FORMULATION AND FABRICATION OF A NOVEL SUBCUTANEOUS IMPLANT FOR THE ZERO-ORDER RELEASE OF SELECTED PROTEIN AND SMALL MOLECULE DRUGS

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ABSTRACT

Diabetes is a leading cause of death and disability in the United States. Diabetes require s a lifetime medical treatment. Some diabetes drugs could be taken orally, while others require daily injection or inhalation to maximize bioavailability and minimize toxicity. Parenteral delivery is a group of delivery routes which bypass human gastrointestinal track. Among all the parenteral methods, we chose subcutaneous implant based on its fast act and high patient compliance.

When using subcutaneous implant, drug release needs to be strictly controlled. There are three major groups of controlled release methods. Solvent controlled system is already used as osmotic implant. Matrix controlled system is used in Zoladex® implant to treat cancer. Membrane controlled systems is widely used in coating tablets, but not that popular as an implant. Based on the research reported by previous scientists, we decided to build a hybrid system using both matrix and membrane control to delivery human insulin and other small molecule drugs.

Subcutaneous environment is different from human GI track. It has less tolerance for external materials so many polymers cannot be used. From the FDA safe excipient database, we selected albumin as our primary polymer and gelatin as secondary choice. In our preliminary insulin diffusion study, we successfully found that insulin mixed with albumin provided a slower diffusion rate compared with control. In addition, we added zinc chloride, a metal salt that can precipitate albumin. The insulin diffusion rate is further reduced. The

preliminary study proved that matrix control using albumin is definitely feasible and we might add zinc chloride as another factor.

In order to fabricate an implant with appropriate size, we use lyophilisation technology to produce uniformly mixed matrix. Apart from albumin and human insulin, we added sucrose as protectant and plasticizer. The fine powder after freeze-dry was pressed as a form of tablet. The tablets were sealed in Falcon® cell culture insert. Cell culture insert provide a cylinder shape and 0.3 cm^2 surface area for drug release. Insulin release study provided a zero order kinetics from prototypes with zinc chloride or 0.4 micron pore size membrane.

Caffeine was used as a model drug to investigate the releasing mechanism. Three pore size membranes (0.4, 3 and 8 micron) were tested with same formulation. While 0.4 micron prototypes provided the slowest release, 3 micron ones surprisingly released caffeine faster than 8 micron implants. We calculated the porosity with pore size and concluded that the percentage of open area on a membrane is the key point to control caffeine release. 0.4 micron membranes were used for future research. We increased the percentage of albumin in our excipient, and achieved a slower caffeine release. However, the zero order release could only last for 3 days. After we replaced sucrose with gelatin, a 5 day zero order release of caffeine was achieved. With all the results, we proposed our "Three Phase" drug release mechanism controlled by both membrane and matrix.

Seven other small molecule drugs were tested using our prototype. Cloudy suspension was observed with slightly soluble drugs. We updated our "Three Phase" drug release mechanism with the influence of drug solubility. Data shows that releasing rate with same

formulation and membrane follows the solubility in pH 7.4. This result proves that our prototype might be used for different drugs based on their solubility.

Finally, with all the information of our prototype, we decided to build a “smart insulin implant” with dose adjustment. We proposed an electrical controlled implant with different porosity membranes. Solenoid was used as the mechanical arm to control membrane porosity. 3-D printing technology was used to produce the first real prototype of our implant. Finally, insulin implant with clinically effective insulin release rate was achieved.

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CHAPTER I

INTRODUCTION OF DIABETES, INSULIN THERAPY AND SUBCUTANEOUS IMPLANT

1.1.Abbreviation

API: Active pharmaceutical ingredient

BSA: Bovine serum albumin

BSA-Zinc-Insulin: complex formed by albumin, insulin and zinc ion

DI water: Deionized water

HAS: Human serum albumin

PBS: Phosphate buffered saline

1.2. Current U.S. diabetes challenge

Diabetes is a serious burden for the United States community. According to American Diabetes Association®, 1.4 million Americans are diagnosed as diabetic every year. In 2012, 29.1 million American people, which occupies 9.3% of national population, were diagnosed with diabetes[1]. The cost to treat diabetes is very expensive. In 2012, $245 billion were spent in diagnose, and $176 billion dollars were expended for treatment. Health related professionals believe that the number of diagnosed patients will increase dramatically in the future through 2050[2–4] (Figure 1.1).

1.3. Pathogenesis and treatment of diabetes

The main symptom of diabetes is the unregulated high glucose level in human blood. Untreated diabetes may cause heart disease, stroke, kidney failure, foot ulcers, and vision damage[5]. Diabetes has two types: type I and type II. Type I diabetes, also named insulin dependent diabetes, requires external insulin supply as the main treatment method.

Differently, type II diabetes, widely known as insulin resistance, can be controlled through change of diet, administration of oral anti-diabetic agents and physical exercise[6–12]. However, advanced and severe type II diabetes still need insulin therapy to control blood glucose[13].

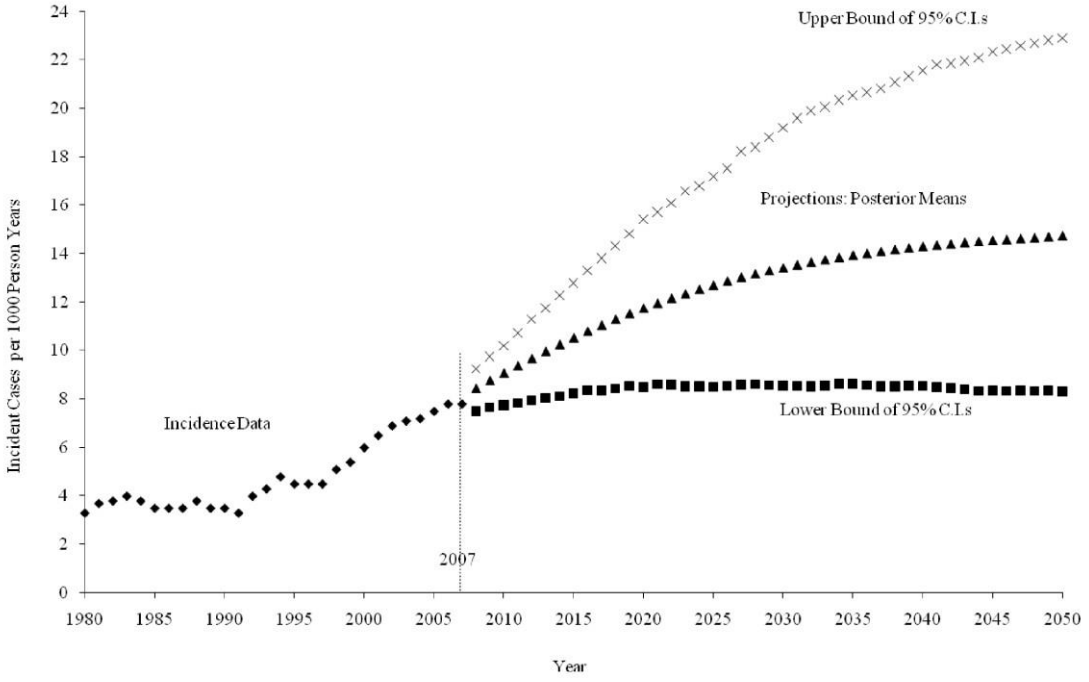

Figure 1.1: Incident cases of diagnosed diabetes per 1,000 people, 1980-2007, and three scenarios for projected cases per 1,000, 2008 -2050[4].

1.3.1. Type I diabetes and treatment

The main pathogenesis of type I diabetes is the destruction of human insulin-producing β cells in pancreas, which mainly comes from autoimmune attack of T cells and macrophages[14–17]. As a result of this metabolic issue, type I patients have significantly lower level of insulin and higher glucose level compared with healthy people (Figure 1.2). This type accounts for approximately 10% of all diabetic patients. In addition, among all

patients under the age of 16, type I diabetes takes more than 95% of all the cases[3,18,19]. Scientists believe the incident of type I diabetes is strongly associated with genes, while the mechanism of generic cause is not fully understood. Other scientists believe environmental factors also have influence, including natural environment, mental health, diet and living style[14].

Currently, insulin therapy is the main solution to treat type I diabetes[20]. External supply of insulin functions as an alternative for human pancreas. Details of insulin administration will be introduced in the later part of this chapter.

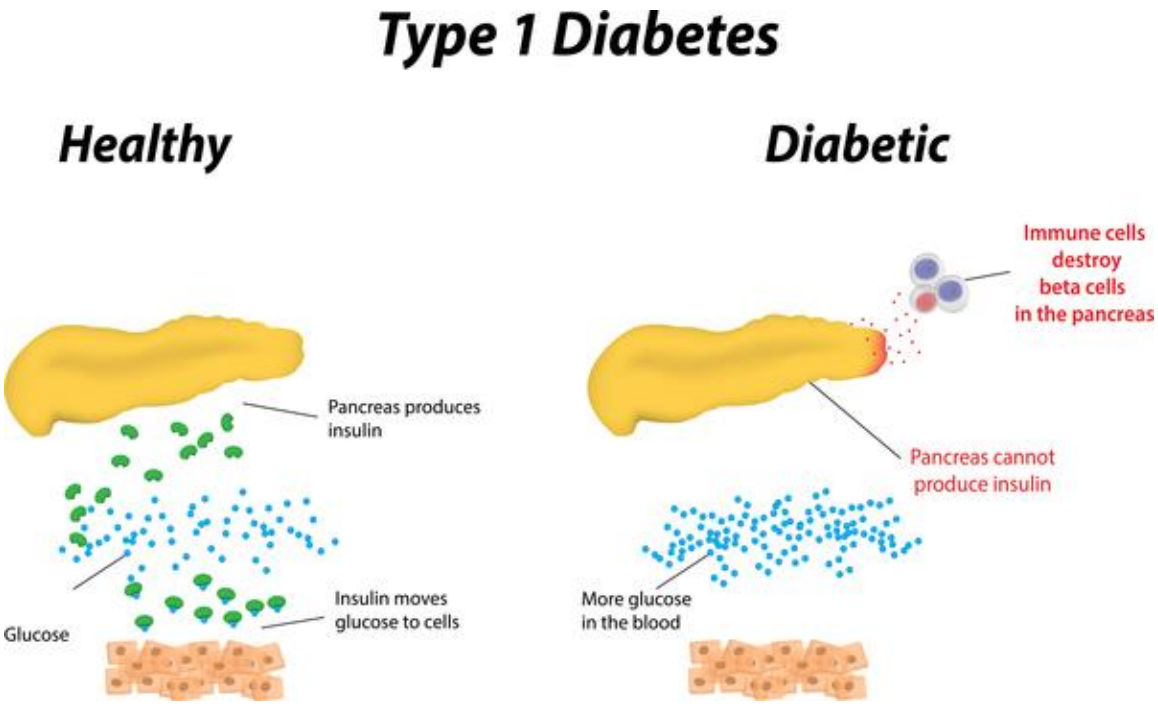

Figure 1.2: Pathogenesis of type I diabetes[21]

1.3.2. Type II diabetes and treatment

The pathogenesis of type II diabetes is different from type I, while the symptom is similar as high blood glucose level. Even though scientists could not fully understand all the causes of type II diabetes, they already made huge progress in past decades. There are two major theories of type II diabetes. The first one is insulin resistance. Scientists believe that

patients' cells fail to respond to normal level insulin (Figure 1.3). The other theory is β cell dysfunction[22]. Recently years, scientists believe these two hypothesis may work in concert with each other and become the real pathogenesis of type II diabetes[12]. Most clinicians believe type II diabetes has genetic correlation based on higher incidence within families. Other scientists argue that the similar lifestyle within one family maybe the real reason for this disease. Besides of that, environment and mental health are still within consideration.

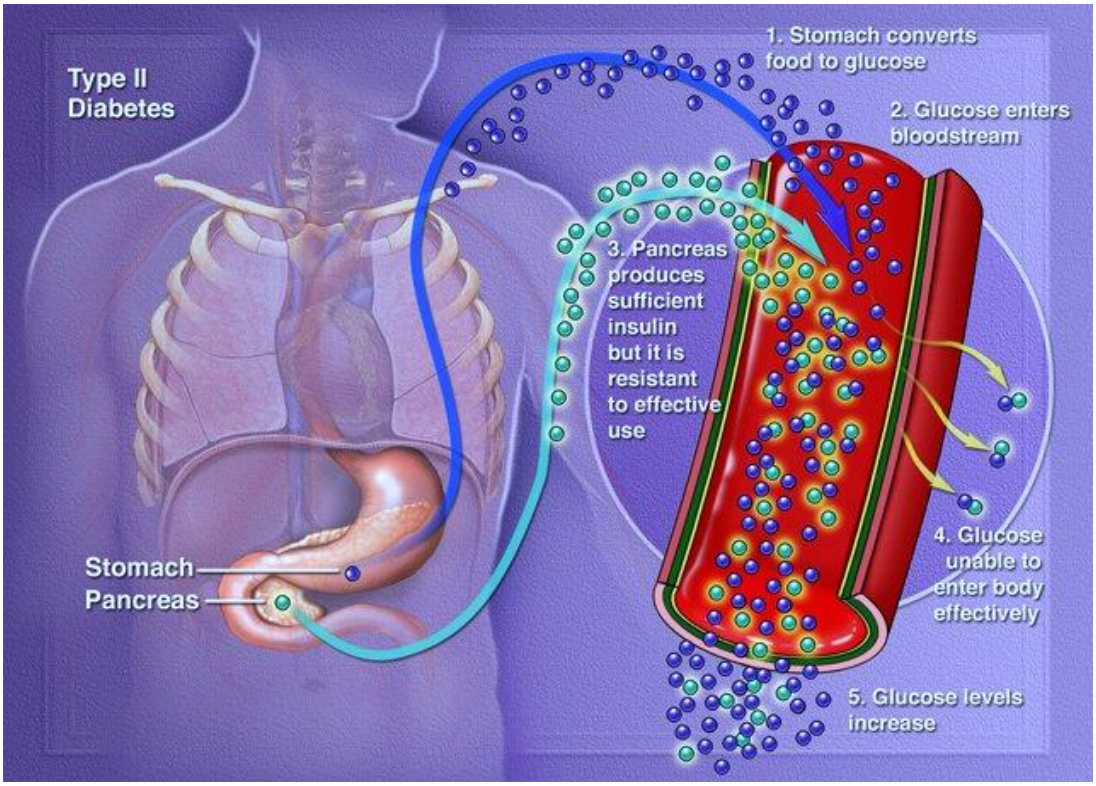

Figure 1.3: Pathogenesis of type II diabetes[23]

There are more options to treat type II diabetes. Clinically, physicians' goal is to control the glycated hemoglobin (HbA1c) level lower than 7%[24–26]. Glycated hemoglobin represents those hemoglobin formed through non-enzymatic glycation reaction with glucose. Newly diagnosed patients are usually required to change life style including diet

and physical exercise. In addition, oral anti-diabetic agents may be prescribed to control the progression of disease[11]. HbA1c should be monitored every three months. If treatment cannot control HbA1c lower than physicians' satisfaction, multiple anti-diabetic agents may be prescribed as combination. Cases are reported that some patients cannot control HbA1c level through all the methods we mentioned above. As a result, insulin therapy will be used. According to Lexicomp® database, initial insulin therapy for type II patients only provide basal level[27], which excludes meal time insulin. If this treatment still fails, then type II patients will be treated similar as type I. Oral anti-diabetic agents may still be prescribed during insulin therapy.

1.3.3. Other types of diabetes

Although scientists define other types of diabetes based on different criteria, the incidence of these types are very uncommon compared with type I and type II. In this thesis, I will only focus on type I and type II diabetes and its treatment through insulin therapy. Other types of diabetes are listed just for readers' reference[28].

Gestational diabetes

Latent autoimmune diabetes of adults (LADA)

Congenital diabetes

Cystic fibrosis-related diabetes

Steroid diabetes

Monogenic diabetes

Type 3 diabetes

1.4. Introduction of insulin, insulin production and insulin administration

1.4.1. Human insulin

Human insulin is secreted by β cells of human islets in pancreas. Insulin is the hormone that adjust human glucose level and cell glucose usage[29]. In human biochemical pathways, insulin increases cell glucose intake and decrease blood glucose level. Insufficient insulin will result in hyperglycemia and diabetes.

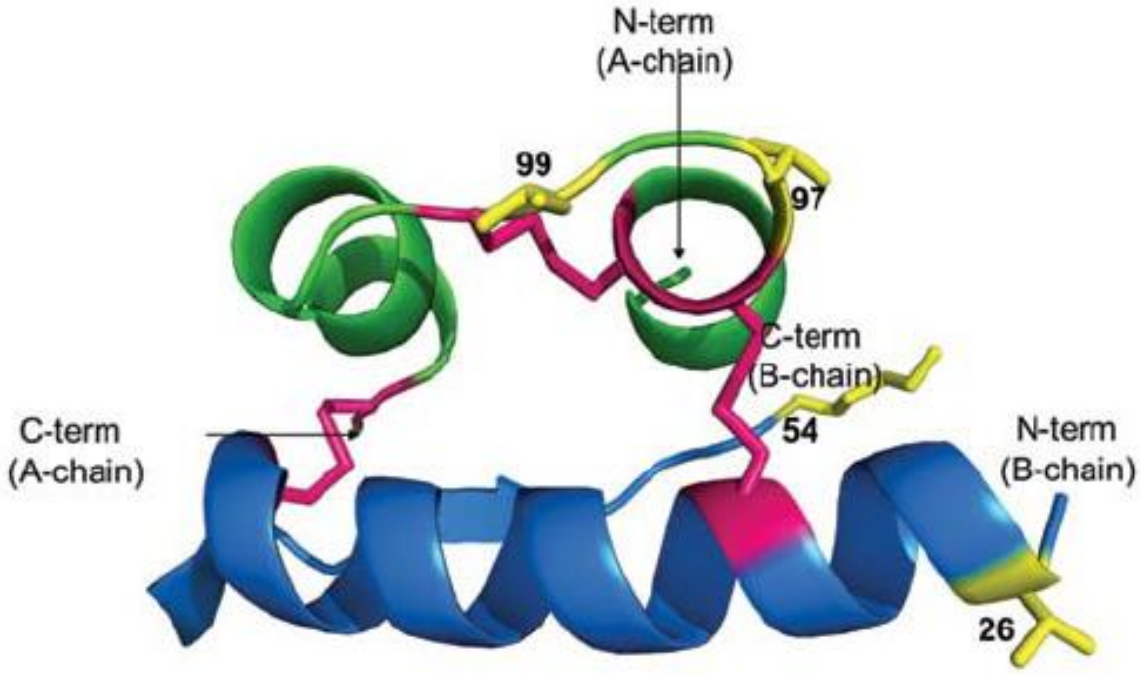

Figure 1.4: Human insulin 3-D structure[30]

Insulin is a peptide composed of 2 amino acids chains. Chain A and chain B are connected through disulfide bonds[31]. Insulin contains 51 amino acids with molecular weight 5808 Dalton (Figure 1.4)[29,32]. Current insulin products are mainly recombinant DNA product. Scientists insert human insulin gene code into bacteria's DNA and let bacteria to produce insulin (Figure 1.5)[33]. The insulin produced will go through certain purification before packaging.

1.4.2. Current insulin products

Currently, most insulin products are designed in the form of solution for subcutaneous injection or infusion (Figure 1.6), while only one product is designed as inhaler.

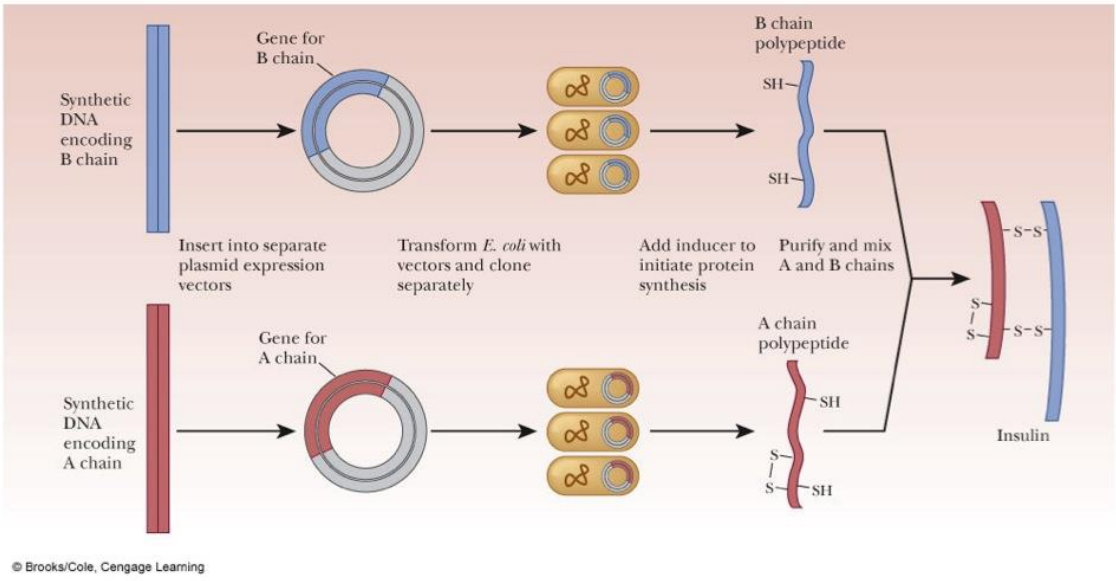

Figure 1.5: Human insulin produced by recombinant DNA technology[33]

Figure 1.6: Humulin® products produced by Eli Lilly and Company®[34]

1.4.2.1.Insulin injection solution

Rapid-acting

Rapid-acting insulin includes insulin Lispro, insulin Aspart, and insulin Glulisine[35]. All of them have quick onset time within half an hour, and reach peak concentration within an hour. Rapid insulin only covers bolus level insulin need during meal time. The duration of rapid-acting insulin is 3-5 hours[36,37].

Short-acting

Shorting-acting insulin is widely known as regular insulin[35]. Regular insulin has 100 units per ml and 500 units per ml concentration. This type of insulin usually onsets between 30 minutes to 1 hour, and reaches peak concentration between 2-5 hours. Usually, short-acting insulin only covers meal time need.

Intermediate-acting

NPH insulin stands for neutral pH protamine insulin. Scientists add protamine into insulin solution to form precipitation based on the interaction between negatively charged insulin and positively charged protamine[38]. NPH insulin onsets between 1-2 hours, and reaches its peak concentration between 4-12 hours[39–41]. This type of insulin can cover basal insulin need for half a day, but it needs to be used with short-acting or rapid-acting insulin as combination.

Long-acting

Long-acting insulin includes insulin glargine, insulin detemir, and insulin degludec. The onset time for them is between 1-2 hours. Insulin glargine and insulin degludec have no peak concentration, while insulin determir reaches its peak between 6-8 hours[42–46]. Similar to NPH insulin, long-acting insulin covers basal insulin need for the whole day, but they need to be used with rapid-acting or short-acting insulin as combination[47–49].

Pre-mixed insulin

Pre-mixed insulin usually contains one rapid-acting or short-acting insulin, and one intermediate-acting or long-acting insulin[50]. As I discussed above, intermediate-acting or long-acting insulin can only cover basal level insulin needs. As a result, patients used to inject rapid-acting or short-acting insulin before meals. In order to reduce injection frequency, manufacturers and scientists premix 2 types of insulin: one covers basal need and the other covers bolus need. Pre-mixed insulin has different combinations with various ratio. Physician should prescribe different products for each patient based on individual condition[39,51–53].

1.4.2.2.Continuous Subcutaneous Insulin Infusion (CSII)

Continuous subcutaneous insulin infusion is also named as insulin pump (Figure 1.7). Insulin pump is a device that delivers insulin into patients' subcutaneous tissue based on individual's need with different rate[54]. Insulin pump contains a pump case and a catheter. Catheter is a plastic tube with needle head that connects pump case to human body. The catheter should be attached to human skin all the time in order to infuse insulin. The needle head will stick into human fat tissue with less pain compared with injection[55]. Insulin pump is not an artificial pancreas, so patients still need to monitor their blood glucose level. Most insulin pumps use regular insulin in their cartridge. Some pump requires manual refill of insulin into cartridge, while others provide ready-to-use cartridges[56]. Insulin pump should be programmed by providers or physicians to accurately release insulin based on patients' need[57]. Some pumps require patients to press a certain button before meal to increase insulin release, others may have glucose sensor to adjust insulin release rate automatically. Some latest version pump can be connected to smart phones to provide on-time insulin release rate, glucose level and other human body data[58].

Insulin pump has many advantages. Compared with insulin injection, insulin pump has tighter control of insulin level and blood glucose level. Patients will benefit from this advantage to avoid hypoglycemia during exercise, sleeping and fasted time[59]. Additionally, insulin pump can avoid daily injections to better protect the integrity of human skin. Long term frequent injection may cause irritation and permanent damage of skin[20]. As a summary, insulin pump provides flexibility for patients in many cases[60].

Figure 1.7: The Minimed® VEO™ Insulin Pump[61]

Insulin pump is not perfect. Insulin pump is a very precise electronic instrument. The maintenance and production is complicated, resulting in the high price compared with insulin injection pens[62]. Another disadvantage is that insulin pump can lower patients' glycated hemoglobin level, meaning more glucose are utilized by human body. The energy absorbed by patients' body may result in quick and huge weight gain[63]. This number can be as large as 75 pounds in 2 years. Since insulin pump requires continuous connection

between human body and catheter, patients may feel uncomfortable and bothering because of weak pain and itchiness from the needles. Dangerous situation may happen if catheter disconnects from patient by accident[25,57]. This is also one reason that insulin pump requires long time training to educate patients where to put the pump case during shower, exercise, sleep and other scenarios when disconnection may happen.

1.4.2.3. Afrezza® inhaler

Back in 2006, Pfizer launched the first insulin inhaler name Exubera®[64]. One year later, Pfizer was forced to withdraw all Exubera® based on bad sales result[65]. Exubera® was proved to be no more effective than insulin injection with much higher expenses[66]. In 2014, Mannkind Corperation launched a brand new insulin inhaler name Afrezza®. Afrezza® was believed to be safer and cheaper than Exubera® but the sale amount is still struggling[67]. Afrezza® use fumaryl diketopiperazine, polysorbate 80 as excipient to form powder[68]. Afrezza® was released as an alternative to rapid-acting and short-acting insulin injection. Afrezza® must be used as combination with intermediate-acting or long-acting insulin[69].

1.5. Current issues with insulin therapy and blood glucose control

1.5.1. Glucose control

Human blood glucose level should be controlled in a very small range. For healthy population, fasting blood glucose while awake should be under 100 mg/dl. Before-meal normal glucose are 70–99 mg/dl. “Postprandial” glucose taken two hours after meals should be less than 140 mg/dl. Optimally, insulin therapy should bring patients’ glucose back to the range above for the best therapeutic effect[70]. Figure 1.8 is the duration and peak times for the most common insulin [71]. Figure 1.9 is the change of insulin level in a

healthy person [72]. If compare these 2 figures, current insulin products can barely provide a perfect glucose control. The mostly reported side effect from patients is hypoglycemia[71]. Patients using insulin pump report less hypoglycemia because pump slowly infuse insulin into human body without peak concentration. Premix insulin was first invented to narrow the glucose window. Currently, low-ratio premix insulin, the premix insulin with equal or less than 30% rapid-acting or short-acting ones, is one of the most widely prescribed insulin products globally[53]. Individualized premix insulin prescription is still not feasible. So patients that cannot control glucose tightly with available insulin products will have no choice but to use insulin pump.

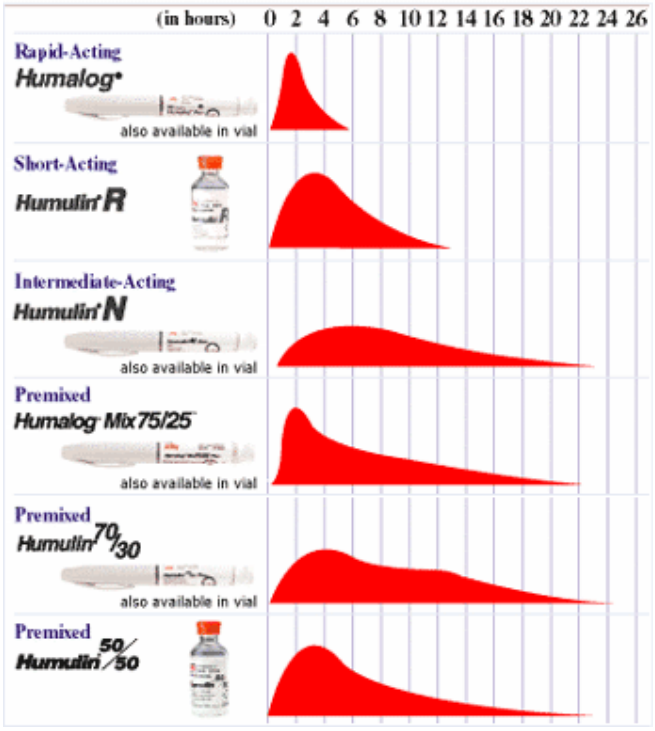

Figure 1.8: Duration and peak times for some insulin products[72]

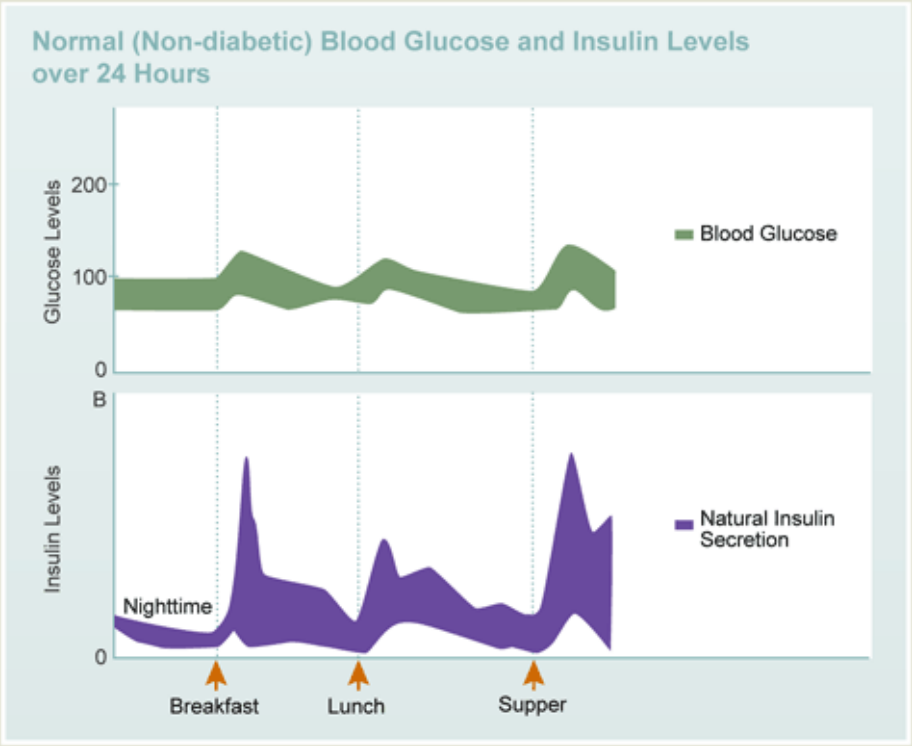

Figure 1.9: Daily blood glucose and insulin level in healthy population[73]

1.5.2. Injection side effects

Patients using insulin injection need to perform at least one injection every day. Local side effects reported include hypertrophy and lipoatrophy, both of which result from long-term and frequent injection[74]. Daily injection also has negative influence on patients' mental health and social activity. Increasingly obvious injection orifice on the skin may also bother patients during shower, dressing and sleep[75].

Insulin pump is an efficient way to avoid injections. Small needle patch connected with catheter can attach to human skin with minimized pain. One risk using insulin pump is the accidental disconnection of catheter. Many cases have been reported, especially from children's patients and elderly patients, that insulin pumps disconnect without patients' notice. This issue becomes a common one for almost all insulin pump manufactures. A

patent technology was filed in 2014 by Yodfat and Shapira as a solution. Roche Diagnostics Operations, Inc. currently is in possession of it[57].

1.5.3. Cost efficiency

1.5.3.1. Insulin injection though KwikPen®

KwikPen® is a registered insulin injection device invented by Ely Lilly and Company®. The cost of this device is $120 on average. Injection needle is approximately $0.2 each. Cooling case for insulin and KwikPen® is around $30. Humulin® R U-100 is priced as $16 per 10 ml, while U-500 is $1,679.64 per 20 ml. Daily usage of insulin depends on patients' condition, while device price should be the same for every patient. All price data is provided from Lexicomp® database.

1.5.3.2. Insulin pump

Insulin pump price is different for different brands and designs. Price usually starts with $5,000[58]. Latest version of pumps has continuous glucose monitor, disconnection sensor and smart device connection. Expensive pumps can be more than $10,000. In addition, insulin pump needs to be calibrated and serviced regularly. Overall cost of insulin pump is much more expensive than insulin injection. Insulin pump use regular insulin, so the price of insulin should be similar with insulin injection.

1.5.4. Conclusion of current insulin therapy

Insulin injection is a widely used insulin administration method globally, especially in low income countries. Disadvantages of this method include injection pain and irritation, large blood glucose window and other systemic side effects. Most issues related to insulin injection can be resolved perfectly through switching to insulin pump technology, but

insulin pump is expensive, may cause quick weight gain, and may disconnect from human body.

A better technology is needed to solve these problems and provide a better insulin therapy. The requirements for a better technology include affordable device cost, less pain and skin damage, tight glucose control and easy application.

1.6.**Current subcutaneous implant and insulin implant**

1.6.1. Introduction of subcutaneous implant technology

Figure 1.10: Nexplanon® [76]

Subcutaneous implant is a solid form of medicine to be inserted into human subcutaneous tissue. Drugs slowly release from implant and diffuse into human body fluid[77]. Human body fluid will carry drug molecules into blood circulation. The most famous subcutaneous implant is known as Nexplanon® (Figure 1.10). Nexplanon® is designed for female birth control by releasing etonogestrel. The release of etonogestrel can be effective as long as 3

years[78]. The release rate of etonogestrel decreases with time goes, shown as Figure 1.11. Another example of subcutaneous implant is Zoladex®. Zoladex® is designed to release goserelin to treat prostate cancer. Figure 1.12 is the package and syringe of Zoladex®. Zoladex can last as long as 3 months[79].

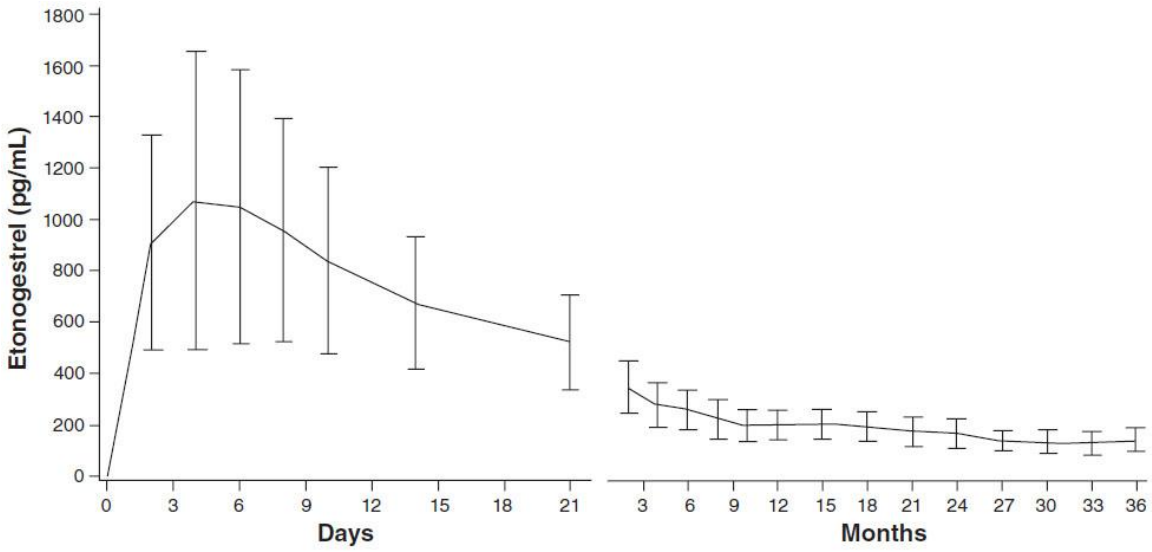

Figure 1.11: Serum concentration of etonorgestrel (pg/ml) over time during 3 years of Nexplanon® use[80]

Figure 1.12: Zoladex® implant and syringe [79]

1.6.2. Potential market for insulin implant

In section 1.5., I introduced some issues with current insulin therapy. If insulin implant can replace current device, there will be less pain and irritation because the injection frequency will be once every several weeks. Implant can be programmed or manually controlled to change insulin release rate. Also, this implant should be much cheaper than insulin pump.

1.7.Introduction of Controlled Release System

1.7.1. Therapeutic Window

Almost all drug molecules have toxicity against human body. In most cases, drug molecules will provide a therapeutic effect within a certain concentration. Higher concentration will cause side effect and toxicity. Lower concentration will result in inefficient treating effect. Control the drug concentration in human system, mainly plasma, within this therapeutic window is extremely important. Ideal control may maximize therapeutic effect, as well as minimize toxicity[81].

1.7.2. Controlled Release System

Controlled release dosage forms are designed mainly for several reasons. The first rationale to use controlled release is to minimize toxicity or side effects, maintaining drug amount within therapeutic window. In addition, controlled release system may deliver drug molecules with least amount of waste[82]. For example, some sustained release tablets are designed to reduce the amount of drug to be eliminated without absorption.

Controlled release systems either provide long-term delivery over days / weeks / months / years (birth control implant, cancer implant treatment), or provide a one-time target delivery (colon targeting tablets)[83]. In most cases, a controlled release system should

release drug molecules in a known and predictable kinetics[84]. The most ideal situation is to provide a uniform drug concentration as a function of time[85].

1.7.3. Classification of controlled release systems

Even though controlled release systems have different types, they do use similar principles to delivery drugs into targets[86]. Typically, drug molecules need to be released from the delivery systems and diffuse into surrounding environments. In ideal situation, the diffusion from delivery system should be the rate limiting step in order to provide a controlled drug delivery. As a result, diffusion is the key point in controlled release system. The classification is based on different types of diffusion. The membrane of diffusion can be classified as macroporous membrane, microporous membrane and nonporous membrane. These membranes are identified by pore size and pore formation[87].

1.7.3.1. Diffusion Controlled

Diffusion is dominant in drug delivery system and it exists in all controlled release systems. Diffusion can be divided into serval types[88].

Reservoir Systems

This type of dosage form is separated from target environment by a membrane. In order to maintain a constant flux, the concentration gradient should maintain constant. In most design, the constant concentration gradient is achieved by adding excess amount of solid drug inside of the reservoir to maintain saturated solution of drug molecules. This kind of system usually provide zero-order release of drugs. Coated tablet is the most common dosage form in this classification.

Matrix Systems

In this type of systems, drug molecules are either dissolved in matrix, or dispersed in matrix. Two factors will contribute to the kinetics of drug release. One is the dissolution of drugs, and the other one is the diffusion path of drugs through water filled pores. This type of systems usually do not provide zero-order released kinetics. In some cases, the release profile follows square of root of time. Wax tablet is one example of this system.

1.7.3.2. Chemically Controlled

Chemically controlled systems can be divided into two subtypes.

Erosion Matrix Systems

In this system, drug molecules are disbursed into matrix. The matrix will erode layer by layer to release drugs with eroded polymers. The rate of erosion determines the release rate of drugs. Erosion system can be added in both reservoir systems and matrix systems. If added with reservoir systems, membrane effect should be considered. The slower step, either erosion or diffusion through membrane, will control the drug release rate. Erosion has two types: heterogeneous erosion and homogeneous erosion. Most hydrophobic polymers tend to erode heterogeneously. Vice versa, hydrophilic ones prefer homogeneous erosion. Erosion tablet is a good example of this system. Dr. Owaisat in our group invented a erosion tablet using alginate polymer to provide universal release of different drugs.

Pendant Chain Systems

In this type of system, drug is chemically bonded to polymers, and will be released through the hydrolytic cleavage.

1.7.3.3. Solvent Activated

Osmotic Pump

Osmotic pump delivery system is a closed system with an orifice. Water comes into the system through orifice and build osmotic pressure. Drug molecules will be pumped out based on osmotic pressure.

Swelling Controlled System

Swelling controlled system is a polymer based system where drug molecules dispersed in it. Drugs will be released by swelling of polymers.

1.7.3.4. Magnetically Controlled

Magnetic beads are coated with drugs. Drug molecule can release while dissolved into solvent. However, the release rate will be faster if an external magnetic field was applied.

1.8. Parenteral drug delivery

Parenteral drug delivery systems are designed to bypass human GI track, and delivery drug molecules directly into human system[89]. Parenteral systems are extremely helpful for poor bioavailable drugs and drugs with narrow therapeutic windows. In addition, parenteral dosage forms are good choices for long term drug delivery. Some drugs expect frequent or life time usage. Daily injection or oral administration maybe inconvenient or uncomfortable. As a result, some parenteral formulations have been invented for long term drug delivery.

Parenteral drug delivery systems can be divided into several types: injectable, implantable and infusion devices.

1.8.1. Injectable

Injectable systems are the most straightforward systems. Solutions, suspensions and emulsions are three major groups that fit this subtype. Drug molecules are formulated based on its physiochemical characterizations. Most drug molecules are delivered directly into

human blood vessel to achieve high therapeutic effect. In recent years, innovative formulation using nano technologies start to become popular in injectables. Liposomes, niosomes, micelle technology, nanoparticles and macroparticles are frequently proposed[90]. The general purpose of these technologies is to improve bioavailability, keep drug concentration within therapeutic window, or provide targeting delivery.

1.8.2. Implantable

Implantable technology was first used to administer crystalline hormone in the form of solid. Implants are inserted under the skin. Implantable medical device has many advantages. Implants designed for local drug administration can provide higher local concentration. This advantage is very useful during cancer treatment. In addition, implants designed for systemic drug delivery can provide 100% bioavailability of drug molecules. More importantly, implants can provide sustained drug release[91–93]. This is critical to narrow therapeutic window drugs. Lastly, implants can provide higher patients' compliance than oral dosage forms and injections.

1.8.3. Infusion pump

Among all infusion devices, infusion pump is the most widely used device for the controlled delivery of nutrition and medication. Infusion pump requires professional training and usage, which results in frequent visit to healthcare facilities. Self-administration is strictly controlled for infusion pump due to the high risk of over dosing[20,54–56,94]. Latest version of infusion pump use digital screen to control dose and rate. Insulin and other drug molecules now can be administered through mobile phone applications and Bluetooth technology. Accuracy and on-time warning is very common

now. However, mechanical problems and safety concerns are still linked with infusion pumps. More design and work needs to be done to eliminate such doubt[59,91,94].

1.8.4. Topical and Transdermal

Human skin is the largest organ on human body. Topical and transdermal drug delivery use human skin as its target to bypass gastrointestinal track and maximum bioavailability. Dosage form used on human skin including but not limited to cream, gel, suspension, spray solution and patch. Topical dosage forms usually prefers high local drug concentration, while transdermal dosage forms deliver drug into blood circulation. Both methods are widely used in many scenarios in modern medicine[95–99].

1.9. Hypothesis and specific aims

1.9.1. Hypothesis

A cylinder-shape subcutaneous implant might be fabricated with biocompatible excipients to provide controlled drug release.

If formulated and/or fabricated correctly, this implant might provide zero-order drug release.

Drug releasing rate might change after insertion with certain preset during formulation and fabrication.

1.9.2. Specific aims

Formulate and fabricate a prototype with cylinder-alike shape with biocompatible materials and excipients.

Investigate the drug releasing mechanism of this prototype and establish zero-order drug release for at least 24 hours.

Apply this prototype to multiple drugs with different physical, chemical and biological characteristics.

Propose a feasible and practical method for mass production. 1.10. Conclusion of this chapter

A large group of population in the United States are suffering from diabetes. Insulin therapy is the routine treatment but current insulin delivery devices are not perfect. They either bring pain, irritation with other side effects, or are too expensive to afford. These issues are not special for diabetes but also happen to other chronic diseases. Most chronic diseases require lifetime medical treatment. The goal of this project is to follow the idea of Nexplanon® and Zoladex® by using a subcutaneous implant to deliver insulin and other lifetime medication. In the future chapters, I will introduce the detailed experimental design and technologies I used to address my specific aims.

CHAPTER 2

PRELIMINARY STUDY: RELATED FACTORS IN DRUG DIFFUSION

2.1. Abbreviation

API: Active pharmaceutical ingredient

BSA: Bovine serum albumin

BSA-Zinc-Insulin: complex formed by albumin, insulin and zinc ion

DI water: Deionized water

HAS: Human serum albumin

PBS: Phosphate buffered saline

2.2. Introduction

2.2.1. Fick's Law

Drug diffusion is a critical step in drug release from delivery system. Understand drug diffusion process and investigate factors that influence drug diffusion rate is important in designing drug delivery systems. Drug diffusion process is usually divided into two types: passive diffusion and active transportation. Passive diffusion is purely powered by concentration gradient between two sides of a membrane. Fick's Law of Diffusion described the relationship between diffusion flux and concentration. It is the first guidance in diffusion based drug delivery system[100]. Equation 2.1 is Fick's First Law. J is diffusion flux; D is diffusion coefficient determined by chemical structure of molecules; C is concentration and X is position.

$$J_{D,\alpha} = -D_{\alpha} \frac{dc_{\alpha}}{dx}$$

Equation 2.1: Fick's First Law of Diffusion

2.2.2. Porous and Non-Porous Membrane

Porous membrane can be defined as its name: membrane with pores or channels through its surface. Porous membrane is semipermeable. The main goal is to separate solutes based on membranes' pore size. The pore size of porous membrane can vary from 0.001 to 10 μm. In most cases porous membrane are used in filtration systems. Formulation scientists use porous membranes for controlled drug release based on its semi-permeable characteristics. Meanwhile, medical providers use membrane to purify drug products and human-related fluid. Human dialysis is one method using semi-permeable membranes to separate human plasma from impurities and metabolites[101].

