Glucose-Responsive Insulin Release from Poly(vinyl alcohol)-Blended Polyacrylamide Membranes Containing Glucose Oxidase

THOMAS CHANDY and CHANDRA P. SHARMA*

Biosurface Technology Division, BMT Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojapura, Thiruvananthapuram-695 012, India

SYNOPSIS

Glucose-sensitive membranes that can increase their permeability in the presence of glucose have been developed. Membranes are fabricated by free-radical polymerization of acrylamide and poly(vinyl alcohol) blends, containing glucose oxidase (GOD). The polymers are hydrogels, with water content in the range of 85–95%, depending on the pH or glucose concentration. The gluconic acid produced by an enzymatic reaction between glucose oxidase and glucose induces a decrease in pH value of the medium. This may causes the protonation of the amino groups in the membrane, resulting in an increase in water content of the polyamine membrane or which changes the solubility of insulin and the diffusional driving force. The *in vitro* retention of the enzyme activity by the membrane is also reported. It appears that the problem of membrane rupture may be alleviated by the blending of polyacrylamide (AA) with poly(vinyl alcohol) (PVA), since they have demonstrated an improved wet strength, without altering their insulin-transport properties. This preliminary report proposes the possibility of developing glucose-sensitive membranes for controlled delivery of insulin and also benefits from ongoing research on biosensors.

INTRODUCTION

The widespread occurrence of diabetes together with the recognized shortcomings of conventional therapy with daily injections of insulin have prompted an examination of alternative strategies for improved delivery of insulin.[1] Drug delivery at a constant rate is often preferable to the conventional methods of drug administration, typified by the injection or ingestion of a bolus. However, a better method may be drug delivery in response to the physiologic needs of the body.

For many years, controlled-release systems have been capable of slowly releasing drugs of only low molecular weight (< 600). Large molecules such as proteins have not been considered feasible candidates, because polypeptides have been considered too large to slowly diffuse through most polymeric materials, even after swelling of the polymer.[2,3] The development of protein-delivery systems is a real challenging problem, since the encapsulated proteins may denature or aggregate as a result of exposure to moisture at 37°C, causing the loss of biological activity and possible changes in immunogenicity.[2,4] However, numerous polymeric systems have recently been developed, which demonstrated the effective delivery of a variety of proteins, polysaccharides, and polynucleotides.[4,5]

The controlled release of insulin is of major interest with regard to the treatment of diabetes, and many studies in this field have been reported.[1,6] Langer et al.[7] demonstrated that ethylene/vinyl acetate copolymers impregnated with insulin can maintain diabetic blood glucose levels near normal values for 1 month. A glucose-controlled insulin-delivery system has been proposed by Brownlee and Cerami,[3] based on competitive binding between glucose and glycosylated insulin, for carbohydrate-specific binding sites on the plant lectin, concanavalin A. Previous studies have shown that the hydrogels

* To whom correspondence should be addressed.

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such as poly(hydroxy methacrylate), polyacrylamide or poly(vinyl alcohol) can be utilized for developing glucose-sensitive membranes with entrapped glucose oxidase enzyme[4,8] for controlled insulin-delivery from a reservoir system.

The objective of this study is the development of a polymeric membrane, which is capable of delivering insulin, at rates dependent on the external glucose concentration. In this paper, we describe the preparation and properties of glucose-responsive membranes based on polyacrylamide and poly(vinyl alcohol) blends. The macroporous membranes containing amine groups and entrapped glucose oxidase have been found to alter insulin permeability, in response to external glucose concentration.

MATERIALS AND METHODS

Materials

Acrylamide, *N-N'*methylenebisacrylamide (Bis), tris(hydroxymethyl)aminomethane (Tris), *N-N-N'-N'*-tetramethylethylenediamine (TEMED), riboflavin, and glucose oxidase (GOD) (Type VII, 125,000 U/g solid) were from Sigma Chemicals, U.S.A. Poly(vinyl alcohol) (PVA) (88% mol hydrolyzed having weight-average mol. wt. 125,000), and paraformaldehyde were obtained from BDH Chemicals Ltd., Poole, England. Insulin (I.P. 40 U per mL, the Boots Company, India), and all other chemicals used were analytical grade.

