EVALUATION OF THE EFFECTS OF NON-MEDICINAL INGREDIENTS ON THE IN VITRO CHARACTERISTICS AND IN VIVO BIOAVAILABILITY OF A SUBLINGUAL TABLET FORMULATION OF EPINEPHRINE

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DEDICATION

I would like to dedicate this work to my father Mahmoud Rachid and my mother Asmaa Al Atrash. Words cannot repay their endless and heroic support and encouragement. This work is also dedicated to my lovely home country, Syria.

ABSTRACT

Objectives: To review, develop, and validate appropriate methods for quality control testing of sublingual (SL) tablets; to formulate and characterize new generations of SL tablets of epinephrine (E) for the potential first-aid treatment of anaphylaxis; and to evaluate the effects of non-medicinal ingredients (NMIs) on the in vitro characteristics and in vivo bioavailability of the formulated tablets. ***Methods:*** A custom-made apparatus and a novel method that simulates SL conditions were evaluated for dissolution testing of SL tablets. An electronic tongue (e-Tongue) was used to assess the degree of E bitterness and to demonstrate the masking effects of sweetening and/or flavoring agents. The effect of several NMIs in various properties on the in vitro characteristics of new generations of E SL tablets was evaluated. Formulations with the best in vitro characteristics, containing E 30 mg and 40 mg, were evaluated in vivo using our validated rabbit model and compared with placebo SL tablets (negative control) and E 0.3 mg intramuscular (IM) injection (positive control). ***Results:*** The novel in vitro dissolution testing resulted in accurate and reproducible data and was capable of detecting the effect of minor changes in formulations. Using the e-Tongue, E bitartrate had an extremely bitter taste which was masked to various degrees by the addition of aspartame, acesulfame potassium, and citric acid alone or in combination. Citric acid alone masked the bitter taste by >80%. The evaluation of NMIs revealed that the best formulation contained specific proportions of mannitol and coarse and fine grades of microcrystalline cellulose. Appropriate comparative testing resulted in the selection of a

taste-masked E SL formulation with optimum in vitro characteristics. This formulation containing E 40 mg resulted in similar bioavailability to E 0.3 mg IM. This formulation containing E 30 mg had higher bioavailability than placebo, but lower bioavailability than E 40 mg tablets. ***Conclusions:*** Grades and proportions of NMIs carefully selected using appropriate in vitro testing resulted in successful formulations. The results of these in vitro tests enabled the development of the optimum E SL tablet formulation which was bioequivalent to the EpiPen. These tablets are potentially suitable for Phase 1 studies in humans and might transform the first-aid treatment of anaphylaxis in community settings.

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GLOSSARY

ANCOVA	analysis of covariance
ANOVA	analysis of variance
API	active pharmaceutical ingredient
ASK	acesulfame potassium
ASP	aspartame
AUC	area under the plasma epinephrine concentration versus time curve
AV	acceptance value
BF	breaking force
BPM	bitterness prediction module
CA	citric acid
$C_{basline}$	baseline plasma concentration (endogenous epinephrine)
C_{max}	maximum plasma epinephrine concentration
CF	compression force
CU	content uniformity
CV%	coefficient of variation (%)
DR%	percent of drug released
DT	disintegration time
E	epinephrine
E-auto	epinephrine autoinjector
EB	epinephrine bitartrate

EC	electrochemical detection
EP	European Pharmacopeia
e-Tongue	electronic tongue
F	friability
FDA	food and drug administration
GIT	gastrointestinal tract
H	hardness
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
IM	intramuscular
ISDN	isosorbide dinitrate
IVIVC	in vitro in vivo correlation
JP	Japanese Pharmacopeia
L-HPC	low-substituted hydroxypropyl cellulose
MCC	microcrystalline cellulose
MSG	monosodium glutamate
NaCl	sodium chloride
NCE	new chemical entity
NMI	non-medicinal ingredient
PC	principle component
PCA	principle component analysis
PLS	partial least squares

R^2	correlation of coefficient
RDST	rapidly-disintegrating sublingual tablet
rpm	rotations per minute
SAP	sensory analysis panel
SD	standard deviation
SEM	standard error of the mean
SL	sublingual
T	thickness
T_{max}	time of maximum plasma epinephrine concentration
USP	United States Pharmacopeia
UV	ultraviolet detection
WAO	World Allergy Organization
WHO	World Health Organization
WT	wetting time
WV	weight variation

CHAPTER I: INTRODUCTION

1.1. Anaphylaxis

Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death (Sampson et al., 2006). It is under-recognized by patients and under-diagnosed by health care professionals (F. E. R. Simons & Sampson, 2008). The prevalence of anaphylaxis reported in the literature is 0.05% to 2%. The rate of occurrence is increasing, especially in young people (Lin, Anderson, Shah, & Nurruzzaman, 2008; F. E. R. Simons, 2010).

Common triggers of anaphylaxis include foods, medications, and insect stings. The most common food triggers are peanut, tree nuts, shellfish, fish, milk, and egg (Jarvinen, 2011; F. E. R. Simons, 2006; F. E. R. Simons, 2009). Medication triggers include β-lactam and other antibiotics; aspirin, ibuprofen, and other analgesics; biologic agents such as cetuximab, infliximab, and omalizumab; and allergens used in immunotherapy (F. E. R. Simons, 2010; F. E. R. Simons, Edwards, Read, Clark, & Liebelt, 2010; Warrington & Silviu-Dan, 2011). Anaphylaxis can also be triggered by stinging insects venom or by saliva from biting insects (Freeman, 2004; Peng et al., 2004). Other triggers include natural rubber latex, animal dander, grass pollen, exercise, cold air or water and heat (F. E. R. Simons, 2009).

Allergens are the most common triggers of anaphylaxis. Upon exposure to an allergen or other triggers, two key cells, mast cells and basophils, play major role in

initiating and amplifying anaphylaxis. This leads to the release of mediators of inflammation, including histamine and tryptase, which account for the signs and symptoms of anaphylaxis (Ewan, 1998; P. Lieberman, 2003b; Ring, Brockow, & Behrendt, 2004). These usually begin within minutes to a few hours. In severe or fatal episodes, cardiorespiratory arrest occurs at median times of 5 to 30 minutes after allergen exposure (Pumphrey, 2000). The organs involved in anaphylaxis include the skin, respiratory tract, gastrointestinal tract, cardiovascular system and central nervous system. The most common symptoms experienced by patients with anaphylaxis involve the skin and include flushing, urticaria, pruritus, and angioedema (Kemp & Lockey, 2002; P. Lieberman, 2003b; Sampson, 2003). Respiratory manifestations are also common and include wheezing, chest tightness, cough, rhinitis, sneezing, congestion, and rhinorrhea (P. Lieberman, 2003b; Lockey, 2006; Sampson, 2003). Other manifestations may include gastrointestinal symptoms (e.g., abdominal pain, nausea, vomiting, and diarrhea) and cardiovascular symptoms (e.g., hypotension, distributive shock, arrhythmias, syncope, and chest pain) (P. Lieberman, 2003b; Lockey, 2006; Sampson, 2003).

The initial pharmacologic treatment of anaphylaxis focuses on the prompt administration of epinephrine (adrenaline). Delay in giving epinephrine (E) may lead to biphasic or protracted anaphylaxis and fatality (Kemp, Lockey, Simons, & World Allergy Organization ad hoc Committee on Epinephrine in Anaphylaxis, 2008). For first-aid treatment of anaphylaxis in community settings, E should be administered by intramuscular (IM) injection in the mid-outer thigh. The recommended initial dose is

0.01 mg/kg to a maximum of 0.5 mg in adults and 0.3 mg in children (P. Lieberman et al., 2010).

1.2. Epinephrine

Epinephrine is an agonist for all adrenergic receptors including α_1- and β_2-adrenergic receptors found in organs of the cardiovascular and respiratory systems (Westfall & Westfall, 2011). It is life-saving due to its potent vasoconstrictor and bronchodilator effects and is considered the first-line medication of choice in the treatment of anaphylaxis according to the World Allergy Organization (WAO) anaphylaxis guidelines (Kemp et al., 2008; Liberman & Teach, 2008; F. E. R. Simons, 2009; F. E. R. Simons et al., 2011). Epinephrine has a narrow therapeutic index and despite its potential adverse effects including anxiety, fear, restlessness, headache, dizziness, palpitations, pallor, and tremor, there is no absolute contradiction to E use in anaphylaxis (Joint Task Force on Practice Parameters, American Academy of Allergy,Asthma and Immunology, American College of Allergy,Asthma and Immunology, & Joint Council of Allergy,Asthma and Immunology, 2005; F. E. R. Simons, 2004; Waserman et al., 2010). As noted previously, the recommended initial E dose in anaphylaxis is 0.01 mg/kg to a maximum of 0.3 mg in children and 0.5 mg in adults. These doses are based on clinical consensus rather than prospective, randomized, double-blind, placebo-controlled clinical trials of E in individuals actually experiencing anaphylaxis. Such trials are difficult to standardize because of the nature of the disease and, of greater importance, they would be unethical to perform because E administration is life-saving in anaphylaxis (Kemp et al., 2008).

Most anaphylaxis episodes occur unexpectedly in non-medical settings. For the first-aid, prehospital treatment in community settings, E is available in autoinjectors including EpiPen Jr®, EpiPen® (Dey LP, Nappa, CA, USA), Twinject® 0.15 mg, Twinject® 0.3 mg (Sciele Pharma, Inc., a Shionogi Company, Atlanta, GA, USA), Anapen® 0.15 mg, Anapen® 0.3 mg, Anapen® 0.5 mg (Lincoln Medical, Salisbury, UK), Jext® 0.15 mg, Jext® 0.3 mg (ALK-Abelló Ltd., Berkshire, UK), Auvi-Q™ 0.15 mg, Auvi-Q™ 0.3 mg, Allerject™ 0.15 mg, and Allerject™ 0.3 mg (Sanofi-aventis, Bridgewater, NJ, USA).

Epinephrine autoinjectors (E-autos) are underutilized for many reasons. Fear and anxiety about needles and injections are major concerns in many patients, especially children, experiencing anaphylaxis (Nir, Paz, Sabo, & Potasman, 2003; Rosen, 2006). Many patients, care givers and health professionals are uncertain about the correct technique of E administration by using autoinjectors (Frew, 2011). Many at-risk patients fail to carry their E-autos with them due to bulky shape and large size (Frew, 2011), compounded by the recommendation to carry two doses (two E-autos) at all times (Kemp et al., 2008). Lack of response to E-autos was reported to be due to incorrect route of administration/injection site (F. E. R. Simons, Gu, & Simons, 2001), poor absorption (Korenblat, Lundie, Dankner, & Day, 1999), and suboptimal dose with the currently available two fixed E doses, 0.15 mg and 0.3 mg, which are not suitable for infants and very young children, or for large teenagers and adults. Other drawbacks include poor stability of the E solution in the autoinjector, with consequent short shelf life and the need to replace autoinjectors annually even when they have not been used, and unintentional E injections from autoinjectors, with or without associated injuries (F.

E. R. Simons et al., 2010; K. J. Simons & Simons, 2010). Autoinjectors must not be stored under refrigeration and must not be frozen. Epinephrine in solution potentially degrades rapidly if exposed to heat or light. It should ideally be handled and stored at ambient temperature of 25° C, which is often impractical during daily life activities.

Alternatives to E-autos, such as E ampules supplied with syringes and needles, E in unsealed prefilled syringes, or E from metered-dose inhalers are impractical with regard to rapid, accurate dosing and stability of the E formulation (M. Rawas-Qalaji, Simons, Collins, & Simons, 2009; F. E. R. Simons, Gu, Johnston, & Simons, 2000; F. E. R. Simons, Chan, Gu, & Simons, 2001).

There is considerable interest in the development of novel, non-invasive E dosage forms that will achieve plasma E concentrations similar to those obtained after use of an E-auto. Ideally, in addition to being small, unobtrusive, easy to carry, and needle-free, these formulations should be easy to use, be available in a wide range of doses, and have a long shelf-life.

Oral administration of E is not feasible because of rapid metabolism by catechol-O-methyltransferase in the gastrointestinal tract (GIT) and by monoamine oxidase in the GIT and liver, with excretion of E mainly as 3-methoxy-4-hydroxyphenylethyleneglycol and 3-methoxy-4-hydroxymandelic acid (Lefkowitz, Hoffman, & Taylor, 1996).

The oral mucosa is an attractive alternative route for E delivery during anaphylaxis, a medical emergency. This accessible and convenient route has long been used for self treatment in other medical emergencies, such as initial nitroglycerine treatment for angina. Oromucosal preparations may be intended for either buccal or

sublingual (SL) administration. Drug delivery through the buccal area is mostly utilized for modified-release formulations. For example, mucoadhesive systems are designed to adhere to the gum or inner cheek to provide a controlled- and sustained-release of medication through the buccal mucosa. Epinephrine needs to be formulated in an immediate-release formulation, as prompt dosing and prompt absorption are life-saving in anaphylaxis.

Sublingual delivery of E has potential usefulness in the first-aid treatment of anaphylaxis. The thin oral mucosal layer and abundant blood supply in the SL area facilitate rapid E absorption and systemic distribution. Epinephrine tablets intended for SL administration can potentially be formulated in a wide range of doses, and can be removed from the SL cavity, if necessary, to discontinue further drug absorption. They should be easy to administer, palatable, and will have a long shelf-life, as E is more stable in the dry state in solid dosage forms than in solution. The production of E tablets should be in a heat- and moisture-free environment, as E degrades when exposed to such conditions. For this reason, manufacturing processes such as wet granulation, freeze-drying (lyophilization), molding, and cotton candy are not appropriate. Similarly, fast-melt tablet dosage forms are not a viable option for heat- or moisture-sensitive medications such as E. In addition, fast-melt tablet ingredients are mostly intended to be swallowed after melting, which is not an option for E because it is inactivated in the GIT and in the liver. Accordingly and despite the manufacturing challenges, direct compression is the first choice in the formulation of SL tablets of E.

1.3. Sublingual Delivery

Drug delivery via the oral mucosa is a preferred route of systemic administration due to ease of dosing, non-invasiveness, and patient compliance. The mucosa of the oral cavity has rapid cellular recovery after local stress or damage with turnover time period of 3-8 days compared to 30 days in the skin (Washington, Washington, & Wilson, 2001). Dosage forms intended for SL administration are less expensive to manufacture than injectable dosage forms which must obviously be sterile to ensure patients' safety. As noted previously, drugs absorbed through the SL mucosa bypass the first-pass effect and avoid the pH changes, digestive enzymes and presystemic elimination within the GIT.

1.3.1. Structure of Sublingual Cavity

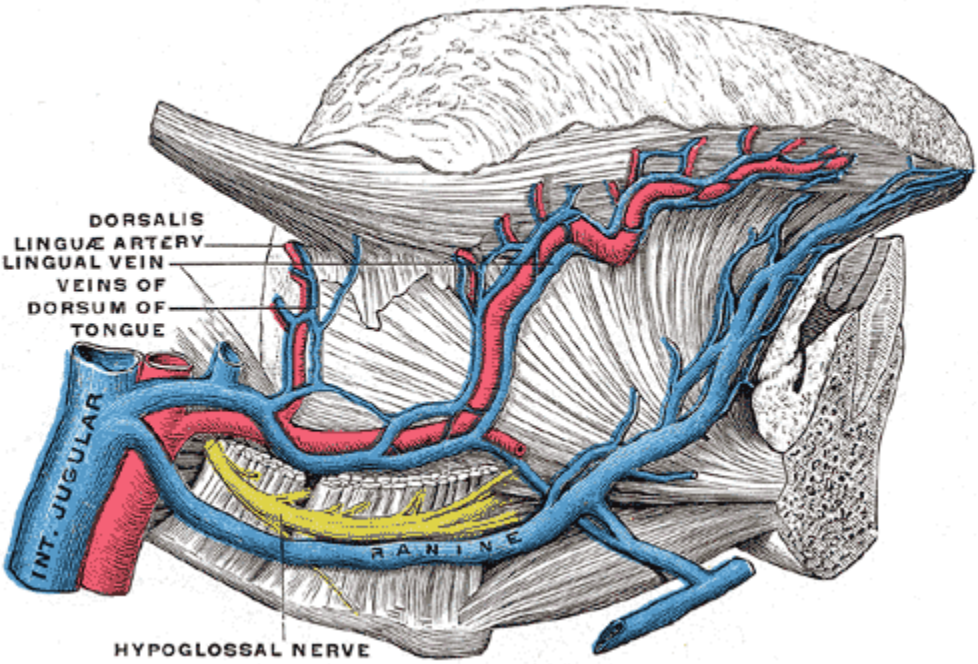
The oral cavity consists of the lips, cheeks, tongue, hard palate, soft palate and floor of the mouth. The oral mucosa is the lining of the oral cavity that includes the labial, buccal, SL, gingival and palatal mucosa. The surface area of the oral mucosa is about 50-200 cm^2, 16-20% of which accounts for the floor of the mouth and the ventral surface of the tongue (Collins & Dawes, 1987; Moore & Catlin, 1967). It is relatively small when compared with the GIT and skin, but is highly vascularised and permeable (Lesch, Squier, Cruchley, Williams, & Speight, 1989) and drugs absorbed enter directly into the systemic circulation. The permeability of the oral mucosa increases in the order of palate, buccal, lateral border of tongue, and floor of the mouth. The latter is more permeable than any other region in the oral mucosa having a permeability constant of 22 times that of skin towards water (Lesch et al., 1989; C. A. Squier & Hall, 1985). The differences in permeability depend partly on the degree of keratinization among the

different regions of the oral mucosa which can be divided broadly into two types: keratinized masticatory (30%), which includes the hard palate, dorsum of tongue and gingivae, and non-keratinized (50%), which includes the remaining regions including the SL mucosa (Collins & Dawes, 1987; C. A. Squier, Johnson, & Hackemann, 1975). The SL, or under the tongue, cavity is simply referred to as the area that is visible when the tip of the tongue is turned upwards, i.e., the cavity and floor of the mouth. Both permeability and surface area are important factors of determining the absorptive capabilities of different regions of the oral mucosa. The specific permeability of each region in the oral mucosa will also depend on the thickness of mucosa in that specific region. It has been determined that the thickness of the mucosa in the buccal region (500-800 μm) is about 4-5 times that of the floor of the mouth and the ventral surface of the tongue (100-200 μm) (Harris & Robinson, 1992).

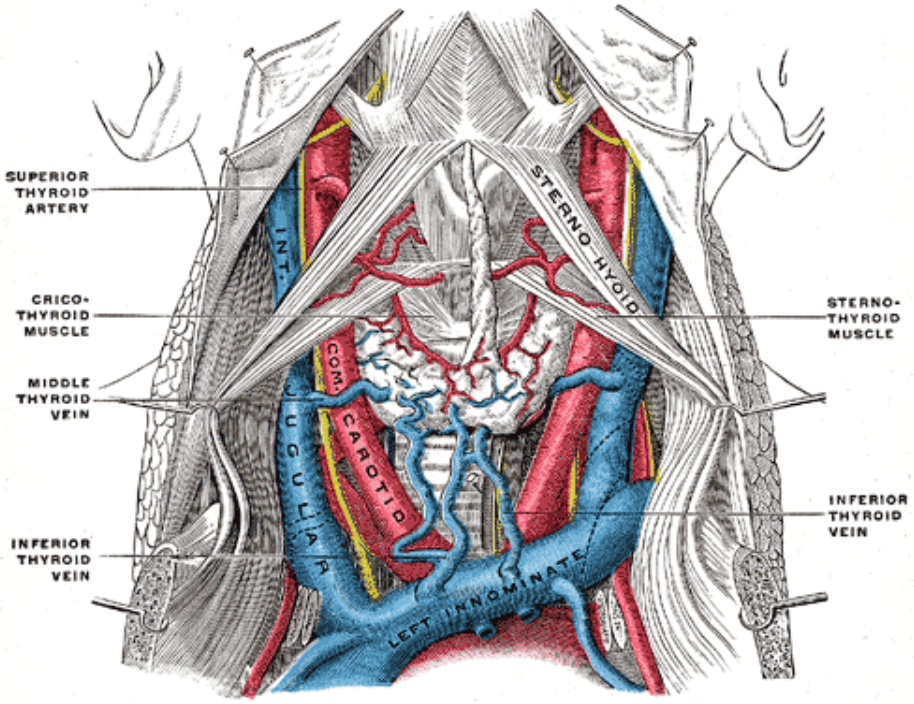
The venous drainage of the SL area occurs via the sublingual veins (ranine) and lingual veins (Figure 1a). They empty into the internal jugular veins (Figure 1b). The SL mucosa is highly vascular facilitating rapid absorption of drug directly into the systemic circulation (Gray, 1918; Netter, 2006). This has the potential of bypassing the presystemic metabolic conversion within the GIT and hepatic first-pass metabolism. Therefore, drugs reach the systemic circulation in a pharmacologically active form, resulting in a faster pharmacological response than orally administered drugs (Bredenberg et al., 2003; Cunningham, Baughman, Peters, & Laurito, 1994; Kroboth, McAuley, Kroboth, Bertz, & Smith, 1995; Motwani & Lipworth, 1991; Price et al., 1997).

***Figure 1**: **a)** The sublingual vessels, and **b)** the internal jugular vein* (Gray, 1918).

a



b



The main constituent of the oral mucosa is the epithelium which is composed of squamous stratified epithelial cells, compacted at the surface of the mucosa. The submucosal blood vessels and nerves are located under the epithelial basement membrane and lamina propia (Harris & Robinson, 1992; McElnay, 1990).

About 5000 taste buds spread over the human tongue in clusters of 50-100 sensory cells. Qualities of taste are defined as sweet, sour, salty, and bitter. Umami, the taste sensation caused by monosodium L-glutamate (MSG) found mainly in protein-rich food, is now classified as a fifth quality of taste. The sensory cells specific to these qualities are distributed over the whole tongue, but differ in densities (Iwatsuki & Uneyama, 2012). Sweetness is mainly tasted with the tongue tip, saltiness with the tip and margins, sourness with the margins, and bitterness with the posterior part of the tongue.

Taste is one major determinant of residence time of a dosage form in the oral mucosa, especially in children (Ayenew, Puri, Kumar, & Bansal, 2009; Baguley, Lim, Bevan, Pallet, & Faust, 2012; Buchanan et al., 2012; Tucker et al., 2002). The unpleasant or bitter taste, common in most drugs, should be masked in order to achieve high levels of patient adherence to any medication regimen administered orally.

The SL, lingual, submandibular, and parotid glands are responsible for the secretion of saliva in the oral cavity. The submandibular and carotid glands secrete about 95% of the daily secretions ranging from resting to stimulated volumes of 0.5 to 1.5 L of saliva with a rate of 0.1 to 4 mL per minute (Sreebny & Schwartz, 1997; Sreebny, 2000).

1.3.2. Mechanisms of Sublingual Absorption

Compared with the numerous studies published on the mechanisms of drug absorption through the gastrointestinal mucosa, very few studies are available on the transport mechanisms that may exist in the oral mucosa. The cellular absorption of molecules through the epithelial mucosa is governed by two main mechanisms: the transcellular pathway, i.e. through cells, and the paracellular pathway, i.e. between cells. Both pathways behave as a gatekeeper that governs the passage of various molecules into the systemic circulation (Washington et al., 2001). The oral epithelium is stratified, so both transcellular and paracellular routes are likely involved in the transport of molecules across mucosa (Shojaei, 1998). The intercellular spaces and cytoplasm are hydrophilic, whereas the cell membranes are lipophilic. The affinity of molecules towards a hydrophilic or lipophilic environment depends on their partition coefficients and affects the mechanism of transport across the mucosa. A small lipophilic molecule which has the physico-chemical characteristics of many drugs such as propranolol and ketoprofen, crosses the cellular membrane by passive diffusion in a concentration gradient manner following the Fick's first law of diffusion (Artursson, 1990; Corti, Maestrelli, Cirri, Zerrouk, & Mura, 2006). Very small molecules such as water and ethanol, and gases such as oxygen and carbon dioxide move across cellular membrane also by passive diffusion. The paracellular pathway differs from all other absorptive pathways as it facilitates the transport of molecules through the aqueous pores between cells, which comprise only 0.01% of the total surface area of the

epithelia. This type of transport is important for ions such as calcium and for sugars, amino acids, and peptides at concentrations above the capacity of their carriers.

1.3.3. Factors Affecting Sublingual Absorption

According to Fick's law of diffusion (Equation 1), drug molecules diffuse from a region of high drug concentration to a region of low drug concentration (Shargel, Wu-Pong, & Yu, 2012).

Equation 1:

$$\frac{dQ}{dt} = \frac{DAK}{h}\left(C_{GI} - C_p\right)$$

where dQ/dt = rate of diffusion (mg/sec), D = diffusion coefficient (cm^2/sec), A = surface area of membrane (cm^2), K = lipid-water partition coefficient of drug in the biological membrane that controls drug permeation (a ratio), h = membrane thickness (cm), and $C_{GI} - C_p$ = difference between the concentrations of drug in the GIT and in the plasma (mg/cm^3 or mg/mL).

The factors affecting drug absorption (Equation 2 and 3) through the SL mucosa can be derived from Fick's law of diffusion (Equation 1), which can be used to describe the drug absorption via the oral mucosa in general (Zhang, Zhang, & Streisand, 2002).

Equation 2:

$$P = \frac{DK}{h}$$

Equation 3:

$$Q = PCAt = \frac{DKCAt}{h}$$

where *P* = the permeability coefficient (cm^3/sec or mL/sec), *D* = the diffusion coefficient (cm^2/sec), *K* = lipid-water partition coefficient of drug in oral mucosa that controls drug permeation (a ratio), *h* = the thickness (cm) of the region of oral mucosa where drug absorption occurs, *Q* = the amount of drug absorbed (mg), *C* = the free drug concentration (mg/cm^3 or mg/mL), *A* = the surface area (cm^2) of the region of oral mucosa where drug absorption occurs, *t* = the duration of drug residence time (sec) in the oral mucosa.

Parameters such as diffusion coefficient, partition coefficient, and oral mucosa membrane thickness are beyond control. The surface area of the mucosal membrane depends on its location in the oral mucosa and is smaller compared with the surface area of the GIT mucosa. For a drug intended for SL administration, the surface area would be even smaller and the recommended duration of residence time is short (e.g., 2 minutes for SL nitroglycerin tablets). The major determinant, then, of drug permeation would be its concentration in the absorption site. In small surface area and short residence time, the drug should be administered in a high dose to create a large concentration gradient and boost absorption through the SL mucosa directly into systemic circulation.

Lipophilic compounds have higher permeability coefficients than hydrophilic compounds. However, the aqueous solubilities of lipophilic compounds are usually lower than those of hydrophilic compounds. Therefore, a fine balance between partition coefficient and solubility is required for a drug to be administered through the SL mucosa. Due to these challenges, a drug intended for SL administration should be potent because only few milligrams of a drug can be absorbed through the SL mucosa (Zhang et al., 2002).

1.4. Tablets

Solid dosage forms account for about two-thirds of all prescriptions, half of which are tablets (Hiestand, 2003; Qiu, Chen, Liu, & Zhang, 2009). Tablets remain the most popular dosage form because of their advantages towards both patients (e.g., dose accuracy, portability, and ease of administration) and manufacturers (e.g., high throughput, economy, stability, convenience in packaging, shipping, and dispensing) (Rudnic & Schwartz, 1995). Tablet types include uncoated, sugar-coated, film-coated, enteric-coated, controlled-release (also named modified-, prolonged- or sustained-release), effervescent, buccal and SL tablets (Rudnic & Schwartz, 1995).

An orally disintegrating tablet (ODT) is one type of uncoated tablets and it is defined as a solid dosage form that disintegrates quickly in the oral cavity without chewing or the need for water administration. ODTs are also known as fast melts, quick melts, fast or rapidly disintegrating tablets and orodispersible systems.

1.4.1. Preparation of Tablets

Tablets are prepared by compacting a powder or granulated mixture into solid dosage forms. They can be formulated into different shapes, sizes, and weights that can be administered through different routes including the oral, buccal, SL, rectal, and vaginal routes. Substance materials that make up tablets can be divided into two types: the active pharmaceutical ingredients (APIs) and the non-medicinal ingredients (NMIs). Ingredients are carefully selected, blended, and compacted to produce solid dosage forms with specified characteristics. Tablets may contain one or be comprised of more APIs for some medicinal indications and a number of NMIs each with a specific function.

