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Covalent coupling of concanavalin A to a Carbopol 934P and 941P carrier in glucose-sensitive gels for delivery of insulin

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Abstract

A novel glucose-sensitive gel formulation, containing concanavalin A and specific polysaccharides, was stabilised via covalent coupling to two structurally different carbomers. The bonding was done to minimise leaching of gel components thereby preventing toxicity and preserving the working mechanism of the gel. Increased gel stability was introduced by covalently bonding amine groups present on the lysine residues of concanavalin A to carboxylic moieties on Carbopol 934P NF and 941P NF using carbodiimide chemistry. The introduction of dextran then produced a glucose-sensitive formulation that transformed from gel to sol in the presence of free glucose. Rheological examination of glucose-sensitive gels stabilised in this way and containing varying concentrations of glucose was conducted with a cone and plate viscometer used in continual rotation mode. A decrease in viscosity over the chosen glucose concentration range was exhibited by both carbomer-stabilised formulations. The subsequent testing of such formulations in in-vitro diffusion experiments revealed that the leaching of concanavalin A from the covalently coupled gels is restricted significantly with respect to non-coupled formulations. In addition, insulin delivery in response to glucose in the physiologically relevant glucose concentration range has been demonstrated using the carbomer-stabilised gels at 37°C. The performance of this self-regulating drug delivery system has been improved in terms of increased gel stability with reduced component leaching.

Introduction

A self-regulating or closed-loop insulin delivery system would improve the management of type 1 diabetes (Baudys & Kim 1999; Adams et al 2000). Conventional subcutaneous therapy using commercially available insulin does not adequately maintain normoglycaemia because the pharmacokinetics following injection do not match physiological secretion of pancreatic insulin. The resulting hyperglycaemia is responsible for the long-term macrovascular and microvascular complications associated with diabetes (Brange & Volund 1999; Graves & Eisenberth 1999). Improved glycaemic control in type 1 diabetes has been proven efficient in preventing such complications and the key to strict glycaemic control with the use of exogenous insulin lies in the creation of delivery methods that emulate physiological insulin secretion (Baudys & Kim 1999; Emilien et al 1999; Jeandidier & Boivin 1999). A self-regulating insulin delivery system would more closely resemble the normal physiological process in which the amount of drug released is affected according to physiological needs and thereby minimise, if not prevent, the complications of diabetes which are a result of poor glycaemic control. In self-regulated devices, the controlled variable is detected and as a result the system output is adjusted accordingly; drug release rate is controlled by feedback information, without any external intervention (Kost & Langer 2001). Stimuli-sensitive gels that undergo changes in state in response to changes in environmental conditions have potential applications as smart biomaterials for polymeric controlled drug delivery (Wang et al 1999; Yuk & Bae 1999; Qiu & Park 2001; Miyata et al 2002). Development of glucose-sensitive gels may therefore advance the construction of a self-regulating drug delivery system for the management of diabetes (Kost & Langer 2001). Experimental self-regulated insulin

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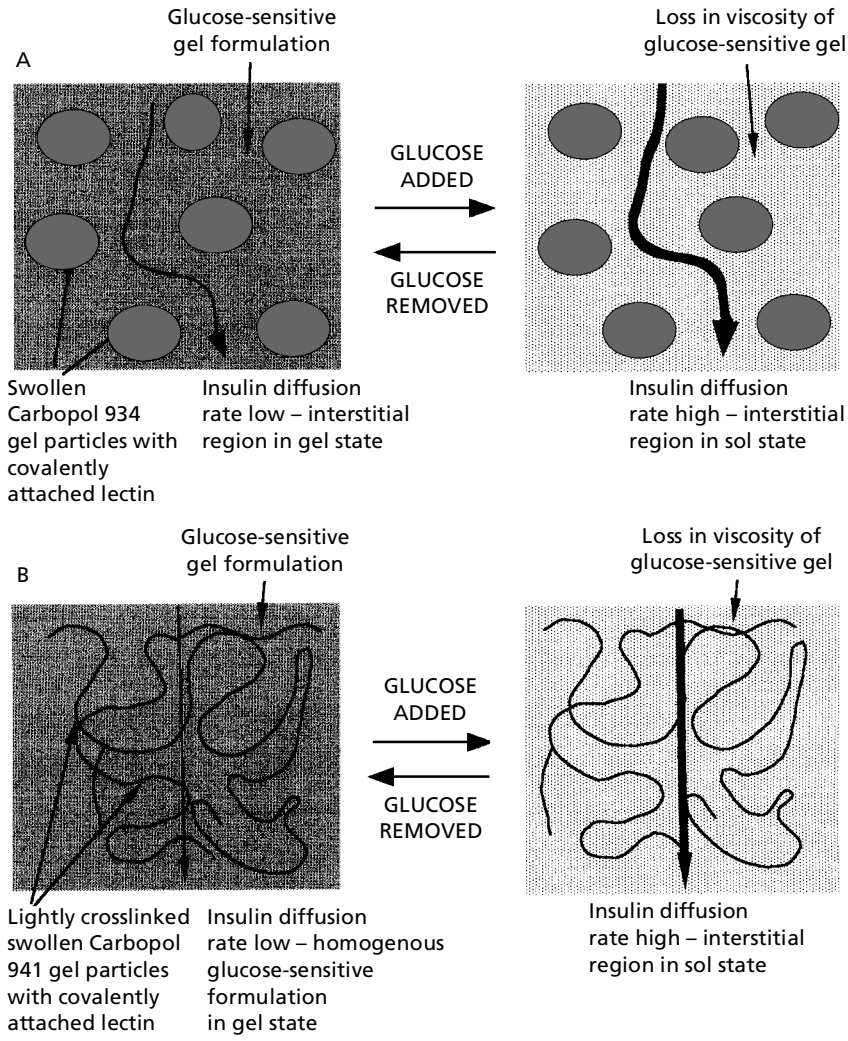

