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Masking the bitter taste of injectable lidocaine HCl formulation for dental procedures

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Masking the Bitter Taste of Injectable Lidocaine HCl Formulation for Dental Procedures

by

Yangjie Wei

Submitted to **the Graduate Faculty** as partial fulfillment of the requirements for the

The Master of Science Degree in Pharmaceutical Sciences, Industrial Pharmacy Option

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An Abstract of

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Lidocaine HCl (LID), an amino amide-type local anesthetic, is a bitter drug that is widely used in dental procedures. It is administered in the form of injections in the oral cavity. This study aims to mask the bitter taste of injectable LID formulation using hydroxypropyl-β-cyclodextrin (HP-β-CD) and sodium saccharin. Inclusion complexes of LID and HP-β-CD were prepared by the solution method in 1:1 and 1:2 molar ratios. Inclusion complexes in solution were studied using phase solubility in phosphate buffer solutions (50 mM, pH 8, 9, and 10). Freeze dried inclusion complexes were characterized using differential scanning calorimetry (DSC), X-ray analysis (X-ray), Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), and *in vitro* release. Injectable formulations were prepared using inclusion complexes and characterized for stability using HPLC and for taste using an Alpha MOS ASTREE ETongue. The association constants of HP-β-CD with lidocaine free base and its ionized form were found to be 26.23 ± 0.00025 M^{-1} and 0.8694 ± 0.00045 M^{-1}, respectively. Results obtained from the DSC, X-ray, FT-IR, and SEM confirmed the inclusion of LID within the non-polar cavity of HP-β-CD. HP-β-CD did

not show a significant decrease in the release of LID at the 1:1 molar ratio, but slightly delayed its release at the 1:2 molar ratio. The drug content of LID in inclusion complexes was found to be stable for up to 6 months when stored at 4°C, 25°C, and 40°C. The taste evaluation study indicated that HP-β-CD (1:1 and 1:2 molar ratio) significantly improved the bitter taste of LID injectable formulation. In conclusion, inclusion complex in the 1:1 molar ratio with 0.09% sodium saccharin was considered to be optimum in masking the bitter taste of LID.

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Chapter 1

Introduction

1.1. Local anesthetics

Local anesthetics act by blocking the sensory neuronal conduction of noxious stimuli. Local anesthetic molecules can bind to voltage-gated sodium channels to prevent passage of sodium ions in nerve membranes and thereby inhibit action potentials in axon [1, 2]. In clinics, local anesthetics have a variety of applications including: 1) treatment of pain during labor; 2) analgesia during operative and postoperative period; 3) management of chronic pain; and 4) treatment of cardiac arrhythmias (e.g., lidocaine HCl) [3].

1.1.1. Structure and properties of local anesthetics

A local anesthetic molecule is composed of three moieties: 1) aromatic ring; 2) intermediate ester or amide chain; and 3) terminal chain [3]. Depending on the type of

aromatic chain, local anesthetic can be divided into two main classes: amide (e.g., articaine, bupivacaine, lidocaine, ropivacaine) and ester (e.g., benzocaine, cocaine, proparacaine, and tetracaine). Fig. 1.1 shows the difference in chemical structure between amide and ester local anesthetics. Compared to ester anesthetics, amide local anesthetics are more commonly used in clinics because of relatively lower allergic reactions to human associated to amide local anesthetics [4, 5]. Besides, amide local anesthetics have better lipid solubility, higher potency and longer duration of action than the ester type [6].

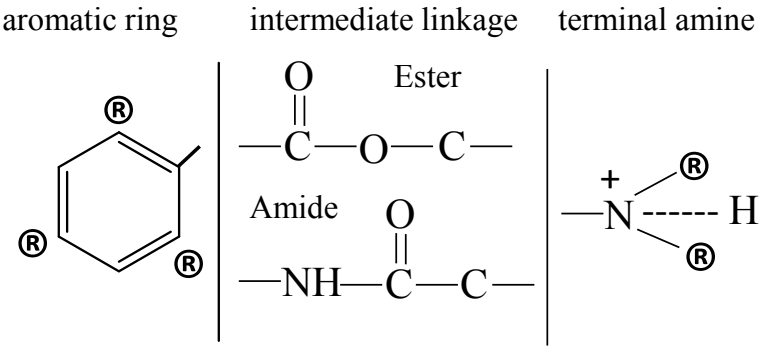


Fig. 1.1: Local anesthetic structure. Modified from Becker et al., [7].

Local anesthetics are administered in different concentrations typically ranging from 0.5% to 4% depending on their anesthetic potency. The anesthetic potency of local anesthetic largely depends on its lipophilicity. A local anesthetic with a greater lipophilicity has higher potency. For example, bupivacaine is more lipid soluble than lidocaine, therefore bupivacaine is commonly formulated as a 0.5% concentration while lidocaine as a 2% concentration. Local anesthetics are commonly formulated as their salt form, e.g., hydrochloride salt, to be soluble and stable in aqueous solution. Therefore, there are two forms of local anesthetic present in aqueous environment after

administration: water-soluble or ionized form and lipid-soluble or unionized form. Only lipid soluble form other than water soluble form can penetrate the neuron. Therefore, the onset of anesthesia of local anesthetic directly depends on the percentage of the unionized form which is determined by its ionization constant (pKa) value. All the local anesthetics clinically available have pKa higher than physiologic pH (7.4). Considering local anesthetics are weak bases, more than half of drugs exist in ionized form under the physiological conditions. Theoretically, local anesthetics with higher pKa have fewer molecules available in lipid soluble form at pH 7.4 and thereby resulting in longer onset time.

1.1.2. Local anesthetics in dentistry

Local anesthetics are used to achieve adequate local anesthesia during dental procedures so that dental practitioners can focus solely on operative procedures without worrying about pain-induced movements. Local amide anesthetics available for dental use include lidocaine (Xylocaine®), mepivacaine (Carbocaine®), articaine (Septocaine®), prilocaine (Citanest®), and bupivacaine (Marcaine®). To reduce the systemic toxicity and improve anesthetic effect, local anesthetic formulations usually contain vasoconstrictor, which is preserved using bisulfate.

1.1.3. Use of lidocaine hydrochloride in dental procedures

Lidocaine or xylocaine hydrochloride (lidocaine HCl), an amino amide-type local anesthetic, is one of the most widely used local anesthetics. Lidocaine was first synthesized by a Swedish chemist Nils Lofgren and Lundquvist in 1943 and introduced clinically in 1947 [8, 9]. Chemically lidocaine is diethylamoinoacetyl-2-6-xylidine. The chemical structure of lidocaine HCl is shown in the Fig. 1-2.

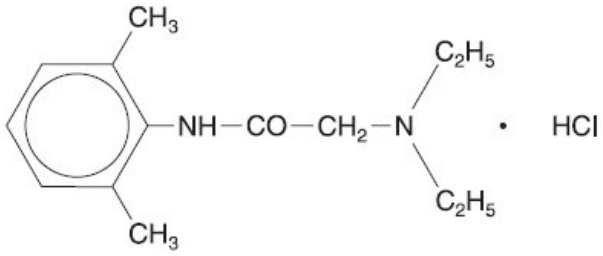


Fig. 1.2: Chemical structure of lidocaine HCl

Lidocaine HCl reversibly blocks the sensory neuronal conduction of noxious stimuli from reaching the central nervous system by binding to voltage-gated sodium channel on excitable membranes [1, 2]. Epinephrine, a vasoconstrictor, is usually combined with lidocaine HCl to improve the efficacy of anesthesia. Epinephrine can reduce the bleeding at the injection site, reduce the systemic toxicity of lidocaine HCl, and prolong the duration of anesthesia [7]. Commonly, lidocaine HCl solution in concentrations ranging between 0.5% to 2% is administered to provide intense sensory and motor nerve blockade. Higher concentration of lidocaine HCl (5%) can be used for spinal anesthesia [3]. Lidocaine HCl is a fast-acting local anesthetic with intermediate duration of action due to its relatively low protein binding [1]. Many dosage forms of lidocaine HCl are available on the market including: injection, dermal patch, nasal spray, gel, ointment, oral

liquid, and topical solution. For dental use, lidocaine HCl is commonly administered in the form of injections inside the oral cavity. A solution of lidocaine HCI (2%) with epinephrine bitartrate (1:100,000 or 1:50,000) marketed under the name Xylocaine® is widely used in routine dentistry as a local anesthetic during dental procedures. Epinephrine is essential for establishing satisfactory anesthesia. Approximately, 300 million dental cartridges are administered each year in the US, totaling more than 900 million worldwide [10].

1.1.4. Patient non-compliance associated with the use of lidocaine HCl in dental procedures

Dental fear and anxiety is a multifactorial problem that exists in most children and adolescents during the dental treatment. Dental fear can arise due to several factors including: past trauma in the dental surgery or during other medical procedures, fear of injection and drill, fear of local anesthetic not working, and the bitter taste of local anesthetics [11]. Dental repairs are performed under the local anesthesia induced using a 2% Xylocaine® injection in the oral cavity. Volumes ranging from approximately 0.5 to 2.0 ml are injected into the oral cavity [12]. Most likely, a part of the injected volume refluxes back onto the oral cavity and tongue because of improper injection techniques and resistance from patients, resulting in patients experiencing the bitter taste of the drug. The tertiary amine group of lidocaine HCl is considered responsible for its bitter taste. The extremely bitter and unpalatable taste of lidocaine HCl is not well-received by the patients leading to a poor medication tolerance. However, the issue of the bitter taste of

lidocaine HCl injection has been neglected until now. Today, the attitude of patients has dramatically changed and patients now expect liquid medications that are pleasant and tolerable. Eliminating the bitter taste of dental products would partially enhance patient tolerance, change the patient's perspective towards the dental procedures and render oral care visits more pleasant [13].

1.2. Masking the bitter taste

The need of development of pharmaceutical formulations with improved palatability has been rising in recent years. Taste of a pharmaceutical formulation is of significant importance for patient compliance, especially for children patients who are more sensitive to bitter taste and patients who are suffering from chronic diseases [14]. The bitter and unpalatable taste of a formulation is not well-received by the patients and this leads to poor medication tolerance. Therefore, taste-masking of formulation with unpleasant tastes will have a significant clinical impact.

1.2.1. Mechanism of taste

Taste bud contains an onion-shaped bundle of 50 to 100 taste cells, which are responsible for the sensation of taste [15]. Taste buds are mainly located on the tongue and soft palate. Chemicals from food or medication first dissolve in the saliva and then enter the taste buds via taste pores, which is a small opening at the top of the taste bud (Fig. 1-3). They stimulate the taste bud through interactions with either the pore-like proteins known as the ion channels or the surface proteins known as the taste receptors. These

interactions generate electrical changes in the taste cells followed by the transduction of the taste signals to the central nervous system (CNS) in the brainstem, and finally resulting in the sensation of the taste of chemicals.

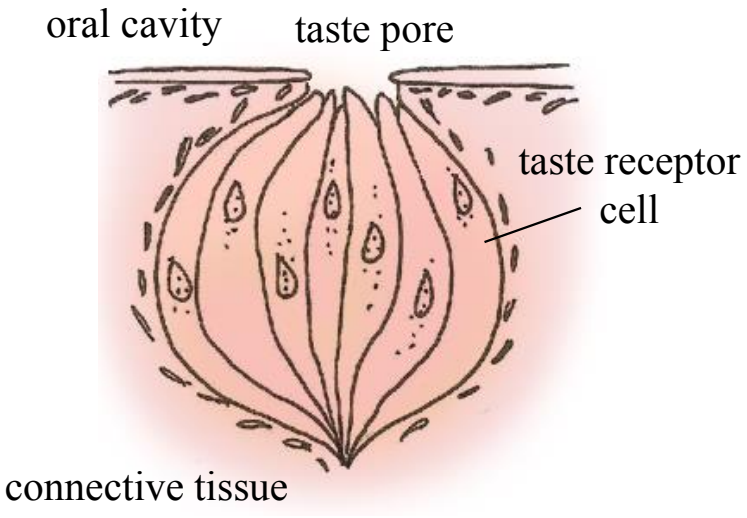


Fig. 1.3: Physiology of taste buds. Modified from http://en.wikipedia.org/wiki/File:Smagsloeg.png

Regardless of the location on the tongue, all the taste buds can respond to every type of fundamental sensation of tastes, including sweet, sour, bitter, salt and umami. Bitter sensation is a taste receptor mediated response and is associated with a surface protein. Taste receptors are intrinsically G-protein coupled receptors located on the surface of the taste cell. Binding of the bitter chemicals to the taste receptors triggers a split in the subunits of G-protein resulting in the activation of a nearby enzyme which produces a "second messenger". The "second messenger" helps in the release of calcium ion from the endoplasmic reticulum to the cytoplasm of taste cells. The continuous accumulation of calcium ions causes a depolarization of taste cells and transmission of taste signal into brain, which is then interpreted as a bitter taste [16].

1.2.2. Relevance of taste masking of bitter injectable drugs

Injectable formulations are generally administered via intravenous or intramuscular or subcutaneous routes, hence, the need for masking the bitter taste of drugs used in injectable formulations does not arise. In the recent years, dental procedures such as the number of fillings, root canals, crowns, and extractions that children and adolescents undergo increased significantly [17]. Dental repairs are performed under local anesthesia and most local anesthetics are bitter in taste. Dentists are left with no options other than injecting the bitter drugs as local anesthetics in the oral cavity, which is associated with patient intolerance. A taste masked injectable formulation would enhance patient tolerance and change the patient's perspective towards the dental procedures.

1.2.3. Taste masking of bitter injectable drugs

Several techniques have been used in the taste masking of bitter drugs. These include: addition of sweeteners, microencapsulation [18], polymer coating [19], formulation of inclusion complex [20], ion exchange resins [21], designing prodrugs [22], and solid dispersion [23]. However, most of these techniques cannot be implemented for an injectable formulation due to the stringent policies of the FDA for injections. The concept of taste-masking of an injectable formulation is uncommon. Only two of the above mentioned taste masking technologies could be used in injectable formulations, namely (i) addition of sweeteners and (ii) formation of inclusion complexes with cyclodextrins.

1.3. Addition of sweeteners such as sodium saccharin

Sodium saccharin, an artificial sweetener, has an intense sweet taste and is commonly used in beverages, foods and pharmaceutical preparations [24]. The chemical structure of sodium saccharin is shown in Fig. 1-4. Compared to saccharin, sodium saccharin has a better solubility and therefore sodium saccharin is more frequently used in the pharmaceutical research. The sweetness of 1 g of sodium saccharin is equivalent to that of 300 g of sucrose [25]. Sodium saccharin is stable in solution form when stored at normal conditions. Sodium saccharin is considered as a safe excipient when used as a sweetener in pharmaceutical formulations. Sodium saccharin can be taken both orally and parenterally. The IV injection of saccharin sodium (0.9% w/v) is used for the measurement of arm-to-tongue circulation time. Sodium saccharin (0.9 % w/v) is also used in Prochlorperazine Edisylate Injection (Compazine®), which is indicated for the treatment of schizophrenia and controlling severe nausea and vomiting. Sodium saccharin has been employed in a variety of dosage formulations including oral solutions, oral suspensions, tablets, powders, mouthwashes, and dental paste/gel [24]. Sodium saccharin has been used as a secondary taste masking agent to further improve taste masking efficiency of sulfobutyl ether-β-CD [26].

