



Assessment of epinephrine sublingual stability and permeability pathways to enhance its permeability for the treatment of anaphylaxis

Mutasem Rawas-Qalaji^{a,b,c,*}, Rawan Bafail^d, Roberta Cagliani^b, Mohamed Haider^{a,e}, Zahid Hussain^{a,b}

^a College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates

^b Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates

^c Dr. Kiran C. Patel College of Allopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL 33326, USA

^d College of Pharmacy, Taibah University, Medina, Saudi Arabia

^e Faculty of Pharmacy, Cairo University, Cairo 71526, Egypt

ARTICLE INFO

Keywords:

Anaphylaxis
Epinephrine
Fast disintegrating tablets
Stability
Permeability
Sublingual

ABSTRACT

Prompt epinephrine (Epi) injection using auto-injectors is the initial life-saving out-of-hospital treatment for anaphylaxis. However, patients and healthcare providers are eagerly awaiting a more convenient alternative dosage form that would overcome auto-injectors drawbacks.

Previously, we extensively evaluated multiple alternative fast-disintegrating sublingual Epi tablet (FDSTs) formulations. However, the sublingual stability of Epi and effect of modifying the sublingual microenvironment pH on its stability and transport pathways were not yet fully investigated.

Results depicted that Epi remained sufficiently stable at various pHs in human saliva and porcine sublingual tissue's extract. Epi permeability (EP) through excised porcine sublingual membrane was greatest at pH 8.0 ($p < 0.05$), 11-fold higher than the negative control (Epi at pH 6.8). Sodium carbonate (Na Carb) 0.75% was the most efficient buffer to modify Epi solution pH to 8.0. Both sodium dodecyl sulfate (SDS) 0.075% and palmitoyl-DL-carnitine chloride (PCC) 1.2% increased paracellular EP 10-fold and 3-fold, respectively; however, both demonstrated a delayed enhancement (>5 min). Meanwhile, Na Carb and SDS combination increased EP 23-fold without a delay.

It is evident that pH-modifiers or their SDS combination showed promising potential to enhance Epi sublingual permeability and further reduce the required Epi dose using FDSTs as a feasible alternative to Epi auto-injectors.

1. Introduction

For out-of-hospital treatment of anaphylaxis, where most anaphylactic episodes occur, epinephrine (Epi) auto-injectors are universally recommended and prescribed for self-administration (Lieberman et al., 2010; Simons et al., 2011). Prompt administration of these Epi auto-injectors is required for efficient treatment of anaphylaxis, which results in fewer hospitalization and deaths (Chooniedass et al., 2017; Fleming et al., 2015).

However, the ability of these auto-injectors to consistently deliver accurate dosing and function reliably is sometimes questionable. There have been several recalls for these devices from the market due to mechanical problems (Brown, 2015; Palmer, 2017). Additionally, there were several supply shortages for these devices due to manufacturing

delays and recalls (Scutti, 2018), which led to the recommendation to use them beyond their expiry dates when there was a shortage in the market (Palmer, 2018). Furthermore, auto-injectors are being underutilized due to the challenges associated with their administration requirements and availability. The length of the auto-injector's needle intended for administration in underweight or overweight children may either be too long and penetrate to the bone or too short and fail to deliver the drug into the muscle tissues (Dreborg et al., 2017; Kim et al., 2014). Administration errors causing injuries including lacerations and embedded needles have been previously reported as well and can add to the challenges associated with self-administration (Brown et al., 2016).

Due to aforementioned issues associated with Epi auto-injectors as well as their short shelf-life, bulky size, and the fears associated with needles, we developed a novel fast-disintegrating sublingual tablet

* Corresponding author at: College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates.

E-mail address: mqalaji@sharjah.ac.ae (M. Rawas-Qalaji).

<https://doi.org/10.1016/j.ejps.2021.106025>

Received 11 May 2021; Received in revised form 20 July 2021; Accepted 26 September 2021

Available online 29 September 2021

0928-0987/© 2021 Elsevier B.V. All rights reserved.

(FDST) of Epi that has been undergone extensive preclinical evaluation for the first-aid treatment of anaphylaxis in out-of-hospital settings (Rachid et al., 2011, 2012, 2018a; Rawas-Qalaji et al., 2013, 2006a, 2006b, 2007, 2006c). Epi FDSTs offer greater administration convenience, dosing flexibility and significantly longer stability than Epi auto-injectors (Rachid et al., 2018b; Rawas-Qalaji et al., 2013, 2007, 2006c). Our Epi FDSTs went through further development and evaluation to mask the bitter taste of Epi following their disintegration (Rachid et al., 2013, 2010) and to enhance the sublingual bioavailability of Epi through micronization using a novel microfluidics method. Such optimizations resulted in a 50% reduction in the required sublingual Epi dose to achieve pharmacokinetics parameters similar to Epi auto-injectors (Rachid et al., 2018b; Rawas-Qalaji et al., 2015a, 2015b).

To further optimize the sublingual absorption and delivery of Epi, which can be limited by its intrinsic characteristics such as lipid solubility, degree of ionization, and molecular size, it was critical to understand the sublingual permeation pathways that can be impacted by altering the pH of the saliva and the application of penetration enhancers (Sattar et al., 2014). For the sublingual route, drug permeability occurs through the sublingual mucosa and its absorption into the systemic circulation undertakes two major transport pathways, the paracellular and the transcellular routes (Wang and Chow, 2014). The degree of ionization of the administered drug at the absorption site and the extent of the membrane's resistance to drug permeation determine the preferred pathway for most ionizable drugs (Chen et al., 1999). Basic drugs with high pKa such as Epi (pKa 8.59) can be highly ionized at the pH of the saliva that ranges between 5.5 and 7.0, which would alter the extent of its sublingual absorption (Chen et al., 1999; Goswami et al., 2013; National Library of Medicine, 2004).

Therefore, we hypothesized that the use of a pH-modifying agent in the Epi formulation will modify the pH of the sublingual microenvironment, thus, enhancing the sublingual permeability of Epi. Moreover, stabilizing the pH of the absorption microenvironment at a pH that is optimal for drug absorption would reduce drug absorption variabilities due to variations in the oral pH. Furthermore, transbuccal resistance to drug permeability and systemic delivery can be significantly reduced by various penetration enhancers (Hassan et al., 2010). Therefore, we hypothesized that the use of a penetration enhancer in Epi formulation along with the pH modifier can further enhance Epi sublingual permeability and absorption.

The objectives of this study were to investigate the significance of altering the pH of the absorption medium on Epi stability and permeability, the stability of Epi during its sublingual administration, and the significance of using penetration enhancers for enhancing Epi sublingual permeability and confirm its transport pathways. Thus, a proper selection and utilization of a pH-modifying excipient and a penetration enhancer would allow for more efficient Epi sublingual delivery and further reduction in the required sublingual Epi dose for the treatment of anaphylaxis.

2. Materials and methods

2.1. Materials

All the following chemicals were purchased from Sigma-Aldrich (St. Louis, MO): (-)-epinephrine (+) bitartrate (EpiBit) as the active ingredient, disodium phosphate and citric acid to prepare a citrate-phosphate buffer (McIlvaine buffer), perchloric acid and sodium metabisulfite to stabilize epinephrine aqueous samples, and protease inhibitor cocktail for tissue homogenization. Methanol, sodium phosphate monobasic, 1-octanesulfonic acid sodium salt monohydrate, and ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate were purchased to prepare the mobile phase for HPLC analysis. Sodium bicarbonate (Na Bicarb), sodium carbonate (Na Carb) and sodium citrate (Na Citr) were purchased as pH-modifiers, and sodium dodecyl sulfate (SDS) and palmitoyl-DL-carnitine chloride (PCC) as penetration

enhancers.

