CONTROLLED SYSTEMIC DELIVERY OF INSULIN THROUGH THE OCULAR ROUTE

by

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1996
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Pahala Simamora entitled Controlled Systemic Delivery of Insulin Through the Ocular Route and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Pahale Simonon
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TO MY PARENTS
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The purpose of this research project is to develop biodegradable delivery devices for the controlled systemic delivery of insulin via the ocular route. Commercially available Gelfoam® absorbable gelatin sponge is used as the insulin carrier.

In the first part of this project, a simple gelfoam sponge is utilized in the fabrication of a matrix system for the delivery of insulin. The matrix system is developed by dispersing insulin in the interstitial pores of the gelfoam sponge. The \textit{in vivo} results show that the simple gelfoam device produces a prolonged delivery of insulin in rabbits when placed in the lower conjunctival cul-de-sac. The device yields a substantial improvement in insulin bioavailability and a significant prolongation in the duration of the pharmacological response (blood glucose lowering) than the eyedrops.

In the second part of this project, the gelfoam sponge is modified to obtain a more prolonged delivery of insulin. The modification is achieved by embedding a retardant into the pores of the gelfoam matrix. The \textit{in vivo} results show that the modified gelfoam device produces a longer duration of insulin delivery than the corresponding simple gelfoam device.

Overall, the proposed ocular devices make it feasible to obtain prolonged systemic delivery of insulin with the desired therapeutic levels without the risk of hypoglycemia. The application of the gelfoam device also significantly reduces the required frequency of dosing.
CHAPTER I. BACKGROUND

Insulin

Insulin consists of two polypeptide chains, A and B, which contain 21 and 30 amino acid residues, respectively. The two chains contain three cysteine linkages to form a bicyclic system with a 20-membered and an 80-membered ring (1). There is a slight difference in the amino acid composition of insulin from different animal species. The primary structures of bovine, human, rabbit, and porcine insulins are shown in Figure 1.1.

Insulin is the primary hormone responsible for controlling the storage and utilization of cellular nutrients. An important function of insulin is to facilitate the transport of glucose from the bloodstream into certain cells (2). Insulin provides a passageway for the glucose molecules to pass through cell barriers which are otherwise not penetrable.

Insulin controls the storage and utilization of cellular nutrients in a number of ways, including: acceleration of the transport of glucose from the blood into the cells, especially muscle cells, acceleration of the conversion of glucose into glycogen in the liver, retarding the conversion of glycogen into glucose, retarding the production of glucagon, stimulating the conversion of glucose and other nutrients into fatty acids, stimulating amino acid uptake and protein synthesis and inhibiting protein degradation in muscle and other tissues (3).
The exact mechanism by which insulin facilitates the transport of glucose molecules into cells is not completely understood. However, available information suggests that molecules of insulin somehow interact with receptors at the surface of certain cells and facilitate the transport of glucose into some cells but not into others (2). These insulin receptors have been found to be present in virtually all mammals (4).

Certain cells such as heart muscle, skeletal muscle, diaphragm muscle and adipose tissue require insulin for efficient glucose transport, while some other cells such as red blood cells, brain cells, and cells of the intestine, liver and kidney tubules permit the transport of glucose molecules across their barriers without the aid of insulin (2).

Insulin is produced and secreted by the islet β-cells of Langerhans of the pancreas from its precursor, proinsulin, and circulates in the blood as the free monomer. The monomer is believed to be most likely the biologically active form of insulin (5). The regulation of insulin secretion is directly determined by the level of sugar in the blood and is primarily based on a negative feedback mechanism. This mechanism involves the pancreatic β-cells and the concentration of glucose in the blood flowing to them (5).

In healthy humans, the pancreas detects changes in the concentration of blood glucose, and responds by altering its rate of secretion of insulin into the bloodstream. Insulin production by a normal, thin, healthy person is generally between 0.75 and 1.7 mg per day or approximately 8 to 21 µg/kg of body weight per day. Approximately half of this is secreted in the basal state and about half in response to meals (6).
Diabetes Mellitus

Diabetes mellitus or carbohydrate intolerance is "a group of syndromes characterized by high blood glucose concentration (hyperglycemia); altered metabolism of lipids, carbohydrates and proteins; and by an increased risk of complications from vascular disease" (5). There are two types of diabetes mellitus generally known: type I and type II.

Type I diabetes mellitus is also called insulin-dependent diabetes mellitus (IDDM). This condition is characterized by a lack of insulin secretion. This type of diabetes is also known as juvenile-onset diabetes because it occurs most commonly in people at young age (younger than 20) and it persists throughout life. Although people with type I diabetes appear to have certain genes that make them more susceptible, some triggering factor is required. Viral infection seems to be such a factor. β-cells of the pancreas are apparently destroyed by autoimmune damage (7). The trigger for the immune response still remains unknown.

Type I diabetic patients have to take exogenous insulin for their survival. The average therapeutic dose of insulin is 25 to 29 μg/kg of body weight per day with a range of 8 to 40 μg/kg per day (5). Obese patients generally require a higher dose.

Unless patients with type I diabetes get treatment, the symptoms can become severe. The low insulin level in the blood would accelerate the breakdown of the body's reserve of nutrients especially fat which is used by cells as an alternative energy source. This would in turn result in the production of organic acids called ketone bodies such as
acetoacetate and β-hydroxybutyrate. These ketone bodies cause ketosis, which lowers the pH of the blood and if severe enough can lead to diabetic coma and death. The catabolism of reserve fats and proteins also results in progressive weight loss.

Type II diabetes is also called non insulin-dependent diabetes mellitus (NIDDM). It is more common than type I and often occurs in people who are over 40 and generally overweight. Because it usually occurs later in life, it is also referred to as maturity-onset diabetes. Unlike type I diabetics, patients with type II diabetes are able to produce insulin, however, the cells in many parts of their bodies have become less sensitive to the presence of insulin (8). The clinical symptoms of these patients are usually mild and their hyperglycemia can usually be treated by dietary regulation, oral hypoglycemic agents as well as by insulin injection (5).

Treatment of Diabetes Mellitus

The most commonly practiced means of administering insulin to Type I diabetics (and some Type II diabetics) is by subcutaneous injection. Because of the immediate release of insulin administered by injection, patients must take food before or immediately after the injection to avoid hypoglycemia. It is unfortunate that insulin cannot be administered orally. This route of administration is not feasible due to its extensive enzymatic degradation in the gastrointestinal tract.
Insulin is usually injected into the subcutaneous tissues of the abdomen, buttock, anterior thigh, or dorsal arm. The fastest absorption is usually obtained from the abdominal wall, followed by the arm, buttock, and thigh (9). Injection into the same region (preferably to the abdominal wall) is currently favored to minimize day-to-day variability in the rate of absorption.

Although many patients are satisfied with their current insulin treatment, others are not. Some patients are unable or unwilling to inject themselves several times a day. A number of drawbacks associated with this route of delivery include pain, redness, itching, irritation, swelling and stinging at the site of injections. Atrophy of subcutaneous fat tissue at the injection site may also occur (5,10). Furthermore, the subcutaneously injected insulin shows significant individual variability in absorption (9,11).

The self-administration of insulin sometimes results in an overdose, which causes an abrupt decrease in blood glucose concentration (hypoglycemia). It is not uncommon that diabetes patients take too large a dose of insulin or forget to take food after an insulin injection. Mild hypoglycemia can be relieved by oral glucose. However, in severe cases it must be treated by glucose infusion and/or glucagon injection. If this condition is left untreated, it could result in coma and even death. On the average, in the U.S., one out of 20 cases results in death because the patient is alone and unable to get medical help (12).

