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Original Article

Development of Fast Dissolving Sublingual Wafers of Promethazine Hydrochloride

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Abstract

Promethazine hydrochloride, a 5-HT3 antagonist is a powerful antiemetic drug with an oral bioavailability of 25% due to extensive hepatic first pass metabolism and is extremely bitter in taste. To overcome the above draw backs, the present study was carried out to formulate and evaluate fast dissolving taste masked wafers of Promethazine hydrochloride for sublingual administration. Taste masking was achieved by inclusion complexation with β-Cyclodextrin, confirmed by e-tongue evaluation. The wafers were prepared by lyophilization, using polymers such as Gelatin, Xanthan gum and Methyl cellulose in different ratios. The IR spectral studies showed no interaction between drug and β-Cyclodextrin or with other additives. Satisfactory results were obtained when the wafers were subjected to tests such as uniformity of weight, thickness, surface pH, uniformity of drug content, disintegration time, moisture uptake, moisture loss, moisture content, and *in vitro* drug release studies. *Ex vivo* drug permeation studies were carried out using porcine membrane model and a drug permeation of 33-99% was observed through porcine mucosa within 6 min. *In vitro* release studies indicated 98-100% release within 6 min. A higher percentage of drug release was observed from wafers containing a combination of Gelatin and Xanthan gum. The stability studies conducted for a period of 12 weeks showed no appreciable change in drug content, surface pH, and *in vitro* drug release and *ex vivo* permeation for the first 8 weeks. Thus stable, porous, uniformly loaded fast disintegrating, taste masked Promethazine HCL lyophilized sublingual wafers with good compatibility and stability were achieved.

***Key words*:** Dissolution, Disintegration time, e-tongue, lyophilization, Permeation, Promethazine HCL, Taste masking, Wafers

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1. Introduction

The sublingual route is capable of producing a rapid onset of action because of the high permeability and the rich blood supply, thus making it an appropriate route for

drugs with short delivery period and frequent dosing regimen. This route overcomes first pass metabolism thus increasing the bioavailabilty of the drug.

Wafers are paper-thin polymer films used as carriers for pharmaceutical agents. This dosage form is taken orally but does not require water for swallowing. It dissolves rapidly in the oral cavity and the active ingredient can be absorbed into the blood stream via the oral mucosa. Hepatic first-pass effect is avoided thus improving bioavailability[1, 2].The active ingredient may be dissolved, emulsified or dispersed within the polymer matrix and the typical size of wafers is between 1cm^2 to 10 cm^2, with a thickness of 20 μm to 500 μm. [1, 3, 4].

Lyophilization or Freeze Drying is the most frequently used process for the manufacture of wafers. Here, the water present in the sample is frozen, followed by its removal, initially by sublimation (primary drying) and then by desorption (secondary drying). After lyophilization, the moisture content of the product is reduced to such a low level that it does not support biological growth or chemical reactions that makes the formulation unstable. This technique is useful in the development of drugs which are thermolabile and/or unstable in aqueous medium. Freeze dried products are porous because of which rehydration will be rapid leading to quick disintegration. The possibility of damage due to high drying temperatures is considerably reduced and the appearance of the product is not altered [1, 5, 6, 7, 8]. Some of the fast dissolving/ disintegrating technologies that are currently available in the market include ZYDIS and LYOC(R.P. Scherer, Inc.),WOWTAB (Yamanouchi Pharma Technologies, Inc.) , ORASOLV and DURASOLV (Cima Labs, Inc.), FLASHDOSE (Fuisz Technologies, Ltd.), FLASHTAB (Prographarm Group) and ORAQUICK (KV Pharmaceutical Co., Inc.) [9].

Kinetosis also known as motion sickness is a very common disturbance of the inner ear that is caused by repeated motion and can develop from the movement of a car, movement of a boat, or from turbulence in an airplane. The symptoms of motion sickness are nausea, vomiting, dizziness, fatigue and headache [10]. A wide range of drugs have proven to be effective against nausea and vomiting. These include anti- histamines, anti-cholinergics, dopamine receptor antagonists, 5–HT_3 receptor antagonists and prokinetic agents. Promethazine hydrochloride (PMT) is one of the most effective agents for treating motion sickness and other balance disorders. Promethazine is also indicated for inducing light sedation and for treating various allergic conditions. This drug is a first generation anti-histamine of the phenothazines family and acts as a strong antagonist of the H_1 receptor (antihistamine) and moderate mACh receptor antagonist, thus blocking the action of acetylcholine on the receptors (anticholinergic effect). Promethazine is well absorbed after oral and intramuscular administration and has a half-life of 16-19 hours and Peak plasma

concentrations are attained 2 to 3 hours after a dose by these routes. But its systemic bioavailability after oral doses is very low (about 25%) which is mainly due to extensive first-pass metabolism in the liver. To overcome these problems and for effective drug delivery, Promethazine may be administered via the sublingual route [11].

Literature survey revealed that extensive work has been carried out on orodispersible tablets of Promethazine, but no work on lyophilized wafers has been reported so far. Hence the objective of the present research work is to formulate and evaluate fast dissolving sublingual wafers of Promethazine hydrochloride by lyophilization process to achieve a safe, rapid and effective dosage form with enhanced oral bioavailability. The wafer will be is instantly wetted by saliva when placed sublingually, following which the wafer will rapidly hydrate and adhere onto the site of application. It will then rapidly disintegrate and dissolve (in less than a minute), to release the medication for oramucosal absorption. Since Promethazine hydrochloride is a bitter drug, taste masking will be attempted in order to overcome the bitter taste, by using various techniques.

2. Materials and Methods

Promethazine Hydrochloride (Promethazine HCl) was obtained from Yarrow Chem., India. Gelatin, Xanthan Gum, Methyl Cellulose A-15, β-Cyclodextrin (β-CD) was purchased from Yarrow Chem., India. All other chemicals were of analytical grade and were used without additional purification.

2.2. Taste Masking of Promethazine Hydrochloride

Taste Masking of Promethazine hydrochloride was achieved by the following methods.

2.2.1. Physical Mixture of Promethazine HCl and Sweetener

Promethazine HCl and aspartame were weighed accurately in the ratios 1:1 and 1:2. These were mixed properly by triturating them in a mortar and pestle and then passing through sieve no.120.

2.2.2. Solid Dispersion of Promethazine HCl with Mannitol

The drug (Promethazine HCl) and carrier (Mannitol) were weighed accurately in the ratios, 1:1 and1:2. The carrier was melted in a china dish and the drug was incorporated into the molten carrier and mixed thoroughly. The resultant mass was then solidified by gradually reducing the temperature and the resultant solid dispersion was passed through sieve No. 16.

2.2.3. Promethazine Hydrochloride – β-Cyclodextrin Inclusion Complex

Promethazine Hydrochloride – β-Cyclodextrin inclusion complexes was prepared by co-evaporation method. 1:1 and 1: 2 molar ratios of β-Cyclodextrin (β-CD) and Promethazine hydrochloride were dissolved in 10 ml of 50% aqueous ethanol. The mixture

Table 1. Scale for e – tongue analysis.

Scale	Taste
1	Very sweet
2-3	Sweet
4-5	Acceptable
10	Very bitter

was stirred till a clear solution was obtained and the resulting solution was evaporated under vacuum at a temperature of 45 ^{0}C, until completely dry. The dried complex was pulverized into a fine powder and passed through sieve no. 100. The resulting sample was stored in a desiccator until further use.