Tablets can be prepared by three main methods: wet-granulation, dry-granulation, and direct compression (Gennaro, 2000). 1) Wet-granulation method involves a number of separate steps: weighing, mixing, granulation, mass screening, drying, dry screening, lubrication, and compression. Granulation of powder mixtures is often necessary to overcome the poor compressibility and flow properties of certain APIs and NMIs. However, it should be avoided when moist- and heat-sensitive APIs are used. Although widely used, the major disadvantage of wet-granulation is cost due to labor, time, equipment, energy, and space requirements. 2) Dry granulation involves the aggregation of ingredients under high pressure. This can be approached by two processes: slugging, producing large tablets by heavy-duty tablet press; and roller compaction, producing a sheet material by squeezing ingredients between two rollers. The intermediates produced are then milled into granules. This method is mainly used

for APIs that are sensitive to moisture or heat, or if direct compression is not an option. 3) Direct compression is the process by which tablets are compressed directly from powder mixtures of APIs and NMIs without modifying their physical nature. It involves no heat or moisture and becomes available with the development of specially designed NMIs that are free-flowing and highly compressible.

ODTs can be prepared by the above mentioned conventional preparation methods and also by other manufacturing processes such as spray-drying (a granulation method), freeze-drying (lyophilization), molding, hot-melt extrusion, sublimation and cotton candy process (Badgujar & Mundada, 2011; Goel, Rai, Rana, & Tiwary, 2008).

1.4.2. Tablet Non-Medicinal Ingredients

In addition to the APIs, tablets contain a number of NMIs or excipients. Both terms are used interchangeably in literature. These can include diluents, binders or granulating agents, glidants (flow aids), and lubricants to ensure efficient tabletting; disintegrants to facilitate tablet break-down into smaller aggregates and powder; flavoring agents to mask unpleasant taste; and coloring agents to make the tablets visually attractive and easy to identify. Tablet coating with certain polymers can be used for controlled-release tablets and to enhance taste, texture and appearance for easy administration.

Although the term "inert materials" is sometimes referred to NMIs, it is becoming apparent that those NMIs can have major impact on tablet characteristics.

Many studies have shown the NMIs' influence on stability and bioavailability of APIs in tablets (Rowe, Sheskey, & Weller, 2003a).

1.4.2.1. Types of Non-Medicinal Ingredients

Diluents are added when the quantity of API is small and difficult to compress alone. They are sometimes called fillers, used to make up the convenient total weight of the tablet. Common tablet diluents include lactose, mannitol, sorbitol, starch, and microcrystalline cellulose (MCC). Microcrystalline cellulose is used in most tablets prepared by direct compression methods due to its dry binding efficacy (United States Pharmacopeial Convention, 2009a).

Binders are used to provide cohesiveness to the powdered mixture of ingredients to form granules and to insure that tablets remain intact after compression. Commonly used binders include starch, gelatin, sugars such as sucrose, glucose, dextrose, lactose, and gums such as acacia, sodium alginate, carboxymethylcellulose, and methylcellulose (Rowe, Sheskey, & Weller, 2003b). Microcrystalline cellulose, microcrystalline dextrose, and polyvinylpyrrolidone have free-flowing and sufficient cohesive properties to act as binders in the formulation of tablets by direct compression.

Lubricants are hydrophobic NMIs used in concentrations of less than 1% of tablet weight to reduce interparticle friction and yield even flow, to prevent adhesion of tablet ingredients to the surface of dies and punches during tabletting, and to facilitate ejection of tablets from the die cavity in tablet machines. Studies have shown that the

order and duration of mixing of lubricants and other NMIs have major effects on the characteristics of tablets including hardness, disintegration and dissolution (Rowe, Sheskey, & Weller, 2003b). Magnesium stearate, sodium lauryl sulfate, and magnesium lauryl sulfate are the most widely used lubricants.

Disintegrants are added to tablets to facilitate their break down into small particles following administration. Their mechanism of action depends on their swelling abilities and capillary action when moistened, facilitating the rupture of tablet matrix. Disintegrants may include starches, clays, celluloses, algins, gums, and cross-linked polymers. Other disintegrants can be added in relatively low concentrations (2 – 4%) and are commonly referred to superdisintegrants including croscarmelose, crospovidone, sodium starch glycolate, and low-substituted hydroxypropyl cellulose. Despite the significant role of disintegrants, other NMIs and the tablet hardness have been shown to influence disintegration time (Rudnic & Schwartz, 1995).

FDA-approved coloring agents can be used to enhance appearance, serve as a means of identification, and match the flavor of tablets. Natural and artificial/synthetic flavoring agents can be added to mask the bitter taste inherent in most APIs. An acceptable taste becomes critically important for buccal/SL tablets which have relatively longer residence time in oral cavity compared to conventional oral tablets designed to be swallowed.

Co-processing of NMIs is widely accepted as a method of combining two or more established NMIs by an appropriate process. Co-processed NMIs result in superior

properties compared to the simple physical mixtures of NMIs. Co-processing is mostly used to produce directly compressible NMIs (M. C. Gohel & Jogani, 2005). Examples of co-processed NMIs can be found in Table 1 in the Appendix.

1.4.2.2. Selection of Non-Medicinal Ingredients

As can be seen by the examples of each group of NMIs, many can be used for different purposes, sometimes for contradicting roles even within the same formulation. For example, corn starch is used as both a binder and a disintegrant in certain formulations. Therefore, it is necessary to understand the properties of different NMIs to select the proper types, grades, and proportions that would result in the required characteristics of tablets for specific indication and route of administration.

When the percentage of the API in tablets is high, NMI properties may have minor effects on the characteristics of tablets, and the API physical/chemical properties will be critically important. In contrast, when the percentage of the API in tablets is small, the overall properties of tablets are based mainly on the physical/chemical properties of the NMIs used. The formulator must be aware of these properties and their effect on the in vitro and in vivo characteristics of tablets.

For example, NMIs differ in terms of solubility, ranging from freely water-soluble to water-insoluble. The NMI solubility should be carefully considered in tablet formulations since it might compete with the dissolution media available for the API. Solubility becomes very important in the limited volumes of saliva, the dissolution media in the oral cavity for SL tablets, in situations when water is not available to

facilitate tablet administration, or for people suffering from xerostomia and hyposalivation.

Another factor to consider when selecting an NMI is its safe use to the larger population. The formulator should consider the level of allerginicity the NMIs might cause to patients. Lactose, for example, should be avoided whenever possible as some patients might be intolerant to it and mannitol, for example, can be used alternatively.

Consideration of the mixing process is as important as the selection of NMIs (Rudnic & Schwartz, 1995). The NMIs incorporated in tablets should be carefully mixed to result in the required characteristics of tablets, such as hardness, friability, disintegration, and dissolution. Non-medicinal ingredients are incorporated in specific order to ensure that the resultant powder mixture possesses high fluidity, compressibility, and physical stability during compression. Non-medicinal ingredients with specific functions such as lubricants are added at pre-calculated percentages during the final stages of mixing. They are also mixed for relatively shorter periods of time to result in external positioning within the powder mixture and consequent high fluidity in the hopper feed of the tablet press. Other NMIs such as disintegrants are added in two separate portions of different ratios to achieve both internal and external positioning of specific quantities and locations in the powder mixture.

Direct compression is the manufacturing process of choice for heat- and moisture-sensitive APIs such as epinephrine. Therefore, directly compressible NMIs should be selected in the formulation of SL tablets of epinephrine. Ideally, these NMIs

must have high compressibility and free flowing properties. They must also result in acceptable tablet strength and at the same time possess super-disintegrating properties to enable the rapid disintegration of tablets in the presence of minimal dissolution mediums. Examples of NMIs used in orally disintegrating tablets and the rationale for the selection of specific NMIs in the formulation of the new-generation of SL tablets of epinephrine in the studies reported in chapter IV can be found in Tables 2 and 3, respectively, in the Appendix.

1.4.3. Characteristics of Tablets

Tablets can be described or evaluated by a number of specific characteristics including size, shape, thickness, weight, hardness, wetting and disintegration times, and dissolution. The punches and dies used in tablet machines control the size and shape of tablets formulated. Tablets generally have a discoid shape. Other shapes include oval, round, cylindrical, and triangular. The upper and lower surfaces can be flat, round, concave, or convex. The tablets can be scored to facilitate breaking into halves to provide smaller doses. The size, shape, and color of tablets are meant mainly for identification purposes. Other characteristics are specified for formulation development and quality control assurance of the manufacturing process to ensure consistency between batches and lots.

Tablet characteristics can be evaluated in vitro using standardized methodologies specified in international pharmacopeias such as the United States Pharmacopeia (USP), European Pharmacopeia (EP), and Japanese Pharmacopeia (JP) and

considered official for many dosage forms. However, a number of studies have shown drawbacks with the currently used testing making it necessary to revisit these official tests. Most importantly is the lack of correlation between the results obtained from in vitro testing of dissolution and in vivo testing of bioavailability, partly due to the lack of suitability of the conditions under which these tests are performed compared with the conditions of the site of administration of these dosage forms. New tests need to be developed and validated for new dosage forms of specific functions. SL tablets, for example, have no suitable pharmacopeial disintegration and dissolution testing that mimics the physiological conditions of the SL cavity. The following sections include the in vitro tests of tablet characteristics, with indication of their suitability to the SL tablets, where applicable.

1.4.3.1. Uniformity of Dosage Units

Tablets need to have consistent weight in order to deliver a consistent dose of API. This is especially important with potent APIs such as digoxin, because any small variation of dose may have major effect on efficacy and toxicity. Weight variation and content uniformity are two required tests for tablets to ensure consistency of dosage units. Each tablet should have consistent API substance content within a narrow range around the label claim.

Uncoated tablets containing ≥25 mg and ≥25% API dose require the weight variation test only (United States Pharmacopeial Convention, 2010b). Content uniformity test is required for tablets containing <25 mg or <25% API dose. For weight

variation test, ten tablets randomly selected are individually weighed. Drug substance content is calculated and expressed as % of label claim. An acceptance value (AV) is calculated using this USP formula (United States Pharmacopeial Convention, 2010b):

$$AV = |M - \bar{X}| + ks$$

where $\bar{X}$ = the mean of individual contents, expressed as a percentage of the label claim, M = a reference value that depends on the value of T, T = the average of the limits specified in the potency definition in the individual monograph, and k = an acceptability constant that depends on n. If n = 10, then k = 2.4. If n = 30, then k = 2.0. s = the sample standard deviation. Definitions, conditions, and values of variables in this formula are detailed in a table in the USP under section (905) Uniformity of Dosage Units (United States Pharmacopeial Convention, 2010b).

Coated tablets are exempt from weight variation requirements, but must conform to the content uniformity requirements.

For content uniformity test, 10 units are individually assayed for drug content and AV is calculated as above using the same formula. A maximum AV of 15.0 was used, according to the harmonized USP method.

The tests for uncoated tablets can be applied to SL tablets.

1.4.3.2. Breaking Force and Friability

Tablets should be hard enough to withstand various forces and stresses during manufacturing, packaging, shipping and handling. International pharmacopeias

recommend two tests which supplement each other: breaking force (United States Pharmacopeial Convention, 2008b) and friability tests (United States Pharmacopeial Convention, 2008c).

The friability test of uncoated tablets is performed using a rotational drum. Tablets are rotated and subjected to tumbling at each turn by a curved projection. Thus, at each turn the tablets are exposed to rolling and repeated shocks from freefalls within the drum. This test determines the tablets' resistance to chipping, capping, lamination and surface abrasion by tumbling them in a rotating cylinder. For tablet weight of ≤ 650 mg, a random sample of whole tablets corresponding to 6.5 g is used. For tablet weight of > 650 mg, a random sample of 10 whole tablets is used. Tablets are carefully dedusted, accurately weighed, and placed in the drum of a friability tester. The drum is rotated 100 times and tablets are removed, dedusted, and accurately weighed. The percentage weight loss after tumbling is referred to as the friability of the tablets. A maximum weight loss of not more than 1.0% is considered acceptable, given that there are no obvious cracked, cleaved, or broken tablets by visual inspection.

Another measure of the mechanical integrity of tablets is their breaking force, which is the force required to cause them to fail or break in a specific plane (United States Pharmacopeial Convention, 2008b). The terms 'hardness' and 'crushing strength' are also used in literature to refer to breaking force, but their meaning can be misleading. In material science, hardness refers to the resistance of a surface to penetration by a probe, and crushing strength implies that tablets are actually crushed

during the test, which is often not the case (United States Pharmacopeial Convention, 2008b). Generally, tablets are placed between two platens, one of which moves gradually to apply sufficient force to a tablet to cause fracture. A minimum of 6 tablets should be tested to achieve sufficient statistical precision. A consistent orientation should be followed especially for scored or irregularly shaped tablets. This test is performed during tabletting to determine the need for compression force adjustments on the tablet machine. If tablets are too hard, they may not disintegrate in the required period of time or meet the dissolution specifications. If they are too soft, they may not withstand manufacturing, shipping, and handling.

Commonly, the minimum acceptable breaking force of tablets is 4 kg, but may be less for tablets intended to be soft for rapid disintegration and dissolution such as the SL tablets.

1.4.3.3. Disintegration

Disintegration of a tablet can be defined as that state in which any residue of the tablet is a soft mass having no firm core (United States Pharmacopeial Convention, 2008a). A tablet should be able to disintegrate in the biological environment from which it is intended to be absorbed. To be absorbed, the API must be in solution and the disintegration test is a measure only for the time required for tablets to break down into particles. Therefore, this test is useful as a quality-control test for conventional, not controlled-release or chewable tablets. It cannot be used to relate to in vivo behavior of tablets.

The official USP method includes the use of an apparatus of a basket-rack assembly, a 1000-mL beaker, and a device for reciprocating the basket into the immersion fluid. One tablet is dropped into the fluid of each one of the six open-ended transparent tubes located in the basket-rack assembly. The bottom end is covered with a 10-mesh screen. Fluid temperature should be maintained at 37±2°C. Tablets should disintegrate completely in the time frame specified in the monograph. If 1 or 2 tablets fail to disintegrate, repeat with 12 additional tablets. At least 16 out of the total 18 tablets should disintegrate to pass the test. The conditions of the test vary for coated, buccal, or SL tablets. For most uncoated tablets the recommended disintegration time is 30 minutes. For coated tablets, up to 2 hours may be accepted; while for SL tablets, 3 minutes is required.

Other methods have been suggested for rapidly disintegrating tablets. They do not mimic the breakdown of tablets after oral administration because the in vitro conditions used did not simulate the in vivo conditions (Abdelbary et al., 2005; Dor, Rogers, & Fix, 1999; el-Arini & Clas, 2002; M. Gohel et al., 2004).

A SL tablet should disintegrate rapidly in the presence of relatively small volumes of saliva. There is no official compendial apparatus and method which simulate such conditions.

To simulate the SL environment, one method was developed (M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji, Simons, & Simons, 2007) to evaluate the disintegration times of SL tablets under rigorous conditions. Six tablets are

randomly selected from each batch. Each individual tablet is dropped into a 10-mL glass test tube (1.5-cm diameter) containing 2 mL distilled water. The time required for complete tablet disintegration without agitation is observed visually and recorded to the nearest second using a stopwatch. The visual inspection is enhanced by gently rotating the test tube at a 45-angle to distribute any tablet particles that might mask any remaining undisintegrated portion of the tablet.

1.4.3.4. Wetting

Although a wetting test is not required by international pharmacopeias, it is useful for quality control evaluation of tablets intended for oral administration. As an alternative to the disintegration test, the wetting test utilizes a minimal volume of medium which may be more representative of the physiological conditions in the oral cavity.

Wetting time of tablets can be measured by a procedure modified from that reported in literature simulating the physiological conditions of a moist tongue surface (Bi et al., 1996). Two layers of absorbent paper fitted into a rectangular plastic dish (11 cm x 7.5 cm) are thoroughly wetted with distilled water. Any excess water is completely drained out of the dish. A tablet is placed at the center of the plastic dish and the time required for the water to diffuse from the wetted absorbent paper throughout the entire tablet is then recorded using a stopwatch. Wetting time of six randomly selected tablets per batch should be determined.

The wetting test is more representative of the in vivo conditions and more appropriate for SL tablets than the official compendia disintegration test.

1.4.3.5. Dissolution

Compared to disintegration, dissolution appears to be a more indicative in vitro parameter for the in vivo behavior of tablets since absorption of an API depends on having it dissolve at the site of administration. Therefore, dissolution testing is a critical and mandatory in vitro quality control procedure for tablets. Such testing confirms that tablets have released the labeled quantity of API into solution within a designated time interval under specific conditions. It demonstrates that the drug will be readily available for absorption after oral administration. Table 1 summarizes the seven different dissolution and drug release apparatus in USP (United States Pharmacopeial Convention, 2009c; United States Pharmacopeial Convention, 2010a).

***Table 1**: A summary of the dissolution and drug release apparatuses in USP.*

Apparatus	Components	Medium Temperature (°C)	Vessel	Specifications	Dosage Position
1 Basket	Vessel, motor, metallic drive shaft, cylindrical basket.	37 ± 0.5	Cylindrical with a hemispherical bottom. 1-4 L capacity.	Transparent apparatus is preferred. Fitted cover to retard evaporation of vessel may be used. Speed-regulating device to select the shaft rotation speed (within ± 4%)	A dosage unit placed in a dry basket at beginning of each test.
2 Paddle	Assembly from Apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element.	37 ± 0.5	Cylindrical with a hemispherical bottom. 1-4 L capacity.	Shaft should rotate smoothly without significant wobble. Small loose piece of non-reactive material (wire helix) may be attached to dosage unit that would otherwise float.	Dosage unit allowed sinking to bottom of vessel before rotation of blade.
3 Reciprocating Cylinder *NOT approved by JP.*	Vessel, set of glass reciprocating cylinders, screens, motor and drive assembly (for vertical reciprocating).	37 ± 0.5	Cylindrical flat-bottomed glass vessel.	Speed-regulating device to select the shaft dip rate (within ± 5%). Transparent apparatus is preferred. Vessels provided with evaporation cap.	Dosage unit placed inside a dry cylinder at beginning of each test.
4 Flow-Through Cell	Reservoir and pump for dissolution medium, flow-through cell.	37 ± 0.5	No vessel.	The pump forces dissolution medium upwards through the cell with a delivery range of 240-960 mL/hour and constant flow rate of 4, 8, and 16 mL/min (± 5%). Pulsating flow of 120 ± 10 pulses/min. Cell is transparent mounted vertically with a filter system preventing escape of undissolved particles from top.	Dosage unit placed in a tablet holder in appropriate cell type.

5 Paddle over Disk	Paddle and vessel of Apparatus 2, stainless steel disk assembly (to hold transdermal system at bottom of vessel).	32 ± 0.5	Cylindrical with a hemispherical bottom. 1-4 L capacity.	Vessel may be covered to minimize evaporation.	Disk holds the dosage unit flat and positioned in a way release surface is parallel with paddle blade bottom.
6 Cylinder	Vessel assembly of apparatus 1, except replace the basket and shaft with a stainless steel cylinder stirring element.	32 ± 0.5	Cylindrical with a hemispherical bottom. 1-4 L capacity.		A dosage unit placed on the exterior of cylinder so that the long axis of unit circumference the cylinder.
7 Reciprocating Holder	Set of volumetrically calibrated or tared solution containers made of glass or inert material, motor and drive assembly (to reciprocate the system vertically), set of sample holders	32 ± 0.5	Cylindrical with a hemispherical bottom. 1-4 L capacity.	Designed for a variety of dosage forms. Angled disk and cylinder holders for transdermal systems. Rod pointed and spring holders for oral extended-release.	Dosage unit placed in the suitable sample holder.

Sublingual tablets, ideally, should release the total quantity of drug within seconds into solution in a limited volume of saliva, for maximum absorption via the SL veins into the systemic circulation. Currently, the available pharmacopeias' dissolution apparatuses and methods (Committee on JP, 2006; Convention on the Elaboration of a European Pharmacopoeia, 2008; United States Pharmacopeial Convention, 2009c; United States Pharmacopeial Convention, 2010a) do not simulate the unique

characteristics for testing SL tablets. For example, the USP dissolution method recommended for isosorbide dinitrate SL tablets uses apparatus 2 (Paddle), 900 mL of water, and 50 rotations per minute (rpm) to achieve not less than 80% of the labeled amount dissolved in 20 min (United States Pharmacopeial Convention, 2009b). Upon reviewing other pharmacopeia such as the EP (Convention on the Elaboration of a European Pharmacopoeia, 2008) and JP (Committee on JP, 2006), it is readily apparent that none of the official compendia dissolution apparatuses or methods are designed to evaluate the release of drug from rapidly-disintegrating tablets, and more specifically the SL tablets under simulated SL conditions.

The few new non-compendial in vitro methods cited for dissolution testing of SL tablets, utilize similar compendia apparatuses under modified conditions (Das, Das, & Surapaneni, 2006; Hunt, Shah, Prasad, Schuirmann, & Cabana, 1981). Smaller volumes of dissolution medium have been proposed, but they are still larger than the volume of saliva secreted in the SL cavity within 2 min. For example, a mini-paddle apparatus, which can accommodate a minimum operational volume of approximately 30 mL of dissolution medium, has been introduced (Crist, 2009; S. Klein, 2006; S. Klein & Shah, 2008). However, the fluid hydrodynamics of these apparatuses are still not appropriate for modeling dissolution within the SL cavity.

Custom-made dissolution apparatuses and more biorelevant methods are needed to evaluate rapidly disintegrating tablets intended for SL administration. In addition, an in vitro dissolution method should be capable of detecting and discriminating among minor changes in SL tablet formulations (Fortunato, 2005).

1.4.3.6. Taste Masking

Medications that are administered into the oral cavity should have an acceptable taste, especially for pediatrics and geriatrics age-groups. Palatability testing of such medications becomes part of the formulation development process and an essential requirement for drug regulatory approval (Lorenz, Reo, Hendl, Worthington, & Petrossian, 2009).

Early-stage taste assessment and masking of bitter APIs is a major challenge to formulators and manufacturers. Taste assessment by human sensory analysis panels (SAPs) is common, but associated with a number of drawbacks. Human SAPs are carefully selected, extensively trained, and continuously monitored, requiring tremendous time, effort, and cost. Moreover, these SAPs cannot be used for new chemical entities (NCEs) before FDA or Health Canada approval is obtained for use in humans. Instead, a multichannel taste sensor instrument commonly named the electronic tongue (e-Tongue) is becoming an alternative method to human SAPs. It can be used in the assessment of the bitterness of NCEs and APIs and for evaluating the masking efficiency of NMIs. In addition, it is used in placebo development, in taste matching of formulations, and in unknown-to-reference comparisons (Kayumba et al., 2007; Lorenz et al., 2009; Zheng & Keeney, 2006).

For a prescription SL tablet, the recommended residence time in the mouth is 2 min or until dissolved (Mennella & Beauchamp, 2008; Repchinsky, 2009). Taste may affect the length of time a patient holds a tablet within the SL cavity which in turn may

affect compliance. In order to achieve optimal compliance, the taste of a SL tablet should be assessed and improved if necessary to ensure that it is palatable, especially for children.

1.5. Research Proposal

1.5.1. Research Rationale

Anaphylaxis is a sudden, severe systemic allergic reaction, which can be fatal within minutes. Most anaphylactic reactions occur unexpectedly in community settings rather than in hospital settings and commonly triggered by foods, insect stings, medications, natural rubber latex, and other allergens (P. Lieberman, 2003a; Sampson et al., 2006; F. E. R. Simons, 2004). There is universal agreement that prompt E injection is the drug of choice for the treatment of anaphylaxis (P. Lieberman, 2003a; McLean-Tooke, Bethune, Fay, & Spickett, 2003; Sampson et al., 2006; F. E. R. Simons, 2004). The recommended E dose for the treatment of anaphylaxis is 0.3 to 0.5 mg in adults and 0.01 mg/kg, to a maximum of 0.3 mg, in children, given by IM injection in the mid-outer thigh (P. Lieberman, 2003a; P. Lieberman et al., 2010; McLean-Tooke et al., 2003; Sampson et al., 2006; F. E. R. Simons, 2004). These recommendations are based on clinical experience and/or studies in healthy volunteers (F. E. R. Simons et al., 2001), rather than on randomized, double-blind, placebo-controlled dose-ranging studies in patients experiencing anaphylaxis, which are impossible to perform for ethical reasons (F. E. R. Simons, 2004).

For out-of-hospital emergency treatment of anaphylaxis, E-autos such as EpiPen Jr®, EpiPen® (Dey LP, Nappa, CA, USA), Twinject® 0.15 mg, Twinject® 0.3 mg (Sciele

Pharma, Inc., a Shionogi Company, Atlanta, GA, USA), Anapen® 0.15 mg, Anapen® 0.3 mg, Anapen® 0.5 mg (Lincoln Medical, Salisbury, UK), Jext® 0.15 mg, Jext® 0.3 mg (ALK-Abelló Ltd., Berkshire, UK), Auvi-Q™ 0.15 mg, Auvi-Q™ 0.3 mg, Allerject™ 0.15 mg, and Allerject™ 0.3 mg (Sanofi-aventis, Bridgewater, NJ, USA) are prescribed; however, self-injectable E is underused when anaphylaxis occurs (Bock, Munoz-Furlong, & Sampson, 2001; Gold & Sainsbury, 2000). The drawbacks of E-autos include high cost ($100 for a single E injection), which limits affordability and availability worldwide (F. E. R. Simons, 2005); perceived large size and bulkiness; limitations on repeated dosing (if required) (Korenblat et al., 1999); fear and anxiety associated with the use of needles (especially in children) (F. E. R. Simons, 2004; F. E. R. Simons, 2006); and dosing errors caused by incorrect technique of administration (Gold & Sainsbury, 2000; Sicherer, Forman, & Noone, 2000). Epinephrine autoinjectors should only be injected IM into the anterolateral aspect of the thigh and accidental injection into the hand finger, thumb, may result in critical loss of blood flow to the affected area. In addition, it is impossible to give an accurate dose to infants and to many young children using currently available autoinjectors, which provide only two different pre-measured, fixed E doses, 0.15 mg and 0.3 mg (F. E. R. Simons, 2004). These limited ranges of doses create a dilemma in the decisions made by clinicians prescribing E for infants and children, and also for large adolescents and adults for whom the 0.3 mg dose is too low (F. E. R. Simons, 2006).

Alternatives to an E-auto, such as an E ampoule/syringe/needle or an E metered-dose inhaler, are impractical with regard to rapid and accurate dosing (F. E. R. Simons et al., 2000; F. E. R. Simons et al., 2001; F. E. R. Simons, 2004). Despite these drawbacks,

new autoinjectors such as Auvi-Q™ and Jext have been developed and approved for use in some countries.

The SL route of administration is a promising alternative route for E administration. Drugs that are absorbed SL bypass potential metabolic conversion in the GIT and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Cunningham et al., 1994; Kroboth et al., 1995; Motwani & Lipworth, 1991; Price et al., 1997). Drugs with a low molecular weight such as E are likely absorbed across the SL mucosa into the venous circulation by transcellular diffusion (Birudaraj, Berner, Shen, & Li, 2005), a mechanism driven by the concentration gradient (Sherwood, 2004). The formulation of SL tablets of E would facilitate the development of tablets with a range of E doses to match the body weights of patients at risk on a mg/kg basis. Tablets will have longer shelf-life because E is more stable in dry solid dosage forms than it is in aqueous solution. Sublingual tablets of E would be easy to carry and self-administer, eliminating the fear and anxiety associated with autoinjector needles, especially in children, as well as provide the capability of repeating the dose if necessary.

In previous in vitro and in vivo studies, first-generation E SL tablet formulations with similar disintegration times had different bioavailability in vivo in the preclinical model (M. M. Rawas-Qalaji, Simons, & Simons, 2006a; M. M. Rawas-Qalaji, Simons, & Simons, 2006c). Upon reviewing these studies, a number of questions rose: 1) What effect do the NMIs have on E bioavailability? 2) What effect does mannitol have on the dissolution of E? 3) Can we develop a method that simulates the conditions in the SL

cavity to measure dissolution in vitro? 4) Can this in vitro method predict in vivo behavior? and 5) Can we improve these first-generation tablets to reduce the E dose?

