Study of Drug Delivery Behavior Through Biomembranes Using Thermal and Bioanalytical Techniques

Hareesha Reddy Venumuddala
Cleveland State University

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STUDY OF DRUG DELIVERY BEHAVIOR THROUGH BIOMEMBRANES USING THERMAL AND BIOANALYTICAL TECHNIQUES

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Bachelor of Science in Pharmacy
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Date: December 3, 2010
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STUDY OF DRUG DELIVERY BEHAVIOR THROUGH BIOMEMBRANES USING THERMAL AND BIOANALYTICAL TECHNIQUES

HAREESHA REDDY VENUMUDDALA

ABSTRACT

This Master of Science thesis encompasses two projects in chemical pharmaceuticals. The first is a study of excipients and the added new information collected beyond Thermal Gravimetric Analysis and Differential Scanning Calorimetry from Dielectric Analysis. These new properties enhance our global knowledge of excipients by thermal analytical methods. Excipients, the inactive ingredients in formulated drugs, aid different functions of the active pharmacy ingredient, the drugs. Low temperature transitions, by DEA including melting of frozen solvents, e.g. water, are more definitive than observed by low temperature DSC.

Millions of dollars are expended annually on pharmaceutical testing to qualify excipients for fully formulated drugs, medicines and active ingredients. To understand the action of the excipients in the human body at body temperature of 37°C, the study of their individual and interactive properties are desirable. DEA DSC and Thermal Gravimetric analysis (TGA) methods are employed to screen the most widely used drug excipients. In this study the following excipients were examined by DEA: cotton seed oil, mannitol, peanut oil, polyethylene glycol, sugar, sodium lauryl sulfate, sodium starch glycolate, sodium stearate, canola oil, and anhydrous lactose, benzoic acid and vanillin. The comparison of DSC and DEA thermal curves for each excipient indicates that major endothermic events have occurred e.g., volatilization or melting of the excipient are viewed as fundamental DEA properties. These properties are the rise in permittivity and
dielectric loss factor. The focus of this project was to learn to prepare, examine and interpret the resulting variations.

The electrical conductivity (\(\varepsilon'' \times \text{frequency} \times \text{constant}\)), permittivity (\(\varepsilon'\)) and tan delta value (\(\varepsilon''/\varepsilon'\)) are used to enhance the characterization of the excipient.

The second, and major project for this thesis, is to evaluate bipolar disorder drug transport with and without an applied electric field of 10V mm\(^{-1}\). Drug delivery was tested with several animal models, dry shed snake skins and a moist pig skin, which will be used to develop a new transdermal patch. Characterization of appropriate drugs by DEA and other thermal analytical methods will aid the understanding of chemical stability and interactions. These properties will aid a Bipolar Disorder (BPD) patient to receive their correct dosage in a timely manner and improve patient compliance. BPD also called as Manic Depressive Disorder results in extreme shifts in mood and behavior that may last for weeks or months, causing severe disturbances in the lives of those affected. A high percentage of BPD is untreated due to the lack of a well-tolerated and effective drug transport therapy. So, the analysis of the selected drugs by thermal analytical techniques gives support to understand the transport properties. Studying the transport properties of these drugs through a transdermal route may pave the way for a novel transdermal drug delivery method. In this study, Dielectric Analysis (DEA) is used to characterize and evaluate the transport properties of the selected drugs (Olanzapine, Risperidone, and Quetiapine Fumarate). Dielectric Analysis implements drug delivery employing an AC frequency, modulating drugs of a wide molecular weight range (e.g. 300 to 30,000 Daltons) with an interdigitated electrode system. In this analysis, the drugs were studied using animal models (Shed snake skin and pig skin). There is a specific
frequency we discovered for each drug, where the change in the electric profile begins which “aids the delivery”. The delivery is measured by enhanced conductivity and is paralleled with increased drug throughput tracked by UV analysis.
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ABBREVIATIONS

BPD- Bipolar Disorder

Mol.Wt. - Molecular Weight

DSC- Differential Scanning Calorimetry

$T_m$ - Onset Melting Temperature

$T_{mp}/T_p$ - Peak Melting Temperature

$\Delta H_f$ - Heat of Fusion

$T_g$ - Glass transition

$T_c$ - Onset Crystallization Temperature

$T_{cp}$ - Crystallization peak temperature

$\Delta H_c$ - Heat of Crystallization

API’s - Active Pharmaceutical Ingredients

DEA- Dielectric Analysis

UV/vis- Ultraviolet-visible spectroscopy
OBJECTIVES

The main objective of this thesis was to characterize the selected drugs using Thermal Analysis and then relate the drug properties to the transport of the drugs transdermally. Later the evaluation of these drugs was accomplished by UV analysis and the data was correlated. The electrical properties of the drugs were studied using Dielectric Analysis. Other physical property attributes were determined by Differential Scanning Calorimetry. Structural features were studied by Fourier Transform Infrared Spectrometry and Macro photography.

The initial phase of this thesis was the characterization of the selected chemical excipients by Thermal Analysis. This project was undertaken as the groundwork for the later part of this study, where the Bipolar Disorder drugs were carefully evaluated. The excipients selected were screened by DEA, DSC and TGA and the data interpreted was compared and ranked. Excipients are the inactive pharmaceuticals that are used to make up a medication. They are used as adhesives, plasticizers, binders, disintegrates, dispersants and fillers. In this study, nine common excipients were measured by DSC and TGA. An additional new Thermal Analytical tool, DEA, added a significant amount of characterization data. DEA uniquely measures the electrical properties of the organic chemicals studied and revealed new physical-chemical properties, for example, electrical conductivity is a function of temperature and time. Detection of water melting at 0°C was observed by DEA and not DSC. The electrical conductivity of mannitol and anhydrous lactose was observed more than a 100°C below their melting temperature.

In the next phase of our study, the Bipolar Disorder drugs were screened by the above cited instrumental methods. The properties of these drugs were determined and were used
to identify and quantify the drugs used by the mentally ill. The major value of this research is to develop an AC electrified patch for Bipolar Disorder dosing. Patient compliance can be improved with knowledge of the drug dielectric transport properties as determined by DEA.
HYPOTHESIS

Transdermal drug delivery is becoming more preferable over the past decades due to its advantages over the oral systemic routes. Pharmacokinetic properties, drug bioavailability and patient compliance can be improved by transdermal procedures. Active ingredients in most of the well-known transdermal products are delivered through the skin by passive diffusion. Recently, an efficient drug delivery method using electric field has been developed as transdermal patches. This field based drug delivery transfers drugs at a controlled rate, which in turn improves patient compliance. Direct current (DC) is the most commonly used in the field based transdermal patches. However, these DC field based methods are associated with several compelling drawbacks including side effects which can potentially cause skin irritation and burning. Replacing the DC field with an AC field would be an ideal solution for these drawbacks. Preliminary studies using AC field in drug delivery by Riga et al yielded promising results and based on these studies we hypothesize that an efficient and effective drug delivery device for Bipolar Disorder (BPD) can be developed using AC field. The major advantage of the new development is that it overcomes the main side-effect of the DC method. Moreover, AC field provides less flux enhancement than DC field.

This new method based on AC field is validated using two model biomembranes, shed snake skin and porcine skin. The focus of this innovative technique is to improve patient compliance in the case of BPD.


PROTOCOL

Drug Characterization by Physical – Analytical Techniques

*Overview*

- Characterization and evaluation of excipients
- Characterizations of Bipolar Disorder drugs and evaluate their transport properties

*Scope*

- Purpose of the techniques: Quantitative and Qualitative (DEA and UV)
- Define physical properties to be defined

*Terminology*

- Define all significant terms
- Define all abbreviations

*Summary of Methods*

- Brief outline of each method
- Brief statement of the test method principle

*Significance in Use*

- Relevance in meaning of each test method
- Development of drug delivery patch
- Development of new transdermal delivery route

*Methods*

- Differential Scanning Calorimetry
- Dielectric Analysis
- Ultraviolet –Visible spectrometry
**Hazards**

- Care should be taken in handling specific drugs

**Calibration**

- Detailed instructions of calibration of the instrument are mentioned

**Procedure**

- Sequenced detailed directions
- Describe successive steps for all the procedures

**Interpretation of results**

- Directions for evaluating the results
- List of all the tables
- Specify significant figures

**Research report**

- Detailed information required in reporting the results
- Correlating the results

**References**

- References to publications supporting or providing needed information
- Keywords: identify words, terms or phrases that best represent the technical information reported

**Conclusions**

- Summarize all collected data
- Compare data from different methods
- Site all relevant information
CHAPTER I
INTRODUCTION

Pre-formulation studies are used for the characterization of drug substances and excipients [1]. There is an ongoing need for technical information that describes physical and chemical properties of drugs and excipients in the development of formulations and manufacturing processes [2]. Pre-formulation studies often include, crystal size and shape; polymorphism; drug permeability; pH-solubility profiles; pH-stability profiles; and screening for suitable excipients.

Thermal analysis and Microcalorimetry are key tools for purity evaluations, thermal and oxidative stability determination, and drug-excipient compatibility studies. Thermal analytical techniques are used to characterize the behavior of pharmaceutical samples as a function of temperature or time [3, 4]. These methods include DSC, DEA, and TGA [5, 6]. DSC and TGA are continually used to study and characterize potential drug-drug, drug-excipient interactions using thermal analysis [7, 8].
Polymorphism is the ability of a compound to crystallize in more than one distinct crystal structure. Polymorphs usually show different physiochemical behavior in properties of pharmacological interest such as: density, hardness, hygroscopic tendency, solubility, velocity, or thermal stability [12]. One polymorph can be converted into another under certain circumstances and can be characterized by DSC, TGA, and other combined techniques. The excipients used are listed in the Table 1.

