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Enhancing the Sublingual Permeability of Atropine Sulfate: Effect of pH and Penetration Enhancers

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ENHANCING THE SUBLINGUAL PERMEABILITY OF ATROPINE SULFATE: EFFECT OF PH AND PENETRATION ENHANCERS

By

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An Abstract of a Dissertation Submitted to Nova Southeastern University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ENHANCING THE SUBLINGUAL PERMEABILITY OF ATROPINE SULFATE: EFFECT OF PH AND PENETRATION ENHANCERS

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Rawan Bafail

(05/10/2019)

Atropine Sulfate (AS) auto-injector (AtroPen®) is being used as an effective and safe antidote for the treatment of organophosphate (OP) pesticides or nerve gas poisoning. The use of AtroPen® is associated with several drawbacks including: bulky size, availability, affordability, invasiveness, and administration errors. Previously, AS fast disintegrating sublingual tablets (FDSTs) were developed and the feasibility of AS sublingual permeability were demonstrated. However, AS permeability was delayed due to the negative impact of higher doses of AS on FDST's physical characteristics. Therefore, the aim in this research project was to optimize the previously developed AS FDSTs. It was hypothesized that optimizing the tablet's filler grade will improve the tablet physical characteristics along with incorporating a pH modifier and penetration enhancers will significantly enhance AS sublingual permeability.

Ten batches of AS FDSTs containing AS 8 mg were manufactured using a highly compressible filler grade of microcrystalline cellulose, MCC UF-702. AS FDSTs with and without a pH modifier (Na Bicarb 2%), or penetration enhancers (sodium dodecyl sulfate (SDS 0.5% or 1%), palmitoyl carnitine chloride (PCC 16%), or sodium glycocholate (Na Gly 15% or 20%)) were manufactured and evaluated.

Several US Pharmacopeia (USP) and non-USP physical tests were performed to evaluate AS FDSTs' characteristics. AS permeability from the ten AS FDST formulations were evaluated using Franz cells through excised porcine sublingual membranes. Results were statistically compared and deemed significant if $p< 0.05$.

All manufactured AS FDSTs passed the quality control tests. MCC UF-702 grade resulted in better powder flowability, higher breaking force, faster disintegration, faster dissolution rate, and higher water uptake. AS sublingual permeability was linear, indicating for a passive transport. Transcellular enhancers had significantly higher AS permeability enhancement in comparison to paracellular enhancer. Incorporating Na Bicarb 2% along with SDS 1% into AS FDSTs resulted in the highest enhancement in AS cumulative

sublingual permeation ($AUC_{0\text{-}90\ min}$), influx, and permeability. These optimized novel AS FDSTs has the potential to deliver therapeutic AS concentrations to the systemic circulation and achieve rapid onset of action for the first-aid treatment of OP toxicity. Further pharmacokinetics studies are recommended to determine the bioequivalence sublingual AS dose to AtroPen®.

Dedication

I dedicate my thesis to almighty Allah,

To my mom, who will always stay loved and remembered,

To my dad, who prayed for me and helped me in all things great and small,

To my lovely husband, who supported me and encouraged me to achieve all my dreams,

To my sweet kids, who colored my life and made me reach the stars,

To my beloved sisters and brothers, who never left my side,

Thank you. My love for you all can never be quantified,

God bless you.

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Contents

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List of Abbreviations and Symbols

AAPCC	American Association of Poison Control Centers
ACh	Acetylcholine
AChE	Acetylcholinesterase
AS	Atropine sulfate
AUC	Area under the curve
AV	Acceptance value
B	8 mg AS FDSTs, using MCC PH-301
BChE	butyrylcholinesterase
BCS	Biopharmaceutics classification system
BF	Breaking force
ºC	Degree Celsius
Ca Carb	Calcium Carbonate
C_{max}	Maximum serum concentration that drug achieves
CU	Content uniformity
D	Tablet diameter
DD	Drug dissolution
DT	Disintegration time
F	Friability
FDSTs	Fast disintegrating sublingual tablets
FDA	Food and Drug Administration
GIT	Gastrointestinal tract
HPLC	High performance liquid chromatography
Ibs	Pounds
IM	Intramuscular injection
IV	Intravenous
J	Influx of cumulatively diffused AS per area per min (μg/cm^2/min)
$AUC_{0–15}$	Cumulative amount of AS permeated per area for 15 min (μg/cm^2)
$AUC_{0–90}$	Cumulative amount of AS permeated per area for 90 min (μg/cm^2)
kgf	Kilogram-force
L-HPC	Low-substituted hydroxypropyl cellulose
LOQ	Limit of quantification
MC	Moisture content
MCC	Microcrystalline cellulose
Na Bicarb	Sodium bicarbonate
Na Cit	Sodium citrate
Na Gly	Sodium glycocholate

ODTs	Orally disintegrating tablets
OP	Organophosphate
P	Membrane area permeability per min
PCC	Palmitoyl carnitine chloride
PF	Powder flow
PSM	porcine sublingual membrane
QbD	a quality-by-design
R1	8 mg AS FDSTs, using MCC UF-702
R2	8 mg AS FDSTs, with pH-modifier Na Bicarb 2%
R3	8 mg AS FDSTs, with pH-modifier Na Bicarb 2% and SDS 0.5%
R4	8 mg AS FDSTs, with pH-modifier Na Bicarb 2% and SDS 1%
R5	8 mg AS FDSTs, with pH-modifier Na Bicarb 2% and PCC 16%
R6	8 mg AS FDSTs, with pH-modifier Na Bicarb 2% and Na Gly 15%
R7	8 mg AS FDSTs, with pH-modifier Na Bicarb 2% and Na Gly 20%
R8	8 mg AS FDSTs, with SDS 1%
R9	8 mg AS FDSTs, with PCC 16%
R10	8 mg AS FDSTs, with Na Gly 20%
RSD%	Relative standard deviation
R^2	Correlation of coefficient
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SLUDGE & DUMBELLS	These two mnemonics that specify AChE inhibitors' toxicity symptoms: salivation, lacrimation, urination, defecation, diaphoresis, gastric upset, emesis, diarrhea, miosis, bradycardia, and bronchospasm
$T_{1/2}$	Half life
T_{max}	Time taken to reach C_{max}
T_0	The withdrawn sample at time 0
TC	Tablet center thickness
TEER	Transepithelial electrical resistance
USP	The United States Pharmacopeia
WHO	World Health Organization
WT	Wetting time
WU	Water uptake

Chapter 1

Introduction

1.1 Research Rationale

Organophosphate (OP) poisoning is reported by the World Health Organization (WHO) to cause 300,000 deaths annually (Chowdhary, Bhattacharyya, & Banerjee, 2014) and 3,000,000 poisoning cases per year (Robb & Baker., 2019). About 60% of the globally reported cases of suicides in agricultural or industrial settings involved the use of OPs. Also, even after the global prohibition of the use, production, and storage of weapons of mass destruction (OPCW, 2005), OP nerve agent such as sarin is still used worldwide in wars. For example, it was reported by the United Nations that 1,300 civilians died due to OP poisoning after the use of sarin nerve agent in Syria in 2013 (United-Nations, 2013). In the United States of America (USA), around 8,000 OP exposure cases are reported every year (Robb & Baker., 2019).

These OP suicide cases, exposures to nerve agents due to war, and accidental OP poisoning cases have high mortality rate in developing countries that lack adequate and

well-equipped medical facilities to manage OP poisoning in a timely manner (Chowdhary et al., 2014).

Organophosphates work by stimulating the continuous production of acetylcholine resulting in the continuous activation of muscarinic and nicotinic receptors leading to the symptoms associated with OP poisoning. Researchers use two mnemonics, "SLUDGE" and "DUMBELLS", to specify AChE inhibitor toxicity symptoms: salivation, lacrimation, urination, defecation, diaphoresis, gastric upset, emesis, diarrhea, miosis, bradycardia, and bronchospasm. The symptoms of OP poisoning can range from mild to severe depending on the length and strength of the exposure.

The immediate initiation of the treatment is very critical to save patients' lives and prevent irreversible neurological complications. Atropine Sulfate (AS) is an effective and safe antidote used alone or in combination with other drugs for the treatment of toxicities due to nerve agent attacks and OP pesticide poisoning (Balali-Mood & Saber, 2012). The therapeutic effectiveness of AS against OP poisoning is based on the rapid bioavailability of therapeutic concentrations of AS in the blood (Vijayaraghavan, 2012). AS auto-injector (AtroPen®) is a pre-filled AS intramuscular (IM) injection that was approved by the Food and Drug Administration (FDA) in 1973 as an antidote for OP poisoning (Vijayaraghavan, 2012). AtroPen® autoinjector is designed to be used out of the hospital with a starting dose of 2 mg for adults then doubling the dose every 5 minutes until atropinization (a term used to refer to the signs and symptoms of atropine toxicity) is achieved. This administration method and regimen despite being inconvenient, has successfully saved many lives following OP poisoning (Karakus et al., 2014). However, the use of the auto-injector is

associated with several limitations and drawbacks. These include but not limited to its availability, cost, and administration convenience. AtroPen® is only available for veterans, which limits its use for farmers (Bentur, Layish, & Krivoy, 2006). The autoinjectors require training for its administration and poisoning can result in some individuals being disoriented, hence may not be able to self-administer the drug as instructed (Topal et al., 2014). The cost of the device limits its availability to many potential patients, especially that more than one device are needed for the treatment of OP poisoning. Additional drawbacks for using AtroPen® auto-injector include, patients' body weight that can reduce medication effectiveness for overweight and obese patients (Palma & Strohfus, 2013) and the fear of the needle that can cause a delay in the administration, which is very critical for the treatment of emergency medical conditions (Altman & Wood, 2014).

A new route for administering AS is being sought in order to overcome the limitations and drawbacks associated with the use of AtroPen® auto-injector and to increase the availability of AS as OP antidote. The sublingual route is one of the non-invasive routes of administration that has been used for the administration of drugs in emergency conditions and for the administration of highly metabolized drugs. However, the significance of the sublingual route depends on the feasibility of the immediate absorption of administered drug following its placement under the patient's tongue. Sublingually administered drugs get absorbed through the reticulated vein in the lining mucosa in the oral cavity, then get transported to the facial, jugular, brachiocephalic veins, and finally to the systemic circulation (Kweon, 2011). AS sublingual administration appears to be a promising solution for most of AtroPen® drawbacks. It requires simpler manufacturing processes that

would be more cost-effectiveness than the auto-injectors currently used for the first-aid treatment of emergency OP poisoning conditions (Aodah, Bafail, & Rawas-Qalaji, 2017). The formulation of AS as fast disintegrating sublingual tablets (FDSTs) may offer better patient accessibility due to their lower cost, administration convenience, non-invasive administration when multiple doses to be administered, and storing and handling flexibility by the patient due to their small size compared to the auto-injectors, especially during emergency conditions (M.M Rawas-Qalaji, Simons, & Simons, 2007). These tablets can also be administered without prior training or assistance by a trained medical professional. Due to all the previous advantages for FDSTs, a preliminary AS FDSTs were developed and evaluated as an alternative, non-invasive dosage form for the treatment of OPs acute toxicities (Aodah et al., 2017).

A range of AS doses, 2 mg, 4 mg and 8 mg, were previously formulated and evaluated as AS FDSTs (Aodah et al., 2017). However, the increase in AS dose up to 8 mg has negatively impacted the FDST's physical characteristics. For example, in an aliquot of 2 mL of water, the FDST's disintegration time was significantly increased up to 3 min and AS dissolution was significantly reduced to 30% during the 1st min of the test when AS dose was increased to 8 mg in formulated FDSTs. Additionally, the sublingual permeation of AS had a lag time of 5 min, which can negatively impact the onset of action of AS (Aodah et al., 2017). Because of these formulation and permeation limitations, the potential of optimizing AS FDSTs formulation using a quality-by-design (QbD) approach were investigated to achieve an efficient AS sublingual delivery.

The absorption of the drug from the sublingual area can be affected by many factors. However, the most important two main factors are: the type of formulation used as well as the drug's physicochemical properties (Meanwell, 2011). The types and grades of the excipients used in a drug formulation, mainly the filler excipient, can significantly impact drug absorption through controlling the rate of tablet disintegration into fine particles and, therefore, controlling the rate of drug release and dissolution necessary for its absorption (W. Brniak, Jachowicz, Krupa, Skorka, & Niwinski, 2013). Also, they are important for localizing the released drug from the tablet formulation at the site of absorption and limiting its loss into the stomach. Different excipients and excipients' grades can perform differently, especially under the very strict conditions in the sublingual cavity that lack any agitation and has a limited volume of the saliva available for tablet disintegration and drug dissolution (Jivraj, Martini, & Thomson, 2000). For a FDSTs formulation, disintegration and wetting times are critical variables and can influence the rate of the drug dissolution (Witold Brniak, Jachowicz, & Pelka, 2015). Therefore, careful selection of the excipients that ensure rapid tablet disintegration is critical to liberate the drug and make it available for dissolution, which can enhance the rate of drug dissolution. Therefore, the effect of changing the filler's grade in the AS FDSTs formulation on the tablets' physical characteristics was investigated in order to optimize the AS FDSTs formulation.

In order to enhance AS sublingual permeation, studying the effect of medium's pH on AS ionization is very important to demonstrate if modifying the sublingual medium's pH using a pH-modifying excipient, incorporated into the AS FDSTs formulation, can affect the sublingual permeability of AS, and therefore, its relative bioavailability. The selection

of excipients to be used depends on the properties of the targeted absorption site and the drug to be administered (Goswami, Li, & Jasti, 2016). The addition of pH modifiers into the tablet formulation to be administered sublingually ensures that the pH of the saliva is controlled within the range that is optimal for drug absorption.

Paracellular or transcellular pathways are the two main transport pathways for any drug to be transported into the systemic circulation through the mucosal membrane. It is crucial to understand the dominant mechanistic transport pathways that characterize the permeation process through the oral mucosa for different molecules. This would assist in the selection and incorporation of the right penetration enhancer at the optimal amount to the AS FDSTs formulation in order to enhance AS sublingual permeability and relative bioavailability. The careful selection for the appropriate enhancer at a suitable concentration is very critical not only to enhance AS permeability but also to ensure their safety profile.

1.2 Research Hypothesis

It was hypothesized that incorporating a pH-modifying pharmaceutical excipient into the FDSTs formulation would reduce the AS ionization in the tablet diffusional layer "microenvironment" and significantly enhance its permeation through sublingual membranes along with the assistance of a permeation enhancer.

1.3 Research Objectives and Specific Aims

The overall objective of this research was to develop a FDSTs of AS as an alternative and effective dosage form for the emergency treatment of OP poisoning. The specific objective in this project was to enhance the sublingual permeability of AS by applying multiple approaches including, optimizing the physical characteristics of AS FDSTs formulation and enhancing AS permeability by altering the absorption microenvironment's pH using a pH-modifying pharmaceutical excipient and incorporating a penetration enhancer. The specific aims to achieve this objective were as follow:

1) Evaluate the effect of the filler's grade on the physical characteristics of AS FDSTs.
2) Evaluate the pH-permeability profile of AS sublingual tablets.
3) Formulate and evaluate optimized AS FDSTs containing a pH-modifier to assess their effect on AS permeability.
4) Formulate and evaluate optimized AS FDSTs containing different permeability enhancers with or without a pH-modifier to assess their effect on AS permeation.

1.4 Significance and Innovation

The wide-spread use of OP pesticides contributes to the high frequency of OP toxicity that occurs worldwide. The onset of the toxicity symptoms is often within minutes, which

can cause a number of long-term and irreversible complications. According to American Association of Poison Control Centers (AAPCC), the number of reported exposures to OP insecticides in the US alone were 1994 cases with 17 major outcomes and one death case in 2016 (Katz & Brooks, 2018). The numbers of reported cases are much higher in developing countries such as India and Nicaragua (Kanchan et al., 2010). These numbers have been increasing every year due to the increase in in the use and exposure to these OP pesticides. The main basic and initial treatment for the treatment of acute OP poisoning is the immediate administration of AS, a drug that inhibits the action of excess acetylcholine (ACh) at parasympathetic nervous system. AtroPen®, an AS auto-injector, has been approved by FDA and considered as an effective and safe antidote used alone or in combination with other drugs for treating OP acute toxicity. In order to overcome the aforementioned drawbacks of using AtroPen® auto-injector and to increase the availability and accessibility of AS as an antidote for OP poisoning, the sublingual route for AS administration has been explored by our group (Aodah et al., 2017).

The development of AS FDSTs will provide an accessible and non-invasive first-aid antidote for the treatment of OP poisoning and reduce the number of fatalities due to nerve gas attacks or OP pesticide poisoning. More people in danger of OP-induced toxicities will have access to the treatment and as a result, less fatalities and less neurological complications will occur if this new treatment was accessible and started early, as a first-aid treatment, until patient is transported to a hospital.

The basics of our research relies on the fact that sublingual lining has a highly networked blood vessels that aids in fast drug absorption to the systemic circulation

(Swarbrick, 2006). Also, using FDST formulations that release drug in 10-30 sec and promote drug dissolution in 1 min will provide non-invasive, user friendly, and more cost-effective alternative treatment for OP toxicity that require no prior training for its administration, which overcome most of the drawbacks associated with AtroPen®. Evaluating the pH-permeability profile of AS and then incorporating a pH-modifier excipient in order to alter the Microenvironment pH to enhance absorption and reduce individual absorption variability, can overcome the sublingual permeability limitations encountered at high AS FDSTs dose. The addition of chemical penetration enhancers are another way that was studied and added to the AS FDSTs formulation to enhance its transcellular and/or paracellular sublingual permeability in order to improve its *in vivo* absorption to deliver therapeutic quantities of AS to the systemic circulation using the sublingual route.

The development of new FDST formulations with enhanced permeability is a promising step to reach the therapeutic blood concentration needed for OP treatment. This novel dosage form can have a clinical significance as an alternative and non-invasive dosage form for treating OP toxicity.

1.5 Assumptions, Barriers and Limitations

1.5.1 Assumptions

Based on the literature, the porcine sublingual membrane (PSM) shares comparable anatomical and physiological properties with human sublingual membrane (Birudaraj, Berner, Shen, & Li, 2005; Goswami, Kokate, Jasti, & Li, 2013; Ong & Heard, 2009; Volz-Zang, Waldhauser, Schulte, & Palm, 1995). Therefore, PSM was selected to be used in our *ex vivo* permeation experiments.

1.5.2 Barriers

In this research project, some of the United States Pharmacopeia (USP) quality control tests were not suitable to be used as standardized tests or methods to evaluate the quality of our developed FDST formulations or to differentiate between them. For example, the USP disintegration time test (DT) is more suitable for regular tablets, which, unlike orally disintegrating tablets (ODTs), require a longer time to disintegrate, a higher volume to dissolve, and vigorous agitation that resemble the stomach motility (USP/NF, 2018a). Therefore, a previously developed apparatus were used to evaluate the DT of AS FDST formulations that was adapted instead of the USP Disintegration test (Aodah et al., 2017). A previously developed and validated drug dissolution (DD) test is another example of a non-USP method used in this research to evaluate the AS dissolution from FDSTs in 60 sec (Rachid, Rawas-Qalaji, Simons, & Simons, 2011).

Membrane integrity was one of the most important variables that can affect the results of the drug permeation. However, in the research, the variability of the membranes due to integrity issue was decreased by increasing the number of the replications in the experiment (n number) and excluding outlier membranes, if any. Each *ex vivo* permeation study was performed using six replicates (n=6), however, only 4 replicates (n=4) were reported by removing data from membranes that showed excessive permeability indicating for membrane integrity issue. If all membranes had good integrity, data from the highest and lowest permeating membranes were excluded to maintain equal n number between experiments. A reasonable sublingual membrane's variability is expected and can reflect the real biological differences between people. Permeability markers like propidium iodide (PI), Yo-Pro-1, and trypan blue have been used before to determine membrane integrity and exclude failing membranes (Bowman, Nesin, Pakhomova, & Pakhomov, 2010). However, this approach requires adding the marker to all the permeability experiments performed, then the quantification of the marker in addition to AS in the collected samples to identify failing membranes. Then relate the experiment for the membranes that had integrity issue. For the large amount of permeability studies performed in the project, this approach will add significant unnecessary work, complexity, analysis, and would consume more time. Therefore, this approach was simpler and achieved similar sensitivity and outcome in detecting membrane integrity issues.

The addition of pH-modifier and penetration enhancers into AS FDSTs formulation can lead to a local irritation of the tissue when the tablets are administered sublingually. Therefore, the excipient were carefully reviewed for their safety profile before being

selected. The amounts or concentrations reported in the literature to be safe and induced no local toxicities were adapted and used in our AS FDST formulations to ensure the safety of developed tablets and decrease any potential irritation or side effects that can affect the site of administration.

1.5.3 Limitations

Incorporating a pH-modifier with or without a penetration enhancer into AS FDSTs formulation to modify the microenvironment's pH and enhance AS sublingual permeability can be a very promising approach to achieve optimal AS sublingual absorption. *In vivo* pharmacokinetic studies using these optimized AS FDSTs can confirm the obtained ex vivo permeability studies, however, due to the lack of the animal facility and the analytical equipment to conduct pharmacokinetic studies and analyze collected blood samples it was not feasible to perform such studies.

1.6 Chapter Summary

The fact that high annual OP poisoning cases due to agricultural and household accidental exposures, military and terrorist use, or suicidal cases was the main rational behind this research project. The aim of the project was to optimize the physical properties of AS FDSTs formulation and enhance the AS sublingual permeability and absorption to achieve our ultimate goal of delivering therapeutic quantities of AS to the blood using the sublingual route. These AS FDSTs will offer a novel approach for the treatment of OP

toxicities due to the significant advantages that the sublingual route offers and the use of a novel delivery system, FDSTs, to disintegrate, release, and promote the dissolution of AS in 1 min or less. Also, the novelty of this dosage form is that it will be the first alternative and non-invasive dosage form designed for self-administration for the treatment of OP poisoning, which will offer more clinical significance compared to AtroPen®.

Chapter 2

Literature Review

2.1 Chapter Overview

In this chapter, literature review was carefully conducted to include the most important and up to date information pertinent to this research project. The mechanism of action for OP, its poisoning effects and symptoms, and the currently available treatment options in the market were reviewed and discussed in detail.

The backbone for all treatment regimens used for acute OP poisoning is AS. AtroPen®, a pre-filled AS auto-injector, is a single use device that can be self-administered intramuscularly. However, the use of AtroPen® is limited due to the challenges associated with its size (range from 10 to 14 cm) that limits the number of devices that can be carried, handled, and stored since multiple injections are required to treat OP toxicity. The use of the auto-injector in countries with low socioeconomic levels that have high risks of OP poisoning is challenging due to their high cost and the required training for their administration. This motivated us to develop a user-friendly alternative dosage form that can offer several advantages and overcome these drawbacks.