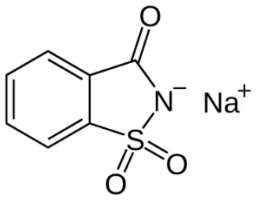


Fig. 1.4: Chemical structure of sodium saccharin

1.4. Application of cyclodextrins in taste masking

Cyclodextrins (CDs) are natural products formed by degradation of starch by bacterial enzyme called cyclodextrin glucanotransferase enzyme [27]. In 1891, cyclodextrin was discovered by French scientist Villiers in alcohol waste [28]. In the early 20th century, the bacteria (i.e. *Bacillus mancrans*) responsible for cyclodextrin synthesis were isolated. Scientists did not figure out cyclodextrin cyclic structure until 1938 [29].

1.4.1. Structure and properties of cyclodextrins

CDs are cyclic oligosaccharides consisting of six or more glucose units linked by α-(1,4) bonds. The cyclic structure of CDs is shown in Fig 1-5.

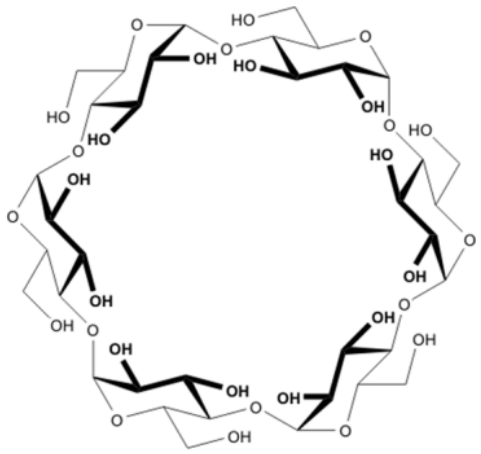


Fig. 1.5: Chemical structure of alpha-cyclodextrin

CDs have a hollow truncated cone shape with a hydrophilic outer surface and a hydrophobic central cavity (Fig 1-6.). The hydrophilic surface of CD molecule enables it soluble in aqueous environment. The hydrophobic cavity of CD is capable of interacting

with lipophilic moiety of large variety of guest molecules and forming interaction complex via non-covalent intermolecular interactions [30].

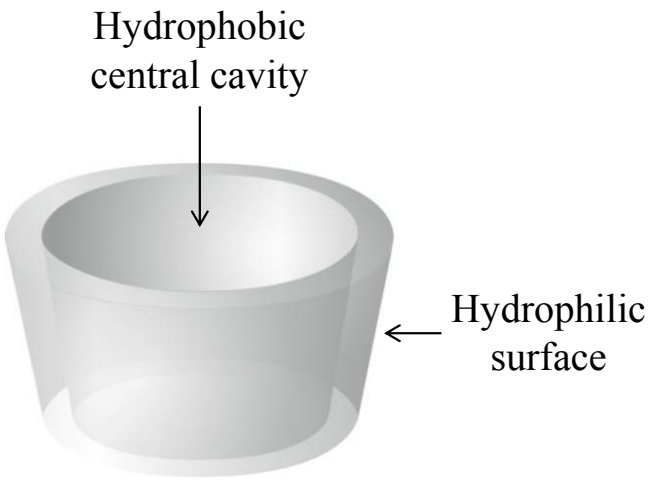
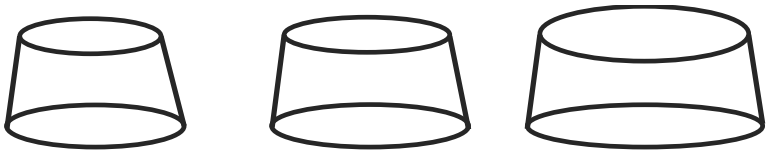


Fig. 1.6: Structure of cyclodextrin. Modified from http://www.chemiedidaktik.uni-wuppertal.de/disido_cy/cyen/info/03_physical_cy.htm

There are three types of most commonly used natural cyclodextrins: α-CD, β-CD and γ-CD which consists of 6, 7, and 8 glucose units, respectively [31]. Those three CDs differ in not only number of glucose units, but also molecule weight, cavity diameter, and molecule size. The properties of α-, β-, and γ- cyclodextrins are listed in Fig 1-7. Out of the three CDs, β-CD is most widely used in pharmaceutical research due to its accessibility and lowest price. The cavity size of α-CD is sometimes insufficient to "fit" drug molecules and γ-CD is highly priced. The relatively low water solubility of β-cyclodextrin due to the strong intermolecular hydrogen bonding limits its use parenteral formulations [32, 33].



	α-CD	β-CD	γ-CD
No. of Glucose Units	6	7	8
cavity diameter (nm)	0.47	0.60	0.75
Height torus (nm)	0.79	0.79	0.79

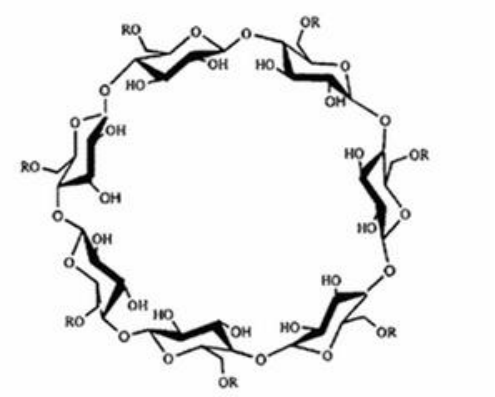
Fig. 1.7: Structure of alpha-, beta-, and gamma-cyclodextrins. Modified from http://www.chem.sci.osaka-u.ac.jp/lab/harada/eng/eng/research/01.html

The multiple hydroxyl groups on β-CD are chemically modified and substituted using a variety of alkylated groups, including methyl (M-β-CD), 2-hydroxypropyl (HP-β-CD), sulfobutylether (SEB-β-CD) for improving its aqueous solubility. Addition of alkylated groups prevents the formation of the intermolecular hydrogen bonding and thereby increases the aqueous solubility of β-CD. For example, β-CD has a low aqueous solubility (18.5 mg/ml), while the aqueous solubility of HP-β-CD is over 600 mg/ml [34].

1.4.2. 2-hydroxypropryl-β-cyclodextrin

2-hydroxypropryl-β-cyclodextrin (HP-β-CD) has attracted growing interest due to its greater water solubility, improved complexation ability, and improved toxicological profiles over native β-CD. The chemical structure of HP-β-CD is shown in Fig. 1-8. HP-β-CD is used in parenteral products due to its improved toxicological profiles. HP-β-CD is listed in both European and US Pharmacopeias and cited in the FDA's inactive pharmaceutical ingredients list. HP-β-CD has been used an excipient in different dosage

forms, including rectal (Propulsid®), Buccal (Dexocort®), IV infusion (Sporanox®), and IV Injection (Mitozytrex®).



R=H; β-CD
R= 2-hydroxypropyl; HP-β-CD

Fig. 1.8: Structure and β-cyclodextrin and 2-hydroxypropryl-β-cyclodextrin

Cyclodextrins are macromolecules with molecule weight ranging from 1000 to 2000 Da. CDs have very low octanol/water partition coefficient with a log P value between -3 and 0. These two factors make CDs less permeable across biological membranes [35, 36]. Oral administration of CDs is practically non-toxic due to poor absorption of CDs from the gastrointestinal tract [37]. Due to toxicological considerations, parent α- and β-CDs are not used in parental formulations. A number of toxicological studies have shown that γ-CD, HP-β-CD and SBE-β-CD are safe for parental use [38-40]. For example, itraconazole is solubilized in Sporanox® using 40% HP-β-CD in an aqueous pH 4.5 solution [41]. Due to its safety to human in parenteral use, HP-β-CD has been chosen as the taste masking agent to improve the taste of lidocaine HCl injectable formulation.

1.4.3. Cyclodextrin inclusion complexes

CDs interact with a wide range of drug molecules in solid, liquid, and even gaseous states to form inclusion complexes. The cavity of CDs is relatively lipophilic in nature. Lipophilic guest molecules of appropriate size enter the cavity resulting in the formation of an inclusion complex [42]. The schematic illustrating the formation of an inclusion complex between a CD and a drug molecule is shown in Fig 1-9. The driving forces that result in the formation of an inclusion complex include: release of enthalpy-rich water molecules from the cavity, hydrophobic interactions, electrostatic interactions, hydrogen bonding, and Van-der-Waals forces [43-45]. During the inclusion interaction, water molecules residing in the inter cavity of CDs are replaced by more hydrophobic guest molecules dissolved in solution resulting in a stable lower energy state [46].

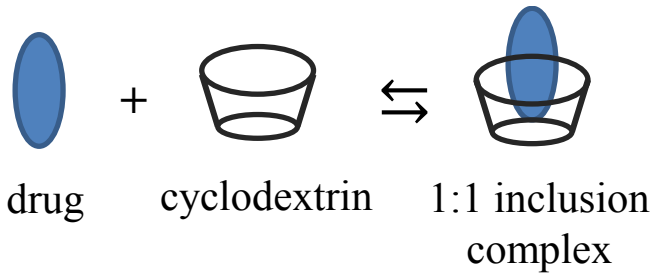


Fig. 1.9: Formation of 1:1 cyclodextrin inclusion complex with a drug molecule

Inclusion complexes can be prepared by a range of methods including: solution mixing [48], kneading [49], coevaporation [50], and freeze-drying [51]. In a solid state, only drug molecules that are in contact with CD molecules are available for complexation, while drug molecules in a solution state are uniformly dispersed and have much more

accessibility to CD molecules. Therefore, a majority of inclusion complexes are prepared in an aqueous environment or in a co-solvent system. Water is the most commonly used solvent in the preparation of CD inclusion complexes as it is non-toxic and could be easily evaporated to obtain a solvent-free inclusion complex. However, not all drug molecules readily dissolve in water, so low boiling point organic solvents such as ethanol and diethyl ether are also commonly used in the preparation of inclusion complexes.

1.4.3.1. Complex formation and solubility

The physicochemical and pharmacokinetics properties of drug molecules can be significantly altered after complexation with CDs, e.g., solubility, chemical stability, UV/Vis absorption spectrum, fluorescence, chromatography retention, NMR chemical shifts, and absorption across biological membranes. In theory, all these techniques can be utilized to study and confirm the formation of inclusion complexes. The inclusion between the CD molecules and guest molecules is a dynamic process [32]:

$$m\ D + n\ CD \leftrightharpoons D_mCD_n$$

Where m drug molecules (D) interact with n cyclodextrin (CD) molecules and form host-guest inclusion complex (D_mCD_n), the association constant ($K_{m:n}$) of the inclusion can be expressed as:

$$K_{m:n} = \frac{[DmCDn]}{[D]^m * [CD]^n}$$

When CD interacts with the drug at an equal molar ratio, they form 1:1 drug/CD inclusion complex and this is the most common type of inclusion complex. The association constant ($K_{1:1}$) can be expressed:

$$K_{1:1} = \frac{[D\ CD]}{[D]*[CD]}$$

Inclusion complexes of the drug/CD in an aqueous environment can be studied using the phase solubility profiles described by Higuchi and Connors [52]. The phase solubility study investigates the effect of the CD on drug solubility. The phase solubility diagram is constructed by plotting the drug solubility against CD concentrations (Fig. 1-10).

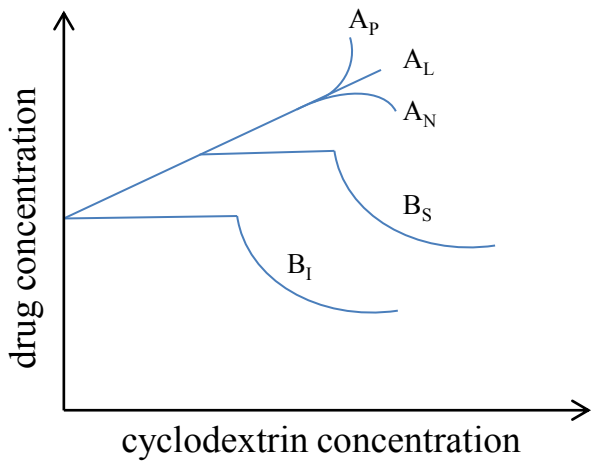


Fig. 1.10: Phase solubility diagram

Phase solubility curves can be categorized in two ways: A-type and B-type. A-type phase solubility profile is obtained when the drug solubility increases linearly with cyclodextrin concentrations. A-type profile can be further classified into two sub-types: A_L - indicates

a linear increase in drug solubility with cyclodextrin, A_P - indicates a positive deviation from linearity, and A_N - indicates a negative deviation from linearity. B-type phase solubility profile is obtained when the drug and CD form inclusion complexes with limited aqueous solubility. In general, less soluble natural parent CDs, e.g., β-CD, give rise to B-type profile due to the poor aqueous solubility of the CD. CDs which can readily dissolve in water, e.g., HP-β-CD and SBE-β-CD, result in A-type profile with drug molecules.

When the guest molecule is a weak acid or a weak base, it exists in both unionized and ionized form in aqueous solutions. Both ionized and unionized species can form an inclusion complex with CD molecules, but unionized species form a more stable complex than its ionized counterpart. This could be attributed to the greater affinity between the hydrophobic inner cavity of CD and lipophilic unionized drug molecule than its corresponding ionized form [45]. Fig. 1-11 shows how CD forms an inclusion complex with unionized and ionized drug forms. K_u and K_i are association constants for the CD/unionized drug inclusion complex and CD/ionized drug inclusion complex, respectively. Extensive research has been done to study the K_u and K_i values of CDs with a variety of weak acidic and basic drugs [54-56]. The mathematical expression for the total solubility of ionizable weak base drug against cyclodextrin concentration is shown below [57].

$$[D_{tot}] = [D_u] + [D_u] * 10^{pKa - pH} + \frac{Ku[Du]}{1 + Ku[Du]}[CD_{tot}] + \{\frac{Ki[Du] * 10^{pKa-pH}}{1 + Ki[Du] * 10^{pKa-pH}}\}[CD_{tot}]$$

Where, $[D_{tot}]$, $[D_u]$, and $[CD_{tot}]$ represent the total aqueous solubility of the drug, solubility of unionized drug (i.e., intrinsic solubility), and total CD concentration, respectively. K_a and K_b are the dissociation constant for weak acid and base, respectively.

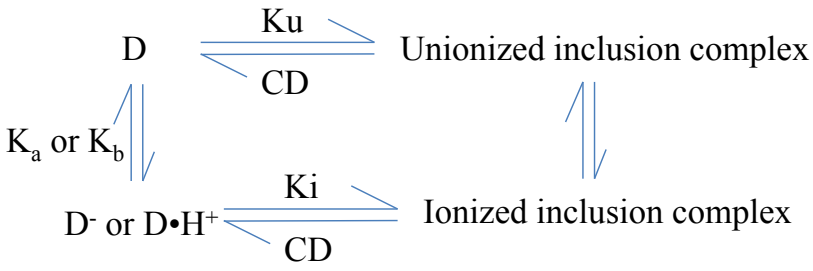


Fig. 1.11: Schematic of interaction cyclodextrin with an ionizable drug

1.4.3.2. Thermodynamics of inclusion complex formation

The formation of inclusion complexes depends on temperature. The thermodynamic parameters including the Gibbs standard free energy (ΔG), the standard enthalpy change (ΔH), temperature (T), and the standard entropy change (ΔS) are used to explain the relation between temperature change and interaction. The Van't Hoff equation can be used to express the correlation between association constant (*k*) and temperature:

$$\log k = -\frac{\Delta H}{2.303\,R}\frac{1}{T} + constant$$

A plot of log *k* versus 1/T is a straight line with slope of $-\frac{\Delta H}{2.303\,R}$

The Gibbs free energy change (ΔG) and entropy change (ΔS) of complexation interaction can be calculated using the following equations:

$$\Delta G = -RTlnk$$

$$\Delta G = \Delta H - T\Delta S$$

1.4.4. Drug release from cyclodextrin complexes

Inclusion complexes of the drug and cyclodextrin stored in a dry form at ambient temperature has a high physical and chemical stability. Energy in the form of heat is needed for the drug molecule to be displaced by another molecule, e.g., water molecule. Upon dissolution of inclusion complexes in water, drug molecules are displaced by the water molecules resulting in the release of drug molecules. Equilibrium will be established between free and complexed drug molecules. Dilution of aqueous media could further force the release drug molecules from the inclusion complex [32]. The following factors can also contribute to the rapid release of drug from inclusion complex: drug-protein binding; drug partitioning from the complex; and competitive binding [30, 58-60].

