

Is there a future in glucose-sensitive, responsive insulin delivery systems?

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Considerable amount of work has been directed towards the development of optimal glucose-responsive devices for insulin release. Hydrogel-based devices offer a wide range of parameters that can be tuned to obtain easy control of the insulin release properties. Taking advantage of their unique swelling and release properties, we may be able to achieve the desirable steady state and dynamic nature of insulin release. We review previous attempts at developing glucose oxidase-based insulin delivery systems, and we conclude that there are significant problems that must be solved for an adequate development of a device for treatment of diabetics.

Key words: Insulin release – Glucose-sensitive devices – Biosensors – Diabetes.

Diabetes mellitus is a disease characterized by an accumulating concentration of glucose in the blood and urine. The breakdown of the glucose regulation in the system can be attributed to several causes, including the death and malfunctioning of β -cells in the pancreas that produce insulin and abnormalities in the control mechanism by which the brain regulates insulin release. There are two types of diabetes, type I and type II. Type I diabetes is an autoimmune disease in which the β -cells die because of the immune action of the body [1]. Juvenile-onset diabetes is a form of this disease found in children and adults below forty years of age. This disease can be treated by insulin therapy only. Type II diabetes is very common in some populations and can be genetic. A special form of this disease, maturity-onset diabetes, is found in older subjects, especially those who are obese. This disease is caused by a malfunctioning of the control pathway of insulin secretion. A strict diet and an exercise regimen are sufficient to keep the disease under control in most cases.

Juvenile-onset diabetes is often called insulin-dependent diabetes mellitus (IDDM) while maturity onset diabetes is also known as non-insulin dependent diabetes mellitus (NIDDM). IDDM and type I diabetes are both characterized by the death of pancreatic cells under influence of antibodies and the two terms are often used interchangeably.

The treatment of diabetes is based on several patient characteristics, such as age, sex, body weight, activity and severity of the disease. Another very important factor is the insulin sensitivity of the patient. It is the ability of the blood glucose to respond to a specific dose of insulin. The insulin insensitivity of a patient goes up steeply with diabetes and continues increasing with time. Thus, the amount of insulin that has to be given to a patient also increases as time passes.

I. INSULIN DELIVERY DEVICES

1. Control methodology of insulin release

The pancreas secretes insulin under the action of a glucose stimulus. The mechanism by which the pancreas senses the pres-

ence of glucose and the β -cells are induced to secrete insulin is complex. The release process is modulated and inhibited by several blood factors that can be grouped under neurotransmitters, hormones and metabolites [1]. Increased activity of the β -cells is induced by glucose and other intermediates in the glucose catabolism cycle.

Modeling of the dynamics of insulin release in response to glucose input is difficult because of the influence of various factors at different stages of the release process. Also, some of the mechanisms involved in the loop are not understood perfectly. In spite of this, several models exist which predict insulin release with reasonable accuracy. Some of these models depended on experimental data obtained from animal experiments or *in vitro* studies on β -cell cultures [2-4]. Another class of models involved the adaptation of parameters to glucose-sensitivity data obtained directly from human subjects [5, 6]. These models have been discussed in details later.

Several control methodologies have been suggested to model insulin dynamics in the human body [7-10]. The primary aim of all these algorithms is to design a controller for insulin delivery that will be able to replicate the natural dynamics of glucose as closely as possible. These strategies are based on the minimization of some performance criterion, which, in most cases, was the deviation in glucose levels from a reference trajectory.

In order for the reader to appreciate (i) how insulin delivery devices (not just simple pharmaceutical systems) are put together; and (ii) appreciate how optimization of such systems is achieved, we will analyze below several such insulin devices.

2. Insulin devices

The multitude of insulin infusion devices can be classified as open-loop and closed-loop systems [11-18]. The most common open-loop device is the patient-programmed external pump [11]. These devices consist of a reservoir loaded with insulin to last for a 24- or 48-h period. A needle is inserted under the skin and attached to the pump by plastic tubing. A basal rate of

release of 1 unit/h is maintained under steady state conditions. The patient decides on the amount of insulin to be infused depending on activity and bolus and sets the pump accordingly. This infusion device has a considerable amount of advantage over injection regimens. However, for close tracking of normoglycaemic levels, it is necessary to monitor blood glucose carefully. This leads to a very demanding insulin program which most of the patients have been unable to sustain for a long time. Moreover, users of this device have reported a very high instance of ketoacidosis, a diabetes-related complication involving an increase in toxic ketones in the blood.

Implantable versions of the open-loop pumps have also generated some interest. These pumps were tested for basal delivery, variable dosing and treatments for ketoacidosis and insulin resistance. Mechanical failures and insulin aggregation and deactivation were the major reasons why these pumps were not marketed commercially. Other pump-related problems are site infections, inflammation and catheter obstruction [19-25].

Closed-loop systems, more specifically feedback-controlled devices such as the Biostator [22], do not require any form of patient intervention. The Biostator contains a system of catheters, one for continuous withdrawal of blood and the other for the infusion of insulin. The blood withdrawal catheter is coated with heparin to prevent clotting. It is also equipped with a glucose-oxidase sensor for *in situ* measurement of blood glucose levels. A computer uses a suitable algorithm for deciding on the amount of insulin to be released.