The sublingual route of administration was proposed as one of the promising solutions for the several drawbacks associated with the use of AS injection. An overview of FDSTs and their benefits to overcome the drawbacks of using AS auto-injectors were discussed.

The role of selected excipients for FDSTs formulation on the physical characteristic of the dosage form were also reviewed. The different characteristics of various filler grades were also reviewed in this section to guide the selection of the appropriate filler grade to achieve optimal FDSTs characteristics for AS sublingual delivery.

The effect of pH on drug ionization and the role of pH-modifying excipients to be incorporated into the FDSTs formulation on altering drug ionization and enhancing its sublingual permeability were explained in detail. Also, the role of various penetration enhancers and their mechanisms to enhance drug permeability were described.

2.2 Organophosphate Poisoning

Organophosphates are one of the most widely used pesticides. Today, organophosphates have variety of uses in agriculture, homes, and as chemical gases. Organophosphates are esters of phosphoric acid. The organophosphorus compounds share the general structure of $O=P(OR)_3$ (

Figure 1) (Newmark, 2004; Zhao & Yu, 2013).

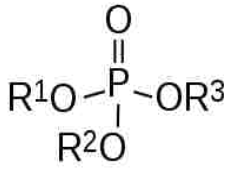

Figure 1. General chemical structure of organophosphates.
R1, R2, and R3 represent the chemical functional groups that define the intensity of OP action, toxicity, and physicochemical properties.

Because of the wide uses of OPs, poisoning due to the exposure to OP is more common to occur, especially for farmers and veterans. Pesticides and nerve agents are the two main sources for OP toxicity. According to WHO, the annual incidence rate of OP poisoning cases to be as much as 35 per 100,000 in general population (Chowdhary et al., 2014). In some of the agricultural countries, OPs are still the most widely used pesticides because of their effectiveness. For example, dichlorvos, malathion, parathion methyl, and chlorpyrifos are some of the pesticides that are used in this area (Chowdhary et al., 2014; WHO, 2004). OP poisoning commonly occur in developing countries that lack of adequate medical care, because they cannot afford safer but more expensive pesticides (Chowdhary et al., 2014; Kanchan et al., 2010). For example, countries such as Sri Lanka (Gunnell et al., 2007) and India (Kanchan et al., 2010) were registered to have the highest mortality cases due to OP pesticides poisoning. This is because these countries have agriculture-based economies and pesticides are commonly used and available (Chowdhary et al., 2014).

OP nerve agents such as tabun, sarin, soman, and VX have also been used as weapons in warfare and terrorist attacks. Sarin is one of the most known OP nerve gas that was used during the first Gulf War in 1988 and resulted in the deaths of over 40,000 people. Sarin

gas was also used by terrorists as a weapon of mass destruction in Tokyo attacks in 1995 (Chowdhary et al., 2014). A recent account of the use of sarin gas was reported in Syria in 2012 where the Assad's regime was accused of poisoning over 1300 people using sarin gas (Tillman et al., 2012).

2.2.1 Organophosphates mechanism of action

Organophosphates mechanism of action relies on inhibiting the acetylcholinesterase (AChE) activity, the enzyme that degrades acetylcholine (ACh) (

Figure 2), leading to the accumulation of ACh (Chowdhary et al., 2014). ACh is one of the most abundant neurotransmitters that is found in both the central and peripheral nervous system. So, the accumulation of ACh due to the inhibition of AChE by OP will overstimulate the parasympathetic nervous system and muscarinic receptors (Figure 3). This overstimulation leads to many symptoms and life threatening respiratory failure, which is the main cause of death in OP poisoning (Eddleston, Buckley, Eyer, & Dawson, 2008; Eddleston et al., 2004). The rate and degree of ACh inhibition is related to the structure of OP compound and its metabolism.

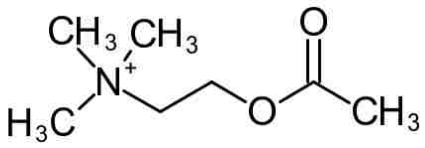

Figure 2. The chemical structure of acetylcholine.
The structure is a combination of acetic acid and choline.

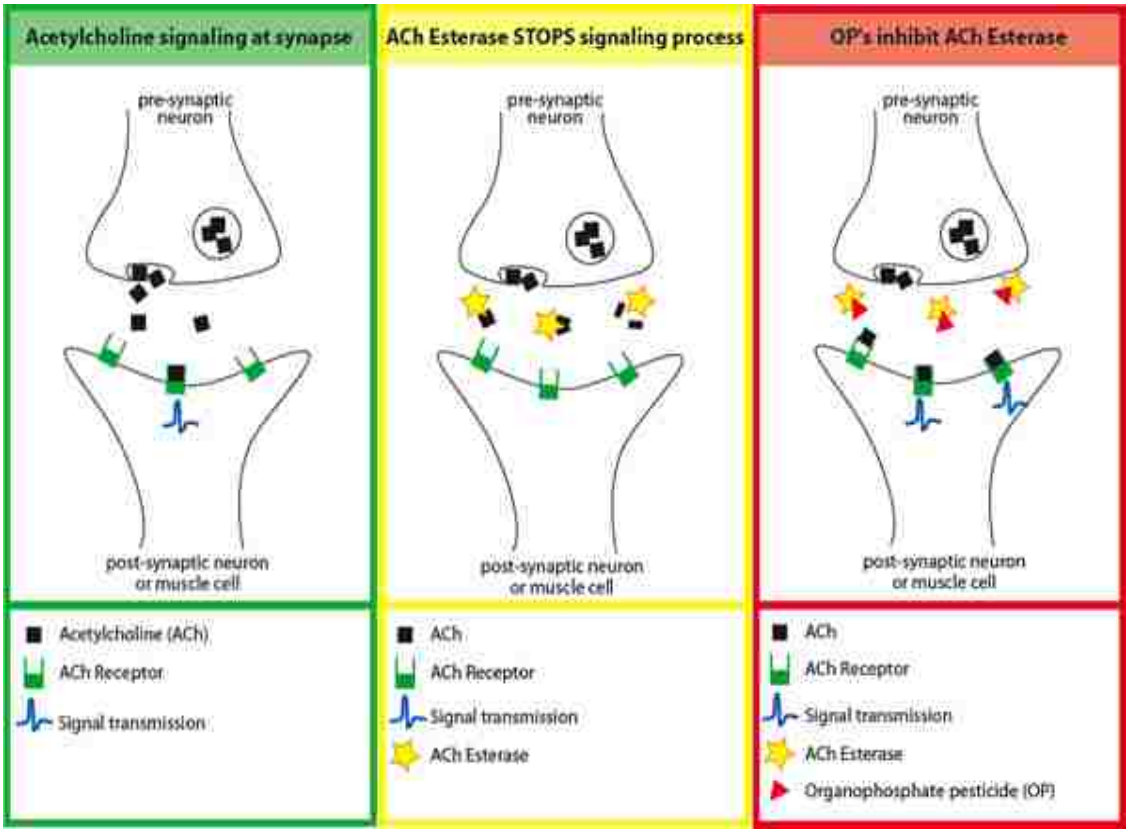

Figure 3. Mechanism of action of organophosphates (PEHSU, 2018).

The severity of the toxicity following OP positing depends on the type of OP compound, as well as, the amount and the period of the exposure. The relationship between the structure of OP and its activity suggests that the severity of the toxicity is directly related to the hydrophobicity of the compound (Zhao & Yu, 2013). The O=P bond in OP competes with the carbonyl bond (C=O) in the acetyl part of ACh for the serine at the esteratic site of AChE (Wiener & Hoffman, 2004; Zhao & Yu, 2013). The nucleophilic hydroxyl group (-OH) on the serine residue of AChE binds to the electrophilic O=P center of the OP that cause the formation of a very strong covalent bond (Westfall & Westfall, 2010; Wiener & Hoffman, 2004; Zhao & Yu, 2013). As a result, the phosphorylated AChE becomes inactive and cannot hydrolyze ACh because ACh binding site is blocked by the formed covalent bonded with OP. Therefore, this causes the inactivation of AChE, which leads to the accumulation of ACh that cause the overstimulation of the parasympathetic nervous system and muscarinic receptors (Sidell & Borak, 1992; Wiener & Hoffman, 2004; Zhao & Yu, 2013) (Figure 4).

2.2.2 Organophosphates toxicity symptoms

The symptoms of different OPs toxicity are similar to symptoms due to the ACh over-stimulation either in nicotinic or muscarinic receptors (Eskenazi, Bradman, & Castorina, 1999). Anxiety, headache, convulsions, general weakness, and depression of respiration are commonly due to the overstimulation of the nicotinic ACh receptors. On the other hand, symptoms like increased salivation, lacrimation, sweating, and urination are due to excess

ACh at the muscarinic ACh receptors (Leibson & Lifshitz, 2008). Bronchoconstriction, rhinorrhea, and diaphragm paralysis symptoms are mainly due to the autonomic nervous system overstimulation by OP, which lead to death.

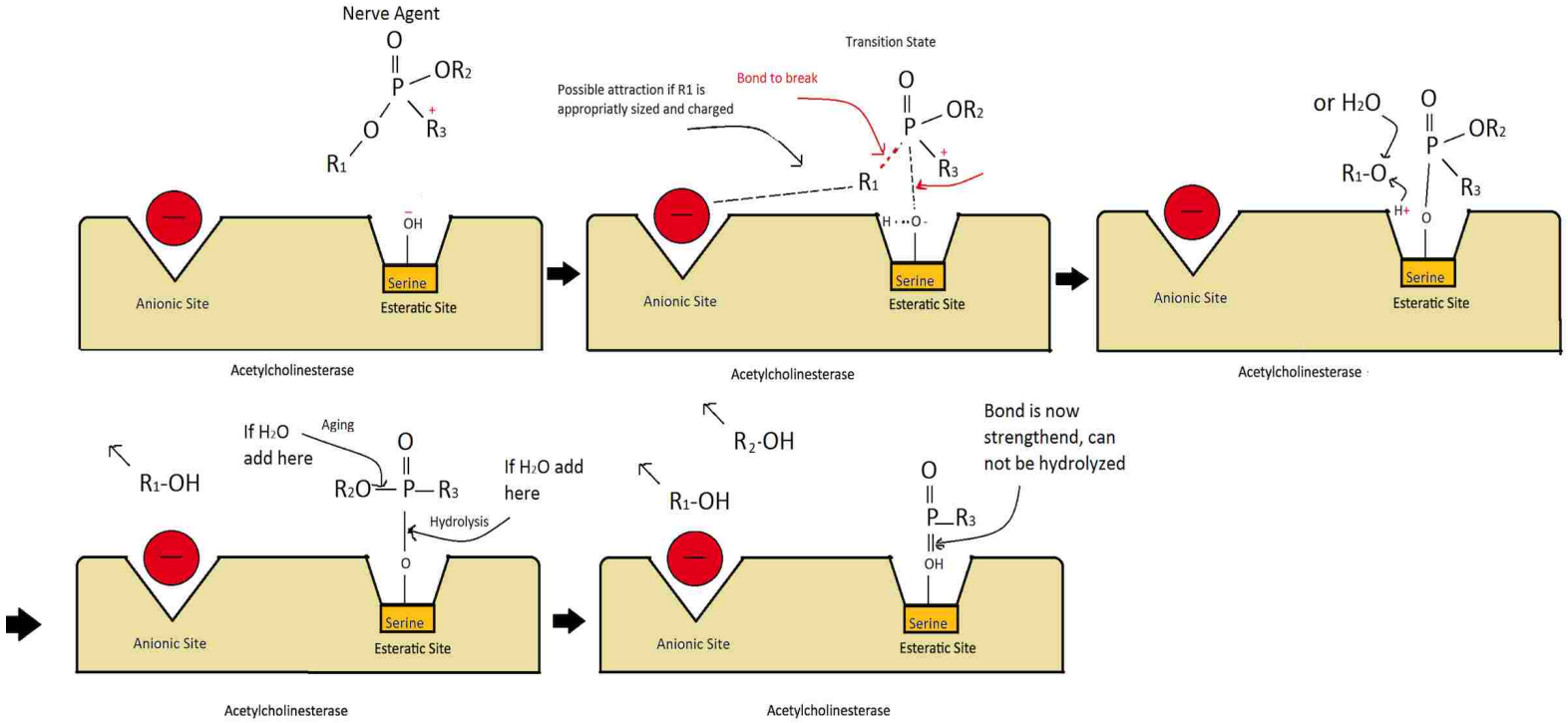

Figure 4. Mechanism of action of organophosphates toxicity.
The nucleophilic (-OH) element of the AChE binds to OP group (O=P), leading to the formation of a covalent bond between the two molecules. This bond is strengthened when it releases an H_2O molecule; this is termed the "aging" process (CDC, 2010).

2.2.3 Organophosphates toxicity treatment

Numerous articles were published and established a guideline and the steps for the treatment of OP toxicity (Eddleston et al., 2008; Eddleston et al., 2004; Mathias & Bannister, 2013; Moshiri, Darchini-Maragheh, & Balali-Mood, 2012; Newmark, 2004; Westfall & Westfall, 2010; WHO, 2004; Wiener & Hoffman, 2004). Since OP poisoning is an emergency medical condition, a prompt treatment of intoxicated patient is very critical. The treatment of OP toxicity depends on two important stages, the emergency treatment stage and the follow-up treatment stage. The treatment steps for the emergency treatment stage should begin at the site of exposure as follows. First, the patient must be removed from the contaminated area and all of the soiled clothing items should be removed. Second, airway control and adequate oxygen should be provided while checking the breathing and heart rate of the patient. Third, the patient should be injected with AS, which is the essential drug in every OP emergency guideline (Eddleston et al., 2008; Eddleston et al., 2004; Wiener & Hoffman, 2004).

Co-administration of oxime derivatives shortly after atropine such as pralidoxime can also be a part of the treatment plan. Oximes can lead to the reactivation of AChE by trapping the phosphate group of OP to release the hydroxyl group from the esteratic site on the AChE enzyme. The selection of the oxime molecule depends on the type OP causing the toxicity. Also, the reactivation of AChE by oxime is only effective in a recent OP exposure, for example, if the OP molecule has already "aged," reactivation is unlikely to

occur (Eddleston et al., 2008; Eddleston et al., 2004; Wiener & Hoffman, 2004). Aging is the conversion of the inhibited enzyme into a non-reactivable form.

Following the administration of AS or the coadministration of AS with pralidoxime, the patient need to be transferred to a medical center to start the follow up treatment stage. Overnight hospitalization is required to monitor the patient (WHO, 2004; Wiener & Hoffman, 2004). In some cases, the treatment can include the coadministration of benzodiazepines with AS injection for the treatment of seizures associated with OP toxicity (WHO, 2004; Wiener & Hoffman, 2004).

As a prophylaxis, butyrylcholinesterase (BChE) can be administered prior to OP exposure. It is the only clinically effective prophylaxis considered for OP toxicity (Iyer, Iken, & Leon, 2015).

2.3 Atropine Sulfate

Atropine sulfate (AS) is the sulfate salt of atropine that is extracted from the *Atropa belladonna* plant (Evans, 2002; Steenkamp, Harding, Heerden, & Wyk, 2004). Atropine is an ester consisting of tropic acid and tropine (Brown & Laiken, 2010). Because atropine has a low water solubility, AS is the active ingredient that is used in the current dosage forms. AS is an alkaloid with a molecular formula of $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$ (Figure 5), and molecular weight of 694.84. Its pK_a is 9.8 and the pH for a 2% AS solution in water is 4.5 to 6.2. An aliquot of 1 mL of water can dissolve up to 2.5 g of AS substance (RSC, 2013).

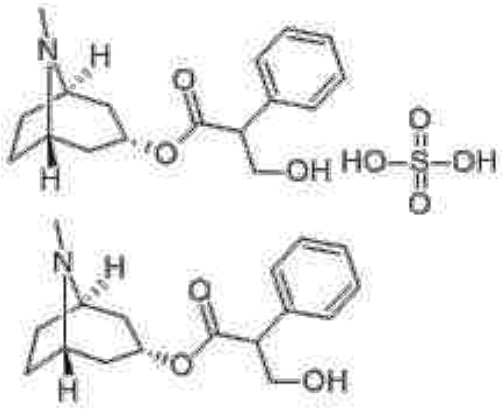

Figure 5. Chemical structure of atropine sulfate.

2.3.1 Atropine sulfate mechanism of action and clinical indications

AS acts as a sympathetic antagonist and binds to the muscarinic cholinergic receptors. It inhibits the parasympathetic nervous system by preventing the activation of the muscarinic receptors by the ACh neurotransmitter (Eddleston et al., 2008; Eddleston et al., 2004). AS has a wide range of clinical uses. It is used in combination with diphenoxylate hydrochloride (2.5 mg Diphenoxylate hydrochloride USP and 0.025 mg Atropine sulfate USP) as a tablet dosage form (Lomotil®) as adjunctive therapy to treat diarrhea or bowl syndrome (RxList, 2018). Atropine 0.4 mg is also administered orally as anticholinergic and antispasmodic agent (MedScape, 2018). As an ophthalmic drop solution, it is used for cycloplegia and to induce mydriasis (Elsevier, 2015). As an injection, it is used preoperatively to reduce salivation and bronchial secretions during surgery (Elsevier,

2015). Also, as injection, it is used as antidote for the treatment of cholinergic toxicity associated with OP exposure (Heath, 2002; Meridian, 2016; Wiener & Hoffman, 2004).

2.3.2 Atropine sulfate pharmacokinetics and pharmacodynamics

According to the clinical studies, AS's distribution kinetics are dose-dependent. As a result, for the emergency treatment of OP toxicity, multiple administrations of AS are required to reach the effective concentration needed for the treatment. Based on the biopharmaceutics classification system (BCS), drugs can be classified depend on their solubility and permeability into four classes, high solubility-high permeability (Class I), low solubility-high permeability (Class II), high solubility-low permeability (Class III), and low solubility-low permeability (Class IV). AS is considered as a class III based on the BCS classification (Custodio, Wu, & Benet, 2008). AS is a highly soluble salt in water (2.5 g/mL) that exhibited first-order elimination kinetics with renal plasma clearance of 660 mL/min (Hinderling, Gundert-Remy, & Schmidlin, 1985; Lindenberg, Kopp, & Dressman, 2004).

After oral administration, atropine appears in plasma after 15 min and the C_{max} is achieved within 1.5 – 4 hours. About 90% of a 2 mg oral dose was found to be absorbed through the gastrointestinal tract (GIT) (McEvoy, 2012). The half-life ($T_{1/2}$) of atropine intravenous (IV) administration is (mean ± SD) 3.0 ± 0.9 hours (NIH, 2016).

For our-of-the hospital treatment of OP toxicity, the recommended starting dose for an adult is 2 mg using AtroPen® auto-injector, and then doubling the dose every 5 minutes

until atropinization is achieved, with a maximum use of 3 auto-injectors. A 2 mg dose will results in a C_{max} of 9.6 ± 1.5 ng/mL (mean ± SEM) and a T_{max} of 3 min (NIH, 2016).

AS can be used alone or in combination with other drugs for the treatment of OP toxicity. However, most of these combinations have more side effects. For example, the coadministration of pralidoxime with AS can lead to increased respiratory complications and higher mortality. Also, the coadministration of benzodiazepine such as diazepam with AS showed a poor intramuscular (IM) absorption (Eddleston et al., 2008; Eddleston et al., 2004; Meridian, 2016).

Most of the adverse effect associated with atropine is due to its antimuscarinic action. These include dry mouth, blurred vision, tachycardia, palpitation, headache, nausea, and vomiting. AS induces changes to the heart rate and respiratory passages based on the dose administered (Elsevier, 2015). After the administration of 0.5 mg AS, the excessive secretions from mouth and skin were stopped and dried up. Doubling the dose to 1 mg AS can increase the heart rate and mildly enlarges the pupils. With a dose of 2 mg AS, sever dry mouth, palpitation, and pupil dilatation accompanied by paralysis of accommodation may occur. A 5 mg AS dose can cause a more intense effects. These include, headache, difficulty in urinating, and slow gut movement. By increasing the dose to 10 mg AS or above, hallucination, arrhythmia, coma, and respiratory depression may occur as a result of the reduction in the secretions in respiratory passages that leads to a constriction and spasm of the respiratory passages, which can lead to death (Brown & Laiken, 2010; Heath, 2002; Meridian, 2016). The administration of AS for children should be used carefully as they are more sensitive to its adverse effects (Elsevier, 2015).

2.3.3 Atropine sulfate auto-injector

The AtroPen® auto-injector is designed for self or caregiver administration. Each unit is composed of a needle inside a cartridge that is 21 mm long for the 2 mg, 1 mg, and 0.5 mg units or 13 mm long for the 0.25 mg unit. The drug delivery begins at the moment the needle emerges from the cartridge. After the use of the AtroPen®, the container should be disposed and cannot be refilled and the protruding needle cannot be retracted (NIH, 2016).

AtroPen® are manufactured as AS 0.25 mg (for infants weighing less than 15 pounds (lbs)), AS 0.5 mg (for Children weighing 15 lbs to 40 lbs), AS 1 mg (for Children weighing 40 lbs to 90 lbs), or AS 2 mg (for Adults and children weighing over 90 lbs) by Meridian Medical Technologies (Meridian, 2016; NIH, 2016) (Figure 6). Each strength provides different amount of atropine in either 0.3 mL or 0.7 mL sterile solution containing glycerin, phenol, citrate buffer and water for injection. AtroPen® 0.25 mg provides 0.21 mg atropine/0.3 mL, AtroPen® 0.5 mg provides 0.42 mg atropine/0.7 mL, AtroPen® 1 mg provides 0.84 mg atropine/0.7 mL, and AtroPen® 2 mg provides 1.67 mg atropine/0.7 mL (NIH, 2016).

Figure 6. Image of atropine sulfate auto-injector devices (AtroPen®) (Hilmas & Hilmas, 2009).

AtroPen® alone is the basic treatment used against acute OP poisoning. This auto-injector is designed to be injected through the IM route to deliver AS. AtroPen® should be administered firmly straight down a 90° angle against the outer thigh. The current dosage form and administration method have successfully saved many lives from organophosphate poisoning. However, it is still inconvenient and unavailable in many developing countries and for farmers (Chowdhary et al., 2014). AtroPen® auto-injectors mainly available for military use in some countries and is not available for public use (Gunnell et al., 2007; Kanchan et al., 2010), which limit their use by farmers and civilians who are at risk of

nerve gas attacks. As stated previously, the use of the auto-injector, when available, is associated with several drawbacks. Their large size makes them difficult to carry and limits the number of devices that can be stored at any given time since multiple injections are required to administer the required doses of AS to achieve atropinization. Due to the multiple AtroPen® administration required to treat OP poisoning, they are considered highly invasive and are associated with increased risks of administration errors and infections, which may limit their effectiveness in practice. Because the needle cannot be retracted after administration this can lead to a possible post administration injuries (NIH, 2016). Many IM injections, including AtroPen® may result in poor absorption which can reduce the drug effectiveness in obese (overweight) patients. This is due to the fixed needle length used in AtroPen®, which may not go deep enough to reach deep into the muscle (Palma & Strohfus, 2013). The use of these auto-injectors is very challenging in countries with low socioeconomic levels and have high risks of OP poisoning due to their high cost ($37 per device), the required prior training for their administration, and the lack of adequate and well-equipped health care facilities in rural areas where majority of the OP toxicity cases occur (Ingle & Agarwal, 2014).