1.4.5. Pharmaceutical applications of cyclodextrins

The formation of cyclodextrin inclusion complexes can be considered as microencapsulation of guest molecules by cyclodextrins at the molecular level. The microencapsulation process improves the physicochemical and pharmacological properties of guest molecules. CDs have numerous pharmaceutical applications including: drug solubilization, drug stabilization, enhancement of bioavailability, penetration enhancer, facilitates the handling of volatile products, and masking the bitter taste and irritant smell of drugs. Cyclodextrins have been used in 30-40 different commercially available pharmaceutical formulations worldwide [29] (Table 1.1).

Table 1.1: Cyclodextrin-containing pharmaceutical products

Cyclodextrin	Drug	Trade name	Dosage form	Country
α-cyclodextrin	Alprostadil	Prostavastin, Rigidur	I.V. solution	Japan, Europe, USA
	Cefotiam hexetil HCl	Pansporin T	Tablet	Japan
β-cyclodextrin	Benexate HCl	Ulgut, Lonmiel	Capsule	Japan
	Dexamethasone	Glymesason	Ointment	Japan
	Nitroglycerin	Nitropen	Sublingual tablet	Japan
	Omeprazol	Omebeta	Tablet	Europe
2-hydroxypropyl-β-cyclodextrin	Itraconazole	Sporanox	Oral and I.V. solutions	Europe, USA
	Cisapride	Propulsid	Suppository	Europe
	Mitomycin	Mitozytrex	I.V. infusion	Europe, USA
Sulfobutylether-β-cyclodextrin	Voriconazole	Vfend	I.V. solution	Europe, USA
	Ziprasidone mesylate	Geodon, Zeldox	IM solution	Europe, USA
2-Hydroxypropyl-γ-cyclodextrin	Diclofenac sodium	Voltaren	Eye drop solution	Europe
	Tc-99 Teoboroxime	Cardiotec	I.V. solution	USA

The information listed in this table is mainly based on Loftsson *et al.* [29]

1.4.5.1. Drug solubilization

Solubilization of poorly water-soluble drugs is the most common application of CDs in the pharmaceutical research. The solubilization effect of CDs has been investigated extensively [61, 62]. Compared to the conventional solubilization techniques like pH adjustment, use of co-solvents, and use of surfactants, the use of cyclodextrin is less toxic to the human body. There are several rules describing the solubilizing effects of CDs on drugs [42]. Firstly, CDs tend to be more effective in enhancing the solubility of poorly water soluble drugs. In the presence of CDs, drugs with solubility in micrograms/liter range usually show a greater enhancement in solubility compared to drugs with solubility in micrograms/milliliter range. Secondly, the charge of CDs has a tremendous effect on solubilization and it is dictated by the proximity of the CD charged groups to the CD cavity. The closer the charged group is located from the central cavity, the less solubilization effect CD possesses. For instance, sulfobutyl ether (SBE)-β-CD has an excellent solubilization effect because the anionic sulfonate group is moved away from the β-CD cavity by the butyl ether spacer [63]. If CDs and drugs have opposite charges in an aqueous environment, the electrostatic attraction can enhance their interaction and further improve the drug solubility. However, the solubilization effect is compromised when both CD and drug carry the same type of charge. Thirdly, the solubilization effect of the CD can be further improved by the addition of water-soluble polymers, e.g., cellulose. Water soluble polymers have been shown to alter the physiochemical properties of CDs by forming inclusion complexes [64]. The polymers also increase the apparent association constants between the CDs and drugs in an aqueous environment. HPMC (0.75%, w/v) has been shown to increase the association constants of

famotidine/β-CD complex from 538 M^{-1} to 15,096 M^{-1} [65]. Water soluble polymers also enhance the aqueous solubility of CDs without compromising their ability to form inclusion complexes. Therefore, there is an increasing interest on how polymers enhance the CD/drug inclusion interactions. Several techniques can be applied simultaneously to optimize the solubilization effect of CDs.

1.4.5.2. Enhancement in dissolution release kinetics

The release of drug from a CD containing formulation involves dissolution of the free drug molecule and drug/CD inclusion complex in the surrounding fluid [66]. The dissolution rate of the neutral drug in the absence of CD can be expressed using the classical Noyes-Whitney dissolution equation [67]:

$$\text{Dissolution rate} = -[A*D_{drug}/l_{bl} * (S_o - [drug]_f)]$$

Where A is the surface area, D_{drug} is the diffusivity of the drug, l_{bl} is the length of the unstirred boundary layer, S_o is drug solubility, which is taken as the drug concentration at the drug particle surface, $[drug]_f$ is the drug concentration at the edge of the boundary layer fluid. $A*D_{drug}/l_{bl}$ is also called the dissolution constant (k_{dis}). An increase in the drug concentration gradient ($S_o - [drug]_f$) will improve the drug dissolution rate. When a CD is added to the formulation, the formation of CD/drug inclusion complex contributes to two additional driving forces: 1) the CD/drug inclusion complex concentration difference in the formulation and the surrounding environment; and 2) enhanced drug concentration gradient due to an increase in the drug solubility. The dissolution expression can be modified to the following equation [67, 68]:

$$\text{Dissolution rate} = -\left[A*D_{drug}/l_{bl} * (S_o' - [\text{drug}]_f) + A*D_{complex}/l_{bl} * ([\text{complex}]_{surface} - [\text{complex}]_f)\right]$$

Where S_o' is the enhanced drug solubility in the presence of the CD; $D_{complex}$ is the diffusivity of the drug in drug/CD inclusion complex; $[\text{complex}]_{surface}$ is the CD/drug inclusion complex concentration in a formulation; $[\text{complex}]_f$ is the CD/drug inclusion complex concentration in the surrounding environment. In addition, formation of a CD/drug complex, especially when derivatized CDs are used, can sometimes change the crystalline drug to its amorphous nature. An amorphous CD/drug inclusion complex has a higher dissolution rate compared to the drug alone because it is much easier for water molecules to break up amorphous complex material than the crystalline drug alone [69]. Also, the formation of an inclusion complex can sometimes increase the surface area (A) to further improve the dissolution rate. Due to a variety of reasons, a drug/CD inclusion complex has higher dissolution rate than the drug alone [66].

1.4.5.3. Drug stabilization

Drug stability in a pharmaceutical preparation is an important concern, especially in aqueous dosage forms. Drugs can undergo many degradation processes including hydrolysis, oxidation, and photodegradation. The degradation process is affected by the microencapsulation of a drug molecule, especially a chemically unstable moiety, inside a CD cavity. The inclusion of drug molecules into a CD cavity accelerates, inhibits, or does not significantly affect the drug degradation. Fig. 1-12 shows the effect of inclusion complex formation on the drug degradation process.

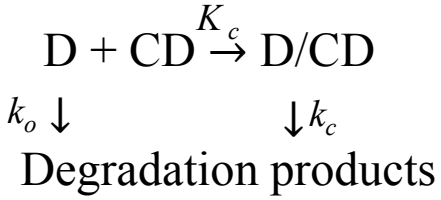


Fig. 1.12: Degradation of drug and its inclusion complex with CD

Where K_c is the association constant of the drug/CD inclusion complex; k_o is the degradation rate constant of the pure drug molecule; k_c is the degradation rate constant of the drug inside the inclusion complex.

When the degradation of a drug and its inclusion complex, both follow an apparent degradation rate constant similar to the drug (k_{app}) in an aqueous solution, we can estimate the k_{app} with the following equation [36]:

$$k_{app} = (ko + kc\ Kc\ [\mathrm{CD}])/\ (1 + Kc\ [\mathrm{CD}])$$

The apparent degradation rate constant of a drug (k_{app}) depends on the cyclodextrin concentration, the association constant of the drug/CD inclusion complex, and degradation rate constant of the pure drug molecule.

In a drug/CD inclusion complex, the CD acts as a molecular shield for protecting the drug molecule from stress factors in the surrounding environment at the molecular level. In this way, the degradation process like hydrolysis, oxidation, and racemization could be significantly reduced. Extensive research work has been done on the stabilization effect of CD on a variety of chemically unstable drugs. For example, the γ-CD is found to be a very effective stabilizer for doxorubicin [70]. HP-β-CD has been shown to significantly enhance the photostability of 2–ethyl hexyl p–dimethyl aminobenzoate in solution [71].

A cyclodextrin can sometimes destabilize a drug molecule through direct catalysis. For example, the hydrolysis of the β-lactam ring can be facilitated by interaction between the functional groups on both CD and β-lactam ring [72].

1.4.5.4. Penetration enhancer

The permeation of a cyclodextrin molecule is negligible because of its large molecular weight, chemical structure, and a very low logP value [35, 73]. Therefore, only the free drug will permeate through biological membranes. Drug molecules residing inside the cyclodextrin cavity should be released prior to permeation [74]. Fig. 1-13 illustrates the drug permeation from a cyclodextrin complex through the biological membranes.

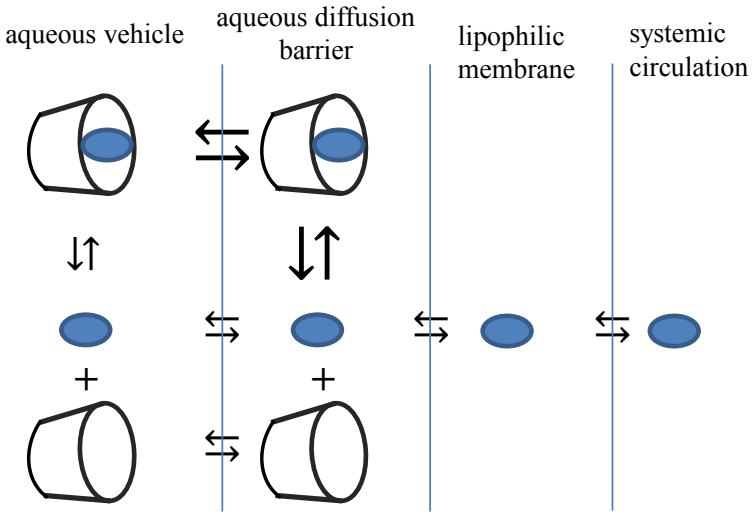


Fig. 1.13: Effect of cyclodextrin inclusion complex on drug permeation. Modified from [36].

Most of the biological membranes consist of two layers: an aqueous exterior and a lipophilic membrane barrier. The aqueous exterior forms a stagnant or an unstirred water layer. In general, a conventional penetration enhancer improves the drug permeation by decreasing the lipophilic barrier properties of the biological membrane. In contrast, a cyclodextrin molecule enhances the drug permeation through the unstirred water layer, but hinders its permeation through the lipophilic membrane barrier. Therefore, the physicochemical properties of the drug (e.g., aqueous solubility and lipophilicity) determine how cyclodextrin molecules effect its permeation. If drug permeation is limited by the unstirred water layer, its permeation through biological membranes can be effectively enhanced by the cyclodextrins. However, cyclodextrins did not show any effect on the permeation of hydrophilic drugs through the lipophilic membrane barrier [75, 76].

1.4.5.5. Effect on biological membrane properties

CDs have the ability to interact and remove the membrane components and disturb the membrane integrity. The effect of CDs on biological membranes is often associated with the CD-induced drug absorption. CDs improve the membrane fluidity and cause membrane invagination by interacting with the membrane cholesterol. CDs can interact with calcium ions which leads the loosening of tight junctions [77]. Unlike the conventional surfactants which penetrate into biological membranes and extract membrane components, CDs extract membrane components without entering into the membrane. Therefore, CDs exert a mild and reversible effect on biological membranes. 2,

6-dimethyl-β-cyclodextrin (DM-β-CD) was proved to enhance the nasal absorption of enoxaparin by temporarily (~ 6 h) opening the tight junctions of the nasal membrane [78].

1.4.5.6. Enhancement of bioavailability

CDs can alter the area under the curve (AUC), the time to reach maximum plasma concentration (T_{max}), and the peak plasma drug concentration (C_{max}) of drugs, and thereby improve its bioavailability [79, 80]. CDs can also improve the drug bioavailability by enhancing its solubility, dissolution, permeation, absorption, and by stabilizing the drug molecule at the absorption site. In general, CDs improve the bioavailability of lipophilic drugs (logP > 2.5), poorly water soluble drugs (less than 1 mg/ml), low dose drugs (less than 100 mg), and drugs with a moderate binding constant with CDs (< 5000 M^{-1}) [67]. Generally, CDs enhance the drug bioavailability by increasing the solubility of drugs. For example, the bioavailability of hydrophobic itraconazole in an oral solution dosage form was improved by enhancing its solubility using cyclodextrins [81]. CDs can also improve drug permeation by either increasing its availability at absorption site or direct interaction with biological membrane. For labile drugs that are prone to hydrolysis in the body fluids or undergo enzymatic degradation, complexation with CDs would improve the stability and increase the contact time at the absorption site. For instance, the salbutamol/β-CD complex was found to be 1.7 times more bioavailable as compared to the pure drug. This can be attributed to the reduced glucuronidation of salbutamol after complexation with CDs in the intestine [82].

1.4.5.7. Effect on drug safety

CDs improve the drug safety and reduce its side effects by making the drug effective at a lower dose. After complexation with the CD, lesser amount of drug is needed to achieve required drug efficacy and potency. Secondly, CDs can also reduce the toxicity associated with the crystallization of the drug in parenteral formulations by forming a more soluble CD/drug inclusion complex. For example, the inclusion complex of phenytoin/HP-β-CD showed lesser tissue irritation as compared to the commercially available phenytoin injectable formulation in mouse model [83]. Thirdly, CD complexation with drugs at the molecular level would decrease the interaction of the drug with the surrounding tissues, and thus reduce its side effects and local irritation. Drug/CD inclusion complex has shown no irritation to precorneal area by reducing free drug concentration to safe level [84].

1.4.5.8. Facilitates the handling of volatile products

A CD/drug inclusion complex can facilitate the handling and formulation of volatile drug substances, e.g., liquids, because complexation with CDs sometimes converts a volatile drug to solid crystals or powders, which is more convenient for handling, manufacturing, and even during administration.

1.4.5.9. Masking of bitter taste

Taste is one of the most important characteristic of an oral formulation development. Cyclodextrins are widely used as excipients in taste masking of bitter drugs by forming inclusion complexes. Cyclodextrins mask the bitter taste of drugs by preventing the interaction of drugs with the taste receptors [85]. The inclusion of the functional group of the drug molecule responsible for bitterness into CD cavity prevents its direct interaction with taste buds on the tongue, which in turn suppresses the bitter taste [85]. Taste masked formulation of a bitter drug leads to better patient compliance, especially for pediatric and geriatric patients. Fig. 1-14 shows the molecular mechanism of taste masking using a CD molecule.

