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Assessment of polysaccharide gels as drug delivery vehicles

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Summary

A range of cross-linked polysaccharide gels were prepared from bacterial sodium hyaluronate (HANa) and dextran using diethylenetriaminepentaacetic acid dianhydride (DTPA) as the cross-linking agent. The physical characteristics of the gels were assessed using a Ferranti-Shirley cone and plate viscometer, a TA-XT2 Universal Texture Analyser and FT-IR spectrometry. FT-IR demonstrated a concentration-dependent incorporation of DTPA to form gels from either polysaccharide with varying properties. The gels exhibited an increase in viscosity and maximal compression force with increasing cross-linking. Dextran gels were stronger and of greater viscosity than comparable HANa gels. The gels are being investigated as potential drug delivery vehicles for parenteral depot preparations.

Introduction

Polysaccharides such as dextran (D) and sodium hyaluronate (HANa) are currently used in medicine, dextran as a plasma volume expander (Reynolds, 1989) and HANa for the treatment of dry eye syndrome (DeLuise and Peterson, 1984; Sand et al., 1989), in viscosurgery (Alpar et al., 1988) and orthopaedics (Weis and Balazs, 1984). In addition, dextran (Paavolainen and Sundell, 1976; Nangia and Hung, 1991) and HANa (Doillon and Silver, 1986; King et al., 1991) have been investigated as wound dressings. Dextran has a low incidence of adverse reaction (Gibby et al., 1989; Reynolds, 1989) and as HANa is an endogenous body chemical it should be biodegradable (Richter, 1974) and non-immunogenic (Richter, 1974; Richter et al., 1979). Polymeric systems are increasingly being investigated and developed as drug release matrices (Juliano, 1980; Chandrasekaran et al., 1983; Joshi, 1988; Langer, 1990) and controlled drug delivery devices such as implants (Heller et al., 1983; Powell et al., 1990) or carriers (Leong et al., 1985; Rydén and Edman, 1991). These two polysaccharides may therefore be useful alternatives to the current excipients employed for parenteral depot preparations.

HANa, which occurs naturally in animals and

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bacteria, is an unbranched linear polysaccharide consisting of repeating units of D-glucuronic acid and *N*-acetylglucosamine linked β1–3 and β1–4. The HANa used here was produced by *Streptococcus equi* in a continuous fermentation procedure which can be rigorously controlled to give reproducible batches with narrow ranges of molecular weight. Dextran is produced by the fermentation of sucrose using a strain of *Leuconostoc mesenteroides* and has no animal source. It was first reported in 1861 and later work identified the structure (Glicksman, 1969) as polymerised glucose units linked α1–6 with a branch every 10–12 units linked α1–3. The molecular weight, which ranges from 10^4 to 10^6, is controlled by altering the fermentation medium (Glicksman, 1969).

The cross-linking of polysaccharides to form a matrix can be produced by radiation (Adler, 1963), or by chemical agents. Starch, for example, is widely used in the food industry cross-linked by a variety of agents including epichlorohydrin and phosphoryl chloride (Whistler et al., 1984). Cross-linked HANa gels have been prepared using 1,2,3,4-diepoxybutane (Laurent et al., 1964), copper sulphate (Schmut and Hofman, 1982), carbodiimide (Sparer et al., 1983; Kuo et al., 1991), formaldehyde (Balazs et al., 1986) and divinyl sulphone (Balazs and Leshchiner, 1986, 1987a,b). Dextran, with a triazine side chain, has been used for targeting antineoplastic agents (Baki and Vaughan, 1982) and as a soluble carrier molecule (Poznansky and Cleland, 1980). It has also been cross-linked using DTPA to provide a macromolecular paramagnetic contrast agent (Gibby et al., 1989).

In this paper the physical properties of cross-linked polysaccharide gels have been investigated as a prelude to utilising these systems for controlled drug delivery. The cross-linking method used here was based on that of Gibby et al. (1989) with the cross-linking ratios expressed as the number of molecules of DTPA to the number of disaccharide units of the polysaccharide. Physical properties of the gels were assessed using FT-IR spectrometry, a Ferranti-Shirley cone and plate viscometer and a TA-XT2 Universal Texture Analyser.