To alleviate problems associated with multiple daily injections, increasing efforts have been directed towards developing implantable delivery systems which can supply insulin continuously for a prolonged period of time. These delivery systems include:
insulin pumps (13-16), microcapsules (17), biodegradable and non-biodegradable matrices (18-20) and the glucose-sensitive devices (17). Although the results obtained via these approaches are promising, several problems need to be solved. These include: (i) instability of the entrapped insulin, (ii) uncontrolled release, (iii) poor biocompatibility of the matrices, (iv) limited amount of insulin embedded in the delivery systems and (v) surgery to implant the devices and to recover non-biodegradable matrices.

Many other attempts have also been carried out to find alternate and more satisfactory pathways of insulin administration that are non-invasive. These alternate routes include rectal (21-25), vaginal (26-28), buccal (29-31), sublingual (32), tracheal (33-40), transdermal (41-46), and nasal (47-62). The latter is considered to be the most promising provided that the insulin is delivered to the nasal meatus which is located in the back of the nose above the turbinate (concha). One significant advantage of the administration of drugs through most of the absorptive mucosa in the various parts of the body is that it by-passes the presystemic degradation associated with gastrointestinal breakdown and hepatic metabolism. Mucosal membranes, particularly the nasal mucosa offer the potential of rapid absorption of drugs. This is especially useful in emergency situations. However, none of these approaches have achieved an acceptable level of practical utility and at present insulin has to be administered by injections.

In addition to these routes, the ocular route can also be considered for the delivery of insulin. The systemic delivery of insulin through the ocular route in animals has been well documented (63-78). Cristie and Hanzal in 1931 were the first to observe the
reduction of blood glucose level in rabbits after the instillation of insulin eyedrops into their conjunctival cul-de-sac (63). The lowering of the blood glucose concentration was found to be proportional to the dose of insulin instilled.

Following their finding, numerous studies on the systemic delivery of insulin through the ocular route have been conducted (64-78). Insulin has been successfully delivered by the instillation of different ophthalmic formulations into the conjunctival cul-de-sac. Yamamoto and coworkers (66) determined the extent and pathway of the systemic absorption of insulin after topical instillation to the albino rabbit eye. They found that the majority of the insulin is absorbed systemically from the nasal mucosa and to a lesser extent from the conjunctival mucosa.

Feasibility of the Ocular Route for Systemic Delivery

The feasibility of the systemic delivery of insulin or any water-soluble drug through the ocular route is based upon the dynamics of the lachrimal system. Anatomically, the lachrimal system consists of four structures: (i) lachrimal glands, (ii) lachrimal canaliculi, (iii) lachrimal sac, and (iv) nasolachrimal duct (79). The tear drainage apparatus of the eye is shown in Figure 1.2.

The dynamic lachrimal process is a highly efficient and rapid means of producing tears and draining them away from the eyes. This process results in the tendency of the eye to maintain the residence volume (steady state volume) of tear in the conjunctival cul-
Tear fluid secreted by the lachrimal glands is emptied on the surface of the conjunctiva of the upper eyelid. It washes over the eyeball and is swept up and collected in the lower conjunctival cul-de-sac by the aid of the wiperlike (blinking) action of the eyelids. From the lower conjunctival cul-de-sac, the fluid is drained into the lachrimal sac through the puncta and the lachrimal canaliculi. This movement is assisted by the blinking of the eye as well as the negative pressure in the lachrimal sac. Fluid from the lachrimal sac drains into the nasolachrimal duct which drains further into the inferior nasal meatus on the back of the nose. The nasal meatus is a highly vascular area, and hence drugs contained in tears are expected to be absorbed into the systemic circulation from this area (79).

Although ocular delivery of insulin from eyedrops is possible, it is currently neither practical nor controllable. One significant drawback of the instillation of insulin eyedrop is the variability of absorption. The primary sources of the variation are due to the differences in application, fluid run-off out of the eye (spillage), fluid loss due to blinking, and differences in the rate of tear production. This results in less than 5 percent bioavailability with a great deal of patient to patient and day-to-day variation. Another drawback of eyedrops is the short duration of activity.
Eyedrops are rapidly washed by tear fluid to the nasal meatus to produce rapid absorption. However, they are also rapidly washed away from this absorptive region by additional tears and the unabsorbed insulin is swallowed and degraded in the gastrointestinal tract.

The problems with high variability, low bioavailability, short duration as well as spillage in ophthalmic delivery can be overcome through the use of an ocular device. The application of insulin via an ocular device would be standardized as the device would simply have to be placed in the eye. In addition, an ocular device can also be utilized to deliver a medication at a rate that will insure complete and prolonged absorption of insulin.

A drug carrier that functions as a controlled release system can optimize the duration of activity of insulin. By delivering the drug at an optimal rate, this formulation can achieve therapeutic efficacy with a minimum amount of insulin and therefore avoid undesirable systemic side effects of hypoglycemia. In addition, with drugs that require chronic administration, such as insulin, the frequency of dosing will be greatly reduced. In practice, the development of the ocular device is achieved by incorporating or encapsulating the drug into a carrier, generally a polymeric material.
Polymers For Controlled Delivery

There are a number of polymers that can be used in controlled release systems (82-85). The ideal polymer should be sterilizable, easily removable, comfortable, stable, inexpensive, and either bioerodible or biodegradable. The latter obviates the need for removal of the device at the end of its therapeutic use.

One particular biopolymer that can be utilized as a drug carrier for ophthalmic drug delivery is gelatin. The use of gelatin and crosslinked gelatin for ocular drug delivery has been suggested previously (86,87). However, there have been limited studies done in characterizing this particular biodegradable polymer. Nadkarni and Yalkowsky successfully utilized gelatin to develop the ocular controlled delivery of pilocarpine (88).

Gelatin is a water-soluble protein which is obtained only from collagen. Chemically, it can be considered as any other protein having the same amino acid sequence as collagen but partially or completely lacking the secondary structure (peptide chain configuration) and the tertiary (triple helical) structure. In general, the approximate amino acid composition of gelatin is glycine (25.5%), proline (18%), hydroxyproline (14.1%), glutamic acid (11.4%), alanine (8.5%), arginine (8.5%), aspartic acid (6.6%), lysine (4.1%), leucine (3.2%), valine (2.5%), phenylalanine (2.2%), threonine (1.9%), isoleucine (1.4%), methionine (1.0%), histidine (0.4%), tyrosine (0.5%), serine (0.4%), cystine and cysteine (0.1%) (89).

Gelatin is widely available commercially and is very inexpensive. It is extensively utilized in the pharmaceutical field, particularly as a coating or shell in solid dosage forms.
It is also used as stabilizer, thickener, and texturizer in foodstuffs. Commercially, gelatin is obtained from animal tissues using simple extraction procedures. This water-soluble protein can be easily converted into the cross-linked, absorbable form either by heat treatment (90) or by chemical treatment (91-93).

The overall objective of this study is to develop a controlled release device for the systemic delivery of insulin via the ocular route in rabbits. The proposed controlled release delivery device utilizes the absorbable gelatin sponge (Gelfoam®, Upjohn Company) as the insulin carrier. The Gelfoam® absorbable gelatin sponge is a medical device intended for application to bleeding surfaces as a hemostatic (94). It is a water-insoluble, off-white, porous, pliable product prepared from purified pork skin gelatin USP granules (95).

Absorbable gelatin sponge swells but does not dissolve in water. It is able to hold many times its weight of water within its interstices. When it absorbs water (or tears) it becomes very soft. Its consistency resembles that of a wet cotton ball. This is an important factor in making it comfortable in the eye. As mentioned previously, Gelfoam absorbable gelatin sponge is biodegradable and thus will not have to be removed from the conjunctival sac at the end of the dosing period. It has been shown by Nadkarni and Yalkowsky to be an excellent drug carrier for controlled ocular pilocarpine delivery (88).