Figure 1 Schematic diagram representing swollen Carbopol 934 (A) and 941 (B) structures incorporating glucose-sensitive lectin–polysaccharide gel.

delivery designs studied have utilised several approaches as rate-control mechanisms : pH-sensitive polymers, enzyme–substrate reactions and competitive binding (Kost & Langer 2001 ; Qiu & Park 2001 ; Miyata et al 2002). The latter forms the basis of the system described in this study.

Formulations of the plant lectin, concanavalin A (Con A) and specific polysaccharides have been shown to produce glucose-sensitive gels (Taylor 1992, 1993). The viscosity of such gels can be lowered reversibly on interaction with glucose. The gel switch mechanism depends on a competitive displacement of a glucose-bearing polysaccharide, by free glucose, from concanavalin A. The three-dimensional gel structure that forms between the lectin and the polysaccharide is dismantled when glucose causes this displacement. Gel viscosity falls as a result, but is restored on glucose removal, thus providing the switch controlling the diffusion of drug held in a reservoir. Dextran (Taylor & Tanna 1994 ; Tanna 1996) and polysucrose (Taylor et al 1995 ; Tanna 1996) have been used as the polysaccharide components. One of the problems encountered in earlier studies is the significant leaching of the lectin from the gel membranes during low-viscosity phases (Tanna 1996 ; Tanna & Taylor 1998a). The loss of concanavalin A from these gels is undesirable for two reasons. Firstly, the material is mitogenic and therefore potentially toxic (Beckert & Al 1970 ; Powell & Leon 1970), and secondly, the repeated action of the glucose-sensitive gel switch mechanism depends on maintaining the components in juxtaposition and critical concentration. This gel has been successfully improved by the covalent coupling of concanavalin A to dextran or glycogen using Schiff's base derivatives. The resulting covalently coupled gels were shown to be both glucose sensitive and able to control the insulin delivery rate in response to glucose (Tanna & Taylor 1998a, b ; Tanna et al 1999). Others have subsequently used a similar approach to show that polymer-bound glucose and concanavalin A could form a gel undergoing reversible gel–sol transition to regulate insulin (Lee & Park 1994 ; Obaidat & Park 1997).

An alternative approach to producing a stable glucose-sensitive gel using a commercially available carbomer has been examined previously (Tanna et al 2001, 2002). Here, the lectin was conjugated to Carbopol 974 (C974), thereby creating a massive molecular structure that could still participate in receptor binding with dextran. This produced a glucose-sensitive gel, which allowed the more rapid diffusion of insulin when challenged with free glucose. Covalent coupling of concanavalin A to C974 was accomplished using the standard carbodiimide method, where the amine groups present on the lysine residues of the lectin were covalently bonded to the carboxylic groups present on the acrylic acid polymer backbone of C974.

In this study, two further carbomer types, Carbopol 934P and Carbopol 941P (C934 and C941) were assessed as potential carriers for the covalent attachment of the mitogenic lectin. Coupling was accomplished using the carbodiimide chemistry described previously (Tanna et al 2001, 2002). These carbomers are structurally different to the C974 studied previously. Analogous to C974, C934 is composed of cross-linked fuzzballs or minigels; however, the density of cross-linking is lower than C974 and it is cross-linked with allyl sucrose (Goodrich 1994). Each minigel, therefore, has a discrete hydrogel structure surrounded by an aqueous interstitial region. The microviscosities are lower than their macroviscosities due to the presence of water in the interstitial microvoids. During carbodiimide conjugation covalent bonding using C934 is expected to be similar to C974 where it is envisaged that bonding of concanavalin A is confined to the periphery of the minigels. The introduction of dextran would then produce a glucose-sensitive region in the interstitial regions around these minigels, which would undergo a gel to sol transformation in the presence of glucose (Figure 1). In contrast, the structure of C941 is composed of linear acrylic acid chains which are cross-linked with allyl pentaethyritol to produce a fishnet-type arrangement (Goodrich 1994). With this more open structural architecture, bonding of concanavalin A is likely to occur throughout the whole structure. A homogeneous glucose-sensitive formulation is proposed because the interstitial spaces between the swollen carbomer gel particles are eliminated (Figure 1). In this formulation glucose sensitivity would not be restricted to certain regions of the gel as with the C934-based formulation.

