Drug Development and Industrial Pharmacy, 2011, 37(3): 351–358

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI:10.3109/03639045.2010.513983

informa
healthcare

ORIGINAL ARTICLE

Effect of varying molecular weight of dextran on acrylic-derivatized dextran and concanavalin A glucose-responsive materials for closed-loop insulin delivery

Tarsem Sahota, Kirsty Sawicka, Joan Taylor and Sangeeta Tanna

Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester, UK

Abstract

Aim: Dextran methacrylate (dex-MA) and concanavalin A (con A)-methacrylamide were photopolymerized to produce covalently cross-linked glucose-sensitive gels for the basis of an implantable closed-loop insulin delivery device. *Methods*: The viscoelastic properties of these polymerized gels were tested rheologically in the non-destructive oscillatory mode within the linear viscoelastic range at glucose concentrations between 0 and 5% (w/w). *Results*: For each cross-linked gel, as the glucose concentration was raised, a decrease in storage modulus, loss modulus and complex viscosity (compared at 1 Hz) was observed, indicating that these materials were glucose responsive. The higher molecular weight acrylic-derivatized dextrans [degree of substitution (DS) 3 and 8%] produced higher complex viscosities across the glucose concentration range. *Conclusions*: These studies coupled with in vitro diffusion experiments show that dex-MA of 70 kDa and DS (3%) was the optimum mass average molar mass to produce gels that show reduced component leach, glucose responsiveness, and insulin transport useful as part of a self-regulating insulin delivery device.

Key words: Closed loop, concanavalin A, dextran methacrylate, glucose responsive, insulin delivery

Introduction

Diabetes is an underestimated disease affecting millions worldwide including many children suffering the acute Type 1 disease[1–5]. Insulin has been available for many decades but, despite its life-saving effects, its delivery does not mimic the kinetics of healthy pancreatic function, which means that most diabetics can suffer the toxic effects of frequently raised levels of glucose. The associated side effects can be severe and include complications such as blindness, cardiovascular disease, kidney failure, and nerve damage, which can reduce both length and quality of life.

Improvements are possible if the shortcomings of current treatment could be addressed. At present for insulin-treated diabetics, an injection is made at least once daily. The size and frequency of dose is calculated from clinical observations of the patient but inevitably is based on the often unpredictable size, absorption, and timing of the next meal. This protects from the immediate threats of the disease by reducing glucose to an acceptable mean but blood glucose is nevertheless often outside normal limits and thus quite different from the case in the nondiabetic. Other therapies include continuous infusion by means of both external and implantable pumps and these have been credited with improving outcomes[6,7]. Although patients can make these systems work to considerable advantage, they need vigilant commitment.

Pancreatic output of insulin is governed by a negative biochemical feedback ('closed-loop') that operates continuously and automatically, causing appropriate response to losses and gain in glucose levels in normal glucose control[8,9]. An artificial glucose-responsive insulin delivery system that works by a similarly automated

Address for correspondence: Dr. Sangeeta Tanna, Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, Leicester, LE, UK. Tel: +44 116 2078274, Fax: +44 116 2577287. E-mail: stanna@dmu.ac.uk

(Received 29 Sep 2009; accepted 3 Aug 2010)

feedback would therefore have obvious advantages over above-mentioned conventional therapies.

Formulations of the plant lectin, concanavalin A (con A), and dextran have been shown to produce glucose-sensitive gels[10,11]. These gels have been used as part of an in vitro self-regulating drug delivery system based on a polysaccharide displacement mechanism. However, one of the obstacles with the design of these gels has been the leaching of the mitogenic lectin. This has been addressed previously by various covalently bonded prototype designs of two main components of this 'smart' biomaterial[12,13]. However, more recently acrylic derivatives of dextran and con A have been produced, which when photopolymerized produce cross-linked glucose-responsive gels[14,15].

Previously it has been shown that a UV-polymerized gel of dextran methacrylate (dex-MA)-con A-methacrylamide (con A-MA) was able to prevent (or minimize) component leach when tested in in vitro diffusion experiments. This cross-linked gel was also able to deliver insulin and has been examined at glucose concentrations between 0.1% and 1%. In this study UV-polymerized gels were prepared using different molecular weight (MW) precursors of dextran ranging between 11 and 500 kDa.

These covalently cross-linked smart gels will be examined rheologically in the presence of glucose in oscillatory experiments to determine their viscoelastic properties and to gauge glucose sensitivity. In order for these cross-linked polymers to be used as part of a self-regulating insulin delivery device, they must also retain their polymerized monomeric components when challenged with free glucose and must be capable of delivering insulin. Candidate gels were tested in in vitro diffusion experiments both for component retention and for glucose responsiveness in terms of differential delivery of insulin.