Such algorithms were developed to administer insulin based on extrapolated glucose concentrations calculated from the rate of change of glucose. The optimization problem solved to calculate the insulin requirement required the minimization of a cost function. The cost function was usually some form of the deviation of glucose concentrations from the reference trajectory. Integral square errors, integral absolute error and integral time average error were the most common form of the cost function. This also took care of a rapidly decreasing blood glucose concentration by leveling out the amount of insulin as hypoglycemia was approached. A mechanical pump was used to transport insulin intravenously. A 5-10 min delay was reported to exist between the withdrawal of blood and the infusion of insulin.

However, the Biostator did not perform as expected because the insulin stored in the device slowly deactivated with time and thus lost its immunoreactivity [25]. Early effluents from the device were reported to reduce glucose levels in a pancreatized dog from 100 to 75 mg/dl after an oral bolus. However, late effluents did not affect the blood glucose level significantly. This confirmed a device-related loss of insulin bioactivity. Added disadvantages included the fact that the machine required very specialized care. The sensor membrane had to be changed every three days and recalibration had to be done frequently. Also, the catheters had to be cleaned frequently to prevent clotting of blood.

The biohybrid artificial pancreas is another class of devices which have been studied. These devices contain β -cells enclosed within biocompatible, semipermeable membranes that are permeable to insulin and glucose [26]. Hollow fibers with

cells implanted on the inside surface are used for this purpose. Special care is taken to exclude immune cells so that the device is not rejected by the body. These devices are still in their early stages of development. They are dogged by the clumping and subsequent death of the islets. Also, there is an acute shortage of donors for healthy human pancreatic islets which makes large-scale culture of β -cells a difficult proposition. The use of porcine islets is a viable option but the immunoactivity of the human body towards these foreign cells has to be suppressed.

To better appreciate the behavior of all these systems, we need to examine how such devices are controlled and optimized to detect inadequacies in glucose absorption and trigger insulin delivery.

3. Insulin release models obtained from experimental evidence

We will examine some of the most important models of analysis of insulin delivery using complex delivery systems. Curry *et al.* [2] studied the dynamics of insulin release from rat pancreatic cells in response to glucose infusion. It was observed that the release rate showed a distinctive biphasic nature, an early fast rate followed by a slowly increasing rate which continued until glucose infusion was stopped. This phenomenon was explained by the presence of existing proinsulin, an inactive form of insulin, which can be converted directly into insulin and released immediately as the pancreas senses glucose. The second phase of response was due to the slow production and secretion of insulin directly from the pancreas.

Nomura *et al.* [3] subjected rat pancreatic cell cultures to step changes in glucose concentration. The responses from these cells were fit to a simple model of release that contained a proportional and a derivative response. This kind of response predicted that at the onset of change, the rate of insulin release would show a spike followed by an asymptotic increase to a maximum. The model suggested is represented in the Laplace domain by:

$$I(s) = \left(\frac{k_p}{1 + \tau_1 s} + \frac{k_d s}{1 + \tau_2 s} \right) G(s) \quad \text{Eq. 1}$$

where $I(s)$ is the insulin output rate and $G(s)$ is the glucose deviation from normal values. The parameters k_p and k_d are non-linear parameters that depend mostly on the size of the step input. The first order release was characterized by a time constant τ_1 of 12 min, while the derivative time constant τ_2 was 2 min.

This model was able to fit the data for the glucose ramp tests, as well. Pacini and Cobelli [4] studied insulin release using the C-peptide tracer studies. The dynamics of release was modeled using an initial Dirac delta function followed by a ramp function, the slope of which was proportional to the deviation of glucose from normal levels. The model suggested by these researchers is:

$$I(t) = I_0 \delta(t) + \gamma(G - G_B) \quad \text{Eq. 2}$$

where G_B is the basal level of glucose in the body.

4. Compartmental modeling

The most common models used to predict insulin release and glucose concentrations are compartmental models. The body is broken down into several compartments, such as the brain, the liver, the muscles and the pancreas. Each compartment is modeled using a set of equations which defines the transport of material in and out of it. Simple compartmental models using three compartments, one each for plasma glucose, remote insulin and insulin were used by Bergman *et al.* [5]:

$$\begin{aligned} \dot{G} &= -p_1 G - I_c(G + G_B) + P(t) \\ \dot{I}_c &= -p_2 I_c + p_3 I \\ \dot{I} &= -n(I + I_B) + u(t)/V_1 \end{aligned} \quad \text{Eq. 3}$$

Here, I and G refer to blood insulin and glucose concentration respectively. I_c represents the remote insulin compartment and the subscript B denotes basal levels. The insulin infusion term is shown as $u(t)$ and $P(t)$ represents the external glucose consumption, for example, a bolus. It can be seen that there was no provision for insulin feedback loop in this model. This means that glucose levels could not directly induce insulin release.

A more detailed model of the body was proposed by Sorenson [6]. Different compartments were used for the brain, peripheral tissue, liver, gut, kidneys, heart, lungs and the pancreas. Within each compartment, differentiation was made between the capillary and interstitial fluid spaces to capture the transport mechanisms and resistances. The parameters used in these models were directly taken from experiments involving normal subjects. Infusion from the pancreas was represented by a hyperbolic tangent function shown as follows:

$$I(t) = \tanh(k_1(G + k_2 \dot{G}^3 + k_3 \dot{G} - G_B)) \quad \text{Eq. 4}$$

There was a trade-off between the number of compartments and parameters (and thus the complexity of the model) against the performance of the model under all circumstances.