Although the disintegration test ensures tablet breakdown into smaller particles, it does not evaluate complete release of the API, in this case E. Therefore, dissolution assessment, a more selective in vitro test, was selected as a potential tool for the prediction of in vivo properties. A unique dissolution apparatus (assembly) was required to simulate the conditions in the SL cavity and overcomes the problems associated with the use of official USP dissolution apparatus for SL tablets (United States Pharmacopeial Convention, 2009c; United States Pharmacopeial Convention, 2010a). There is no single USP apparatus specifically designed to test dissolution of rapidly disintegrating and fast dissolving SL tablets. The limited volume of saliva produced over a short period of time (0.3 mL/min as the average resting flow rate to 1 mL/min as the average stimulated flow rate) (Sreebny & Schwartz, 1997) in relatively static environment are the conditions in the SL cavity that were considered in the design of the novel dissolution apparatus and development of method. This novel apparatus and method should have the ability to discriminate between the E SL formulations of similar in vitro characteristics. It should be able to determine the effect of small changes in formulation on tablet characteristics. This should help in the selection of the best performing E SL formulations for the in vivo studies in our validated animal model to generate in vivo data in an attempt to construct an in vitro in vivo correlation (IVIVC) model.

Epinephrine 40 mg in the first-generation formulations of SL tablets was bioequivalent to E 0.3 mg IM injections from EpiPens (M. M. Rawas-Qalaji, Simons, &

Simons, 2006c). However, the limitations of the first-generation tablets included: the unmasked intrinsic bitter taste of E (a hindrance to patient acceptability), and incomplete information about their disintegration and dissolution times.

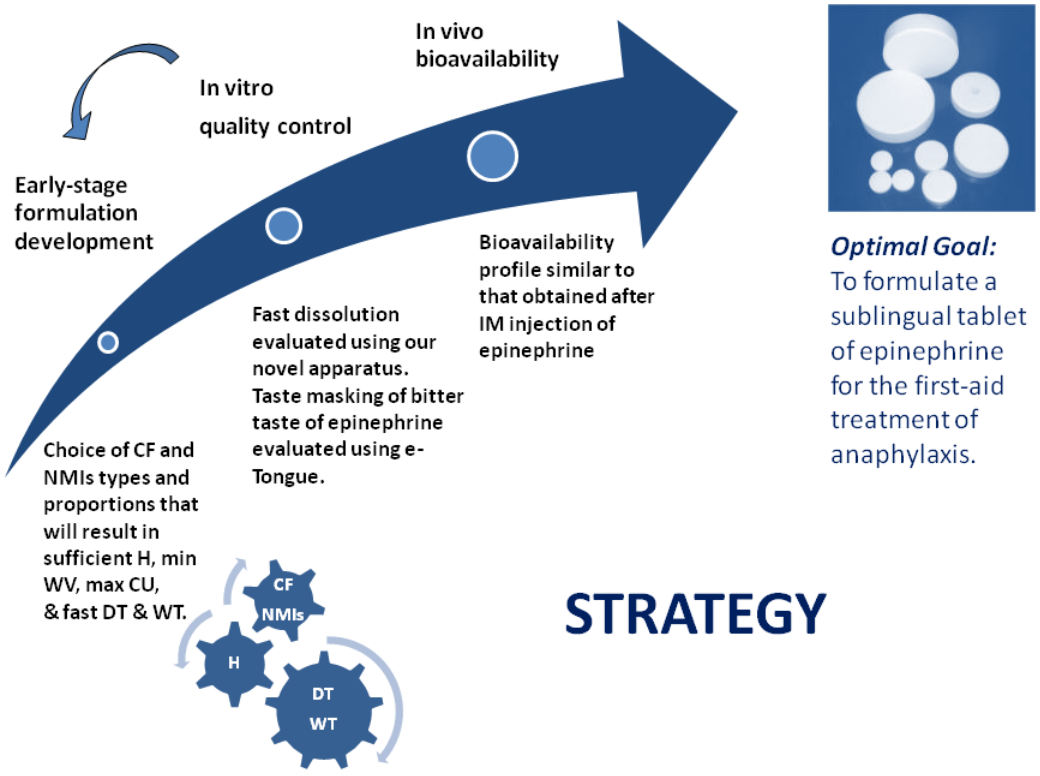
1.5.2. Research Hypothesis

We hypothesized that the type and proportion of NMIs used in a SL tablet formulation of E might have major effects on the in vitro characteristics (e.g., weight variation, content uniformity, hardness, wetting time, disintegration time, taste, dissolution) and in vivo bioavailability (i.e., rate and extent of E SL absorption).

1.5.3. Research Objectives and Strategy

To test this hypothesis, two main objectives were developed and followed throughout the studies. The first of these objective was selection of suitable NMIs including a wide range of grades of insoluble diluents such as MCC, differing with respect to particle size and bulk density; soluble diluents such as mannitol; soluble sweetening and flavouring agents to mask the bitter taste of E; and secretagogue agents that promote saliva secretion, which would enhance tablet disintegration to improve E release and promote E absorption. The second main objective was the selection of suitable in vitro tests and in vivo models for the evaluation of SL tablets of E. Accordingly, the E SL tablets were formulated with the overall goal of developing the best performing tablet in both in vitro and in vivo studies. A strategy was developed to facilitate the development of tablets while testing our hypothesis. This strategy was followed for every SL formulation. Review and assessment steps were implemented to explore the possible effects of NMIs selection on in vitro characteristics (Figure 2).

***Figure 2**: The strategy developed and followed throughout the studies to test our hypothesis with the optimum goal of formulating a sublingual tablet of epinephrine for the first-aid treatment of anaphylaxis. NMIS, non-medicinal ingredients; CF, compression force; H, hardness; WV, weight variation; CU, content uniformity; DT, disintegration time; WT, wetting time; IM, intramuscular.*



Official international pharmaceutical compendia such as the USP, EP, and JP do not include suitable tests or specific standards for SL tablets. Accordingly, to achieve our objectives it was, sometimes, necessary to either follow non-compendial validated tests and standards published in peer-reviewed literature or develop and validate new ones in our research facility. Accordingly, some of the specific aims of the research considered these facts.

The specific research aims were: 1) to review current official compendial quality control tests and standards and evaluate their suitability for SL tablets, 2) to develop and validate novel quality control tests and standards, where needed, 3) to assess the bitter taste of E and the masking capabilities of pre-selected flavoring/sweetening agents, 4) to develop and manufacture new generations of SL tablets of E, 5) to assess these tablets in vitro using suitable quality control tests and standards, e.g., dissolution, 6) to assess the in vivo bioavailability of new generation tablets, which had successful in vitro characteristics, by using a validated rabbit model, and 7) to explore on possible relationship between in vitro dissolution testing and in vivo bioavailability.

1.5.4. Organization of Thesis

This thesis is organized into six chapters as a sandwich thesis (manuscripts within a thesis). The entire thesis has been assigned sequential page numbers and the format and print font are consistent (Faculty of Graduate Studies, 2005). The American Psychological Association (5^{th} edition) bibliography style was used to create citations and references in the entire thesis.

The first chapter "Introduction" includes a literature review on anaphylaxis, epinephrine, sublingual delivery, and tablets, followed by the research proposal, which includes research rationale, hypotheses, objectives and strategy, and this section "organization of thesis".

The second to the fifth chapters include the research studies conducted to pursue the objectives and to prove the hypothesis of this research. Each chapter, from the second to the fifth, stands alone and addresses one or more of the main objectives

and specific aims, in a systematic and sequential manner. Each chapter is organized in a manuscript format, which contains its own Abstract, Introduction, Materials and Methods, Results and Discussion, Conclusions, and References. Each chapter represents an original manuscript in a peer-reviewed journal. These papers are published. Written permission from the copyright holder of each published paper included in this thesis has been obtained and attached before the “Introduction” chapter.

The sixth and final “Conclusions” chapter includes the overall conclusions for all the research studies described in chapters two to five and future directions.

The sandwich thesis format has been selected because all of my research studies have been published (chapters two, three, four, and five).

My contribution to each manuscript in this thesis has been acknowledged, as I am the first author of all the published manuscripts. All of the study designs were based on my evaluation of the literature and in discussion with my co-advisors and co-investigators. I have performed all of the research studies described in each chapter alone or with the assistance of my co-advisors where necessary. All the figures, tables, and photos reported in the Results section of each chapter were designed and drawn by myself and reviewed and modified where necessary following discussion with my co-advisors and/or co-investigators. All the ideas, justifications and explanations, and the literature review in the Discussion section of each chapter were my work alone or with the assistance of my co-advisor and/or co-investigators when necessary. All the papers were written by myself and reviewed, corrected and modified by the coauthors if necessary.

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CHAPTER II: DISSOLUTION TESTING OF SUBLINGUAL TABLETS: A NOVEL IN VITRO METHOD

2.1. Abstract

In the sublingual (SL) cavity, compared with the gastrointestinal tract (GIT), tablets are subjected to minimal physiological agitation, and a limited volume of saliva is available to facilitate disintegration and dissolution. None of the official compendial dissolution apparatuses and methods simulates these SL conditions. In this study, a custom-made dissolution apparatus was constructed, and a novel in vitro method that simulates SL conditions was evaluated. Several epinephrine (E) 40 mg SL tablet formulations under development and two commercial SL tablets, isosorbide dinitrate 5 mg and nitroglycerin 0.6 mg, were studied. The dissolution medium was 2 mL of distilled water at 25°C. Dissolution was measured at 60 and 120 s. The novel in vitro method was validated for accuracy, reproducibility, and discrimination capability, and was compared with the official United States Pharmacopeia (USP) dissolution method using apparatus 2 (Paddle). The data obtained following the novel in vitro method were accurate and reproducible. This method was capable of detecting minor changes in SL formulations that could not be detected using other in vitro tests. Results from the official USP dissolution method and our novel in vitro method were significantly different ($p<0.05$). Results reflecting the dissolution of rapidly disintegrating tablets using simulated SL conditions were obtained using the novel in vitro dissolution method.

2.2. Introduction

Dissolution testing is a critical and mandatory in vitro quality control procedure for solid dosage forms such as tablets. Such testing confirms that a tablet has released the labeled quantity of active pharmaceutical ingredient (API) into solution within a designated time interval. It demonstrates that the API will be readily available for absorption after oral administration. Ideally, SL tablets such as nitroglycerin should release the total quantity of API within seconds, for maximum absorption via the SL veins into the systemic circulation. The SL route is highly useful for an API, such as E, that is inactivated in the GIT due to extensive metabolism (Lefkowitz, Hoffman, & Taylor, 1996). Conversely, buccal formulations are intended to release their APIs over an extended time period. Although buccal and SL delivery both take place within the oral cavity, they differ in aspects such as specific location, mucosa permeability, and intended duration of release of medication (Campisi et al., 2010; Madhav, Shakya, Shakya, & Singh, 2009; Zhang, Zhang, & Streisand, 2002).

The SL route of administration has not been extensively studied because relatively few SL commercial products are currently available. The SL cavity is characterized by unique anatomical and physiological conditions compared with other segments of the GIT such as the stomach and small intestine. A tablet that is swallowed will be subjected to GIT peristalsis in the presence of relatively large volumes of digestive fluids secreted throughout the GIT, facilitating tablet disintegration and drug dissolution. In the SL cavity, tablets are exposed to minimal physiological agitation;

moreover, a limited volume of saliva, 0.3 mL/min resting flow rate up to 1 mL/min stimulated flow rate (Sreebny & Schwartz, 1997), is available to facilitate tablet disintegration and drug dissolution. Currently, the available pharmacopeias' dissolution apparatuses and methods (Committee on JP, 2006; Convention on the Elaboration of a European Pharmacopoeia, 2008; United States Pharmacopeial Convention, 2009d; United States Pharmacopeial Convention, 2010a) do not simulate these unique characteristics for testing rapidly disintegrating SL tablets. For example, the USP dissolution method recommended for isosorbide dinitrate SL tablets uses apparatus 2 (Paddle), 900 mL of water, and 50 rotations per minute (rpm) to achieve not less than 80% of the labeled amount dissolved in 20 min (United States Pharmacopeial Convention, 2009b). Upon reviewing other pharmacopeia such as the European Pharmacopeia (Convention on the Elaboration of a European Pharmacopoeia, 2008) and Japanese Pharmacopeia (Committee on JP, 2006), it is readily apparent that none of the official compendia dissolution apparatuses or methods are designed to evaluate the release of API from a rapidly disintegrating SL tablet dosage form under simulated SL conditions.

The few new non-compendial in vitro methods cited for dissolution testing of SL tablets, utilize similar compendia apparatuses under modified conditions (Das, Das, & Surapaneni, 2006; Hunt, Shah, Prasad, Schuirmann, & Cabana, 1981). Smaller volumes of dissolution medium have been proposed, but they are still larger than the volume of saliva secreted in the SL cavity within 2 min. For example, a mini-paddle apparatus, which can accommodate a minimum operational volume of approximately 30 mL of

dissolution medium, has been introduced (Crist, 2009; S. Klein, 2006; S. Klein & Shah, 2008). However, the fluid hydrodynamics of these apparatuses are still not appropriate for modeling dissolution within the SL cavity.

Custom-made dissolution apparatuses and more biorelevant methods are needed to evaluate rapidly disintegrating tablets intended for SL administration. In addition, an in vitro dissolution method should be capable of detecting and discriminating among minor changes in SL tablet formulations (Fortunato, 2005). Due to the short residence time within the SL cavity, we propose that the minor changes in formulations might have major effects on the rate and the extent of SL absorption (M. M. Rawas-Qalaji, Simons, & Simons, 2006a; M. M. Rawas-Qalaji, Simons, & Simons, 2006c). It is therefore mandatory to develop a dissolution method that meets these requirements.

We designed and constructed a custom-made apparatus suitable for measuring the dissolution of rapidly disintegrating SL tablets under simulated SL conditions. This novel in vitro method was evaluated for accuracy, reproducibility, and discrimination capability, and was compared with an official USP dissolution method.

2.3. Materials and Methods

2.3.1. Parts of the Custom-Made Apparatus Unit

Parts purchased included Nalgene 180 vacuum tubing and automatic shut-off, quick-disconnect coupling inserts (Sigma-Aldrich, Oakville, ON, Canada) and Millipore 25

mm glass microanalysis vacuum filter holders and supports, Whatman 0.45 µm nylon filter membranes, and 4 mL polystyrene disposable plastic tubes (Fisher Scientific, Nepean, ON, Canada).

2.3.2. Active Pharmaceutical Ingredients Used Throughout the Study

Epinephrine bitartrate (EB) (Sigma-Aldrich Inc., St. Louis, MO, USA) was used in the preparation of standard E solutions and E SL tablets formulated in the tablet manufacturing laboratory of the Faculty of Pharmacy (M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji, Simons, & Simons, 2007). Diluted isosorbide dinitrate and diluted nitroglycerin were purchased as standards from USP (Rockville, MD, USA) and used to construct their corresponding calibration curves. The commercial generic isosorbide dinitrate 5 mg (Apo-ISDN) and brand nitroglycerin 0.6 mg (Nitrostat®) were purchased from the University Centre Pharmacy (Winnipeg, MB, Canada).

2.3.3. Components of Epinephrine Sublingual Tablets under Development

All E SL tablet formulations (E^{a-g}) evaluated were formulated in our laboratory. Non-medicinal ingredients (NMIs) incorporated into the E SL tablets included microcrystalline cellulose (MCC) (Asahi Kasei Chemicals Corp, Tokyo, Japan), mannitol (Roquette America Inc., Keokuk, IA, USA), citric acid (Fisher Scientific Co., Fair Lawn, NJ, USA), low-substituted hydroxypropyl cellulose (Shin-Etsu Chemical Co., Tokyo, Japan), and magnesium stearate (Mallinckrodt Baker, Phillipsburg, NJ, USA).

2.3.4. Construction of One-Unit and Six-Unit Custom-Made Apparatus

For each dissolution test, a 0.45-µm nylon filter membrane was pre-wetted with 50 µL of distilled water and placed between the 15 mL glass funnel and the fritted glass base, which were clamped and inserted into Büchner flask (Fig. 1a). A 4-mL disposable plastic collection tube was placed at the outlet tip of the clamped unit to collect the filtrate. The Büchner flask was connected to a vacuum line controlled by automatic shut-off, quick-disconnect coupling inserts (on/off switches).

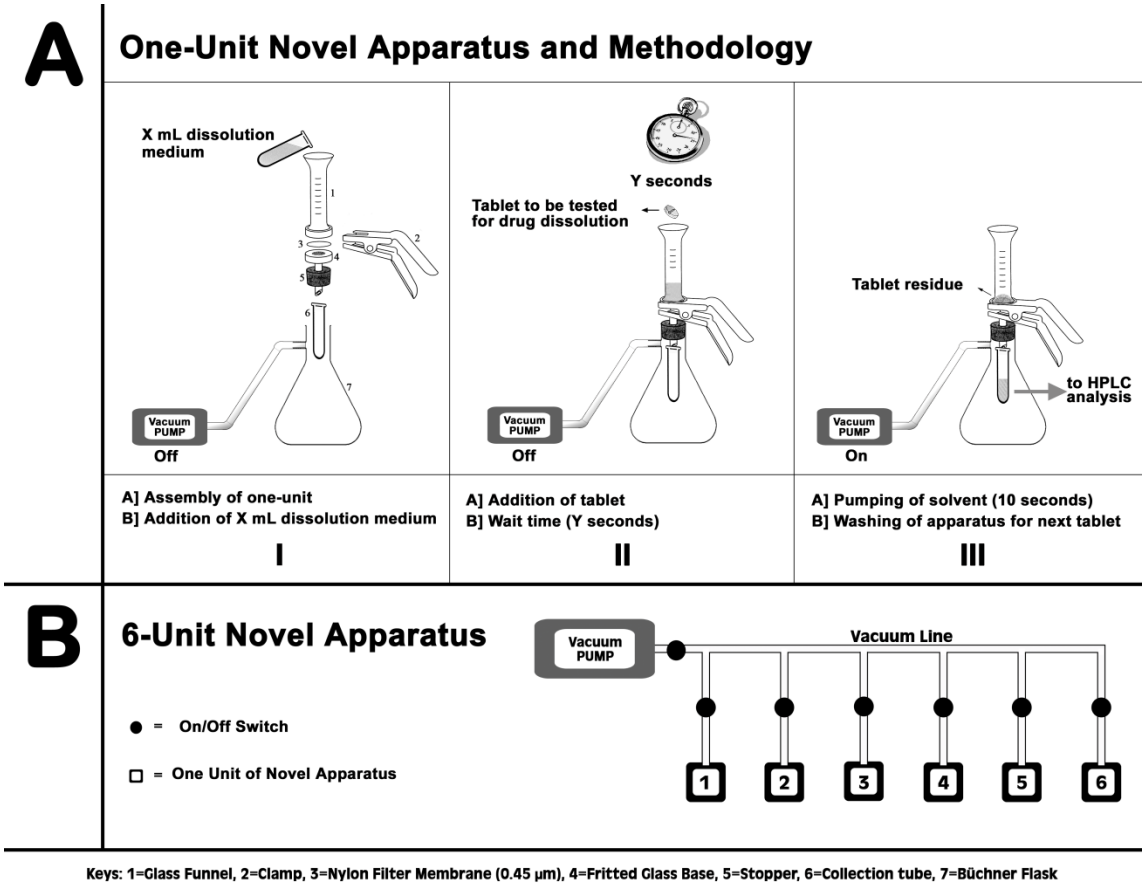
A six-unit custom-made apparatus was created by joining six individual assemblies to a common vacuum line. The vacuum for each individual unit was controlled by its own on/off switch (Fig. 1b).

2.3.5. The Novel In Vitro Dissolution Method

A volume of 2 mL of distilled water, as the dissolution medium, was measured into the 15 mL glass funnel at 25°C. The tablet was placed into the dissolution medium undisturbed for each specified time. The time-points, ranging from 15 to 120 s (stopwatch), were used initially to assess the dissolution profile of a representative formulation of E 40 mg SL tablets. Based on these results, the 60 and 120 s timepoints were selected for subsequent experiments. At each appropriate time-point, full vacuum was applied by opening the on/off switch causing the total volume of dissolution medium to be withdrawn instantly through a 0.45-µm filter membrane into the collection tube and terminating any further dissolution. The membrane prevented the passage of any undissolved particles and was replaced by a new membrane for each

dissolution analysis. The API content in each sample was measured by HPLC with UV detection (Waters Corp) according to the official USP assays for E injection (M. Rawas-Qalaji, Simons, Collins, & Simons, 2009; United States Pharmacopeial Convention, 2009a), nitroglycerin sublingual tablets (United States Pharmacopeial Convention, 2009c), and isosorbide dinitrate sublingual tablets (United States Pharmacopeial Convention, 2009b). To obtain the percent of drug released (DR%), the API content (mg) in the filtrate was compared with the mean content uniformity of ten individual dosage forms of the SL tablets being tested.

***Figure 1**: Schematic diagram showing the **a)** one-unit, and **b)** six-unit custom-made apparatus with brief description of the novel in vitro dissolution method.*



2.3.6. Assessment of E Adsorption to Apparatus Components

Using EB, the standard solutions equivalent to E 5, 10, 20, and 40 mg/mL (E^5, E^{10}, E^{20}, and E^{40}, respectively) were prepared; and 10 mL of each was filtered through the apparatus corresponding to 50, 100, 200, and 400 mg of E passing through the filter membrane and the fritted glass base to evaluate any loss of E through adsorption to apparatus components. The E content in the four standard solutions before and after filtration was measured. The 0.45-µm filter membrane was soaked in 10 mL distilled water to extract any E residues retained in the membrane for quantification. In addition, the fritted glass base was washed with five 10 mL aliquots of distilled water to detect any E residue remaining from the E^5 and E^{40} solutions after filtration.

2.3.7. Formulation of Epinephrine Sublingual Tablets and Evaluation of All Tablets

The representative E SL tablet formulations ($E^{a–g}$) under development using direct compression (M. M. Rawas-Qalaji, Simons, & Simons, 2006b) were available for dissolution testing. In all E formulations, EB was used to prepare SL tablets equivalent to E 40 mg. Non medicinal ingredients incorporated into these formulations included several grades of MCC (diluent), mannitol, citric acid (for taste-masking) (Rachid, Simons, Rawas-Qalaji, & Simons, 2010), low-substituted hydroxypropyl cellulose (disintegrant), and magnesium stearate (lubricant). These formulations differed by grade and proportion of MCC, by proportion of mannitol, and by the compression forces used to maintain uniform hardness (H) and disintegration times (DTs) (M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji et al., 2007). All the representative

E SL tablet formulations, as well as the commercial Apo-ISDN and Nitrostat®, were evaluated for weight variation (WV) and content uniformity (CU) according to USP specifications (United States Pharmacopeial Convention, 2010b).

2.3.8. Assessment of Dissolution Profile Using Representative Epinephrine 40 mg Sublingual Tablets

The dissolution testing of a representative formulation of E 40 mg SL tablets was evaluated at 15, 30, 45, 60, 75, 90, 105, and 120 s. The minimum time evaluated was 15 s, the time required for complete disintegration of the E SL tablets irrespective of E dose (M. M. Rawas-Qalaji, Simons, & Simons, 2006c); the maximum time evaluated was 120 s, the time recommended for patients to retain the commercially available SL tablets under the tongue (Repchinsky, 2009). Appropriate time-points were selected accordingly for subsequent experiments.

2.3.9. Assessment of Reproducibility

To assess day-to-day variability, dissolution testing at 60 and 120 s was performed and compared between day 1 and day 2 using one commercial SL tablet, Nitrostat®, and three representative E SL tablet formulations, E^a, E^b, and E^c.

2.3.10. Assessment of Discrimination Ability

To assess the ability to discriminate among SL tablet formulations, dissolution testing at 60 and 120 s was performed using two commercial SL tablets, Nitrostat® and Apo-ISDN, and one representative E 40 mg SL tablet formulation, E^d. The dissolution

testing of Apo-ISDN was also evaluated in 10 mL of dissolution medium due to the limited solubility of ISDN. The dissolution testing at 60 s of ten representative E 40 mg SL tablet formulations with DT of ≤15 s was also evaluated.

2.3.11. Dissolution Testing Using the Six-Unit Apparatus

The six-unit apparatus (Fig. 1b) was constructed and used to test the dissolution of six tablets simultaneously, using three E SL tablet formulations, E^e, E^f, and E^g. Results were compared with previous dissolution data collected using the one-unit apparatus.

2.3.12. Dissolution Testing Using the Official USP Apparatus and Method

The official USP apparatus 2 (Paddle) and method for ISDN SL Tablets (United States Pharmacopeial Convention, 2009b) were used as a control for dissolution testing of two E SL tablet formulations, E^c and E^d, and for two commercial SL tablets, Apo-ISDN and Nitrostat®. The dissolution medium was 900 mL of distilled water at 37±1°C. The paddle rotations were set at 50 rpm, and the samples were withdrawn as recommended at the 20 min time-point for analysis of the API content.

2.3.13. Data Analysis

Results were presented as means ± standard errors of means (SEM) of at least three replicate experiments and statistically analyzed by one-way ANOVA using Microsoft Excel software. The differences were considered significant at $p<0.05$.

2.4. Results

2.4.1. Assessment of Epinephrine Adsorption to Apparatus Components

The E content was slightly lower after filtration of 10 mL standard E solutions than before filtration (Table 1). The difference in E content before and after filtration, which represents the E retention in both the filter membrane and the fritted glass base, increased with increasing E concentration but did not exceed 10%. After filtration of E^5, >88% of the retained E residue in the fritted glass base was washed out after the first wash. The following washes removed >97% of the E residue in the fritted glass base. When filtering standard E solution with higher concentration (E^{40}), >94% of the E residue was removed from the fritted glass base after the first wash and >99% after the fourth wash.

2.4.2. Evaluation of All Sublingual Tablets Used in This Study

The compression forces applied in the manufacturing of representative E SL tablet formulations resulted in a uniform H of 1.31±0.04 kg (mean ± SEM). All SL tablets, including the commercial Apo-ISDN and Nitrostat® SL tablets, resulted in a DT of ≤15 s and were within USP limits of WV and CU.

***Table 1**: Epinephrine (E) retention (mg) in the custom-made apparatus following the filtration of 10 mL of standard E 5, 10, 20, and 40 mg/mL solutions (E^5, E^{10}, E^{20}, and E^{40}, respectively).*

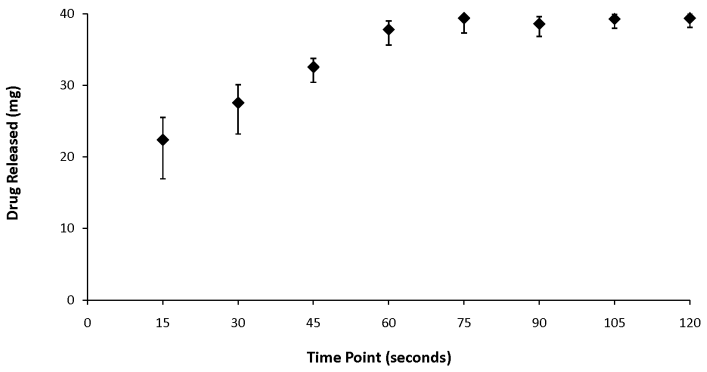
	Standard E Solutions			
Mean ± SEM[a]	E^5	E^{10}	E^{20}	E^{40}
E before filtration	48.8 ± 1.3	100.5 ± 0.4	200.9 ± 1.5	399.9 ± 1.2
E after filtration	45.9 ± 0.9	90.8 ± 5.1	187.6 ± 1.5	360.2 ± 2.1
E difference[b] (%)[c]	2.9 (5.9)	9.7 (9.7)	13.3 (6.6)	39.7 (9.9)
E residue in filter	0.9 ± 0.1	6.7 ± 1.7	11.4 ± 1.4	34.8 ± 2.5

[a] Means ± standard error of means (SEM) of E content (mg) of at least three replicates.
[b] E difference = mean E before filtration – mean E after filtration.
[c] Percent difference = (E difference / mean E before filtration) x 100

2.4.3. Assessment of Dissolution Profile Using Representative Epinephrine 40 mg Sublingual Tablets

More than 50% of E (22.40±3.14 mg) was released from the SL tablet and dissolved in the dissolution medium after 15 s and >90% (37.78±1.22 mg) after 60 s (Fig. 2). The DR% increased linearly with time from 15 to 60 s. The DR% appears to reach an asymptote after more than 75 s, achieving the values of 96% (39.38±1.18 mg) at 75 s and 98% (39.38± 0.72 mg) at 120 s

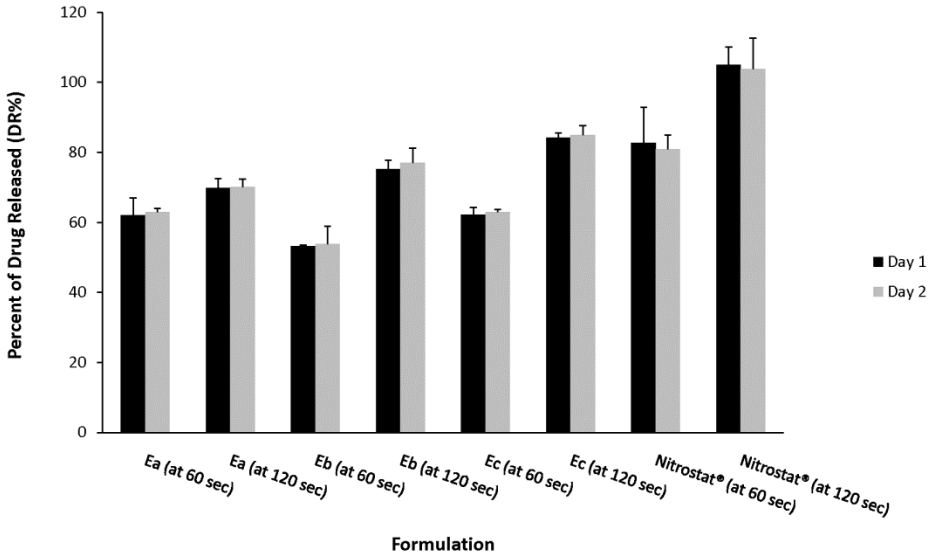
***Figure 2**: Dissolution profile of a representative epinephrine 40 mg sublingual tablet formulation over 120 s. Data represent means ± standard error of means (SEM) of at least three replicates.*



2.4.4. Assessment of Reproducibility

The DR% of E at 60 s from formulations E^a, E^b, and E^c ranged from 53.16% to 62.18% on day 1 and from 53.77% to 63.01% on day 2 (Fig. 3). The DR% of E at 120 s from formulations E^a, E^b, and E^c ranged from 69.81% to 84.14% on day 1 and from 70.15% to 84.96% on day 2. Following dissolution testing of Nitrostat®, the DR% at 60 s was 82.70% and 80.95% for day 1 and day 2, respectively, and 105.06% and 103.89% at 120 s. No significant difference was found between the results from days 1 and 2 of any SL tablet formulation.