Preparation of Membranes

Polyacrylamide (AA)–PVA-blended membranes were prepared by polymerizing the monomer solutions and PVA in various ratios. In summary, a PVA solution in distilled water was prepared and mixed well with paraformaldehyde, which was dissolved in 5 g % NaOH solution and kept for cross-linking. The excess paraformaldehyde was removed by dialyzing the solution against water overnight. A known amount of each monomer solutions, acrylamide, Bis, Tris, TEMED, and riboflavin, were well mixed in presence of PVA solution at various ratios at room temperature. This was spread over a clean glass plate separated by shims and the polymerization was allowed to proceed. The membranes were removed from the plate and allowed to swell in buffer. The membranes were never dehydrated. Similarly, AA gel–PVA-blended membranes incorporating glucose oxidase (GOD) enzyme was also prepared by polymerizing the monomer solutions and PVA.

Swelling Measurements

The membranes were immersed in distilled water for several days and the water on the membrane surface was blotted off prior to weighing. The weights of the dry membranes were determined by drying them to constant weight at about 100°C. The water content was expressed as the ratio of the weight of water in the water-swollen membrane to that of the wet membrane.

The mechanical properties of the wet membranes were also determined by the ASTM standard method protocol using a Chatillon Universal test stand Model UTSE-2.[9] The membranes were dipped in water overnight and were cut in the form of standard dumbbell-shaped specimens, having a length between the grips of 2.5 cm and a width of 0.5 cm. A crosshead speed of 1 in./min was employed. The tensile stress and the tensile strength (percentage of elongation) were calculated.

Enzyme Assay

Titration of glucose-sensitive membranes were done using 0.01*N* NaOH for measuring the rate of formation of gluconic acid due to GOD and glucose solution as reported elsewhere.[5] Briefly, 15 mL of glucose solution of interest was adjusted to pH 7.4 and placed for pH measurements. When the solution temperatures reached the room temperature ($\sim$ 30°C), a membrane disk (10 mm diameter) was placed in the solution and the assay was initiated. The pH was maintained at 7.4 $\pm$ 0.02 during the reaction by automatic addition of 0.01*N* NaOH. The amount of 0.01*N* NaOH required to neutralize the generated gluconic acid was noted for 28 min contact, which has direct correlation with the enzyme activity. Stability of the enzyme in the enzyme-incorporated disks were also performed in a similar fashion after keeping them in 0.05*M* PBS for various intervals of time.

Measurement of the Amount of Insulin Permeated through the AA–PVA Membrane

A dialysis chamber was used for determining the permeability of insulin through glucose-sensitive membranes as described earlier.[10] The GOD-incorporated AA–PVA membrane was swollen in buffer solution of given pH at room temperature (30°C). After the water content of the membrane reached equilibrium, the membrane was clamped between the two compartments using multiple supporting and sealing devices. One compartment was filled

Table I Percent Water of Hydration and Mechanical Properties of Various AA–PVA Blended Membranes

Membranes[a]	% Water of Hydration[b] ± SD	Tensile Strength[c] (kg/cm^2) ± SD	Tensile Strain[c] (% elongation) ± SD
AA	91.7 ± 1.0	0.72 ± 0.02	2.35 ± 0.6
AA : PVA (90 : 10)	92.2 ± 0.8	3.38 ± 0.40	87.95 ± 21.8
AA : PVA (80 : 20)	90.8 ± 0.7	5.99 ± 0.50	134.70 ± 39.0
AA : PVA (75 : 25)	89.6 ± 0.9	9.82 ± 0.90	174.9 ± 22.9
AA : PVA (70 : 30)	88.7 ± 1.9	10.76 ± 1.40	178.40 ± 24.2
AA : PVA (60 : 40)	88.2 ± 0.4	12.45 ± 1.20	182.30 ± 38.1
AA : PVA (75 : 25) Enz: entrapped	90.4 ± 1.5	11.01 ± 1.32	141.3 ± 26.9

[a] Paraformaldehyde cross-linked PVA was well mixed with various acrylamide monomer solutions in different ratios and acrylamide–PVA-blended membranes were developed.

