Introduction

According to the Center for Disease Control, diabetes is the seventh leading cause of death in the United States and over 29 million Americans currently have this disease, which translates to about 1 in every 11 people [1]. Two types of diabetes exist, including type 1 and type 2. Type 1 diabetes is usually present at birth, and is caused by insulin deficiencies that prevent the pancreas from producing enough insulin. Patients with type 2 diabetes typically contract the disease over time. Type 2 diabetes is caused by insulin resistance and occurs when the pancreas produces insulin, though the body does not appropriately react to the protein [2]. Type 2 diabetes increases one’s risk for many other health problems such as heart and blood vessel diseases, kidney damage, vision degeneration, nerve damage, and foot damage (even leading to amputation in some cases) [3]. Patients with type 2 diabetes continuously alternate between a hyperglycaemic and hypoglycemic state, which refers to high blood glucose and low blood glucose levels, respectively [4]. It is imperative for these patients to monitor their blood glucose levels in order to remain in good health.

Oral drug delivery and liquid injections are currently the two most common forms of diabetes treatment [5]. However, both methods implicitly present limitations. Patients who take oral anti-diabetic medications typically require high dosages in order for sufficient efficacy due to low bioavailability in drugs of this class. Patients also tend to experience negative side effects, such as vomiting and digestive pain from taking high-dose oral anti-diabetic medications. Patients who take hypodermic insulin injections are required to inject themselves with insulin up to three times a day; this method is very painful and inconvenient for patients who are dependent on this life-saving protein [6].

A feasible treatment option for diabetes lays in transdermal drug delivery approaches. It has been shown that the skin is a barrier that can be exploited for drugs to enter the body, so transdermal patch or cream formulation has potential for development as an alternative to the typical diabetes treatments. Furthermore, the use of transdermal patches can provide sustained drug release over hours, and even days. This novel methodology for diabetes treatment would have the ability to enable patients to lead quality lives without the pain from the numerous hypodermic insulin injections or the negative side effects caused by oral anti-diabetic medications [7]. However, this delivery method possesses limitations of its own, such as the necessity of selecting a drug small enough (with appropriate chemical properties) to facilitate transdermal diffusion [8-11].

Sitagliptin was an ideal candidate in considering transdermal diabetic drug transport treatment due to its ability to effectively treat diabetes [12] and small overall size. Past studies have concluded that Sitagliptin does not cause hypoglycaemia, and it requires a low effective dosage for a minimum effective concentration in the body. Sitagliptin’s mechanism of action in type 2 diabetes is driven by inhibiting the dipeptidyl peptidase 4 (DPP-4) enzyme [13]. One of the main hormones that
involve in the process of glucose removal by stimulating the release of insulin from the pancreas is glucagon-like peptide-1 (GLP-1) [14,15]. In addition to stimulate insulin secretion, GLP-1 can lower blood glucose levels via several other mechanisms including a reduction in glucagon concentrations, a delay of gastric emptying, and potential induction of satiety. GLP-1 may also play a role in the proliferation of beta cells and the decrease in beta-cell apoptosis [16]. DPP-4 is an enzyme that can cleaves peptides with the amino acid alanine or proline in position 2 of the N-terminus of the peptide chain. Active GLP-1 can be inactivated and degraded by DPP-4 [17-19]. DPP-4 inhibitors, such as Sitagliptin, inhibit the degradation of GIP, and potentially other peptides involved in regulating glucose homeostasis which results in an increase in the circulating levels, of biologically active GLP-1, can be an alternate therapeutic treatment approaches for type-2 diabetes [20].

The minimum effective concentration of Sitagliptin required in the blood is 100nM [21]. When Sitagliptin is taken orally, there are many negative side effects that have been reported which include severe digestive discomfort, general pain, upper respiratory tract infections, and headache [22]. However, by delivering Sitagliptin transdermally, there is potential for these side effects to be circumvented through avoiding digestive overload.

