Development of a Liposomal Formulation for the Transdermal Delivery of Liothyronine

A Capstone Report
presented to the faculty of the
School of Engineering and Applied Science
University of Virginia

by

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with

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May 12, 2023

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Abstract

Hypothyroidism describes the underproduction of the two thyroid hormones, triiodothyronine and thyroxine. Over 100 million Americans suffer from hypothyroidism and are treated solely with levothyroxine, the synthetic version of thyroxine. Fifty-six percent of these patients are unsatisfied with this treatment and studies have shown that patients could benefit from the additional administration of liothyronine, the synthetic form of triiodothyronine. However, liothyronine has a short half-life leading to transient peaks and thyrotoxicity. This project aimed to begin to overcome the limitations of liothyronine administration through the development of a time-sustained release delivery system. Utilizing a weighted selection criteria system, a liposomal formulation within a transdermal patch was selected based on factors such as hypersensitivity reactions, bioavailability factors, and patient compliance. To demonstrate proof of concept, liothyronine was encapsulated within liposomes, and the liposomes were injected into an ultrasound gel that acted as a permeation enhancer. The formulation was then applied to a skin mimic within a vertical diffusion cell, and samples were collected over 24 hours to analyze the transdermal drug permeation. This project provides a novel approach to address the limitations of liothyronine administration and could have significant implications for the treatment of hypothyroidism. Further studies are needed to optimize the formulation and delivery system for liothyronine and to evaluate the effectiveness of the transdermal patch for sustained release delivery of liothyronine. This experiment serves as a proof of concept that liothyronine can be delivered transdermally utilizing a liposomal formulation.

Keywords: transdermal liothyronine, thyroid, hypothyroidism, liposomes, drug delivery

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Introduction

Hypothyroidism is a prevalent endocrinopathy affecting nearly 100 million Americans, and typically results from insufficient thyroid gland activity.[1,2] The thyroid gland secretes two hormones, thyroxine (T4) and triiodothyronine (T3), with the latter serving as the active form of thyroid hormone (TH). The current standard of care for hypothyroidism involves daily oral administration of levothyroxine (LT4).[3] However, a significant percentage of patients (30-50%) experience over- or under-treatment and remain vulnerable to the adverse effects of thyroid dysfunction.[1] This treatment approach is grounded in the notion that deiodinase enzymes can rectify T3 production deficiencies and compensate for the small quantities of T3 generated by the thyroid gland. However, accumulating pre-clinical and clinical data suggest that deiodinases do not fully restore T3 production, which raises the possibility that liothyronine (LT3) may be beneficial for treating certain hypothyroid patients. Despite this promise, orally administered LT3 tablets result in transient thyrotoxic peaks of circulating T3 that rapidly dissipate over the next several hours, which significantly differs from the relative

stability of T3 levels observed in healthy individuals. As a result, there is a pressing need for innovative strategies to address this gap in hypothyroidism treatment, including the development of novel LT3 delivery methods that surmount the drug's low bioavailability and brief half-life.[4]

Numerous research initiatives have attempted to develop alternative drug delivery systems for thyroid hormones, but so far, none have been successful in clearing clinical trials or becoming commercially available. Despite this, many innovative approaches to new delivery strategies have been pursued, with some showing relative success. These approaches include both enteral and parenteral routes, with efforts made subcutaneously, intravenously, intraperitoneally, and this innovative approach, which incorporates transdermal delivery as an alternative parenteral route.

Among the significant enteral approaches are T3-Sulfate and Poly-Zinc Liothyronine. T3-Sulfate involves the sulfation of the phenolic hydroxyl within the molecule, which inactivates T3, but animal studies support the claim that sufficient T3-S can be reactivated as T3 via de-sulfation by sulfatases in the liver, ultimately triggering systemic thyromimetic effects.[5–7] While this approach remains uncommercialized, further research is required before it can be sent into clinical trials and practice. Poly-Zinc Liothyronine, on the other hand, involves the formation of a supramolecular complex between T3 and Zinc, resulting in higher bioavailability due to superior mucoadhesive properties. When combined with hydrolysis behavior in the gut, it creates a controlled or slow-release formulation. However, this formulation is not currently commercially available and still requires further research, although preliminary experimentation in animal models has shown promising results.[8]

Significant parenteral approaches include an ethylene-vinyl acetate rod and an osmotic pump/pellet. The ethylene vinyl acetate rod from Titan Pharmaceuticals delivers LT3 subcutaneously utilizing proprietary technology. In preliminary studies, the subcutaneous rod had initial transient peaks in the serum T3 levels followed by a sustained period of relatively stable levels of serum T3. Another subcutaneous initiative utilizes osmotic pumps/pellets to release fixed amounts of T3 daily for a predetermined number of days, utilizing an osmotic driving force to push the pharmaceutical solution into the dermis, where it enters the circulating serum via the dermal capillaries.[9] However, subcutaneous delivery methods introduce invasivity factors as these devices are implanted beneath the skin.

While other developmental efforts towards new delivery strategies for LT3, such as liquid formulations, chewable LT3-resin complex gum, intravenous injections, tissue-targeted T3-hybrid molecules, nanoparticles containing T3, and stem cells for de novo development of the thyroid gland, have not been as successful as the aforementioned approaches.[4] It is important to note that enteral approaches require strict patient adherence, which is an essential factor in determining treatment outcomes.