[b] Water content was expressed as the ratio of the weight of water in the water-swollen membrane to that of the wet membrane.

[c] Tensile strength and strain were noted after dipping the membranes in water for 24 h (wet membranes).

with insulin and the other with 0.05 *M* phosphate-buffered saline (PBS) pH 7.4, or with varying concentrations of glucose in PBS. The permeability of insulin through the membrane was analyzed spectrophotometrically using Folin and Ciocalteu's method.[11] The permeability percentages were then calculated from triplicate experiments.

RESULTS

Table I gives the percent water of hydration and mechanical properties of various AA–PVA-blended membranes. The water content of PVA-blended AA membranes remained the same at various blend ratios, as is evident from Table I. Thus, it appears that all these membranes have high swellability and the blending of AA with PVA did not alter their water content significantly. However, the tensile strength and the percent elongation increased with increase in PVA content. The AA-to-PVA ratio of 75 : 25 had substantial wet mechanical strength without altering the water content and had been chosen for further studies.

The generation of acid by immobilized glucose oxidase in the presence of glucose was measured by

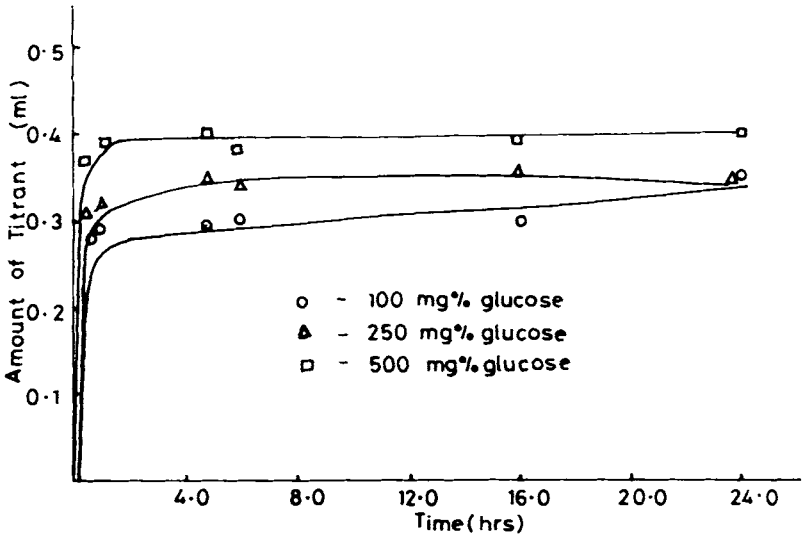

Figure 1 Acid formation from glucose by GOD-incorporated AA–PVA membrane. Data shown are for a single membrane disc but are representative of many similar measurements.

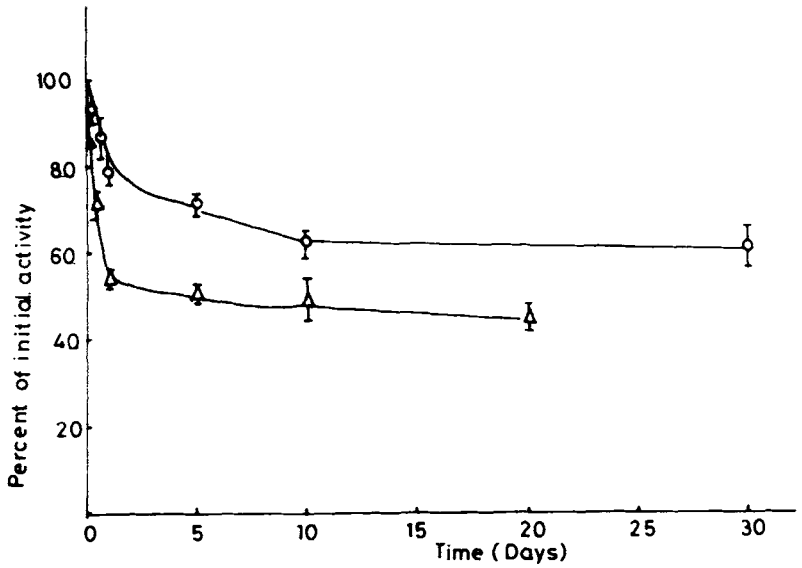

Figure 2 Stability of GOD in the AA–PVA membrane: (— O —) in buffer, (— Δ —) in calf plasma. Bar indicates 95% confidence limits.