2.2. Assessment of pH-stability profile of Epi for sublingual administration

Epi Bitartrate (EpiBit) equivalent to 20 mg Epi was dissolved by vortexing in 2 mL of McIlvaine buffer ($n = 3$) prepared at pH 1.2, 3.5, 4, 4.5, 5, 5.5, 6, 6.8 (average saliva pH), 7.4, and 8. EpiBit dose was selected based on the results from our previous *in vivo* pharmacokinetics studies (Rawas-Qalaji et al., 2015a, 2015b). Different volumes of 0.2 M disodium phosphate stock solution and 0.1 M citric acid stock solution were used to prepare McIlvaine buffers at the required pH values according to McIlvaine TC, 1921 (McIlvaine, 1921). Aliquot samples were withdrawn at 2 min (maximum expected time for sublingual administration), 4 min, 6 min, 10 min, 15 min, and 30 min. Withdrawn samples were immediately diluted with a stabilizing solution containing 0.1 M perchloric acid and 0.1 mM sodium metabisulfite and frozen by liquid nitrogen. Samples were analyzed using HPLC with UV detection to calculate Epi recovery (%) at the various time intervals and assess the short-term stability of Epi.

2.3. Assessment of Epi stability in saliva and sublingual tissue extracts

2.3.1. Stability in human saliva

For sublingual administration potential, the Epi short-term stability was assessed in human saliva. Saliva was collected from a human volunteer that has been instructed to abstain from eating or drinking any acidic, alkaline or spicy food or beverages and to rinse his mouth before saliva collection. The saliva was collected in a beaker over ice and then air bubbles were removed by aspiration.

EpiBit equivalent to 20 mg Epi was dissolved by vortexing in 2 mL saliva ($n = 3$) to assess its stability over time. Aliquot samples were withdrawn at the different time intervals, diluted with the stabilizing solution, and analyzed by HPLC as previously described to calculate Epi recovery (%) and assess its short-term stability in human saliva. Data were compared to Epi recovery (%) at pH 6.8 (average saliva pH) as a negative control.

2.3.2. Stability in porcine sublingual mucosa extract

Excised porcine sublingual membrane described hereafter was homogenized in a protease inhibitor cocktail to extract any potential enzymes and medium within the sublingual mucosa that may negatively impact Epi stability and concentration during its sublingual permeation. Briefly, the excised sublingual mucosa was cut into small pieces while frozen and immersed in a freshly prepared protease inhibitor cocktail in a phosphate buffer at pH 6.8 (average saliva pH). The mixture was homogenized over an ice bath and then centrifuged at 4 °C to collect the supernatant. Aliquots (2 mL) of the collected supernatant were then spiked with EpiBit equivalent to 20 mg Epi ($n = 3$). Samples were withdrawn at the different time intervals, diluted with the stabilizing solution, and analyzed by HPLC as previously described to assess Epi stability during its permeation through the sublingual mucosa. Data were also compared to Epi recovery (%) at pH 6.8 (average saliva pH) as a negative control.

2.4. Assessment of sublingual pH-permeability profile for Epi

Assessment of the permeability of Epi through excised sublingual membranes at various pH were performed using Franz cells with an OD of 20 mm and a reservoir volume of 20 ± 1 mL (PermeGear Inc., Hellertown, PA). Pigs' lower jaws were purchased from a local butcher. The sublingual membranes were surgically dissected to remove the underlying connective tissue and fat using scalpel and tweezers according to our previously reported procedure (Aodah et al., 2019; Bafail et al., 2019; Rawas-Qalaji et al., 2015b). Dissected membranes were then stored at -20 °C in PBS to be used as diffusional membranes.

The receptor cell with a magnetic stirrer was filled with a phosphate

buffer prepared at pH 7.4 to represent the pH of the blood. The dissected membranes were defrosted at room temperature and then mounted between the vertically oriented donor and receptor compartments of the Franz cells. Before the beginning of the experiments, the mounted membranes were left to equilibrate for 30 min with the diffusion medium at 37 °C. A water bath was used to circulate the heated water through the jacketed Franz cells. Cells were checked and adjusted for any leaks and air bubbles were removed. At the beginning of each experiment, EpiBit equivalent to 20 mg Epi was dissolved in 2 mL of McIlvaine buffer prepared at the desired pH (pH 5, 6, 6.8, 7.4, and 8) and pipetted into the donor cell ($n = 4$). Epi permeability at pH 6.8, the average saliva pH, was used as a negative control.

Aliquot samples of 200 μ L were collected from the receptor cell by aspiration at 5, 10, 15, 20, 30, 45, 60, 75, and 90 min using 6-inch needles (Popper & Sons, Inc., New Hyde Park, NY) fitted into 1 mL syringes. The sampled volumes were replenished using fresh phosphate buffer. The collected samples were filtered, analyzed using HPLC-UV system according to the USP product monograph (USP/NF, 2020), and statistically compared to determine the optimal pH for the sublingual permeability of Epi.

2.5. Selection of excipient for altering the pH of the sublingual microenvironment

Three different alkalinizing salts, sodium bicarbonate (Na Bicarb), sodium carbonate (Na Carb), or sodium citrate (Na Citr) were selected to modify the pH of the sublingual absorption medium. Their *in vitro* ability to modify the pH of the medium upon their incorporation with Epi was determined and the effect of Epi on their buffering capacity was assessed.

Na Bicarb, Na Carb, or Na Citr at various concentrations (0.15% and up to 1.9% w/v) were dissolved in 2 mL of deionized water ($n = 3$) or in EpiBit solution equivalent to Epi 20 mg. The pH readings were recorded using a lab pH meter and results were statistically analyzed. The selection of the pH-modifying excipient to be used for further studies was based on its ability to raise the pH of the Epi solution using the lowest possible concentration, up to the pH at which Epi permeability was found to be the highest based on results from Section 2.4.

2.6. Effect of penetration enhancers on the sublingual permeability of Epi

The effect of two different transcellular and paracellular penetration enhancers, SDS and PCC, on the sublingual permeability of Epi was evaluated using static vertical jacketed Franz cells. Porcine sublingual membranes were excised, stored, and used as a diffusional membrane as previously described.

EpiBit equivalent to 20 mg Epi was dissolved with 1.5 mg SDS or 24 mg PCC in 2 mL (0.075% or 1.2%, respectively) of McIlvaine buffer prepared at pH 6.8, which is the average pH of saliva, and pipetted into the donor cell ($n = 4$). Aliquot samples were withdrawn at the various time intervals, withdrawn volumes were replenished, and samples were filtered and analyzed as described in Section 2.4.

Epi permeability with the penetration enhancers was statistically compared to its permeability at pH 6.8 as a negative control to determine the predominant Epi transport pathway and the penetration enhancer with the greatest effect on enhancing Epi permeability. Results were also compared to Epi permeability at the pH that achieved the greatest Epi permeability to compare the two approaches for their efficiency in enhancing Epi permeability.

2.7. Combined effect of pH-modifier and penetration enhancer on the sublingual permeability of Epi

The combined effect of incorporating both a pH-modifier and a penetration enhancer on the enhancement of the sublingual permeability of Epi was investigated. Franz cells and excised porcine

sublingual membranes were prepared and used as previously described in Section 2.4. The selection of pH-modifier and penetration enhancer to be combined for the evaluation of their combined enhancement effect was based on results obtained from previously described studies in Sections 2.5 and 2.6, respectively.

EpiBit equivalent to 20 mg Epi was dissolved with 1.5 mg SDS and 15 mg Na Carb in 2 mL (0.075% and 0.67%, respectively) of deionized water instead of McIlvaine buffer and was immediately pipetted into the donor cell ($n = 4$). Water was used as a diffusion medium instead of the buffer to evaluate the pH-modification effect by Na Carb on Epi sublingual permeability. Samples were withdrawn, filtered and analyzed as previously described in Section 2.4.

The permeability of Epi with SDS and Na Carb was statistically compared to its permeability at pH 6.8, as a negative control, pH 8, and with the corresponding penetration enhancer alone to evaluate the potential of their combined enhancing effect over their individual enhancing effect on Epi sublingual permeability.

