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Topical Formulation For Stimulating Sweat Secretion as an Alternative to Iontophoresis

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TOPICAL FORMULATION FOR STIMULATING SWEAT SECRETION AS AN ALTERNATIVE TO IONTOPHORESIS

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ABSTRACT

Sweat test or pilocarpine iontophoresis test is the existing method for diagnosis of cystic fibrosis that is recommended to perform at 48 hours after birth. However, the current method is painful, with several side effects such as full thickness skin burn, skin rash, erythema etc. Therefore, the purpose of this study is to develop a pilocarpine topical formulation which can stimulate sweat secretion when applied on skin without iontophoresis. Several formulations with Transcutol®, polyethylene glycol 400 (PEG 400), Polyethylene glycol 200 (PEG 200), menthol and salicylic acid (SA) each at varying concentrations were screened as penetration enhancers. In vitro penetration test (IVPT) was performed on these formulations to compare the influence of these ingredients on the penetration of pilocarpine. The results from the preliminary studies indicate that the formulation with SA showed better penetration into porcine skin after both 10 minutes (120.29 ± 27.54 µg/cm2) and 40 minutes (158.97 ± 20.15 µg/cm2) compared to others. Based on the preliminary studies, the lead formulation was decided to have pilocarpine nitrate, ethanol and water as solvents, salicylic acid as penetration enhancer, PEG 200 as a hydrating agent to minimize any dehydration due to ethanol, menthol as a cooling agent and sodium hydroxide as a pH modifier. The penetration of pilocarpine nitrate on application of lead formulation into the porcine skin was compared to that of iontophoresis technique by both IVPT as well as tape stripping techniques. The IVPT results indicate that total amount of pilocarpine nitrate recovered from skin with passive formulation (152.04 ± 52.23 µg/cm2 after 10 min, 210.27 ± 53.72 µg/cm2 after 40 min) is higher than that of iontophoresis with pilocarpine solution (97.05 ± 27.93 µg/cm2 after 10 min, 140.56 ± 88.66 µg/cm2 after 40 min) at both 10

and 40 minutes. The tape stripping data showed a correlation with the IVPT results. The amount of pilocarpine recovered from the tape strips used in the lead formulation (78.46 µg/mg at 10 min and 53.32 µg/mg at 40 min) was significantly higher than the pilocarpine recovered from tape strips used for iontophoresis (13.32 µg/mg at 10 minute and 7.38 µg/mg at 40 minutes). The lead formulation was also investigated for its effectiveness by a clinical study on 20 human volunteers to determine if the developed formulation was efficient enough to stimulate the sweat production. The average amount of the sweat secreted due to passive formulation was found to be 77.28 ± 18.97 mg when applied on an area of 38.46 cm2. Based on these results, it can be concluded that the passive formulation was successful in delivering pilocarpine and to stimulate sweat secretion which can be a compliant alternative technique to iontophoresis.

DEDICATION

I dedicate my thesis to my wonderful family. I am grateful to my parents, Dr. Rouf Sarder and Dr. Habiba Khatoon, who provide unconditional support throughout this master's program. I am also grateful to my younger brother Dr. Mahmud Mohammad Sarder for his immense help and continuous emotional support during this program. I am especially thankful to my loving husband, Tanzil Rahman, for encouraging me and giving me constant motivation throughout the program.

LIST OF ABBREVIATIONS

CF: Cystic Fibrosis

CFTR: Cystic Fibrosis Transmembrane Conductance Regulator

EPI: Exocrine Pancreatic Insufficiency

ASL: Airway Surface Liquid

Tr: Transcutol

P200: Polyethylene glycol 200

P400: Polyethylene glycol 400

M: Menthol

SA: Salicylic acid

API: Active Pharmaceutical Ingredient

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INTRODUCTION

Disease Overview

Cystic fibrosis (CF) is a progressive genetic disease that is inherited in autosomal recessive manner (1). According to the Cystic Fibrosis Foundation Patient Registry, more than 30,000 people have cystic fibrosis in the United States and more than 70,000 people worldwide. Each year, approximately 1,000 new cases of CF are diagnosed and more than 75 percent of patient with CF are diagnosed by age two (2). It is usually manifested as persistent lung infection, aquagenic wrinkling of palm as shown in figure 1 (3), exocrine pancreatic insufficiency and excessive amount of salt in the sweat. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a membrane protein and chloride channel that is found in sebaceous and exocrine gland (5), lung, liver, pancreas and it is encoded by CFTR gene (5, 6). CFTR gene is located at chromosome 7 (6) and genetic mutation on chromosome 7 results in cystic fibrosis. In healthy individual, this CFTR chloride channel regulates the transport of sodium, chloride and bicarbonate ions. This genetic mutation results in abnormality in production and function of protein CFTR that leads to reduced transport of sodium chloride, defect in reabsorbing chloride of sweat duct (7, 8) and excretion of large amount of sweat electrolytes (9).

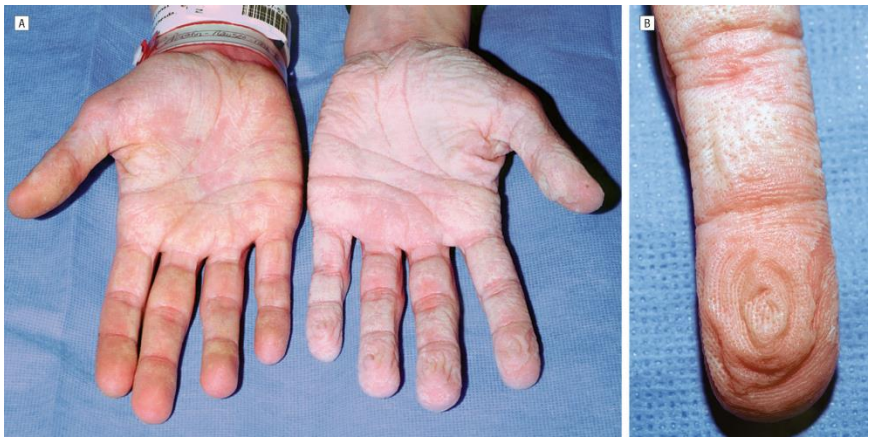

Figure 1: Aquagenic wrinkling of palm

Sweat gland

CFTR is highly expressed in sebaceous gland. In eccrine glands and ducts, CFTR is located in apical membrane of the cells lining the sweat duct. Sweat gland is located in hypodermis of the skin and it helps to maintain the body temperature by releasing the sweat from hypodermis onto the skin surface. Sweat consists of water and salt (Na^+ and Cl^-), generated by sweat gland and is carried through the sweat duct onto the skin surface. In healthy individuals, salt and water are reabsorbed into the skin. However, in cystic fibrosis patients, defective CFTR protein does not allow the sodium and chloride ions to reabsorb into the cells lining the sweat duct which results in the deposition of salt in the skin (10,11). Salty skin as shown in figure 2 (12) is one of the most common symptoms of cystic fibrosis and it causes severe itchiness and redness of the skin (12).

Figure 2: Salty skin in patient of cystic fibrosis

Lungs

CFTR is located in the epithelial cells inside the lung. Lung alveoli are lined up by the alveolar cells that produces mucus. This thin mucus layer or airway surface liquid (ASL) coats the airway and protects the airway by trapping the bacteria and foreign particle. Moreover, constant movement of cilia wipes the foreign particle up and out of the airways. In cystic fibrosis individual, defected CFTR protein does not allow the chloride ions to reach the chloride channel and it also increases the transport of sodium along with the water out of the surface liquid (13). As a result, the ASL becomes thick and thick layer of mucus hamper the function of cilia that leads to entrapment of bacteria within the airway and chronic infection of the lung (11).

Pancreas and the Gastrointestinal (GI) tract

Cystic fibrosis is one of the most common cause of exocrine pancreatic insufficiency (EPI). In cystic fibrosis, the thick mucus layer clogs the pancreas and blocks the pancreatic enzyme from entering the small intestine that leads to EPI (14). As the pancreatic enzyme cannot enter the small

intestine, this affects the digestion of food particularly fat and protein leading to malnutrition (11, 15).

Management of Cystic Fibrosis

There is no cure for cystic fibrosis. However, early treatment and close monitoring by the specialist can reduce the complications from multisystem involvement. The management of CF includes initial diagnosis by genetic and sweat test, prompt treatment and close follow up of the patient. The goal of CF treatment is to maintain the lung function, prevent the intestinal obstruction and to provide nutritional supplement. Frequent clearance of airway, inhalation of the bronchodilator and intake of oral antibiotic help to reduce the pulmonary exacerbations (16, 17). Inhalation of hypertonic saline increases the volume of ASL and remove thick mucus layer from the airway and improve the lung function (18). Cystic fibrosis patient suffering from exocrine pancreatic insufficiency are treated with oral pancreatic enzyme. Ivacaftor (19), lumacaftor-ivacaftor (20) are used to improve the function of defected CFTR protein.

Methods for diagnosis of cystic fibrosis includes sweat testing or genetic test. The sweat test is relatively less expensive and easily deployable in any place. The sweat test includes electrically mediated application of pilocarpine for inducing sweat.

Pharmacology of Pilocarpine

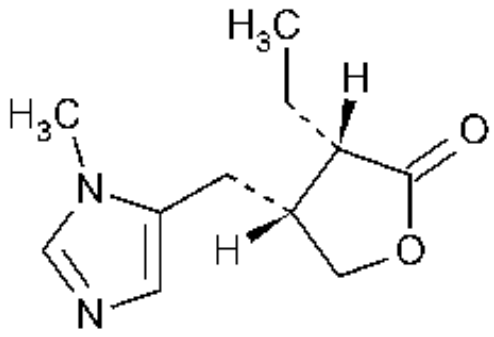

Figure 3: Structure of Pilocarpine

Pilocarpine is an alkaloid derived from the plant of genus *Pilocarpus* (21, 22). Pilocarpine is a cholinergic agonist or muscarinic receptor agonist that increases the effect of acetylcholine in the body. Pilocarpine is available in market as pilocarpine hydrochloride tablet (5 mg, 7.5 mg and 10 mg). The mean elimination half-life is 0.76 hours and maximum plasma concentration (C_{max}) is 15 ng/ml (23). It requires 1.25 hours to reach peak plasma level. For 10 mg dose, the mean elimination half-life is 1.35 hours, the maximum plasma concentration (C_{max}) is 41 ng/ml and peak plasma level reaches 0.85 hours (23). Pilocarpine metabolizes to pilocarpic acid by serum esterase at neuronal synapse and excreted in urine (21). Pilocarpine is also available as pilocarpine nitrate eye drop (2.0% w/v).