Generally, porous membrane's diffusion ability is determined by pore size and porosity for the same solute. Membrane with larger pore size and higher porosity would provide faster diffusion and less selectivity during separation. Vice versa[102].

Non-porous membrane is usually a dense film without specific holes or pores (Figure 2.1). Substances diffuse though non-porous membrane by pressure, concentration gradient or electric potential gradient[103]. The diffusion process depends on the solubility of substances in that membrane, as well as the partition coefficient. For example, transdermal drug molecules need to diffuse through human skin. The diffusion process is highly

dependent on drug's partition coefficient. Topical drug molecules need to diffuse into human skin and stay there. So molecules with high lipophilicity are preferred.

In conclusion, both porous membranes and non-porous membranes are widely used in drug delivery and health care service. When using membranes, several factors will contribute to the drug diffusion rate, including drug solubility in media, drug solubility and binding in membrane, pore size and porosity.

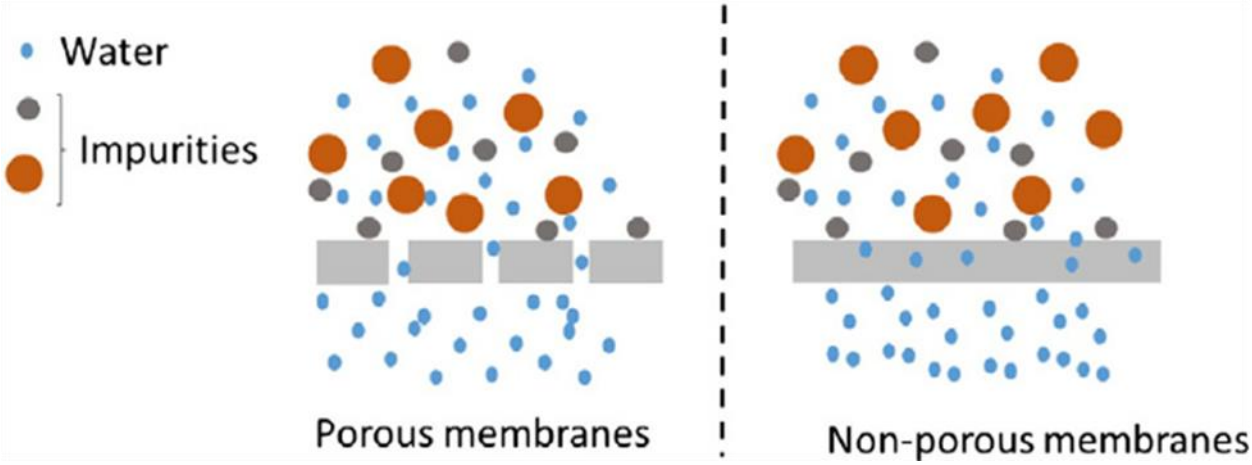

Figure 2.1: Diffusion Mechanism of Porous and Non-porous Membranes[103]

2.2.3. Osmotic Pressure

When using a semi-permeable membrane, researchers often refer to osmotic pressure. Osmotic pressure is defined as the critical pressure to prevent water from flowing into the matrix through a semi-permeable membrane. The final stage is the equal concentration of the same solute on both sides of a semipermeable membrane. Water has a tendency to flow from low osmotic pressure to high osmotic pressure environment[93]. This principle is strictly enforced while performing IV infusion. Figure 2.2 is a general description about osmotic pressure in human body related fluid.

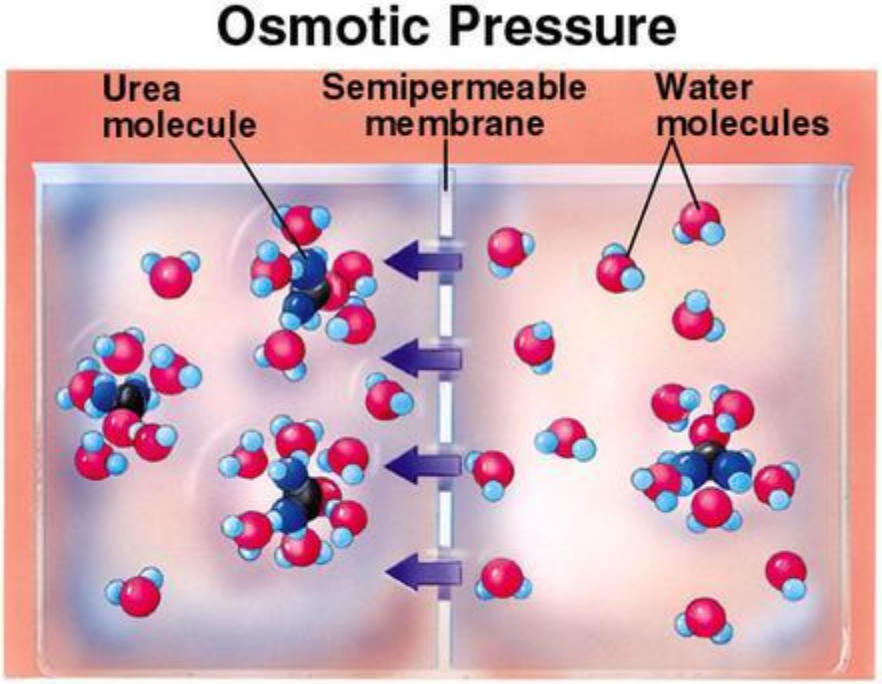

Figure 2.2: Description of Osmotic Pressure in Human Urine (McGraw-Hill Learning Center)

2.2.4. Magnetophoresis

Materials can be attracted or repelled by external magnetic field. Paramagnetism is a symptom when materials are attracted by external magnetic field. These materials usually have unpaired electrons in their electron cloud. Paramagnetic materials include aluminum, oxygen, titanium and iron oxide. On contrary, diamagnetism is defined in the opposite way. Diamagnetic materials are repelled by external magnetic field. This phenomenon comes from the induced dipole moments. Diamagnetism is a quantum mechanical effect and weak force[104,105]. Figure 2.3 is a description of these three types of magnetism. If paramagnetism or ferromagnetism applies, diamagnetism is difficult to be observed. However, in Nijmegen High Field Magnet Laboratory, a 16 tesla magnetic field successfully lifted a frog into the air[106]. This extreme experimental setting proves the existence of diamagnetism.

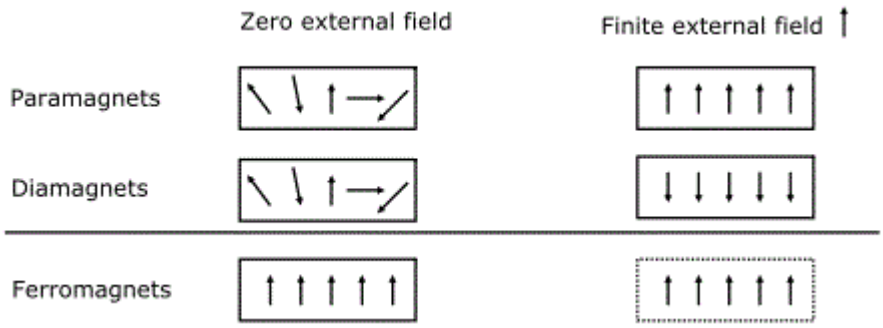

Figure 2.3: Comparison of Different Magnetism in Electron Cloud (Courtesy of Gerrit-Jan Linker)

Figure 2.4: A live frog levitates inside a 32 mm vertical bore of a Bitter solenoid in a magnetic field of about 16 teslas at the Nijmegen High Field Magnet Laboratory (Berry and Geim)

Murthy et al applied magnetophoresis on transdermal drug delivery[105]. They achieved significant increase in drug penetration using lidocaine with appropriate formulation. Murthy's achievement proves that magnetophoresis can be used as an external force to affect drug diffusion through non-porous membrane. It is highly possible that magnetophoresis also works for porous membrane.

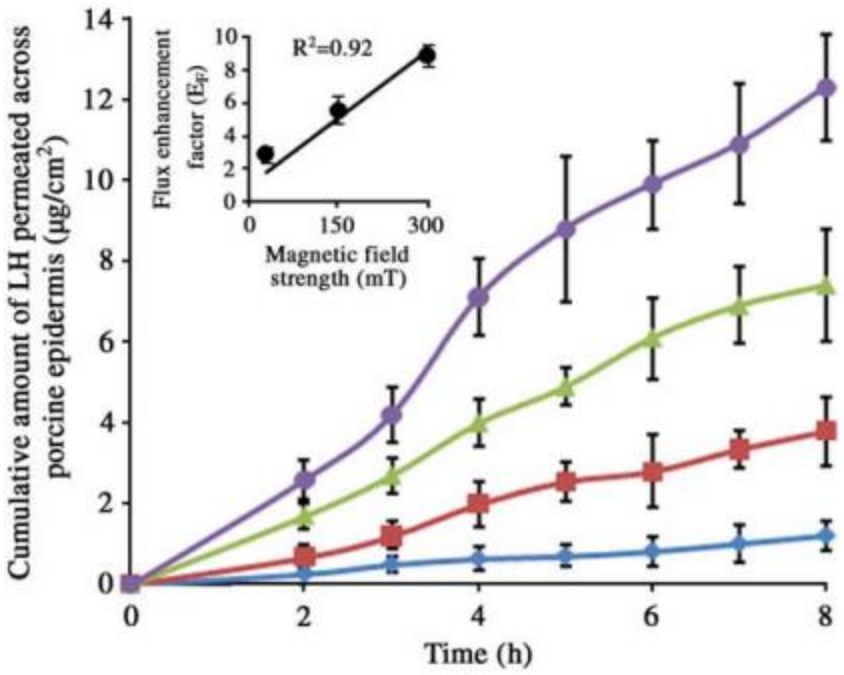

Figure 2.5: Cumulative permation of LH across porcine epidermis in case of passive (♦), magnetic field strength of 30 mT (■), 150 mT (▲) and 300 mT (●). Insert graph shows plot of magnetic field strength vs. flux enhancement factor[105].

2.2.5. Iontophoresis

Drug molecules usually carry ionizable groups. In pharmaceutical sciences, ionization of compound may alter molecules' physical behavior, as well as macro characterizations. Solubility and lipophilicity are typical examples. For ionized molecules, their movement might be controlled by electrical field. Iontophoresis is one physical method to control the movement of molecules based on their charge[107–109]. In pharmaceutical delivery system, iontophoresis might be used to deliver drug molecules through a membrane (Figure 2.6), or trigger an environmental change in drug matrix to alter drug release rate[87].

Iontophoresis may alter drug diffusion through two mechanisms. One mechanism is named electromigration. According to Coulomb's Law and the definition of electric field intensity, Cations move in the same direction as electrical field while anions move in the opposite

direction[108]. The charge of drug molecules can be controlled through pH value of formulation. The other mechanism is named electroosmosis. Cations, such as sodium, potassium, calcium ions, always follow electrical field's direction. As a result, the cations forms cation flow in solution. The large volume flow would carry water molecules to form water flow. Water flow would move every component in solution in that direction. In most cases, electromigration dominates in iontophoresis and it is preferred in drug delivery systems[110–112].

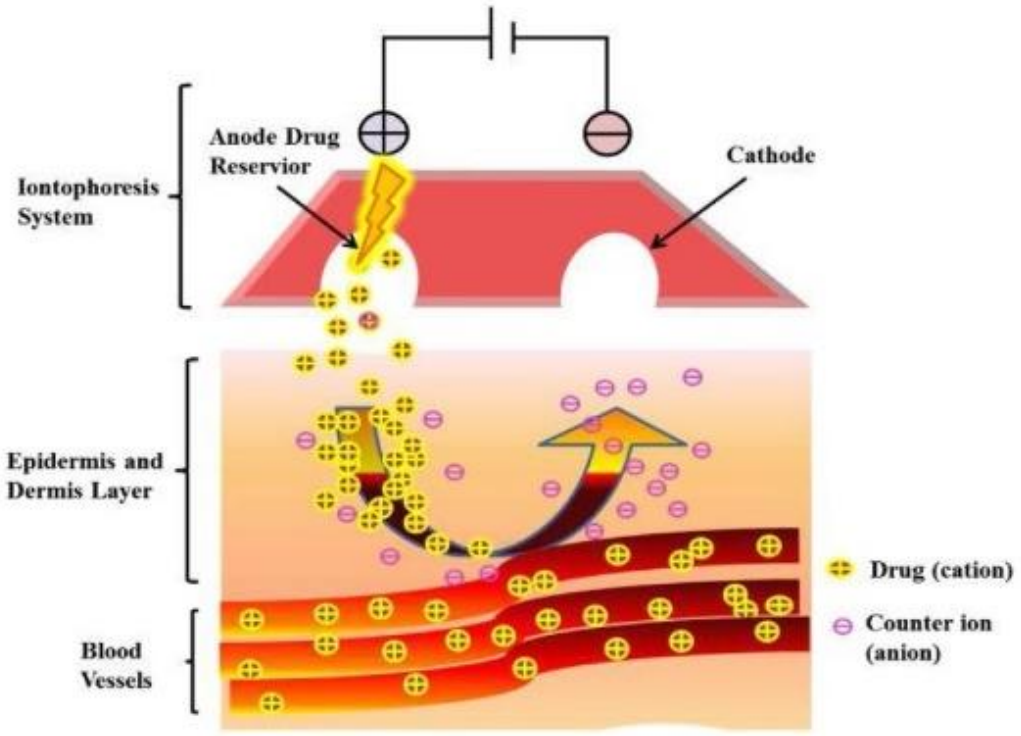

Figure 2.6: Principle of Iontophoresis in Transdermal Cation Drug Delivery (Courtesy of Mohmmad Rasool Sakhawarz)

2.2.6. In Vitro Drug Releasing Test

The measurement of drug release is fundamental for a given dosage form. Solid dosage forms, including tablets and capsules, are tested in dissolution studies. For semisolids,

patch, implant and other non-solid dosage forms, Franz Cell or Side-bi-Side Cell systems are preferred for drug releasing test.

2.2.7. Franz Cell and Side-bi-Side Cell Systems

Figure 2.7 is the structure of one Franz Cell (Particle Sciences®). In Franz diffusion cell, dosage forms will be placed in donor chamber. One synthetic membrane or biological membrane will be immobilized underneath donor chamber. Both the donor chamber and membrane will be locked firmly onto receptor chamber. The whole system is controlled at constant temperature by heater or circulator. Samples can be pulled though sampling port by needles and syringes.

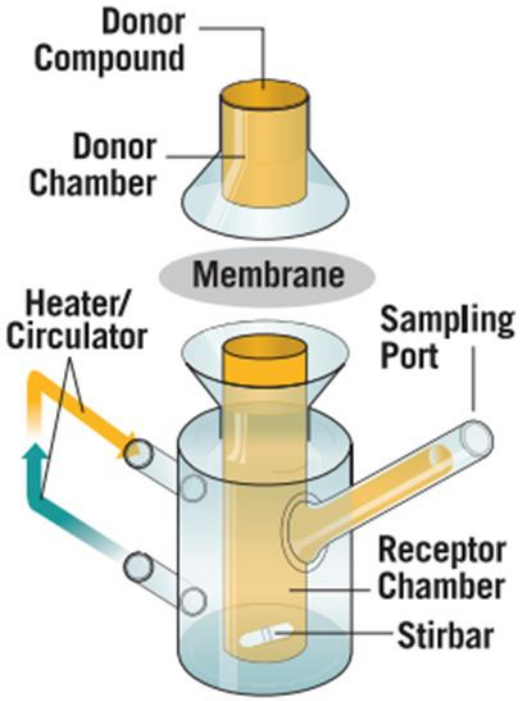

Figure 2.7: Franz Diffusion Cell Structure (Partical Sciences®)

Figure 2.8: Franz Diffusion Cell ordered from PermeGear (PermeGear Product Page)

Different from Franz Cell diffusion system where molecules move in vertical direction, Side-bi-Side diffusion system only allows molecules to move laterally. Figure 2.9 is a cross-sectional view of a Side-bi-Side cell. There is no specific selection of donor or receptor chamber. Researchers can choose based on their preference. Membranes should be put between two chambers and immobilized for the best experimental result. Samples can be pulled from sampling port. Temperature of the system can also be controlled. In addition, Side-by-Side cells have an extra port for iontophoresis operation (Figure 2.10). Electrodes can be inserted through additional port while sampling will not be influenced.

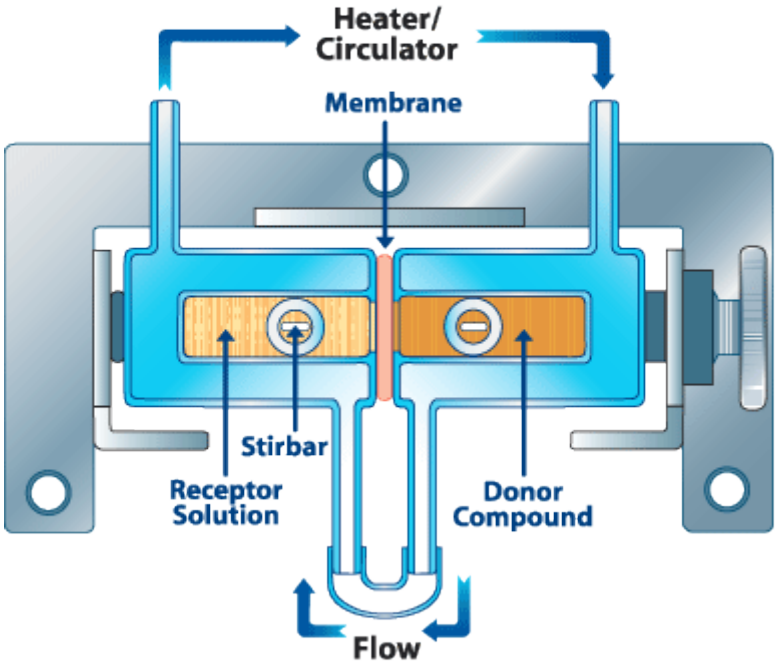

Figure 2.9: Cross-sectional View of Side-bi-Side Diffusion Cell (PermeGear Product)

Figure 2.10: Side-bi-Side Diffusion Cell ordered from PermeGear (PermeGear Product)

2.3. Materials and Devices

Humulin® R U-100 was purchased from Eli Lilly and Company (Indianapolis, IN, USA). CPO was purchased from Spectrum Chemical Mfg. Corp., Edison, New Brunswick, USA. Zinc wire (1.0mm diameter, extruded, 99.9%), Potassium phosphate monobasic (99.99%), sodium hydroxide (ACS reagent, ⩾97.0%), dimethyl sulfate (⩾99.8%), and triethylamine (for HPLC, 99.0%) were bought from Sigma Aldrich. Supelco Nylon 66 Membranes (0.45 μm x 47 mm) and Millipore Ultracel® 1 KDa Ultrafiltration Discs were purchased from Millipore Sigma. Sodium phosphate dibasic (Anhydrous, ACS reagent, ⩾99%) were obtained from Acros Organics. Alfa Aesar™ Bradford Dye Reagent was purchased from Fisher Scientific. Franz diffusion cells and Side-bi-Side diffusion cells were purchased from PermeGear. Permanent magnets was purchased from Macmaster Carr. Nanopure water was used for the preparation of 10 mM pH 7.4 phosphate-buffered saline (pH 7.4 PBS). HPLC grade acetonitrile from Fischer Scientific was used for the preparation of mobile phase. All the chemicals were used as received without any additional purification.

2.4. Experimental Methods

2.4.1. High Performance Liquid Chromatography

Ciclopirox Olamine (CPO)

Derivatization was performed before HPLC assay. Escarrone et al reported a successful derivatization method[113]. To perform derivatization, add 200 μL dimethyl sulfate into 1 mL sample solution. The mixture was mixed by vortex for 30 seconds and incubated in 37 °C for 30 minutes. After the incubation, 200 μL triethylamine was added to stop the reaction.

CPO content in the samples was assayed using an Agilent 1100 series HPLC with dual wavelength detector. The mobile phase composition was acetonitrile/water (50:50 v/v). The column we used was 150 × 4.6-mm Eclipse Plus C18 column (Agilent). The flow rate was 1 mL/min and the injection volume was 50 µL. The wavelength of detection was 298 nm.

Humulin® R U-100

Insulin was assayed in the same system. Acetonitrile : 50 mM pH 2 Sodium Phosphate (20:80 v/v) was used as the mobile phase. Column was purchased from Millipore as a 25 mm by 4.6 mm Chromolith® Phenyl column. The flow rate was 1.5 mL/min and the injection volume was 50 µL. The wavelength of detection for insulin was 214 nm. In this method, pH 2 was chosen based on insulin stability and solubility.

2.4.2. Bradford Assay for General Protein Amount

General protein amount was assayed through Bradford assay. Bradford assay is a non-specific assay based on amount of amino acids. In our research, a three-tier prototype was used.

i. 1.25 - 10 µg/ml protein sample: add 1 volume of sample solution and 1 volume Bradford reagent.
ii. 125 - 1,000 µg/ml protein sample: add 1 volume of sample solution and 30 volume Bradford Reagent.
iii. 10 - 125 µg/ml protein sample: add 1 volume sample and 5 volume Bradford Reagent

iv. It is also possible that standard curve in each range may not provide linear relationship. In that case, a Point-to-Point plot is recommended for more accurate result.

2.4.3. Franz Cell Diffusion Study of CPO using Magnetophoresis

Formulation: saturated CPO solution in PBS 7.4. Approximate concentration was more than 1.41 mg/mL. Formulation was set to be oversaturated by adding additional CPO and ensure vision of undissolved CPO solid. Membrane: Millipore Ultracel® 1 KDa Ultrafiltration Discs. Receiver buffer: phosphate buffered saline. Magnetophoresis: permanent magnets was used to on top of the donor chamber.

In this experiment, same formulation was used for both control group and magnetophoresis group. 1 ml saturated solution was added into donor chamber. 3 mL phosphate buffered saline was added into receiver chamber. During the experiment, the temperature was controlled as 32 °C. 0.5 mL was pulled every hour. Another 0.5 mL pure PBS was added as refill to control total volume as 3 mL. Samples were assayed by HPLC method.

2.4.4. Side-bi-Side Diffusion Study of Ibuprofen using Iontophoresis

Formulation: saturated ibuprofen solution in pH 3 buffer system. Approximate concentration is higher than 21 mg/L. Membrane: Millipore Ultracel® 1 KDa Ultrafiltration Discs. Receiver buffer: phosphate citrate buffer in hypertonic (2.7%) NaCl solution at pH 7. Iontophoresis: Zinc wire was used for both anode and cathode. Direct current was applied through the cathode in the donor chamber. Reverse current was applied though the anode in the receiver chamber. For all experiments 5 milliamps was applied.

In this experiment, same formulation was used for both control group and iontophoresis group. 3 ml saturated solution was added into donor chamber. 3 mL phosphate citrate

buffer was added into receiver chamber. During the experiment, the temperature was controlled as 37 °C.

2.4.5. Side-bi-Side Diffusion Study of BSA using Dialysis Tubing Membrane

Formulation: 5% BSA in PBS. Membrane: SpectrumLab® Dialysis Tubing 50 KDa. Receiver buffer: PBS at pH 7.4. Iontophoresis: Zinc wire was used for both anode and cathode. Direct current was applied through the cathode in the donor chamber. Reverse current was applied though the anode in the receiver chamber.

In this experiment, same formulation was used for both control group and iontophoresis group. 3 ml saturated solution was added into donor chamber. 3 mL PBS was added into receiver chamber. During the experiment, the temperature was controlled as 37 °C.

2.4.6. Side-bi-Side Diffusion Study of Insulin Related Formulation

Preparation of insulin related dosage forms

Insulin water solution: 1 ml Humulin® R U-100 (Figure 2.11) was dissolved directly into 11.5 ml DI water to form 8 UI/ml insulin diluted solution. Solution was stirred to keep uniform mixture.

Figure 2.11: Humulin® R U-100 from Eli Lilly and Company

Insulin albumin solution: 1.25 gram BSA was added into 11.5 ml DI water. After complete dissolution, 1 ml Humulin® R U-100 was added into BSA solution. The final concentration was 10% (0.1 g/ml) for BSA and 8 UI/ml for insulin.

Insulin albumin suspension: 1.25 gram BSA was added into 11.287 ml DI water. After complete dissolution, 1 ml Humulin® R U-100 was added into BSA solution. After mixing uniformly, 0.213 ml 20% zinc chloride solution was added into the mixture. White cloudy suspension formed immediately after adding zinc chloride. The whole suspension was stirred until a uniform, cloudy, non-yellowish suspension formed.

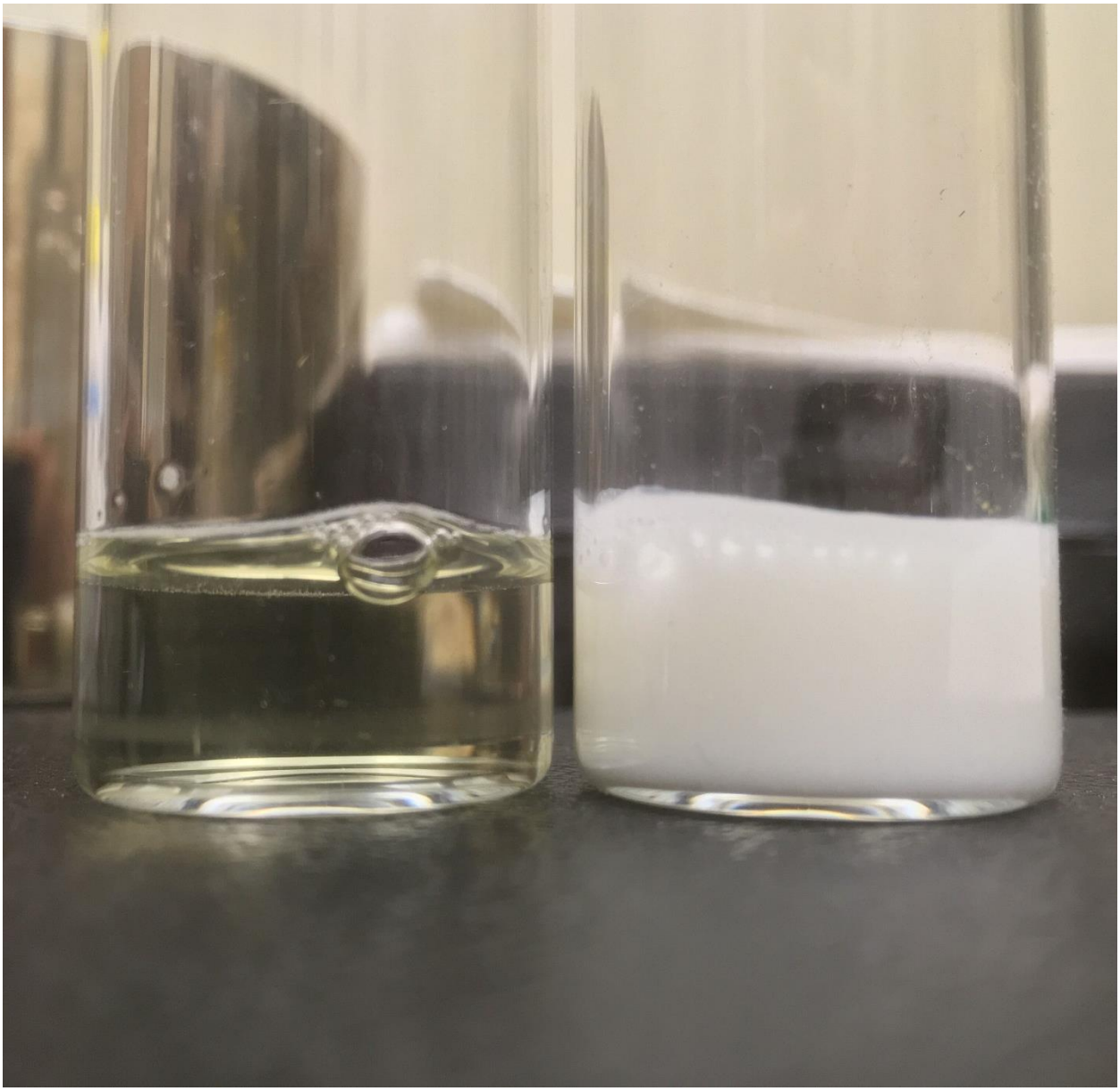

Figure 2.12: 10% albumin insulin solution (Left), 10% insulin albumin suspension (Right)

Some other formulations were prepared based on Table 2.1. Preparation method is the same is insulin albumin suspension. Different concentration of BSA and zinc chloride were chosen as comparison to investigate the influence of different ingredients.

Number #	BSA Concentration (%)	Zinc Chloride (mM)	Insulin concentration (UI/ml)
1	5	25	6.67 UI/ml
2	10	25	6.67 UI/ml
5	5	100	6.67 UI/ml
6	10	100	6.67 UI/ml
7	15	100	6.67 UI/ml
8	20	100	6.67 UI/ml
9	5	500	6.67 UI/ml
10	10	500	6.67 UI/ml
11	15	500	6.67 UI/ml
12	20	500	6.67 UI/ml

Table 2.1: Preparation of different formulations of insulin suspension

The diffusion study helps to compare the insulin release rate for different formulations. This diffusion study can provide a general function of different ingredients. It can also guide the future design of formulation to increase or decrease insulin diffusion rate.

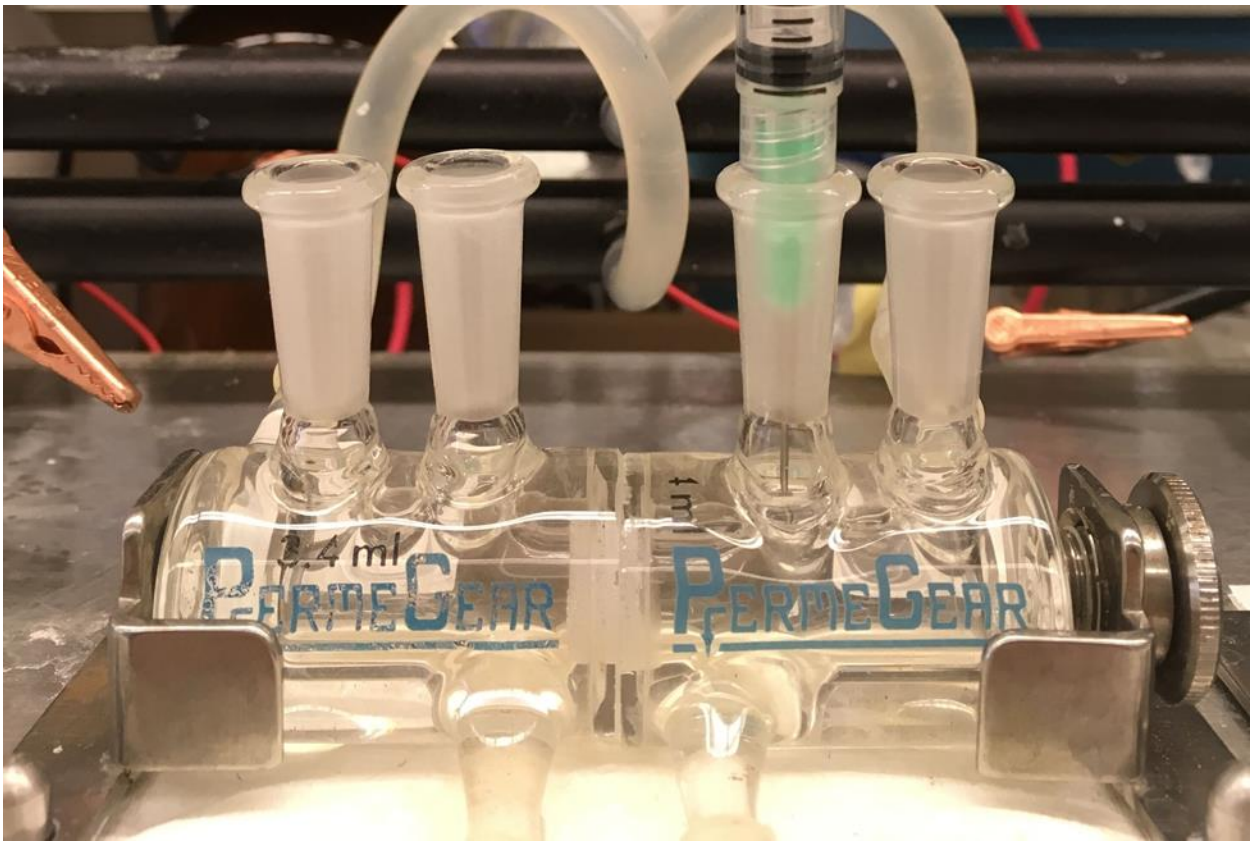

Figure 2.13: Side-bi-Side Diffusion Cells with Solution and Sampling Needle (PermeGear)

In the diffusion study, different dosage forms were added into donor side with volume 3.4 ml. PBS solution (pH 7.4) was added into receiver compartment as same volume. Nylon membrane with 0.45 µm pore size was used between two compartments. The orifice diameter is 9 mm. Total diffusion area is approximately 63.62 mm^2.

The diffusion study lasts 4 hours. 0.5 ml of the receiver compartment buffer was sampled and analyzed through HPLC insulin assay. 0.5 ml fresh PBS was refilled to receiver compartment after each sample. Samples were taken at the time point 1, 2, 3 and 4 hour. The accumulative amount of insulin was calculated. Insulin diffusion release profile was also plotted as accumulative insulin amount (UI) versus time (Hour). The slope is the

average diffusion rate as UI/Hour. Flux rate should be diffusion rate divided by surface area, which is 63.62 mm^2.

2.4.7. Statistical Analysis of Experimental Data

All the experiments were performed in triplicate and the data was reported as mean value ± standard error.

2.5. Results and Discussion

2.5.1. Franz Cell Diffusion Study of CPO using Magnetophoresis

Magnetic field strength of permanent magnets was done by mimic the distance of permanent magnets to the membrane. The magnetic field in this study was 8.42 mTesla (Figure 2.15). However, the top layer of solution in donor chamber may be influenced by 80.3 mTesla strength (Figure 2.16).

Figure 2.14 is the releasing profile of CPO as passive diffusion and under magnetophoresis. As we can see, the releasing of both groups are not zero order. The donor chamber was set to maintain saturated concentration. The solution in receiver chamber would increase CPO concentration with time moves forward. The decrease of concentration gradient was the reason of this "Rectangular Hyperbola" curve. This curve is linear at the beginning, but becomes non-linear when it reaches to its maximum. However, magnetophoresis group has a significant increase in drug diffusion. The general increase in drug diffusion is three folds. There are two explanation of this phenomenon. One is the diamagnetism effect of CPO molecule itself. The other is the diamagnetism of all molecules including water. Diamagnetism has no selection. If it applies to CPO, theoretically it should apply to water. In addition, in 1 ml of saturated CPO solution, there were more water molecule than CPO.

This experiment proved the existence of diamagnetism. In addition, it also provide one method to provide controlled drug release. One proposal we made was to use magnetophoresis to increase transungual drug delivery. If diamagnetism can provide non-specific increase in diffusion, then it could help drug molecules to diffusion through human nail made of keratin protein. Meanwhile, water molecule could also diffuse into human nail to function as a plasticizer. The disadvantage of magnetophoresis is the relatively weak effect. Diamagnetism is a weak force. Huge increase in drug diffusion may require a high power electro-magnets.

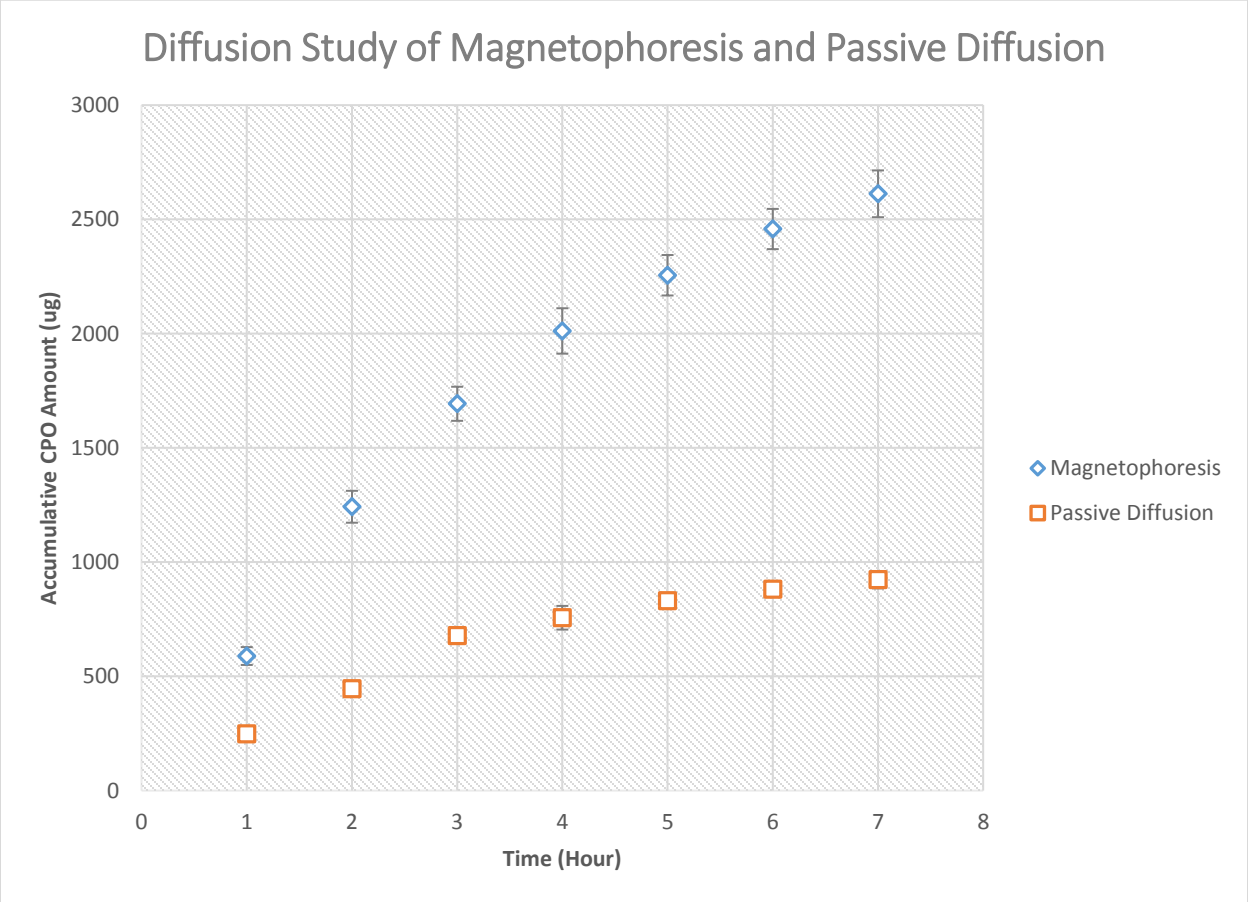

Figure 2.14: Diffusion Profile of Saturated CPO Solution in PBS at pH 7.4 with and without Magnetophoresis

Figure 2.15: Magnetic Field Strength of Permanent Magnet

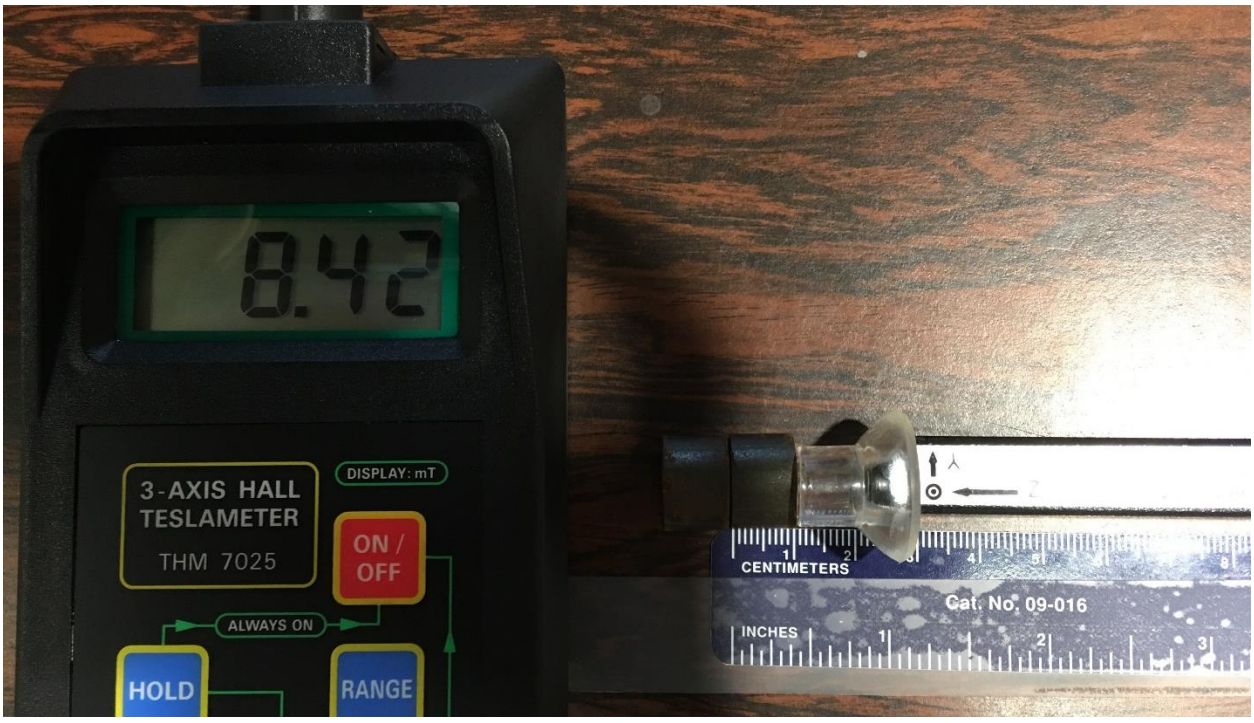

Figure 2.16: Magnetic Field Strength of Permanent Magnet with Distance of a Chamber

2.5.2. Side-bi-Side Diffusion Study of Ibuprofen Using Iontophoresis

As described in the introduction part, iontophoresis includes electromigration and electroosmosis. Ibuprofen has a pKa at 4.91. It is neutral under pH 3. While performing iontophoresis, the cathode starts to electrolyze water. In the anode, zinc metal was oxidized into zinc oxide, while water was reduced to release hydrogen gas. With time moved forward, the pH environment in the donor compartment started to rise and ibuprofen started to carry negative charge. Once ibuprofen became negatively charged, electromigration started to dominate this iontophoresis. Figure 2.17 is the diffusion profile of ibuprofen while performing iontophoresis. The lag time and zero order drug diffusion proved the effectiveness of iontophoresis in drug movement. Figure 2.18 is the cumulative amount of ibuprofen diffused after six hours in this setting.

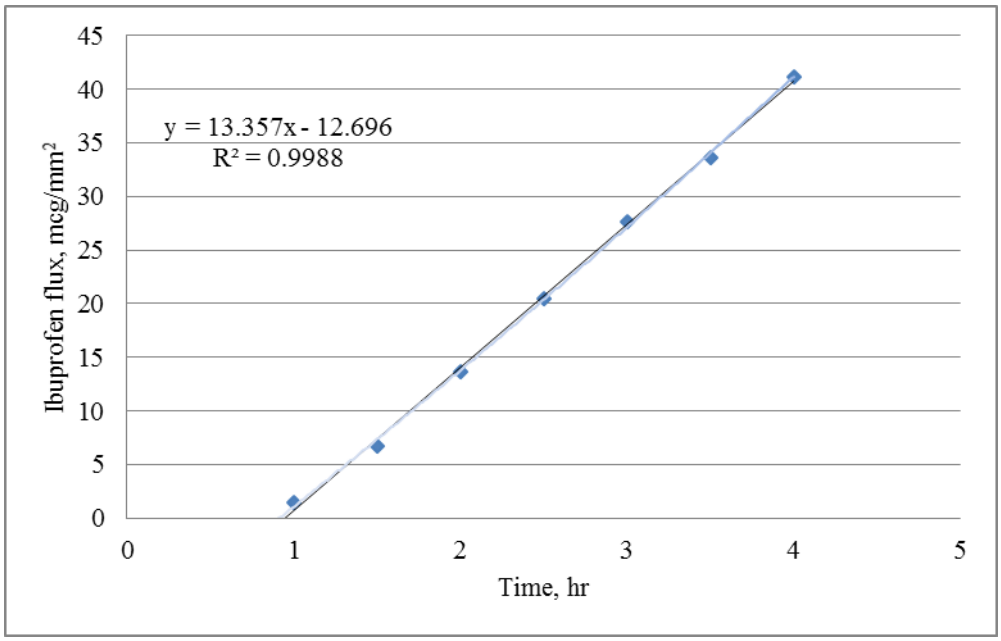

Figure 2.17: Diffusion Profile of ibuprofen when 10 mM pH 3 phosphate citrate buffer solution was used in the donor, and pH 7 phosphate citrate buffer was used in the receiver. Cathode was connected to the donor side[114].

