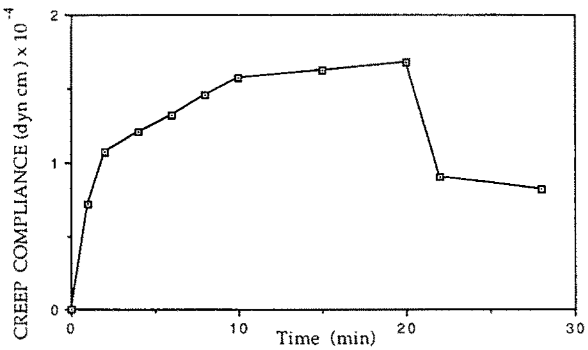

Fig. 8. Creep compliance curve for 1% w/w Gelrite dispersion.

such as K^+. The co-ordination of divalent cations between the double-helices is so strong that aggregation can occur readily even in the presence of very low ionic concentrations.

Creep testing

Creep testing is essentially non-destructive and yields valuable information on the structure of a semi-solid at the molecular level. Prior to the determination of creep curves, the linear viscoelastic region was determined for 1% w/w Gelrite dispersions and a shear stress corresponding to the mid-point of the narrow range obtained was used in the study. The creep compliance curve is plotted as shown in Fig. 8 from a number of points obtained from replicate experimental traces, and the data used for the mathematical analysis of the resultant curve. $J_0 \times 10^{-4} = 0.125$ $\text{dyn}^{-1}\ \text{cm}^2$, $J_R \times 10^{-5} = 0.84$ $\text{dyn}^{-1}\ \text{cm}^2$ and $J_N \times 10^{-4} = 0.75$ $\text{dyn}^{-1}\ \text{cm}^2$, which represent the instantaneous compliance, retarded compliance and Newtonian compliance respectively, as determined by the discrete spectral method described by Warburton and Barry (1968). As J_R represents the part of the structure of the Gelrite dispersion in which secondary bonds break and reform at different rates during the experiment, the value determined represents a large number of shear compliances in a single composite term.

From inspection of the creep compliance curve and the value of 1.2×10^6 P calculated for the residual viscosity, the Gelrite dispersion appears to behave as a viscoelastic liquid. On removal of the applied shear stress the material did not exhibit complete recovery in the time scale of the experiment. The presence of unretarded viscosity indicates that the yield value observed in the continuous shear experiments at comparable polymer concentration is only apparent, as any stress will cause an observable flow provided a sufficient period of time is allowed.

Conclusions

Gelrite is capable of forming optically clear dispersion in water at low concentration, whose apparent viscosity can be easily increased by increasing the polymer concentration. A heat treatment is needed for the full dispersion of the polymer, which can include autoclaving to sterilize without loss of apparent viscosity. Reduction in apparent viscosity observed with increase in storage temperature over normal ranges is modest. Both increase in pH and cation concentration, particularly divalent metallic ions, can markedly increase the apparent viscosity of dispersions. Yield values observed in dispersions of low polymer concentration are only apparent. Apart from the few applications reported to date, the results of this study indicate that Gelrite is a very interesting biocompatible polymer, which should have many other exciting uses in areas such as suspension or topical formulation and the design of sustained drug delivery systems for both parenteral and oral use.

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