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Research Article

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

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Abstract. Several attempts have been made to mask the bitter taste of oral formulations, but none have been made for injectable formulations. This study aims to mask the bitter taste of dental lidocaine HCl (LID) injection 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 M ratios. Inclusion complexes in solution were studied using phase solubility in phosphate buffer solutions (pH 8, 9, and 10). Freeze-dried inclusion complexes were characterized using differential scanning calorimetry (DSC), X-ray, Fourier transform infrared (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 and for taste using an Alpha MOS ASTREE electronic tongue (ETongue). The association constants of HP-β-CD with lidocaine-free base and its ionized form were found to be 26.23± 0.00025 and 0.8694±0.00045 M^{-1}, respectively. Characterization studies confirmed the formation of stable inclusion complexes of LID and HP-β-CD. Injectable formulations were found to be stable for up to 6 months at 4°C, 25°C, and 40°C. The taste evaluation study indicated that HP-β-CD (1:1 and 1:2 M ratios) significantly improved the bitter taste of LID injectable formulation. In conclusion, inclusion complex in the 1:1 M ratio with 0.09% sodium saccharin was considered to be optimum in masking the bitter taste of LID.

KEY WORDS: bitter taste; HP-β-CD; inclusion complex; injectable; lidocaine HCl; taste masking.

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 USA, totaling to more than 900 million worldwide (3).

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 (4). Consequently, providing a favorable environment that allows treatment has become challenging for dentists, especially in children (5–7). 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®, which 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 toward the dental procedures, and render oral care visits more pleasant (8). Limited attempts have been made to mask the taste of dental products, e.g., oral rinse and mouthwash (8–10), 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

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extractions that children and adolescents undergo increased significantly (11,12). 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 (13). 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 (14). 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 Food and Drug Administration (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 (15–17). 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 (18). 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 (19–21). 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 (22). Sodium saccharin has been employed for a variety of dosage formulations, including oral solutions, oral suspensions, tablets, powders, mouthwashes, and dental pastes/gels (22). 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 (SEM), and *in vitro* drug release. Injectable formulations of LID were prepared using inclusion complexes and tested for stability and taste. Alpha MOS ASTREE electronic tongue (ETongue) analyzer (Alpha M.O.S., France) was used to assess the bitterness of formulations.

MATERIALS AND METHODS

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.

Methods

Phase-Solubility Study

The phase-solubility study was carried out as reported by Higuchi and Connors (23). 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 pK_a value of lidocaine-free base were calculated by the linear least squares fitting technique (24). The pK_a value and intrinsic water solubility of LID were calculated using the following equation:

$$S = S_0(1 + [H^+]/K_a)$$

Where [*S*] represents total lidocaine solubility at different pH values in the absence of HP-β-CD. The Y-intercept obtained by plotting [*S*] against [H^+] indicates the intrinsic solubility (S_0) 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 (25):

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

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. K_a 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 nonlinear least squares regression method (GraphPad Prism, V5.00, San Diego, CA) to determine K_u and K_i values (24). This study was conducted in triplicate.

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 M 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 M ratios.

Differential Scanning Calorimetry

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.

Fourier Transform Infrared 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 FT-IR spectrometer (Varian Excalibur Series UMA 600 FT-IR, 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 4000 cm^{-1}.

Powder X-ray Diffractometry

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 V2.1 software.

Nuclear Magnetic Resonance 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 s, and number of transients of 16.

Scanning Electron Microscopy

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 s and viewed with an SEM at an accelerating voltage of 5 kV.

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 1000 Da). Required amounts of LID and 1:1 and 1:2 LID/HP-β-CD inclusion complexes were dissolved in deionized water,

Table I. Compositions of Lidocaine HCl Injectable Formulations

Ingredients	Percentage composition				Role
	FC (control)	F1 (1:1 M ratio)	F2 (1:2 M 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[a]	0.17[a]	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

[a] Amount of NaCl in test formulations was adjusted according to isotonicity calculation