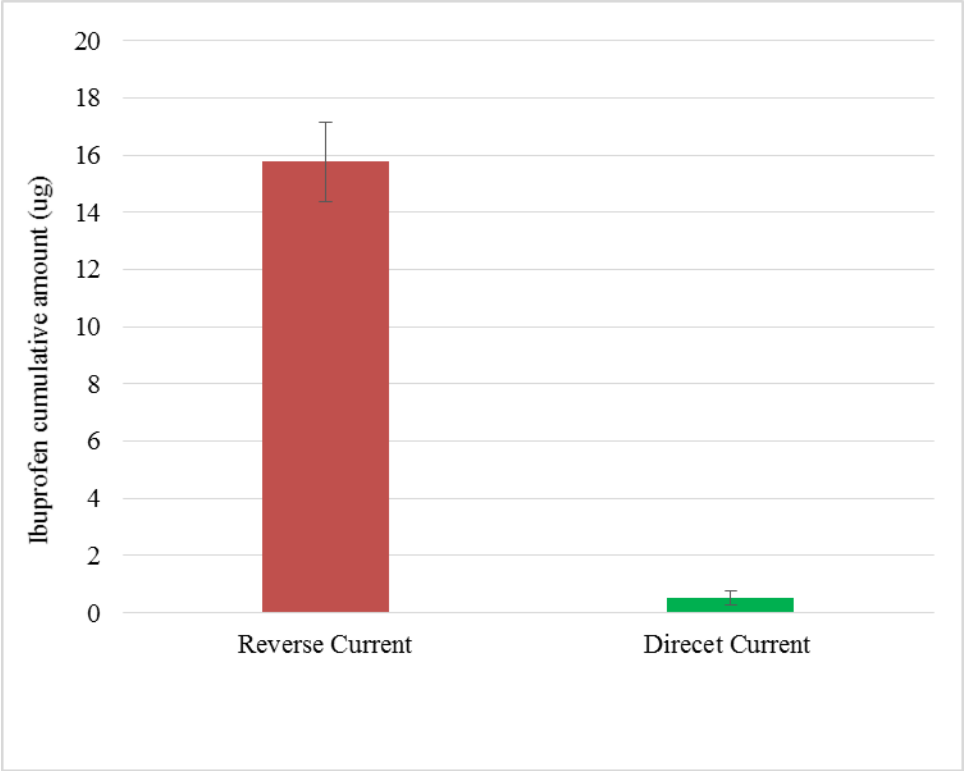

Figure 2.18: Cumulative amount of ibuprofen released when pH 3 phosphate citrate buffer was used in the both compartments donor and receiver. Cathode was connected to the donor side for direct current and anode was connected to the donor side of the reverse current. The duration of the experiment was six hours[114].

2.5.3. Side-bi-Side Diffusion Study of Bovine Serum Albumin Using Iontophoresis

In previous studies, we built a general idea about how iontophoresis and magnetophoresis influence molecules diffusion through a semi-permeable membrane. In this section, we picked albumin as a target for two reasons. Albumin has a molecular weight that is 66 KDa. Its diffusion profile is a very important guidance for future formulation on peptides and proteins. In addition, most small molecule drugs, even some peptides, bind albumin. This binding effect could be used to delivery other molecules through controlled release of albumin matrix.

In Figure 2.19, we could see that reverse current did not provide a significant increase in albumin diffusion while direct current did. The relatively large structure of albumin might

result in this difference. Under physiological pH, albumin is negatively charged. Theoretically, albumin should have an increase in diffusion with reverse current. However, in protein, different domains carry different charges. Even though overall albumin carries negative charge, it may still have residues that carries positive charge. The increase in direct current might come from the positive charge on albumin's surface while most negative charge was encapsulated in an inner structure.

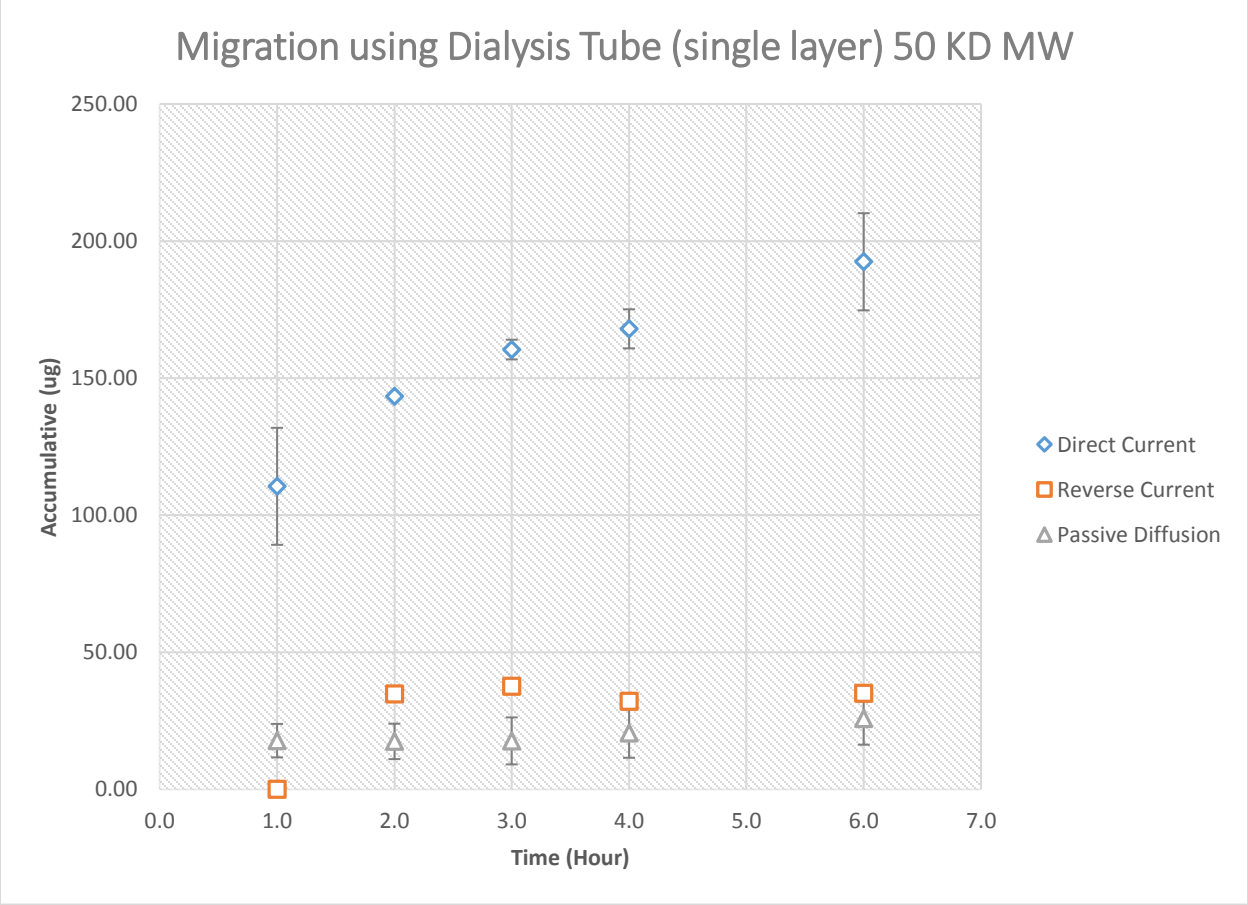

Figure 2.19: Diffusion Profile of 5% Albumin in PBS at pH 7.4 with/without Iontophoresis

2.5.4. Side-bi-Side Diffusion Study of Insulin (Humulin) Using Iontophoresis

2.5.4.1. General function of albumin and zinc chloride

Based on sample assay, a plot is drawn to show the relationship between accumulative insulin amount (mUI) and time (Hour) (Figure 2.20). Diluted insulin solution, as marked blue in the figure, has the highest diffusion rate. Insulin albumin solution is relatively slower with a roughly 43% diffusion rate. Insulin albumin suspension shows the slowest diffusion profile with a 23% diffusion rate comparted with insulin in water. Flux rate needs to be calculated based on diffusion area. In our experiment using side-bi-side design, the diameter of orifice for all cells are 9 mm. So the percentage relationship among different formulation does not change. Figure 2.21 is the relationship of diffusion rate of 3 formulations. Flux rate of insulin in water, insulin in 10% BSA and BSA-Insulin-Zinc suspension is 20.57±0.07, 8.59±0.33, 4.64±0.31 mUI/mm^2/hour correspondingly.

The data shows us the influence of albumin and zinc chloride in controlling insulin diffusion. The huge difference between insulin in water and insulin in 10% albumin solution proves the idea that insulin may bind to albumin reversibly. Both albumin and insulin are negatively charged under biological pH, while albumin is a large protein so it may carry positive charge in one regimen. Insulin may bind to positive regimen only. In addition, zinc chloride precipitate the mixture of albumin and insulin. As reported by other literature, insulin also forms hexamer with zinc ions[41]. Hence, the idea to continue this project is to use albumin as a vehicle to bind insulin, and use zinc chloride as an addictive to control the degree of precipitation. Free insulin concentration is decreased in this suspension to provide a slower diffusion.

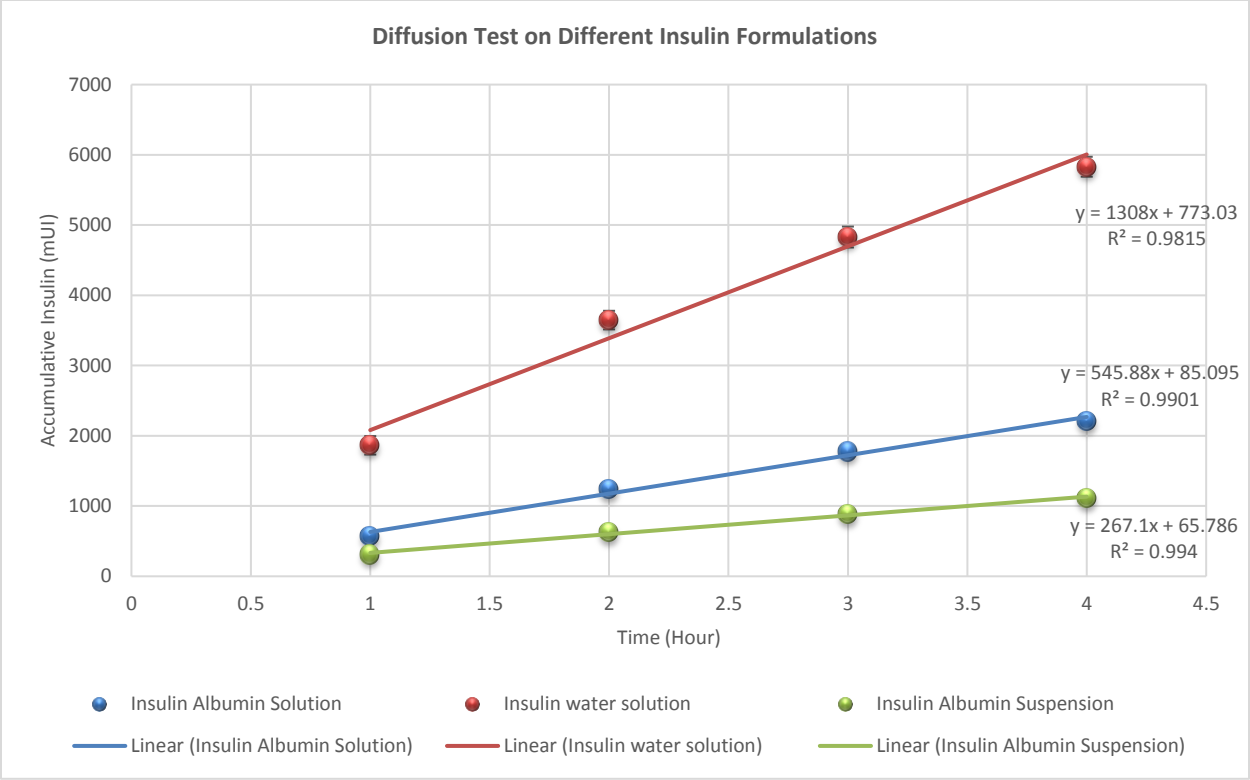

Figure 2.20: Diffusion Profile of Insulin, Insulin-Albumin Solution and Suspension

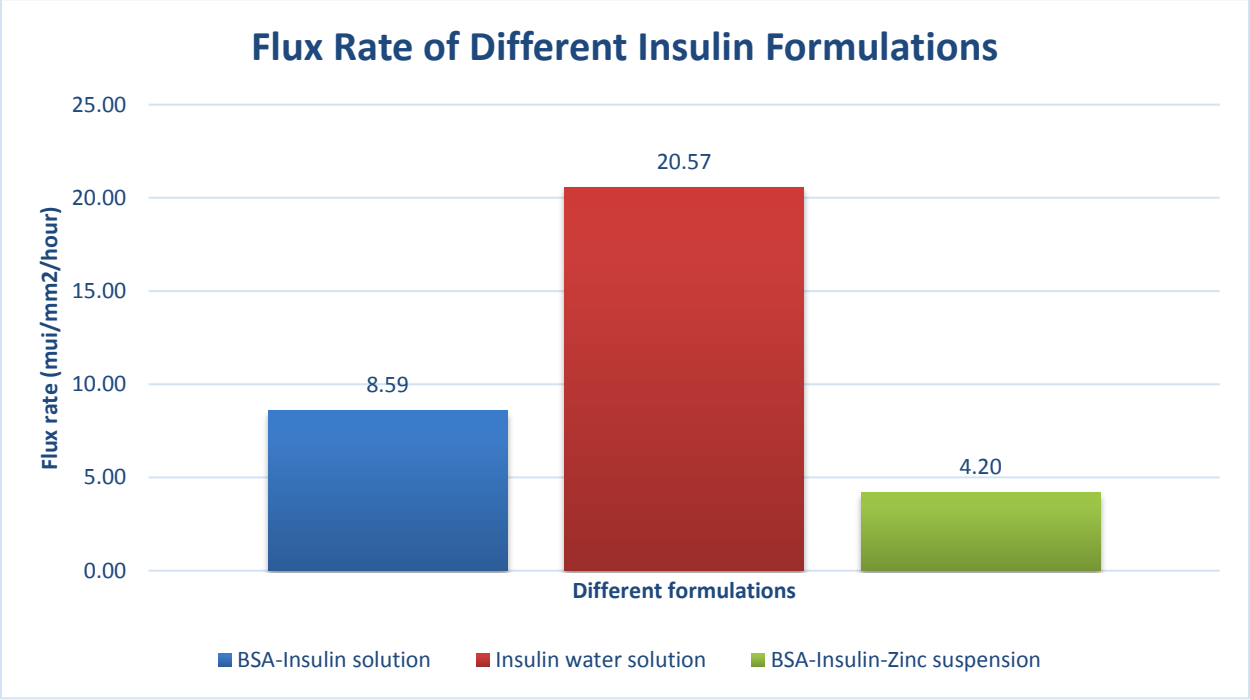

Figure 2.21: Flux Rate of Insulin Solution, Insulin-Albumin Solution and Suspension

2.5.4.2. Optimization of Ingredients to Control Insulin Diffusion Rate

In order to further investigate the influence of albumin and zinc chloride on insulin diffusion rate, 12 formulations were screened according to table 2.1. Figure 2.22 is the comparison of formulation 5, 6, 7 and 8. With the same concentration of zinc chloride (0.1 M), different amount of albumin (5%, 10%, 15% and 20%) was used to form suspension. Data shows diffusion rate decreases with increasing amount of albumin. Similarly, the comparison of formulation 9, 10 and 11 was also performed as Figure 2.10. The zinc chloride was controlled as 0.5 M, albumin was prepared as 5%, 10% and 15%. Similar trend was observed as Figure 2.23.

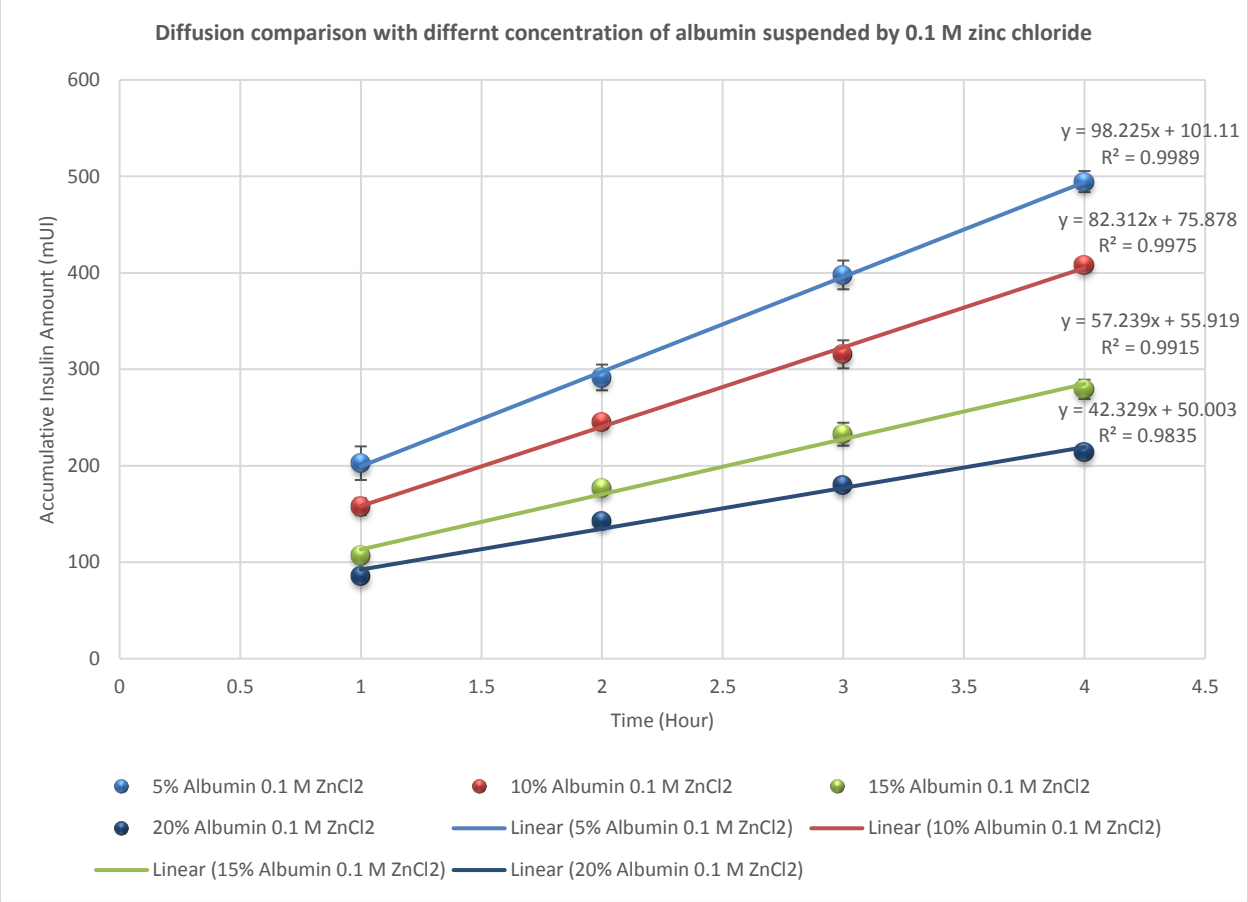

Figure 2.22: Comparison of formulations prepared with different amount of albumin and 0.1 M $ZnCl_2$

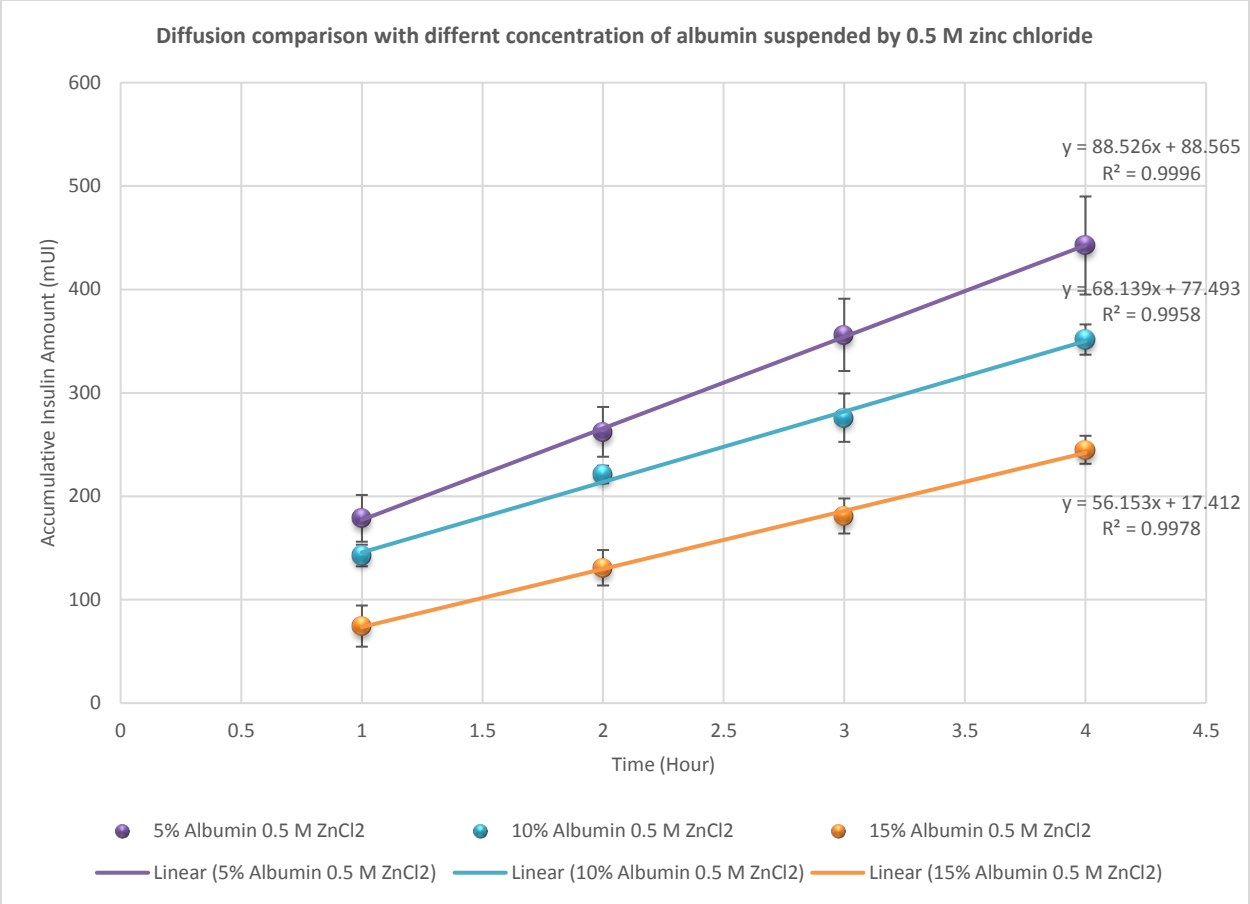

Figure 2.23: Comparison of formulations prepared with different amount of albumin and 0.5 M $ZnCl_2$

Another series of comparisons were also finished: 5 versus 9 (Figure 2.24), 6 versus 10 (Figure 2.25), and 7 versus 11 (Figure 2.26). This comparison was designed to investigate the influence of zinc chloride with the same amount of albumin. In each figure, 0.5 M zinc chloride group with the same amount of albumin, always have slower diffusion rate than 0.1 M group. This trend is the same for all three groups, despite the narrow difference in 15% albumin group. In addition, zinc chloride can form hexamer with insulin molecules, which reduces insulin's diffusion rate significantly.

Based on the comparison of different formulations, we can conclude that both albumin and zinc chloride can decrease free insulin concentration and insulin diffusion rate. Data shows

albumin has stronger influence on diffusion, compared with zinc chloride. This conclusion will guide the future formulation with higher insulin loading. Albumin will be adjusted as primary factor and zinc chloride will be the secondary one.

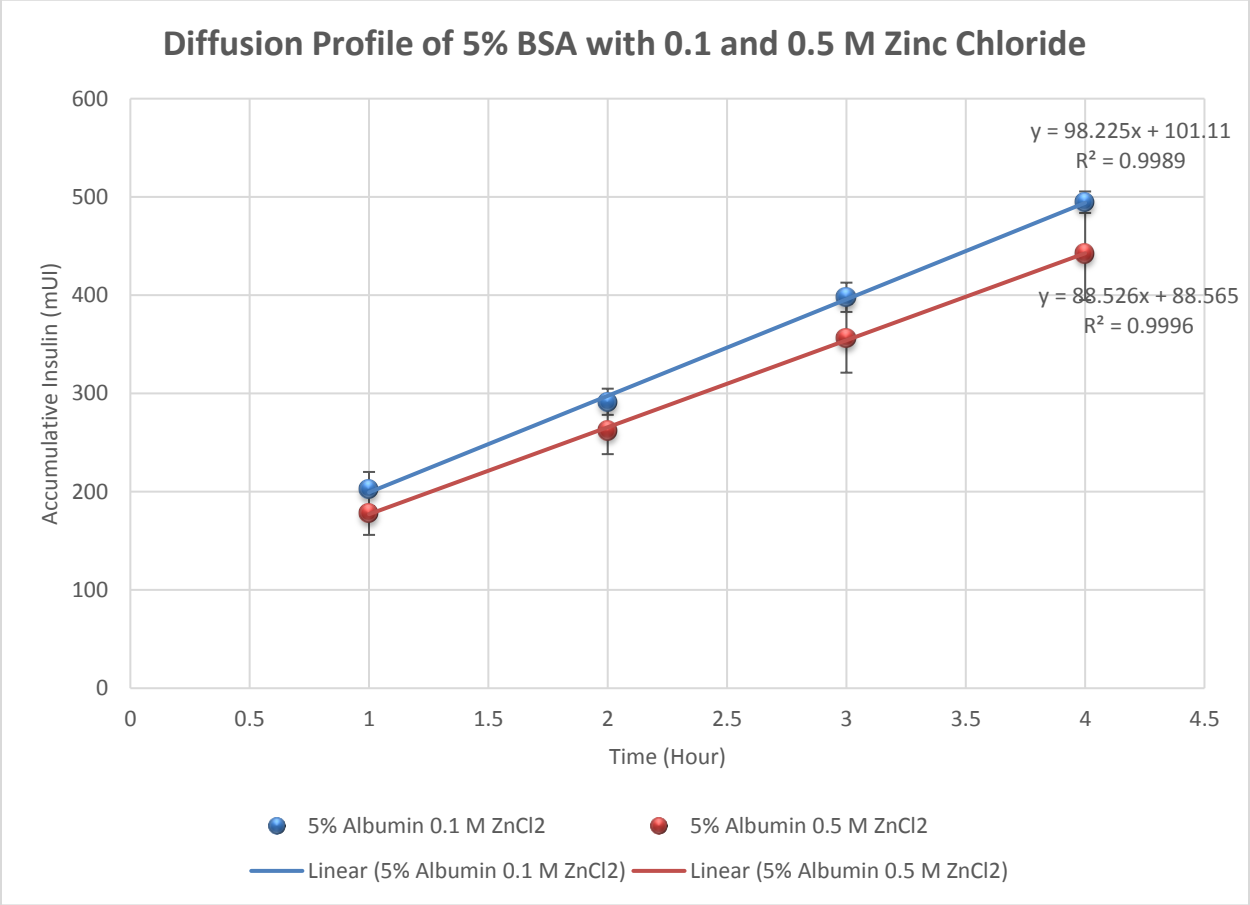

Figure 2.24: Comparison of formulations with different amount of $ZnCl_2$ with 5% albumin

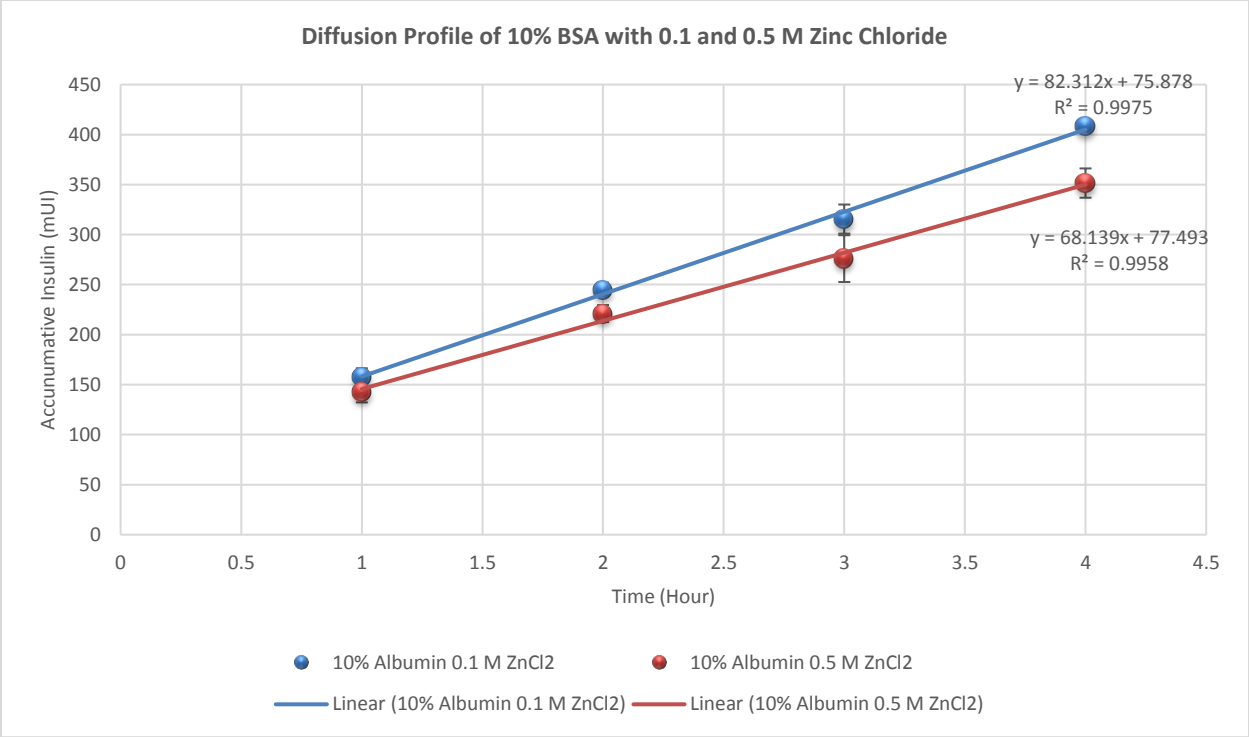

Figure 2.25: Comparison of formulations with different amount of $ZnCl_2$ with 10% albumin

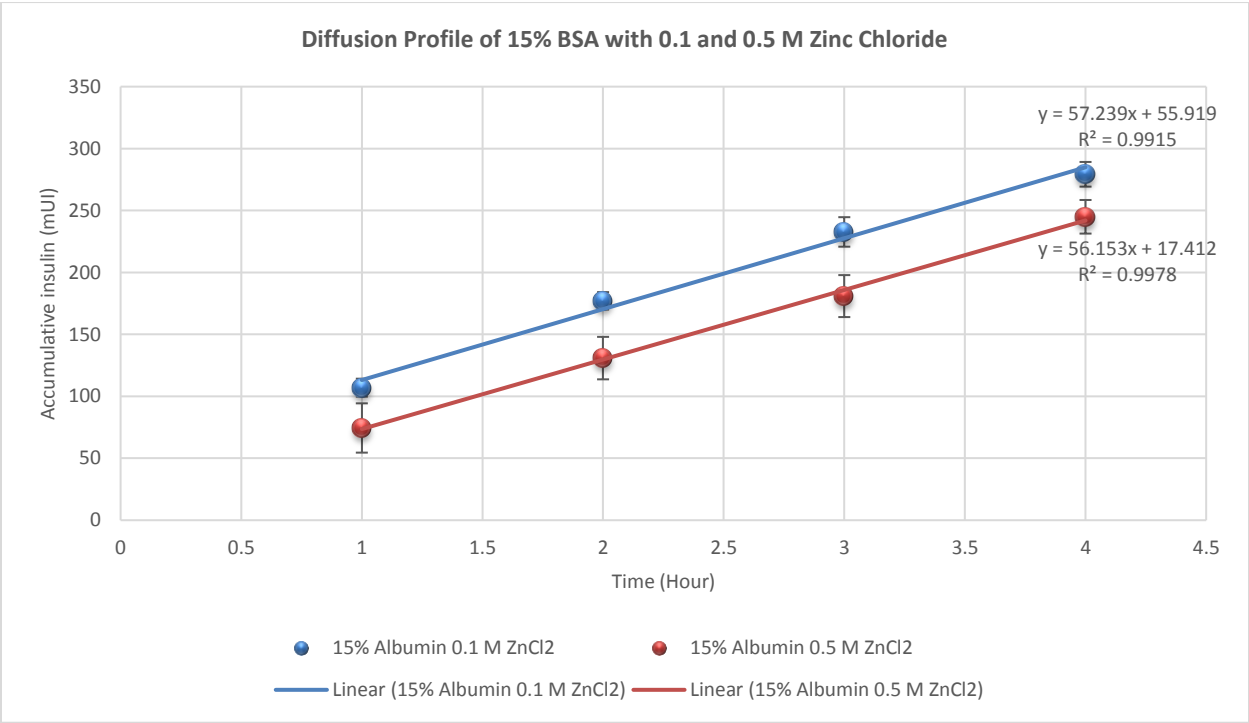

Figure 2.26: Comparison of formulations with different amount of $ZnCl_2$ with 15% albumin

2.6. Conclusion of this chapter

This chapter is a combination of preliminary studies of four different projects. The goal for this chapter is to understand thoroughly how diffusion works in drug delivery system. In addition, we need to understand how matrix modification would influence drug diffusion. Drug diffusion is critical in most cases. Understand the controlling factors of diffusion and physical methods which might influence diffusion rate could be helpful in the progress of thesis project.

In this chapter, two physical methods were used: magnetophoresis and iontophoresis. Theoretically, magnetophoresis has less potential side effects based on its weak force classification. As a result of that, the improvement of drug delivery might not be that significant. On contrary, iontophoresis could provide a much higher drug diffusion with appropriate setting. But human has a tolerance limit of electricity. In addition, the electrolysis reaction might change system's pH environment and cause other potential risk. The goal of this thesis is to discover a potential parenteral prototype for subcutaneous delivery of human insulin. Based on experimental data. The concentration gradient of insulin between two sides of a membrane is the only driving force. According to Fick's law, the flux rate is proportional to free insulin concentration in donor compartment, as shown in Equation 2.2 where J is flux rate, D is diffusion coefficient, L is the thickness of membrane, C_d is concentration in donor compartment, and C_r is concentration in receiver compartment. As a result, in order to control the diffusion rate, free insulin concentration of insulin must be controlled. In this design, insulin albumin suspension is not prepared as a static form. Contrarily, it is a dynamic form with continuous exchange between precipitation form and the solution form of albumin, insulin and zinc ions. The advantage

of this design is, once the free insulin concentration in solution decreases in the donor compartment, the insulin, albumin and zinc chloride complex will dissociate to release more free insulin. This precipitation can function as an insulin reservoir. As shown in Equation 2.3, the decrease of insulin in the right side will contribute to the right shift of the equivalence.

$$J = \frac{D(Cd - Cr)}{L}$$

Equation 2.2: Fick's Second Law after Integration (Cr=0 if sink condition)

$$\text{BSA} - \text{Insulin} - \text{Zinc} \downarrow \leftrightarrow \text{BSA} + \text{Insulin} + \text{Zinc Ions}$$

Equation 2.3: dynamic association and dissociation of BSA-Insulin-Zinc precipitation

Those 12 formulations that we prepared and tested are some representatives of feasible formulations. Initial assumption was to use zinc chloride precipitate insulin albumin solution and form hexamer with insulin molecules. So zinc chloride should be critical to control the diffusion rate. Contrarily, data shows that with the same amount of albumin, extra zinc has very limited influence of insulin diffusion rate. The increase of albumin concentration actually dominates the control of insulin release.

This chapter covers all the preformulation research. All the experiments were finished with very low insulin concentration and amount in order to decrease expenses. Meanwhile, this chapter does not propose a real shape and fabrication process of our implant. In the next chapter, we will focus on the fabrication of implant with a feasible 3-D dimension. In addition, processing methods will be discussed in detail starting next chapter.

CHAPTER 3

FABRICATION PROCESS OF SUBCUTANEOUS IMPLANT

3.1. Introduction of Current Subcutaneous Implant

Long-acting therapy has an advantage to bypass frequent drug administration, including oral dosage forms, injection, infusion and inhalation. Among all long-acting therapies, subcutaneous implants can provide the longest duration of drug delivery and stable plasma drug concentration.

3.1.1. Nexplanon® and Zoladex®

Nexplanon® is a contraceptive implant. The API in this implant is etonogestrel. Nexplanon® implant can last up to three years to provide therapeutically effective etonogestrel for female. Nexplanon® implant is a flexible polymeric rod. Its size is similar to a matchstick: 4 cm in length and 2 mm in diameter. Nexplanon's drug release profile is shown in Figure 3.1. In Figure 3.1, we could see that Nexplanon® would provide a burst release for thirst week. I believe this burst comes from the water flow into the dosage form and initial dissolution of matrix. After the first week, Nexplanon's drug release rate would start to decrease slowly. However, the release of etonogestrel could still maintain minimum therapeutic concentration (MTC) in plasma. Zoladex® is a chemotherapy implant. The API of Zoladex® is goserelin. Zoladex® implant has two strength. The 3.6 mg implant is designed to last for 28 days while the 10.8 mg one could function for 12 weeks. Zoladex® is a 1.5 mm diameter cylinder with injection needle in the package. The matrix of Zoladex® is D,L-lactic and glycolic acids copolymer. AstraZeneca filed a patent for Zoladex® based on its sustained release formulation of API. Figure 3.2 is the release profile of Zoladex®

with different loading. Zoladex® can provide zero order drug release with different loading. This patent technology is a degradable matrix made of polymer. Similar technology has been reported by other researchers for oral dosage forms[87].

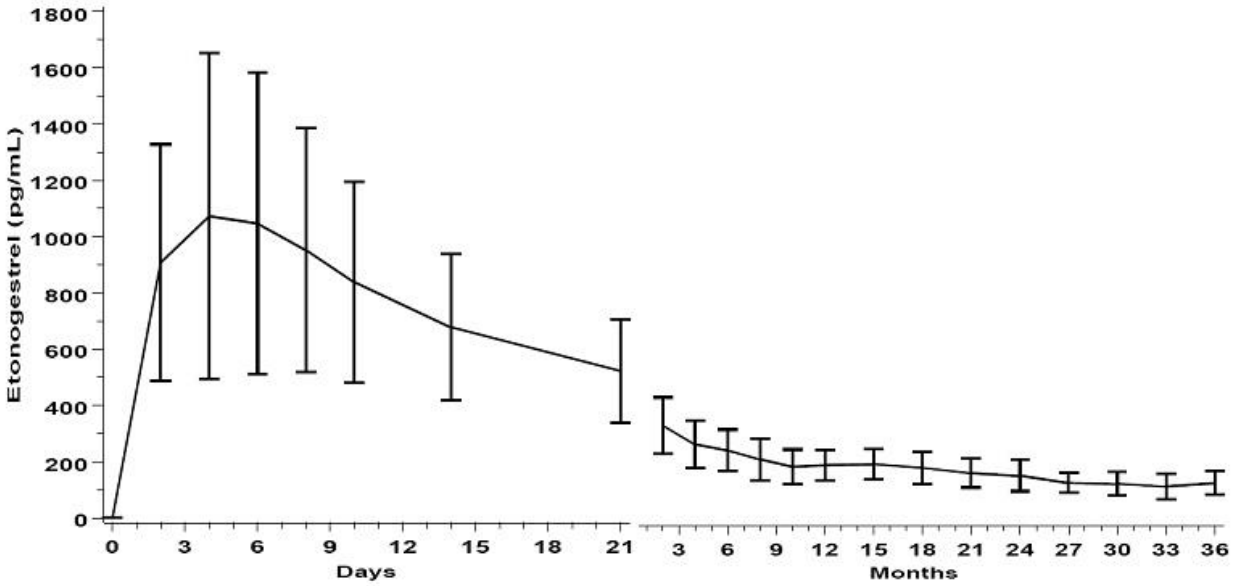

Figure 3.1: Mean (± SD) Serum Concentration-Time Profile of Etonogestrel After Insertion of Nexplanon During 3 Years of Use (FDA Public Data)

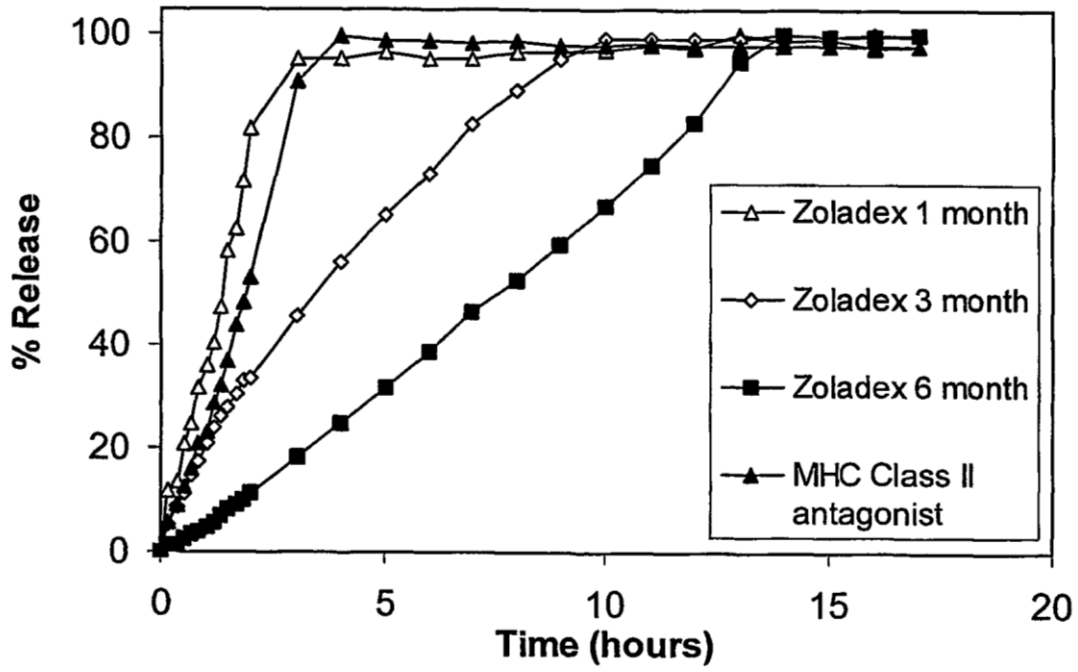

Figure 3.2: Releasing Profile of Goserelin from Zoladex® 10.8 mg (US Patent 7247491)

3.1.2. Dissolvable and Retractable Subcutaneous Implant

The first usage of biodegradable materials happened in 1984 for a treatment of bone fracture. After that, biodegradable materials started to improve health care in many aspects. Subcutaneous implant is one of them. Most biodegradable polymers degrade through either hydrolysis reaction or enzymatic degradation. Most materials used are short-chain hydroxyl acids, sugars, amino acids and organic monomers. The philosophy is to take advantage of human body to degrade polymer back to monomer slowly. Commonly used biodegradable systems are:

i. Polyglycolic Acid (PGA)

ii. Poly-L-Lactic Acid (PLLA)

iii. Poly-DL-Lactic Acid (PDLLA)

iv. PGA/Trimethylene carbonate compolymers (PGA/TMC)

v. Poly-P-Dioxanone (PDS)

vi. Poly-Beta-Hydroxybutyric Acid (PBHBA)

Biodegradable implants have many advantages. The main advantage is the removal process. There will be no removal related pain, bleeding or infections. In addition, polymers usually do not interfere with radiological imaging. However, polymer matrix may require chemical reaction or synthesis[90,115,116]. The production process could be more expensive. While metal or inert materials are easy to produce, store and transport. Moreover, degradable polymers may not be strong enough. Broken rod was reported for Nexplanon® and a retrieve surgery is the only method to solve the issue. In addition, polymer implants have a higher possibility to trigger local inflammation reaction than inert materials.

Retractable implant needs to be removed from human tissue after usage. Vantas® is an implant manufactured by Endo Pharmaceuticals as a dosage form of histrelin to treat cancer. It is a diffusion-controlled hydrogel polymer reservoir. Vantas® is a 3.5 cm by 3 mm, cylindrical-like small thin flexible tube. Because of this design, Vantas® needs to be removed from human body after its 52 week treatment duration. Figure 3.3 is the releasing profile of Vantas® implant. The general drug release still has a burst effect after immediate surgery. In 52 week duration, Vantas® tends to decrease its drug release rate with time moves forward. One possible explanation is the hydrogel matrix. Hydrogel matrix is a swelling matrix[117]. After insert, the matrix absorb human body fluid and start to swell. The burst release may come from this initial swelling. Drug molecules diffuse out of the matrix to enter human circulation. With time moves forward, drug molecules in the inner matrix need to diffuse through a longer path in order to reach the matrix surface. As a result of that, the drug release tends to decrease with time.

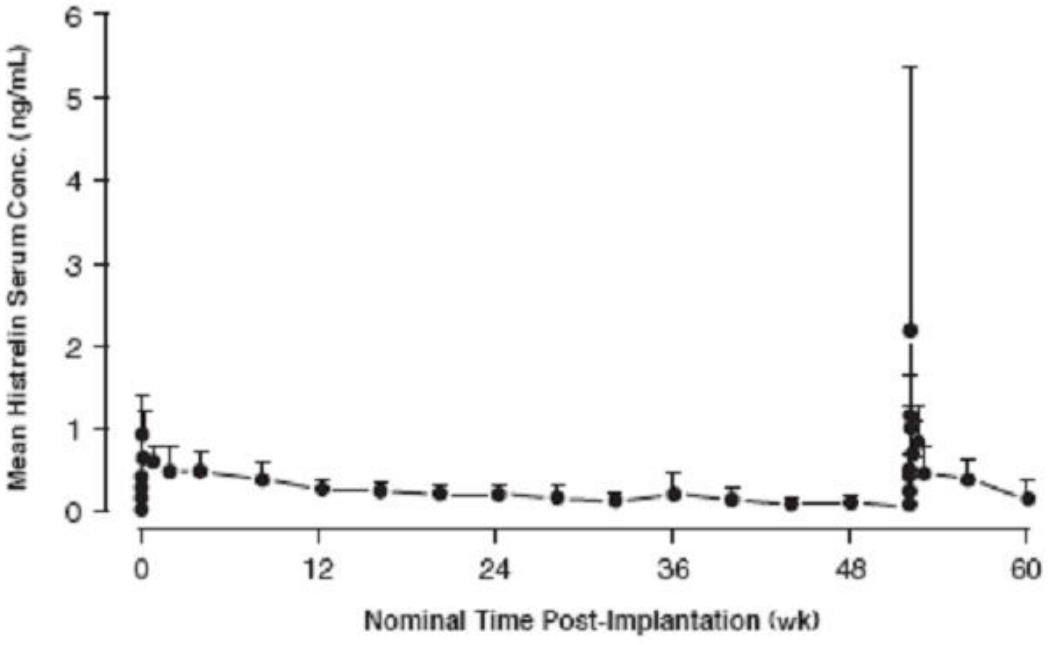

Figure 3.3: Releasing Profile of Histrelin from Vantas® (Vantas FDA File)

3.2. Excipient Selection in Compliance of FDA

FDA provides a database for the safety consideration of all excipients. In this database, the maximum potency allowed for each excipient is clearly described.