5. Control of insulin release

Several studies have proposed control methodologies for insulin delivery in the body. Ollerton [7] used the Bergman minimal model to propose a discrete optimal control strategy for closed-loop insulin infusion. The performance index used was an integral square error of the glucose deviations. Fisher [8] used a semi-open loop along with optimal control design for the same three-compartment model. Using the same cost criterion, the author reported that the best control was obtained with one initial injection followed by hourly injections of insulin. Sorenson [9] used an internal model control to design a controller based on a linear version of the previously stated compartmental model.

Parker *et al.* [10] suggested the use of model predictive control to design the dynamics of an insulin infusion pump. The basic model used is a 19th order compartmental model of the glucose-insulin system. Constraints on rates of release and low levels of glucose were taken into account. The controller

was reported to handle disturbances, such as, bolus input, effectively.

II. HYDROGEL-BASED, GLUCOSE SENSITIVE DEVICES FOR INSULIN DELIVERY

A novel method of insulin delivery in response to changes in glucose absorption is a series of devices based on hydrogels and usually containing glucose oxidase or other glucose-sensitive compounds.

1. Hydrogels as carriers

Hydrogels are cross-linked polymeric matrices that absorb large amounts of water and swell. These materials may be physically or chemically cross-linked to maintain their structural integrity. Hydrogels have the potential of being the materials of the future because of their remarkable compatibility with blood and tissue [27]. They have often been used in the preparation of soft contact lenses, coating dressings, superabsorbents, ion-exchange membranes and many other products that are used in our day-to-day life [28-30]. Scientists have been studying the possibility of using these materials as tissue substitutes, such as blood, cartilage, skin and muscles [31]. These materials have also been successfully used as scaffolds for the growth of live cells to be implanted into the human body [32].

Hydrogels have been studied extensively as drug delivery devices by many researchers [28, 31, 33- 35]. Environmentally sensitive hydrogels have been studied extensively as viable alternatives to traditional methods of drug delivery in the body. Site-specific drug delivery and tissue targeting are also some areas in which hydrogels have been tested successfully [36]. Vaginal [37] and other mucosal implants maybe used to transfer drug to areas rich in capillaries. As a result, the drug is released at a low rate but takes action within seconds of release. In short, such devices help to maintain a non-toxic, yet effective, amount of drug in the system at all times.

Hydrogels can be broadly classified into neutral and ionic depending on the type of repeating units or side chains on the polymer backbone. Neutral polymers, for example, poly(vinyl alcohol) (PVA), do not have any ionizable groups on their side chains. They can imbibe water and swell without being environment-sensitive. These materials can be used as drug delivery matrices, bioadhesives and superabsorbents. The swelling, dissolution and release properties of physically cross-linked PVA gels were studied extensively by Mallapragada and Peppas [38, 39]. Korsmeyer and Peppas [40] studied diffusion and release of water-soluble drugs through chemically cross-linked PVA. Ionic hydrogels are by far the more interesting class of hydrogels because of their stimuli-specific properties. These hydrogels swell or deswell depending on certain external conditions, such as temperature, pH, ionic strength [41] and buffer composition [42]. These changes can be also brought about by external force fields, for example, pressure [43], ultrasonic energy and electromagnetic radiation [44]. Changes in the configuration of the hydrogel are guided by thermodynamic principles. Swelling/deswelling may be due to phase changes [27], hydrophobic-hydrophilic interchanges or ionizations [35] that occur due to variations in the environmental conditions (see also *Figure 1*).

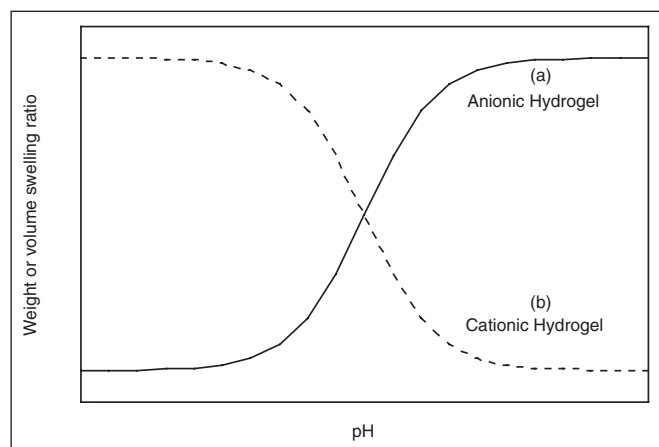


Figure 1 - Equilibrium swelling characteristics of anionic and cationic hydrogels. Anionic hydrogels swell in high pH solutions and remain collapsed in low pH (a), whereas cationic hydrogels show the opposite behavior, swelling at low pH and collapse at high pH (b).