Materials and methods

Materials

Dextrans (produced by Leuconostoc mesenteroides) having mass average molar mass: 8500–11,500 (D11), 35,000–45,000 (D40), 65,000 (D70), 100,000–200,000 (D200), and 500,000 (D500), con A (Type VI), methacrylic anhydride, dimethylaminopyridine,1,4-dioxane, dimethylsulfoxide (DMSO), boric acid, bovine pancreas insulin, and Sephadex G25 were all purchased form delta-Aldrich Chemical Company Ltd. (Poole, Dorset, UK). N-α-Acetyl lysine was obtained from Nova Biochem (Geneva, 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 (MWCO 12,000–14,000 Da) were obtained from Medicell International Ltd., Liverpool, UK. All other chemicals were of analytical reagent grade and double-distilled water was used throughout.

Methods

Methacrylation of con A

Methacrylic anhydride (0.65 mmol) and con A (1 g) were dissolved in phosphate buffered saline (PBS) (pH 7.4) and stirred at 50°C for 2 hours under nitrogen atmosphere. The solution was then diluted with distilled water and dialyzed against distilled water for 3 days at 4°C after which it was lyophilized to yield a white powder of con A-MA, which 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[16,17]. Briefly, boric acid buffer (pH 9.5), fluorescamine solution, and the sample solution were rapidly agitated. The emitted fluorescence at 480 nm was then measured for the fluorescamine-labeled protein.

Comparison of extinction coefficients for con A and con A-MA reveals the extent of coupling. All con A-MA batches synthesized contain ~60% substituted amine groups.

Methacrylation of dextran

The following method for preparing dex-MA was based on a theoretical degree of substitution (DS) of 4.5%.

Dimethylaminopyridine (0.273 mmol) and methacrylic anhydride (3 mmol) were added to a solution of dextran (10 g), which had been dissolved in dimethylsulfoxide. The reaction mixture was stirred at 50°C for 24 hours under nitrogen atmosphere after which it was precipitated in acetone. The reaction product was dialyzed against distilled water for 6 days and then freeze-dried, which resulted in a white powder of dex-MA.

Dex-MA was analyzed by ^{1}H NMR (Bruker Avance 400, QMP probe, Coventry, UK). The integrated peaks at δ5.6 and δ6.1 ppm are attributed to CH_2=C and at δ1.8 ppm to methyl groups of the methacrylic anhydride moieties[18]. The integrated signals of δ4.9 ppm and between δ3.25 and δ3.82 ppm were assigned to anomeric and remaining protons of dextran molecules. Accordingly, the DS of each of dex-MA calculated as $(x/3y) \times 100$ where x and y are the integrated areas of proton peaks at δ1.8 and δ4.9 ppm. The actual degree of modification for the above synthesis was 3%.

Dex-MA having a DS of 8% in correspondence to the theoretical DS of 10% was prepared using the same method with appropriately decreased amounts of reagents. This method was repeated for other MW dextrans.

Purification by gel permeation chromatography

After synthesis, both dex-MA and con A-MA were further purified to remove impurities by elution with distilled water through a Sephadex G25 column with a flow rate

of 2 mL/min (BUCHI 688 chromatography pump, BUCHI 686 peak detector, BUCHI 686 fraction collector, and Knauer differential refractometer all supplied by Fisher Scientific, Loughborough, UK). Dex-MA or con A-MA was dissolved in distilled water and injected onto the column. The fractions were then lyophilized to produce white powder.

Cross-linking of acrylic monomers

Dex-MA-con A-MA polymerized gels were prepared by free radical polymerization of the acrylic derivatives. For example, con A-MA (100 mg) was dissolved in PBS (pH 7.4) and Irgacure® (0.178 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-μm thick gasket. The mixture was irradiated under UV light (365 nm, 10 mJ cm^{-2}) for the chosen irradiation time. Samples were stored at 4°C for at least 24 hours before use.

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

Dynamic rheological measurements for polymerized glucose-responsive gels

Dynamic rheological properties of the UV-polymerized gels were evaluated using a Physica MCR501 rheometer (Anton Paar, Graz, Austria) using cone and plate geometry (cone characteristics: 24.915 mm, 1.003° angle, truncation 47 μm, Plate: 63 mm diameter) in oscillatory mode at 37°C. Using a spatula 0.2 g of sample was placed onto the centre of the plate and the cone was lowered to a distance 10% above the measuring gap (47 μm). Excess sample was then removed and the cone was lowered to the measuring position and the test started. Tests were conducted in controlled strain amplitude performed within the linear viscoelastic region (LVR) so that the storage (G′) and loss (G″) moduli were independent of strain. Firstly, a strain sweep between 0.1% and 100% was conducted at a frequency of 1 Hz for a cross-linked gel containing no glucose and also containing 5% (w/w) glucose and a strain value that was within the LVR for both samples was selected for the subsequent frequency sweeps. The strain value selected for all cross-linked gels was 1%. Frequency sweeps at this constant strain were then conducted between 0.01–50 Hz and a solvent trap was used to prevent sample dehydration during the test. All tests were done in triplicate using fresh sample for each test. The rheological parameters examined were processed with the dedicated Physica 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. 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 (1 mL volume), whereas 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[15]. The gel thickness (path length through the gel) was dictated by a spacer gasket between the filters and was set at 0.4 mm.

