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Research paper

Rheological characterisation of dextran–concanavalin A mixtures as a basis for a self-regulated drug delivery device

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Abstract

The rheological characterisation of glucose sensitive mixtures containing dextran and concanavalin A (con A) with and without glucose, was undertaken using oscillatory rheometry at 20 and 37 °C so that comparative data could be gathered in the linear viscoelastic (LVE) range. Measurements for a series of mixtures showed that complex viscosity is a function not only of the con A concentration but of the content and molecular weight of the dextran used. The extent of liquefaction on addition of glucose also depended on these factors. The tan delta profiles confirmed the change from semi-solid towards fluid behaviour. This occurs when glucose effects dismantling of the three-dimensional structure of the dextran–con A system by competitive binding to the glucose receptors in the protein. For the mixtures studied, the changes occurred between contents of 0 and 1% (w/w) glucose at 20 and 37 °C and form a useful basis for the formulation of a self-regulating delivery device for the control of hyper-and hypoglycaemia in diabetes.

Keywords: Insulin delivery; Formulation; Concanavalin A; Viscosity; Glucose; Diabetes; Self-regulating; Dextran

1. Introduction

A self-regulating drug delivery system with a reversible gel to sol transformation has been the focus of research in recent years [1–4]. In this system the gel has been used to modulate the delivery of insulin as a function of ambient free glucose. Prototype gels were formed between a glucose selective lectin, con A and a branched glucose-based polysaccharide, dextran. The temporary cross-linking of the polysaccharide by the tetravalent lectin is selectively responsive to free glucose because of the competition for the glucose specific sites on the lectin. This formulation strategy has been used to form a variable permeability, rate-determining membrane for an insulin reservoir and shown to be capable of delivery of low and high molecular weight dyes as well as of insulin itself [5]. Gels used to make these membranes have a tendency to lose con A to their surroundings, especially when in the glucose-rich sol state, but related work has shown that when con A is covalently bonded to periodate oxidised dextran, the resulting material retains its ability to form a reversible sol and can deliver insulin with negligible con A leaching [6]. It is the competitive nature of the temporary interaction between receptor and glucose moieties that is responsible for the reversible sol mechanism and it was shown that this occurs independently from the covalent stabilisation bonding between the two components, dextran and con A. In this paper, the rheological evidence for the glucose-specific interaction and transition will be examined in simple dextran–con A mixtures for future comparison with other covalently stabilised analogues so that the differential influences of the two types of bonding can be better understood.

When in controlled shear stress oscillatory mode (constant torque) a rheometer measures two independent variables, those being the deflection angle and the phase shift angle [7]. From these two raw data, the elastic (storage modulus) and viscous (loss modulus) components of

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a viscoelastic material can be obtained as well as complex viscosity and tan delta.

2. Materials and methods

2.1. Materials

Dextran (produced by *Leuconostoc mesenteroides*, strain No. B512; average relative molecular weight 65,000 (D70), 473,000 (D500) and 2,000,000 (D2M) and Concanavalin A (Type V) were purchased form Sigma-Aldrich Chemical Company Ltd (Poole, Dorset, UK). All chemicals were analytical grade and were used as received. Double distilled water was used throughout.

2.2. Formulation of gels for the three dextran types

Aqueous formulations of dextran-con A (8.3%, w/w) were made in distilled water and adjusted to pH 7.0 at 20 °C by mixing solutions of the components. The glucose inclusion over the range 0–1% was accomplished by adding glucose concentrates (for example, 5 μL glucose 50% (w/w) glucose to 0.5 g of gel would produce ∼0.5% (w/w) glucose) with a control for any possible dilution effect. The mixtures were then thoroughly stirred and immediately rheologically tested.

2.3. Rheological testing of gels

The rheological testing of the gels was conducted on a Physica MCR 300 rheometer (Anton Paar, Germany) with cone and plate geometry (CP 25-1) and Peltier temperature control. The tests were conducted in oscillation mode using controlled stress. Gels were formulated with con A, dextrans of three molecular weights and in the presence of glucose from 0 to 1% (w/w) and have been compared at 20 and 37 ± 0.1 °C using stress sweeps. Frequency sweeps within the range of 0–10 Hz and at a stress within the LVE were undertaken in particular cases as described. All rheological parameters were obtained in triplicate using fresh sample for each test, having been processed using the Anton Paar software.