A typical transdermal patch consists of three different layers. They include an impermeable backing layer, a drug reservoir layer, and a release liner. The drug reservoir layer consists of three basic components, including polymers, drugs, and/or penetration enhancers. This most significant layer is responsible for carrying the drug molecules, making it vital for the drug reservoir layer to effectively control the drug release. The properties of this layer must be nontoxic, non-irritating, and compatible with other patch layers [23,24].

To optimize the transdermal patch described by the manuscript herein, a mathematical model was applied to gain insight toward the relationships between the independent and dependent variables of the patch design. In this study, MATLAB (v. R2017a) was used to identify specifications for the components of a reservoir transdermal patch design. These models have an impermeable backing, drug reservoir, rate controlling membrane, and adhesive material. A MATLAB representation was used to calculate the amount of the drug should be infused with the patch based on the desired plasma concentration. Release rate specifications were used to determine the necessity and appropriate properties of a rate controlling membrane. Both of these factors were incorporated to discern an overall patch area. A series of mathematical model helped researchers reduce the number of necessary experiments and in turn, the amount of time needed to complete research projects [25-30].

The aim of this study is to develop a transdermal drug delivery system to deliver Sitagliptin, a small chemical that can effectively treat diabetes. By delivering Sitagliptin transdermally, negative side effects induced by oral administration of Sitagliptin can be circumvented. To do this, the optimized transdermal patch design was proposed by a mathematical model, and tested in vitro using Franz cell apparatus.

Materials and Methods

Materials

Sitagliptin was purchased from Sigma Aldrich (CAS Number: 654671-77-9, St. Louis, MO, USA). The Franz cell diffusion system and skin-mimicking membranes, durapore, were kindly donated by PermeGear, Inc. (Catalogue #: 4G-01-00-09-05 Hellertown, PA, USA) Standard transdermal patch rate control membranes were kindly donated by 3M (Catalogue #: 3M™9702 & 97012, USA). Natrasol and acelullose powder were purchased from Hercules Inc. (Wilmington, Delaware, USA) by Aqualon division.

Methods

Standard curve of Sitagliptin: A direct result of Beer-Lambert’s law is that a standard curve of a given compound can be generated by measuring the absorbance at a fixed wavelength of the pure material at varying concentrations. Absorbance values of different known concentration (2.5mM, 5 mM, 7.5 mM and 10 mM) of Sitagliptin were read with a UV spectrophotometer at 266 nm, and a standard curve of Sitagliptin was generated by using best-fit-line function in excel to fit a linear curve to the data points. An equation describing this linear relation over the range of absorbance and concentration were then be generated. Using this relation, the concentration of that compound in an unknown sample can be determined by comparing the absorbance value of the unknown at the selected wavelength with values found in the standard curve.

Characterization of drug properties through in vitro studies using Franz Cell apparatus: The properties of Sitagliptin as it permeates through the skin were determined experimentally with in vitro skin permeation studies. These studies were performed using water-jacketed Franz cells (PermeGear, Inc), which have receptor capacities of 20mL. The skin mimicking membrane used in all permeation studies was a synthetic polymer, durapore, with a pore size of 0.45 μm. The receptor compartment was filled with 20mL of deionized water, and the durapore membrane was placed over the receptor’s aperture. The donor compartment was then mounted on top of the membrane and clamped in place. Three concentrations of Sitagliptin (1.25mM, 2.5mM, and 5mM) were tested. The donor compartments each contained 5mL of the respective Sitagliptin solutions. All cells were connected to a water heater/circulator to maintain the cells at 37°C in order to mimic normal body temperature throughout testing. The donor compartment and sampling port were sealed with parafilm to prevent evaporation. The whole assembly was kept on top of a magnetic stirrer and the solution inside the receptor was stirred continuously throughout the entire experiment using magnetic stir bars.