Temperature-sensitive hydrogels have been studied by several researchers for uses in bioseparations, drug delivery and diagnostic uses [45, 46]. Temperature sensitivity of hydrogels is attributed to their lower critical solution temperatures (LCST) [47]. At temperatures above LCST, the hydrogel deswells and releases the solvent from its matrix. Hoffman [27] has reported the behavior of one such material, poly(*N*-isopropyl acrylamide) (PNIPAm) around its LCST. In this study, the effect on LCST of the copolymerization of PNIPAm with various hydrophobic and hydrophilic monomers has been noted. Polymers with LCST greater than 37°C can be used to carry ligands for antibodies into the human system for diagnostic purposes [48-50]. Once out of the body, the antibodies can then be released from the carrier by increasing the temperature.

2. Equilibrium and dynamic response of anionic hydrogels

pH-Sensitive hydrogels can revert between the swollen and the collapsed states with changes in pH of the surrounding medium. The swelling takes place via one of the following mechanisms: (i) changes in the hydrophobic-hydrophilic nature of the chains [41], and (ii) inter- and intramolecular complexation by hydrogen bonding [50]. Both of these processes involve protonation of the ionizable moieties on the polymer backbone or the side chains. In the first case, ionization results in the hydrophobic polymer network becoming more hydrophilic in nature and thus imbibing water into the matrix. In the second case, ionization results in the breaking up of hydrogen bonds that exist in the network in the unionized state. In both of these cases, the thermodynamics and the kinetics of the swelling process is affected considerably by several other factors, such as ionic strength of the medium, buffer composition, presence of salts [51].

pH-Sensitive hydrogels can be classified into two categories, anionic and cationic hydrogels depending on the nature of ionizable moieties on their backbones. Anionic gels usually contain acid groups which form -COO^- when the pH of the surrounding medium rises above its pK_a . Complexes formed by hydrogen bonds exist throughout the polymer maintaining it in the collapsed state. At high pH values, the polymer swells as

a result of the breaking up of complexes [50, 52, 53]. Notable examples of such polymers are poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAA). Copolymers of PMAA and PAA with poly(ethylene glycol) (PEG) [50] and poly(hydroxyethyl methacrylate) (PHEMA) [52] have been characterized for use as drug-releasing matrices. Copolymers of PMAA with PNIPAm show an interesting coupling of the temperature and pH-dependent swelling behavior [38].

3. Cationic hydrogels

A vast amount of work has been done on cationic hydrogels by Siegel and associates over the past two decades [41, 43-50]. The hydrogels that were used are copolymers of cationic monomers, dimethylaminoethyl methacrylate (DMAEM) [43, 44] or diethylaminoethyl methacrylate (DEAEM) [50], with methyl methacrylate (MMA). At pH values over the pK_a of the cationic groups, the copolymers are very hydrophobic and exclude water from the system. However, at pH values lower than the pK_a , the amine groups protonate to form NH_3^+ from -NH_2 . As a result, the gel becomes hydrophilic and absorbs water into its matrix. The formation of charged groups on the polymer backbone affect the osmotic balance between the hydrogel and the surrounding medium. Thus the swelling dynamics is very dependent on the ionic strength and the type of ions in the surrounding medium [47]. It has been observed that Donnan exclusion of hydrogen ions from the gel is a rate-limiting factor in water sorption. Exclusion can be overcome by the incorporation of weakly acidic buffering ions in the surrounding medium [48]. The unionized form of the buffer is capable of carrying the hydrogen ions to the swollen gel overcoming Donnan equilibrium. Common examples of such buffers are citrates and phosphates.

The equilibrium swelling characteristics of these materials show a sharp transition between the swollen and the relatively collapsed states [41, 43]. The effect of the methacrylate chain length on the equilibrium swelling was studied. Increasing the chain length amounts to an increase of the hydrophobic nature of the polymer. Though the critical pH was not affected, the maximum amount of water imbibed decreased significantly with the increase in hydrophobicity. Also, an increase in the MMA:DEAEM ratio affected both, the value of the critical pH and the uptake of water considerably.

Hariharan and Peppas [51] and am Ende *et al.* [52] studied the swelling behavior of cationic hydrogels for application as a drug-delivery material. DEAEM and diethylaminoethyl acrylate (DEAEA) were used as the cationic materials copolymerized with HEMA. Equilibrium swelling studies showed a gradual transition. The equilibrium water uptake was observed to be a strong function of the ionic strength of the medium, decreasing as the ionic strength was increased. Dynamic swelling studies showed that the rate of uptake of water increased with the decrease of pH.

4. Solute release from pH-sensitive hydrogels

The pH dependence of swelling of hydrogels can be used to deliver drug under specific condition in the human body [34]. Oral drugs are sometimes encapsulated or embedded in pH-sensitive hydrogels for taste-masking or for protection against

stomach acids. Blood has a weak buffering action. As a result, abnormalities involving small changes in blood pH can be sensed easily by hydrogels. Release of entrapped drug ensues immediately and at the right place for maximum effect.

Am Ende and Peppas [52] looked into the viability of poly(hydroxyethyl methacrylate-co-acrylic acid) as an oral drug carrier. It was found that the diffusion coefficients of drugs, such as oxprenolol, theophylline and proxiphylline were higher in hydrogels containing acrylic acid than those containing methacrylic acid. The rate of release increased with the increase in drug loading. They studied the release of several solutes from poly(methacrylic acid-g-ethylene glycol) hydrogels. For each solute, the permeation coefficients were calculated and related to the solute radii. Release was triggered by varying the pH between 7 and 4. Complexation was found to be a slow process requiring the polymer chains to rearrange. As a result, the release was not totally cut off at the low pH values.