3. Results and discussion

For materials that undergo solid (or semi-solid) to liquid-like behaviour, changes in complex viscosity and tan delta provide useful information. The complex viscosity is the angular velocity-dependent function of G^*, the complex shear modulus and differs from shear viscosity in that it is determined during forced harmonic oscillation of shear stress. Tan delta is the ratio of the loss modulus to the storage modulus. It is therefore a quantification of the elastic and viscous contributions, where a value above 1 is indicative of liquid like viscous behaviour and below 1 signifies elastic behaviour. The mixtures in this case are produced from temporary interactions between dextran and con A. They respond to glucose by an obvious liquefaction measured by the loss in complex viscosity [6].

Fig. 1(a) and (b) shows the tan delta values for mixtures formulated using D70, D500 and D2M at glucose concentrations between 0 and 1% (w/w) at 20 and 37 °C, respectively. These mixtures contained 8.3% (w/w) for both dextran and con A (unless otherwise stated) to enable a future comparison with analogous covalently bonded mixtures and were all assessed in the LVE range at 5 Pa from stress sweep data at 1 Hz. At 20 °C. the lowest values for tan delta can be seen for the mixtures formulated using D2M and D500 which were both clearly much more viscous than those made with D70 (see also below). The D500 and D2M mixtures became more fluid with increasing glucose concentration and showed a gradual rise in tan delta by

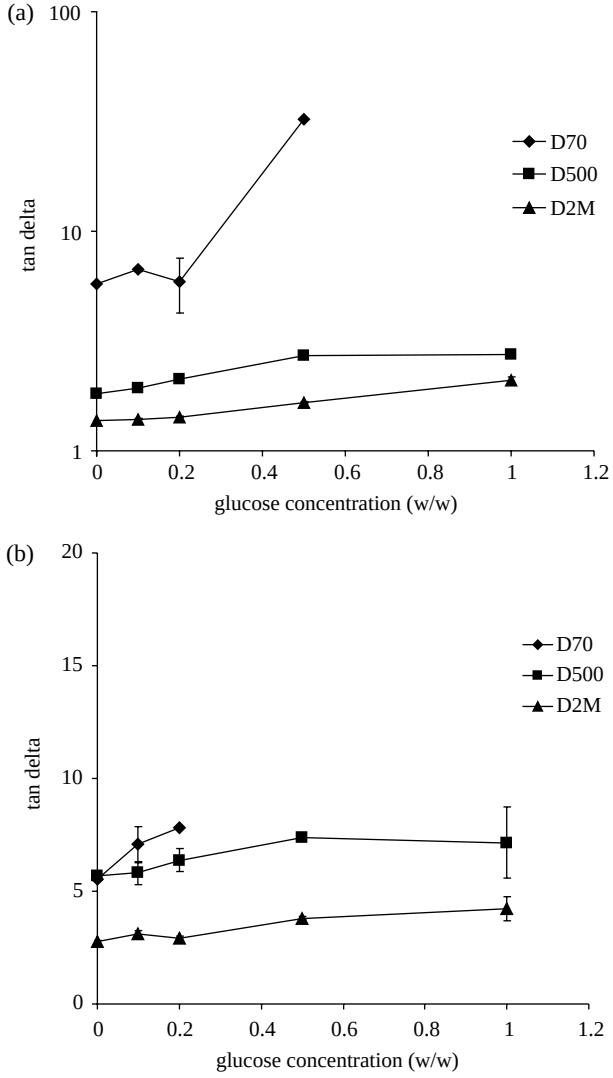

Fig. 1. Tan delta at different glucose concentrations for D70, D500 and D2M–con A gels at 20 °C (a) and (b) 37 °C from stress sweeps at 5 Pa and 1 Hz. (Data represent mean ± SD of three measurements.)