2.8. HPLC analysis

All collected samples from stability and permeability experiments were filtered using 0.22 μ m single-use syringe filters before injecting into an HPLC system with UV detection. The USP method for Epi injections was used to quantify samples for their Epi content (USP/NF, 2020). Briefly, a reversed phase Nova-Pack C-18 column, 3.9 $\times$ 150 mm (Waters, Milford, MA) was used for the separation of Epi at flow rate of 1 mL/min and wavelength of 280 nm. The mobile phase used for the elution of Epi was composed of buffer and methanol at a ratio of 85:15. The buffer was composed of 0.05 M sodium phosphate monobasic, 519 mg 1-octanesulfonic acid sodium salt monohydrate, and 45 mg EDTA adjusted to pH 3.8 using phosphoric acid.

Four calibration curves were prepared with seven standards each ranging from 0.025 mg/mL to 1 mg/mL (Fig. 1). All curves were linear with a coefficient of variation (R^2) of > 0.99 . The intra-assay ($n = 2$) and inter-assay ($n = 4$) coefficient of variations (RSD%) were $< 0.2\%$ and 1.6% at the lowest concentration and 0.1% and 2.7% at the highest concentration of the standard curves, respectively.

2.9. Data calculation and statistical analysis

The means ($\pm$ SD) of the cumulative amount of Epi permeated (μ g/cm²) over time were plotted, and the area under the curve for 90 min (AUC₀₋₉₀) was calculated for each tested Epi formulation. The mean steady-state influx of Epi, J (μ g/cm².min), was determined by calculating the average of the individual slopes ($n = 4$) of the linear portion of the amount of drug permeated versus time curve. The permeability of Epi, P (cm/min), was determined by calculating the mean of the individual steady-state influx of Epi ($n = 4$) divided by its initial concentration used in the donor cell.

Results were statistically analyzed by one-way or two-way ANOVA and Tukey-Kramer tests. The statistical analysis tests were performed using NCSS statistical analysis software (NCSS, Kaysville, UT). A statistically significant of $p < 0.05$ was used to detect differences.

3. Results

3.1. pH-stability profile of Epi for sublingual administration

The mean ($\pm$ SD) for Epi recovery (%) versus pH at various time intervals was plotted in Fig. 2. At pH 1.2, Epi recovery (%) was not significantly different ($p > 0.05$) from its initial concentration at the different sampling time intervals. Additionally, Epi recovery (%) was only significantly lower ($p < 0.05$) than its initial concentration after 15 min at pH 3.5 and 8, and after 10 min at a pH between 5.5 and 7.4. However, between pH 4 and 5, Epi recovery (%) declined significantly ($p < 0.05$) and very quickly as early as 2 min.

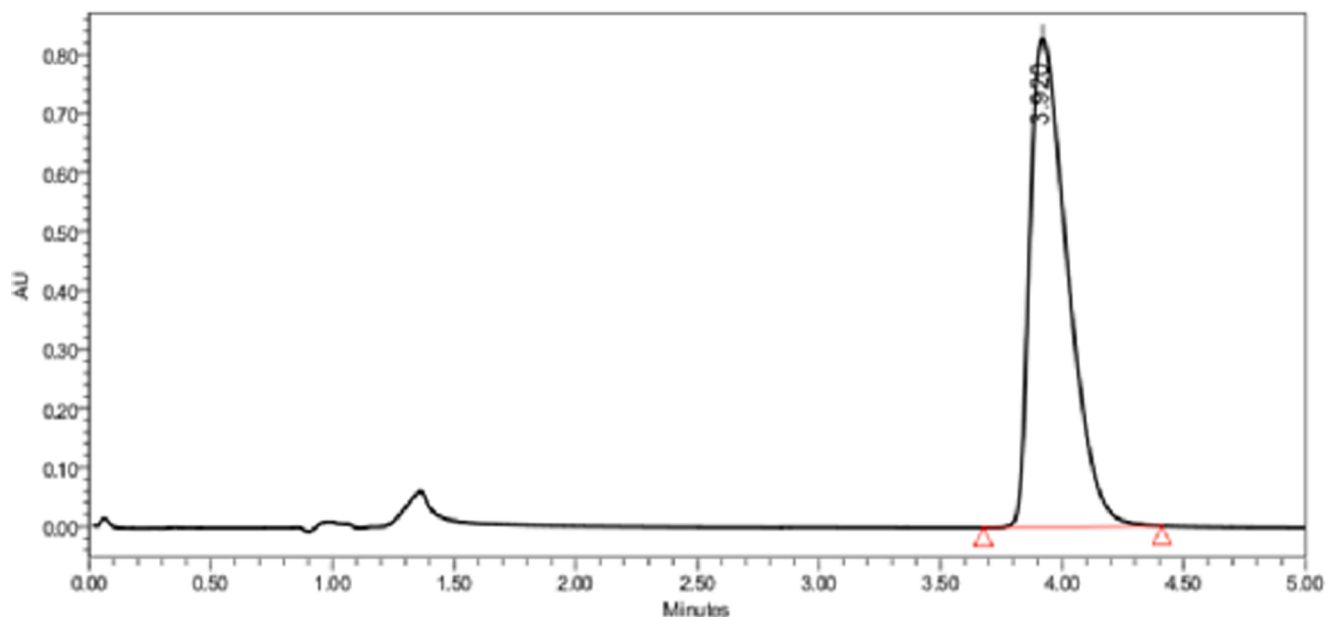


Fig. 1. HPLC chromatogram of epinephrine.

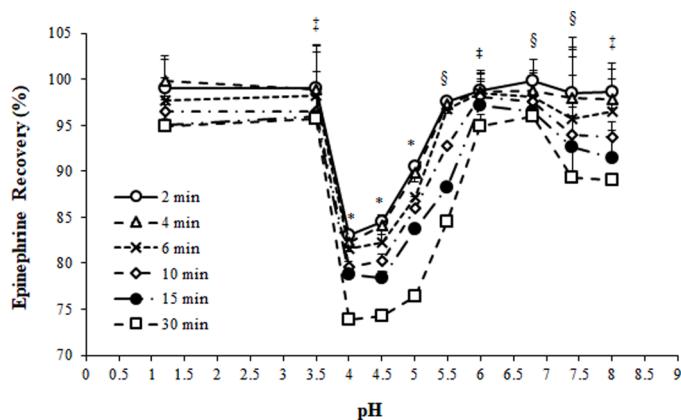


Fig. 2. Mean ($\pm$ SD) epinephrine recovery (%) over 30 min at a range of pH media ($n = 3$). *Significantly different from the initial concentration during the whole 30 min. †Significantly different from initial concentration after 10 min. §Significantly different from initial concentration after 15 min.

3.2. Epi stability in saliva and sublingual tissue extracts

The mean ($\pm$ SD) for Epi recovery (%) in saliva and sublingual tissue extract over time were plotted in Fig. 3. Epi recovery (%) in both human saliva and sublingual tissue extract was not significantly lower ($p > 0.05$) than its initial concentration for at least up to 15 min. Additionally, Epi recovery (%) became significantly lower ($p < 0.05$) than the negative control (Epi at pH 6.8) only at 30 min for Epi in saliva.

3.3. Sublingual pH-permeability profile for Epi

The mean ($\pm$ SD) cumulative amount of permeated Epi over time at various pH media was plotted in Fig. 4. The AUC_{0-90} for the cumulative Epi permeated, influx (J), and permeability (P) were increased ($p < 0.05$) as the pH of the diffusion medium increased. The highest values were achieved at pH 8, which resulted in an 11-fold permeability enhancement compared to the negative control (Fig. 5). On the other hand, the lowest values were obtained at pH 5 and 6 (Table 1).