Materials and Methods

Materials

DTPA and anhydrous DMSO were purchased from Aldrich Chemical Co. Ltd, Gillingham, Dorset. Dextran (average molecular weight 74 200, 162 000, 503 000, 2 000 000 and 5 000 000) was purchased from Sigma Chemical Co. Ltd, Poole, Dorset. HANa (molecular weight range 1.34–1.88 $\times 10^6$) was supplied by Fermentech Ltd, Herriot Watt University Science Park, Riccarton Campus, Edinburgh. The TA-XT2 Universal Texture Analyser was kindly provided by Stable Micro Systems, Haslemere, Surrey.

Methods

Gel ratios

A range of gels was prepared with cross-linking ratios ranging from 1 : 1 to 1 : 10. The cross-linking ratio refers to the number of molecules of DTPA to the number of disaccharide unit of the polysaccharide. The molecular weights of DTPA (Mol. Wt 357) and the disaccharide units (dextran, Mol. Wt 324; hyaluronate Mol. Wt, 377) are similar, so for a gel of ratio 1 : 1, approximately equal weights of DTPA and polysaccharide were used.

Gel preparation

Gels were prepared according to the method of Gibby et al. (1989) using 1–2 g of polysaccharide. The solids were mixed dry with DTPA, then anhydrous DMSO (20 ml) was added and the mixture stirred with heating for up to 15 min. The yield of gel was 20–30 g depending on the weights of starting materials used. The time required for gel formation was recorded for dextrans of each molecular weight at ratios of 1 : 1, 1 : 3 and 1 : 5.

FT-IR analysis

DTPA incorporation was assessed by FT-IR spectrometry using Nujol mulls and a Nicolet 20SXB spectrometer. A Nicolet 510P FT-IR with PC-IR software and a Spectra-Tech In-Compartment Horizontal ATR with gripper sample clamp was used to obtain the HATR spectra. The sam-

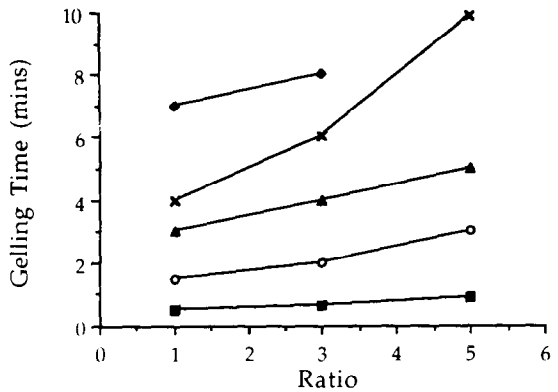

Fig. 1. Gelling times for dextrans of different molecular weights at ratios of 1:1, 1:3 and 1:5. Dextran molecular weight: (♦) 74200; (×) 162000; (▲) 503000; (○) 2×10^6; (■) 5×10^6.

ples were spread onto a zinc selenide crystal and light pressure applied using the clamp attachment to ensure a good contact. Scans were collected (64 at 4 cm^{-1} resolution) over a total collection time of approx. 1 min. KBr discs were prepared using approx. 5 mg of sample and 200 mg of spectroscopic grade KBr. They were pressed for 1–2 min at a pressure of 10 tons. The spectra were recorded at 4 cm^{-1} resolution with 64 scans collected.

Viscometric analysis

Gels were assessed on a Ferranti-Shirley cone and plate viscometer serial number 756 with a Bryans 25000 XY recorder. Measurements were conducted at 25°C using a sweep time of 600 s. HANa gels and solutions were tested using a large cone (7 cm diameter) at a maximum shear rate of 1684 s^{-1} and a small cone (2 cm diameter) was used for the dextran gels at a maximum shear rate of 1598 s^{-1}.