Before testing in the diffusion experiment each glucose-responsive gel formulation was loaded in the small experimental cell and washed in situ with 1% (w/v) glucose solution for 24 hours followed by PBS (pH 7.4) for 24 hours. This washing procedure was to remove any nonbonded components from the cross-linking procedure. The washings were assayed using reverse-phase HPLC to separate and identify the leached components.

During each diffusion experiment anhydrous glucose was added to the bulk receptor solution of a test run to produce concentrations of either 0.5% (w/v) (~28 mM) or 1.0% (w/v) (~55 mM) 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. Solute delivery was monitored spectrophotometrically for insulin at 276 nm. The insulin-release gradients for both the glucose-triggered test and the nonglucose-triggered control were determined, enabling calculation of the ratio of fluxes from the test and the control. This was termed the flux factor increase (FI value). The FI value is a measure of the effect of glucose on insulin delivery from the glucose-responsive gel.

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 and therefore in addition to the use of these data for assessing component escape from the glucose-sensitive gels, they were used to correct the insulin-release profile. Absorbance at 276 nm versus time plots was plotted for the component release experiments for the glucose-sensitive gels because all of the mixture components and the UV photoinitiator have an absorption maximum at 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. Separation and identification of the leached nonbonded material was carried out using the subsequent reverse-phase HPLC analysis of the bulk receptor solutions.

HPLC analyses of washings and experiment receptor solutions

Chromatographic analysis was performed with a Shimadzu Prominence HPLC system (Milton Keynes, UK) consisting of an in-line degasser, quaternary pump, autosampler, column oven, and diode array detector. A Jupiter 30 nm C_5 column (250 × 4.6 mm) from Phenomenex, Cheshire, UK, was used for the separation preceded by a 0.5-µm in-line filter and a widepore C_5 4 × 3 mm guard column. Mobile phase A consisted of 95:5 10 mM ammonium acetate buffer pH 7.4/acetonitrile and mobile phase B was acetonitrile. Elution was isocratical with 100% A to 1 minute, followed by a gradient over 9 minutes to 90% B. The flow rate was 1.5 mL/min and the gel components and insulin were detected by their absorbance at 215 nm.

Statistical analysis

For rheological experiments values of mean ± SD for each sample were obtained from at least three separate experiments. Statistical analysis of the differences between two mean values was assessed using the Mann–Whitney U test; a value of $P < 0.05$ was considered significant for diffusion experiments.

Results and discussion

Rheology

Dynamic rheological experiments on UV-polymerized gels were performed in the nondestructive oscillation mode of the rheometer to monitor their glucose sensitivity. These experiments were conducted in controlled strain across a varying frequency range within the LVR; the viscoelastic behavior of the gel material being a combination of an elastic and viscous response.

Effect of changing dex-MA MW

Figures 1 and 2 show the storage and loss modulus (G′ and G″) of gels cross-linked for 50 minutes using D40-MA, D70-MA, D200-MA, and D500-MA with DS of 3%. The storage modulus is defined as a measure of the deformation energy stored in the sample during the shear process[19]. After the load is removed, this energy is completely available and acts as the driving force for the elastic recovery of the deformed matrix. It can be seen that as the glucose concentration was increased G′ becomes lower, implying that less energy was available for structural recovery of the deformed matrix. The addition of glucose dismantles the gel network by competitively displacing dextran in the con A-MA receptor sites.

The loss modulus is a measure of the deformation energy used in the sample during the shear process and lost to the sample afterward[19]. This energy is either used up during the process of changing the sample's structure or dissipated to the surrounding environment in the form of heat. Therefore, a decreasing G″ as glucose concentration was increased shows that more energy was lost and viscous flow was promoted [20].