150% of their original values. However, the D70 mixture changed at 0.5% (w/w) glucose with a much larger and more sudden tan delta increase (>500%). Further addition of glucose to D70 mixtures gave readings at 5 Pa outside the LVE range whereas the other two systems were well within the LVE for all glucose concentrations studied, despite their obvious liquefaction. When comparisons were made for each dextran type at the two temperatures tested, mobility as gauged by tan delta, was always higher at 37 °C, as is the case with many polymers including dextran solutions. This temperature dependent behaviour of these mixtures can be seen in Fig. 1b and shows that at 37 °C, tan delta increased by 126 and 154% within the glucose concentration range 0–1% (w/w) for mixtures made with D500 and D2M, respectively. For D70 mixtures at 37 °C, glucose concentrations higher than 0.2% (w/w) again produced responses outside the LVE at 5 Pa.

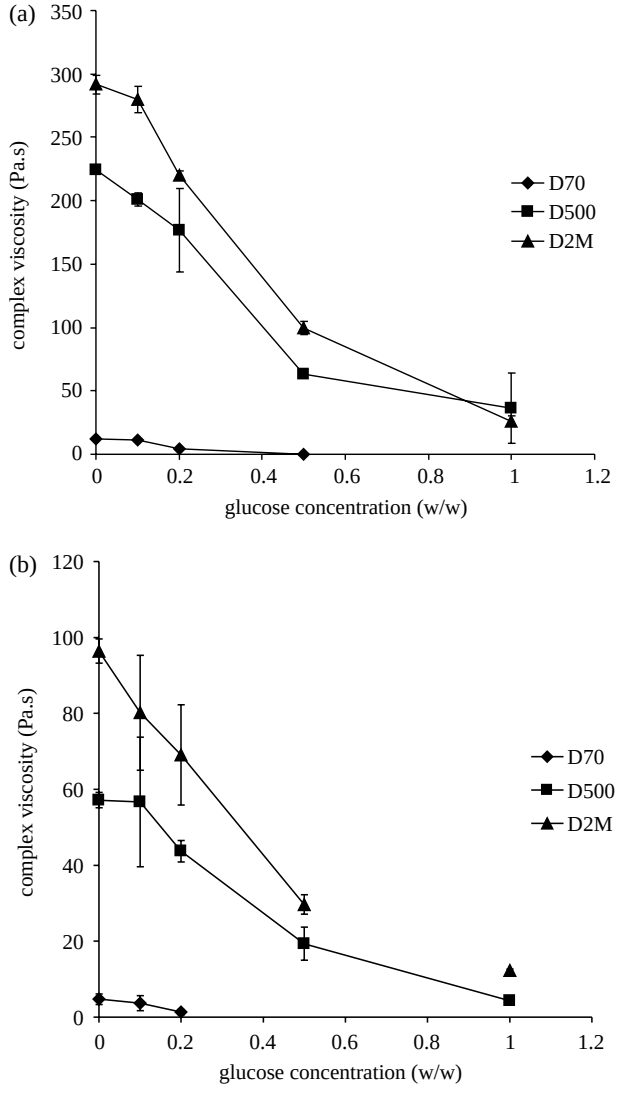

Fig. 2. Complex viscosity at different glucose concentrations for D70, D500 and D2M–con A gels at 20 °C (a) and (b) 37 °C from stress sweeps at 5 Pa and 1 Hz. (Data represent mean ± SD of three measurements.)

Fig. 2(a) and (b) shows complex viscosity changes at both temperatures with increasing glucose content for mixtures made with the three dextran types. D2M mixtures gave the highest complex viscosity throughout the glucose range and even the sols in this series were more viscous than any systems in the D70 range. The complex viscosity of the glucose free systems at 37 °C were much lower than for 20 °C but for all three dextran types, falls of ∼90% in complex viscosity at the two temperatures were observed at the highest glucose concentrations.

Ballerstadt and Ehwald [8], Ehwald et al. [9] and Beyer et al. [10] have all shown that increasing glucose concentration lowered the viscosity of their con A–dextran mixtures. Ballerstadt's glucose-specific viscosity changes were measured with mechanical viscometric devices involving glass capillaries. Further work by Ballerstadt and Schultz [11] used surface plasmon resonance with a 1% con A–5% D2M probe. The change in resonance angle reported the sugar-induced dissociation reactions in the sol network because of the lamellar separation of the sol layer from the sensing region.