In contrast, this innovative approach incorporates transdermal delivery as an alternative parenteral route. Several factors were considered in the selection criteria, including bioavailability, continuous and steady delivery of the drug, patient usage and/or compliance, and hypersensitivity reactions. Ultimately, a liposomal transdermal patch was selected as the most effective delivery system for LT3. This approach aims to overcome the limitations of current LT3 delivery methods by providing a non-invasive and convenient mode of

administration with improved bioavailability and sustained release.

This research project introduces a novel solution for administering liothyronine through a reservoir transdermal patch that utilizes a liposomal formulation to provide controlled-release delivery over a 7-day period. Transdermal patches offer a non-invasive drug delivery method that administers medication through the skin and into circulation in a sustained and consistent manner. To accomplish this, the patch will consist of four layers - an adhesive, a polymer membrane, a drug reservoir, and an impermeable backing (as illustrated in Figure 1). This design will prioritize patient adherence, minimize invasiveness, and ensure a steady and continuous drug delivery. The adhesive layer enables the patch to be discreetly adhered to the patient's body, while the polymer membrane controls the rate of drug delivery. The drug reservoir layer encapsulates the drug to be delivered, and the impermeable backing layer protects the patch from environmental contaminants while adhered to the skin. This approach stands out from previous initiatives by emphasizing continuous drug delivery over a seven-day period, which is expected to enhance patient adherence, and by using a liposomal formulation to provide optimal drug delivery rate control. In order to optimize the device, the drug delivery rate will be quantified and the polymer membrane adjusted to account for this rate. The development of a novel drug delivery system that will administer the time-sustained release of LT3 will be accomplished through two aims, determine the optimal liothyronine delivery system and prototype, test, and iterate the selected liothyronine delivery system.

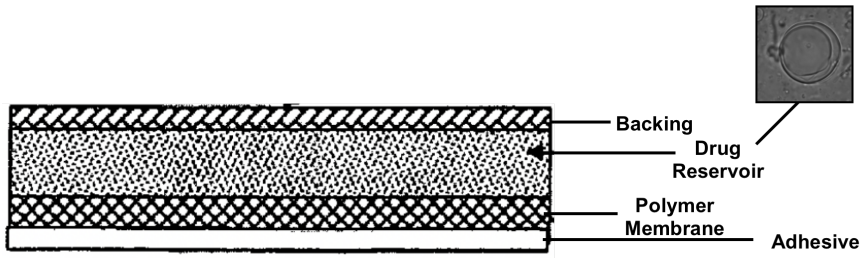

Figure 1: Proposed Time-Sustained and Rate-Controlled Liothyronine Transdermal Patch

Results

Prior to commencing the experiments, thorough research was conducted to identify the most suitable administration route. A comprehensive weighted selection process was employed, taking into account various critical factors, such as hypersensitivity reactions, bioavailability, patient compliance, and killer criteria. The killer criteria focused on the ability to maintain a steady and continuous drug delivery, as well as the ability to dose within the therapeutic range (Figure S1). Based on the selection criteria, it was determined that a liposomal transdermal patch was the optimal administration route.

Microscopy of each formulation was performed to ensure the presence of liposomes (see Figure 2).

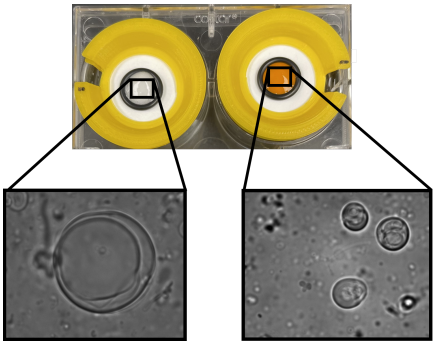

Figure 2. Microscopy of Liposome Formulation.

Fluorescence measurements of calcein were conducted at each time point to assess transport trends (refer to Figure 3). Permeability was calculated by dividing the mass flow rate by the concentration difference.

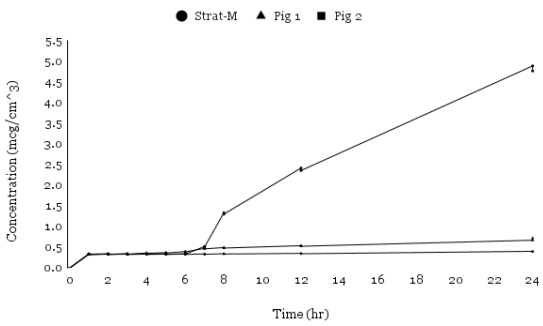

Figure 3. Calcein Mass Flow Rate.

Effective diffusivity was calculated by multiplying the permeability by the thickness of either the skin sample or mimic. The mass flow rate was calculated by dividing the cumulative mass flux by 24 hours, the length of the experimental trial. The calculated values of the mass flux, permeability, and effective diffusivity of each of the samples, Strat-M and porcine skin, can be seen in Table 1 and Figure S5.