The viscosity of the gels formulated in this way, using the two carbomers, will be considered in terms of their response to glucose to predict the characteristics of the formulation in the drug diffusion mechanism. A comparison with non-covalently coupled gels will be made. Gel formulations will be also tested in in-vitro experiments both for concanavalin A retention and for glucose responsiveness in terms of differential delivery of insulin.

Materials and Methods

Materials

Dextran (Type no. B-512; average relative molecular mass 2000000), concanavalin A (Type V), bovine pancreas insulin, and sodium azide were purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, UK). [1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] (EDAC) and [2-(*N*-morpholino)ethanesulfonic acid] (MES) and ethylenediaminetetraacetic acid (EDTA) were also purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, UK). Carbopol 934P NF and 941P NF (C934 and C941) were obtained from B. F. Goodrich (Brussels, Belgium). All other chemicals were of analytical reagent grade and double-distilled water was used throughout.

Synthesis of covalently coupled gels

Dispersions of C934 and C941 (1% w/w) based on a dry weight of 200 mg were made in 0.1 M MES buffer with pH adjusted to neutral and stirred until clear. The same dry weight of concanavalin A was then dissolved in 2 mL of 0.1 M MES buffer (pH 7) and added to each carbomer dispersion to produce a final concanavalin A concentration of approximately 0.9% w/w. The systems were then conjugated using 50 mM EDAC for 3 h at room temperature and then quenched with phosphate-buffered saline (PBS; pH 5.9) and centrifuged. To determine the coupling efficiencies, the supernatants were assayed at 276 nm. The gelatinous pellets were re-suspended in PBS (pH 5.9, 15–20 mL) and centrifuged. After each centrifugation the supernatants were assayed at 276 nm until no further concanavalin A was detected. The carbomer–lectin conjugates were then precipitated by lowering the pH values to approximately 4.5 with 1 M HCl. The precipitates were removed and neutralised with 1 M NaOH to yield tight gels. A 1-g quantity of 20% w/w aqueous dextran (average MW: 2000000) was added to each of the neutralised conjugates and mixed thoroughly. The pH values of the final products were adjusted to 7.4 to yield opaque gels. The compositions of the conjugated gels were 2.6% w/w of carbomer and dextran. The percentage dry weights of concanavalin A present in the carbomer-based gels was 2.4% w/w for C934 conjugated gels and 2.6% w/w for C941 conjugated gels, giving a coupling efficiency for both formulations of 92% and 100%, respectively.

To produce enough gel for both viscosity and diffusion experiments, further batches were made in exactly the same way and of exactly the same composition. These batches were thoroughly mixed together to produce a stock gel for use in all experiments.

Synthesis of non-covalently coupled control gels

C934 (0.13 g) was dispersed in PBS (2 g, pH 7.4) to form an opaque viscous solution. Addition of concanavalin A (0.112 g) resulted in the formation of a white precipitate, to which 1 M NaOH was added drop-wise until a viscous opaque gel resulted. Dextran (0.13 g) was then added and mixed to yield a tight elastic gel. The pH of this gel was adjusted to 7.4 with NaOH and PBS (pH 7.4) until a final weight of 5 g was attained. A gel formulation based on C941 was made in exactly the same way with appropriate adjustments made for concanavalin A.

Further batches of carbomer gels were made in exactly the same way to produce a stock of gel, which was thoroughly mixed before use in all experiments.

Rheological studies

Rheological properties of the carbomer-stabilised glucose-sensitive gels were evaluated using a Haake Rheostress RS75 rheometer (Haake, Germany) using cone and plate geometry (C35 with 2° angle) in continuous rotation mode. The rheometer was used in this mode to elucidate information on the characteristics of bond rupture leading to flow from which the viscosity could be measured. The sample viscosity was measured from a viscosity curve with a shear rate ramped from 0 to 5 s^{-1}. Viscosity values corresponding to a rate of shear of 5 s^{-1} were used to compare the gels at 20 and 37 $\pm$ 0.5°C with approximately 0.5 g of sample. The gels to be tested were formulated with a range of glucose concentrations between 0 and 1% w/v but otherwise equivalent content. Glucose solutions were added as the same small volume of appropriate concentrate made with PBS (pH 7.4). Glucose-free and non-buffered controls were also tested to monitor the effect of the buffered vehicle. Values for the rheological parameters examined were processed from the RheoWin software provided with the rheometer.