Figure 3*: Percent of drug released (DR%) at 60 and 120 s from E^a, E^b, E^c, and Nitrostat® sublingual tablets on days 1 and 2. Data represent means ± standard error of means (SEM) of at least three replicates. No significant differences (p>0.05) were found between the adjacent bars.*



2.4.5. Assessment of Discrimination Ability

The DR% of E from E^d was 96.41±0.75% at 60 s and 102.62±0.86% at 120 s (Fig. 4). The DR% of nitroglycerin from Nitrostat® SL tablets was 75.1±1.12% at 60 s and 89.94±0.46% at 120 s. Only 0.90±0.06% of Apo-ISDN was released after 60 s and 1.71±0.14% after 120 s in 2 mL of dissolution medium. Compared to the DR% of ISDN from Apo-ISDN in 2 mL, the DR% in 10 mL increased significantly ($p<0.0001$) to 8.04±0.22% at 60 s and to 7.62±0.57% at 120 s (Fig. 4). Ten representative formulations with similar DTs (11 to 15 s) were evaluated for dissolution at 60 s which resulted in a DR% ranging from 58% to 104% (Fig. 5).

2.4.6. Dissolution Testing Using the Six-Unit Apparatus

Results obtained using the six-unit apparatus did not differ significantly ($p>0.05$) from previous dissolution results of the same three E SL tablet formulations (E^e, E^f, and E^g) using the single-unit apparatus.

2.4.7. Dissolution Testing Using the Official USP Apparatus and Method

The DR% was >90% after 20 min for all E^c, E^d, and Apo-ISDN (Fig. 6). In contrast, DR% differed significantly ($p<0.05$) between E^c, E^d, and Apo-ISDN (62.2%, 96.4%, and 1%, respectively) when dissolution was tested using the custom made apparatus and the novel in vitro method. Using the official USP apparatus and method, the API released from Nitrostat® was not detected by UV, but was >80% after 60 s using the novel in vitro method.

***Figure 4**: Percent of drug released (DR%) at 60 and 120 s from E[d], Apo-ISDN, and Nitrostat® SL tablets. In addition, DR% from Apo-ISDN SL tablets was tested in 10 mL of dissolution medium. Data represent means ± standard error of means (SEM) of at least three replicates.*

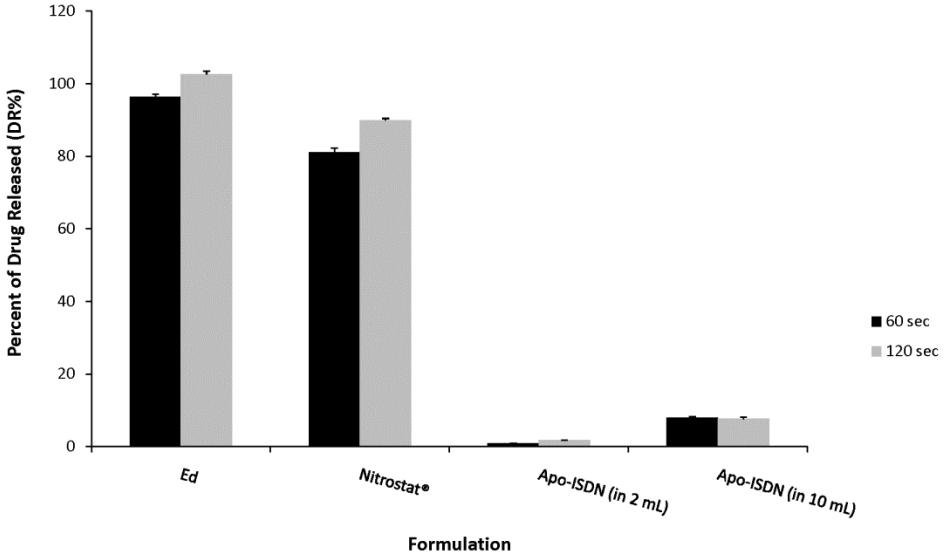


***Figure 5**: A comparison between the disintegration times (DT) and corresponding percent of drug released in 60 s (DR%) of ten different E 40 mg SL tablet formulations.*

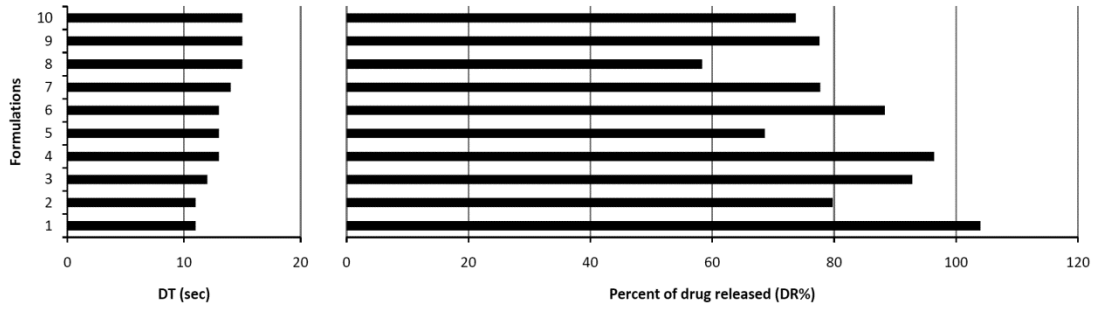
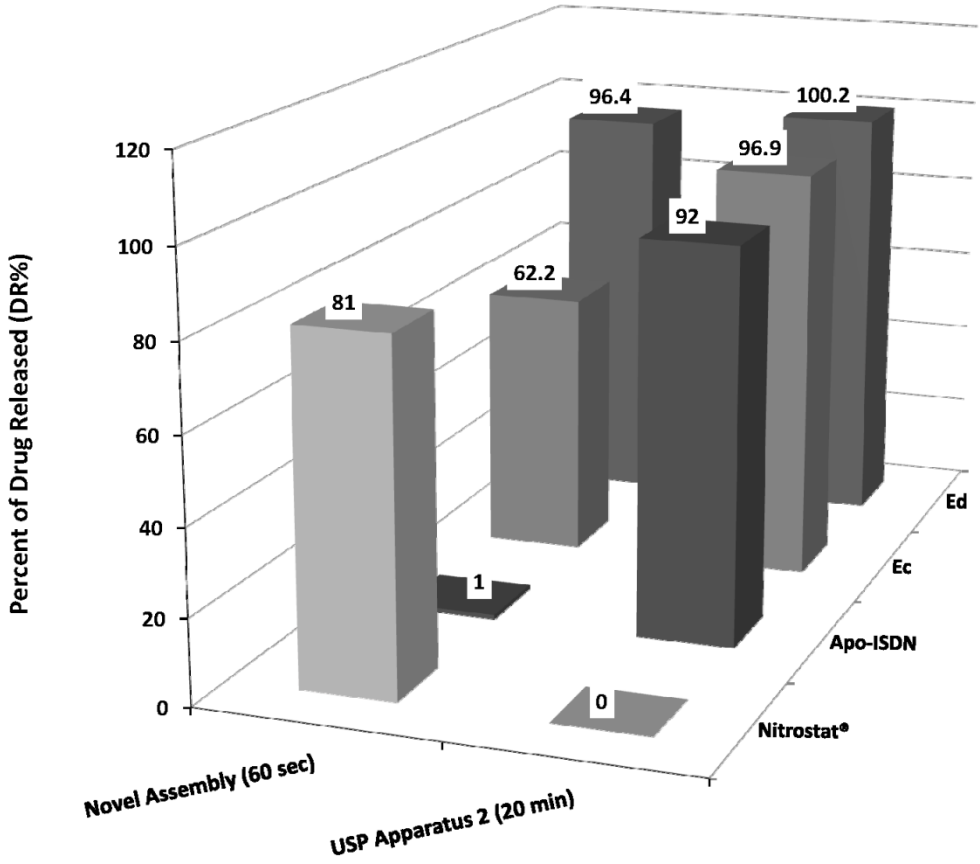


***Figure 6**: Percent of drug released (DR%) from E[c], E[d], Apo-ISDN, and Nitrostat® SL tablets at 60 s using the custom-made apparatus and novel in vitro method, and at 20 min using the official USP apparatus 2 (Paddle) and method. Data represent means of at least three replicates.*



2.5. Discussion

The official compendia apparatus and method for testing dissolution, and even disintegration (Abdelbary et al., 2005), of SL tablets includes the use of large volumes of dissolution medium with constant agitation surrounding the tablets. These conditions do not simulate those in the SL cavity. More bio-relevant dissolution tests for SL tablets were proposed in the 1970s using methods based on in situ concentration

measurement (Gaglia, Lomner, Leonard, & Chafetz, 1976), but few advances have been reported in recent years. Due to the limited number of SL tablet formulations now commercially available, there is limited focus for dissolution testing of this dosage form. In contrast, buccal formulations have been studied extensively, leading to advances in dissolution apparatus, procedures, and techniques (Azarmi, Roa, & Lobenberg, 2007). Although both buccal and SL formulations are intended to be administered within the oral cavity, there are significant differences in design of these formulations which in turn lead to different physicochemical and release characteristics. It is "bio-irrelevant" to apply one single apparatus and method to test the API release of two or more different dosage form categories, i.e., oral versus SL tablets. Instead, the guidelines of the International Pharmaceutical Federation/American Association of Pharmaceutical Sciences recommend that "different apparatus should be employed on a case-by-case basis, and the method should be specific to the dosage form category, formulation type, or even to a particular, individual product" (Siewert et al., 2003). The custom-made apparatus and the novel in vitro method proposed here were specifically designed to evaluate the dissolution of rapidly disintegrating SL tablets.

The dissolution testing consists of two main steps: the dissolution of the dosage form when the API is released and dissolved into the medium, and the measurement of the API content in the samples. These two steps are separated by a filtration step (Crist, 2009) which ensures that the sample for analysis contains only the API in solution, dissolved in the specified time period. The design of our apparatus was based on these concepts. The API was released and dissolved in the dissolution medium inside the glass

funnel, and the samples were instantaneously collected at the specified time-point in the collecting tube following filtration to prevent the passage of undissolved API solid particles and to terminate dissolution.

The reusable parts that came into contact with the API including the glass funnel and the fritted glass base (Fig. 1) were thoroughly cleaned between tests. Any API remaining in the dissolution apparatus was totally removed prior to the next dissolution test in order to maintain the integrity of testing.

Five washings were sufficient to remove virtually 100% of any residual E left in the fritted glass base after the filtration of either low or high concentrations of standard E solutions. Similarly, the potential problem of minimal amounts of E that might have been adsorbed to the nylon filter membrane (Kiehm & Dressman, 2008) was solved by replacing the membrane after each test to prevent carry-over to the next test. Other filter membrane materials (Lindenberg, Wiegand, & Dressman, 2005) could be evaluated for possible reduction in the adsorption of API. The recovery of API could also be improved by discarding a partial fixed volume of filtrate before analysis. This step insures that the available active adsorption sites in the filter membrane are saturated and the subsequent filtration does not further decrease the API concentration in the filtrate (Kiehm & Dressman, 2008).

The percent of drug released and dissolved increased with time from 15 to 60 s and was virtually 100% at 120 s for the representative E 40 mg SL tablet (Fig. 2). The source of variability among time-points could be partially due to variability in tablet

content. Since individual SL tablets had to be used for each time-point as replicates, greater variability was expected due to content variability among tablets, which were always within the USP limits. However, use of the novel in vitro method resulted in reproducible dissolution data, with a coefficient of variation of 5.6% at 60 s and 3.2% at 120 s. Time-points ≥60 s were associated with less variability than those <60 s; consequently, the 60 and 120 s intervals were selected for dissolution testing of SL tablets.

Between-days reproducibility was achieved for both the commercial tablets and the E 40 mg SL tablets under development (Fig. 3). This ensured that the dissolution testing variability among different tablets within the test and among different test days resulted in acceptable consistency using the novel in vitro method.

Our custom-made apparatus required a different tablet to test dissolution at each time-point. Dissolution testing at a range of time-points is required only for modified release formulations and the demonstration of an API release profile over time (Garbacz, Blume, & Weitschies, 2009). This apparatus is intended to evaluate SL tablets at the single time-point recommended for SL tablet administration (Repchinsky, 2009). When assessing drug release from rapidly disintegrating SL tablets, the major objective is to ensure virtually total release of the API for absorption within 120 s or less after insertion into the SL cavity.

The sensitivity of the novel in vitro method to evaluate different SL tablet formulations was tested using both commercial tablets and representative E SL tablets

under development (Fig. 4). It was shown that Apo-ISDN SL tablets released about 1% of the label content after 60 s and not more than 2% after 120 s. The low DR% of Apo-ISDN could result from a poor SL formulation as reported previously for one of the ISDN SL products of slow and/or incomplete disintegration resulting in lack of therapeutic effectiveness (Weda, van Riet-Nales, van Aalst, de Kaste, & Lekkerkerker, 2006). The Apo-ISDN disintegration time was less than 10 s; therefore, the low DR% of Apo-ISDN was anticipated because of the low water solubility of ISDN. Subsequently, the volume of dissolution medium for Apo-ISDN was increased from 2 mL to 10 mL, a volume that is more than sufficient to dissolve the 5 mg dose of ISDN, but is still below "sink" conditions. Even in the presence of 10 mL of dissolution medium, only 8% (0.4 mg out of 5 mg) of ISDN was dissolved (Fig. 4) and would theoretically be available for absorption within the SL cavity. The remaining dose is swallowed and metabolized for ongoing activity via the ISDN metabolites, isosorbide mononitrates, following oral administration. The systemic availability of ISDN after SL and oral tablets was previously reported to be similar, based on plasma levels and area under the plasma concentration versus time curve of the ISDN metabolites (Straehl & Galeazzi, 1985). The only advantage of a SL tablet of ISDN therefore seems to be the rapid disintegration and fast onset of action of the initial 8% of the dose.

The novel in vitro method was also evaluated for its ability to discriminate among representative E 40 mg SL tablets which have similar in vitro DTs. In previous in vitro and in vivo studies, E SL tablet formulations with similar DTs had different bioavailabilities (M. M. Rawas-Qalaji, Simons, & Simons, 2006a). Although the DT test

ensures tablet breakdown into smaller particles, it does not evaluate the rate and extent of the API release, and in general, disintegration has been proved to be a poor indicator of bioavailability (Kumar, 2005). The dissolution assessment is a more selective in vitro test than disintegration for prediction of in vivo behavior. For tablets showing a narrow range of DTs (11–15 s), the novel in vitro method was sufficiently sensitive to identify a significant difference in DR% at 60 s ranging from 58% to 104% (Fig. 5). For example, formulations 4, 5, and 6 showed identical DT (13 s), but the novel in vitro method was able to detect formulation differences resulting in DR% that ranged from 69% to 96% after 60 s.

Using the official USP dissolution method, the concentrations of nitroglycerin could not be detected for Nitrostat® (nitroglycerin 0.6 mg), due to the sensitivity limit of the UV detector for a 0.6 mg in 900 mL dissolution medium (Fig. 6). In 900 mL, a minimum of 50 Nitrostat® SL tablets would be required for UV detection, which is impractical. This small volume custom-made apparatus has the advantage of detecting low dose APIs in commercial SL tablets like Nitrostat®. Using the novel in vitro method, the quantitative levels of nitroglycerin from individual tablets were obtained that could be quantified by UV analysis.

With regard to Apo-ISDN (ISDN 5 mg), a procedure for testing a pooled sample of six tablets in the same dissolution vessel was followed by using the USP dissolution apparatus 2 (United States Pharmacopeial Convention, 2009b). This resulted in 92% of the drug being released after 20 min, which does not necessarily represent the

dissolution within 120 s in 2 mL of saliva in the SL cavity. Using the novel in vitro method, individual Apo-ISDN SL tablets were tested in replicate, and 1% of the drug was released at 60 s (Fig. 6). Since only 2 mL of dissolution medium was available for Apo-ISDN in the custom-made apparatus, ISDN solubility was considered to be the rate limiting step in this process. To provide acceptable "sink" conditions for ISDN 5 mg, at least 15 mL of saliva should be available within the SL cavity which is larger than the normal physiological secretions of saliva in 2 min.

This small-volume custom-made apparatus offers the advantage of testing dissolution in volumes of dissolution medium as low as 2 mL, similar to the average volume of saliva normally secreted over 2 min (Sreebny & Schwartz, 1997). After administration, the SL tablet is maintained in a relatively quiescent environment under the tongue as simulated in this apparatus. The lack of agitation of dissolution medium in our apparatus eliminates the problem of unstable hydrodynamics of small-volume dissolution apparatus, which is a major concern with USP Basket or Paddle apparatuses (Brown et al., 2009; S. Klein & Shah, 2008; Scholz, Kostewicz, Abrahamsson, & Dressman, 2003; Wu, Kildsig, & Ghaly, 2004).

The current design of the apparatus only permits the operation of dissolution testing at room temperature (25°C), but it could be modified to provide the testing of physiological temperatures of 37°C. However, since SL tablets are only exposed to the 2 mL of dissolution medium for ≤120 s, the increased temperature effect on drug dissolution is anticipated to be minimal.

The multi-unit apparatus (Fig. 1b) facilitates testing the dissolution of six SL tablets simultaneously similar to the official USP dissolution apparatus. This novel in vitro method demonstrated day-to-day reproducibility and discrimination among formulations.

2.6. Conclusion

A novel in vitro method is proposed specifically for the assessment of dissolution of rapidly disintegrating SL tablet dosage forms. Data obtained were accurate, reproducible, and significantly different from the data obtained by using the USP method. The effects of minimal changes in formulations on tablet dissolution were readily detected and measured. This novel in vitro method is potentially useful for dissolution testing of rapidly disintegrating tablets using simulated SL conditions.

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CHAPTER III: AN ELECTRONIC TONGUE: EVALUATION OF THE MASKING EFFICACY OF SWEETENING AND/OR FLAVORING AGENTS ON THE BITTER TASTE OF EPINEPHRINE

3.1. Abstract

An epinephrine (E) tablet is under development for sublingual (SL) administration for the first-aid treatment of anaphylaxis; however, the inherent bitterness of E may hinder acceptability by patients, especially children. To assess the degree of E bitterness and to predict the masking effects of sweetening and/or flavoring non-medicinal ingredients (NMIs), the potential usefulness of an electronic tongue (e-Tongue) was evaluated. The e-Tongue sensors were conditioned, calibrated, and tested for taste discrimination. Six standard active pharmaceutical ingredients (APIs) were used to build and validate a bitterness model which was then used to assess E bitartrate (EB) solutions from 0.3–9 mM. Taste masking efficiency of aspartame (ASP), acesulfame potassium (ASK), and citric acid (CA) each at 0.5 mM was evaluated. Using EB 9 mM, the bitterness score was 20 on a scale of 20 (unacceptable) down to 1 (not detected). When NMIs 0.5 mM were added, neither ASK (17.2, unacceptable) nor was ASP (14.0, limit acceptable) effective in masking the bitter taste. When the combination of ASK and ASP was used, the bitterness score was reduced to 9.2 (acceptable). However, the addition of CA alone resulted in the best reduction of the bitterness score to 3.3 (not detected). Using the e-Tongue, the incorporation of a variety of sweetening and/or flavoring NMIs

into a SL tablet of E could be shown to mask its bitter taste by up to 80%. These results should be confirmed by in vivo studies.

3.2. Introduction

Medications that enter the oral cavity, whether orally administered, sublingually administered, or inhaled, should have an acceptable taste. One of the major barriers that prevent patients from following a prescribed medication regimen has been identified as the unpleasant taste of APIs in these dosage forms (Ayenew, Puri, Kumar, & Bansal, 2009).

For a prescription SL tablet, the recommended residence time in the mouth is 2 min or until dissolved (Repchinsky, 2009). Taste may affect the length of time a patient holds a tablet within the SL cavity which in turn may affect compliance. In order to achieve optimal compliance, the taste of a SL tablet should be assessed and improved if necessary to ensure that it is palatable, especially for children (Mennella & Beauchamp, 2008). Taste assessment is usually performed in the early stages of drug development of a new chemical entity (NCE). The taste of the NCE or API may require the addition of sweetening and/or flavoring NMIs to the final formulation.

Epinephrine, a potent vasoconstrictor and bronchodilator with a narrow therapeutic index, is the drug of choice for the treatment of anaphylaxis (Liberman & Teach, 2008; Simons, 2009). For the first-aid, prehospital treatment of anaphylaxis, E is available in autoinjectors including EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA, USA), Twinject 0.3 mg®, Twinject 0.15 mg® (Sciele Pharma, Inc., a Shionogi Company, Atlanta,

GA, USA), Anapen 0.15 mg ®, Anapen 0.3 mg®, and Anapen 0.5 mg® (Lincoln Medical, Salisbury, UK). A fast disintegrating SL tablet formulation of E has been successfully formulated in our laboratory (Rawas-Qalaji, Simons, & Simons, 2006b). The bioavailability profile of this E formulation is similar to that of an intramuscular (IM) injection of E (Rawas-Qalaji, Simons, & Simons, 2006a; Rawas-Qalaji, Simons, & Simons, 2006c). The SL tablet formulation has not been approved for administration to humans. Accordingly, at this early stage of development, assessment of its taste by using human sensory analysis panels (SAPs) is not an option.

Taste assessment using a multichannel taste sensor, an instrument commonly named the electronic tongue (e-Tongue), is becoming established as a novel alternative to human SAPs. A number of pharmaceutical laboratories around the world are using this instrument to assess the bitterness of NCEs/APIs and the masking efficiency of NMIs. In addition, it is used in placebo development, in taste matching of formulations, and in unknown-to-reference comparisons (Kayumba et al., 2007; Li, Naini, & Ahmed, 2007; Lorenz, Reo, Hendl, Worthington, & Petrossian, 2009; Sadrieh et al., 2005; Takagi, Toko, Wada, & Ohki, 2001; Tokuyama et al., 2009; Zheng & Keeney, 2006). The e-Tongue consists of an array of liquid electrochemical sensors coated with an organic membrane that governs the sensitivity and selectivity of each individual sensor. The αAstree e-Tongue (Alpha M.O.S., France) is a fully automated taste analyzer equipped with a seven-sensor probe assembly that is based on the chemical modified field-effect transistor (ChemFET) technology for liquid sample analysis (Alpha MOS, 2004; Mifsud & Lucas, 2003).

The degree of bitter taste of the E SL tablet has not yet been evaluated. The purpose of this study was to assess the potential of the e-Tongue to determine the degree of E bitterness and to evaluate the taste-masking effect of sweetening and/or flavoring NMIs.

3.3. Methods

3.3.1. Materials

Epinephrine bitartrate and ASP were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aspartame and CA anhydrous were purchased from Fisher Scientific (Nepean, ON, Canada). Paracetamol, caffeine monohydrate, quinine hydrochloride (HCl), loperamide HCl, and famotidine were purchased from MP Biomedicals (Solon, OH, USA). Prednisolone metasulfobenzoate sodium was purchased from Science Lab (Houston, TX, USA). Hydrochloric acid (HCl 0.1 and 1 M), sodium chloride (NaCl 0.1 and 1 M), and monosodium glutamate (MSG 0.1 M) solutions were provided by Alpha M.O.S. All chemicals were of analytical grade and used without further purification.

3.3.2. Equipment

The αAstree e-Tongue (Alpha M.O.S., France) used in all experiments is equipped with a 48-position auto-sampler, a bitterness prediction module (BPM) software, and a seven-sensor probe assembly (reference number 803-0070: sensors BD, EB, JA, JG, KA, OA, and OB), specifically developed to detect and predict bitter taste, with the Ag/AgCl reference electrode from Metrohm AG.

3.3.3. Selection of an Appropriate Concentration Unit Based on the Molecular Assumption

Since potentiometric differences created by the sensors and the Ag/AgCl reference electrode are based on molecular interactions between the molecules in solution and the molecules of the sensor membrane material, concentrations were presented as millimole per liter (mM). One mole of any substance contains the Avogadro's number of atoms or molecules. By calculating the quantities of samples based on molar concentrations, precise molecular ratios can be calculated giving accurate estimates of quantities of flavors and/or sweeteners needed for masking effects.

3.3.4. Sample Preparation and e-Tongue Operational Conditions

Each series of experiments consisted of three main procedures: e-Tongue preparation and training, sample preparation and analysis, and data processing and statistical analysis (Table 1). All samples were weighed using an analytical balance (±0.5 mg precision) and completely dissolved in appropriate volumes of non-deionized distilled water at 25°C to obtain the desired concentrations and taste attributes (Table 2). Each of the e-Tongue testing beakers was loaded with 25 mL of the appropriate, particle-free solution. The reference electrode and the seven-sensor assembly were immersed into each testing beaker for an acquisition time of 120 s. This was followed by sequential immersion into two rinsing beakers containing fresh non-deionized distilled water for 10 s each to prevent any cross-contamination or carry-over residues from

previous samples. This series of tests was repeated six times in rotation. The first two replicate measurements of the test solution were for sensor training purposes and the readings from the last four replicates were used for data analysis. The potentiometric difference created between each individual sensor and the reference electrode was measured and recorded by the e-Tongue BPM software. All samples were analyzed at room temperature.

***Table 1**: Summary of the procedure followed for each series of experiments.*

Major Steps	Sub-steps
I] e-Tongue preparation and training	A] Sensors conditioning and calibration. B] Sensors taste discrimination ability. C] Building and validating the bitterness standard model.
II] Sample preparation and analysis	D] Preparation of EB (0, 0.3, 3, 9 mM) solutions. E] Predicting EB bitter taste. F] Preparation of EB 9 mM + NMIs 0.5 mM (ASP, ASK and CA) solutions. G] Assessment of NMIs masking effect on EB.
III] Data processing and statistical analysis	H] Building data libraries. I] Data analysis using multivariate algorithms: 1. Principle component analysis (PCA). 2. Partial least-squares (PLS).