Hariharan and Peppas [51] studied the release of solutes from P(DEAEM-co-HEMA) hydrogels. Rates of release were dependent on the pH of the medium. For example, insulin release at a pH of 10 was practically zero, whereas at a pH of 6 release occurred at a high rate. As the concentration of ionizable groups increased, the rate of release increased due to the increased repulsion between the charges. Release of caffeine from poly(DMEAEM-co-MMA) gels was reported by Siegel *et al.* [49]. The release data obtained at different pH was collapsed into a single master curve indicating that the release was primarily swelling controlled.

III. GLUCOSE-SENSITIVE POLYMERIC INSULIN INFUSION DEVICES

1. Development of environmentally- and glucose-sensitive hydrogel carriers

The aim of the traditional approach to drug delivery has been to maintain a constant amount of drug in the body. The delivery of drug in the body is controlled in such a way that its concentration in the body is high enough to have therapeutic effect without being toxic. Usually, it is desirable for the release rates to attain a steady rate irrespective of the concentration of drug within the device. This is called zero-order release.

However, for the release of insulin, a zero-order release is not optimal. As seen from the work of Nomura *et al.* [3], the optimal release (as observed from pancreatic cells) was biphasic. It is difficult to achieve a repeatable biphasic response from simple non-swelling polymeric devices. Also, the release of insulin should occur under elevated glucose conditions. Therefore, a glucose-sensitive swelling gel is most promising for the delivery of insulin.

The development of insulin delivery devices based on triggering of the delivery mechanism by the concentration of glucose and other components in the blood has become a subject of significant research interest. Development of insulin delivery devices and analysis of their response in various patient groups is an important subject for current medical research.

Glucose-sensitive insulin delivery devices have been studied for the self-regulatory release of insulin in the body. The most important component in such a device is a glucose sensor

that can sense the concentration of glucose in the blood and possibly, the rate of change of concentration. One method in which glucose-sensing membranes have been made is by the immobilization of Concanavalin A (Con A) on a glycosylated matrix of poly(glycosyloxyethyl methacrylate) [53, 54]. When glucose was absent in the medium, Con A forms complexes with the polymer backbone. In the presence of glucose, these complexes are broken up to release insulin. However, the gels lose their activity with time due to the leakage of Con A from the mesh. A second method is to use phenylboronic acid gels with hydroxylated insulin. Insulin forms a complex with the phenylboronic acid polymer [55]. The complex formed between phenylboronic acid and glucose is unstable. With the presence of amine groups in the polymer backbone enhances the formation of complexes and thus helps in the tuning of insulin release to a certain extent [56].

A different and more common principle behind glucose-sensitive matrices is the harnessing of the pH-sensitivity of hydrogels for glucose-responsive purposes [57, 58]. This is done by the incorporation of glucose oxidase (GOD) into the polymer matrix. GOD reacts with glucose to form gluconic acid which triggers the pH-sensitive swelling/deswelling of the hydrogel. These glucose-responsive materials were tested under different conditions. Bioerodible poly(ortho esters) were used by Heller *et al.* [59] for the release of insulin. It was observed that though the glucose-sensitivity of these membranes was not significant, an improvement could be brought about by incorporating tertiary amines into the structure. Blends of polyacrylamide and poly(vinyl alcohol) with GOD immobilized on the backbone were used by Chandy and Sharma [60] for studying insulin release. However, the stability of GOD in plasma reduces to 40% after 5 days. The authors suggest a membrane-bound reservoir device taking into consideration that the system has to be mechanically stable to support blood pressure.

Albin *et al.* [61, 62] studied polyacrylamide gels and poly(dimethylaminoethyl methacrylate) gels as glucose-sensitive matrices. Both of these hydrogels are cationic in nature. It has been found that macroporous gels showed greater glucose sensitivity compared to nonporous and microporous gels [61]. A model of release has been developed taking into account the kinetics of glucose reaction [62]. The kinetics of transport in the membranes has been found to be limited by the solubility of oxygen in the surrounding medium. Klumb and Horbett [63] have proposed a new geometry for the device which will overcome oxygen dissolution limitations. Insulin delivery from a pressurized chamber through a glucose-sensitive membrane has also been studied [64]. The flow through the membrane has been characterized as a function of the pH. However, the basal flow rate of insulin through the membrane is too high for practical purposes.

Further characterization of GOD-immobilized copolymers of hydroxyethyl methacrylate (HEMA) and dimethylaminoethyl methacrylate (DMAEM) cross-linked with tetraethylene glycol dimethacrylate (TEGDMA) has been done by Goldrath and Kost [65]. The dependence of swelling properties on cross-linking ratio and comonomer ratio has been found. It has been observed that in the absence of glucose there is very little

release of insulin. However, the gels are not truly reversible as collapse was a slow process compared to swelling. Therefore, insulin release will not be abruptly cut off in the absence of glucose.