In-vitro diffusion experiments

Insulin solutions (5 mg mL^{-1}) were prepared by dissolution in a minimum volume of HCl and diluted to volume with PBS (pH 7.4) containing 5 mM EDTA (Quinn & Andrade 1983; Brange 1987). For the diffusion experiments a small experimental cell was used to hold a thin layer of a glucose-sensitive gel. In this arrangement the gel was confined between two cellulose nitrate filter disks (0.2 μm pore size; 13 mm diameter) to form a barrier membrane for a solute reservoir (1 mL), while the other side was exposed to a temperature-controlled buffered bulk receptor medium (10 mL) to which glucose was 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 experiment anhydrous glucose was added to the bulk solution of a test run to produce concentrations of either 0.1% w/v ($\sim$ 5.5 mM), 0.2% w/v ($\sim$ 11 mM), 0.5% w/v ($\sim$ 28 mM) or 1.0% ($\sim$ 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. Solute delivery was monitored spectrophotometrically for insulin at 276 nm.

Concomitant release of concanavalin A from the gel membrane was assessed by use of an identical arrangement by using a solute-free reservoir solution. Concanavalin A was also assayed at 276 nm and therefore in addition to the use of these data for assessing lectin escape from the gels, they were used to correct the insulin-release profile.

Statistical analysis

Values of mean $\pm$ s.d. for each sample were obtained from 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.

Results and Discussion

Depending on concentration and hydration conditions, glucose-sensitive gels resulted when aqueous dextran solutions were mixed with concanavalin A covalently coupled to C934 and C941. The carbomer-based gels, like the carrier-free counterparts, were shown to be glucose-sensitive visibly and mechanically upon mixing with anhydrous glucose. This is evidence that after covalent coupling with each polymeric carrier, the lectin receptor sites have retained their capacity to interact reversibly with glucose, implying that free glucose could still displace the terminal glucose units in dextran, dismantling the temporary cross-linking between the lectin. These resultant sols were visibly more viscous than those seen in the covalently modified dextran–concanavalin A gels studied previously (Tanna & Taylor 1998a). It is postulated that this is because the gelatinous polymeric carrier provides a certain contribution to the macroviscosity despite there being a change in microviscosity provoked by the glucose-responsive gel-to-sol switch. The gel formulations could only transform between gel and sol in response to glucose if the lectin receptor sites remain freely accessible and the resulting structure is not so tight that glucose ingress is impeded. As a consequence, a change in the complexity of the gel structure would arise due to the dismantling of the additional smart polymeric gel lattice in the interstitial regions.

Results from viscosity controls for the C934 and C941-based covalently coupled and non-coupled gels, tested neat and when triggered with water and PBS (pH 7.4), are shown in Table 1. As the vehicle for the glucose solutions is PBS (pH 7.4) it was necessary to consider any effect its presence would have on the gel viscosity. Gels made using both carbomers, when triggered with distilled water, exhibited a fall in viscosity implying that, as expected, a 10% dilution of liquid concentrate or control solution produces a looser gel. With PBS (pH 7.4) as the diluent vehicle, a further reduction in viscosity was observed. This suggested that an additional influence on the gel viscosity was provided by the presence of phosphate ions and saline, although the viscosity fall with glucose was considerably greater. A similar trend was observed for the non-coupled gels. It can also be seen from Table 1 that the covalent coupling of the lectin to the carbomer carriers produces lower viscosity values than for non-coupled gels, implying

Table 1 Viscosity of covalently coupled and non-coupled Carbopol 941P- and 934P-based glucose-sensitive gels when tested neat and triggered with distilled water and PBS (pH 7.4), at 20 and 37°C.

Diluent	Temp.	Viscosity (Pa s)			
		C941 coupled	C934 coupled	C941 non-coupled	C934 non-coupled
Neat	20°C	181.2±6.72	188.2±5.09	—	—
Distilled water	20°C	153.4±2.8	175.0±10.46	—	—
PBS (pH 7.4)	20°C	150.9±7.74	152.86±7.76	—	—
Neat	37°C	151.2±6.54	119.0±7.89	280.0±28.48	447.4±53.75
Distilled water	37°C	135.4±3.96	110.7±7.69	268.6±29.21	308.9±30.95
PBS (pH 7.4)	37°C	124.9±14.28	91.9±7.82	253.5±19.29	305.8±47.91

Data represent mean±s.d. of three measurements. $P < 0.05$ for comparisons with covalently coupled and non-covalently coupled formulations.