2.3.4 Alternative dosage forms for atropine sulfate administration

In order to increase the availability of AS as an OP antidote, new routes of administration for the systemic delivery of AS are being sought that can avoid the drawbacks associated with the use of AtroPen® auto-injectors. One key aspect of the

selection of these new routes is to offer alternative noninvasive administration methods that can be used for multiple drug administrations. Also, these alternative administration methods or developed dosage forms have to be accessible to the individuals in low-income regions, farms, rural areas, and countries with ongoing armed conflicts. Upon reviewing the literature, three different dosage forms have been investigated and tested for this purpose as follow:

1. *AS respiratory inhaler:*

    As respiratory inhaler was one of the dosage forms that were designed and evaluated (Corcoran, Venkataramanan, & Hoffman, 2013). According to the study, five puffs of AS inhaler were needed to deliver AS dose equivalent to AS 2 mg IM injection dose. The study concluded that an AS inhaler can be used only as an adjunctive therapy after the auto-injector (Corcoran et al., 2013). Another inhaler using Nano-AS dry powder was designed and evaluated by Ali et al. (2009). The authors conducted a clinical trial, and based on their results, a 6 mg of AS delivered via an inhaler was had a pharmacokinetic profile equivalent to AS 2 mg IM injection (Ali, Jain, & Iqbal, 2009).

2. *AS nasal aerosol spray:*

    According to the study performed by Kumar et al. (2001), AS was delivered using a nasal spray in rats to study the cardiovascular and respiratory variables for OP toxicity. The results concluded that using AS nasal spray is as effective as an intraperitoneal injection (Kumar, Vijayaraghavan, & Singh, 2001).

3. *AS sublingual injection:*

AS sublingual absorption was evaluated for the emergency treatment of OP toxicity by injecting AS eye drop solution formulation (Minims® 1%) under the tongue. The results showed that the time needed to reach the maximum concentration after sublingual injection (T_{max}) was less than the time after IM injection of AS (Rajpal, Ali, Bhatnagar, Bhandari, & Mittal, 2010). In spite of the promising results this administration method is perceived as very invasive and not practical for self-administration.

However, AS sublingual administration appears to be a promising solution for most of AtroPen® drawbacks. Therefore, preliminary AS FDSTs for the potential treatment of OP toxicity were developed, as reported previously (Aodah et al., 2017).

2.4 Sublingual Route of Administration

The sublingual route of administration is one of the efficient routes that can be used for treating emergency conditions. The significance of this route is due to the feasibility of the drug's immediate absorption after its placement under the tongue. The blood in the reticulated veins in the oral mucosal lining absorbs and transports the drug to the facial, jugular, brachiocephalic veins and finally to the systemic circulation (Kweon, 2011) (Figure 7). The sublingual mucosa is the thinnest mucosal lining of all oral mucosal area, highly vascularized, has low membrane's thickness (100 to 200 μm), and low

keratinization, which promotes rapid drug absorption and onset of action, bypassing the first hepatic metabolism (Teubl et al., 2013) (Figure 8).

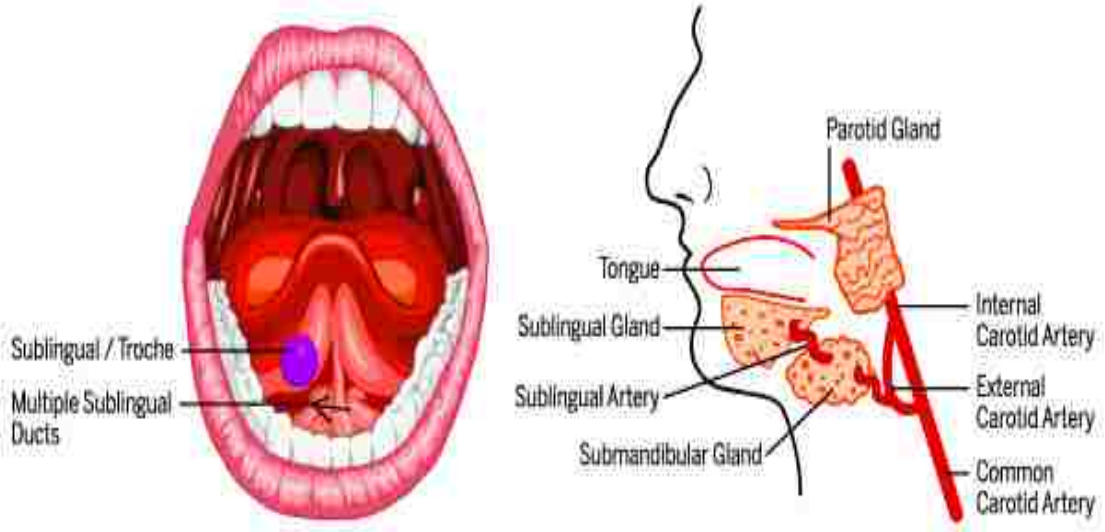

Figure 7. The Sublingual Region (Ardent, 2018).

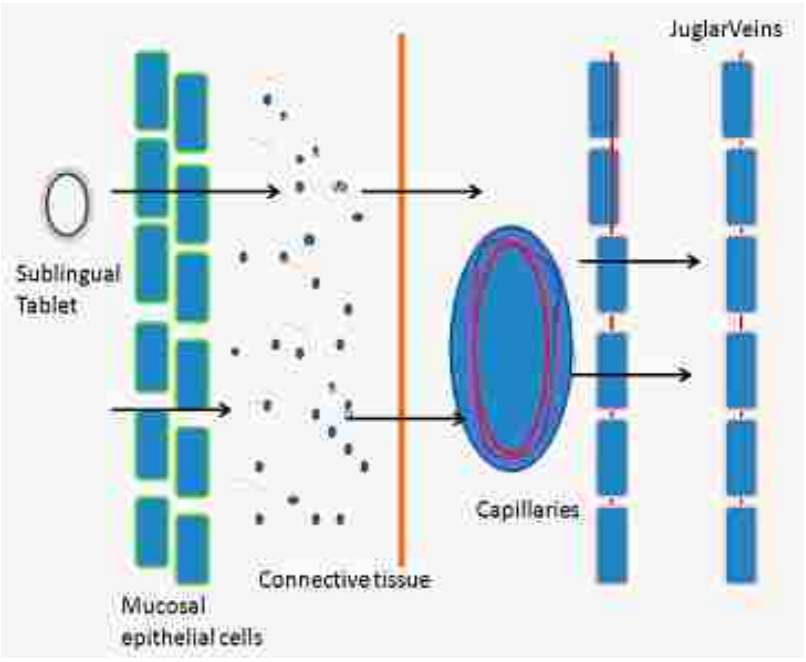

Figure 8. Movement of Drug Across Sublingual Mucosa (Dev, Mundke, Pawar, & Mohanty, 2016).

2.4.1 Fast disintegrating sublingual tablets

Fast disintegrating sublingual tablets are solid dosage form that dissolve or disintegrate under the tongue without water within 1 min or less (USB/NF, 2018). ODTs in general are a user-friendly drug delivery system that helps patients such as geriatrics and children, with swallowing problems, by combining the advantages of the ease of the oral administration of liquids and the practicality of tablets administration (Senel, Rathbone, Cansiz, & Pather, 2012). Sublingual administration through FDSTs offers many advantages when it comes to treating emergency conditions. The tablets can be administered immediately as a first-

aid treatment right after the occurrence of the incident and until patient is transported to an emergency room or an equipped health care facility.

The first-aid administration of these tablets would allow for an early initiation of the treatment and reduction in complications and patient death. These tablets need no water to disintegrate or drug to dissolve, which can avoid a critical limiting step for their first-aid administration in emergency treatment. They offer more administration convenience and storing and handling flexibility for patients due to their small size (M.M Rawas-Qalaji et al., 2007). These tablets also can be administered without prior training or the assistance of a trained medical professional (Singh et al., 2012). Sublingual tablets production is similar to production of most of other solid dosage forms that require simple and cost-effectiveness manufacturing processes (Aodah et al., 2017). Formulating drugs to be administered sublingually as FDSTs ensures rapid tablet disintegration and drug release, which is important in the emergency conditions.

One of the earlier examples of sublingual drug administration for the treatment of an emergency clinical condition is nitroglycerin sublingual tablet, which is used for the treatment of angina (Divakaran & Loscalzo, 2017). It relies on rapid drug release and onset of action. The sublingual route for the administration of nitroglycerin sublingual tablet elicits a drug response within 1-3 minutes after its administration (Divakaran & Loscalzo, 2017). Verapamil is another example used for the treatment of angina that has shown to elicit a quick response when administered sublingually (Al-Waili & Hasan, 1999; John, Fort, Lewis, & Luscombe, 1992).

2.4.2 Atropine sulfate fast disintegrating sublingual tablets

The formulation of the aforementioned AS FDSTs (Aodah et al., 2017) was adapted from a previously prepared epinephrine FDSTs formulation (M. M. Rawas-Qalaji, Simons, & Simons, 2006). The doses used in the previous AS FDSTs ranged from 2 mg to 8 mg with a total tablet weight of 50 mg (Aodah et al., 2017). These tablets were developed as potential alternative dosage form for OP acute toxicity treatment. All the AS FDSTs batches passed the quality control test. However, when the AS dose was increased from 2 mg to 8 mg, the tablet formulation's properties deteriorated. For example, the disintegration time for the tablet was increased up to 3 min. Also, only 30% of the drug dissolved in 1 min (Aodah et al., 2017). Therefore, the compression force used to manufacture 8 mg AS tablets was reduced from 130 -150 kgf to around 90 kgf in order to improve tablet disintegration and AS dissolution (Aodah et al., 2017). Also, the sublingual permeability of AS using this preliminary tablet formulation resulted in a lag time of 5 min. This means that the start of AS sublingual permeability was delayed by 5 min, which can negatively impact the potential of using these tablets as antidote for OP toxicity, which require a fast onset of action (Aodah et al., 2017). Due to these limitations, the previous preliminary tablet formulation was optimized in this work and then the optimized FDSTs were characterized using a quality-by-design approach to overcome the aforementioned limitations and to increase the potential of using AS FDSTs as a non-invasive, user-friendly, and cost-effective AS dosage form for the treatment of emergency OP poisoning.

2.5 Excipients

Pharmaceutical excipients are pharmacologically inactive ingredients added to the drug formulation and form part of the finished product (Chen, Chetty, & Chien, 1999). The absorption of the drug from the sublingual area is affected by many factors. Some of the main factors include the type of formulation used, including excipients, as well as the drug's physicochemical properties (Meanwell, 2011). Excipients in a drug formulation play an important role in determining the rate of drug absorption through the mucosa by controlling the rate of tablet disintegration into small particles and, therefore, controlling the rate of drug release and dissolution necessary for its absorption (W. Brniak et al., 2013). Also, in comparison to a liquid formulation, excipients in a sublingual tablet formulation are important for localizing the formulated drug at the site of absorption and limiting its loss into the stomach.

2.5.1 Effect of excipients on the physical characteristics of FDSTs

Different excipients can perform differently under very strict conditions like the sublingual cavity that lacks any agitation and has limited volume of saliva to facilitate tablet disintegration and drug dissolution (Jivraj et al., 2000). For FDSTs formulation, disintegration and wetting times are critical attributes that can influence the rate of drug dissolution (Witold Brniak et al., 2015). Therefore, selecting excipients that ensure rapid

tablet disintegration is critical to liberate the drug and make it available for dissolution, which can lead to enhancing the rate of drug absorption.

Most of the physical characteristics of FDSTs such as hardness, disintegration time, and powder flowability can be affected by the type of excipients used and their percentages. For example, Watanabe et al. (1995) used microcrystalline cellulose (MCC) grade PH-301 and Low-substituted hydroxypropyl cellulose (L-HPC) grade LH-11 in their studies. Their results showed that increasing the percentage of LH-11 from 10% to 30% resulted in a reduction in the tablet hardness from 8 kgf to 6 kgf, and an increase in their disintegration time. Also, increasing the percentage of LH-11 (beyond 30%) reduced the powder's flowability (Watanabe et al., 1995). Additionally, excipients selected for FDSTs formulation have to have low moisture content and low water solubility to ensure drug stability and enhance tablet disintegration and drug dissolution (Alyami et al., 2017).

2.5.2 Microcrystalline cellulose

The MCC and L-HPC were the two cellulose excipients used in our AS FDSTs formulation (Aodah et al., 2017). MCC is a filler that is produced in wide variety of grades with different range of particle sizes and shapes, moisture contents, angle of reposes, and porosities (Guy, 2009). Each grade offers various set of properties that can affect the overall characteristics of the formulation. For example, MCC's particle size and shape are the two important variables that can influence the entire powder flowability. MCC the PH grade is one of the most widely used grade in tablet formulations. Later, the MCC UF grade was

introduced as a new highly compressible filler grade. The different properties of different grades of MCC are shown in Table I (Asahi, 2018).

For FDSTs formulation, the filler is one of the most important excipient that represents the highest percentage incorporated into the tablet formulation (Moolchandani et al., 2015). Since the preliminary AS FDSTs formulation resulted in unfavorable tablet's hardness and disintegration time characteristics at high AS dose, it has been proposed that altering the filler type or grade in this project can improve the overall physical characteristics of the tablet, which can influence drug dissolution and absorption (Horio, Yasuda, & Matsusaka, 2014).

Table I. Characteristic of different MCC Filler Grades (Asahi, 2018)

MCC Grade	**Average Particle Size (μm)**	**Bulk Density (g/cm^3)**	**Loss on Drying (%)**	**Repose Angle (degree)**
UF-702	90	0.29	2.0-6.0	34
UF-711	50	0.22	2.0-6.0	42
PH-101	50	0.29	2.0-5.0	45
PH-102	90	0.30	2.0-5.0	42
PH-200	170	0.35	2.0-6.0	36
PH301	50	0.41	2.0-6.0	41
PH302	90	0.43	2.0-6.0	38

2.6 Role of pH in Enhancing AS Sublingual Permeability

The pH of the drug and its degree of ionization are critical parameters that can affect the drug permeation and absorption through the mucosal membrane. The pKa of the drug and the pH at the site of drug absorption in the sublingual region affect the extent of drug ionization and therefore, its permeability and absorption. The physiological pH in the sublingual area ranges between 5.8 to 7.5 (Sattar, Sayed, & Lane, 2014). Therefore, drugs that are unionized or partially ionized at this pH and with acceptable lipophilicity and low molecular weight can be readily absorbed through the sublingual mucosa (Wang & Chow, 2014). The less the drug is ionized the more it can easily pass through the sublingual mucosal phospholipid layer (Chen et al., 1999). In general, most of the drugs are either weak basic or weak acidic, which means that they are partly ionized, hence, can attract water molecules, forming large complexes that cannot pass through the pores in the semipermeable membrane (Goswami et al., 2016). However, the degree of ionization of weak basic or acidic drugs is based on the prevailing pH at the site of absorption.

According to Lee et al. (2005), the permeability of different compounds were tested using different apical pH buffer. The permeability of the basic compounds such as propranolol and timolol was decreased when the apical pH changed from 7.4 to 6.5 (Lee et al., 2005). Also, when the permeability of cimetidine (pKa= 6.8) and alfentanil (pKa= 6.5) were tested at different pH in Caco-2 cells monolayers, their permeability were increased 30 – 60 fold at pH 8.0 in comparison to pH 5.0 (Palm, Luthman, Ros, Grasjo, & Artursson, 1999).

Altering the absorption medium's pH in order to enhance drug bioavailability of basic drugs can be achieved by incorporating alkalinizing excipients into the drug formulation. The selection of the excipients to be used depends on the properties of the target absorption site (Goswami et al., 2016). Additionally, the addition of an alkalinizing excipient, also called a pH-modifier, to the tablet formulation to be administered sublingually ensures that the pH of the saliva is controlled within the range that is optimal for drug absorption and reduces absorption variability due to individual differences or food effect. The selection of these excipients is particularly important for AS as a weak base (Hassan, Ahad, Ali, & Ali, 2010). Its extent of absorption can be greatly dependent on its degree of ionization, which is mainly affected by the pH of the saliva (Goswami et al., 2016). Therefore, the evaluation of various pH-modifying excipients is critical for enhancing and optimizing AS permeability.

2.6.1 Calcium carbonate

Calcium carbonate (Ca Carb), $CaCO_3$, is a white inorganic water soluble salt. It forms a moderately alkaline solution when it dissolves in water. Its molecular weight is 100.09 g/mol, with a melting point of 1571°F (825°C) (USP/NF, 2018i). Ca Carb is used therapeutically as a buffer in hemodialysis. It can be used as antacid for temporary heartburn relief. Ca Carb is also frequently used as a calcium supplement for osteoporosis treatment (MeSH, 1965).

2.6.2 Sodium citrate

Sodium citrate (Na Cit), $Na_3C_6H_5O_7$, is a white water soluble salt. It can be prepared as mon, di, or tri sodium citrate. Its molecular weight is 214.10 g/mol, with a melting point of 414°F (212°C) (USP/NF, 2018k). It can be used to increase the free sodium load. Na Cit is widely used as a buffer to adjust the pH of weak acidic or weak basic drugs (Ugwu & Apte, 2004).

2.6.3 Sodium bicarbonate

Sodium bicarbonate (Na Bicarb), $NaHCO_3$, is a white solid powder usually known as baking soda. It is a strong base that generates sodium carbonate when it heats over 200°F in oven for about an hour. Its molecular weight is 84.01 g/mol, with a melting point of 122°F (50°C) (USP/NF, 2018j). It is one of the most commonly used pH buffering agent. Na Bicarb is also used as antacid to treat heartburn, indigestion, and upset stomach by neutralizing the excess stomach acid (MeSH, 1994).

2.7 Role of Penetration Enhancers in Enhancing AS Sublingual Permeability

Properties of drugs such as lipid solubility and molecular weight have been reported to affect the absorption of the drug (Gao & Morozowich, 2006). It is very important to understand the mechanistic analysis and the characteristics for the permeation process in the oral mucosal for drugs of interest to optimize their drug delivery. The paracellular and

the transcellular routes are the two major pathways for any drug in order to pass through the mucosal membrane. The preferred pathway for most ionizable molecules will depend on two factors, the charge status of the molecule and the resistance encountered during the permeation process (Chen et al., 1999).

2.7.1 Transcellular transport

The drug movement via the transcellular route involves the following: drug transport across the luminal membrane, diffusion through the cytosol, transport across the basolateral membrane, and movement through interstitial fluid and capillary (Patel & Misra, 2011). Transcellular permeation enhancers work by promoting the disruption in the cellular membrane. These enhancers, such as surfactants, partition into the cellular membranes and disrupt the packing of the lipids which results in defects in the structural integrity of the membrane (

Figure 9). However, their concentrations to be used are critical to avoid associated cytotoxicity.

2.7.2 Paracellular transport

Paracellular transport is a passive transport where the substance transfers across the epithelium cells through the intercellular spaces between cells (Maiti, 2017). Tight junctions between cells play an important role in paracellular permeation. Unlike transcellular transport, paracellular transport is less selective with respect to size, charge,

and hydrophobicity (Preusch, 2007). It is the suitable way for hydrophilic substances that are not able to permeate through transcellular transport. Paracellular penetration enhancers have the ability to enhance drug absorption through transient widening of the tight junctions of the cells in the membrane leading to reducing in their resistance and increasing permeability while decreasing cell viability (Goswami et al., 2016) (

Figure 9). It is also known that the paracellular pathway is more selective to positively charged molecules than negatively charged molecules (Caon, Jin, Simoes, Norton, & Nicolazzo, 2015). Some of these enhancers can work as a mucoadhesive as well, which can potentially prolong the retention of the drug at the site of absorption and minimize drug loss by salivary secretions in sublingual area.

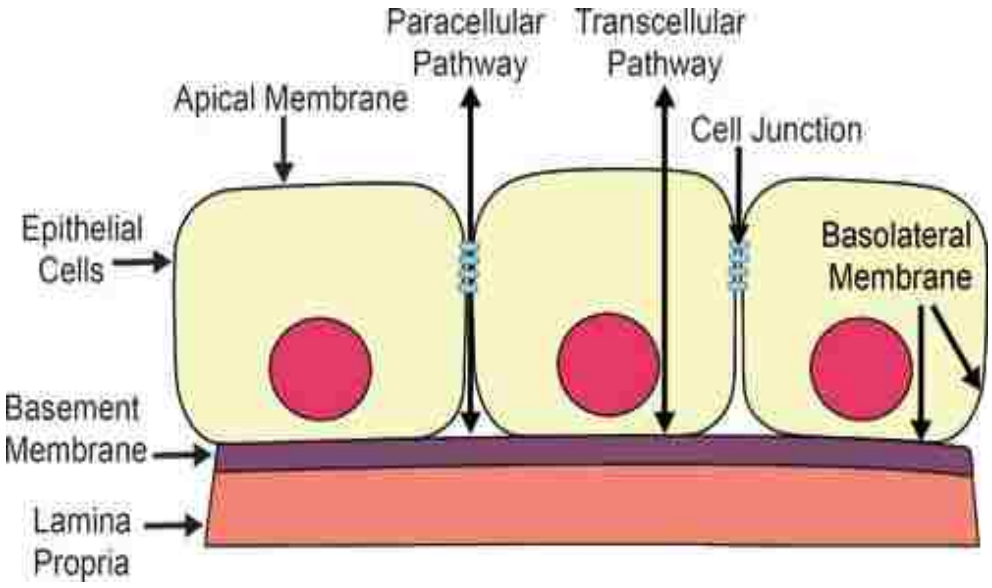

Figure 9. Transcellular and Paracellular Transport (Levendoski, Leydon, & Thibeault, 2014).

2.7.3 Sodium dodecyl sulfate

Sodium dodecyl sulfate (SDS) is a synthetic surfactant that enhances the absorption of the drugs through the sublingual mucosa by modifying the permeability of biological membranes and through the interaction with the drug (Goswami et al., 2016). SDS works as a transcellular enhancer that enhances the absorption of the drug due to protein denaturation, enzyme inactivation, swelling of tissue, and extraction of lipid components (Goswami et al., 2016). It also works as a paracellular enhancer by increasing the absorption of the hydrophilic drugs through the paracellular route through the solubilization of the intracellular lipids that form a barrier to paracellular permeant. The effects of SDS as penetration enhancer depend mainly on the lipophilicity of the permeant. It showed a very promising effect when used for the buccal drug delivery (Nicolazzo, Reed, & Finnin, 2004). On the other hand, SDS is a powerful irritant at high concentrations, however, a 1% SDS concentration was reported as the maximum concentration that can be used without causing cytotoxicity ("Final Report on the Safety Assessment of Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate," 1983).