Ishihara and associates have studied GOD immobilized polyamides [66, 67] and polymethacrylates [68] for glucose sensitivity and insulin permeability. The collapse of the hydrogels is again a slow process requiring about 50 h for total collapse. Also, as the variation of pH within the material is only between 6.2 and 6.6, insulin secretion is not stopped totally when glucose is removed from the medium.

Ito *et al.* [69] have studied the permeability of cellulose membranes grafted with poly(acrylic acid)(PAA). PAA has been further grafted with GOD to impart glucose sensitivity. In the absence of glucose, the grafts of PAA are uncomplexed and spread out blocking the pores of the cellulose membrane. As a result insulin is unable to diffuse out. Glucose induces the protonation of PAA and thus interchain complexes form. The chains coil revealing the pores for insulin diffusion.

2. Recent work from our laboratory

The specific goals of many of these newer devices are the development of new regimens for the treatment of diabetes in two specific cases:

- (i) a closed-loop feedback system which responds to changes in blood glucose concentration, the latter being based upon an implantable glucose sensor, and permits adjustment of the rate of insulin release provided by the pump; and
- (ii) a feedback system which responds to changes in blood glucose concentration and pH, leading to abrupt swelling and deswelling of a controlled drug delivery device based upon a glucose- and pH-sensitive hydrogel which releases insulin at triggered intervals.

We have developed [70-79] a series of novel self-regulated, glucose- and pH-sensitive gels for insulin delivery. In preparation for these systems, we have experimented with novel hydrogels in which the swelling ratio and the resulting mesh size change reversibly as a function of environmental parameters such as pH or temperature. These reversible changes allow for the release of drugs or the permeation of solutes depending on surrounding environmental conditions. Poly(methacrylic acid) (PMAA) exhibits interpolymer complexation with poly(ethylene glycol) (PEG) as the protons of the carboxylic acid groups on PMAA form hydrogen bonds with the ether groups on the PEG chain. This complexation forms only at pH low enough to insure substantial protonation of the carboxylic acid groups. Complexation of free chains of PMAA with PEG in solution has been studied. We have also shown that poly(dimethyl aminoethyl methacrylate) (PDMAEM) and PEG (henceforth designated as P(DMAEM-g-EG) and shown in *Figure 2*) exhibit the same type of hydrogen bonding, except that the pH dependence is such that the systems decomplex at low pH and complex at high pH values.

In our early research, Doyle *et al.* [80] have studied GOD-immobilized poly(methacrylic acid-g-ethylene glycol) (henceforth designated as P(MAA-g-EG)) hydrogels for pH sensitivity. In the hydrogels mentioned so far, insulin is released by diffusion. In this study, however, the hydrogels are expected to release

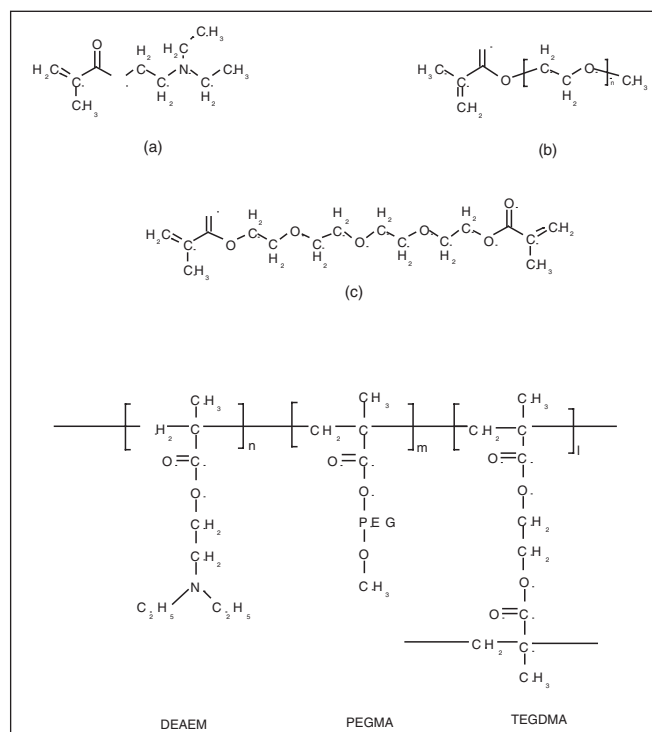


Figure 2 - Comonomers and cross-linking agents used in the formation of the hydrogel network. The pH-responsive monomer, diethylaminoethyl methacrylate (DEAEM, a), poly(ethylene glycol) monomethacrylate (PEGMA, b) for the grafts and tetra(ethylene glycol) dimethacrylate (TEGDMA, c) as the cross-linking agent are the main components of the network.

insulin by a rapid squeezing effect. Oscillatory pH-swelling studies show a promising rapid collapse which can be expected to release insulin by squeezing. Also, a model-based design procedure has been suggested to obtain a hydrogel having the most desirable release characteristics. In another paper [81], the authors have characterized the equilibrium and the dynamic swelling behavior of these anionic grafted hydrogels. The characteristic swelling behavior has been attributed to the formation of reversible complexes within the matrix.

