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UV Cross-Linked Dextran Methacrylate—Concanavalin A Methacrylamide Gel Materials for Self-Regulated Insulin Delivery

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In this study, the successful acrylic derivatization of dextran and concanavalin A (con A) to form dextran methacrylate and con A methacrylamide is shown. These derivatized acrylic monomers are then photopolymerized in the presence of a water soluble photoinitiator Irgacure® under various conditions to form covalently bonded glucose-responsive gel materials, which undergo a transformation to sol in the presence of free glucose. Rheological data have revealed that as the degree of substitution for dextran methacrylate is increased, a more elastic material is produced due to the increased covalent linkages. Some of these gel systems show negligible component loss in in vitro diffusion experiments used to simulate the behavior of the cross-linked gel, as would be used in a self-regulated insulin delivery device.

Keywords dextran methacrylate; concanavalin A; closed loop; insulin delivery; UV-polymerization; rheology

INTRODUCTION

A self-regulating (closed-loop) insulin delivery system, where insulin can be delivered automatically to match blood glucose levels, would be important in the treatment of diabetes (Adams, Clark, Sahota, Tanna, & Taylor, 2000; Taylor, Clark, Tanna, & Sahota, 2003; Taylor, Tanna, & Sahota, 2004).

Insulin activity in diabetics needs to be managed carefully because hypo- or hyperglycemic events lead to acute and chronic effects of the disease (Brange & Volund, 1999; Graves & Eisenberth, 1999). Current insulin treatment for diabetics is delivered subcutaneously, usually by several subcutaneous bolus doses of insulin per day or by continuous infusion with a pump. The plasma drug concentration in a diabetic at a particular time is less dependent on compliance with the prescribed routine than for many medicines because of the unpredictability of absorption of insulin and the uptake and use of glucose. Many diabetics are chronically poorly controlled. It would be clinically useful, therefore, to have responsive systems that would more closely resemble the normal physiological process in which the amount of insulin released can be affected according to the physiological needs (Kost & Langer, 2001; Taylor et al., 2003, 2004).

In previous work, we have described a basis for a self-regulating insulin delivery device that could be implanted in the intraperitoneal cavity (Tanna & Taylor, 1995; Tanna, Taylor, & Adams, 1999; Taylor, Tanna, Taylor, & Adams, 1995). This delivery system is a reservoir device in which the rate control resides in a membrane comprising a glucose-sensitive gel material. The material is, in the simplest system, a mixture of the lectin concanavalin A (con A) and a polysaccharide bearing terminal glucose units (often dextran). This forms a molecular complex in which the polysaccharide acts as a temporary cross-linker for the lectin, which has receptors specific for glucose. The result is a material with a characteristic viscoelastic character dependent on content. Addition of free glucose displaces the polysaccharide reversibly from the con A receptor sites, dismantling the connections, and producing a sol. The rheology of the material is dependent on the glucose content and has been described previously (Beyer, Ehwald, & Fleischer, 1997). Variations are documented in which the polysaccharide has been replaced by semisynthetics such as polysucrose and glucose-substituted acrylics (Valuev, Chupov, Stayton, & Plate, 1997). These materials have been used in sensor designs (Ballerstadt & Ehwald, 1994; Ehwald, Ballerstadt, & Dautzenberg, 1996) and as rate-determining membranes and matrices in potential drug delivery devices (Kim & Park, 2001c; Obaidat & Park, 1996; Taylor et al., 1995). In delivery systems, the drug leaves the device at a rate that is dependent on the receptor-polysaccharide linked status of the viscous layer and, thus, on the glucose concentration (Taylor et al., 1995).

The glucose-triggered sol stage is vulnerable to component loss, and various strategies have been devised to deal with that.

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These include examples where the lectin and (poly) saccharides are bonded to each other (Kim & Park, 2001a; Tanna et al., 1999), as in a general case for antigens and antibodies described by Miyata and colleagues (1999, 2002), or where the components are each bonded to carriers such as Carbopol® (Tanna, Sahota, Clark, & Taylor, 2002). The aim is to prevent escape of components, since toxicity or loss of interactivity may ensue. This strategy of covalent bonding of both con A and the polysaccharide was used in vivo such that devices containing a Schiff's base stabilized gel material were shown to maintain the blood glucose of diabetic rats within a normal range (Taylor et al., 2003, 2004). Pegylated components (Kim & Park, 2001b) and imprinting methods of producing artificial lectins, similar to those described for use with glycosylated insulins, have also been described with a possible application to this system (Li, Lee, & Park, 1998; Seong, Lee, & Park, 2002).

In this study we describe other polymeric conjugates that involve the synthesis and photopolymerization of acrylic derivatives of dextran and con A. It has been shown by others that gelatin (Van Den Bulcke et al., 2000) and dextran (Kim & Chu, 2000; Van Dijk-Wolthius et al., 1995) derivatized with acrylic ligands can be UV-polymeriized to produce thin films and appear attractive candidates for drug release due to their tissue biocompatibility. In this study, similar synthetic procedures will be utilized, and gel materials resulting from the derivatization of acrylic groups on dextran and con A that can be cross-linked in the presence of UV light to produce glucose-responsive gel materials, as shown in Schematic 1, will be assessed. The dextrans in this work were methacrylated to various degrees of substitution (DS), varying irradiation times, and photoinitiator concentrations in order to vary the complexity of the polymeric gel materials produced, such that the product could be optimized by reaching a compromise between retention of glucose-sensitive rheology and prevention of component loss. It is important that these prototype gel materials retain their glucose-sensitive viscous component and not be so highly cross-linked to prevent the relevant degree of relaxation and impede insulin diffusion.

MATERIALS AND METHODS

Materials

Dextran (produced by *Leuconostoc mesenteroides*, strain No. B512; average relative molecular weight 65,000 [D70]), con A (type V), methacrylic anhydride, dimethylaminopyridine, 1,4-dioxane, dimethyl sulfoxide (DMSO), and boric acid were all purchased from Sigma-Aldrich Chemical Company Ltd. (Poole, Dorset, UK). Nα-acetyl lysine was obtained from Nova Biochem (Switzerland) and fluorescamine from Lancaster Chemicals (Lancaster, UK). The radical photo initiator 1-(4-[2-hydroxyethoxy]-phenyl)-2-hydroxy-2-methyl-1-propane-1-one (Irgacure® 2959) was a generous gift from Ciba Speciality Chemicals (Cheshire, UK). Dialysis membranes (molecular weight cut off [MWCO] 12,000–14,000 Da) were obtained from Medicell International Ltd. (Liverpool, UK). Double-distilled water was used throughout.