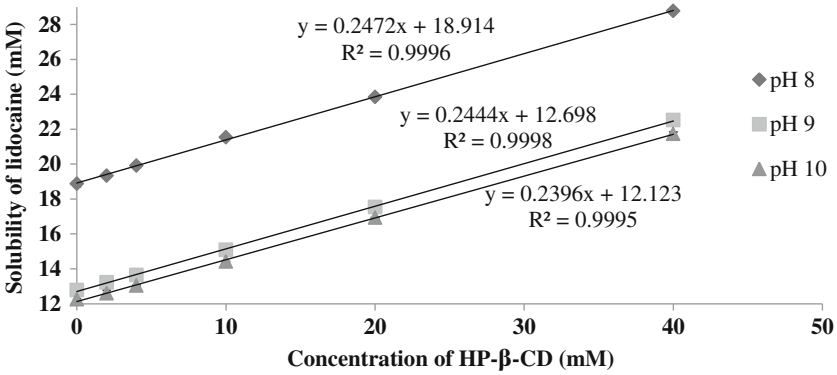

Fig. 1. Phase-solubility diagram of lidocaine in the presence of increasing HP-β-CD concentrations at different pH values, at 25°C (n=3, mean±standard deviation values). *Invisible error bars* are smaller than the symbols.

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 263 nm after suitable dilution.

Preparation of Injectable Formulations Using Inclusion Complexes

The final injectable formulations (control formulation (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 I, and the final volumes were adjusted to 100 ml. The solutions were filtered through 0.2 μm Millex (Millipore Corporation, Billerica, USA) and used for stability and taste perception studies.

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 FC (without HP-β-CD and sodium saccharin). Sterile samples were placed 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 high-performance liquid chromatography system (HPLC).

Assay of LID

A HPLC (Waters Alliance 2695 separation module, Milford, MA) equipped with a Waters C18 column (75×4.6 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 min/% 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

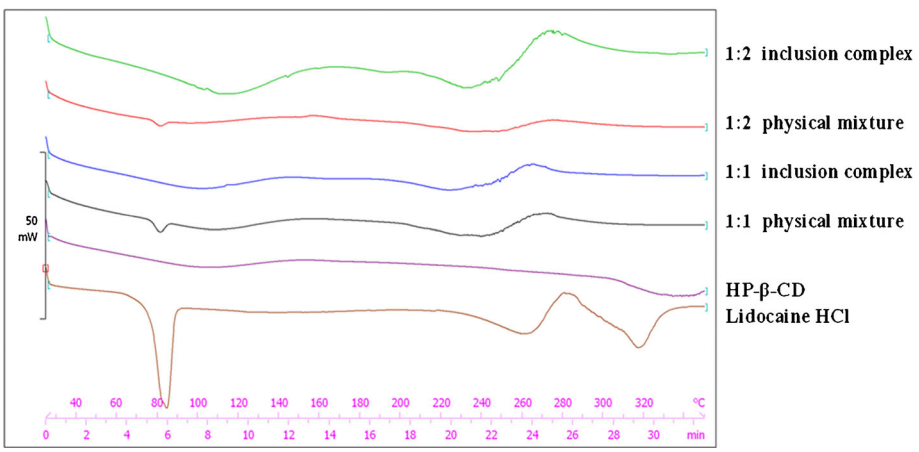

Fig. 2. DSC curves of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes.

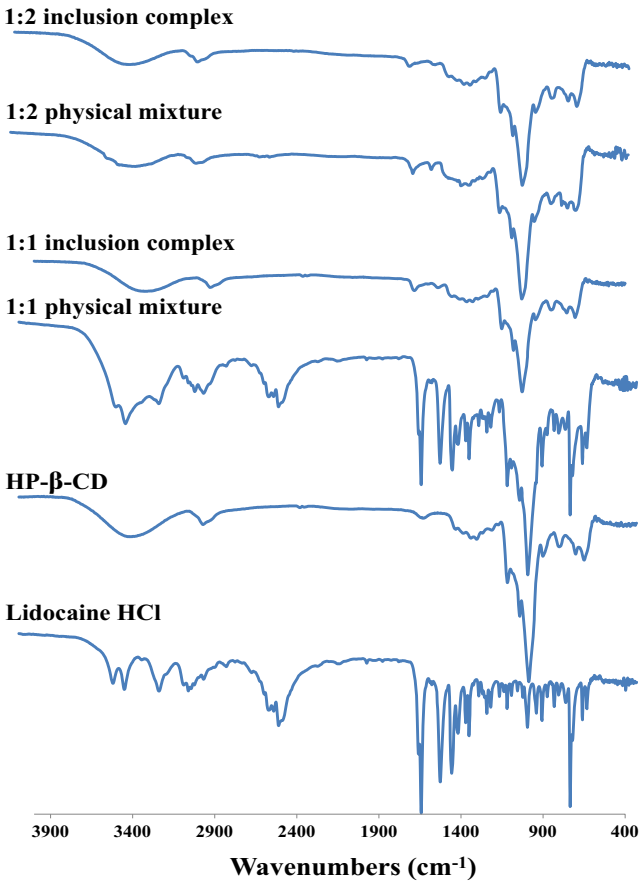

Fig. 3. FT-IR spectra of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes.