Table 1. Mass Transport of Calcein Liposome Formulation

Skin Sample	24 hr Transport Results		
	Mass Flux ($\mu g \cdot cm^2 \cdot hr^{-1}$)	Permeability ($cm \cdot s^{-1}$)	Effective Diffusivity ($cm^2 \cdot s^{-1}$)
Strat-M	2.04E-01	2.47E-07	7.42E-09
Swine 1	1.39E-02	1.72E-08	3.15E-09
Swine 2	1.70E-02	2.14E-08	1.16E-08

The *in-vitro* permeation testing of the liothyronine formulation trials were quantified through absorbance and optical density measurements of the tyrosine rings of liothyronine at 280 nm. 5.6 µL of Tween-20 was added to each experimental sample to disrupt the liposomes and release encapsulated LT3. However, the detection limit, 0.1 mg/mL, of the Nanodrop 2000c limited the accuracy of these readings. To calculate the concentration of the liothyronine in the experimental samples, the Beer-Lambert law, $A = \varepsilon bC$, was used with the molar absorptivity of liothyronine being 4.09 mM, and the length of the light path being 10 mm. The calculated concentrations (refer to Figure 4) were then used to calculate the mass flow rate, mass flux, effective diffusivity, and permeability. The mass flux of samples 1 and 2 were 5.84E-01 and 4.10E-07 $\mu g \cdot cm^2 \cdot hr^{-1}$, respectively. The effective diffusivity of samples

1 and 2 were 1.23E-08 and 1.20E-08 $cm^2 \cdot s^{-1}$, respectively. The permeability of samples 1 and 2 were 4.10E-07 and 4.00E-07 $cm \cdot s^{-1}$ (as shown in Table 2 and S6).

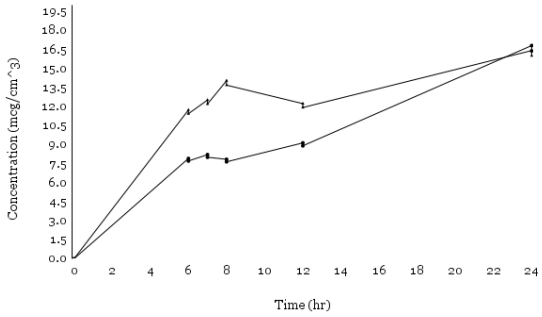

Figure 4. LT3 Mass Flow Rate.

Table 2. Mass Transport of Liothyronine Liposome Formulation.

Skin Sample	24 hr Transport Results		
	Mass Flux ($\mu g \cdot cm^2 \cdot hr^{-1}$)	Permeability ($cm \cdot s^{-1}$)	Effective Diffusivity ($cm^2 \cdot s^{-1}$)
Strat-M	5.84E-01	4.10E-07	1.23E-08
Strat-M	5.70E-01	4.00E-07	1.20E-08

Discussion

Interpretation of Experimental Results

In the *in-vitro* permeation tests, the LT3 liposomal transdermal formulation was found to have the ability to permeate the skin for a period of 24 hours. Analysis of the cumulative mass flow of LT3 through the Strat-M skin mimic revealed an average of 16.6 µg of LT3 that permeated the skin mimic over 24 hours, resulting in a delivery efficiency of 92.1% of the initial 18.03 µg of LT3. Notably, LT3 demonstrated a higher permeation efficacy compared to calcein, with 30.1% and 3.49% of the delivered 17.15 µg of calcein permeating through the Strat-M and porcine skin, respectively. These findings suggest that the LT3 liposomal transdermal formulation has potential for effective and efficient drug delivery through the skin. Furthermore, the LT3 formulation demonstrated consistent transport properties, including mass flux, permeability, and effective diffusivity. These findings suggest that the experiment can be repeated in future studies to obtain statistically significant results.

The observed variations in the transport results of the calcein formulation through the porcine skin sample and Strat-M are likely due to the unavailability of a dermatome to remove all the fat and muscle layers from the porcine samples. It is possible that the formulation may have been trapped within those layers, leading to inconsistent results.

Table 3. Average Cumulative Mass Flow.

Skin Sample	Total Delivered (μg)	Total in Receptor Compartment (μg)	Mass Delivered:Mass Recovered (%)
		LT3	
Strat-M	18.03	16.6	92.1
		Calcein	
Strat-M	17.15	5.16	30.1
Swine	17.15	0.599	3.49

Challenges and Limitations

The LT3 concentrations were synthesized from optical density absorbance readings using a Nanodrop 2000c. However, the spectrophotometer had a detection limit of 0.1 mg/mL and concentrations were predicted to be below this limit. Therefore, protein quantification assays, HPLC, or fluorescent labeling of the drug should be carried out to confirm the LT3 concentration outcomes.

Additionally, while the 3D-printed vertical diffusion cell apparatus did not display any observable leakage, it is imperative to authenticate the consistency of the outcomes and the efficiency of the setup through the use of Franz vertical diffusion cells. Further studies should aim to gauge the formulation evaporation within the apparatus through an experiment with an impermeable membrane in place of the skin mimic, and a comparison should be made between the amount of formulation at the end of 24 hours and its initial quantity. Lastly, to verify the requirement for liposome encapsulation for transdermal drug permeation, an experiment should be performed using LT3 that is not encapsulated in liposomes.

Implications for Future Research and Considerations

To reach the final goal of a time-sustained liothyronine delivery system with a rate-controlled transdermal patch, an optimized formulation would be used in a seven-day *in-vitro* permeation test experiment. Then determine the transdermal patch parameters by choosing a polymer membrane to achieve the desired delivery rate of the drug to the skin. Iterations of the polymer membrane would then be tested using in vitro release tests. And finally, animal studies should be conducted where serum and plasma are collected to evaluate the pharmacokinetics and pharmacodynamics of the drug and delivery system.

Materials and Methods

Selecting a Delivery System

In order to reach the long-term goal of more effectively treating hypothyroidism, a rubric was developed that evaluated various treatment options and determined the optimal delivery system that best meets the needs and treatment constraints defined by a comprehensive literature review. Treatment requirements were based on a tactfully-chosen initial subset of hypothyroidism patients requiring the additional administration of liothyronine to simplify the number of factors influencing the experimental design process, with the future hope of testing its efficacy on broader groups of hypothyroidism patients. Both current and potential delivery systems were evaluated with the selection criteria rubric to ensure the selected delivery system would be advantageous.