Methacrylation of Con A

Methacrylic anhydride (0.1 ml, 0.65 mmol) and con A (1 g) were dissolved in phosphate buffered saline (pH 7.4) and stirred at 50°C for two hours in a nitrogen atmosphere. The solution was then diluted with distilled water and dialyzed against distilled water for three days at 4°C, after which it was lyophilized to yield a white solid of con A methacrylamide (con A-MA) and was stored at 4°C.

Determination of Free Lysine Residues

The number of free lysines of con A-MA was determined by the fluorescamine method (De Bernardo et al., 1974; Felix, Toome, De Bernardo, & Weigele, 1975). Briefly, boric acid buffer (1 ml, pH 9.5), fluorescamine solution (0.5 ml, 0.8 mg/ml made in 1,4-dioxane), and the sample solution (0.5 ml) were rapidly agitated. The absorbance at 390 nm was then measured for the fluorescamine-labeled protein.

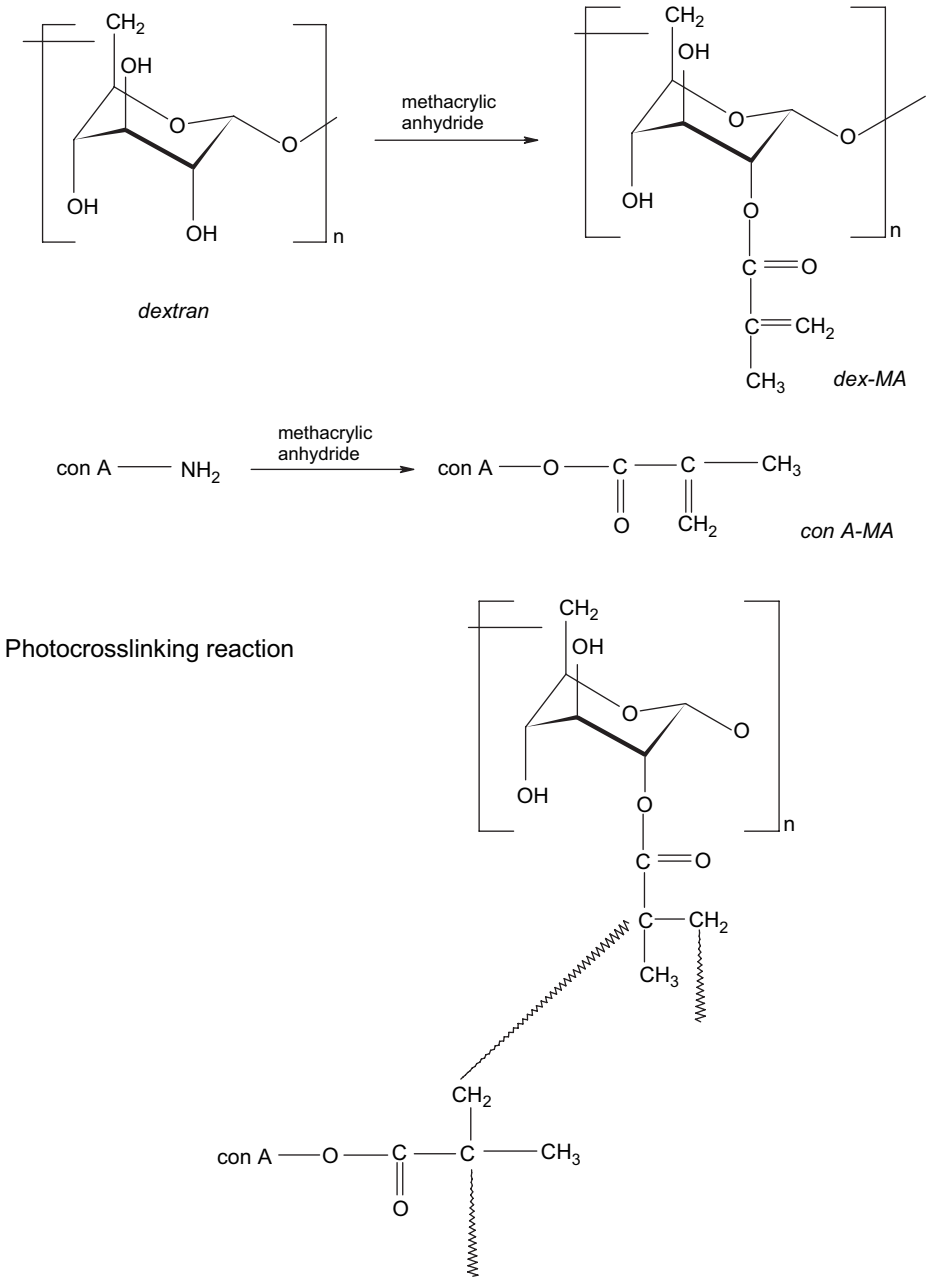
This method, based on the comparison of extinction coefficients between Nα-acetyl lysine and the protein provides an experimental value for the number of lysine residues present. The con A protein sequence shows that there are 12 lysine residues present per monomer (Hardman and Ainsworth 1972). However at pH 9.5, con A exists as a tetramer and would have 48 lysine residues. Comparison of extinction coefficients for con A and con A-MA reveals the extent of coupling. All con A-MA batches synthesized contain ≈ 55% to 60% substituted amine groups.

Methacrylation of Dextran (DS 30%)

The following method for preparing dextran methacrylate (dex-MA) was based on a theoretical DS of 30%.

Dimethylaminopyridine (0.2 g, 1.64 mmol) and methacrylic anhydride (2.77 ml, 18 mmol) were added to a solution of dextran (10 g) which had been dissolved in DMSO (100 ml). The reaction mixture was stirred at 50°C for 24 hours under nitrogen atmosphere, after which it was precipitated in a 1:1 mixture of methanol:acetone. The reaction product was dialyzed against distilled water for six days and then freeze-dried, which resulted in a white solidified preparation of dex-MA.