9.1 min. 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 intraday 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 h and oxygen (2% H_2O_2), heat (70°C), and light (UV light) for 21 h. Stressed samples were analyzed using the HPLC method (26). The LID peaks of stress samples were examined for any interference with degradant peak.

Taste Assessment Using an Electronic Tongue

Taste was assessed using an Alpha MOS ASTREE ETongue system equipped with an Alpha M.O.S. sensor set no. 2 (for pharmaceutical analysis) composed of seven specific sensors (ZZ, AB, GA, BB, CA, DA, and 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 FC, LID/HP-β-CD in 1:1 M ratio with 0.09% sodium saccharin (F1), LID/HP-β-CD in 1:2 M ratio with 0.09% sodium saccharin (F2), and the FC with 0.09% sodium saccharin (F3) was carried out using an ETongue. The taste of F3 (FC 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 I. Twenty milliliters of

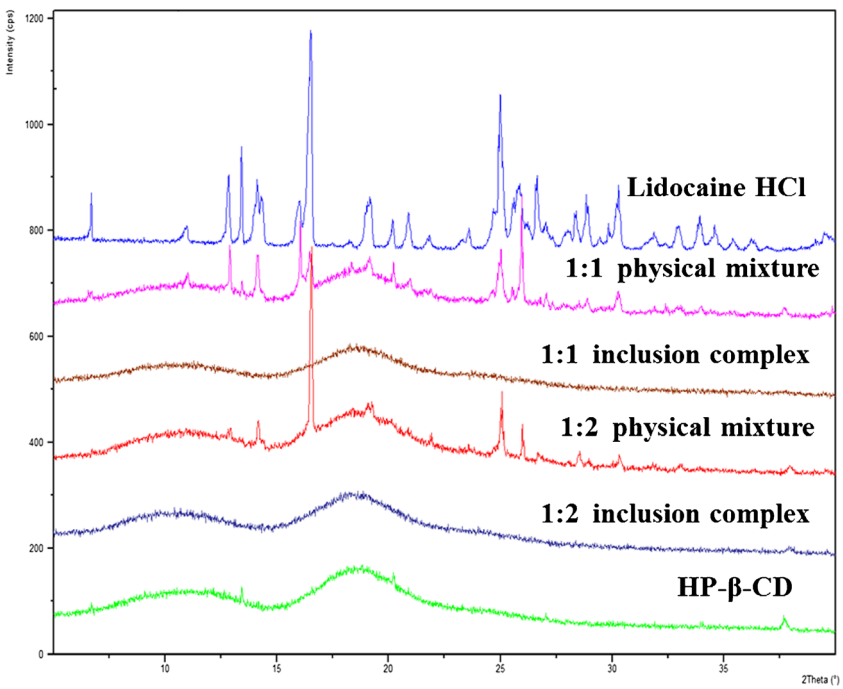

Fig. 4. X-ray diffractograms of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes.

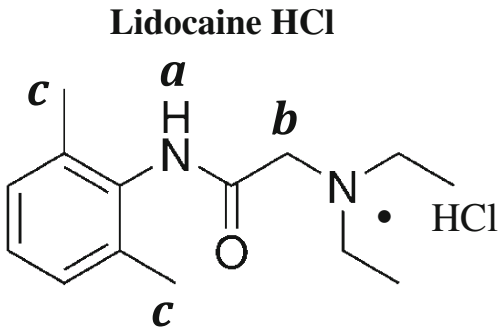

Fig. 5. Chemical structure of lidocaine HCl.

each sample was placed directly into a beaker and analyzed by the Alpha MOS ASTREE ETongue. Acquisition time and time per analysis were set at 120 and 180 s, respectively. The ETongue 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 Alpha MOS ASTREE ETongue.