The difference in the AUC_{0-90} for the cumulative Epi permeated at pH 8 became significant from all as early as 5 min. At pH 6.8 and 7.4,

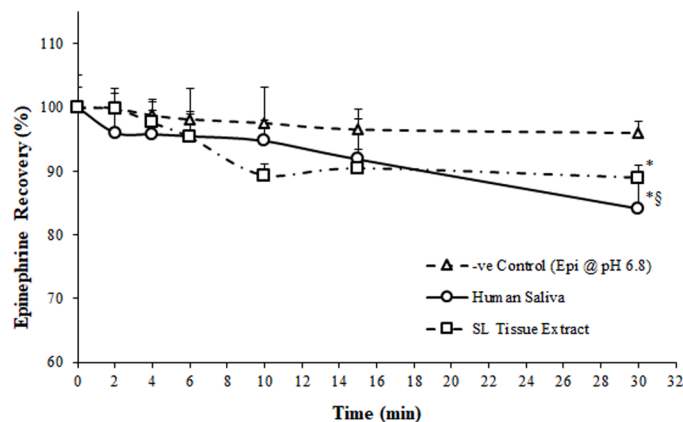


Fig. 3. Mean ($\pm$ SD) epinephrine recovery (%) over 30 min in human saliva and sublingual (SL) tissue extract ($n = 3$). *Significantly lower than its initial concentration. §Significantly lower than negative control (Epi at pH 6.8).

however, it took 45 min to demonstrate a significant difference ($p < 0.05$) from pH 5 and 6, which were not significantly different ($p > 0.05$) from each other throughout the entire time.

3.4. Selection of excipient for altering the pH of the sublingual microenvironment

The mean ($\pm$ SD) pH readings for the different pH-modifying excipients at various concentrations with or without Epi are listed in Table 2. Na Carb with or without Epi resulted in the highest pH of 8.6 ± 0.2 and 11.7 ± 0.0 , respectively, at the lowest concentration used (0.75%) compared to other pH-modifying excipients. This difference in pH was significantly higher ($p < 0.05$) than all other buffers used at the same concentration.

3.5. Effect of penetration enhancers on the sublingual permeability of Epi

The mean ($\pm$ SD) cumulative amount of Epi permeated over time with various penetration enhancers, PCC and SDS, was illustrated in Fig. 6.

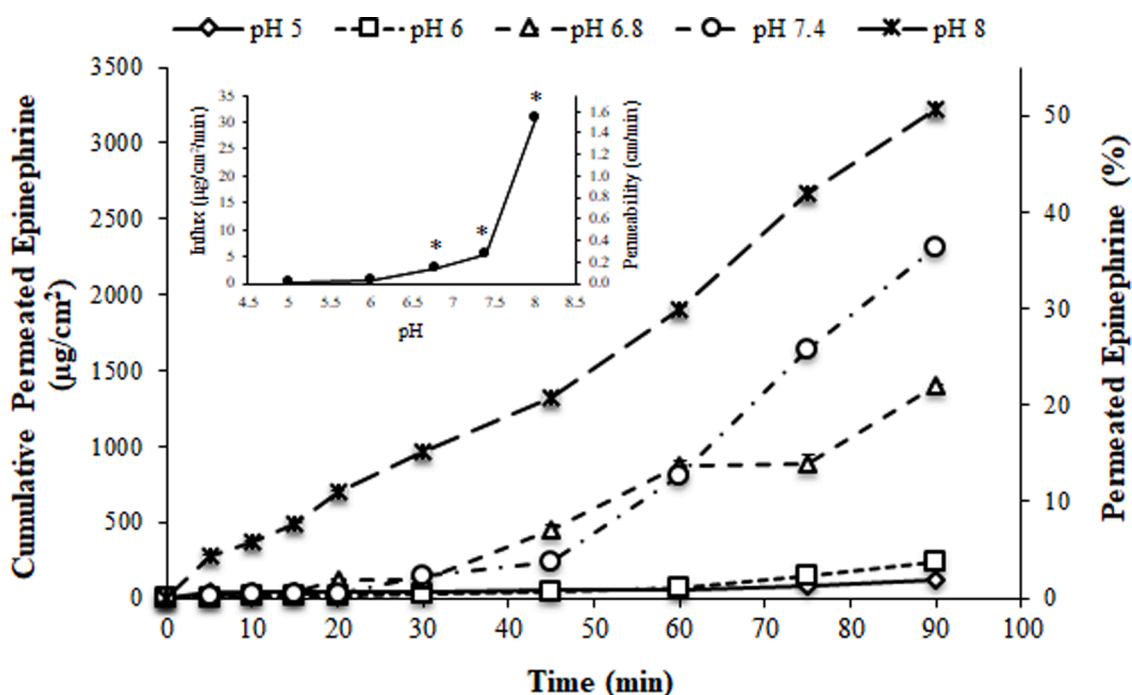


Fig. 4. Mean ($\pm$ SD) cumulative epinephrine permeation per area ($\mu\text{g}/\text{cm}^2$) versus time at a various pH media ($n = 4$). *Significantly different from all.

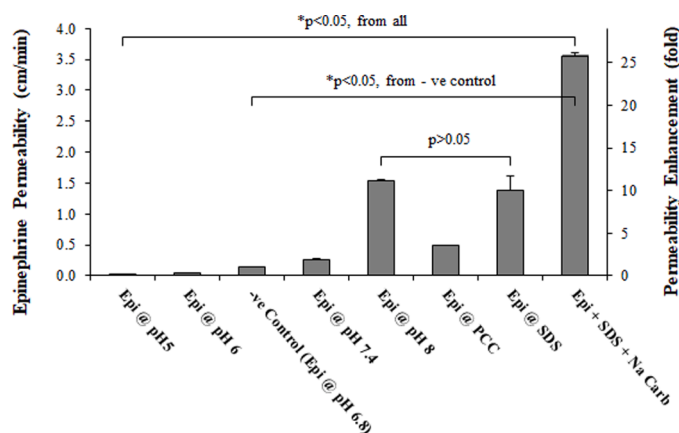


Fig. 5. Mean ($\pm$ SD) epinephrine permeability and permeability enhancement at different conditions and formulations ($n = 4$). Permeability enhancement was calculated by dividing epinephrine permeability at the different conditions by the negative control (Epi permeability at pH 6.8). *Significantly different.

Table 1
Epinephrine sublingual permeability (mean $\pm$ SD) at different pH media.

	pH 5	pH 6	pH 6.8	pH 7.4	pH 8
Area under the curve, $\text{AUC}_{0-90 \text{ min}}$ ($\mu\text{g}/\text{cm}^2 \cdot \text{min}$)	5169 $\pm$ 255 [§]	6191 $\pm$ 756 [§]	46,562 $\pm$ 3450*	60,099 $\pm$ 6576*	135,689 $\pm$ 439*
Influx, J ($\mu\text{g}/\text{cm}^2 \cdot \text{min}$)	0.4 $\pm$ 0.1 [§]	0.8 $\pm$ 0.2 [§]	2.8 $\pm$ 0.2*	5.3 $\pm$ 0.2*	30.9 $\pm$ 0.4*
Permeability coefficient, P (cm/min)	0.02 $\pm$ 0.0 [§]	0.04 $\pm$ 0.01 [§]	0.14 $\pm$ 0.01*	0.26 $\pm$ 0.01*	1.54 $\pm$ 0.02*

* $p < 0.05$ from all.

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

The AUC_{0-90} (mean $\pm$ SD) of the cumulative Epi permeated, J and P with the transcellular enhancer, SDS, were significantly higher ($p < 0.05$) than the negative control (Epi at pH 6.8) (Table 3). The difference

Table 2

pH measurements (mean $\pm$ SD) using various pH-modifying excipients at different concentrations ($n = 3$) with and without epinephrine (Epi).