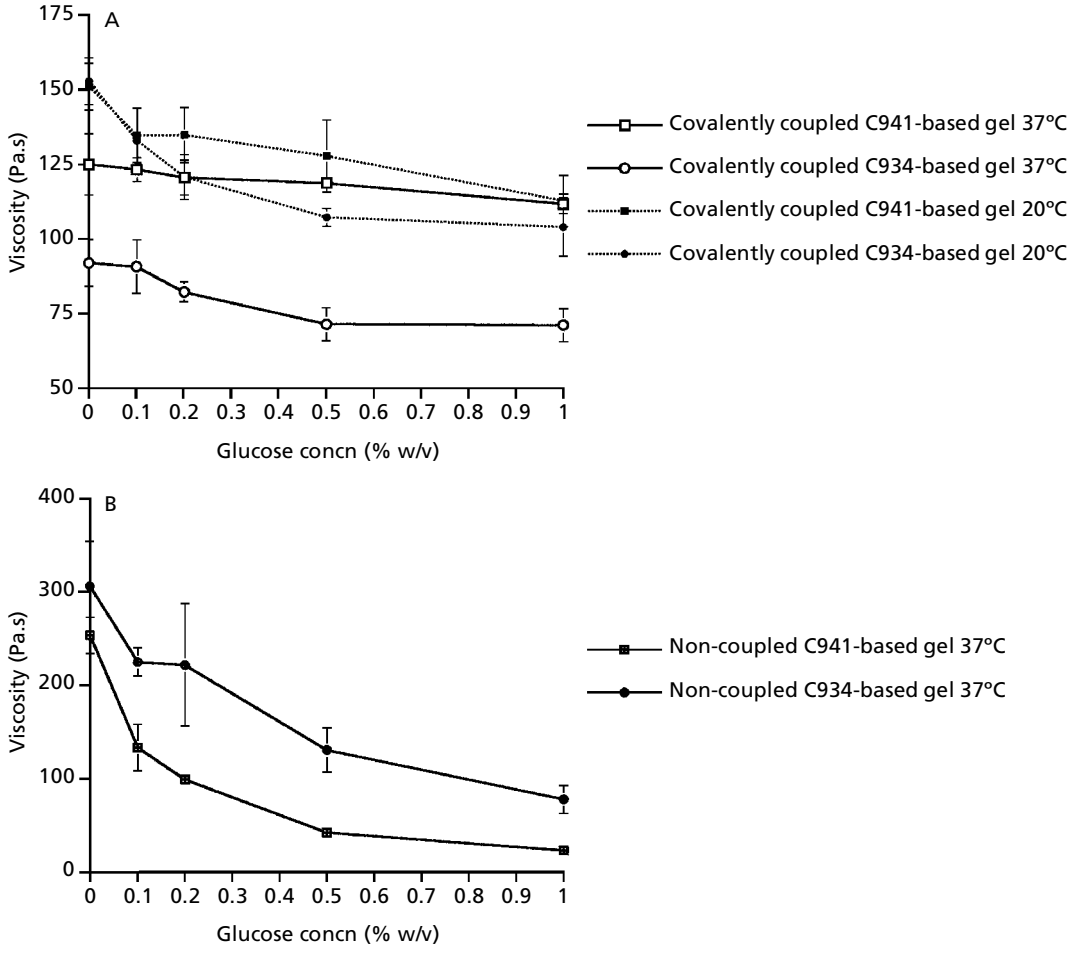

Figure 2 Viscosity profiles for covalently coupled C941- and C934-based formulations with a range of glucose concentrations added, at 20°C and 37°C (A) and for non-covalently coupled C941- and C934-based formulations with a range of glucose concentrations added, at 37°C (B). Data represent mean±s.d. of three measurements. $P < 0.05$ when comparing the covalently coupled with the non-covalently coupled formulations up to glucose concentrations of 0.5% w/v, at 37°C.

that there could be less electrostatic repulsion between the carbomer acrylic acid monomers which have a covalent attachment of the lectin. At 20°C, viscosity tests could only be carried out for covalently coupled gels. The non-coupled gels proved resistant to shear for any viscosity data within the shear rates chosen.

Dynamic rheological measurements show that the covalently coupled and non-coupled carbomer-based gels