RESULTS AND DISCUSSION

Phase-Solubility Study

The S_0 and pK_a 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 (27,28). S_0 and pK_a were used for subsequent calculations of association constants between lidocaine and HP-β-CD. Figure 1 shows the correlation between lidocaine solubility and varying HP-β-CD concentrations at pH values 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. As a matter of fact, phosphate ions in phosphate buffers do not interfere with solubility of lidocaine in the phase-solubility study. The solubility product constant (K_{sp}) of lidocaine phosphate is ~0.043 M^2 (29), and the K_{sp} of lidocaine dihydrogen phosphate was not exceeded (data not shown) in all experimental conditions. This suggests the non-interference of lidocaine dihydrogen phosphate salt in the phase-solubility study. Lidocaine solubility increased linearly with increasing HP-β-CD concentrations at all three pH values 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 (30). The slopes of the phase-solubility lines at pH values 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±0.00025 and 0.8694±0.00045 M^{-1}. The higher value of K_u compared with 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 with its corresponding ionized form (31). 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.

Xylocaine®, a sterile injectable lidocaine HCl (2%) with or without epinephrine, has a pH ranging between 3.3 and 5.5 (32). Considering the pK_a 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

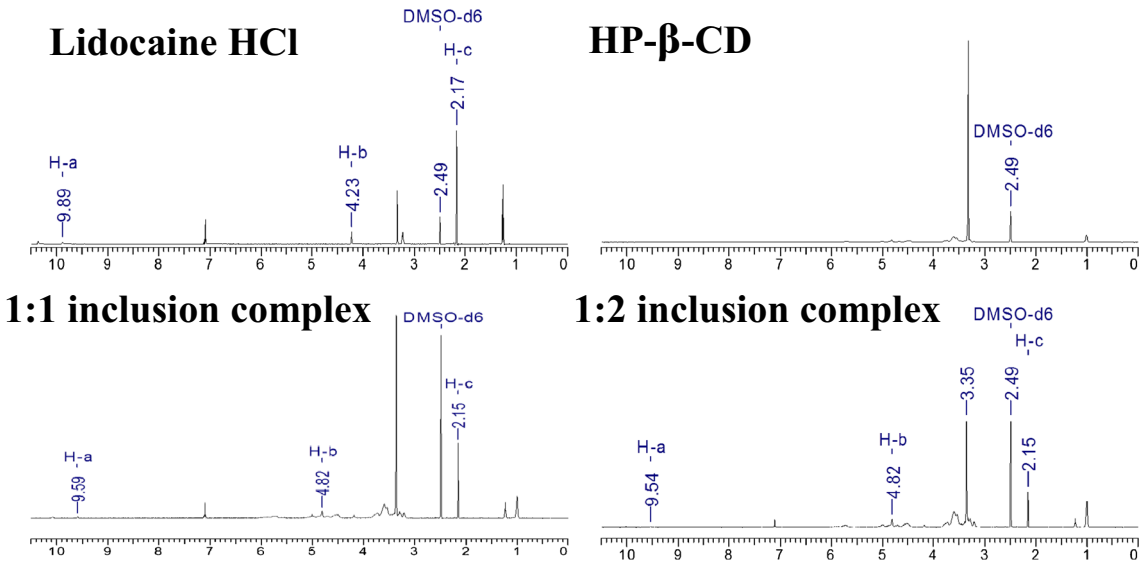

Fig. 6. Nuclear magnetic resonance spectra of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes.

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. Furthermore, 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 (33).

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. 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°C and 100°C due to the release of water molecules present in the HP-β-CD cavity (34). When HP-β-CD interacts with a guest molecule, their melting peaks, boiling peaks, or sublimation peaks in DSC thermograms usually shift or disappear (35). 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 (85°C) 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.

Fourier Transform Infrared 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. The FT-IR spectrum of LID showed the presence of the following characteristic peaks: N–H stretching at 3451 and 3385 cm^{-1}, C=O stretching at 1655 cm^{-1} (36). FT-IR spectrum of HP-β-CD showed O–H stretching at 3353 cm^{-1} (36). 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.

Table II. Change in ^{1}H NMR Spectra of Lidocaine HCl After Complexing with HP-β-CD

	Chemical shift		
Hydrogen	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[a])	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)

[a] The difference of the chemical shift of protons between free lidocaine HCl and its inclusion complex