recording the base required to maintain constant pH (pH 7.4). As shown in Figure 1, the rate of titrant addition required increased with increasing glucose concentration. The data demonstrated that the enzyme activity of the membrane was not saturated with respect to glucose at physiologic glucose levels and that variations in acid production within the membrane needed to produce the changes in swelling occurred in response to changes in glucose concentration. However, keeping the membrane for prolonged periods in glucose solutions did not alter the subsequent acid formation significantly as compared to the initial values.

The stability of GOD activity during storage in buffer and in calf plasma is depicted in Figure 2. Membranes stored in buffer for 30 days retained 61% of their initial activity. The membranes stored in calf plasma for 20 days had also retained 44% of

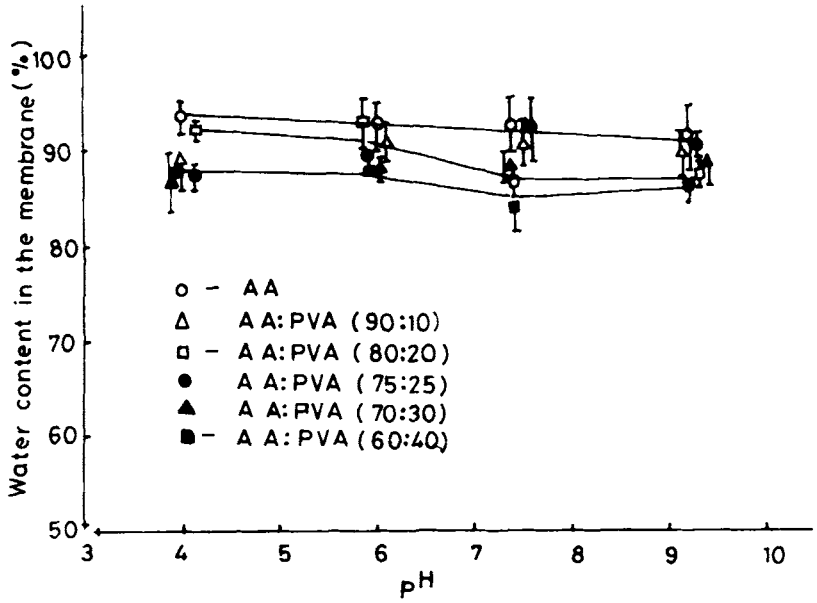

Figure 3 pH effect on the swelling of AA–PVA membranes. Bar indicates 95% confidence limits.

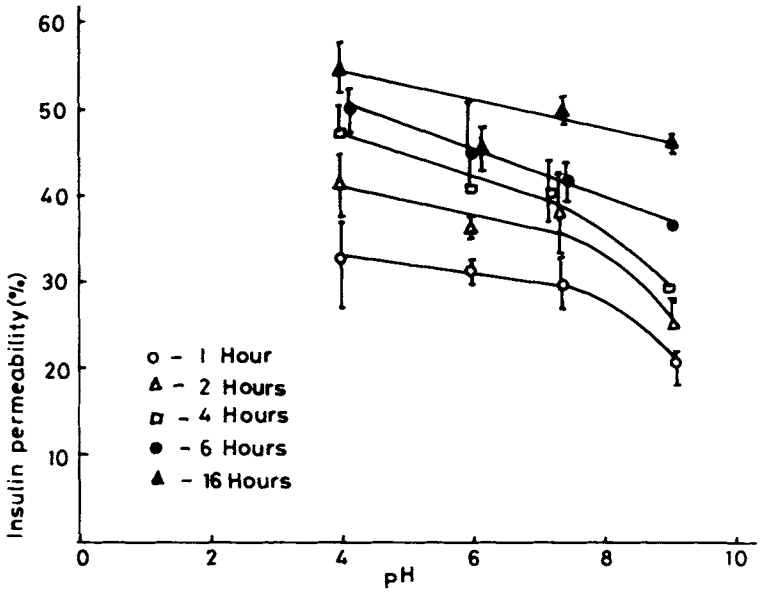

Figure 4 The effect of pH on the permeability of insulin through GOD-incorporated AA–PVA membrane. Bar indicates 95% confidence limits.