As pilocarpine is a parasympathomimetic drug that mimics the action of acetylcholine released by parasympathetic nervous system and acetylcholine decreases the heart rate, so pilocarpine increases the effect of parasympathetic nervous system and decreases the heart rate (24). As a muscarinic receptor agonist, pilocarpine produces vascular relaxation and conjunctival vasodilation (25). Pilocarpine causes contraction of ciliary muscle, that opens the trabecular meshwork. Opening of the meshwork accelerates the drainage of aqueous humor resulting in

decrease intraocular pressure (26). Therefore, pilocarpine is used to treat glaucoma. Pilocarpine stimulates the muscarinic receptor (M1) of myoepithelial cells that surrounds the secretory coils of sweat gland and cause contraction of myoepithelial cells and facilitate secretion of sweat (27, 28). Pilocarpine (2% solution) also stimulates the salivary gland and stimulates the salivary flow (29).

Side Effects of Pilocarpine

Therapeutic dose of pilocarpine ranges from 5 mg to 10 mg orally per day. Dose of pilocarpine should not exceed more than 30 mg/day. Overdose leads to headache, visual disturbance, hallucination, sweating, abnormal heart rate, hypotension, vomiting, nausea and diarrhea (30). Sweating, nausea running nose, increased salivation and lacrimation, frequent urge to urinate may occur as side effects of pilocarpine (31).

Physiochemical Properties of Pilocarpine nitrate

Physicochemical property	Value
Molecular weight	271.273 g/mol
Melting Point	173.5-174° C
Log P	1.1
pKa	1.6, 1.7

Table 1: Physicochemical properties of pilocarpine

Physicochemical properties of pilocarpine are shown in table 1. Pilocarpine is soluble in water, alcohol and chloroform. It is sparingly soluble in ether, benzene (32). Pilocarpine is hygroscopic in nature (32) and sensitive to light (33).

Reason to choose pilocarpine nitrate over pilocarpine hydrochloride

Pilocarpine hydrochloride containing formulation was applied to the filter paper to check if the chloride ions of pilocarpine hydrochloride would interfere with chloride concentration of sweat. Following the application, chloride ions from pilocarpine hydrochloride solution was getting stuck into the filter paper and these chloride ions were detected in chloride analyzer which may interfere with chloride concentration of sweat during future study and the exact chloride concentration of sweat may not be detected by the chloride analyzer. This filter paper was dissolved into distilled water and that solution was run into HPLC. A sharp peak for pilocarpine HCL was found confirming that pilocarpine hydrochloride was getting stuck into the filter paper. As one of the aim of this study to measuring the chloride concentration of the sweat; interference of chloride ions of pilocarpine hydrochloride was not favorable for this study. Formulations containing pilocarpine nitrate showed no chloride ion interference with the chloride analyzer. Enhancers were kept same while making the formulations. So, pilocarpine nitrate was used as an API to make the topical formulation.

Current Diagnostic Method of Cystic Fibrosis

Sweat test or pilocarpine iontophoresis test is the current method that is used as a screening test for diagnosis of cystic fibrosis. The quantitative pilocarpine iontophoresis test includes the stimulation of sweat by iontophoresis, collection of sweat with the use of gauze, filter paper or macroduct coils followed by quantification of chloride in sweat (34, 35). Pilocarpine is used as a

diagnostic agent during this iontophoresis. In this test, a gauge of 5.1x 5.1- cm is soaked into 2 or 3 ml of 0.2- 0.5% of pilocarpine solution and the electrodes are placed over the gauge (30). The current of 2.5 to 4 mA is applied on 26 cm^2 area of the forearm to stimulate the sweating and the sweat is collected for 30 minutes (36). Then the amount of chloride concentration in sweat is quantified by using chloride analyzer (37).

The Need for Passive Formulation as New Diagnostic Method of Cystic Fibrosis

Iontophoresis method causes pain to children as the current of 2.5 to 4 mA is applied to deliver the pilocarpine. There are incidences of patient's skin being burned during iontophoresis for sweat induction. It also has been reported to cause several side effects such as skin burn, skin rash, erythema etc. (38). It has been reported that a seven week old child develop four semicircular area of burn from the electrode during the iontophoresis. There is a report of another incidence where a child experienced burn from iontophoresis that produce two mm diameter of circular punched out lesion, that penetrate completely through all the layer of the skin (38). There are some other incidences such as redness of the skin, blisters with or without scaring, frank electrical burn that have been reported while performing the iontophoresis test on adults (38). Most of the time burn occurs during iontophoresis due to application of current. To avoid this, it was suggested to incorporate an automatic current limiter to shut down the iontophoresis if the current exceeds more than 1.75 mA (38). But this safety feature does not significantly reduce the incidence of burn (38). Hence developing a passive formulation has an advantage over iontophoresis. Moreover, sweat test costs between $200-$500 (39, 40, 41).

In passive formulation method, very small amount of pilocarpine formulation (24 mg of pilocarpine) is applied on the skin for 10 minutes. The amount of pilocarpine that penetrates the

skin is negligible and may reach the systemic circulation but would not cause any toxicity or side effects at such minute dose. So there are absolutely no chances for any systemic side effects using topical pilocarpine formulation. Moreover, an adult can intake 5 mg or 7.5 mg of pilocarpine orally per day, but it should not exceed more than 30 mg per day (42). As per FDA, the prescribing dosage of pilocarpine is 5 mg or 7.5 mg per day (43). So the amount that is expected to penetrate the skin in 10 minutes is likely not more than a quantity that is about 1/100 th of recommended oral dose.

As this screening test is commonly performed on neonates and is recommended at 48 hours after birth, there is a need to develop an efficient, alternative, painless method for the same. Therefore, the purpose of this study is to develop a pilocarpine topical formulation which will generate enough quantity of sweat without the use of current.

OBJECTIVE OF THE STUDY

The objective of the present study was to develop a pilocarpine containing topical formulation that penetrates the skin and stimulates sweat production. The extent of perspiration and the concentration of chloride in sweat were estimated followed by sweat stimulation. Pilocarpine containing topical formulation with different enhancers were prepared and characterized. *In vitro* and clinical study were performed for the formulation.

As a part of preliminary testing, *In- vitro* skin penetration of the drug was investigated on porcine skin using the passive pilocarpine formulation and iontophoresis technique to quantify the amount of drug penetrated into the skin. estimation of extent of perspiration has been performed on human subjects where the developed pilocarpine topical formulation is applied directly on the skin and the sweat stimulation.

MATERIALS AND METHODS

Materials

Pilocarpine nitrate (≥98.0%), Pilocarpine hydrochloride (≥98.0%), Polyethylene Glycol 200, Polyethylene Glycol 400, Menthol, Salicylic acid (99%), Sodium chloride Gran USP-FCC, Coomassie brilliant blue G-250, Grade 4 qualitative filter paper Standard grade circle 70 mm were purchased from VWR, USA. TRANSCUTOL HP (diethylene glycol monoethyl ether) was obtained gift sample from Gattefosse, USA. Combined acid buffer and chloride standard (100 mmol/l of chloride) were purchased from Nelson Jameson Inc. USA. Adult Electrodes (3M Red Dot^{TM}) and adhesive tape (Transpore ^{TM}) were purchased from 3M Science, USA. Heat bandage (Therma Care Heatwraps) was purchased from Walmart, USA. All solvents used in the analysis of were of HPLC grade. The formulation that was used for clinical study, was prepared in cGMP lab and the ingredients of this formulation were also prepared in cGMP grade.

Methods

Development of Pilocarpine Containing Topical Formulation

Different enhancers and ingredients used during development of topical formulation are mentioned in table 2 and table 3 respectively.

Name of Enhancers	Abbreviation	Mechanism of Action
Transcutol HP (diethylene glycol monoethyl ether)	Tr	Improving the solubility of drug within stratum corneum (44)
Polyethylene glycol 400 (PEG 400)	P400	Enhances the solubility of the drug within stratum corneum (45)
Polyethylene glycol 200 (PEG 200)	P200	Enhances the solubility of the drug within stratum corneum (45)
Menthol	M	Enhancing the hydration of stratum corneum lipid (46)
Salicylic acid	SA	Keratolytic action (47)

Table 2: The list of the enhancers with mechanism of action

Ingredients	Purpose
Pilocarpine nitrate	API
Water	Solvent
Ethanol	Solvent
Enhancers	Penetration Enhancer

Table 3: The list of ingredients used in topical formulation

Screening of the penetration enhancers

Pilocarpine containing topical formulations were prepared with different enhancers. Compositions of the formulations containing individual enhancer are mentioned in table 4

Formulation	Enhancer (w/w%)	Pilocarpine nitrate (w/w%)	Ethanol (w/w%)	Water (w/w%)
Tr 5%	5%	5%	27%	q.s.
Tr 10%	10%	5%	27%	q.s.
P400 5%	5%	5%	27%	q.s.
P400 10%	10%	5%	27%	q.s.
P200 5%	5%	5%	27%	q.s.
P200 10%	10%	5%	27%	q.s.
M 2.5%	2.5%	9%	45%	q.s.
M 5%	5%	9%	45%	q.s.
SA 5%	5%	5%	59.5%	q.s.
SA 10%	10%	5%	59.5%	q.s.

Table 4: Composition of the formulations containing individual enhancer

Compositions of the formulations containing combination of enhancers are mentioned in table 5

Formulation	PG 200 (w/w%)	M (w/w%)	SA (w/w%)	Pilocarpine (w/w%)	Ethanol (w/w%)	Water (w/w%)
P200 + M	10%	4%	--	6%	45%	q.s.
P200 + M + SA (pH was adjusted to 4 by NaOH)	10%	4%	10%	6%	45%	q.s.

Table 5: Composition of the formulations containing combination of enhancers

Five individual enhancers were used to prepare the formulation: Transcutol®, polyethylene glycol 400, polyethylene glycol 200, menthol and salicylic acid. For each enhancer except for menthol, two formulations were prepared with the enhancer concentration of 5% and 10%. Pilocarpine nitrate concentration was 5% for each of the solution. For menthol, two formulations were prepared with the enhancer concentration of 2.5% and 5% and pilocarpine nitrate concentration was 9% for each of the solution. Concentration of ethanol was 27% for all the formulations except for the one with salicylic acid and the one with menthol. For the formulation with SA, the ethanol concentration was 59.5% and for the formulation with menthol, it was 45%. Quantity sufficient water was added in all the formulations.