EB = Epinephrine Bitartrate, ASP = Aspartame, ASK = Acesulfame Potassium, CA = Citric Acid, NMIs = Non-Medicinal Ingredients

***Table 2**: Formulations prepared for taste analysis by the e-Tongue.*

Samples	Contents (concentration in mM)	Taste attribute(s) in order
API	EB (0.3, 3, or 9)	Bitter
Formulation 1	EB (9), ASK (0.5)	Bitter, sweet
Formulation 2	EB (9), ASP (0.5)	Bitter, sweet
Formulation 3	EB (9), ASK (0.5), ASP (0.5)	Bitter, sweet, sweet
Formulation 4	EB (9), ASK (0.5), ASP (0.5), CA (0.5)	Bitter, sweet, sweet, sour
Formulation 5	EB (9), CA (0.5)	Bitter, sour

API = Active Pharmaceutical Ingredient, EB = Epinephrine Bitartrate, ASK = Acesulfame Potassium, ASP = Aspartame, CA = Citric Acid

3.3.5. Sensor Array Conditioning and Calibration

The best long-term storage environment for the sensitive e-Tongue sensors is in the dry state so they must be conditioned and hydrated before each use. Sensor conditioning is needed to check the signal stability of each individual sensor. Following a procedure prescribed by Alpha M.O.S., three beakers each containing 25 mL of 10^{-2} M HCl reference solution were used to condition the sensors and the reference electrode for 300 s in each immersion. The pass criterion was to achieve stable signals for all seven sensors with minimal or no noise or drift. This was a prerequisite prior to the calibration procedure. Due to the chemical nature of the samples and the sensitivity of the sensor array used in this study, the conditioning step was repeated 12 times at the beginning of every working week following ≥2 days of sensor storage in the dry state.

To ensure consistency and reproducibility of data produced from the e-Tongue, each individual sensor was calibrated to a known numerical value before use. Each sensor required its own target value and a previously defined error limit. The calibration step ensured that the output response of each sensor did not exceed the maximum error allowed. According to the calibration procedure prescribed by Alpha M.O.S., one beaker containing 25 mL of 10^{-2} M HCl reference solution was used to calibrate the sensors for 120 s for each immersion. The calibration step was performed after every successful conditioning step. The pass criterion for the calibration step was to have all sensors adjusted to their target values within the specified error limit.

3.3.6. Taste Discrimination Ability of the Sensor Array

The e-Tongue must be trained to identify distinctive tastes to ensure it is working optimally. A diagnostic procedure using HCl, NaCl and MSG each at a concentration of 10^{-1} M representing sourness, saltness, and umami tastes, respectively, was performed. The pass criterion required a discrimination index of at least 0.94 with compound clusters being visibly separated from each other on a principal component analysis (PCA) map.

3.3.7. Building and Validating a Bitterness Standard Model

A 1 to 20 range was used to associate the bitterness intensity of different APIs with scores (Table 3). The specific type of sensors used in this study was designed to detect the bitter taste of APIs and correlate their measurements with the bitterness intensities of these standardized APIs. For this purpose, several APIs as references have been tasted in vivo at several concentrations by human SAPs and the bitterness scores were provided by Alpha M.O.S. (Table 4). To examine the correlation between in vivo measurements and the e-Tongue measurements, the same APIs were analyzed by the e-Tongue in the current experiments. The correlation of both data measurements was achieved using an inverse standard model based on partial least-squares (PLS) analysis. This bitterness standard model should have a correlation coefficient (r^2) of 0.8 or more (Alpha MOS, 2004). As shown in Table 4, caffeine, paracetamol, prednisolone, and quinine each at two different concentrations were used to build the bitterness standard

model. This model was validated using loperamide and famotidine, each at two different concentrations (Table 4).

***Table 3**: Bitterness intensity levels with corresponding scores used in building the bitterness standard model.*

Bitterness Intensity level	Corresponding Score	
	From	*To*
Taste not detected	1	4.5
Slight taste	4.5	8.5
Acceptable	8.5	12.5
Limit Acceptable	12.5	16.5
Not Acceptable	16.5	20

***Table 4**: The in vivo sensory analysis panel (SAP) scores obtained for reference active pharmaceutical ingredients (APIs) at each concentration used either to build or validate the bitterness standard model as provided by Alpha M.O.S.*

Reference APIs	Used to Build*	Used to Validate*	Concentration (mM)	In vivo Score
Caffeine	√		0.24	2.5
			2.36	8.5
Paracetamol	√		3.31	4
			19.85	11
Quinine	√		0.03	9
			0.12	15.5
Prednisolone	√		0.44	13.5
			0.88	17
Loperamide		√	0.002	7.5
			0.01	14
Famotidine		√	0.06	4.2
			0.15	9

**The reference active pharmaceutical ingredients (APIs) that were used either to build or to validate the bitterness standard model.*

3.3.8. Predicting the Bitter Taste of Epinephrine

The E base was only slightly soluble in water (Keef, 2005) therefore E water-soluble salts (hydrochloride and bitartrate) were used instead for medical applications (Sciarra, Patel, & Kapoor, 1972). The bitartrate salt of E (EB) was used in our continuing

studies because it is readily obtainable as the pure L-isomer, the pharmacologically active form used in the E SL tablet formulations. Numerous studies of various concentrations of EB were carried out to determine the e-Tongue threshold of EB. Ultimately in order to assess the degree of E bitterness, three solutions with increasing concentrations of EB (0.3, 3, and 9 mM) were analyzed by the e-Tongue and compared to a negative control of water containing no EB. Analysis of each solution was repeated at least three times.

3.3.9. Bitterness Masking of Epinephrine

To mask the bitter taste of EB, different NMIs were added to EB solutions. Based on critical and extensive review of the available NMIs used for taste-masking/improvement, ASP and ASK were selected as artificial sweeteners and CA as a flavor. Numerous studies of EB 9 mM plus various concentrations of NMIs were carried out to select the optimal ratio of these agents. In the definitive studies, all NMIs, each alone or in combination, were used at a concentration of 0.5 mM and added to EB 9 mM using the same sample analysis procedure described in the e-Tongue operational conditions. Analysis of each solution was repeated at least three times.

3.3.10. Data Processing and Statistical Analysis

Due to the complexities of analyzing the output data from several sensors for more than two samples, all data were processed and analyzed using the αAstree software provided by Alpha M.O.S. except for some primary data interpretation that was done using Microsoft Excell software following Alpha M.O.S. recommendations. The

αAstree software reduces the number of variables created by the sensors when analyzing a given sample. Data reduction allows responses of the seven sensors to be processed and displayed in two- or three-dimensional maps. These tools are known as multivariate statistic algorithms to determine which of the differences between samples are important to identify unknown samples, to predict sensory intensities, or to quantify substance concentration of unknown samples. The PCA and the PLS multivariate statistic techniques were used in this study. The PCA technique was used to assess discrimination performances of the sensors when examining their taste discrimination abilities. The PCA summarizes the information contained in the database into individual principle components (PCs) which are linear combinations of the original variables. For every sample analysis, the two PCs with most informative results are used to create the PCA map. The efficiency of the PCA map of a given group of samples is measured with the discrimination index. The closer the index to 100%, the more efficient the PCA map is. The PLS technique was used to quantify the intensity of the bitter taste of the samples assessed including the references and the samples. The PLS map is considered valid if the correlation coefficient is greater than 0.8 (Alpha MOS, 2004). This PLS map is then used to predict the bitterness intensities of unknowns. To obtain reproducible data, the relative standard deviations of each individual sensor type and in every analysis and experiment were confirmed to be below 3%.

3.4. Results and Discussion

3.4.1. Sensor Array Conditioning and Calibration

The organic coating membrane of the sensors must be completely hydrated in order to allow possible interactions between the sample molecules dissolved in liquid and the sensitized molecules of the coating membrane covalently bound to the solid electrochemical sensor. All sensors showed stable signals (Fig. 1a) in the sensor array conditioning step and among the experiments with minimal noise and drift. The output from the seven sensors was successfully adjusted to the default target intensity value (Fig. 1b). These are predetermined values that were set by default for every individual sensor (Table 5).

3.4.2. Taste Discrimination Ability of the Sensor Array

The human tongue can recognize five basic tastes: salt, sour, sweet, bitter, and umami. The umami taste is commonly referred to the taste of MSG first described by Kikunae Ikeda (Ikeda, 2002) and widely used as a flavor enhancer. These taste attributes were tested using the e-Tongue in order to determine its ability to differentiate between tastes: salt, sour, and umami. A mean discrimination index of 97.8% was obtained from 11 repetitions throughout all experiments performed to achieve the objectives of this study using the e-Tongue (Fig. 2).

***Figure 1**: Sensor array conditioning and calibration (repeated 12 times at the beginning of each working week and 3 times for each analysis). **a)** Example of a stable signal for the sensor array used in this study. **b)** Example of a successful calibration (hydration) step in which the numerical values of all sensors were adjusted to their target values. BD, EB, JA, JG, KA, OA, and OB are sensor types (Letter designations are Alpha M.O.S. identification codes).*

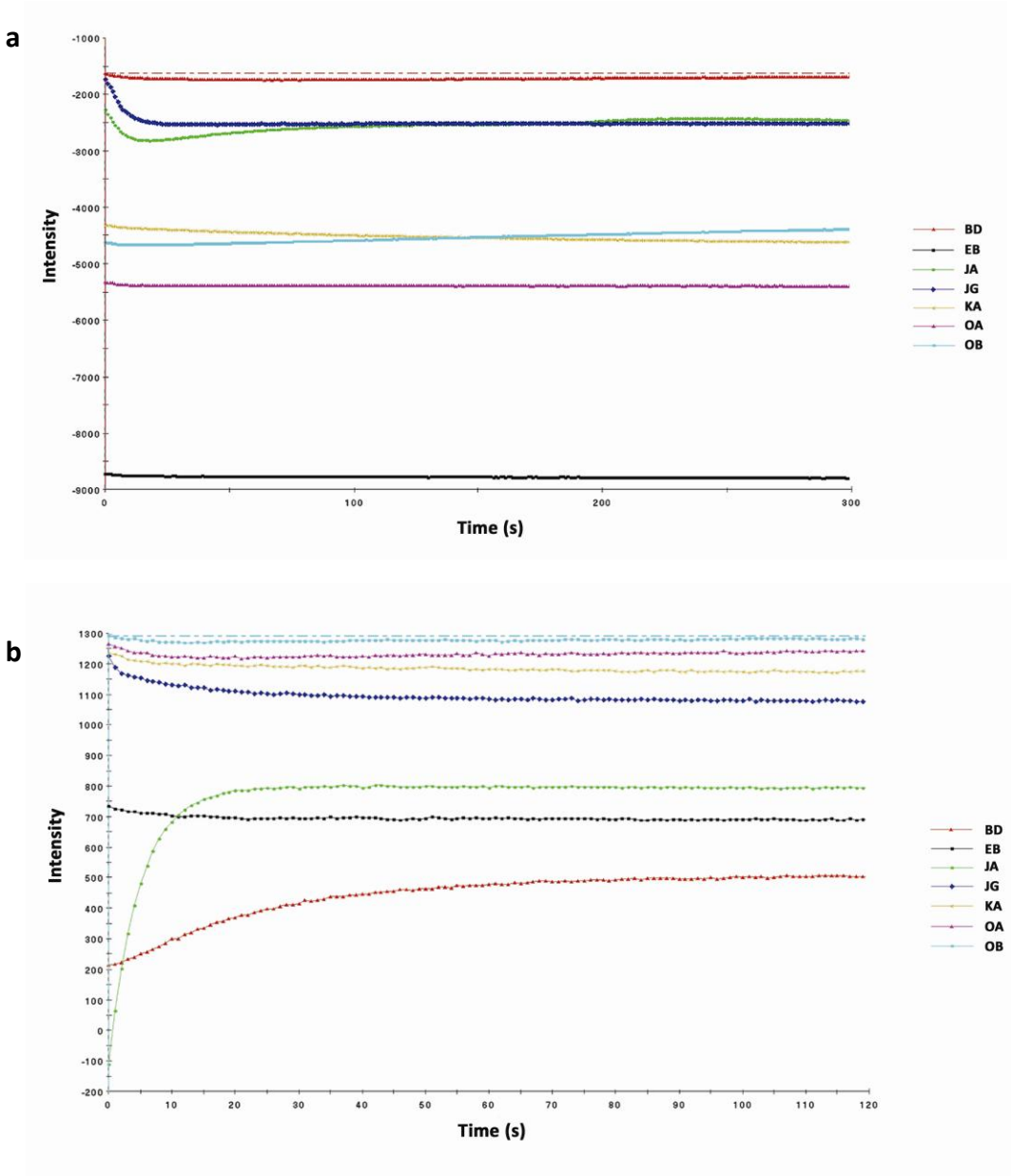


***Figure 2**: Example of a successful taste discrimination test (repeated 11 times throughout the study) having a discrimination index of 97.2%. The three different taste compounds (NaCl, HCl, and MSG) were discriminated from each other into separate space locations in a two-dimensional principal component analysis (PCA) map.*

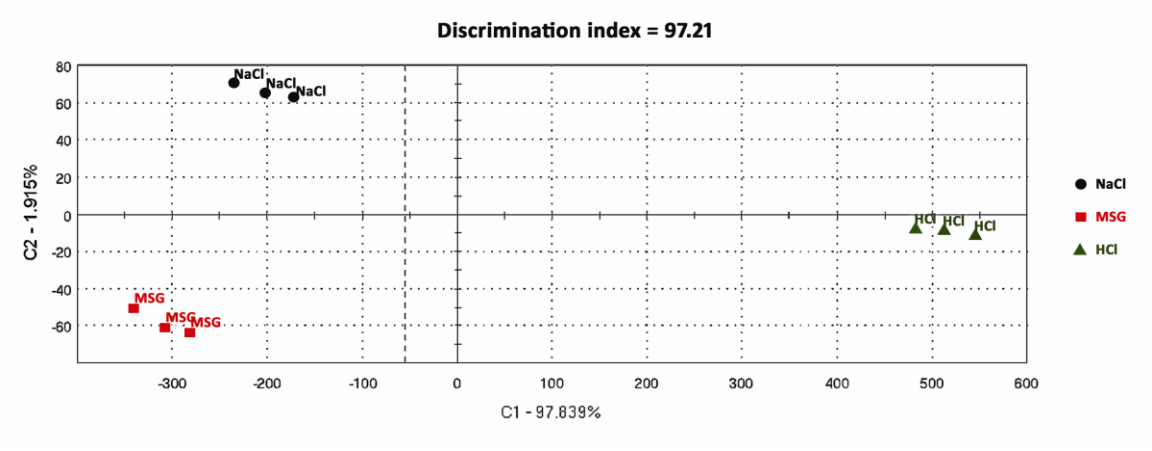


***Table 5**: The target and actual achieved values for each individual sensor used in this study.*

Sensor type*	Target value (mV)	Achieved value (mV)	Difference (mV)	Error (%)	Pass/Fail
BD	500	504.00	-4	0.80	Pass
EB	700	690.30	9.7	1.39	Pass
JA	800	794.68	5.32	0.67	Pass
JG	1080	1079.05	0.95	0.09	Pass
KA	1200	1174.52	25.48	2.12	Pass
OA	1250	1239.74	10.26	0.82	Pass
OB	1300	1281.54	18.46	1.42	Pass

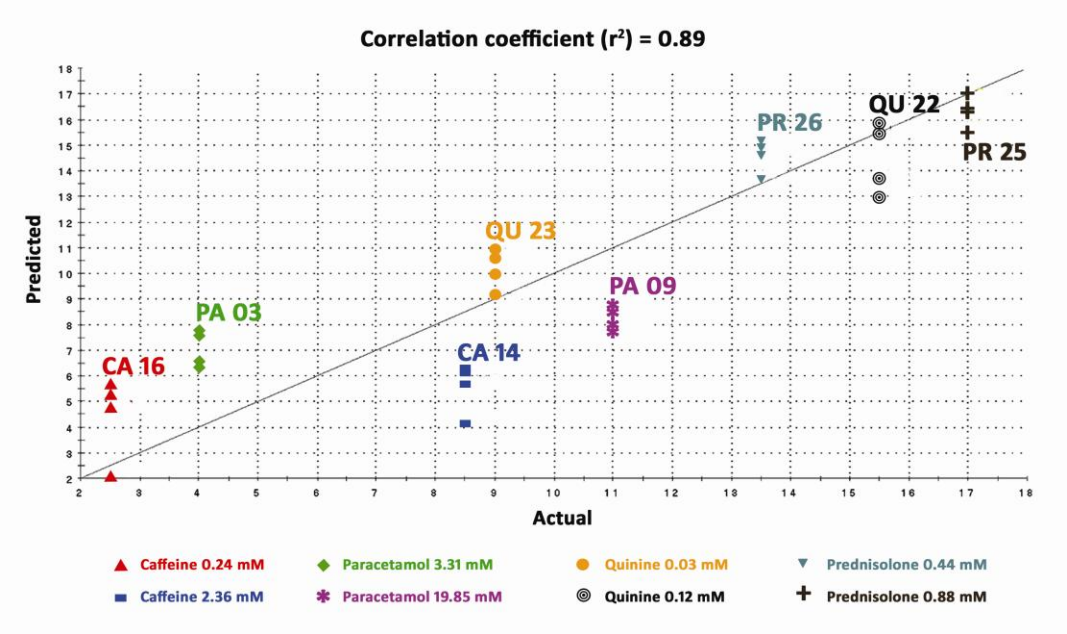
**Letter designations are company identification codes*

3.4.3. Building and Validating a Bitterness Standard Model

Actual bitterness scores (Table 4) from in vivo studies (Alpha M.O.S.) were compared to predicted bitterness scores from the e-Tongue (Fig. 3). In all standard models built throughout the experiments, the correlation coefficient (r^2) obtained was always above 80% which is the acceptable criterion for a successful model (Alpha MOS,

2004). Absolute differences (Δ) between actual and predicted scores were calculated and were always within the limits specified by Alpha M.O.S. For the four standard drugs used to build the bitterness standard model, Δ was always <2.5; and for those two standard drugs used to validate the model, Δ was always <5.

***Figure 3**: Example of a successful bitterness standard model (r^2=89%). The straight line shown represents an ideal 100% correlation and the colored points are the predicted e-Tongue measurements in comparison with the actual in vivo sensory analysis panel (SAP) measurements for four standards (caffeine, paracetamol, quinine, prednisolone). The other two standards (loperamide, famotidine) were used to validate the bitterness standard model (validation results are not shown). The model was repeated 3 times in each analysis.*



3.4.4. e-Tongue Threshold and Concentration Determination of Epinephrine Bitartrate

A number of studies were performed to determine the threshold of the e-Tongue sensors to an appropriate range of EB concentrations that could be evaluated

using this instrument. Based on the results from studies of other bitter APIs, an initial concentration of EB 60 mM was tested using the e-Tongue which resulted in consistent bitterness scores of >20, above the maximum level, indicating that EB has an intensely bitter taste. The concentration was reduced systematically until within-range bitterness scores of ≤20 were obtained for EB. These relatively low concentrations (0.3, 3, 9 mM) were selected for evaluation. The EB 9 mM concentration was selected as the maximum strength to evaluate a series of bitterness-masking NMIs.

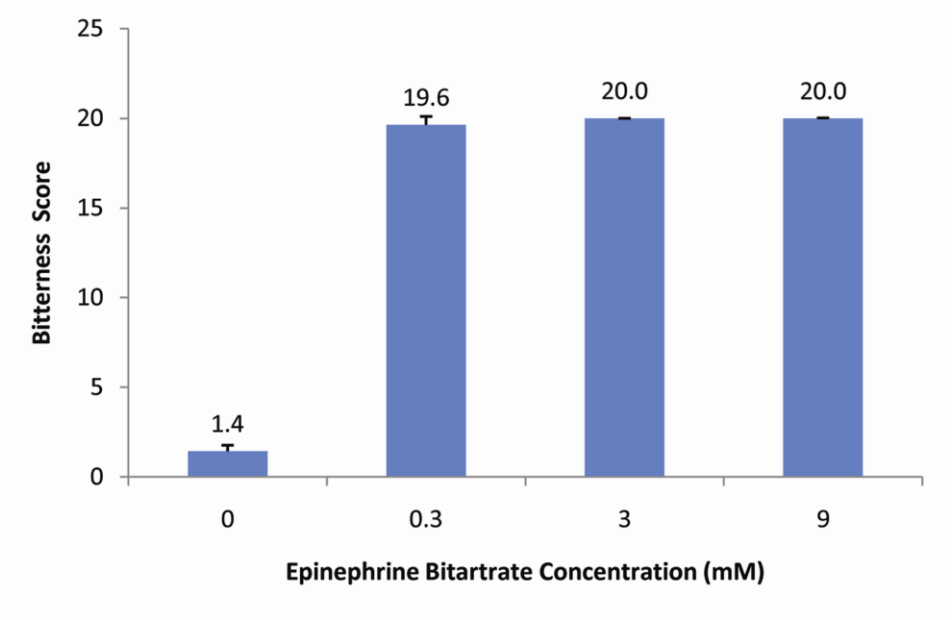
3.4.5. Reproducibility and Method Modifications

Lack of reproducibility of results obtained from the e-Tongue was observed after several taste-masking studies of EB 9 mM with all NMIs. In order to obtain reproducible results from the e-Tongue, major adjustments were made to several procedural steps. The one time hydration or conditioning of e-Tongue sensors was increased to 12 times. Instead of immersion in one rinsing beaker of water between active samples for evaluation, the sensors were sequentially immersed in two beakers of water for 10 s in each, following each sample analysis. The intense bitterness of EB almost overwhelmed the sensors and the offset sensor values had to be readjusted using strict, default, and large calibration levels. These levels were evaluated for the parameters that best reproduced the results from the e-Tongue. The parameters define the maximum allowed dispersion or drift of within and across each sensor's responses.

3.4.6. Predicting the Bitter Taste of Epinephrine Bitartrate

As expected, EB solutions resulted in high scores of bitterness (Fig. 4). Even the lowest, EB 0.3 mM, resulted in a 19.6±0.5 bitterness score which indicated an unacceptable bitter taste. The intensely bitter taste of EB required an efficient taste masking approach to enable the formulation of palatable SL tablets. This approach should lack any heating or moistening process that may affect the chemical stability of the heat and moisture-labile EB. The addition of intense sweeteners and/or flavors to EB was identified as the most suitable approach to mask EB bitterness and was assessed in this study.

Figure 4*: Bitterness scores (n=6) for epinephrine bitartrate (EB) at three different concentrations. A blank of water (containing no EB) was used as a negative control.*



3.4.7. Bitterness Masking of Epinephrine

Two artificial sweeteners (ASP and ASK) were selected to mask the unacceptable highly bitter taste of EB. ASP and ASK have an approximate sweetening power of 180–200 times that of sucrose (Rowe, Sheskey, & Weller, 2003). Aspartame, a first-generation artificial sweetener, enhances flavor systems and can be used to mask some unpleasant taste characteristics. It is widely used in medications including Feldene Melt (piroxicam), Maxalt-MLT (rizatriptan), Pepcid RPD (famotidine), Zyprexa Zydis (olanzapine), Zofran ODT (ondansetron), and Nulev (hyoscyamine) (Goel, Rai, Rana, & Tiwary, 2008). Acesulfame potassium, a second- or new-generation artificial sweetener, is widely used as a sugar substitute in compounded formulations and as a toothpaste sweetener (Rowe et al., 2003). Although artificial sweeteners have been reported to show toxic, mutagenic, or carcinogenic effects, results are inconsistent due to poor study design (Hagiwara, Fukushima, Kitaori, Shibata, & Ito, 1984; Ishii, 1981; B. Magnuson & Williams, 2008). Toxicities related to ASP or ASK were observed at doses many fold greater than those proposed here (Bandyopadhyay, Ghoshal, & Mukherjee, 2008; B. A. Magnuson et al., 2007; Whitehouse, Boullata, & McCauley, 2008). Minute quantities of these sweeteners could be safely incorporated into a SL tablet formulation of E developed in our laboratory with minimal effect on the in vitro characteristics of these tablets.

Citric acid was selected as a flavoring agent to be added to EB due to its wide use, safety, and acceptance by children who prefer the sour “lemon” taste over the

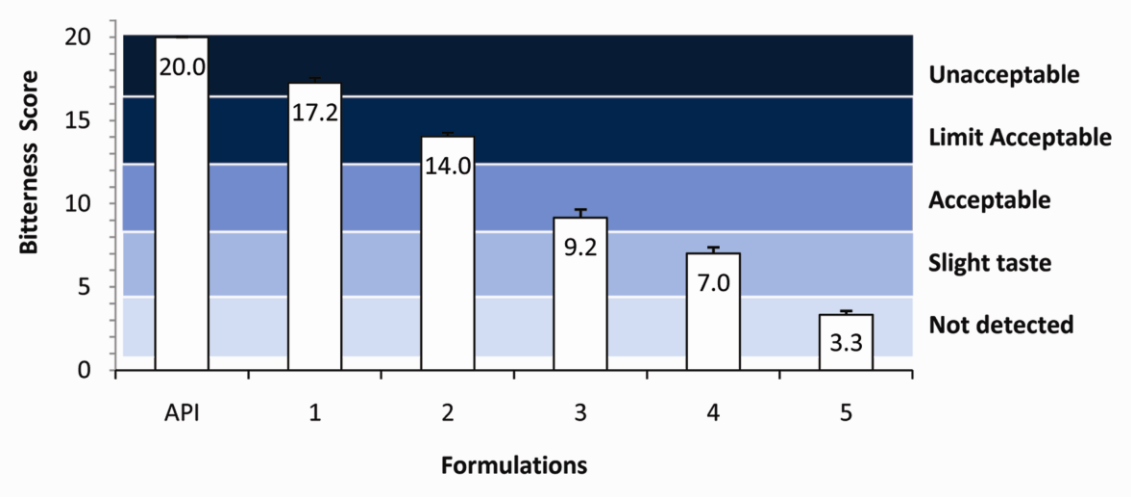
sweet (Liem, Westerbeek, Wolterink, Kok, & de Graaf, 2004). Citric acid is widely used in a number of FDA-approved products available in the market like Remeron Soltab (mirtazepine) and Zoming ZMT (zolmitriptan) (Goel et al., 2008).

The ratio of EB to these NMIs must be appropriate for use in formulation of E SL tablets. Accordingly, ASP was first used for taste-masking studies at 0.1, 0.5, and 5 mM reducing the bitterness score of EB 9 mM to 14.4, 14.0, and 13.9, respectively. From these results, it seemed that ASP 0.1 mM partially masked the bitterness score of EB 9 mM but there was no apparent increased effect upon increasing the concentration of ASP. Similar concentration-independent masking trends were observed with either ASK or CA alone when added to EB 9 mM. The NMI 0.5 mM concentration was selected for further studies because when tested against EB 9 mM, a ratio of around 1:30 (NMI:API) was achieved for either CA:EB or ASK:EB and of around 1:20 for ASP:EB, (based on a milligram scale) which was feasible to achieve in the tablet formulation.

Aspartame and ASK, alone or in combination, at a concentration of 0.5 mM were added to a EB 9 mM solution resulting in a maximum bitterness-masking effect of more than 54% when both sweeteners (Formulation 3) were used (Fig. 5). Neither of the sweeteners alone at the concentration 0.5 mM (Formulations 1 and 2) improved the bitterness intensity from “unacceptable” to “acceptable” but did in combination (Formulation 3) suggesting a synergistic effect. This combination of ASP and ASK was much more effective in reducing the bitterness score of EB 9 mM than increasing the concentration of either of them when used alone.

When CA 0.5 mM was added to the combination of ASP and ASK (Formulation 4) the masking effect increased to almost 65% reducing the bitter taste of EB 9 mM from "unacceptable" to "slight taste". Citric acid alone (Formulation 5) was able to inhibit the intense bitter taste of EB 9 mM by more than 80% from "unacceptable" to "not detected" (Fig. 5). The CA results were consistent with previous reports in that bitter taste-masking effects of acidic substances are pH- rather than concentration-dependent. It was also found that acidic substances have an inhibitory effect on one of the human bitter taste receptors found in the tongue (Sakurai et al., 2009).