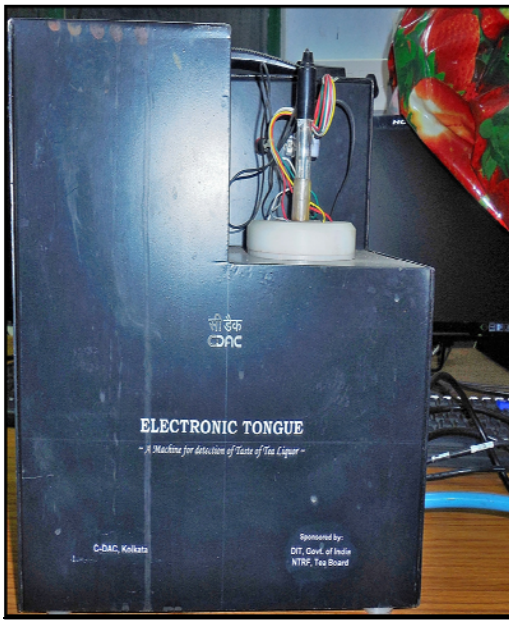


Figure 1. Electronic Tongue.

2.3. Determination of Extent of Taste-Masking

The products obtained from the three different taste masking methods were analyzed for the degree of taste masking with the help of an electronic tongue (as shown in Figure1) developed by the Centre of Development of Advanced Computing, Kolkata, West Bengal at the Tea Testing Laboratory, Ambasa, Tripura. The samples were dissolved in 10 ml distilled water and placed in the beaker attached with electrodes of the instruments. The instrument was allowed to run for 10 min. before recording the result. The results were generated on a scale of 1-10. The values of the scale are interpreted in Table 1.

2.4. Characterization of Promethazine HCl – β-C yclodextrin inclusion complex

Promethazine HCl – β-Cyclodextrin inclusion complex prepared in the molar ratio 1:2 was characterized by the following methods:

2.4.1. X-Ray Diffractometry (XRD)

X-ray diffraction (XRD) experiments were performed using AXS D8 Advance (Bruker, USA) diffractometer. The radiation used was nickel filtered CuKα, which was generated using an acceleration voltage of 40Kv and a cathode current of 35 mA. The samples were scanned over a 2 θ range of 3-80°, with a counting time of one second per 0.02°.

2.4.2. Nuclear Magnetic Resonance Studies

1H Nuclear magnetic resonance spectra were recorded on NMR (Minispec mq- one series, Bruker, USA) spectrometer. The samples were scanned at frequency of 300 mHz. Chemical shifts (δ) were reported in parts per million (ppm) downfield from

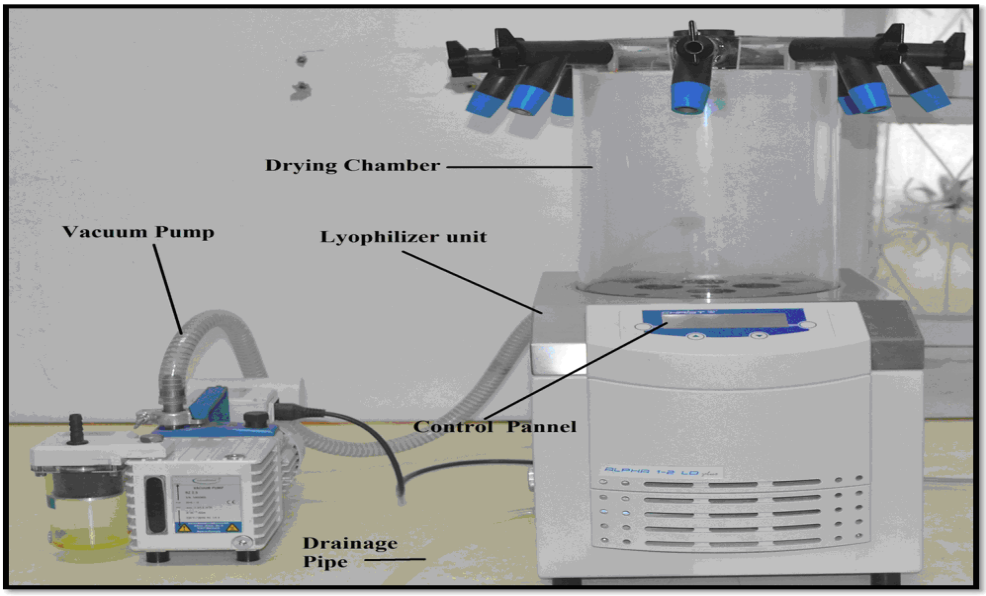


Figure 2. Lyophilizer.

internal reference standard Tetra Methyl Silane (TMS).

2.4.3. Mass Spectroscopic Studies

The molecular weight of the inclusion complex was determined by Electron Spray Ionization -Mass spectroscopy (ESI-MS). The mass spectra were recorded on LCMS 2010 (Shimadzu, Japan).

2.5. Selection of Wafer Forming Polymer

Placebo wafers were prepared by using different polymers (Gelatin, Xanthan Gum, Hydroxypropyl Cellulose, Locust Bean Gum, Methyl cellulose A-15) in different concentrations ranging from 1 to 3% w/v. The suitability of polymers was investigated by evaluating different parameters like wafer forming ability, integrity and disintegration time. From the preliminary trials carried out, Gelatin and Xanthan Gum were selected for the final formulations.

2.6. Formulation of Promethazine Hydrochloride Wafers

Accurately weighed amount of Promethazine HCl-β cyclodextrin inclusion complex was dissolved in 10 ml of distilled water with continuous stirring. To this accurately weighed amount of polymer(s) according to the formulation requirements were added with minimum heating. Aspartame and Methyl Paraben were added and the stirring was continued to obtain a gel like consistency. Strawberry flavour was added to it and stirring continued for some more time. The resultant gel was poured into moulds and placed in a deep freezer for a period of 24 hours. The frozen samples were then dried in a lyophilizer (Alpha 1-2 LD plus, Martin Christ, Germany) for a period of another 24 hours at

Table 2. Formulation table for Promethazine Hydrochloride wafers.

Ingredients	G1	G2	G3	X1	X2	X3	GX1	GX2	GX3
PMT-βCD*	818mg	818mg	818mg	818mg	818mg	818mg	818mg	818mg	818mg
Gelatin	0.1 g	0.2 g	0.3 g	-	-	-	0.05 g	0.1 g	0.15 g
Xanthan gum	-	-	-	0.1 g	0.2 g	0.3 g	0.05 g	0.1 g	0.15 g
Methyl cellulose	-	-	-	-	-	-	-	-	-
Methyl Paraben	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g
Aspartame	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g	0.02g
Strawberry flavour	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml

-85°c maintaining a vacuum of 0.015 mBar (as shown in Figure 2). The resultant wafers were taken out of the moulds, double wrapped in aluminium foil and stored in a desiccator. The details of the formulation are presented in Table 2.

2.7. Drug and Polymer Compatibility Studies

2.7.1. Fourier Transform Infrared (FTIR) Spectroscopy Studies

In this technique, a solid sample of approximately 2-3 mg was mixed with about 0.5-1 g of KBr (which is transparent to IR) and then thoroughly ground in a mortar. The mixture was pressed in a pellet die manually and placed in a Fourier Transform Infrared (FTIR) spectrophotometer (FTIR 8400S, Shimadzu, Japan). FTIR spectrum of the samples (Promethazine HCL, β-Cyclodextrin, Gelatin, Methyl Cellulose, Xanthan gum, Inclusion complex and physical mixture of inclusion complex with polymers) were recorded.