Two formulations were prepared by combination of enhancers. The first formulation constituted PG 200 and Menthol and the second formulation comprised of PG 200, Menthol and Salicylic acid. The pH of the formulation was adjusted to pH 4 by adding NaOH.

Preparation of topical formulation with pilocarpine nitrate

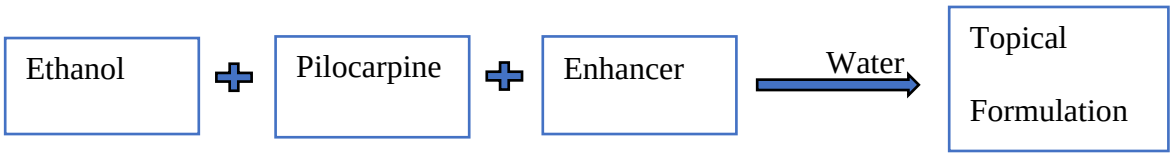

Figure 4: Steps of Preparing Topical Formulation

As shown in figure 4, each of the ingredients were weighed and added to the formulation in sequence of ethanol, enhancer/enhancers, pilocarpine nitrate. Water was added to dissolve the other ingredients of formulation. The final formulations were stored in amber color glass containers away from light.

HPLC Analysis

Pilocarpine concentrations in the samples were quantified using a reversed phase HPLC method. The analysis was carried out on Waters HPLC system (Water 600 Controller, USA) equipped with a 600-pump unit, a 717 plus auto sampler with an injection valve with a sample loop of 50 µl, and a 2487 dual absorbance UV detector. The mobile phase consists of pH 3 buffer and methanol in the ration 90:10. pH 3 buffer is prepared using 10 mM of potassium phosphate solution adjusted with o-phosphoric acid. The mobile phase was filtered by using filter paper (Whatman, Grade 4, Qualitative circles, 0.2 micron). A flow rate of 0.6 ml/min^{-1} was used to elute pilocarpine. 30 µl of injection was eluted in a LUNA 5µ C18 (2) 250 x 4 60 mm column (Phenomenex, USA) and the column was maintained at 27°C. The UV detection was set at 215 nm and the retention time of pilocarpine was 4.6 min. Water was used as a solvent for preparation of standard concentrations of pilocarpine and calibration curve was plotted in the range of 0.5 µg -10 µg.

Drug Recovery Study

Drug recovery studies were performed to validate the *In vitro* penetration study. Validation study was conducted by using three doses (13 $µl/cm^2$, 19 $µl/cm^2$, 25 $µl/cm^2$). The effective area for this study was 0.94 cm^2. Fresh full thickness porcine skin was brought from a local slaughterhouse. The abdominal skin regions were taken and shaved using an electric shaver and subcutaneous fat was removed completely. The skin was cut into medium size rectangular pieces and the pieces were covered with aluminum foil. The skin was stored immediately in a refrigerator at 5°C for future use. Fat free full thickness skin was used (n=3) for each dose. Followed by application of each dose, the skin was allowed to dry for 40 minutes. After 40 minutes of drying, each piece of skin was washed with 15 ml of water so as to remove the

superficial drug. Followed by washing for first time, the same piece of skin was washed again with another 15 ml of water to make sure that no superficial drug was present in the skin. The same piece of skin was placed into another vial containing 15 ml of water. The vials were covered with aluminum foil and left in a bio shaker for overnight, so that the pilocarpine that has been penetrated into the skin gets extracted into the water. Four different extraction procedures were tried in order to validate the extraction procedure of pilocarpine to precipitate the protein. The solution was taken into 4 Eppendorf tubes of which acetonitrile was added to two of them and methanol into other two. The acetonitrile and methanol added solutions were either acidified or basified to precipitate the proteins. The Eppendorf tubes were kept for centrifugation and the supernatant solutions were analyzed for pilocarpine using validated HPLC method.

Dose (µl/cm2)	% Recovery
13 µl/cm^2	91.33 ± 2.3
19 µl/cm^2	93.18 ± 3.1
25 µl/cm^2	98.46 ± 2.8

Table 6: Drug recovery for different doses

***In vitro* penetration study (IVPT)**

The IVPT was performed for screening of enhancers. The in vitro skin penetration study was performed using Franz diffusion cells with an effective diffusion area of 0.94 cm^2. The full

thickness skin was sandwiched between the donor and receiver compartment with the stratum corneum side facing the donor compartment.

17 µl/cm^2 of developed topical formulation was loaded into 0.94 cm^2 area of donor compartment. There were total twelve topical pilocarpine formulations with different enhancers of different concentrations. IVPT was performed on the formulation with each enhancer of two different concentration.

For each solution, there were 6 trials. For first 3 trials, duration of study were 10 minutes. Full thickness skin was collected at 10th minute of time point. The duration of study was 40 minutes for last 3 trials and full thickness skin was washed after 40 minute. Each skin that was collected form trials was washed with distilled water in a beaker, cut into small pieces and immersed into 15 ml of water. The vials were covered with aluminum foil and left in a bio shaker for overnight, so that the pilocarpine formulation that has been penetrated into the superficial skin extracts into the water. 300 µl of solution was collected from each vial into the Eppendorf tube and 600 µl of basified Acetonitrile was added to each of the tube. The Eppendorf tubes were kept for centrifugation and the supernatant solutions were analyzed for pilocarpine using validated HPLC method.

Followed by the IVPT with individual enhancer, IVPT was performed for the second time with the two formulations with combination of enhancers by following the same procedures as mentioned above.

Drug Retention Study

IVPT with the final passive formulation and iontophoresis with 0.5% pilocarpine solution were performed as the part of the study.

IVPT for screening of penetration enhancers

Six trials were performed for final formulation. For first 3 trials, duration of study were 10 minutes. Full thickness skin was collected at 10th minute of time point. The duration of study was 40 minutes for last 3 trials and full thickness skin was collected at 40^{th} minute time point. The full thickness skin was sandwiched between donor and receiver compartment with the stratum corneum side facing the donor compartment. 17 µl/cm^2 of final formulation was loaded into 1.74 cm^2 area of donor compartment. Last 3 cells of 40^{th} minute time point were placed in an incubator at a temperature of 40° C. Each skin that was collected form trials was washed with distilled water in a beaker, cut into small pieces and immersed into 15 ml of water. The vials were covered with aluminum foil and left in a bio shaker for overnight, so that the pilocarpine formulation that has been penetrated into the superficial skin will come out into the water. 300 µl of solution was collected from each vial into the corresponding Eppendorf tube and 600 µl of basified Acetonitrile was added to each of the tube. The Eppendorf tubes were kept for centrifugation and the supernatant solutions were analyzed for pilocarpine using validated HPLC method.

Pilocarpine Iontophoresis on Porcine Skin

Iontophoresis was a control study and it was performed by using the Chattanooga Ionto™ iontophoresis device. Six trials were performed for iontophoresis. 113 µl/cm^2 of 0.5% pilocarpine solution was applied on 1.76 cm^2 area of the full thickness porcine skin. The full thickness skin

was sandwiched between two 3M Red Dot^{TM} Adult Electrodes. The positive electrode was attached to the electrode placed on dorsal side of the skin and the negative electrode was attached to the electrode placed on the ventral side of the skin as shown in figure 8. 0.5 mA of current was applied for 10 minutes as shown in figure 5. For first 3 trials, skin was collected after current application and left for 10 minutes and for last 3 trials, skin was collected after current application and left for 40 minutes. Each skin that was collected form trials was washed with distilled water in a beaker, cut into small pieces and immersed into 15 ml of water. The vials were covered with aluminum foil and left in a bio shaker for overnight, so that the pilocarpine formulation that has been penetrated into the superficial skin will come out into the water. 300 µl of solution was collected from each vial into the corresponding Eppendorf tube and 600 µl of basified Acetonitrile was added to each of the tube. The Eppendorf tubes were kept for centrifugation and the supernatant solutions were analyzed for pilocarpine using validated HPLC method.

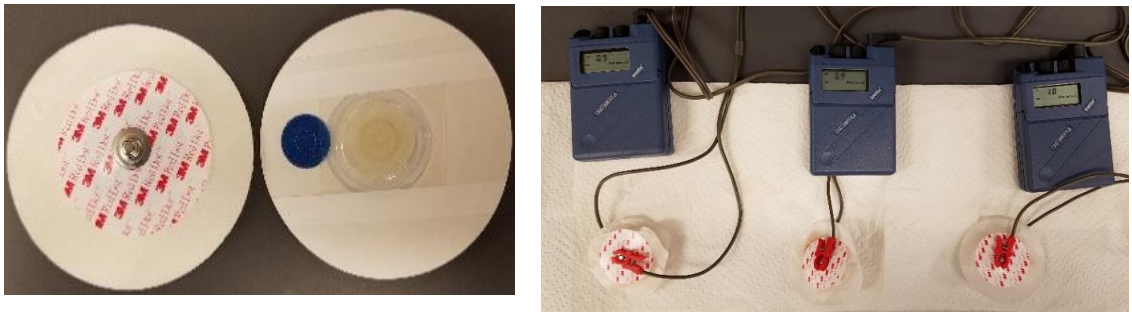

Figure 5: Steps of Iontophoresis

Penetration Depth Profile

Tape stripping procedure was performed to check the depth of penetration of pilocarpine. Tape stripping is a long established and minimally invasive technique to remove the stratum corneum for determining the depth of penetration of drug (48). This procedure involves repeated application and pulling off a pre-weighed adhesive tape on an area of skin surface where the drug

is applied (48) and quantification of the drug per mg of epidermis from the tape strips by validated HPLC method.

Tape stripping with the passive formulation

Six trials were performed for final formulation. For first 3 trials, duration of study were 10 minutes. Full thickness skin was collected at 10th minute of time point. The duration of study was 40 minutes for last 3 trials and full thickness skin was collected at 40^{th} minute time point. The full thickness skin was sandwiched between donor and receiver compartment with the stratum corneum side facing the donor compartment. 17 µl/cm^2 of final formulation was loaded into 1.74 cm^2 area of donor compartment. Last 3 cells of 40^{th} minute time point were placed in an incubator at a temperature of 40° C. Each skin that was collected form trials was washed with distilled water in a beaker. For each piece of skin, three pre weighed adhesive tapes were used and each tape strip was repeatedly applied and pulled off for three times to remove the stratum corneum in the 1.76 cm^2 area where the pilocarpine formulation was applied. After the procedure, weight of the tapes were noted again and first weight was subtracted from the second weight to get the weight of epidermis as shown in table 8.