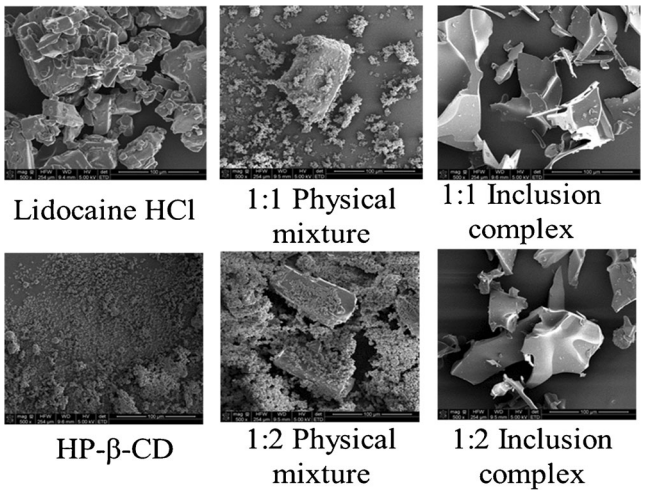

Fig. 7. Scanning electron microscopy images of lidocaine HCl, HP-β-CD, 1:1 and 1:2 lidocaine HCl/HP-β-CD physical mixtures, and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes, ×500 magnification, *bar*=100 μm.

Powder X-ray Diffractometry

The diffractograms of samples are represented in Fig. 4. The presence of a variety of sharp peaks at different diffraction angles of 2θ in its powder X-ray diffractometry (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

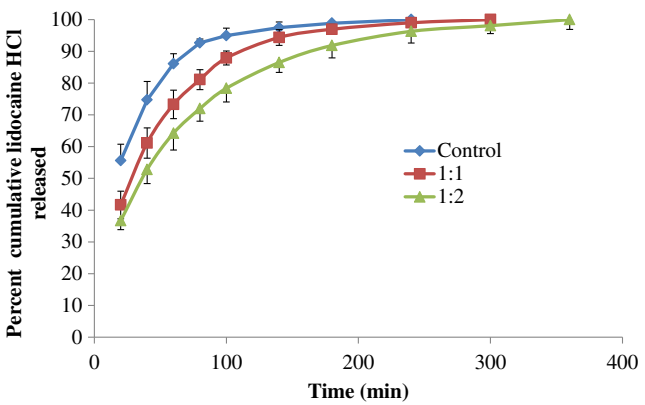

Fig. 8. *In vitro* release profiles of lidocaine HCl from pure drug and 1:1 and 1:2 lidocaine HCl/HP-β-CD inclusion complexes (n=3). *Invisible error bars* are smaller than the symbols.

Table III. Comparision of *In Vitro* Drug Release Profiles of Lidocaine HC1 from Pure Drug and 1:1 and 1:2 Lidocaine HCl/HP-β-CD Inclusion Complexes

Formulations	Release constants (min^{-1})	Sampling time ($t_{60\ min}$)	Dissolution efficiency ($DE_{60\ min}$)	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

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.

Nuclear Magnetic Resonance Study

NMR is an analytical technique extensively used for structural elucidation of organic compounds and also to investigate intra-/inter-molecular interactions (37). 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. Figure 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. 6. Change of proton chemical shift in ^1H NMR spectra of LID after complexation with HP-β-CD is listed in Table II. The changes in chemical shift can be attributed to the inclusion of LID within the HP-β-CD cavity.

Scanning Electron Microscopy

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. 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.

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 (50 mM, pH 7.4) at 37°C are shown in Fig. 8. The release of LID from inclusion complexes was compared with the pure drug. The release profile of pure drug indicates immediate availability of drug for action. Release of LID followed a first-ordered pattern from 1:1 and 1:2 inclusion complexes. Parameters calculated from the dissolution studies are presented in Table III. 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 (f_1) and similarity factor (f_2) (38). Factors f_1 and f_2 are widely used to compare the *in vitro* dissolution profiles (39). The difference in release profile is considered significant when f_1 is greater than 15 or when f_2 is less than 50. The f_1 and f_2 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 f_1 and f_2 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.

Table IV. Chemical Stability of Lidocaine HCl in FC, F1, and F3 Injectable Formulations

	Lidocaine HCl recovery (%) after 6 months of storage		
Formulation	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 M ratio with 0.09% sodium saccharin, *F2* lidocaine HCl/HP-β-CD in 1:2 M ratio with 0.09% sodium saccharin