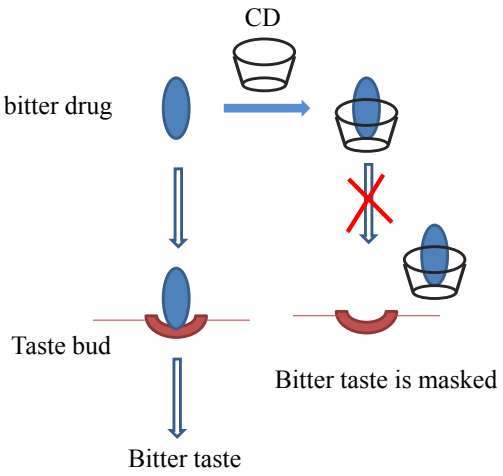


Fig. 1.14: Schematic of taste masking with cyclodextrins

The taste masking application of CDs in the pharmaceutical field has been widely exploited in recent years. CDs have been used successfully in masking the bitter taste of famotidine [65], ibuprofen [86], and femoxetine [87].

1.4.6. Use of cyclodextrins in parenteral formulations

HP-β-CD and SBE-β-CD have been extensively used in parenteral formulations. Applications of CDs in parenteral formulations include: drug solubilization, drug stabilization [88], reduction of irritation at administration site [89]. CDs are relatively safer and non-toxic compared to the conventional solubilizers like surfactants or other organic solvents. In addition, unlike prodrugs which are commonly used for drug solubilization, CDs have a negligible effect on drug pharmacokinetics [77]. Drug are rapidly released from the drug/CD complex after administration due to dilution effect and competitive replacement of drugs [90]. Scientists have been working on synthesizing safer CD derivatives with a lower hemolytic effect. E.g., heptakis (2,6 di-O-methyl-3-O-acetyl)-β-CD (DMA-β-CD) [91].

1.5. *Taste assessment*

Taste sensation is subjective and differs from person to person. Taste assessment is an important quality-control parameter for taste-masked pharmaceutical dosage forms. There are several techniques available for quantitatively evaluating the taste of drugs and oral formulations, which include: (1) human taste panel [92], (2) electronic tongue [93], (3) drug dissolution [94], and (4) spectrophotometric method [95].

1.5.1. Human taste panel

A human taste panel or gustatory sensation study is performed by evaluating the taste of samples on healthy human volunteers based on their gustatory sensation responses.

Usually a randomized, single blind study is conducted to evaluate the taste in healthy adult volunteers with an informed consent signed by every volunteer before starting the study. Each sample is then tasted by volunteers and rated on an intensity scale (e.g., Hedonic Rating Scale). The average rating for each sample is calculated and statistically compared.

A human taste panel is well established and more commonly considered for taste analysis. It is the standard method for taste assessment of pharmaceutical formulations [96]. However, it is still facing lots of challenges including: 1) human variations, 2) time-consuming, 3) safety and toxicity issues; especially for new chemical entities with unknown toxicities, and 4) ethical consideration especially for children [93].

1.5.2. Electronic tongue for taste assessment

Electronic tongue (ETongue) is an automated instrument used to assess the bitterness of a single or multiple drug substances. The ETongue approach has shown a very good correlation with the human taste panel study [97-99]. In addition, it is able to overcome the challenges of using the human taste panel and could offer a safer alternative, therefore ETongue has attracted more attention in the taste assessment. There are two types of commercially available ETongue equipments: the Insent taste sensing system and Astree ETongue.

1.5.2.1. Main principle and general setup

ETongue equipped with a probe consists of artificial multichannel taste sensors for transferring taste responses to electric signals [100]. The other elements of an ETongue include a robot arm which is connected to the probe, a sample table, an amplifier, and a data recording system. Fig. 1-15 demonstrates the components of an ETongue.

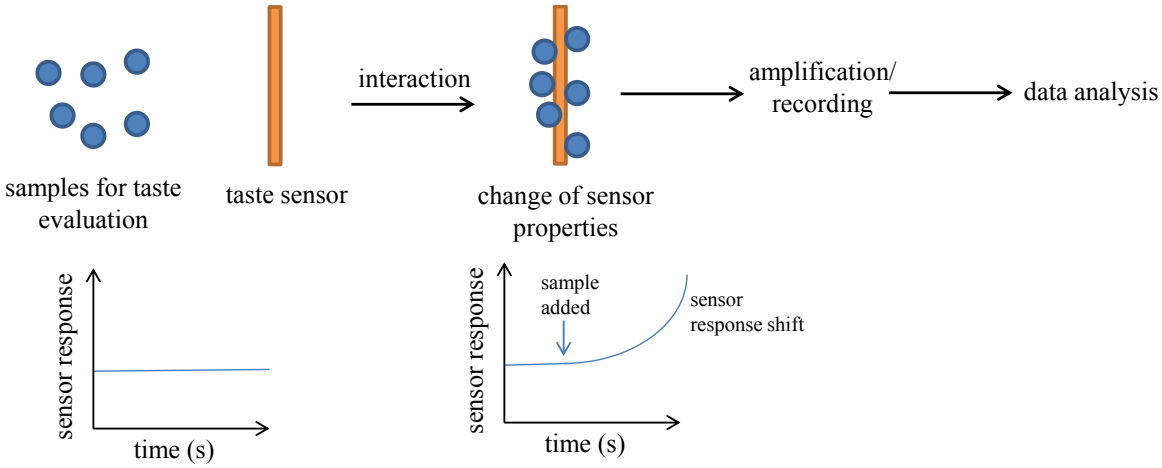


Fig. 1.15: Schematic of an ETongue

Basically, an ETongue system utilizes the taste sensors to interact with molecules dissolved in the sample in a similar manner as the taste buds in the mouth. The signal is represented by the changes in the electric potentials (voltage, mV) from the taste sensors. These signals are recorded in the computer system and evaluated by comparing with existing data base based on the taste sensor responses, which is comparable with human taste perception [101].

There are several kinds of taste sensors depending on the type of sample for analysis. The most commonly used sensor consists of a charged lipid membrane along with plasticizers. Other sensors are made up of alcoholic compounds or with inorganic salts.

The ETongue works on the principle of potentiometry and voltage values (mV) are recorded using Ag/AgCl as a reference electrode. Samples for the ETongue analysis need to be in liquid form, therefore non-liquid samples should be dissolved in a proper solvent before the ETongue analysis. In addition, the particles which might damage the taste sensors must be removed before immersing a sensor into the sample.

1.5.2.2. Insent taste sensing system

The Insent taste sensing system was invented by Japanese scientists, and is distributed by the Intelligent Sensor Technology Inc. The latest commercially available model till today is TS-500Z (Fig. 1-16 a). The system can carry up to eight different sensors with each one corresponding to a specific gustatory stimulus like saltiness, sourness, astringency, sweetness, umami, and three types of bitterness [101]. The taste sensing system is based on the potentiometry and the response is recorded as voltage change in membrane potential (mV) using Ag/AgCl as a reference electrode. It is also possible to measure the aftertaste using this taste sensing system. The default measurement time is 30 s but can be changed if required.

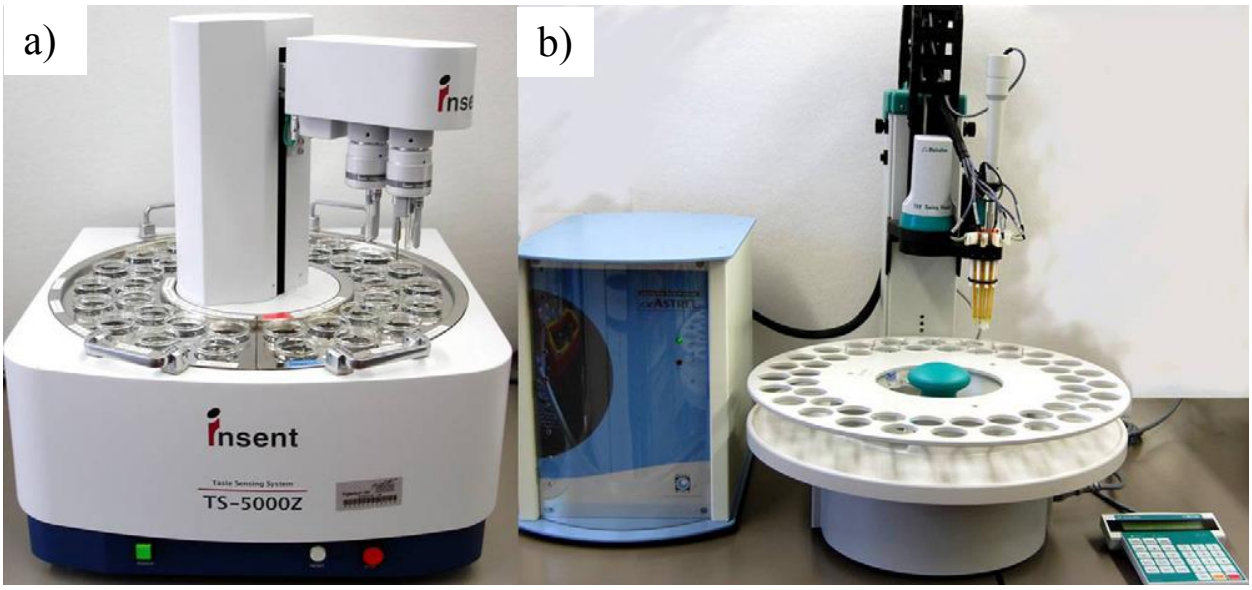


Fig. 1.16: Commercially available electronic tongues: a) Taste sensing system TS-5000Z (Insent Inc., Japan); b) αAstree (Alpha MOS, France)

1.5.2.3. αAstree electronic tongue

The αAstree electronic tongue is a potentiometric based instrument distributed by the Alpha MOS (Toulouse, France) (Fig. 1-16 b). It is equipped with a probe consisting of seven sensors, an Ag/AgCl reference electrode, and a sample holder. There are three types of probes with different combination of sensors and are for food application, pharmaceutical application, and bitterness intensity measurement, respectively. The sensor probe for pharmaceutical application composes of sensors ZZ, AB, BA, BB, CA, DA, and JE. The difference between the Insent taste sensing system and αAstree e-tongue is that the latter one measures the sample in a cross-selective manner, which means that the sensors in the αAstree electronic tongue are not assigned to specific gustatory feelings. The sensors are coated with special materials which are not disclosed, but they

are known to interact with molecules in a sample via Van deer Waals interactions, and hydrogen bonding.

Astree ETongue is commercially available and widely used in food and beverage, nutraceutical and pharmaceutical industry. Astree ETongue (Alpha M.O.S., France) was chosen to assess the bitterness of different formulations in this study. ETongue analysis can integrate the signal collected by each sensor and reveal the taste map of each formulation sample based on the Principle Component Analysis (PCA). The distance between two taste maps is measured and indicates the taste discrimination between two formulation samples. Shorter distance reveals more similarity in taste between two formulations.

1.5.2.4. Pharmaceutical applications of ETongue

ETongue has gained more attention in the pharmaceutical research in recent years. It has been widely used for taste analysis during the research and development in the pharmaceutical and food industries. The applications of ETongue include:

- Quality control [102]
- Characterization of active pharmaceutical ingredients with unpleasant tastes
- Formulation development (e.g., selecting proper taste masking agents)
- Analysis of flavor ageing in beverages
- Identification of toxic substances
- Taste comparison between product

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Chapter 2

Significance of the Thesis Research

Lidocaine hydrochloride (lidocaine HCl), an amino amide-type local anesthetic, is a bitter drug that is widely used in dental procedures. It is administered in the form of injections in the oral cavity. Lidocaine HCl is a fast-acting local anesthetic that blocks certain functions of the nervous system and thus prevents the transmission of pain impulses from the treated area to the brain. A solution of lidocaine HCI (2%) with epinephrine (1:100,000) is widely used in routine dentistry to provide relief from pain during procedures. Epinephrine is essential for establishing satisfactory anesthesia. Approximately, 300 million dental cartridges are administered each year in the US, totaling more than 900 million worldwide. In addition to the fear and anxiety from injection, the extremely bitter and unpalatable taste of lidocaine HCl is not well-received by the patients and this leads to poor medication tolerance. Therefore, taste-masking of injectable lidocaine HCl formulation will have a significant clinical impact. A taste masked injectable formulation would enhance patient tolerance and change the patient's

perspective towards the dental procedures. This work aims to mask the bitter taste of lidocaine HCl for dental applications using a two-step approach:

(i) to prepare an inclusion complex of lidocaine HCl with hydroxypropyl-β-cyclodextrin (HP-β-CD) which prevents the initial interaction of drug with taste receptors,

(ii) addition of sodium saccharin to overcome the bitter aftertaste of the formulation, leaving behind a lingering sweet taste.

Chapter 3

Masking the Bitter Taste of Injectable Lidocaine HCl Formulation for Dental Procedures

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3.1. Abstract

Purpose: Lidocaine HCl (LID), an amino amide-type local anesthetic, is a bitter drug that is widely used in dental procedures. It is administered in the form of injections in the oral cavity. This study aims to mask the bitter taste of injectable LID formulation using hydroxypropyl-β-cyclodextrin (HP-β-CD) and sodium saccharin. **Methods**: Inclusion complexes of LID and HP-β-CD were prepared by the solution method in 1:1 and 1:2 molar ratios. Inclusion complexes in solution were studied using phase solubility in phosphate buffer solutions (50 mM, pH 8, 9, and 10). Freeze dried inclusion complexes were characterized using differential scanning calorimetry (DSC), X-ray analysis (X-ray), Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), and in vitro release. Injectable formulations were prepared using inclusion complexes and characterized for stability using HPLC and for taste using an ASTREE ETongue. **Results**: The association constants of HP-β-CD with lidocaine free base and its ionized form were found to be 26.23 ± 0.00025 M-1 and 0.8694 ± 0.00045 M-1, respectively. Results obtained from the DSC, X-ray, FT-IR, and SEM confirmed the inclusion of LID within the non-polar cavity of HP-β-CD. HP-β-CD did not show a significant decrease in the release of LID at the 1:1 molar ratio, but slightly delayed its release at the 1:2 molar ratio. The drug content of LID in inclusion complexes was found to be stable for up to 6 months when stored at 4°C, 25°C, and 40°C. The taste evaluation study indicated that HP-β-CD (1:1 and 1:2 molar ratio) significantly improved the bitter taste of LID injectable formulation. **Conclusions**: In conclusion, inclusion complex in the 1:1 molar ratio with 0.09% sodium saccharin was considered to be optimum in masking the bitter taste of LID.

3.2. INTRODUCTION

Lidocaine hydrochloride (LID), an amino amide-type local anesthetic, is widely used in dental procedures. For example, a 2% solution of LID with epinephrine bitartrate (1:100,000 or 1:50,000) is widely used in routine dentistry to provide relief from pain during procedures and is marketed under the name Xylocaine®. LID reversibly blocks sensory neuronal conduction of noxious stimuli from reaching the central nervous system by binding to the voltage-gated sodium channel on excitable membranes [1, 2]. LID is a fast-acting local anesthetic with an intermediate duration of action [1]. Epinephrine, a vasoconstrictor, is essential for establishing satisfactory anesthesia via decreasing the removal of local anesthetics through blood flow to systemic circulation. Approximately 300 million dental cartridges are administered each year in the US, totaling more than 900 million worldwide [10].