2.7.4 Palmitoyl carnitine chloride

Palmitoyl carnitine chloride (PCC) is a fatty acid derivative of L-carnitine that works as an enhancer of hydrophilic molecules (Duizer, van der Wulp, Versantvoort, & Groten, 1998). It enhances the absorption of the drug by distributing the epithelial tight junctions,

which reduces the resistance and increases the permeability while decreasing cell viability. It also causes dilation in the paracellular spaces. Duizer et al. (1998) studied the correlation between the absorption enhancing effect of PCC and its effect on tight junction morphology and cytotoxicity on the intestinal epithelium. They found that PCC was able to decrease the transepithelial electrical resistance (TEER) for hydrophilic molecules and increase their absorption and permeation (Duizer et al., 1998). The effect of PCC as an enhancer is a dose dependent, which means that by increasing the concentration of PCC, its effect as absorption enhancer increases (Duizer et al., 1998; Sutton, LeCluyse, Engle, Pipkin, & Fix, 1993). The maximum reported concentration that can be used without causing cytotoxicity is 1mM (Duizer et al., 1998).

2.7.5 Sodium glycocholate

Sodium glycocholate (Na Gly) is a bile salt that have been shown to be effective as a penetration enhancer, especially in buccal epithelial mucosa. Na Gly works both as a transcellular and a paracellular penetration enhancer. Its transcellular enhancement works by interacting with the epithelial lipids, which cause a destruction in the lipid packing and formation of micelles that overcomes the resistance at the aqueous diffusion layer of epithelial cell membrane (Senel, Duchene, Hincal, Capan, & Ponchel, 1998). However, the paracellular enhancement works by disrupting the cell-cell junction to cause widening in tight junctions between cells (Mahaling & Katti, 2016). According to the study done by Williams et al. (2004), 0.5% Na Gly was able to increase the permeability of

polycaprolactone nanoparticles in the anterior part of the eye (Mahaling & Katti, 2016). Also, Senel et al. (1998) studied the effect of Na Gly as a permeation enhancer for morphine hydrochloride (MPH) across the porcine buccal mucosa. Two concentrations were tested (10 mM and 100 mM). The results showed that 100 mM concentration was able to significantly enhance the permeability of morphine at porcine buccal mucosa but not the 10 mM concentration (Senel et al., 1998).

2.8 Chapter Summary

In this chapter, the mechanism of action of OP poisoning and their signs and symptoms were discussed. The steps for OP poisoning treatments were described. AS autoinjector is the basic and initial pharmacological treatment for OP poisoning. However, because of the drawbacks associated with its use, alternative route of administration was proposed. The advantages of the sublingual route anatomy and physiology for sublingual drug delivery were explained and the formulation of AS FDSTs was discussed. The role of the excipients and filler grades in enhancing the physical characteristics of FDSTs formulation was described. Finally, two different approaches to enhance the sublingual permeation of FDSTs were reviewed. These included the use of pH-modifying agents and penetration enhancers.

Chapter 3

Methodology

3.1 Chapter Overview

In order to address the objectives and aims of this research project, the methods used were developed and performed according to the following five main steps:

1. Validating the analytical method for the quantification of AS.
2. Formulating and manufacturing various AS FDST formulations.
3. Evaluating the characteristics of AS FDSTs using various quality control tests.
4. Evaluating the *ex vivo* pH-permeability profile for AS FDSTs.
5. Evaluating the potential of incorporating different pH-modifiers into AS FDSTs and their effect on AS FDSTs *ex vivo* permeability.
6. Evaluating the potential of incorporating different penetration enhancers into AS FDSTs and their effect on AS FDSTs *ex vivo* permeability.

3.2 Analytical Method of Atropine Sulfate

The validation of the analytical method was required for the accurate and reliable analysis of AS samples. AS samples obtained from the quality control tests and Franz cells permeability studies were analyzed and quantified using high pressure liquid chromatography (HPLC) system as the analytical equipment used in this project (USP/NF, 2018h). Several AS calibration curves were prepared. Intra- and inter-assay variation, instrument and method reproducibility, instrument injection volume accuracy, and the minimum limit of quantification were determined to ensure that reliability of the analytical method used.

3.2.1 HPLC system and detection method

In this project, an HPLC system, model e2695, Waters Corporation (Milford, MA) was used. The system was equipped with a UV photodiode array (PDA) detector, model 2998, a pump, a column oven, a degasser, and an auto sampler. The column used was the reversed-phase μBondapak C_{18} Column, 125Å, 10 μm, 3.9 mm X 300 mm, which was purchased from Waters Corporation (Milford, MA). A sample injection volume of 20 μL with a pump flow rate of 2 mL/min, and a detection wavelength of 254 nm were used for AS analysis (USP/NF, 2018g).

3.2.2 Materials

The mobile phase was prepared by dissolving 5.1 g of tetrabutyl ammonium hydrogen sulfate, purchased from Sigma Aldrich (Saint Louis, MO), in a 50 mL acetonitrile, purchased from EMD Millipore Corp. (Billerica, MA), in a 1 L volumetric flask. The final volume of 1 L was then completed by adding acetate buffer that was previously prepared by adding 5.9 g of sodium acetate, purchased from Sigma Aldrich (Saint Louis, MO), in 1 L volumetric flask. Acetic acid 3 mL, purchased from Sigma Aldrich (Saint Louis, MO), was added and the final volume of 1 L was then completed by adding deionized water to prepare acetate buffer at a pH of 5.5. The mobile phase pH was adjusted to 5.5±0.1 with 5N sodium hydroxide, purchased from EMD Millipore Corp. (Billerica, MA), and then filtered using 0.2 µm 47mm Supor®-200 filter (Pall Corporation, Mexico).

3.2.3 Calibration curves

Calibration curve is a general method used to understand the response of the instrument to the analyte and to determine the concentration of an unknown sample analyte. The calibration curves were prepared and used for method qualification and AS quantification. A stock solution of AS (2 mg/mL) was prepared by dissolving 20 mg of AS monohydrate, purchased from Sigma Aldrich (Saint Louis, MO), in 10 mL deionized water in a 10 mL volumetric flask. A series of different AS standards were then prepared using the stock solution, including 200 µg/mL, 160 µg/mL, 80 µg/mL, 40 µg/mL, 20 µg/mL, 10 µg/mL,

and 5 µg/mL. Calibration factor was calculated from the slope obtained from plotting the area under the curve (AUC) of the different AS standards against their concentrations (n=5).

3.2.4 Instrument reproducibility

Measuring the instrument reproducibility is very important as it measures the ability of the instrument to produce the same result if the same input was used. The reproducibility of HPLC instrument was evaluated by injecting and analyzing multiple AS standards of a high and low concentrations of AS, 20 µg/mL and 200 µg/mL (n=5). The relative standard deviation percentage (RSD%) for the injected and analyzed AS standards were calculated.

3.2.5 Injection volume accuracy

The accuracy of the injection volume can measure the error that can result when using a specific instrument (instrument error). Usually, for each instrument, there is a range of error that can be acceptable. In order to evaluate the accuracy of the autosampler, injection linearity test was performed. The linearity of injecting increasing volumes of 10 µL, 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL of AS standard solution 20 µg/mL was evaluated by calculating the correlation of coefficient (R^2) of the slope obtained from plotting the area under the curve (AUC) of the different injections against their injection volumes.

3.2.6 Intra and inter-assay variation system

In order to evaluate the method and personal variability, intra and inter-assay variation of AS calibration curves were performed. This test can express the precision and repeatability of the results, which are the two important factors for instrumental and analytical method qualification. Different calibration curves were used from three days at different times of the day (morning and evening). For intra-assay variability, 3 sets of AS standards for 3 different calibration curves (n=3) were prepared on the same day but at different times and used to determine variations between the results analyzed on the same day. For inter-assay variability, 3 sets of AS standards were prepared at different days (n=3) and used to determine variations from day to day analysis. The RSD% for the analyzed AS standards were calculated.

3.2.7 The limit of quantification

The limit of quantification (LOQ) is defined as the minimum concentration that can be quantified accurately and reproducibly. LOQ was measured to determine method's sensitivity and qualify the analytical method used. Low AS standard concentrations including: 0.1 µg/mL, 0.5 µg/mL, 1.25 µg/mL, 2.5 µg/mL, and 5 µg/mL, were injected and analyzed (n=5). The lowest AS concentration that was detected and analyzed with an RSD% of ≤ 5%, was considered the LOQ for AS using this analytical method.

3.3 Formulation and Manufacturing of AS FDSTs

The previously prepared and manufactured AS FDST formulations were adapted (Aodah et al., 2017) and optimized to address our aims.

For this research project, 10 different AS FDST formulations were formulated and manufactured. All the AS FDST batches contained 8 mg AS as the active ingredient and had a total tablet weight of 50 mg.

3.3.1 Materials

Atropine sulfate monohydrate (AS) was purchased from Sigma Aldrich (Saint Louis, MO). Magnesium stearate was used as a lubricant and purchased from Alfa Aesar (Heysham, Lancs, UK). Two grades of Ceolus® fillers MCC (PH-301) and (UF-702) were generously provided by Asahi Kasei Chemicals Co. (Tokyo, Japan). The superdisintegrant L- HPC (LH-11) was provided by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan).

3.3.2 Formulation preparation

All the powders used were sieved before mixing using an electrical sieve shaker (Cole-Parmer, Vernon Hills, IL) with a sieve number 140 (106 μm) to ensure all excipients and active ingredient had uniform particles size distribution. AS was manually mixed with MCC by geometric dilation method. L-HPC, two-third the quantity, was mixed with the other powder mixture for 4 min using a three-dimensional manual mixer (Inversina,

Bioengineering AG, Wald, Switzerland). Magnesium stearate and the remaining one-third of L-HPC were manually mixed and then added to the powder mixture to be mixed for additional 30 sec. This mixing procedure was used to achieve both internal and external positioning of the superdisintegrant.

The composition of the ten formulations are shown in Table II. The optimized AS FDST formulation contained the highly compressible filler grade, MCC UF-702, and was compared to the previously used filler grade, MCC PH-301. A pH modifier, Na Bicarb, a pH modifier and penetration enhancers, SDS, PCC, Na Gly, and enhancers alone, SDS, PCC and Na Gly, were incorporated in the optimized AS FDST formulations (Table II).

3.3.3 FDSTs' manufacturing

The mixed powder from each formulation of the ten batches was compressed and manufactured by direct compression method using a rotary Minipress I (Glob Pharma, NJ) at a compression force of 130-150 kgf using 3"/16" concave punches (Natoli Engineering Company, Inc., St. Charles, MO).

Table II. Composition of AS FDST Formulations

Ingredients	AS FDSTs Formulations									
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**
Atropine sulfate, mg (%)	8 .00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)	8.00 (16.0)
Microcrystalline cellulose (Ceolus® UF-702), mg (%)	37.35 (74.7)	36.45 (72.9)	36.22 (72.45)	36.00 (72.0)	29.25 (58.5)	29.7 (59.4)	27.45 (54.9)	36.90 (73.8)	30.15 (60.3)	28.35 (56.7)
Low-substituted hydroxypropyl cellulose (LH-11®), mg (%)	4.15 (8.3)	4.05 (8.1)	4.02 (8.0)	4.00 (8.0)	3.25 (6.5)	3.30 (6.6)	3.05 (6.1)	4.10 (8.2)	3.35 (6.7)	3.15 (6.3)
Magnesium stearate, mg (%)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)	0.50 (1.0)
Sodium bicarbonate, mg (%)	0.00 (0)	1.00 (2.0)	1.00 (2.0)	1.00 (2.0)	1.00 (2.0)	1.00 (2.0)	1.00 (2.0)	0.00 (0)	0.00 (0)	0.00 (0)
Sodium dodecyl sulfate (SDS), mg (%)	0.00 (0)	0.00 (0)	0.25 (0.5)	0.50 (1.0)	0.00 (0)	0.00 (0)	0.00 (0)	0.50 (1.0)	0.00 (0)	0.00 (0)
Plamitoyl carnitine chloride (PCC), mg (%)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	8.00 (16.0)	0.00 (0)	0.00 (0)	0.00 (0)	8.00 (16.0)	0.00 (0)
Sodium glycholate (Na Gly), mg (%)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	7.5 (15)	10.00 (20.0)	0.00 (0)	0.00 (0)	10.00 (20.0)
Total weight (mg), mg (%)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)	50.0 (100)

R1: AS FDSTs using MCC UF-702; R2: AS FDSTs with Na Bicarb 2%; R3: AS FDSTs with Na Bicarb 2% and SDS 0.5%; R4: AS FDSTs with Na Bicarb 2% and SDS 1%; R5: AS FDSTs with Na Bicarb 2% and PCC 16%; R6: AS FDSTs with Na Bicarb 2% and Na Gly 15%; R7: AS FDSTs with Na Bicarb 2% and Na Gly 20%; R8: AS FDSTs with SDS 1%; R9: AS FDSTs with PCC 16%; R10: AS FDSTs with Na Gly 20%.

3.4 Evaluation of The Physical Characteristics and Quality Control Testing of AS FDST Formulations

The mixed powder from each batch was tested for its flowability (PF) and moisture content (MC) before compression. Then, the manufactured tablets were tested for their breaking force (BF), friability (F), and content uniformity (CU) using the United States Pharmacopeia (USP) standard tests and limits. Due to the lack of an accurate USP test that can discriminate small differences between FDSTs, tablets' disintegration time (DT) and drug dissolution (DD) were tested using the previously developed and published apparatuses and procedures that can detect small differences between tablets (Aodah et al., 2017; Rachid et al., 2011). FDSTs' wetting time (WT) and water uptake (WU) were tested as well using modified procedures.

3.4.1 Powder flowability (PF) or the angle of repose test

Powder's flow behavior is an important factor that has a significant impact on tablets manufacturability (Prescott & Barnum, 2000). Powder flowability has a direct effect on weight variability and content uniformity. A poor powder flowability results in huge weight and content variation (Prescott & Barnum, 2000). Therefore, the flowability of the powder mixture of each batch was tested before compressing the tablets. The mixed powder from each formulation was poured into a clean funnel with a diameter of 7 cm at a height of 30 cm and allowed to freely flow on a flat stainless steel surface and form a cone shape. This

process was repeated three times (n=3). The angle of repose was determined by using a special protractor to measure the angle between the wall of the cone side and the flat surface (Figure 10) (USP/NF, 2018d).

The USP powder flow properties and its corresponding angles of repose are presented in Table III. The lower the angle of repose, the better the powder flowability (USP/NF, 2018d).

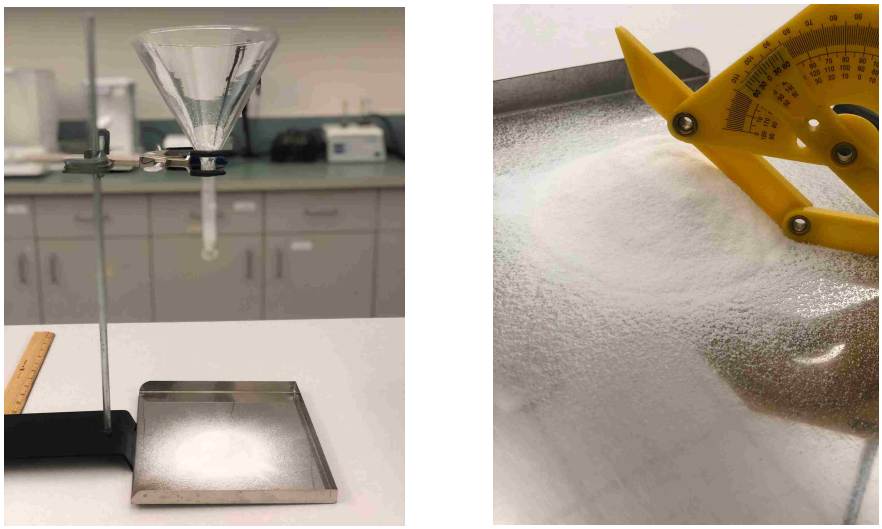

Figure 10. Measurement of the powder's angle of repose using a goniometer angle finder, miter gauge arm, measuring ruler protractor.

Table III. Flow properties and corresponding angles of repose according to USP (USP/NF, 2018d)

Flow Property	Angle of Repose (degree)
Excellent	25–30
Good	31–35
Fair—aid not needed	36–40
Passable—may hang up	41–45
Poor—must agitate, vibrate	46–55
Very poor	56–65
Very, very poor	>66

3.4.2 Moisture content (MC) test

Measuring the MC of the mixed powder is one of the important tests that can affect drug stability and powder flowability. High percentage of powder MC indicates difficulties, especially for FDST formulations due to increasing the ability to uptake moisture from the surrounding which may negatively affects the tablet's disintegration (Alyami et al., 2017). A specific amount of the mixed powder from each formulation, usually 1 g (n=3), was spread on the heating pan of a Halogen Moisture Analyzer HE73, METTLER TOLEDO® (Sonnenbergstrasse, Schwerzenbach, Switzerland). The temperature in the analyzer reached 300°C to evaporate all the moisture in the powder. The MC (%) of the powder was recorded after heating based on the weight of powder used. The powder samples were discarded after testing.

3.4.3 Measurement of tablet's dimensions

The tablet dimensions were measured to ensure the uniformity of the tablets dimension and manufacturing. In this test, ten tablets were randomly selected from each formulation and the diameter (D) and thickness at the tablet's center (TC), were measured using a digital caliber (VWR, Randor, PA) (Figure 11). The mean (± SD) was calculated and recorded.

Figure 11. Measurement of the tablet's dimensions using a digital caliber.

3.4.4 Breaking force (BF) test

Breaking force "hardness" test was performed to test the amount of force required to break up the tablet. It is critical to form hard FDSTs during manufacturing that pass the friability test but without retarding the tablet's disintegration. The test was performed according to the USP guideline (USP/NF, 2018f). The BF of six randomly selected tablets was measured using Vanguard Hardness Tester LIH-3 (Vanguard Pharmaceutical Machinery, INC, Spring, TX). The mean (± SD) was calculated and recorded.

3.4.5 Friability (F) test

The friability (F) test is a required test according to the USP guidelines to ensure that manufactured tablets can stand shipping and handling. The test involved testing 130 dedusted tablets equivalent to 6.5g using a USP friability tester (Vanguard Pharmaceutical Machinery, INC, Spring, TX). Tablets were weighed before the test and then placed in the drum and rotated for 100 rounds at 25 rpm. At the end of the test, the tablets were dedusted and weighed again. The percentage of weight loss was calculated using the following equation:

$$\text{Weight Loss \%} = \frac{(\text{Weight before} - \text{Weight after})}{\text{Weight before}} \text{ X } 100$$

The maximum allowed weight loss according to the USP criteria is less than or equal to 1.0% (USP/NF, 2018e).

3.4.6 Content uniformity (CU) test

Content uniformity is a quality control test used to assess the individual content of the active ingredient in each tablet (Vranić & Uzunović, 2008). According to USP, content uniformity test is required for tablets that contain less than 25 mg or less than 25% of the active ingredient. Tablet content was analyzed by randomly selecting 10 tablets and dissolving each one in 10 mL of distilled water by vortexing for 1 min. Aliquot sample from each solution was collected, diluted, and then filtrated using 0.45 μm nylon syringe filters (VWR, Randor, PA). Samples were analyzed by HPLC with UV detection (Waters Corporation, Milford, MA) using the standard USP procedure for analyzing AS injection (USP/NF, 2018g). The USP acceptance value (AV) of L1 (15% or less) was calculated for each formulation (USP/NF, 2018c).

3.4.7 Tablet's disintegration time (DT) test

Disintegration time test is an important test for ODTs and used to assess the time the tablet takes to liberate its active ingredients to be available for absorption (Al-Gousous & Langguth, 2015). An alternative non-USP disintegration test method was previously developed and published for FDSTs (Aodah et al., 2017). The developed apparatus included a rotating shaft (8 ± 2 mm diameter, 220 ± 20 mm height), a stainless-steel round USP basket (38.5 ± 1 mm diameter, 23 ± 2 mm height) with a stainless-steel wire screen (0.36 - 0.44 mm apertures and 0.22 - 0.31 mm wire diameter) attached at the base of the

rotating shaft, and a glass beaker (30 ± 10 mm diameter, 40 ± 10 mm height, 20 mL volume).

The test was performed by partially immersing the rotating basket that contains one tablet rotated at a speed of 60 rpm into the glass beaker containing 2 mL of warmed water to 37 ± 2 ºC to facilitate tablet disintegration. The time (in seconds) required for each tablet (n=6) to disintegrate completely and for the fine particles to pass through the basket screen into the beaker was determined using a stopwatch (Aodah et al., 2017) (Figure 12).

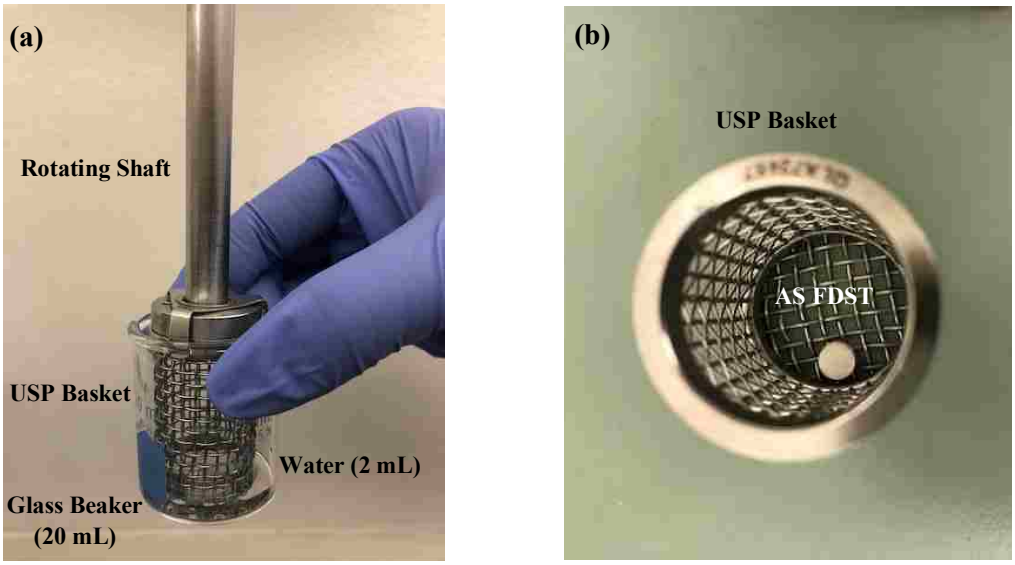

Figure 12. (a) Disintegration apparatus; (b) USP stainless-steel basket.