The selection criteria were developed as a literature-backed rubric that evaluates the therapeutic ability, hypersensitivity reactions, bioavailability factors, and patient usage and compliance factors. The delivery systems were evaluated by the design criteria with weighted averages. The therapeutic ability criterion was determined to be “killer criteria.” Therefore, the system was eliminated if the delivery system did not meet these needs. The remaining three categories of criteria were weighted according to significance in the order of hypersensitivity reactions (45%), bioavailability factors (35%), and patient usage and compliance factors (20%). Each criterion was then evaluated on a scale of -1, 0, 1, where each score has a specific range of factors that the delivery system must meet. Six potential and current treatment options were evaluated with the selection criteria rubric, biphasic vesicle transdermal patch, liposome transdermal patch, unencapsulated transdermal patch, intradermal cannula patch, infusion pump, microneedle transdermal patch, oral liothyronine tablets, and compounded pharmacy liothyronine for extended, sustained release (see Figure S1 for selection criteria rubric).

The liposomal transdermal patch scored the highest at 5.65, and oral liothyronine tablets and the compounded pharmacy liothyronine for extended sustained release did not meet the “killer criteria” and were initially eliminated. Each of the three non-invasive transdermal patch delivery systems scored over 5, and the two minimally invasive systems, intradermal cannula patch and microneedle transdermal patch scored over 4. Of the delivery systems that met the killer criteria, the infusion pump scored the lowest at 3.95 (see Figure S1 for selection criteria scores). Evidence was compiled to rationalize the selected criterion from published literature, but one may interpret this system as subjective. However, there are many references that point to these as necessary criteria for developing an optimal delivery system. Along with the evidence supporting the criterion, the experimenter's judgment was used to determine which criteria were most significant and ultimately weighted higher.

Designing 3-D Vertical Diffusion Cell Inserts

Before experimentation, the vertical diffusion cell inserts for a six-well plate were designed using Autodesk Fusion360 as a computer-aided design platform. The inserts were designed for a six-well plate to maximize the number of trials that could be run at once. The vertical diffusion cell inserts utilized three pieces: the base, an o-ring, and a tamper (see Figure S2). The base

acted as a piece to hold either the skin sample or Strat-M membrane with a diameter of 25 mm above the receptor of the six-well plate. The o-ring acted as a seal to hold the skin sample or mimic as well as the formulation on the base. The tamper acted to seal the formulation onto the skin sample or mimic. Additionally, each of these pieces were designed with a notch that would be aligned in placement to act as a sampling port for pipetting during experimentation. The designs were then printed using a Lulzbot TAZ 5 3-D printer with ABS filament.

Developing a Transdermal Liposome Formulation

A transdermal calcein dye formulation was prepared by encapsulating calcein dye, 100 mg/ml aqueous solution, into multilamellar vesicles. The multilamellar vesicles were prepared using the thin-film hydration technique. This method involved mixing distearoylphosphatidylcholine (20 mg/mL), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (10 mg/mL), and cholesterol (20 mg/mL) with a ratio of 1:0.7:0.5, respectively, before removing the organic solvent, chloroform, through evaporation in a glass vial to create a thin lipid film on the bottom. Then 1,873 µL of calcein dye was added to the liposome film and agitated to combine in a hot water bath. The solution was then transferred into a centrifuge tube, vortexed, and centrifuged. After the initial centrifugation, the supernatant was removed and replaced with a milliliter of saline. This process was repeated until the supernatant was clear indicating the removal of all free calcein dye. Once the supernatant was clear it was pulled off the top and a Coulter counter was used to determine the concentration and number of liposomes in a sampling of the pellet, overall confirming the presence of liposomes. Next, 800 µL of the multilamellar vesicles were then put into a 1 mL syringe and mixed into 1500 µL of ultrasound gel containing propylene glycol, a transdermal permeation enhancer, using a 3 mL syringe and a three-way stop cock (see Figure 6).

Porcine Skin Harvest and Preparation

Due to the limited availability of the Strat-M membranes, porcine skin samples were utilized to maximize trials in initial formulation testing with calcein dye. Porcine skin samples were acquired from the Lung Transplant and Injury Research Lab in the Department of Surgery at the University of Virginia. Samples were harvested from the abdomen of the pig and skin was shaven and excess fat was removed using a scalpel. However, it is important to note that these samples were acquired without a dermatome.

In-Vitro Permeation Testing of Liposomal Formulation

After the calcein dye liposomal formulation was prepared, 200 µL was applied to three porcine skin samples of varying thicknesses (2.448 cm, 2.583 cm, 2.46 cm) and 3 Strat-M membranes in the vertical diffusion cells. Each receptor of the six-well plate was then filled with 12 mL of DPBS and the vertical diffusion cell inserts were placed on top of the receptors. The plate was then wrapped in parafilm to prevent excess evaporation throughout the experiment. Next, the six-well plate was placed into a shaking incubator at 50 RPM and 37 degrees Celsius to mimic the physiological conditions of transdermal drug delivery. During the *in-vitro* permeation testing, 300 µL samples were taken at 1, 2, 3, 4, 5, 6, 12, and 24 hours and stored at 2°C (see Figure 7). After the completion of the experiment, the samples were analyzed using fluorescence.