The samples from the in vitro testing were withdrawn (200 μL) at two hour intervals for 10 hours. The receptor compartment was replenished with an equal amount of fluid medium (deionized water). The cumulative mass of Sitagliptin in the receptor compartment over time was calculated using the standard curve previously determined and plotted for each trial. This procedure was performed in triplicates for each concentration. Permeability coefficients were determined at the end of the experiment. The mathematical model was derived from the result of initial testing.

Mathematical model derivation and validation: There are several parameters that are essential to characterizing the diffusive properties of a specific drug through a selectively permeable membrane such as the skin, including the permeability coefficient ($K_p$ or $P$), the diffusion constant ($D$), lag time ($\tau$), the diffusive area ($A$), the initial concentration in the patch or donor ($C_o$), the concentration in the blood or receptor ($C_i$), the membrane thickness ($x$), and the initial mass of drug in the donor or patch ($Q_{d0}$). The value of each of these parameters affects the experimental diffusive properties of any drug in a given system. Experimental testing was necessary to obtain results required to “train” the mathematical model and determine parameters such as permeability coefficient, since this was the first time Sitagliptin has been tested for transdermal delivery. The parameters such as the diffusion constant ($D$) will be found in the literature. The parameters such as lag time ($\tau$), the diffusive area ($A$), the initial concentration in the patch or donor ($C_o$), the concentration in the blood or receptor ($C_i$), the membrane thickness ($x$), and the initial mass of drug in the donor or patch ($Q_{d0}$) will be generated and optimized using mathematical model described below. When conducting this experiment in the Franz diffusion cell, there are two possible conditions that can be considered: an infinite dose condition (often used in drug permeation studies to obtain a steady-state and quantify theoretical values such as diffusion constant and lag time), or a finite dose condition (less ideal but more representative of a patch on the skin). In this study, a finite dose condition was used to establish steady-state conditions based on the computations guided by Equation 1 & 2.

**Finite dose condition:** The characterization of a finite dose condition with the use of experimental data was guided by the following expression.

$$\ln \left( \frac{Q_d}{Q_{d0}} \right) = \frac{PA_t}{V_d} \rightarrow P = \frac{V_d}{At} \ln \left( \frac{Q_d}{Q_{d0}} \right) \quad \text{Eqn 1}$$

$$K_p = \frac{Q}{\left[ A \cdot t \cdot (C_o - C_i) \right]} D = \frac{K_p x}{K_{m\tau}} \tau = \frac{x^2}{6D} \quad \text{Eqn 2}$$

Where $Q_d$ is the current mass of the drug in the donor or patch, $Q_{d0}$ is the initial mass of the drug in the donor or patch, $P$ is the permeability coefficient of the given drug through the specific membrane tested, $A$ is the diffusive area, $t$ is the time elapsed, and $V_d$ is the volume of the donor cell or the volume of solution to be delivered.

The properties of Sitagliptin that were determined during permeation testing were used in the mathematical model. From this model, the permeability coefficient of Sitagliptin was determined. Parameters such as donor solution volume and donor area were established in the mathematical model to replicate the experimental testing conditions.

**Optimal patch design development and testing:** After the successful establishment of a mathematical model, the computation was used to develop and test an optimal patch design. The desired patch design would include a gel matrix containing a calculated concentration of Sitagliptin that would be capable to reach and maintain the minimum effective concentration of 100nM Sitagliptin in the blood to help sustain glucose control for a 24-hour period. A general patch schematic was provided by 3M™ and used to construct the optimal patch prototype. This prototype included an occlusive backing to prevent drug evaporation. The active drug ingredient was covered by a membrane for the facilitation of diffusion into the skin. An adhesive was also included, and finally an optional release liner was considered for the control the drug diffusion rate. These various components are highlighted in Figure 1. For patch testing purposes, only the occlusive backing and drug-release membrane were used to confine the drug to the patch.