Texture analysis

A TA-XT2 Universal Texture Analyser was used in the compression mode, with a probe of 13 mm diameter. The probe travelled a pre-set distance of 10 mm in the gel at a set rate of 3 mm/s, was held for 2 s and then withdrawn at the same rate. The force required to compress the gel and remove the probe was measured and recorded electronically.

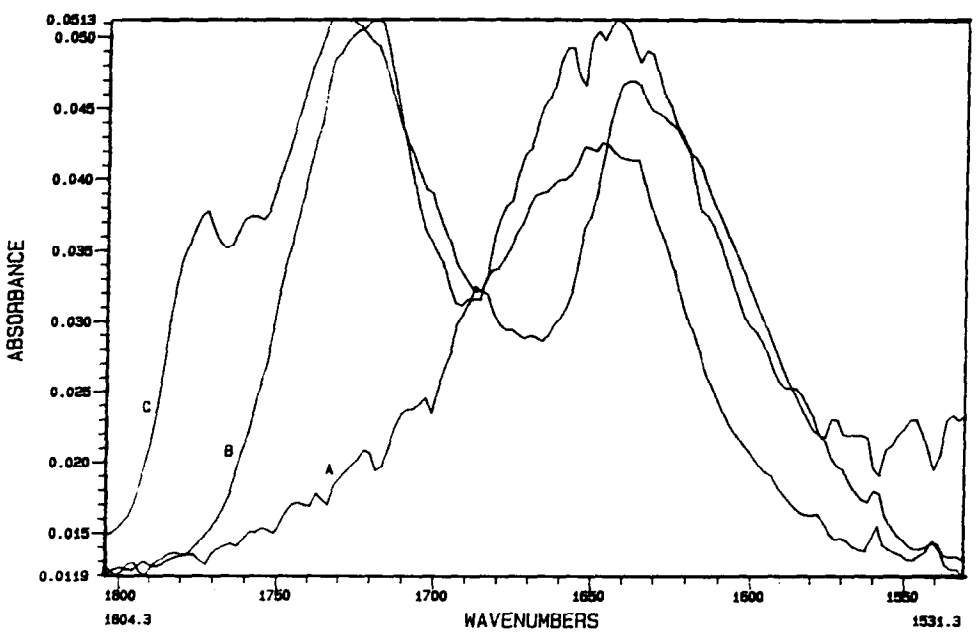

Fig. 2. FT-IR spectra of DTPA, dextran and dextran gel. (A) Dextran powder, Mol. Wt 162000; (B) dextran gel ratio 1:1, Mol. Wt 162000; (C) DTPA powder.

Results and Discussion

Results

Gel preparation

The HANa used varied between batches and was not entirely suitable for the gel formation method; HANa gels did not form suddenly as did the dextran gels and were not reproducible. Dextran, however, was easily mixed with DTPA and formed reproducible gels. Gels formed more quickly with higher molecular weight dextrans and were firmer than lower molecular weight dextrans with a similar ratio. The times required for gel formation are presented in Fig. 1, showing a trend of increased gelling time as cross-linking and molecular weight decrease; with lower molecular weights the difference in gelling times is more marked. Dextran of average Mol. Wt 74200 and ratio 1:5 did not form a gel even after stirring for 60 min. Using dextran of average Mol. Wt 162000, the more cross-linked gels (ratio < 1:5) were firmer, drier and could be cut with a sharp edge. Less cross-linked gels (ratio $\geqq$ 1:3) became progressively less rubbery through to a sticky jelly.

FT-IR analysis

DTPA incorporation in the gels was confirmed by FT-IR analysis. Dextran gels of average Mol. Wt 162000 (ratio 1:1, 1:3, 1:5) were run and compared to the starting materials. DTPA has an absorption band at 1725 cm^{-1} which was also found in the gels but not in any of the other starting materials (Fig. 2). A possible assignment of the band is a carbonyl stretching vibration and its intensity increased with a trend in relative peak height with increasing cross-linking ratio (Fig. 3). The band was also found to be present in the HANa gels (data not shown) and its presence confirms that DTPA incorporation has occurred.