Concentration	pH Reading					
	Na Bicarb		Na Carb		Na Citr	
	Without Epi	With Epi	Without Epi	With Epi	Without Epi	With Epi
0.15%	8.7 $\pm$ 0.1	4.5 $\pm$ 0.2	10 $\pm$ 0.0	4.7 $\pm$ 0.3	8.0 $\pm$ 0.3	3.7 $\pm$ 0.1
0.375%	8.8 $\pm$ 0.1	4.9 $\pm$ 0.2	11.7 $\pm$ 0.1	6.8 $\pm$ 0.1	8.6 $\pm$ 0.1	4.1 $\pm$ 0.1
0.75%	8.9 $\pm$ 0.0	6.9 $\pm$ 0.0	11.7 $\pm$ 0.0	8.6 $\pm$ 0.2 [§]	9.3 $\pm$ 0.1	4.8 $\pm$ 0.0
1.125%	8.9 $\pm$ 0.1	7.9 $\pm$ 0.1			9.4 $\pm$ 0.1	5.0 $\pm$ 0.1
1.5%					9.8 $\pm$ 0.0	6.6 $\pm$ 0.2
1.875%					9.8 $\pm$ 0.1	7.1 $\pm$ 0.1

Na Bicarb: sodium bicarbonate; Na Carb: sodium carbonate; Na Citr: sodium citrate.

* Percentages (w/v) were calculated for EpiBit dose equivalent to 20 mg Epi in 2 mL water.

[§] $p < 0.05$ from all at the same concentration.

in their AUC_{0-90} became significant after 15 min (Fig. 6). On the other hand, the AUC_{0-90} of Epi permeated with SDS was significantly lower ($p < 0.05$) than Epi permeated at pH 8 but only after 60 min. Additionally, their J and P were not significantly different ($p > 0.05$) from each other.

Similarly, the AUC_{0-90} (mean $\pm$ SD) of the cumulative Epi permeated, J and P with the paracellular enhancer, PCC, were significantly higher ($p < 0.05$) than the negative control (Table 3). Also, the difference in the AUC_{0-90} became significant after 20 min (Fig. 6). The AUC_{0-90} of Epi permeated with PCC was also significantly lower ($p < 0.05$) than Epi permeated at pH 8, however in contrast to SDS, this difference was immediately evident after the first 5 min. Their J and P were also significantly lower as well ($p > 0.05$).

The transcellular enhancer, SDS 0.075%, resulted in a 10-fold increase in Epi sublingual permeability when compared to the negative

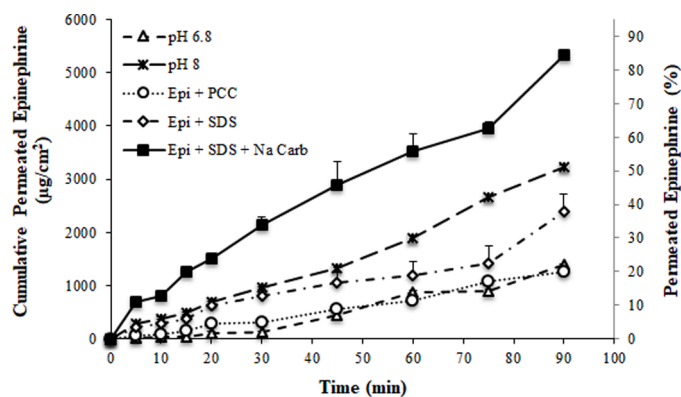


Fig. 6. Mean ($\pm$ SD) cumulative epinephrine (Epi) permeation per area ($\mu\text{g}/\text{cm}^2$) versus time at different conditions and formulations ($n = 4$).

Table 3

Effect of pH, penetration enhancers, or their combination using pH-modifier on epinephrine (Epi) sublingual permeability (mean $\pm$ SD).

	Epi @ pH 6.8 (-ve control)	Epi @ pH 8	PCC (1.2%)*	SDS (0.075%)*	SDS (0.075%) + Na Carb (0.75%)*
Area under the curve, $\text{AUC}_{0-90 \text{ min}}$ ($\mu\text{g}/\text{cm}^2 \cdot \text{min}$)	46,562 $\pm 3450^{\ddagger}$	135,689 $\pm 439^{\ddagger}$	52,127 $\pm 670^{\ddagger}$	92,365 $\pm$ 16,594 [†]	247,123 $\pm$ 11,900 [†]
Influx, J ($\mu\text{g}/\text{cm}^2 \cdot \text{min}$)	2.8 $\pm$ 0.2 [‡]	30.9 $\pm$ 0.4 [‡]	8.7 $\pm$ 0.8 [‡]	29 $\pm$ 4.3 [‡]	71.6 $\pm$ 0.6 [‡]
Permeability coefficient, P (cm/min)	0.14 $\pm$ 0.01 [‡]	1.54 $\pm$ 0.02 [‡]	0.4 $\pm$ 0.0 [‡]	1.4 $\pm$ 0.2 [‡]	3.6 $\pm$ 0.0 [‡]

* Percentages (w/v) were calculated for EpiBit dose equivalent to 20 mg Epi in 2 mL water.

[†] $p < 0.05$ from all.

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

control, which was very similar to its permeability enhancement at pH 8 (Fig. 5). On the other hand, the paracellular enhancer, PCC 1.2%, resulted in only a 3.5-fold increase in Epi sublingual permeability.

3.6. Combined effect of pH-modifier and penetration enhancer on the sublingual permeability of Epi

The mean ($\pm$ SD) cumulative amount of Epi permeated over time for the formulation combining the transcellular enhance (SDS 0.075%) with the pH-modifier (Na Carb 0.75%) was plotted in Fig. 6 and compared to their individual effects and the negative control. The AUC_{0-90} (mean $\pm$ SD) of the cumulative Epi permeated, J and P from the SDS with Na Carb combined were significantly higher ($p < 0.05$) than using the SDS or a pH 8 medium alone (Table 3). The significant difference in the AUC_{0-90} was immediately evident within the first 5 and 10 min when compared to either SDS or pH 8, respectively (Fig. 6).

Furthermore, the combined effect of SDS with Na Carb resulted in a 25.8-fold enhancement in Epi sublingual permeability when compared to the negative control (Fig. 5).

4. Discussion

The development of an alternative and more user-friendly dosage form to current Epi auto-injectors would better facilitate and promote its early self-administration as a life-saving dosage form for the treatment of anaphylaxis (Chooniedass et al., 2017; Fleming et al., 2015). Patients can be then transported to a healthcare facility for further assessment and treatment. Previously, we developed and extensively evaluated, *in*

vitro and *in vivo*, the potential of our novel fast-disintegrating sublingual Epi tablets for the first-aid treatment of anaphylaxis as an alternative dosage form to Epi auto-injectors (Rachid et al., 2012, 2018a, 2013; Rawas-Qalaji et al., 2015a, 2013, 2006a, 2006b, 2007, 2006c, 2015b).

These tablets were designed to overcome the challenges associated with the utilization of currently available Epi auto-injectors including the dilemma of the appropriate needle length for obese versus thin individuals, administration errors and injuries during their self-administration, and the patient's fear of self-injection (Brown et al., 2016; Dreborg et al., 2017; Kim et al., 2014; Noimark et al., 2012; Simons et al., 2009). Some of the previously reported advantages of these tablets include their portability due to their smaller size and discreet handling and administration in public. They are more affordable due to lower manufacturing costs and longer shelf-life that does not require annual replacement as with Epi auto-injectors. Additionally, they are more convenient to administer and non-invasive even when a second dose is needed, and they do not require assistance or prior training to administer (Rachid et al., 2018b; Rawas-Qalaji et al., 2013, 2007, 2006c).

With use of these tablets, there may be the need for a relatively high Epi dose to achieve pharmacokinetic parameters similar to Epi auto-injectors. Our early *in vivo* pharmacokinetics studies showed that we need to administer a 40 mg dose sublingually to achieve pharmacokinetic parameters similar to Epi auto-injectors and overcome permeability and local vasoconstriction challenges induced by Epi (Rachid et al., 2013; Rawas-Qalaji et al., 2006a, 2006b). Through further formulation optimization and Epi micronization, we were able to reduce the required sublingual Epi dose to 20 mg (Rachid et al., 2018b; Rawas-Qalaji et al., 2015a, 2015b).

In this study, we evaluated the stability of Epi in media that simulated the sublingual environment, the effect of modifying the sublingual medium pH on Epi stability and permeability, and the use of various penetration enhancers to enhance Epi permeability to further reduce the required sublingual Epi dose and maximize the efficiency of its sublingual delivery.