Fluorescence Analysis of Calcein In-Vitro Permeation Testing

In order to quantify the concentrations of calcein dye in the experimental samples, a calcein dye fluorescence standard curve was performed (with calcein concentrations of 0.0025, 0.001, 0.0009, 0.00075, 0.0005, 0.00025, 0.0001, 0.00009, 0.000075, 0.00005, and 0.000025 mg/ml). Utilizing the fit equation, y = 1.82*10^7 - 555, of the calcein dye standard curve, fluorescence values of the experimental samples were able to be converted into concentration values over time (see Figure 5).

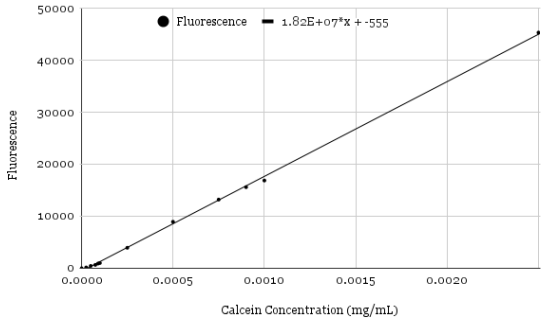

Figure 5. Calcein Concentration Standard Curve.

Five microliters of Triton-X was added to each experimental sample to disrupt the liposomes and release encapsulated calcein. The fluorescence values of each experimental sample were serially diluted to concentrations of 0.5, 0.25, 0.125, and 0.0625 mg/ml, ensuring that the expected linearity was maintained to minimize absolute error. Experimental fluorescence values were the input into the calcein dye standard curve equation to determine the concentration of the receptor at each time point and the cumulative mass flux. The mass flux was then calculated by dividing the mass flow rate by the surface area of the sample.

Development of Liothyronine Transdermal Liposome Formulation

After validating the calcein dye formulation as a transdermal formulation, a transdermal liothyronine formulation was prepared by encapsulating a liothyronine solution into multilamellar vesicles. A 0.1 mg/mL liothyronine solution was prepared with 1.13 mg of liothyronine, 1.13 mL DMSO, and 10.17 mL of deionized water. Liothyronine has a solubility of 100 mg/mL in DMSO. While this is not the highest solubility of liothyronine in an organic solvent, DMSO was compatible with the Strat-M membranes that were used for the *in-vitro* permeation testing. The multilamellar vesicles were then prepared using the thin-film hydration technique. This method involved mixing distearoylphosphatidylcholine (20 mg/mL), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (10 mg/mL), and cholesterol (20 mg/mL) with a ratio of 1:0.7:0.5, respectively, before removing the organic solvent, chloroform, through evaporation in a glass vial to create a thin lipid film on the bottom. Then 936.5 µL of the liothyronine solution was added to the lipid film and agitated to combine in a hot water bath. The solution was then transferred into a centrifuge tube, vortexed, and centrifuged until liposomes formed a pellet. After the initial centrifugation, the supernatant was removed and replaced with a milliliter of saline and centrifuged. This process was repeated until all the free liothyronine and liposomes were at the bottom of the centrifuge tube forming a pellet to remove all free liothyronine solution. A sample of the liothyronine liposome pellet was then put into a Coulter counter to determine the concentration and number of liposomes in a sampling of the pellet, overall confirming the presence of liposomes. Next, 350 µL of the liothyronine multilamellar vesicles were then mixed into 900

µL of ultrasound gel containing propylene glycol (see Figure 6).

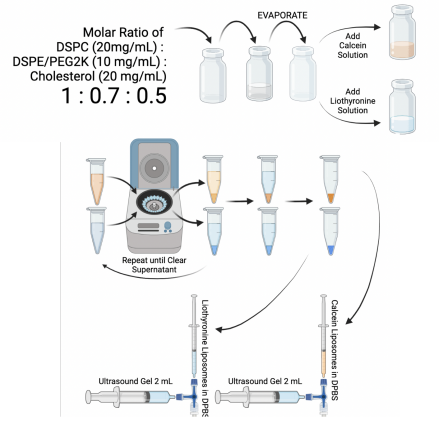

Figure 6. LT3 and Calcein Formulation Methods.

In-Vitro Permeation Testing of Liothyronine Liposomal Formulation

After the liothyronine liposome formulation was prepared, 200 µL was applied to four Strat-M membranes within the diffusion cell inserts. The six-well plate receptors were then filled with 12 mL of DPBS and the diffusion cell inserts were placed on top of the receptors. The six-well plate was then wrapped/sealed with parafilm to prevent excess evaporation during the experiment. Next, the six-well plate was placed into a shaking incubator at 50 RPM and 37 degrees Celsius to mimic the physiological conditions of transdermal drug delivery. During the *in-vitro* permeation testing, 500 µL samples were taken at 1, 2, 3, 4, 5, 6, 12, and 24 hours and stored at 2°C (see Figure 7). After the completion of the experiment, the samples were analyzed using a spectrophotometer (Nanodrop 2000c) to measure the optical density of the tyrosine rings of liothyronine at 280 nm.