Overall, the proposed ocular controlled release device for the systemic delivery of insulin offers several advantages. These include:

1. Avoids gastrointestinal breakdown and first-pass metabolism
2. Reduces frequency of dosing

3. Improves patient compliance

4. The delivery of insulin can be terminated anytime simply by removing the delivery device from the eye.

Animal Model for In-Vivo Evaluation of Ocular delivery systems

The albino rabbit is the most frequently used animal model in ocular drug delivery research primarily because of ease of handling, relatively low cost, comparable size of its eyes to human and the abundance of information regarding its ocular biochemistry and physiology. The precorneal characteristics of the rabbit eye are similar to those in the human eye (96). The volume of tear fluid (7.5 µL) and the tear protein concentration (0.5 %) in albino rabbit eye are comparable to those in human subjects (7.0 µL and 0.7 %, respectively). The cornea does not seem to differ greatly in dimensions between rabbit and human. The diameter of the rabbit eye (15 mm) is relatively close to the diameter of human eye (12 mm). Table 1.1 compares the precorneal characteristics of rabbit and human (97).

On the other hand, rabbits have a lower tear volume turnover rate and retain aqueous solution somewhat longer than humans in the precorneal area (97). Rabbits also have lower frequency of blinking. Consequently, topically instilled formulation would be retained somewhat longer in the precorneal area of the rabbit for drug absorption to take place.
Assessment of Efficacy of the Ocular Device

The in-vivo evaluation of ocular drug delivery systems intended for systemic absorption involves topical instillation of the device in the lower conjunctival cul-de-sac of the rabbit followed by the quantitation of drug release. The drug release can be quantitated either by measuring the plasma concentration or by monitoring the changes in the pharmacological response of the drug. The latter is limited only to drugs that produce changes in physiological parameters such as blood glucose level, pupil size, body temperature, calcium level, blood count, etc. For these compounds, the measurement of the biological response appears to be much easier technically and more reliable therapeutically (37).

Many previous insulin absorption studies through various routes of administration did not attempt to measure the plasma insulin concentration directly. Instead, the pharmacological activity of insulin was monitored in assessing the efficacy of the formulations with respect to blood glucose levels (31,32,36,38,97-100). In this study, the same method is carried out to assess the relative efficacy of the ocular insulin delivery systems.
Table 1.1. Comparison of some precorneal characteristics in rabbit and human*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rabbit</th>
<th>Human</th>
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<tr>
<td>Tear volume</td>
<td>7.5 μL</td>
<td>7.0 μL</td>
</tr>
<tr>
<td>Tear turnover rate</td>
<td>0.53 μL/min</td>
<td>1.2 μL/min</td>
</tr>
<tr>
<td></td>
<td>(7 %/min)</td>
<td>(16 %/min)</td>
</tr>
<tr>
<td>Solution drainage rate</td>
<td>0.545/min</td>
<td>1.45/min</td>
</tr>
<tr>
<td>constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blinking frequency</td>
<td>2-5/min</td>
<td>15-20/min</td>
</tr>
<tr>
<td>Protein content</td>
<td>0.5 %</td>
<td>0.7 %</td>
</tr>
</tbody>
</table>

* from ref. 97
Figure 1.1. Primary structure of bovine insulin. Human (h), porcine (p) and rabbit (r) insulins differ from this in positions A8-10, human and rabbit insulins also in position B30. (from ref. 1).
Figure 1.2. The tear drainage apparatus of the eye.
CHAPTER II. IN VIVO EVALUATION OF SIMPLE GELFOAM DEVICES

MATERIALS AND METHODS

Materials

Gelfoam® sponge (absorbable gelatin sponge, USP, size 100) was obtained from the Upjohn Company (Kalamazoo, MI). Sodium bovine insulin (26.0 IU/mg) was purchased from Calbiochem Corporation (La Jolla, CA). Polyoxyethylene-20-stearyl ether (Brij-78) was obtained from Aldrich Chemical Company (Milwaukee, WI) and was used as an enhancer. A sterile 0.9% sodium chloride solution (normal saline) for intravenous use (Abbott Laboratories, North Chicago, IL) was used for the replacement of blood volume taken during sampling. Heparin sodium injection, USP (1000 U/mL) was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). A commercially available blood glucose monitoring system (ONE TOUCH® BASIC™) was obtained from Lifescan Company (Mountain View, CA) as a gift and was used to analyze the blood glucose concentration. All other solvents and chemicals were of reagent or HPLC grade and were used as received from commercial suppliers.

Preparation of Insulin Delivery Systems

Insulin eyedrop formulations of different concentration were prepared by dissolving either 0.5 mg or 1.0 mg of sodium insulin along with 20 μg of Brij-78 in a 30
μL solution of 50 mM isotonic phosphate buffer pH 7.4. Eyedrop solutions were prepared fresh immediately before the experiment.

A gelfoam disc of approximately 4 mm diameter and 2 mm height was punched from a slab of gelfoam sponge with a hole punch and accurately weighed using a Metler (Model AE163) analytical balance. To prepare matrices, either 0.5 mg or 1.0 mg of sodium insulin and 20 μg of Brij-78 were dissolved in a 30 μL of a solution of 30% (v/v) ethanol in water. The mixture was then placed on and sorbed into the gelfoam sponge. The wet devices were then stored under vacuum for 72 hours to evaporate the solvents. The dried matrices were weighed to confirm their insulin content.

In vivo Evaluation

Animals: Fourteen New Zealand white rabbits of either sex (Myrtle’s Rabbitry, Inc., Thompson Station, TN) of approximately equal weight (3.0 kg) were used in the in-vivo experiments. Each experiment was carried out in three rabbits. All animals were fasted overnight prior to the experiment and during the experiment but had free access to water. They were neither anaesthetized nor restrained throughout the experiment.

Dosing: The four insulin delivery systems evaluated in the in-vivo study are summarized in Table 2.1. For instillation, the lower eyelid was pulled slightly away from the globe and either the solution or the eye device was instilled in the center of the lower conjunctival cul-de-sac with care to avoid direct contact with the eye. Eyedrops were delivered from a PipetteMan pipettor using a plastic disposable pipette tip. The lower
eyelid was returned to its normal position immediately following instillation of either eyedrops or the eye device. The baseline response values for the device were obtained by instilling a placebo containing Brij-78 alone.

Sampling Procedure: The rabbit jugular vein was cannulated with polyethylene tubing (PE-90) for the collection of blood samples and for replacement of blood volume with heparinized normal saline. The glucose concentration was first determined prior to the application of the delivery systems. After dosing, approximately 0.1 mL of blood was collected at predetermined times. The volume of the blood taken was replaced with an equal volume of heparinized normal saline.

Blood glucose assay: One drop of the fresh blood sample obtained from the jugular vein was carefully applied onto a ONE TOUCH® test strip containing glucose-sensitive reagents. The intensity of the blue color formed correlates with the concentration of glucose in the blood sample. The color was quantitated by ONE TOUCH® BASIC™ blood glucose meter, which gives a reading of glucose concentration (mg/dL) in the blood. This type of blood glucose analyzer can determine blood glucose concentrations in the range of 0-600 mg/dL with a ±3% precision.

Statistical analysis of data: All data were analyzed with Student’s t-test for two values and analysis of variance (ANOVA) for more than two values. A p value of 0.05 or less was considered significant.
RESULTS AND DISCUSSION

The hypoglycemic response to insulin is quantitated by the measured blood glucose concentration after dosing as a percentage of the initial concentration. The advantages of using pharmacological response data with insulin are the simplicity of the blood glucose determination and the fact that this parameter is the most relevant to therapy.

Figure 2.1 shows the blood glucose levels of three individual rabbits for 12 hours after receiving placebos. The average deviation of the values from the initial values is about 20 percent and there is no systematic change with time. Figures 2.2 and 2.3 show the blood glucose levels of rabbits for 4 hours after receiving eyedrops 1 and 2 containing 0.5 mg and 1.0 mg of insulin, respectively. In each case, the three rabbits responded in a similar manner. Within 2 hours after an initial drop, the blood glucose levels returned to their baseline values. For purposes of comparison, the mean values of Figures 2.1 through 2.3 are given in Figure 2.4.