Based on the mathematical modelling data, three types of patches that had 1% gel solutions with 1.25mM concentration of Sitagliptin were realized. Two milliliters of gel solution were inserted into each patch design, and each was tested over a 24-hour period. The first patch presented no membrane, simulating the gel solution in direct contact with the skin. The second and third patches each had different rate-controlling membranes covering the gel solution. These membranes varied in diffusive characteristics. Patch 2 incorporated a 3M™ 9702 membrane, which is 2mm thick and has a 9% EVA (Ethylene Vinyl Acetate) composition. Patch 3 included a 3M™ 9712 membrane, which is 2mm thick and has 18.5% EVA. Lower EVA percentages correlate to longer diffusion times. The results of this patch testing...
indicated that not only is Sitagliptin suitable for transdermal delivery, but also that this drug can be used in a patch for sustained glucose control for over 24 hours.

**The drug delivery profile of an optimized transdermal patch:**
The three types of patches mentioned above were tested on a Franz cell over a 24-hour period. The studies utilized the synthetic skin mimicking membranes with a pore size of 0.45 µm. The membrane was placed over the receptor’s aperture which contained 20 mL of deionized water. The patch with the backing material was pressed onto the center of the cell over the membrane. The donor and receptor compartment were held together by parafilm. The temperature of the Franz cell was kept at 37°C. The whole assembly was placed on top of the magnetic stirrer and the solution inside the receptor was constantly stirred. Samples were withdrawn (200 µL) at two hour intervals and were replenished with equal amounts of deionized water. Absorbance values of the samples were read with the spectrophotometer at 266 nm.

**Statistical analysis:** A student’s t-test was used for comparing between mathematical model results to experimental results. One-way ANOVA was used for comparing more than two means with single factor. These statistical analyses were performed using Sigma Plot 12.3 (San Jose, CA). p<0.05 was considered significant.

**Results**

**Characterization of drug properties through initial experimentation**

As previously discussed, it was necessary to study the diffusive properties of Sitagliptin prior to designing a patch for therapeutically relevant delivery. A standard curve (Figure 2) was created to relate absorbance readings to that of various known concentrations of Sitagliptin. An equation (Eqn 3) was determined from the linear regression to relate the absorbance and concentration values. This equation was used to determine unknown concentration values for future absorbance readings found through diffusive testing. Equation 3 depicts the relationship, where y is absorbance and x is concentration (mM).

\[
y = 2.192x + 0.011
\]

(Eqn 3)

Once the standard data were generated, diffusion of Sitagliptin at three different concentrations (1.25, 2.5, and 5 mM) was tested using Franz Cell analysis, and the results are reflected by Figure 3.

Cumulative mass delivered to the receptor chamber was determined by taking trial-to-trial concentration readings and accounting for mass lost due to dilution from sampling (200 µL of solution was taken and replaced with pure deionized water). Finally, the data illustrated in Figure 3 shows the average diffusion of Sitagliptin at three different initial concentrations across the Franz Cell membranes.

It can be noted that the averaged results of the various tests produced a clear trend of delivery that was logarithmic in nature and indicates that a substantial portion of the drug was delivered in the first two hours (~30%). After the 10-hour testing period, delivery tapered off, revealing that approximately 48% of the initial drug was delivered and available to the bloodstream. This experimental testing supported that Sitagliptin can be delivered transdermally, and that this route may provide higher bioavailability than the original oral mode of delivery.

Once these data were obtained, the observed results were applied to a mathematical model to calculate parameters for a transdermal patch that would be able to deliver the clinically established concentration of 100 nM Sitagliptin to the bloodstream (assuming an average blood volume of 4.7 L) [21]. The details of these calculations and the resulting values can be found in following section using the equations in the method section.