Table 1 List of excipients studied

<table>
<thead>
<tr>
<th>Excipient</th>
<th>CAS</th>
<th>Phase</th>
<th>Melting Point (°C)</th>
<th>Source</th>
<th>Functional Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic Acid</td>
<td>65-85-0</td>
<td>Solid</td>
<td>122</td>
<td>Fisher Scientific Company</td>
<td>Preservative</td>
</tr>
<tr>
<td>Mannitol</td>
<td>69-65-8</td>
<td>Solid</td>
<td>165</td>
<td>Atlas Chem.</td>
<td>agent, Diluent</td>
</tr>
<tr>
<td>Anhydrous Lactose</td>
<td>63-42-3</td>
<td>Solid</td>
<td>223</td>
<td>Eastman Organic Chemical</td>
<td>Diluent, Filler</td>
</tr>
<tr>
<td>Calcium Phosphate</td>
<td>7756-87-4</td>
<td>Solid</td>
<td>Decomp&gt;100</td>
<td>Amend Drug Chemicals</td>
<td>Buffer, Glidant, Diluent</td>
</tr>
</tbody>
</table>

*Decomp = Decomposition
In the next phase, we studied the drug delivery behavior of the antipsychotic drugs used in Bipolar Disorder Dosing.

Bipolar disorder (BPD) is a widespread chronic disorder with recurrent episodes of mania and depression [13]. It accounts for 25% of all mental disorders. For many years, the conventional mood stabilizers such as lithium (as carbonate and citrate) and antidepressants have been used as a first choice of medication for the treatment of BPD patients before the evolution of newer medications [14-16]. Due to their long-term side effects, recently a new class of antipsychotic drugs, the atypical antipsychotics, has become effective with fewer side effects and better mood stabilizing properties [17]. It is very important for a bipolar disorder patient to take medications in a timely manner. Untimely discontinuation of the medications is the major complication to positive outcomes in individual bipolar disorder patients. Treatment adherence is a very important aspect to be considered and it is affected by various factors like age, gender, medication side effects and other environmental factors [13, 18 and 19]. Another important factor to be considered is the route of administration. This treatment adherence problem can be overcome by transdermal drug delivery method which will be advantageous to insure far greater patient compliance [20]. Due to improved pharmacokinetic properties, transdermal drug delivery is becoming more popular and preferable [21, 22]. Further, the transdermal route is beneficial since it avoids one pass through the liver where other undesirable biochemical transformations and deactivation take place [23-25]. Transdermal studies have used a variety of model skins such as hairless mouse skin, pig skin, human cadaver skin and some synthetic membranes [26]. In the present study, shed snake skin and excised pig skin are used as a model membranes. The rationale for opting
snake skin is that its epidermal layer is considered to have a stratum corneum similar to that of human stratum corneum [27]. Its profuse availability, negligible biohazard, its easy storage and handling makes it more advantageous [28]. But due to the lack of hair follicles, in addition to the shed snake skin, pig skin was also used. The drugs used for the study are mentioned in Table 2. All the drug samples selected were analyzed by DEA and the data was correlated with the UV studies. The drug throughput and structure were confirmed by UV analysis (Absorbance vs. Concentration & maximum peak wavelength). The drug purity was set by Differential Scanning Calorimetry. The results were interpreted for the analyzed samples.

**Table 2** List of BPD drugs studied

<table>
<thead>
<tr>
<th>Drug (Trade name)</th>
<th>CAS No.</th>
<th>Melting Point (°C)</th>
<th>Mol. Wt. (g/mol)</th>
<th>Structure</th>
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<tr>
<td>Olanzapine (Zyprexa)</td>
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<td>195</td>
<td>312.44</td>
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</tr>
<tr>
<td>Risperidone (Risperidal)</td>
<td>106266-06-2</td>
<td>170</td>
<td>410.49</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Quetiapine Fumarate (Seroquel)</td>
<td>11974-72-2</td>
<td>174</td>
<td>883.11</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
CHAPTER II
INSTRUMENTS AND METHODS

2.1 Differential Scanning Calorimetry

This is one of the most employed Thermal Analytical techniques for the characterization of pharmaceutical solids. It measures the heat flow into and out of a sample relative to a reference sample as a function of time and temperature. DSC provides test data for a wide range of materials which include polymers, plastics, foods, pharmaceuticals, biological samples and more. It measures the following: sample purity (Melting points), Heat capacity ($c_p$), Crystallization temperature ($T_c$), Glass transition temperature ($T_g$), drug stability, and oxidative stability.
2.1.1 Instrument

DSC operates over a temperature range of -50 to +300°C. It compares differences between the heat flow rate of the test sample and known reference materials at a constant temperature. DSC plots are obtained as the differential heating rate (in units of watts sec\(^{-1}\), joules sec\(^{-1}\) or calories sec\(^{-1}\)) verses temperature. Calibration parameters, flow rate, purge gas are some of the important experimental parameters. Heating rates range from 1°C min\(^{-1}\) to 100°C min\(^{-1}\).
2.1.2 Calibration

Proper calibration of the instrument is very important to obtain consistent results. Following calibration, the characteristic temperatures and the enthalpy associated with a phase change can be measured for any sample. High purity calibration standards and clean DSC sensors play an important role. Standard reference materials are used to calibrate the temperature and energy scales of DSC. Well defined standards and calibration procedures should be taken into consideration as crucial factors in comparing the results from different instruments. Laboratory-grade chemicals are not suitable for use as calibration standards due to the presence of trace amounts of impurities which may alter the melting temperature. Calibration should be done as close to the transition temperature of interest as possible with the commonly used standards such as indium, tin and lead. Standard procedures can be obtained from the American Society for Testing Materials (ASTM).

2.1.3 Sampling

A reliable DSC curve can be obtained if there is a good thermal contact of sample with the base of the sample pan. The heating a sample to a temperature higher than the melting or glass transition temperature makes it possible to obtain a dependable DSC curve. Proper choice of experimental conditions leads to a successful experiment. DSC uses pans made of aluminum, platinum, stainless steel or silver to minimize the reaction with the sample. The experiment can be conducted with an open pan, covered pan or sealed pan and the reference pan with similar configuration. The sample size of 2-10mg is employed. Samples should be weighed before and after the experiment to observe any mass loss upon heating. If powdered samples are used, distribution of the sample evenly
throughout the pan ensures good thermal contact. To obtain accurate quantitative results
the mass of sample and reference pans should match.

2.1.4 Procedure

Calibration is a basic technique for any instrument in order to obtain accurate results.
The calibration of this instrument was done based on the Standard Test method for
temperature calibration of Differential Scanning Calorimetry, adaption ASTM method
E967-03. Sample was first equilibrated to 40°C below the melting point and then was
heated at 3°C min\(^{-1}\), in an Aluminum pan (open and closed); 10 mgs sample size. The
samples used were the APIs and Excipients as powders. As mentioned in ASTM method
the APIs were placed in a DSC standard aluminum pan except for caffeine, which was
placed in closed aluminum pan, as it sublimes. Nitrogen gas was supplied in order to
keep the DSC cell inert. Some of the calibration materials/samples used was Acetanilide,
Acetophenetidin, Caffeine, and Vanillin. A DSC scan usually provides us the data of the
following thermal properties- The heat of fusion ($\Delta H_f$), melting temperature ($T_m$) peak
melt temperature ($T_{mp}$), heat of crystallization ($\Delta H_c$), crystallization temperature ($T_c$),
peak crystallization temperature ($T_{cp}$) and glass transition temperature ($T_g$). The drug
profile of Acetanilide is illustrated in fig 3.
**Figure 3** Heat-Cool-Heat cycle of Acetanilide with $T_m$, $T_{mp}$, $\Delta H_f$, $T_c$, $T_{cp}$, $\Delta H_c$

### 2.2 Dielectric Analysis

It measures the electrical properties of a material with the respect of time and temperature. DEA measures two fundamental electrical characteristics of a material or fluid: Capacitance and Conductance. Unlike DSC, DEA can be used to identify the secondary relaxations present in the pharmaceuticals as long as the secondary group has a net dipole moment.
The capacitive nature of a material is its ability to store electrical charge, and the conductive nature is its ability to transfer an electric charge. This component becomes more important when the material is heated above the melting temperature to the amorphous phase. These electrical properties are related to molecular activity, allowing for probing the chemistry, rheology and molecular mobility of drugs and polymers. DEA measures three main parameters over a wide range of frequencies (e.g. 0.10 to 10,000 Hz): 1. **Permittivity** ($\varepsilon'$) is a measure of the alignment of molecular groups (dipoles) in the electric field. 2. **Loss factor** ($\varepsilon''$) is a measure of the energy required to move the molecular groups or ions and is proportional to ion conductivity. 3. **Tan delta** is the ratio of the loss factor divided by the permittivity.

2.2.1 **Calibrating temperature of Dielectric Analyzer**

Dielectric analyzers are used to characterize a broad range of materials that possess dielectric moments. They can be used over a wide range of temperatures from -100 to 300°C. One of the desired values to be assigned by the measurements is the temperature at which significant changes occur in the properties of test specimen. In order to obtain
consistent results from one period of time to another and from one laboratory to another, the temperature from the apparatus must be calculated accurately over the temperature range of interest. This method covers the temperature range from -100 to 300°C. The calibration is performed by observing the melting transition of standard reference materials having known transition temperatures within the temperature range of use.