Figure 5*: Bitterness scores (n=6) and intensity levels of the formulations in comparison with epinephrine bitartrate (EB) 9 mM as a positive control. Formulations contained EB alone or in combination with acesulfame potassium (ASK), aspartame (ASP), and/or citric acid (CA) as following: API (EB 9 mM), 1 (EB 9 mM, ASK 0.5 mM), 2 (EB 9 mM, ASP 0.5 mM), 3 (EB 9 mM, ASK 0.5 mM, ASP 0.5 mM), 4 (EB 9 mM, ASK 0.5 mM, ASP 0.5 mM, CA 0.5 M), 5 (EB 9 mM, CA 0.5 mM).*



Based on the assumption that interactions occur among molecules in solution and with molecules of the sensor coatings, every molecule of EB could interact with one molecule of the masking agent for complete masking efficacy, e.g., EB 9 mM would require NMI 9 mM for 100% masking effect. However, this assumption alone cannot explain the results reported above, so other mechanisms of masking effect are likely involved. In addition to the molecular interaction assumption, the masking effects could be explained by the different affinities EB and the NMIs might have toward each other and toward the sensor coatings.

From our results, it can be seen that neither ASK (Formulation 1) nor ASP (Formulation 2) alone at 0.5 mM was effective in masking the bitter taste of EB 9 mM. Even the combination of ASK and ASP (Formulation 3) did not reduce the bitterness intensity level of EB 9 mM to "not detected". These results could be explained by the slightly bitter aftertaste these sweeteners have (Rowe et al., 2003) which appeared to be masked by the addition of CA 0.5 mM (Formulation 4). However, CA 0.5 mM alone (Formulation 5) resulted in the best masking of the bitterness of EB 9 mM of >80% from "unacceptable" to "not detected".

3.5. Conclusion

We have demonstrated that the e-Tongue is a useful tool in taste assessment, enhancement, and masking studies for an intensely bitter substance such as EB. The e-Tongue has the potential to screen different NMIs to determine the agent that best masks the unpleasant taste of the API, especially in the early stage of drug and

formulation development. To our knowledge, this is the first study showing the degree of EB bitterness and the taste-masking effect of sweetening and/or flavoring NMIs using the e-Tongue.

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CHAPTER IV: RAPIDLY-DISINTEGRATING SUBLINGUAL TABLETS OF EPINEPHRINE: ROLE OF NON-MEDICINAL INGREDIENTS IN FORMULATION DEVELOPMENT

4.1. Abstract

Epinephrine (E) is the drug of choice in the management of anaphylaxis. For first-aid treatment in the community, E autoinjectors (E-autos) are commonly prescribed, but are underutilized. In our laboratory, we developed a series of first-generation rapidly disintegrating sublingual tablets (RDSTs) containing E 40 mg. One RDST had similar bioavailability to E 0.3 mg from an autoinjector, as confirmed in a validated rabbit model, while other formulations containing different non-medicinal ingredients (NMIs) and with similar in vitro characteristics demonstrated much lower bioavailability. Subsequently, we evaluated the effect of changing the grade and proportion of NMIs, specifically mannitol and microcrystalline cellulose (MCC), on the in vitro characteristics of second and third-generation RDSTs. Weight variation, content uniformity, breaking force, and friability were tested using official United States Pharmacopeia (USP) methods. Novel validated methods that simulate ambient conditions of the sublingual (SL) cavity were developed to test disintegration time, wetting time, and dissolution. Using these methods, it was possible to measure the effects of making small changes in NMIs on the in vitro characteristics of the formulations. The RDST formulation that resulted in the best in vitro characteristics contained the optimum proportion of

mannitol and a specific ratio of coarse and fine particle grades of MCC. Appropriate comparative testing resulted in the selection of the RDST with the optimum in vitro characteristics.

4.2. Introduction

Anaphylaxis is a severe allergic reaction that is rapid in onset and may lead to death (Simons et al., 2011). In the first-aid treatment of anaphylaxis in the community, E is commonly administered through an autoinjector in the mid-outer thigh. Epinephrine autoinjectors are under-utilized for a number of reasons including the limited number of fixed prefilled doses available, short shelf-life, anxiety associated with use of needles, errors, unintentional injections, and injuries due to incorrect administration technique (Sicherer, Forman, & Noone, 2000; Simons, 2004; Simons, 2005). Currently, E-autos are only available in two fixed prefilled doses (E 0.15 mg and 0.3 mg); therefore, no optimal dose exists for infants or young children weighing less than 15 kg, or for large adult patients at risk of anaphylaxis in the community. Unused E-autos need to be replaced every year due to the poor stability of E in aqueous solution.

There is increased interest in developing novel, non-invasive E dosage forms that will provide E plasma concentrations equivalent to those obtained after use of E-autos, will be available in a range of doses, will have a long shelf-life, and be free from needle anxiety and the possibility of error, unintentional injection, and injury.

Sublingual administration of E has the potential to fulfill these requirements. The thin mucosal layer and abundant blood supply in the SL area facilitate rapid E absorption

and systemic distribution. Epinephrine tablets intended for SL administration can potentially be formulated in a wide range of doses. They will be easy to administer, palatable, and will have a long shelf-life, as E is more stable in solid dosage form than in solution.

The design and development of a series of RDSTs of E using direct compression is a major focus of our research (M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji, Simons, & Simons, 2007). One first-generation (G1) E 40 mg tablet administered sublingually in a validated rabbit model resulted in plasma E concentrations not significantly different to those obtained following E 0.3 mg IM dose from an E-auto (M. M. Rawas-Qalaji, Simons, & Simons, 2006c). The bioavailability of E following SL administration of E 40 mg dose from other G1 RDSTs with similar in vitro characteristics was significantly reduced by variations in the composition of NMIs in the tablets (M. M. Rawas-Qalaji, Simons, & Simons, 2006a). The effect of the NMIs was not predicted based on then current in vitro testing (M. M. Rawas-Qalaji, Simons, & Simons, 2006a; M. M. Rawas-Qalaji, Simons, & Simons, 2006c). Therefore, there was a need to develop an in vitro test to measure the effect that small changes in formulation might have on in vivo bioavailability (Rachid, Rawas-Qalaji, Simons, & Simons, 2011).

The selection of type, grade, and proportion of NMIs is critically important in tablet formulations manufactured by direct compression. This is especially true of diluents, which constitute the largest proportion of the powder matrix used in tablet preparation (Shangraw, 1991). A successful tablet formulation has acceptable weight

variation and content uniformity to ensure consistent dosing; sufficient hardness and minimal friability to withstand manufacturing, shipping, and handling; rapid disintegration and high dissolution rates. Such RDSTs must release the active pharmaceutical ingredient (API) for absorption after its dissolution within the SL cavity, in the presence of relatively small volumes of saliva.

The purpose of the study reported here was to evaluate the effect of a range of selected NMI grades and proportions on the rate and extent of E release from a series of second-generation (G2) RDSTs, using reliable in vitro assessment. Additionally, the specific NMI grades and proportions evaluated in G2 tablets were combined with taste-masking ingredients, which were evaluated using an electronic tongue (Rachid, Simons, Rawas-Qalaji, & Simons, 2010) before developing and evaluating third-generation (G3) RDSTs.

4.3. Materials and Methods

4.3.1. Materials

The pharmacologically active pure L-isomer (-)-epinephrine (+) bitartrate used in all E tablet formulations was purchased from Sigma-Aldrich (St. Louis, MO). The following NMIs were purchased from or kindly supplied by the manufacturers and used as received: Ceolus® (MCC), grades PH-301, PH-M-06, and KG-802 (Asahi Kasei Chemicals Corp, Tokyo, Japan), Pearlitol® (100% mannitol), grade 400 DC (Roquette America, Inc., Keokuk, IA), and Ludiflash® (~88% mannitol) (BASF The Chemical Company, Ludwigshafen, Germany) as fillers; low-substituted hydroxypropyl cellulose,

grade LH11 (Shin-Etsu Chemical Co., Tokyo, Japan) as superdisintegrant; citric acid monohydrate (Mallinckrodt Specialty Chemicals Co., Paris, KY) for taste-masking; and magnesium stearate (Mallinckrodt Baker, Phillipsburg, NJ) as lubricant.

4.3.2. Preparation of Rapidly-Disintegrating Sublingual Tablets of Epinephrine

4.3.2.1. Powder Compositions

The ratio, proportion, and composition of the powder mixtures used to manufacture the G2 and G3 RDSTs (F_n, where n is the formulation number) are shown in Tables 1 and 2, respectively. Epinephrine bitartrate 72.77 mg, equivalent to E 40 mg, was used in all formulations (M. M. Rawas-Qalaji, Simons, & Simons, 2006a). The ratio of total MCC to low-substituted hydroxypropyl cellulose was always maintained at 9:1 in each tablet formulation to achieve the optimal disintegration times as reported previously (Y. Bi et al., 1996; Y. X. Bi, Sunada, Yonezawa, & Danjo, 1999; Ishikawa, Mukai et al., 2001; Watanabe et al., 1995). Magnesium stearate was always maintained at 2% in a total tablet weight of 150 mg for the G2 RDSTs, and 200 mg for the G3 taste masked RDSTs, as reported previously.

***Table 1**: Composition of the second generation (G2), rapidly-disintegrating sublingual tablet formulations of epinephrine. Tablet weight was maintained at 150 mg.*

Ingredient (mg)	Formulations								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Epinephrine bitartrate	72.77	72.77	72.77	72.77	72.77	72.77	72.77	72.77	72.77
MCC (Ceolus® KG-802)	66.81	53.31	46.56	39.81	33.06	-	-	-	-
MCC (Ceolus® PH-301)	-	-	-	-	-	46.56	33.06	-	-
MCC (Ceolus® PH-M-06)	-	-	-	-	-	-	-	46.56	33.06
Man (Pearlitol® 400DC)	-	15.00	22.50	30.00	37.50	22.50	37.50	22.50	37.50
L-HPC (LH11)	7.42	5.92	5.17	4.42	3.67	5.17	3.67	5.17	3.67
Magnesium stearate	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00

MCC, microcrystalline cellulose; L-HPC, low-substituted hydroxypropyl cellulose; Man, mannitol.

***Table 2**: Composition of the third generation (G3), taste-masked rapidly-disintegrating sublingual tablet formulations of epinephrine. Tablet weight was increased and maintained at 200 mg.*

Ingredient (mg)	Formulations			
	F_{10}	F_{11}	F_{12}	F_{13}
Epinephrine bitartrate	72.77	72.77	72.77	72.77
Microcrystalline cellulose (Ceolus® PH-M-06)	81.66	60.32	40.83	11.17
Microcrystalline cellulose (Ceolus® PH-301)	-	21.34	40.83	66.80
Mannitol (Pearlitol® 400DC)	30.00	30.00	30.00	-
Mannitol (Ludiflash®)	-	-	-	34.10
Citric acid	2.50	2.50	2.50	2.50
Low-substituted hydroxypropyl cellulose (LH11)	9.07	9.07	9.07	8.66
Magnesium stearate	4.00	4.00	4.00	4.00

4.3.2.2. Powder Mixing

A two-stage mixing procedure using a three-dimensional manual mixer (Inversina®, Bioengineering AG, Wald, Switzerland) was used to achieve both internal and external positioning of the low-substituted hydroxypropyl cellulose (super-disintegrant), and external positioning of citric acid (taste-masking) and magnesium stearate (lubricant). The E bitartrate, MCC, mannitol, and two-thirds of the quantity of low-substituted hydroxypropyl cellulose were blended in Stage I for 4 min, timed by

stopwatch. In Stage II, the remaining one-third of the quantity of low-substituted hydroxypropyl cellulose, the magnesium stearate, and the citric acid were added to the mixture from Stage I and blended for 30 s.

4.3.2.3. Direct Compression

An 11/32 inch die with flat face, bevel edge, bisect upper punch and flat face, bevel edge, lower punch; and an 13/32 inch die with flat face upper and lower punches (Natoli Engineering Company, Inc., St. Charles, MO) were used in the direct compression of the G2 and G3 RDSTs, respectively. A total powder mixture of 45–60 g enough to make 300 tablets in each batch was compressed using a Manesty®-F3 single-punch tablet press machine (Liverpool, UK) at a preselected range of compression forces (CF, 18.5–23.25 kN). Tablets were placed in a Canadian Standard Sieve (Combustion Engineering, St. Catharines, ON) with a mesh size No. 10 (with openings of 2.00 mm), dedusted for 1 min using Wet/Dry Vac (Emerson Electric Co., St. Louis, MO), and stored at 4 °C in opaque containers with desiccants. The dimensions, diameter and thickness, of the tablets compressed were measured using a 6 inch Dial Caliper (Hempe Manufacturing Co., Inc., New Berlin WI). The dimensions were used to calculate the tablet surface area (A) and volume (V) using Eqs. (1) and (2), respectively

$$A = 2\pi r^2 + 2\pi rh \quad (1)$$

$$V = \pi r^2 h \quad (2)$$

where A is the surface area; V, volume; r, radius of tablet; and h is the thickness of tablet. The minimal effect of the bevel edge and bisect dies on the tablet shape was not considered in the calculation of A and V.

4.3.3. In vitro Characterization of Rapidly-Disintegrating Sublingual Tablets

4.3.3.1. Weight Variation and Content Uniformity

Tablet weight variation (WV) and drug content uniformity (CU) were measured using the USP methods and criteria (United States Pharmacopeial Convention, 2010). A dosage form containing ≥25 mg and ≥25% API dose requires the WV test only (United States Pharmacopeial Convention, 2010). We also tested CU for further investigation on uniformity of dosage units. Ten tablets randomly selected out of 30 were individually weighed on an analytical balance (Mettler-Toledo Inc., Columbus, OH) to determine tablet WV. Drug content was analyzed using high-performance liquid chromatography (HPLC) system with ultraviolet (UV) detection at 280 nm (Waters Corp., Milford, MA). A maximum acceptance value (AV) of 15.0 was used, according to the harmonized USP method.

4.3.3.2. Breaking Force and Friability

The breaking force (BF) of six tablets randomly selected from each batch was measured using an Erweka hardness tester (Heusenstamm, Germany).

The friability (F) test was performed according to the USP guidelines to measure friability of compressed, uncoated tablets (United States Pharmacopeial

Convention, 2008). Since the tablet weight (150 mg or 200 mg) was always less than 650 mg, a random sample of whole tablets corresponding to 6.5 g was carefully dedusted, accurately weighed, and placed in the drum of a friability tester (Pharma Test Apparatebau GmbH, Hainburg, Germany). The drum was rotated 100 times and tablets were removed, dedusted, and accurately weighed. A maximum weight loss of not more than 1.0% was considered acceptable.

4.3.3.3. Disintegration Test

A method was developed (M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji et al., 2007) to evaluate the disintegration times (DT) of RDSTs under rigorous conditions. Six tablets were randomly selected from each batch. Each individual tablet was dropped into 10-mL glass test tube (1.5-cm diameter) containing 2 mL distilled water. The time required for complete tablet disintegration was observed visually and recorded to the nearest second using a stopwatch. The visual inspection was enhanced by gently rotating the test tube at a 45-angle, with minimal agitation, to distribute any tablet particles that might mask any remaining undisintegrated portion of the tablet.

4.3.3.4. Simulated Wetting Test

The wetting time (WT) was measured by a procedure that simulates the physiological conditions under a moist tongue surface (Y. Bi et al., 1996; M. M. Rawas-Qalaji, Simons, & Simons, 2006b; M. M. Rawas-Qalaji et al., 2007). Two layers of absorbent paper fitted into a rectangular plastic dish (11 x 7.5 cm) were thoroughly

wetted with distilled water. Any excess water was completely drained out of the dish. The tablet was placed at the center of the plastic dish and the time required for the water to diffuse from the wetted absorbent paper throughout the entire tablet was then recorded using a stopwatch. The WT of six randomly selected RDSTs per batch was determined.

4.3.3.5. Dissolution Test

Using a validated novel in vitro method that simulates SL conditions, dissolution was measured by a custom-made apparatus constructed in our laboratory (Rachid et al., 2011). A volume of 2 mL of distilled water, as the dissolution medium, was measured into a 15-mL dissolution container. The tablet was placed into the dissolution medium and remained undisturbed for 60 or 120 s, after which dissolution was terminated instantaneously by withdrawing the total volume of dissolution medium using a vacuum, through a 0.45 µm filter membrane into the collection tube. The membrane prevented the passage of any undissolved particles and was replaced by a new membrane for each dissolution analysis. The drug content in each sample was measured by HPLC with UV detection (Waters Corp) according to the official USP assay for E injection (M. Rawas-Qalaji, Simons, Collins, & Simons, 2009; United States Pharmacopeial Convention, 2009). To obtain the percent of drug released (DR%), the drug content (mg) in the filtrates of six individual RDSTs was compared with the mean content of ten individual tablets of the batch being tested.

4.3.4. Statistical Analysis

Results were presented as means ± standard deviations of at least six replicate experiments and statistically analyzed by one way ANOVA using Microsoft Excel software. Differences were considered significant at $p < 0.05$.

4.4. Results and Discussion

4.4.1. Formulation and Characterization of the Second Generation Rapidly-Disintegrating Sublingual Tablets

The first part of the study was designed to formulate the G2 RDSTs (F_1–F_9 in Table 1) and to determine the optimum grades and proportions of mannitol and MCC. All G2 tablets, irrespective of grade and proportion of NMIs, resulted in a mean ± SD diameter of 8.5 ± 0.1 mm and thickness of 1.75 ± 0.1 mm. Each tablet had a surface area (A) of 160 mm^2 and a volume (V) of 99 mm^3.

4.4.1.1. Effect of Mannitol Proportion on Rapidly-Disintegrating Sublingual Tablet Characteristics

Mannitol is widely used in tablet formulations, primarily as a diluent/binder and sweetening agent at 10–90% w/w. It is not hygroscopic (Rowe, Sheskey, & Weller, 2003) and is therefore suitable for use with moisture sensitive APIs such as E. This is important because hygroscopicity can cause poor powder flow, caking, and sticking during tableting, adversely affecting breaking force, dissolution, and bioavailability (Aulton, 2007). Another advantage of mannitol that makes it an appealing

NMI in RDSTs is the negative heat of solution that imparts a cooling sensation when it dissolves in saliva (Rowe et al., 2003). The water-solubility of mannitol helps in wetting the RDSTs; however, the proportion of mannitol needs to be carefully determined to prevent potential competitive dissolution, as mannitol may compete with epinephrine bitartrate for dissolution in the limited volume of saliva in the sublingual cavity.

Tablets (*F_1, F_2, F_3, F_4, and F_5*) containing 0% (0 mg), 10% (15 mg), 15% (22.5 mg), 20% (30 mg), and 25% (37.5 mg) mannitol, respectively, (Table 1) were formulated to determine the optimum mannitol proportion that would result in the best RDSTs. All G2 formulations passed the USP specifications (acceptance value ≤15) for WV and CU, except for *F_1* (0% mannitol) which resulted in a CU acceptance value of 16.53 (Table 3). The presence of mannitol (Pearlitol® 400DC) in formulations *F_2–F_5* can improve flow properties of the powder mixture during manufacturing [20]. Formulations, *F_1–F_5* (0–25% mannitol), resulted in similar breaking force (range 1.17 ± 0.05–1.23 ± 0.04 kg) and friability (range 0.94–1.72%). Only *F_2* (10% mannitol) and *F_3* (15% mannitol) passed the USP friability requirement of <1% (Table 4).

Mannitol crystals are needle-shaped. On compaction, these particles can form mechanical interlocking bonds (Aulton, 2007; Rowe et al., 2003) when they hook with each other and with particles of other NMIs. Friability was acceptable when mannitol at 10% and 15% was incorporated in tablets which might have resulted in the best ratio for creating inter-particulate hooking. Higher mannitol proportions of 20%

and 25% or the absence of mannitol in these tablets resulted in an unacceptable friability.

***Table 3**: Weight variation and content uniformity of all rapidly-disintegrating sublingual tablet (RDST) formulations of epinephrine [mean ± SD (n = 10)*]. F_1 to F_9: RDSTs (tablet weight = 150 mg), F_{10} to F_{13}: taste-masked RDSTs (tablet weight = 200 mg). Acceptance values ≤15.00 were considered acceptable.*

Formulation	Tablet weight (mg)*	Acceptance value	Drug content (%)*	Acceptance value
F_1	150.37±1.48	2.37	99.97±6.89	16.53
F_2	148.64±0.72	1.15	99.89±1.85	4.44
F_3	150.83±1.36	2.17	99.98±2.32	5.58
F_4	151.17±1.03	1.65	99.90±1.67	4.00
F_5	152.70±0.68	1.09	103.88±2.85	8.21
F_6	148.36±0.98	1.57	99.64±5.75	13.79
F_7	152.32±1.04	1.67	100.03±4.15	9.95
F_8	148.39±0.64	1.03	100.08±3.42	8.22
F_9	149.55±1.10	1.76	98.04±7.27	17.90
F_{10}	202.80±21.58	25.90	60.33±15.56	75.51
F_{11}	194.50±12.73	16.33	62.84±25.62	97.15
F_{12}	199.40±2.17	2.60	98.55±1.76	4.22
F_{13}	202.00±2.58	3.10	97.82±3.23	8.43

The tablets had DTs (range 14.83±0.98 – 32.50±2.95 s) and WTs (range 14.33±0.52 – 27.33±1.21 s) of less than 60 s (Table 4). Formulations containing 0–15% mannitol resulted in DTs of <20 s, which increased significantly ($p < 0.001$) to >30 s when the mannitol load was increased to 25%. Conversely, a shorter WT of <20 s was observed when mannitol percentage was ≥15%. Accordingly, mannitol at 15% was regarded as the optimum proportion that resulted in the best DT and WT (both <20 s). The dissolution of mannitol facilitated water penetration into the tablet (wetting) but delayed its disintegration. Briefly, tablet porosity created by other NMIs that are insoluble, such as MCC and low-substituted hydroxypropyl cellulose, allow for capillary diffusion of water that causes the disintegration of RDSTs (Ishikawa, Watanabe,

Utoguchi, & Matsumoto, 1999). We assume that the presence of mannitol retards the capillary diffusion by absorbing water for dissolution, which enhances wetting but delays disintegration. The disintegration method used in this study enabled us to distinguish these small differences in DTs among fast-disintegrating tablets. These differences could not be measured using the official disintegration method described in USP.

Rawas-Qalaji et al. (M. M. Rawas-Qalaji, Simons, & Simons, 2006a) suggested that E dissolution could be the rate-limiting step for absorption after SL administration and that the presence of water-soluble NMIs like mannitol in RDSTs reduces E dissolution in the limited volume of saliva present in the SL cavity and therefore reduces its bioavailability (M. M. Rawas-Qalaji, Simons, & Simons, 2006a). Although the same findings were reported with other APIs (Koizumi, Watanabe, Morita, Utoguchi, & Matsumoto, 1997), the exact proportion of mannitol that would not significantly affect E dissolution or bioavailability required further investigation. Based on these results, API's dissolution appears to be a more indicative in vitro parameter to study in the development of RDSTs, provided all tablets have similar DTs. However, there is no USP dissolution method that can discriminate among the different dissolution rates of various RDSTs.

***Table 4**: Breaking force, friability, disintegration time, and simulated wetting time of all rapidly-disintegrating sublingual tablet (RDST) formulations of epinephrine [mean ± SD (n = 6)*]. F_1 to F_9: RDSTs (tablet weight = 150 mg), F_{10} to F_{13}: taste-masked RDSTs (tablet weight = 200 mg).*

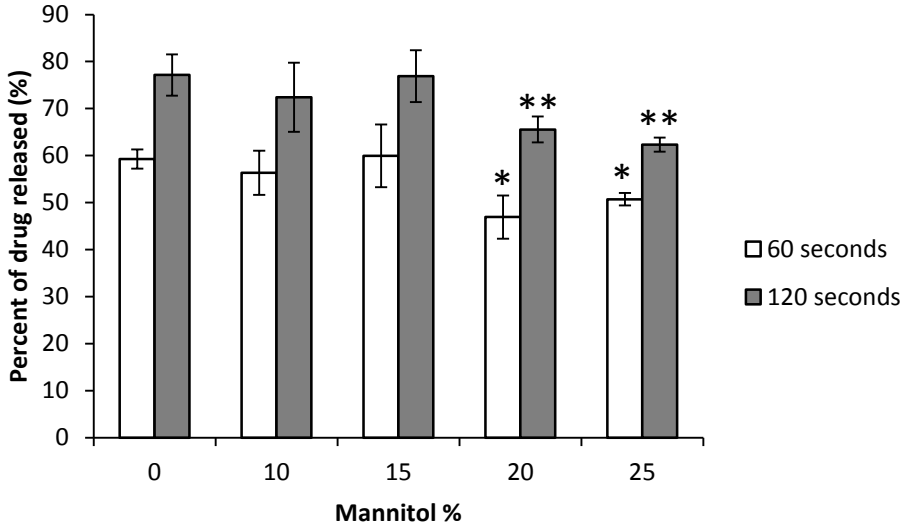
Formulation	Breaking force	Friability	Disintegration time	Simulated wetting time
F_1	1.17±0.05	1.52	16.33±0.82	26.33±1.03
F_2	1.22±0.04	0.95	14.83±0.98	27.33±1.21
F_3	1.23±0.03	0.94	17.50±0.84	15.33±0.52
F_4	1.23±0.04	1.08	24.67±0.82	15.33±0.52
F_5	1.22±0.05	1.72	32.50±2.95	14.33±0.52
F_6	1.23±0.03	0.82	10.50±0.55	21.33±1.97
F_7	1.23±0.03	7.63	13.17±0.75	11.33±0.52
F_8	1.45±0.08	18.7	12.67±0.52	55.67±6.71
F_9	1.23±0.03	27.17	14.67±0.52	43.50±2.17
F_{10}	2.28±0.17	21.56	71.67±3.01	160.17±3.54
F_{11}	1.94±0.28	16.96	10.00±1.41	83.33±3.08
F_{12}	2.67±0.26	0.77	13.00±1.67	104.17±2.86
F_{13}	3.03±0.17	0.74	13.50±1.87	104.33±3.61

In order to determine the maximum proportion of mannitol that does not affect E dissolution, we therefore constructed a custom-made apparatus and used a novel dissolution method to simulate SL conditions, as previously described and validated (Rachid et al., 2011). The percentages of drug released (DR%) from RDST formulations containing 0%, 10%, and 15% mannitol were 59.26%, 56.74%, and 59.92% at 60 s and 77.15%, 72.42%, and 76.92% at 120 s, respectively. When the mannitol proportion was increased to 20% and 25%, the DR% decreased significantly ($p<0.05$) to 46.91% and 50.71% at 60 s and to 65.55% and 62.31% at 120 s, respectively (Table 5 and Fig. 1). In addition, there was no significant difference in the DR% at either 60 or 120 s among the RDST formulations containing 0%, 10%, 15% mannitol. Therefore, we were able to confirm that the incorporation of up to 15% mannitol into these RDSTs did not prolong E dissolution.

***Table 5**: Dissolution [percent of drug released (DR%)] at 60 and 120 seconds of all rapidly-disintegrating sublingual tablet (RDST) formulations of epinephrine [mean ± SD (n = 6) compared to the mean content uniformity of 10 tablets from each formulation]. F_1 to F_9: RDSTs (tablet weight = 150 mg), F_{10} to F_{13}: taste-masked RDSTs (tablet weight = 200 mg).*

Formulation	Time (sec)	
	60	120
F_1	59.26±2.06	77.15±4.40
F_2	56.74±4.69	72.42±7.36
F_3	59.92±6.68	76.92±5.52
F_4	46.91±4.60	65.55±2.76
F_5	50.71±1.35	62.31±1.49
F_6	79.77±3.15	99.84±5.15
F_7	68.65±2.15	88.57±2.55
F_8	96.41±1.49	102.62±1.72
F_9	77.59±4.32	95.87±2.39
F_{10}	52.43±11.42	73.93±7.20
F_{11}	77.87±32.03	87.85±24.76
F_{12}	88.39±5.27	94.06±7.31
F_{13}	99.80±1.17	104.21±7.50

***Figure 1**: Dissolution at 60 and 120 seconds [percent of drug released (DR%)] of rapidly-disintegrating sublingual tablet (RDST) formulations of epinephrine containing 0, 10, 15, 20, 25% mannitol (F_1, F_2, F_3, F_4, F_5, respectively) [mean ± SD (n = 6)].*



**Significantly different from DR% at 60 seconds of 15% mannitol (F3).*

*** Significantly different from DR% at 120 seconds of 15% mannitol (F3).*

4.4.1.2. Effect of Microcrystalline Cellulose Grade on Rapidly-Disintegrating Sublingual Tablet Characteristics

Microcrystalline cellulose in concentrations of 20–90% is widely used as a binder/diluent in tablet and capsule formulations. In concentrations of 5–15%, it can also be used as a tablet disintegrant. It is commercially available in various grades in a range of particle sizes, bulk densities, and repose angles that can produce different tablet properties and characteristics. The MCC grade used in formulations F_1–F_5 was Ceolus® KG-802. Two other grades of MCC, Ceolus® PH-301 and Ceolus® PH-M-06 (Table 6), were also examined for their possible effects on the in vitro characteristics of RDSTs

of E. Tablets containing these different grades of MCC with either 15% (F_3, F_6, F_8) or 25% mannitol (F_5, F_7, F_9) were formulated and compared to determine the optimum MCC grade that would result in the best RDSTs. All formulations passed the USP specifications for WV and CU, except for F_9 (MCC Ceolus® PH-M-06 and 25% mannitol) which resulted in a CU acceptance value of 17.90 (Table 3).