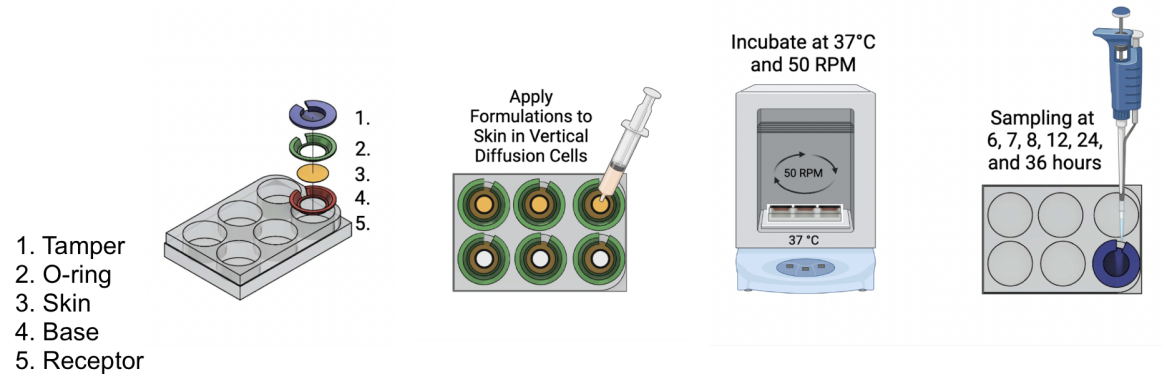

Figure 7: Six-Well Plate Vertical Diffusion Cell Insert for *In-Vitro* Permeation Testing

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Supplementary Information

Figure S1: Design Criteria Rubric and Scoring

Selection Criteria	Concepts							
	Biphasic Vesicle Transdermal Patch	Liposome Transdermal Patch	Intradermal Cannula Patch	Infusion Pump	Microneedle Transdermal Patch	Oral Liothyronine Tablets	Compounded Pharmacy Liothyronine for Extended/Sustained-Release	Unencapsulated Transdermal Patch
Killer Criteria								
Continuous/Steady Drug Delivery	(+) No or Minimal Transient Peaks with a Near-zero time derivative of changes in T3 serum/plasma (0) Transient Peaks within Therapeutic Range (-) Transient Peaks outside of the therapeutic range							
Dosing within the Therapeutic Range	(+) never exceeds therapeutic range (0) 1-30% of time outside of therapeutic range (-) >30% time outside of the therapeutic range							
Hypersensitivity Reactions								
Gastrointestinal	(+) administration of the drug has no effect on the gastrointestinal system (0) administration of the drug has little to no effect on the gastrointestinal system (-) administration of the drug has noticeable effects on the gastrointestinal system							
Dermatologic	(+) administration of the drug has no dermatologic effect (0) administration of the drug has little to no dermatologic effects (-) administration of the drug has noticeable dermatologic effects							
Bioavailability Factors								
Therapy administration	(+) once a week administration at specificied time with no dietary restricitions (0) everyday same time administration with no dietary restrictions (-) must be administered at the same time everyday on empty stomach with water							
Organs with rapid D3-mediated TH catabolism limit bioavailability	(+) the rapid D3-mediated TH catabolism in the skin has no effect on drug bioavailability (-) the rapid D3-mediated TH catabolism slightly affect drug bioavailability (-) the rapid D3-mediated TH catabolism significantly affect drug bioavailability							
Preexiting absorption disorders limiting bioavailability	(+) absorption disorders (such as: coeliac disease, pernicious anaemia, gastritis, malabsorption or diabetic gastropathy) have no effect on drug bioavailability (0) absorption disorders (such as: coeliac disease, pernicious anaemia, gastritis, malabsorption or diabetic gastropathy) slightly affect drug bioavailability (-) absorption disorders (such as: coeliac disease, pernicious anaemia, gastritis, malabsorption or diabetic gastropathy) significantly impair drug bioavailability							
Nephritis and Nephrotic syndrome limitation on bioavailability	(+) bioavailability of drug is not affected when patient has nephritis and nephrotic syndrome (0) bioavailability of drug is slightly impaired when patient has nephritis and nephrotic syndrome (-) bioavailability of drug is significantly impaired when patient has nephritis or nephrotic syndrome							
Dietary choices limiting bioavailability	(+) bioavailability of drug is not affected when therapy is administered with food, beverages, cereals, milk, soy, alcohol and fruit juices. (0) bioavailability of drug is slightly impaired when therapy is administered with food, beverages, cereals, milk, soy, alcohol and fruit juices. (-) bioavailability of drug is significantly impaired when therapy is administered with food, beverages, cereals, milk, soy, alcohol and fruit juices.							
other Medications limiting bioavailability (due to impaired absorption)	(+) Medications such as antacids, iron compounds and proton pump inhibitors have no effect on the bioavailability of drug (0) Medications such as antacids, iron compounds and proton pump inhibitors slightly impair bioavailability of drug (-) Medications such as antacids, iron compounds and proton pump inhibitors significantly impair bioavailability of drug							
Factors affect Proper Treatment based on Patient Usage and/or Compliance								
Therapy's affect on patient discomfort	(+) Drug delivery system causes no patient discomfort (0) Drug delivery system causes patient discomfort at time of administration (-) Drug delivery system causes prolonged patient discomfort							
Device water immersion durability	(+) Therapy is not affected at all by interactions with water (0) Therapy can withstand small amounts of water (-) Therapy is interupted when in contact with any water							
water vapor permeability	(+) device can withstand a moisture uptake ranged from 5.27±0.012 to 7.89±0.019% (0) device can withstand a moisture uptake less than 5.27±0.012% (-) device cannot withstand any moisture uptake							
Incresed peripheral blood flow affect on delivery rate of deivce	(+) Therapy is not affected at all by the patient's physcial activity or other causes of increased peripheral blood flow (0) Therapy can withstand small amounts of physcial activity or other causes of increased peripheral blood flow (-) Therapy is interupted when patient is physically active or other causes of increased peripheral blood flow							
Lightweight	(+) No external device required for drug administration (0) external device <=250 mg required for drug administration (-) external device >250mg required for drug administration							
Folding Endurance	(+) Device is able to move with all of the patient's movements without affecting or interrupting treatment (0) Device is able to move with some or most of the patient's movements with little affect and/or interuption of treatment (-) Device is unable to move with all of the patient's movements and treatment is affected and/or interrupted							
Durability	(+) delivery systems is equally as effective in wet, hot, humid, and cold conditions (0) delivery systems is less effective in wet, hot, humid, and cold conditions (-) delivery systems must be removed in wet, hot, humid, and cold conditions							
Minimize Invasivity	(+) Drug administration does not require skin penetration (0) Drug administration requires minimal penetration of the skin (not past dermal layer of skin) (-) Drug administration requires penetration of the skin (deeper than dermal layer)							
>= 7 day drug reservior	(+) Device capable of holding a 7-day drug quantity based on applicable drug formulation with a relatively small reservoir (0) Device is capable of holding 7 day drug quantity based on applicable drug formulation with a relatively large reservior (-) Device cannot hold a 7 day drug quantity							
Self Administration	(+) Patient can administer therapy by themselves in any location (0) Patient needs an additional device to safely administer the therapy (-) Patient must have a doctor administer the therapy in a clinical setting							
Cost of Drug Delivery System	(+) Drug delivery system has no upfront costs and costs less than $30 for a month of treatment (0) Drug delivery systems has minimal upfront costs and/or costs $30 for a month of treatment (similar to current treatment) (-) Drug delivery system has a large upfront cost and is more than $30 a month							