Time Point	Trial	Number of Adhesive Tape	1st Weight (mg)	2nd Weight (mg)	Weight of Epidermis (mg)
10 minute	**1**	1-3	434.18	435.52	1.34
		4-6	570.74	571.28	0.54
		7-9	556.66	556.89	0.23
	2	1-3	510.49	510.72	0.23
		4-6	551.87	551.17	0.3
		7-9	577.46	577.63	0.17
	3	1-3	556.46	546.95	0.49
		4-6	599.10	599.45	0.35
		7-9	531.75	532.06	0.31
40 minute	**1**	1-3	531.97	533.63	1.66
		4-6	585.60	586.08	0.48
		7-9	535.31	535.81	0.50
	2	1-3	516.33	517.16	0.83
		4-6	528.27	528.95	0.68
		7-9	533.41	533.97	0.56
	3	1-3	524.23	524.83	0.60
		4-6	528.41	529.62	1.21
		7-9	503.23	504.58	1.35

Table 7: Weight of adhesive tape and epidermis for passive formulation

Iontophoresis followed by tape stripping

0.5% pilocarpine solution was used and six trials were performed for iontophoresis. 113 µl/cm^2 of 0.5% pilocarpine solution was applied on 1.76 cm^2 area of the full thickness porcine skin. Full thickness skin was sandwiched between two 3M Red Dot^{TM} Adult Electrodes. The positive electrode was attached to the electrode placed on dorsal side of the skin. The negative electrode was attached to the electrode placed on the ventral side of the skin. 0.5 mA of current was applied for 5 minutes. For first 3 trials, skin was collected after current application and left for 10 minutes. For last 3 trials, skin was collected after current application and left for 40 minutes. Each skin that was collected form trials was washed with distilled water in a beaker. For each piece of skin, pre weighed adhesive tape was repeatedly applied and pulled off for 9 times in the 1.76 cm^2 area where the pilocarpine formulation was applied. After the procedure, weight of the tape was noted again and first weight was subtracted from the second weight to get the weight of epidermis as shown in table 9.

Quantification of pilocarpine followed by tape stripping

Each adhesive tape that was collected form trials of IVPT with passive formulation and iontophoresis with 0.5% pilocarpine solution were cut into small pieces and immersed into 15 ml of water. The vials were covered with aluminum foil and left in a bio shaker for overnight, so that the pilocarpine formulation will come out into the water. 1 ml of the solution was collected from each vial into corresponding Eppendorf. The Eppendorf tubes were kept for centrifugation and the supernatant solutions were analyzed for pilocarpine using validated HPLC method.

Time Point	Porcine Skin	Number of Adhesive Tape	1st Weight (mg)	2nd Weight (mg)	Weight of Epidermis (mg)
10 minute	**1**	1-3	495.56	498.66	3.1
		4-6	591.68	593.77	2.09
		7-9	579.33	580.24	0.91
	2	1-3	542.83	545.25	2.42
		4-6	543.15	544.21	1.06
		7-9	549.98	550.73	0.75
	3	1-3	554.14	556.76	2.62
		4-6	546.17	547.64	1.47
		7-9	520.22	521.23	1.01
40 minute	**1**	1-3	513.21	516.93	3.72
		4-6	557.49	559.36	1.87
		7-9	498.34	499.94	1.6
	2	1-3	554.19	557.28	3.09
		4-6	526.16	527.26	1.1
		7-9	527.92	528.57	0.65
	3	1-3	500.16	502.07	1.91
		4-6	513.16	514.36	1.2
		7-9	500.09	500.87	0.78

Table 8: Weight of adhesive tape and epidermis for iontophoresis

Mechanistic Study

Surface tension study

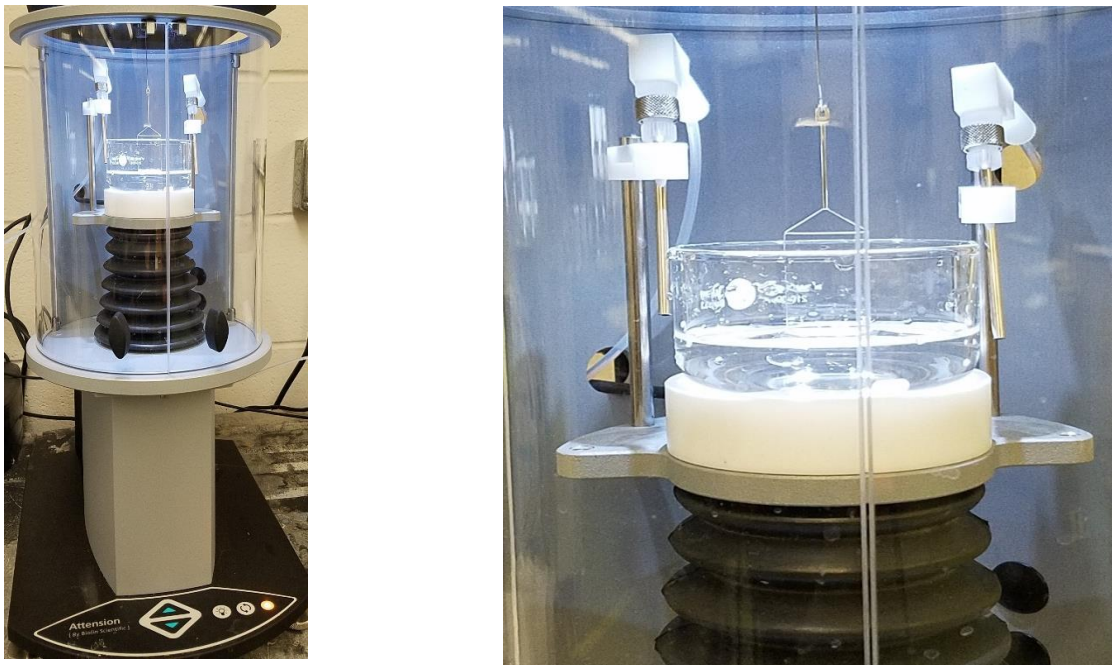

Figure 6: Tensiometer (Biolin Scientific)

The surface tension of all the formulations was measured by using Tensiometer (Biolin Scientific) as shown in Figure 6. All the measurements were performed at 20° C. The sample of the formulation was taken into the beaker and placed on the sample stage. The surface tension of the formulation was measured by using a platinum ring probe hanged on balance as shown in Figure 6. Platinum ring probe was immersed in the formulation and meniscus was formed pulling down the ring while the ring was raising from the formulation. The force applied on the meniscus was used to measure the surface tension. For each sample, it took five minutes to measure surface tension and the values were recorded twenty times.

Partitioning into octanol

Partition coefficient is the ratio of concentration of unionized drug molecule in water and octanol phase at equilibrium**.** So the partition coefficient was determined for pilocarpine solution and the passive formulation containing octanol and water.

In vivo Evaluation in Human Subjects

The formulation was evaluated in healthy human volunteers to assess the ability of lead topical pilocarpine formulation to stimulate sweat production. Sweat secretions and its chloride concentration of sweat from small regions of arm was monitored in response of topical pilocarpine formulation. Twenty human subjects were assigned in the protocol. In this study, one circular area (12.2 cm in diameter) was marked on subject's each of the forearms: one area on subject's right forearm and one area on subject's left forearm.

The application site of subject's right forearm was washed with distilled water and warm topical pilocarpine formulation (kept at 34°C) on the demarcated area was applied on subject's skin. A pre-weighed filter paper was placed after the pilocarpine formulation was allowed to dry for 10 minutes. The forearm covering the demarcated area was wrapped with plastic wrap, heat bandage, paraffin film. After 30 minutes, filter paper was collected and weighed to determine the amount of sweat produced. At the end of the procedure, the application site was washed with soap and running water.

Similarly, on the left forearm, same procedures will be performed using placebo solution instead of pilocarpine formulation. At the end of the study, treated areas will be washed with soap and water.

Chloride Concentration Measurement

Figure 7: Chloride Analyzer (CIBA Corning 925 NSN 6630-01-137-8460)

Chloride concentration of sweat was measured by using CIBA Corning 925 NSN 6630-01-137-8460 chloride analyzer as shown in Figure 9. The chloride analyzer was calibrated with 20 µl of chloride standard.

The chloride concentration of the sweat collected in the filter paper was determined by using the formula as mentioned below:

Concentration of chloride= (20 X R * Density of sweat)/ weight of the sweat

R: Reading on the analyzer, Density of sweat: 1.0047 g/ml

The beaker (provided with the device) and the petri dish were washed with combined buffer, and this combined buffer was conditioned by chloride analyzer to neutralize any pre-existing chloride ions in the beaker and petri dish. Followed by the trial with topical pilocarpine

formulation, the filter paper was collected and chloride ions from the filter paper were eluted by immersing it in conditioned combined buffer in the petri dish for 10 to 15 seconds. And this combined buffer was titrated by chloride analyzer.

RESULTS AND DISCUSSION

Screening of penetration enhancers

Results

Formulation	Amount penetrated after 10 minutes (µg)	Amount penetrated after 40 minutes (µg)
Control (Pilo 9%)	43.48 ± 6.94	58.07 ± 15.91
Tr-5%	108.23 ± 46.70	89.27 ± 35.63
Tr-10%	115.54 ± 54.08	82.58 ± 13.23
P400-5%	79.81 ± 31.35	74.99 ± 13.04
P400-10%	68.99 ± 0.57	80.20 ± 33.93
P200-5%	69.81 ± 6.25	106.14 ± 22.42
P200-10%	57.38 ± 47.65	69.54 ± 8.06
M-2.5%	68.76 ± 8.97	73.43 ± 15.94
M-5%	71.26 ± 19.82	78.92 ± 10.18
SA-5%	106.29 ± 4.46	139.65 ± 34.39
SA-10%	120.29 ± 27.54	158.97 ± 20.15

Table 9: Amount of pilocarpine penetrated into the skin (individual enhancer)