It is clear from Figure 2.4 that the instillation of both eyedrops 1 and 2 results in a rapid decrease in the blood glucose level followed by a rapid return to normal. Eyedrop 1 produces a relatively small blood glucose reduction while eyedrop 2 with higher insulin concentration produces a greater blood glucose depression. The reduced sugar level of device 2 is also slightly longer. However, the time required to reach the minimum level remains about the same.
The insulin response profiles obtained in this study from eyedrops are similar to those observed by numerous investigators (63-70,74,77). It should be noted that, at present, the only means of administering insulin systemically via the ocular route is by the instillation of a solution in the lower conjunctival cul-de-sac. In general, eyedrops have several limitations such as pulse entry and a short duration of activity. Pulse entry of insulin can produce hypoglycemia while a short duration of activity requires a high dosing frequency in order to obtain a uniform blood sugar level. For chronic diseases such as diabetes mellitus, this is not acceptable.

Figures 2.5 and 2.6 show the blood glucose levels for 12 hours after instillation of devices 1 and 2 containing 0.5 and 1.0 mg of insulin, respectively. As in the case of the eyedrop data, the mean values of these data are given in Figure 2.7. A slight discomfort was observed initially after the device was instilled (animals blinked two to three times in about 4 seconds). This is believed to be due to the instillation of the dry device. However, as the device becomes hydrated it softens and the animals stopped blinking after 4 seconds. There were no signs of irritation such as redness and lachrimation observed during the remainder of the experiment. In all cases, the gelfoam insert degraded in the eye within 24 hours.

Figure 2.7 clearly shows that when the gelfoam device is used, it produces a more gradual initiation and a more substantial prolongation of the glucose lowering response than the eyedrops. As shown in the figure, device 1 produces a somewhat large blood glucose decrease approximately one hour after instillation of the device. It is also clear
that the reduced blood sugar level is steadily maintained for nearly 6 hours. Figure 2.7 also shows that device 2 with higher insulin concentration yields an even greater blood glucose reduction as well as a longer duration of the response. The reduced sugar level is maintained for approximately 8 hours in near steady-state manner.

Overall, the application of the gelfoam device in administering insulin systemically via the ocular route produces a less abrupt decrease in blood glucose while maintaining steady reduced glucose levels for a significantly longer period of time than the eyedrops. The ability of the device to prolong the activity of insulin greatly reduces the required dosing frequency of insulin while producing more uniform blood sugar levels and greater patient convenience. Also the device can be removed at will by the patient if the blood glucose level becomes too low.

Assessment of In-Vivo Efficacy

The relative effectiveness of the liquid drops and the gelfoam inserts for the systemic administration of insulin were compared based on four indicators of efficacy: (i) the area above the glucose concentration-time curve (AAC), (ii) the time required to reach 80% of initial glucose level (onset time), (iii) the time required for the reduced glucose level to return to 80% of initial (offset time) and (iv) the duration of response (blood glucose lowering). The time during which blood glucose levels are maintained below 80% of the initial glucose level was chosen as a reference point in the calculation of the duration of the response. The indicators of efficacy from eyedrops as well as the devices
are summarized in Table 2.2. The AAC values and the duration of blood glucose lowering are also plotted for comparison in Figures 2.8 and 2.9, respectively.

The AAC is assumed to be an indicator of the extent of systemic absorption (bioavailability) of insulin from the delivery system. The AAC’s for both ocular delivery systems with different insulin concentrations are shown in Figure 2.8. Based on the AAC values, each of the gelfoam devices is about 7 times more effective than its eyedrop counterpart.

It is also obvious from Figure 2.8 that, in all cases, formulations with higher insulin concentration produce larger AAC values, suggesting that the glucose lowering response is dose-dependent. However, the dose-response relationship is not linear. When the amount of insulin is doubled in either eyedrop or device formulation, the AAC is increased by about 40%. This non-linearity was also observed by other investigators (32,37,101).

The onset time as well as the offset time from the eyedrop formulations, as shown in Table 2.2, are significantly faster than the corresponding gelfoam devices. Figure 2.9 shows the duration of blood glucose lowering obtained from the four delivery systems. As shown in the figure, the duration of blood glucose lowering from the gelfoam device containing 0.5 mg of insulin lasted for nearly 7 hours compared to about 0.4 hour from the liquid drops of the same insulin concentration. The response obtained from the device containing 1.0 mg of insulin lasted for about 10 hours compared to 0.9 hour from the corresponding eyedrops (see also Table 2.2). Undoubtedly, the gelfoam devices are capable of prolonging the duration of the ocular delivery of insulin.
The superiority of the eye device over the eyedrop is probably due to the ability of the device to release insulin in a prolonged manner into the tears in the lower cul-de-sac. Normal tears and insulin contained in tears in the cul-de-sac are then drained into the nasal meatus. Since the normal turnover rate of tears is relatively constant, it is believed that the rate of insulin absorption is also relatively constant. This in turn gives an efficient, prolonged, and relatively uniform absorption of insulin.

On the other hand, upon instillation of eyedrops, a substantial fraction of the instilled amount is wasted due to spillage. The fraction that remains increases the fluid volume in the lower cul-de-sac and thus is rapidly washed by tear fluid to the nasal meatus to yield rapid absorption. In the nasal meatus, however, the fraction of instilled solution also rapidly washed away from the absorptive region by additional tears and the unabsorbed insulin is swallowed and destroyed in the gastrointestinal tract. This results in inefficient (low) absorption as well as short duration of activity of insulin.

Overall, the application of the gelfoam absorbable gelatin sponge has been shown to be an effective means of administering insulin systemically in a controlled manner in rabbits when placed in the lower conjunctival cul-de-sac. The gelfoam insert produces substantial improvement in insulin bioavailability and significant prolongation in the duration of blood glucose lowering over the eyedrop formulations.
Table 2.1. Ocular delivery systems of insulin evaluated in the in-vivo study

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Amount of insulin (mg)</th>
<th>Amount of enhancer (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyedrop 1</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>Eyedrop 2</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>Device 1</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>Device 2</td>
<td>1.0</td>
<td>20</td>
</tr>
</tbody>
</table>
Table 2.2. Summary of pharmacological response parameters of insulin administered ocularly in eyedrops and gelfoam devices.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Insulin (mg)</th>
<th>Enhancer (µg)</th>
<th>Retardant (mg)</th>
<th>Area above the curve (AAC, % hr)</th>
<th>Onset time(^1) (hrs)</th>
<th>Offset time(^2) (hrs)</th>
<th>Duration of blood glucose lowering(^3) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyedrop 1</td>
<td>0.5</td>
<td>20</td>
<td>0</td>
<td>54.1 (±11.5)</td>
<td>0.3</td>
<td>0.8</td>
<td>0.4 (±0.1)</td>
</tr>
<tr>
<td>Eyedrop 2</td>
<td>1.0</td>
<td>20</td>
<td>0</td>
<td>81.3 (±10.3)</td>
<td>0.2</td>
<td>1.1</td>
<td>0.9 (±0.3)</td>
</tr>
<tr>
<td>Device 1</td>
<td>0.5</td>
<td>20</td>
<td>0</td>
<td>405.4 (±24.6)</td>
<td>0.6</td>
<td>7.3</td>
<td>6.7 (±0.8)</td>
</tr>
<tr>
<td>Device 2</td>
<td>1.0</td>
<td>20</td>
<td>0</td>
<td>552 (±93.4)</td>
<td>0.9</td>
<td>11.1</td>
<td>10.2 (±0.4)</td>
</tr>
</tbody>
</table>