The main limitations of the current study include the possibility for differences in the sublingual permeability between porcine and human membranes despite the reported anatomical similarities. Also, the optimal formulation obtained from these studies require further *in vivo* confirmation, as physiological factors of live animals such as saliva volume and blood flow may alter drug permeability.

4.1. Epi stability for sublingual administration

Epi is a weak base that is unstable in aqueous solutions and primarily undergoes oxidation degradation reactions (Connors et al., 1986). In a strong sodium hydroxide solution, Epi almost immediately degrades to adrenochrome, which is characterized by its yellowish to pinkish color, and then darkens to a dark reddish to brownish color due to the formation of melanin. However, Epi oxidation and oxygen uptake occurs at different rates in diluted acidic, alkaline, and physiological pH (Yamahara and Lee, 1993). Elevated temperature, catalytic ions and enzymes can also speed up the degradation of Epi (Garhammer et al., 2004; Norris et al., 2018; Yamahara and Lee, 1993). Therefore, it was critical to evaluate Epi stability at various pH and sublingual media before modifying the pH of the sublingual microenvironment to increase Epi permeability.

4.1.1. pH-stability profile

The average saliva pH is 6.8 and any potential pH modification would involve alkalizing the microenvironment medium to reduce Epi ionization and therefore increase its sublingual permeability. However, as previously mentioned, alkalizing the medium may promote Epi degradation. Therefore, to evaluate the effect of modifying the pH of the sublingual microenvironment on enhancing Epi permeability, we had to evaluate first the effect of this pH on Epi stability during the time

required for its sublingual administration.

In this study, our results demonstrated that significant Epi loss in both acidic and diluted alkali media occurs only over an extended period of time beyond the 1–3 min window commonly required for sublingual administration. Epi exhibited good stability at the average salivary pH and up to pH 8 for at least 10 min (Fig. 2). Previous stability studies reported a lag time of 1 h at pH 8 before Epi undergoes a chemical degradation to the pinkish color of adrenochrome (Yamahara and Lee, 1993).

These results were useful for ruling out the potential for any significant Epi loss during its sublingual administration in the oral cavity at the normal saliva pH and, therefore, the need to include an antioxidant in the formulation to control its oxidation. Additionally, the alkalization of the sublingual microenvironment to enhance Epi permeability did not result in any significant loss of Epi. Thus, we can conclude that all the administered Epi would be available for absorption through the sublingual mucosa under these alkaline conditions.

4.1.2. Stability in saliva and sublingual tissue extracts

The results from the Epi stability studies in human saliva and porcine sublingual tissue extract also demonstrated the feasibility of Epi sublingual administration without any significant loss during its administration (Fig. 3). Therefore, all the administered Epi would be available to be absorbed through the sublingual mucosa into the systemic circulation. This is mainly because the composition of the saliva and sublingual tissue extract lacks the required oxidative enzymes and catalysts such as strong basic pH, high temperature, and metallic ions in concentrations that would accelerate Epi's degradation (Garhammer et al., 2004; Humphrey and Williamson, 2001; Norris et al., 2018; Yamahara and Lee, 1993).

4.2. pH-permeability profile of epinephrine Epi

Following the confirmation of the stability of Epi in the oral cavity and alkalized pH, the effect of altering the pH of the sublingual microenvironment on the sublingual permeability of Epi was explored.

Altering the medium pH can drastically affect drug permeability due to its effect on the degree of ionization and molecular charges (Lee et al., 2005). Epi is a weak base with a pKa of 8.59 (National Library of Medicine, 2004) and its sublingual absorption can be greatly enhanced by alkalizing the pH of its absorption microenvironment beyond 6.8 (the average saliva pH). Temporarily altering the pH of the sublingual microenvironment or the saliva to pH 8 is considered safe and within the acceptable range for drinking water according to the water quality standards published by the US Environmental Protection Agency (United States Environmental Protection Agency, 2021).

In this study, the significance of altering the medium pH on Epi permeability and lag time was demonstrated (Fig. 4) and guided the selection of the pH-modifying excipient which modified the medium pH where Epi sublingual permeability was the highest (Table 2). At pH 8, the overall Epi sublingual permeability, AUC_{0-90} , J , and P were significantly increased (Table 1), and Epi permeability enhancement was increased by 11-fold compared to its permeability at pH 6.8 (negative control) (Fig. 5). These findings were in agreement with the pH-partition theory and the suggested permeability route for Epi is through the paracellular pathway due to its low lipophilicity ($\log P -1.37$) (National Library of Medicine, 2004). The permeability of basic drugs with a high pKa were previously reported to be increased as the medium pH was increased (Lee et al., 2005; Streisand et al., 1995).

Furthermore, the increase in Epi AUC at pH 8 was significantly higher than the negative control within the first 5 min (Fig. 4), a time that is critical to cause a clinical difference for the emergency treatment of anaphylaxis. Although the Epi AUC at pH 7.4 was significantly higher than the negative control, the Epi permeability enhancement was increased by less than one-fold (Fig. 5).

4.3. Selection of pH-modifying excipient

The incorporation of a pH-modifying excipient into the Epi FDST formulation would modify the pH of the media following tablet disintegration to the optimal pH at which Epi is least ionized. Moreover, it would help control the pH of the sublingual absorption microenvironment and maintain it at the optimal pH for Epi absorption. This would also reduce the difference in sublingual absorption between patients and minimize the effect of food on the pH of the saliva that would otherwise alter drug absorption.

Na Carb, which was the most efficient pH-modifier in terms of the amount required to alter the pH of the medium to 8, was selected and evaluated for its effect on enhancing Epi sublingual permeability.

4.4. Effect of penetration enhancers on the sublingual permeability of Epi

Two types of penetration enhancers, paracellular and transcellular, were used to explore the sublingual transport pathway for Epi and evaluate their sublingual permeation enhancement effect on Epi permeability. Their effect was compared with a negative control (the permeability of Epi at pH 6.8). It was also compared with Epi permeated at pH 8 to evaluate the efficiency of each approach in enhancing Epi permeability (Fig. 6).

Transcellular penetration enhancers reduce the resistance to drug permeation by disrupting the lipid composition within the cell membrane and intercellularly altering the structure and integrity of the cellular membrane. In contrast, paracellular penetration enhancers loosen tight junctions between the cells within the intracellular space to reduce drug permeability resistance. Charged, larger, or less lipophilic drugs may have higher permeability potential through the paracellular route (Preusch, 2007).

The use of SDS 0.075% as a transcellular enhancer at pH 6.8 was as effective as alkalizing the diffusion medium to pH 8 in enhancing the sublingual permeability of Epi (Fig. 5). However, the permeability enhancement onset by SDS was slower than modifying the medium pH to 8. SDS took 15 min, while modifying the medium pH to 8 took 5 min to demonstrate a significant difference in Epi permeability when compared to the negative control (Fig. 4).

Initially, Epi was almost completely ionized at pH 6.8 and with less potential for permeation through both the transcellular route and paracellular route as well due to the presence of the cell membrane lipids and intercellular lipids, respectively. At high concentrations, SDS can promote the transcellular and paracellular permeability of hydrophilic drugs by extracting or solubilizing the lipids present in both the epithelial membrane and the intercellular spaces (Nicolazzo et al., 2005; Pinto et al., 2020). These lipids are considered the rate-limiting step for hydrophilic drug's permeability across the oral mucosa (Nicolazzo et al., 2005). Since the porcine sublingual membrane were not pretreated with the penetration enhancer to simulate their clinical application, the observed delay in enhancing Epi permeability was mainly due to the time needed for SDS to extract the intercellular membrane-coating granules present in the superficial layer of nonkeratinized sublingual epithelia. Despite the reduction in the sublingual permeability resistance of both routes, it is very unlikely for the ionized Epi at pH 6.8 to be transported transcellularly. Thus, Epi permeability was significantly enhanced mainly due to the reduction in the paracellular resistance to Epi permeation.