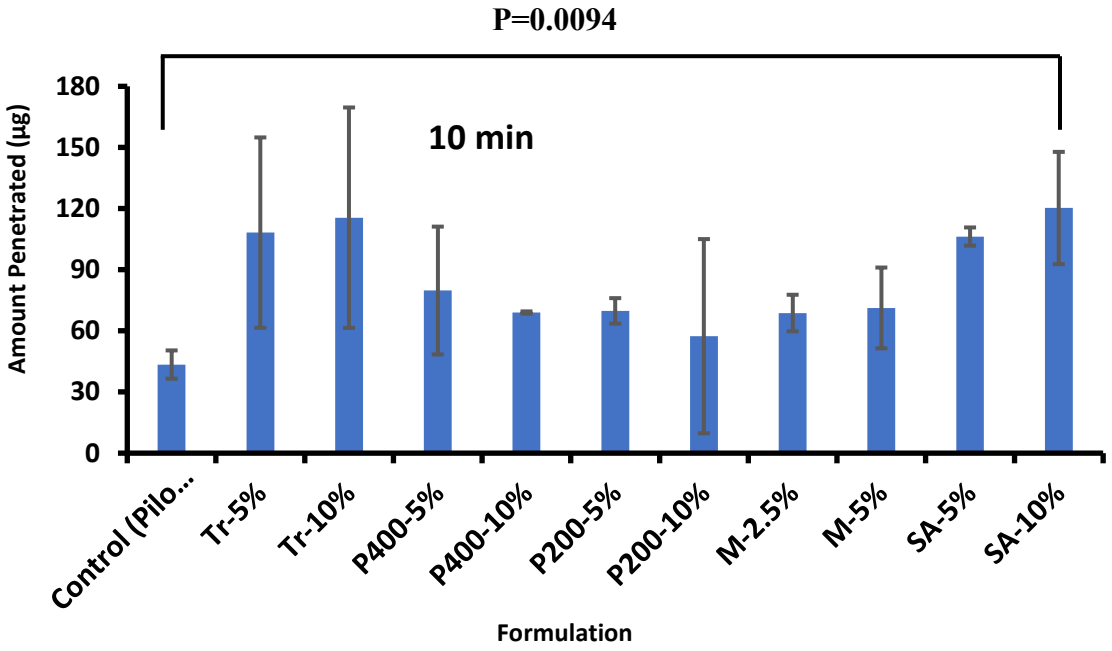

Figure 8:Amount of pilocarpine nitrate penetrated into the skin at 10 minutes time point (individual enhancer)

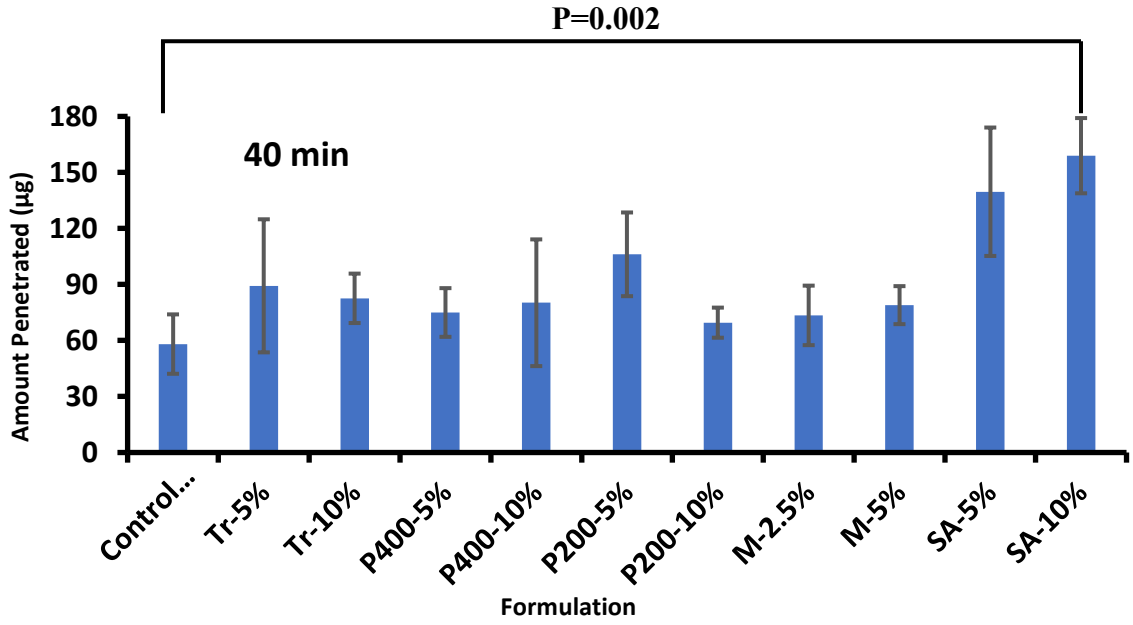

Figure 9: Amount of pilocarpine nitrate penetrated into the skin at 40 minutes time point (individual enhancer)

Discussion

Penetration enhancers are the agents that promotes the penetration of the drug by decreasing the barrier resistance of the skin. So screening of the enhancers is an important introductory step for preparation of the formulation. Five individual enhancers with each of two concentrations were used during preparation of the formulation: Tr, P400, P200, Menthol and SA. P200 and P400 are the hydrating agent and menthol act as a cooling agent. As presented in table 10, all the enhancers resulted in significant enhancement except for P200 10%. As shown in figure 7 and figure 8, for Tr 10%, good penetration was observed after 10 minutes (115.54 ± 54.08 µg) but penetration was moderate after 40 minutes of time point (82.58 ± 13.23 µg). Amount of penetration was moderate both after 10 minutes (68.99 ± 0.57 µg) and 40 minutes (80.20 ± 33.93 µg) for P400 5%. P200 5% showed better penetration after 40 minutes of time point (106.14 ± 22.42 µg). Moderate penetration was observed both after 10 minutes (71.26 ± 19.82 µg) and 40 minutes (78.82 ± 10.18 µg) for M 5%. SA 10% showed more penetration for both 10 minutes (120.29 ± 27.54 µg) and 40 minutes (158.97 ± 20.15 µg) time point compared to all other formulation with individual enhancer. Amount of penetration for SA 10% was statistically significantly higher compared to the control for both 10 minutes and 40 minutes time point.

Combination of enhancers

Results

Formulation	Amount penetrated after 10 minutes (µg)	Amount penetrated after 40 minutes (µg)
Control (Pilo 9%)	43.48 ± 6.94	58.07 ± 15.91
P200+M	105.45 ± 41.58	140.60 ± 38.46
P200+M+SA	145.91 ± 43.07	138.02 ± 23.33

Table 10: Amount of pilocarpine nitrate penetrated into the skin (combination of enhancer)

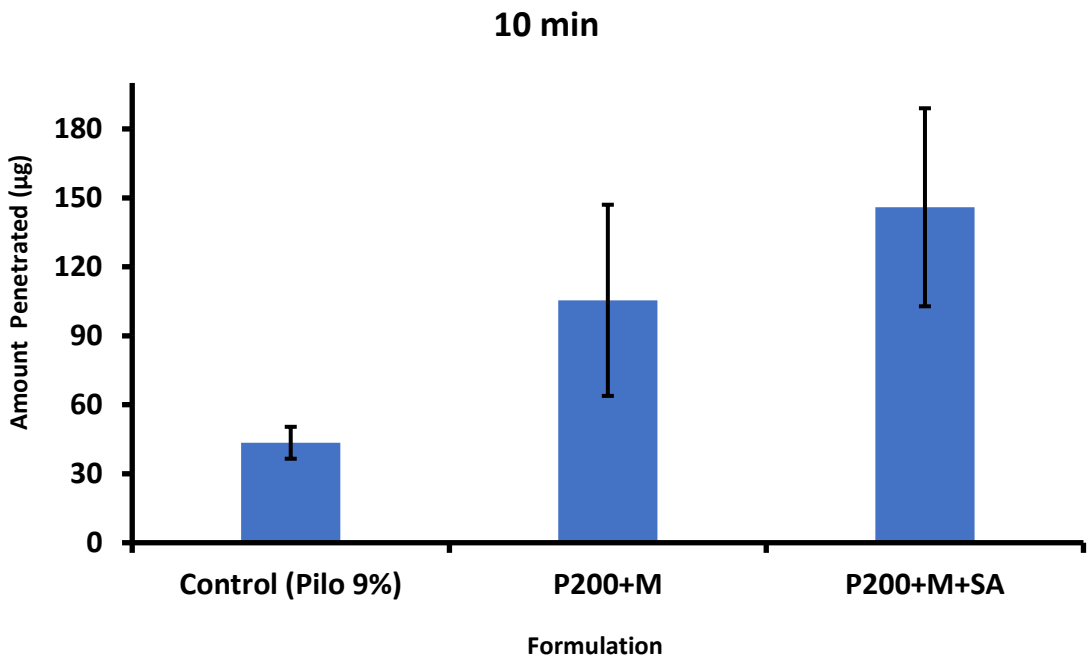

Figure 10: Amount of pilocarpine nitrate penetrated into the skin at 10 minutes time point (combination of enhancer)

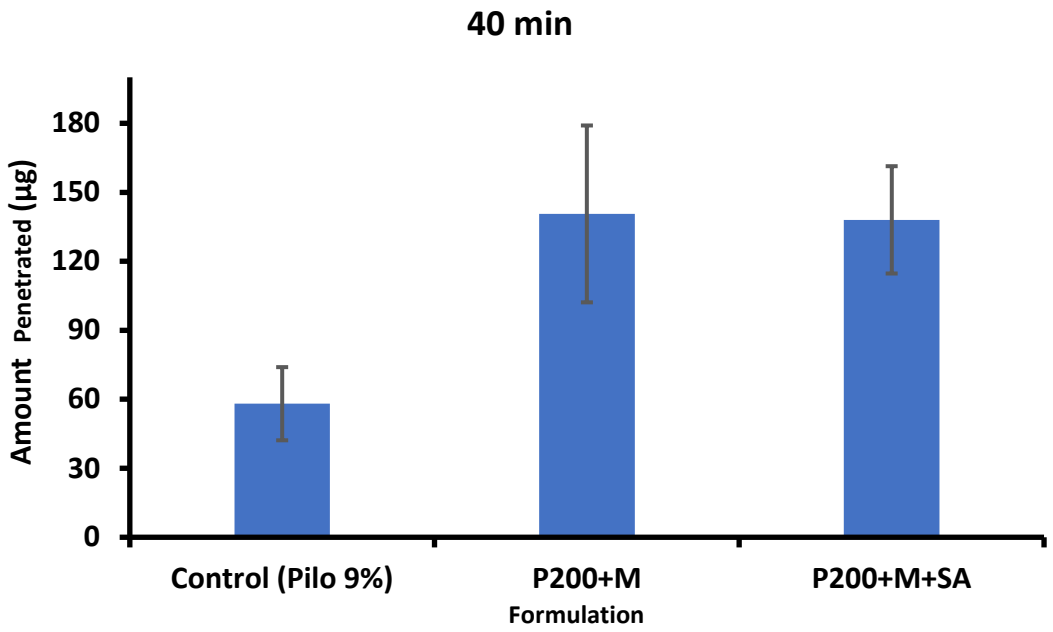

Figure 11: Amount of pilocarpine nitrate penetrated into the skin at 40 minutes time point (combination of enhancer)