Dex-MA was analyzed by ^{1}H NMR (Chu, Wu, & Lin, 2001). The integrated peaks at δ 5.6 and 6.1 ppm are attributed to CH_2=C and at δ 1.8 ppm to methyl groups of the methacrylic anhydride moieties. The integrated signals of δ 4.9 ppm and between δ 3.25 and 3.82 ppm were assigned to anomeric and remaining protons of dextran molecules. Accordingly, the DS of each dex-MA calculated as $(x/3y) \times 100$ where x and y are the integrated areas of proton peaks at δ 1.8 and 4.9 ppm. The actual degree of modification for the above synthesis was 26%.



Schematic 1. Photopolymerization of dex-MA and con A-MA.

Dex-MA having lower degrees of substitution of 2, 5.8, 7.5, and 11.5 in correspondence to theoretical DS of 3, 7, 10, and 15, respectively, were also prepared using the same method with appropriately decreased amounts of reagents.

Cross-Linking of Acrylic Monomers

Dex-MA—con-MA polymerized mixtures were prepared by free radical polymerization of the acrylic derivatives. For example, con-MA (100 mg) was dissolved in phosphate buffered saline [PBS] (pH 7.4), and Irgacure® (0.356 μmol) was added. Dex-MA (100 mg) was added to the mixture and stirred to form a viscous solution. The solution was covered in foil and allowed to stand for 24 hours, after which the mixture was placed between two glass plates separated by a 60-micron thickness gasket. The mixtures was irradiated under UV light for 50 minutes (365 nm, 10 mJ cm^{-2}) for the chosen irradiation time. Samples were stored at 4°C for at least 24 hours prior to use.

The final concentration of dex-MA and con A-MA in the mixtures was 8.3% w/w, and this was used for all polymerized mixtures.

Rheological Measurements

The rheological testing was conducted on a Physica MCR 300 rheometer (Anton Paar, Germany) with cone and plate geometry (CP 25-1) and Peltier temperature control. Tests were conducted in oscillation mode using controlled stress. For stress sweeps, the range selected for experiments was 0 to 1000 Pa at a frequency of 1 Hz and measurements were conducted at 20°C ± 0.1°C and 37°C ± 0.1°C. For frequency sweeps (0–50 Hz) a stress of 1Pa was selected at 37°C. All tests were done in triplicate using fresh samples for each test. The rheological parameters examined were processed with the dedicated Anton Paar software provided with the rheometer.

In Vitro Diffusion Experiments

For in vitro diffusion experiments, a small experimental cell was used to hold a thin layer of a glucose-responsive gel material (Figure 1). In this arrangement, the glucose-responsive gel material was confined between two cellulose nitrate filter disks (0.2-mm pore size; 13-mm diameter) to form a barrier membrane for a solute reservoir (ml volume), while the other side was exposed to a temperature-controlled, buffered, bulk receptor solution of PBS pH 7.4 (10 ml) to which glucose could be added. The gel thickness (path length through the gel) was dictated by a spacer gasket between the filters and was set at 0.4 mm.

During each diffusion experiment, anhydrous glucose was added to the bulk receptor solution of a test run to produce concentrations of 1.0% (≈55 mM) w/v in the receptor. The output from the reservoir was monitored and compared with a glucose-free control for increase in solute flux in response. To create conditions under which glucose is described as having been removed, the experiment was suspended during replacement with a glucose-free solution matched for temperature before resuming readings.

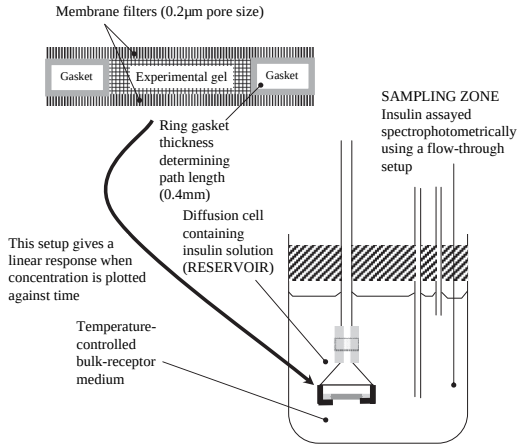


FIGURE 1. In vitro diffusion setup.

Concomitant release of any nonbonded components (derivatized lectin and dextran precursors) from the gel membrane was assessed by use of an identical arrangement with an insulin-free reservoir solution. The leached components were also assayed at 276 nm in addition to the use of these data for assessing component escape from the glucose-sensitive gels. Absorbance at 276 nm versus time plots were plotted for the component-release experiments for the glucose-sensitive gel materials since all of the components and the UV photoinitiator have an absorption maximum of 276 nm and it was therefore not possible to identify the leached components from the in vitro insulin-free diffusion experiments. These experiments were therefore used to indicate the total amount of component leach from the gel material membrane.

FTIR Analysis of Cross-Linked Gels

FTIR spectra were recorded on a Shimadzu FTIR 8300 (Japan). The dry materials were finely ground with KBr powder and pressed into pellets under pressure. For each sample, 20 scans were recorded between 4000 and 500 cm^{-1}, and the dedicated Shimadzu software was used to resolve all peaks from IR absorptions.

After UV irradiation, the polymerized gel was resuspended in distilled water and then lyophilized to produce a white powder, which was then used to produce a disc suitable for FTIR when mixed with KBr.

As shown by others (De Smedt et al., 1995; Pitarressi, Palumbo, Giammona, Casadei, & Moracci, 2003; Stenekes & Hennink, 2000; Zhang et al., 2004), when dextran was derivatized to contain acrylic groups that were then examined by FTIR, they would show the emergence of two new bands: one at 1710 cm^{-1}, attributable to ester bond formations, and one at 813 cm^{-1}, due to the wagging of the vinylidene (C=CH) deformation. Con A-MA did not show the same vinylidene (C=CH) deformation seen with dex-MA in its FTIR spectra, perhaps due to the derivatized lectin having a low DS. A prominent carbonyl peak was observed, which could be attributed to the amide linkages between amino acid residues making up the protein sequence as well as the carbonyl group in the acrylic side chain.

When the acrylic-derivatized dextran (DS 26%) was UV-polymeriized with the similarly acrylic-derivatized con A to produce a cross-linked mixture, FTIR analysis shows that the peak originating from the methacrylate double bond (813 cm^{-1}) disappeared after polymerization.