The competitive occupation of con A receptors by glucose and dextran allows the mixture to exhibit cyclical viscosity and related permeability changes as glucose concentrations are altered and this makes it suitable for governing closed loop insulin delivery. For such a responsive drug delivery system to operate effectively for the management of diabetes, changes in viscosity need to be in the clinically useful glucose range and need to occur in a physiologically relevant timescale. Fig. 3 represents the well-known sigmoidal relationship between tissue glucose concentration and beta cell release of insulin (solid line). This sliding scale physiological sensitivity has been argued to result from either differential beta cell sensitivity or cellular insulin granule content [12]. Under normal conditions the secretion of insulin must be quite low until, post-prandially it is required to control glucose resulting from absorbed food by raising secretion appropriately.

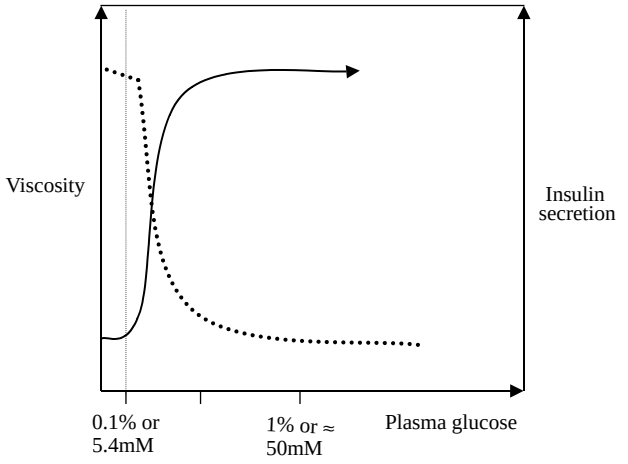

Fig. 3. Profile of viscosity and insulin secretion as a function of plasma concentration.

Although post-prandial secretion involves a gradual ramping, the normal pancreas is capable of high output if glucose should happen to reach abnormal levels. The normal pancreas is also capable of reducing output if hypoglycaemia is a risk for any reason (although counter regulatory systems also contribute to restoring normality). The mixtures here should show rheological characteristics that would govern a delivery device similarly, as is indicated by the dashed line in Fig. 3.

At 37 °C it can be seen that a potentially useful profile was achieved by the D500 mixture (Fig. 2b). This showed little change at 0.1% (w/w) glucose (complex viscosity drop of ∼1%) and then a 92% drop in complex viscosity at 1% (w/w) glucose. For the D2M mixture at 0.1% (w/w) glucose, there was a 15% drop in complex viscosity that progressed further to 88% at 1% glucose. Despite the fall in initial complex viscosity for the D2M mixture, the value at 0.1% (w/w) glucose was higher than the D500 mixture with no glucose.

The structuring of the high viscosity mixture at a glucose concentration of 5.4°mmol (0.1%, w/w) is still significant, on the evidence of our complex viscosity and tan delta results for both temperatures. For example, at 20 °C, the increase in tan delta is 6% and 1.5% for D500 and D2M, respectively, while the analogous figures for 37 °C are 2 and 13%. The tan delta increases for 0.5 and 1% (w/w) glucose are about an order greater, although not large in absolute terms. The complex viscosity data also show that only a small change has occurred in the presence of 0.1% (w/w) glucose. In a drug delivery device this would allow a basal (minimal) delivery of insulin through the high viscosity mixture when it equilibrates with normal glucose levels in fluids with which it is in contact in insulin delivery experiments. Beyer's discussion [10] of similar mixtures but containing 100 mmol (∼1.8%) glucose, provides yield stress evidence that networking can still persist in such mixtures at low stresses. He argued that although this glucose concentration should seriously weaken the con A–dextran network, low stress values probably do not disrupt the juxtaposition of relevant moieties during the cycle of interaction between the receptor, dextran and glucose even when the concentration of the latter is quite high. In the results reported here, the gradual rise in tan delta as glucose was added, for example, also appears to support the theory of progressive weakening by displaced dextran. In addition, on the basis that dextran has interactive terminal glucose moieties on branches that occur about 1 in every 20 linear glucose units, the actual con A receptor valency of dextran is high compared to the figure calculated in terms of the linear terminal glucose unit only. Thus a 10% (w/w) (100 g/l) solution of D2M, is nominally 5×10^{-2} mmol but possibly about 25 mmol (500 branches each with a suitably configured glucose unit) in terms of lectin interaction, assuming no steric hindrance. This outweighs a 0.1% glucose solution (about 5 mmol) by a factor of 5. Thus it is not surprising that the response between 0 and 1% (w/w) glucose is a graded one in terms of viscosity, as found in our work.