Discussion

Two formulations with combination of enhancers were prepared. The first formulation consists of P200 and Menthol. Though P200 and Menthol did not show significant amount of penetration, as several studies have shown the ability of these enhancers to improve the dermal drug delivery of several drugs. As shown in figure 9 and figure 10, formulation with combination of P200 and Menthol showed better penetration than the penetration observed by the formulation with respective individual enhancers. The second formulation is the combination of P200, Menthol and SA. As formulation with SA showed much better penetration compared to all other formulation with individual enhancer, SA was added to the second formulation to have a possible additive effect. As SA is a potential skin irritant, menthol and P200 are added to the second

formulation to minimize the irritation as they are cooling and hydrating agent respectively. Permeation study was done to observe if the ability of SA to enhance the penetration of pilocarpine is compromised because of hydrating and cooling agent. It appeared that the penetration ability of the formulation was improved while Menthol and P200 were added to SA. As shown in table 9 and table 10, the formulation with P200, Menthol and SA showed great amount of penetration for both time points (145.91 ± 43.07 µg, 138.02 ± 23.33 µg) compared to all other formulations. As shown in table 10, the amount of penetration for the formulation with P200, Menthol and SA was better than control (43.48 ± 6.94 µg, 85.75 ± 62.96 µg) and formulation with P200 and M (105.45 ± 41.58 µg, 140.60 ± 38.46 µg). Therefore, the formulation with P200, Menthol and SA was considered to be the lead formulation. In addition to their ability to be supportive enhancers with salicylic acid, P200 is also a hydrating agent and Menthol is a cooling agent in the formulation.

Drug retention study

Results

Name of the Study	Formulation	Amount penetrated after 10 minutes (µg)	Amount penetrated after 40 minutes (µg)
IVPT (42˚C)	P200+M+SA	152.04 ± 52.23	210.27 ± 53.72
Iontophoresis	Pilocarpine Solution	97.05 ± 27.93	140.56 ± 88.66

Table 11: Total amount of pilocarpine nitrate present in the skin Followed by passive delivery and iontophoresis

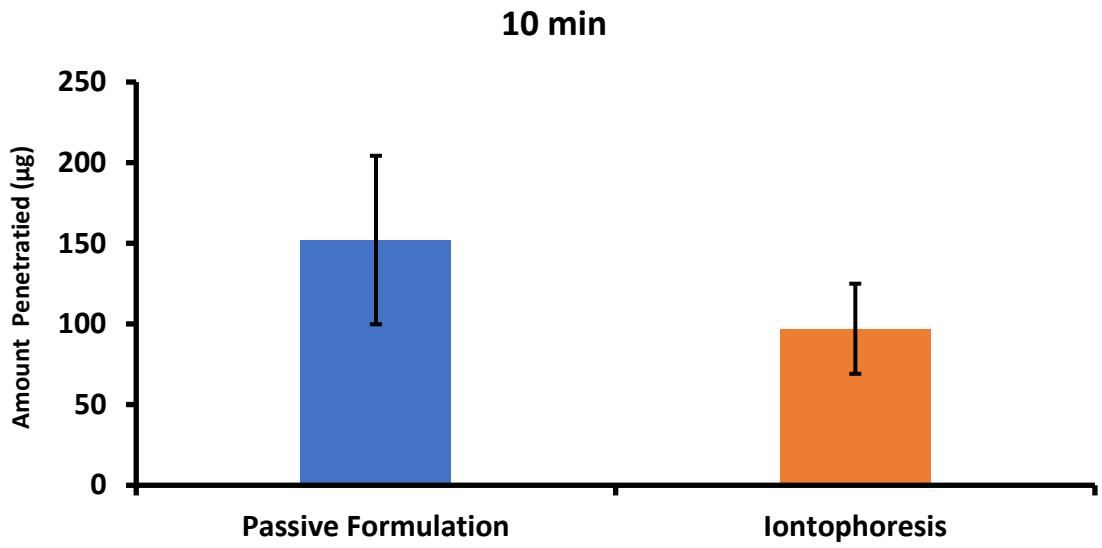

Figure 12: Total amount of pilocarpine nitrate present in the skin after 10 minutes followed by passive delivery and iontophoresis

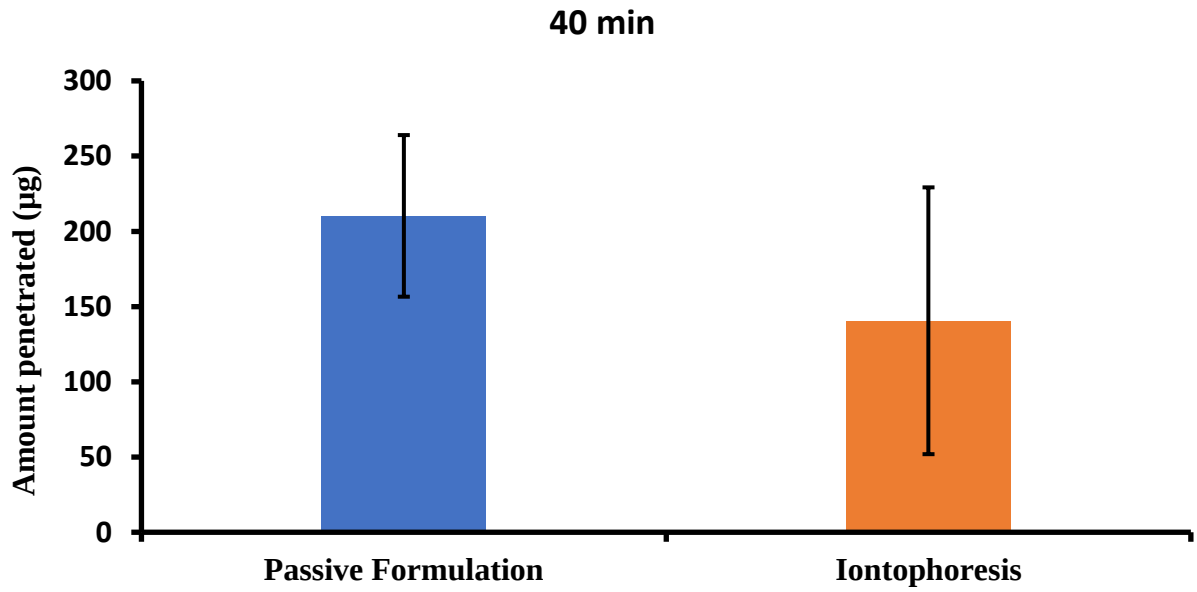

Figure 13: Total amount of pilocarpine nitrate penetrated in the skin after 40 minutes followed by passive delivery and iontophoresis

Discussion

The passive formulation with P200, Menthol and SA performed well and enhance the delivery of large amount of pilocarpine. IVPT with passive formulation and iontophoresis with 0.5% of pilocarpine solution were performed to prove that the passive formulation performs as good and efficient as the iontophoresis. As shown in table 11, the total amount of pilocarpine nitrate present in skin followed IVPT with passive formulation was compared with that of iontophoresis with 0.5% pilocarpine solution.

As shown in figure 11 and figure 12, the total amount of pilocarpine nitrate present in skin followed IVPT with passive formulation is much higher than that of iontophoresis with 0.5% pilocarpine solution for both 10 and 40 minutes time point. As shown in table 11, amount of penetrated drug for passive formulation is 152.04 µg at 10 minutes time point and 210.27 µg at 40 minutes time point. However, for iontophoresis with 0.5% pilocarpine solution, the amount of penetrated drug is 97.05 µg at 10 minutes time point and 140.56 µg at 40 minutes time point.

Effect of Temperature on Penetration

Results

Formulation	Penetration after 10 minutes (µg)	Penetration after 40 minutes (µg)
P200+M+SA (25 °C)	145.91 ± 43.07	138.02 ± 23.33
P200+M+SA (40 °C)	152.04 ± 52.23	210.27 ± 53.72

Table 12: Effect of temperature on amount of penetration of pilocarpine nitrate

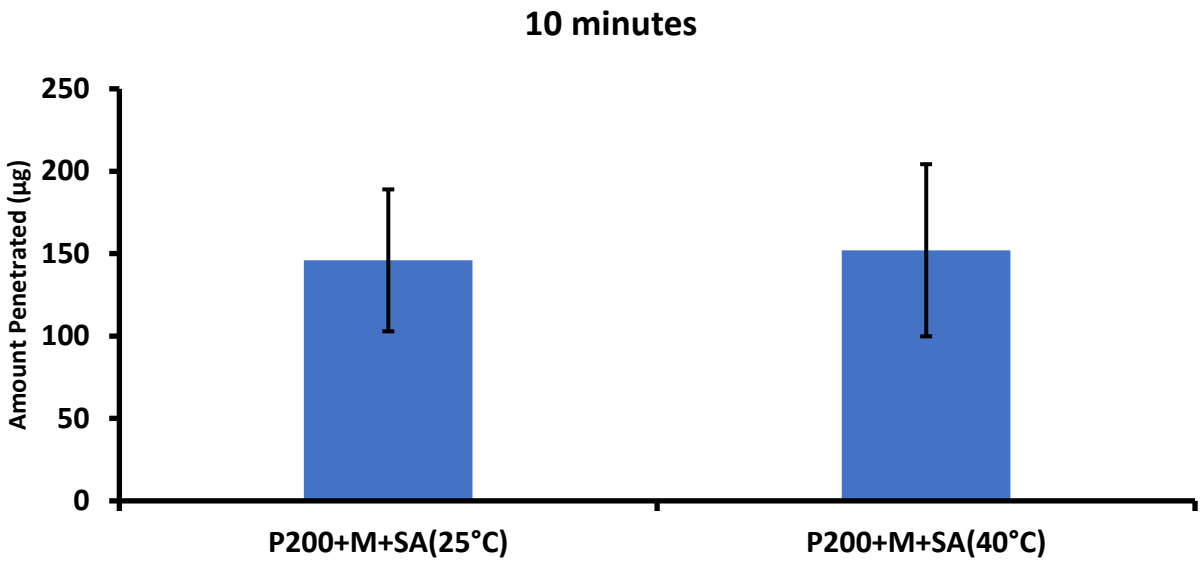

Figure 14: Effect of temperature on amount of penetration of pilocarpine nitrate at 10 minutes time point