Viscometric analysis

A dextran gel (Mol. Wt 162000) at a cross-linking ratio of 1:7 and a dextran solution (20% w/v) were tested on a Ferranti-Shirley cone and plate viscometer, the results of which are presented in Fig. 4. For the solution, the down-curve

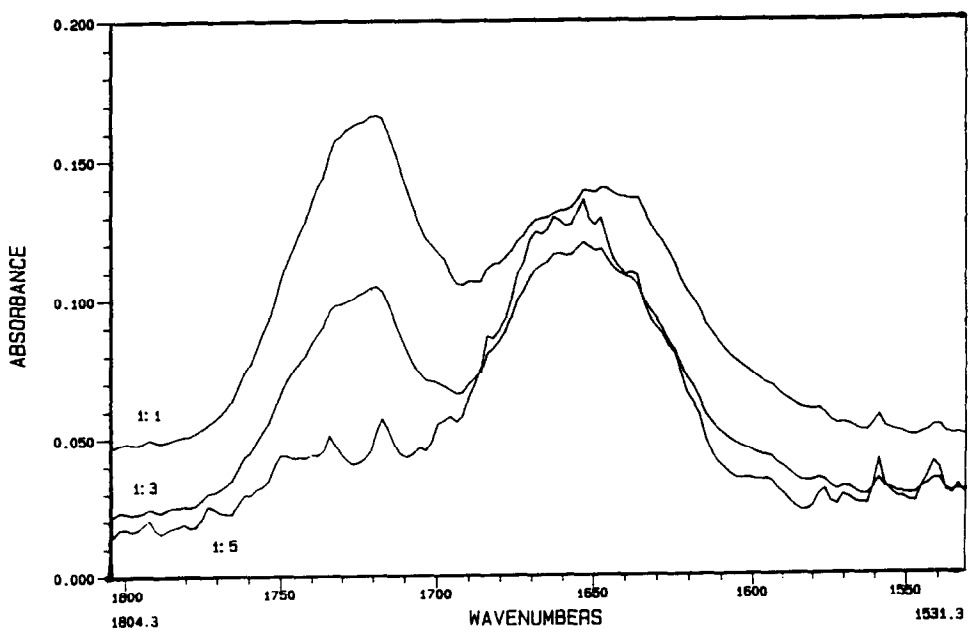

Fig. 3. FT-IR of dextran gels. Gel ratio 1:1, 1:3, 1:5; dextran Mol. Wt 162000.

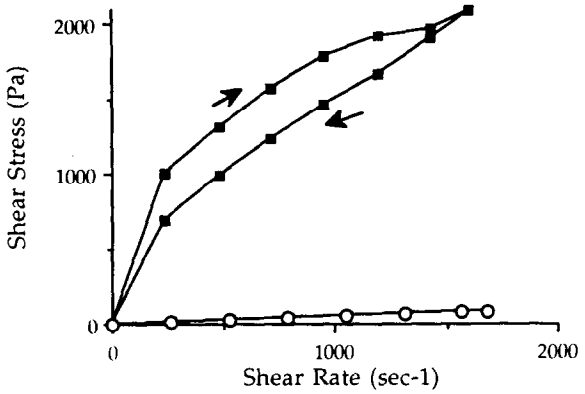

Fig. 4. Rheological properties of dextran solution and dextran gel (Mol. Wt 162000) on Ferranti-Shirley viscosimeter. (■) Dextran gel 1:7 (≡ 10% solution); (○) dextran solution (20% w/v); arrows indicate up-curve and down-curve of hysteresis loop.