Table 1
Formulation of mixtures

Mixture	D2M (w/w)	con A (w/w)
A	5.1	4.9
B	5.2	10.6
C	5.0	16.7
D	11.4	10.4

The drop in complex viscosity beyond 0.1% (w/w) glucose, allows the insulin diffusion coefficient to rise such that a boost dose results, as has been shown by Tanna [5,6]. The glucose concentration at which this occurs should provoke a modest increase in the insulin release up to about 0.2% (w/w) glucose and a steeper increase thereafter if the delivery system is to work as a useful mimic for normal pancreatic control. The almost linear decrease in complex viscosity seen in some of these results may not rule out the optimum profile described (see Fig. 3) especially for large molecules. Insulin diffusion may not increase significantly until the sol state is well established and cross-link lattice larger than the diffusant. The increase in tan delta above the 0.1% (w/w) glucose is evidence that a change of this kind could be occurring. In any case, the major change to a low viscosity sol state for the mixtures described here occurs over 0.1–0.5% (w/w) glucose range that is critical for diabetes control.

Mixtures based on one of the dextran types (D2M) were selected for further investigation and four different mixtures (A–D) were prepared at different con A and dextran concentrations (Table 1). The comparisons from stress sweeps in these experiments were made at 1 Hz and 20 Pa which was within the LVE range. Fig. 4 shows there is a rise in complex viscosity in the mixture subset A–C. These had a

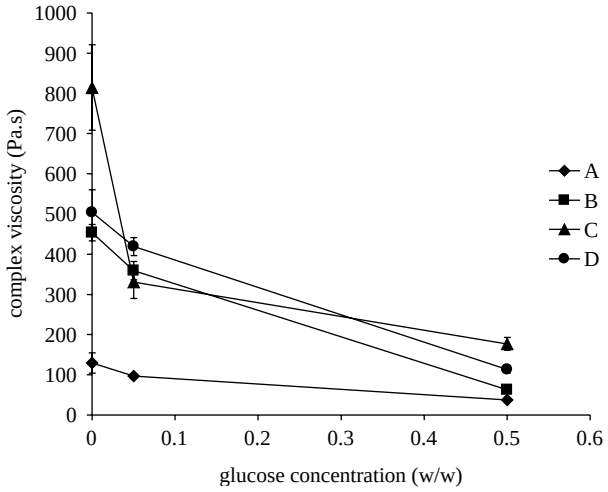

Fig. 4. Complex viscosity at different glucose concentrations for four different dextran–con A mixtures from stress sweeps conducted at 1 Hz, 20 Pa and at 20 °C. (Data represent mean ± SD of three measurements.)

constant dextran content (5.1%, w/w ± 0.1%) but an increasing con A concentration (4.9–16.7%, w/w). Given the molar ratio explanation above regarding the excess dextran ligands, this result was predictable because the increased con A contributes more bridging between dextran chains and leads to a tighter three dimensional structure. Mixtures B and D, on the other hand, are fairly similar in complex viscosity values despite the raised dextran content.