\(^1\) time required to reach blood glucose level of 80% of initial  
\(^2\) time required for the reduced blood glucose level to return to 80% of initial  
\(^3\) time during which blood glucose level is maintained below 80%
Figure 2.1. Blood glucose concentrations after ocular administration of the placebos.
Figure 2.2. Blood glucose concentrations after ocular administration of eyedrop solution containing 0.5 mg of sodium insulin and 20 μg of Brij-78 (Eyedrop 1).
Figure 2.3. Blood glucose concentrations after ocular administration of eyedrop solution containing 1.0 mg of sodium insulin and 20 μg of Brij-78 (Eyedrop 2).
Figure 2.4. Mean blood glucose concentrations after ocular administration of placebo (×), Eyedrop 1 (□), and Eyedrop 2 (○). Each value represents the average of three rabbits.
Figure 2.5. Blood glucose concentrations after instillation of gelfoam device containing 0.5 mg of sodium insulin and 20 μg of Brij-78 (Device 1).
Figure 2.6. Blood glucose concentrations after instillation of gelfoam device containing 1.0 mg of sodium insulin and 20 μg of Brij-78 (Device 2).
Figure 2.7. Mean blood glucose concentrations after instillation of placebo (×), Device 1 (■), and Device 2 (●). Each value represents the average of three rabbits.
Figure 2.8. Area above the glucose concentration-time curve (AAC) of insulin delivered ocularly in different delivery systems.
Figure 2.9. Duration of response (blood glucose lowering) of insulin delivered ocularly in different delivery systems.
CHAPTER III. *IN VIVO* EVALUATION OF THE MODIFIED GELFOAM DEVICES

MATERIALS AND METHODS

Materials

Gelfoam® sponge (absorbable gelatin sponge, USP, size 100) was obtained as a gift from the Upjohn Company (Kalamazoo, MI). Sodium bovine insulin (26.0 IU/mg) was purchased from Calbiochem Corporation (La Jolla, CA). Polyoxyethylene-20-stearyl ether (Brij-78) and cetyl alcohol were obtained from Aldrich Chemical Company (Milwaukee, WI). Brij-78 was used as an enhancer while cetyl alcohol was used as a retardant. A sterile 0.9% sodium chloride solution (normal saline) for intravenous use (Abbott Laboratories, North Chicago, IL) was used for the replacement of blood volume taken during sampling. Heparin sodium injection, USP (1000 U/mL) was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). A commercially available blood glucose monitoring system (ONE TOUCH® BASIC™) was obtained from Lifescan Company (Mountain View, CA) as a gift and was used to analyze the blood glucose concentration. All other solvents and chemicals were of reagent or HPLC grade and were used as received from commercial suppliers.
Fabrication of the Modified Gelfoam Devices

A gelfoam disc of approximately 4 mm diameter and 2 mm height was punched from a slab of gelfoam sponge with a hole punch and accurately weighed. Matrices containing different amount of sodium insulin and 20 μg of Brij-78 were prepared according to the procedure described earlier. The wet matrices were stored under vacuum for about 20 minutes. To prepare matrices containing different amounts of retardant, 20 μL of ethanol solution containing either 0.1 mg or 0.2 mg of cetyl alcohol was placed on and sorbed into the gelfoam sponge containing insulin and enhancer. The wet matrices were then stored under vacuum for at least 72 hours. The dried matrices were weighed using a Metler (Model AE163) analytical balance to verify their weight.

In vivo Evaluation

Animals: Eleven New Zealand white rabbits of either sex (Myrtle's Rabbitry, Inc., Thompson Station, TN) of approximately equal weight (3.0 kg) were used in the in-vivo experiments. Each experiment was performed at least in three rabbits. All animals were fasted overnight prior to the experiment and during the experiment but had free access to water. They were neither anaesthetized nor restrained throughout the experiment.

Dosing: The four modified gelfoam devices evaluated in the in-vivo study are summarized in Table 3.1. For instillation, the lower eyelid was pulled slightly away from the globe and the device was placed in the center of the lower conjunctival cul-de-sac with
care to avoid direct contact with the cornea. The lower eyelid was returned to its normal position immediately following instillation.

**Sampling Procedure:** The rabbit jugular vein was cannulated with polyethylene tubing (PE-90) for the collection of blood samples and for replacement of blood volume with heparinized normal saline. The glucose level was first determined prior to the instillation of the devices. After instillation, approximately 0.1 mL of blood was collected at predetermined time intervals. The volume of the blood taken was replaced with an equal volume of heparinized normal saline.

**Blood glucose assay:** One drop of the fresh blood sample obtained from the jugular vein was carefully applied onto a ONE TOUCH® test strip containing glucose-sensitive reagents. The intensity of the blue color formed correlates with concentration of glucose in the blood sample. The color was quantitated by ONE TOUCH® BASIC™ blood glucose meter, which gives a reading of glucose concentration (mg/dL) in the blood. This type of blood glucose analyzer can determine blood glucose concentrations in the range of 0-600 mg/dL with a ± 3% precision.

**Statistical analysis of data:** All data were analyzed with Student’s t-test for two values and analysis of variance (ANOVA) for more than two values. A p value of 0.05 or less was considered significant.
RESULTS AND DISCUSSION

In the preceding chapter, the gelfoam absorbable gelatin sponge has been shown to be a useful vehicle in prolonging the systemic delivery of insulin via the ocular route. The device alleviates several drawbacks such as pulse entry and short duration of activity that are associated with the eyedrops. Consequently, hypoglycemia is avoided and the frequency of dosing is reduced. In this study, the gelfoam sponge was modified to obtain even more prolonged delivery of insulin. The modification is carried out by embedding a retardant (cetyl alcohol) into the pores of the matrix. The fabrication process described above is very simple and inexpensive.

Observations

Figure 3.1 shows the blood glucose levels of three individual rabbits for 14 hours after instillation of device 3 containing 0.5 mg of insulin embedded with 0.1 mg of cetyl alcohol. The response profiles from the three rabbits are comparable. The blood sugar levels decrease slowly and reach the minimum in about 3 hours. The reduced levels are somewhat steadily maintained for a long period of time and return to normal within 14 hours after the instillation.

Figure 3.2 shows the blood sugar levels for each of two rabbits for 14 hours after the instillation of device 4 containing 0.5 mg of insulin and 0.2 mg of retardant. As can be seen from the figure, there is a comparatively more gradual initiation of blood sugar
reduction than the devices with half the amount of retardant. It is also obvious from the figure that their maximum reduced sugar levels are not as low.

Figure 3.3 shows the blood sugar levels of three rabbits for 24 hours after the instillation of device 5 containing 1.0 mg of insulin and 0.2 mg of retardant. Like the devices containing half the amount of insulin and the same amount of retardant (device 4), their blood sugar levels decrease slowly over time. The time required for the reduced sugar levels to return to normal is longer (20 hours).

Figure 3.4 shows the blood sugar levels for 16 hours after instillation of device 6 containing 2.0 mg of insulin and 0.2 mg of retardant. Again the blood glucose concentration-time profiles from the two rabbits are comparable in magnitude and similar in shape. The blood glucose drops are quite low and the maximum reduced levels are maintained in a near steady fashion for a relatively long period of time. However, compared to the devices containing less insulin and equal amount of retardant, their reduced blood sugar levels return to normal sooner.

A slight discomfort (animals blinked three times in about four seconds) was noticed immediately after the instillation of the devices. Animals stopped blinking after four seconds. The blinking could be due to the irritation by the dry device and disappears as the device becomes hydrated by the tears. In all cases, no physical signs of irritation (e.g., redness and lachrimation) were observed in the animals throughout the experimental period. The modified gelfoam devices were degraded in the eye within 24 hours. This biodegradation time was comparable to that for the simple gelfoam device.
**Effect of Retardant Concentration**

To examine the effect of retardant concentration on insulin delivery, the mean values of Figures 3.1 and 3.2 are shown in Figure 3.5 together with the mean value of the placebo and the device containing 0.5 mg of insulin without retardant (device 1) as determined in the previous study. The figure compares the results of the devices containing equal amount of insulin (0.5 mg) and varying amount of retardant. The amount of retardant ranges from 0 to 0.2 mg. It is obvious from Figure 3.5 that the devices embedded with retardant produce a more gradual initiation of glucose reduction as well as longer duration of the reduced blood glucose levels compared to the device with no retardant. It is also clear that the reduced sugar levels of devices with retardant are not as low as the reduced level produced by the device with no retardant.