their original activity, as is evident from Figure 2. Since the membranes were prepared with an excess of GOD, complete retention of the enzyme activity need not be required for the membrane performance.

Figure 3 shows the pH dependence of the equilibrium water content of the AA–PVA membrane. There were no significant variations in the water content of PVA-blended membranes at different pH levels. However, the percent of insulin permeability increased with decrease in pH of the medium, as is evident from Figure 4. It is also clear that the percent of insulin permeated through the membrane varied reversibly with pH change with all timings examined.

Figure 5 demonstrates the results of insulin permeation through GOD-entrapped AA–PVA mem-

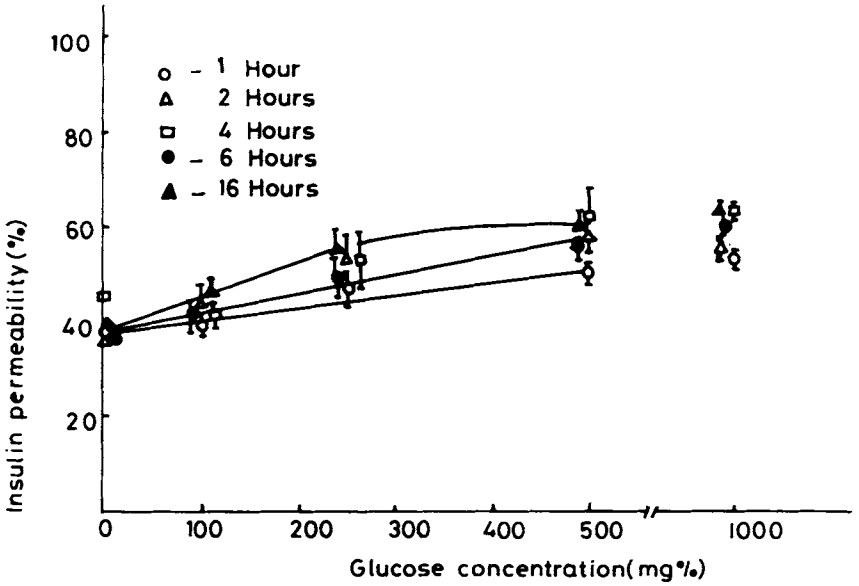

Figure 5 The effect of glucose concentration on the permeability of insulin through GOD-incorporated AA–PVA membrane. Bar indicates 95% confidence limits.