**Mathematical modelling and verification**

Once dosing conditions were established, the system parameters and experimental results were inputted to model the system in question. For each experimental condition, the system parameters listed in Table 1 and Equation 1 display cumulative mass delivered, which was used to calculate the permeability coefficient P, of the drug in the given membrane for each trial. The permeability coefficient results from these calculations can be found in Table 2. The first 4 hours of diffusion was expressed by the linear part of the curve which provided better quantification, which is why the experiments were completed with 1/2-hour test intervals for 4 hours; the data generated during these 4 hours were used for the computational model. It should be noted that an assumption was made that any drug missing from

---

**Table 1: System Parameters Used in Experimental Testing and Mathematical Modeling.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Diameter</td>
<td>2.4</td>
<td>cm</td>
</tr>
<tr>
<td>Membrane Area (Transfer Area)</td>
<td>4.52</td>
<td>cm²</td>
</tr>
<tr>
<td>Membrane Thickness</td>
<td>0.0125</td>
<td>cm</td>
</tr>
<tr>
<td>Donor Volume</td>
<td>5</td>
<td>mL</td>
</tr>
<tr>
<td>Receptor Volume</td>
<td>20</td>
<td>mL</td>
</tr>
<tr>
<td>Donor Concentration (1)</td>
<td>1.25E-03</td>
<td>M</td>
</tr>
<tr>
<td>Donor Concentration (2)</td>
<td>2.50E-03</td>
<td>M</td>
</tr>
<tr>
<td>Donor Concentration (3)</td>
<td>5.00E-03</td>
<td>M</td>
</tr>
<tr>
<td>Donor Mass (1)</td>
<td>3.16</td>
<td>mg</td>
</tr>
<tr>
<td>Donor Mass (2)</td>
<td>6.32</td>
<td>mg</td>
</tr>
<tr>
<td>Donor Mass (3)</td>
<td>12.63</td>
<td>mg</td>
</tr>
<tr>
<td>Molecular Weight of Sitagliptin</td>
<td>505.31</td>
<td>g/mol</td>
</tr>
</tbody>
</table>
the donor cell during experimentation was in fact delivered in the receptor cell). This assumption would later be corrected for by using an adjustment factor. Using the calculated permeability coefficient $P$, the original system parameters that were tested experimentally were placed in the model and the cumulative mass delivered was calculated over time using an adjustment factor $F$ of 0.45 (the average overall percentage of drug delivered over the 10-hour testing periods) for each initial concentration. The results from this model can be found in Figure 4.

The result in Figure 4 provides adequate verification of the mathematical model comparing the experimental data from Figure 3. The results from the mathematical model were consistent with the experimental results, which confirmed the use of such a model to determine an optimal patch design as an alternative for diabetes treatment. With the model verified, it was then necessary to investigate the appropriate parameters for developing an optimal patch design for delivery of the effective drug dosage into the bloodstream.

### Optimal patch design development and testing

Using the model developed above, a parametric study on various patch parameters was conducted to determine feasible combinations of solution concentration and volume that could be placed in a patch. The patch was set on the membrane of the Franz cell in order to deliver the target concentration of Sitagliptin to the blood. The average human has 4.7 L of blood, and therefore a minimum of 0.24 mg of Sitagliptin must be delivered to establish an effective drug plasma concentration of 100nM. A patch with 1.25 mM Sitagliptin solution was selected because it was able to deliver the required mass of drug. The permeability coefficient for 1.25 mM (5.12 x 10^-5 cm/s) was adapted to the mathematical model. Through the model, it was established that 2 mL of 1.25mM Sitagliptin should be loaded onto a 4.52 cm² transdermal patch. Table 3 shows the selected specifications. Figure 5 shows theoretical predictions for the design summarized in Table 3, and it shows that Sitagliptin can reach the targeted effective dosage within one hour.

### Table 2: Results for Permeability Coefficient Using Experimental Results.

<table>
<thead>
<tr>
<th>Group #</th>
<th>Concentration</th>
<th>Description</th>
<th>Permeability Coefficient P (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25 mM</td>
<td>10-hour test, 2-hour test intervals</td>
<td>4.89E-05</td>
</tr>
<tr>
<td>2</td>
<td>2.5 mM</td>
<td>10-hour test, 2-hour test intervals</td>
<td>6.93E-05</td>
</tr>
<tr>
<td>3</td>
<td>5.0 mM</td>
<td>10-hour test, 2-hour test intervals</td>
<td>3.87E-04</td>
</tr>
<tr>
<td>4</td>
<td>1.25 mM</td>
<td>4-hour test, 1/2-hour test intervals</td>
<td>5.12E-05</td>
</tr>
<tr>
<td>5</td>
<td>2.5 mM</td>
<td>4-hour test, 1/2-hour test intervals</td>
<td>7.01E-05</td>
</tr>
<tr>
<td>6</td>
<td>5.0 mM</td>
<td>4-hour test, 1/2-hour test intervals</td>
<td>9.32E-05</td>
</tr>
</tbody>
</table>