2.2.2 Instrument

The apparatus used is the Dielectric Analyzer (DEA) TA instrument (TAI) 2970. The following parameters should be taken into consideration: Sensors, impart the alternating electric field and measure the induced current and phasing in the sample specimen. These sensors may be either parallel plate or a single plate containing a series of interdigitated electrodes. Single surface interdigitated electrodes are used in this experiment, the temperature Programmer and Furnace set the heating rate and operating temperatures below ambient temperatures. Cooling may be by a mechanical refrigerator or other coolant such as liquid nitrogen. Specimen Atmosphere Control System, capable of supplying inert gas, usually nitrogen with a selectable flow rate of 50 mL min⁻¹. The dielectric thermal curve consisting of permittivity on the Y axis (ordinate) and temperature on the X axis (abscissa) was set for the calibration material for e.g. Acetanilide, Vanillin, and Acetophenetidin.

Small sample sizes are used for the analysis. The mixing or stirring of samples prior to the analysis was recommended. Any mechanical or thermal treatment, such as grinding or sieving if applied to the sample before analysis was recorded. The sample must cover the electrode array completely.
2.2.3 Procedure

Load the calibration material into the DEA instrument. Set the initial temperature of the apparatus to a value of about 30°C below the estimated transition temperature of the calibration material. The sample was analyzed at a heating rate of 5°C min⁻¹ in flowing nitrogen at 50 mL min⁻¹ to a temperature above 20°C above the estimated transition temperature of the calibration material. Initiate the measurement of permittivity over frequencies from 0.1Hz to 1000 Hz and single frequency 1000 Hz to record permittivity measurement on a linear scale, as a function of temperature. Remove the residue material and clean the electrode after the experiment.

2.3 Thermal Gravimetric Analysis (TGA)

The primary purpose of everyday routine TGA experiments is to measure moisture content, residual solvent, and polymerization of materials like phenolics that evolve reaction products.

2.3.1 Instrument

The heart of the TGA is the furnace and the area surrounding it. Regular heating of furnace and the sample holder up to 800°C in air for 10-15 minutes should be done to keep them clean from organic residues. The instrumental baseline should be checked occasionally, especially after running a furnace cleaning procedure and where very small weight losses are being recorded. When performing TGA runs, the following experimental conditions should be carefully selected.
2.3.2 Sampling and Procedure

Before loading the sample into the TGA instruments, it has to be weighed into TGA pans. Platinum, aluminum (sealed and open) are usually the commercially available TGA pans. Platinum pans are used to analyze polymers as they can withstand high temperatures (up to 800°C). Aluminum pans can be used for the measurements that leave a stable residue. The sample should be weighed on an electronic microbalance to at least ±0.01% accuracy. Usually minimum sample weights are used to decrease the thermal lag and the internal thermal resistance of the sample. But sometimes larger sample weights are used in order to increase the sensitivity of the measurement. After the sample has been prepared, it is weighed into the TGA pan, usually at room temperature. A variety of purge gas options are available. An inert atmosphere, usually high purity dry nitrogen, should be used when it is important to avoid oxidative processes. If the source of the purge gas is compressed air it is important to use a filter to remove any pump oil. It may be useful to purge the TGA furnace with a gas, usually air or nitrogen that contains a controlled amount of moisture. The flow rate of the purge gas is important, and it should be set as suggested by the manufacturer. Initially the sample pan weight is tared to zero. After this, the sample is carefully loaded into the pan and the experiment is set to run. The sample is heated at a rate of 10°C min⁻¹ with purging of nitrogen at a flow rate of 50 ml min⁻¹. Then the data is analyzed using TA software.
CHAPTER III

Dielectric Analysis Enhances Characterization of Pharmaceutical Excipients.

Hareesha Reddy Venumuddala

(Manuscript submitted for publication and is under review)

(Journal of Thermal Analysis Calorimetry)

3.1 Abstract

Millions of dollars are expended annually on pharmaceutical testing to qualify excipients for fully formulated drugs, medicines, and active ingredients. To understand the action of the excipients in the human body at body temperature, 37°C the study of their individual and interactive properties is desirable. Dielectric Thermal Analysis (DEA), Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TG) methods were employed to screen the most widely used drug excipients. In this study the following chemicals were examined by DEA: calcium phosphate, cotton seed oil, croscarmellose, gelatin, mannitol, peanut oil, polyethylene glycol, pioneer sugar, plasdone, sodium alginate, sodium lauryl sulfate, sodium starch glycolate, sodium
stearate, a sorbitol solution, canola oil, anhydrous lactose, and benzoic acid. The comparison of DSC and DEA thermal curves for each excipient indicates that major endothermic events, e.g. volatilization or melting of the excipient or a drug, are delineated by fundamental DEA properties, with an exponential rise in permittivity and dielectric loss factor. The focus of this research is to study the “thermal event“ response from the DEA, that is, the electrical conductivity (loss factor*frequency*constant), permittivity and tan delta (loss factor/permittivity) vs. frequency.

Key Words: Excipient, DSC, DEA, TGA, Polymorph.

3.2 Introduction

Preformulation studies are used for the characterization of drug substances and excipients [1]. There is an ongoing need for technical information that describes physical and chemical properties of drugs and excipients in the development of formulations and manufacturing processes [2]. Preformulation studies often include, crystal size and shape; polymorphism; drug permeability; pH-solubility profiles; pH-stability profiles; and screening for suitable excipients.

Thermal analysis and Microcalorimetry are key tools for purity evaluations, thermal and oxidative stability determination, and drug-excipient compatibility studies. Thermal analytical techniques are used to characterize the behavior of pharmaceutical samples as a function of temperature or time [3, 4]. These methods include DSC, DEA, and TGA [5, 6]. DSC and TGA are continually used to study and characterize potential drug-drug, drug-excipient interactions using thermal analysis [7, 8].

DEA, employing an interdigitated array of electrodes, is a new innovative physical-analytical method in evaluating real world excipient interactions and possibly predicting
performance. DEA measures two fundamental electrical characteristics of a material or fluid, capacitance and conductance as a function of time, temperature, and frequency. The capacitive nature is the ability of the material to store an electrical charge and dominates the electrical response at low temperatures. The conductive is the ability to transfer an electrical charge and this component becomes important when the material is heated above the melting temperature.

DEA measures three main quantities over a wide range of high frequency: permittivity, loss factor, and tan delta. Permittivity (dielectric constant at high frequency) is a measure of the alignment of the molecular groups to the electric field. Loss factor is a measure of the energy required to move the molecular groups or ions and is proportional to ion conductivity (loss factor*frequency*constant and the energy to align dipoles). Moreover, ion conductivity becomes increasingly sensitive when the material becomes fluid. Finally, tan delta is the ratio of the loss factor to permittivity. Drug degradation and stability are revealed by TGA studies [9].

Thus, DSC, TGA and DEA, were applied to screen the model excipients, which all have specific purposes in the pharmaceutical industry. The assets of these techniques are that one can evaluate critical properties based on very small amounts of chemical in a very short time². Benzoic acid also known as flowers of benzoin is commonly used as an antiseptic in cough medications [10, 11]. Mannitol is a naturally occurring sugar alcohol that is non-hygroscopic and is typically utilized in food applications and therapeutically to decrease cellular edema and increase the urinary output [11]. Anhydrous lactose is milk sugar that serves as a diluent, filler, and bulking agent. It is also provides free flow
ability and avoids segregation in capsule filling [10-13]. Calcium phosphate is binder, filler and sometimes an antacid [11].

Polymorphism is the ability of a compound to crystallize in more than one distinct crystal structure. Polymorphs usually show different physiochemical behavior in properties of pharmacological interest such as: density, hardness, hygroscopic tendency, solubility velocity, or thermal stability [12]. One polymorph can be converted into another under certain circumstances and can be characterized by DSC, TGA, and other combined techniques.

3.3 Experimental

3.3.1 Materials Used

All excipients, except benzoic acid were received from the University of Toledo, College of Pharmacy. The original source and condition of all the samples are in Table 3.

Table 3 List of Excipients

<table>
<thead>
<tr>
<th>Excipient</th>
<th>CAS</th>
<th>Phase</th>
<th>Melting Point (°C)</th>
<th>Source</th>
<th>Functional Category</th>
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</thead>
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<tr>
<td>Benzoic Acid</td>
<td>65-85-0</td>
<td>Solid</td>
<td>122</td>
<td>Fisher Scientific Company</td>
<td>Preservative</td>
</tr>
<tr>
<td>Mannitol</td>
<td>69-65-8</td>
<td>Solid</td>
<td>165</td>
<td>Atlas Chem.</td>
<td>Sweetening agent</td>
</tr>
<tr>
<td>Anhydrous Lactose</td>
<td>63-42-3</td>
<td>Solid</td>
<td>223</td>
<td>Eastman Organic Chemical</td>
<td>Diluent, Filler</td>
</tr>
<tr>
<td>Calcium Phosphate</td>
<td>7756-87-4</td>
<td>Solid</td>
<td>Decomp* &gt;100</td>
<td>Amend Drug Chemicals</td>
<td>Buffer, Glidant, Diluent</td>
</tr>
</tbody>
</table>

*Decomp = Decomposition
3.3.2 Methods

The TA Instruments (TAI) Dielectric Analyzer 2970 was used for dielectric analysis of the samples over a wide range of frequencies from 0.1 to 1000 Hz and temperatures from −50 to 300°C. Other parameters included a heating rate of 10°C min⁻¹, nitrogen gas purge at a flow rate of 50mL min⁻¹, and liquid nitrogen cooling when necessary. The single surface gold ceramic interdigitated sensors were utilized. A Sample size of 10-20mg of either solid or liquid sample was used to cover the plated sensor with a thin layer. DEA calibrated with acetanilide with a T_{mp} 115°C (DSC) and T_{m} (DEA) 114°C.