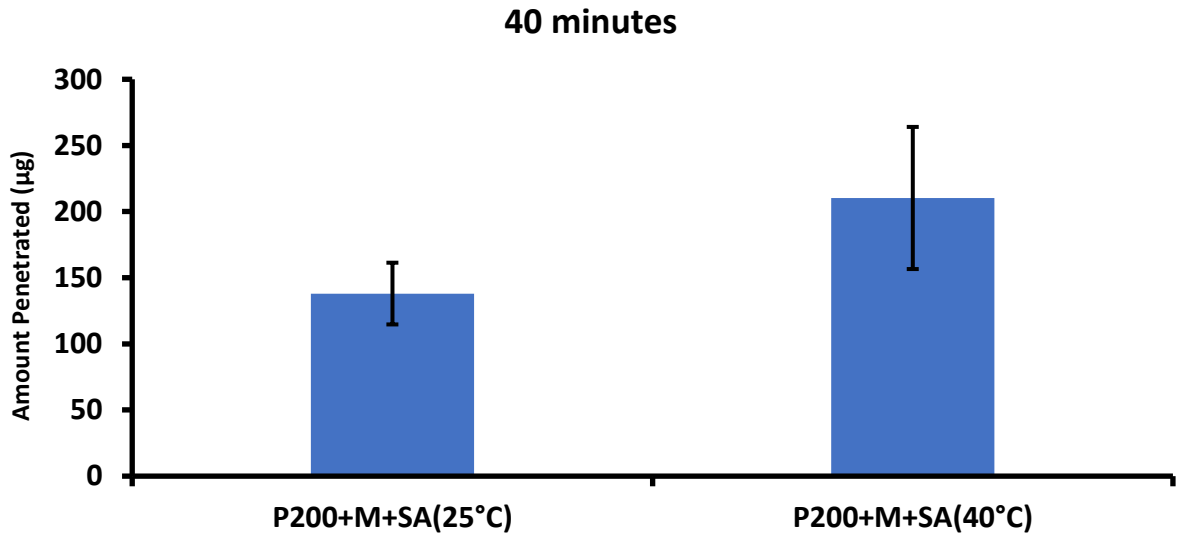

Figure 15: Effect of temperature on amount of penetration of pilocarpine nitrate at 40 minutes time point

Discussion

As the final objective is to apply the passive formulation for 10 minutes and wrap the application site with heat bandage for next 30 minutes; it was observed if the amount of penetration is increased due to application of heat. So, one penetration study was performed with passive formulation where last three trials were kept in the incubator at 43°C and in another penetration study was performed at 25° C. As shown in table 11, for the penetration study at 40° C, the amount of penetration is relatively higher than the one was performed at 25° C for both time point.

Penetration Depth profile

Results

Formulation	Tape Stripping	Amount of drug in epidermis after 10 minutes (µg/mg)	Amount of drug in epidermis after 40 minutes (µg/mg)
P200+M+SA	1-3	30.93 ± 20.25	20.38 ± 13.92
	4-6	22.50 ± 10.10	16.16 ± 7.81
	7-9	25.03 ± 7.77	16.78 ± 11.42
	Total (1-9)	138.09 ± 64.66	93.85 ± 30.83
Iontophoresis	1-3	4.15 ± 1.29	1.40 ± 0.67
	4-6	3.67 ± 1.33	1.98 ± 0.41
	7-9	5.50 ± 1.95	4.00 ± 0.88
	Total (1-9)	23.44 ± 6.13	12.98 ± 0.85

Table 13: Total amount of drug present in the tape strip followed by IVPT and iontophoresis

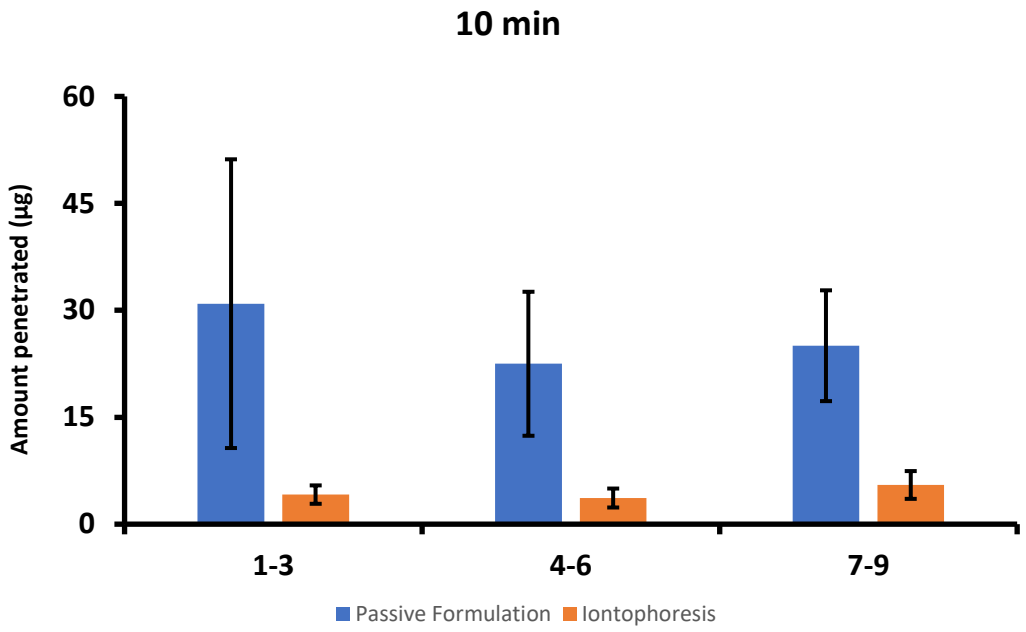

Figure 16: Total amount of drug present in the tape strip after 10 minutes followed by passive delivery and iontophoresis

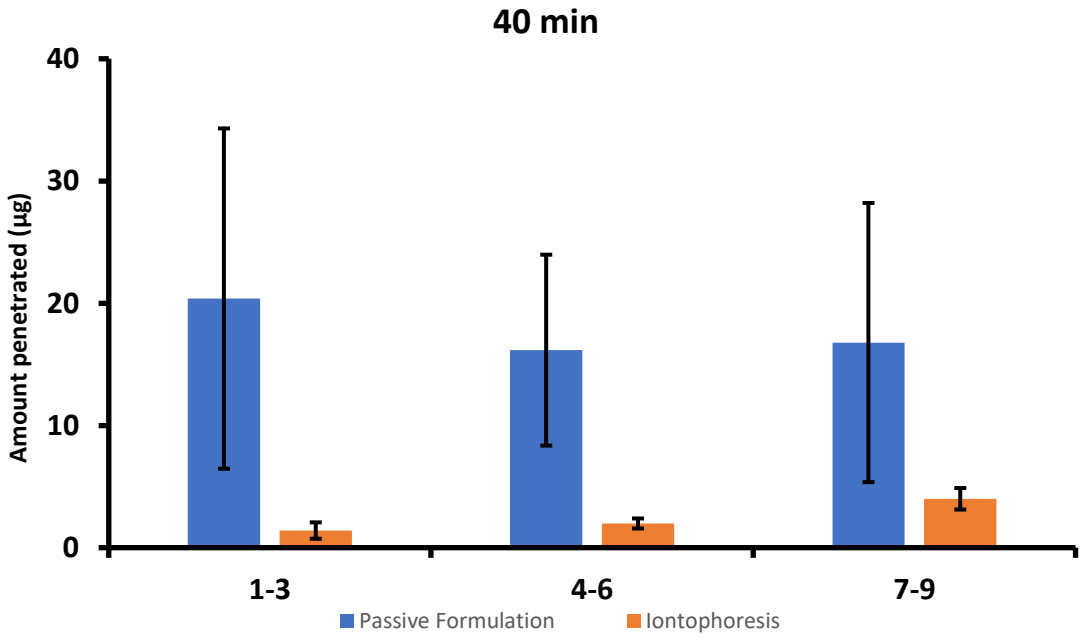

Figure 17: Total amount of drug present in the tape strip after 40 minutes followed by passive delivery and iontophoresis

Discussion

Though it is shown in table 11, more amount of pilocarpine was getting penetrated during IVPT with passive formulation compared to the iontophoresis; the determination of the depth of penetration of pilocarpine into the epidermis is also important. Therefore, the tape stripping procedure was performed on 1.76 cm^2 area of porcine skin followed by the IVPT with passive formulation and the iontophoresis with 0.5% pilocarpine solution.

As shown in table 12, large amount of drug was present in tape strips (78.46 µg/mg) at 10 minutes time point for passive formulation. On the other hand, for pilocarpine iontophoresis, very small amount of drug was present in the tape strips at the same time point (13.32 µg/mg). For 40 minutes time point, the amount of drug presented in tape strips was significantly higher (53.32 µg/mg) in case of passive formulation compared to that of the iontophoresis (7.38 µg/mg).

Mechanistic Study

Surface tension study

Result

Formulation	Surface Tension (mN/m)
Control (Pilo 9%)	71.74 ± 0.19
P200+M+SA	28.25 ± 0.03

Table 14: Surface tension of control and passive formulation

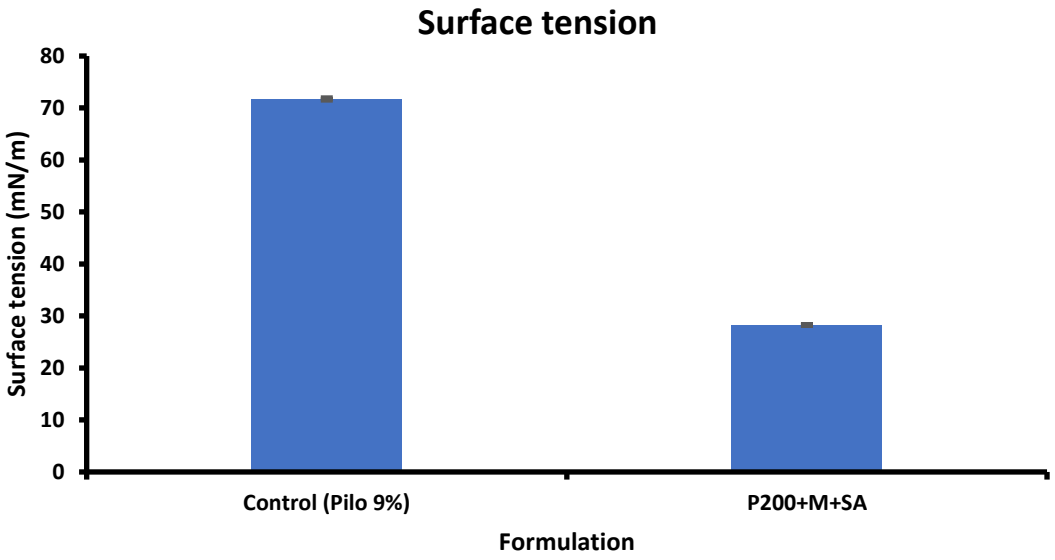

Figure 18: : Surface tension of control and passive formulation

Discussion

Surface tension is the cohesive attraction between liquid molecule that allow the liquid to resist the external force and to maintain the elastic tendency of liquid surface. Surface tension of the passive formulation (28.25 ± 0.03 mN/m) is half of that of the control (71.74 ± 0.19 mN/M). Convective transport is the criteria that depends on the surface tension of the liquid. The lower the surface tension, the better the convective transport and the better ability of liquid to penetrate into pores. As the passive formulation (P200+M+SA) has lower surface tension, it can be concluded that the amount of penetration that was observed in first 10 minutes was significantly contributed by convective transport.