3.4.8 Drug dissolution (DD) test

Drug dissolution is critical and can be the rate limiting step for its absorption. Dissolution test is a quality control test that can determine the extent and the rate of the drug absorption (Kraemer, Gajendran, Guillot, Schichtel, & Tuereli, 2012). Dissolution test was measured according to our previously developed and validated non-USP dissolution test designed to simulate the low fluid volume and static environment available in mouth cavity and to discriminate between small differences in the dissolution of different AS FDST formulations (Rachid et al., 2011).

Tablets were randomly selected (n=6) and tested according to our previously published procedure. Each tablet was dropped into the donor chamber that contained 2 mL of water and connected to a sampling tube under vacuum. The donor chamber and the sampling tub were separated by 0.45 μm filter membrane (Figure 13). After 60 sec, the vacuum valve was activated and only the drug released and dissolved from each tablet was sucked into the receiving sampling tube through the filter membrane, while the undissolved drug and excipients were retained on the membrane (Rachid et al., 2011). Collected samples were diluted and analyzed by HPLC with UV detection (Waters Corporation, Milford, MA) using the standard USP procedure for analyzing AS injection to quantify and calculate the percentage of AS dissolved from the tablet within 60 sec (USP/NF, 2018g).

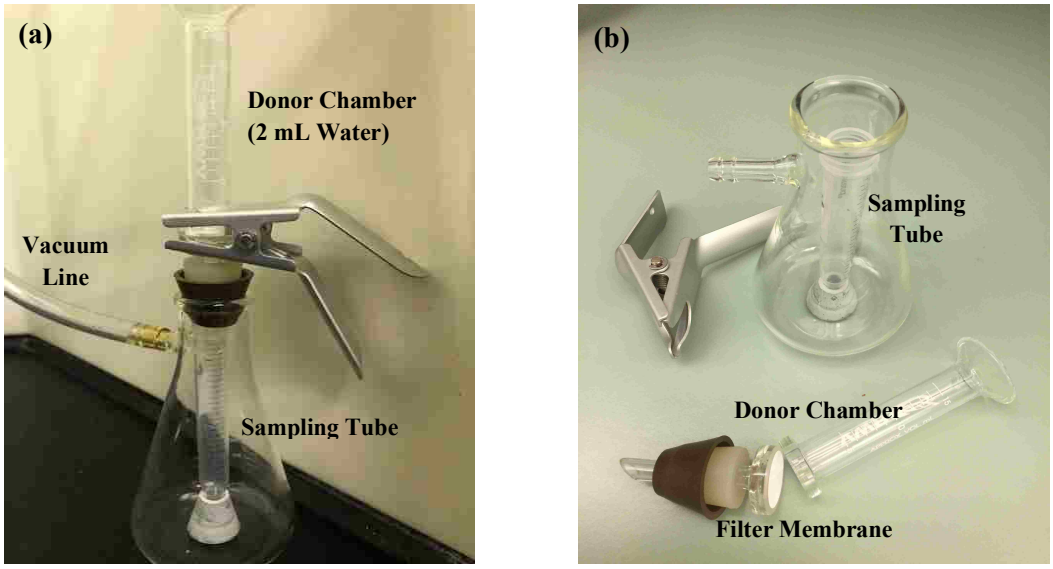

Figure 13. (a) An illustrative dissolution apparatus (b) Disassembled dissolution apparatus.

3.4.9 Water uptake (WU) test

For FDSTs, it is important to determine how well the tablet can absorb and hold water to facilitate drug dissolution. In this non-USP test developed by Aodah and coworkers (Aodah et al., 2017), the dry weight of each tablet (n=6) was measured using an analytical balance (d=0.01 mg). Then, while the tablet was still on the balance, water was added dropwise on the top of the tablet. Once the tablet could not hold more water and water started to ooze out, its wet weight was recorded (Figure 14). The percentage of how much the tablet can absorb and hold water was calculated using the following equation:

$$\text{Water Uptake (\%)} = \frac{(Wet\ Weight - Dry\ Weight)}{Dry\ Weight} X\ 100$$

Figure 14. Measurement of AS FDST's water uptake.

3.4.10 Wetting time (WT) test

Similar to the dissolution test, wetting time was necessary to measure how fast water can diffuse throughout the tablet to dissolve the drug and, therefore, measure small differences in FDST formulations using a previously published non-USP method (Aodah et al., 2017). Wetting time was recorded using a stopwatch right after placing each tablet (n=6) on a wetted but drained paper towel to remove excess water before each test. The time was recorded when the water penetrated throughout the entire tablet (Figure 15).

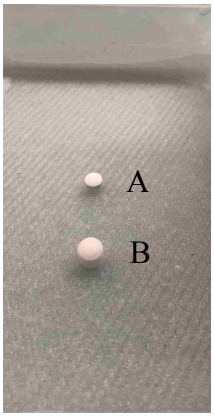

Figure 15. Wetting time test of FDSTs.
A: an image for AS 50 mg FDST when placed on a wet paper towel at t_0; B: an image of FDST placed on a wet paper towel at the end of wetting test.

3.5 *Ex vivo* Permeability Studies

The *ex vivo* permeation of AS from each FDSTs formulation batch was performed to evaluate and measure the sublingual permeability of AS, and the effect of various excipients incorporated into the tablet formulation to optimize tablets' physical characteristics and AS sublingual permeability.

3.5.1 Franz cells preparation

Static vertical jacketed Franz cells containing donor and receiver chambers with an OD of 20 mm, a reservoir volume of 20 ± 1 mL, and a magnetic stirrer at the bottom of the receiver chamber (PermeGear Inc., Hellertown, PA) were used to perform the *ex vivo*

permeability studies (Figure 16). The temperature of the circulating water was set at 37°C ± 1°C. The surgically excised thin sublingual epithelial membrane from the underlying connective and fat tissues of a porcine lower jaw was used as the diffusional membrane (n=4) as previously established and reported (Rachid et al., 2011; M. M. Rawas-Qalaji, Werdy, Rachid, Simons, & Simons, 2015) (Figure 17). The integrity of the membranes were visually examined and experimentally assessed for any significant variability in AS permeability within each study. The excised membranes were stored at -20°C in phosphate buffer, pH 6.8, which represent the average pH of the saliva (pH 5.8 – 7.5), until being used within three months of their storage (Zhang, Zhang, & Streisand, 2002).

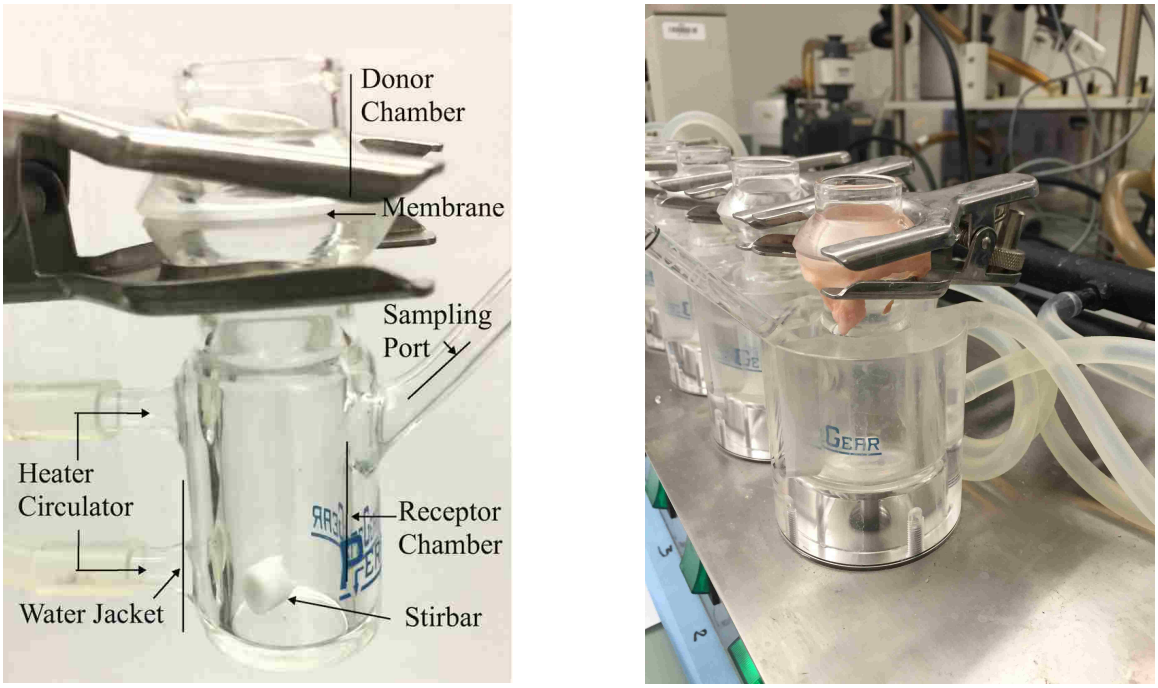

Figure 16. An illustrative scheme for a Franz cell.

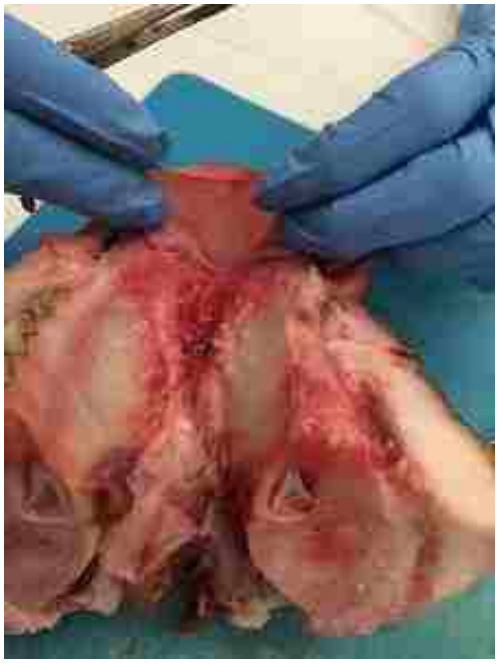

Figure 17. An image of excised porcine sublingual membrane.

3.5.2 Evaluation of the effect of MCC filler grade on the sublingual permeability of AS FDSTs formulation

For each study, excised sublingual porcine membranes were thawed at room temperature and mounted on Franz cells for 30 min to equilibrate with the diffusion medium from both sides. Air bubbles were removed from the receptor chambers and cells were checked for leaks. The water bath was set at 37^{o}C and water was circulated in the jacketed Franz cells. A receiver chamber with a magnetic stirrer was filled with phosphate buffer, pH 7.4 (which represent the pH of the blood). Deionized water 2 mL was used in the donor chamber to facilitate tablet disintegration and dissolution. Tablets from FDSTs formulation containing MCC UF-702 (formulation R1) (n=4) were placed at the center of the mounted sublingual membrane in the donor chamber at time 0 (T_0). Aliquots, 200 µL, were withdrawn from the receptor chamber using of 22 G and 6 inches needles (Cadence Inc., Cranston, RI) and 1 mL syringes at several time intervals, 5, 10, 15, 20, 30, 45, 60, 75, and 90 min. The volumes withdrawn were replenished with fresh phosphate buffer. Samples were filtered and transferred into HPLC vials for HPLC analysis using UV detector (Waters Corporation, Milford, MA) according to the standard USP method for analyzing AS injection (USP/NF, 2018g).

The result was then compared to the previously prepared and published AS FDSTs formulation (formulation B), that contained MCC PH-301 (Aodah et al., 2017).

3.5.3 Evaluation of the ex vivo pH-permeability profile of AS FDSTs formulation

The e*x vivo* permeability of the AS FDSTs formulation using MCC UF-702 (formulation R1) was prepared and performed as described in section 3.5.2. However, instead of using water in the donor chamber as a diffusion medium, Mcvilian buffer (phosphate acetate buffer) was prepared at pH 5, 6.5, 6.8, or 8 and 2 mL of the prepared buffer was placed in the donor chamber to establish a pH-permeability profile for AS and to allow for tablet disintegration and AS dissolution.

The pH that facilitated the highest AS permeability from FDSTs was selected as the optimal pH for AS sublingual permeability.

3.5.4 Evaluation of the effect of different pH-modifiers on the pH of AS solution

Three pH-modifiers were selected: Na Bicarb, Ca Carb, and Na Cit, used in two concentrations (1 or 2% of tablet weight, 50 mg) to evaluate *in vitro* their ability to modify the pH of deionized water as a diffusion medium, which has almost the same pH of human saliva. Various concentrations from each pH-modifier were dissolved in 2 mL of deionized water (n=3) and the pH of the solution was measured using a pH meter (Orion Star®, Thermo Scientific, Waltham, MA). The pH-modifier that was able to modify the pH of the diffusion medium to the optimal pH for AS sublingual permeation based on the *ex vivo* pH-permeability studies (section 3.5.3) was then tested again with the addition of AS FDST in 2 mL of deionized water to evaluate their effect along with AS in FDSTs.

3.5.5 Evaluation of the effect of incorporating a pH-modifier and/or penetration enhancers on the sublingual permeability of AS FDST formulations

Different AS FDST formulations containing a pH-modifier and/or a penetration enhancer (Table II) were formulated and manufactured according to section 3.3 and evaluated according to section 3.4. The e*x vivo* permeability of AS from these different AS FDST formulations was performed as described in section 3.5.2. A 2 mL of deionized water was used in the donor chamber to facilitate tablet disintegration and dissolution. AS FDSTs permeated at pH 6.8 medium, the average saliva pH, was used as a control to evaluate the effect of incorporating a pH-modifier and/or various penetration enhancers on AS sublingual permeability.

3.6 Statistical Analysis

The mean (± SD) of the results from the physical tests including, PF, MC, BF, F, CU, DT, DD, WU, and WT for each FDSTs formulation were calculated AS FDSTs formulation without pH-modifier and penetration enhancers were statistically compared to the previously prepared and published AS FDSTs formulation (formulation B) (Aodah et al., 2017) using student's t-tests. Al the rest of AS FDST formulations prepared in this project were statistically compared within each other by a one-way analysis of variance (ANOVA) and Tukey-Kramer tests.

The mean (± SD) of cumulative amount of AS ($\mu g/cm^2$) permeated over time was

plotted and the area under the curve of AS permeated for 90 min ($AUC_{0–90}$) and 15 min ($AUC_{0–15}$) were calculated for each AS FDST formulation. The mean of AS influx, *J* (μg/cm^2.min), was determined from the slope of the linear portion of the amount of drug permeated versus time curve. The AS permeability coefficient, *P* (cm/min), was calculated by dividing *J* by the initial AS concentration in the donor chamber at $T_{0,}$ which is 8 mg in 2 mL (4 mg/mL). The Lag time (LagT), which is the time required for AS before it started to diffuse through the sublingual membrane to the receiving chamber, was calculated by extra-plotting the slope line to intersect with the X-axis. Data were statistically compared by student's t-tests or one-way ANOVA and Tukey-Kramer tests.

All the statistical analysis tests were performed using NCSS statistical software (NCSS, Kaysville, UT). Differences were considered to be statistically significant at $p<0.05$.

3.7 Chapter Summary

In this chapter, the methods and the designs of the studies performed in order to achieve the objectives of this project were discussed in detail. Ten different formulations of AS FDSTs weighing 50 mg were prepared and manufactured using a new filler grade (UF-702), a pH-modifier (Na Bicarb), and/or a penetration enhancers (SDS, PCC, or Na Gly). Each formulation was evaluated for its physical characteristics. The PF, MC, CU, and F tests were performed and evaluated using the USP standard tests. However, due to lack of an accurate USP testing method that can discriminate small differences between FDSTs,

DT, DD, WT, and WU tests were performed using our developed apparatuses and procedures to detect small differences between tablets.

Testing AS permeability at a range of pH values to evaluate the pH-permeability profile of AS sublingual tablets were used as a guideline for choosing the optimal pH and pH-modifier to be incorporated into the tablet's formulation. In order to evaluate the AS sublingual permeation and the effect of incorporating a pH-modifier and/or a penetration enhancer into the tablet formulation, various *ex vivo* permeation studies were performed for each formulation using static vertical jacketed Franz cells. All the results and data from the quality control tests as well as the *ex vivo* permeation studies were analyzed using HPLC system with a UV detector and statistically analyzed.

Chapter 4

Results

4.1 Chapter Overview

In this chapter, the results for the described studies in the methods section were presented in detail. These include the results from HPLC qualification method tests, quality control and physical tests for AS FDST formulations, AS permeability studies to establish the pH-permeability profile, the pH results from using different pH-modifiers, and results from evaluating the effect of a pH-modifier and or various penetration enhancers on enhancing AS sublingual permeability from various AS FDST formulations. In this chapter, the most significant results were illustrated and emphasized using tables and figures to easily compare the results for the different FDST formulations. Mean ± SD was calculated and compared by *T-tes*t, one-way ANOVA and Tukey-Kramer tests using NCSS statistical software (NCSS, Kaysville, UT). Differences were considered to be statistically significant at $p < 0.05$.

4.2 HPLC Method Qualification

4.2.1 Atropine sulfate calibration curves

Calibration curves ranging from 5 to 200 µg/mL (n=5) were linear with a correlation of coefficient (R^2) of > 0.9998. The retention time of AS was at 8 min. The mean of the performed calibration curves was plotted in Figure 18.

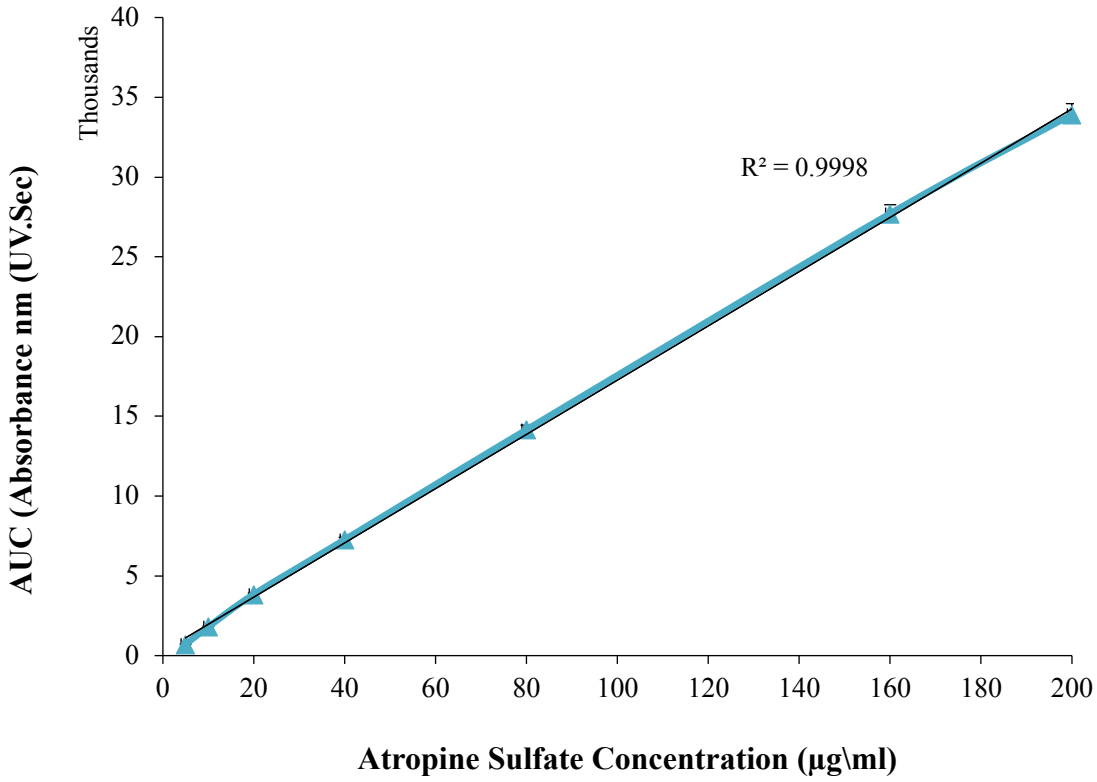

Figure 18. AS calibration curves (n=5).

4.2.2 Instrument and method reproducibility

The HPLC system reproducibility was evaluated by analyzing the lowest and highest concentrations of AS (n=5), 20 µg/mL and 200 µg/mL. The RSD% for the injected concentrations were 0.7% and 0.2%, respectively.

4.2.3 Injection volume accuracy

The instrument accuracy was evaluated by injecting 20 µg/mL AS standard, using increasing injection volumes starting from 10 µL. The AUC of the injected volumes (n=6) resulted a linear correlation with a correlation of coefficient of R^2= 0.999 (Figure 19).

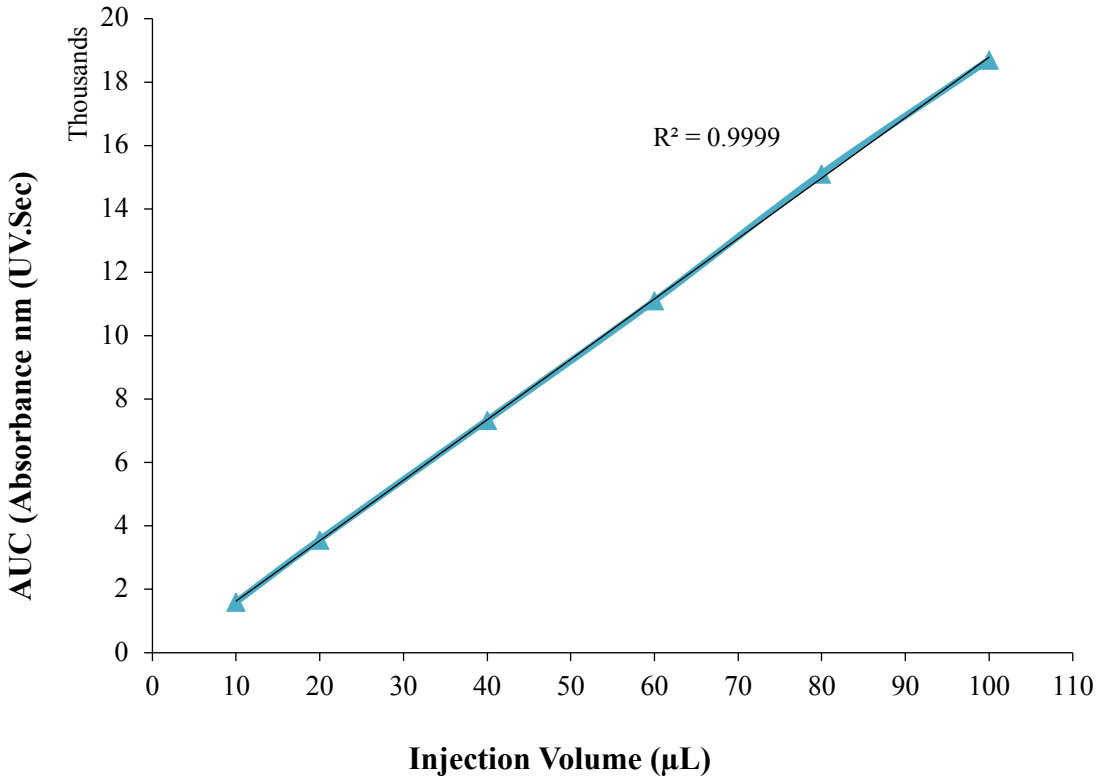

Figure 19. The linearity of the autosampler's injection volume.