With the advancements in dentistry, most treatments are done with high patient comfort and perfection. However, the fear and anxiety from injections and the extremely bitter and unpalatable taste of LID are not well-received by patients, leading to poor medication tolerance [103]. Consequently, providing a favorable environment that allows treatment has become challenging for dentists, especially in children [104-106]. Although significant progress has been made in dental care, there is still room for improvement in the visit to an oral care provider or a dentist. In particular, numerous dental products, such as Xylocaine®, that are used during dental care have an undesirable bitter and metallic taste. Eliminating the bitter taste of dental products would partially enhance patient tolerance, change the patient's perspective towards the dental procedures and

render oral care visits more pleasant [13]. Limited attempts have been made to mask the taste of dental products, e.g., oral rinse and mouthwash [13, 107, 108], but none have been made for injectable formulations.

The concept of taste-masking for an injectable formulation is uncommon. As they are generally administered via intravenous or intramuscular or subcutaneous routes, the need for masking the bitter taste of drugs used in injectable formulations does not arise. In recent years, dental procedures such as the number of fillings, root canals, crowns and extractions that children and adolescents undergo increased significantly [109, 110]. Dental repairs are performed under local anesthesia induced using a 2% Xylocaine® dental injection in the oral cavity. Volumes ranging from approximately 0.5 to 2.0 ml are injected into the oral cavity [12]. Part of the injected volume refluxes back onto the oral cavity and tongue because of resistance from patients and improper injection techniques, resulting in patients experiencing the bitter taste of the drug. Therefore, taste-masking of the injectable LID formulation will have a significant clinical impact and enhance patient tolerance toward dental procedures.

Several techniques such as coating with insoluble polymers, addition of sweeteners, use of ion exchange resins, complexing with cyclodextrins, addition of flavors, and use of prodrugs have been implemented to reduce the bitterness and obnoxious taste of orally administered drugs [111]. Taste masking with these techniques is achieved by preventing drug substances from interacting with taste buds. However, most of these techniques cannot be implemented in an injectable formulation due to the stringent requirements and

policies of the FDA. We intend to mask the bitter taste of injectable LID using 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sodium saccharin. HP-β-CD is an alkylated derivative of β-cyclodextrin with greater aqueous solubility, improved complexing ability, and less toxicity [32, 39, 112]. Hence, HP-β-CD has been used as an excipient in various injectable formulations in order to increase aqueous solubility of drugs. For example, itraconazole is solubilized in Sporanox® using 40% HP-β-CD in an aqueous pH 4.5 solution [41]. Cyclodextrin complexes of LID reported in the literature have been, for the most part, prepared for oral and topical deliveries with an intention to improve its physicochemical and biopharmaceutical properties [113-115]. However, the ability of HP-β-CD to mask the bitter taste of LID in an injectable formulation remains unknown. Sodium saccharin, an artificial sweetener, has an intense sweet taste and is commonly used in beverages, foods and pharmaceutical preparations [24]. Sodium saccharin has been employed for a variety of dosage formulations including oral solutions, oral suspensions, tablets, powders, mouthwashes, and dental pastes/gels [24]. Sodium saccharin is approved by the FDA for use in injectable formulations up to a concentration of 0.09% w/v. In this project, sodium saccharin was used as a secondary taste masking agent along with HP-β-CD to give the LID injectable formulation a pleasant taste.

The aim of this study was to prepare inclusion complexes of LID and HP-β-CD and characterize them using phase solubility study, X-ray diffractometer, Fourier Transform Infrared (FT-IR), differential scanning calorimetry (DSC), ^{1}H nuclear magnetic resonance (NMR) spectroscopy, scanning electron microscopy, and *in vitro* drug release.

Injectable formulations of LID were prepared using inclusion complexes and tested for stability and taste. ASTREE ETongue analyzer (Alpha M.O.S., France) was used to assess the bitterness of formulations.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Lidocaine HCl was purchased from MP Biomedicals, LLC (Ohio, USA). HP-β-CD, sodium saccharin and epinephrine bitartrate were purchased from Acros Organics (New Jersey, USA). All solvents used were of analytical grade. Deionized water was used throughout the experiments.

3.3.2. Methods

3.3.2.1. Phase solubility study

The phase solubility study was carried out as reported by Higuchi and Connors [116]. A series of phosphate buffer solutions (50 mM, pH 8, 9, and 10) containing varying concentrations of HP-β-CD (0 to 40 mM) were prepared. LID is freely soluble in water and therefore a free base is chosen for the phase solubility study. Lidocaine is known to alter the pH of solutions when used as a free base or as a salt, which in turn affects its solubility. In an attempt to maintain the pH and minimize the effects of pH on drug solubility, phosphate buffers were used in the phase solubility study. An excess amount of lidocaine free base was added to these solutions and equilibrated on a nutator (Fisher Scientific, Pittsburgh, PA) for 96 h at room temperature. Samples were then centrifuged

at 14,000 rpm for 10 min by Centrifuge 5430R (Eppendorf AG, Hamburg, Germany). Supernatants were taken and suitably diluted for analysis. The drug content was determined by spectrophotometry (Agilent 680 UV-Visible Spectrophotometer) at 263 nm against appropriate blanks so as to nullify the absorbance of HP-β-CD. The phase solubility diagram was constructed by plotting the total drug dissolved against total HP-β-CD concentration. The intrinsic water solubility and pKa value of lidocaine free base were calculated by the linear least squares fitting technique [117]. The pKa value and intrinsic water solubility of LID were calculated using the following equation:

$$S = S_o (1 + [H^+]/K_a)$$

Where [S] represents total lidocaine solubility at different pHs in the absence of HP-β-CD. The Y-intercept obtained by plotting [S] against $[H^+]$ indicates the intrinsic solubility (S_o) of lidocaine free base. The association constants for the inclusion complexes of HP-β-CD/lidocaine free base and HP-β-CD/ionized lidocaine were estimated according to the following equation [57]:

$$[LID_{tot}] = [LID_u] + [LID_u] * 10^{pKa - pH} + \frac{Ku[LIDu]}{1 + Ku[LIDu]}[CD_{tot}] + \left\{\frac{Ki[LIDu]*10\text{^}(pKa-pH)}{1 + Ki[LIDu]*10\text{^}(pKa-pH)}\right\}[CD_{tot}]$$

Where $[LID_{tot}]$, $[LID_u]$, and $[CD_{tot}]$ represent total lidocaine aqueous solubility, solubility of unionized lidocaine (i.e., S_0), and total HP-β-CD concentration, respectively. Ka is the dissociation constant of lidocaine. K_u and K_i are association constants for inclusion complexes of HP-β-CD/lidocaine free base and HP-β-CD/ionized lidocaine, respectively. The data was analyzed using a non-linear least squares regression method (GraphPad

Prism, version 5.00, San Diego, CA) to determine K_u and K_i values [117]. This study was conducted in triplicate.

3.3.2.2. Preparation of LID/HP-β-CD inclusion complex and physical mixtures

Inclusion complexes were prepared by dissolving LID and HP-β-CD (1:1 and 1:2 molar ratios) in deionized water at room temperature (25 ± 1°C) and stirring for 24 h. The resulting solutions were freeze dried (LABCONCO, Freeze Dry System Freezone 2.5® MO, USA) and the inclusion complexes were stored at -20 °C until further use. Physical mixtures (PM) were obtained by simple mixing of LID and HP-β-CD powders in 1:1 and 1:2 molar ratios.

3.3.2.3. Differential scanning calorimetry (DSC)

DSC analysis was carried out for LID, HP-β-CD, 1:1 and 1:2 LID/HP-β-CD physical mixtures, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes, to study the change in the rate of heat absorbed by LID after complexation with HP-β-CD. The samples (5-10 mg) were placed and sealed in aluminum crucibles using the Mettler MT 5 microbalance. DSC studies were performed at a 10°C/min heating rate over a wide range (20 - 350°C) using a DSC 822^e Mettler Toledo instrument (*Mettler Toledo* GmbH, Schwerzenbach, CH) fitted with a TSO801RO sample robot and a TSO800GCI Gas control attached to a Nitrogen gas cylinder. A Star e software V8.10 was used to obtain the scans. Nitrogen gas was purged at a rate of 20 ml/min.

3.3.2.4. Fourier Transform Infrared (FT-IR) spectroscopy

FT-IR spectra of LID, HP-β-CD, 1:1 and 1:2 LID/HP-β-CD physical mixtures, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes were obtained using a FTS 4000 FTIR spectrometer (Varian Excalibur Series UMA 600 FTIR, Digilab, USA) equipped with germanium crystal. A resolution of 2 cm^{-1} was used and 64 scans were co-added for each spectrum in the range of 400 to 4,000 cm^{-1}.

3.3.2.5. Powder X-ray diffractometry (PXRD)

The X-ray diffraction patterns of LID, HP-β-CD, 1:1 and 1:2 LID/HP-β-CD physical mixtures, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes were obtained using an X-ray diffractometer (PANanlytical's X'pert Pro Tokyo, Japan) equipped with X'Celerator high speed detector and CuKα source with a voltage of 45 kV, and a current of 40 mA. The samples were crushed, placed in an aluminum sample holder, and packed smoothly using a glass slide. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40° and the results were evaluated using the X-Pert Data collector version 2.1 software.

3.3.2.6. Nuclear magnetic resonance (NMR) studies

^{1}H NMR spectra were obtained by Varian Unity Inova 600 MHz instrument with a Penta probe. Twenty milligrams of LID, HP-β-CD, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes were dissolved in 600 µl of DMSO-d_6 in 5 mm NMR tubes and left overnight for equilibration before NMR analysis. The probe temperature was regulated at 295 K.

Typical acquisition parameters consist of sweep width of 8000 Hz, acquisition time of 3 seconds, and number of transients of 16.

3.3.2.7. Scanning electron microscopy (SEM)

The morphology of LID, HP-β-CD, 1:1 and 1:2 LID/HP-β-CD physical mixtures, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes was determined by using Hitachi S-4800 High Resolution Scanning Electron Microscope (Hitachi High-Technologies Corp., Tokyo, Japan). Samples were attached to a double-sided tape, spray-coated with gold at 0.6 kV for 10 seconds and viewed with an SEM at an accelerating voltage of 5 kV.

3.3.2.8. In vitro drug release study

In vitro release of LID from inclusion complexes was performed in a two-compartment dialysis system using a cellulose membrane (Spectra pore, MWCO 1000Da). Required amounts of LID, 1:1, and 1:2 LID/HP-β-CD inclusion complexes were dissolved in deionized water, and the solutions were placed in dialysis bags and sealed. The dialysis bags were introduced into vials containing 40 ml of phosphate buffer (50 mM, pH 7.4). The vials were placed in a shaker bath at 37±0.5°C and 60 oscillations/min. Two milliliters of samples were withdrawn at predetermined time points and replaced with an equal volume of fresh buffer. LID concentration was determined by UV spectrophotometer at 263nm after suitable dilution.

3.3.2.9. Preparation of injectable formulations using inclusion complexes

The final injectable formulations (FC, F1, F2, and F3) were prepared by dissolving LID inclusion complexes and epinephrine in 50 ml of distilled deionized water. To these solutions, required quantities of sodium saccharin, sodium metabisulfate, and sodium chloride were added as shown in Table 3.1, and the final volumes were adjusted to 100 ml. The solutions were filtered through 0.2μm Millex (Millipore Corporation, Billerica, USA) and used for stability and taste perception studies.

Table 3.1: Compositions of lidocaine HCl injectable formulations

Ingredients	**Percentage composition**				**Role**
	FC (control)	F1 (1:1 molar ratio)	F2 (1:2 molar ratio)	F3	
Lidocaine HCl	2	2	2	2	Anesthetic
Epinephrine bitartrate	0.002	0.002	0.002	0.002	Vasoconstrictor
Potassium metabisulfite	0.12	0.12	0.12	0.12	Antioxidant
EDTA disodium	0.025	0.025	0.025	0.025	Chelating agent
Sodium chloride	0.65	0.41*	0.17*	0.65	Tonicity agent
HP-β-CD	-	11.385	22.77	-	Complexing agent
Sodium saccharin	-	0.09	0.09	0.09	Sweetener
Water	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	Solvent

*amount of NaCl in test formulations was adjusted according to isotonicity calculation

3.3.2.10. Stability of injectable formulations

Stability studies were carried out for the injectable formulations (F1 and F2) prepared using LID inclusion complexes and compared with the control formulation (FC, without HP-β-CD and sodium saccharin). Sterile samples were placed in in vials and stored at 4°C, 25°C, and 40°C for up to 6 months without light. Samples were taken at regular time intervals, and LID was analyzed using HPLC.

3.3.2.11. Assay of LID

A high-performance liquid chromatography system (HPLC) (Waters Alliance 2695 separation module, Milford, MA) equipped with a Waters C18 column (75 X 4.6 mm, Symmetry®) and photodiode array (Waters 2998) detector was used for analysis. LID was analyzed using aqueous mobile phase containing 0.1% 1-octanesulfonic acid sodium salt monohydrate (pH 2.5 adjusted by acetic acid) in a gradient combination with acetonitrile pumped at a flow rate of 1.0 ml/min. The column temperature was maintained at 25˚C. The HPLC gradient program (run time in minutes/% aqueous phase) was set as follows: 0/87%, 5/87%, 10/40%, 10.01/87% and 20/87%. The retention time of LID (λ = 254 nm) was found to be 9.1 minutes. Different calibration standards of LID were prepared in the mobile phase. For the calibration curve, each standard was analyzed in triplicate and the average peak area was plotted against concentration. The drug content was determined quantitatively by plotting a calibration curve. The assay method was found to be linear in the range of 0.2-25 mg/ml with a correlation coefficient of 0.9999. The percentage recovery of LID ranged from 99.62% to 100.62%. The intra- day precision (measured by %RSD) was found to be in the range of 0.08% to 0.29%. Stress studies were performed to test the stability-indicating efficiency, i.e. ability to effectively resolve drug from its degradants, of the HPLC method. LID (100 mg/ml) solution was stressed with acid (1 N HCl), and base (1 N NaOH) for 8 hours, oxygen (2% H_2O_2), heat (70°C), and light (UV light) for 21 hours. Stressed samples were analyzed using the HPLC method [118]. The LID peaks of stress samples were examined for any interference with degradant peak.

3.3.2.12. Taste assessment using an electronic tongue (ETongue)

Taste was assessed using an ASTREE ETongue system equipped with an Alpha M.O.S. sensor set #2 (for pharmaceutical analysis) composed of seven specific sensors (ZZ, AB, GA, BB, CA, DA, JE) on a 48-position autosampler using 25 ml-beakers. All the data generated on ASTREE system were treated using multidimensional statistics on AlphaSoft V14 software. Taste analysis of control formulation (FC), LID/HP-β-CD in 1:1 molar ratio with 0.09% sodium saccharin (F1), LID/HP-β-CD in 1:2 molar ratio with 0.09% sodium saccharin (F2), and the control formulation with 0.09% sodium saccharin (F3) was carried out using an electronic tongue. The taste of F3 (control formulation with 0.09% sodium saccharin) was also assessed to identify the taste masking effect of HP-β-CD. The exact composition of each formulation is presented in Table 3.1. Twenty milliliters of each sample was placed directly into a beaker and analyzed by the ASTREE ETongue. Acquisition time and time per analysis were set at 120 seconds and 180 seconds, respectively. The e-tongue signal of each solution was measured at equilibrium using seven sensors (ZZ, AB, BA, BB, CA, DA, and JE). Solutions were analyzed in triplicate. ASTREE sensors were cleaned with deionized water between measurements. The distance on the taste map between FC and other formulations was measured by the ASTREE ETongue.