RESULTS AND DISCUSSION

Rheological Results

All measurements were taken from stress sweeps at 5 Pa, which was within the linear viscoelastic region (LVR) for these materials. Using the rheometer in controlled-stress mode to detect the deflection angle and the phase shift angle (Mezger, 2002), the elastic (storage modulus) and viscous (loss modulus)

components of a viscoelastic material were measured and further parameters such as complex viscosity and tan delta derived.

Figures 2a and 2b show viscosity profiles of five UV cross-linked dextran-con A copolymeric materials differing in the degree of methacrylation of the D70 dextran. The complex viscosity is shown as a function of the concentration of added glucose at 20°C (Figure 2a) and 37°C (Figure 2b). For all gel materials studied at varying glucose concentrations, there was a fall in viscosity as the glucose concentration rose.

In these glucose-sensitive gel materials, dextran has been modified to contain acrylic groups that form covalent bonds with similarly modified con A in the polymerization process, during which both intra- and intermolecular dex-MA bonding must occur in addition to the physical receptor interactions between dextran and con A. Despite the methacrylation of lysine residues on the con A, its receptors still show an affinity for dex-MA in a similar way to that shown by native con A (Tanna & Taylor, 1995; Tanna et al., 1999, 2002; Taylor et al., 1995) and the interaction to form a viscous material persists following polymerization.

In the polymerized materials, the glucose-sensitive loss in complex viscosity was expected to be suppressed by the extent of superimposed polymer bonding. For example, the product formed using dex-MA (DS 26%) was more densely cross-linked than that made with the dex-MA (DS 2%) analogue. The permanent bonds limit relative structural movement, resulting in the effect of the loss of glucose-sensitive contribution.

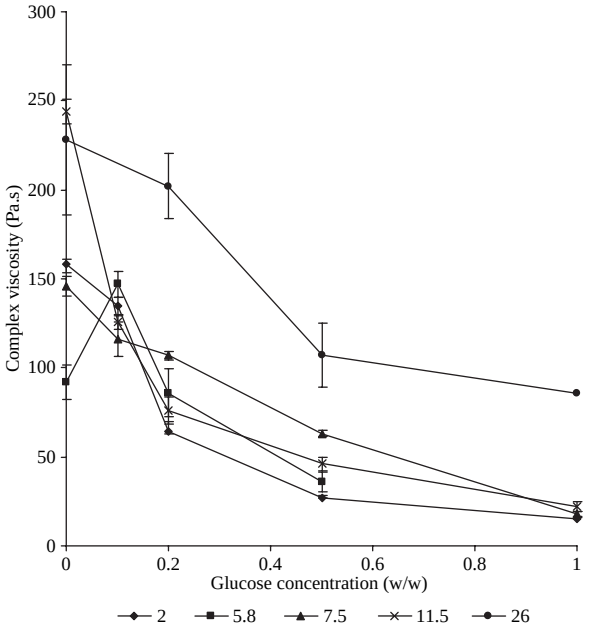


FIGURE 2a. Complex viscosity profiles of UV–cross-linked dex-MA—con A-MA gel materials with dex-MA having a different DS when challenged with varying glucose concentrations at 20°C ($n = 3 \pm SD$).

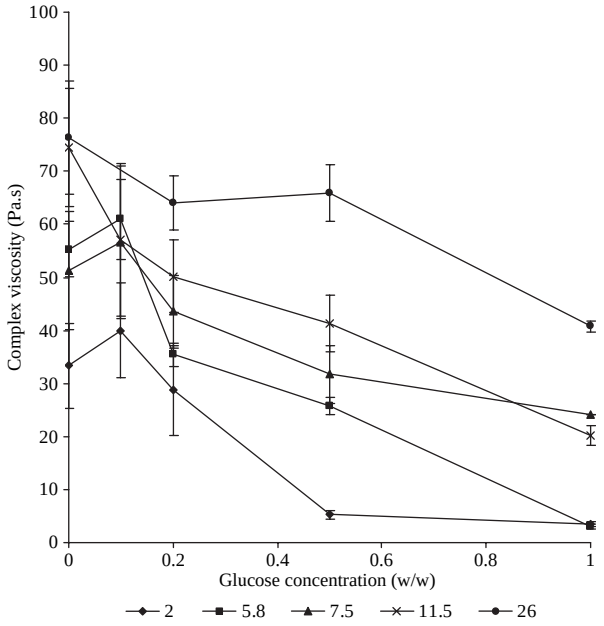


FIGURE 2b. Complex viscosity profiles of UV–cross-linked dex-MA—con A-MA gel materials with dex-MA having a different DS when challenged with varying glucose concentrations at 37°C ($n = 3 \pm SD$).

The more highly substituted material would therefore be predicted to give products that show a viscosity differential less markedly than sparsely cross-linked polymers, even when challenged with a high glucose concentration.

This behavior was evident at the two temperatures (Figures 2a and 2b) for the cross-linked preparations. At a DS of 26%, the viscosity profile across the glucose range was highest, and even after challenging with 1% glucose, its viscosity had only fallen by 62% at 20°C and 47% at 37°C, compared with a gel material having the lowest DS (2%) whose viscosity had fallen by 90% at both temperatures.

Chu and colleagues (2001) have shown that high degrees of dextran substitution with dex-MA hydrogels limit the aqueous mobility of resulting polymer chains. This correlates with our results for DS 26% profiles at both temperatures where, although glucose sensitivity was observed, high viscosity values persist even when triggered with the highest glucose concentration. Chu and colleagues also found that at high polymer concentrations (150 mg/ml), there was an enhanced physical entanglement of polymer chains that reduced swelling capability. Radius of gyration results from small angle X-ray scattering [SAXs] studies for plain aqueous dextran D70 show randomly coiled structures that are progressively more associated as the concentration rises (Hirata et al., 2003), suggesting that at the concentrations used in both our own observations, as well as those of Chu and colleagues, the conditions for substantial reinforcement of tangled structures would exist during polymerization. For the highly substituted dextrans, this fostered intermolecular polymerization would be considerable.

Figures 3a and 3b show tan delta values for the UV cross-linked materials described previously at 20°C and 37°C. At 20°C, tan delta values are above one, except those cross-linked using the highest DS (26%) dex-MA. This indicates that as a series, these materials are high viscosity liquids until the cross-link density reaches a critical value at which they become gels.