2.7.2. Differential Scanning Calorimetry (DSC) Studies

Samples of about 1-3 mg were weighed and placed in aluminium pans and the lids were crimped using a crimper. An empty pan sealed in the same way as for the sample was used as a reference. Thermal behaviour of the samples was investigated under nitrogen gas at scanning rate of 20°C/min, covering a temperature range of 30-300°C. The instrument was calibrated with an indium standard. Thermograms of the samples (Promethazine HCL, β- Cyclodextrin, Inclusion complex and physical mixture of inclusion complex with excipients) were recorded using the instrument DSC-60(Shimadzu, Japan).

2.8. Evaluation Studies of Promethazine HCl Wafers

2.8.1. Uniformity of Weight

20 wafers were individually weighed, and the average weight and relative standard deviation was calculated.

2.8.2. Thickness of Wafers

Three random wafers were selected from each batch and the thickness was measured with the help of a screw gauge. The average thickness was recorded.

2.8.3 Surface pH Determination

The surface pH of fast dissolving wafers was determined in order to investigate the possibility of any side effects *in vivo*. As an acidic or alkaline pH may cause irritation to the oral mucosa, it is important to keep the surface pH as close to neutral as possible. The wafer to be tested was placed in a petridish and was moistened with 0.2 ml of distilled water. The electrode of pH meter (Elico, India) was placed on the surface of wafer to determine the surface pH.

2.8.4. Percentage moisture uptake

The percentage moisture absorption test was carried out to check physical stability or integrity of the wafer at humid condition. The wafers were weighed and placed in a desiccator containing 100 ml of saturated solution of potassium chloride to maintain 75±5% R.H. After three days, the films were taken out and reweighed. The percentage moisture content was calculated by the following formula:

$$\%\ Moisture\ uptake = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

2.8.5. Percentage moisture loss

The percentage moisture loss was carried out to check the integrity of the wafer at dry condition. The wafers were weighed and kept in desiccators containing anhydrous calcium chloride. After three days, the wafers were taken out and reweighed. The percentage moisture loss was calculated using the formula.

$$\%\ Moisture\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

2.8.6. Residual moisture content

Moisture content of wafers after freeze drying was analyzed by Karl Fischer Titration using an Auto-Titrator -TITRASYS 352 (Systronics, INDIA). The solvent DMSO was standardized with the help of Karl Fischer (KF) reagent to determine and eliminate moisture content. The wafers were pulverized and solubilised in the DMSO. To the standardized solvent, fixed volume of sample solution was added and titrated with Karl Fischer Reagent. The percentage moisture content was determined by the formula.

$$\text{KF factor} = \frac{\text{weight of standard} * 0.1556}{\text{ml of KF reagent required}}$$

$$\text{Moisture content (\%)} = \frac{\text{KF factor} * \text{ml of KF reagent}}{\text{Volume (ml) of sample solution}}$$

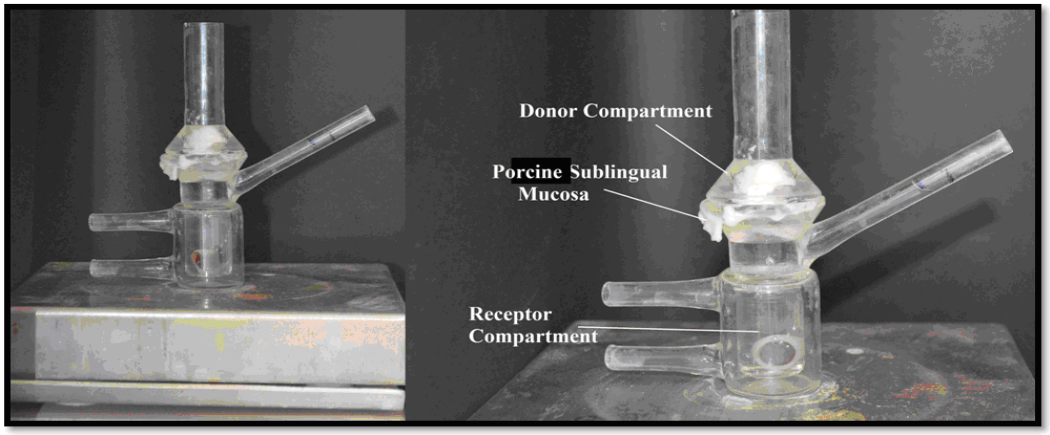


Figure 3. Fabricated Keishary Chein cell.

2.8.7. In vitro disintegration time

In vitro disintegration time was determined by placing the wafer in a petridish containing 10ml distilled water with swirling every 10 sec. The time at which the wafer disintegrated was noted.

2.8.8. Drug Content Estimation

The distribution of active ingredient in the solution is important to achieve dose uniformity. Randomly three wafers were selected and dissolved in 100 ml of pH 6.8 buffer. The resulting solution was filtered through Whatmann filter paper # 41 and diluted suitably with pH 6.8 buffer. The absorbance of the resulting solution was measured spectrophotometrically at 224 nm using pH 6.8 buffer as blank.

2.8.9. Surface Morphology determination by Scanning Electron Microscopy

The surface morphology of a randomly selected wafer was observed by Scanning Electron Microscopy (SEM). The sample was

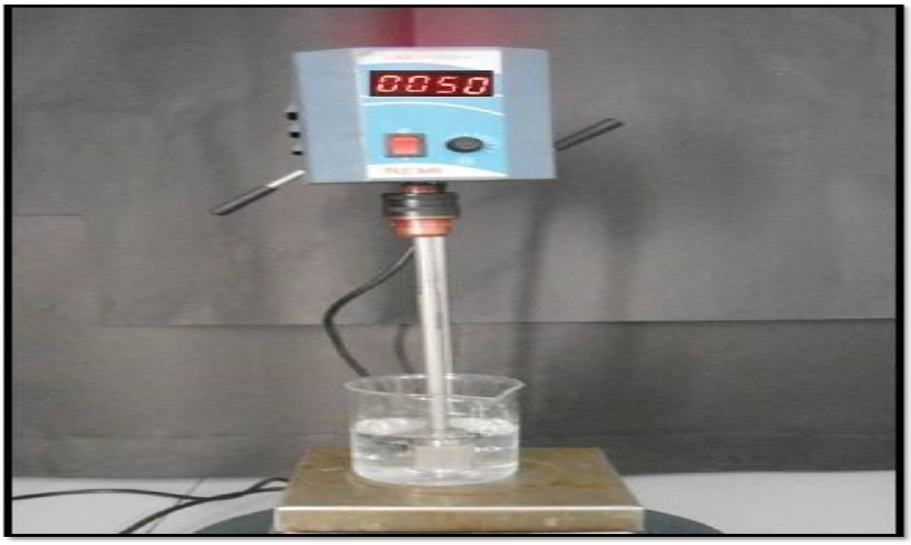


Figure 4. Modified i*n-vitro* dissolution test apparatus (basket type).

prepared by attaching the wafer onto a slab with a double sided adhesive tape and then coated with gold prior to examination. The acceleration voltage was 15 KV. The scanning electron photomicrograph was taken at 40X and 75X magnifications.

2.8.10. Ex- vivo permeation studies

The *ex-vivo* permeation drug release studies were carried out in a fabricated Keishary Chein cell (as shown in Figure 3). Porcine sublingual mucosa was used as the permeating barrier. Simulated salivary fluid was used as the receptor media and the wafer was placed in the donor compartment with a few drops of simulated salivary fluid. The receptor compartment was agitated constantly at 50 rpm and maintained at a temperature of 37^0C ± 0.5^0C. 2 ml of samples were withdrawn from the receptor compartment at predetermined intervals (1 min.) and replaced with the same amount of fresh media to maintain sink conditions. The samples were suitably diluted and the absorbance was measured at 224 nm using UV-Visible spectrophotometer (Shimadzu-1700, Japan).