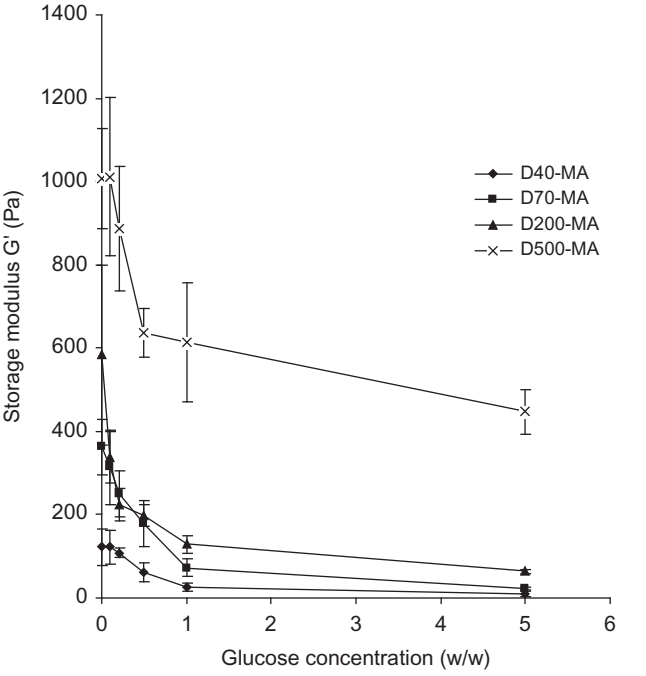

Figure 1. Storage modulus profiles for D40-MA, D70-MA, D200-MA, and D500-MA (DS 3%)-con A-MA gels irradiated for 50 minutes at different glucose concentrations at 37°C. Data were taken at a frequency of 1 Hz from frequency sweeps conducted in the range 0.01–50 Hz.

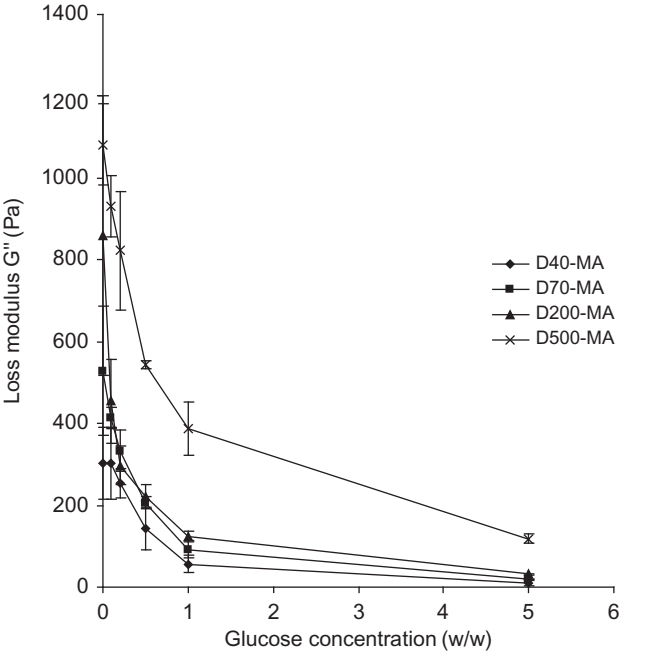

Figure 2. Loss modulus profiles for D40-MA, D70-MA, D200-MA, and D500-MA (DS 3%)-con A-MA gels irradiated for 50 minutes at different glucose concentrations at 37°C. Data were taken at a frequency of 1 Hz from frequency sweeps conducted in the range 0.01–50 Hz.

As glucose concentration increased there was a fall in complex viscosity for these cross-linked gels (Figure 3) suggesting that despite permanent covalent linkages being formed by the UV polymerization of acrylic-derivatized con A and dextran there was still a dismantling of the three-dimensional network. This is evidence that after covalent stabilization the lectin receptor sites have retained their capacity to interact reversibly with glucose. It is also apparent from Figures 1–3 that as the glucose concentration was raised above 1–5% (w/w) the decrease in the viscoelastic parameters G′ and G″ and complex viscosity did not reduce as steeply as that seen between glucose concentrations of 0.1–1% (w/w). This suggests that temporary cross-links between the glucose units in dextran and the con A-MA receptor sites may have been dismantled and that remaining viscoelastic contribution can be attributed to the covalent polymerization.

Also, although the DS of the polymer chains was the same, an increase in MW of dex-MA produces a mixture that was more viscous in the absence of polymerization. The complex viscosity profiles are lower with decreasing MW of dextran produced in the same irradiation time.

The viscoelastic properties of these cross-linked gels were also examined with dex-MA of DS 8% as shown by the complex viscosity profiles in Figure 4. As expected the complex viscosity profiles are higher for gels produced with D40 and D70-MA than those seen in Figure 3