3.4. RESULTS AND DISCUSSION

3.4.1. Phase solubility study

The intrinsic solubility (S_o) and pKa of lidocaine free base at room temperature were found to be 0.0121 M and 7.74, respectively. These two values are close to the reported values in the literature [3]. S_o and pKa were used for subsequent calculations of association constants between lidocaine and HP-β-CD. Fig. 3.1 shows the correlation between lidocaine solubility and varying HP-β-CD concentrations at pHs of 8, 9 and 10. Lidocaine is known to alter the pH of solutions when used as a free base or as a salt, which in turn affects its solubility. In an attempt to maintain the pH and minimize the effects of pH on drug solubility, phosphate buffers were used in the phase solubility study. Lidocaine solubility increased linearly with increasing HP-β-CD concentrations at all three pHs corresponding to the A_L-type profile. A_L-type phase-solubility profile indicates that the complex is first or higher order with respect to HP-β-CD and first order with respect to lidocaine. From the phase solubility data, we can conclude that lidocaine forms a 1:1 inclusion complex with HP-β-CD [52]. The slopes of the phase solubility lines at pHs of 8, 9 and 10 are 0.2472, 0.2444, and 0.2396, respectively. This indicates an increase in lidocaine solubility at the lower pH. The slope of the phase solubility line and the corresponding pH were used to determine the association constants for the inclusion complex of HP-β-CD/lidocaine free base (K_u) and HP-β-CD/ionized lidocaine (K_i). K_u and K_i were found to be $26.23 \pm 0.00025\ M^{-1}$ and $0.8694 \pm 0.00045\ M^{-1}$. The higher value of K_u compared to K_i can be attributed to the greater affinity between the hydrophobic inner cavity of HP-β-CD and lipophilic lidocaine free base as compared to its corresponding ionized form [45]. This study indicated that HP-β-CD can form a weak yet stable inclusion complex with both lidocaine free base and its ionized form in an aqueous solution.

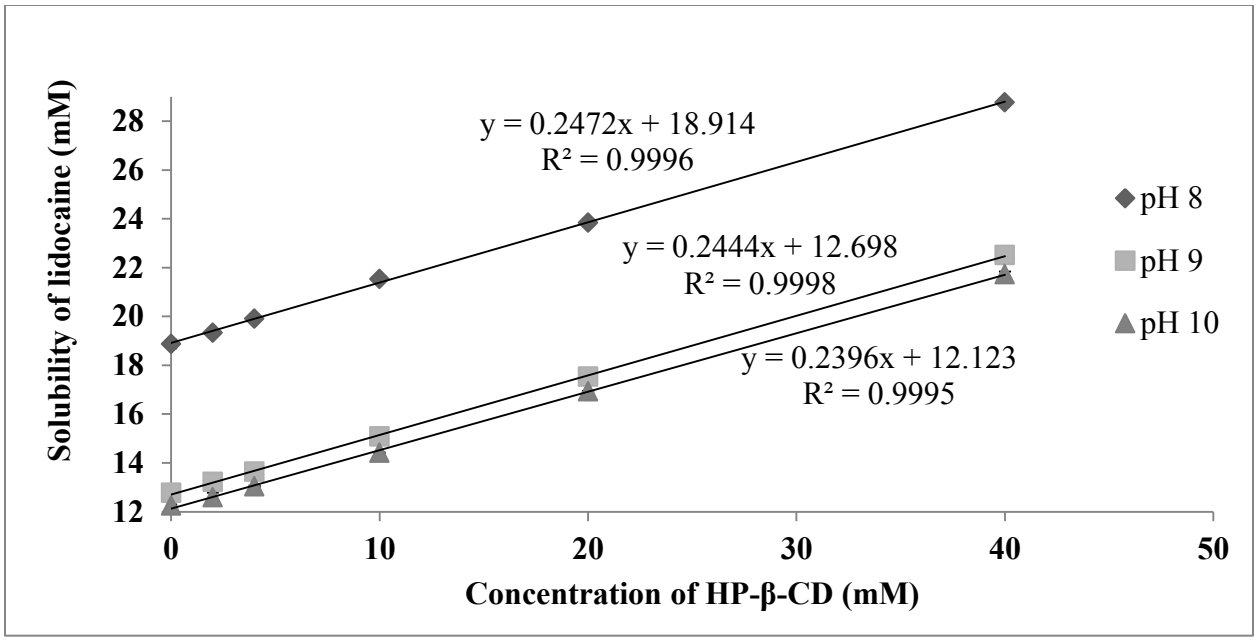

Figure 3.1: Phase-solubility diagram of lidocaine in the presence of increasing HP-β-CD concentrations at different pHs, at 25°C. (n=3, mean ± standard deviation values). Invisible error bars are smaller than the symbol

Xylocaine®, a sterile injectable lidocaine HCl (2%) with or without epinephrine, has a pH ranging between 3.3 to 5.5 [119]. Considering the pKa value of lidocaine to be 7.74, most lidocaine molecules remain ionized in the formulation. Therefore, we are interested in the association constant between the HP-β-CD and ionized lidocaine rather than its free base. Moreover, it was not possible to completely solubilize 2% of lidocaine free base in a 20% HP-β-CD solution (data not shown). Hence, we have chosen the ionized form of lidocaine (LID) for taste masking studies. Although the interaction between the HP-β-CD and LID seems to be weak (with association constant of 0.8694 M^{-1}) when compared with its free base, it is desirable to know the level of taste masking effect. Further, strong interaction between cyclodextrin and drug molecules is not always desired as the inclusion complex delays or alters the release and availability of the free drug. Drug molecules that form strong inclusion complexes with cyclodextrins display high retention

times, while drug molecules that form weak inclusion complexes display low values. If the interaction is too strong, the release profile of drug might be significantly delayed and the therapeutic effect of drug would be altered [120].

3.4.2. Differential scanning calorimetry

DSC thermograms of LID, HP-β-CD, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes are illustrated in Fig. 3.2 LID exhibited a characteristic sharp endothermic peak at 83.24 °C, which corresponds to its melting point. The thermogram of HP-β-CD showed a very broad endothermic peak between 70-100°C due to the release of water molecules present in the HP-β-CD cavity [121]. When HP-β-CD interacts with a guest molecule, their melting peaks, boiling peaks, or sublimation peaks in DSC thermograms usually shift or disappear [122]. DSC curves of LID/HP-β-CD physical mixtures (1:1 and 1:2) showed a decrease in endothermic peaks at 85°C, which indicated the presence of the free form of LID in physical mixtures. A decrease in LID melting peak in the physical mixture was due to the dilution effect of HP-β-CD. However, the complete disappearance of melting point peak (85ºC) in 1:1 and 1:2 LID/HP-β-CD inclusion complexes represent the absence of the free form of inclusion complexes, which confirms the inclusion of the LID molecule into the HP-β-CD cavity.

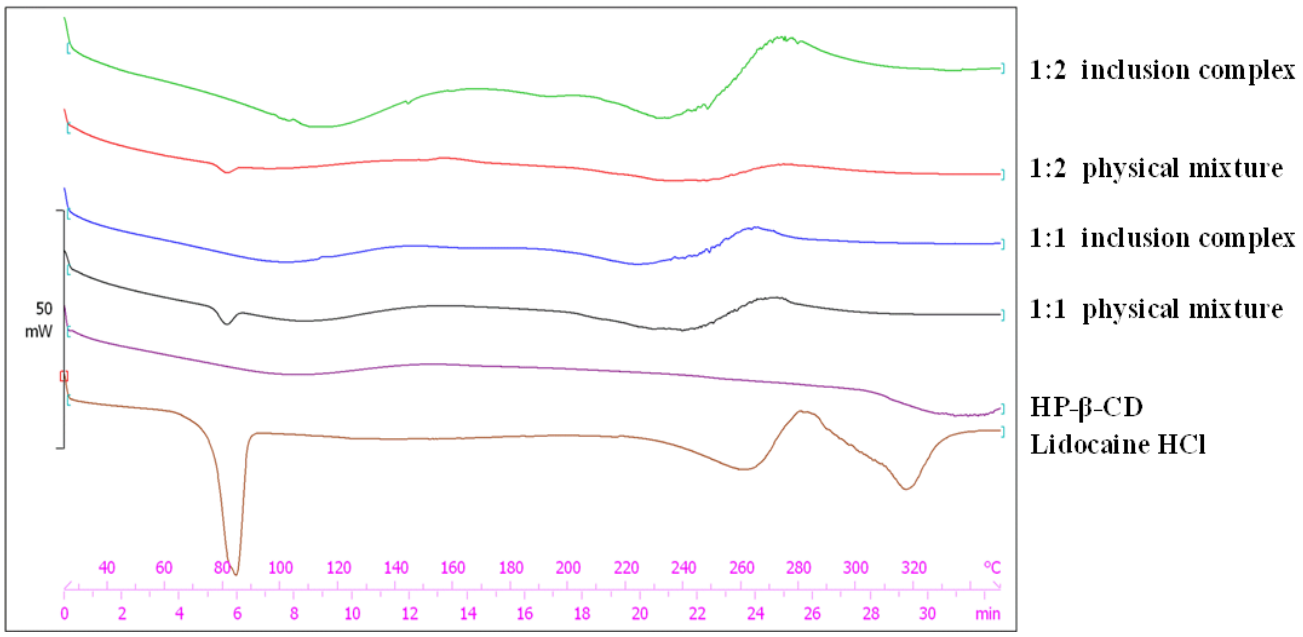

Figure 3.2: DSC curves of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

3.4.3. Fourier Transform Infrared (FT-IR) spectroscopy

FT-IR is one of the commonly used analytical techniques to characterize inclusion complexes of cyclodextrins. FT-IR spectra of the samples are represented in Fig. 3.3 The FT-IR spectrum of LID showed the presence of the following characteristic peaks: N-H stretching at 3451 cm^{-1} and 3385 cm^{-1}, C=O stretching at 1655 cm^{-1} [123]. FT-IR spectrum of HP-β-CD showed O-H stretching at 3353 cm^{-1} [123]. In physical mixtures (1:1 and 1:2), the LID characteristic peak at 3451 and 3385 cm^{-1} can still be detected, which indicates the presence of uncomplexed LID in physical mixtures. The complete disappearance of the LID characteristic peak at 3451, 3385, and 1655 cm^{-1} in inclusion complexes (1:1 and 1:2) can be attributed to the inclusion of functional groups of LID into the HP-β-CD cavity.

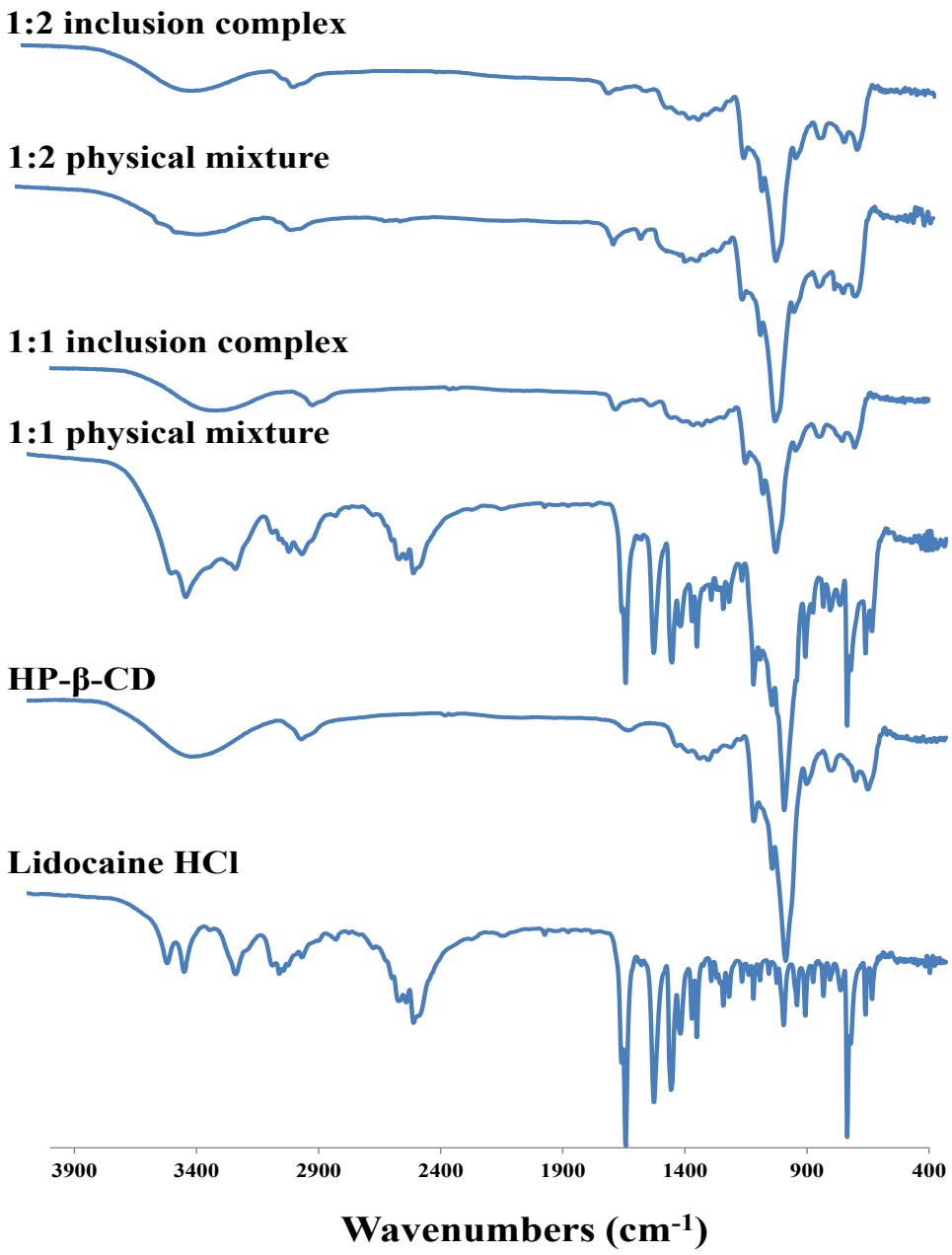

Figure 3.3: FT-IR spectra of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

3.4.4. Powder X-ray diffractometry (PXRD)

The diffractograms of samples are represented in Fig. 3.4 The presence of a variety of sharp peaks at different diffraction angles of 2θ in its PXRD pattern clearly indicates the crystalline nature of LID. In contrast, HP-β-CD was present in an amorphous form. The LID/HP-β-CD physical mixtures (1:1 and 1:2) showed distinct sharp peaks that matched the PXRD pattern of LID. This confirmed the presence of LID in crystalline form in physical mixtures. The diffractograms of LID/HP-β-CD inclusion complexes (1:1 and 1:2) indicated the loss of LID crystallinity in the inclusion complexes. These results may be attributed to the possible inclusion of LID molecules into the HP-β-CD cavity.