### Table 3: Proposed patch parameters for targeted delivery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel Solution</td>
<td>1%</td>
</tr>
<tr>
<td>Initial Conc.</td>
<td>1.25 mM</td>
</tr>
<tr>
<td>Initial Volume</td>
<td>2 mL</td>
</tr>
<tr>
<td>Patch Area</td>
<td>4.52 cm²</td>
</tr>
</tbody>
</table>
Experimental mass delivered over time from proposed patch for the regression was 0.9898, showing an exceptional fit. Therefore, a linear regression was applied to identify Equation 3. The $r^2$ value was 0.9898, indicating a strong linear relationship was established between absorbance and concentration.

The calculated values from the mathematical model were used to design an optimal patch. The proposed schematics were applied to the Franz device to evaluate the design experimentally. Figure 6 shows the cumulative delivery of Sitagliptin over time from the Franz Cell. Figure 6 depicts that the data supported the ability of the proposed design to deliver the target drug quantity. The results from the experimental results were consistent with the mathematical model, which confirmed the optimal patch design developed using the mathematical model.

To further mimic the human body response to the drug, the body clearance of Sitagliptin was introduced to the mathematical model. Figure 7 shows the plasma concentration of Sitagliptin over 24 hours with clearance considered. It has been proven that this patch design can successfully deliver Sitagliptin with effective dosage for at least 24 hours, and is possible to serve as an alternative for diabetes treatment.

Mathematical modelling was carried out and is reflected by Equation 1 & 2. As seen in the equation, a permeability coefficient of $5.12 \times 10^{-5}$ cm/s was found and applied to determine an appropriate donor concentration of Sitagliptin. The average human has 4.7 L of blood, and therefore a minimum of 0.24 mg of Sitagliptin must be delivered to establish a drug concentration of 100 nM. Through the model, it was established that 2 mL of 1.25mM Sitagliptin should be loaded onto a 4.52 cm² transdermal patch. By scaling these predictions to suit a Franz Cell analysis, a patch was loaded and put in place of the Franz donor cell. After the in vitro testing was carried out, the data supported the ability of the proposed design to deliver the target drug quantity. This study is a preliminary in vitro study to prove of a concept that Sitagliptin can be successfully delivered to the body via transdermal delivery system. One of the limitations is that further in vivo study of safety and effectiveness is missing. The future work will be in vivo animal tests of this patch to further demonstrate the safety and effectiveness of this transdermal delivery system.

Conclusion

Sitagliptin delivery by optimization of a transdermal patch configuration was carried out by using a mathematical model to determine the various parameters of the patch design that provide the optimum cumulative drug release amount for at least 30 hours. The results obtained by this study suggest high potential for further experimentation in vivo. Due to the fact that the adverse side effects of Sitagliptin mainly related to GI system, therefore the transdermal method of delivery of Sitagliptin can be effectively and efficiently delivered, providing for extended, side-effect minimizing glucose management treatment. This treatment method has potential to be more reliable than commonly used treatments such as the oral mode of delivery as well as hypodermic insulin injections, which produce unwelcome side effects that disrupt the quality of life for patients. The success of this research shows promising results that indicate the quality of life can be enhanced for the millions of people suffering from type 2 Diabetes.

Acknowledgements

This study was supported by American Heart Association.

References

2. Diabetes: Differences between Type 1 and 2-Topic Overview. WebMD.