For those formulations cross-linked with dex-MA substituted at 5.8% and above, a fall in tan delta was observed with increasing glucose content. Initially, this appears counterintuitive because the glucose concentration caused a visible loss in consistency in these materials, as was reflected in the viscosity plots (Figure 2a and 2b) described above. This apparent tendency to liquefy might be expected to produce a rise in tan delta, but this occurred only with the lowest degree of substitution (2%). A critical dex-MA DS may be required below which liquid-like behavior produces a corresponding increase in tan delta. It may also be that higher dex-MA DS gel mixtures may exhibit similar behavior when challenged with higher glucose concentrations (>1% w/w).

At 37°C the behavior is analogous. For the three highest substitution values, tan delta again falls progressively with increasing glucose. The value for the very fluid DS 2% based formulation does not actually rise at this temperature, but has a much higher tan delta than at 20°C, while the DS 5.8% has an intermediate profile in which glucose reduces tan delta until the highest glucose concentration, where there is a sharp rise. Examination of the profiles for the contributory factors—storage modulus G′ and loss modulus G″—for these glucose-sensitive gel materials reveals an interesting rationale.

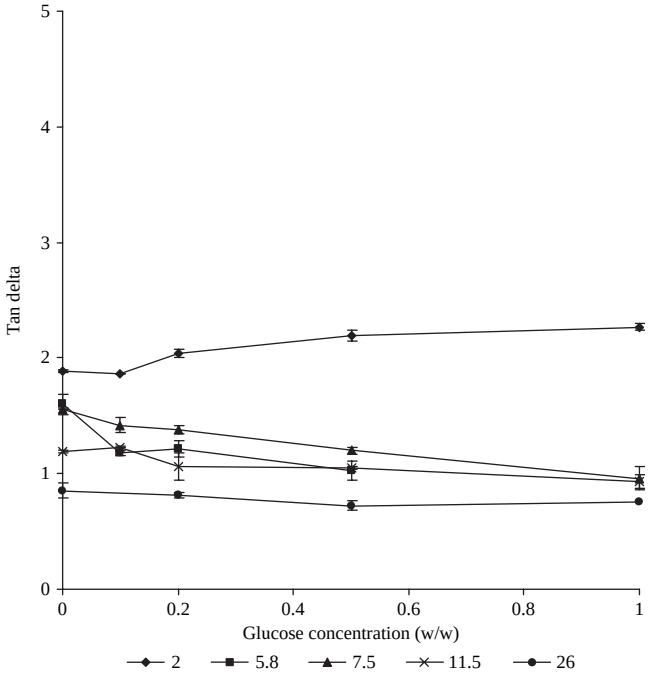


FIGURE 3a. Tan delta profiles of UV–cross-linked dex-MA—con A-MA gel materials with dex-MA having a different DS when challenged with varying glucose concentrations at 20°C ($n = 3 \pm SD$).

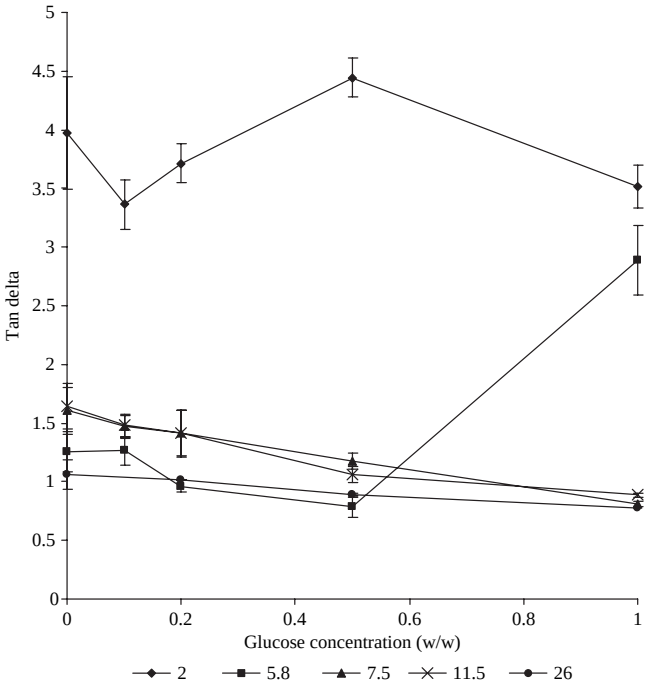


FIGURE 3b. Tan delta profiles of UV–cross-linked dex-MA—con A-MA gel materials with dex-MA having a different DS when challenged with varying glucose concentrations at 37°C ($n = 3 \pm SD$).

Figures 4a, 4b, and 4c show G′ and G″ values for representative cross-linked gel materials with dex-MA substituted at 2%, 7.5%, and 26%, respectively, at 20°C. All modulus values decreased as glucose was added. G″ was larger than G′ for the lowest substitution value (2%) throughout the glucose-concentration range (Figure 4a). Similar behavior has been seen with nonacrylic-modified dextran-con A gel materials formulated using dextran of varying molecular weight (70, 500, and 2000 kDa; Taylor, Tanna, Sahota, & Voermans, 2006). For this formulation at 20°C, although the G″ and G′ profiles converge with increasing glucose, their ratio (G″/G′ = tan delta) becomes larger and reflects an increase in fluidity.

When the dextran substitution was raised to 7.5%, the G′ value increased and the G″ value decreased, each by small margins for the glucose-free gel material in comparison to dex-MA (DS 2%) cross-linked gel material. The small drop in tan delta as the DS is changed from 2% to 7.5% is influenced more by the 12% reduction in G″ than by the 6% rise in G′. Presumably, this reflects a slight loss in viscous adjustment attributable to reduced slippage of long dextran chains and not that this level of bonding by polymerization has imposed three-dimensional solidity.