A subcutaneous implant is in direct contact with human body fluid. As a result, several routes might provide equivalent guidance in excipient selection. Subcutaneous (SC), Intramuscular (IM), Intradermal (ID), Intravenous (IV) are several routes that might be helpful in finding useful information.

Considering the function of potential excipient, a high human tolerance is preferred. Figure 3.4 is a list of selected products of carboxymethylcellulosse. According to the database, carboxymethylcellulose can be used in injection dosage form. Similarly, it may also be used as a subcutaneous implant. Unfortunately, in the column of maximum potency, most dosage form only allows less than 1%. 1% excipient in a matrix is not very ideal. Figure 3.5 is the list of usage of gelatin. Different from carboxymethycellulose, gelatin has a 16% maximum potency as SC injection, which is very promising for an implantable dosage form. Figure 3.6 is the same list for silicone. Silicone is widely used as inert material in many plastic and orthopedic surgeries. Silicone's maximum potency is up to 60 mg, which is way enough for an implant. After research in this database, we conclude that most chemically synthetic polymers have a relatively low potency allowed, while natural substances, including human albumin, sucrose, lactose, gelatin, have much higher tolerance. This result provides a hint that we might need some other technology besides matrix control in our drug delivery system.

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency	Record Updated
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRA-ARTICULAR	INJECTION, SUSTAINED ACTION	9004324	K679OBS311	NA	
CARBOXYMETHYLCELLULOSE	INTRABURSAL	INJECTION	9000117	05JZI7B19X	NA	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRABURSAL	INJECTION	9004324	K679OBS311	0.76%	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRADERMAL	INJECTION	9004324	K679OBS311	NA	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRADERMAL	INJECTION, SUSTAINED ACTION	9004324	K679OBS311	NA	
CARBOXYMETHYLCELLULOSE	INTRALESIONAL	INJECTABLE	9000117	05JZI7B19X	0.75%	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRALESIONAL	INJECTION	9004324	K679OBS311	NA	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRALESIONAL	INJECTION, SUSTAINED ACTION	9004324	K679OBS311	NA	
CARBOXYMETHYLCELLULOSE	INTRAMUSCULAR	INJECTION	9000117	05JZI7B19X	0.9%	
CARBOXYMETHYLCELLULOSE SODIUM, UNSPECIFIED FORM	INTRAMUSCULAR	INJECTABLE	9004324	K679OBS311	0.75%W/V	

Figure 3.4: List of Pharmaceutical Products Using Cellulose as Excipient (FDA Database)

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency	Record Updated
GELATIN, UNSPECIFIED	BUCCAL	GUM, CHEWING	9000708	2G86QN327L	4.27MG	
GELATIN, CROSSLINKED	DENTAL	TABLET		Pending	3.44MG	
GELATIN, UNSPECIFIED	DENTAL	PASTE	9000708	2G86QN327L	180MG	
GELATIN, UNSPECIFIED	IM - IV - SC	INJECTION	9000708	2G86QN327L	16%	
GELATIN, UNSPECIFIED	IM - IV - SC	POWDER, FOR INJECTION SOLUTION	9000708	2G86QN327L	1.4%	
GELATIN, UNSPECIFIED	IM - SC	INJECTION	9000708	2G86QN327L	16%	
GELATIN, UNSPECIFIED	IM - SC	INJECTION, SUSTAINED ACTION	9000708	2G86QN327L	16%	
GELATIN, UNSPECIFIED	INTRA-ARTICULAR	SUSPENSION	9000708	2G86QN327L	0.03%	
GELATIN, UNSPECIFIED	INTRAMUSCULAR	INJECTION	9000708	2G86QN327L	0.26%	
GELATIN, UNSPECIFIED	INTRAMUSCULAR	INJECTION, MICROSPHERES	9000708	2G86QN327L	0.13%	

Figure 3.5: List of Pharmaceutical Products Using Gelatin as Excipient (FDA Database)

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency
SILICONE	IM - IV	INJECTION	63394025	NA	NA
SILASTIC MEDICAL ADHESIVE,SILICONE TYPE A	IMPLANTATION	PELLET, IMPLANT		Pending	13MG
SILICONE	INTRAUTERINE	INTRAUTERINE DEVICE	63394025	NA	50.44MG
SILICONE	INTRAUTERINE	SUPPOSITORY, INSERT, CONTROLLED RELEASE	63394025	NA	60MG
SILICONE	ORAL	CAPSULE	63394025	NA	15MG

Figure 3.6: List of Pharmaceutical Products Using Silicone as Excipient (FDA Database)

3.3. Implant Fabrication Processing Development

3.3.1. Film Casting and Membrane Control Establishment

The easiest way to implant a device into human subcutaneous is to make a thin but sharp film. In that case, a film casting process is needed. An excipient only film casting process was tried using water-soluble polymers, such as polyethylene oxide, hydroxypropyl methylcellulose, methylcellulose, carboxymethylcellulose, polyvinyl pyrrolidone, gelatin, pectin, and pullulan. In addition, several plasticizers, such as glycerin, propylene glycol, and polyethylene glycol, can be added to the formulation to alter the mechanical properties of the final film. Substances with low tolerance from FDA website were eliminated from the list.

Solvent casting method was used based on its gentle operation environment and less aggressiveness. However, due to the water solubility of polymers, films disintegrated immediately after disintegration test started. Owaisat et al mentioned provided one method to crosslink polymers to achieve erosion model of matrix. Her method could provide a more rigid matrix that lasts longer in disintegration test[87]. However, film still completely dissolved within one hour.

Osmotic pump tablet invented by Alza Corporation use semipermeable membrane to inflow water from human gastrointestinal system and pump out drug molecules through an orifice. Osmotic pump tablet provides us a hint that using semipermeable membrane can preserve drug with matrix.

Spectrum®Labs provide dialysis tubing. Figure 3.6 is a picture of dialysis tubing patented under name Float-A-Lyzer®. The diffusion rate of drugs are related to the cut-off molecular weight and surface area of the tubing. In order to provide a tightly controlled

release implant, we used the idea of this dialysis tubing but in a different way. Float-A-Lyzer® tubing is not permeable through bottom but permeable through cylinder surface. We modified that idea to make it non-permeable through side surface but permeable through bottom. In this case, the surface area is tightly controlled and the erosion of matrix is direction oriented, which is from the membrane side (bottom) to the top side (vertically). With this idea, we acquired the Felcon® cell culture insert from Fisher Scientific. Figure 3.7 is Falcon cell culture insert. Till this step, the dosage form successfully stepped into membrane controlled delivery system. Falcon® cell culture insert is a cylinder-like shape plastic tubing. The membrane on the bottom is made of polyethylene terephthalate (PET).

Figure 3.6: The Spectra/Por® Float-A-Lyzer® G2 Dialysis Tubing (Product Profile)

Figure 3.7: Falcon® Cell Culture Insert (ThermoFisher™)

3.3.2. Matrix Manufacturing Approach

Initially, we added Humulin R U-100 solution into our cell culture insert for diffusion study. The diffusion rate was absolutely fast. Based on our result in chapter 2, we start to add Insulin-Albumin solution and/or Insulin-Albumin-Zinc suspension. We received controlled diffusion rate of our dosage form. However, as a subcutaneous implant, it is almost impossible to support 5 ml solution or suspension with a reasonable size. We decided to remove water from our dosage form. Initially, we use low pressure evaporation technology with high temperature. However, considering the brittleness of insulin, we decided to use freeze-dry technology.

Even though with membrane control, the matrix need to be uniform in dissolution. Irregular packing will result in tighten and/or loosen matrix. In order to provide a uniform matrix, we decided to use tablet press as our main tool.

Our matrix needs to be a uniform mixture of all ingredient with no solid concentration difference. Before freeze dry process, a complete dissolution is necessary. After freeze dry, different ingredient should be mixed with same proportion. This method is useful for high water soluble drugs. However, for slightly soluble drugs, we recommend blending as matrix processing method. Traditional blending requires minimum 250 grams of blender. In our experiments, we acquired small batch blending method from Emerson Resources, Inc (Norristown, PA, 19401). All ingredients are pre-weighted and stored in one polymeric bag. The bag is inflated with air and sealed. A Fisher Brand Vortex is used to provide shaking. The polymeric bag is immobilized on the blender. Figure 3.8 is the Figure for blending process.

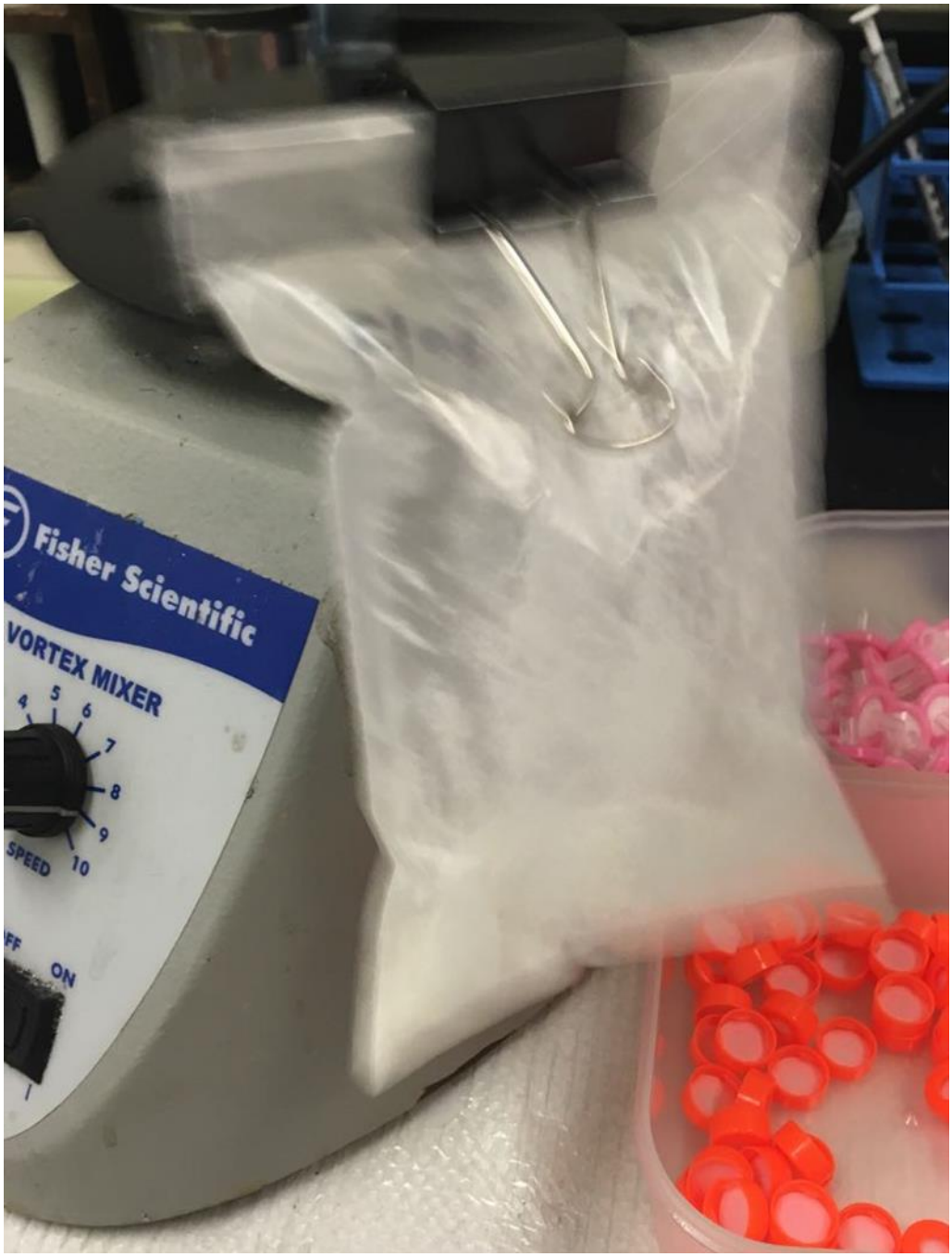

Figure 3.8: Small Batch Blending Using Polymeric Bag and Fisher Vortex

3.4. Materials

BSA, fraction V, 97% pH 7, Lyophilized powder is purchased from Alfa Aesar. Zinc Chloride solution (20%) is purchased from Aqua Solutions, Inc. Humulin® R U-100 is provided by Eli Lilly and Company. Sucrose (Ultra Centrifugation Grade) is provided by

Fisher Biotech. Deionized water is purified from tap water through Thermal Fisher DI Water system. Corning® cell culture insert (24 well, 8 and 0.4 µm pore size) is ordered from Fisher Scientific. Vankel® 7010 dissolution bath with auto sampler is provided my Agilent Technologies.

3.5. Experimental Methods

3.5.1. High Performance Liquid Chromatography

Insulin in all samples was assayed using an Agilent 1100-series HPLC with dual wavelength detector. Acetonitrile : 50 mM pH 2 Sodium Phosphate (20:80 v/v) was used as the mobile phase. Column was purchased from Agilent as a 25 mm by 4.6 mm Chromolith® Phenyl column. The flow rate was 1.5 mL/min and the injection volume was 50 µL. The wavelength of detection for insulin was 214 nm.

3.5.2. Preparation of insulin freeze dry powder

Humulin R U-100® solution was used as source of insulin. Sucrose was added as filler for the ease of tablet pressing. After forming a clear and transform solution, albumin was added and dissolved. A magnet stir bar was used for mixing process. After complete dissolution of albumin, a yellowish solution was formed. Zinc chloride was added and a cloudy suspension formed after mixing.

The whole suspension was prepared in stainless steel beaker. Suspension was stored in -80 °C freezer for 1 hour before freeze dry. Labconco® FreeZone 1 dreeze dryer (Figure 3.9) was used to prepare powder. The temperature was controlled as -50 °C. Freeze dry process took 24 hours. After the process, we should achieve white and dry insulin formulation for tablet press.

Figure 3.9: Labconco FreeZone 1 Freeze Dryer

3.5.3. Preparation of insulin tablets

600 mg of freeze powder prepared from section 3.5.2 was used to press one tablet. In the process of tablet press, Carver® Lab Press model C was used (Figure 3.10). Natoli® tablet tooling set was used for punch and die. During tablet press, the pressure was controlled as

zero, which means there is no additional application of force to lower the thickness of tablets. Tablet size and shape are shown in Figure 3.12.

Figure 3.10: Carver® Lab Press Model C

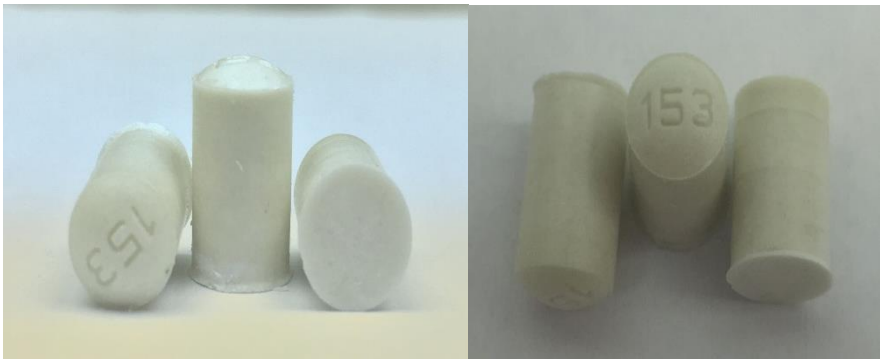

Figure 3.11: Insulin tablets (600 mg)

3.5.4. Preparation of insulin implants

Corning® cell culture insert was used as the form of implant. Insulin tablet from section 3.5.3 was put in the insert. The top of insert was covered and sealed by Parafilm®. Substance should only be available at the membrane end of the insert (Figure 3.12).

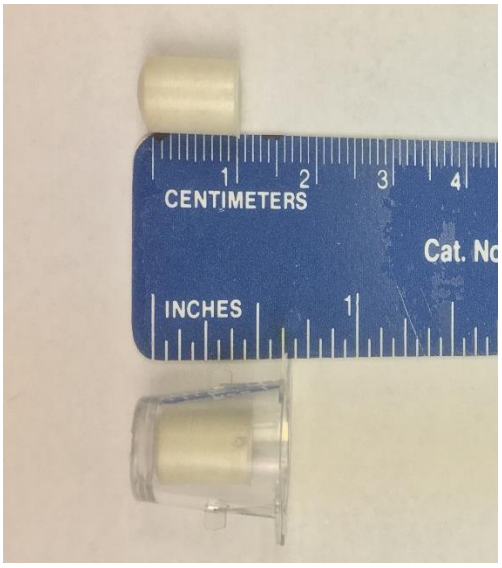

Figure 3.12: Implant fabrication: “Tablet-in-Insert” system

3.5.5. Insulin Releasing Study with USP Basket Apparatus

We use USP basket apparatus to perform diffusion experiments. In this series of experiments, Vankel® 7010 dissolution bath with auto-sampler was used (Figure 3.13). In this dissolution test, 900 ml phosphate buffer saline was used with pH 7.4. PBS was used to mimic human subcutaneous environment. Temperature was controlled as 37 °C. Basket rotation speed was 50 RPM. Auto sampler was set to withdraw 0.5 ml sample every time. The time point of sampling was set to be: 1, 2, 4, 6, 10, 12, 14, 16, 20, and 24 (hour). For those experiments more than one day, samples were withdrawn every 12 hours.

Figure 3.13: Vankel® 7010 dissolution system with heater and auto-sampler

3.14: USP basket apparatus (right)