Graft P(MAA-g-EG) and P(DMAEM-g-EG) copolymers were synthesized by free radical solution polymerization of MAA and DMAEM purified by vacuum distillation, and poly(ethylene glycol) monomethacrylate (PEGMA). Tetra-ethylene glycol dimethacrylate (TEGDMA) was added as a cross-linking agent in the amount of 2 wt% monomer. The reaction mixture was diluted with a 50:50 ethanol:water mixture and a 50:50 mixture of ammonium persulfate, and sodium metabisulfite was used as a redox initiator in the amount of 0.025 wt% monomer. The reaction was carried out in polypropylene vials under nitrogen for 24 hours at 37 °C. The cylindrical shaped polymer samples were dried in air and cut into 0.5 mm thick disks using a diamond blade rotary saw. All disks were washed in distilled/deionized water for a week and dried in air. In addition we have been able to carry out the same reaction under UV irradiation conditions at room temperature, using 0.5 % dimethylphenyl acetophenone as a photoinitiator. The latter provides free radicals for the reaction, is partially incorporated in the growing chains but does not affect the swelling behavior, and is known to be a non-toxic initiator [76, 77, 82, 83].

Hydrogels of the previous comonomers containing glucose oxidase have been prepared (see also *Figure 3*). Glucose oxidase was first functionalized. The ensuing monomer was reacted with DEAEM and PEGMA under the same conditions as above. In the preliminary studies, we have concentrated only on copolymers consisting of 1:1 ratio of DEAEM and PEG monomethacrylate. Upon polymerization such systems exhibit cationic behavior with hydrogen bonding characteristics similar to those of P(MAA-g-EG), albeit with a reversal in behavior. Swelling studies were performed in 50 ml of constant pH solution at 37 °C; the pH was adjusted by adding sodium hydroxide, hydrochloric acid, or sodium acetate [84-86]. Dynamic and equilibrium swelling was determined gravimetrically until an equilibrium value was reached. The swelling response of the gels under varying pH conditions was obtained by equilibrating samples in an acidic solution at 37°C then placing them into a basic solution (still at 37°C) for 45 min following weight changes gravimetrically. The cycle was repeated several times.

To examine the pH sensitivity, the equilibrium swelling behavior of P(MAA-g-EG), P(DEAEM-g-EG) and glucose oxidase-containing hydrogels was investigated as a function of pH. *Figure 4* shows the typical swelling behavior of complexing gels. The presence of hydrogen bonding in the complexes caused the network to be less hydrophilic because the carboxylic acid groups on the PMAA main chains were used in the complexes. A detailed behavior of glucose oxidase-containing P(DEAEM-g-EG) hydrogels under different degrees of cross-linking is presented in *Figure 5*. Clearly, these gels expand in acidic environment but collapse at pH values higher than about 6.8. This swelling ratio change from q of 1.2 (17% water) at pH 7.2 to $q = 17.5$ at pH 4.2 (95% water) indicates a change of the insulin diffusion coefficient by about two orders of magnitude. This means that the associated flux will be about 100 times higher at the pH values where gluconic acid is produced.

When analyzing the behavior of glucose-sensitive hydrogels for their response to varying glucose concentrations [86-90], the glucose oxidase of the hydrogel reacts with glucose to produce gluconic acid. At high concentrations of glucose, the pH of the glucose solution may be decreased at about 3.0. But even with low concentrations of glucose, enough gluconic acid is produced to decrease the pH of the glucose solution to 3.0.

The glucose-sensitive hydrogels synthesized were tested to determine if the glucose oxidase remained active with the synthesis techniques used. A disk of 3 mm thickness was cut with a razor blade from the cylindrical polymer sample before it was dried. The sample caused a drop of 3 pH units when placed in a 100 mg/dl solution of glucose for 15 min. Over this 15-min interval, the glucose oxidase of the polymer reacted with the glucose in the surrounding solution to produce gluconic acid (see also *Figure 6*). Thus, glucose oxidase remained active throughout the activation of the enzyme and polymerization procedures.

We have shown that P(MAA-g-EG) and P(DEAEM-g-EG) hydrogels exhibited pH-sensitive swelling behavior due to the formation and dissociation of complexes [68-74]. This inter-polymer complexation was due to hydrogen bonding between the hydrogels on the carboxylic acid groups of the MAA or the

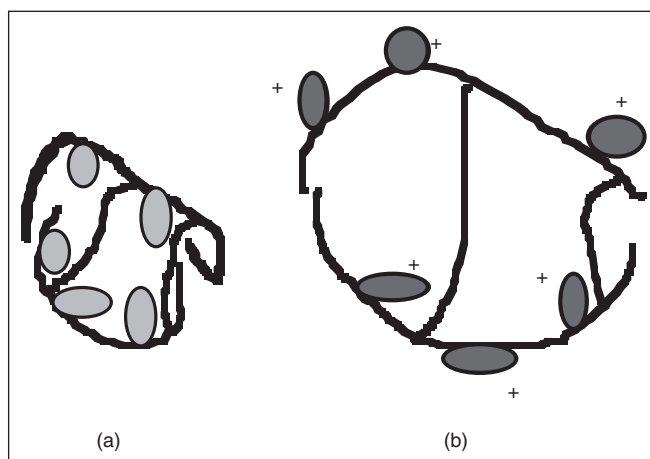


Figure 3 - Swelling mechanism in a cationic polymer. (a) at a high pH, the hydrophobic nature of the pendent side group prevent water from entering the system, (b) at a low pH, ionization of the side groups induces swelling in the system.