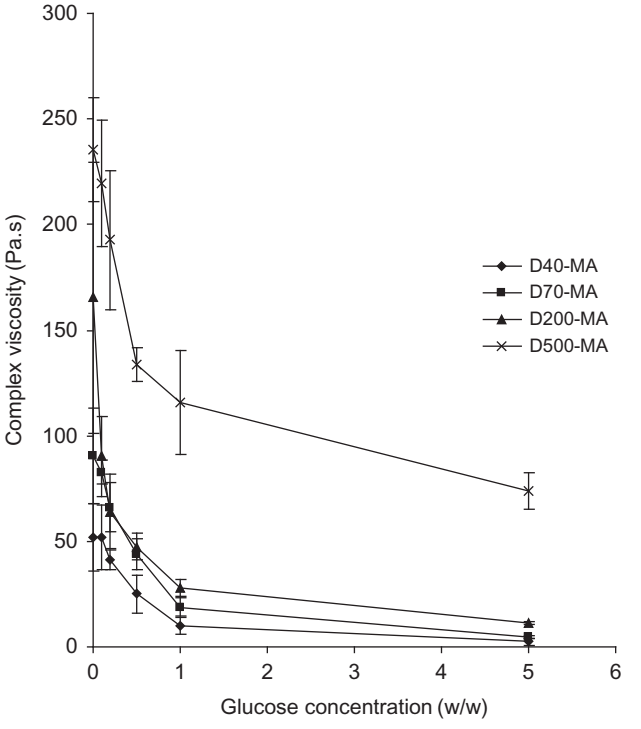

Figure 3. Complex viscosity profiles for D40-MA, D70-MA, D200-MA, and D500-MA (DS 3%)–con A-MA gels irradiated for 50 minutes at different glucose concentrations at 37°C. Data were taken at a frequency of 1 Hz from frequency sweeps conducted in the range 0.01–50 Hz.

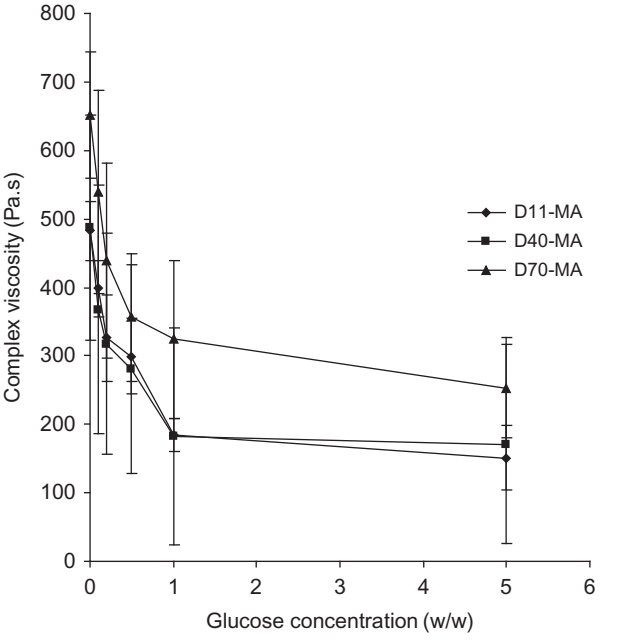

Figure 4. Complex viscosity profiles for D11-MA, D40-MA, and D70-MA (DS 8%)-con A-MA gels irradiated for 50 minutes at different glucose concentrations at 37°C. Data were taken at a frequency of 1 Hz from frequency sweeps conducted in the range 0.01-50 Hz.

where the DS was 3%. We have shown previously[15] that an increasing DS produces a more tightly cross-linked structure because of the increased number of acrylic side chains present on the dextran backbone. The complex viscosity profiles for the D11- and D40-MA-based cross-linked gels are very similar suggesting that no real change was seen in the viscoelastic properties of these gels; however, increasing the MW to 70 kDa produces a higher viscosity profile. Gels were not characterized for the D200 and D500-MA dextran types at this high DS because they would produce materials with higher complex viscosity profiles than seen with D70-MA, which when tested in the diffusion setup have shown a slow release of insulin[15].

HPLC studies and in vitro diffusion experiments to monitor component leach and insulin transport

Polymerized glucose-responsive gel formulations made with derivatized dextran of different MWs were assessed for monomer incorporation and leach after challenge with glucose in in vitro diffusion experiments.

Figure 5 shows the amount of component (dex-MA and con A-MA) leached from the gels during their in situ wash with glucose solution before the diffusion experiments determined by HPLC analysis. The effect of increasing the DS of the dex-MA to 8% for D11, D40, and D70-MA was compared to leach from gel formulations containing D40 and D70-MA (DS 3%). It can be seen that as the dex-MA MW was increased from D11 to D70-MA there was reduced dex-MA leach. In these gel formulations increasing the dex-MA chain length and DS would

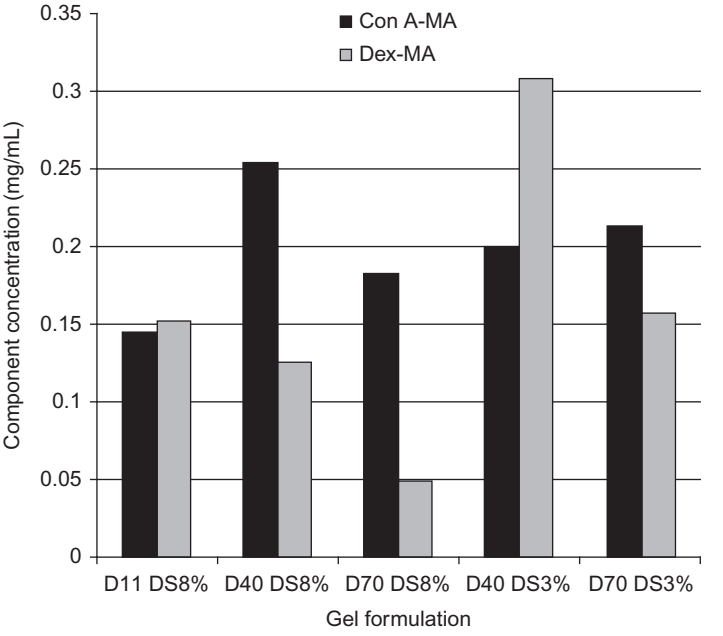

Figure 5. Leached component (dex-MA and con A-MA) concentrations in the glucose solutions used to wash the gels before in vitro diffusion experiments as determined by HPLC.

produce a more complicated network in the same irradiation time. At the lower DS a similar trend in terms of component leach was observed except at higher concentrations. In contrast the leach of con A-MA although variable showed no correlation to the dex-MA chain length or DS.