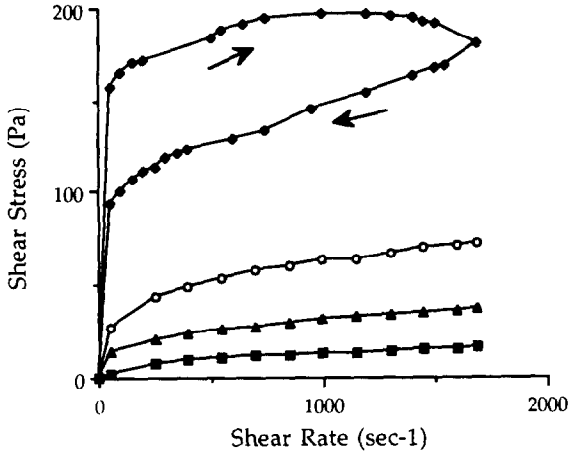

Fig. 5. Rheological properties of HANa gel and solutions on Ferranti-Shirley viscosimeter. (♦) HANa gel 1:5 (≡ 0.5% solution); (○) HANa solution (1% w/v); (▲) HANa solution (0.5% w/v); (■) HANa solution (0.2% w/v); arrows indicate up-curve and down-curve of hysteresis loop.

was superimposed on the up-curve, showing no evidence of hysteresis. The gel (ratio 1:7), however, provided a different profile with a hysteresis loop and an increased apparent viscosity of 1.13 Pa s (1598 s^{-1}) when compared with a similar concentration of dextran in solution (Table 1). A range of HANa solutions (0.2–1.0% w/v) measured using the Ferranti-Shirley viscosimeter produce a series of curves with an increase in apparent viscosity as concentration increases (Fig. 5). As with the dextran solution, the down-curve for the HANa solutions was superimposed on the up-curve with no evidence of hysteresis. An HANa gel (ratio 1:5) with a concentration equivalent to a 0.5% solution, however, had a hysteresis loop (Fig. 5) and an apparent viscosity similar to a 2% solution (Table 1).

TABLE 1

Viscosities of solutions and gels measured using the Ferranti-Shirley (viscosity of HANa measured at 1684 s^{-1}; dextran measured at 1598 s^{-1})

Solution/gel	Concentration/ratio (% w/v)	Viscosity (Pa s)
HANa solution	0.2	0.01
HANa solution	1.0	0.05
HANa solution	1.6	0.09
HANa solution	2.0	0.12
HANa solution	2.5	0.14
HANa gel (≡ 0.5% w/v)	1:5	0.12
Dextran solution	20	0.05
Dextran gel (≡ 10% w/v)	1:5	2.62
(≡ 10% w/v)	1:7	1.13
(≡ 10% w/v)	1:9	0.44

Texture analysis

The more cross-linked dextran gels (ratio ≦ 1:3) were too firm to be tested on the Ferranti-Shirley viscosimeter and were assessed using a TA-XT2 Universal Texture Analyser (Fig. 6). The probe depresses the gel through a pre-set distance (10 mm) and as the more cross-linked gels are firmer, the compression force required is greater, giving a larger value for the maximal compression force (Table 2). Less cross-linked gels become progressively softer, are more easily compressed and so have lower values for maximal compression force (Table 2). During the holding period of 2 s, the compression force decreased slightly for each of the gels, although this was less in the more cross-linked samples. Some HANa gels were also measured on the TA-XT2 analyser showing a similar trend although the maximal compression force required was less than for a

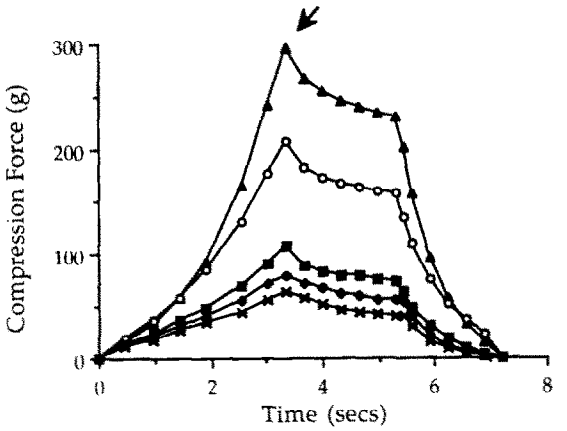

Fig. 6. Compression behaviour of dextran gels (Mol. Wt 162000) on TA-XT2 analyser. (▲) Gel ratio 1:0.6; (○) gel ratio 1:1.2; (■) gel ratio 1:3; (♦) gel ratio 1:6; (×) gel ratio 1:9; gel concentration equivalent to a 10% w/v solution. Arrow indicates maximal compression force.