The TAI DSC 2920 was used to verify heat flow properties as a function of temperature. The heating rate was 10°C min⁻¹ from −50 to 255°C with nitrogen gas purge at 50mL min⁻¹. Sample size was between 4 and 6mg.

The TAI TGA 2950 was also used to verify mass loss properties as a function of temperature. The heating range was 10°C min⁻¹ from ambient to 500°C with nitrogen gas purge at 50mL min⁻¹ and nitrogen gas balance purge at 40mL min⁻¹. Sample size was between 7 and 12mg.

3.4 Results and Discussion

Tan delta, permittivity, and ion conductivity curves at 0.1, 10, and 100Hz respectively, were specifically interpreted for evaluation. The 0.1Hz tan delta is very sensitive to molecular relaxations and changes in chemical behavior at the surface of the electrode. The DEA sensor evaluates chemicals within 300µ from the electrode surface. This is based on the envelope of chemicals being evaluated that is 3 times the distance between the interdigitated electrodes or 100 microns.
The 10Hz permittivity was selected based on its repeatability and correlation with other permittivity measurements. Permittivity is very sensitive to chemistry variations, that is, molecular chemical changes at the surface of the electrode and not related to bulk changes in the chemicals. The 100Hz conductivity (also a near surface measurement) was selected based on its repeatability and correlation with other real world conductivity measurements. This is based on preparing non-aqueous solutions of known conductivity and measuring it by several methods including the DEA. The 100Hz was the most reliable and comparable with real world conductivity values.

**Benzoic Acid**

Benzoic acid is considered as a calibrant in this study. In the overlay of the DSC and 100Hz ion conductivity DEA curve (Fig. 5), a strong endothermic peak is evident at 124°C for the DSC and corresponds to a peak on the DEA at 118°C. The finding is supported by the known standard melting point of benzoic acid at 122°C [11]. The endothermic DSC peak and the rise of slope in the DEA curve following their respective melting point peaks are probably due to the sublimation stages of the material and the TGA curve (Fig. 6) indicates complete weight loss due to sublimation and is reached around 160°C. The DSC gave sharp melting peaks while the DEA gave broad diffuse peaks in the same general temperature range. Therefore, the DEA is less sensitive than the DSC to melting, but is more sensitive to polar-dipole constituents. However, the DEA is more sensitive to overall events.
Figure 5 Benzoic acid DSC and 100Hz Ion Conductivity DEA

Figure 6 TGA of Benzoic acid
Mannitol

The mannitol overlay of the DSC, TGA, and the 100Hz ion conductivity DEA (Fig 7), reveals a significant endothermic DSC peak at 168°C and the DEA confirms this with its peak at 167°C. The peaks represent the melting point of the sample as no weight loss is recorded from the TGA at the equivalent temperature. The ionic conductivity becomes increasingly sensitive when the material becomes fluid and amorphous. The standard melting temperature of mannitol is 164 to 168°C depending on the type of mannitol [11, 14]. The resulting DEA/DSC temperature is accurate when compared to the standards; however, polymorphism may have some effect on this property.

Figure 7 Mannitol TGA, DSC and 100Hz DEA Ionic Conductivity
Next, from a magnified image the DEA curve (Fig 9), a second peak occurs at 1°C that has no corresponding DSC peak. An obvious advantage of the DEA over the DSC is now evident. The origin of this DEA peak is most likely from beta transitions or water fusion. The transitions involve the rotation of short-chain OH side groups on the mannitol structure and therefore, occur below.

**Figure 8** Mannitol structure

**Figure 9** Mannitol DSC and 100Hz DEA Ionic conductivity
**Anhydrous Lactose**

In the overlay of the DSC, TGA, and 10Hz permittivity DEA (Fig. 10), a major endothermic DSC peak at 235°C is exhibited and the DEA curve is also increasing to reach a peak around 235°C. Both the DSC and DEA suggest that 235°C is the melting point and the decomposition of the sample begins shortly thereafter, as supported by the TGA. The standard melting point is actually 223°C and again, the temperature difference is most likely due to polymorph behavior\(^\text{10}\). In addition, the DEA exhibits a beta transition peak with the OH side chains on the molecule at -7°C and the DSC has no indication of any activity, similar to mannitol.

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**Figure 10** Anhydrous Lactose DSC, TGA and 10Hz Permittivity DEA
Calcium Phosphate

The overlay of DSC, TGA, and 10Hz ion conductivity DEA (Fig. 11) indicates a DSC exothermic peak at 13°C and corresponds to the DEA peak at roughly 11°C. The cause is likely due to the crystallization of the sample.

The TGA curve shows about twenty percent weight loss of the sample over a span of roughly three hundred degrees, from 100 to 400°C. The standard melting point of pure calcium phosphate is over 1000°C. Thus, the weight loss is probably not due to properties of calcium phosphate. Consequently, impurities in the sample are the likely cause of the activity. The Therm Ex-GC/MS was again used for the sample. Results identify two main compounds in the first partition between 25 and 150°C: propanal, 2-oxo- (C₃H₄O₂), which has a boiling point of 72°C; and 1-methoxy-2-propyl acetate (C₆H₁₂O₃). The decomposition of a combination of these two is probably responsible for the DSC endothermic peak at 144°C, which corresponds to the DEA peak at 143°C and the first weight loss from the TG around 113°C. The second partition from 150 to 300°C shows 1-methoxy-2-proyl (C₆H₁₂O₃) and is probably the remainder of the compound from the earlier decomposition.
As far as the excipients are concerned, when comparing the complexity of the analysis results of the liquid versus solid, the liquid samples are more difficult to interpret due to the fact that liquid samples themselves are more complicated. Low temperature properties of excipients reveal endothermic and conductivity changes as a result of various transitions. Higher temperature properties designate the relative thermal stability or a property change as a function of temperature. The DEA is more sensitive to polar versus non-polar chemicals, overall thermal events, and to polar-dipole constituents; however, the DEA is less sensitive than the DSC to melting. Even so, the DEA serves a valuable tool when it comes to analyzing the thermal properties of excipients used for pharmaceutical purposes. Nevertheless, using a variety of instruments to complement the DEA, DSC, TGA are necessary to assist in interpreting accumulated data and to ensure detailed analysis.
3.6 Further Studies

Future work includes more research and testing of excipients to determine the exact origin of certain indeterminate peaks for selected samples, interpreting the data of the remaining excipients, and applying excipients data to assist in the development or modification of pharmaceutical drugs. We also want to blend excipients into a two or three component system for analysis to collect data over a wide concentration range, thus aiding in the development of a computer simulation model that could predict the interactive behavior of the forty most prominent excipients.

3.7 References


4.1 Abstract

Atypical antipsychotics are emerging as effective drugs for the treatment of Bipolar Disorder (BPD), a common and recurrent mood disorder. Historically, this class of drugs has been specifically used to treat Schizophrenia. More recently, their mood stabilizing properties and reduced extra pyramidal effects have made them treatments of choice for BPD consumers. Studying the transport properties of these drugs through a transdermal route may pave the way for a novel transdermal drug delivery method. This will aid BPD patients to receive their dosage in a timely manner and improve patient compliance. In this study, Dielectric Analysis (DEA) is used to characterize and evaluate the transport properties of the selected drugs (Olanzapine, Risperidone, and Quetiapine Fumarate).
DEA implements drug delivery employing an AC frequency, modulating drugs of a wide molecular weight range (e.g. 300 to 30,000 Daltons) with an interdigitated electrode system. In this analysis, the drugs were studied using an animal model (shed snake skin). There is a specific range of critical frequencies discovered for each drug associated with active drug transport. Drug transport is measured by observed increases in conductivity of the shed snake skin and is confirmed with drug throughput measured by UV spectroscopy.

Key Words: atypical antipsychotics; bipolar disorder; dielectric analysis; ultraviolet-visible spectroscopy; differential scanning Calorimetry.

### 4.2 Introduction

Bipolar disorder (BPD) is a widespread chronic disorder with recurrent episodes of mania and depression [1]. It accounts for 25% of all mental disorders. For many years, the conventional mood stabilizers such as lithium (as carbonate and citrate) and antidepressants have been used as a first choice of medication for the treatment of BPD patients before the evolution of newer medications [2-4]. Due to their long-term side effects, recently a new class of antipsychotic drugs, the atypical antipsychotics, has become effective with fewer side effects and better mood stabilizing properties [5]. It is very important for a bipolar disorder patient to take medications in a timely manner. Untimely discontinuation of the medications is the major complication to positive outcomes in individual bipolar disorder patients. Treatment adherence is a very important aspect to be considered and it is affected by various factors like age, gender, medication side effects and other environmental factors [1, 6 and 7]. Another important factor to be considered is the route of administration. This treatment adherence problem can be
 overcome by transdermal drug delivery method which has been advantageous to insure far greater patient compliance [8]. Due to improved pharmacokinetic properties, transdermal drug delivery is becoming more popular and preferable [9, 10]. Further, the transdermal route is beneficial since it avoids one pass through the liver where other undesirable biochemical transformations and deactivation take place [11-13]. Transdermal studies have used a variety of model skins such as hairless mouse skin, pig skin, human cadaver skin and some synthetic membranes [14]. In the present study, shed snake skin is used as a model membrane. The rationale for opting snake skin is that its epidermal layer is considered to have a stratum corneum similar to that of human stratum corneum [15]. Its profuse availability, negligible biohazard, its easy storage and handling makes it more advantageous [16].

Most transdermal drug delivery platforms rely on passive diffusion. Commercial transdermal patches have also been developed to deliver the drugs using an electric field. The prime objective of this research is to achieve a controlled release delivery FDA-approved BPD drugs into and through the skin using Dielectric Analysis (DEA). This thermal technology uses a series of optimum AC signals to transfer the drug through the skin at a controlled rate. The drug throughput was confirmed by UV analysis. The drug purity was set by Differential Scanning Calorimetry.