***Table 6**: Physical properties of the microcrystalline cellulose grades used in the formulation of the rapidly-disintegrating sublingual tablets of epinephrine.*

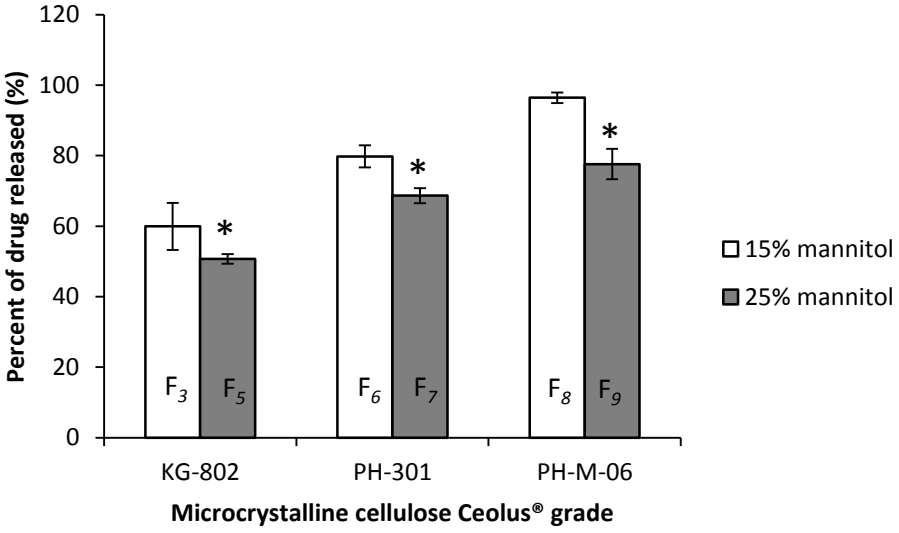
MCC Grade	Mean particle size (μm)	Bulk density (g/cm^3)	Repose angle (degrees)	Formulations
KG-802	50	0.21	49	F_1, F_2, F_3, F_4, F_5
PH-301	50	0.41	41	F_6, F_7, F_{11}, F_{12}, F_{13}
PH-M-06	7	0.48	58	F_8, F_9, F_{10}, F_{11}, F_{12}, F_{13}

The formulations had similar BF (range 1.22±0.05 – 1.45±0.08 kg), but variable F (range 0.94 – 27.17%), indicating that BF does not precisely correlate with F. Only F_3 (MCC Ceolus® KG-802 and 15% mannitol) and F_6 (MCC Ceolus® PH-301 and 15% mannitol) passed the USP F requirement of <1% (Table 4). None of the two formulations containing the fine MCC Ceolus® PH-M-06, F_8 and F_9, passed the F test due to the poor compressibility of this specific grade of MCC (Ishikawa, Mukai et al., 2001). However, these two formulations were the best in terms of tablet texture and mouth feel, in accordance with previous reports stating that this grade of MCC, Ceolus® PHM-06, is superior in texture to other grades (Ishikawa et al., 2001).

Tablets had DTs (range 10.50±0.55 – 32.50±2.95 s) and WTs (range 11.33±0.52 – 55.67±6.71 s) of less than 60 s (Table 4). Tablets with relatively low

porosity, F_8 and F_9 that comprise fine MCC Ceolus® PH-M-06 grade, would be expected to require more time to be completely wetted, as demonstrated in our results, compared to more porous tablets comprised of coarser MCC grades. Despite the longer WTs of the tablets containing MCC Ceolus® PH-M-06, they still had fast DTs of ≤15 s. The incorporation of this MCC grade into the RDSTs resulted also in the best E dissolution (DR%) of 96.41% at 60 s and 102.62% at 120 s when 15% mannitol was used (Table 5 and Fig. 2). The DR% decreased significantly ($p < 0.05$) when the mannitol load was increased to 25%, which further confirms our previous findings in Fig. 1 and in our previous study (M. M. Rawas-Qalaji, Simons, & Simons, 2006a) on the effect of mannitol on E dissolution.

***Figure 2**: Dissolution at 60 seconds [percent of drug released (DR%)] of rapidly-disintegrating sublingual tablet (RDST) formulations of epinephrine containing the following microcrystalline Ceolus® grades: KG-802, PH-301, PH-M-06 with either 15% or 25% mannitol [mean ± SD (n = 6)].*



**Significantly different from DR% at 60 seconds of 15% mannitol in equivalent formulations.*

4.4.2. Formulation and Characterization of the Third Generation Rapidly-Disintegrating Sublingual Tablets

Based on our previous studies (Rachid et al., 2011; M. M. Rawas-Qalaji, Simons, & Simons, 2006a), E dissolution within the SL cavity is the rate-limiting step for its absorption and should be considered as one of the most important parameters in the formulation of G3 tablets (F_{10} – F_{13} in Table 2). Accordingly, mannitol at 15% was selected based on our results presented earlier for G2 RDSTs. The MCC grade Ceolus® PH-M-06 resulted in the best %DR of E compared to Ceolus® KG-802 and PH-301. The high friability encountered using the fine particle MCC grade Ceolus® PH-M-06 was resolved by increasing the compression forces during tableting and/or incorporating a second coarser MCC grade, F_{12} and F_{13} (Table 4).

Epinephrine has an extremely bitter taste (Rachid et al., 2010). When a tablet of E dissolves in the SL cavity, the taste experience might be disagreeable or even unpleasant. In the G3 tablets, in order to overcome this problem, we added citric acid 2.5 mg, a taste-masking agent that masked the bitter taste by >80% (Rachid et al., 2010). Citric acid also has a secretagogue effect that potentially induces saliva secretion and increases the volume of liquid for tablet disintegration and drug dissolution. This attribute is highly useful, given that water intake is discouraged when a SL tablet is administered, in order to prevent the possibility of swallowing the tablet. Additionally, citric acid potentially facilitates transport of molecules across the SL mucosa (Pohl & Steiner, 2007).

All G3 tablets, irrespective of grade and proportion of NMIs, resulted in a mean ± SD diameter of 10.0 ± 0.1 mm and thickness of 2.0 ± 0.1 mm. These larger tablet dimensions resulted in a surface area (A) of 220 mm^2 and a volume (V) of 157 mm^3 (37.5% and 58.6% increase in A and V, respectively, compared to G2 tablets) in order to enhance drug absorption.

4.4.2.1. Effect of Using Single versus Multiple Microcrystalline Cellulose Grades on Rapidly-Disintegrating Sublingual Tablet Characteristics

Mannitol 15%, combined with citric acid as a taste-masking agent and MCC Ceolus® PH-M-06 as the main filler, were used to make the F_{10} tablets (Table 2) in the larger G3 RDSTs. Due to the poor flow of the powder mixture, F_{10} tablets resulted in high variability in tablet weight and drug content and did not pass the acceptance value of ≤15 (Table 3). This was attributed to the differences in particle size between the very fine MCC grade, Ceolus® PH-M-06, and other ingredients which may have lead to unblending of the powder mixture in the hopper or feed frame of the tablet press. Despite the higher compression force used for manufacturing G3 RDSTs, F_{10} tablets were fragile, with a high F of 21.56%. Disintegration and wetting times were >60 s (Table 4) and dissolution was 52% at 60 s and 74% at 120 s (Table 5). The failure of this formulation was mainly due to use of very fine MCC grade Ceolus® PH-M-06, which has the highest percentage of NMI (41%) in the F_{10} tablets.

The problem of unblending of the powder mixture and resultant high variability in tablet weight and drug content was solved by keeping particle sizes of

tablet NMIs as close as possible to each other. The proportion of the very fine MCC grade, Ceolus® PH-M-06, was gradually decreased and compensated for with a second coarser MCC grade, Ceolus® PH-301, to produce tablets with multiple MCC grades in formulations, F_{11}–F_{12}–F_{13} (Table 2). The void spaces between larger particles of APIs or other NMIs were filled by smaller particles. Compacting such a mixture should create stronger MCC interparticulate hydrogen bonding leading to harder tablets and better packing density (Shangraw, 1991), which will also disintegrate upon exposure to dissolution medium.

We found that by decreasing the proportion of the very fine MCC grade, the in vitro characteristics of G3 RDSTs improved and was maximal when the ratio of the coarse to fine MCC grades was ≥1:1 (F_{12} and F_{13}). The variability in tablet weight and drug content decreased gradually and reached acceptable values in formulations, F_{12} and F_{13} (Table 3). Also, the BF increased gradually and F reached acceptable values in formulations, F_{12} and F_{13}. Despite the longer WTs (>60 s), disintegration was relatively rapid (≤15 s) in formulations, F_{11}, F_{12}, and F_{13} (Table 4). Neither a positive nor a negative correlation could be found between DT and WT. Dissolution at 60 and 120 s improved gradually in formulations, F_{11}, F_{12}, and F_{13}, resulting in almost complete dissolution in F_{13} (Table 5). Mannitol 15% was incorporated in F_{13} as Ludiflash®, commercial co-processed NMIs for rapidly-disintegrating solid oral dosage forms. Although the Ludiflash® particle sizes are relatively large (45–90% range from 60 to 200 μm) compared to the other ingredients (Table 6), they do not cause a chalky or sandy sensation in the mouth, but rather have a pleasant-creamy mouth feel (BASF, personal communication).

4.5. Conclusion

The selection of NMIs in the process of formulating RDSTs by direct compression is critically important. The proportion and grade of NMIs should be well-studied to optimize the in vitro properties of RDSTs. Water-soluble NMIs such as mannitol are often used in rapidly-disintegrating and fast-dissolving tablet formulations. Water-insoluble fillers such as MCC are also used to make the matrix of tablet formulations. However, we have demonstrated that only carefully controlled proportions of NMIs with specific grades result in successful formulations. We conclude that the properties of each and every NMI and the powder flow and particulate bonding of their mixtures are critical in formulating RDSTs by direct compression.

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CHAPTER V: EPINEPHRINE (ADRENALINE) ABSORPTION FROM NEW-GENERATION, TASTE-MASKED SUBLINGUAL TABLETS: A PRECLINICAL STUDY

5.1. Abstract

The recommended first-aid treatment of anaphylaxis in community settings is prompt injection of epinephrine (E) using an autoinjector. Many patients at risk of anaphylaxis fail to carry their E autoinjectors (E-autos) because of bulky shape and large size. If anaphylaxis occurs, some patients fail to use their E-autos because they fear needles. Previously, in a proof-of-concept study, we showed that E is well-absorbed through the sublingual (SL) mucosa (M. M. Rawas-Qalaji, Simons, & Simons, 2006a). Subsequently, we have developed new-generation, taste-masked E SL tablets by modification of non-medicinal ingredients (NMIs). The objective of the study reported here was to compare the bioavailability of E from the new-generation SL tablets with E by intramuscular (IM) injection from autoinjectors in a preclinical study. The rate and extent of E absorption from the new-generation SL E 30 mg and E 40 mg tablets were compared with absorption after E 0.3 mg by IM injection in the thigh (positive control) and SL placebo tablets containing identical NMIs but no epinephrine (negative control). Epinephrine concentrations were measured using an HPLC-EC assay. Pharmacokinetic parameters were calculated using WinNonlin. The mean ± SD maximum plasma concentrations (C_{max}) achieved were 31.7±10.1 ng/mL (E 40 mg tablets) versus 27.6±7.0

ng/mL (E-autos) ($p>0.05$). The peak E concentrations were achieved at 20±7.1 min (E 40 mg tablets) versus 30±0.0 min (E-autos) ($p>0.05$). The areas under the curve (AUC) were 678±149.0 ng/mL/min (E 40 mg tablets) versus 592.0±122.3 ng/mL/min (E-autos) ($p>0.05$). Epinephrine 30 mg tablets resulted in significantly higher AUC and C_{max} than placebo, but lower than the E 40 mg tablets and E-autos. In this preclinical study, new-generation, taste-masked E tablets administered sublingually and E injected intramuscularly in the thigh had similar bioavailability. These SL E tablets are potentially suitable for Phase 1 studies in humans.

5.2. Introduction

In anaphylaxis, timely administration of E is life-saving. The recommended first-aid treatment of anaphylaxis in community settings is prompt injection of E through an autoinjector (Boyce et al., 2010; Golden et al., 2011; Lieberman et al., 2010; F. E. R. Simons et al., 2011). When anaphylaxis occurs in such settings, E-autos are under-utilized, even by patients with life-threatening respiratory or cardiovascular symptoms (Noimark et al., 2012; F. E. R. Simons, Clark, & Camargo, 2009).

Many patients at risk fail to carry their E-autos with them at all times because of bulky shape and large size. If anaphylaxis occurs, some patients fail to inject E promptly and correctly because of fear of getting a needle (Frew, 2011). Patients who have used E-autos and survived anaphylaxis have expressed concerns about following instructions correctly (for example, choosing the injection site on the mid-outer thigh) (F. E. R. Simons et al., 2009), and mis-firings have been reported (Noimark et al., 2012). Other

issues include availability of only two fixed doses (0.15 mg and 0.3 mg) of E in autoinjector formulations (F. E. R. Simons, Gu, Silver, & Simons, 2002; F. E. R. Simons, 2004; K. J. Simons & Simons, 2010), availability of only a single dose in each autoinjector (K. J. Simons & Simons, 2010), poor stability of the E solution in the autoinjector (F. E. R. Simons, Gu, & Simons, 2000; K. J. Simons & Simons, 2010) leading to a short shelf-life and need to replace devices frequently, and unintentional E injections with or without associated injuries from the autoinjectors (F. E. R. Simons, Edwards, Read, Clark, & Liebelt, 2010; K. J. Simons & Simons, 2010).

Alternatives to E-autos, such as E ampules supplied with syringes and needles (F. E. R. Simons, Chan, Gu, & Simons, 2001), E in unsealed prefilled syringes (M. Rawas-Qalaji, Simons, Collins, & Simons, 2009), or E inhalation from metered-dose inhalers (F. E. R. Simons, Gu, Johnston, & Simons, 2000) are impractical with regard to fast, accurate dosing and stability of the E formulation. Oral administration of E is not feasible because of rapid metabolism by catechol-O-methyltransferase in the gastrointestinal tract (GIT) and by monoamine oxidase in the GIT and liver, and excretion mainly as 3-methoxy-4-hydroxyphenylethyleneglycol and 3-methoxy-4-hydroxymandelic acid (Westfall & Westfall, 2011).

There is interest in developing novel, non-invasive E dosage forms that will achieve plasma E concentrations similar to those obtained after use of an E-auto. Ideally, in addition to being needle-free, small, and unobtrusive to carry, these formulations need to be easy to use and available in a wider range of doses, and to have

a long shelf-life. Sublingual administration of E has the potential to meet all these requirements. In the SL area, the mucosa is thin and the blood supply is abundant. This facilitates rapid E absorption by passive diffusion across a concentration gradient into the SL veins, followed by rapid systemic distribution (M. M. Rawas-Qalaji et al., 2006a; Sherwood, 2004).

The development of E tablets for SL administration is a major focus of our research (M. M. Rawas-Qalaji et al., 2006a). The first-generation SL E 40 mg tablets we developed are small, unobtrusive to carry, and easy to use. Additionally, these tablets have a shelf-life of 7 years or longer (M. M. Rawas-Qalaji, Rachid, Simons, & Simons, 2012 (in press)) because E is more stable in dry solid dosage forms than it is in aqueous solution (F. E. R. Simons et al., 2000). In a proof-of-concept study in a preclinical model, after SL administration of first-generation tablets, the plasma E concentrations measured after E 40 mg SL tablet were similar to those measured after IM injection of E 0.3 mg from EpiPens (M. M. Rawas-Qalaji et al., 2006a). However, the limitations of the first-generation tablets included: the unmasked intrinsic bitter taste of E (a hindrance to patient acceptability), and incomplete information about their disintegration and dissolution times.

Subsequently, new-generation E SL tablets were developed. Modification of excipients (NMIs) by addition of citric acid led to successful masking of the intrinsic bitter taste of E. Further modification by changing the proportion of mannitol, microcrystalline cellulose, and a "super-disintegrant" in the tablets, and by increasing

tablet surface area, led to disintegration within 13 seconds and dissolution of the E within 60 seconds (Rachid, Simons, Rawas-Qalaji, & Simons, 2010; Rachid, Rawas-Qalaji, Simons, & Simons, 2011; Rachid, Rawas-Qalaji, Simons, & Simons, 2012; M. M. Rawas-Qalaji, Simons, & Simons, 2006c; M. M. Rawas-Qalaji, Simons, & Simons, 2007). Modification of tablet excipients potentially affects medication bioavailability significantly and indeed, has been reported to decrease E bioavailability (M. M. Rawas-Qalaji, Simons, & Simons, 2006b).

The purpose of the study reported here was to evaluate the SL absorption of E 30 mg and E 40 mg from new-generation tablets in comparison with the SL placebo tablets and IM E 0.3 mg from EpiPen® in a validated preclinical model, to evaluate the feasibility of a SL residence time of just 2 minutes, and to evaluate a possible relationship between in vitro dissolution and in vivo absorption testing.

5.3. Methods

The research was conducted according to guidelines published by the Canadian Council on Animal Care (Olfert, Cross, & McWilliam, 1993) and was approved by the University of Manitoba Protocol Management and Review Committee.

New-generation rapidly disintegrating tablets containing E 0 mg, 30 mg, or 40 mg were formulated without using heat or water in the latex-free manufacturing laboratory of the Faculty of Pharmacy at the University of Manitoba. The tablets did not contain sodium metabisulfite or lactose and met United States Pharmacopeia (USP) standards for weight variation, content uniformity, and friability (United States Pharmacopeial

Convention, 2008; United States Pharmacopeial Convention, 2010). They disintegrated in less than 15 sec, as evaluated using a novel in vitro disintegration test developed to simulate the SL environment (M. M. Rawas-Qalaji, Simons, & Simons, 2004a; M. M. Rawas-Qalaji, Simons, & Simons, 2004b). Epinephrine 30 mg and 40 mg tablets resulted in total in vitro release of E within 60 sec, as tested using a validated novel dissolution apparatus and method (Rachid et al., 2011).

Using a prospective, randomized, placebo-controlled, parallel-dose study design, eleven New Zealand female white rabbits (mean ± SD weight 3.6 ± 0.1 kg) were investigated on four study days at least four weeks apart, using a protocol described previously (M. M. Rawas-Qalaji et al., 2006a). Before the studies were initiated and between the studies, each rabbit was kept in a private room and accessed food and water ad libitum. During each 1-hour study, they were kept in a restrainer cage (Nalgene, Rochester, NY, USA). They received either E 30 mg or 40 mg as a SL tablet, E 0.3 mg by IM injection in the thigh from an EpiPen as a positive control, or a placebo SL tablet (no epinephrine) as a negative control.

The technique of administration of SL tablets in this animal model has been described in detail previously (M. M. Rawas-Qalaji et al., 2006a). Briefly, the rabbit's mouth was opened and two smooth wooden rods of soft surface were inserted between the jaws behind the front incisors. The two wooden rods were spaced from each other and the tablet was placed underneath the tongue using a pair of forceps. A 0.1-0.2 mL volume of water was administered immediately after dosing to facilitate

tablet disintegration. One wooden rod was removed from the mouth and the rabbit's mouth was gently, but firmly held shut for 2 minutes with the second wooden rod in place to prevent it from chewing or swallowing the tablet. After the 2-minute SL residence time, the oral cavity was rinsed with 35 mL of water in order to remove any tablet residue. Epinephrine was measured in the washouts.

Epinephrine 0.3 mg was injected intramuscularly in the thigh using an EpiPen, after which the solution remaining in the EpiPen was evacuated into a plastic tube, frozen at –20°C, and later analyzed for E content using a reverse phase high performance liquid chromatography (HPLC) system (Waters Corp., Milford, Mass.) with ultraviolet detection (UV) (F. E. R. Simons et al., 2000).

5.3.1. Measurement of Plasma Epinephrine Concentrations

On each study day, an indwelling catheter (22G 1", BD, Ontario, Canada) was inserted into an ear artery at least 30 min before dosing. Two mL blood samples for measurement of plasma E concentrations were obtained and transferred into Vacutainer plasma separation tubes containing EDTA (BD, Ontario, Canada) at baseline, immediately before dosing and at 5, 10, 15, 20, 30, 40, and 60 minutes afterwards.

Blood samples were refrigerated within 1 hour of sampling and centrifuged at 1600g, 4°C. Plasma was frozen promptly and stored at -20°C. Before analysis, plasma was thawed at room temperature and E was extracted by a solid-phase extraction process, with an efficiency of 78% - 83%. Epinephrine concentrations were measured using HPLC system (Waters Corp., Milford, Mass.) with electrochemical detection (EC)

(M. M. Rawas-Qalaji et al., 2006a; F. E. R. Simons, Roberts, Gu, & Simons, 1998). Two calibration curves were constructed. The low range calibration curve was linear over the range of 0.1 to 1.0 ng/mL with a coefficient of variation of 0.4% at 0.1 ng/mL and 0.1% at 1.0 ng/mL. The high range calibration curve was linear over the range of 1.0 to 10.0 ng/mL with a coefficient of variation of 0.1% at 1.0 ng/mL and 0.1% at 10.0 ng/mL (M. M. Rawas-Qalaji et al., 2006a; F. E. R. Simons et al., 1998).

5.3.2. Data Analysis

The maximum plasma E concentration (C_{max}), the time at which C_{max} was achieved (T_{max}), and the area under the plasma concentration versus time curves (AUC) 0 to 1 hour, were calculated from the plasma E concentration versus time plots using WinNonlin 5.3 (Pharsight, Mountain View, CA) and linear trapezoidal calculation methods. The C_{max}, T_{max}, and AUC values for each animal were compared using repeated measures ANCOVA and Tukey-Kramer multiple comparison tests (NCSS Statistical Analysis Software, Kaysville, Utah). Differences were considered to be significant at $p < 0.05$.

5.4. Results

The mean ($\pm$SD) E dose injected using EpiPen autoinjectors was 0.29 ± 0.02 mg, calculated by multiplying the E concentrations in the solutions remaining in the EpiPens after injection by the stated injected volume (0.3 mL).

Mean (±SD) plasma E concentration versus time plots after administration of E 30 mg and 40 mg SL tablets, E 0.3 mg by IM injection, and placebo SL tablet are shown in Figure 1 and Figure 2. Mean (±SD) $C_{baseline}$ (endogenous E), C_{max}, T_{max}, and AUC values after the administration of E 30 mg and 40 mg sublingual tablets, E 0.3 mg intramuscularly, and placebo SL tablets are shown in Table 1.

Mean (±SD) C_{max} values after E 40 mg SL tablets (31.7 ± 10.1 ng/mL) and E 0.3 mg intramuscularly (27.6 ± 7.0 ng/mL) did not differ significantly from each other, but were significantly higher than after placebo SL tablets (7.5 ± 3.0 ng/mL). C_{max} after E 30 mg SL tablets (16.7 ± 6.3 ng/mL) was significantly higher than after placebo, but significantly lower than after the E 40 mg SL dose.

Mean (±SD) T_{max} after administration of E 30 mg (21.0 ± 5.5 min), 40 mg SL tablets (20.0 ± 7.1 min), E 0.3 mg intramuscularly (30.0 ± 0.0 min), and placebo SL tablets (33.3 ± 17.5 min) did not differ significantly.

Mean (±SD) AUC after the administration of E 40 mg SL tablets (678.0 ± 149.0 ng/mL/min) and E 0.3 mg intramuscularly (592.0 ± 122.3 ng/mL/min) did not differ significantly, but were significantly higher than after placebo SL tablets (220.1 ± 78.0 ng/mL/min). AUC after the administration of E 30 mg SL tablets (372.3 ± 48.6 ng/mL/min) was significantly higher than after placebo, but significantly lower than after the E 40 mg SL dose.

The E dose remaining in the SL cavity after dose administration of the tablets ranged from 33% to 78%.