3.6 Experiment Results and Discussion

3.6.1. High Performance Liquid Chromatography

A linear plot was attained in the range of 0.015625 to 1 UI/mL with a correlation coefficient of 0.9999. The retention time of insulin was 2.15 min.

```
Data File C:\CHEM32\...ING ZHI 2016-12-26\INSULIN FOR K ZHI 2016-12-26 19-32-10\003-0501.D
Sample Name: STD

    ======================================================================
    Acq. Operator   : Kaining Zhi                    Seq. Line :   5
    Acq. Instrument : Instrument 1                   Location : Vial 3
    Injection Date  : 12/26/2016 8:12:44 PM               Inj :   1
                                                  Inj Volume : 50.0 µl
    Acq. Method     : C:\CHEM32\1\DATA\KAINING ZHI 2016-12-26\INSULIN FOR K ZHI 2016-12-26 19-32-10\
                      PEPTIDES.M
    Last changed    : 12/26/2016 8:01:20 PM by Kaining Zhi
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KAINING ZHI 2016-12-26\INSULIN FOR K ZHI 2016-12-26 19-32-10\
                      PEPTIDES.M (Sequence Method)
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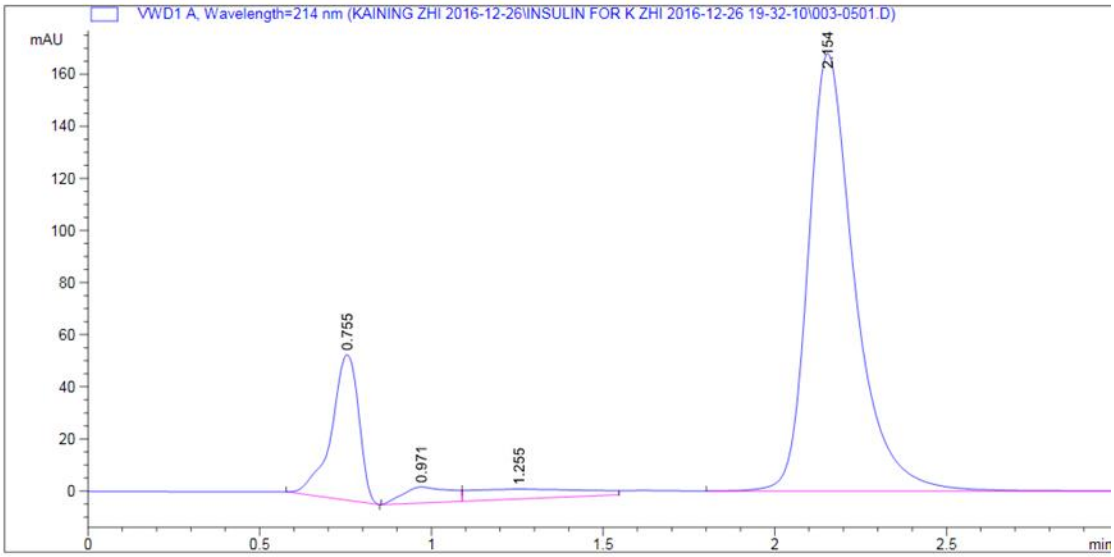

Figure 3.15: HPLC chromatogram of insulin with 0.25 UI/ml concentration

3.6.2. Diffusion study of implants

Implant formulation was listed in Table 3.1. Factors for each implant include membrane pore size, sucrose amount, albumin amount and zinc chloride amount. Figure 3.16 displays implants with formulation # 1 in Table 3.1. Figure 3.17 is the data of all four formulations. Based on the data, we can conclude that implants made from formulation 1, 2 and 3 have

significantly slow release of insulin. The reason for this phenomenon is definitely the existence of zinc chloride, which decreases the dissolution of the whole matrix (Figure 3.17).

#	Pore Size	Tablet Weight	Insulin	Albumin (bovine)	Sucrose	Zinc Chloride
1	8 µm	600 mg	500 UI	250 mg	250 mg	10 mg
2	8 µm	600 mg	500 UI	250 mg	250 mg	5 mg
3	8 µm	600 mg	500 UI	250 mg	250 mg	2.5 mg
4	8 µm	600 mg	500 UI	250 mg	250 mg	0 mg

Table 3.1: Formulation of implants

Figure 3.16: Implants with formulation #1 before sealing

Figure 3.17 is the comparison of formulation #1, 2 and 3. The formulations with zinc chloride provide zero order release of insulin. The zero order release indicates, based on Fick's law, a constant insulin concentration in the implant. The target insulin release speed of this project is 1-2 units every hour. Formulation 1 is definitely acceptable for this project. Formulation 2 and 3 are also useful for type II diabetes patients. Some type II diabetes do not need large amount of insulin to control their glycated hemoglobin level. So a release

rate lower than 1 unit every hour is still helpful. Extended experiments were finished for maximum 3 days. Data of 3-day diffusion study are displayed in Figure 3.18. In this scatter figure, we can conclude that zinc chloride amount in implant is critical to insulin release rate. In long term, more zinc chloride in formulation will result in slower insulin diffusion rate. For all the three formulations we have, #1 provides insulin release as 1.24 units every hour. #2 formulation is 0.95, while #3 formulation is 0.70 unit every hour. #1 can definitely provide a stable basal insulin for type I patients. # 2 formulation is considered effective for some type I patients and most type II patients. #3 formulation is designed for type II patients who do not need large dose of insulin therapy.

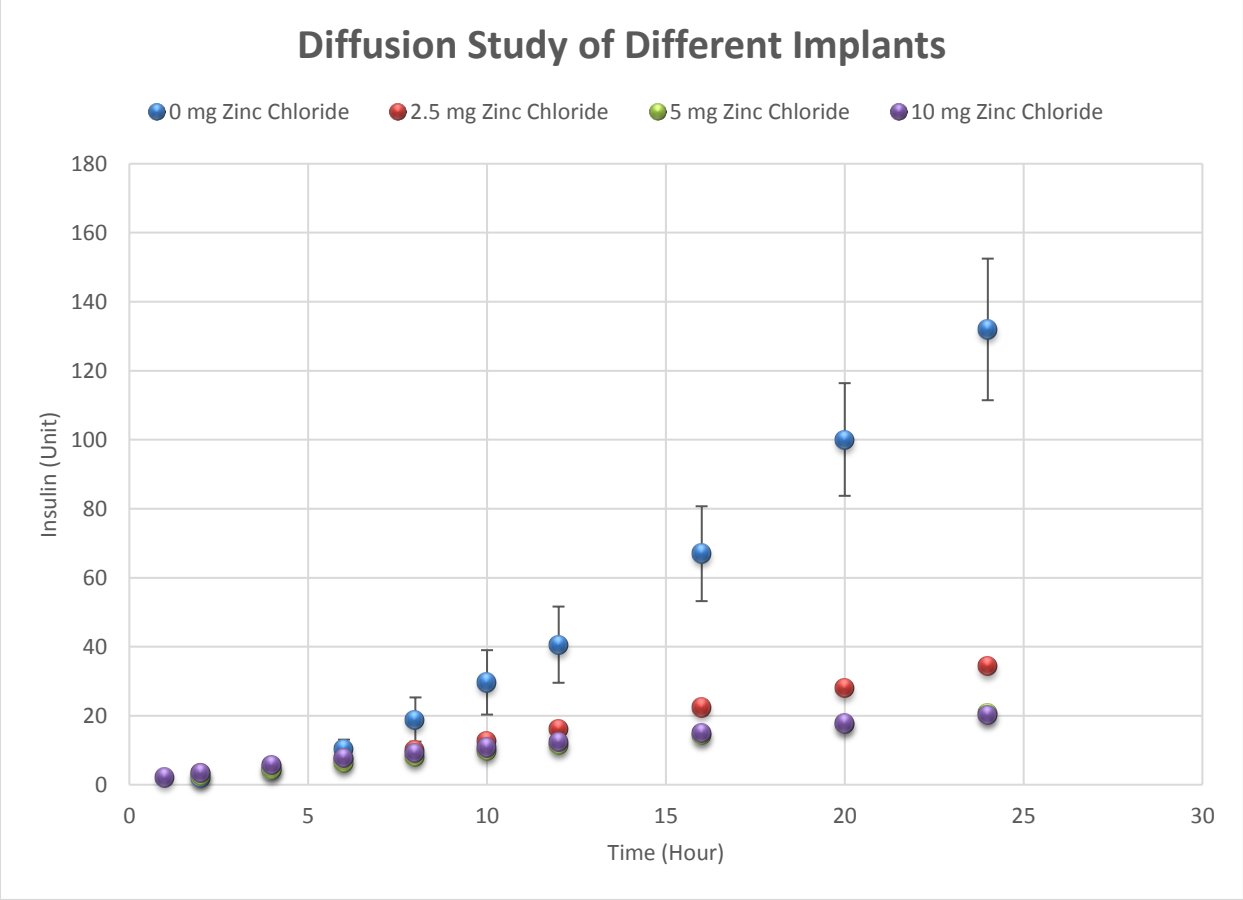

Figure 3.17: Diffusion study of different implants (Formulation #1, 2, 3 and 4)

In Chapter 2, we discussed how matrix might influence drug diffusion with albumin and zinc ions. However, we never discussed the membrane effect. Figure 3.17 is a similar diffusion study using different pore size membranes. In this study, we use the same formulation (#4) with 0.4 micron and 8 micron membranes. The insulin releasing result is as expected. Prototypes with 0.4 micron provided a slower release with a zero order kinetics. This releasing kinetics remind us that our prototype is a combination of membrane control and matrix control.

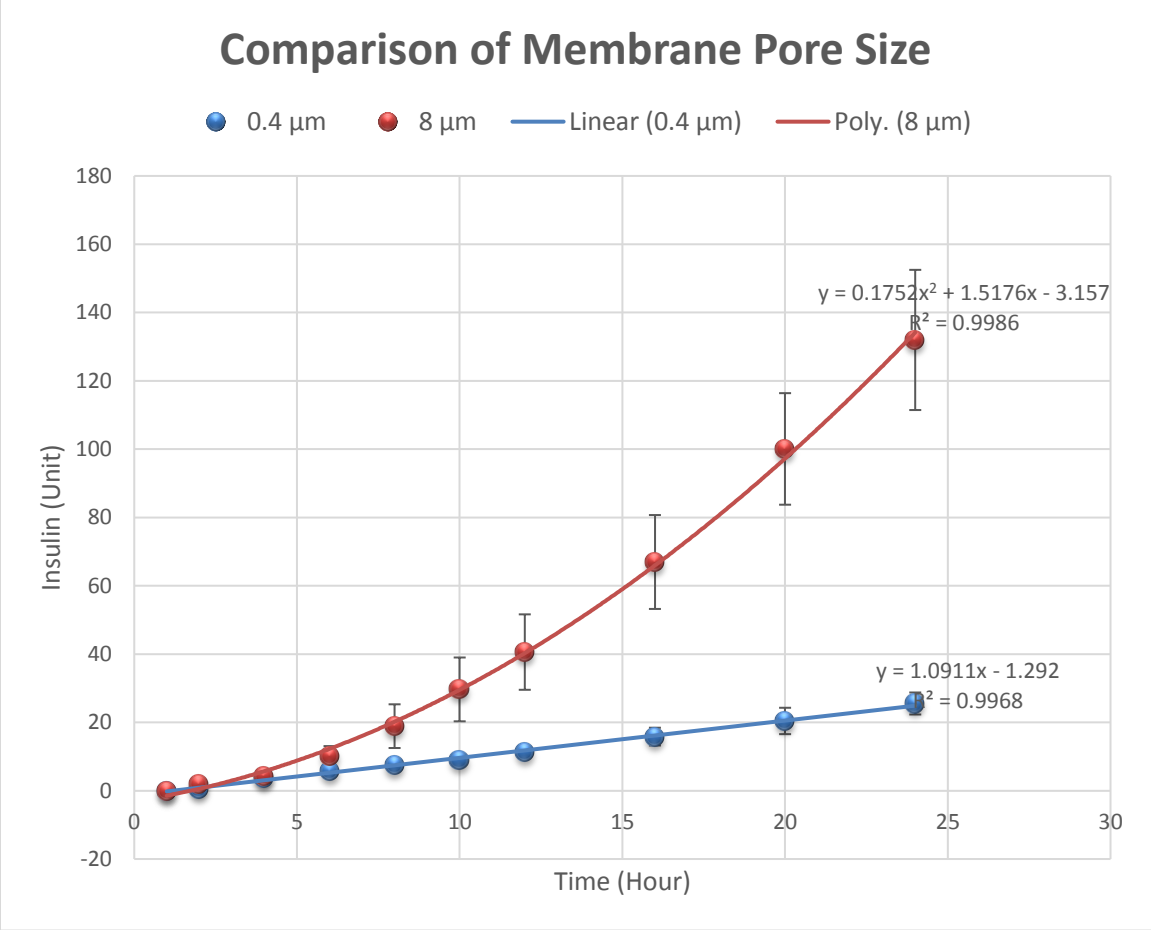

Figure 3.18: Diffusion Study of Formulation #4 using Different Pore Size Membrane

3.7. Conclusion

In this chapter, we initially build the components of this implant. This insulin implant includes a cell culture insert as its shell and membrane, and an insulin tablet formulated with albumin and zinc chloride. The implant can provide zero order release of insulin with variable rate based on the amount of zinc chloride. Implants with high rate can serve as a basal insulin level for type I patients, while those with slow rate can help control the glycated hemoglobin level of type II patients.

In addition, we did an initial screening for membrane effect. We compared the releasing rate between 0.4 micron and 8 micron membranes within 24 hours using formulation #4. As we expected, 0.4 micron membrane provided a stronger control of insulin release. More importantly, 0.4 micron membrane provided a zero-order release.

Based on the data we acquired in this chapter, we made some hypothesis. First of all, we believe that if the drug release rate from our prototype is controlled strictly, we might achieve zero-order release for long term. In addition, we could either use membrane control or choose matrix control to build our system. Similarly, if we want to change drug releasing rate, we could either enlarge/decrease pore size, or change amount of zinc chloride in our system.

Future research will focus on the kinetics of this prototype. We wish to fully understand the mechanism of this implant and the possibility to apply this prototype on other drug molecules. In addition, we hope to propose a method to switch the drug releasing rate after the administration of this implant. Also, we hope to provide a guidance of implant manufacturing method or blueprint of fabrication.

CHAPTER 4

INVESTIGATION OF RELEASING MECHANISM USING CAFFEINE AS MODEL DRUG MOLECULE

4.1. Abbreviation

TII: Tablet-in-Insert, a name for our innovative prototype

4.2. Introduction

Previous research provides a very useful formulation and fabrication processing method of this tablet-in-insert (TII) prototype. However, the releasing kinetics is not fully understood. We hypothesize that drug molecules need to experience two steps in order to be released: dissolution of matrix and diffusion through membrane. In this chapter, different formulations have been designed and tested in order to understand the releasing kinetics of TII prototype.

In this chapter, three cell culture inserts have been tested. All cell culture inserts were ordered from the same vendor with same quality. The only difference is pore size and porosity. Pore size is the open room for an intact membrane. Porosity is defined as the ratio of empty spaces in the membrane. Generally, membrane with larger pore size and/or higher porosity would have more space for molecules to pass though. In our TII prototype, we need to prove either porosity or/and pore size that influence our drug release rate.

In addition, we also need to understand how the tablet matrix works inside of a insert. Unlike a tablet which disintegrates in human gastrointestinal track, our tablet should have a specific pattern of dissolution. It was hypothesized that the dissolution has a direction which is from the source of buffer, toward the distal side of membrane. It was also

hypothesized that formulation of matrix and should effect the drug releasing rate. In this chapter, three excipients were used to examine the effect from matrix in drug release.

4.3. Materials

BSA, fraction V, 97% pH 7, Lyophilized powder is purchased from Alfa Aesar. Zinc Chloride solution (20%) is purchased from Aqua Solutions, Gelatin, type A, 175 Bloom Powder was purchased from Alfa Aesar. Sucrose (Ultra Centrifugation Grade) is provided by Fisher Biotech. Caffeine Monohydrate was acquired from MP Biomedicals. Deionized water is purified from tap water through Thermal Fisher DI Water system. Corning® cell culture insert (24 well, 8, 3 and 0.4 μm pore size) is ordered from Fisher Scientific. Vankel® 7010 dissolution bath with auto sampler is provided my Agilent Technologies.

4.4. Experimental Methods

4.4.1 Preparation of Freeze-Dry Powder

10 ml DI water was added into stainless steel beaker. Caffeine powder was added and completely dissolved. Sucrose was added later. Both chemicals should dissolve completely with no precipitation. The solution is transparent with no color. Albumin was added gradually with gentle stir. Fast adding of albumin will cause bulky lump and result in bubbles. In protein solution, any form of bubbles should be avoided. After complete dissolution, the whole solution is transparent with yellowish color. The whole solution was frozen under -80 °C for 1 hour and dried under Freeze-Dry equipment overnight. The exhaust temperature of Freeze-Dry was -50 °C.

4.4.2. Preparation of Tablet Matrix

The freeze-dry powder would be separated into different weight boats equally for tablet press. In the process of tablet press, Carver® Lab Press model C was used. Natoli® tablet

tooling set was used for punch and die. During tablet press, the pressure was controlled as zero, which means there is no additional application of force to lower the thickness of tablets.

4.4.3. Fabrication of Tablet-in-Insert (TII) Prototype

Corning® cell culture insert was used as the form of implant. Tablet from previous steps was put in the insert (Figure 4.1.). The top of insert was covered and sealed by plastic plumps. Substance should only be available at the membrane end of the insert (Figure 4.2.)

Figure 4.1.: Falcon® Cell Culture Insert (Courtesy of Fisher Scientific)

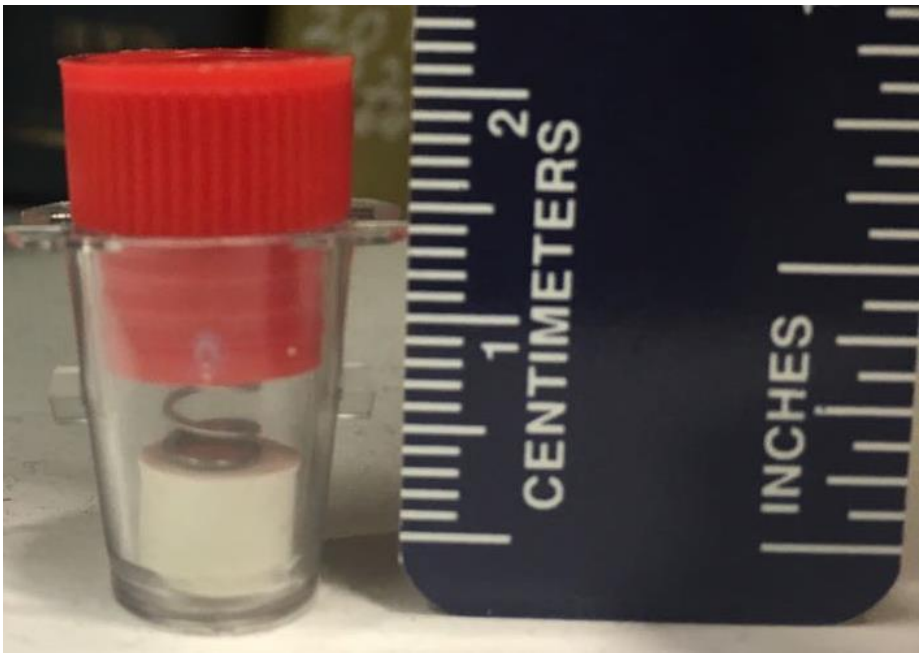

Figure 4.2.: Prototype Appearance and Size after Fabrication

4.4.4. Dissolution and Diffusion Test of TII Prototype

All formulations tested were listed in Table 4.1. We use USP basket apparatus to perform diffusion experiments. In this series of experiments, Vankel® 7010 dissolution bath with auto-sampler was used. In this dissolution test, 1000 ml phosphate buffer saline was used with pH 7.4. PBS was used to mimic human subcutaneous environment. Temperature was controlled as 37 °C. Basket rotation speed was 50 RPM. Auto sampler was set to withdraw 0.5 ml sample every time. The time point of sampling was set to be: 1, 2, 4, 6, 10, 12, 14, 16, 20, and 24 (hour). For those experiments more than one day, samples were withdrawn every 6 hours.

4.4.5. High Performance Liquid Chromatography

HPLC with dual wavelength detector. The HPLC method was provided by Owaisat et al. The mobile phase is composed of 60% acetonitrile and 40% aqueous solution (60:40 v/v). The aqueous solution was prepared by adding 0.5% formic acid into pure deionized water.

The wavelength was 275 nm. The flow rate was 1 ml/min. Injection volume was 20 µL. Column was purchased from Agilent as a 150 mm by 4.6 mm Eclipse® C18 column.

4.4.6. Data Collection and Analysis

All data is reported as "Average ± Standard Error" with triplicate setting.

4.5. Results and Discussion

4.5.1. Vertical Erosion Mechanism

Tablet 4.1 is a list of selected prototypes that have been tested in this chapter. All prototypes were loaded with 25 mg caffeine. In this section, our prototypes were fabricated with two different matrix, 2 different membranes and 1 spring. Figure 4.3 is the appearance of our prototype.

Formula #	Sucrose	Albumin	Membrane (µm)	Spring
KZ-CF-01	125 mg	125 mg	8	Long
KZ-CF-02	125 mg	125 mg	8	Short
KZ-CF-03	125 mg	125 mg	3	Long
KZ-CF-04	125 mg	125 mg	3	Short
KZ-CF-05	187.5 mg	62.5 mg	8	Long
KZ-CF-06	187.5 mg	62.5 mg	8	Short
KZ-CF-07	187.5 mg	62.5 mg	3	Long
KZ-CF-08	187.5 mg	62.5 mg	3	Short

Table 4.1.: Formulation and Fabrication of Selected Prototypes-1

Figure 4.3: Relative Size Appearance of TII Prototype

It hypothesized that the drug release from our prototype is a two-step process. The first step is buffer comes into the implant and starts to dissolve tablet vertically. The second step is dissolved drugs diffuse out of the implant through porous membranes. For half of all prototypes, I inserted a long spring to immobilize tablet by using compressive force, while the other half groups only have a short spring without compression as steric hindrance. With long compressive spring, tablets would not dissolve with vertical erosion but disintegration. Figure 4.4 to Figure 4.7 are experimental data of different prototypes. From these 4 figures, we noticed that the existence of a compressive spring brings significant difference in caffeine release from our prototypes, even though the matrix formulation and membrane are the same. Visual observation noticed that prototypes with none-compressive springs showed a vertical dissolution and erosion of tablet, while prototypes with long springs simply disintegrated under the pressure of spring force. Even though after 24 hours of experiment, all prototypes showed completely dissolved matrix as a form of yellowish solution, the process is different. Data result from Figure 4.4 to Figure 4.7 also proved our hypothesis. We concluded that matrix erosion rate contributes to drug releasing rate from out prototypes.

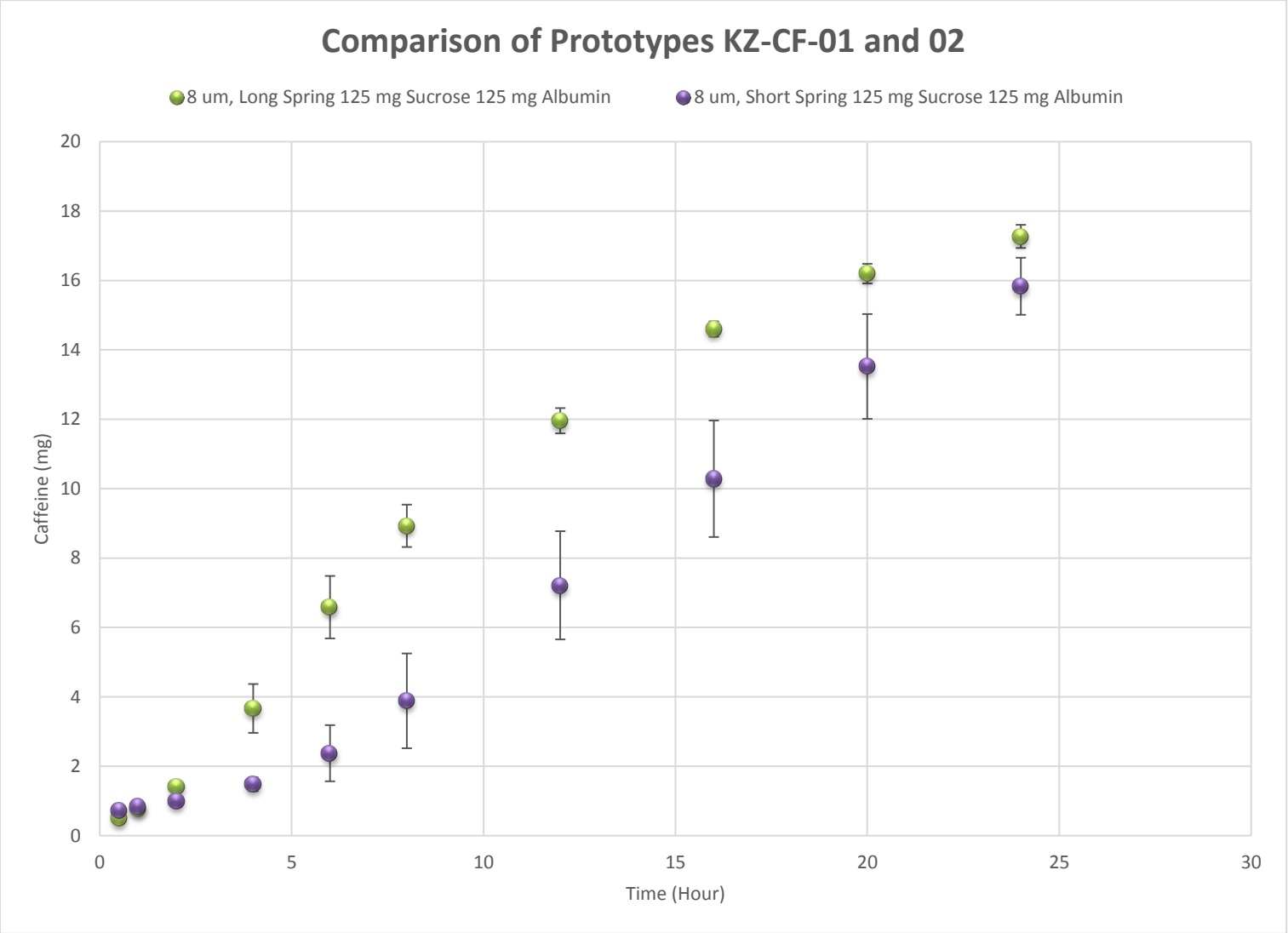

Figure 4.4.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-01 and KZ-CF-02 (KZ-CF-01 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Long Spring; KZ-CF-02 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Short Spring)

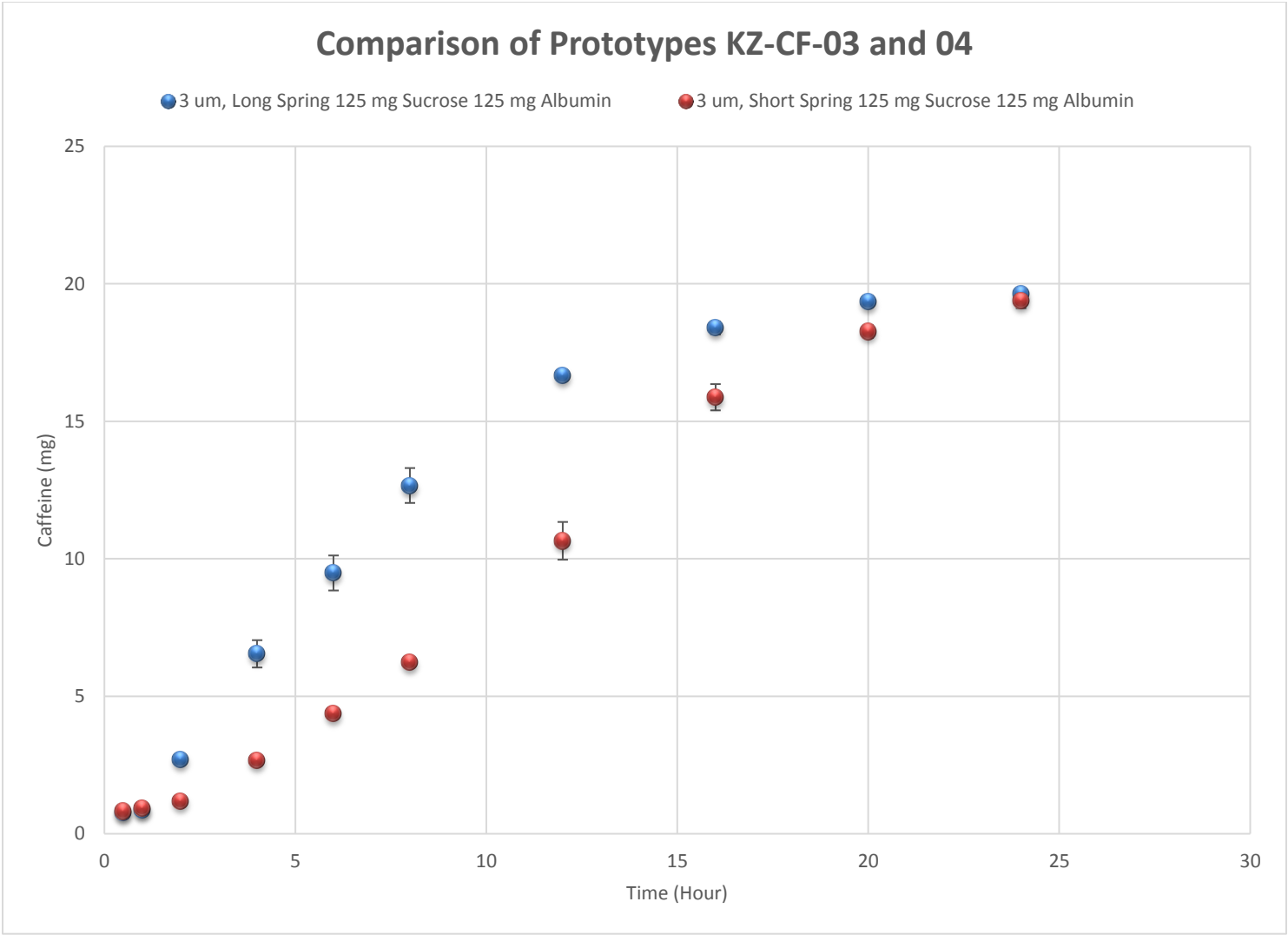

Figure 4.5.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-03 and KZ-CF-04 (KZ-CF-03 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Long Spring; KZ-CF-04 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Short Spring)

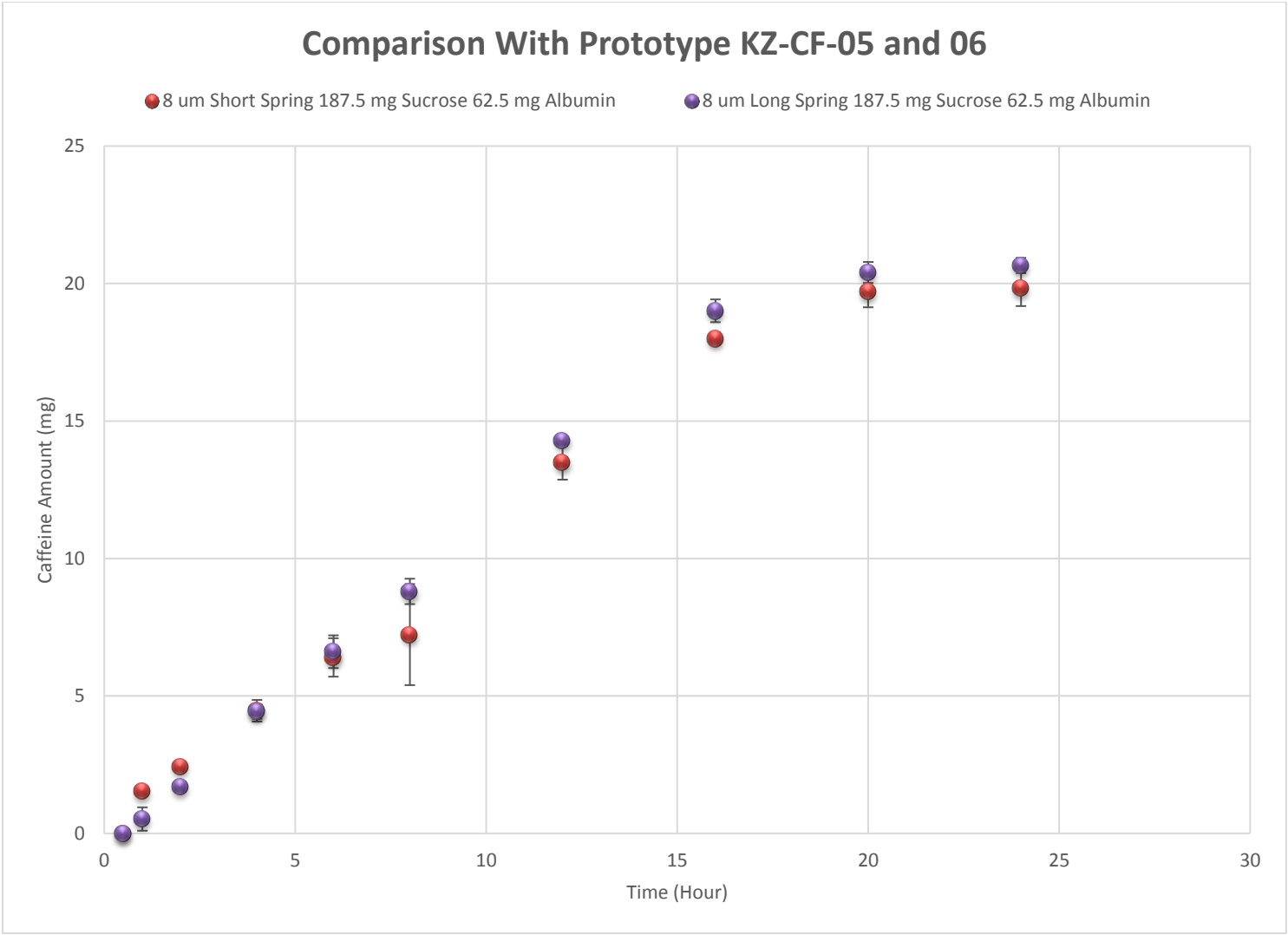

Figure 4.6.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-05 and KZ-CF-06 (KZ-CF-05 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Long Spring; KZ-CF-06 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Short Spring)

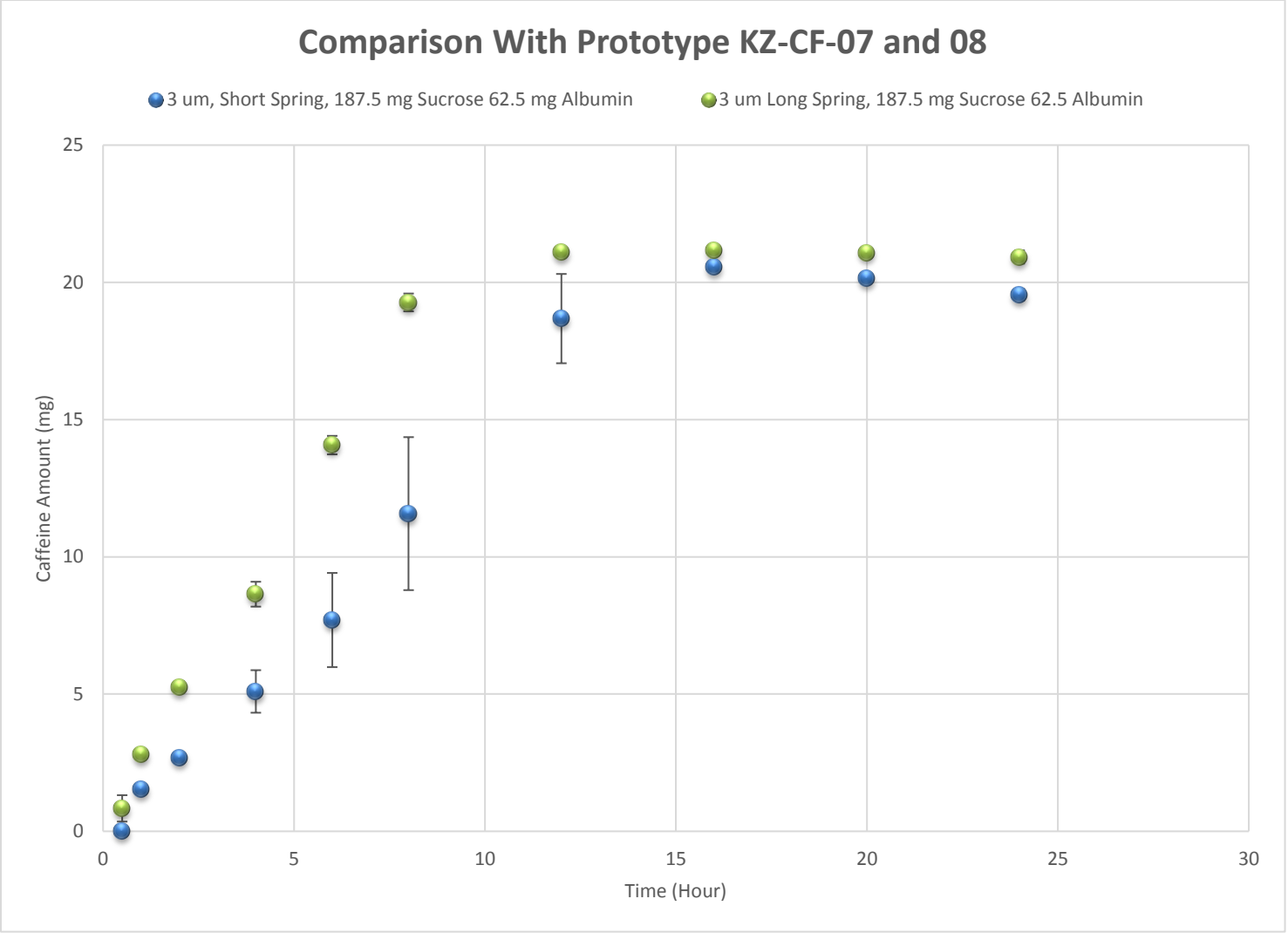

Figure 4.7.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-07 and KZ-CF-08 (KZ-CF-07 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Long Spring; KZ-CF-08 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Short Spring)

4.5.2. Membrane Effect in Drug Releasing Rate

Figure 4.8 to Figure 4.11 are experimental results as a comparison study for different membranes. It was hypothesized that for the same matrix with different membranes, 8 µm groups should have faster drug release compared to 3 µm groups. It was out of expectation to see results below that all 3 µm groups with same formulations actually provided faster drug release. Based on the theory of how porous membrane works, this result is considered abnormal. However, we looked into the parameter of all Falcon® Cell Culture Inserts in Table 4.2 and we realized that 3 µm groups have larger porosity than 8 µm ones. Despite the smaller pore size, 3 µm cell culture inserts still provide larger "empty surface" than 8 µm ones. This explained that why 3 µm prototypes provide faster drug release and proved that porosity is more important than pore size for these prototypes.

CATALOG NO.	MEMBRANE MATERIAL	PORE SIZE (MICRON)	PORE DENSITY (PORES/SQ. CM)	OPTICAL QUALITY	TC PLATE (NO. OF WELLS)
353090	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	6
353180	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	12
353095	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	24
353102	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	6
353103	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	12
353104	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	24
353091	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	6
353181	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	12
353096	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	24
353093	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	6
353182	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	12
353097	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	24
353493	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	6
353494	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	12
353495	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	24
353092	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	6
353292	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	12
353492	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	24

Table 4.2.: Guidelines for Falcon® Cell Culture Inserts

Based on the porosity and pore size provided by Falcon®, we could list a ranking of all 24 well cell culture inserts which might be used in future chapters for our research (Table 4.3.). 3 μm ones have the largest empty surface area. 8 μm ones are the second. 1 μm one is smaller than previous two types while 0.4 μm one has the smallest empty surface area. As a result of this ranking, we decide to move on with 0.4 μm cell culture inserts to achieve a highly controlled drug release prototype. Our goal is to achieve zero order caffeine release in long terms.