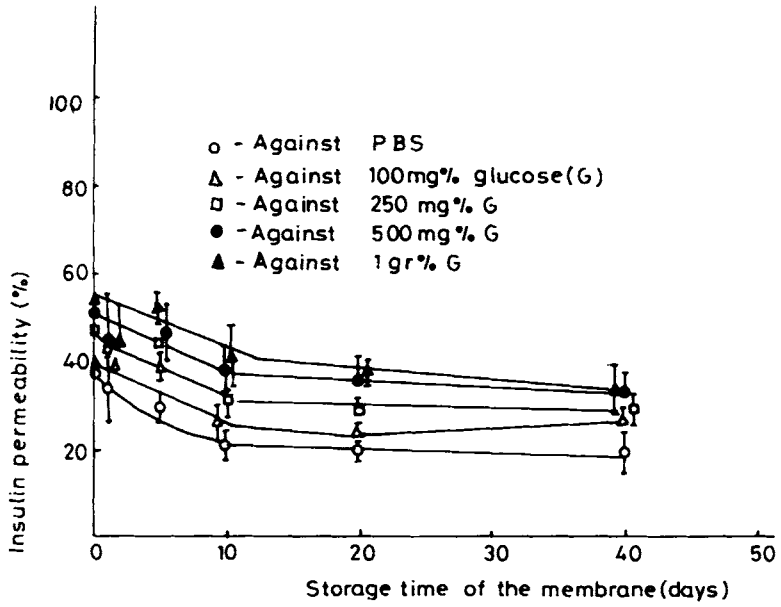

Figure 6 Insulin permeability of GOD-incorporated AA–PVA membrane for 1 h as a function of membrane storage time in PBS. Bar indicates 95% confidence limits.

brane as a function of glucose concentration at various time intervals. It appears that the presence of glucose had enhanced the rate of insulin permeation through the membrane, when compared with a glucose-free system. It is also evident that the insulin permeability increased as a function of dialysis time, with all glucose levels studied, as depicted in Figure 5.

GOD-entrapped AA–PVA membranes were stored in PBS at 5°C for various time intervals. The insulin permeability through such membranes as a function of glucose concentration was carried out, and the results of such studies are shown in Figure 6. Percent of insulin permeability decreased with storage time of membranes. However, an increased amount of insulin permeated through the membrane

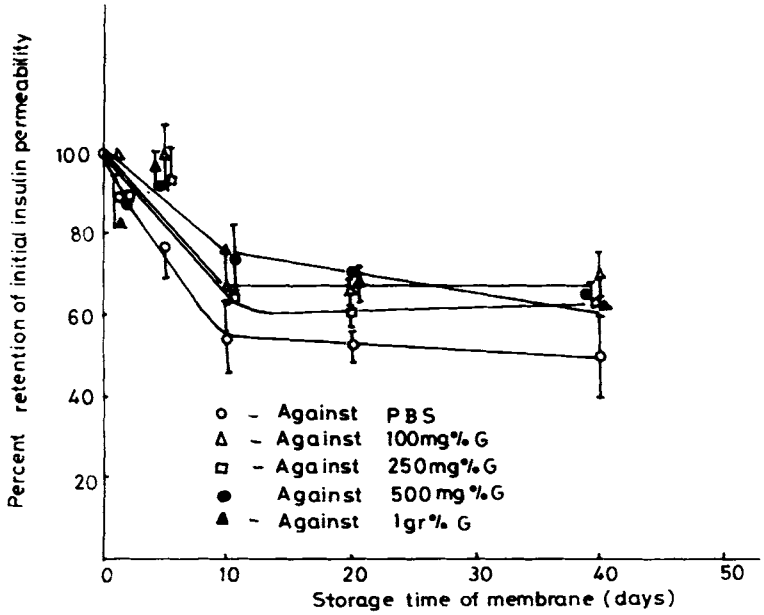

Figure 7 Stability of GOD-incorporated AA–PVA membrane as a function of storage time in PBS. Bar indicates 95% confidence limits.

against higher concentrations of glucose in all these cases, as is evident from Figure 6.

Percent retention of initial insulin permeability through GOD-incorporated AA–PVA membranes as a function of storage time was plotted against glucose concentration as represented in Figure 7. Membranes stored in buffer for 40 days retained 50% of their initial insulin permeability when dialyzed against PBS.

However, higher amounts of insulin permeability was observed with increase in glucose concentration as demonstrated in Figure 7. About 65–70% retention of initial insulin permeability had been indicated with various levels of glucose content in the dialysate. Thus, it is conceivable that the increase in glucose concentration had demonstrated an enhanced insulin transport through the membranes.

DISCUSSION

It is well known that a polymer having tertiary amino groups is protonated by a decrease in the pH value of a medium and, subsequently, the hydrophilicity of the polymer increases.[12,13] As expected, for AA-bare substrates, there is a slight increase in the water content of the membranes as pH decreases. However, the water content of PVA-blended membranes remained the same at various pH levels, as is evident from Figure 1. Thus, it appears that all these membranes have high swellability and the blendings of AA with PVA have not altered their water content significantly.