Selection Criteria	Concepts							
	Biphasic Vesicle Transdermal Patch	Liposome Transdermal Patch	Intradermal Cannula Patch	Infusion Pump	Microneedle Transdermal Patch	Oral Liothyronine Tablets	Compounded Pharmacy Liothyronine for Extended/Sustained-Release	Unencapsulated Transdermal Patch
Killer Criteria								
Continuous/Steady Drug Delivery	+'	+'	+'	+'	+'	-'	-'	+'
Dosing within the Therapeutic Range	+'	+'	+'	+'	+'	-'	0	+'
Hypersensitivity Reactions								
Gastrointestinal	+'	+'	+'	+'	+'	-'	-'	+'
Dermatologic	0	0	0	0	-'	+'	+'	-'
Bioavailability Factors								
Therapy administration	+'	+'	+'	+'	+'	-'	-'	+'
Organs with rapid D3-mediated TH catabolism limit bioavailability	-'	-'	-'	+'	-'	+'	+'	-'
Preexiting absorption disorders limiting bioavailability	+'	+'	+'	+'	+'	-'	-'	+'
Nephritis and Nephrotic syndrome limitation on bioavailability	+'	+'	+'	+'	+'	-'	-'	+'
Dietary choices limiting bioavailability	+'	+'	+'	+'	+'	-'	-'	+'
other Medications limiting bioavailability (due to impaired absorption)	+'	+'	+'	+'	+'	-'	-'	+'
Factors affect Proper Treatment based on Patient Usage and/or Compliance								
Therapy's affect on patient discomfort	+'	+'	0	-'	0	+'	+'	+'
Device water immersion durability	+'	+'	-'	-'	+'	+'	+'	+'
water vapor permeability	+'	+'	-'	-'	+'	+'	+'	+'
Incresed peripheral blood flow affect on delivery rate of deivce	0	0	+'	+'	0	+'	+'	0
Lightweight	+'	+'	0	-'	+'	+'	+'	+'
Folding Endurance	+'	+'	0	-'	+'	+'	+'	+'
Durability	0	0	+'	+'	0	+'	+'	0
Minimize Invasivity	+'	+'	0	-'	0	-'	-'	+'
>= 7 day drug reservior	+'	+'	+'	+'	+'	-'	-'	+'
Self Administration	+'	+'	+'	-'	+'	-'	+'	+'
Cost of Drug Delivery System	0	+'	-'	-'	0	+'	0	+'
Killer Criteria	2	2	2	2	2	-2	-1	2
Hypersensitivity Reactions	0.45	0.45	0.45	0.45	0	0	0	0
Bioavailability Factors	1.4	1.4	1.4	2.1	1.4	-1.4	-1.4	1.4
Factors affect Proper Treatment based on Patient Usage and/or Compliance	1.6	1.8	0.4	-0.6	1.2	1.4	1.4	1.8
Total Sum	**5.45**	**5.65**	**4.25**	**3.95**	**4.6**	**0**	**0**	**5.2**