4.2.4 Intra and inter-assay variation system

The RSD% for the intra and inter-assay (n=3) of different AS concentrations were calculated and the results were shown in Table IV.

Table IV. Intra and inter-assay variation

RSD%	AS Concentration (µg\ml)						
	5	**10**	**20**	**40**	**80**	**160**	**200**
Intra-assay	0.5	0.5	0.9	0.6	0.9	0.9	0.4
Inter-assay	0.4	0.4	0.9	0.2	0.3	0.8	0.2

RSD%: relative standard deviation percentage.

4.2.5 The limit of quantification

The minimum amount of AS that could be detected and quantified using the HPLC-UV detector system was 125 ng with an RSD% of 1.6% (n=5).

4.3 Physical Characteristics and Quality Control Testing of AS FDST Formulations

4.3.1 The effect of the MCC filler grade on the physical characteristics of AS FDSTs formulation

The current new formulation of AS FDSTs (formulation R1), that contained MCC UF-702, was evaluated based on the various previously described physical tests in section 3.4 and was compared to the previously prepared and published AS FDSTs formulation (formulation B) (Aodah et al., 2017). Results comparing the physical characteristics of the

new AS FDSTs formulation containing MCC UF-702 to the previously developed and published AS FDSTs formulation containing MCC PH-301 were summarized in Table V.

Mean (± SD) angle of repose of the powder mixture for formulation R1 was 32° ± 0.5°, which was significantly lower (p<0.05) than formulation B, 42° ± 2°. The tablet BF of formulation B (1.9 ± 0.6 kgf) was significantly lower (p<0.05) than formulation R1 (2.5 ± 0.1 kgf). AS FDSTs from formulation R1 had significantly faster DT (p<0.05), higher WU and DD compared to AS FDSTs from formulations B (Table V). However, the WT was significantly (p<0.05) faster in formulation B AS FDSTs compared to formulation R1 AS FDSTs.

Table V. Quality control tests of atropine sulfate 8 mg FDST formulations

Test	**AS FDST Formulations**	
	B	**R1**
Powder Flow (repose angle)	42 ± 2	32 ± 0.5*
Breaking Force (kgf)	1.9 ± 0.6	2.5 ± 0.1*
Friability (loss %)	0.09	0.05
Disintegration Time (sec)	14.0 ± 0.4	5.0 ± 0.6*
Drug Dissolution (%)	88.5 ± 14	99.5 ± 6.2*
Water Uptake (%)	229 ± 12	303 ± 16*
Wetting Time (sec)	11 ± 1	17.0 ± 0.9*

Results were presented as mean (± SD)
B: AS FDSTs using MCC PH-301; R1: AS FDSTs using MCC UF-702.
* p<0.05

4.3.2 Powder flowability (PF) or angle of repose test

According to the USP criteria (USP/NF, 2018d), all the powder blends developed for AS FDST formulations in this project had either a passable or a good angle of repose based on Table III. The angle of repose results for the powder blends from the different formulations were presented in Table VI.

4.3.3 Moisture content (MC) test

Moisture content was tested for all the powder blends from the different AS FDST formulations. MC can influence the tablets stability and powder flowability. The addition of the pH-modifier and/or transcellular penetration enhancers (formulation R2, R3, R4, R6, R7, R8, R9, and R10) showed a significantly higher (p<0.05) MC than AS FDSTs formulation with no pH-modifier and/or penetration enhancers (formulation R1). However, the difference was higher when the transcellular enhancers were added alone to AS FDSTs (formulation R8, R9, R10). The results of MC from the different AS FDST formulations were presented in Table VI.

4.3.4 Tablets' dimensions measurement

All the tablets prepared for the different formulations of AS FDSTs had the same tablet's size. The mean (± SD) of tablet's dimensions including, tablet's diameter and tablet's central thickness for different AS FDST formulations were shown in Table VI.

4.3.5 Breaking force (BF) test

At the same compression force used (130 – 150 kgf), different formulations showed different tablets' hardness. Breaking force of the manufactured tablets ranged from 2.0 to 2.4 kgf. However, these differences between the different AS FDST formulations were not significantly different (p>0.05). The tablet hardness results for each AS FDSTs formulation were presented in Table VI.

4.3.6 Friability (F) test

According to the USP, the maximum weight loss allowed for a tablet dosage form is no more than 1% (USP/NF, 2018e). All the manufactured AS FDST formulations passed the friability test. The friability results for each AS FDSTs formulation were shown in Table VI.

4.3.7 Content uniformity (CU) test

According to the USP criteria for dosage form's content uniformity (USP/NF, 2016), all AS FDST formulations passed the acceptance value (AV) for CU, with AV of ≤ 15. The mean (± SD) of tablets' CU% and AV for each FDSTs formulation were presented in Table VI.

4.3.8 Tablet's disintegration time (DT) test

The addition of the pH-modifier (Na Bicarb) and/or penetration enhancers (SDS, PCC, Na Gly) significantly increased (p<0.05) the tablet's disintegration time compared to the AS FDSTs formulation with no pH-modifier and/or penetration enhancers (formulation R1). Despite the addition of the pH-modifier and/or the penetration enhancers into the tablet formulations containing the new MCC filler grade UF-702, most of the AS FDST formulations (formulation R2, R3, R4, R6, R7, R8, and R10) had a short disintegration time of less than 12 sec. AS FDST formulations containing the paracellular enhancer PCC 16% with or without a pH-modifier (formulation R5 and R9) resulted in the longest disintegration time (p<0.05) of 12 sec and 16 sec, respectively. All the disintegration results for the different AS FDST formulations were shown in Table VI.

4.3.9 Drug dissolution (DD) test

The percentage of dissolved AS from AS FDSTs in the first 60 seconds for all the AS FDST formulations were almost complete. The addition of the pH-modifier and/or penetration enhancers (formulation R2, R3, R4, R6, R7, R8, and R10) showed no negative effect (p>0.05) on the percentage of AS dissolved per minute. The addition of the 16% paracellular enhancer PCC with or without a pH-modifier (formulation R5 & R9) resulted in significantly less (p<0.05) AS percentage dissolved per min (86% and 88) compared to the other formulations. The mean (± SD) of DD% for all AS FDST formulations were shown in Table VI.

4.3.10 Water uptake (WU) test

The WU by the AS FDSTs formulation with no pH-modifier and/or penetration enhancers (Formulation R1) was significantly higher (p<0.05) than the formulations that had a pH-modifier, a pH-modifier with or without penetration enhancers, and penetration enhancers alone (formulation R2, R3, R4, R5, R6, R7, R8, R9, and R10). Also, the addition of the transcellular enhancer Na Gly 20% with or without the pH-modifier resulted in the lowest (p<0.05) percentage of WU (247% and 240%). The mean (± SD) percentage of tablet's WU for all AS FDST formulations were shown in Table VI.

4.3.11 Wetting time (WT) test

The WT of FDSTs was only affected by the addition of the penetration enhancers alone without the pH-modifier including, SDS 1% (formulation R8), PCC 16% (formulation R9), and Na Gly 20% (formulation R10). They all significantly increased (p<0.05) the tablet's WT compared to the other AS FDST formulations (Table VI). Also, AS FDSTs formulation with the pH-modifier and paracellular enhancer PCC 16% (formulation R5) resulted in the longest WT (P<0.05) compared to the all other formulations. The WT results for all AS FDST formulations were shown in Table VI.

Table VI. Quality control tests of AS FDST formulations

Test		FDSTs Formulations									
		R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**
Powder Flowability (angle)		32.0±0.6	35.0±0.0	33.0±0.6	33.0±1.1	34.0±0.0	35.0±0.6	37.0±0.6	36.0±0.6	36.0±1.1	35.0±0.0
Moisture content (%)		7.0±0.0	9.0±0.0[#]	8.0±0.0[#]	8.0±0.0[#]	7.0±0.0	9.0±0.0[#]	9.0±0.0[#]	15.0±0.0[$]	14.0±0.0[$]	12.0±0.0[$]
Tablet Dimensions	Thickness	3.15±0.07	3.1±0.0	3.1±0.0	3.1±0.1	3.1±0.05	3.1±0.0	3.1±0.0	3.1±0.0	3.1±0.02	3.1±0.0
	Diameter	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0
Breaking Force (kgf)		2.4±0.1	2.4±0.2	2.3±0.2	2.1±0.2	2.0±0.1	2.1±0.2	2.0±0.9	2.2±0.3	2.4±0.4	2.0±0.2
Friability (loss%)		0.05±0.0	0.02±0.0	0.02±0.0	0.04±0.0	0.4±0.0	0.02±0.0	0.05±0.0	0.09±0.0	0.06±0.0	0.08±0.0
Content Uniformity (%) (AV)		102.8±3.7 (10.3)	106.0±2.2 (9.8)	95.3±2.2 (8.4)	107.6±0.4 (7.2)	98.3±3.4 (8.4)	95.2±4.3 (13.7)	111.4±1.0 (12.3)	109.7±1.9 (12.7)	105.2±3.6 (11.2)	97.1±2.2 (6.7)
Disintegration Time (sec)		5.0±0.6[*]	9.0±0.0	7.0±1.0	8.0±1.0	12.0±1.1[*]	9.0±0.0	10.0±0.6	10.0±0.6	16.0±0.5[*]	7.0±0.0
Drug Dissolution (%)		99.5±6.2	100.0±1.4	106.0±5.3	96.6±6.0	86.4±8.2[$]	90.0±9.7	107.0±6.3	93.0±6.0	88.0 ±4.1[$]	91.1±4.8
Water Uptake (%)		303.0±16.4[*]	281.0±8.4	281.0±3.6	285.0±3.8	276.0±12.3	274.0±6.0	247.0±9.8[$]	284.0±6.5	274.0±3.0	240.0±9.7[$]
Wetting Time (sec)		17.0±0.9	17.0±1.1	17.0±1.2	10.0±0.6[*]	35.0±0.9[*]	13.0±1.2[*]	19.0±0.8	38.0±1.0[$]	40.6±0.7[$]	38.0±2.2[$]

Results were presented as mean (± SD)

R1: AS FDSTs using MCC UF-702; R2: AS FDSTs with Na Bicarb 2%; R3: AS FDSTs with Na Bicarb 2% and SDS 0.5%; R4: AS FDSTs with Na Bicarb 2% and SDS 1%; R5: AS FDSTs with Na Bicarb 2% and PCC 16%; R6: AS FDSTs with Na Bicarb 2% and Na Gly 15%; R7: AS FDSTs with Na Bicarb 2% and Na Gly 20%; R8: AS FDSTs with SDS 1%; R9: AS FDSTs with PCC 16%; R10: AS FDSTs with Na Gly 20%.

[*] $p<0.05$ from all.

[$] $p<0.05$ from all but not than each other.

[#] $p<0.05$ from AS FDSTs using MCC UF-702 (R1), i.e. AS FDSTs without a pH-modifier and penetration enhancers.

4.4 *Ex Vivo* Permeability Studies

4.4.1 The effect of the MCC filler grade on the sublingual permeability of AS FDSTs formulation

The mean (± SD) cumulative amount of AS permeated over time from both formulations, the previously developed AS FDSTs formulation (Formulation B) and the current formulation (Formulation R1) were plotted in

Figure 20. Formulation R1 AS FDSTs resulted in a significantly higher (p<0.05) mean (± SD) cumulative amount of AS permeated (AUC) over 90 and 15 min, AS influx, and significantly decreased the permeation lag time in comparison to formulation B AS FDSTs (Table VII).

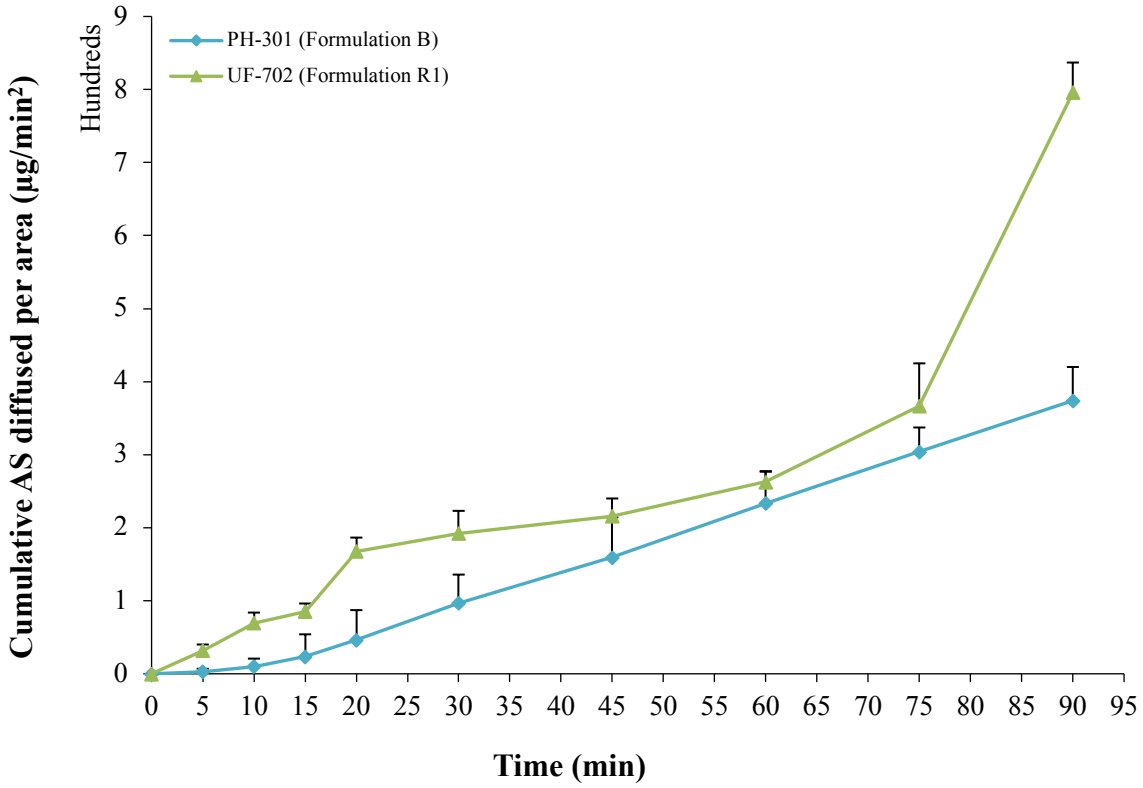

Figure 20. The cumulative AS permeated per area (µg/cm^2) versus time from FDST using MCC PH-301 (formulations B) and UF-702 (Formulation R1).

Table VII. *Ex vivo* permeability of atropine sulfate 8 mg FDST formulations in water as a diffusion medium

	AS FDSTs Formulations	
	B	**R1**
Area under the curve, $AUC_{0\text{-}90\ min}$ (μg/cm²/min)	14995 ± 3184	23239 ± 550*
Area under the curve, $AUC_{0\text{-}15\ min}$ (μg/cm²/min)	122 ± 150	722 ± 134*
Influx, *J* (μg/cm²/min)	4.6 ± 0.9	7.7 ± 0.8*
Lag time, LagT (min)	11.2 ± 4.7	0.0 ± 0.0*

Results were presented as mean (± SD)
B: AS FDSTs using MCC PH-301; R1: AS FDSTs using MCC UF-702.
* $p<0.05$.

4.4.2 The ex vivo pH-permeability profile of AS FDSTs formulation

The mean (± SD) cumulative amount of AS permeated over time from AS FDSTs at various pH media were plotted in

Figure 21. During the first 20 min of the permeation studies, AS permeation from formulation R1 AS FDSTs at different pH media did not show any significant difference ($p>0.05$). However, the mean (± SD) of $AUC_{0\text{-}90}$ of AS permeated, *J*, and *P* from formulation R1 AS FDST in a diffusion medium of pH 8 were statistically higher ($p<0.05$) than at all other pH media (5, 6.5 and 6.8). Also, the AUC, *J*, and *P* from formulation R1 AS FDST at pH 6.8 medium, which represented the average saliva pH, were statistically higher ($p<0.05$) than at pH 5 medium (Table VIII).

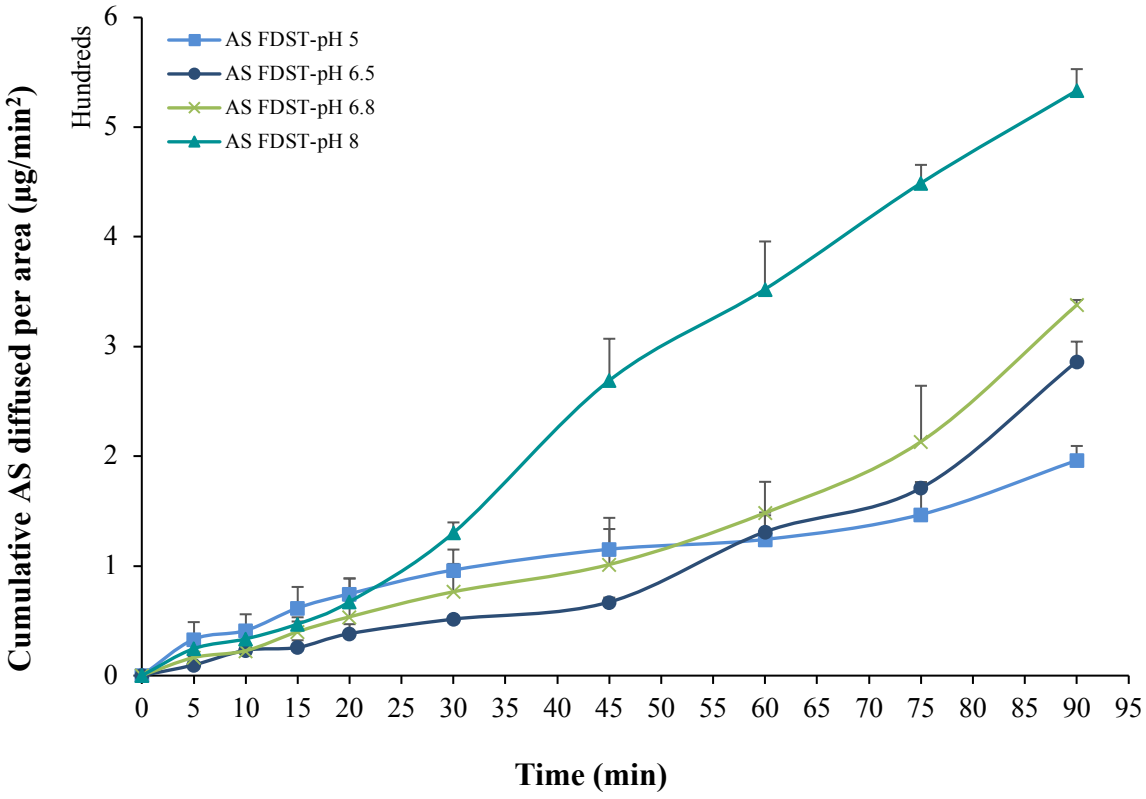

Figure 21. The cumulative AS permeated per area (µg/cm^2) versus time from FDSTs formulation R1 at different pH medium

Table VIII. *Ex vivo* permeability of AS FDSTs at different pH

	AS FDSTs Formulation (R1) at Different pH Medium			
	pH 5	**pH 6.5**	**pH 6.8 (control)**	**pH 8**
Area under the curve, $AUC_{0\text{-}90\ min}$ (µg/cm^2/min)	9708 ± 13530	9908 ± 14330	11232 ± 12006	22715 ± 524*
Influx, *J* (µg/cm^2/min)	3.0 ± 0.5	3.3 ± 0.3	4.5 ± 0.4$^\$$	8.4 ± 1.6*
Permeability coefficient, *P* (cm/min)	6.0×10^{-4} ± 1.2×10^{-4}	8.2×10^{-4} ± 8.6×10^{-5}	1.1×10^{-3} ± 9.7×10^{-5} $^\$$	2.1×10^{-3} ± 4.1×10^{-4}*

Results were presented as mean (± SD)
* $p<0.05$ from all.
$^\$$ $p<0.05$ from pH 5.

4.4.3 The effect of different pH-modifiers on the pH of AS solution

Na Bicarb 2% resulted in a pH of 8.1 ± 0.3 that was significantly higher (p<0.05) than all other buffers at the various concentrations used. Also, incorporating Na Bicarb 2% into AS FDSTs formulation resulted in a similar (p>0.05) pH value of 7.9 ± 0.1. The mean (± SD) pH values for the different buffers used at different concentration were shown in Table IX.

Table IX

Concentration (%)	**pH-Modifiers**		
	Na Bicarb	**Ca Carb**	**Na Cit**
1%	7.2 ± 0.2	6.6 ± 0.1	6.2 ±0.3
2%	8.1 ± 0.3*	7.6 ± 0.1	7.5 ± 0.1
2% with AS FDSTs	7.9 ± 0.1	-	-

Table IX. pH measurements of different pH-modifiers at different concentrations

Results were presented as mean (± SD)
* p<0.05 from all except 2% Na Bicarbonate incorporated into AS FDSTs.

4.4.4 The effect of incorporating a pH-modifier with or without penetration enhancers on the sublingual permeability of AS FDST formulations

The mean (± SD) cumulative amount of AS permeated over time from AS FDSTs that contained the pH-modifier (2% Na Bicarb) with or without penetration enhancers (SDS,

PCC, or Na Gly at different concentrations) were plotted in Figure 22. The mean (± SD) $AUC_{0\text{-}90}$ of cumulative drug permeated, *J*, and *P* from AS FDST formulations (formulation R2, R3, R4, R5, R6, and R7) were statistically higher ($p<0.05$) than the control (formulation R1 at pH 6.8) (Table X). The mean (± SD) $AUC_{0\text{-}90}$ of cumulative AS permeated, *J*, and *P* from AS FDSTs with the pH-modifier and transcellular enhancers (formulation R3, R4, R6, and R7) were significantly higher ($p<0.05$) than with the paracellular enhancer (formulation R5) and the control (Table X). Incorporating the pH-modifier Na Bicarb 2% with SDS 1% (formulation R4) achieved the highest enhancement in AS sublingual permeability ($p<0.05$) and increased AS permeability 13-fold compared to the control. The *ex vivo* results for the different AS FDST formulations were shown in Table X.

Also, the *J* and *P* of AS FDST formulations with the pH-modifier and paracellular enhancers (formulation R5) showed similar results ($p>0.05$) compared to the AS FDST formulations with the pH-modifier alone (formulation R2) (Table X).