		Porous Surface Area (cm2/cm2)	
Pore Size (μm)	Porosity Density (Pores/cm2)	Pore as Circle	Pore as Square
3	**8.00E+05**	**0.22608**	**0.072**
8	6.00E+04	0.120576	0.0384
1	1.60E+06	0.05024	0.016
0.4	2.00E+06	0.010048	0.0032
3HD	2.00E+05	0.05652	0.018
0.4HD	1.00E+08	0.5024	0.16

Table 4.3.: Porosity Calculation of Different Types of Falcon® Cell Culture Inserts

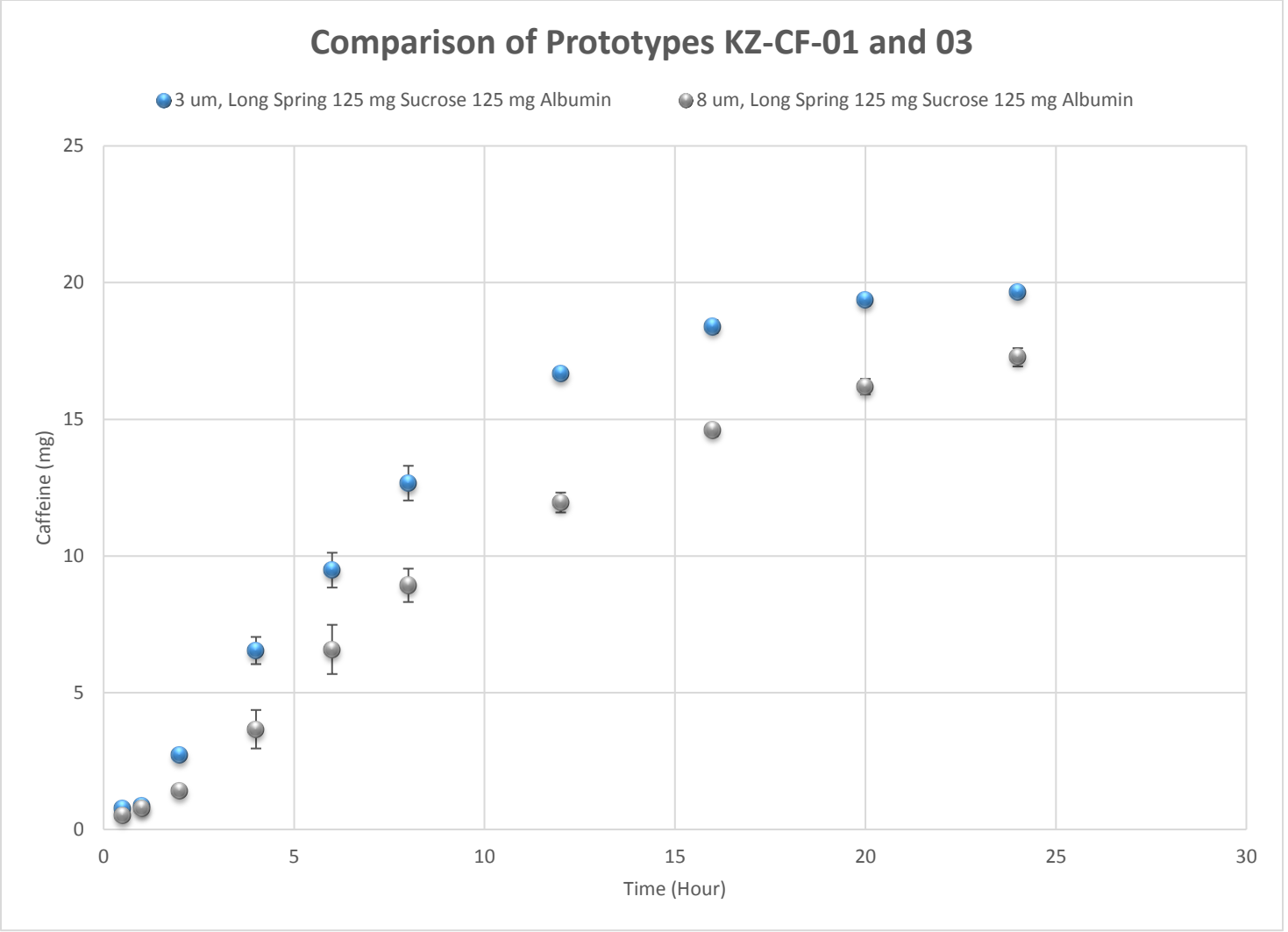

Figure 4.8.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-01 and KZ-CF-03 (KZ-CF-01 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Long Spring; KZ-CF-03 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Long Spring)

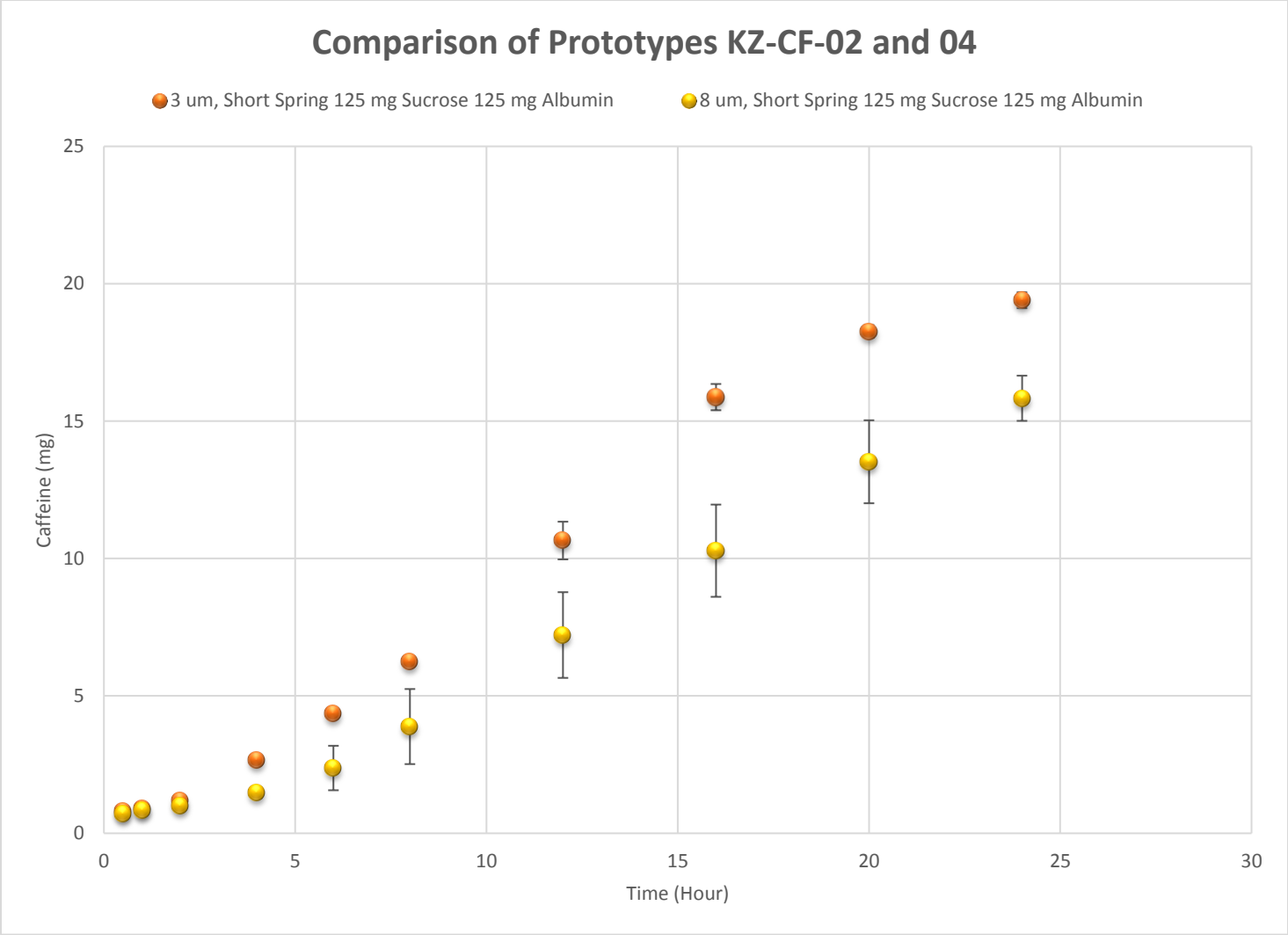

Figure 4.9.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-02 and KZ-CF-04 (KZ-CF-02 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Short Spring; KZ-CF-04 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Short Spring)

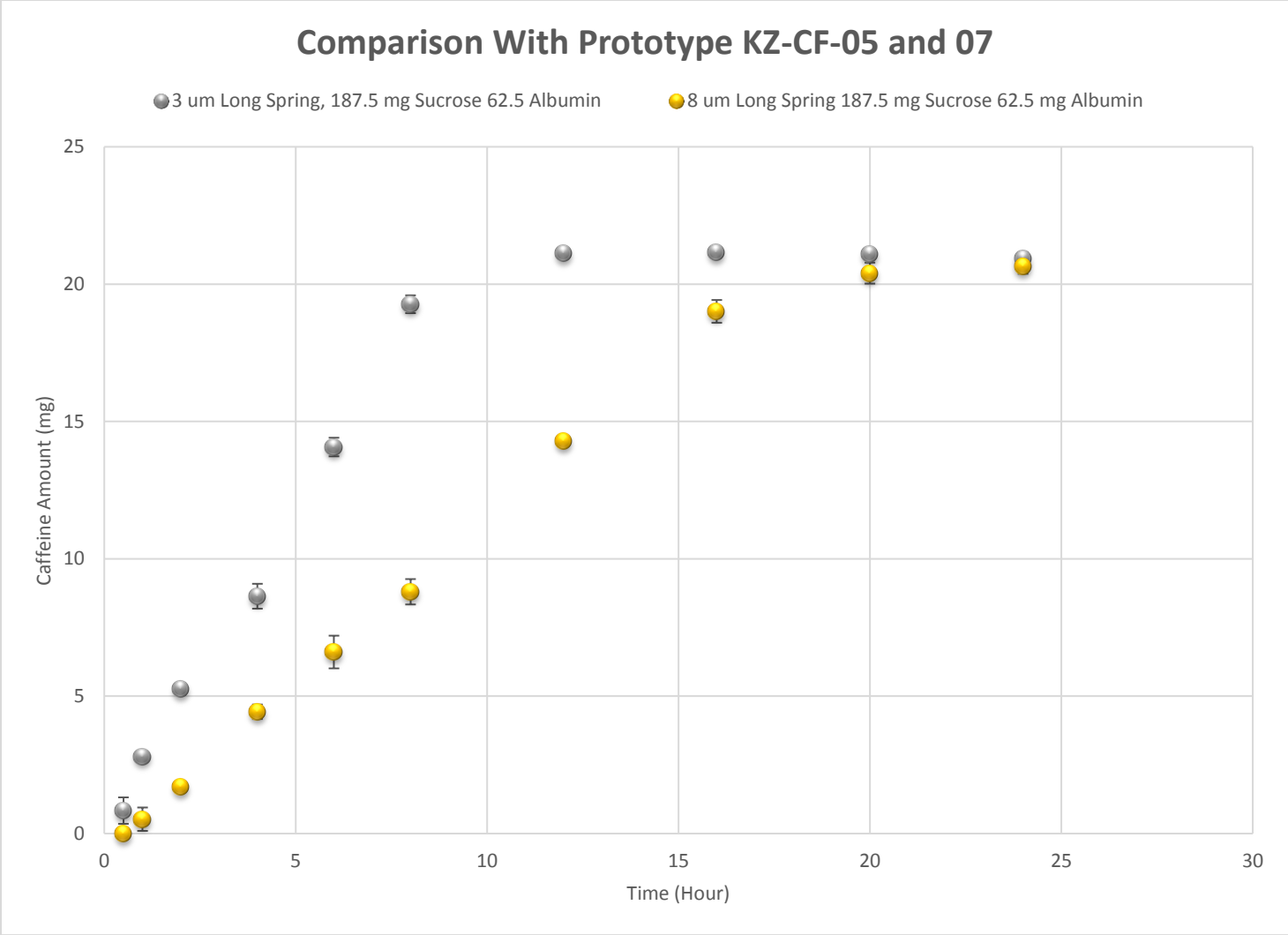

Figure 4.10.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-05 and KZ-CF-07 (KZ-CF-05 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Long Spring; KZ-CF-07 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Long Spring)

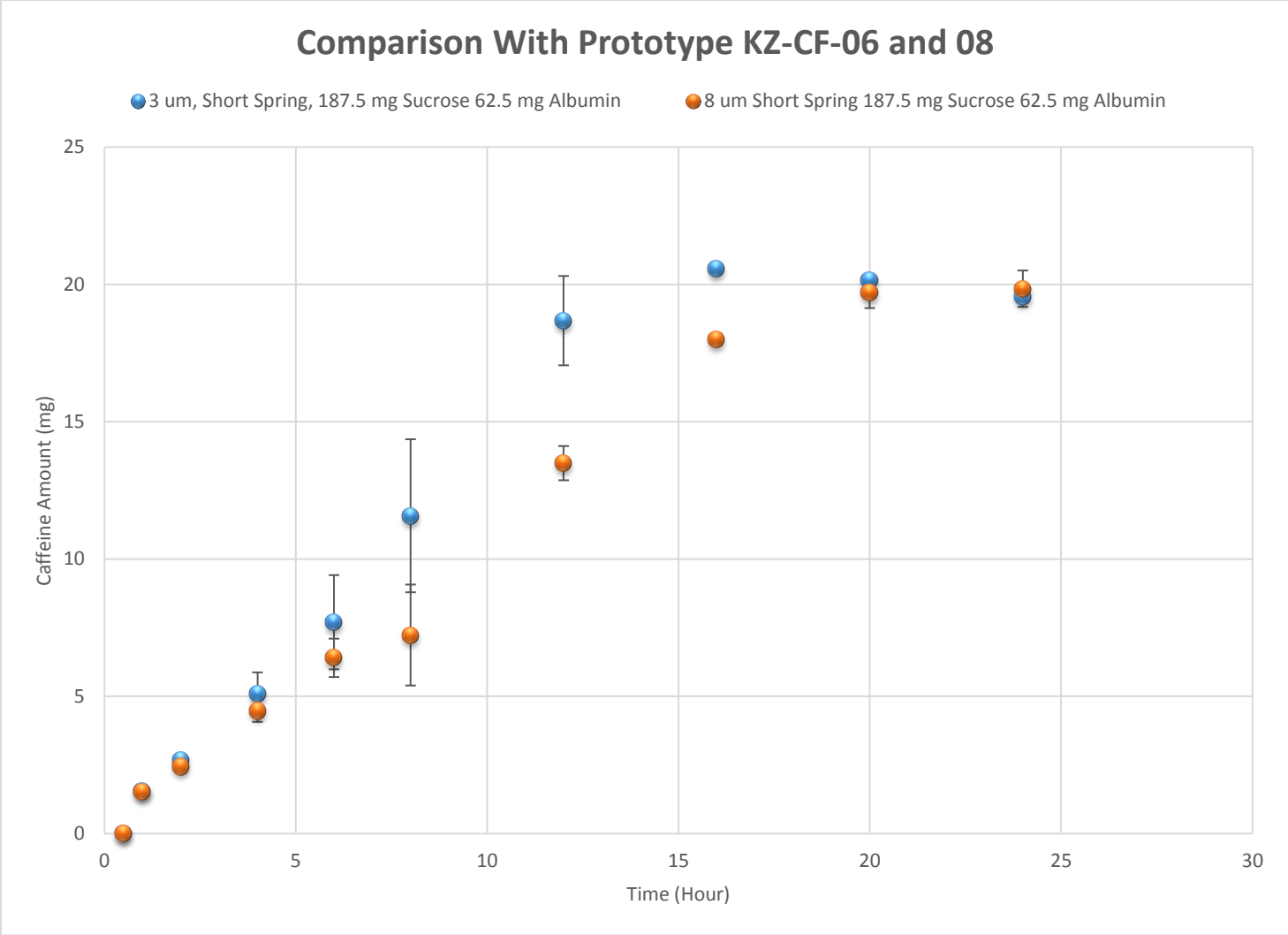

Figure 4.11.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-06 and KZ-CF-08 (KZ-CF-06 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Short Spring; KZ-CF-08 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Short Spring)

4.5.3. Matrix Effect in Drug Releasing Rate

The last factor that might influence drug release rate in this prototype is the formulation of matrix. As we already proved in previous sections that drug needs to dissolve first in order to diffuse out though porous membrane. In erosion model, the erosion rate is determined by the formulation of matrix. Owaisat et al used crosslinked alginate polymer with ethyl cellulose to provide erosion matrix. In her prototype, the tightness of cross linking determines the erosion rate. Similarly, in our prototype, the dissolution rate of our matrix could definitely influence drug releasing rate. Table 4.4 is a list of commonly used excipients for controlled release dosage forms. However, our prototype is a parenteral dosage form which requires minimum toxicity.

Name	Dosage Form	Route of Administration	FDA Maximum Potency
Carboxymethyl cellulose	Injectable	Intra-Articular	0.75%
Methyl Cellulose	Injection	Intra-Articular	0.10%
Microcrystalline Cellulose	Injection	Intra-Articular	0.05%
Sucrose	**Solution, Injection**	**IM, IV**	**90%**
Lactose Monohydrate	**Injection**	**IM, IV, SC**	**20.35%**
Albumin Human	**Injection**	**IV**	**80%**
Gelatin	**Injection**	**IM, IV, SC**	**16%**
Alginate	Tablet, Suspension	Oral Only	N/A
Stearic Acid	Pellet	Implantation	0.2 mg
Magnesium Stearate	Delivery System	Implantation	0.1 mg
Glyceryl palmitostearate	Injection	IM, ID	1.60%

Table 4.4: List of Commonly Used Excipients and FDA Maximum Potency

Based on FDA's guidance, we decided to choose sucrose, lactose, human albumin and gelatin as our excipient. For current research, we used bovine albumin for budget consideration.

Figure 4.12 to Figure 4.15 experimental result of porotypes with different matrix. From the result, we could easily conclude that high dosage of sucrose would definitely faster drug release based on its high water solubility (2000 g/L at 25 °C). Albumin is relatively slower in dissolution (200 mg/mL). This trend is the same for both membranes and both spring settings and that proves our hypothesis. The next step is to reduce the amount of sucrose used in our prototype till we achieved a satisfactory long-term zero order drug release.

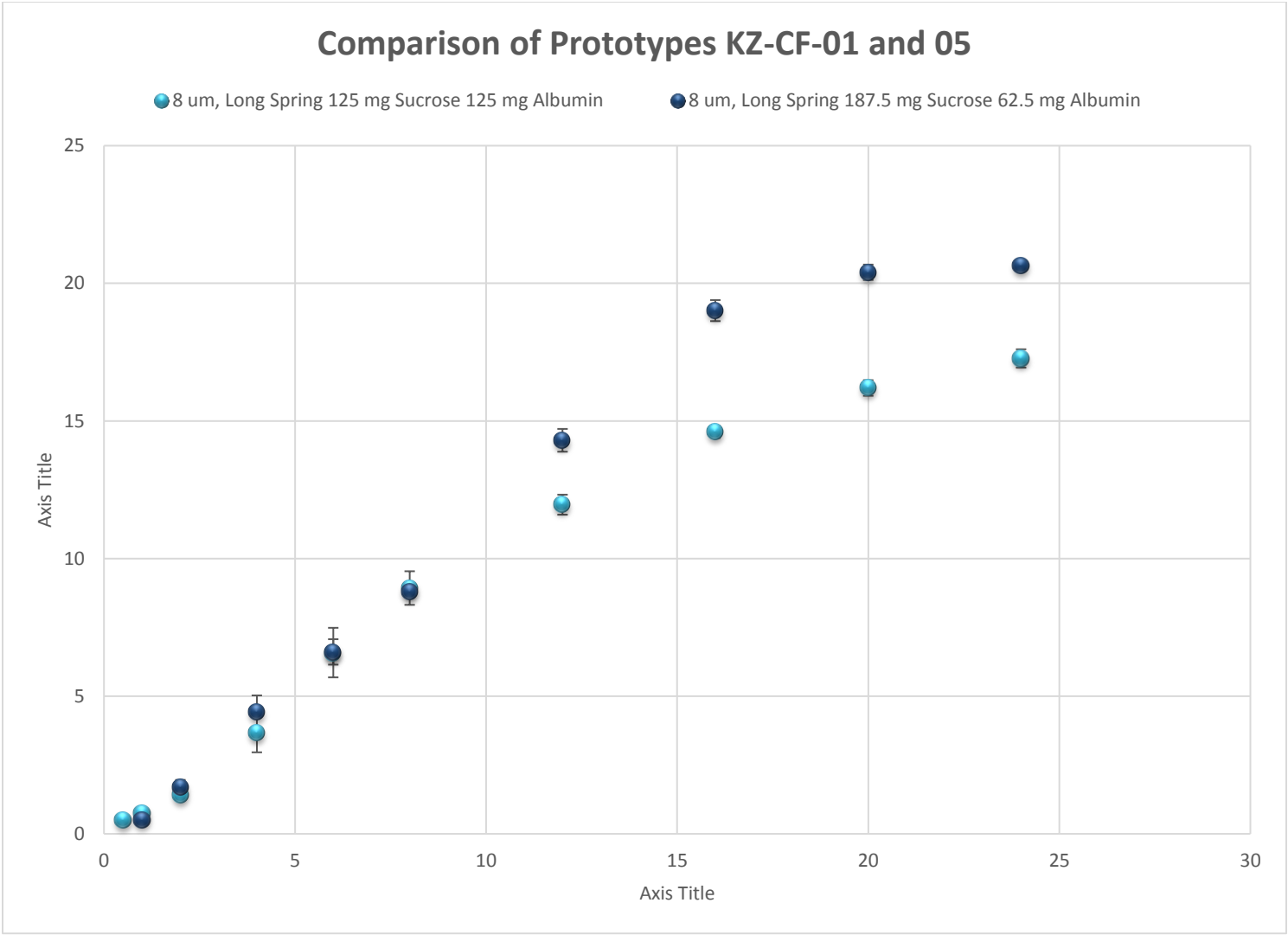

Figure 4.12.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-01 and KZ-CF-05 (KZ-CF-01 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Long Spring; KZ-CF-05 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Long Spring)

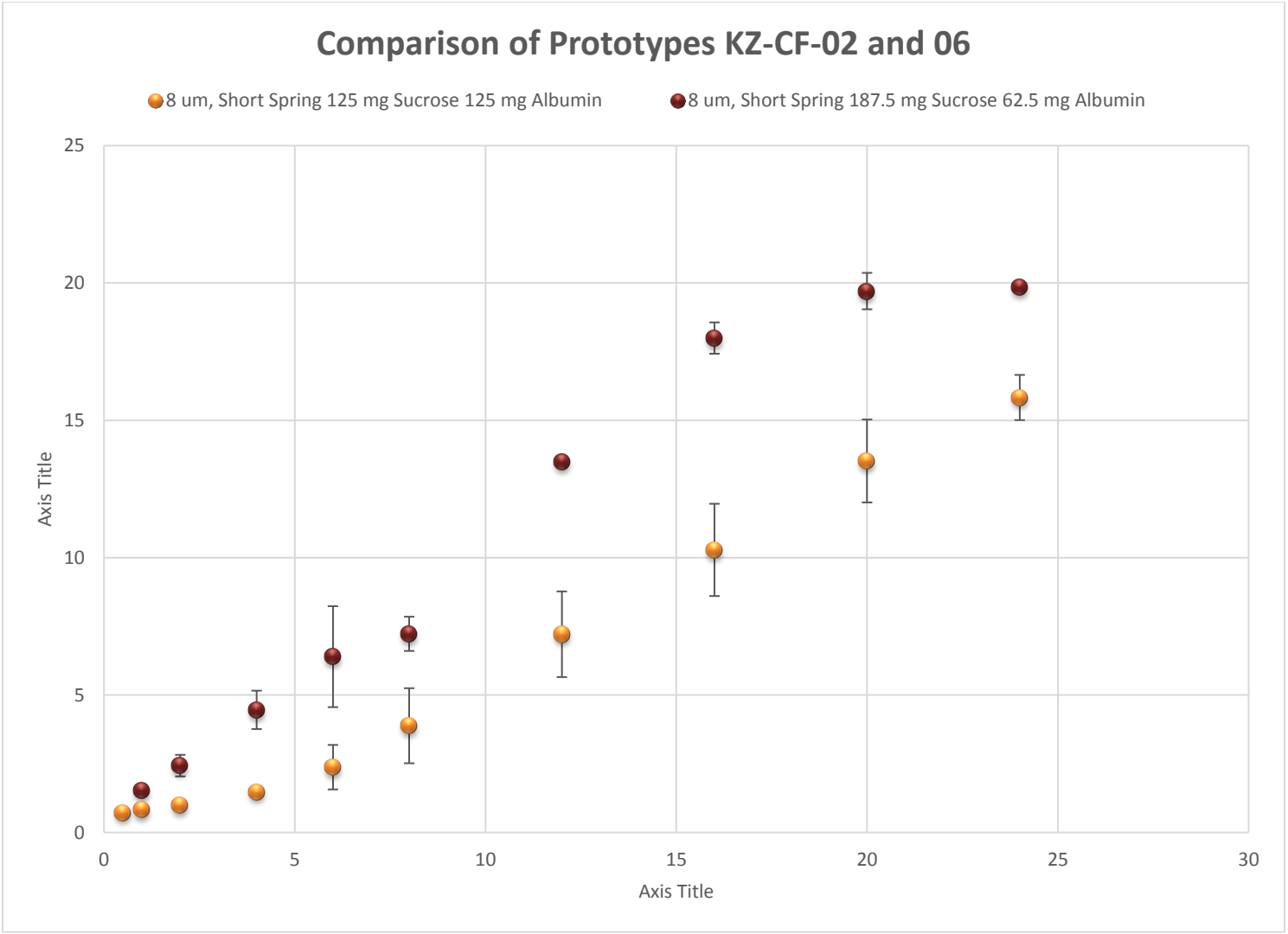

Figure 4.13.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-02 and KZ-CF-06 (KZ-CF-02 Formulation: 125 mg Sucrose, 125 mg Albumin, 8 µm Membrane, Short Spring; KZ-CF-06 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 8 µm Membrane, Short Spring)

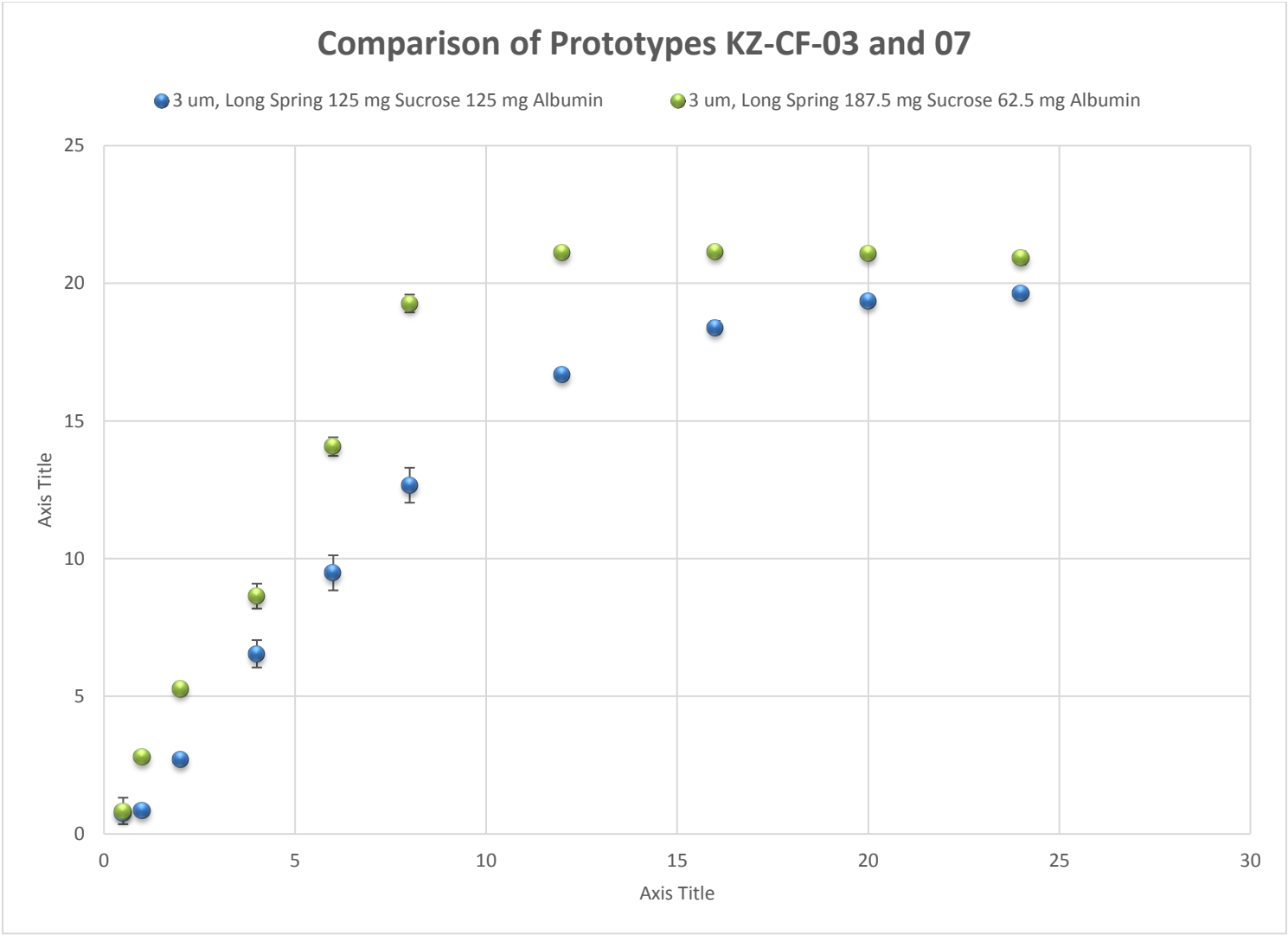

Figure 4.14.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-03 and KZ-CF-07 (KZ-CF-03 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Long Spring; KZ-CF-07 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Long Spring)

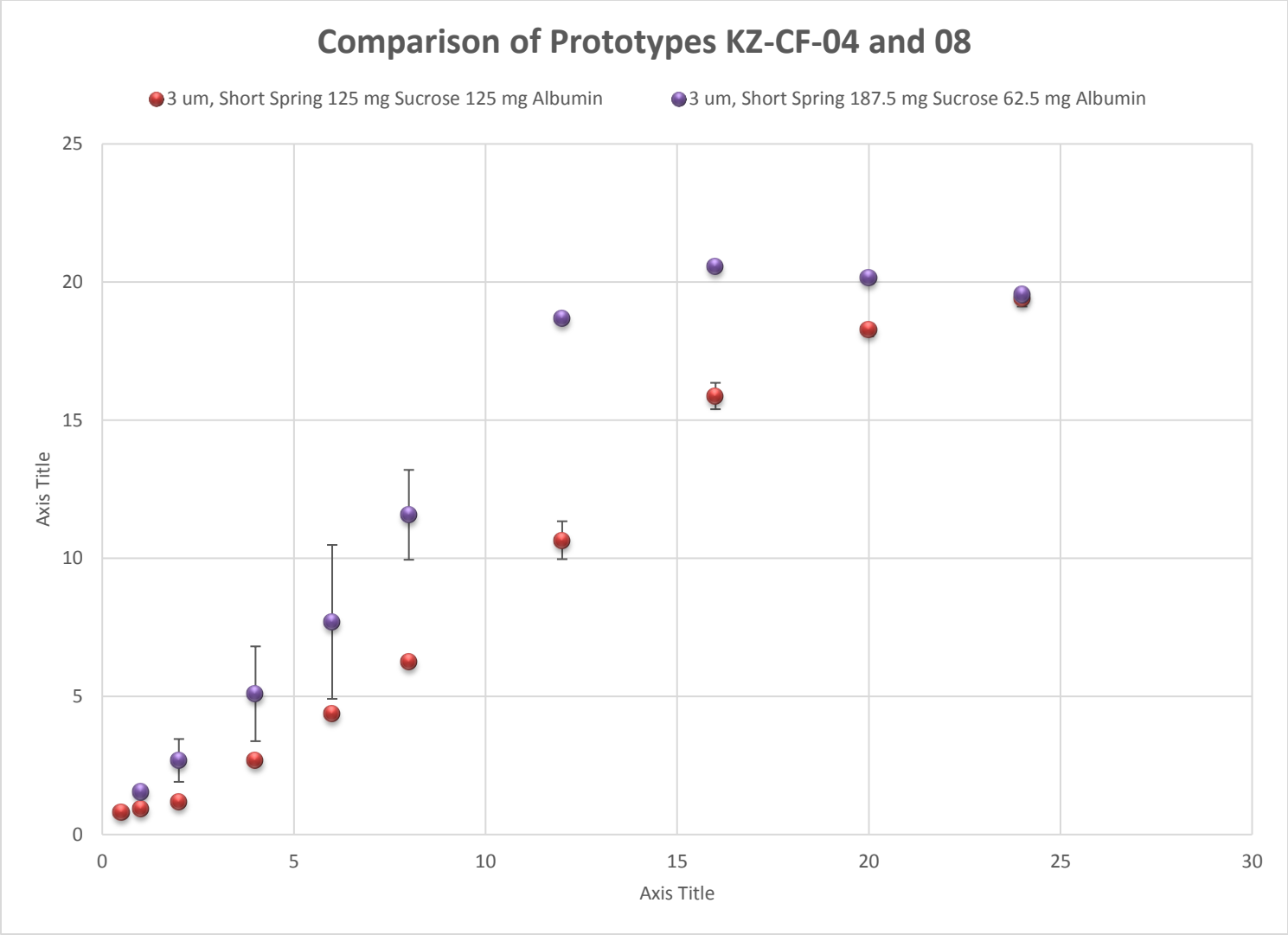

Figure 4.15.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-04 and KZ-CF-08 (KZ-CF-04 Formulation: 125 mg Sucrose, 125 mg Albumin, 3 µm Membrane, Short Spring; KZ-CF-08 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 3 µm Membrane, Short Spring)

4.5.4. The Best Controlled Prototype with Current Parameters

Starting this section, we were dedicating to achieve a zero-order drug release with caffeine for long term. The idea is to use both membrane control and matrix control to slow down the burst release. Based on previous data, we could easily acquire zero-order drug release when we have constant drug concentration inside of the membrane. Previous results also provided some comparisons among different matrix compositions. In order to provide a long-term zero-order release, we decided to use 0.4 μm membranes to provide the slowest membrane effect. In addition, we would gradually reduce the amount of sucrose added into our matrix. Table 4.5 is a list of formulations we tested. We still kept the option for spring to ensure that this factor is not excluded.

KZ-CF-09	125 mg	125 mg	0.4	Short
KZ-CF-10	125 mg	125 mg	0.4	No
KZ-CF-11	187.5 mg	62.5 mg	0.4	Short
KZ-CF-12	187.5 mg	62.5 mg	0.4	No
KZ-CF-13	150 mg	100 mg	0.4	Short
KZ-CF-14	150 mg	100 mg	0.4	No
KZ-CF-15	175 mg	75 mg	0.4	Short
KZ-CF-16	175 mg	75 mg	0.4	No

Table 4.5.: Formulation and Fabrication of Selected Prototypes-2

Figure 4.16 to Figure 4.19 are experimental results for the comparison of spring effect. To our surprise, prototypes without spring provide a relatively higher drug release while prototypes with short spring provided a slower caffeine release. While we still could not understand why this phenomenon happened, Figure 4.17 provided us a potential answer. Prototype KZ-CF-11 with 187.5 mg sucrose and 62.5 mg albumin stopped releasing caffeine starting 40th hour. At the same time, a picture of KZ-CF-11 after 84 hours showed

a recrystallization of sucrose inside of the prototype (Figure 4.20). Meanwhile, other prototypes provided similar drug release rate with same formulations, but prototypes with spring had a tendency to slow down drug release. Most prototypes without spring provided a very good zero-order drug release for 72 hours, then they start to slower the drug release. The data in this section provided us several important information about the drug release in our prototype:

1. For small porosity prototypes, diffusion is the slow step of drug release. Formulation can change drug releasing rate, but there is a limit about the maximum releasing rate that it could provide.
2. With 0.4 μm membrane, all ingredients will diffuse out without selection. However, since the membrane has a relatively low porosity, highly concentrated sucrose recrystallize and could not diffuse our quickly. This proves that diffusion is the slow step in this series of drug releasing system.
3. Even though diffusion is the slow step, we could still achieve a slower diffusion rate with better linearity by using a less soluble matrix. The spring is not needed in this prototype because dissolution is no longer the slow.
4. Some prototypes provide very good zero-order release while others could only provide linear release for less duration of time. We believe if the dissolution is further slowed down, we could have a better linearity based on the constant drug concentration inside of our TIIprototype.
5. For the consideration of a real subcutaneous implant, there should not be any empty room inside of the implant. So we decided to exclude adding a spring in

our future prototypes. And we will design the blueprint of this implant with a completely sealed matrix with no air.

In order to achieve a slower drug release rate, we decided to add gelatin A as another excipient. Gelatin is a very commonly used protein in food and pharmaceutical industry. Gelatin does not have a fine molecular weight. Our 175 bloom gelatin is probably in the smaller side of 40,000-50,000 as molecular weight. Also, under 35-40 °C, gelatin usually absorbs water and swells but does not dissolve completely. Gel strength and viscosity gradually weaken upon prolonged heating. Table 4.6 are formulations we prepared in order to test the function of Gelatin.

Formula #	Sucrose	Albumin	Gelatin	Membrane (μm)
KZ-CF-17	50 mg	200 mg	0	0.4
KZ-CF-18	25 mg	200 mg	25 mg	0.4
KZ-CF-19	0	200 mg	50 mg	0.4
KZ-CF-20	0	175 mg	75 mg	0.4

Table 4.6.: Formulation and Fabrication of Selected Prototypes-3

Figure 4.22 is the experimental result of Table 4.6. As we can see from the result, with graduate replacement of gelatin for sucrose, the drug diffusion rate slows down stably. In addition, the effect from gelatin has a limit. For 250 mg excipient, after 20% to 30% gelatin is the maximum amount we could formulate. KZ-CF-19 and KZ-CF-20 are the most successful prototypes that meet the need of this design of experiment. We achieved a 114 hour continuously zero-order release of 0.055-0.058 mg/hour caffeine from these two prototypes. At the same time, the R square value of both prototypes are higher than 0.99.

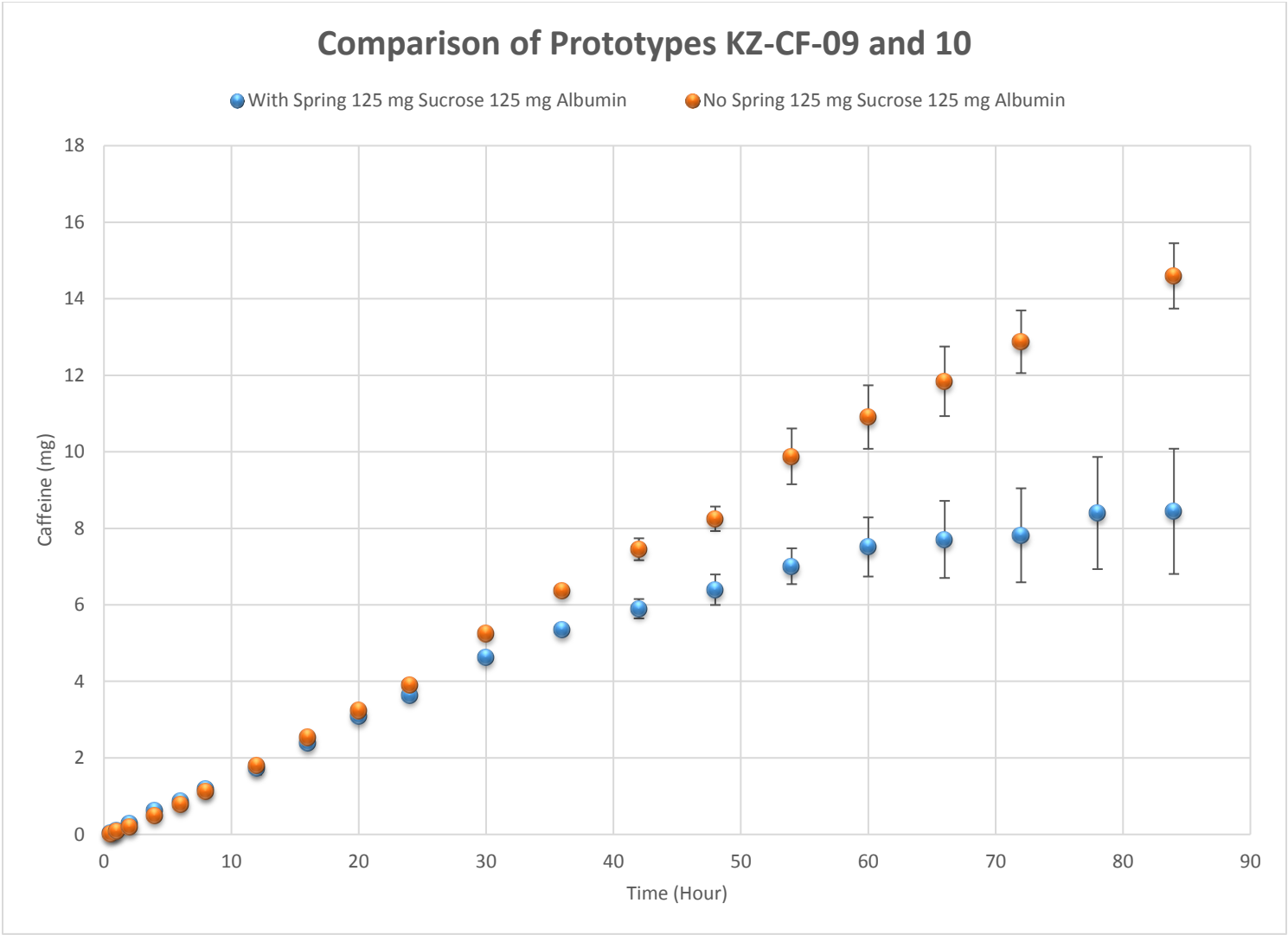

Figure 4.16.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-09 and KZ-CF-10 (KZ-CF-09 Formulation: 125 mg Sucrose, 125 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-10 Formulation: 125 mg Sucrose, 125 mg Albumin, 0.4 µm Membrane, No Spring)

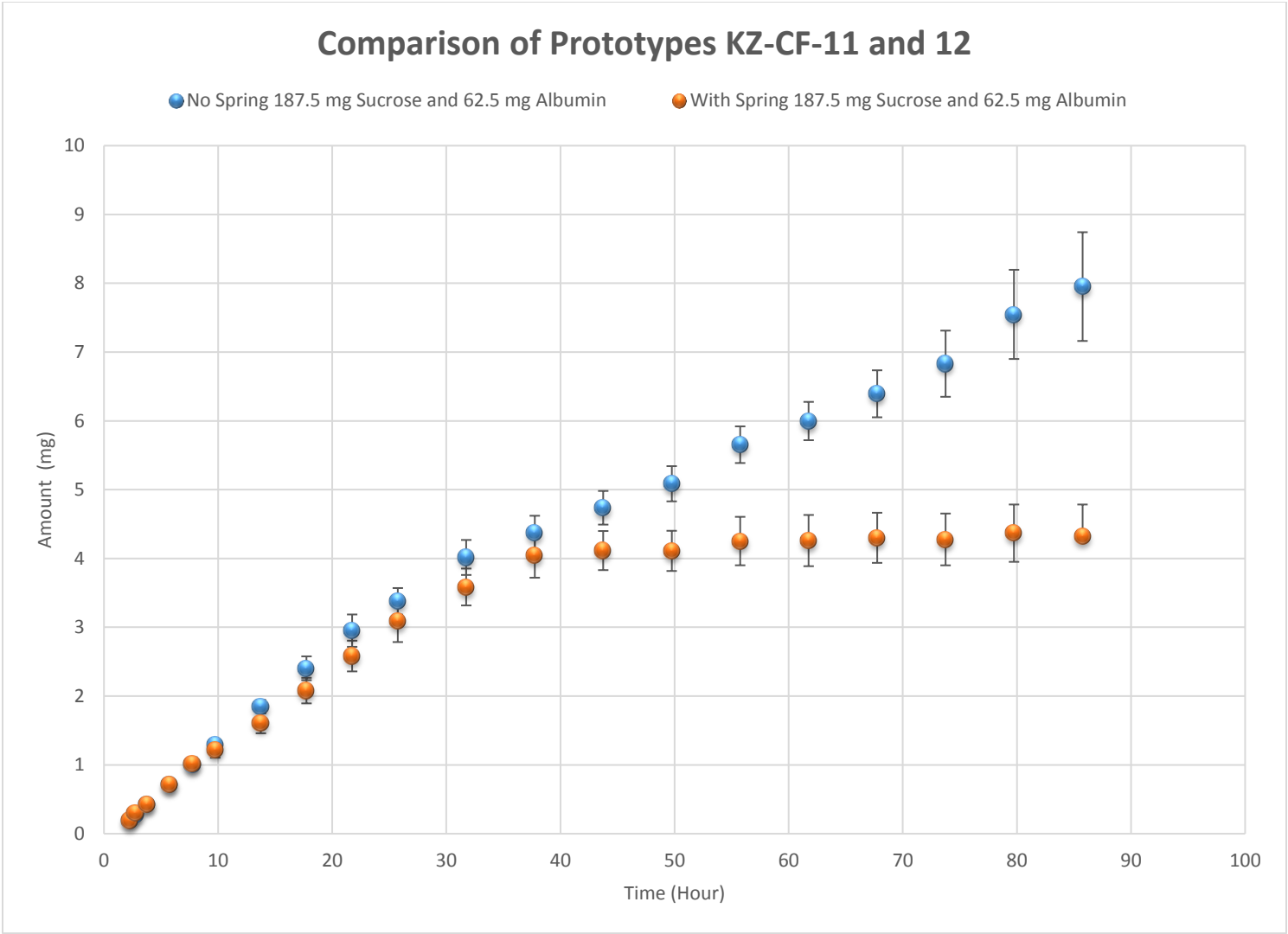

Figure 4.17.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-11 and KZ-CF-12 (KZ-CF-11 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-10 Formulation: 187.5 mg Sucrose, 62.5 mg Albumin, 0.4 µm Membrane, No Spring)

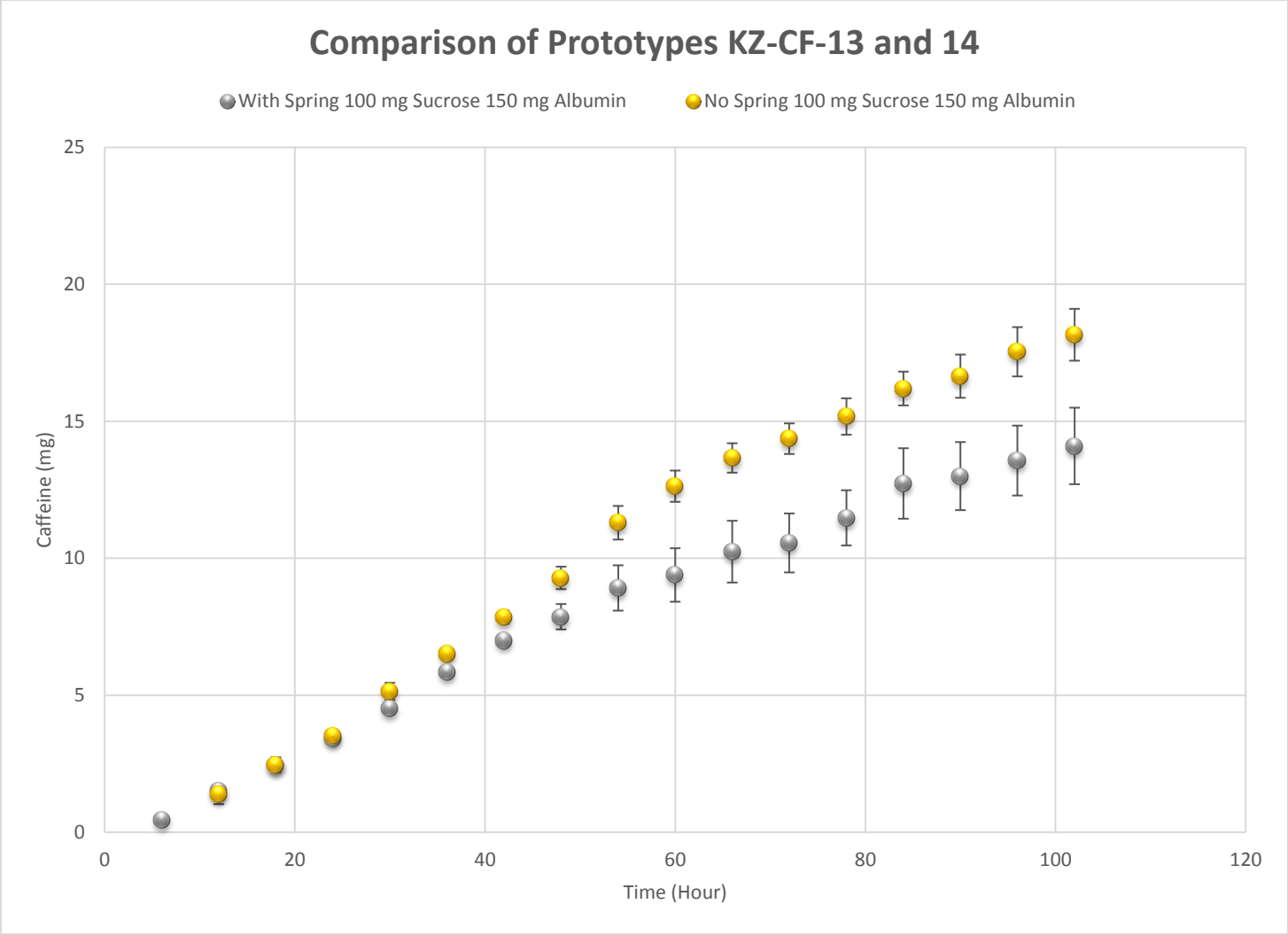

Figure 4.18.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-13 and KZ-CF-14 (KZ-CF-13 Formulation: 100 mg Sucrose, 150 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-14 Formulation: 100 mg Sucrose, 150 mg Albumin, 0.4 µm Membrane, No Spring)

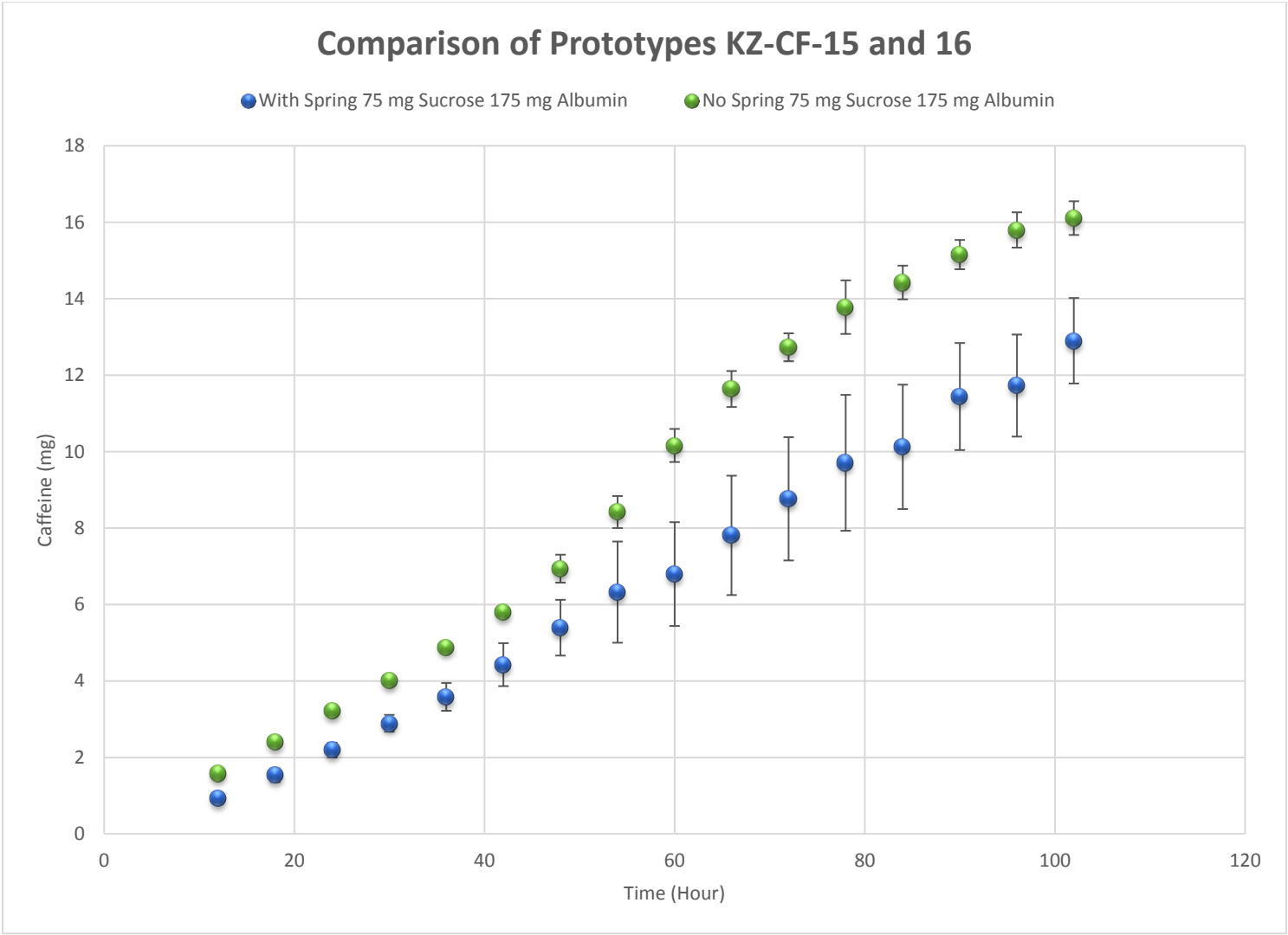

Figure 4.19.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-13 and KZ-CF-14 (KZ-CF-13 Formulation: 75 mg Sucrose, 175 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-14 Formulation: 75 mg Sucrose, 175 mg Albumin, 0.4 µm Membrane, No Spring)

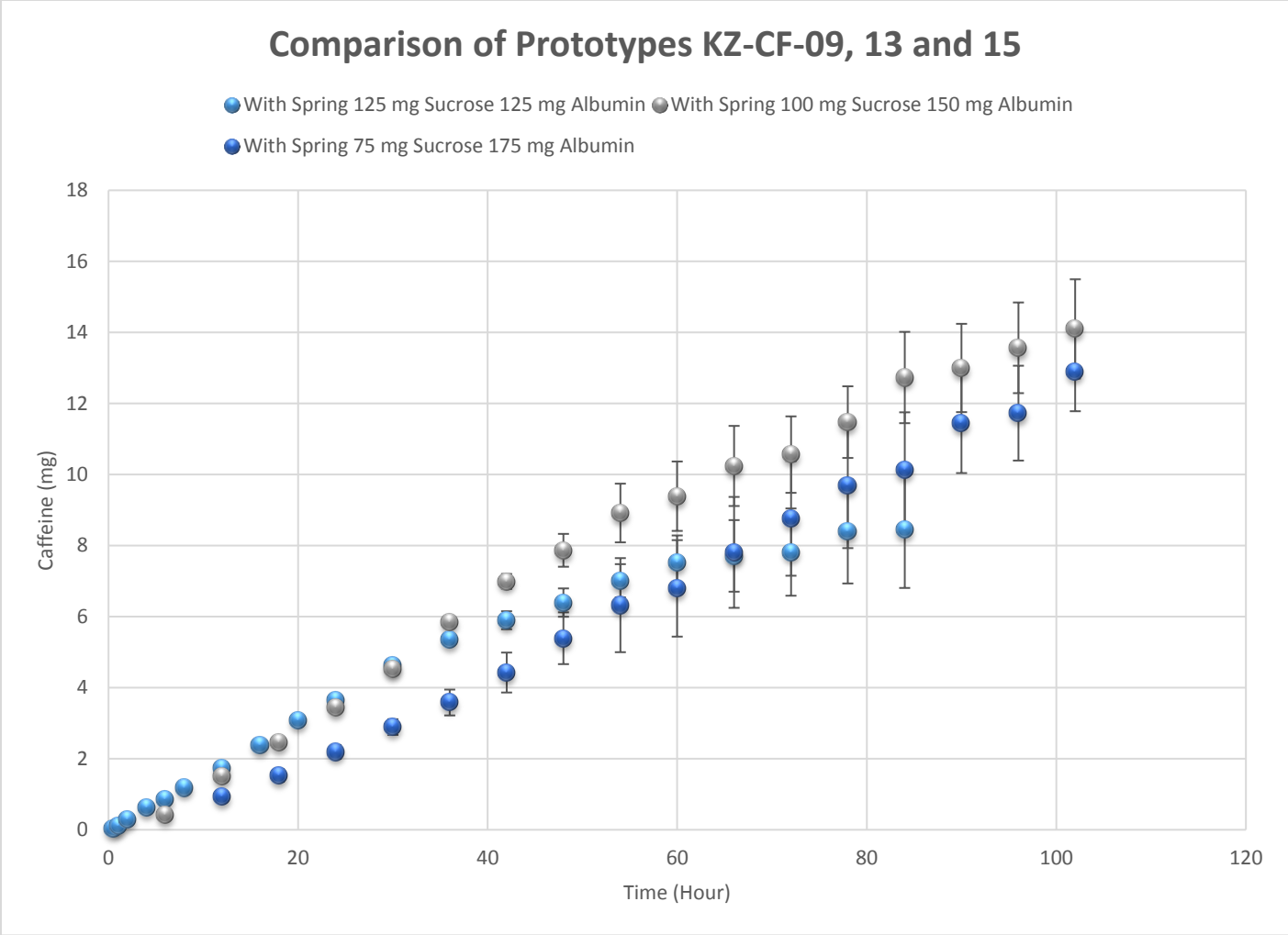

Figure 4.20.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-09, KZ-CF-13 and KZ-CF-11 (KZ-CF-09 Formulation: 125 mg Sucrose, 125 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-13 Formulation: 100 mg Sucrose, 150 mg Albumin, 0.4 µm Membrane, Short Spring; KZ-CF-15 Formulation: 75 mg Sucrose, 175 mg Albumin, 0.4 µm Membrane, Short Spring)

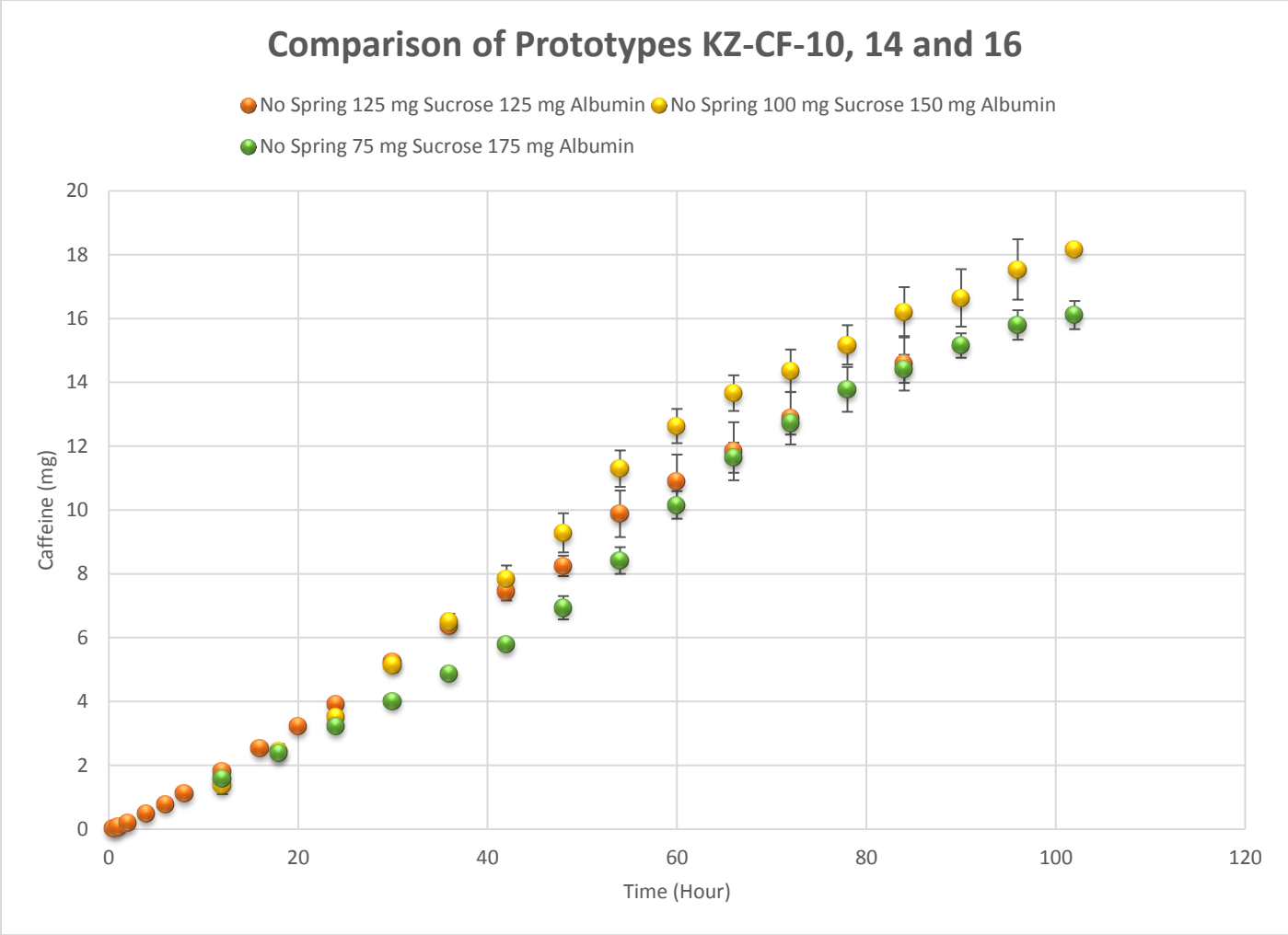

Figure 4.21.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-10, KZ-CF-14 and KZ-CF-16 (KZ-CF-10 Formulation: 125 mg Sucrose, 125 mg Albumin, 0.4 µm Membrane, No Spring; KZ-CF-14 Formulation: 100 mg Sucrose, 150 mg Albumin, 0.4 µm Membrane, No Spring; KZ-CF-16 Formulation: 75 mg Sucrose, 175 mg Albumin, 0.4 µm Membrane, No Spring)

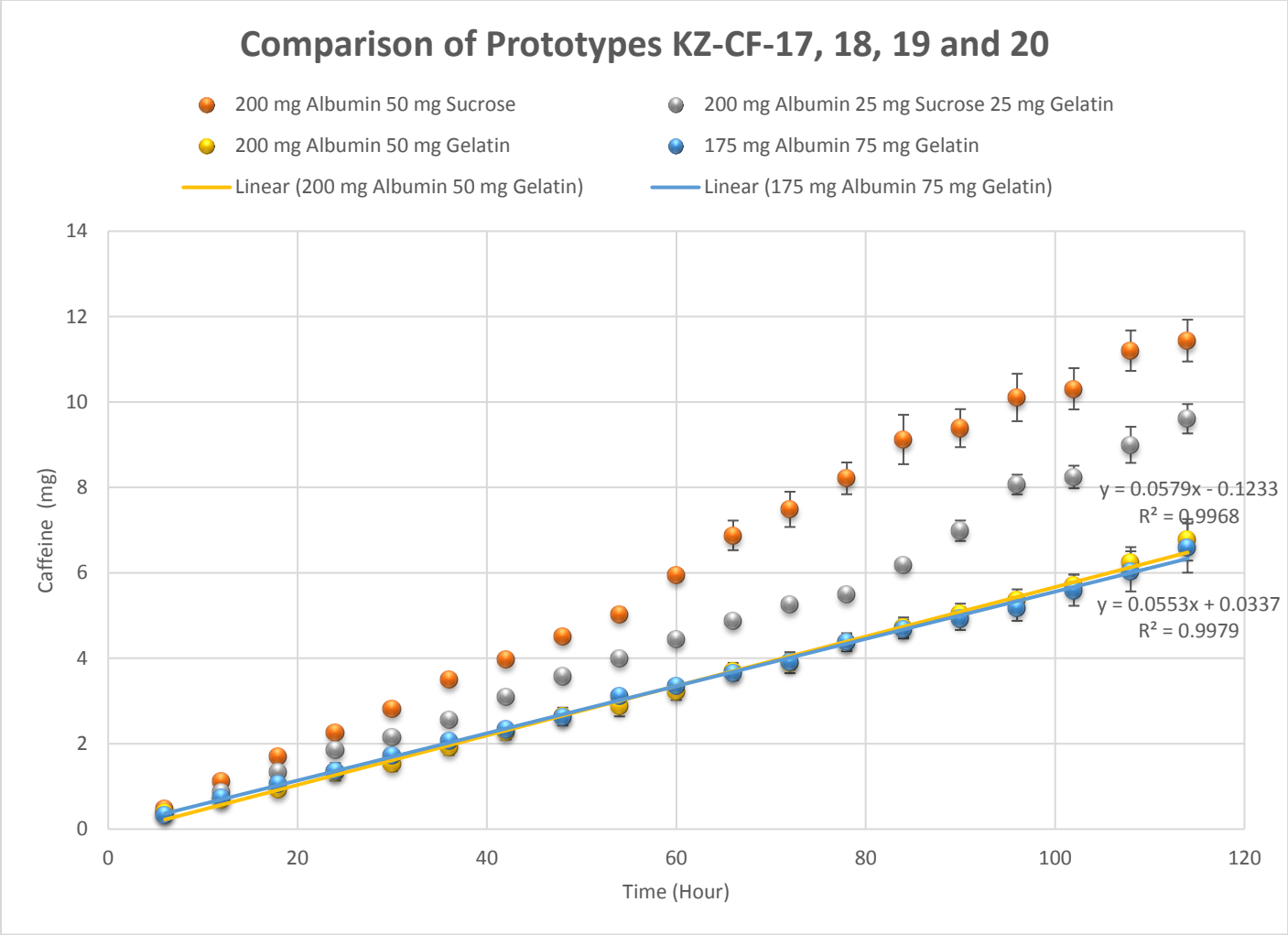

Figure 4.22.: Release Profile of Caffeine in pH 7.4 Phosphate Buffer Saline for KZ-CF-17, 18, 19 and 20 (KZ-CF-17 Formulation: 50 mg Sucrose, 200 mg Albumin, 0.4 µm Membrane, No Spring; KZ-CF-18 Formulation: 25 mg Sucrose, 25 mg Gelatin, 200 mg Albumin, 0.4 µm Membrane, No Spring; KZ-CF-19 Formulation: 50 mg Gelatin, 200 mg Albumin, 0.4 µm Membrane, No Spring; KZ-CF-20 Formulation: 75 mg Gelatin, 175 mg Albumin, 0.4 µm Membrane, No Spring)

4.5.5. Explanation of Drug Releasing Mechanism

Phase I: Buffer Moves into Implant

In this phase, Phosphate Buffer Saline moves into the prototype from dissolution vessel and start to dissolve the very bottom layer of matrix. (Figure 4.23 and Figure 4.24). At the same time, drug concentration inside of the implant start to rise from 0 to highest concentration (C_{max}) based on the drug loading. High drug loading prototypes should have higher C_{max} than low drug loading ones.

Phase II: Dissolution of Matrix

The matrix inside of the implant keep dissolving and the height of dissolved matrix solution keep rising. At this stage, the drug concentration inside of the implant will remain C_{max}. Meanwhile, the diffusion rate for this stage should be zero-order.

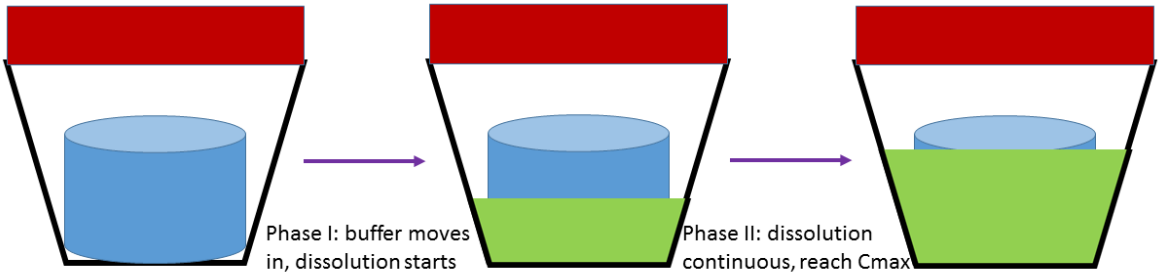

Figure 4.23: Erosion Model of TII Prototype

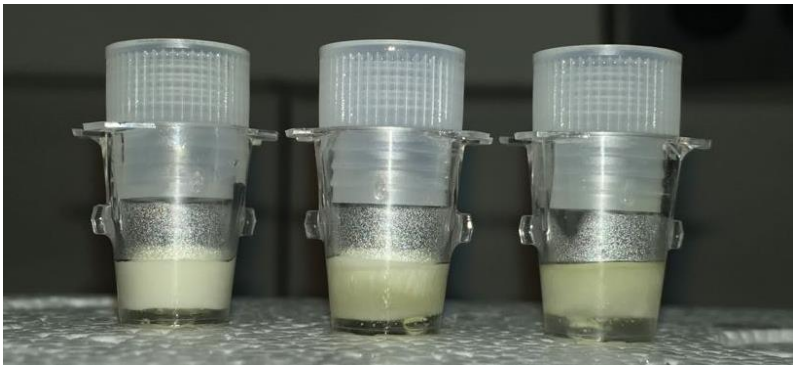

Figure 4.24: Erosion Process of Prototype KZ-CF-17

Phase III: Decrease of Inner Concentration

In this phase, additional buffer would be absorbed into the implant due to the hypertonic environment inside of the implant (Figure 4.25). As a result, the concentration of drug molecules will drop from C_{max} toward a balance concentration. When the concentration for both sides of the membrane reaches the same, drug diffusion will stop.

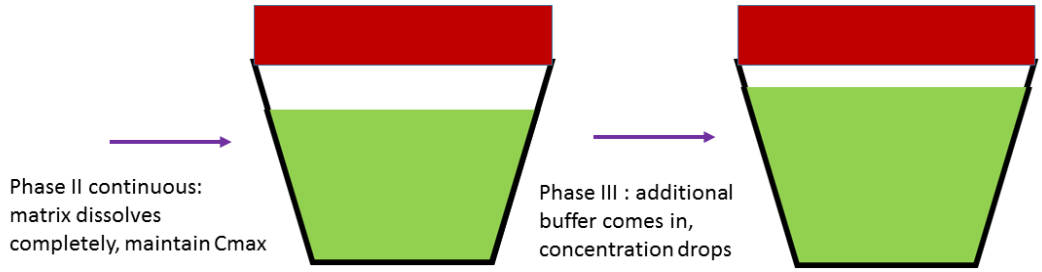

Figure 4.25: Absorption of Additional Buffer after Complete Dissolution

4.6. Conclusion

In this chapter, we hoped to have a thorough understanding of our Tablet-in-Insert (TII) prototype. We chose caffeine as a model drug based on its high water solubility and assay easiness. While testing different porous membranes, we found that solubility of matrix is critical to drug release. In addition, we found that low porosity membranes could provide a better control on drug release. Meanwhile, we added gelatin to further slowdown the dissolution rate and brought in higher viscosity and gelling effect. Finally we achieved our best prototype to control the drug release. This prototype used 0.4 μm cell culture insert as its shell. The matrix was prepared by 175 mg albumin and 75 mg gelatin. The caffeine drug loading is 20 mg. Total releasing rate was 0.055 mg/hour. The duration of zero order drug release is 114 hour.

In this chapter, we could make a conclusion that in our TII prototype, both membrane and matrix matter in drug release. This conclusion is critical to our future application on insulin. Our final goal is to provide a prototype that can change insulin releasing rate after implantation. In order to achieve that, we could either change the matrix composition, which is difficult, or change the open surface area of our membrane. Both ideas need to be investigated in future research.

In addition, we still could not fully understand the drug releasing mechanism. In Figure 4.23 and 4.25, we proposed an explanation of the drug releasing process. However, what if this drug is not very water soluble and form a suspension? Will it still provide zero order release based on the constant Cmax? Can we apply this idea into other drugs and provide the same drug release or different releasing rate? These questions will be discussed and answered in detail in next chapter.

CHAPTER 5

TII PROTOTYPE FOR THE DELIVERY OF SELECTED DRUG MOLECULES

5.1. Abbreviation

TII: Tablet-in-Insert, a name for our innovative prototype

PBS: Phosphate buffered saline

5.2. Introduction

In chapter 4, we performed a detailed study to investigate the drug releasing mechanism of our Tablet-In-Insert (TII) prototype. We concluded that both matrix erosion and membrane controlled diffusion contribute to the releasing rate of drug molecules. In this chapter, we are emphasizing apply our TII prototype to other small molecule drugs as a parenteral dosage form. Currently, many chronic diseases, such as diabetes, kidney malfunction and autoimmune diseases, request long term even lifetime medication. In this chapter, we selected several drug molecules (Table 5.1) to be delivered by our TII prototype. We selected water soluble drugs, medium soluble drugs and slightly soluble drugs. The goal is to understand whether this prototype can provide uniform drug release. If not, what parameter(s) would contribute to the different drug releasing rate? The result of this study would be helpful to further design a prototype for other drug molecules, even unknown compound. We also listed other physical and chemical characteristics in Table 5.1 as references.

Drug	Solubility (mg/mL)	Classification	Disease Apply
Prednisolone	0.223	Steriod	Auto-immune, COPD
Benzoic Acid	0.29	Anti-Bacterial, Anti-Fungal	Infectious Disease
Ibuprofen	0.021	NSAID	Auto-immune
Propranolol HCl	0.0794	β Blocker	Hypertension
Acetaminophen	14	Anti-Fever, Pain-Relief	Pain, Fever
Guaifenesin	16.9	Respiratory Tract Relief	COPD
Metoprolol Tartrate	50	β Blocker	Hypertension

Table 5.1: Selected Drug Molecules for the Releasing Study of TII Prototype

5.3. Materials