Figure 4b shows that with the addition of glucose, there was a greater convergence between G″ and G′ profiles than for the

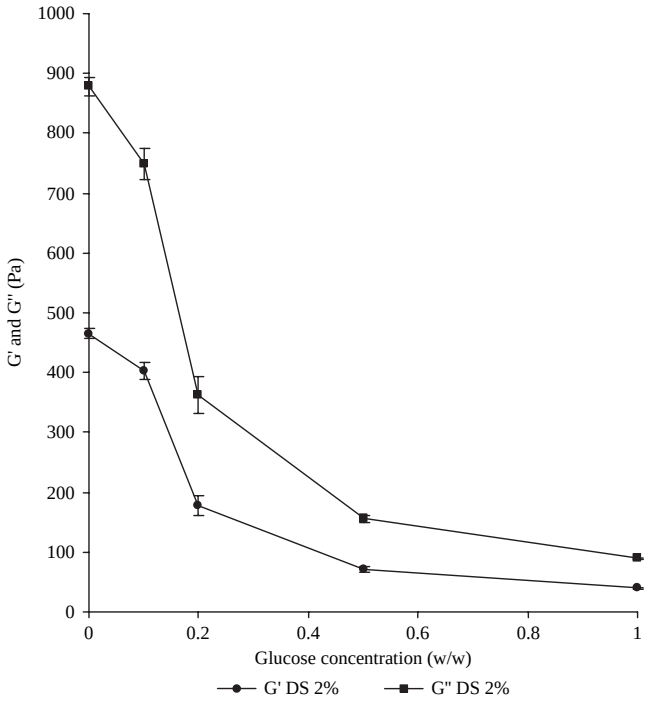


FIGURE 4a. Elastic modulus (G′) and loss modulus (G″) of UV–cross-linked dex-MA (DS 2%)—con A-MA gel material when challenged with varying glucose concentrations (0%–1% w/w) at 20°C ($n = 3 \pm SD$).

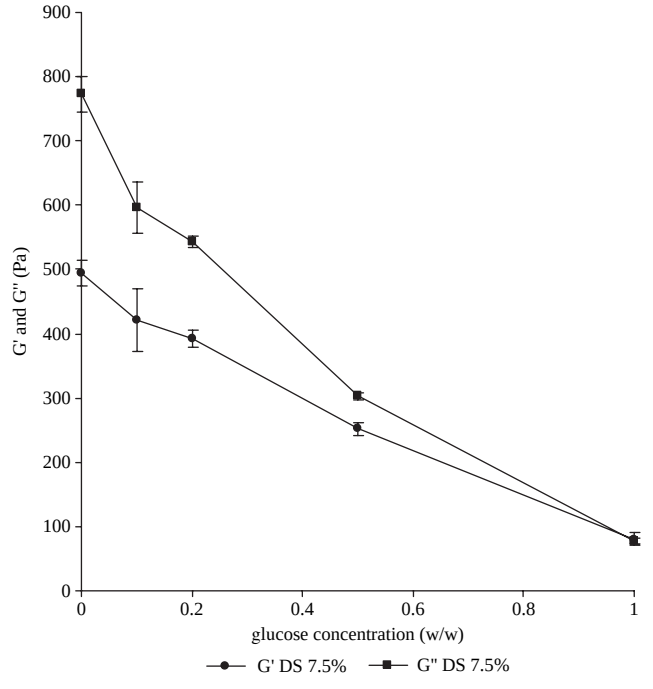


FIGURE 4b. Elastic modulus (G′) and loss modulus (G″) of UV–cross-linked dex-MA (DS 7.5%)—con A-MA gel material when challenged with varying glucose concentrations (0%–1% w/w) at 20°C ($n = 3 \pm SD$).

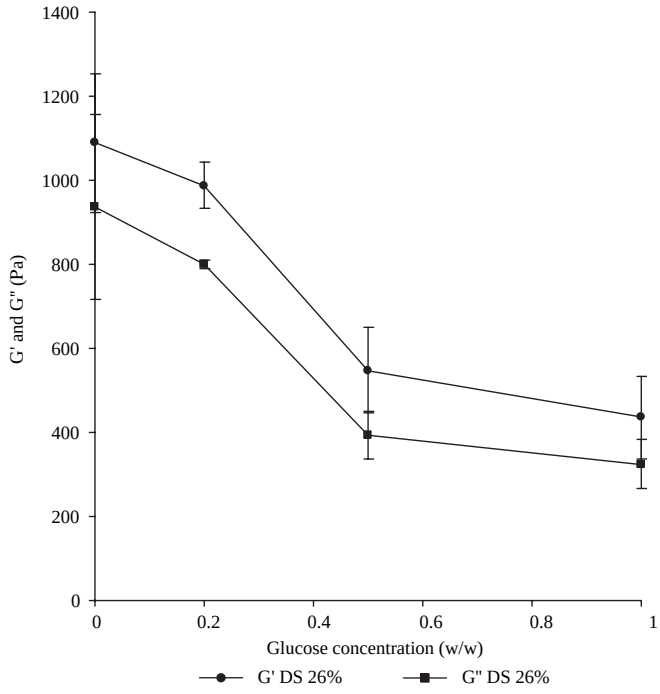


FIGURE 4c. Elastic modulus (G′) and loss modulus (G″) of UV–cross-linked dex-MA (DS 26%)—con A-MA gel material when challenged with varying glucose concentrations (0%–1% w/w) at 20°C ($n = 3 \pm SD$).

polymeric gel materials made with 2% substituted dextran. A crossover of the profiles was observed at 1% glucose content. Elastic behavior was therefore just dominant at this point despite the loss of viscosity and the low values for both moduli.

Figure 4c shows that for the materials made with 26% substituted dextran, the G′ and G″ profiles are higher than for polymers made with the 2% and 7.5% substituted precursors, but as the degree of substitution is further increased, it becomes the G′ dominance that contributes more to the tan delta reduction. As with the other polymeric gel materials, both G′ and G″ fall when glucose is then added, but much less steeply. The plots are in fact almost parallel as they decrease with glucose concentration, although the ratio tan delta, which is always below 1 in this case, is not constant but falls progressively as glucose is added. Similar to the case of the 7.5% dextran material, the material showed predominantly elastic behavior despite the loss of complex viscosity. This differs from the 2% case where G″ is always greater than G′.