Although increasing the DS of the dex-MA is desirable in terms of retaining the gel components, these gels are not suitable for use in the device presented here as their complex viscosity profiles across the glucose concentration range (Figure 4) were much higher than the D500-MA at lower DS (Figure 3), which as stated earlier has been found to be too cross-linked for insulin transport. They were therefore not tested for insulin diffusion.

Extensively washed polymerized candidate gel formulations[14] of dex-MA-con A-MA irradiated for 50 minutes with dex-MA of MW 40, 70, 200, and 500 kDa with DS of 3% were assessed to monitor the efficiency of the cross-linking process by monitoring leach of material. Figure 6 shows that the MW of the dextran precursor does affect the extent of component leach in the in vitro diffusion experiments in a similar manner to that observed during the gel washing. The sample made with D40-MA exhibits marginally greater component loss on addition of glucose. The negligible amount of component leach for the D500-MA-based gel may be because of the tightly cross-linked nature of the formulation that may be impeding glucose ingress into the gel membrane layer and to the lectin receptor sites. Evidence for this may seen in Figure 3 that shows that the complex viscosity profile for the D500-MA-based gel was much higher than the other MW dextrans.

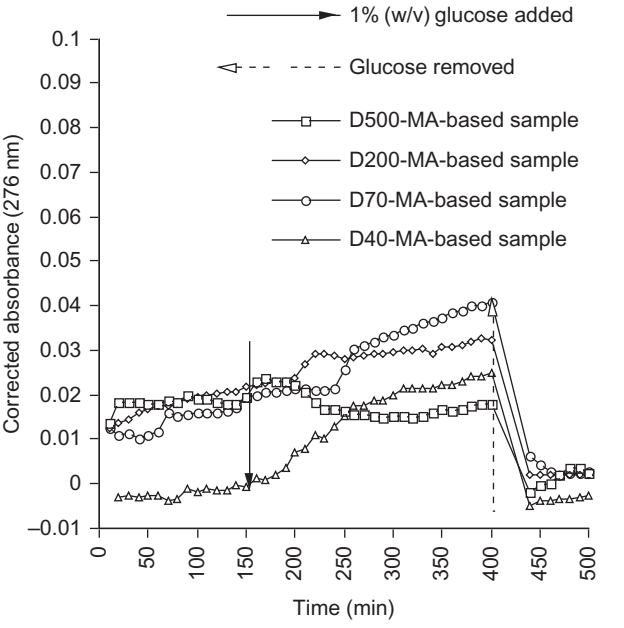

Figure 6. Gel component release profiles for a polymerized gel of dex-MA DS 3% (MW 40, 70, 200, and 500 kDa)-con A-MA irradiated for 50 minutes, under conditions of glucose triggering at 37°C. Data represent mean of three experiments.

Figure 7 (A)–(C) show the insulin delivery characteristics for polymerized gels of differing dex-MA MW when investigated in diffusion experiments with a 5 mg/mL bovine insulin reservoir (pH 7.4) at 37°C. From the insulin-release profiles the insulin-release gradients for both the glucose-triggered test and the nonglucose-triggered control were determined, enabling calculation