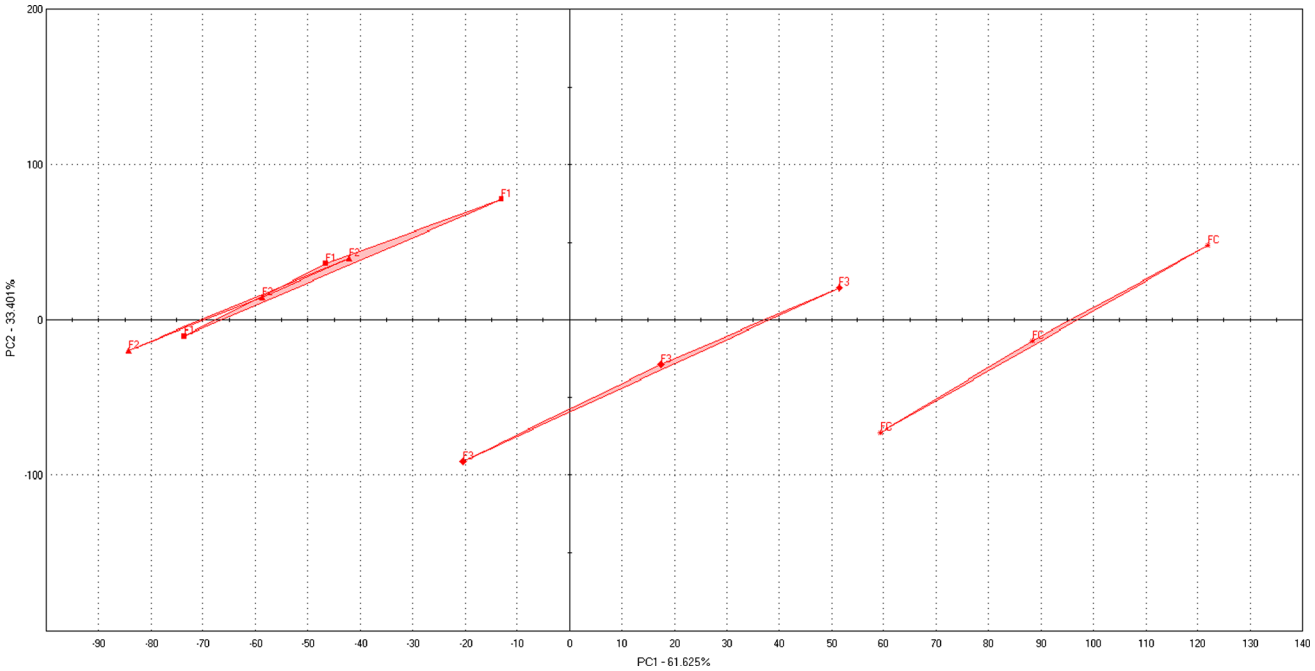

Fig. 9. Taste map based on principal component analysis (PCA) of formulations, control formulation (FC), lidocaine HCl/HP-β-CD in 1:1 M ratio with 0.09% sodium saccharin (F1), lidocaine HCl/HP-β-CD in 1:2 M ratio with 0.09% sodium saccharin (F2), and control formulation with 0.09% sodium saccharin (F3).

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 IV shows that LID was chemically stable for at least 6 months at 4°C, 25°C, and 40°C. The study confirms the chemical compatibility between LID and HP-β-CD in solution.

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 (40–42). 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 (43). 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. 9). 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 (44). As shown in Fig. 9, 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 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 FC, 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. 10, the distance between F3 and

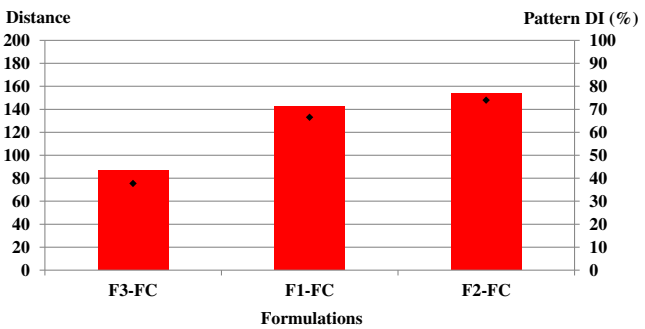

Fig. 10. Distance between control formulation (FC) and active formulations, control formulation (FC), lidocaine HCl/HP-β-CD in 1:1 M ratio with 0.09% sodium saccharin (F1), lidocaine HCl/HP-β-CD in 1:2 M ratio with 0.09% sodium saccharin (F2), and control formulation with 0.09% sodium saccharin (F3).

FC is less than that of the distances between F1 and FC and F2 and FC. This indicated that the formulations containing both HP-β-CD and sodium saccharin (F1 and F2) have a better taste compared with 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 with 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.

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 M ratios were confirmed using DSC, XRD, ^{1}H NMR, SEM, and FT-IR analyses. 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 M 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.

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