Similar to SDS, the delay in the onset of Epi permeability enhancement was also demonstrated with PCC, which took about the same time (20 min). However, unlike SDS, this enhancement was insufficient to be equivalent to the permeability enhancement achieved by modifying the medium pH to 8 (Fig. 5). Although, PCC was able to significantly increase Epi permeability by widening the tight junctions between the epithelial cells, it lacks the ability to extract the intercellular lipids that continue to pose major resistance to the paracellular permeability of the ionized Epi at pH 6.8. This ultimately resulted in less permeability

enhancement efficiency than SDS.

The significant permeability enhancement achieved by both penetration enhancers further confirms a paracellular sublingual transport pathway for Epi. Despite the 10-fold and 3.5-fold permeability enhancement by SDS and PCC, respectively, the significant delay in their onset limits their clinical significance for the treatment of anaphylaxis.

4.5. Combined effect of buffer and penetration enhancer on the sublingual permeability of Epi

For the emergency treatment of anaphylaxis, both the rate and extent of Epi sublingual absorption are critical and should resemble the currently available intramuscular Epi auto-injectors. To reduce the lag time caused by SDS and maximize the permeability enhancement for Epi, Epi permeability with SDS was investigated in combination with the pH-modifier to increase the pH to 8.

The combined effect of both Na Carb and SDS achieved a synergistic enhancement in the sublingual permeability of Epi (Fig. 5). Furthermore, the onset of this synergistic enhancement was significant within the first 5 min in comparison to the negative control. This increase in the sublingual permeability can translate to clinically significant outcomes for the treatment of emergency conditions, such as anaphylaxis, where rapid elevation of the plasma epinephrine concentrations is critical to saving patients' lives.

This synergistic effect was mainly due to the significant reduction in Epi ionization and molecular charges and was further augmented by the extraction of intercellular lipids facilitating the paracellular permeation of higher concentrations of the unionized hydrophilic Epi. Both the anatomical changes induced by SDS and the chemical changes in Epi's polarity caused by altering the medium pH contributed to increasing Epi permeability by almost 26-fold compared to their individual 10- and 11-fold enhancement effects, respectively.

5. Conclusion

In this study, we further demonstrated the feasibility of using the sublingual route of delivery for Epi. We were able further to demonstrate the stability of Epi in human saliva and in porcine sublingual membrane extract simulating its permeability environment. We measured Epi stability at various pH with the aim of modifying the absorption's micro-environment pH and reduce Epi ionization. Altering the pH of the sublingual absorption's microenvironment to pH 8 using Na Carb was shown to be a very effective approach to reducing Epi ionization and increasing the sublingual permeability.

The paracellular diffusion pathway was shown to be the main transport pathway for Epi. Both SDS and PCC significantly enhanced Epi permeability, however SDS was more efficient than PCC, but may have limited clinical significance due to their delayed onset. On the other hand, the combined effect of Na Carb with SDS has the potential for increasing Epi sublingual permeability and therefore reducing the required sublingual dose.

Future pharmacokinetic animal studies using Epi FDSTs containing Na Carb 0.7% and SDS 0.07% will be explored to further improve the pharmacokinetic parameters (AUC, Cmax and Tmax) of Epi FDSTs and reduce the required sublingual Epi dose. These novel Epi FDSTs will offer an affordable, easy-to-administer, non-invasive, and portable alternative dosage form for the out-of-hospital treatment of anaphylaxis.

CRediT authorship contribution statement

Mutasem Rawas-Qalaji: Conceptualization, Methodology, Formal analysis, Visualization, Supervision. **Rawan Bafail:** Investigation, Data curation, Writing – original draft, Visualization. **Roberta Cagliani:** Investigation, Data curation. **Mohamed Haider:** . **Zahid Hussain:** .

References

- Aodah, A., Rawas-Qalaji, M., Bafail, R., Rawas-Qalaji, M., 2019. Effect of fast-disintegrating tablets' characteristics on the sublingual permeability of atropine sulfate for the potential treatment of organophosphates toxicity. *AAPS PharmSciTech.* 20, 229.
- Bafail, R., Rawas-Qalaji, M., Rawas-Qalaji, M., Aodah, A., 2019. Effect of the filler grade on the characteristics and the sublingual permeability of atropine sulfate fast disintegrating sublingual tablets. *Drug Dev. Ind. Pharm.* 45, 1617–1623.
- Brown, C., 2015. Initial confusion after massive Allerject recall. *CMAJ* 187, E509–E510.
- Brown, J.C., Tuuri, R.E., Akhter, S., Guerra, L.D., Goodman, I.S., Myers, S.R., Nozicka, C., Manzi, S., Long, K., Turner, T., Conners, G.P., Thompson, R.W., Park, E., 2016. Lacerations and embedded needles caused by epinephrine autoinjector use in children. *Ann. Emerg. Med.* 67, 307–315 e308.
- Chen, L.L., Chetty, D.J., Chien, Y.W., 1999. A mechanistic analysis to characterize oramucosal permeation properties. *Int. J. Pharm.* 184, 63–72.
- Chooniedass, R., Temple, B., Becker, A., 2017. Epinephrine use for anaphylaxis: too seldom, too late: current practices and guidelines in health care. *Ann. Allergy Asthma Immunol.* 119, 108–110.
- Connors, K.A., Amidon, G.L., Stella, V.J., 1986. *Chemical Stability of Pharmaceuticals: A Handbook For Pharmacists*, 2nd Ed. Wiley-Interscience Publication, New York.
- Dreborg, S., Wen, X., Kim, L., Tsai, G., Nevis, I., Potts, R., Chiu, J., Dominic, A., Kim, H., 2017. Do epinephrine auto-injectors have an unsuitable needle length in children and adolescents at risk for anaphylaxis from food allergy? *Allergy Asthma Clin. Immunol.* 13, 33.
- Fleming, J.T., Clark, S., Camargo, C.A., Rudders, S.A., 2015. Early treatment of food-induced anaphylaxis with epinephrine is associated with a lower risk of hospitalization. *J. Allergy Clin. Immunol. Pract.* 3, 57–62.
- Garhammer, P., Hiller, K.A., Reitering, T., Schmalz, G., 2004. Metal content of saliva of patients with and without metal restorations. *Clin. Oral Investig.* 8, 238–242.
- Goswami, T., Kokate, A., Jasti, B.R., Li, X.L., 2013. In silico model of drug permeability across sublingual mucosa. *Arch. Oral. Biol.* 58, 545–551.
- Hassan, N., Ahad, A., Ali, M., Ali, J., 2010. Chemical permeation enhancers for transbuccal drug delivery. *Expert Opin. Drug Deliv.* 7, 97–112.
- Humphrey, S.P., Williamson, R.T., 2001. A review of saliva: normal composition, flow, and function. *J. Prosthet. Dent.* 85, 162–169.
- Kim, L., Nevis, I.F., Tsai, G., Dominic, A., Potts, R., Chiu, J., Kim, H.L., 2014. Children under 15kg with food allergy may be at risk of having epinephrine auto-injectors administered into bone. *Allergy Asthma Clin. Immunol.* 10, 40.
- Lee, K.J., Johnson, N., Castelo, J., Sinko, P.J., Grass, G., Holme, K., Lee, Y.H., 2005. Effect of experimental pH on the *in vitro* permeability in intact rabbit intestines and Caco-2 monolayer. *Eur. J. Pharm. Sci.* 25, 193–200.
- Lieberman, P., Nicklas, R.A., Oppenheimer, J., Kemp, S.F., Lang, D.M., Bernstein, D.I., Bernstein, J.A., Burks, A.W., Feldweg, A.M., Fink, J.N., Greenberger, P.A., Golden, D.B.K., James, J.M., Ledford, D.K., Sheffer, A.L., Blessing-Moore, J., Cox, L., Khan, D.A., Lang, D., Portnoy, J.M., Randolph, C., Schuller, D.E., Spector, S.L., Tilles, S., Wallace, D., 2010. The diagnosis and management of anaphylaxis practice parameter: 2010 update. *J. Allergy Clin. Immunol.* 126, 477–480 e442.
- McIlvaine, T., 1921. A buffer solution for colorimetric comparison. *J. Biol. Chem.* 49, 183–186.
- National Library of Medicine, 2004. PubChem Compound Summary for CID 5816, Epinephrine. National Library of Medicine (US), Bethesda, MD <https://pubchem.ncbi.nlm.nih.gov/compound/Epinephrine>. (accessed 4/30/2021).
- Nicolazzo, J.A., Reed, B.L., Finnis, B.C., 2005. Buccal penetration enhancers—how do they really work? *J. Control. Release* 105, 1–15.
- Noimark, L., Wales, J., Du Toit, G., Pastacaldi, C., Haddad, D., Gardner, J., Hyer, W., Vance, G., Townshend, C., Alfaham, M., Arkwright, P.D., Rao, R., Kapoor, S., Summerfield, A., Warner, J.O., Roberts, G., 2012. The use of adrenaline autoinjectors by children and teenagers. *Clin. Exp. Allergy* 42, 284–292.
- Norris, H.L., Friedman, J., Chen, Z., Puri, S., Wilding, G., Edgerton, M., 2018. Salivary metals, age, and gender correlate with cultivable oral *Candida* carriage levels. *J. Oral Microbiol.* 10, 1447216.
- Palmer, E.FiercePharma, 2017. FDA slams Pfizer unit responsible for EpiPens that failed during fatal emergencies. <https://www.fiercepharma.com/pharma/fda-slams-pfizer-unit-made-epipens-failed-during-fatal-emergencies> (accessed 4/1/2021).
- Palmer, E., 2018. FDA fights EpiPen shortage by extending expiration date for some devices. FiercePharma, <https://www.fiercepharma.com/pharma/fda-fights-epipen-shortage-by-extending-expiration-date-for-some-devices> (accessed 4/1/2021).
- Pinto, S., Pintado, M.E., Sarmento, B., 2020. *In vivo*, *ex vivo* and *in vitro* assessment of buccal permeation of drugs from delivery systems. *Expert Opin. Drug Deliv.* 17, 33–48.
- Preusch, P., 2007. Equilibrative and concentrative transport mechanisms. In: Atkinson, A., Abernethy, D., Daniels, C., Dedrick, R., Markey, S. (Eds.), *Principles of Clinical Pharmacology*, 2nd Ed. Academic Press, Burlington, pp. 197–227.
- Rachid, O., Rawas-Qalaji, M., Simons, F.E.R., Simons, K.J., 2011. Dissolution testing of sublingual tablets: a novel *in vitro* method. *AAPS PharmSciTech* 12, 544–552.
- Rachid, O., Rawas-Qalaji, M., Simons, F.E.R., Simons, K.J., 2012. Rapidly-disintegrating sublingual tablets of epinephrine: role of non-medicinal ingredients in formulation development. *Eur. J. Pharm. Biopharm.* 82, 598–604.
- Rachid, O., Rawas-Qalaji, M., Simons, K.J., 2018a. Epinephrine in anaphylaxis: preclinical study of pharmacokinetics after sublingual administration of taste-masked tablets for potential pediatric use. *Pharmaceutics* 10, 24.
- Rachid, O., Rawas-Qalaji, M.M., Simons, F.E., Simons, K.J., 2013. Epinephrine (adrenaline) absorption from new-generation, taste-masked sublingual tablets: a preclinical study. *J. Allergy Clin. Immunol.* 131, 236–238.