Figure 3.5 also shows that device 1 (with no retardant) produces the largest blood sugar depression as well as the shortest period for the reduced sugar level to return to normal. Device 3 with 0.1 mg of retardant produces somewhat less depression but longer duration of reduced sugar level while device 4 with the highest amount of retardant (0.2 mg) yields the least blood glucose depression and the longest duration of reduced glucose. Note that the blood glucose of device 3 remains below 80% of normal for 14 hours after instillation of the device.

As in the case of the previous chapter, the relative efficacies of the devices are compared on the basis of four quantitative parameters: (i) the area above the glucose
concentration-time curve (AAC), (ii) the time required to reach 80% of initial level (onset time), (iii) the time required for the reduced glucose level to return to 80% of initial level (offset time) and (iv) the duration of blood glucose lowering. The AAC is utilized as an indicator of the extent of systemic absorption (bioavailability) of insulin from the devices. The overall results of the biological response parameters of insulin from the devices are summarized in Table 3.2. The response parameters from devices without retardant (determined in chapter 2) are included in the table for comparison.

Table 3.2 shows that the three devices containing equal amounts of insulin and varying amounts of retardant yield similar AAC values suggesting similar bioavailability from the three devices. It is also apparent from the table that device 1 with no retardant produces the shortest onset and offset times while both devices 3 and 4 with retardant yield longer onset and offset times. Device 4 with the highest amount of retardant unexpectedly yields a similar onset time to device 3 which has a lower amount of retardant. However, the offset time of device 4 is longer than device 3. Table 3.2 clearly shows that the duration of blood glucose lowering increases as the amount of retardant increases. The increase is in the following order: device 1 without retardant, device 3 with 0.1 mg of retardant and device 4 with 0.2 mg of retardant.

Figure 3.6 shows the mean of the values of Figure 3.3 (device 5) together with the mean values for the placebo and the corresponding device containing 1.0 mg of insulin alone (device 2) determined in the previous study. As shown in the figure, device 2 without retardant produces a quicker blood glucose decrease and a shorter duration of
reduced glucose level. On the other hand, device 5 shows a more gradual blood glucose decrease and a much longer duration of the reduced blood glucose level. It is also obvious that the reduced blood glucose level of device 5 is not as low. Both device 2 and device 5 (shown in Table 3.2) yield similar AAC, suggesting also similar bioavailability. It is also clear from the table that device 5 yields longer onset and offset times than device 2. Likewise, the duration of blood glucose lowering is longer for device 5 than device 2. In general, the addition of retardant produces no significant loss in bioavailability while increasing the duration of activity.

**Effect of Insulin Concentration**

Figures 3-7 presents the mean of the values of Figure 3.4 for the 2 mg insulin device with 0.2 mg of retardant (device 6) together with the mean values of device 4 and device 5. The figure compares the response profiles of devices with varying insulin concentration and equal amount of retardant. Device 4 which contains the least amount of insulin (0.5 mg) produces the least blood glucose depression. Device 5 with twice the amount of insulin (1.0 mg) gives somewhat larger blood glucose depression while device 6 with the highest insulin concentration (2.0 mg) produces the largest reduction. Apparently, the blood glucose level is progressively depressed as the insulin concentration increases. This increase in AAC with increasing insulin concentration is shown in Table 3.2. Again note that the dose-response relationship is not linear. The duration of response is the shortest for the device with the smallest amount of drug (device 4).
Although both device 5 and device 6 produce longer duration of response than device 4, the long duration of device 5 is unexpected and unexplained.

Overall, the modification of the gelfoam sponge by embedding a retardant in the pores of the matrix is an effective means to further prolong the delivery of insulin. The modified gelfoam device produces a more gradual blood glucose reduction and a comparatively longer duration of insulin delivery than the simple gelfoam matrix while maintaining similar bioavailability.
Table 3.1. Modified gelfoam ocular devices of insulin evaluated in the in-vivo study

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Amount of insulin (mg)</th>
<th>Amount of Brij-78 (μg)</th>
<th>Amount of cetyl alcohol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device 3</td>
<td>0.5</td>
<td>20</td>
<td>0.1</td>
</tr>
<tr>
<td>Device 4</td>
<td>0.5</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>Device 5</td>
<td>1.0</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>Device 6</td>
<td>2.0</td>
<td>20</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 3.2. Pharmacological response parameters of insulin administered ocularly in gelfoam delivery devices

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Insulin (mg)</th>
<th>Enhancer (µg)</th>
<th>Retardant (mg)</th>
<th>Area above the curve (AAC, % hr)</th>
<th>Onset time¹ (hrs)</th>
<th>Offset time² (hrs)</th>
<th>Duration of blood glucose lowering³ (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device 1⁴</td>
<td>0.5</td>
<td>20</td>
<td>0</td>
<td>405.4 (±24.6)</td>
<td>0.6</td>
<td>7.3</td>
<td>6.7 (±0.8)</td>
</tr>
<tr>
<td>Device 2⁴</td>
<td>1.0</td>
<td>20</td>
<td>0</td>
<td>552 (±93.4)</td>
<td>0.9</td>
<td>11.1</td>
<td>10.2 (±0.4)</td>
</tr>
<tr>
<td>Device 3</td>
<td>0.5</td>
<td>20</td>
<td>0.1</td>
<td>413.4 (±62.5)</td>
<td>1.6</td>
<td>9.7</td>
<td>8.1 (±0.2)</td>
</tr>
<tr>
<td>Device 4</td>
<td>0.5</td>
<td>20</td>
<td>0.2</td>
<td>372.9 (±88)</td>
<td>1.4</td>
<td>11.1</td>
<td>9.7 (±0.8)</td>
</tr>
<tr>
<td>Device 5</td>
<td>1.0</td>
<td>20</td>
<td>0.2</td>
<td>530.8 (±69.5)</td>
<td>4.1</td>
<td>16.3</td>
<td>12.2 (±1.4)</td>
</tr>
<tr>
<td>Device 6</td>
<td>2.0</td>
<td>20</td>
<td>0.2</td>
<td>585.2 (±56.7)</td>
<td>1.7</td>
<td>12.9</td>
<td>11.2 (±0.2)</td>
</tr>
</tbody>
</table>

¹ time required to reach blood glucose level of 80% of initial

² time required for the reduced blood glucose level to return to 80% of initial

³ time during which blood glucose level is maintained below 80%

⁴ determined in chapter 2
Figure 3.1. Blood glucose concentrations after instillation of gelfoam device containing 0.5 mg of sodium insulin, 20 µg of Brij-78 and 0.1 mg retardant (Device 3).
Figure 3.2. Blood glucose concentrations after instillation of gelfoam device containing 0.5 mg of sodium insulin, 20 µg of Brij-78 and 0.2 mg retardant (Device 4).
Figure 3.3. Blood glucose concentrations after instillation of gelfoam device containing 1.0 mg of sodium insulin, 20 μg of Brij-78 and 0.2 mg retardant (Device 5).
Figure 3.4. Blood glucose concentrations after instillation of gelfoam device containing 2.0 mg of sodium insulin, 20 μg of Brij-78 and 0.2 mg retardant (Device 6).
Figure 3.5. Mean blood glucose concentrations after instillation of placebo (x), Device 1 (■), Device 3 (Δ) and Device 4 (▲).
Figure 3.6. Mean blood glucose concentrations after instillation of placebo (x), Device 2 (●) and Device 5 (○).
Figure 3.7. Mean blood glucose concentrations after instillation of placebo (×), Device 4 (▲), Device 5 (○) and Device 6 (◆).
CHAPTER IV. CONCLUSIONS

One of the alternative routes for the non-invasive systemic delivery of insulin is the ocular route. This is commonly done by the instillation of insulin eyedrops in the lower conjunctival cul-de-sac. At present, however, this means of delivery is not practical due primarily to the variability of absorption and a short duration of activity.