***Figure 1**: Plasma epinephrine concentration versus time plots after administration of epinephrine sublingually, epinephrine by intramuscular injection, or placebo sublingually (n=5). Mean (±SD) C_{max}, T_{max}, and $AUC_{0\text{-}1\ hr}$ after administration of epinephrine 40 mg sublingual tablets and epinephrine 0.3 mg intramuscularly did not differ significantly (p>0.05). These pharmacokinetic parameters were, however, significantly higher after epinephrine sublingually or intramuscularly than after placebo sublingual tablets (p<0.05).*

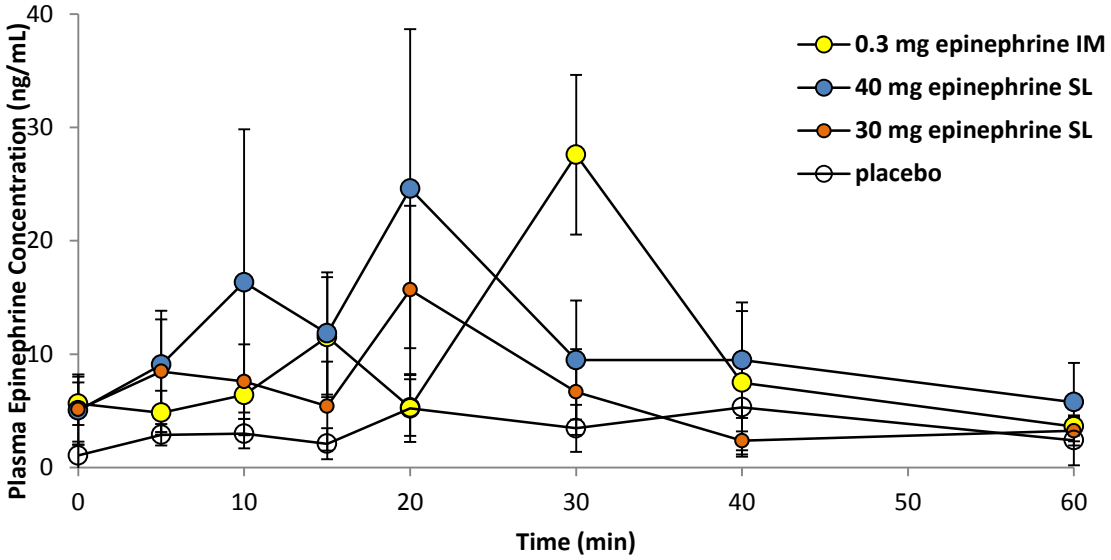


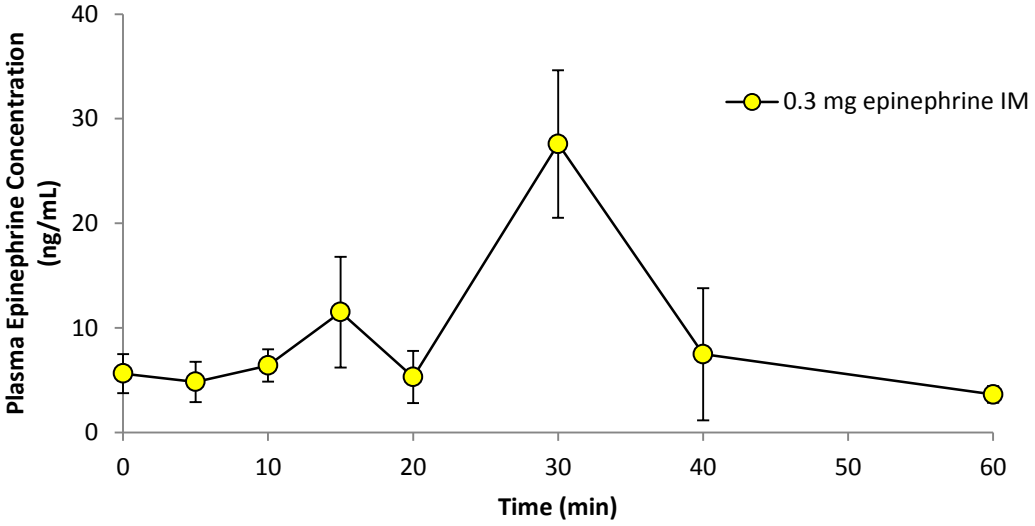
***Table 1**: Epinephrine bioavailability after sublingual administration of epinephrine and placebo tablets and epinephrine intramuscular injection in the thigh.*

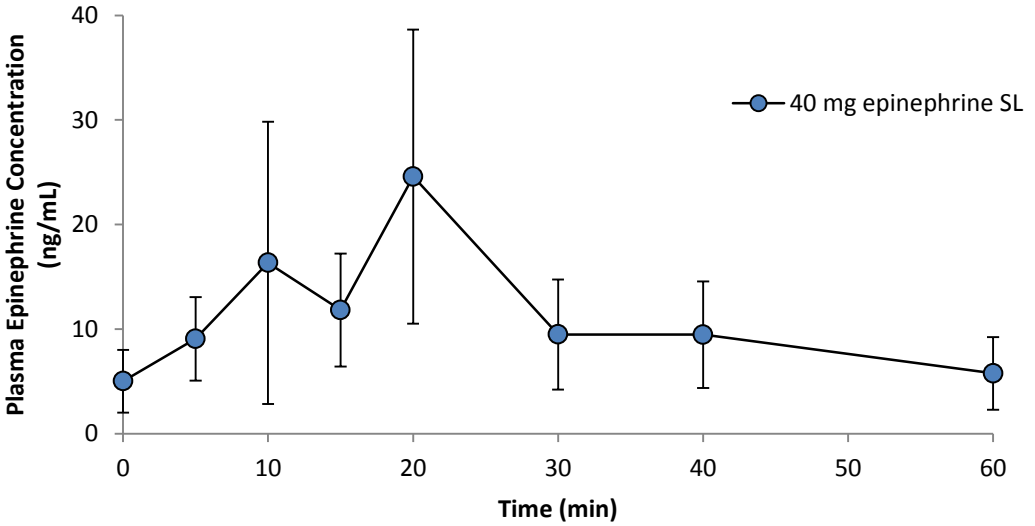
Mean±SD*	Epinephrine from Sublingual Tablets	Epinephrine from Sublingual Tablets	Epinephrine from EpiPens	Placebo Sublingual Tablets
Epinephrine dose (mg)	30	40	0.3	0
$C_{baseline}$ (ng/mL)	5.1 ± 3.1	5.0 ± 3.0	5.6 ± 1.9	1.1 ± 1.2
C_{max} (ng/mL)	16.7 ± 6.3†	31.7 ± 10.1†	27.6 ± 7.0†	7.5 ± 3.0
T_{max} (min)	21.0 ± 5.5	20.0 ± 7.1	30.0 ± 0.0	33.3 ± 17.5
$AUC_{0\text{-}1\ hr}$ (ng/mL/min)	372.3 ± 48.6†	678.0 ± 149.0†	592.0 ± 122.3†	220.1 ± 78.0

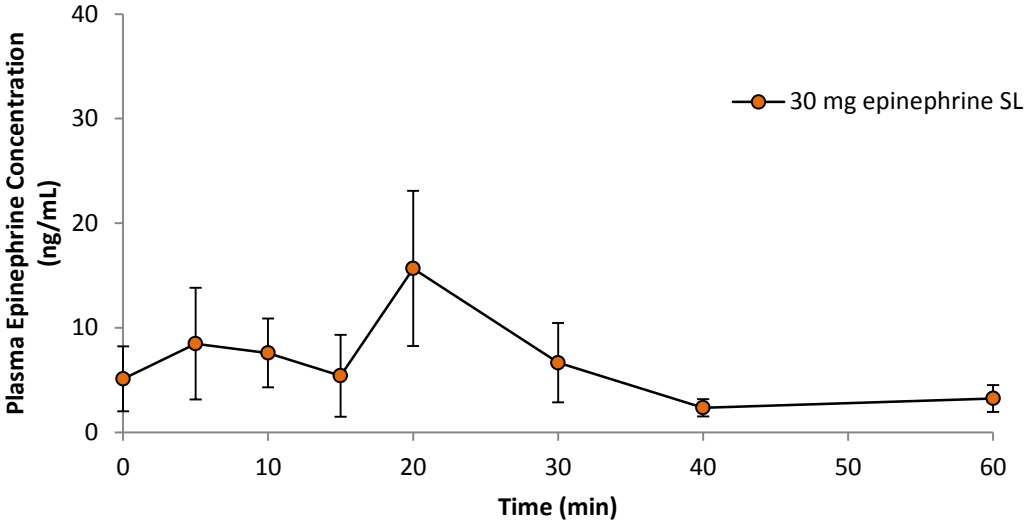
** n=5, † p<0.05 (significantly different from placebo)*

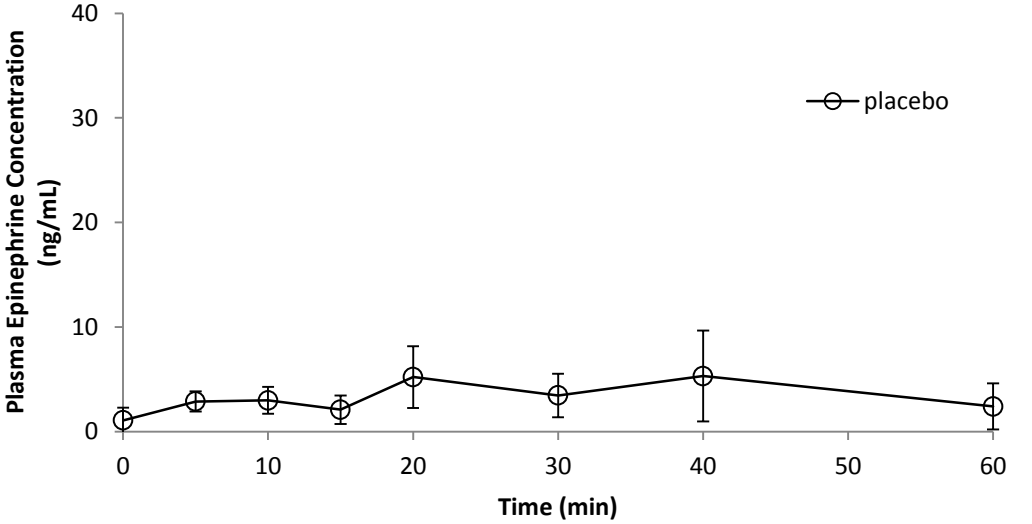
Cbaseline: Baseline plasma concentration reflecting endogenous epinephrine; Cmax: maximum plasma concentration (mean ± SD of individual Cmax values from each rabbit, regardless of the time at which Cmax was achieved); Tmax: time at which maximum plasma epinephrine concentration was achieved (mean ± SD of individual Tmax values from each rabbit); AUC: area under the plasma concentration versus time curve (mean ± SD of individual AUC values from each rabbit).

***Figure 2**: Individual plasma epinephrine concentration versus time plots after administration of epinephrine sublingually, epinephrine by intramuscular injection, or placebo sublingually (n=5). Mean (±SD) C_{max}, T_{max}, and $AUC_{0\text{-}1\ hr}$ after administration of epinephrine 40 mg sublingual tablets and epinephrine 0.3 mg intramuscularly were not significantly different (p>0.05).*









5.5. Discussion

The readily accessible, convenient SL route of administration has long been used to administer medications in medical emergencies; for example, nitroglycerine is self-administered sublingually for the initial treatment of angina. The high vascularity of the

SL mucosa facilitates rapid drug absorption directly into the venous circulation through the SL veins, bypassing the GIT, the hepatic portal circulation, and hepatic first-pass metabolism (Motwani & Lipworth, 1991).

Drugs that are absorbed sublingually reach the systemic circulation in a pharmacologically active form. Ongoing drug absorption can be terminated if necessary by removal of the tablet or other formulation from the SL space. Hydrophilic drugs with a low molecular weight, for example, E bitartrate, need to be given sublingually in a relatively high dose compared to the doses given by other routes, in order to drive the concentration gradient across the mucosa, leading to passive diffusion and absorption into the venous circulation (Sherwood, 2004).

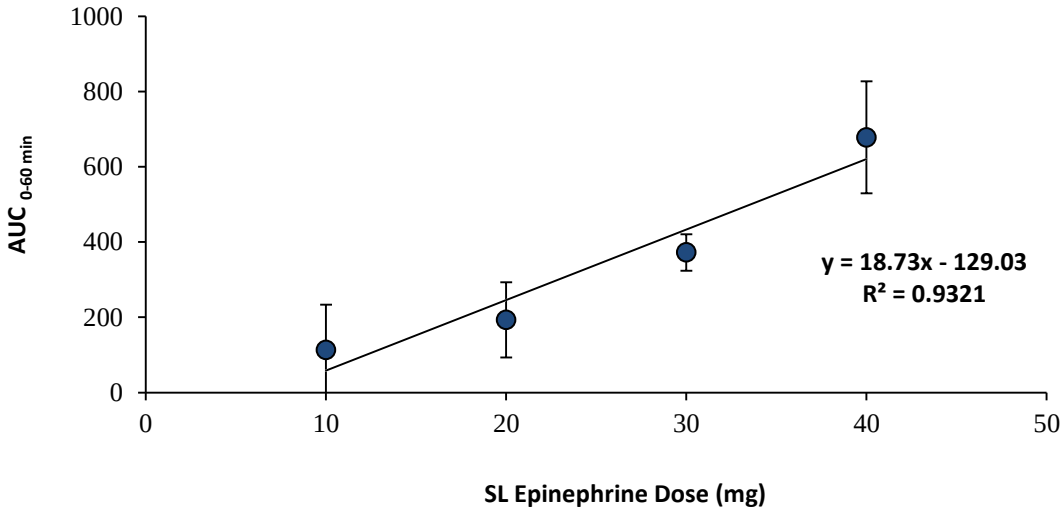
This study confirms that the SL route holds promise for E administration in the first-aid treatment of anaphylaxis in community settings. In vitro, the new-generation tablets disintegrated in less than 15 seconds and released their total E content in 60 seconds. In vivo, we have confirmed that sufficient E is released in 2 minutes and absorbed to achieve plasma concentrations similar to those achieved after IM injection of E 0.3 mg. The C_{max}, T_{max}, and $AUC_{0\text{-}1hr}$ values did not differ significantly between the E 40 mg tablets and the E-autos; however, after administration of E by either the SL route or the IM route, the C_{max} and $AUC_{0\text{-}1hr}$ were significantly higher than the endogenous E concentrations measured after placebo administration.

In our previous studies (M. M. Rawas-Qalaji et al., 2006a; M. M. Rawas-Qalaji, Simons, & Simons, 2006b), the SL E 40 mg tablets were kept under the tongue for 5

minutes and blood samples were collected up to 180 minutes. When the AUC and C_{max} were recalculated for the first 60 minutes only (882 ± 827 ng/ml/min and 31.7 ± 29.0 ng/ml, respectively), we found no significant difference to the AUC (678.0 ± 149.0 ng/ml/min) and C_{max} (31.7 ± 10.1 ng/ml) reported here for the SL E 40 mg tablets which were kept under the tongue for only 2 minutes. Based on this comparison, we concluded that most of E absorption occurred during the first 2 minutes of SL administration and there is no need to keep the tablet residues for a longer time, i.e., a 2-minute of SL residence time will result in the same E bioavailability as the 5-minute SL residence time.

It was also confirmed that the SL absorption pattern of E is dose dependent. In this study, a lower E dose of 30 mg resulted in lower absorption compared to E 40 mg, but still higher than placebo (no E). In a previous study (M. M. Rawas-Qalaji et al., 2006a), even lower E doses of 20 mg and 10 mg in SL tablets were evaluated in vivo. When plotting the first-hour AUCs of all doses (E 10 mg to 40 mg), a clear dose-dependent absorption pattern is shown (Figure 3).

***Figure 3**: Area under plasma epinephrine concentration versus time curve of tablets containing 10, 20, 30, and 40 mg epinephrine given sublingually.*



After SL E administration, plasma E concentrations increased immediately to produce an initial peak, followed rapidly by another increase to produce a second and higher peak. This intermittent absorption pattern is attributed to the strong vasoconstrictor effect of E in the SL mucosa. Initially, E absorption is almost instantaneous due to rapid transport across the single epithelial cell layer of the mucosa into the interstitial fluid on the basolateral side of the epithelial cells, then into the venous circulation across the concentration gradient according to Fick's law (Sherwood, 2004). This initial absorption leads to local vasoconstriction and a temporary reduction in absorption that is followed subsequently by vasodilation and a second, higher plasma epinephrine peak due to absorption of the E that has accumulated in the interstitial fluid. As previously reported, the magnitude of the peak E concentration in the systemic circulation depends on the E dose and the concentration gradient created (M. M.

Rawas-Qalaji et al., 2006a). Similarly, after IM injection of E, the initial plasma E peak concentration was followed by a second and higher peak.

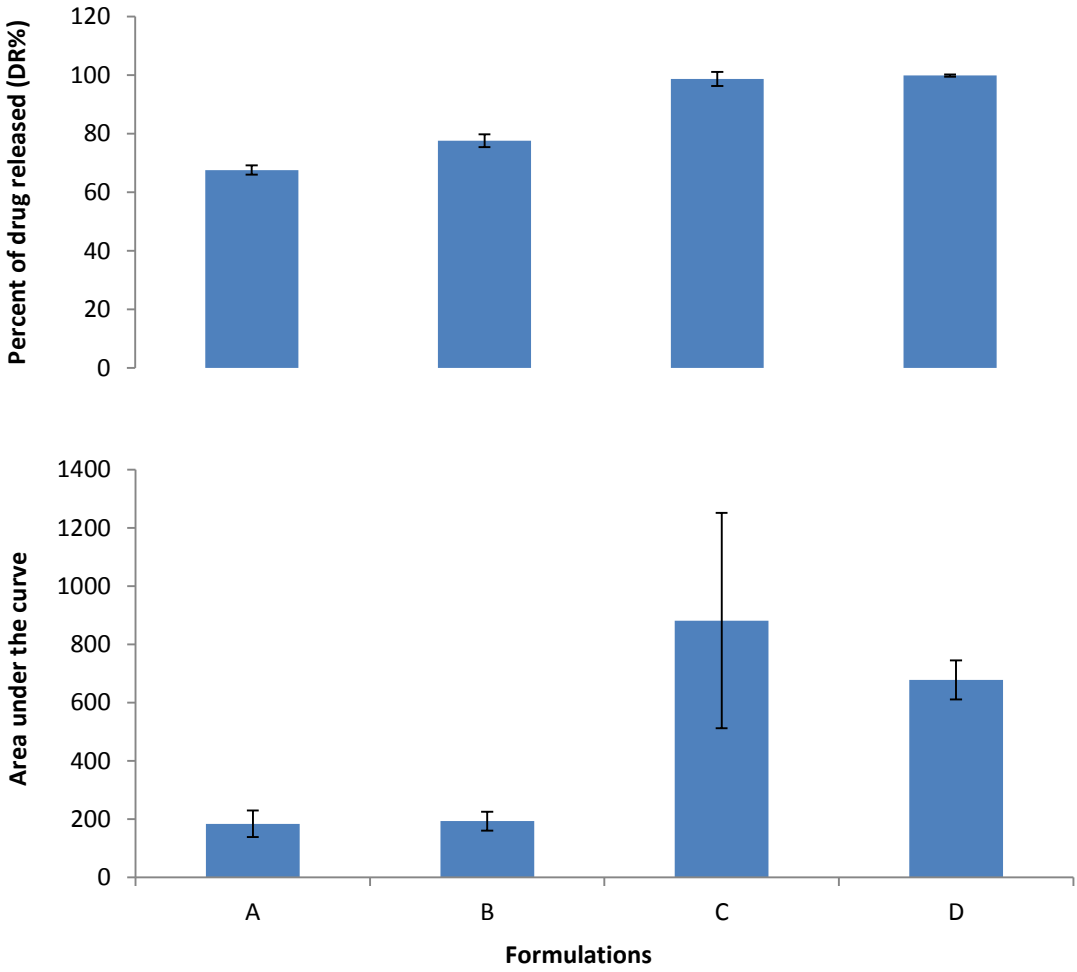
In E pharmacokinetic studies in preclinical models and in humans this intermittent pattern of E absorption has consistently been observed. In preclinical models, it has been reported after SL administration (Gu, Simons, & Simons, 1999; Gu, Simons, & Simons, 2002; M. M. Rawas-Qalaji et al., 2006a), IM injection (Gu et al., 1999; Gu et al., 2002; M. M. Rawas-Qalaji et al., 2006a), subcutaneous injection (Gu et al., 1999), and inhalation (Gu et al., 1999). In humans, it has been reported after IM injection (Edwards et al., 2012; F. E. R. Simons et al., 1998; F. E. R. Simons, Gu, & Simons, 2001), subcutaneous injection (F. E. R. Simons et al., 1998; F. E. R. Simons et al., 2001), and inhalation from a metered-dose inhaler (F. E. R. Simons et al., 2000).

In this study, we showed that E is rapidly absorbed in the first 20-30 minutes after administration by either the SL or the IM route. Rapid absorption is critical because the pharmacologic effects of E are both concentration- and time-dependent, i.e. high E concentrations need to be achieved rapidly to decrease release of inflammatory mediators (Bautista et al., 2002; Vadas & Perelman, 2012) and prevent escalation of symptoms. Failure to administer E in a timely manner and achieve the high concentrations promptly potentially increases the risk of hypoxic-ischemic encephalopathy, permanent central nervous system damage, or fatality (Pumphrey, 2000; Sampson, Mendelson, & Rosen, 1992). Median times to respiratory or cardiac

arrest in anaphylaxis have been reported as 5-30 minutes, depending on the etiology of the episode (Pumphrey, 2000).

For the purpose of predicting the extent of E absorption and bioavailability after E administration via SL rapidly-disintegrating tablets, we have designed a custom-made dissolution apparatus and developed and validated a novel method in our lab to test dissolution of E from SL tablets (Rachid et al., 2011). The dissolution of E from the current tested new-generation taste-masked E 40 mg SL tablet (Formulation D in Figure 4) has been tested along with several other rapidly-disintegrating tablets (Formulations A, B, and C in Figure 4) including the previously in vivo-evaluated E 40 mg tablets (M. M. Rawas-Qalaji, Simons, & Simons, 2006b) to establish a correlation between in vitro dissolution testing using our novel dissolution apparatus and in vivo absorption. The tablet formulations that resulted in complete in vitro dissolution (100% of drug released in 60 seconds), i.e. E 40 mg tablets from this study (Formulation D in Figure 4) and a previous study (Formulation C in Figure 4) (M. M. Rawas-Qalaji, Simons, & Simons, 2006b), resulted in successful absorption and bioavailability similar to that obtained following EpiPen® injections. On the other hand, E 40 mg tablet formulations that had lower in vitro dissolution (60-70% of drug released in 60 seconds) resulted in lower bioavailabilities (Formulations A and B in Figure 4) compared to EpiPen® injections, despite the rapid disintegrating properties of these tablets (less than 15 seconds).

***Figure 4**: Rank correlation between in vitro dissolution testing and in vivo bioavailability of four different sublingual tablet formulations containing 40 mg epinephrine.*



The use of our novel dissolution apparatus and method enabled us to determine the tablet formulation that provides an optimal E dissolution and therefore predict the formulation that will achieve similar bioavailability as EpiPen® injections.

The new-generation, taste-masked SL E tablets are non-invasive and have similar bioavailability to E injected intramuscularly in the thigh. Additionally, they can

potentially be formulated in a range of doses to provide accurate dosing for all patients regardless of age or body mass (weight). They have a long shelf-life (M. M. Rawas-Qalaji et al., 2012 (in press)) and contain no sodium metabisulfite. They are also practical for administration of a second or third dose of E, which is necessary in 6% to 35% of patients experiencing anaphylaxis in community settings (Huang, Chawla, Jarvinen, & Nowak-Wegrzyn, 2012; Korenblat, Lundie, Dankner, & Day, 1999). They are potentially suitable for Phase 1 studies in humans and might be useful for the first-aid treatment of anaphylaxis in community settings.

5.6. Conclusion

New-generation, taste-masked E SL tablets have been developed by modification of tablet excipients. In this preclinical model, E bioavailability from these tablets was similar to E bioavailability after IM injection in the thigh. These tablets are potentially suitable for phase I studies in humans.

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CHAPTER VI: CONCLUSIONS AND FUTURE PERSPECTIVES

In the first-aid treatment of anaphylaxis in community settings, epinephrine autoinjectors are life-saving. However, when anaphylaxis occurs, they are under-utilized by patients due to drawbacks such as anxiety associated with use of needles, availability of only two fixed doses (0.15 mg and 0.3 mg), and errors in administration technique potentially leading to unintentional injection and injury. For these reasons, there is increasing interest in developing alternative dosage forms that reduce or even eliminate these problems. Sublingual administration of epinephrine has the potential to fulfill all the requirements of an ideal dosage form for administration in life-threatening anaphylaxis in community settings.

In proof of concept studies in a validated animal model, first-generation sublingual tablets containing epinephrine 40 mg were bioequivalent to intramuscular injections of epinephrine 0.3 mg. However, the limitations of using these first-generation tablets included the unmasked intrinsic bitter taste of epinephrine and incomplete information about tablet disintegration and dissolution. Subsequently, a new generation of taste-masked, rapidly-disintegrating sublingual epinephrine tablets was developed. These tablets were evaluated in a systematic series of in vitro experiments in order to characterize the parameters that might affect the bioavailability of sublingual epinephrine. Standardized and validated official tests, as well as newly developed tests, were used in these evaluations.

Official pharmaceutical compendia do not include suitable methods for in vitro dissolution testing of the tablets in small volumes of liquid. We therefore developed a novel in vitro dissolution apparatus and method for this purpose. Results reflecting the dissolution of sublingual tablets containing different medications using simulated sublingual conditions were obtained by using this novel apparatus and method. Data obtained were accurate, reproducible, and significantly different from data obtained using the official compendia methods. Small changes in formulation of tablets could be readily detected and quantitated. This enabled the selection of specific grades and proportions of non-medicinal ingredients that resulted in the best in vitro results. This method has the potential to serve as surrogate for in vivo testing.

This novel apparatus and method can be applied to any rapidly-disintegrating tablets for the measurement of dissolution in a short time frame. Dissolution results under simulated conditions of the sublingual cavity are more representative and in vitro-in vivo correlations are more likely to be obtained. The current design of the custom-made dissolution apparatus could be adapted to accommodate larger size tablets, to control the temperature of the dissolution medium, to test dissolution at several time points for the same tablet, and to facilitate rapid cleanup cycles in between dissolution tests.

Sublingual tablets must have an acceptable taste; specifically, the bitter taste of epinephrine needs to be masked by sweetening and/or flavoring agents. Taste assessment by human sensory analysis panels is not possible for pharmaceutical

formulations that are not yet approved for use in humans. Taste assessment was therefore conducted using a multi-channel sensor, an electronic tongue. Different non-medicinal ingredients can be screened for their abilities to mask the inherent bitter taste of most active pharmaceutical ingredients, especially in the early stage of drug discovery and formulation development. The electronic tongue revealed that a specific proportion of citric acid masked the intense bitter taste of epinephrine by more than 80%. The electronic tongue is a viable tool for taste assessment of new chemical entities in early drug development when toxicological profiles are not yet available and assessment by human sensory analysis panels is unethical. It can also be utilized in the formulation of placebos for taste-matching to dosage forms containing the active pharmaceutical ingredients in placebo-controlled clinical trials.

Epinephrine needs to be formulated in an environment free of heat and moisture. Direct compression is the most suitable process for this purpose. However, carefully selected grades and proportions of non-medicinal ingredients must be used if epinephrine sublingual tablets are to be successfully formulated by this method, because the mean particle size, repose angle, and other characteristics of ingredients in the powder mixture can have a major impact on the resulting compressed tablets. In the new-generation sublingual tablets of epinephrine, mannitol at 15% and a specific ratio of coarse and fine particle grades of microcrystalline cellulose resulted in optimal in vitro characteristics.

In a preclinical study, the new-generation taste-masked, rapidly-disintegrating epinephrine 40 mg tablets administered sublingually and epinephrine 0.3 mg injected intramuscularly in the thigh had similar bioavailability. A lower dose of epinephrine 30 mg sublingual tablets resulted in a significantly higher bioavailability than placebo sublingual tablets, but lower bioavailability than the sublingual epinephrine 40 mg dose. The lower dose of epinephrine was incorporated in the same new-generation tablets by maintaining the types and ratios of non-medicinal ingredients, thus confirming the potential of the formulation for up-scaling or down-scaling.

The studies reported in this thesis open new directions for future research in this field. A lower dose of epinephrine injected intramuscularly using EpiPen Jr (0.15 mg) can be evaluated in our validated preclinical model to compare with the lower dose of epinephrine (30 mg) administered sublingually as a potential pediatric dose. The establishment of in vitro-in vivo correlations using the novel dissolution apparatus and method can be evaluated in future studies. Most importantly, the new-generation, taste-masked epinephrine sublingual tablets are potentially suitable for phase I studies in humans.

Moving these novel tablets to clinical testing and eventual approval by health regulatory agencies has the potential to transform the first-aid treatment of anaphylaxis in community settings. The availability of non-invasive, small, easy-to-administer sublingual epinephrine tablets will provide patients who experience anaphylaxis in the community with an alternative way to administer their life-saving medication.

APPENDIX

Table 1: *Examples of co-processed non-medicinal ingredients (NMIs):*

NMIs included	Brand name (manufacturer, country)
MCC, lactose	Cellactose (Meggle, Germany)
Lactose, PVP, crospovidone	Ludipress (BASF, Germany)
Mannitol, polyvinyl acetate, crospovidone, povidone	Ludiflash (BASF, Germany)
Lactose, maize starch	Starlac (Roquette, France)
MCC, calcium phosphate	Celocal (FMC, USA)
MCC, colloidal silica	Prosolv (Penwest, USA)
Fructose, starch	Advantose FS 95 (SPI Polyols, France)
Mannitol, sorbitol	Compressol SM (SPI Pharma, USA)

MCC = microcrystalline cellulose, PVP = polyvinyl pyrrolidone.

Table 2: *Examples of non-medicinal ingredients (NMIs) used in orally disintegrating tablets (ODTs):*

NMI Type	**Examples**
Filler/diluent	Lactose (α- or β-, monohydrate or anhydrous, spray-dried, agglomerated), mannitol, sorbitol, dextrose, maltose, microcrystalline cellulose, pregelatinized starch, dicalcium phosphates, tricalcium phosphate, calcium sulfate dehydrate.
Disintegrant	Starch and modified starches (carboxymethylstarch, sodium starch glycolate, pregelatinized starch), cross-linked polyvinyl pyrrolidone (PVP), crospovidone, modified celluloses (cross-linked sodium carboxymethylcellulose, low-substituted hydroxylpropyl cellulose, crosscarmellose sodium), microcrystalline cellulose, sodium alginate.
Lubricant	Magnesium stearate, sodium lauryl sulfate

Table 3: *Rationale for the selection of specific non-medicinal ingredients (NMIs) in the formulation of the new-generation of SL tablets of epinephrine in the studies reported in chapter IV:*

NMI	Rationale selection
Microcrystalline cellulose	1. Widely used and available in different grades which enables testing of the effect of NMI properties such as particle size on tablet characteristics. 2. Deforms plastically upon compression leading to relatively higher tablet strength. 3. Also has disintegrating properties making it appealing for orally disintegrating tablets.
Mannitol	1. Preferred in individuals allergic to lactose. 2. Has a negative heat of solution imparting a cooling sensation when dissolved in saliva. 3. Water soluble, overcoming the undesirable "grittiness" oral cavity sensation of insoluble NMIs. 4. Not hygroscopic making it suitable for moisture-sensitive APIs such as epinephrine.
Citric acid	1. Has a superior taste-masking effect on epinephrine compared to artificial sweeteners such as aspartame and acesulfame potassium which are associated with bitter aftertaste. 2. Has a lemon taste which is preferred in children compared to sweet taste.
Low-substituted hydroxypropyl cellulose	1. Has a super-disintegrant property due to its superior swelling capacity. 2. Highly compatible with APIs susceptible to oxidation.

APIs = active pharmaceutical ingredients