Thermal analytical techniques have been used in this study to reveal the transport properties of the selected drugs. Dielectric Analysis (DEA) is known to be a versatile tool for material characterization. It is used to evaluate the electrical properties of a wide range of materials, drugs and pharmaceuticals available in different forms. It is used over a wide range of frequencies, from 0.1Hz to 100,000Hz and temperatures from 50 to
250°C and measures three main properties: permittivity, loss factor and tan delta. Permittivity (dielectric constant at high frequency) is a measure of the alignment of the molecular groups to the electric field. Loss factor is a measure of the energy required to move the molecular groups or ions and is proportional to ion conductivity (loss factor*frequency*constant (2π) and the energy to align dipoles). Moreover, ion conductivity becomes increasingly sensitive when the material becomes fluid. Finally, tan delta is the ratio of the loss factor to permittivity. All the drug samples selected were analyzed by DEA and the data was correlated with the UV studies. The results were interpreted for the analyzed samples.

The antipsychotic drugs used in this study are Olanzapine, Risperidone, and Quetiapine Fumarate. Olanzapine belongs to the class of thienobenzodiazepine and is chemically referred to as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thienol [2, 3-b] [1, 5] benzodiazepine [17]. It is the most widely studied drug of this class. It has significant evidence of efficacy in the treatment of manic phase as well as depressive phase of Bipolar Disorder [18]. It is a yellow crystalline solid and is taken through an oral route of administration [19]. It is an FDA approved drug in the U.S and is approved for use in Europe [20]. Olanzapine is also effective at delaying or preventing relapse during long-term maintenance therapy in treatment responders, and is currently the only atypical antipsychotic approved for this indication. Risperidone is a white powder which is practically insoluble in water. It belongs to the chemical class of benzisoxazole derivatives which is chemically designated as 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one [21]. It is usually administered as tablets, liquids, injection and orodispersible tablets. Quetiapine
Fumarate which is chemically designated as 2-[2-(4-dibenzo [b, f] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy]-ethanol fumarate (2:1) (salt) and belongs to the class of dibenzothiazepines. The FDA has approved Quetiapine monotherapy for the acute treatment of depressive episodes associated with bipolar disorder and as maintenance treatment of bipolar disorder as an adjunct to lithium or divalproex. It is an off-white crystalline powder, slightly soluble in water [22].

4.3 Materials and Methods

4.3.1 Drugs

All the drug samples were ordered from Haorui Pharma-Chem Inc. 100 Menlo Park Dr., Suite 316, Edison, NJ 08837, USA.

<table>
<thead>
<tr>
<th>Drug (Trade name)</th>
<th>CAS No.</th>
<th>Melting Point (°C)</th>
<th>Mol. Wt. (g/mol)</th>
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<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

4.3.2 Skin Membranes

The shed skin of a Cuban Boa (*Epicrates angulifer*), a black snake, is used in this permeation study. It mainly comprises of two portions: the core and the boundary. The
core thickness is 20 μ and the boundary is 150 μ thick (Fig. 12). The latter portion is densely porous through which the drug diffuses. The skin is always sealed in plastic bags and refrigerated at temperature of -20°C.

![Figure 12 Shed skin of Boa](image)

**Figure 12** Shed skin of Boa

### 4.3.3 Experimental Methods

The TAI DSC 2920 was used to verify drug purity and heat flow properties as a function of temperature. DSC scans were run over a temperature range of –50 to 255°C with nitrogen as purge gas at 50mL min⁻¹. Sample size was between 2 to 6mg. DSC uses pans made of aluminum, platinum, stainless steel or silver to minimize the reaction with the sample. The experiment can be conducted with an open pan, covered pan or sealed pan and the reference pan with similar configuration. In this study, commercial solid fat index (SFI) aluminum pans (Fig. 13) were used and the experiment was conducted with an open pan. The samples were weighed and loaded into the pans. The sample is subjected to a Heat-Cool-Heat (HCH) cycle. We started at 28°C and heated to 40°C above the melt temp, i.e. the extrapolated onset temperature and cooled to 40°C below the melt temperature at a rate of 10°C min⁻¹. The data is analyzed using TAI Universal Analysis software.
Figure 13 Solid fat index (SFI) pans

The TA Instruments (TAI) Dielectric Analyzer 2970 was used for dielectric analysis of the samples over a wide range of frequencies from 0.1 to 100,000 Hz and temperatures from –50 to 300°C. Other parameters of the instrument included: the heating rate, purge gas, and liquid nitrogen cooling when necessary. The single surface gold ceramic interdigitated sensors were used (Fig. 14). A sample size of 2 to 6mg of each drug was evaluated. The sample was properly weighed and the shed snake skin is cut according to the size of the sensor. The skin was soaked in pH buffer 7.4 for 10 to 12hrs before the experiment. The weighed sample is then evenly loaded on to the top portion of the skin which is carefully placed on the electrode (Fig. 15). Then the sample is subjected to heating at a rate of 10°C min$^{-1}$ up to 37°C and then kept isothermal for 40 min at that temperature with nitrogen as a purge gas at a flow rate of 50mL min$^{-1}$. The frequency range was selected from 0.1 to 30,000 Hz. The electrical response of the sample is recorded and the samples were analyzed. After each experiment, the skin is safely discarded and the electrode is soaked in an appropriate solvent for the drug (Olanzapine and Quetiapine Fumarate in Methanol and Risperidone in 0.1M H$_2$SO$_4$) for an hour. The residue in solution is then collected in a glass vial for UV analysis. For each drug, two separate DEA studies were conducted and two separate residue samples were collected from those studies. Similarly, two negative controls were run in the DEA apparatus with
no applied electric field and these samples were also collected using the same method described above.

Figure 14 Single surface gold ceramic sensor

Figure 15 Drug on top of snake skin

The Cary 50 B10 UV-Visible spectrophotometer is used to evaluate the concentration of the drug throughput. For this study stock solutions of known drug concentration were prepared using approximately 2 to 6 mg of each drug in a drug soluble solvent. A calibration curve was developed from the above stock solutions with measured absorbance vs. concentration of each drug at a particular wavelength (Olanzapine at 363 nm, Risperidone at 280 nm and Quetiapine Fumarate at 303 nm). Two test samples and two control samples of unknown drug concentration of each drug collected in the vials were screened by UV spectroscopy. These samples were scanned and the absorbencies measured were fit into the standard calibration curve. Thus the unknown concentration of the sample is interpolated from the calibration curve or can be derived from the Beer-Lambert law and is compared with the negative control sample.
4.4 Results and Discussion

4.4.1 DSC Evaluation

DSC revealed the purity of the three drugs tested in our study. In comparison of the melt temperature ($T_m$) measured to the literature value, the relative purity can be ascertained. The three drugs in this study, Olanzapine, Risperidone and Quetiapine Fumarate were observed to have melting temperatures same as that of literature with the correlation co-efficient of 0.98. The DSC protocol also aids in defining the ability of the crystalline solid to recrystallize or remain amorphous and produce a glass transition temperature. The DSC profile of Risperidone shown below (Fig. 16) illustrates the Heat-Cool-Heat cycle in which it happens to recrystallize upon cooling and melting upon reheating which confirms it to be a crystalline material. Whereas, Olanzapine and Quetiapine Fumarate do not recrystallize upon cooling, thus exhibiting their amorphicity. The heat of fusion ($\Delta H_f$), melting temperature ($T_m$) peak melt temperature ($T_{mp}$), heat of crystallization ($\Delta H_c$), crystallization temperature ($T_c$), peak crystallization temperature ($T_{cp}$) and glass transition temperature ($T_g$) were determined for all the three drugs and listed in Table 5. The drugs used in this study are probably >99%w pure.
**Figure 16** DSC profile of Risperidone with melting temperature

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Values</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine Fumarate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Literature Value</td>
<td>195</td>
<td>170</td>
<td>174</td>
</tr>
<tr>
<td><strong>Heat</strong>&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Tm (°C)</td>
<td>194</td>
<td>170</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>Tmp (°C)</td>
<td>196</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>ΔHf</td>
<td>107</td>
<td>90</td>
<td>122</td>
</tr>
<tr>
<td><strong>Cool</strong></td>
<td>Tc (°C)</td>
<td>-</td>
<td>138</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Tcp (°C)</td>
<td>-</td>
<td>133</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ΔHc</td>
<td>-</td>
<td>73</td>
<td>-</td>
</tr>
<tr>
<td><strong>Heat</strong>&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Tm (°C)</td>
<td>-</td>
<td>168</td>
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<td></td>
<td>Tmp (°C)</td>
<td>-</td>
<td>171</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Tg (°C)</td>
<td>73</td>
<td>-</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>ΔHf</td>
<td>-</td>
<td>80</td>
<td>-</td>
</tr>
</tbody>
</table>
**4.4.2 Dielectric Analysis**

From the dielectric profiles of each drug obtained, the log conductivity ($\text{pS}\text{cm}^{-1}$) values were plotted against log frequency (Hz). In this Debye plot, it is observed that the three drugs exhibited a marked change in the slope at about a frequency of 100 Hz. This frequency is called the critical frequency and this change is interpreted as a measure of the drug throughput using the shed snake skin as the animal model. The Debye plot of Olanzapine is illustrated in Fig. 17, Fig. 18 and Fig. 19 indicates the slopes before and after the critical frequency. Olanzapine and Risperidone have an increased percentage of rates of change of log conductivity vs. log frequency which is interpreted as a positive sign of drug transport (see Table 6).