It is also clear (Fig. 4) that the percent of insulin permeability has increased with a reduction of pH in the medium. Thus, it appears that the transport of soluble polypeptides through the hydrogel systems are not wholly dependent upon the total water content alone. Rather, the ratio of free water in the total water content may be important for solubility and the subsequent diffusive permeability of such molecules through the membranes. Hence, it is conceivable that the amount of free water allowing the diffusive permeation of insulin may be different in the AA–PVA-blended systems, although the total water content is the same at different pH levels, proposing a varied membrane permeability. However, further studies are needed to understand the relationship between the diffusive permeability of peptides through the membranes at different states of water in them.

To determine whether the membrane's sensitivity to glucose concentration was sufficient to allow its use in an insulin-delivery system, the effect of glucose concentration on permeability of the membranes was investigated. Figure 5 demonstrates the permeability of PVA-blended AA membrane (AA : PVA = 75 : 25), which contains entrapped glucose oxidase, to insulin in PBS buffer, pH 7.4, against various concentrations of glucose. It is evident that as the glucose concentration of the system increases the insulin transport also enhances through the membrane. The membranes stored in PBS for prolonged periods have also demonstrated glucose-sensitized insulin permeability (Figs. 6 and 7).

The generation of acid by immobilized GOD in the presence of glucose has been measured by titrating with the base required to maintain constant pH. It appears that the rate of titrant addition has increased with glucose concentration (Fig. 1). Thus, this study demonstrates that the swelling and permeability of ionizable polymeric membranes containing GOD are sensitive to glucose concentrations in the physiologic range.

Therefore, it is conceivable that the production of gluconic acid catalyzed by the entrapped GOD is altering the pH of the microenvironment within the membrane, thereby causing the swelling or deswelling and consequent changes in permeability. These results are significant from two standpoints: that the membrane responds to glucose in a physiological concentration range and, second, that the membrane shows a graded response as a function of glucose concentration rather than an all-or-nothing response.

Several closed-loop polymeric systems are being developed for the increased release of insulin in the presence of excess glucose.[3,5,14] In one case, GOD has been immobilized within an insulin-containing polyamine membrane, where the enzyme converts glucose to gluconic acid, the acid protonating amine groups within the membrane. The electrostatic repulsion of the positively charged amine groups may cause an expansion to the membrane, resulting in an enhanced delivery of insulin. As the physiologic glucose concentration decreases in response to the released insulin, the membrane contracts, decreasing the rate of insulin release.[5,15] In another approach, the acid formed when external glucose reacts with the immobilized enzyme lowers the pH, which changes the solubility of insulin and the diffusional driving force.[2,16,17] It seems that similar mechanisms may hold true for the changes in insulin permeation to the present system as well.

The problem of membrane rupture, which could result in complete evacuation of insulin, is one of the drawbacks reported with AA systems.[5,6] It appears (from Table I) that the blending of PVA with

AA, which can improve the wet strength without altering their insulin-transport properties, may alleviate this problem. Further studies with resolved techniques and *in vivo* correlation remains before a suitable device based on these membranes can be developed. The preliminary reports presented here are encouraging and support the continued development of glucose-sensitive membranes for controlled delivery of insulin and may also benefit from ongoing research in biosensors.

CONCLUSION

Thus, it may be concluded that the permeation control of insulin in response to glucose concentration is possible by using a complex membrane, which consists of entrapped glucose oxidase, within the membranes of AA : PVA blends. The permeation of insulin through the blended membrane is reversed in response to the pH of the medium. Hence, it is conceivable that the reversible permeation control of insulin is possible due to the changes in glucose concentration by this complex membrane. Hydrogels like poly(vinyl alcohol), polyacrylamide, etc., are extensively studied for biomedical applications, due to their good biocompatibility and versatility.[4,18] However, there are many remaining questions about this polymer system, such as the biocompatibility or toxicology of these blends, which have to be evaluated. Further studies with animal models are also essential in the future to come to confirm these *in vitro* observations. Hence, much additional work remains before a usable device based on these membranes can be fabricated, but the system represents an interesting area of research for developing intelligent, self-regulating delivery devices.

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