BSA, fraction V, 97% pH 7, Lyophilized powder is purchased from Alfa Aesar. Zinc Chloride solution (20%) is purchased from Aqua Solutions, Gelatin, type A, 175 Bloom Powder was purchased from Alfa Aesar. Sucrose (Ultra Centrifugation Grade) is provided by Fisher Biotech. Caffeine Monohydrate was acquired from MP Biomedicals. Guaifenesin, propranolol and ibuprofen are provided by Spectrum Chemicals. Prednisolone and Metoprolol tartrate are provided by TCI Chemicals. Acetaminophen is purchased from Sigma-Aldrich Company.

Deionized water is purified from tap water through Thermal Fisher DI Water system. Corning® cell culture insert (24 well, 8, 3 and 0.4 μm pore size) is ordered from Fisher Scientific. Vankel® 7010 dissolution bath with auto sampler is provided my Agilent Technologies.

5.4. Experimental Methods

5.4.1. High Performance Liquid Chromatography

HPLC with dual wavelength detector. The HPLC methods are listed in Table 5.2. The mobile phase is composed of different percentage of acetonitrile and aqueous buffer (v/v). The wavelength, flow rate and injection volume are all listed in Table 5.2. Column was purchased from Agilent as a 150 mm by 4.6 mm Eclipse® C18 column.

Name	Wavelength (nm)	Flow Rate (ml/min)	Injection Volume (uL)	%Methanol	% Acetonitrile	% Aqueous	pH
Acetaminophen	243	1	10	75		25	7
Benzoic Acid	230	1	10		75	25	3
Guaifenesin	275	1	20		30	70	3
Ibuprofen	224	1	20		60	40	3
Metoprolol	223	1.5	20		25	75	3
Prednisolone	244	1.5	20		40	60	3
Propranolol	208	1.5	10		40	60	3

Table 5.2: HPLC Methods for Different Drugs

5.4.2. Preparation of Blender

In chapter 4, we dissolved all ingredients in 10 ml deionized water to create a uniformly disbursed matrix. However, many of the drugs in Table 5.1 have relatively lower water solubility than caffeine. As a result of that, small batch blending method was used. This technology was provided by Emerson Resources, Inc located in Norristown, PA. Sucrose and albumin were blended and milled by Warning Commercial Blender (Figure 5.1.). After that, all ingredients in Table 5.3 was weighted accurately and put into a 4 inch x 6 inch size plastic bag. The plastic bag was immobilized on Thermo Fisher Vortex. The vortex process took 10 minutes to ensure uniform mix (Figure 5.2). The formulation of different drugs are the same: 5 gram sucrose, 5 gram albumin and 1 gram drug molecules. Each tablet will be around 265 mg to contain 25 mg different drug molecules.

Figure 5.1: Warning® Commercial Blender, Fulfilled by Thermo Fisher

5.4.3. Preparation of Tablet Matrix

The blender from previous step would be separated into different weight boats equally for tablet press. In the process of tablet press, Carver® Lab Press model C was used. Natoli® tablet tooling set was used for punch and die. During tablet press, the pressure was controlled as zero, which means there is no additional application of force to lower the thickness of tablets.

5.4.4. Fabrication of Tablet-in-Insert (TII) Prototype

Corning® cell culture insert was used as the form of implant. Tablet from previous step was put in the insert. The top of insert was covered and sealed by plastic plumps. Substance

should only be available at the membrane end of the insert. Inserts used in this chapter are all 8 μm pore size.

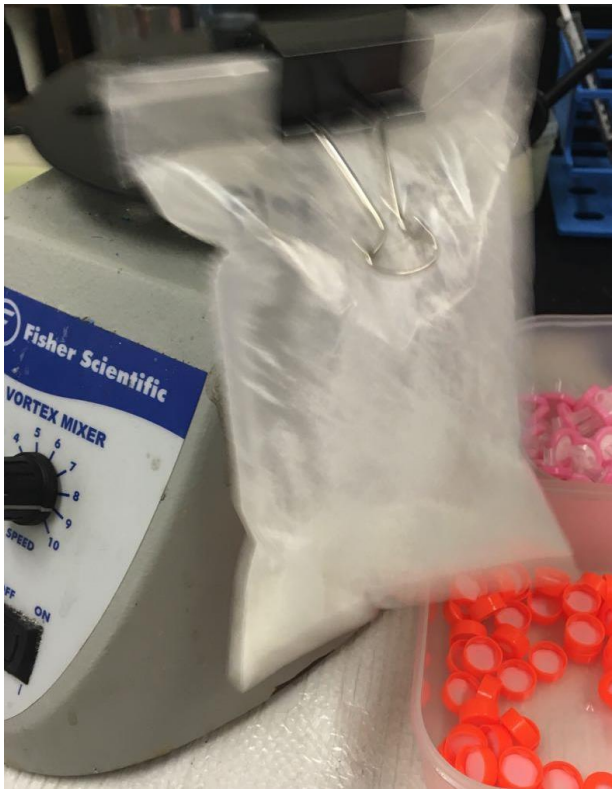

Figure 5.2: Small Batch Blending using Plastic Bag and Fisher Scientific Vortex Mixer (Equipment is turned on during the photographing)

5.4.5. Dissolution and Diffusion Test of TII Prototype

All formulations tested were listed in Table 5.1. We use USP basket apparatus to perform diffusion experiments. In this series of experiments, Vankel® 7010 dissolution bath with auto-sampler was used. In this dissolution test, 1000 ml phosphate buffer saline was used with pH 7.4. PBS was used to mimic human subcutaneous environment. Temperature was controlled as 37 °C. Basket rotation speed was 50 RPM. Auto sampler was set to withdraw 0.5 ml sample every time. The time point of sampling was set to be: 0.5, 1, 2, 4, 6, 8, 16, 20, and 24 (hour).

5.4.6. Data Collection and Analysis

All data is reported as "Average ± Standard Error" with triplicate setting.

5.5. Results and Discussion

5.5.1. Releasing Profile of Different Drugs

Figure 5.4 to Figure 5.11 are releasing profiles of different prototypes. It was observed that most drug molecules showed a burst release for the first three time points (0.5, 1 and 2 hour). In previous chapter, we discussed about the vertical erosion of matrix. It was hypothesized that during the Phase I of dissolution, the drug concentration inside of the implant needs some time to increase from zero to C_{max}. At the same time, the matrix near the membrane dissolved and diffused out faster. This burst of drug release proved our hypothesis.

After the burst release, all drug molecules started to provide zero order drug release. This phenomenon was hypothesized based on the releasing mechanism of this prototype. However, guaifenesin and metoprolol (Figure 5.6 and 5.8) started to decrease the drug release at the time of 20 hours. Considering the water solubility of these two drugs and the amount released are both over 20 mg (80%), we believe the decrease of concentration gradient is the main reason of this curve.

5.5.2. Solubility Effect

All prototypes were fabricated with same drug loading, excipients and membrane. The huge release difference was not fully understood. Table 5.3 is a list of physiochemical characteristics of all drugs tested in this prototype. Water solubility of different drugs are reported by literature. As shown in the table, Acetaminophen, guaifenesin and metoprolol are the most water soluble drugs among all molecules. In addition, these three drugs

provided the highest drug releasing rate (Figure 5.4, 5.6, and 5.8). Metoprolol and guaifenesin's water solubility are 16.9 and 50 mg/mL accordingly. Their release rate from our prototype are 0.8954 and 0.8852 mg/hour accordingly. Acetaminophen provided a 0.7522 mg/hour releasing rate with its solubility as 14 mg/mL. Benzoic acid, ibuprofen and propranolol provided releasing rate between 0.2 mg/hour to 0.3 mg/hour. Their water solubility is below 1 mg/mL. In addition, a cloudy suspension was observed for benzoic acid and ibuprofen prototypes which indicates an incomplete dissolution of matrix (Figure 5.3.). Prednisolone provided the slowest drug release rate among all seven drugs. Generally, the releasing trend follows the water solubility ranking. However, exception occurs and that indicates other factors need to be considered.

Figure 5.3: Cloudy Vision of Prototype for Ibuprofen after 24 Hour Dissolution Study

Name	Solubility (mg/mL)
Acetaminophen	14
Benzoic Acid	0.29
Guaifenesin	50
Ibuprofen	0.021
Metoprolol	16.9
Prednisolone	0.223
Propranolol	0.0617

Table 5.3: Physiochemical Characteristics and Releasing Rate of Selected Drug Molecules without Buffer or Salt Modification

5.5.3. Ionization and pH Effect

Despite the difference in water solubility, acetaminophen, guaifenesin and metoprolol provided similar drug release rate. Considering the effect of membrane porosity, this phenomenon is reasonable. However, Benzoic acid has a 10-fold higher water solubility than ibuprofen, while the release rate is almost the same. Similar trend was observed with propranolol. In addition, prednisolone has similar solubility with benzoic acid, but the release rate is 75% slower. Since all dissolution studies were performed in phosphate buffered saline (PBS) with a pH 7.4, it is reasonable that the ionization of molecules contributed to this difference. Among all four drug molecules mentioned above, prednisolone is the only one that is not charged under pH 7.4. Detailed solubility of drugs under pH 7.4 is listed in Table 5.4. The drug releasing rate follows the trend as solubility.

Drug	Release Rate (mg/Hour)	Solubility (mg/mL)	Charge at 7.4	Charge Unit	pKa Type	pKa
Prednisolone	0.0517	0.223	0	0	Acidic	12.58
Benzoic Acid	0.2109	3.4	-1	1	Acidic	4.08
Ibuprofen	0.2492	3.91	-1	1	Acidic	4.85
Propranolol	0.2537	10	+1	1	Basic	9.42
Acetaminophen	0.7522	14	0	0	Acidic	9.46
Guaifenesin	0.8852	50	0	0	Acidic	13.62
Metoprolol Tartrate	0.8954	50	+1	1	Basic	9.67

Table 5.4: Physiochemical Characteristics in pH 7.4 of Selected Drug Molecules with Salt Modification

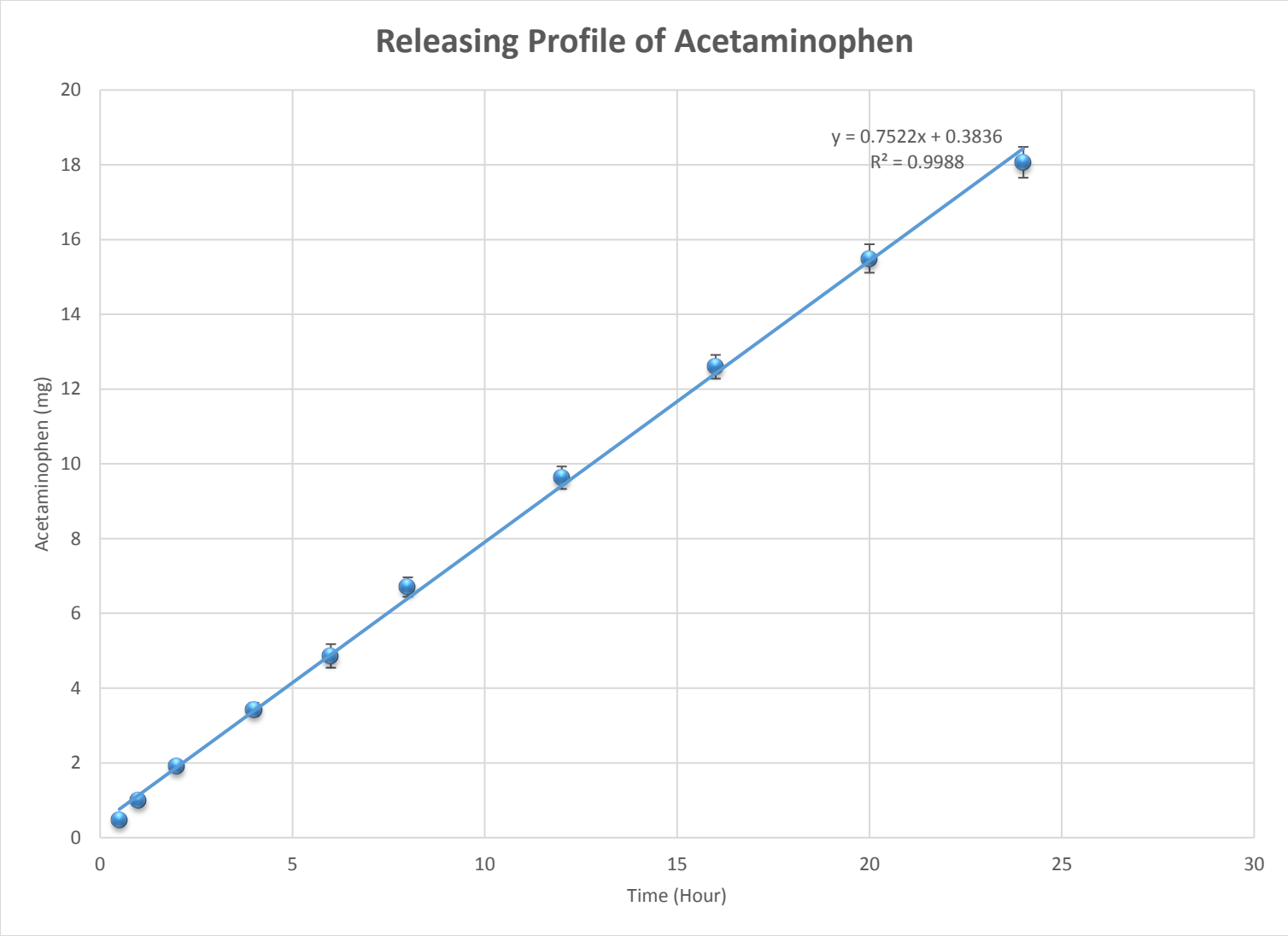

Figure 5.4: Release Profile of Acetaminophen in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Acetaminophen, 8 µm Membrane)

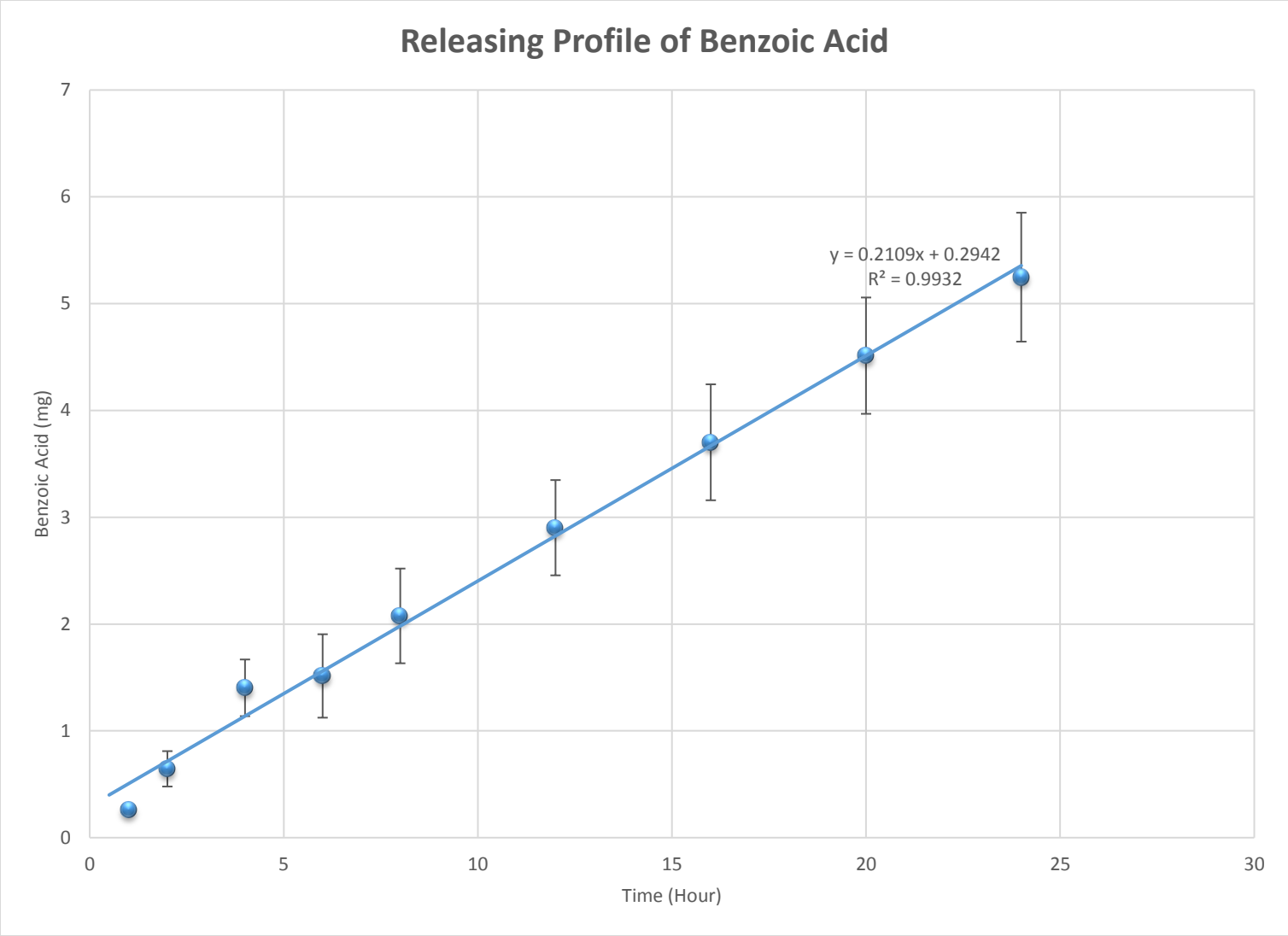

Figure 5.5: Release Profile of Benzoic Acid in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Benzoic Acid, 8 µm Membrane)

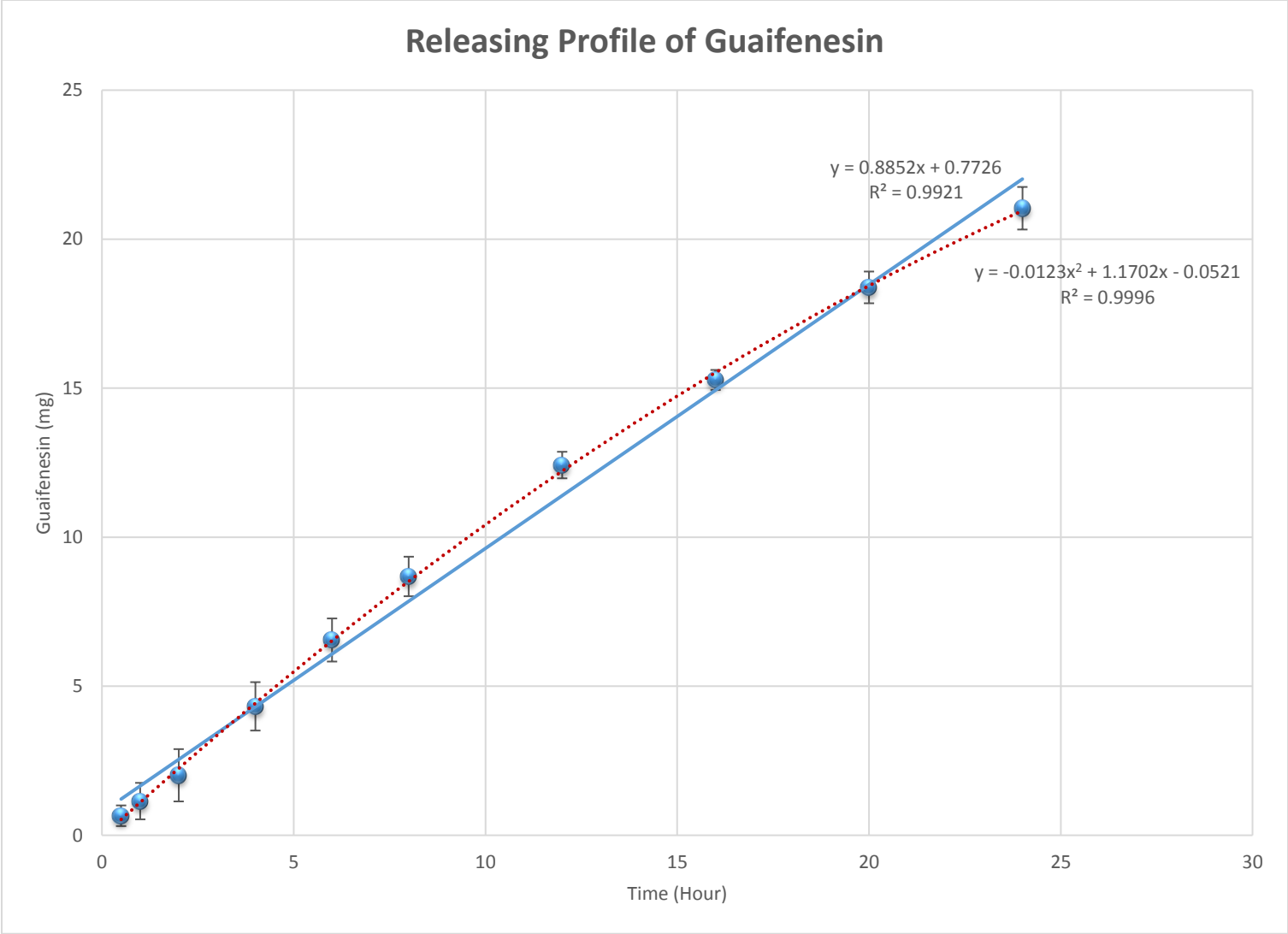

Figure 5.6: Release Profile of Guaifenesin in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Guaifenesin, 8 µm Membrane)

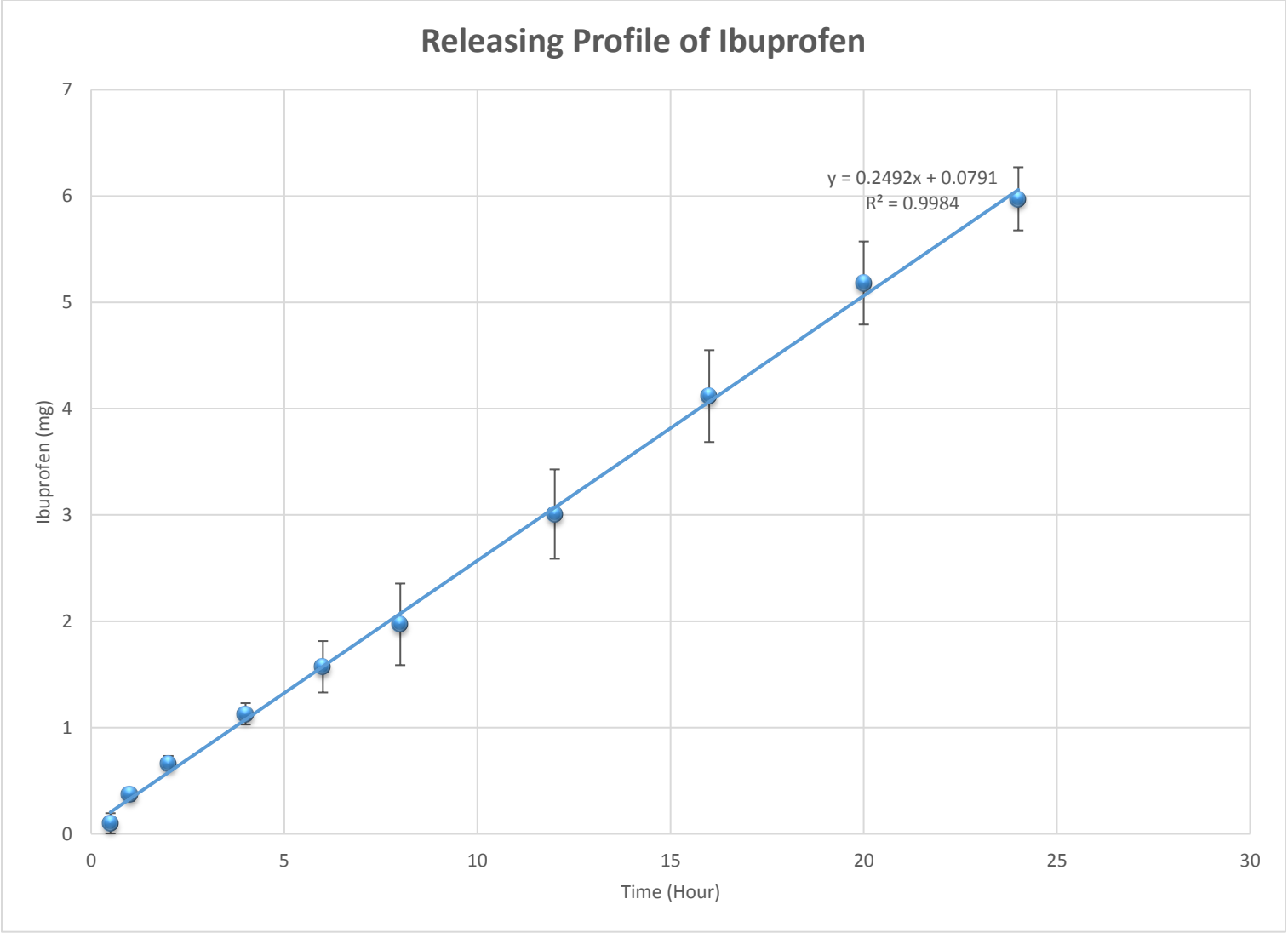

Figure 5.7: Release Profile of Ibuprofen in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Ibuprofen, 8 µm Membrane)

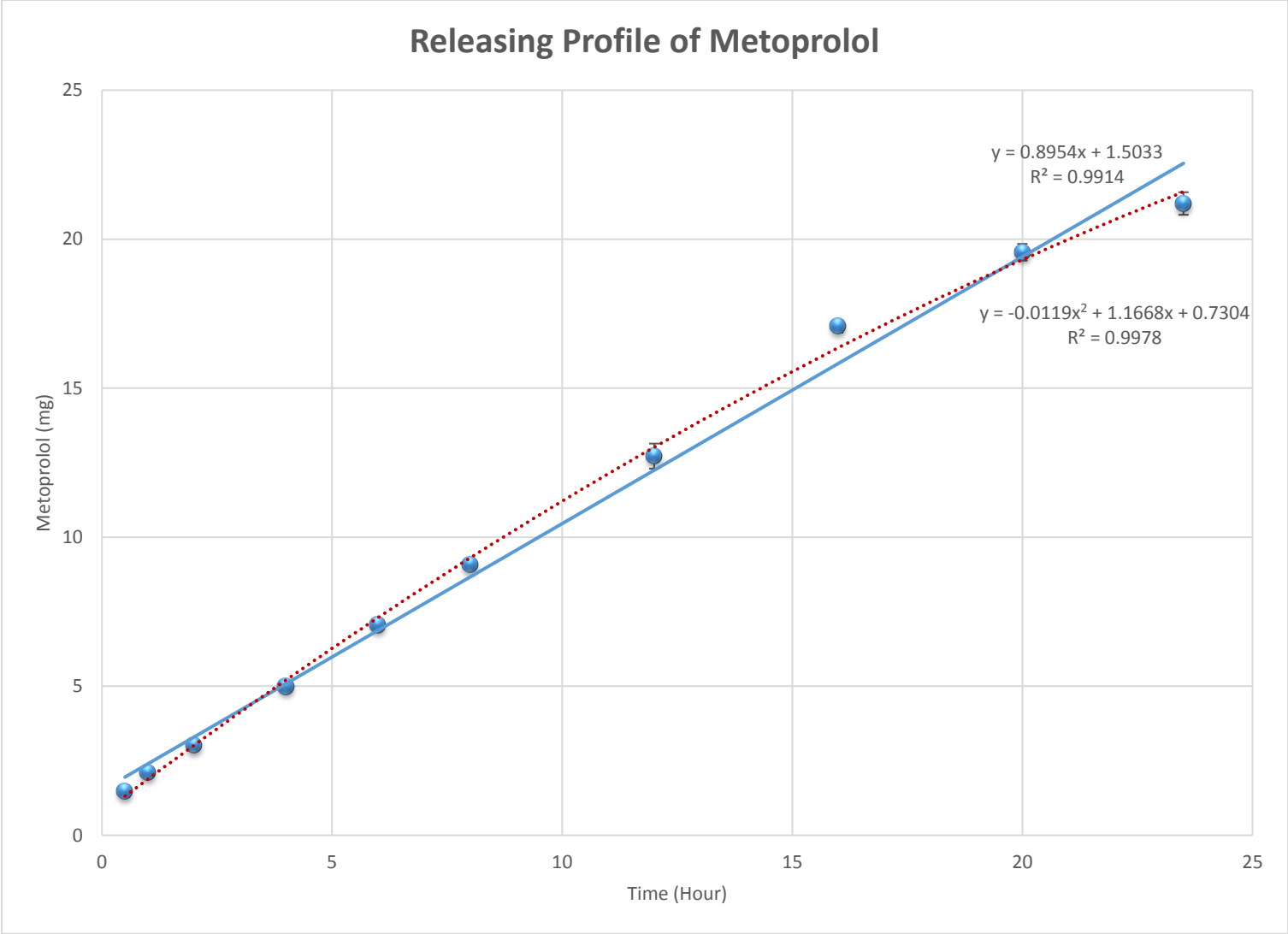

Figure 5.8: Release Profile of Metoprolol in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Metoprolol Tartrate, 8 µm Membrane)

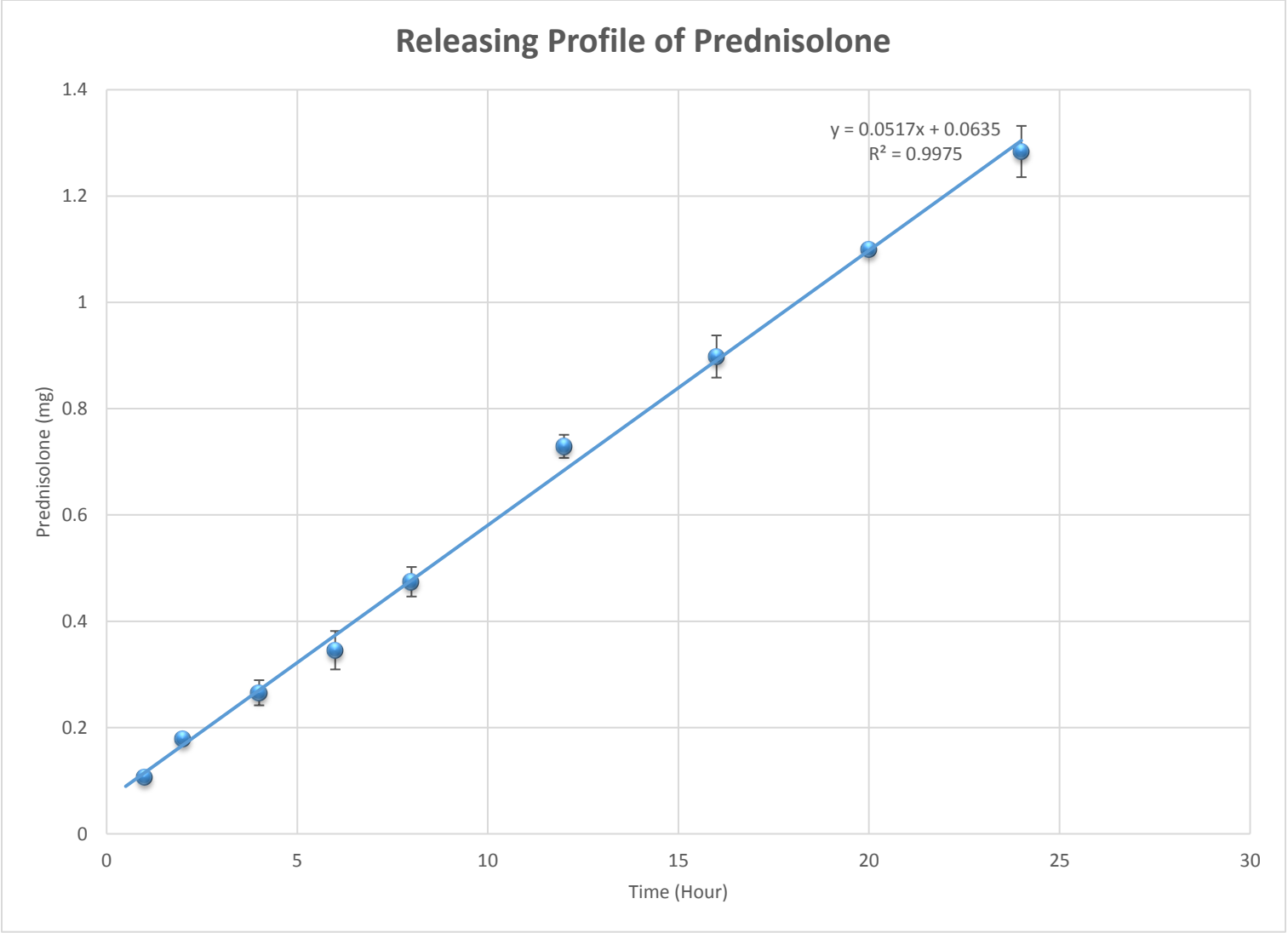

Figure 5.9: Release Profile of Prednisolone in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Prednisolone, 8 µm Membrane)

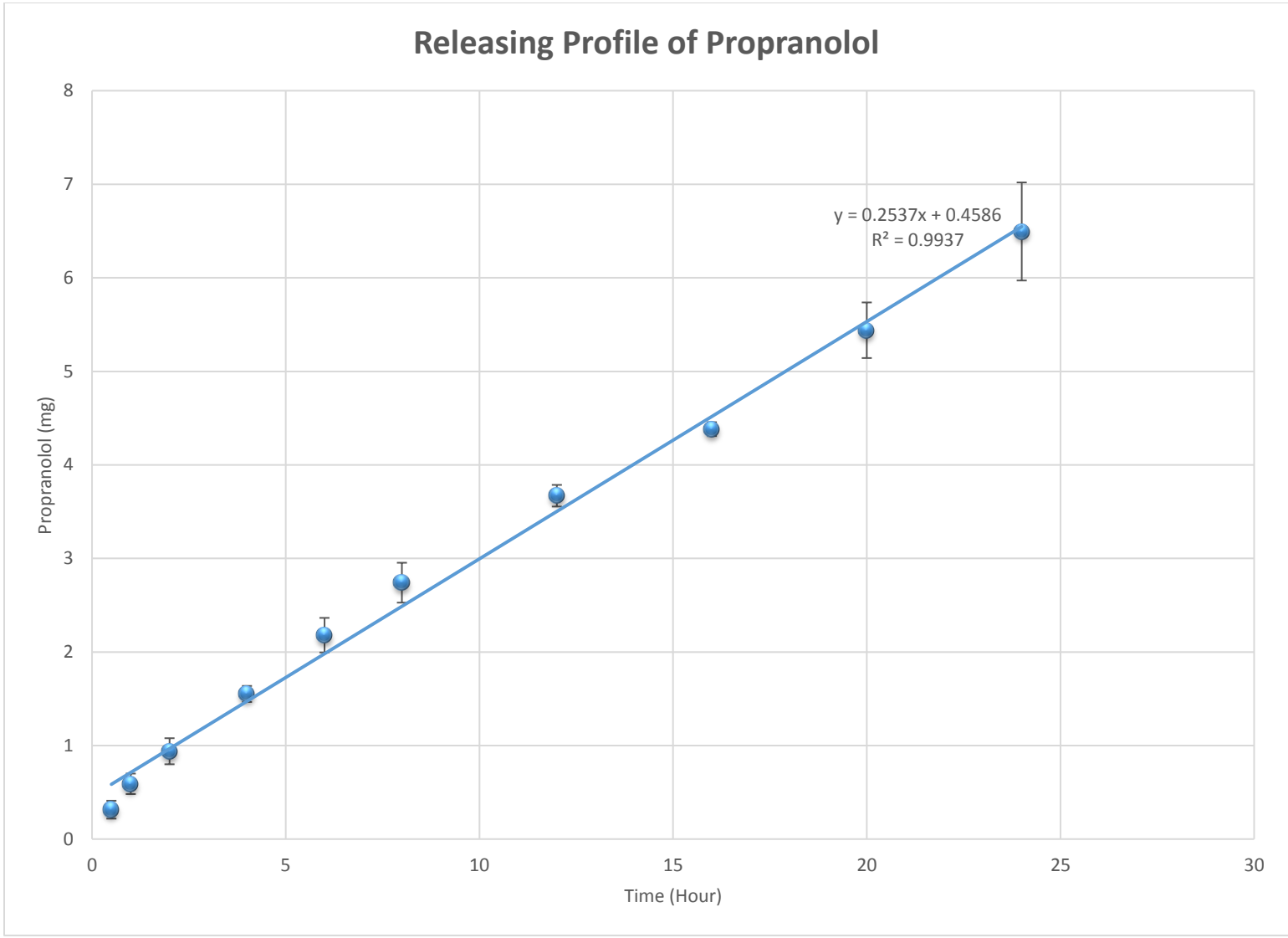

Figure 5.10: Release Profile of Propranolol in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Propranolol, 8 µm Membrane)

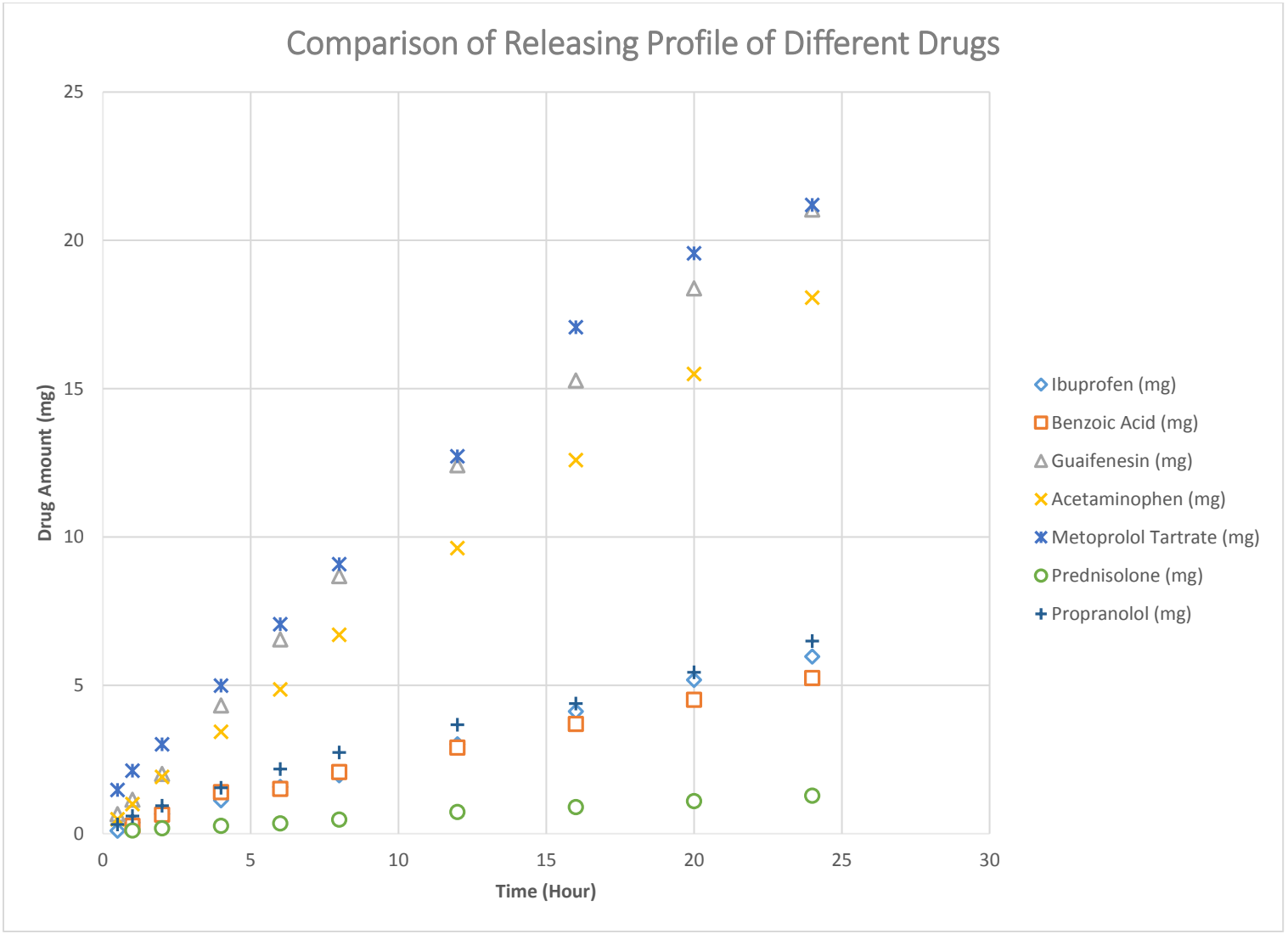

Figure 5.11: Release Profile of Different Drugs in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Drugs, 8 µm Membrane)

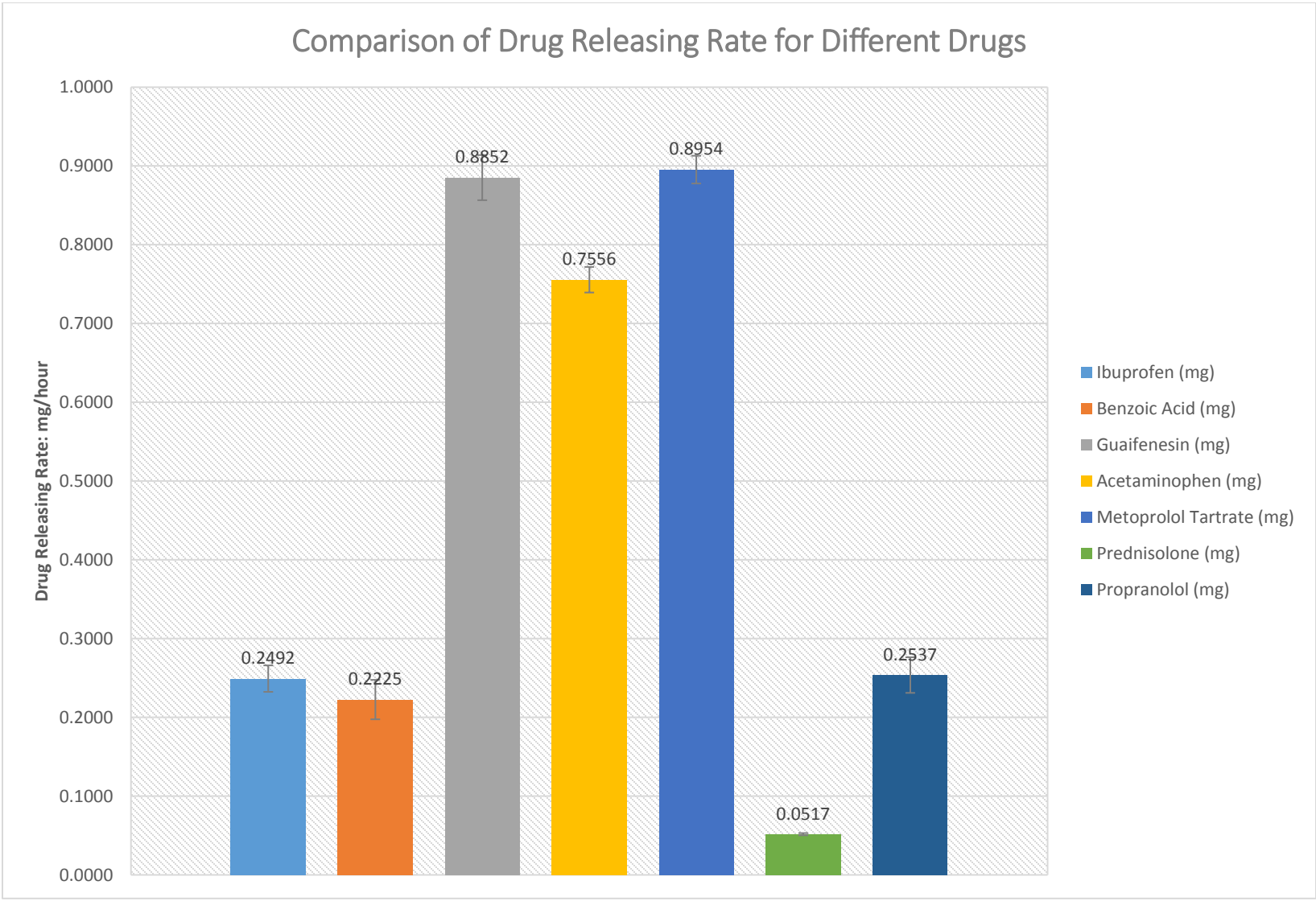

Figure 5.12: Release Rate of Different Drugs in pH 7.4 Phosphate Buffer Saline (Formulation and Fabrication: 125 mg Sucrose, 125 mg Albumin, 25 mg Drugs, 8 µm Membrane)

5.5.4. Adjusted Explanation of Drug Releasing Mechanism

Phase I: Buffer Inflow

In this phase, Phosphate Buffer Saline moves into the prototype from dissolution vessel and start to dissolve the very bottom layer of matrix. At the same time, drug concentration inside of the implant start to rise from 0 to highest concentration (C_{max}) based on the drug loading and drug water solubility. Drug with higher water solubility should have higher C_{max} than the ones with lower solubility (Figure 5.11 and Figure 5.12). Meanwhile, less water soluble drugs may form suspension due to the oversaturation inside of the implant.

Phase II: Dissolution of Matrix

The matrix inside of the implant keep dissolving and the height of dissolved matrix solution keep rising. At this stage, the drug concentration inside of the implant will remain C_{max}. Hence, the diffusion rate for this stage should be zero-order. Visual change of the matrix should be observed. Drugs with high water solubility would form transparent solution, while drugs with low water solubility would form cloudy suspension (Figure 5.12).

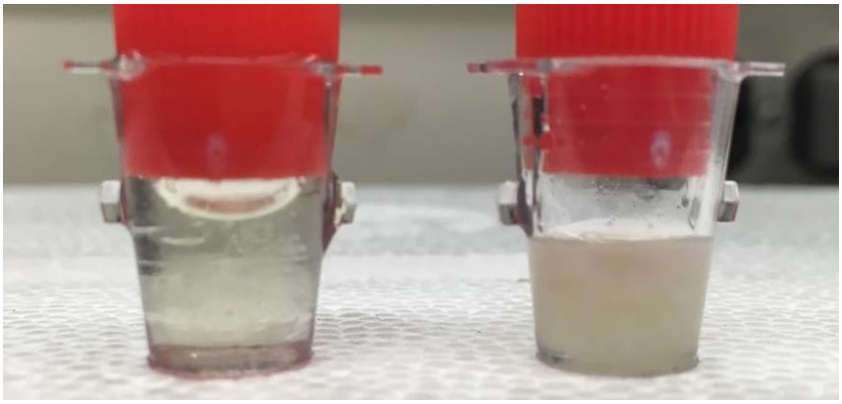

Figure 5.13: Appearance of Prototype for Guaifenesin (Left) and Benzoic Acid (Right) after 24 Hour Dissolution Study

Phase III: Decrease of Inner Concentration

In this phase, additional buffer would be absorbed into the implant due to the hypertonic environment inside of the implant. As a result, the concentration of drug molecules will drop from C_{max} toward a balance concentration. When the concentration for both sides of the membrane reaches the same, drug diffusion will stop. The timing of phase III is different based on drugs' water solubility. Drugs with lower water solubility should maintain longer period of time of phase II while those with higher solubility usually reach phase III faster. A height difference was observed between Acetaminophen and prednisolone as well as transparency in Figure 5.13.

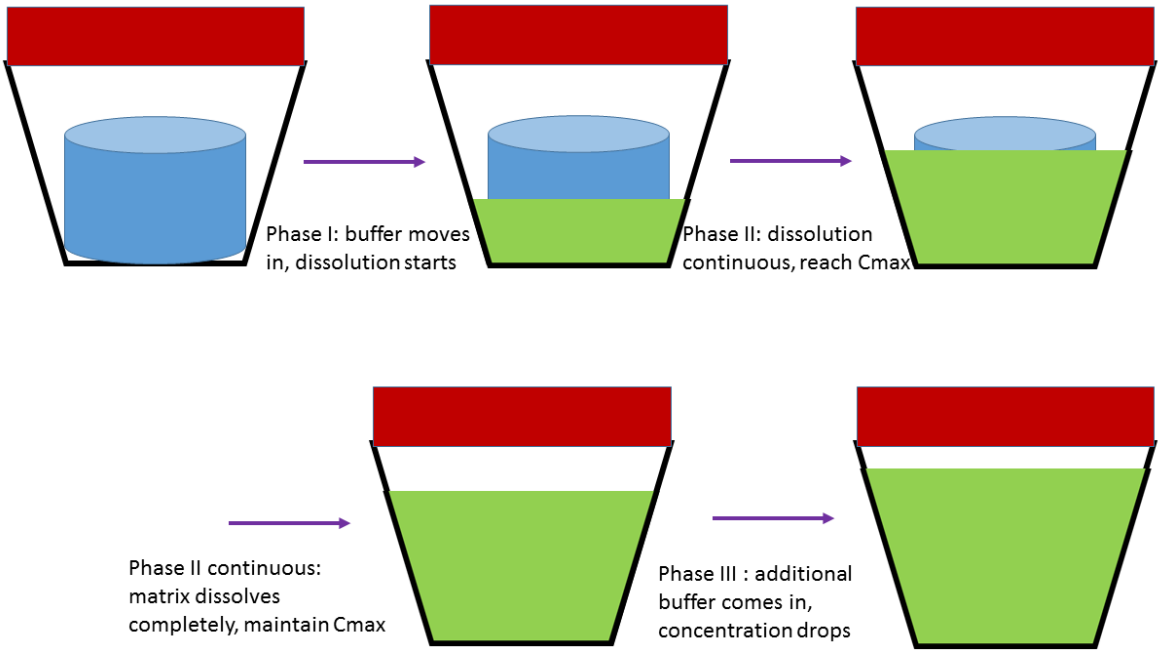

Figure 5.14: Three Phase Model of Drug Release from TII Prototype

5.5.6. Guidance for Long Term Drug Release

In Chapter 4, our TII prototype successfully provided a five day drug release with zero order kinetics for caffeine. In this chapter, we selected 7 drugs with different solubility, pKa and protein binding to expend the usage of our TII prototype. In this section, we would like to provide some general guidance for long-term controlled release of different drugs.

i. High water soluble drugs: caffeine, guaifenesin, metoprolol, acetaminophen, etc.

High solubility drugs dissolve quickly in PBS. Hence, strong membrane control is the priority. In our TII prototype, 0.4 μm membrane is recommended based on its low porosity. In addition, low solubility matrix is recommended. In Chapter IV, we use gelatin and albumin as our final matrix. Gelatin can provide high viscosity gel matrix at human body temperature, while albumin can bind drug to provide a uniform matrix.

ii. Slightly soluble drugs: benzoic acid, ibuprofen, propranolol, etc.

This group of drugs usually suspend inside of our TII prototype. We recommend using 3 and 8 μm membrane based on its high porosity. The matrix usually dissolves very quickly while drug molecules will for suspension. As a result of that, undissolved drug molecules will serve as a reservoir system. The drug concentration will be maintained at Cmax before complete dissolution. For these type of drugs, high sucrose matrix is recommended for its fast dissolution. However, a detailed study is needed for specific drug molecule to ensure zero order drug release.

iii. Almost insoluble drugs: prednisolone, econazole, etc.

This group of drugs should be formulated and fabricated same as slightly soluble drugs. However, protein binding effect may contribute to faster drug release if using high content albumin matrix. A screening study is needed to determine the matrix composition using sucrose and albumin. Gelatin is not recommended unless protein binding is very helpful to increase drug release.

iv. Maintain zero order release

It is believed that for membrane controlled drug release, the release profile would maintain zero order until more than 80% of loaded drug is released. After that, drug release would decrease and a curve profile should be expected.

5.6. Conclusion

In this chapter, we expend the usage of our TII prototype to 7 other small molecule drugs. All of the 7 drugs follow the general drug release procedure provided in Chapter 4. A burst drug release was observed for the first 2 hours; a zero order drug release was maintained between 2-20 hours; and highly soluble drugs decrease drug release after releasing 80% drug loading. A long term release prototype is feasible for these seven drugs based on the design of matrix and membrane.

CHAPTER 6

ELECTRICAL CONTROLLED TII PROTOTYPE FOR THE DELIVERY OF INSULIN WITH CLINICAL EFFECTIVENESS

6.1. Introduction of Insulin Needs

6.1.1 Type I Diabetes

According to Lexi-comp®, initial insulin titration should start with 0.2 to 0.6 units/kg/day in divided doses. Conservative titration should focus on 0.2 to 0.4 units/kg/day to avoid the potential for hypoglycemia. For initial titration, only fast-acting or short-acting insulin should be used. Intermediate and Long-acting insulin is not recommended.

Maintenance insulin titration should control dose from 0.5 to 1 units/kg/day in divided doses. People without obesity is recommended to dose between 0.4 to 0.6 units/kg/day while people with obese should increase dose to 0.8 to 1.2 units/kg/day.

Human needs more insulin during and after meal to decrease glucose level back to normal. Usually, 50% to 75% of the total daily dose (TDD) is given as a basal level using intermediate-acting or long-acting insulin. The remaining portion of the TDD is administered before or at mealtimes as bolus level. Bolus level insulin is usually short-acting or fast-acting insulin.

If we set a normal patient's weight to be 80 kg, this patient will need approximately 16 to 32 units/day for initial conservative titration, and 32 to 48 units/day for maintenance. That equals to 0.67 to 1.5 units/hour and 1.5 to 2 units/hour accordingly. As a result of that, 0.67 to 2 units/hour is our target drug delivery rate.

6.1.2. Type II Diabetes

Type II diabetes is different from type I. The goal of treatment is to control HbA_{1c} <7%. HbA_{1c} is the shortcut for glycated hemoglobin. The dose for type II patients is dependent on the disease condition. However, most type II patients do not need bolus level insulin during or before meal time. As a result of that, type II patients only need basal insulin for treatment.

6.2. Introduction of Current 3-D Printing Technology

3-D printing technology was firstly invented back in 1986 by Dr. Chuck Hull. 3-D printing can be understood as an extension of normal printing, which is 2-D printing. 2-D printing print ink on paper as its function, while 3-D printing can print several materials in three dimensional scale. 3-D printing can print many models, cars, human organ, pharmaceutical dosage forms and many other solid shape products.

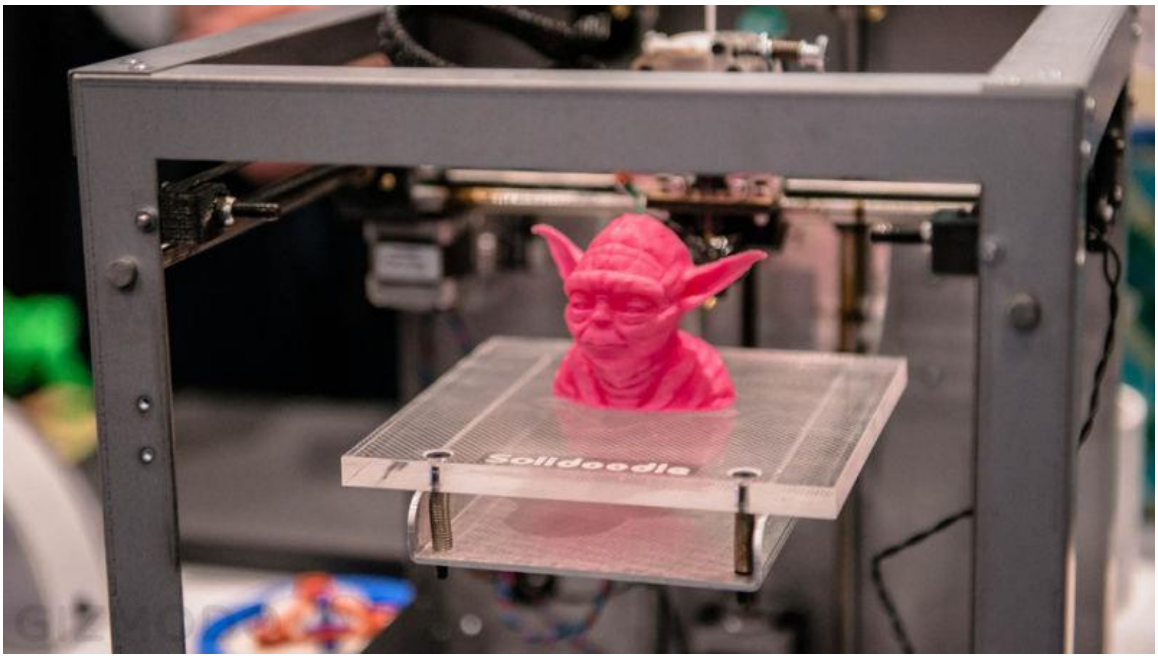

Figure 6.1: 3-D Printing of a Toy Model

6.2.1. General Principle of 3-D Printing