exhibited an overall decreasing trend in viscosity with increasing formulation glucose content giving an indication of the gel glucose-sensitivity at 20 and 37°C (Figure 2). For the design of a self-regulating drug delivery device for the management of diabetes, the changes occurring in the proposed gel in response to glucose need to be appropriate for diabetic physiological glucose levels. This means that such a delivery device would have to be relatively unreactive at normal glucose levels (0.1% w/v, ~ 5.5 mM) but would need to be triggered within or just above post-prandial levels (0.2% w/v, ~ 11 mM). For the system under study, this means that the glucose-sensitive gel must be able to tolerate normal glucose levels up to 0.15% w/v without considerable physical change, but respond at glucose levels higher than this. The viscosity profiles for both covalently coupled carbomer-based gels reveal that, as expected, the viscosities at 20°C were higher than at 37°C throughout the glucose concentration range (Figure 2A). At 20°C the fall in viscosity was greater for the C934-conjugated gel than the C941-conjugated gel, throughout the glucose concentration range. At 37°C the change in viscosity between a glucose concentration of 0 and 0.1% w/v appeared negligible, indicating that the structure is able to tolerate low glucose levels (Figure 2A). The viscosity profiles also show that the C934-based gel produced the greatest change in viscosity at glucose concentrations above 0.2% w/v. A similar trend was observed with the C974-based gels studied previously (Tanna et al 2001, 2002), although the fall in viscosity was more pronounced. The C941-based gel required a glucose concentration of 0.5% w/v and above to show a clear reduction in viscosity. These results suggest that when the glucose-sensitive dextran–lectin mixture is located in the interstitial areas, as with the C934-based gel, a greater change in viscosity is apparent than with the C941-based gel. This is possibly because the interactive constituents are concentrated into the small interstitial volume. As the structure of C934 is a looser fuzzball than C974, it is possible that during coupling some of the lectin will have bonded inside the minigel structure, which could lead to a smaller change in interstitial microviscosity. These types of structures might also allow fast percolation of glucose both through and between the minigels. In the C941-based gel it is envisaged that the bonded lectin would be distributed more homogeneously because of unhindered access throughout the looser structure. In this case a greater macroviscosity is seen which would not change much with low concentrations of glucose.

For the non-coupled carbomer-based gels, a greater viscosity change compared with the covalently coupled gels is observed at 37°C throughout the glucose concentration range (Figure 2B). This would suggest that covalent coupling to these carbomers imposes a reduced freedom of movement of particles and thus allows smaller gel–sol viscosity differentials by comparison. In non-coupled gels the movement of particles is more predominant and therefore the reverse is seen (i.e. larger changes in viscosity because the lectin is not constrained due to covalent bonding).

Insulin-free control diffusion experiments for the covalently stabilised gels showed that for the formulations

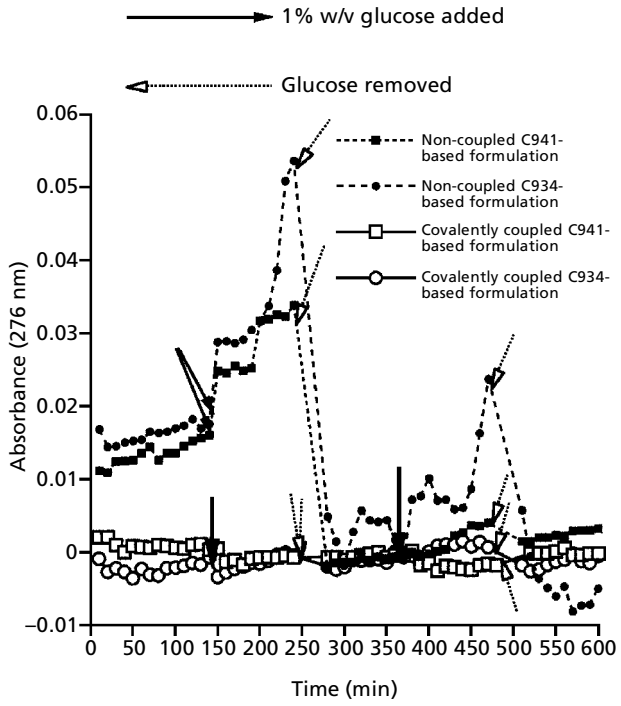

Figure 3 A comparison of the concanavalin A release profiles of a covalently coupled and a non-coupled C941- and C934-based glucose-sensitive formulation, at 37°C. Data represent mean of three experiments. $P < 0.05$ following triggering with glucose when comparing covalently coupled with non-covalently coupled formulations.

studied concanavalin A escape was restricted with respect to non-coupled formulations, suggesting that the covalent anchoring of the mitogenic and cytotoxic lectin to the polymeric carrier had been successful (Figure 3). Thus the gel formulation has been improved in terms of concanavalin A leaching by covalent coupling to a suitable polymeric carrier. The extent of concanavalin A leaching did not differ considerably between the different carbomer types. The possibility of further non-covalent interactions independent of the glucose receptor and due to the complexation of the lectin to the polymeric carrier also exists. This is because of the precipitation observed upon mixing dispersed carbomer to a solution of concanavalin A during the preparation of the gels. The mechanism of interaction is not known, neither is its specificity for this protein, but it could possibly be attributed to electrostatic interactions between the protein and the large number of polyacrylic acid ligands on each polymeric carrier. The higher binding affinity of poly(acrylates) for calcium ions could also account for any non-covalent interactions (Leußen et al 1995), because concanavalin A contains trace levels of this bivalent cation, which is required for carbohydrate binding (Kalb & Levitzki 1968). Further evidence of non-covalent interactions between the lectin and the carbomer carrier is that the cumulative amount of lectin released from the non-covalently-coupled carbomer-based formulations is $< 5\%$ of the total amount.