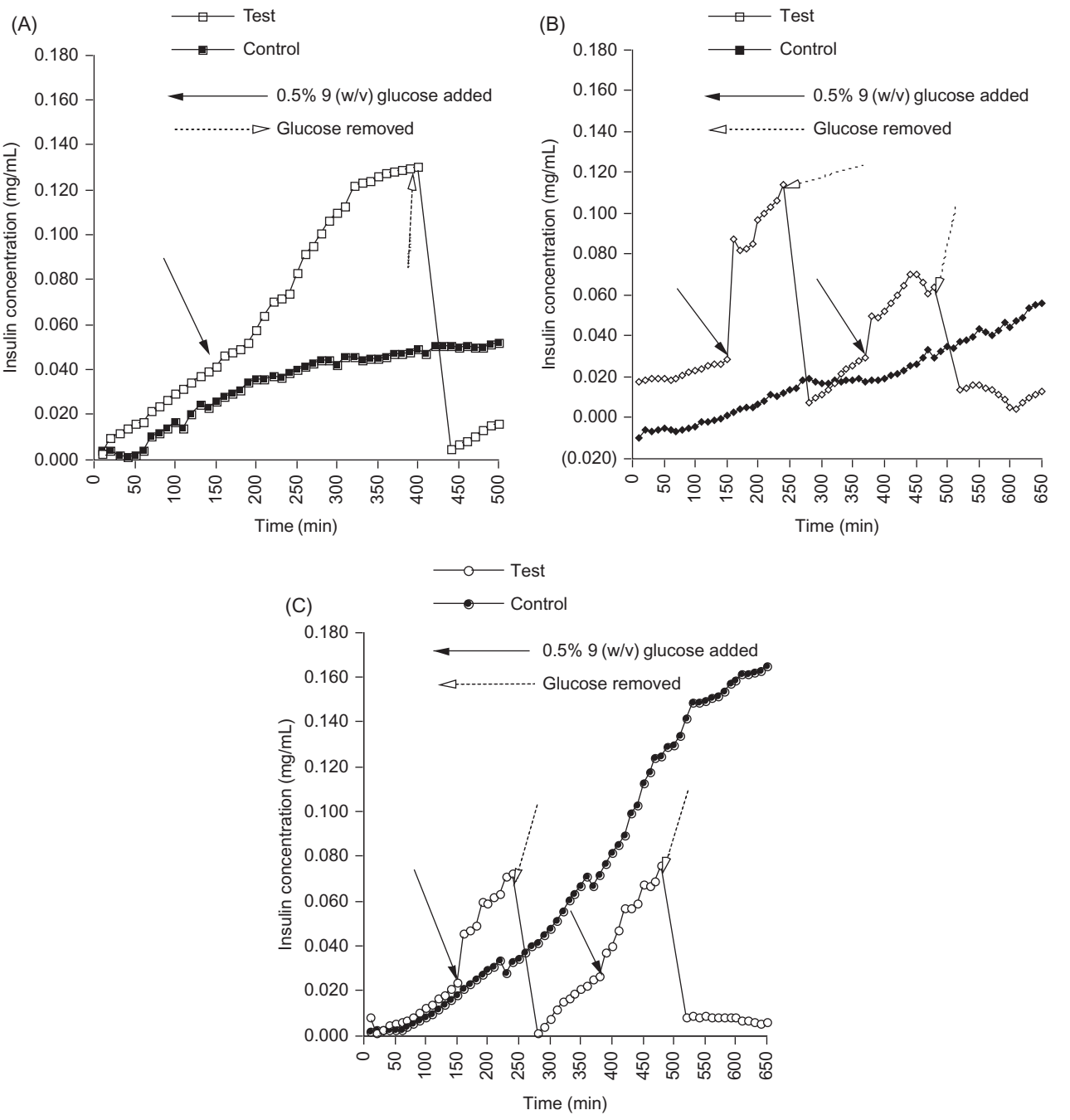

Figure 7. Insulin-release profiles across a gel formulation of (A) D200-MA, (B) D70-MA, and (C) D40-MA (DS 3%)-con A-MA (t = 50 min) in a glucose trigger experiment at 37°C.

Table 1. Glucose-response times and FI values for gel formulations made using dex-MA of MW 40, 70, 200, and 500 kDa with DS 3%.

Dex-MA MW (kDa)	Glucose-response time (min)	FI value for trigger 1
D500	~90	1.4
D200	~70	3.2
D70	immediate	5.5
D40	immediate	1.3

of the ratio of fluxes from the test and the control. This was termed the flux factor increase (FI value); the FI value being a measure of the effect of glucose on insulin delivery from the glucose-sensitive gel formulation. The glucose response times and the glucose-induced FI values after the first glucose trigger from the insulin delivery experiments are summarized in table 1 for gel formulations made using dex-MA of MW 40, 70, 200, and 500 kDa with DS 3%.

These results show that the UV polymerization of D70-MA and con A-MA to produce a covalently stabilized gel has maintained the activity of the glucose-sensitive physical cross-links of the con A and dextran in terms of controlling the diffusion of insulin as a function of glucose content. It is apparent that as the MW of the

derivatized dextran in the polymerized gel was raised from D70-MA to D500-MA a decrease in the FI value coupled with a slower glucose response time was evident. For the D500- and D200-MA-based gel formulations, it was envisaged that a more tangled and tightly cross-linked polymeric network with a small pore size would result although only the D500-MA gel formulation shows a much higher complex viscosity profile across the glucose concentration range. This tangled polymeric network may provide a more tortuous path for insulin diffusion and also impede glucose access to the lectin receptor sites thus resulting in lower levels of insulin transport coupled with low glucose-induced FI values and response times. Conversely for the D40-MA-based polymerized gel formulation although an immediate response was possible, the magnitude of the glucose response falls as there is an increase in insulin flux across the glucose-free control with time. This consequently lowers the differential between the glucose-triggered test and the glucose-free control, resulting in lower FI values. The results suggest that for the experimental conditions chosen there is an optimum MW for the dex-MA of D70-MA in terms of reduced component leach, glucose responsiveness, and insulin transport. For the development of a glucose-sensitive gel formulation for an implantable insulin delivery device a compromise between a high glucose-induced FI value and a quick glucose response time would be desired.