- Rachid, O., Simons, F.E., Rawas-Qalaji, M., Simons, K.J., 2010. An electronic tongue: evaluation of the masking efficacy of sweetening and/or flavoring agents on the bitter taste of epinephrine. *AAPS PharmSciTech.* 11, 550–557.
- Rachid, O., Simons, K.J., Rawas-Qalaji, M., 2018b. *In vivo* evaluation of taste-masked fast-disintegrating sublingual tablets of epinephrine microcrystals. *AAPS PharmSciTech.* 19, 3886–3894.
- Rawas-Qalaji, M., Rachid, O., Mendez, B.A., Losada, A., Simons, F.E., Simons, K.J., 2015a. Adrenaline (epinephrine) microcrystal sublingual tablet formulation: enhanced absorption in a preclinical model. *J. Pharm. Pharmacol.* 67, 20–25.
- Rawas-Qalaji, M.M., Rachid, O., Simons, F.E., Simons, K.J., 2013. Long-term stability of epinephrine sublingual tablets for the potential first-aid treatment of anaphylaxis. *Ann. Allergy Asthma Immunol.* 111, 568–570.
- Rawas-Qalaji, M.M., Simons, F.E., Simons, K.J., 2006a. Epinephrine for the treatment of anaphylaxis: do all 40mg sublingual epinephrine tablet formulations with similar *in vitro* characteristics have the same bioavailability? *Biopharm. Drug Dispos.* 27, 427–435.
- Rawas-Qalaji, M.M., Simons, F.E., Simons, K.J., 2006b. Sublingual epinephrine tablets versus intramuscular injection of epinephrine: dose equivalence for potential treatment of anaphylaxis. *J. Allergy Clin. Immunol.* 117, 398–403.
- Rawas-Qalaji, M.M., Simons, F.E., Simons, K.J., 2007. Fast-disintegrating sublingual epinephrine tablets: effect of tablet dimensions on tablet characteristics. *Drug Dev. Ind. Pharm.* 33, 523–530.
- Rawas-Qalaji, M.M., Simons, F.E.R., Simons, K.J., 2006c. Fast-disintegrating sublingual tablets: effect of epinephrine load on tablet characteristics. *AAPS PharmSciTech.* 7, E72–E78.
- Rawas-Qalaji, M.M., Werdy, S., Rachid, O., Simons, F.E., Simons, K.J., 2015b. Sublingual diffusion of epinephrine microcrystals from rapidly disintegrating tablets for the potential first-aid treatment of anaphylaxis: *in vitro* and *ex vivo* study. *AAPS PharmSciTech.* 16, 1203–1212.
- Sattar, M., Sayed, O.M., Lane, M.E., 2014. Oral transmucosal drug delivery—current status and future prospects. *Int. J. Pharm.* 471, 498–506.
- Scutti, S., 2018. ‘Short-term’ EpiPen shortage, anticipates FDA. Cable News Network. A Warner Media Company.
- Simons, F.E.R., Arduoso, L.R.F., Bilo, M.B., El-Gamal, Y.M., Ledford, D.K., Ring, J., Sanchez-Borges, M., Senna, G.E., Sheikh, A., Thong, B.Y., 2011. World Allergy Organization anaphylaxis guidelines: summary. *J. Allergy Clin. Immunol.* 127, 587–593 e522.
- Simons, F.E.R., Clark, S., Camargo, C.A., 2009. Anaphylaxis in the community: learning from the survivors. *J. Allergy Clin. Immunol.* 124, 301–306.
- Streisand, J.B., Zhang, J., Niu, S., McJames, S., Natte, R., Pace, N.L., 1995. Buccal absorption of fentanyl is pH-dependent in dogs. *Anesthesiology* 82, 759–764.
- United States Environmental Protection Agency, 2021. Causal Analysis/Diagnosis Decision Information System (CADDIS) - pH, Volume 2 ed. United States Environmental Protection Agency <https://www.epa.gov/caddis-vol2/caddis-volume-2-sources-stressors-responses-ph>. (accessed 4/1/2021).
- USP/NF, 2020. Official monograph: epinephrine injection, USP43-NF38 ed. United States pharmacopeial convention, Inc., Rockville, MD.
- Wang, Z., Chow, M.S., 2014. Overview and appraisal of the current concept and technologies for improvement of sublingual drug delivery. *Ther. Deliv.* 5, 807–816.
- Yamahara, H., Lee, V.H.L., 1993. Drug metabolism in the oral cavity. *Adv. Drug Deliv. Rev.* 12, 25–39.