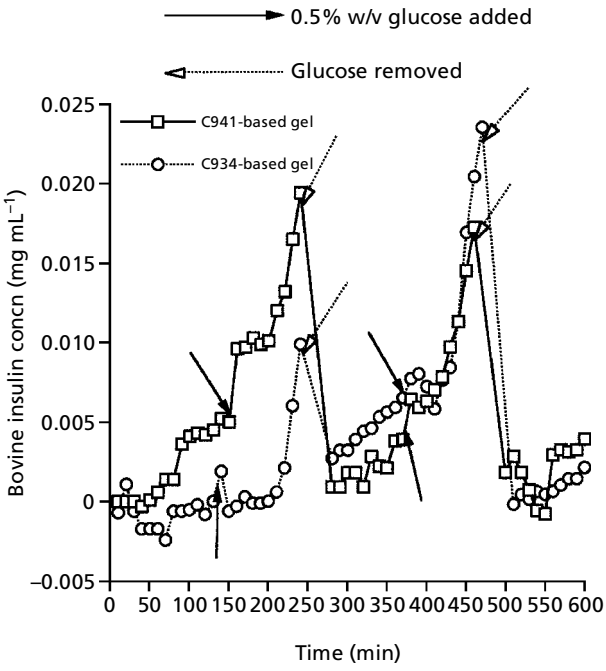

Figure 4 Insulin release profile across a C941- and C934-stabilised glucose-sensitive formulation in conditions of repeated glucose triggering with 0.5% w/v glucose at 37°C. Data represent mean of three experiments.

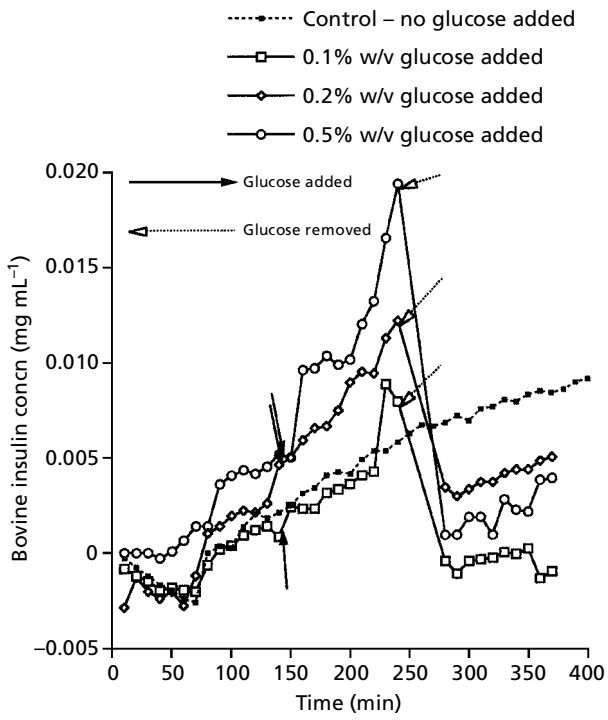

Figure 5 Insulin release profiles in glucose dose response studies of a C941-stabilised glucose-sensitive formulation at 37°C. Data represent mean of three experiments. $P < 0.05$ following triggering with glucose when compared with the control in which no glucose is added.

Insulin delivery in response to glucose in the physiologically relevant glucose concentration range, with the flux reverting to a low level on removal of glucose from the receptor solution, has been demonstrated using the carbomer-based gels at 37°C under conditions of repeated glucose triggering (Figure 4). Analogous to the C974-based formulations studied previously (Tanna et al 2001), it is thought that the interstitial regions and not the particle interiors are the regions controlling insulin transport in the heterogeneous assembly of the C934-based formulations. In this type of structure the viscosity change in response to glucose would be limited to the interstices of the gel structure. A significant difference in the rate of insulin transport across the glucose-sensitive gel membranes was not apparent between the different types of carbomer-based gels. This is probably because diffusion of insulin is controlled by the additional smart polymeric gel lattice formed from lectin–polysaccharide interactions and not affected greatly by the carrier type. In the absence of critical glucose levels, the passage of insulin will be relatively obstructed and in the presence of glucose the additional smart polymeric gel would be dismantled, increasing the passage of insulin through the gel. The change in structure and the resulting fall in viscosity are induced by the exchange of free glucose at specific receptors, with the terminal glucose moieties of the polysaccharide which form part of the formulation. Despite the complexity of the C934-based gel, it is envisaged that the individual fuzzball-type minigel structures are considerably dense so that large solutes diffuse through the interstitial areas between the minigels. For the homogeneous C941-based formulation, insulin transport is proposed to occur throughout the whole structure. The glucose response time and magnitude of this response was affected by the trigger glucose concentration (Figures 5 and 6). For the C934-based gel, it was found that in the set-up used, a concentration of 0.2% w/v (~ 11 mM) glucose was required before a response was triggered. A slow response to a 0.1% w/v (~ 5.5 mM) glucose dose could be elicited in the set-up with a C941-based gel (Figure 5). This suggests that the looser and homogeneous assembly facilitates the passage of insulin when challenged with a low glucose dose. However, the viscosity profile for this formulation at 37°C (Figure 2A) shows that the challenging of the smart polymeric gel lattice at low glucose concentrations does not affect the overall viscosity of the formulation greatly. Thus, the glucose sensitivity could be altered by manipulating the formulation of the glucose-sensitive membrane in the diffusion experiments. This is useful from the point of view of design of a self-regulating insulin delivery device.