Conclusion

This study has shown that it is possible to manufacture polymerized stable glucose-responsive gels differing in terms of the MW of the acrylic-derivatized dextran and lectin precursors. Rheological studies and in vitro diffusion experiments demonstrate the glucose sensitivity and also show that the loss of active components from the polymerized gels is formulation dependent on the MW of the dex-MA. The performance of candidate polymerized gels made with dex-MA of varying MW was investigated with a bovine insulin reservoir. Rheology and in vitro diffusion studies show that D70-MA of low DS (3%) produces gels that show reduced component leach, glucose responsiveness, and insulin transport useful for a prototype implantable self-regulating insulin delivery device.

Acknowledgments

The work was financially supported by a grant from the Department of Health-New and Emerging Applications of Technology (NEAT) programme (NEAT Project E056). The authors also thank Dr. Mike Needham for NMR experiments.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Gomez-Perez FJ, Rull JA. (2005). Insulin therapy: Current alternatives. Arch Med Res, 36(3):258–72.
2. Yamagishi S, Imaizumi T. (2005). Diabetic vascular complications: Pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des, 11(18):2279–99.
3. The Diabetes Control and Complications Study Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N Eng J Med, 329:977–87.
4. UK Prospective DiabetesStudy Group. (1998). Intensive blood glucose control with sulphonylureas or insulin compared with conventional therapy and risk of complications in patients with Type 2 diabetes mellitus: (UKPDS 33). Lancet, 352:827–53.
5. Taylor MJ, Tanna S, Sahota TS. (2004). The race for closed loop delivery of insulin self-regulation of insulin delivery: Progress to date for diabetes mellitus therapy. Am J Drug Deliv, 2(1):1–13.
6. Renard E. (2002). Implantable closed-loop glucose-sensing and insulin delivery: The future for insulin pump therapy. Curr Opin Pharmacol, 2(6):708–16.
7. Selam JL. (2001). External and implantable insulin pumps: Current place in the treatment of diabetes. Exp Clin Endocrinol Diabetes, 109: S333–40.
8. Steil GM, Rebrin K. (2005). Closed-loop insulin delivery—what lies between where we are and where we are going? Exp Opin Drug Deliv, 2(2):353–62.
9. Adams G, Clark J, Sahota TS, Tanna S, Taylor MJ. (2000). Diabetes mellitus and closed-loop insulin delivery. Biotechnol Genet Eng Rev, 17:455–96.
10. Taylor MJ, Tanna S. (1994). A self regulated delivery system using unmodified solutes in glucose sensitive gel membranes. J Pharm Pharmacol, 46:1051a.
11. Taylor MJ, Tanna S, Taylor PM, Adams G. (1995). The delivery of insulin from aqueous and non-aqueous reservoirs governed by a glucose sensitive gel membrane. J Drug Target, 3:209–16.
12. Tanna S, Taylor MJ. (1998). Characterization of model solute and insulin delivery across covalently modified lectin-polysaccharide gels sensitive to glucose. Pharm Pharmacol Commun, 4:117–22.
13. Tanna S, Sahota TS, Clark J, Taylor MJ. (2002). A covalently stabilised glucose responsive gel formulation with a Carbopol® carrier. J Drug Target, 10:411–8.
14. Tanna S, Sahota TS, Taylor MJ, Sawicka K. (2006). Glucose-responsive UV polymerised dextran-concanavalin A acrylic derivatised mixtures for closed loop delivery. Biomaterials, 27:1586–97.
15. Tanna S, Sahota TS, Sawicka K, Taylor MJ. (2006). A smart biomaterial for closed-loop insulin delivery: Effect of dextran and concanavalin A acrylic derivatisation. Biomaterials, 27:4498–507.
16. De Bernardo S, Weigele M, Toome V, Manhart K, Leimgruber W. (1974). Studies on the reaction of fluorescamine with primary amines. Arch Biochem Biophys, 163:390–9.
17. Felix AM, Toome V, De Bernardo S, Weigele M. (1975). Colorimetric amino acid analysis using fluorescamine. Arch Biochem Biophys, 168:601–8.
18. Chu H-C, Wu A-T, Lin Y-F. (2001). Synthesis and characterisation of acrylic acid containing dextran hydrogels. Polymer, 42:1471–9.
19. Mezger T. (2002). Oscillatory tests. In: Zorll, U ed. The rheology handbook. Hanover, Germany: Vincentz Verlag, 112–163.
20. Chamberlain EK, Rao MA. (2000). Effect of concentration on rheological properties of acid-hydrolyzed amylopectin solutions. Food Hydrocoll, 14:163–71.