In 1989, Lee and coworkers carried out a thorough study in determining the extent and pathway of the systemic absorption of insulin after topical instillation of insulin eyedrops to the albino rabbit eye (66). They found that the majority of insulin molecules are absorbed systemically from the nasal mucosa and to lesser extent from the conjunctival mucosa. In their report they concluded that “because topically applied drugs will eventually contact the nasal mucosa, a suitable vehicle may someday be designed that will rest in the conjunctival sac while leaking peptides and proteins to the nasal mucosa for absorption”.

In this project, we have fabricated an ocular device using Gelfoam® absorbable gelatin sponge as an insulin carrier for the controlled systemic delivery of insulin. We believe that the proposed ocular device is such a vehicle. The device is fabricated in the form of a matrix system. This type of delivery system is easy to fabricate and very simple and inexpensive to manufacture.
The matrix system is developed by dispersing sodium insulin in the interstitial pores of the gelfoam sponge. The dispersion process involves the following steps: (i) solubilization of sodium insulin in an appropriate solvent mixture (ii) sorption of mixture into the gelfoam sponge and (iii) evaporation of the solvents under vacuum.

Gelfoam absorbable gelatin sponge is available commercially and is used in the medical field as a hemostatic. It is made from ordinary gelatin and is inexpensive to manufacture. One unique property of the absorbable gelatin sponge is that it swells but does not dissolve in water. Because it becomes very soft after it is inserted and wet with tears the device is quite comfortable and the wearer will not be aware of its presence.

Due to its biodegradability, the absorbable gelatin device will not have to be removed from the conjunctival sac at the end of the dosing period. However, it can be easily removed if desired. The proposed ocular device is much smaller than the commercial ophthalmic pilocarpine delivery device (Ocusert®). In fact it is sufficiently small and soft that it can be used in eyes that have contact lenses.

The efficacy of the various ocular insulin delivery systems are summarized in Table 4.1. The simple gelfoam matrix is clearly shown in the table to produce prolonged delivery of insulin when placed in the lower cul-de-sac of the rabbits. It produces a substantial improvement in insulin bioavailability and significant prolongation in the duration of response over the eyedrop formulations.

Another advantage of using the gelfoam sponge for controlled delivery purposes is that it can be modified to alter the duration of insulin delivery. This modification is done
simply by embedding a retardant in the pores of the matrix. It is also clear from Table 4.1 that the modified gelfoam device produces a more gradual blood glucose reduction and longer duration of activity than the simple gelfoam matrix while maintaining similar bioavailability.

It should be noted that, upon inspection of the figures in chapters 2 and 3, the response profiles obtained from the same devices are somewhat different. The exact cause is not known. However, several factors may contribute to the difference. These include differences in response from different animals, the imperfection of the fabrication technique, the non-linearity of the dose-response relationship, and the possibility that the gelfoam sponge might disintegrate in the cul-de-sac to form small particles which are then drained by the tears into the gastrointestinal tract.

Despite the difference in the response profiles, the gelfoam devices, as clearly shown in Table 4.1, are superior to the eyedrops. The devices produce a substantial increase in bioavailability as well as a longer duration of activity than the corresponding eyedrop formulations.

The application of the gelfoam device makes it feasible to obtain the prolonged systemic delivery of insulin with the desired therapeutic levels without the risk of hypoglycemia. The device also significantly reduces the required frequency of dosing.

We believe that the proposed device could be a significant supplement to parenteral insulin therapy in humans. The device can also be utilized to deliver other drugs including proteins with minimal modification.
Table 4.1. Pharmacological response parameters of insulin administered ocularly in different delivery systems

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Insulin (mg)</th>
<th>Enhancer (μg)</th>
<th>Retardant (mg)</th>
<th>Area above the curve (AAC, % hr)</th>
<th>Onset time&lt;sup&gt;1&lt;/sup&gt; (hrs)</th>
<th>Offset time&lt;sup&gt;2&lt;/sup&gt; (hrs)</th>
<th>Duration of blood glucose lowering&lt;sup&gt;3&lt;/sup&gt; (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyedrop 1</td>
<td>0.5</td>
<td>20</td>
<td>0</td>
<td>54.1 (± 11.5)</td>
<td>0.3</td>
<td>0.8</td>
<td>0.4 (± 0.1)</td>
</tr>
<tr>
<td>Eyedrop 2</td>
<td>1.0</td>
<td>20</td>
<td>0</td>
<td>81.3 (± 10.3)</td>
<td>0.2</td>
<td>1.1</td>
<td>0.9 (± 0.3)</td>
</tr>
<tr>
<td>Device 1</td>
<td>0.5</td>
<td>20</td>
<td>0</td>
<td>405.4 (± 24.6)</td>
<td>0.6</td>
<td>7.3</td>
<td>6.7 (± 0.8)</td>
</tr>
<tr>
<td>Device 2</td>
<td>1.0</td>
<td>20</td>
<td>0</td>
<td>552 (± 93.4)</td>
<td>0.9</td>
<td>11.1</td>
<td>10.2 (± 0.4)</td>
</tr>
<tr>
<td>Device 3</td>
<td>0.5</td>
<td>20</td>
<td>0.1</td>
<td>413.4 (± 62.5)</td>
<td>1.6</td>
<td>9.7</td>
<td>8.1 (± 0.2)</td>
</tr>
<tr>
<td>Device 4</td>
<td>0.5</td>
<td>20</td>
<td>0.2</td>
<td>372.9 (± 88)</td>
<td>1.4</td>
<td>11.1</td>
<td>9.7 (± 0.8)</td>
</tr>
<tr>
<td>Device 5</td>
<td>1.0</td>
<td>20</td>
<td>0.2</td>
<td>530.8 (± 69.5)</td>
<td>4.1</td>
<td>16.3</td>
<td>12.2 (± 1.4)</td>
</tr>
<tr>
<td>Device 6</td>
<td>2.0</td>
<td>20</td>
<td>0.2</td>
<td>585.2 (± 56.7)</td>
<td>1.7</td>
<td>12.9</td>
<td>11.2 (± 0.2)</td>
</tr>
</tbody>
</table>

<sup>1</sup> time required to reach blood glucose level of 80% of initial

<sup>2</sup> time required for the reduced blood glucose level to return to 80% of initial

<sup>3</sup> time during which blood glucose level is maintained below 80%
APPENDIX A

ABSTRACTS OF SIMAMORA PUBLICATIONS
Quantitative Structure Property Relationship in the Prediction of Melting Point and Boiling Point of Rigid Non-Hydrogen Bonding Organic Molecules

P. Simamora and S.H. Yalkowsky

*SAR and QSAR in Environmental Research, 1, 293-300 (1993)*

Abstract

Simple technique to predict the melting point ($T_m$) and boiling point ($T_b$) of non-hydrogen bonding rigid molecules have been developed. The compounds used include halogen, methyl, cyano, and nitro derivatives of benzene and polycyclic compounds. Melting point prediction uses additive constitutive properties e.g., molecular fragments and a non-additive non-constitutive property, molecular symmetry. Boiling point estimation employ only additive constitutive properties. It was found that symmetry affects the entropy of melting which in turn affects the melting point.
Melting Point and Normal Boiling Point Correlations: Application to Rigid Aromatic Compounds

P. Simamora, A. H. Miller and S.H. Yalkowsky

*Journal of Chemical Information and Computer Sciences, 33, 437-440 (1993)*

Abstract

Simple group contribution approaches have been developed to estimate the boiling point and melting point of rigid organic molecules based on their chemical structures. The boiling point estimation requires only additive constitutive properties, whereas melting point prediction employs additive constitutive properties and symmetry. The effects of intramolecular hydrogen bonding and the presence of ortho substituents in biphenyls on the boiling and melting temperatures are discussed.
AQUAFAC: Aqueous Functional Group Activity Coefficients

P. Myrdal, G.H. Ward, P. Simamora and S.H. Yalkowsky

SAR and QSAR in Environmental Research, 1, 53-61 (1993)