2.8.11. In vitro dissolution studies

A modified *in-vitro* "Dissolution apparatus" (as shown in Figure 4) was fabricated [12] for the *in-vitro* release studies of wafers. The apparatus consisted of a beaker of 500 ml capacity containing simulated saliva fluid. The beaker was placed on a thermostat heater to maintain the temperature 37^0C± 0.5^0C. A basket was fixed to the mechanical stirrer and rotated at 50 rpm.

2.9. Stability Studies

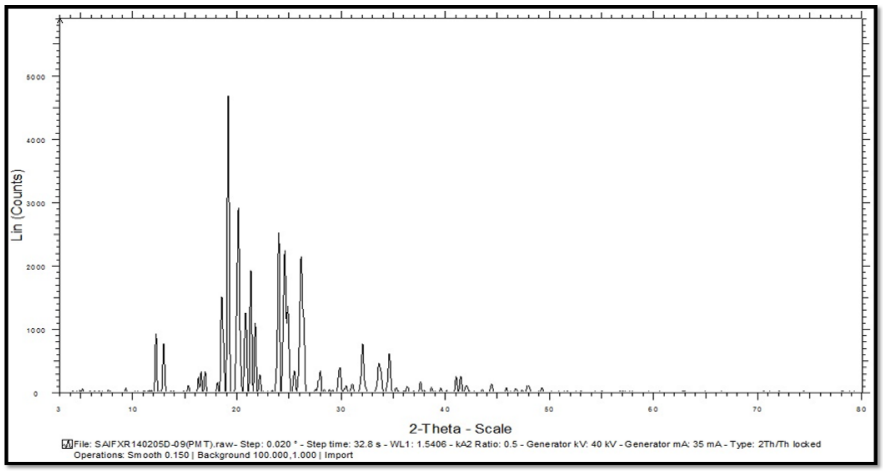
Stability study was carried out at accelerated condition (40ºC temperature and 75% relative humidity) in the humidity chamber for the three months. At the end of 3 months, the wafers were checked for change in appearance, moisture uptake, disintegration time, drug content, permeation studies and *in vitro* drug release.

3. Results and Discussion

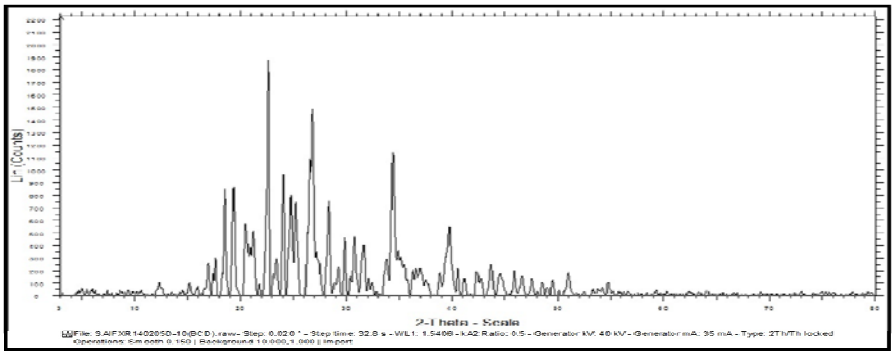
3.1. Taste masking of Promethazine Hydrochloride

Since Promethazine HCl is a very bitter drug, the following methods were carried out for its taste masking:

- Physical mixture of Promethazine HCl and sweetener in the ratios 1:1 and 1:2
- Solid dispersion of Promethazine HCl with Mannitol in the ratios 1:1 and 1:2
- Promethazine Hydrochloride – β-Cyclodextrin Inclusion complex in molar ratios 1:1 and 1:2, prepared by co-evaporation method.



(a)



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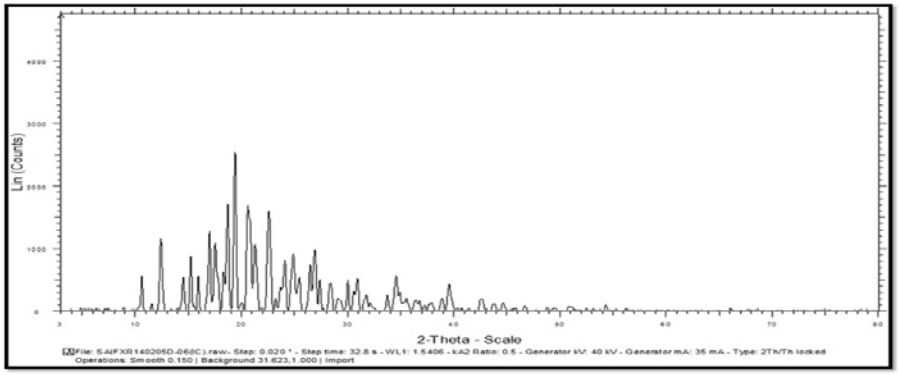


(c)

Figure 5. X-ray diffractograms. (a) X-ray diffractogram of Promethazine Hydrochloride (b) X-ray diffractogram of β Cyclodextrin (c) X-ray diffractogram of Promethazine Hydrochloride- β Cyclodextrin inclusion complex.

3.2. Determination of Extent of Taste masking – using Electronic Tongue

The products obtained from four different taste masking methods were analyzed for the degree of taste masking using Electronic Tongue equipment. The results were generated on a scale of 1-10. The analysis indicated that the inclusion