Ion pairing

Ion pairing is the partial bonding between a positive and a negative ion driven by electrostatic force of attraction between them and this partial association mostly occurs in concentrated solution of electrolytes. In high concentration of sodium chloride solution, the positively charged sodium ion associates with negatively charged chloride ion and thereby makes an ion pair (49).

Hydrophilic or ionized drugs are relatively less permeable through lipid layer of stratum corneum which leads to poor trans-epidermal bioavailability. Incorporation of penetration enhancers has long been used to improve the permeability of the ionized drug. Moreover, ion pair has also been used to increases the lipophilisation of ionic drugs thereby increase the skin permeability of ionized molecule (50). It has been used to promote the percutaneous penetration of ionized molecule using artificial and biological membrane (51).

Permeation of diclofenac is increased by ion pair formation between negatively charged diclofenac and positively charged alkylamine or benzylamine. Ion pair between diclofenac and n-hexylamine improves the permeation of diclofenac by 7.3 folds (52). Ion pairing is also used as a tool to improve the transdermal delivery of ionized drugs such as salicylic acid (51, 50). Skin permeability of salicylic acid is increased by forming an ion pair between anionic salicylic acid and cationic alkyl amines.It was assumed that anionic and hydrophilic SA will pair with positively charged hydrophilic pilocarpine and will form unionized lipophilic SA-pilocarpine ion pair. The unionized SA- pilocarpine ion pair facilitates the penetration of pilocarpine into the skin. To prove this hypothesis, partition coefficient study was performed.

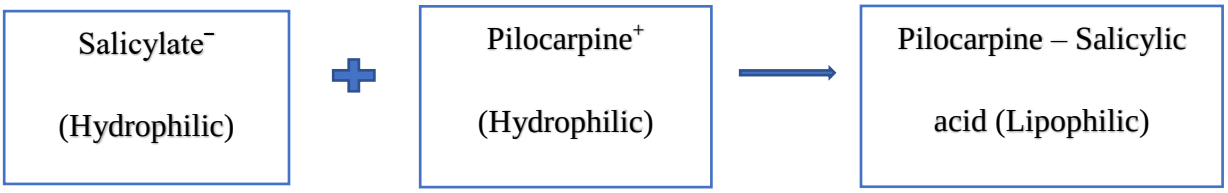

Figure 19: Formation of Pilocarpine -Salicylic acid ion pair Partitioning into Octanol

Results

Solution	Amount in octanol phase (µg/ml)
Pilocarpine solution (used in iontophoresis)	1.07
Passive formulation	1.72

Table 15: Partitioning of pilocarpine in octanol phase for passive formulation and iontophoresis

Discussion

Partition coefficient is the ratio of concentration of unionized drug molecule in octanol and water phase at equilibrium. During partition coefficient study, octanol phase is the representative of biological membrane lipid. As SA is forming an ion pair with pilocarpine, the unionized lipophilic SA-pilocarpine ion pair was hypothesized to partition into octanol (represents stratum corneum lipid phase) compared to its hydrophilic counterpart. It was observed if ion pairing is happening or not; if it is happening more partitioning of pilocarpine was expected in octanol phase compared to aqueous phase.

In vivo Evaluation in Human Volunteers

Results

Subject	Formulation	Initial Wt filter paper	Final Wt filter paper	Sweat collected	Reading	Cl Conc.
1	Test	368.8	421.9	53.1	16	6.04
	Control	374.8	381.6	6.8	0	0.00
2	Test	364.5	409	44.5	17	7.65
	Control	365	392.9	27.9	4	2.87
3	Test	364.8	417	52.2	40	15.35
	Control	363.3	403	39.7	18	9.08
4	Test	381.9	430	48.1	41	17.08
	Control	373.3	388.5	15.2	5	6.59
5	Test	374.8	442.3	67.5	37	10.98
	Control	372	383.8	11.8	1	1.70
6	Test	372.5	441	68.5	39	11.41
	Control	380.5	413	32.5	7	4.32
7	Test	377.8	459.9	82.1	22	5.37
	Control	371.1	393.8	22.7	0	0.00
8	Test	370.2	465	94.8	24	5.07
	Control	375.3	392.5	17.2	0	0.00
9	Test	363.3	451.7	88.4	30	6.80
	Control	371.5	401.5	30	3	2.00
10	Test	369.6	469	99.4	38	7.66
	Control	370.3	391	20.7	1	0.97
11	Test	365	477.4	112.4	106	18.89
	Control	361.9	371.9	10	0	0.00
12	Test	373.4	455.1	81.7	64	15.69
	Control	361.9	368.9	7	0	0.00
13	Test	362.4	464.1	101.7	39	7.68
	Control	373.9	394	20.1	1	1.00
14	Test	372	454.2	82.2	22	5.36
	Control	371	376.9	5.9	0	0.00
15	Test	373.3	472.6	99.3	109	21.99
	Control	366.8	380.8	14	2	2.86
16	Test	375	456.8	81.8	69	16.90
	Control	355.9	359.9	4	0	0.00
17	Test	371.5	440.5	69	86	24.97
	Control	370	386.4	16.4	5	6.11
18	Test	366.6	442.2	75.6	62	16.43
	Control	366.4	377.1	10.7	1	1.87

19	Test	362.4	428.1	65.7	19	5.79
	Control	363.4	373.7	10.3	1	1.95
20	Test	356.4	433.9	77.5	24	6.20
	Control	371	383.1	12.1	1	1.66

Table 16: Results of clinical study (n=20)

Formulation	Sweat Collected (mg)	Chloride Concentration (mmol/l)
Test	77.28 ± 18.97*	11.67 ± 6.22
Control	16.75 ± 9.71	

Table 17: Average weight of collected sweat and average chloride concentration

Discussion

Followed by the clinical study, filter paper was collected and chloride concentration was measured using chloride analyzer (CIBA Corning). As shown in table 17, average weight of the sweat for the test (passive formulation) was statistically significantly higher compared to that of the control. Some sweat was produced for control. However, the amount of the sweat was not enough to determine the chloride concentration. Though heating pad was applied for both test and control, very small amount of sweat was produced for control. So it was observed that the amount of sweat produced for test formulation was significantly contributed to passive formulation.

Following formula was used to determine the chloride concentration

Concentration of chloride= (20R * Density of sweat)/ weight of the sweat

R= Reading from chloride analyzer

Density of the sweat= 1.00176 gm/ml

POTENTIAL APPLICATION OF SWEAT COLLECTION USING PASSIVE FORMULATION

Sweat analysis can be used as an important diagnostic tool for lots of diseases such as cystic fibrosis, diabetes, lung cancer. In cystic fibrosis, qualitative and quantitative sweat analysis are usually performed for chloride concentration in sweat. Chloride concentration of ≥60 mmol per liter indicates that the patient is consistent with cystic fibrosis. And the chloride concentration of 30-60 mmol per liter of sweat is an indication of borderline cystic fibrosis (37).

Moreover, sweat analysis can also be used as diagnostic technique for diabetes as the diabetic biomarkers such as correlation between the blood glucose and the sweat glucose, change in rate of sweating (53, 54) and the composition of sweat (53, 55) can be determined by sweat analysis. In this test, foot sweat is collected in a patch and is analyzed based on color change of the patch from blue to pink due to addition of 6 molecules of water into it (53, 56).

Furthermore, sweat sample is also used as a screening tool for lung cancer. In this method, metabolites of sweat are analyzed to discriminate between patient with lung cancer and healthy individuals (53).

Sweat also plays a significant role in the field of genomics and proteomics. Sweat contains an amino acid called dermcidin (DCD) that is also a tumor marker in breast carcinoma. Elevated level of DCD and overexpressed receptor for DCD have been observed in patient of breast carcinoma (53). Drug contents and ethanol can be detected by sweat analysis. Qualitative detection of drugs from the sweat sample is done by immunochromatographic test and patch technology.

Immunochromatographic test is applicable for the drugs that are ingested within 24 hours and this method requires collection of sweat sample for single time point to detect individual under the influence of drug (53). And the drug ingested within 168 hours can be detected by patch technology. In this method, sweat sample is collected for single time point to follow up the previous drug abusers who are under abstinence treatment (53). Moreover, quantitative detection of drugs such as opiates, buprenorphine, cocaine, amphetamines, cannabinoids is done from the sweat sample (53, 57). Moreover, ethanol can also be detected by the sweat analysis which can be useful as a valuable tool in alcohol abuse (53, 58).

CONCLUSION

Passive formulation method can be used as an alternative to iontophoresis as the new diagnostic method can overcome the limitations involved in the current one. Passive formulation can increase the patient compliance as it does not involve the application of current to deliver the pilocarpine. Moreover, the menthol and P200, which are the ingredient of the passive formulation gives the cooling sensation and maintain the hydration of the skin. It was observed form the IVPT that the passive formulation can enhance the delivery of more amount of pilocarpine into the skin compared to iontophoresis. In addition, tape stripping method confirms that new alternate diagnostic method can deliver the more pilocarpine compared to iontophoresis to the deepest layer of epidermis without any driving force such as application of current. Moreover, as very small amount of pilocarpine (1 mg) is applied on the skin, it would not go far enough down to reach the systemic circulation. So there less chances of systemic side effects or toxicity. Based on these observations, it can be inferred that passive formulation is an efficient, alternative method to iontophoresis.

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VITA

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