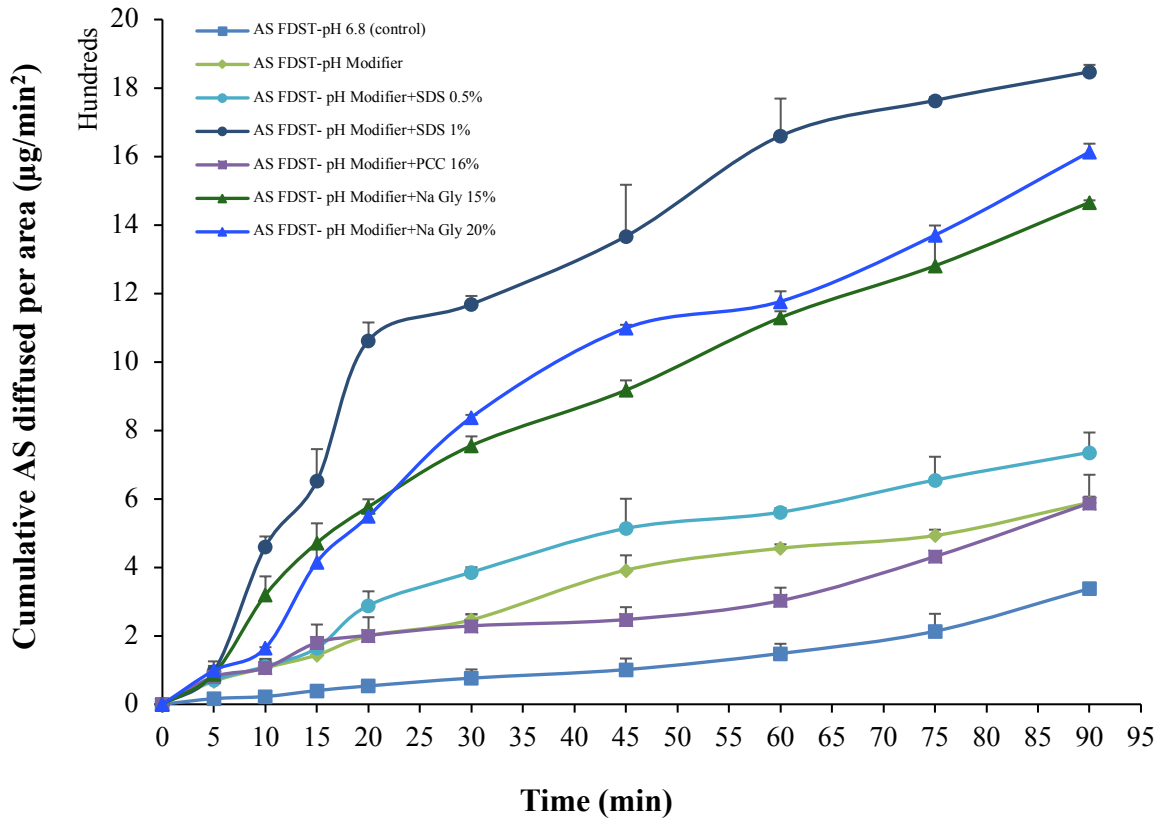

Figure 22. The cumulative AS permeated per area (µg/cm^2) versus time from FDST formulations with pH-modifier and penetration enhancers.

Table X. *Ex vivo* permeability of different AS FDST formulations containing a pH-modifier and penetration enhancers

	AS FDSTs Formulations						
	R1 (control)	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**
Area under the curve, $AUC_{0\text{-}90\ min}$ ($\mu g/cm^2/min$)	11232 ± 12006*	30696 ± 510*	40173 ± 1396*	114334 ± 3413*	25339 ± 1527*	79071 ± 1429*	84775 ± 908*
Influx, *J* ($\mu g/cm^2/min$)	4.5 ± 0.4*	9.6 ± 1.6$	19.3 ± 1.0*	56.1 ± 4.3*	9.5 ± 2.0$	26.4 ± 0.5*	31.0 ± 0.2*
Permeability coefficient, *P* (cm/min)	$1.1x10^{-3}$ ± $9.7x10^{-5}$*	$2.4x10^{-3}$ ± $4.0x10^{-4}$$	$5.0x10^{-3}$ ± $2.2x10^{-4}$*	$1.4x10^{-2}$ ± $1.1x10^{-3}$*	$2.4x10^{-3}$ ± $5.1x10^{-4}$$	$6.6x10^{-3}$ ± $1.2x10^{-4}$*	$7.7x10^{-3}$ ± $6.5x10^{-5}$*

Results were presented as mean (± SD)
R1: AS FDSTs at pH 6.8 medium using MCC UF-702; R2: AS FDSTs with Na Bicarb 2%; R3: AS FDSTs with Na Bicarb 2% and SDS 0.5%; R4: AS FDSTs with Na Bicarb 2% and SDS 1%; R5: AS FDSTs with Na Bicarb 2% and PCC 16%; R6: AS FDSTs with Na Bicarb 2% and Na Gly 15%; R7: AS FDSTs with Na Bicarb 2% and Na Gly 20%.
* $p<0.05$ from all.
$ $p<0.05$ from all but not each other.

4.4.5 The effect of incorporating penetration enhancers on the sublingual permeability of AS FDST formulations

The mean (± SD) cumulative amount of AS permeated over time from AS FDSTs that contained a penetration enhancer (SDS, PCC, or Na Gly) were plotted in Figure 23. The mean (± SD) $AUC_{0\text{-}90}$ of cumulative drug permeated and *J* from AS FDSTs with transcellular enhancers (formulation R8 and R10) were significantly higher ($p<0.05$) than AS FDSTs with paracellular enhancer (formulation R9) and the control (Table XI) (Figure 23). Also, the *P* of AS FDSTs formulation with the transcellular enhancer (SDS)

showed a significantly higher (p<0.05) result compared to the other enhancers and control (Table XI).

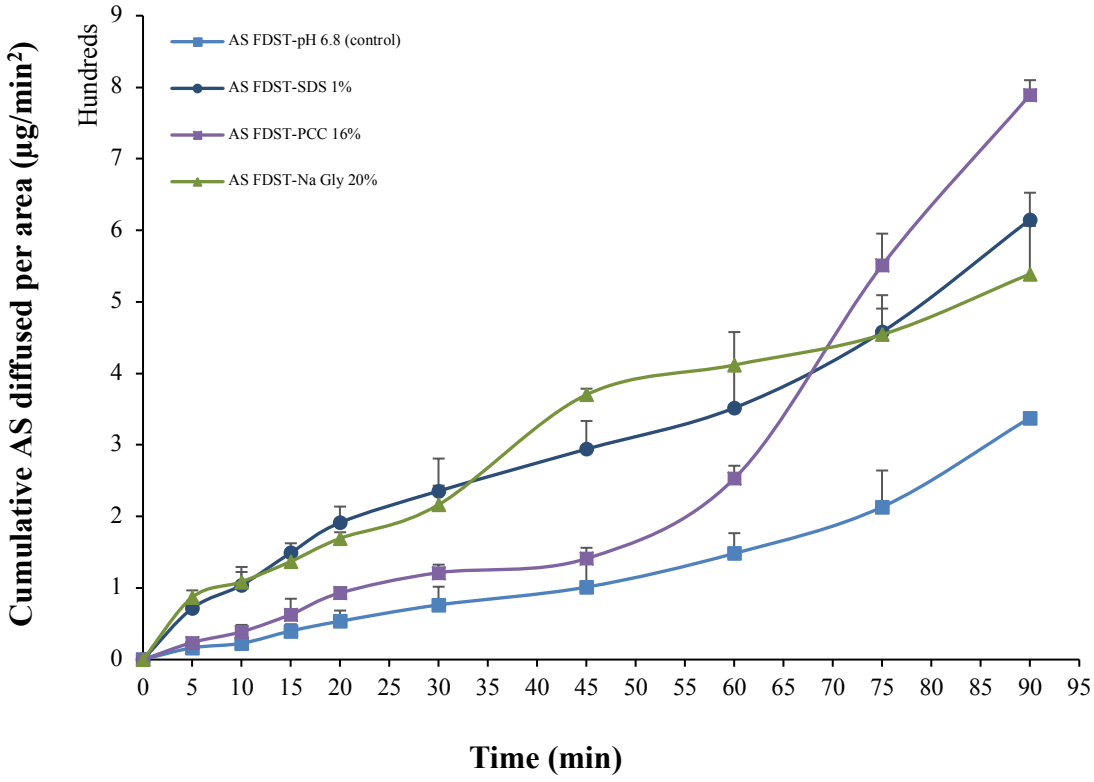

Figure 23. The cumulative AS permeated per area (µg/cm^2) versus time from FDST formulations with penetration enhancers.

Table XI. *Ex vivo* permeability of different AS FDST formulations containing only penetration enhancers

	AS FDST Formulations			
	R1 (control)	**R8**	**R9**	**R10**
Area under the curve, $AUC_{0\text{-}90\ min}$ ($\mu g/cm^2/min$)	11232 ± 12006*	27180 ± 2726$	22947 ± 1210*	28228 ± 1488$
Influx, *J* ($\mu g/cm^2/min$)	4.5 ± 0.4*	8.5 ± 1.0$	3.0 ± 0.2*	6.0 ± 0.7$
Permeability coefficient, *P* (cm/min)	$1.1x10^{-3}$ ± $9.7x10^{-5}$	$2.1x10^{-3}$ ± $2.6x10^{-4}$*	$8.0x10^{-4}$ ± $6.5x10^{-5}$	$1.8x10^{-3}$ ± $2.0x10^{-4}$

Results were presented as mean (± SD)
R1: AS FDSTs at pH 6.8 medium using MCC UF-702; R8: AS FDSTs with SDS 1%; R9: AS FDSTs with PCC 16%; R10: AS FDSTs with Na Gly 20%.
* $p<0.05$ from all.
$ $p<0.05$ from all but not each other.

4.5 Chapter Summary

In this chapter, AS calibration curves were successfully created using the standardized USP analytical method. Both the analytical instrument and the quantification method demonstrated high accuracy and reproducibility. All the physical characteristics and quality control tests, USP and non-USP tests, for the different manufactured FDST formulations were successfully performed. The results of the physical characteristic as well as the *ex vivo* permeability test for the optimized AS FDST formulations using the new MCC filler grade were reported and resulted in more optimal characteristics. The pH-permeability profile for AS was determined and the relationship between the medium's pH and AS sublingual permeation was established. The different AS FDST formulations prepared with the addition of pH-modifier and/or penetration enhancers and their effect on

AS sublingual permeability were also investigated and compared to the control to demonstrate enhancement in AS sublingual permeation from the optimized AS FDSTs.

Chapter 5

Discussion

5.1 Overview

In this project, different ways and strategies for enhancing the physical characteristics as well as the sublingual permeation and absorption of AS for a previously prepared AS FDSTs (Aodah et al., 2017), were explored and evaluated. The tablets characteristics for the sublingual delivery of AS for the treatment of OP toxicity is very critical since the tablet should disintegrates rapidly to release the drug into the sublingual area. Also, complete and rapid drug dissolution should be obtained in the small saliva volume to ensure rapid and efficient drug absorption (Nayak & Manna, 2011; Wang & Chow, 2014; Washington & Washington, 2001; Zhang et al., 2002).

The overall objective of this project is to develop a new dosage form for AS that can overcome the many drawbacks associated with the use of current AS auto-injector, AtroPen®. The proposed AS FDSTs, as alternative first-aid dosage form, should possess the physical qualities that allow them to withstand the USP criteria and function to deliver a bioequivalent dose of AS comparable to AtroPen® in order to attain a clinical significance.

In order to achieve this goal, optimizing the AS FDSTs formulation by changing the MCC filler grade was one of our strategies to enhance the quality and the physical characteristics of the tablet. The standard USP tests including, CU, F, and BF, and in-house developed and modified tests including, DT, WT, WU, and DD, were used to evaluate the effect of changing the filler grade on tablet characteristics and ensure the stringent criteria for sublingual drug delivery are met.

In order to achieve optimal AS permeation and absorption, AS permeability at various pH media was explored to evaluate the effect of altering the microenvironment's pH of the sublingual cavity and the potential of incorporating a pH-modifier into the tablet formulation.

Additionally, the effect of various penetration enhancers at various concentrations on AS sublingual permeability were also explored by incorporating various penetration enhancers with or without a pH-modifier into the tablet formulation and evaluating their effect on AS sublingual permeability in order to enhance AS sublingual permeability from FDSTs.

5.2 The Effect of MCC Filler Grade on the Physical Characteristics and Sublingual Permeability of AS FDSTs Formulation

Microcrystalline cellulose is a widely used filler that is manufactured by different companies and available in various grades of different properties to be used in different pharmaceutical formulations and dosage forms. Fillers with different properties can perform differently when compared at discriminating conditions like the very strict

conditions of the sublingual route and when the sublingual dosage form is subjected to low-volume static conditions under the tongue that does not aid dosage form disintegration or drug dissolution. MCC PH grade is one of the commonly used conventional grades in tablet formulations and MCC UF grade is one of the more recent highly compressible grades manufactured by Asahi Kasei Corp that were used in the formulation of our AS FDSTs. In this project, changing the conventional MCC filler grade, PH-301, in formulation B to a highly compressible MCC UF-702 grade in formulation R1 resulted in significant changes in AS FDST's physical characteristics and AS permeability.

MCC UF-702 grade is spherical in shape, has an average particles size of 90 μm, and a repose angle of 34°. On the other hand, MCC PH-301 grade has an irregular particles' shape, an average particles size of 50 μm, and a repose angle of 41° (Table I) (Asahi, 2018). The particles size distribution, the shape, and the density of the filler's particles are important variables that can influence the entire powder flowability, since the filler constitutes the majority of the tablet composition (Kucera, DiNunzio, Kaneko, & McGinity, 2012). The larger particles size of MCC UF-702 grade provided a less frictional contacting surface area between the MCC UF-702 particles and the surface they are flowing against. Also, the spherical shape of MCC UF-702 particles facilitated a better flow and resulted in a significantly lower angle of repose (32˚ ± 0.5˚) for formulation R1 powder mixture compared to the angle of repose (42˚ ± 2˚) for formulation B powder mixture which contained MCC PH-301 that has irregularly particle shaped (Table V) (Horio et al., 2014).

MCC UF-702 grade has a more porous structure as demonstrated by its lower bulk density of 0.29 g/cm^3 compared with a bulk density of 0.41 g/cm^3 for MCC PH-301 grade

(Table I) (Asahi, 2018). The porous structure of UF-702 grade can result in a higher plastic deformation, which means better compatibility, and faster tablet disintegration compared to PH-301 grade at similar compression forces (Dinunzio et al., 2012). It allows for better and faster water penetration by capillary action through the pores, which promotes tablet swelling and disintegration (Thoorens, Krier, Leclercq, Carlin, & Evrard, 2014). This correlated well with the hardness and disintegration results from formulation R1 and formulation B FDSTs. Harder tablets were obtained for formulation R1 FDSTs without negatively affecting the tablet disintegration (Table V) despite being manufactured at similar compression forces. The difference in the tablet porosity created due to using UF-702 grade in formulation R1 FDSTs resulted in a harder tablet compact and faster tablet disintegration compared to formulation B FDSTs. The disintegration time for AS FDSTs was measured using our previously developed and published method that resembles the statics and low solution volume conditions in the human mouth (Aodah et al., 2017). This method was able to measure the time required in seconds for the AS FDSTs to disintegrate into fine particles and to discriminate between different formulations (formulation R1 and B FDSTs), which cannot be measured by the current official USP standard disintegration test (Aodah et al., 2017; USP/NF, 2018a).

Tablet's WT and WU measure the speed and extent of water absorption by the tablet to initiate drug dissolution and disintegrate the tablet. The number of bonds formed between the particles during compression is one of the variables that can affect tablet porosity and excipients swelling extent, which affects the speed and extent of water penetration into the tablet (Thoorens et al., 2014). Wetting test utilized a very small amount of water just enough to wet the paper towel used in this test representing a stringent testing

conditions, which can be correlated to a dry mouth clinical condition that can negatively impact the tablet's disintegration. Because formulation R1 FDSTs had a higher tablet hardness, they showed a longer (p<0.05) wetting time compared to formulation B FDSTs (Table V). It seems that the amount of water used in the wetting test may not have been sufficient to overcome the stronger bonds formed within formulation R1 FDSTs, therefore, significant wetting time difference was achieved. On the other hand, when more water was presented in the disintegration and water uptake tests representing normal or excess salivary secretions manifested in case of OP toxicity, tablet's disintegration and water uptake in formulation R1 FDSTs were significantly better (p<0.05) when compared to formulation B FDSTs (Table V).

In order to confirm the significance of improving tablet characteristics (disintegration, wetting, and water uptake) on the rate of drug dissolution, the amount of drug dissolved within only 60 sec were measured using our previously validated and published apparatus and method, since it cannot be measured by the current official USP standard dissolution test (Aodah et al., 2017; USP/NF, 2018b). This apparatus and method were developed to simulate the short time and static and low volume conditions available for drug disintegration and dissolution following a sublingual drug administration (Aodah et al., 2017; Rachid et al., 2011) The significant impact of changing MCC PH-301 grade to MCC UF-702 grade was well demonstrated not only on the characteristics of FDSTs but also on the rate AS dissolution (Table V). Formulation R1 FDSTs promoted complete AS dissolution without any agitation within 60 sec (99.5 ± 6.2%) that was significantly higher than formulation B FDSTs (88.5 ± 14%), an indication for the release of more amount of AS from the tablet due to better AS FDSTs characteristics.

These results can be very meaningful and critical when they are correlated with a significant increase in sublingual AS permeation and absorption in order to demonstrate a clinical significance. Therefore, the sublingual permeation of AS from these FDSTs were evaluated through excised porcine sublingual membranes using Franz diffusion cells. Replacing MCC PH-301 grade with MCC UF-702 grade resulted in a significant increase in the overall AS permeation over time, AS influx, and reduced AS permeation lag time (Table VII).

For the treatment of emergency conditions, the rate of drug absorption is as critical as the amount of drug being absorbed in order to achieve the fast onset of action required for treating these conditions. For this reason, the amount of drug diffused for 15 min, (AUC_{0-15}) ($\mu g/cm^2$) was also calculated for further analysis.

The lag time and the amount of AS permeated during the initial 10-20 min (Figure 20) can be useful in guiding the AS FDSTs development. Changing the filler grade to UF-702 in formulation R1 FDSTs reduced the lag time from 11.2 ± 4.7 min to zero min and increased the $AUC_{0\text{-}15min}$ almost 6-fold (Figure 20). The reduction in lag time was mainly due to the significant increase in tablet disintegration and AS dissolution that permitted for immediate and significantly higher AS permeation through the sublingual membrane.

Further drug absorption beyond the applicable time for sublingual absorption, although increased the overall AS permeated over time, may have no clinical significance for the treatment of emergency conditions that require fast onset of action during the short administration time required for sublingual route. The delayed increase in the AS permeated from formulation R1 FDSTs after 75 min of the permeability study (Figure 22) was mainly due to the accumulation of AS in the donner cell that was able to overcome the

permeability resistance of the sublingual membrane. The delayed increase in AS permeation, despite of its clinical insignificance for the sublingual treatment of OP toxicity, is another confirmation that formulation MCC UF-702 was superior to MCC PH-301 and resulted in faster drug release, higher dissolution, higher concentration, and higher drug permeation over time. A similar increasement were expected to be achieved by formulation B FDSTs, despite of its insignificance, if permeability studies were extended beyond 90 min.

In summary, the results from this research support that the filler grade can play a critical role in changing the characteristics of AS FDSTs, which can have significant implications on the permeation and potentially the absorption of sublingually administered AS from FDSTs formulation, and therefore, may increase the potential of developing an alternative non-invasive dosage form for the treatment of OP toxicity.

5.3 The Ex Vivo pH-Permeability Profile of AS FDSTs Formulation

The permeability of any drug is known to be affected by three main factors. These include charges, lipophilicity, and molecular weight of the drug molecules (Lee et al., 2005). The extent of sublingual absorption of AS FDSTs can be greatly dependent on its degree of ionization, which is mainly affected by the pH of the saliva. Therefore, the evaluation of various pH medium is critical for enhancing AS sublingual permeability. Mcvilian buffer was prepared at pH 5, 6.5, 6.8, and 8 to establish a pH-permeability profile for AS FDSTs. The amount of AS permeated from FDSTs (formulation R1) at various pH medium was measured. The results from this test were very important as to evaluate the

effect of AS degree of ionization, as a result of changing the medium pH, on AS permeability from FDSTs. Also, it guided the determination of the optimal pH for AS sublingual permeability and, therefore, the criteria for the ideal pH-modifier that is needed to be incorporated into AS FDSTs formulation to modify the sublingual microenvironment pH to that optimal pH.

AS is a weak base with a pKa of 9.4 and a logP of 2.19 (NIH, 2018). This means that at pH 9.4, 50% of the drug will be in the unionized form, which allow for better absorption for the lipid soluble unionized portion of the drug through cell membrane (Lee et al., 2005). The results from our Franz cells permeability studies indicated that the initial AS permeated, due to the fast DT of the tablet and fast release and dissolution of AS, was not impacted by the medium pH. However, following the initial AS permeation, which seems to have saturated the sublayers of the sublingual membranes, the less ionized AS at higher pH resulted in higher permeation than the ionized AS at lower pH.

The mean (± SD) of $AUC_{0\text{-}90}$ of AS permeated, *J*, and *P* from AS FDSTs (formulation R1) in a diffusion medium of a pH of 8 were statistically higher ($p<0.05$) than at all other pH media (pH 5, 6.5 and 6.8) (Table VIII). The *P* of AS FDSTs was increased 2-fold at pH 8 compared to pH 6.8, which represented the average saliva pH (Table XII).

According to Lee et al., 2005, they found that for basic drugs with a high pKa, the permeability through Caco-2 monolayers was increased as the pH increased (Lee et al., 2005). These results were in agreement with our findings for AS and comply with the pH-partition theory. Therefore, by modifying the microenvironment pH of the saliva to pH 8, the unionized form of AS would be increased and hence its sublingual absorption will be increased.

Table XII. The enhancement in AS permeability coefficient (*p*) for all AS FDST formulations compared to control *p* value (R1 at medium pH 6.8)

	AS FDST Formulations								
	R2	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**
Enhancement in Permeability coefficient, *P* (fold)	2	2	13	2	6	7	2	-	1.5

R2: AS FDSTs with Na Bicarb 2%; R3: AS FDSTs with Na Bicarb 2% and SDS 0.5%; R4: AS FDSTs with Na Bicarb 2% and SDS 1%; R5: AS FDSTs with Na Bicarb 2% and PCC 16%; R6: AS FDSTs with Na Bicarb 2% and Na Gly 15%; R7: AS FDSTs with Na Bicarb 2% and Na Gly 20%; R8: AS FDSTs with SDS 1%; R9: AS FDSTs with PCC 16%; R10: AS FDSTs with Na Gly 20%.