Figure 5a shows G′ values from the three cross-linked gel material formulations across the dex-MA DS range of 2%, 7.5%, and 26% at glucose concentrations of 0% and 1% w/w. A′ represents the change between the two G′ values and also the structure produced by the interaction between the lectin receptor sites and dextran. This would be competitively challenged by free glucose, resulting in a loss in elasticity. The remaining change (B′) for the profile challenged with 1%

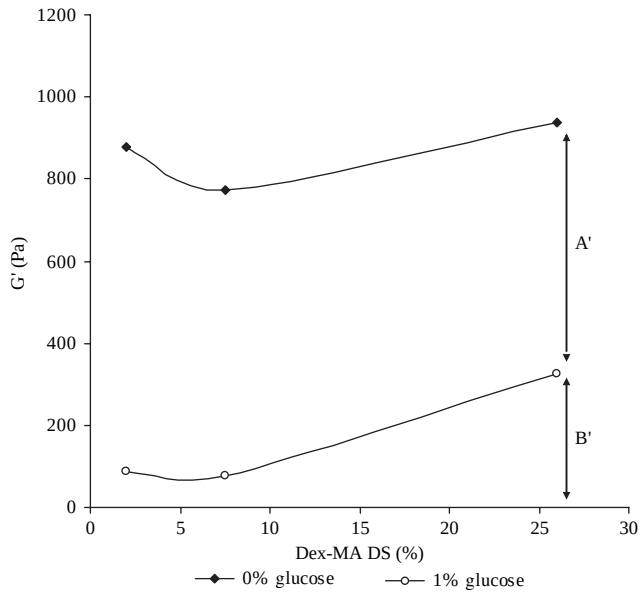


FIGURE 5a. The contributions from each of the bonding types to G′ at 20°C as a function of DS.

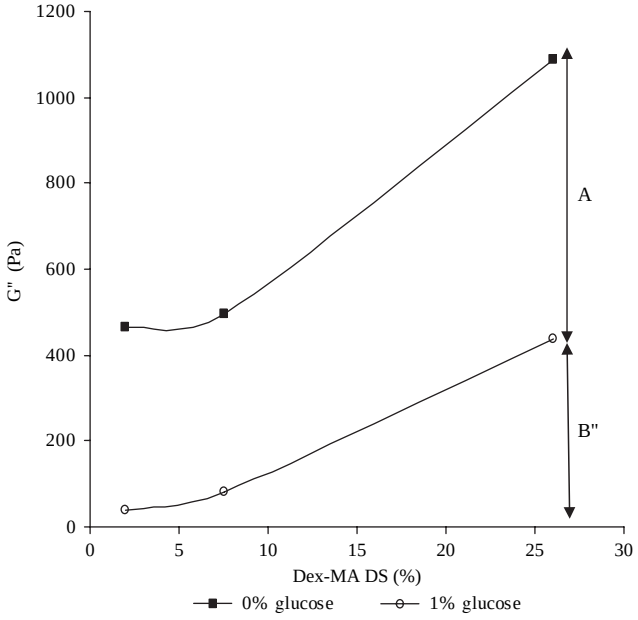


FIGURE 5b. The contributions from each of the bonding types to G″ at 20°C as a function of DS.

glucose can therefore be attributed to the elasticity that remains as a result of the cross-linked gel mixture. As this contribution increases with increasing DS, it can be assumed that elasticity is a representation of the permanent cross-links (i.e., covalent bonds formed), which would increase with increasing DS, as can be seen in Figure 5a.

In the analogous plot for G″ (Figure 5b), that contribution to the glucose free condition does not increase sharply like the G′ value, but does recover from a minimum at DS 7.5%. Again the larger contribution is from the glucose-dependent bonds (A″ on the graph). The viscous component (B″) comes from the cross-links formed by the polymerization and are largest at the higher DS. While cross-links might be expected to confer solid structure, it should be remembered that these products have not progressed to the formation of a rigid hydrogel, but remain capable of flow (being hydrated colloidal particulates). The process of polymerization produces materials of higher viscosity and, presumably, this is a result of the restricted disentanglement of a glucose-sensitive structure. This is reflected in the increased B″ value of G″ for the 26% substituent-based polymer.

Figure 6 shows the glucose-triggered viscosity profiles for dex-MA (DS 7.5%)—con A–MA gels at 20°C and 37°C which had not been irradiated by UV light. Clearly the viscosity was much lower for these gel materials than for the cross-linked versions. At 20°C and 37°C there was a significant loss in viscosity at glucose concentrations between 0% to 0.2%, while further addition of glucose did not produce results in the LVR, indicating that these noncross-linked gel materials seem to have lost all structure and liquefied completely at glucose levels below those at which the polymerized gel materials are still responsive.

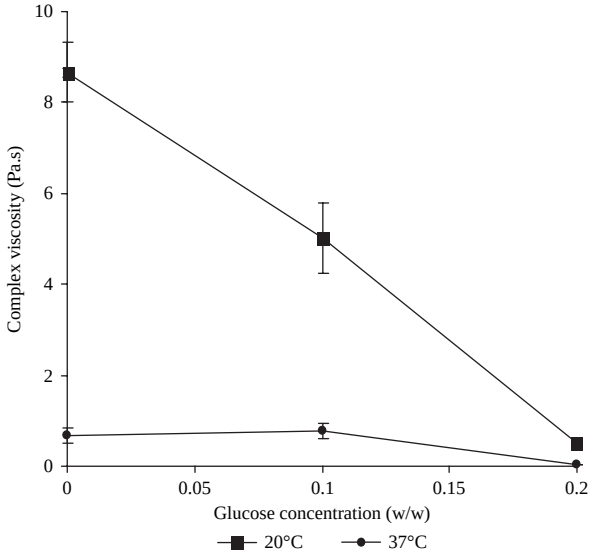


FIGURE 6. Complex viscosity profiles of noncross-linked dex-MA (DS 7.5%)—con A-MA gel materials challenged with different glucose concentrations (0%–0.2%) at 20°C and 37°C ($n = 3 \pm SD$).

In Vitro Diffusion Studies

Previously tested, covalently coupled gel materials showed component loss in the receptor fluid, as monitored at 276 nm in the in vitro diffusion experiments (Taylor et al., 1995; Tanna &

Taylor, 1995; Tanna et al., 1999), and cross-linking to prevent this was the main purpose of polymerizing dextran and con A in this study.