![Graph](image)

**Figure 17** Dielectric profile of Olanzapine, isothermal at 37°C for 40 min
Figure 18 Olanzapine DEA profile before change

y = 0.500x - 1.344
R² = 0.999

Figure 19 Olanzapine DEA profile after change

y = 0.827x - 2.041
R² = 0.994
Table 6 Analysis of DEA data to rank drug transport for the three drugs in shed snake skin

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Slope 1</th>
<th>Slope 2</th>
<th>Ratio (S₂/S₁)</th>
<th>% Increase of S₂ vs. S₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine</td>
<td>0.500</td>
<td>0.827</td>
<td>1.70</td>
<td>70</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.681</td>
<td>0.988</td>
<td>1.45</td>
<td>45</td>
</tr>
<tr>
<td>Quetiapine Fumarate</td>
<td>0.732</td>
<td>0.817</td>
<td>1.12</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: S₁-Slope 1, S₂-Slope 2; S₂ vs. S₁ = Delta log conductivity/delta log frequency

4.4.3 Spectroscopic evidence of drug delivery through the animal model membrane:

Monitoring the transport of the drug through the animal membrane was accomplished by UV Analysis. The calibration curve of Olanzapine is illustrated below in the Fig. 20. The three drugs scanned before measuring absorbencies confirmed peaks at about same wavelengths as that of the original drug (Olanzapine at 362 nm, Risperidone at 282 nm and Quetiapine Fumarate at 303 nm). The transport of drugs through the shed snake skin membrane illustrated a throughput of apparently 12 to 70% with an average of 42% (seen above in Table 6). The relationship between the end-point ionic conductivity and the measured concentration of membrane throughput by the drug was a cause and effect relationship, i.e., the conductivity (52.3 ±5.3 pS/cm or ±10%) at 35 min was related to the average drug transport (0.114 ±0.020 mg/mL or ±17%). Table 7 summarizes the results, with the two studies run for each drug designated with subscript 1 and subscript 2.
Figure 20 Olanzapine calibration curve

Table 7 End-point conductivity and throughput

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample (Abs.)</th>
<th>Control (Abs.)</th>
<th>Difference (Abs.)</th>
<th>Average of Difference</th>
<th>End point conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine₁</td>
<td>0.0935</td>
<td>0.0026</td>
<td>0.0909</td>
<td></td>
<td>54.3</td>
</tr>
<tr>
<td>Olanzapine₂</td>
<td>0.1048</td>
<td>0.0089</td>
<td>0.0959</td>
<td>0.093</td>
<td></td>
</tr>
<tr>
<td>Risperidone₁</td>
<td>0.0898</td>
<td>0.0277</td>
<td>0.0621</td>
<td>0.134</td>
<td>46.2</td>
</tr>
<tr>
<td>Risperidone₂</td>
<td>0.2301</td>
<td>0.0245</td>
<td>0.2056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quetiapine F.₁</td>
<td>0.0667</td>
<td>0.0155</td>
<td>0.0512</td>
<td>0.117</td>
<td>56.3</td>
</tr>
<tr>
<td>Quetiapine F.₂</td>
<td>0.2031</td>
<td>0.1950</td>
<td>0.1840</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.2031</td>
<td>0.1950</td>
<td>0.1840</td>
<td>0.114</td>
<td>52.3</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td></td>
<td></td>
<td>0.020</td>
<td>5.34</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td></td>
<td></td>
<td>17%</td>
<td>10%</td>
</tr>
</tbody>
</table>

End point conductivity of the drugs is 30,000 Hz.
4.5 Conclusion

This study produced varying results of drug throughput across a bio membrane. It is our opinion that a positive slope change in the log conductivity vs. log frequency curve implies good drug transport. This fact has been substantiated by determining the drug throughput of 12 to 70% by UV spectroscopy on the delivery side of the membrane.

UV analysis of the drug samples compared against the control samples yielded positive results of drug delivery. A calibration curve prepared from the stock solutions of each drug resulted in an $R^2$ value of about 0.99. The test samples analyzed were found to contain the drug delivered through the skin. The enhanced slope above the critical frequency is evidence that this protocol for drug transport is effective. The variation in the mg mL$^{-1}$ in the observed throughput is ±17% and has a cause and effect relationship with the conductivity. Future studies will replicate DEA controlled release in a pig skin animal model.

4.6 References


CHAPTER V
TRANSDERMAL DELIVERY: CHARACTERIZATION OF BIPOLAR DISORDER DRUGS AND STUDY OF THEIR TRANSPORT THROUGH BIOMEMBRANES

5.1 Introduction

All the drug samples studied were ordered from Haorui Pharma-Chem Inc. 100 Menlo Park Dr., Suite 316, Edison, NJ 08837, USA (see Table 8).
### Table 8 BPD drugs studied

<table>
<thead>
<tr>
<th>Drug</th>
<th>CAS No.</th>
<th>Melting Point (°C)</th>
<th>Mol. Wt. (g/mol)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine</td>
<td>132539-06-1</td>
<td>195</td>
<td>312.44</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>(Zyprexa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>106266-06-2</td>
<td>170</td>
<td>410.49</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>(Risperidal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td></td>
<td></td>
<td></td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>Fumarate</td>
<td>11974-72-2</td>
<td>174</td>
<td>883.11</td>
<td></td>
</tr>
<tr>
<td>(Seroquel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Olanzapine*

Olanzapine belongs to the class of thienobenzodiazepine and is chemically referred to as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thienol [2, 3-b] [1, 5] benzodiazepine (29). It is a yellow crystalline solid and is usually taken through an oral route of administration only.
It is the widely studied drug of this class. It has significant evidence of efficacy in the treatment of manic phase as well as depressive phase of Bipolar Disorder from the earlier studies (30). It is an FDA approved drug in U.S and Europe (31). Olanzapine is also effective at delaying or preventing relapse during long-term maintenance therapy in treatment responders, and is currently the only atypical antipsychotic approved for this indication. This is a faster oral disintegrating drug than others. Olanzapine is believed to exert its antimanic and antipsychotic effects predominantly by binding to and blocking dopamine D2 and serotonin (5-hydroxytryptamine) 5-HT2A receptors though the exact mechanism of the disorder is not known (32).

*Risperidone*

Risperidone belongs to the chemical class of benzisoxazole derivatives which is chemically designated as 3-[2-[4-(6-fluoro-1, 2-benzisoxazol-3-yl) 1-piperidinyl] ethyl]-6, 7, 8, 9-tetrahydro-2-methyl-4H-pyrido [1, 2-a] pyrimidin-4-one (33, 34). It is a white powder which is practically insoluble in water.
Figure 22. Chemical structure of Risperidone

Risperidone is usually used to help treat illnesses or conditions such as psychosis, schizophrenia, mania and hypomania. The main pharmacological activities of risperidone include serotonin 5-HT2 receptor blockade and dopamine D2 antagonism (Megens et al, 1994) (35). It is usually administered as Tablets, Liquid, Injection, Orodispersible tablets (36). At dosages £8 mg/day, risperidone is generally associated with a lower risk of extrapyramidal symptoms than conventional antipsychotics and may have a more favorable effect on cognitive functioning of the brain.

Quetiapine Fumarate

Quetiapine Fumarate which is chemically designated as 2-[2-(4-dibenzo [b, f] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy]-ethanol fumarate (2:1) (salt) which belongs to the class of dibenzothiazepines. It is an off-white crystalline powder, slightly soluble in water (37).
Figure 23 Chemical structure of Quetiapine Fumarate

It is an atypical antipsychotic agent widely used as a first line drug for the treatment of schizophrenia. Moreover, the FDA has approved quetiapine monotherapy for the acute treatment of depressive episodes associated with bipolar disorder and as maintenance treatment of bipolar disorder as an adjunct to lithium or divalproex.

Materials Used

The shed snake skin and excised pig skin are used as models of human skin. The shed skin of a Cuban Boa (*Epicrates angulifer*), a black snake, is used in this permeation study. It mainly comprises of two portions: the core and the boundary. The core thickness is 20μ and the boundary is 150 μ thick (Fig 24). The latter portion is densely porous through which the drug diffuses.

Figure 24 Shed snake skin
Pig skin samples with thickness ranging from 0.1cm to 0.2cm were obtained from adult Yucatan pigs. The skin is always sealed in plastic bags and refrigerated at temperature of -20°C. The surface areas of both the skins were cut according to the size of the electrodes.

5.2 Procedure

The TAI DSC 2920 was used to verify drug purity and heat flow properties as a function of temperature. DSC scans were run over a temperature range of –50 to 255°C with nitrogen as purge gas at 50mL min⁻¹. Sample size was between 2 to 6mg. DSC uses pans made of aluminum, platinum, stainless steel or silver to minimize the reaction with the sample. The experiment can be conducted with an open pan, covered pan or sealed pan and the reference pan with similar configuration. In this study, commercial solid fat index (SFI) aluminum pans were used and the experiment was conducted with an open pan. The samples were weighed and loaded into the pans. The sample is subjected to a Heat-Cool-Heat (HCH) cycle. We started at 28°C and heated to 40°C above the melt temp, i.e. the extrapolated onset temperature and cooled to 40°C below the melt temperature at a rate of 10°C min⁻¹. The data is analyzed using TAI Universal Analysis software.