5.4 The Effect of Different pH-modifiers on the pH of AS Solution

The addition of pH-modifiers to the tablet formulation to be administered sublingually ensures that the pH of the saliva is controlled within the range that is optimal for drug absorption. In order to change the microenvironmental pH of the saliva and sublingual area, different pH-modifiers were tested in different concentrations to assess their ability to modify the AS solution pH to pH 8. As per our previous results in section 4.4.2, pH 8 was found to be the pH at which the cumulative amount of AS permeated, *J*, and *P* through the sublingual membrane were the highest.

Various pH-modifying excipients were tested. All the pH-modifiers used were a non-irritating salts that can be used safely in sublingual area. At a 1% initial concentration, Na Bicarb, Ca Carb, or Na Cit were able to modify the pH of water. However, this pH modification was not statistically different ($p>0.05$) from the pH of the water. By

increasing the concentration of pH-modifying excipients from 1% to 2%, Na Bicarb was able to show a significantly higher pH (p<0.05) from water and the rest of pH-modifying excipients tested (

Table IX). This means that Na Bicarb has the highest potential to modify the microenvironment of the saliva, however, an appropriate concentration of Na Bicarb had to be used in order to achieve the desired pH (Badawy & Hussain, 2007).

In order to asses any potential interference for AS or the excipients used in the tablet formulation on the functionality of Na Bicarb in modifying the pH to the desired optimal pH, Na Bicarb 2% was tested again with the addition of one AS FDST. The solution's overall pH was measured. Similar pH results were obtained for the solution (

Table IX), which indicated that AS and the excipients used in AS FDSTs formulation had no negative impact on modifying the pH to pH 8 and they did not interfere with the intended function for incorporating Na Bicarb in the tablet formulation.

Concentration (%)	pH-Modifiers		
	Na Bicarb	**Ca Carb**	**Na Cit**
1%	7.2 ± 0.2	6.6 ± 0.1	6.2 ±0.3
2%	8.1 ± 0.3*	7.6 ± 0.1	7.5 ± 0.1
2% with AS FDSTs	7.9 ± 0.1	-	-

5.5 The Effect of incorporating a pH-modifier and/or Penetration Enhancers on the Physical Characteristics of AS FDST Formulations

In order to evaluate the effect Na Bicarb 2% as a pH-modifier and/or penetration enhancers on AS SL permeability, these excipients need to be of incorporated into the AS

FDSTs formulation, However, these changes in the tablet formulation my negatively impact the tablets' characteristics.

When direct compression method is used to manufacture tablets, powder flowability becomes a critical parameter to control for. It can be determined by measuring the angle of repose and the MC of the powder (Alyami et al., 2017). High MC can result in variable tablet characteristics and performance. All prepared and tested AS FDST formulations had good flowability according to the USP (USP/NF, 2018d) (Table VI). However, when only the transcellular and paracellular enhancers were incorporated in to AS FDST formulations (formulation R8, R9, R10), the MC was statistically higher ($p<0.05$) compared to the previously tested AS FDST formulations with a pH-modifier alone, or with a pH-modifier and a penetration enhancer (Formulation R2, R3, R4, R5, R6, and R7) (Table VI).

Even though the addition of penetration enhancers had negatively affected the MC of the powder formulations, these changes did not impact the powder angle of repose and the tablets' breaking force. All AS FDST formulations were compressible within similar compression forces (120 – 140 kgf) and resulted in the formation of hard compact that passed the friability test with less than 1% weight loss. Therefore, although the incorporation of pH-modifier and/or penetration enhancers resulted in an increase in the powder MC, this increase was not significant enough to negatively impact the tablet characteristics (Table VI).

The different AS FDST formulations that have been prepared were able to pass the AV of the CU test according to the USP criteria (USP/NF, 2018c) (Table VI). This showed that incorporating Na Bicarb 2% as a pH-modifier and/or the addition of penetration enhancers

did not influence the flow and the uniformity of the blend during mixing, which resulted in a uniform distribution of AS between the AS FDSTs.

The DT can influence the rate and extent of AS sublingual dissolution and absorption. Testing the DT is very critical for ODT formulations because rapid disintegration is the key of a successful ODT formulation. Since the USP DT is unable to detect small difference between different ODT formulations, a disintegration test developed in our lab was used to evaluate the DT of different AS FDST formulations. This test was designed to take in consideration the critical key attributes and environment in the sublingual area, including the small amount of saliva available for tablet disintegrate and static environment under the tongue (Aodah et al., 2017). Using this DT as described previously, the incorporation of penetration enhancers into the AS FDSTs formulation containing MCC UF-702, which found to enhance tablet characteristics and AS sublingual permeability, retarded tablet's disintegration and significantly increased its DT ($p<0.05$) in comparison to formulation R1 and formulations with only Na Bicarb (Table VI). This can be explained mainly by the reduction in overall table porosity. Previously, It has been demonstrated that highly water-soluble excipients used at high concentrations could absorb and retain the limited available water to dissolve them, which limits the water from traveling within the tablet through the capillary channels created by MCC to induce the swelling of the superdisintegrant that cause tablet's disintegration. Also, the reduction in MCC content due to the addition of non-porous and less water-soluble excipients would lower the overall tablet porosity and therefore would delay tablet disintegration due to reduction in the extent of capillary pathways within the tablet that water uses to travel through to induce fast disintegration. However, the incorporation of Na Bicarb along with penetration enhancers improved the

DT, which can be explained by the high aqueous solubility of Na Bicarb and its low concentration used. As a result, the dissolution of Na Bicarb created more porous tablet without limiting water penetration and compensated for the reduction in tablet porosity due to the incorporation of penetration enhancers on the expense of MCC.

Paracellular enhancers PCC 16% was found to significantly increase (p<0.05) the disintegration time of AS FDSTs when incorporated to the formulation either with Na Bicarb 2% or alone compared to all other formulations (Table VI). This mean that Na Bicarb was not able to reduce the PCC negative effect on tablet characteristics to the same extent as with SDS and Na Gly.

The results of WT test relies on the results of tablets' disintegration time. The wetting time needed for FDSTs is another critical parameter that is more sensitive to FDST formulations' differences than DT since it demonstrates the ability of the tablet to withdraw water into the tablet from the sublingual cavity under extreme conditions like in dry mouth. The tablet relies on the penetration of saliva by capillary diffusion to allow for tablets' disintegration and dissolution. In the previously described WT test, the tablet is in contacted with the wetted tissue from one side only, therefore, water penetrated mainly from one side of the tablets to the entire tablet. A significant increase in the tablet's WT was only observed for AS FDSTs containing SDS or Na Gly without Na Bicarb, and PCC with and without Na Bicarb, which can be related, as previously explained, to the reduction in the overall tablet porosity due to the incorporation of penetration enhancers (Table VI). On the other hand, the addition of the pH-modifier Na Bicarb 2% in the various AS FDSTs (formulation R3, R4, R6, and R7) did not negatively affect the WT (P<0.05) in comparison to formulation R1 (Table VI). This can be due to the high solubility of Na Bicarb as

previously discussed, which did not retard water penetration at the concentration used and was able to create more porous tablet after its dissolution and balanced out the reduction in tablet porosity by the incorporation of less water-soluble penetration enhancers.

Water uptake is a test that was used for FDST to assess the tablet's swelling and its capacity to absorb and hold water in order to facilitate drug dissolution. The results from WU test for formulation R1, which contained no pH-modifier and no penetration enhancer, was significantly higher ($p<0.05$) than all other formulations (Table VI). The incorporation of additional excipients on the expense of MCC and L-HPC in the rest of the AS FDST formulations lowered the ability of the suprdisitegrant, L-HPC, to expand or swell to the same extent as in formulation R1; and lowered the filler's ability, MCC, to create a similar level of tablet porosity as in formulation R1 to accommodate similar amount of absorbed water within the tablet.

The DD % was measured to determine the amount of the drug released and dissolved from FDSTs in 1 min. A previously developed method was used to simulate AS FDSTs dissolution in the oral cavity (Rachid et al., 2011). The addition of a pH-modifier Na Bicarb 2% and penetration enhancers did not negatively impact ($p>0.05$) the percentage of AS dissolved in 1 minute (Table VI). However, PCC 16% with or without a pH-modifier (formulation R5 & R9) had significantly less ($p<0.05$) drug percentage dissolved in comparison to the other formulations (Table VI).

In spite of the differences between these AS FDST formulations, all of these tablets possessed the attributes for a good AS FDST and were within the acceptable and expected ranges, except for AS FDST with PCC 16% with or without a pH-modifier (formulation R5 & R9).

5.6 The Effect of Incorporating a pH-modifier and/or Penetration Enhancers on The Sublingual Permeability of AS FDST Formulations

AS FDSTs were designed to be administered sublingually. Therefore, the processes for complete drug delivery of the therapeutic dose should not take longer than 1–2 minutes for the treatment of emergency conditions and for minimizing the swallowing of the tablet or its components into the GIT. During this time, the tablet should have been disintegrated, the drug dissolved, and a therapeutic drug amount immediately permeated and absorbed through the sublingual mucosa. Any remaining amount of the drug after it has been permeated but not yet necessarily absorbed based on drug permeability coefficient may accumulates in the submucosal layers of the sublingual membrane and result in further drug absorption (Wang & Chow, 2014). Excess drug released from the tablet and dissolved in the sublingual area beyond the sublingual epithelial cells' absorption capacity, it will not be absorbed and can be lost into the GIT. This explanation was also adopted in the previous sublingual animal studies for epinephrine (M. M. Rawas-Qalaji et al., 2006; M. M. Rawas-Qalaji et al., 2015). Tablet's DT and DD% can be considered the main limiting tablet's physical characteristics that can impact AS sublingual permeation, absorption, and relative bioavailability. Tablet's DT can control the rate of drug release and indirectly the rate of drug dissolution (DD%). Therefore, both DT and DD can control the amount of drug available for absorption, i.e. drug concentration, during the short and limited time of sublingual administration. According to Fick's law, altering the initial drug concentration will alter the rate of diffusion, i.e. drug influx.

Considering the importance of the period right after the sublingual drug administration

for AS permeation and absorption for the treatment of emergency condition, *ex vivo* permeability studies were performed for all AS FDST formulations. In order to understand and evaluate the mechanism for enhancing drug permeation, the *ex vivo* permeability studies for AS FDSTs formulation containing a pH-modifier Na Bicarb 2% alone (formulation R2), AS FDST formulations containing a pH-modifier Na Bicarb 2% and penetration enhancers, SDS, PCC, and Na Gly (formulations R3, R4, R5, R6, R7), and AS FDST formulations with penetration enhancers alone, SDS, PCC, Na Gly (formulations R8, R9, and R10) were compared to AS FDSTs formulation (R1). The mean (± SD) area under the curve ($AUC_{0\text{-}90}$) of cumulative drug permeated from AS FDST formulations containing a pH-modifier with penetration enhancers (formulations R3, R4, R5, R6, and R7) were statistically higher ($p<0.05$) than AS FDST formulations containing penetration enhancers only (formulations R8, R9, and R10), pH-modifier only (formulation R2), and control (formulation R1) (Table X). Incorporating SDS 1% with Na Bicarb 1% (formulation R4) achieved the highest enhancement in AS sublingual permeability ($p<0.05$) and increased AS permeability 13-fold compared to control (formulation R1) (Table XII).

Our studies demonstrated that the enhancement in AS sublingual permeability was correlated with the concentration of penetration enhancers used. The higher the concentration, the higher the amount of drug permeated. However, the maximum allowed concentrations to be used in AS FDST formulations were limited to their safety profile. Also, the results from our studies indicated that the addition of transcellular penetration enhancers with a pH-modifier had a synergistic effect on AS sublingual permeability.

The mean (± SD) $AUC_{0\text{-}90}$ and *J* from AS FDSTs with transcellular enhancers alone, SDS and Na Gly (formulation R8 and R10) were significantly higher ($p<0.05$) than with paracellular enhancer PCC (formulation R9) (Table XI). However, the mean (± SD) AS *P* from AS FDSTs with SDS transcellular enhancers were significantly higher ($p<0.05$) than AS FDSTs with Na Gly transcellular enhancers, PCC paracellular enhancer, and control. These findings demonstrated that AS follow the transcellular transport pathway for its sublingual absorption.

The amount of AS permeated from AS FDSTs formulation with paracellular enhancer PCC (formulation R9) increased at 75 min compared to AS FDSTs with transcellular enhancers, SDS and Na Gly (formulation R8 and R10). The reason for this increasement was due to the accumulation of AS in the donor cell to the extent that was able to overcome the permeability resistance in the sublingual membrane. This is probably irrelevant to emergency treatment situations, especially during the short sublingual administration time.

Also, our previous dose escalating *ex vivo* permeability studies using formulation B showed that increasing AS dose resulted in a linear increase of AS permeability (Aodah et al., 2017). These results are an indication for a passive sublingual AS transport mechanism. Therefore, It can be concluded that AS transport is mainly by passive transcellular transport pathway, which is in agreement with previously suggested transport mechanism.

These studies confirmed the potential and the benefits of modulating the absorption's microenvironment pH, to reduce AS ionization, as a promising approach for enhancing AS permeation through sublingual epithelial cells. This enhancement can be further increased by the addition of a transcellular penetration enhancer.

5.7 Recommendations for Future Studies

In the interests of developing AS FDSTs as a new dosage form to treat the emergency condition of OP toxicity, future in vivo pharmacokinetics animal studies are recommended. *In vivo* animal studies will evaluate and confirm the effect of changing the microenvironment pH on drug ionization as well as the effect of penetration enhancers on enhancing AS sublingual permeability. A does-escalating animal studies are recommended to determine the sublingual AS dose bioequivalent to AtroPen®. These preclinical animal studies will guide the dose selection for any future clinical studies.

It is known that improving or masking the bad taste of drugs intended for oral administration can improve patient compliance. Atropine sulfate is known for its bitter taste when administered orally (Maggs, 2008). Therefore, various taste masking approaches for AS FDSTs are recommended to mask the bitter taste of AS before conducting human studies.

5.8 Conclusion

In this project, different AS FDST formulations were successfully manufactured and evaluated. The quality control methods used in this project were able to successfully discriminate and detect formulations differences. The newer highly compressible MCC filler grade UF-702 was able to successfully alter the properties of AS FDSTs and improve the AS dissolution rate, and therefore, the rate and extent of AS sublingual permeation. Reducing AS ionization through altering the diffusion medium's pH by incorporating an

appropriate pH-modifier into the AS FDSTs formulation can be a useful approach to enhance AS sublingual permeation. This approach enhanced AS sublingual permeability 2-fold compared to the control (Table XII).

Combining a transcellular penetration enhancer along with a pH-modifier into the AS FDST formulations, enhanced AS sublingual permeation between 7 to 13-fold (Table XII). This study, therefore, innovatively improved the permeability of sublingually administered AS FDSTs through altering the medium pH and the addition of penetration enhancers.

This novel AS FDSTs are expected to significantly improve the pharmacokinetic parameters (AUC, C_{max}, and T_{max}) in future animal studies and reduce the bioequivalent sublingual AS dose. The successful development of these novel AS FDSTs as alternative dosage form for AtroPen® will ensure the sublingual delivery of therapeutic AS concentrations to the systemic circulation and the rapid onset of action for the treatment of OP toxicity as a first-aid treatment until patients are transported to appropriate health facility. This new dosage form will offer an affordable, easy-to-administer, non-invasive, and portable alternative dosage form for the treatment OP toxicities.

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Figure 6. Image of atropine sulfate auto-injector devices (AtroPen®) (Hilmas & Hilmas, 2009)

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Figure 9. Transcellular and Paracellular Transport (Levendoski et al., 2014).

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List of Scholarly Works

Articles:

- **Aodah, A., Bafail, R., & Rawas-Qalaji, M.** (2016). Formulation and evaluation of fast-disintegrating sublingual tablets of atropine sulfate: The effect of tablet dimensions and drug load on tablet characteristics. *AAPS PharmaSciTech*. 2017 Jul;18(5):1624-1633. Epub 2016 Sep 20

- **Bafail, R., Aodah, A., & Rawas-Qalaji, M.** (2019). Effect of the Filler Grade on The Characteristics and the Sublingual Permeability of Atropine Sulfate Fast Disintegrating Sublingual Tablets. Submitted to *Drug Dev Ind Pharm*.

- **Aodah, A., Bafail, R., & Rawas-Qalaji, M.** (2019). Effect of Fast Disintegration Tablets' Characteristics on the Sublingual Permeability of Atropine Sulfate for Potential Treatment of Organophosphates Toxicity. Submitted to *AAPS PharmaSciTech.*

Patents:

- **Rawas-Qalaji, M. M. & Bafail, R.** (2018). Atropine Sulfate Rapidly-Disintegrating Sublingual Tablets, Methods for Manufacture Thereof, and Methods for Use Thereof for Treatment of Acute Organophosphate Toxicity. International Patent Publication (WIPO) # PCT/US17/50030 (Appl No

PCT/US2017/050030). Filed September 5, 2017 and published March 8, 2018. Assignee: Nova Southeastern University.

- **Rawas-Qalaji, M. M. & Bafail, R.** (2019). Atropine Sulfate Rapidly-Disintegrating Sublingual Tablets of Improved Formulation, Methods for Manufacture Thereof, and Methods for Treatment of Acute Organophosphate Toxicity. US Patent Pub (USPTO) # US2019/ Appl No 16/329299). Filed February 28, 2019. Assignee: Nova Southeastern University.

Abstracts:

- **Bafail, R. & Rawas-Qalaji, M. M.** Effect of Penetration Enhancers on Sublingual Permeability of Epinephrine, *AAPS*J2018; M1230-10-078 – Washington, DC (Nov. 04–07)

- **Bafail, R. & Rawas-Qalaji, M. M.** Evaluation of the Sublingual pH-Permeability Profile of Epinephrine, *AAPS*J2018; T0930-10-080 – Washington, DC (Nov. 04–07)

- **Bafail, R. & Rawas-Qalaji, M. M.** Assessment of the pH-Stability Profile of Epinephrine for Sublingual Administration, *AAPS*J2018; T0930-11-084– Washington, DC (Nov. 04–07)

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** Evaluation of the Effect of pH on Enhancing the Sublingual Permeability of Atropine Sulfate Fast Disintegrating Sublingual Tablets (FDSTs), NSU College of Pharmacy's HPD Research Day, Feb. 16, 2018.

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** Evaluation of the Effect of Combining a Penetration Enhancer to a pH Modifier on Enhancing the Sublingual Permeability of Atropine sulfate from Fast Disintegrating Sublingual Tablets, NSU College of Pharmacy's HPD Research Day, Feb. 16, 2018.

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** The Combined Effect of a Penetration Enhancer and an Alkalizing Agent on the Sublingual Permeability of Atropine from Fast Disintegrating Sublingual Tablets, *AAPS*J2017; T703 – San Diego, CA (Nov. 12–15)

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** Microenvironment's pH Modification and Its Effect on Enhancing the Sublingual Permeability of Atropine from Fast Disintegrating Sublingual Tablets (FDSTs), *AAPS*J2017; T703 – San Diego, CA (Nov. 12–15)

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** The effect of MCC grade on the physical properties of atropine sulfate rapidly disintegrating sublingual tablets, NSU College of Pharmacy's HPD Research Day, Feb. 12, 2016.

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** Development and Validation of a Simple Disintegration Test for Rapidly Disintegrating Tablets, NSU College of Pharmacy's HPD Research Day, Feb. 12, 2016.

- **Aodah, A. H., Bafail, R., & Rawas-Qalaji, M. M.** Effect of atropine sulfate load on rapidly disintegrating sublingual tablets' physical properties. *AAPS*J2016; T703 – Denver, CO (Nov. 13–17)

- **Aodah, A. H., Bafail, R., & Rawas-Qalaji, M. M.** Effect of changing tablet dimensions on the characteristics of fast disintegrating sublingual tablets of atropine sulfate. *AAPS*J2016; T704 – Denver, CO (Nov. 13–17)

- **Bafail, R., Aodah A. H., & Rawas-Qalaji, M. M.** The effect of pH on the sublingual permeability of atropine sulfate tablets. *AAPS*J2016; 384 – Denver, CO (Nov. 13–17)

- **Bafail, R., Aodah, A. H., & Rawas-Qalaji, M. M.** The effect of the filler grade on the characteristics of fast disintegrating tablet formulation. *AAPS*J2016; 589 – Denver, CO (Nov. 13–17)

- **Aodah, A. H., Bafail, R., & Rawas-Qalaji, M. M.** Development and validation of a simple disintegration test for rapidly disintegrating tablets. *AAPS*J2015; R6138 – Orlando, FL (Oct. 25–30)

- **Aodah, A. H., Bafail, R., & Rawas-Qalaji, M. M.** Sublingual permeability of atropine sulfate using novel rapidly disintegrating tablets for the potential treatment of acute organophosphates toxicity. *AAPS*J2015; W5147 – Orlando, FL (Oct. 25–30)

Vita

Dr. Rawan Bafail earned her PharmD degree from King Abdulaziz University, Jeddah, Saudi Arabia, in 2010. Dr. Bafail worked as a teaching assistant in the College of Pharmacy, Taibah University, Medina, Saudi Arabia, in 2010 in the Department of Pharmaceutical Sciences. Then, she received a scholarship from the Ministry of Education in Saudi Arabia to complete her graduate education. Dr. Bafail earned her Master degree in Industrial pharmacy from Long Island University, Brooklyn, NY, in 2014. Then, she joined the pharmaceutical sciences Ph.D. program at Nova Southeastern University in 2014.

In 2015, she joined Dr. Mutasem Rawas-Qalaji's laboratory team at the College of Pharmacy, Nova Southeastern University. Dr. Rawas-Qalaji and his team has developed atropine sulfate fast disintegrating sublingual tablets (AS FDSTs). Under his supervision, Dr. Bafail enhanced the physical characteristics as well as permeability of this AS FDSTs formulation. This work has been filed as a patent application through the World Intellectual Property Organization (WIPO) and Patent Cooperation Treaty (PCT) under international publication number (WO 2018/045367 A1) on March 8, 2018 and United States Patent and Trademark Office (USPTO) under application number (16/329299) field on February 28, 2019.

Also, Dr. Bafail research results have been published or submitted for publication as one original peer-reviewed research article published in *AAPS PharmSciTech* 2016, one original research manuscript in *Drug Development and Industrial Pharmacy Journal* 2019, and one original research manuscript in *AAPS PharmSciTech* 2019. Dr. Bafail has presented her research results through 15 peer-reviewed and published abstracts and poster

presentations in the prestigious national pharmaceutical organization, AAPS, and institutional research events, NSU Research Day.

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