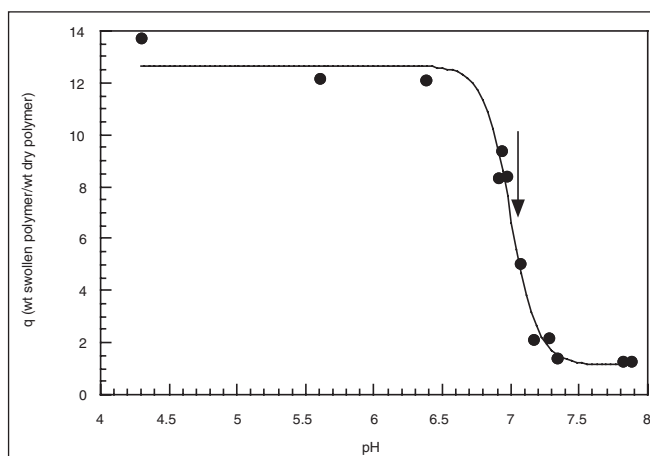


Figure 4 - Typical equilibrium swelling characteristic obtained from poly(diethylaminoethyl methacrylate-g-ethylene glycol) hydrogels immobilized with glucose oxidase and catalase. The arrow indicated the location of the transition pH. This characteristic curve is specifically for a hydrogel sample containing $X = 0.015$, 50 mol% DEAEM to PEGMA 200 and 5.413×10^{-4} g of GOD/g of polymer.

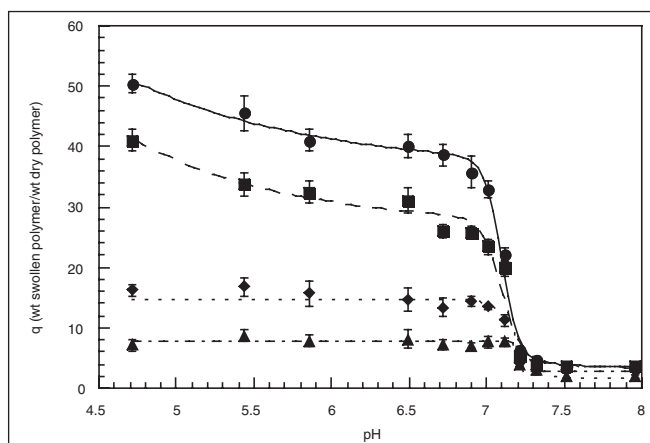


Figure 5 - Variation of equilibrium swelling characteristics based on the cross-linking ratio using in the formation of the network. The graphs shown are for $X = 0.005$ (●), 0.01 (■), 0.02 (◆) and 0.04 (▲).

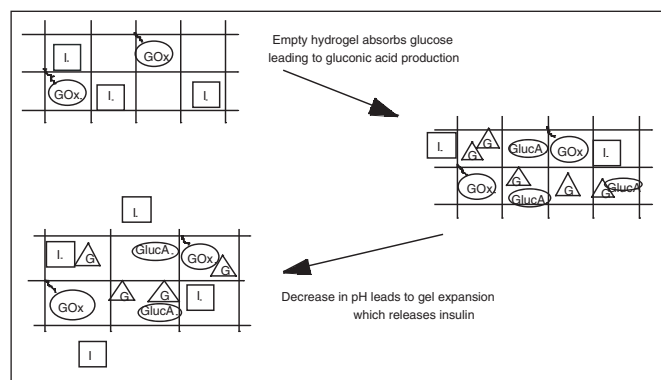


Figure 6 - Release mechanism from cationic hydrogels (a) the gel is a cross-linked network with glucose oxidase immobilized in it and insulin is physically entrapped into the system, (b) in the presence of glucose gluconic acid is produced, (c) increase in mesh size results in release of insulin.

amino groups of DEAEM and the etheric groups on the PEG chains and resulted in pH sensitive swelling behavior.

*
* *

The systems described above have a series of unique characteristics that make them exceptional candidates for insulin delivery:

- (i) They respond fast and abruptly to glucose concentration.
- (ii) They can respond to the rate of glucose production by incorporation of a polysaccharide/PEG moiety that entangles/disentangles due to interaction with glucose.
- (iii) They expand and contract abruptly due to pH changes.
- (iv) They are mechanically strong.
- (v) They contain PEG which can prevent protein aggregation.
- (vi) They can be prepared in a simple way using UV polymerization and can contain large amounts of insulin dispersed uniformly in the gel.

Yet, we believe that such systems, due to their natural complexity, are far away from any possible commercialization. Our work as well as that of others have shown that some of the major problems of these systems are:

- (i) Proof of reproducibility of glucose response over hundreds and thousands of cycles.
- (ii) Ability of oxygen to diffuse adequately into the glucose-sensitive gels.
- (iii) Biocompatibility of the carriers.
- (iv) Ability of the carriers to release the same amount of insulin even under decreasing insulin concentration gradients.
- (v) Final biodegradability of the carriers.

While such systems are promising from an academic point of view, we believe that they will require at least ten more years of intensive *in vitro* and *in vivo* studies before any serious discussion of their commercialization.

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