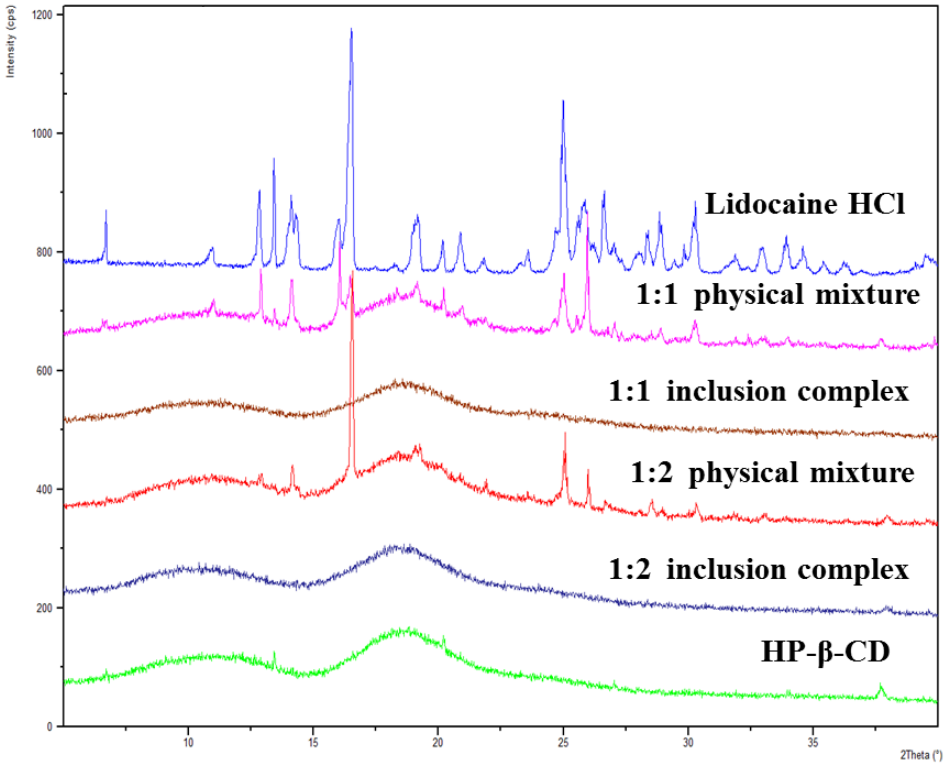

Figure 3.4: X-ray diffractograms of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

3.4.5. Nuclear magnetic resonance (NMR) study

NMR is an analytical technique extensively used for structural elucidation of organic compounds and also to investigate intra/inter-molecular interactions [124]. For example, the proton chemical shifts between a free guest molecule and its inclusion complex with cyclodextrin can be observed and compared to investigate the possible interaction between the guest molecule and cyclodextrin. Fig. 3.5 shows the chemical structure of LID. The NMR spectra of LID, HP-β-CD, 1:1 and 1:2 inclusion complexes are shown in Fig 3.6 Change of proton chemical shift in ^{1}H NMR spectra of LID after complexation with HP-β-CD is listed in Table 3.2. The changes in chemical shift can be attributed to the inclusion of LID within the HP-β-CD cavity.

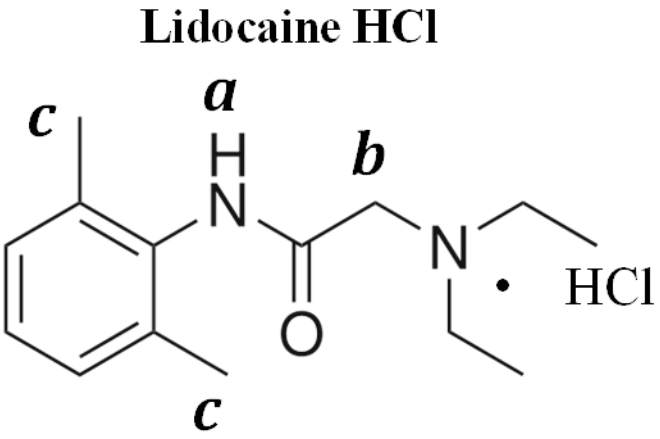

Figure 3.5: Chemical structure of lidocaine HCl

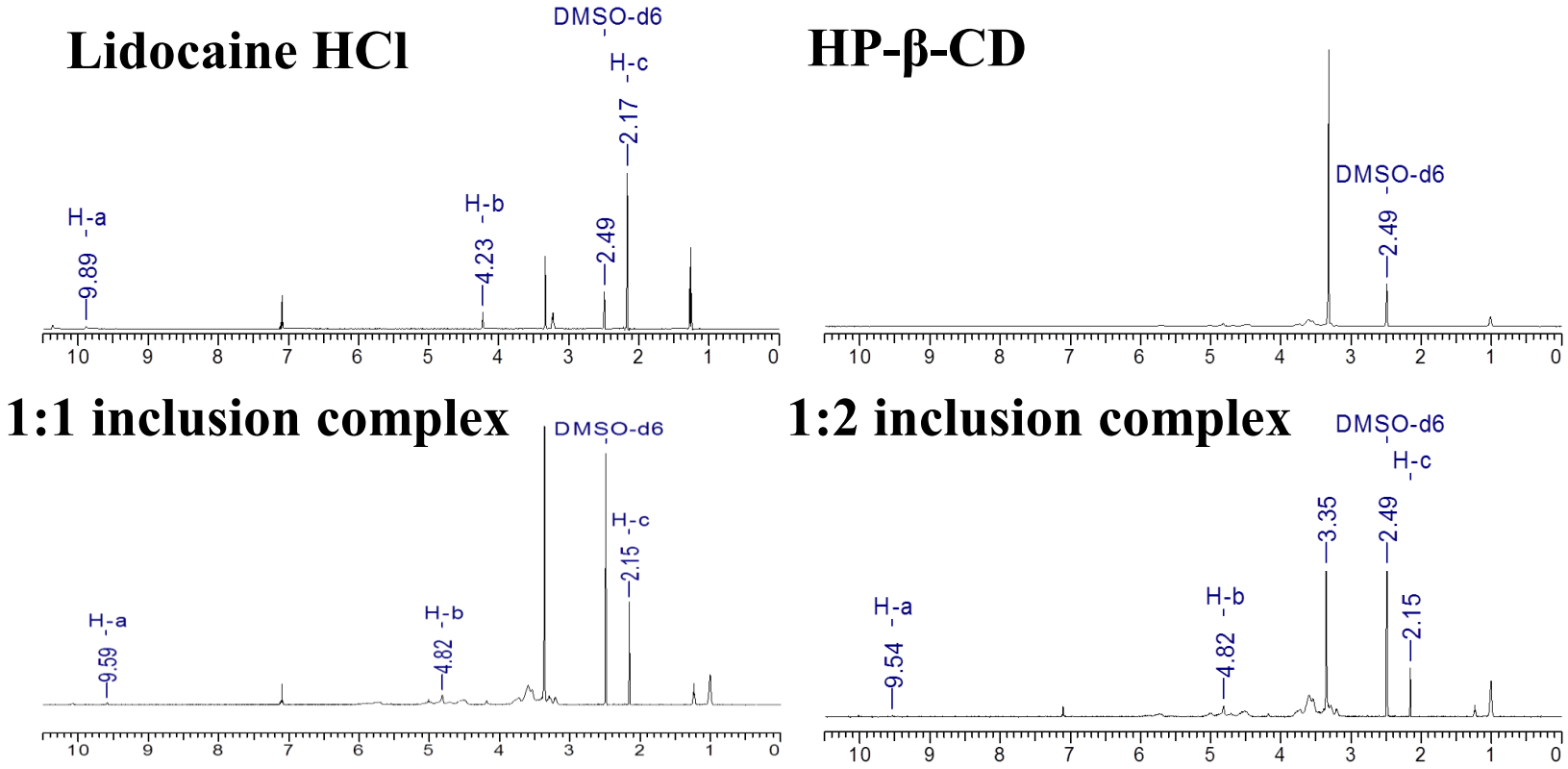

Figure 3.6: Nuclear Magnetic Resonance spectra of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

Table 3.2: Change in ^{1}H NMR spectra of lidocaine HCl after complexing with HP-β-CD

Hydrogen	Chemical Shift		
	Lidocaine HCl	Lidocaine HCl/HP-β-CD inclusion complex (1:1)	Lidocaine HCl/HP-β-CD inclusion complex (1:2)
a	9.89	9.59 (-0.30*)	9.54 (-0.35)
b	4.23	4.82 (+0.59)	4.82 (+0.59)
c	2.17	2.15 (-0.02)	2.15 (-0.02)

*represents the difference of the chemical shift of protons between free lidocaine HCl and its inclusion complex

3.4.6. Scanning electron microscopy (SEM)

SEM is a qualitative method commonly used in visualizing the morphology of drugs and inclusion complexes. The SEM images of LID, HP-β-CD, 1:1 and 1:2 LID/HP-β-CD physical mixtures, and 1:1 and 1:2 LID/HP-β-CD inclusion complexes, are illustrated in Fig. 3.7 LID particles were seen as a homogeneous crystal structure, which indicates its crystalline nature. HP-β-CD existed as loose and spherical particles. In 1:1 and 1:2 LID/HP-β-CD physical mixtures, both LID and HP-β-CD particles could be easily differentiated from each other in the SEM images. In contrast, inclusion complexes were observed as homogenous and flake-like structures. The dramatic change of structural morphology and shape of LID in inclusion complexes confirms the formation of inclusion complexes of LID and HP-β-CD.

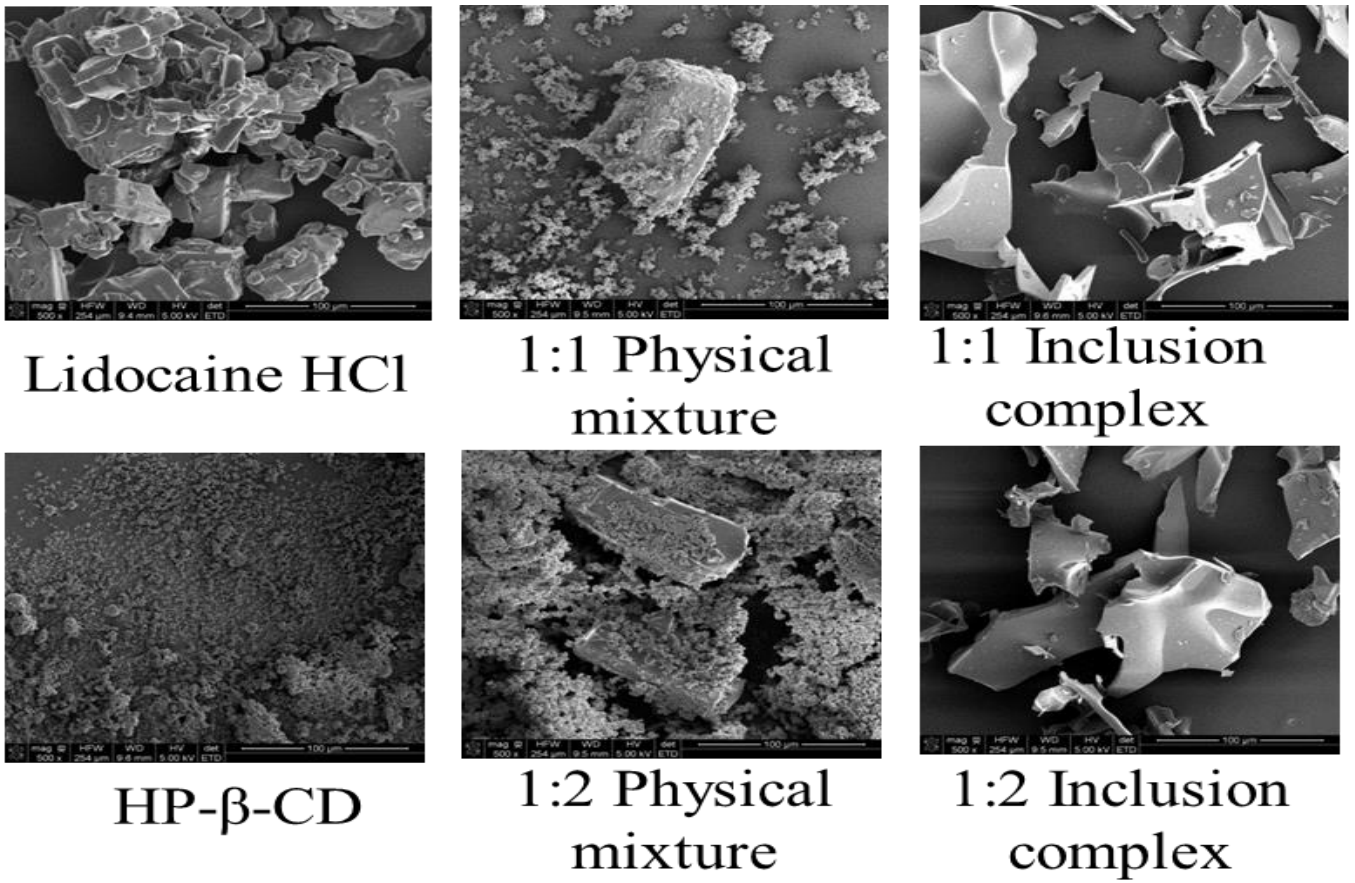

Figure 3.7: Scanning electron microscopy images of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes, 500 X magnification, bar = 100 μm

3.4.7. In vitro drug release study

LID, a fast-acting local anesthetic, reversibly blocks sensory neuronal conduction of noxious stimuli from reaching the central nervous system by binding to the voltage-gated sodium channel on excitable membranes [1]. Only the uncomplexed LID, which is in equilibrium with the complexed form, is capable of eliciting the anesthetic action. Upon injection of inclusion complexes in the oral cavity, we expect the free drug to be released from inclusion complexes for immediate action. In the *in vitro* release study, we compared the release rate of LID from the inclusion complexes with the pure drug. A cellulose membrane with a low molecular weight cutoff (1000 Da) was selected for the study. This cellulose membrane is selectively permeable to uncomplexed LID (Mol.wt.= 270.80 Da) and retains the complex (Mol.wt.= 1812.33 Da) as well as free HP-β-CD (Mol.wt.= 1541.53 Da) inside the dialysis bag. *In vitro* release profiles of LID from 1:1 and 1:2 inclusion complexes in phosphate buffer (50mM, pH 7.4) at 37°C are shown in Fig. 3.8. The release of LID from inclusion complexes was compared to the pure drug. Release of LID followed a first-ordered pattern from 1:1 and 1:2 inclusion complexes (Fig. 3.9). Parameters calculated from the dissolution studies are presented in Table 3.3 LID release from 1:1 and 1:2 inclusion complexes were compared with the pure drug using sampling time (amount of drug dissolved in that time), dissolution efficiency (area under the dissolution curve up to a certain time), and pairwise procedures, i.e. difference factor (f1) and similarity factor (f2). The difference between two drug release profiles is proportional to f1 value, but inversely proportional to f2 value. The difference in release profile is considered significant when f1 is greater than 15 (or) when f2 is less than 50 [125]. The f1 and f2 values of 1:1 inclusion complex in comparison with the pure drug

were found to be 9.27 and 51.99, respectively. For 1:2 inclusion complex, the f1 and f2 in comparison with the pure drug were found to be 17.37 and 40.24, respectively. There is no significant difference in the release of LID between the pure drug and 1:1 inclusion complex, while the 1:2 inclusion complex slightly delayed the release of LID from the dialysis bag.