Fig. 4 also shows the complex viscosity changes at 20 °C and measured at 1 Hz and 20 Pa for mixtures A–D when challenged with increasing glucose levels. For all four mixtures there was a typical decrease in viscosity as glucose concentration increased. Mixture A with the least con A, had the lowest complex viscosity and its viscosity did not change much after 0.05% glucose, suggesting limited clinical usefulness. Mixture B, which had an approximately 2:1 ratio of con A to D2M, showed a small decrease in complex viscosity when challenged with a 0.05% glucose but a much larger fall in complex viscosity was observed at 0.5% (w/w) glucose. Mixture D, with higher dextran content, showed a similar profile to B. Such a trend in complex viscosity, if reproduced at 37 °C, could be useful because only modest changes occur at near normal levels of glucose, becoming much greater where an insulin response must rise to deal with more abnormal glucose levels as proposed in Fig. 3. Mixture C, which had a >3:1 con A to dextran ratio, had a much higher initial complex viscosity value and was very responsive to even a small glucose challenge at 20°C, although at 0.05% (w/w) glucose its complex viscosity was similar to mixtures that contained less con A. The tighter three-dimensional structure in this particular system might, however, be detrimental in three ways if used in a delivery system. First, it may slow the entry of glucose and make the response slow; second, it may restrict insulin transport for practical basal delivery rates. Third, a sharp rheological change in the peri-normal region might lead to inappropriately large burst doses of insulin. The latter, while open to being engineered to mimic the normal physiological pancreatic primary surge, must not be capable of delivering even temporary overdose, especially in the peri-normal range. These factors are being investigated for practical importance and a product should be achievable that combines appropriate rheological change with optimal solute transmission characteristics. In fact the intention is to use covalently cross-linked or polymerised versions of these gels and similar cautions apply to their design.

Fig. 5 shows an increase in the tan delta values with glucose content for all four mixtures at 20 °C. The tan delta values for all mixtures are above 1 and therefore under the conditions of measurement, these materials have more viscous than elastic behaviour. The mixtures (A–C) containing dextran (5 ± 0.1%) with a variable con A concentration all show a graded response to glucose. The profiles are all very similar but the magnitude of the profile is a function of the con A concentration at 20 °C. Tan delta

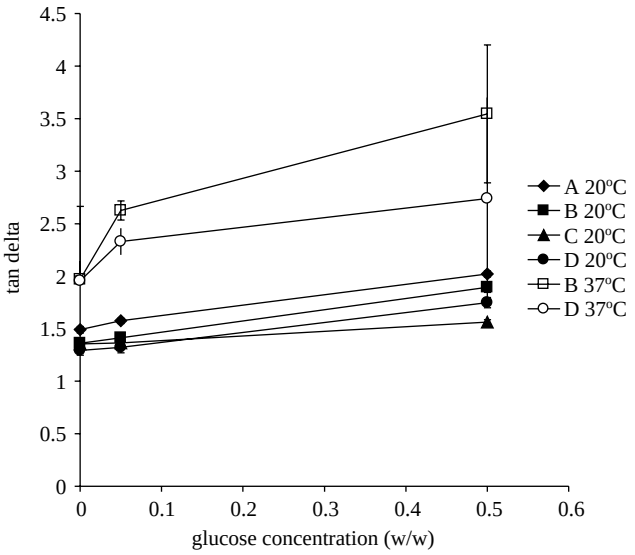

Fig. 5. Tan delta at different glucose concentrations for the four mixtures from stress sweeps conducted at 1 Hz, 20 Pa at 20 and 37 °C. (Data represent mean ± SD of three measurements.)

values at 37 °C are also shown on this graph. Only mixtures B and D were within the LVE range at 20 Pa when tested at 37 °C, the other mixtures showing no clear viscoelastic linearity even at very low stresses, suggesting lower and upper limits of the lectin concentration for effective gel formation. Again, the mixtures were more fluid than at 20 °C particularly when containing glucose, as indicated also in the analogous complex viscosities profiles for mixtures B and D (Fig. 6).

The viscoelastic properties of the mixtures are interesting. Beyer et al. [10] showed that when D2M–con A mixtures were pre-sheared at high stress prior to

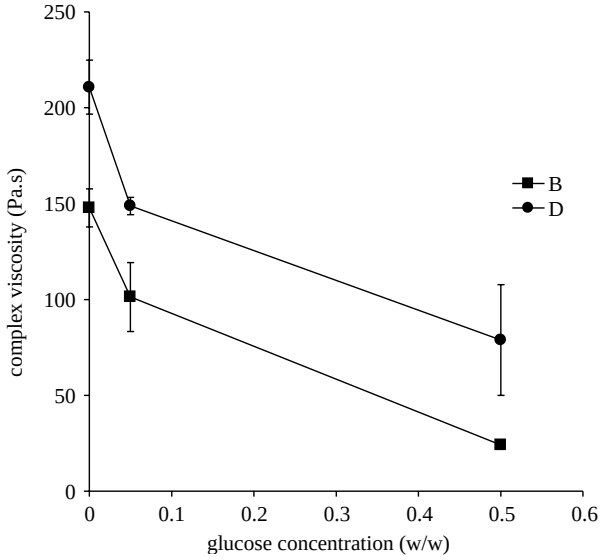

Fig. 6. Complex viscosity at different glucose concentrations for two dextran–con A mixtures from stress sweeps conducted at 1 Hz, 20 Pa and at 37 °C. (Data represent mean ± SD of three measurements.)