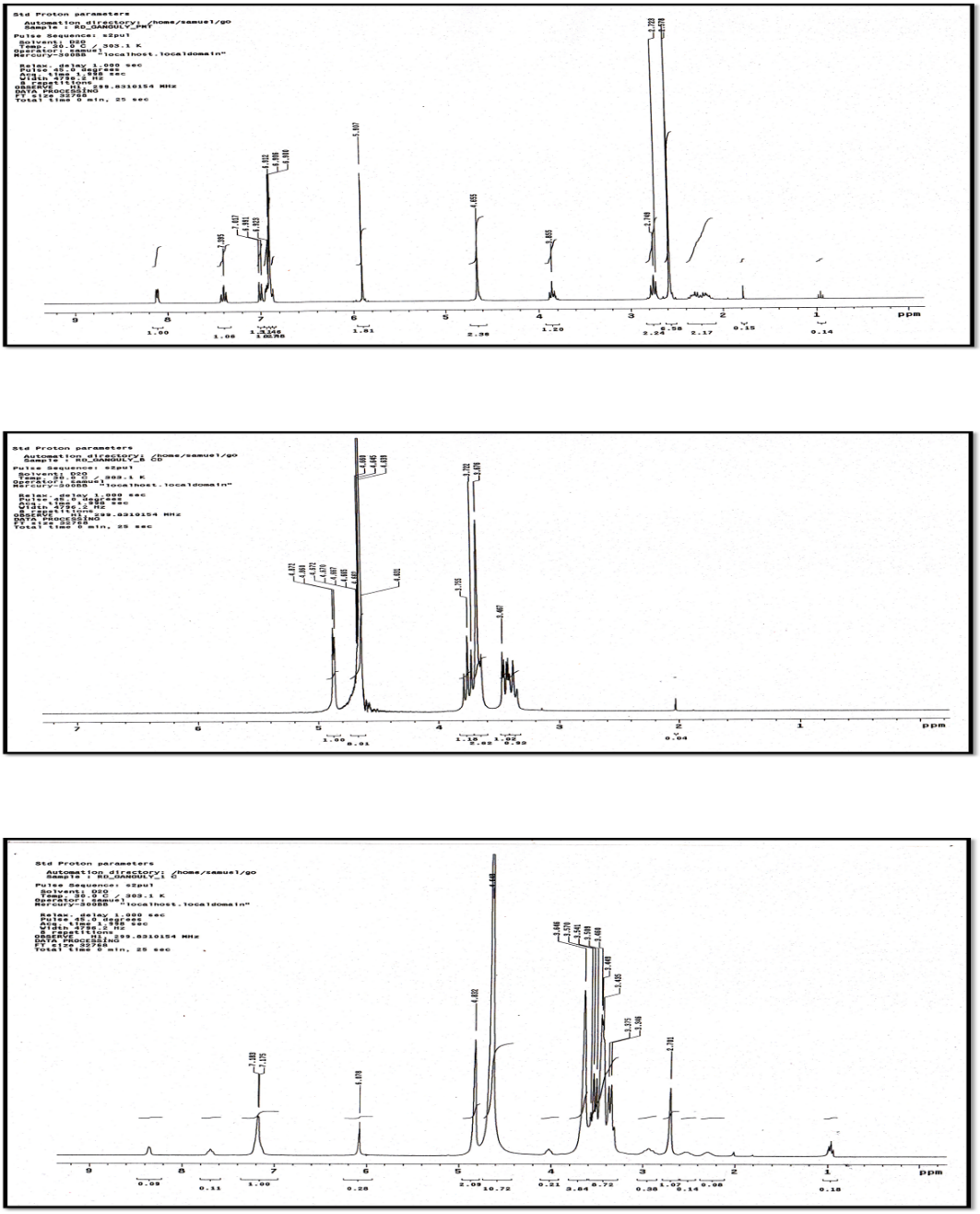


Figure 6. NMR spectra. (a) NMR spectra of Promethazine HCL (b) NMR spectra of β Cyclodextrin (c)NMR spectra of Promethazine HCL- β Cyclodextrin inclusion complex.

complex of Promethazine HCl – β-Cyclodextrin in molar ratio 1:2 was effectively taste masked as it gave a score of 2.0.

3.3. Characterization of Promethazine HCl-β-Cyclodextrin Inclusion Complex

Promethazine HCl – β-Cyclodextrin inclusion complex was prepared by co-evaporation method as described in section 2.2.3. The inclusion complex prepared in the

molar ratio 1:2 was selected for further studies as it showed excellent taste masking of Promethazine HCL. The complexes were characterized by the following methods.

3.3.1. X- Ray Diffraction Studies

The XRD results were in good agreement with the thermal analysis data. The X-Ray diffraction spectra of pure Promethazine HCl (as shown in Figure 5 a), revealed that the drug was in crystalline state as it showed sharp distinct peaks notably at 2θ diffraction angles (in order of intensities) of 12.163°, 12.897°, 19.107°, 20.087°, 23.973° and 26.130°. X-Ray diffraction spectra of β -Cyclodextrin (as shown in Figure 5 b) revealed that it was in crystalline state with sharp distinct peaks at 2θ diffraction angles (in order of intensities) of 22.559°, 24.005°, 18.461°, 19.294°, 28.333° and 34.390°.

The X-Ray diffraction spectra of Promethazine HCl – β-Cyclodextrin inclusion complex (as shown in Figure 5 c) revealed the presence of sharp distinct peaks at 2θ diffraction angles (in order of intensities) of 12.338°, 19.353°, 22.544°, 18.662° and 26.878°. The complex formation

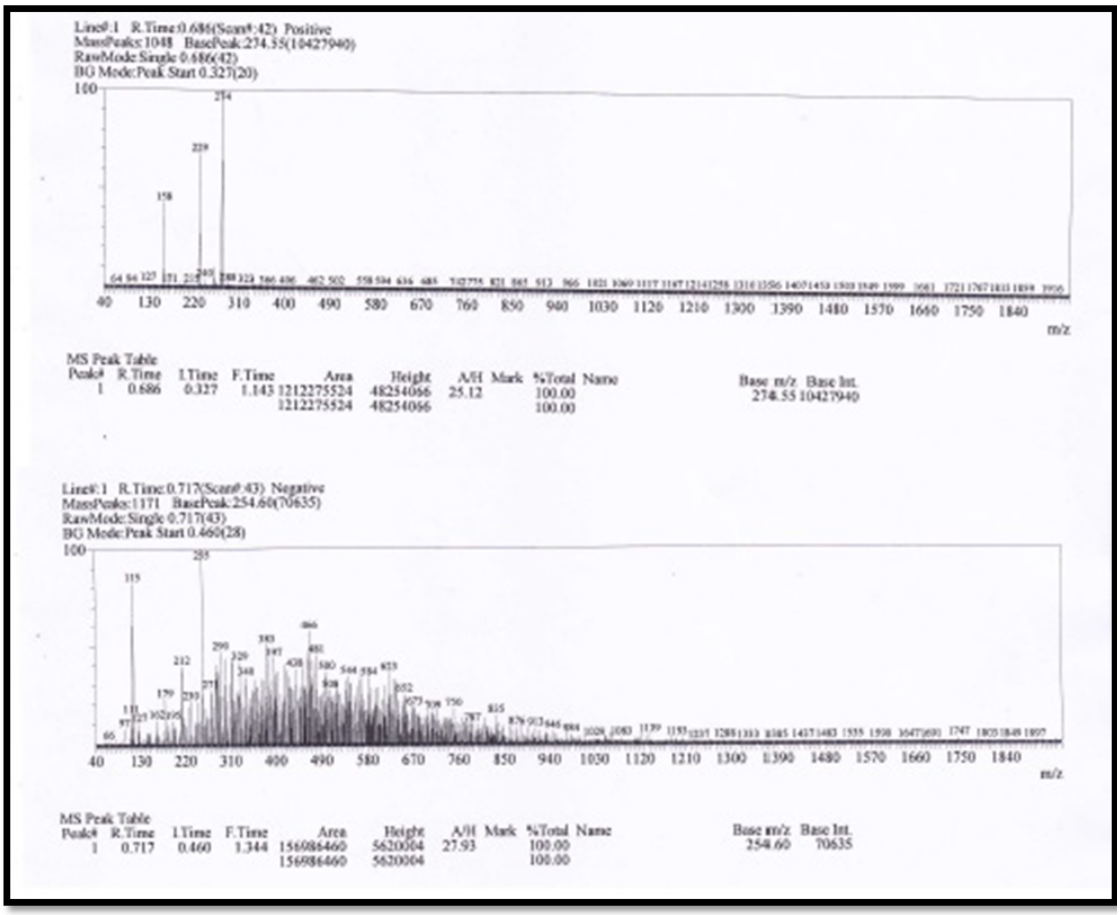


Figure 7. Mass spectra of Promethazine HCL – β Cyclodextrin inclusion complex.