Conclusions

This study has shown that it is possible to formulate stabilised glucose-sensitive gels with covalently coupled carbomer carriers differing in terms of their structure. Rheological studies show that the carbomer-based lectin–polysaccharide gels are glucose sensitive and formulation

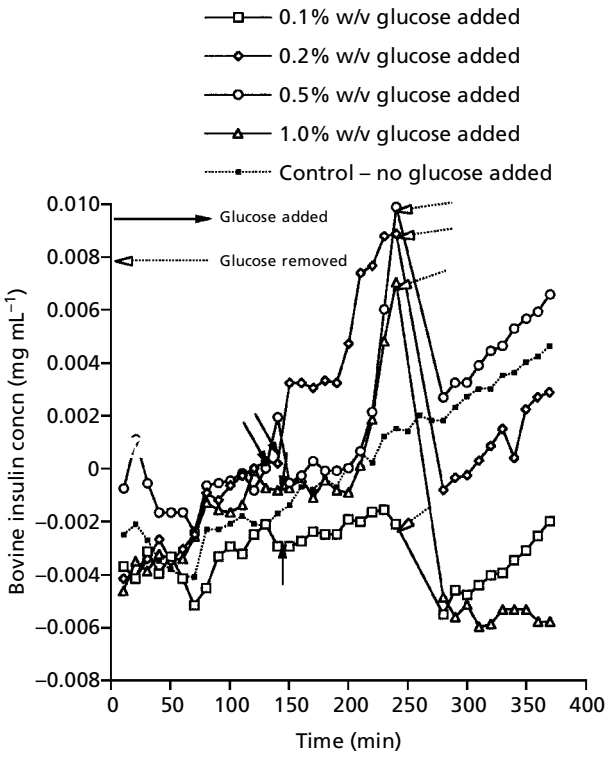

Figure 6 Insulin release profiles in glucose dose response studies of a C934-stabilised glucose-sensitive formulation at 37°C. Data represent mean of three experiments. $P < 0.05$ following triggering with glucose when compared with the control in which no glucose is added, except for the 0.1% w/v glucose-triggered formulation.

dependent. They also show that the inclusion of the polymeric carrier results in a relatively high gel viscosity and it is probably the critical change in the microviscosity upon challenging with glucose that results in changes in viscosity. It was of interest in this work to see whether conditions could be found in which the drop in viscosity of candidate gels was gradual through the glucose levels important in the control of diabetes. Such a gradual drop would form part of the criterion for producing a device response that responds to physiological glucose levels. Suitable changes have been exhibited in the gels studied. A drop to very low viscosity values within the clinically useful range seemed unlikely because of the intrinsic nature of the polymeric carrier and this, in fact, proved to be the case for the gels studied. Further rheological studies using non-destructive oscillatory measurements are being conducted to characterise the viscoelastic properties of the carbomer-based glucose-sensitive gel formulations. The performance of such glucose-sensitive gels in terms of concanavalin A leaching has been significantly improved in the covalently stabilised formulations incorporating the different carbomer carriers. The performance of this gel system was investigated using a bovine insulin reservoir system. In this design, chemically unmodified insulin could be delivered differentially in a specific response to glucose and the increase in insulin delivery was reversible, glucose-dose related and could be triggered repeatedly by glucose. The feasibility of the novel gel formulation as the basis for the design of a self-regulating drug delivery device for therapeutic agents used for the management of diabetes mellitus has been highlighted. This system has advantages over some other self-regulating dosage prototypes that have necessitated the derivatisation of insulin to bear sugar structure which then participates in competition for receptors on concanavalin A (Brownlee & Cerami 1979, 1983; Sato et al 1984) because of the applicability to unmodified insulin or other antihyperglycaemics if desired.

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