Gels were prepared as for the rheological studies (0.356 μmol) with irradiation times between 40 and 50 minutes. Figure 7 shows that when the dex-MA (DS 7.5%)—con A-MA gel was irradiated for 50 minutes, there was negligible component release when triggered with 1% glucose. As irradiation time was increased, the component loss seen at 276 nm when triggered with a 1% glucose solution (grey-shaded area) became less. A glucose concentration of 1% (≈54 mM) used in these experiments is higher than would be present in blood plasma for routine diabetic patients, but has been selected as an extreme to test the structural stability of the glucose-sensitive gel material.

Figure 8 shows component release profiles (276 nm) from dex-MA—con A-MA gels irradiated between 5 and 20 minutes with twice the Irgacure® levels (0.712 μmol) used previously. These experiments were conducted to assess if gels of similar consistency could be obtained after polymerization in shorter irradiation times that would demonstrate minimal component loss. There was very little component loss for the gel material irradiated for 20 minutes. Kim and Chu (2000) have shown that raising the photoinitiator content produced an increasing concentration of cross-links resulting in a tighter more densely cross-linked network for their dex-MA hydrogels. They described the reduced availability of free hydroxyl groups, which contributed to the hydrophilicity of their hydrogel. The results in Figure 7 show that doubling the photoinitiator level and decreasing the irradiation time to 20 minutes resulted in a gel material which showed negligible component loss similar to the gel material previously irradiated for 50 minutes in Figure 6. Further experiments are currently ongoing to assess which of these candidate formulations would be more suitable for insulin delivery.

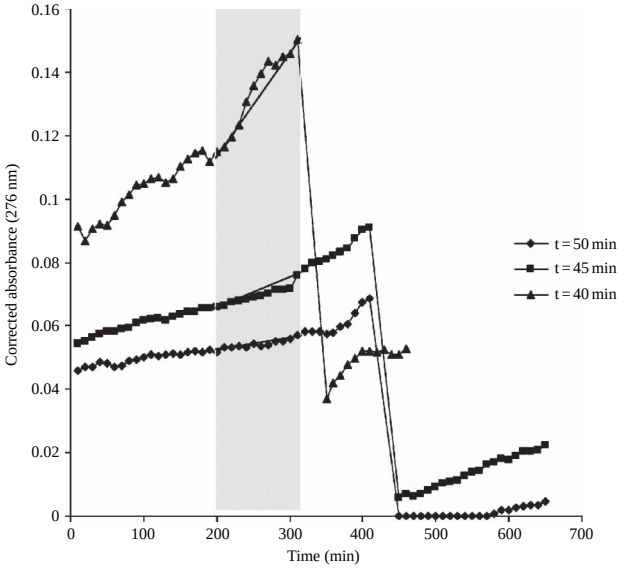


FIGURE 7. Con A-MA leach from dex-MA (DS 7.5%)—con A-MA UV–cross-linked gel materials irradiated for 40, 45, and 50 minutes (Irgacure® 0.356 μmol) from diffusion experiments.

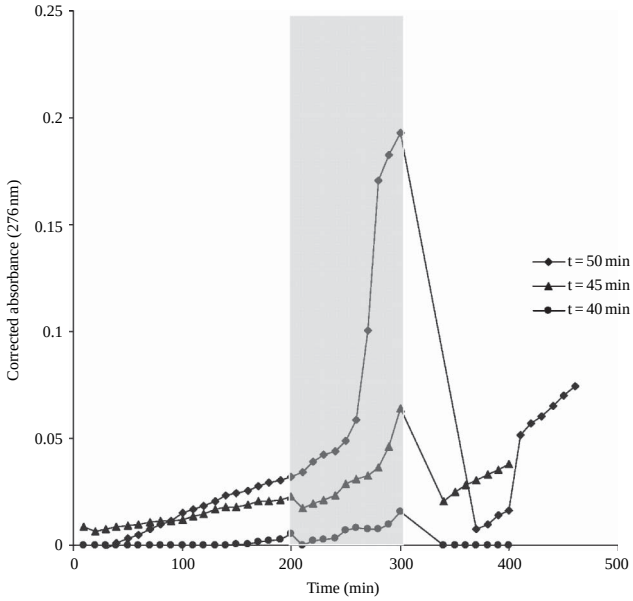


FIGURE 8. Con A-MA leach from dex-MA (DS 7.5%)—con A-MA UV–cross-linked gel materials irradiated for 5, 15, and 20 minutes (Irgacure® 0.712 μmol) from diffusion experiments.

CONCLUSION

Polymerized gel materials have been produced by cross-linking acrylic derivatives of dextran and con A and have been shown to retain the glucose sensitivity of materials made by mixing plain dextran and con A. Rheological studies show that raising the DS by derivatizing more acrylic groups on dextran chains results in correspondingly more densely cross-linked and elastic dex-MA—con A-MA polymerized gel material. They retain flow, however, and are therefore viscoelastic materials, probably comprising large entangled hydrated particulates that retain receptor interaction.

Irrespective of the extent of methacrylation and, therefore, the resulting cross-link density, these viscous materials respond to increasing glucose concentration with a graded decrease in viscosity. However, the more highly cross-linked gel materials showed a decreased tan delta as glucose was added, suggesting that as the glucose-sensitive bonds were disrupted by the presence of glucose, the elasticity conferred by cross-linking became dominant. This differed for the noncross-linked gel materials studied previously (Taylor et al., 2006), in which tan delta increased as glucose concentrations were raised.

Diffusion studies have illustrated that component loss can be controlled when gel materials are polymerized using the methods described here. This is important because con A is potentially toxic to the immune system containing it within the insulin

delivery device is critical to its acceptability in a closed-loop system. In addition, the switch mechanism for which it is responsible would fail to operate if component loss became critical. Failure to operate could also be expected if phase separation occurs, and although both components are miscible with the aqueous base in which they are formulated, such a separation could occur, as has been shown in a variety of aqueous polymeric two-phase systems such as polyethylene glycol and dextran. Thus the polymerization process used here as a stabilization procedure for the gel material is important. Clearly it is equally important that the process does not disable reversible viscosity change mechanism. It is possible that useful glucose-sensitive gel materials might be predicted from the rheology such that a limiting tan delta may be found for the useful polymerization level.

The feasibility of the UV-polymerized dextran—con A acrylic derivatized gel material as the basis for the design of an implantable closed-loop insulin delivery device has been highlighted. Further development work will include the incorporation of the polymerized gel material into a device for in vivo studies and biocompatibility studies.

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