The TA Instruments (TAI) Dielectric Analyzer 2970 was used for dielectric analysis of the samples over a wide range of frequencies from 0.1 to 100,000 Hz and temperatures from –50 to 300°C. Other parameters of the instrument included: the heating rate, purge gas, and liquid nitrogen cooling when necessary. The single surface gold ceramic interdigitated sensors were used (Fig 25). A sample size of 2 to 6mg of each drug was evaluated. The sample was properly weighed prior to the experiment. The shed snake
skin was soaked in pH buffer 7.4 for 10 to 12hrs before the experiment. The weighed sample is then evenly loaded onto the cut portion of pig skin and top portion of the shed snake skin which is carefully placed on the electrode (Fig 26). Then the sample is subjected to heating at a rate of 10°C min\(^{-1}\) up to 37°C and then kept isothermal for 40 min at that temperature with nitrogen as a purge gas at a flow rate of 50mL min\(^{-1}\). The frequency range was selected from 0.1 to 30,000 Hz. The electrical response of the sample is recorded and the samples were analyzed. After each experiment, the skin is safely discarded and the electrode is soaked in an appropriate solvent for the drug (Risperidone and Quetiapine Fumarate in Methanol and Olanzapine in 0.1M H\(_2\)SO\(_4\)) for an hour. The residue in solution is then collected in a glass vial for UV analysis. For each drug, two separate DEA studies were conducted and two separate residue samples were collected from those studies. Similarly, two negative controls were run in the DEA apparatus with no applied electric field and these samples were also collected using the same method described above.

![Figure 25 Single surface electrode](image-url)
Figure 26 Shed snake skin with drug

The Cary 50 B10 UV-Visible spectrophotometer is used to evaluate the concentration of the drug throughput. For this study stock solutions of known drug concentration were prepared using approximately 2 to 6 mg of each drug in a drug soluble solvent. A calibration curve was developed from the above stock solutions with measured absorbance vs. concentration of each drug at a particular wavelength (Olanzapine at 363 nm, Risperidone at 280 nm and Quetiapine Fumarate at 303 nm). Two test samples and two control samples of unknown drug concentration of each drug collected in the vials were screened by UV spectroscopy. These samples were scanned and the absorbencies measured were fit into the standard calibration curve. Thus the unknown concentration of the sample is interpolated from the calibration curve or can be derived from the Beer-Lambert law and is compared with the negative control sample.

5.3 Results and Discussion

5.3.1 DSC Evaluation

DSC revealed the purity of the three drugs tested in our study. In comparison of the melt temperature ($T_m$) measured to the literature value, the relative purity can be ascertained. The three drugs in this study, Olanzapine, Risperidone and Quetiapine fumarate were observed to have melting temperatures same as that of literature with the
correlation co-efficient of 0.98. The DSC protocol also aids in defining the ability of the crystalline solid to recrystallize or remain amorphous and produce a glass transition temperature. The DSC profiles of the three drugs are listed below. Risperidone shown below (Fig 28) illustrates the Heat-Cool-Heat cycle in which it happens to recrystallize upon cooling and melting upon reheating which confirms it to be a crystalline material. Whereas, Olanzapine (Fig 27) and Quetiapine Fumarate (Fig 29) do not recrystallize upon cooling, thus exhibiting their amorphicity. The heat of fusion ($\Delta H_f$), melting temperature ($T_m$) peak melt temperature ($T_{mp}$), heat of crystallization ($\Delta H_c$), crystallization temperature ($T_c$), peak crystallization temperature ($T_{cp}$) and glass transition temperature ($T_g$) were determined for all the three drugs and listed in Table 9. The drugs used in this study are probably >99% w pure.

Figure 27 DSC profile of Olanzapine
Figure 28 DSC profile of Risperidone

Figure 29 DSC profile of Quetiapine Fumarate
The Risperidone $T_m$ from the literature is identical to that measured in this study, i.e. 170°C. This measurement implies that Risperidone is very pure. The difference between the Tmp and Tm is 2°C which also is interpreted as a measurement of a pure chemical. The variation of the Tm and Tc is 32°C is a physical property of Risperidone, that is, the super cooling of this chemical. The variation of the melt and crystallization heats identifies the crystallization of only 81% of the melt. In the second heat of this sample the heat of fusion was 89% of the first heat. Crystallization is a kinetic time dependent process and with more time in this DSC protocol more material crystallized from the amorphous phase and produced a higher crystalline content.

**Table 9** DSC thermal properties of BPD drugs

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Values</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine Fumarate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Literature Value</td>
<td>195</td>
<td>170</td>
<td>174</td>
</tr>
<tr>
<td>Heat₁</td>
<td>$T_m$ (°C)</td>
<td>194</td>
<td>170</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>$T_{mp}$ (°C)</td>
<td>196</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>$\Delta H_f$ (°C)</td>
<td>107</td>
<td>90</td>
<td>122</td>
</tr>
<tr>
<td>Cool</td>
<td>$T_c$ (°C)</td>
<td>-</td>
<td>138</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$T_{cp}$ (°C)</td>
<td>-</td>
<td>133</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\Delta H_c$</td>
<td>-</td>
<td>73 (81%)</td>
<td>-</td>
</tr>
<tr>
<td>Heat₂</td>
<td>$T_m$ (°C)</td>
<td>-</td>
<td>168</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$T_{mp}$ (°C)</td>
<td>-</td>
<td>171</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$T_g$ (°C)</td>
<td>73</td>
<td>-</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>$\Delta H_f$</td>
<td>-</td>
<td>80 (89%)</td>
<td>-</td>
</tr>
</tbody>
</table>
The Olanzapine and Quetiapine melting temperatures were also identical to those published in the literature and are highly pure (>99%w) drugs. Both of these active pharmacy ingredients APIs did not recrystallize and upon further cooling and heating produced an amorphous phase only with glass transitions measured at 73°C and 54°C respectively for Olanzapine and Quetiapine Fumarate.

5.3.2 Dielectric Analysis:

From the dielectric profiles of each drug obtained, the log conductivity (pScm⁻¹) values were plotted against log frequency (Hz). The frequency range was selected from 0.1 to 30,000 Hz. For each drug, the conductivity & log frequency values were taken at 10, 20 and 35 min and were plotted. In this Debye plot, it is observed that the three drugs exhibited a marked change in the slope at about a frequency of 100 Hz (shed snake skin) which is called the critical frequency and 1000 Hz (pig skin) where the drug is almost delivered and levels off. The change is interpreted as a measure of the onset of drug throughput using the shed snake skin and pig skin as the animal models.

5.3.2.1 Snake skin:

![Figure 30 DEA profile of Olanzapine at 10 min; 30,000Hz; Iso at 37°C for 40 min](image)

\[ y = 0.623x - 1.343 \]
\[ R^2 = 0.984 \]
Figure 31 DEA profile of Olanzapine at 10 min; before change

Figure 32 DEA profile of Olanzapine at 10 min after change
The Debye plot of Olanzapine plotted log frequency vs. log conductivity is illustrated in Fig 30 at 10 min, with a correlation coefficient of 0.98. Fig 31 and Fig 32 indicate the slopes before and after the critical frequency at 10 min.

![Graph of Olanzapine Iso at 37°C-20min](image)

**Figure 33** DEA profile of Olanzapine; 30,000Hz, Iso at 37°C for 40 min; 20 min

![Graph of Olanzapine Iso at 37°C-20; before change](image)

**Figure 34** DEA profile of Olanzapine at 20 min; before change
Figure 35 DEA profile of Olanzapine at 10 min; after change

Figure 36 DEA profile of Olanzapine at 35 min; 30,000Hz; Iso at 37°C for 40 min
Figure 37 DEA profile of Olanzapine at 35 min; before change

Figure 38 DEA profile of Olanzapine at 35 min; after change
Figure 39 DEA profile of Risperidone at 10 min; 30,000Hz; Iso at 37°C for 40 min

Figure 40 DEA profile of Risperidone at 10 min; before change
Figure 41 DEA profile of Risperidone at 10 min; after change

Figure 42 DEA profile of Risperidone at 20 min; 30,000Hz; Iso at 37°C for 40 min
Figure 43 DEA profile of Risperidone at 20 min; before change

Figure 44 DEA profile of Risperidone at 20 min; after change
**Figure 45** DEA profile of Risperidone at 35 min; 30,000Hz; Iso at 37°C for 40 min

**Figure 46** DEA profile of Risperidone at 35 min; before change
Figure 47 DEA profile of Risperidone at 35°C; after change

\[ y = 0.988x - 2.839 \]
\[ R^2 = 0.995 \]

Figure 48 DEA profile of Quetiapine Fumarate at 10 min; 30,000Hz; Iso at 37°C for 40 min

\[ y = 0.755x - 1.614 \]
\[ R^2 = 0.998 \]
**Figure 49** DEA profile of Quetiapine Fumarate at 10 min; before change

**Figure 50** DEA profile of Quetiapine Fumarate at 10 min; after change
Figure 51 DEA profile of Quetiapine Fumarate at 20 min; 30,000Hz; Iso at 37°C for 40 min

Figure 52 DEA profile of Quetiapine Fumarate at 20 min; before change
Figure 53 DEA profile of Quetiapine Fumarate at 20 min; after change

Figure 54 DEA profile of Quetiapine Fumarate at 35 min; 30,000Hz; Iso at 37°C for 40 min
Figure 55 DEA profile of Quetiapine Fumarate at 35 min; before change

Figure 56 DEA profile of Quetiapine Fumarate at 35 min; after change
From the data taken at 10, 20 and 35 min, data at 35min showed improved results. Olanzapine and Risperidone have an increased percentage (12 to 70%) of rates of change of log conductivity vs. log frequency which is interpreted as a positive sign of drug transport (see Table 10).