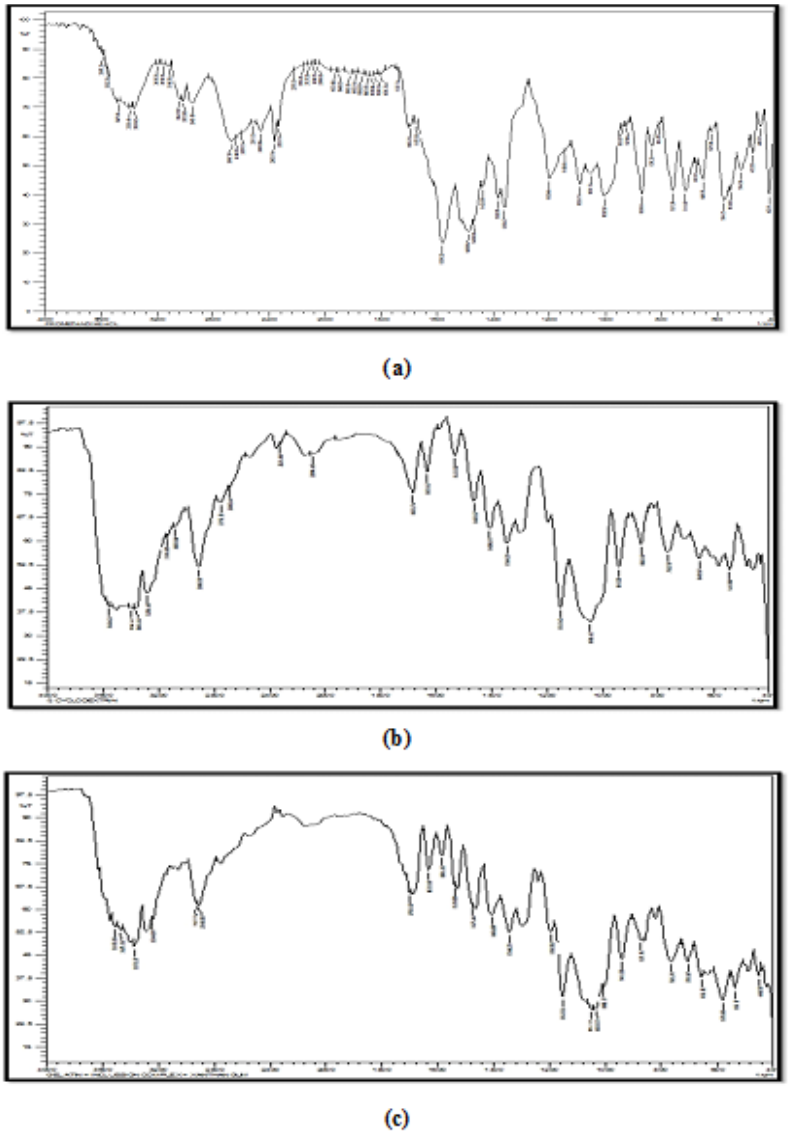


Figure 8. FTIR spectra. (a) IR spectrum of Promethazine HCl (b) IR spectrum of β-Cyclodextrin (c) IR spectrum of Formulation

had lead to the appearance of new peaks, sharpening of existing peaks and shifting of certain peaks observed as in the case of Promethazine HCl.

3.3.2. NMR spectral studies

The 1H-NMR chemical shift for each proton of Promethazine HCl and β-CD was recorded for the inclusion complex of Promethazine HCl and β-CD (as shown in Figures 6a- 6c). Both the pure Promethazine HCl and β-CD gave sharp peaks with high intensity. The chemical shifts for different protons in Promethazine HCl were seen at 2.7, 3.8, 4.6, 5.9, 6.9-6.99, 7.01-7.39 ppm. The chemical shifts for different protons for β-CD

were seen at 3.46-3.75, 4.63-4.87 ppm. The chemical shifts for the protons present in both Promethazine HCl and β-CD were observed in the inclusion complex also. Broadening of peaks was evident at 3.3, 4.0 and 7.2 ppm. The chemical shifts and broadening of peaks in the 1H NMR spectra of the inclusion complex can be accounted to the inclusion complexation of Promethazine HCl into the cavity of β-Cyclodextrin.

3.3.3. Mass spectroscopic studies

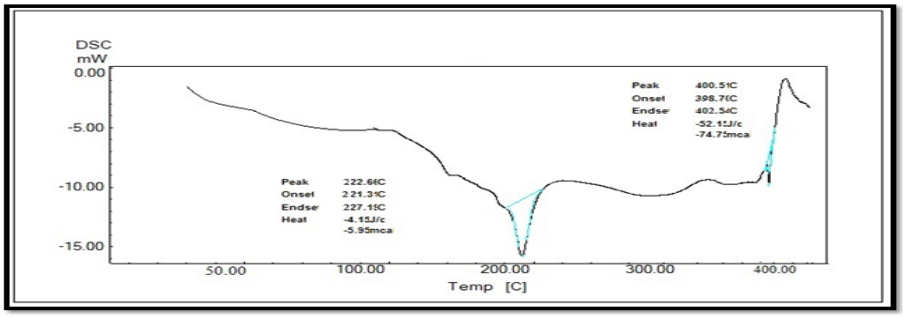
Mass spectroscopic studies were carried out to find the molecular weight of Promethazine HCl – β-CD inclusion complex. The molecular weight of the inclusion complex was found to be 1453 on m/z scale as evident from the mass spectra (as shown in Figure 7). Since no prominent peak was visible at this frequency, it is understood that the product is highly unstable. The base peak at 274.55 on m/z scale corresponds to the free base Promethazine. Extensive fragmentation seen in the mass spectra may be attributed to the large molecular weight of Promethazine HCl – β-CD inclusion complex.

3.4. Formulation of Promethazine Hydrochloride Wafers

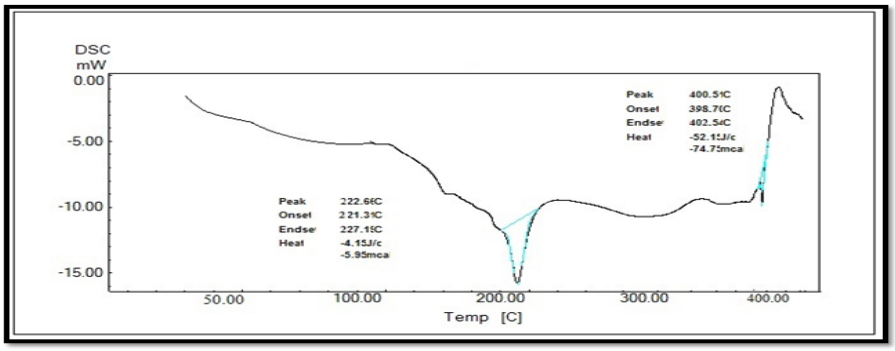
Promethazine HCl wafers were prepared as described in section 2.6. Gelatin and Xanthan gum were used as wafer forming polymers. Aspartame was used as sweetener. Strawberry flavour was used to impart taste to the formulation. A total of 09 formulations were prepared with varying concentrations of polymers. Formulations G1, G2 and G3 contained 1, 2 and 3% of Gelatin as the wafer forming polymer, respectively.