The modeling of printing is the first step. Programmers need to draw the modeling in software layer by layer. The software will control printer to print in a certain direction. Usually it is along Z axil. The printers would print out layers by liquid, powder or pellets. Different layers would stick together by glue or adhesive compounds. The image resolution in DPI is calculated in μm level. This resolution is small enough in most cases. The product after printing usually needs modification or polishing as the last step.

6.2.2. Application of 3-D Printing

3-D printing is used in many fields. On July 1st of 2017, the United States navy used some parts for their ship craft made from 3-D printing. The goal is to reduce the cost of maintenance and repair of military boats. In September of 2016, NASA assembled the telescope from 3-D printing parts. Back in August 2016, NASA did a test to use 3-D printing parts for rockets assembly. This idea came from a British group's accomplishment to print out a small rocket back in 2014. In Russia, a company printed out a small drone.

3-D printing is not designed for military only. It has makes a huge difference in health care. On July 8th of 2015, a Japanese research group successfully printed a human liver model. On August 28th of 2014, a group of Chinese physicians printed a human skull for an injured patient. In the same month, another group of Chinese physicians in Beijing successfully printed vertebral column with very good biocompatibility. On October 13th of 2014, New York physician Dr. Emile Bacha printed a human heart to save an infant's life.

In pharmaceutical industry, 3-D printing also makes contribution. Aprecia pharmaceuticals patented their 3-D printing technology named ZipDose. Their oral disintegration tablet got approved by FDA to launch in the market. The extreme short disintegration time of this

tablet comes from the built-in porosity during 3-D printing. More importantly, 3-D printers may become an alternative in pharmacy store. Patients may bring in their prescription to the store and pharmacists can just turn on the printer to produce drug on site.

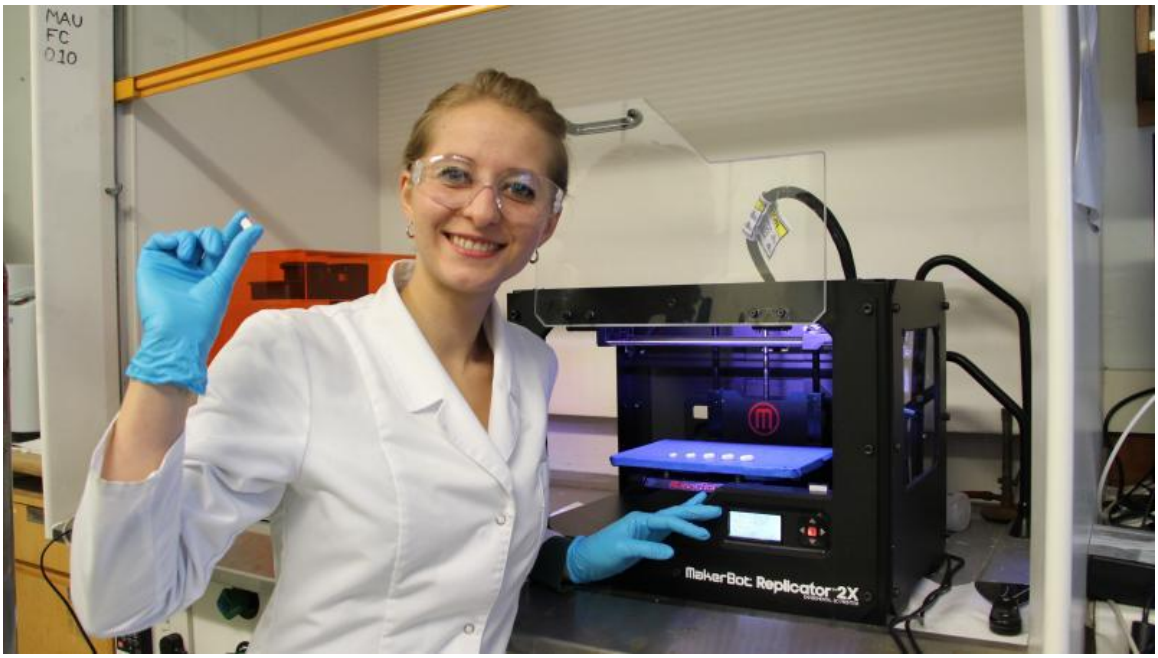

Figure 6.2: 3-D Printing of Theophylline Tablet (Britain's University of Central Lancashire)

6.3. 3-D Printing of Subcutaneous Implant

A subcutaneous implant designed in previous chapters contain several parts:

- A non-permeable tubing body
- A semi-permeable membrane tubing body
- A matrix with drugs and other excipient
- A electronic unit with mechanical control

A non-permeable tubing body will function as a container of matrix. The most ideal material for this part is silicone tubing. Silicone tubing is a flexible and biocompatible material. It can bend, twist and squeeze without damaging the structure. In addition,

silicone tubing may cause minimum or no inflammation. Silicone tubing is not permeable for water or human body fluid. As a result of that, silicone tubing may protect drug matrix from erosion of human body fluid.

A semi-permeable membrane tubing body is designed for drug release. Porous membrane with ideal porosity and pore size will be used for this part. Ideal materials to print membrane include polyethylene terephthalate (PET), regenerated cellulose, cellulose ester and polycarbonate.

The drug matrix will contain insulin, human albumin and sucrose. The matrix will be mixed uniformly in water as a concentrated solution. An electronic control of mechanical valve was printed along with membrane. The valve could be controlled to block partially or the whole membrane. As a result, drug release would be strictly controlled by this valve.

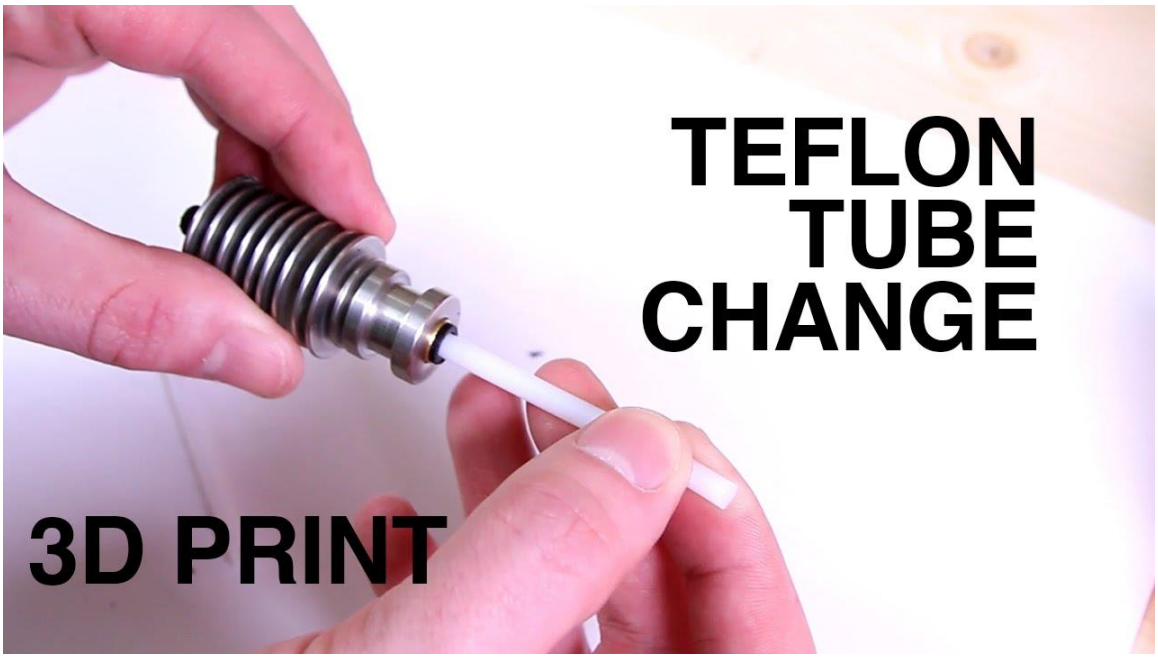

Figure 6.3: 3-D Print of Teflon Tube by Josef Prusa from YouTube

6.4. Implant Functioning Condition and Related Releasing Profile

Most of current commercial implants are cylinder shaped. Cylinder shape implant is easy to insert and may provide a better compatibility in subcutaneous tissue. Human muscle movement usually does not influence the implant. Nexplanon® and Zoladex® have similar 3-D dimension. The length of implant is between 40 to 50 mm, while the diameter is between 2 to 3 mm. Total volume of implant could be 350 mm^3 with 485 mm^2 surface area. Our Falcon cell culture implant has a surface area as 300 mm^2. With all these information, I decided to design our implant with 150 mm^2 surface area for basal insulin release, 150 mm^2 for bolus insulin release, and 150 mm^2 for non-permeable tubing, and 35 mm^2 for electronic controlling module.

Figure 6.4 is the design of this implant. As shown in this picture, the red circle represents the electronic controlling module. The blue part means the non-permeable tubing made of silicone. Silicone is biocompatible with minimum inflammation. The yellow part with large porous dashes is the high porosity/drug releasing section. This section is made of 3 μm membrane. The estimated release rate is 4 units/hour. The green part with small porous dashes is the low porosity/drug releasing section. This section is made of 0.4 μm membrane from Falcon® cell culture insert. The estimated release rate is 1.5-2 units/hour.

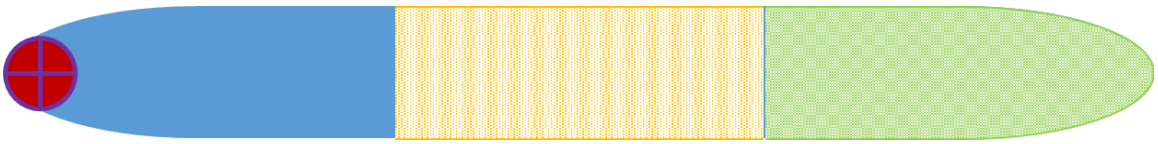

Figure 6.4: Design of Subcutaneous Implant

6.5. Electrical Controlling of Implant

The red circle is the electronic controlling unit to control the 3 μm membrane. This part is designed to control the surface area of tubing to change the drug release.

6.5.1. Electrical Actuation Membrane

Jeon et al reported their innovation in electrical control in membrane actuation. Jeon use weak electricity to open and close membrane pores in nm and μm level. Figure 6.5 is the experimental result of their membrane. As you can see from Figure 6.5, Jeon's team could enlarge the pore size with electrical pressure. After they removed the electrical pressure, the pores reset back to original state. The drug diffusion rate proved by them is also an evidence of this achievement. Figure 6.6 is the SEM images of membrane. A very clear enlarge process was observed from microscope. Similar technology has been reported by other research groups. Jiang et al reported other methods might be used for membrane actuation, including heat, light, electricity and magnetic field. Harris et al reported biocompatible silicone based membrane actuated by electricity back in 1998. However, actuation of membrane requires specially fabricated membrane. Ordering such membrane in large scale could be expensive to afford.

With the budget consideration, we decide to seek a more feasible method to fabricate our prototype, but external actuation of membrane worth consideration in industry level manufacturing.

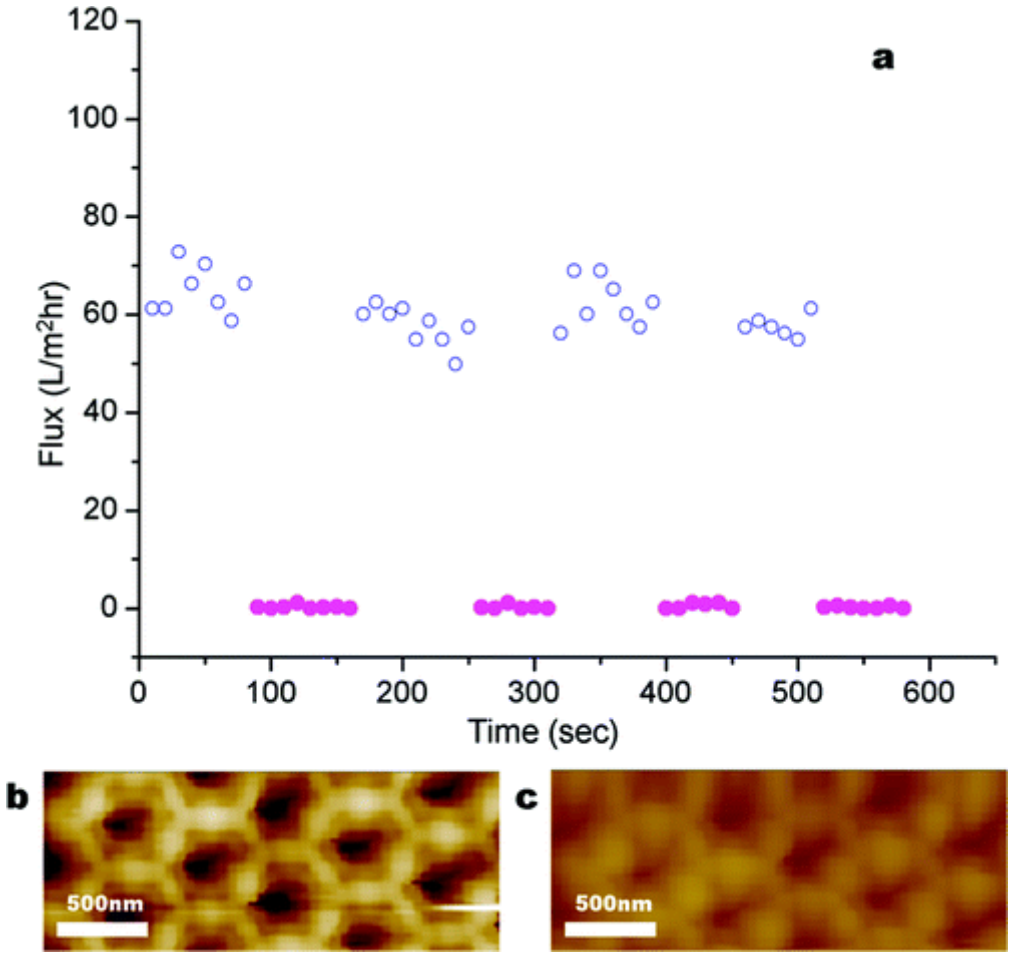

Figure 6.5. (a) Flux versus time for a membrane with a pore diameter of 110 nm at the oxidation (blue open circles) and the reduction state (magenta closed circles). A data point was collected every 10 s. (b,c) In-situ AFM height images corresponding to the oxidation and the reduction states, respectively[102].

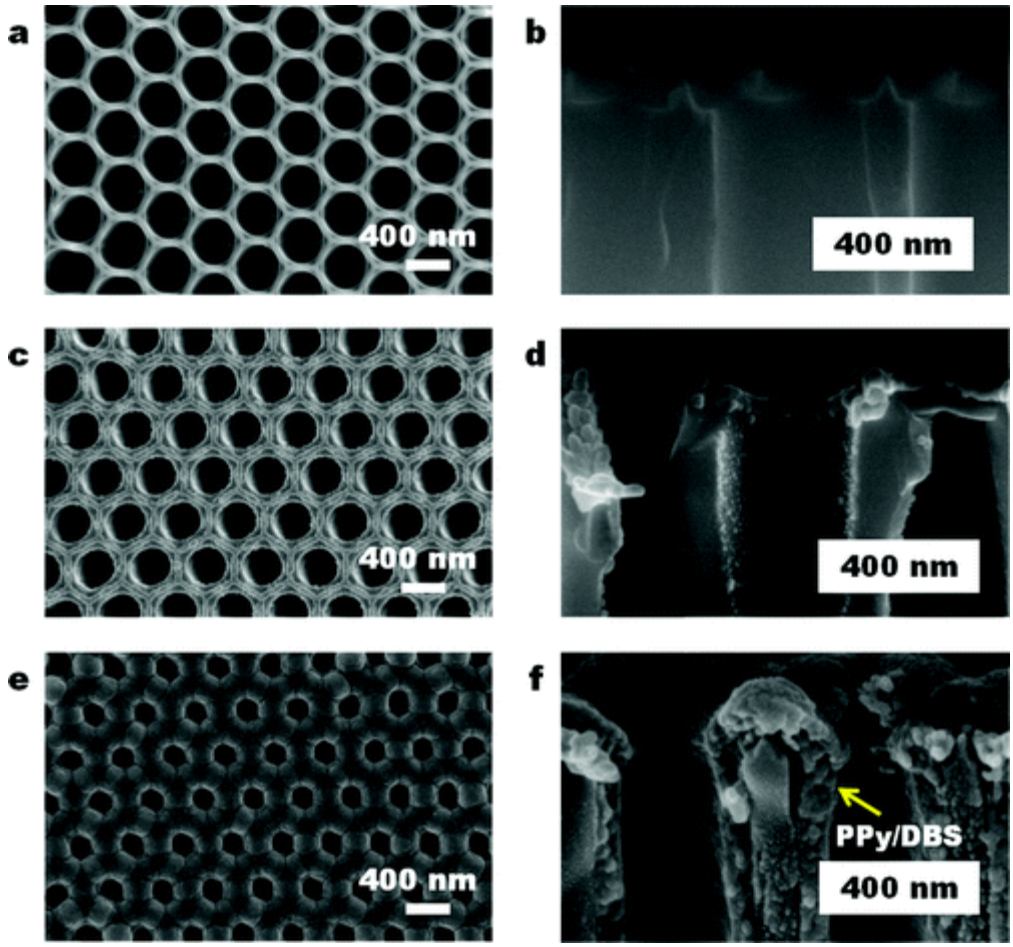

Figure 6.6: Top (left panel) and cross-sectional (right panel) SEM images of the membranes at various fabrication steps. (a,b) AAO membrane. (c,d) Gold deposited membrane. (e,f) After electropolymerization of PPy/DBS for 60 s[102].

6.5.2. Solenoid and Mechanical Arm

Solenoid is a cylinder-shape electromagnets. According to Ampere's law, electric current creates magnetic field around the wire. If the wire forms a loop shape, the magnetic field will follow a natural magnet. Figure 6.7 is a picture of how electromagnet forms magnetic field. Figure 6.8 is a working mechanism of solenoid. As we can see in Figure 6.8, a solenoid is one electromagnet connected with a sprint reset mechanical arm. Solenoid arm will move when the electricity is applied, and reset based on the spring's force without electricity. Solenoid is relatively cheaper and we decided to add it into our prototype.

The working mechanism of solenoid provides us a feasible method to fabricate our implant with a preset switch to change the porosity.

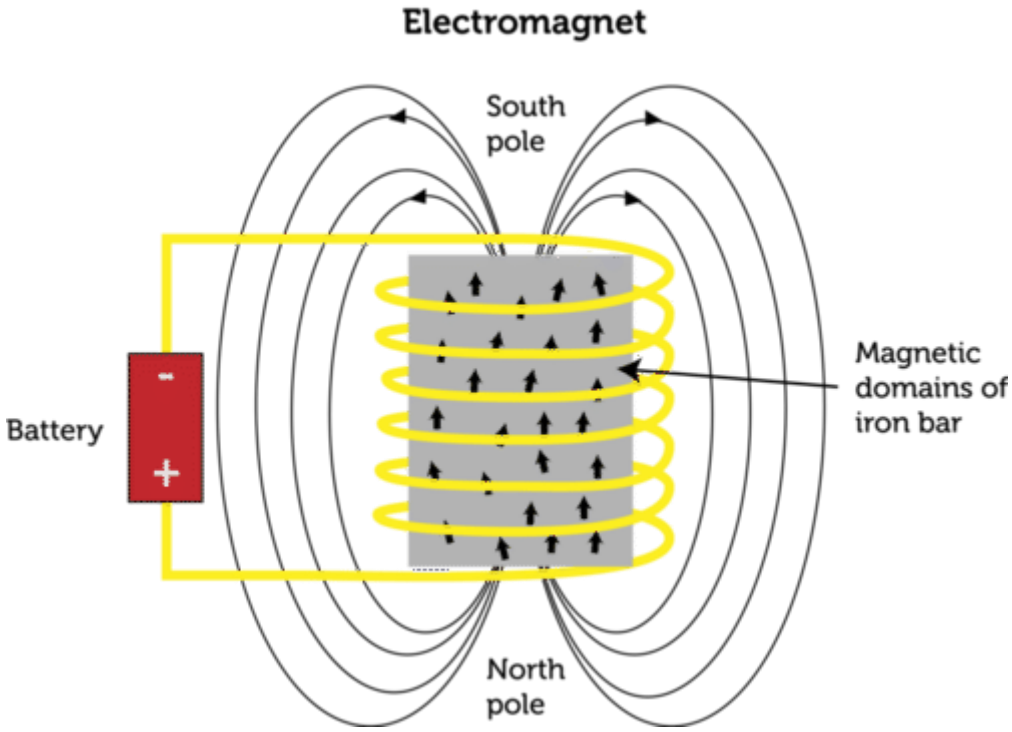

Figure 6.7: Working Mechanism of Electrical Magnet (CK-12 Foundation Org)

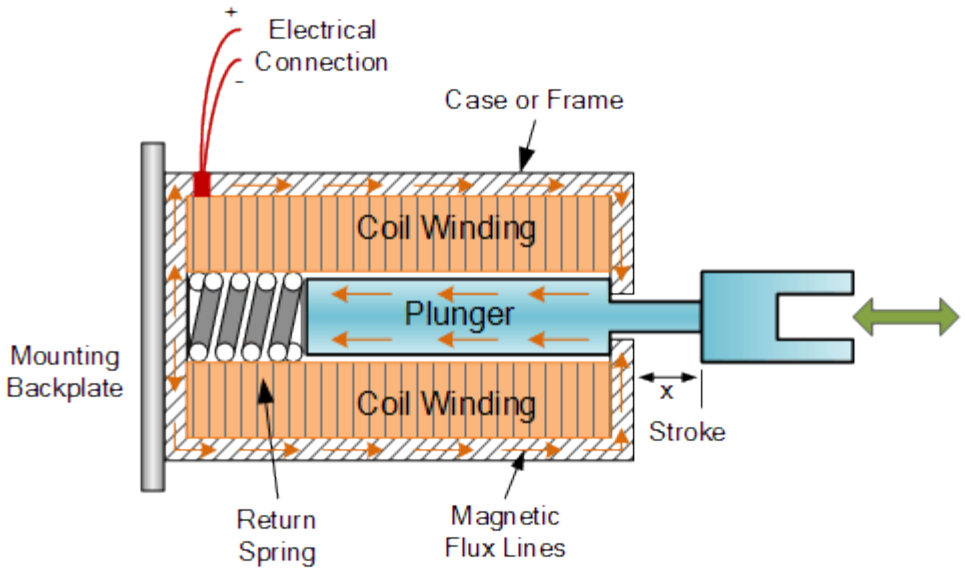

Figure 6.8: Working Mechanism of Solenoid Mechanical Punch (Electronics Tutorials)

6.6. Materials

BSA, fraction V, 97% pH 7, Lyophilized powder is purchased from Alfa Aesar. Humulin® R U-100 is provided by Eli Lilly and Company. Sucrose (Ultra Centrifugation Grade) is provided by Fisher Biotech. Deionized water is purified from tap water through Thermal Fisher DI Water system. Corning® cell culture insert (24 well, 8 and 0.4 μm pore size) is ordered from Fisher Scientific. Vankel® 7010 dissolution bath with auto sampler is provided my Agilent Technologies. Iso-Insulin ELISA Assay Kit was purchased from Thermo Fisher®. Solenoid, electrical switch and insulated wires were purchased from McMaster Carr.

6.7. Experimental Method

6.7.1. Fabrication of TII Prototype

The fabrication process is the same reported as chapter 3. Humulin R U-100 solution was used as the source of insulin. Sucrose and albumin was added according to different formulations. The mixture was freeze-dried to achieve fine powder. Powder was manually pressed in Carver® tablet press with no additional pressure. Tablets were put into Falcon cell culture inserts for the insulin releasing test. All fabrication and processing steps are the same as previous chapters.

6.7.2. Fabrication of Solenoid Controlled Mechanical Arm

Wires, switch, battery and solenoid were connected as a general electrical circulation. Switch was tested to control the circulation. A “pull mode” solenoid was tested and pulling motion was acquired with switch was on, while the mechanical arm will be reset back to original position while switch is off. Figure 6.9 is a picture of the electrical circulation of solenoid, switch and battery.

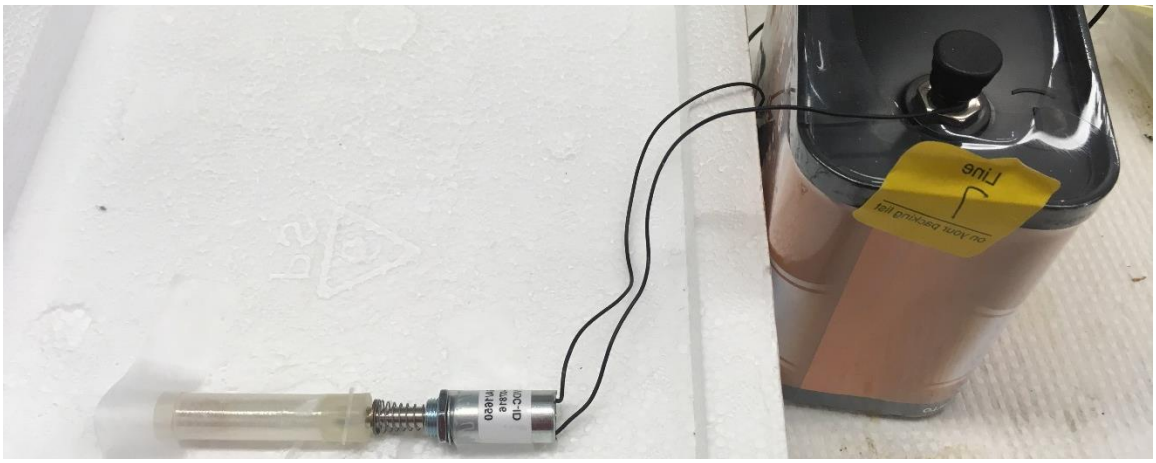

Figure 6.9: Electrical Circulation of Solenoid, Battery and Switch

6.7.4. 3-D Printing of "Tubing with Sleeve"

The tubing in Figure 6.4 was fabricated through 3-D printing technology. Manual fabrication was also feasible but could not provide a very consistent quality of final product. 3-D printing technology was supported by Dr. Joseph Steele in New Jersey Center for Biomaterials. Figure 6.10 and Figure 6.11 are the final product of this tubing. Porous membrane is very expensive to print so we decide to print out a mesh tubing to prove the principle. The tubing has a non-permeable sleeve and a permeable mesh. The sleeve and mesh are designed to fit perfectly with no path for liquid. As a result of that, the tubing is considered "Fail-safe" for insulin. In a real implant for the patient, a porous membrane would be inserted on the right surface of tubing to allow insulin release and basal level.

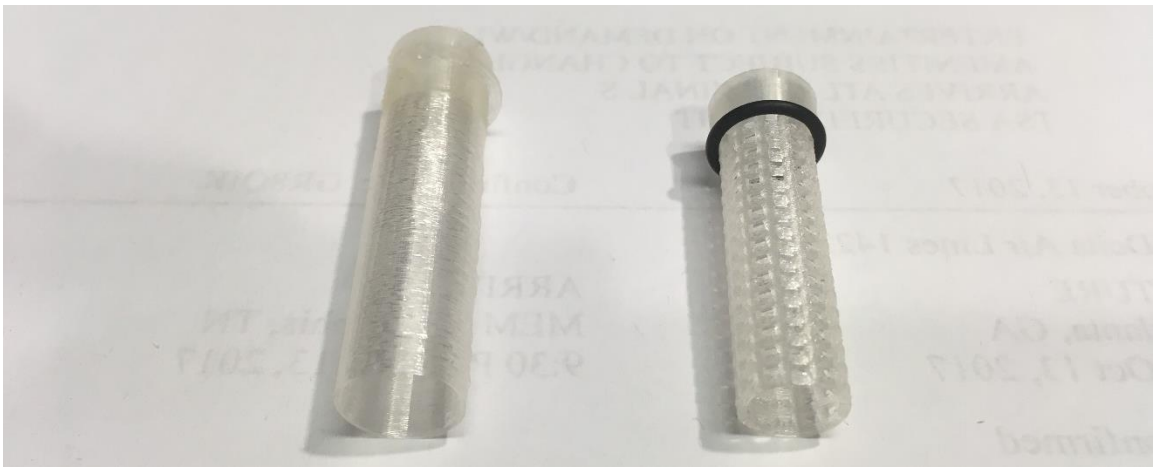

Figure 6.10: Insulin Implant Tubing, Sleeve and Inner Mesh

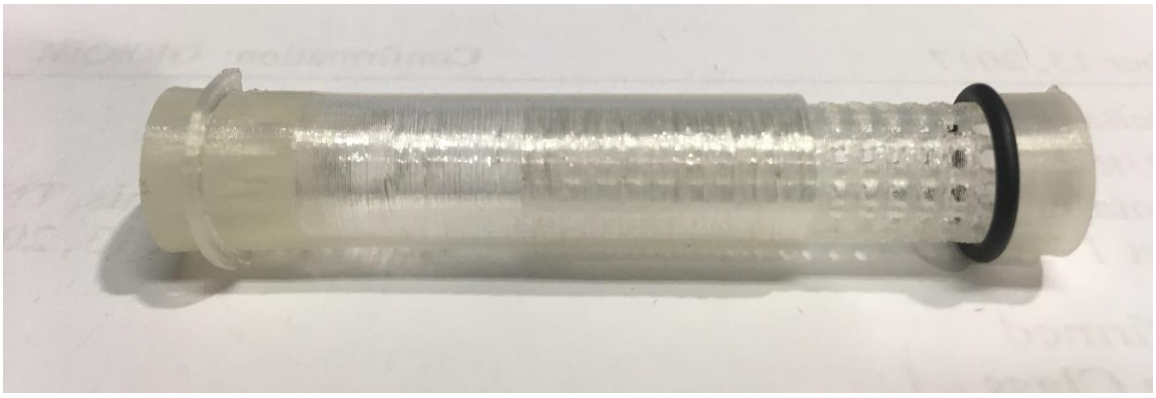

Figure 6.11: Insulin Implant Tubing,

6.7.5. Insulin Releasing Test

Insulin releasing test was performed in Vankel 7010 dissolution system. The media used was 1 liter phosphate buffered saline. All samples were assayed with Iso-Insulin ELISA Kit for bioactivity. All operations were the same as previous chapters.

6.8. Result and Discussion

6.8.1. Insulin Releasing Test of Selected TII Prototype

Two TII prototypes were tested in this study. 0.4 micron and 3 micron cell culture inserts were tested while matrix is the same. Tablet matrix contains 125 mg sucrose, 125 mg albumin and 166.67 units Humulin® R. This releasing study was designed to prove the

bioactivity of insulin from our prototype. Figure 6.12 is the insulin releasing data of this study. As shown Figure 6.12, 0.4 micron prototype could provide 4 units/hour insulin release, while 3 micron ones could provide 8 units/hour. With 0.3 cm^2 membrane surface area, we could easily adjust back to 2 units/hour and 4 units/hour by reducing surface area. Hence, clinical insulin need could be meet by this adjustment. In addition, based on the size of an implant, a maximum 530 mm^2 surface area could be provided by a cylinder shape implant. The idea is to use 150 mm^2 for basal insulin delivery, and another 150 mm^2 for additional insulin. During meal time, total 300 mm^2 surface area would be releasing insulin to meet the increased blood glucose level. A total insulin releasing rate of our TII prototype was displayed in Figure 6.12 as 12 units/hour. Figure 6.13 is a releasing rate design for a real implant using half of the surface area. Actually releasing rate might be different but close.

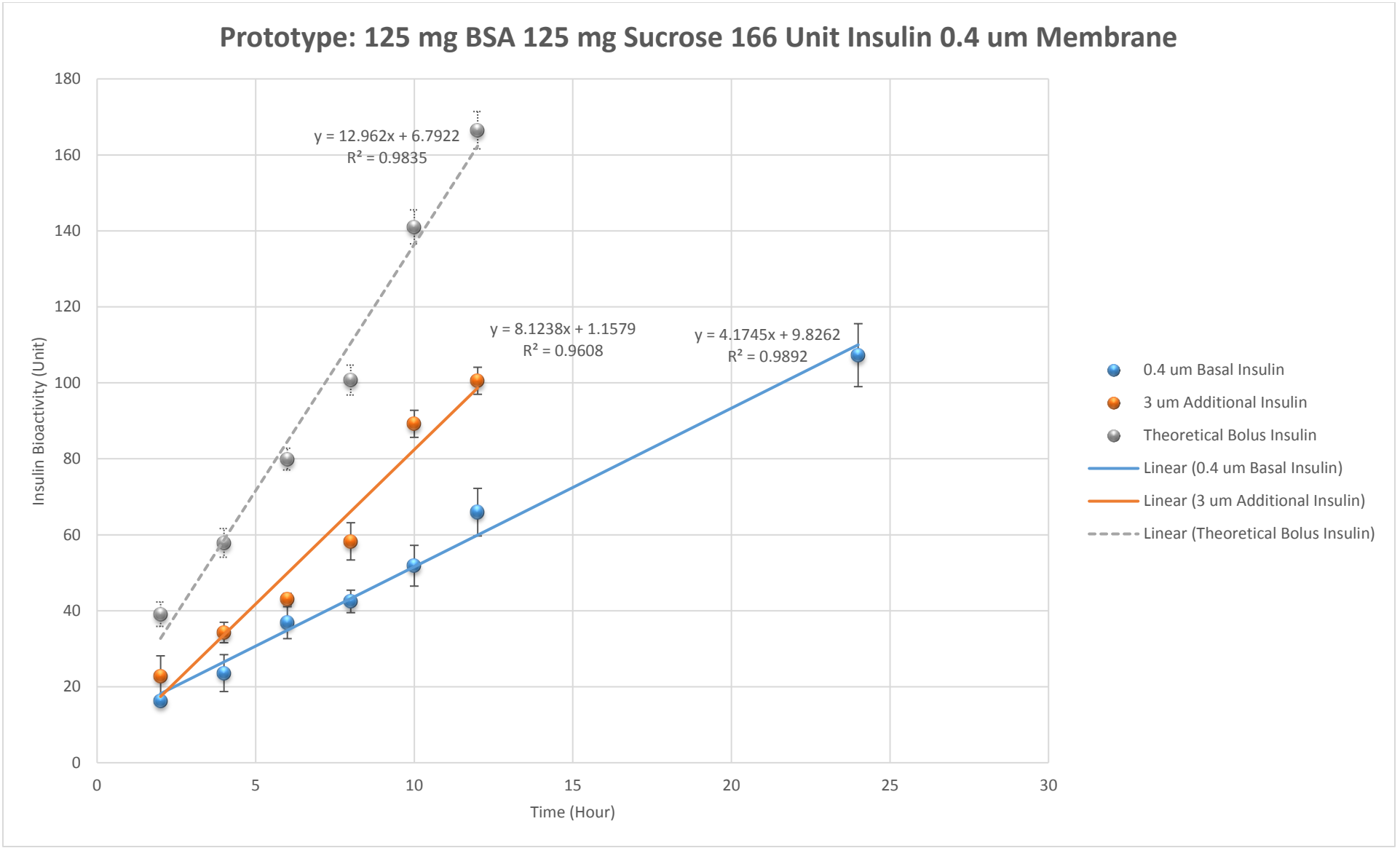

Figure 6.12: Insulin Releasing (Bioactivity) from in pH 7.4 Phosphate Buffer Saline: 125 mg Sucrose, 125 mg Albumin, 0.4 µm Membrane and 3 µm Membrane Mimicking Basal and Bolus Insulin Release

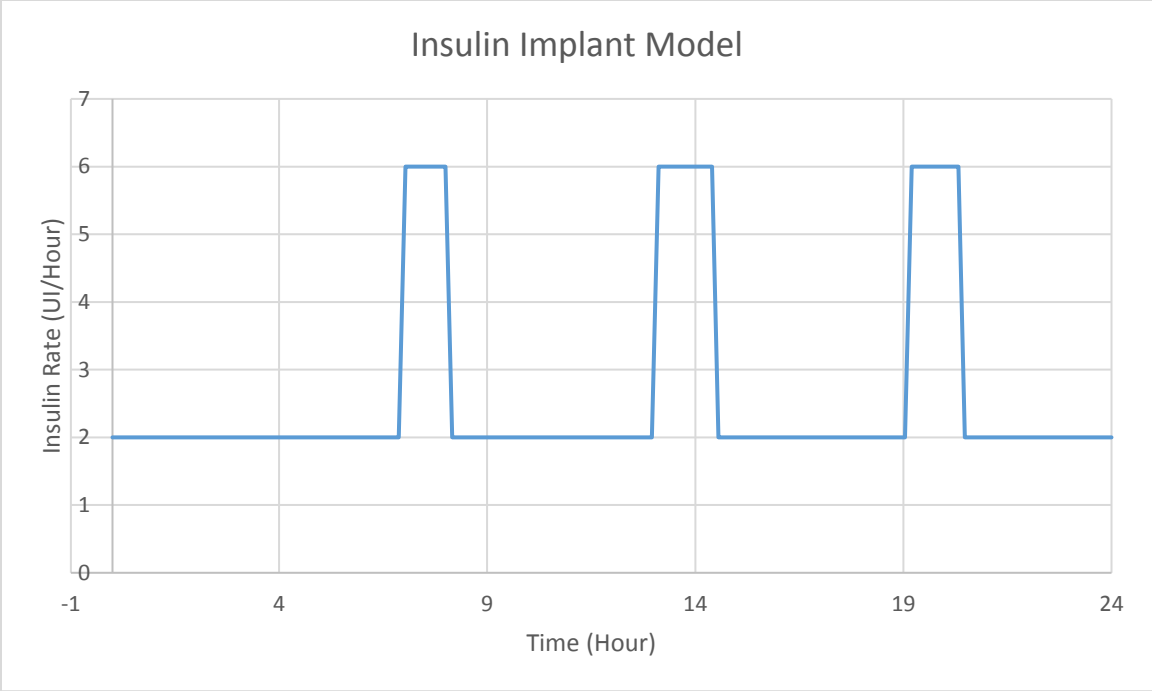

Figure 6.13: Theoretical Insulin Rate-Time Profile of Designed Implant Based on Experimental Data

6.8.2. Electrical and Mechanical Testing of Solenoid Implant Prototype

Section 6.8.1 provides a releasing data of our implant. In order to accomplish the membrane change, a sleeve-tubing model was acquired through 3-D printing. Figure 6.9 is the electrical setting of solenoid and tubing. The goal is to use electricity to control the opening and closing of sleeve, resulting in the change of surface area of tubing. Figure 6.14 is the closing phase with sleeve completely locking opening surface. Figure 6.15 is the opening phase while electricity is turned on. A 5 mm wide opening was acquired with electrical control. If this mesh would be replaced by porous membrane as a real implant, the surface area would be more than 100 mm^2. In this section, we just proved the feasibility of our design.

Figure 6.14: Closing Phase of Implant with Sleeve Covering the Whole Tubing

Figure 6.15: Opening Phase of Implant with Sleeve Retrieving from the Tubing

6.9. Conclusion

In this chapter, we used the conclusion from previous chapters to formulate and fabricate an insulin prototype. We successfully achieved insulin releasing for 24 hours with satisfactory rate. In addition, we created a model of our prototype using 3-D technology and solenoid electrical control. With the budget and laboratory setting, we believed this is the most satisfactory result of this project. We really wish future scientists could micronize this prototype to a relatively feasible size and test the insulin releasing on animals or human subject.

CONCLUSION OF DISSERTATION

In conclusion, a novel subcutaneous implant was designed using a drug matrix and porous membrane. Drug matrix could be prepared through two methods. For highly water soluble drugs, all ingredients could be dissolved, frozen and lyophilized together to acquire uniformly mixed dry powder. For slightly soluble drugs, a small batch blender process is preferred. Tablet press should be done without additional force to avoid potential disintegration issue.

The porous membrane part was provided by using Falcon® cell culture inserts. Tablets prepared could fit into the inert shell perfectly. A compressible spring might be used to immobilize tablet matrix. The inserts were sealed by plastic caps to prevent liquid flow. This prototype was named Tablet-In-Insert (TII) prototype.

Several drugs were tested using this TII prototype. Caffeine, ibuprofen, benzoic acid, guaifenesin, metoprolol tartrate, propranolol HCl, acetaminophen and prednisolone were tested. In addition, human insulin was tested as a model protein drug. All drugs could provide a zero order release from TII prototype. When reaching 80% of the drug loading, drug releasing rate started to decrease.

In order to explain the releasing mechanism, a three-phase philosophy was proposed. The first phase is the initial dissolution of matrix. In this phase, drug concentration inside of the implant start from zero to reach its highest concentration (Cmax). Cmax could be determined by drug loading and drug solubility. Phase II is the zero order releasing phase. In this phase, drug concentration remains at Cmax and continuously provide zero-order drug release. The last phase is called declining phase. In this phase, drug concentration starts to decrease from Cmax towarding zero. As a result of that, drug releasing rate starts to decrease.

In order to propose the feasible method to produce TII prototype, we contacted New Jersey Center for Biomaterials and Rutgers University for 3-D printing. With their technology, we could print out a cylinder tubing. The inner tubing is mesh structured and the outer tubing is non-permeable. The outer tubing could be moved to control the exposed surface area. An electrical solenoid was connected to control the movement of outer tubing. The open area could be controlled by the punch distance of solenoid.

This dissertation is the start beginning of a universal subcutaneous implant. The goal is to provide a novel understanding of implant. More work is needed to bring this idea into reality.

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