Figure S2: Diffusion Cell Insert

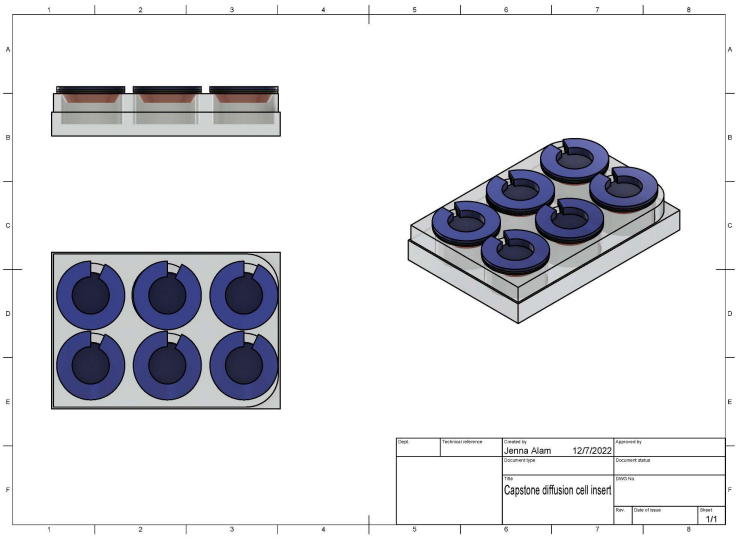


Figure S3: Diffusion Cell Insert Expanded

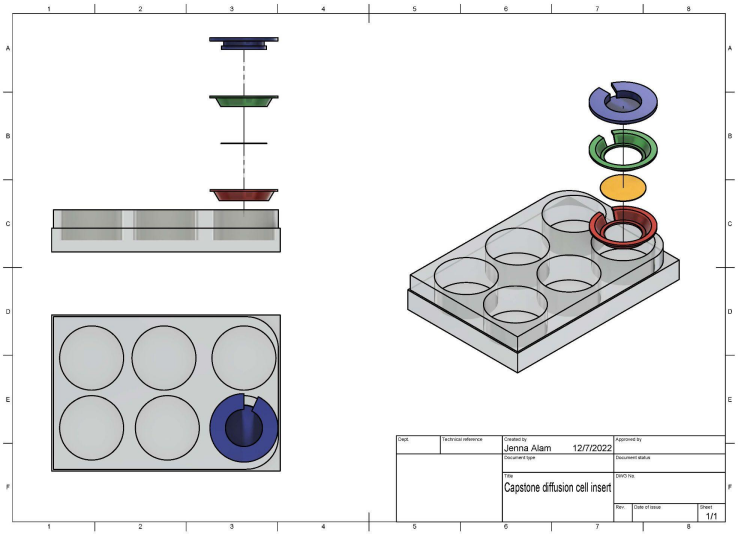
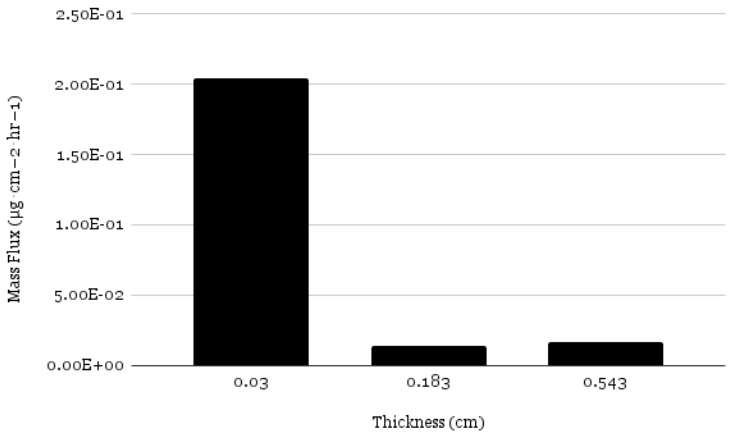
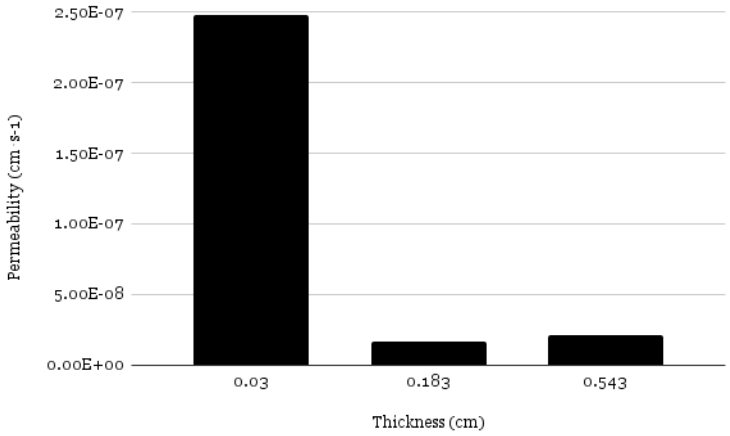


Figure S4: Calcein Formulation Mass Flux, Permeability, & Effective Diffusivity vs Thickness.





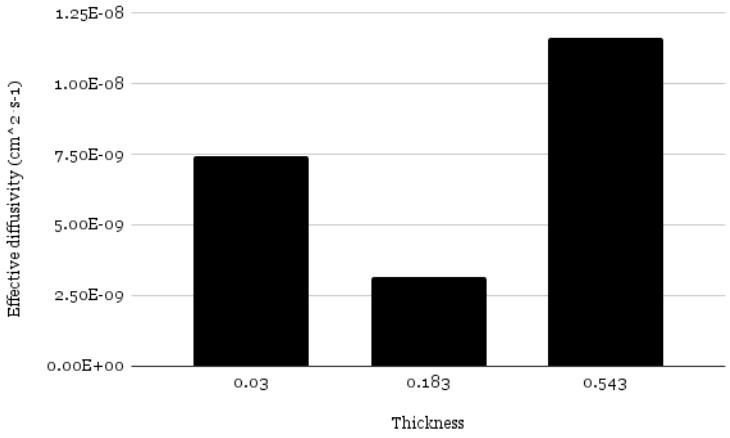


Figure S6: Liothyronine Formulation Mass Flux, Permeability, & Effective Diffusivity vs Thickness.