Table 3. Evaluation parameters of the formulations.*

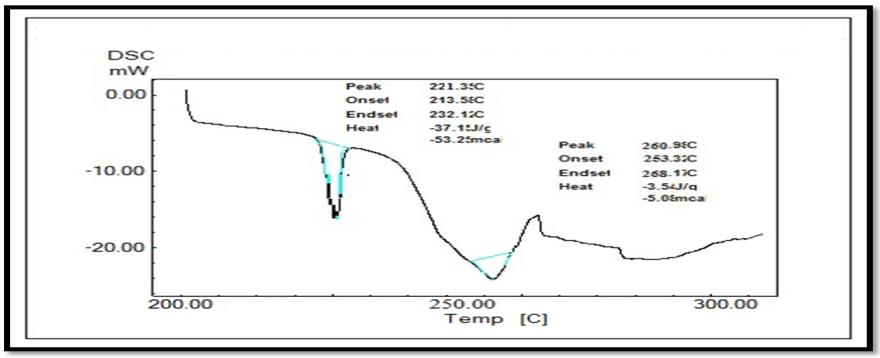
Formulation code	Weight variation (g)	Thickness (mm)	Surface pH	Moisture uptake (%)	Moisture loss (%)	Residual moisture content (%)	Disintegration time (sec)	Drug content (%)
G1	0.18±0.02	4.100 ± 0.0010	6.5	98.56	No loss	0.002	6	97.37
G2	0.20±0.01	4.125 ± 0.0070	6.5	99.10	No loss	0.002	6	96.99
G3	0.18±0.02	4.100 ± 0.0010	6.6	98.23	No loss	0.003	5	99.86
X1	0.23±0.04	4.090 ± 0.0010	6.5	86.28	No loss	0.005	5	99.00
X2	0.22±0.02	4.145 ± 0.0070	6.6	89.90	No loss	0.002	7	102.00
X3	0.24±0.01	4.085 ± 0.0070	6.6	87.54	No loss	0.003	6	96.80
GX1	0.14±0.02	4.120 ± 0.0010	6.4	96.02	No loss	0.002	5	98.98
GX2	0.17±0.012	4.100 ± 0.0010	6.5	99.50	No loss	0.001	2	102.07
GX3	0.17±0.02	4.100 ± 0.0010	6.4	100.00	No loss	0.002	3	101.00



(a)



(b)



(c)

Figure 9. DSC thermograms. (a) DSC thermogram of Promethazine HCl (b) DSC Thermogram of β Cyclodextrin (c) DSC Thermogram of Formulation GX_2.

Formulations X1, X2 and X3 contained 1, 2 and 3% of Xanthan gum as the wafer forming polymer, respectively. Formulations GX1, GX2 and GX3 contained 1, 2 and 3% of Gelatin + Xanthan gum as the wafer forming polymers, respectively.

3.5. Drug-polymer compatibility studies

3.5.1. Fourier Transform Infrared Spectroscopy studies

FTIR was performed for pure Promethazine HCL, β-Cyclodextrin, Gelatin, Xanthan gum, Inclusion complex, and physical mixture of inclusion complex with polymers to detect any sign of interaction which would be

reflected by a change in the position or disappearance of any characteristic peaks of the compound. The IR spectra are shown in the Figures 8a-8c. From the infrared spectral analysis, it was clear that the characteristic absorption peaks of Promethazine HCl were found in physical mixture of drug and polymers, indicating that there was no interaction between drug and polymers.

3.5.2. Differential Scanning Calorimetry studies

One of the classic applications of DSC is the determination of possible interactions between the drug and its excipients in a formulation. The DSC thermograms shown in Figure 10 revealed the thermal behaviour of the drug together with its excipients. The thermogram of Promethazine HCl showed a sharp endothermic peak at 222^0C (as shown in Figure 9a) corresponding to the melting point of the drug in the crystalline form, indicating relative purity. A broad characteristic endothermic peak was observed at 261^0C as in the case of β-Cyclodextrin (as shown in Figure 9b).

The DSC thermogram of the Promethazine HCl- β-Cyclodextrin inclusion complex demonstrated the presence of two endothermic peaks both at melting range of Promethazine HCl and β-Cyclodextrin (as shown in Figure 9c), thus indicating the absence of interaction between them.

3.6. Evaluation Studies of Promethazine HCl Wafers

3.6.1. Weight variation

The formulated PMT wafers were subjected to weight variation test and the wafers showed a weight variation between 0.17g - 0.24g (as shown in Table 3).

3.6.2. Thickness

The PMT wafers were casted in a mould and were found to have almost uniform thickness in the range of 4.085 – 4.130 mm with a minimum amount of deviation (as shown in Table 3).

3.6.3. Surface pH

The surface pH of all the formulations were determined in order to investigate the possibility of any kind of side effects in the oral cavity as acidic or alkaline pH is bound to cause irritation in the oral mucosa. The pH of the formulated wafers was found to be in the range of 6.4-6.7. Thus, it can be considered that the PMT wafers will cause no irritation in the oral cavity (as shown in Table 3).

3.6.4. Percentage Moisture uptake

Lyophilized formulations, being totally devoid of water, tend to absorb even the trace amount of moisture present in the surroundings. The same was depicted for the formulated PMT wafers which showed a water uptake percentage in the range of 86% -100% (as shown in Table 3).