Table 2
Frequencies at crossover for mixtures B and D at 37 °C from frequency sweeps (0–10 Hz) at a stress of 20 Pa at different glucose concentrations

Mixture	No glucose	0.05% (w/w)	0.5% (w/w)
B	4	7	10
D	5	5	9

viscometric testing they then exhibited sharp increases in viscosity at subsequent low measuring stresses both in the presence and absence of glucose. This post-stress thickening was not dilatance, which is an increase in viscosity with an increasing shear rate and in fact their material was reported to be shear thinning. It was claimed that this pre treatment produced more reproducible and stable results and might be attributable to metastable networking that could occur when recoverable aggregates of structure are disturbed by a period of shearing. Their evidence for this was that G' become much more significant (though not dominant) in frequency sweeps only after this kind of shearing. In the present work, where mixtures were each tested immediately after being strongly stirred (and observed to thicken), similar conditions have probably been created for the low stress, low frequency oscillatory measurements collected for the systems investigated in this section, such that reproducibility was promoted. We also examined our systems for evidence of elastic behaviour. Table 2 shows the G'' to G' crossovers from frequency sweeps (0–10 Hz) at 20 Pa for mixtures B and D at 37 °C with varying glucose content. For structured materials, the lower the frequency at which the elastic modulus becomes dominant, the more cross linked the gel, in this case with the temporary links formed as dextran interacts with the lectin receptors in a gel where the interactions have been optimised, possibly augmented by the coiling of dextran. For mixture B the shift in frequency as the crossover point was from 4 Hz with 0% glucose to 7 Hz when 0.05% glucose was present and further movement to 10 Hz when the glucose concentration was raised to 0.5%. For mixture D, which has the same con A content but double the dextran concentration, the frequency at crossover in the absence of glucose was 5 Hz and this did not change at a glucose concentration of 0.05%. However, when the glucose concentration was raised to 0.5% there was a shift in frequency at crossover to 9 Hz. These results support the networks proposed by Beyer [10] and appear to imply that a high dextran content confers a greater resistance to their dismantling with glucose. At 20 °C, where the gels are more viscous, the crossover was always at around 2 Hz, i.e. at a frequency just above the observation point of 1 Hz for the systems A–D described above. The shear values used in this part of the study are high by comparison to those used by Beyer, but so also is the con A content and thus it is not surprising that networking is obvious in the gels we tested. For low shear conditions as in delivery devices where the gel works as an undisturbed gateway layer, the selection of lower con A mixtures may be advantageous in this regard, since extensive networking may mitigate against the increase in insulin flux at high glucose levels. We intend to compare the viscoelasticity profile of these simple mixtures with those of covalently modified gels to ensure the design of the covalent version is optimised in this respect.

4. Conclusions

It can be seen from these results that mixtures of dextran and con A produce materials which are glucose sensitive and which would be beneficial as part of a self regulated drug delivery device.

The viscoelastic systems studied exhibited an underlying trend characterised by an increase in tan delta, which was always above one, and a fall in complex viscosity with increasing glucose concentration. This concurred with the subjective assessment of a soft semi-solid to liquid change as the structural changes occurred during which glucose competes for the receptors in the lectin that are occupied by dextran in the original formulations. The results tend to support the persistence of networking at low shear values even in the presence of glucose, as suggested by others.

A wide range of gel consistencies is achievable by combination with dextrans of different molecular weights and the inclusion of con A at suitable ratios. This affects the magnitude of response and the profile of change as related to glucose concentration, reflecting the stoichiometric basis upon which the systems depend.

Acknowledgements

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