**Table 10** Analysis of DEA curve slope data to rank drug transport in shed snake skin

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Slope 1</th>
<th>Slope 2</th>
<th>Ratio ($S_2/S_1$)</th>
<th>% increase of $S_2$ vs. $S_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine</td>
<td>0.500</td>
<td>0.827</td>
<td>1.70</td>
<td>70</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.681</td>
<td>0.988</td>
<td>1.45</td>
<td>45</td>
</tr>
<tr>
<td>Quetiapine Fumarate</td>
<td>0.732</td>
<td>0.817</td>
<td>1.12</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: $S_1$-Slope 1, $S_2$-Slope 2; $S_2$ vs. $S_1$ = Delta log conductivity/delta log frequency

5.3.2.2 *Pig skin*

![Graph of DEA profile of Olanzapine at 10 min; 30,000Hz; Iso at 37°C for 40 min](image)

**Figure 57** DEA profile of Olanzapine at 10 min; 30,000Hz; Iso at 37°C for 40 min
Figure 58 DEA profile of Olanzapine at 20 min; 30,000Hz; Iso at 37°C for 40 min

\[ y = 0.727x + 5.120 \]
\[ R^2 = 0.994 \]

Figure 59 DEA profile of Olanzapine at 35 min; 30,000Hz; Iso at 37°C for 40 min

\[ y = 0.73x + 5.045 \]
\[ R^2 = 0.995 \]
**Figure 60** DEA profile of Risperidone at 20 min; 30,000Hz, Iso at 37°C for 40 min

\[ y = 0.669x + 5.091 \]
\[ R^2 = 0.997 \]

**Figure 61** DEA profile of Risperidone at 35 min; 30,000Hz; Iso at 37°C for 40 min

\[ y = 0.684x + 5.074 \]
\[ R^2 = 0.998 \]
**Figure 62** DEA profile of Quetiapine Fumarate at 10 min; 30,000Hz; Iso at 37°C for 40 min

**Figure 63** DEA profile of Quetiapine Fumarate at 20 min; 30,000Hz; Iso at 37°C for 40 min
The Debye plot data at 35 min provided improved results. We discovered that the DEA curve resulting from the drug through put with the pig skin membrane did not have an apparent critical frequency. Therefore the interpretation of the log conductivity vs. log frequency curve slope is related to the delivery of the BPD drugs in our testing. It is our experience with a lidocaine patch and DEA delivery that the drug is essentially delivered when the DEA slope or rate levels off e.g. see fig 64 at 8000 to 10000 Hz. The resulting electrified membrane delivery is significantly enhanced with the moist pig skin model vs. the dry shed snake skin model. The proof of concept is based on the UV analysis where the drug identity is set by the drug absorbance peak value of stock sample not varying from the test solution peak (see fig 67 and fig 68).

**Figure 64** DEA profile of Quetiapine Fumarate at 35 min; 30,000Hz; Iso at 37°C; Iso for 40 min
**Figure 65** Olanzapine stock solution- peak observed at 363nm

**Figure 66** Olanzapine test sample solution- peak observed at 362nm
Figure 67 Risperidone standard stock solution scan - peak observed at 280nm

Figure 68 Risperidone test solution scan - peak observed at 282
The normalized BPD drug results were obtained subtracting the non-electrical field control due to diffusion. The average of normalized drug delivery is taken for all the three drugs with shed snake skin and pig skin. (See table 11 for shed snake skin) and (See table 12 for pig skin).
Table 11 End-point conductivity and drug throughput of shed snake skin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample (Drug Conc. mg ml⁻¹)</th>
<th>Control (Drug Conc. mg ml⁻¹)</th>
<th>Difference</th>
<th>Average of Difference (Drug Conc. mg ml⁻¹)</th>
<th>End point Conductivity (pScm⁻¹) (10¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine₁</td>
<td>0.75</td>
<td>0.031</td>
<td>0.72</td>
<td>0.74</td>
<td>54.3</td>
</tr>
<tr>
<td>Olanzapine₂</td>
<td>0.085</td>
<td>0.081</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risperidone₁</td>
<td>0.103</td>
<td>0</td>
<td>0.103</td>
<td>0.13</td>
<td>46.2</td>
</tr>
<tr>
<td>Risperidone₂</td>
<td>0.156</td>
<td>0</td>
<td>0.156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quetiapine F₁</td>
<td>0.035</td>
<td>0</td>
<td>0.035</td>
<td>0.105</td>
<td>56.3</td>
</tr>
<tr>
<td>Quetiapine F₂</td>
<td>0.176</td>
<td>0</td>
<td>0.176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 12 End-point conductivity and drug throughput in pig skin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample (Drug Conc. mg ml(^{-1}))</th>
<th>Control (Drug Conc. mg ml(^{-1}))</th>
<th>Difference</th>
<th>Average of Difference (Drug Conc. mg ml(^{-1}))</th>
<th>End point Conductivity (pScm(^{-1}))(10(^6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine(_1)</td>
<td>2.75</td>
<td>0.51</td>
<td>2.24</td>
<td></td>
<td>2.40</td>
</tr>
<tr>
<td>Olanzapine(_2)</td>
<td>2.96</td>
<td>0.08</td>
<td>2.50</td>
<td></td>
<td>3.32</td>
</tr>
<tr>
<td>Risperidone(_1)</td>
<td>6.70</td>
<td>0</td>
<td>6.70</td>
<td>6.65</td>
<td>2.77</td>
</tr>
<tr>
<td>Risperidone(_2)</td>
<td>6.60</td>
<td>0</td>
<td>6.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quetiapine F(_1)</td>
<td>0.0085</td>
<td>0</td>
<td>0.0085</td>
<td>0.01</td>
<td>1.81</td>
</tr>
<tr>
<td>Quetiapine F(_2)</td>
<td>0.014</td>
<td>0</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The conductivity associated with drug throughput with a pig skin model was significantly greater (10\(^6\) pS cm\(^{-1}\)) with the shed snake skin (10\(^1\) pS cm\(^{-1}\)) (see table 13). They are to be compared to the modified (subtracting the control values) UV analysis of the measured throughput concentrations (mg ml\(^{-1}\)). In summary, the average throughput conductivity increased to 2.6 \(\ast\) 10\(^6\) pS cm\(^{-1}\) associated with the 3.02 mg ml\(^{-1}\) drug delivered to the underside of the pig skin membrane. The ionic conductivity/average of difference of shed snake skin and pig skin were taken and there was no correlation between the values obtained from two skins (see table 14).
Table 13 Drug throughput and conductivity - shed snake skin and pig skin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Shed snake skin</th>
<th>Pig skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug Conc. mg ml⁻¹</td>
<td>Conductivity (pScm⁻¹) (10⁴)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.74</td>
<td>2.40</td>
</tr>
<tr>
<td>Olanzapine</td>
<td></td>
<td>5.43</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.13</td>
<td>6.65</td>
</tr>
<tr>
<td>Risperidone</td>
<td></td>
<td>4.62</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Fumarate.₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate.₂</td>
<td></td>
<td>5.63</td>
</tr>
</tbody>
</table>
Table 14 Summary of results

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Ionic Conductivity (pS cm(^{-1}))/Average of difference (mg ml(^{-1})) (snake)</th>
<th>Ionic Conductivity (pS cm(^{-1}))/Average of difference (mg ml(^{-1})) (pig)</th>
<th>Structure of the drug</th>
</tr>
</thead>
</table>
| Olanzapine        | 54/0.74=73                                                                      | 32/2.40=0.83                                                                    | ![Olanzapine structure](attachment)
| Risperidone       | 46/0.13=354                                                                     | 2.8/6.6=4.24                                                                    | ![Risperidone structure](attachment)
| Quetiapine Fumarate | 56/0.11=509                                                                     | 18/0.01=1,810                                                                   | ![Quetiapine Fumarate structure](attachment)

5.4 Conclusion

This study for three structurally different drugs produced varying results of drug transport across bio membranes e.g. part one- shed snake skin and part two- pig skin. It is our opinion that a positive slope change, part one- shed snake skin, above the critical frequency in the log conductivity vs. log frequency curve implies good drug transport for this skin model. This fact has been substantiated by determining the drug enhanced...
throughput of 12 to 70% for the three BPD drugs by UV spectroscopy on the delivery side of the membrane.

UV analysis of the drug samples compared against the control samples yielded positive results of drug delivery. A calibration curve prepared from the stock solutions of each drug resulted in an $R^2$ value of about 0.99. The test samples analyzed were found to contain the drug delivered through the skin. The enhanced slope above the critical frequency is evidence that this protocol for drug transport is effective. The variation in the mg mL$^{-1}$ in the observed throughput is ±17% and has a cause and effect relationship with the conductivity.

The study of three structurally different drugs tested with pig skin yielded measured delivery of an average 0.347 mg mL$^{-1}$ in conjunction with an applied field. The pig skin conductivity is influenced by the moist condition of this membrane and shows higher conductivity values and the shed snake skin shows much lesser conductivity values probably related due to the extreme dry nature of this model membrane. Finally the drug delivery through the membrane is observed and confirmed but there is no correlation between the pig skin and snake skin results and the delivery is drug dependent.
CHAPTER VI

FUTURE DIRECTIONS

- Our quest is to correlate drug transport under an electric field with varying animal model skins. Shed Snake Skin and Pig skin have enhanced this study and paved the way for transdermal delivery of these BPD drugs.

- Additional model skins for the immediate future are a mouse bladder skin for cancer drugs and rabbit eye sclera for anti-tumor drug development for age related macro degeneration. The latter is already in progress with the Cleveland Clinic Foundation.

- A skin model variable, that needs exploration, is the skin thickness vs. throughput efficacy as well as time and field.

- Other viable areas of research will include the effect of penetration enhancers, e.g. acetone is currently being used with nitroglycerin transdermal patches for cardiac patients
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37. RxList, Seroquel (Quetiapine Fumarate) drug information: User reviews, side
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