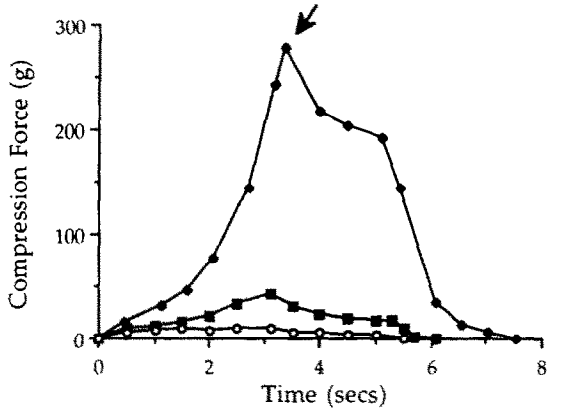

Fig. 7. Compression behaviour of HANa gels on TA-XT2 analyser. (♦) HANa gel ratio 1:2; (■) HANa gel ratio 1:5; (○) HANa gel ratio 1:10, gel concentration equivalent to a 0.5% w/v solution. Arrow indicates maximal compression force.

dextran gel with a similar cross-linking ratio (Fig. 7 and Table 2).

Discussion

A simple method of assessing degree of cross-linking and gel formation is measurement of the time taken for the gel to form (Jullander, 1945). Lower molecular weight gels formed more slowly as did gels with a lower ratio, suggesting that gels formed from higher molecular weights and/or higher ratios are more cross-linked. FT-IR analysis confirmed that DTPA incorporation and therefore cross-linking had occurred with a trend relative to the cross-linking ratio. The solutions of dextran and HANa produced simple curves on the Ferranti-Shirley viscosimeter with increasing viscosity as concentration increases. However, the gels exhibit a hysteresis loop, the presence of which implies an elastic component and shows a complex structure suggesting that cross-linking has occurred (Barnes et al., 1989). The gels show a greatly increased apparent viscosity when compared to simple solutions at the same concentration and this increases as cross-linking increases. A similar trend in properties is obtained on texture analysis of the gels with an increase in maximal compression force as cross-linking increases. Again, as with the viscosity results, the maximal compression force for the gels is greater than the values for solutions at the same concentration. In both sets of measurements, the values for dextran gels are greater than those for HANa gels, suggesting that dextran gels are 'stronger' than HANa gels of the same cross-linking ratio. HANa has different physical properties compared to dextran and due to steric hindrance of side groups in HANa (Gibby et al., 1989), dextran gels are more cross-linked than HANa gels for a similar ratio. This is reflected in the lower viscosity of the HANa gel (Table 1) and lower values for the maximal compression force (Table 2). The texture analyser allowed the firmest and softest gels to be assessed and compared under the same fixed

TABLE 2

Measured maximal compression force for dextran and HANa gels

Polysaccharide	Gel ratio	Maximal compression force (g)
Dextran	1:0.6	316
Dextran	1:1.2	115
Dextran	1:3	107
Dextran	1:6	79
Dextran	1:9	63
HANa	1:2	277
HANa	1:5	42
HANa	1:10	10

conditions which was not possible using the Ferranti-Shirley viscosimeter as the firmer gels were thrown out of the gap at high shear rates.

Dextran gels can be prepared consistently, however, further work is required to ensure reproducibility of the HANa gels. Use of mixtures of different molecular weights could give gels with different characteristics (Gibby et al., 1989). Also, it may be possible to prepare mixed gels of dextran and HANa which may allow the formation of gels with a wide variety of properties. Once the gels have been fully assessed they will be further investigated as potential drug delivery vehicles.

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