Abstract

AQUAFAC, a new group contribution method for estimating aqueous activity coefficients, has been applied to a large set of organic compounds. The current work introduces 27 new group values for hydrocarbon, halogen, and non-hydrogen bond donating oxygen group. Group values (q-values) have been derived from a data set of 621 compounds representing over 1700 individual solubility values. No correction factors were used in generating the current group values. AQUAFAC was found to give acceptable results when applied to some environmentally important compounds.
Precipitation of pH Solubilized Phenytoin

Y. Surakitbanharn, P. Simamora, G.H. Ward and Yalkowsky


Abstract

Precipitation of phenytoin often occurs as it is diluted by blood after intravenous injection. The presence and the amount of precipitate depend upon the initial pH and buffer capacity of the formulation vehicle. The prediction of phenytoin precipitation can be carried out in vitro using isotonic Sorensen's phosphate buffer (SPB) to stimulate blood. An equation is developed to calculate the change in solubility resulting from the change in pH due to dilution. This equation is very difficult to solve analytically because it involves a high order polynomial. However, it can be solved numerically using a spreadsheet program. The relationship between pH or solubility and dilution can then be presented graphically. Therefore, the precipitation of any pH solubilized drug due to dilution under various conditions can be easily predicted. This is illustrated for several aqueous phenytoin solutions.
Group Contribution Methods for Predicting the Melting Points and Boiling Points

of Aromatic Compounds

P. Simamora and S.H. Yalkowsky


Abstract

Simple methods are proposed to estimate the boiling points and the melting points of aromatic compounds from chemical structures. The transition temperatures are determined by the estimation of both the enthalpy and the entropy of transition. The enthalpies of boiling and melting are both estimated as additive constitutive properties. The entropy of boiling is assumed to be constant as described by Trouton’s rule, while the entropy of melting is estimated using a modification of Walden’s rule. The latter utilizes the nonadditive nonconstitutive molecular property, rotational symmetry.
Unified Physical Property Estimation Relationship (UPPER)

S.H. Yalkowsky, R-M. Dannenfelser, P. Myrdal, P. Simamora and D. Mishra

Chemosphere, 28, 1657-1673 (1994)

Abstract

A scheme is presented to evaluate twenty-one molecular properties using known theoretical and semi-empirical equations with geometric descriptors and group contribution values. The geometric descriptors and group contribution values which are derived strictly from molecular structure will be described along with the various physical property calculations. This will allow all twenty-one properties to be estimated from only the structure of the compound.
UPPER II: Calculation of Physical Properties of the Chlorobenzene

S.H. Yalkowsky, P.B. Myrdal, R-M. Dannenfelser and P. Simamora

Chemosphere, 28, 1675-1688 (1994)

Abstract

The twelve chlorobenzenes are used to evaluate the previously described UPPER scheme. Twenty-one physical properties are calculated strictly from molecular structure with no adjustable or fitted parameters and compared with experimental values. The UPPER scheme does reasonably well with estimating all of the considered properties.
Effect of pH on Injection Phlebitis

P. Simamora, S. Pinsuwan, J.M. Alvarez, P.B. Myrdal and S.H. Yalkowsky

*Journal of Pharmaceutical Sciences, 84, 520-522 (1995)*

Abstract

Formulation pH has been reported to be responsible for the incidence of phlebitis. In this study, the effect of pH on injection phlebitis is investigated. Buffers of varying pHs were examined for their ability to produce phlebitis in rabbits. Thermal measurements as well as visual evaluations were used for phlebitis quantitation. The results show that formulation pH between 3.0 and 11.0 does not contribute to the incidence of phlebitis when administered as an iv bolus injection.
Studies in Phlebitis VII: In vitro and In vivo Evaluation of pH Solubilized Levemopamil

P.B. Myrdal, P. Simamora, Y. Surakitbanharn and S.H. Yalkowsky


Abstract

We describe a computational model and an in vitro experiment for assessing whether not a pH-solubilized drug as the potential to precipitate upon dilution with blood. The computational model enables an efficient means of selecting buffer concentration and pH, and the in vitro test provides a simple experimental validation. Both means of screening are applied to the formulation of the weakly basic drug levemopamil HCL. A buffer formulation of levemopamil is chosen from the computational model and shown to be free of precipitation upon dilution in vitro and to not produce phlebitis in the rabbit ear model. In comparison, an unbuffered formulation at the same pH and drug concentration precipitates in vitro and causes significant phlebitis in vivo. The results of this study reinforce the importance of buffering parenteral formulations instead of simply adjusting the pH of the formulation.
Boiling Point and Melting Point Prediction of Aliphatic, Non-Hydrogen Bonding Compounds

J.F. Krzyzaniak, P.B. Myrdal, P. Simamora and S.H. Yalkowsky

*Industrial & Engineering Chemistry Research, 34, 2530-2535 (1995)*

Abstract

Simple group contribution methods which incorporate nonadditive, nonconstitutive properties are proposed to predict normal boiling points and melting points for aliphatic, non-hydrogen bonding compounds. Boiling points and melting points are estimated from the ratio of the enthalpy of transition and the entropy of transition. The enthalpy of transition is assumed to be equal to the summation of group values. The entropy of boiling is estimated using a modification of Trouton’s rule, while the entropy of melting is estimated using a modification of Walden’s rule. The root mean square errors for the estimation of boiling points and melting points are 14.4 K and 34.3 K, respectively.
Studies in Phlebitis VIII: Evaluation of Intravenous Dexverapamil Formulations

P. Simamora, S. Pimsuwan, Y. Surakitbanham and S.H. Yalkowsky


Abstract

Injectable and infusion formulations of dexverapamil are evaluated for their potential to produce phlebitis. The evaluation technique utilize two independent in vitro methods and two in vivo methods to screen a pH solubilized drug for its potential to induce phlebitis due to precipitation at the injection site. One method is based upon a theoretical calculation that simulates the change of solubility upon dilution. Its predictions are confirmed by the second method which consists of an in vitro precipitation experiment. Thermal and visual evaluations are then obtained from an in vivo rabbit ear injection. Dexverapamil formulations that have low buffering capacity are shown to contribute to the incidence of phlebitis to a greater extent than the properly buffered formulations. The calculations and the in vitro precipitation experiment are found to be useful in formulating pH solubilized parenterals. They enable the formulator to optimize pH, drug concentration, and buffer concentration without the need to animal studies. The results of this study shows the importance of selecting a buffer that provides adequate buffer capacity in the formulations.
New Emulsion Formulations for the Intravenous Administration of Taxol

P. Simamora, R-M. Dannenfelser, S.E. Tabibi and S.H. Yalkowsky

Submitted to the NCI for review

Abstract

Paclitaxel is a natural product which has been found to be active against a number of human cancers. This compound is very insoluble in water and contains no groups that are ionizable in an acceptable pH range. It also has a very low solubility in most pharmaceutically acceptable cosolvents. The current FDA approved paclitaxel formulation for intravenous (iv) administration contains an equal amount of nonionic surfactant (Cremophor EL) and ethanol. The former is notorious for producing allergic reactions. In this report, two potential parenteral formulations containing 5 mg/ml and 3.5 mg/ml of taxol for iv administration that are cremophor-free and do not precipitate upon dilution have been developed. Both formulations are found to be chemically and physically stable for at least 3 months when stored at 4 °C.
Molecular Symmetry and Melting Temperatures

S.H. Yalkowsky, R-M. Dannenfelser and P. Simamora

*Journal of Chemical Society. Perkin Transaction. 2. Submitted for publication (1996)*

Abstract

The molecular rotational symmetry number, \( \sigma \), is shown to be related to the entropy of melting and in turn to be an important factor in determining the melting points of isomers. The molecular symmetry number is used to explain some of the trends found in the melting point data for isomeric disubstituted benzenes and as well as isomeric disubstituted naphthalenes which are not accounted for by hydrogen bonding.
REFERENCES