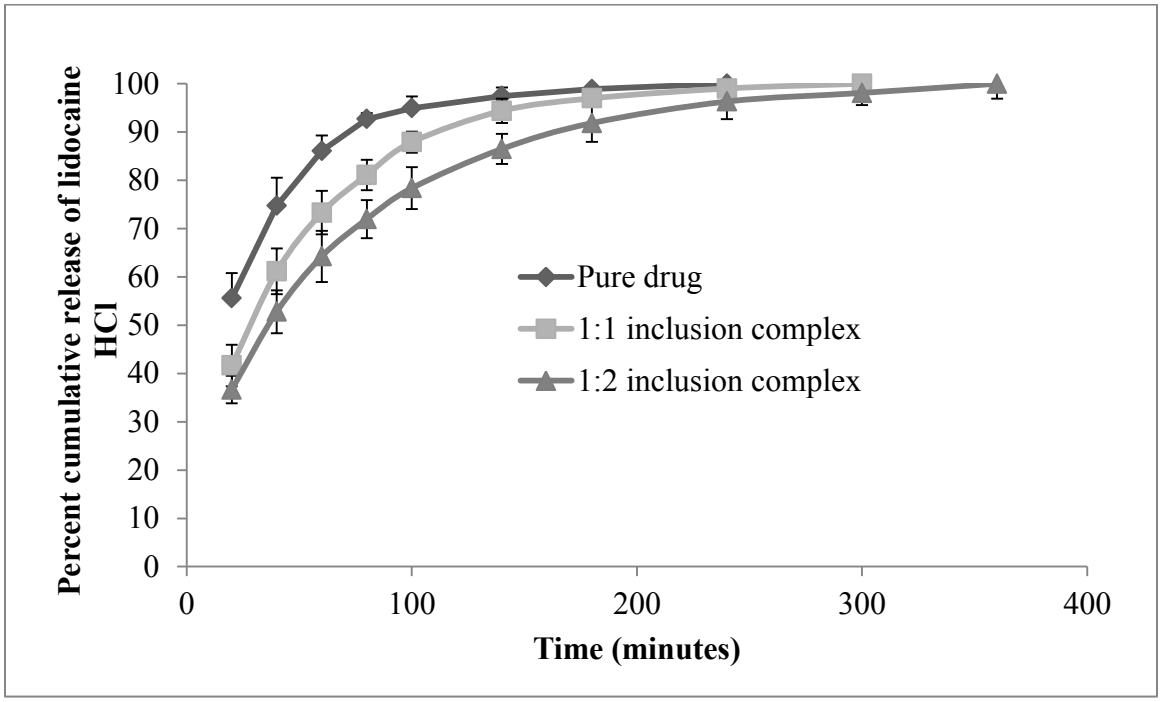

Figure 3.8: *In vitro* release profiles of lidocaine HCl from pure drug, 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes, (n=3)

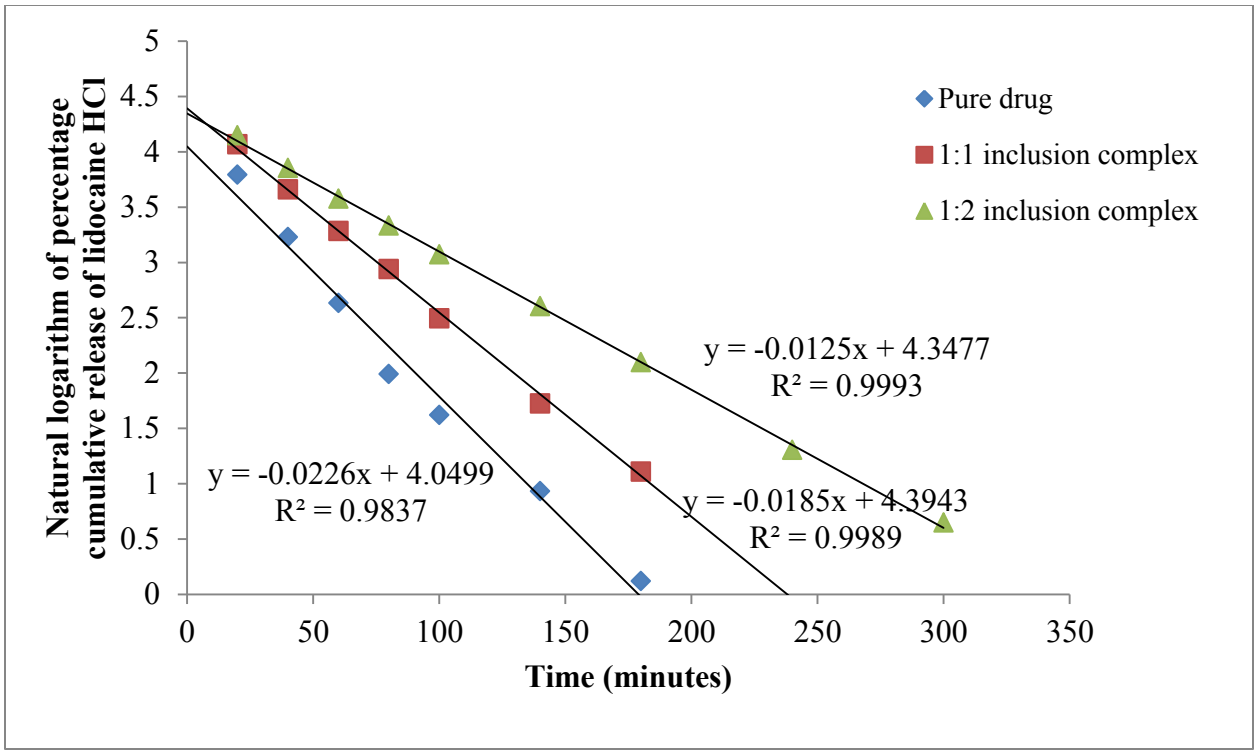

Figure 3.9: *In vitro* kinetic graph of lidocaine HCl from pure drug, 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

Table 3.3: Comparison of *in vitro* drug release profiles of lidocaine HC1 from pure drug, 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes

Formulations	Release constants (min^{-1})	Sampling time (t_{60min})	Dissolution efficiency (DE_{60min})	Difference factor (f_1)	Similarity factor (f_2)
Pure drug	0.0226	86.09%	57.78%	-	-
1:1	0.0185	73.30%	46.48%	9.27	51.99
1:2	0.0125	64.24%	40.53%	17.37	40.24

3.4.8. Stability of injectable formulations

Stability indicating HPLC method was developed for lidocaine for analyzing stability samples. Degradation of lidocaine was observed in the presence of 1 N NaOH. However, no interference between the drug and degradant peaks was observed. Table 3.4 shows that LID was chemically stable for at least six months at 4˚C, 25˚C and 40˚C. The study confirms the chemical compatibility between LID and HP-β-CD in solution.

Table 3.4: Chemical stability of lidocaine HCl in FC, F1, and F3 injectable formulations

Formulation	**Lidocaine HCl recovery (%) after 6 months of storage**		
	4˚C	25˚C	40˚C
Control	102.94 ± 0.04	102.77 ± 0.66	102.47 ± 0.30
1:1 formulation	101.41 ± 0.17	103.10 ± 0.34	102.11 ± 0.27
1:2 formulation	101.71 ± 0.35	102.95 ± 0.17	100.77 ± 0.22

FC: control formulation, F1: lidocaine HCl/HP-β-CD in 1:1 molar ratio with 0.09% sodium saccharin, F2: lidocaine HCl/HP-β-CD in 1:2 molar ratio with 0.09% sodium saccharin

3.4.9. Taste assessment using an electronic tongue

ETongue, an automated instrument to assess the bitterness of single or mixed drug substances, is widely used in the food and beverage, nutraceutical, and pharmaceutical industries. The ETongue approach has shown a very good correlation with the human taste panel study [97-99]. It equips a probe which consists of artificial multichannel taste sensors (ZZ, AB, BA, BB, CA, DA, and JE) for converting taste responses to electric signals [100]. The signal of each sensor after each assay was integrated into a matrix of data that was computed by multidimensional statistic tools. Taste analysis was carried out for formulations F1, F2, and F3. FC was considered a control. A taste map of all four

formulations based on principal component analysis (PCA) was generated using all sensors (Fig. 3.10). PCA was utilized to convert the seven-dimensional data obtained from seven different taste sensors to two-dimensional data for convenience in data analysis. The PCA map with two-dimensional data represents 100% of data information on its two axes [101]. As shown in Fig. 3.10, the PC1 axis and PC2 axis explain 61.625% and 33.401% of data variance, respectively. This indicates that 61.625% of the information obtained from sensors of ETongue lies on PC1 axis and 33.401% of the information lies on PC2 axis. Each sample was analyzed three times and presented as a triangle on the taste map. The distance between two samples is represented by the Euclidean distance between their corresponding triangles. All solutions were clearly differentiated from water (data not shown). The difference with water revealed that the ETongue responds well to measurements in the range of targeted concentrations. The distances between the control formulation (FC) and test formulations (F1, F2 and F3) represent the difference between the taste of FC and that of test formulations (Fig. 10). The larger the distance between the test and control formulations, the greater the taste difference between the formulations. In other words, FC was considered the bitterest of all formulations, so test formulations would have a better taste than the control. In Fig. 3.11, the distance between F3-FC is less than that of the distances between F1-FC and F2-FC. This indicated that the formulations containing both HP-β-CD and sodium saccharin (F1 and F2) have a better taste compared to the formulation containing sodium saccharin (F3) alone. Interestingly, no significant difference in taste was observed between formulations F1 and F2. It is expected that the use of HP-β-CD prevented the interaction of LID with the taste sensor, and addition of sodium saccharin further

enhanced the overall taste of the formulation. Considering that F1 has statistically similar release profile as FC and F1 uses a lower concentration of HP-β-CD compared to F2, F1 is considered the ideal in terms of taste and its ability to readily release the drug for anesthetic action. Such formulation (F1) when injected into the oral cavity is expected to eliminate the bitter taste by a two-step process: (i) inclusion complex of LID with HP-β-CD prevents the initial interaction of drug with taste receptors, and (ii) presence of sodium saccharin would overcome the bitter aftertaste of the formulation, if any, leaving behind a lingering sweet taste.

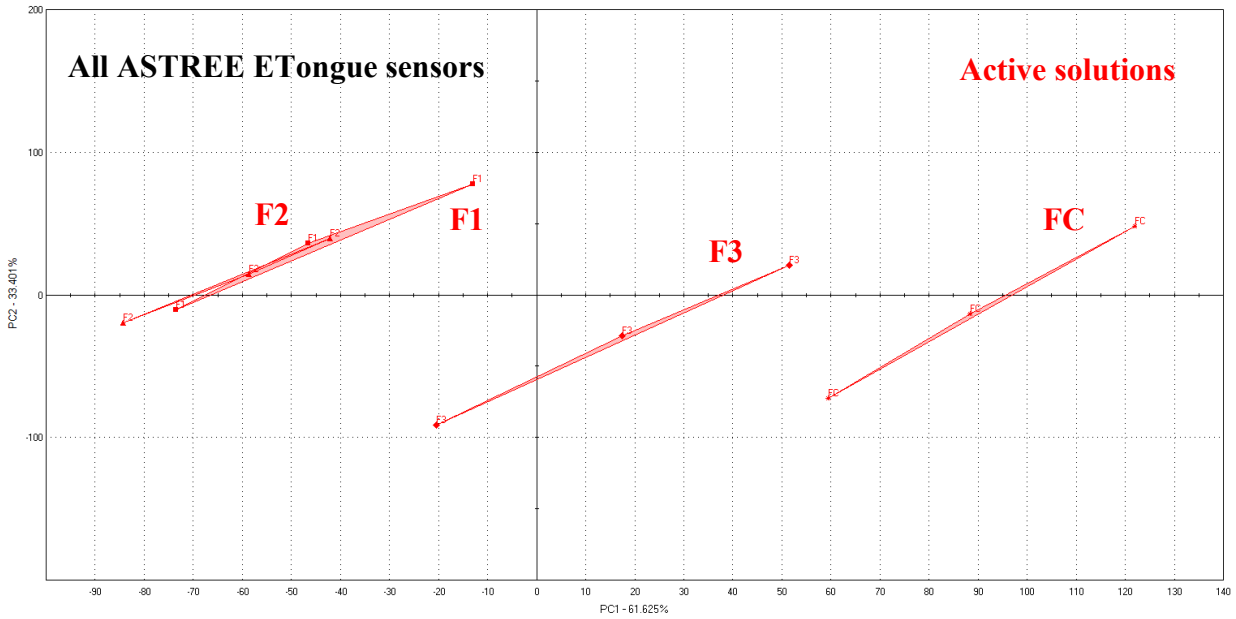

Figure 3.10: Taste map based on principal component analysis (PCA) of formulations, FC: control formulation, F1: lidocaine HCl/HP-β-CD in 1:1 molar ratio with 0.09% sodium saccharin, F2: lidocaine HCl/HP-β-CD in 1:2 molar ratio with 0.09% sodium saccharin, and F3: control formulation with 0.09% sodium saccharin

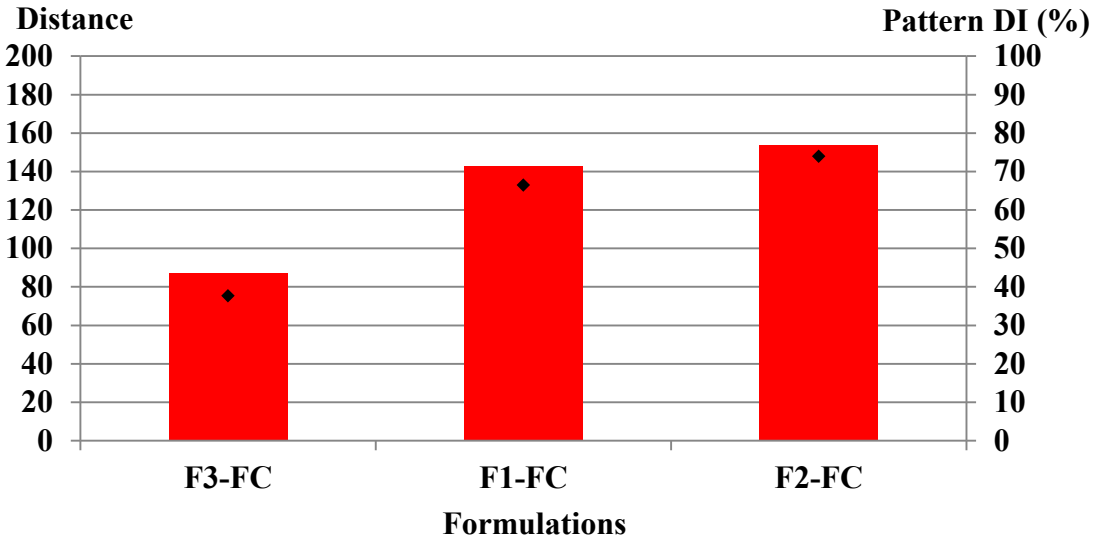

Figure 3.11: Distance between control formulation (FC) and active formulations, FC: control formulation, F1: lidocaine HCl/HP-β-CD in 1:1 molar ratio with 0.09% sodium saccharin, F2: lidocaine HCl/HP-β-CD in 1:2 molar ratio with 0.09% sodium saccharin, and F3: control formulation with 0.09% sodium saccharin

3.5. CONCLUSION

In conclusion, we have successfully prepared and characterized 1:1 and 1:2 inclusion complexes of LID and HP-β-CD. Inclusion complexes of LID and HP-β-CD at 1:1 and 1:2 molar ratios were confirmed using DSC, XRD, ^{1}H NMR, SEM, and FT-IR analysis. LID was found to be stable in both 1:1 and 1:2 cyclodextrin formulations for up to 6 months when stored at 4˚C, 25˚C, and 40˚C. Based on the *in vitro* release profile and data generated by the ETongue, formulation F1 containing LID/HP-β-CD in 1:1 molar ratio with 0.09% sodium saccharin was considered to be optimum. The results presented in this study suggested that taste masking of injectable local anesthetics for dental use can be achieved by utilizing a right combination of HP-β-CD and sodium saccharin, which in turn would improve patient compliance with dental treatment.

3.6. ACKNOWLEDGEMENTS

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