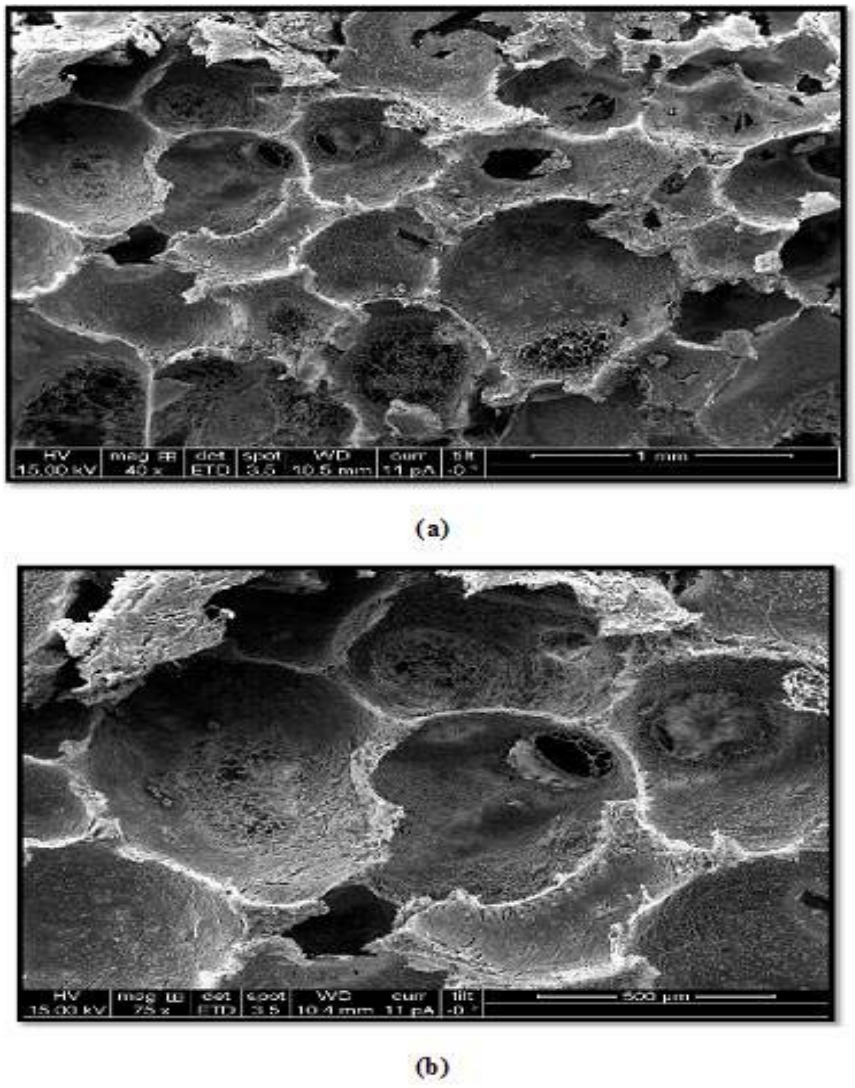


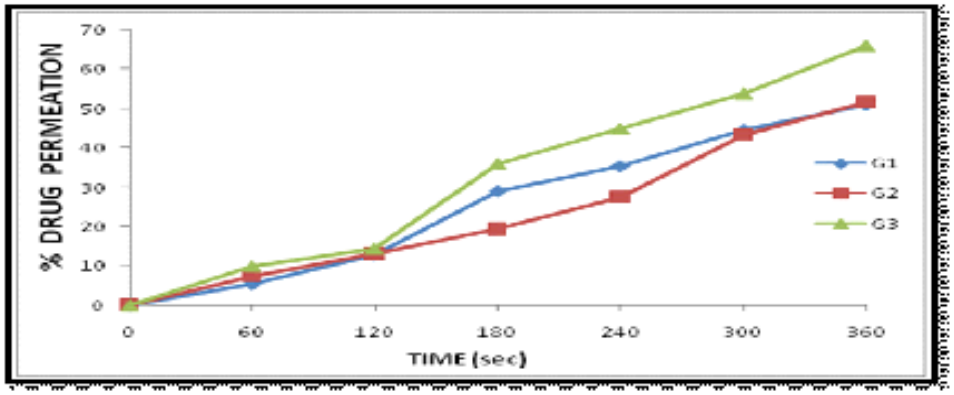
Figure 10. Scanning electron micrographs of Promethazine HCl wafer (a) magnification: 40X (b) magnification: 75X

3.6.5. Percentage Moisture loss

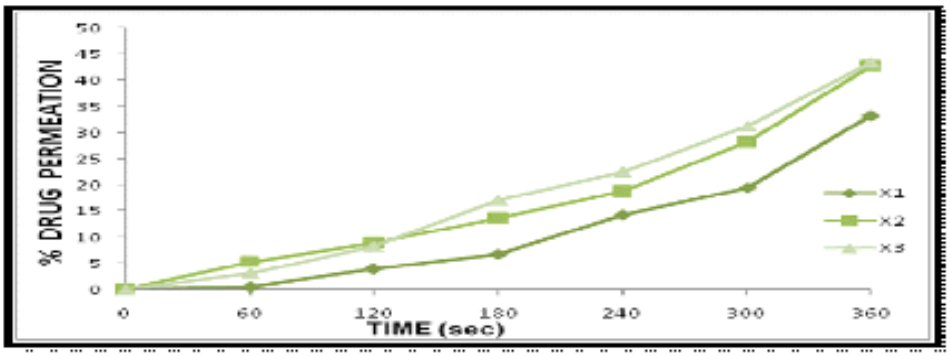
Lyophilized formulations are totally devoid of any kind of moisture when stored properly in a moisture free environment. Thus, when stored in a desiccator, the PMT wafers did not show any kind of moisture loss (as shown in Table 3).

3.6.6. Residual Moisture Content

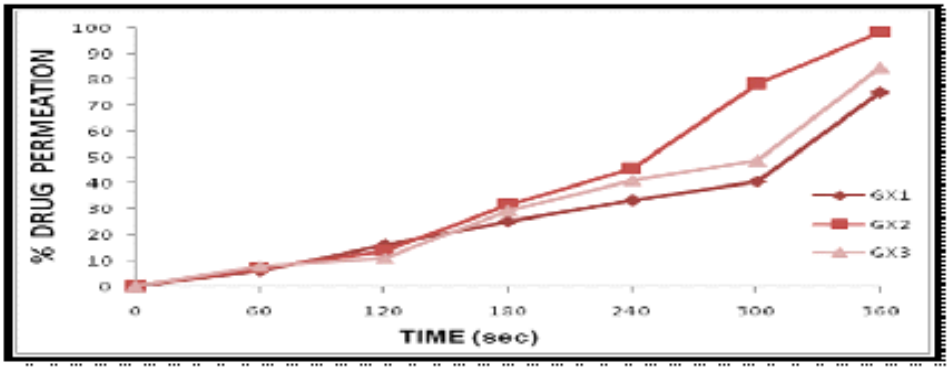
The moisture content of the wafers after freeze drying was analyzed by Karl Fischer titration. The moisture content was found to be within the range of 0.001% - 0.003%, which indicates that water had been removed efficiently by the freeze drying process (as shown in Table 3).



(a)



(b)



(c)

Figure 11. Drug permeation profiles of Formulations

3.6.7. In vitro Disintegration Time

Lyophilized preparations absorb moisture at a very fast rate and they disintegrate as soon as they come in contact with water. The formulated PMT wafers showed a disintegration time in the range of 2-7 sec (as shown in Table 3). Formulation GX2 showed the least disintegration time of 2 sec. Formulations containing only

Table 4. Ex *Vivo* Drug permeation data of Formulations G1- GX3.

Time (sec)	% Drug Permeation								
	G1	G2	G3	X1	X2	X3	GX1	GX2	GX3
0	0	0	0	0	0	0	0	0	0
60	5.282	7.466	9.968	0.556	5.322	3.137	5.957	7.029	7.943
120	12.738	13.029	14.395	3.926	8.930	8.282	15.846	13.884	11.226
180	29.156	19.399	36.074	6.863	13.641	17.194	25.280	31.576	29.063
240	35.476	27.437	45.002	14.371	18.793	22.428	33.122	45.677	41.036
300	44.703	43.437	53.739	19.592	28.234	31.337	40.617	78.381	48.611
360	50.980	51.796	66.111	33.262	42.870	43.482	74.997	98.426	84.531

Gelatin or Xanthan gum showed highest disintegration time of 6-7 seconds.

3.6.8. Drug content

Drug content was analyzed by UV-Visible spectrophotometer at 224.5 nm. The percentage drug content was between 96.99% and 102.00% as shown in Table 3, which proved uniform drug distribution within the PMT wafers.

3.6.9. Surface Morphology Determination by Scanning Electron Microscopy

The scanning electron micrographs (as shown in Figure 10) of the formulated PMT wafers at 40 and 75 X magnification showed a highly porous surface which was attributed to the lyophilization process. The high porosity of the wafers supported rapid disintegration and dissolution.

3.6.10. Ex Vivo drug permeation studies

The main aim of formulating PMT wafers was the immediate release of the drug into the systemic circulation. The *ex-vivo* permeation studies of all nine PMT wafers were carried out as mentioned in section 2.8.10. The first set; G1, G2 and G3 showed a drug permeation of 50.98%, 51.786% and 66.111%.respectively at the end of 360 sec of study period. The comparatively low drug permeation of the set can be accounted to the gellation property of Gelatin even though it is having fast melt properties (as shown in Table 4; Figure 11 a). The second set; X1, X2, and X3 showed a drug permeation of 33.262%, 42.87% and 43.482% respectively at the end of 360 sec of study period. The comparatively low drug permeation of the set can be accounted to the high gellation property of Xanthan gum which leads to the increase in the interstitial path thus increasing the permeation time (as shown in Table 4; Figure 11 b).

The drug permeation increased substantially when used in combination. This was clearly evident from the permeation studies carried out with the remaining two sets of wafers. The third set, GX1, GX2 and GX3 gave a drug permeation of 74.97%, 98.426% and 84.531% respectively at the end of 360 sec of study period. This high permeation of the drug can be accounted to the optimum ratio of

Table 5. *In vitro* drug release data for formulations G1- GX3.

Time (sec)	% Drug Release								
	G1	G2	G3	X1	X2	X3	GX1	GX2	GX3
0	0	0	0	0	0	0	0	0	0
60	27.006	26.370	31.453	30.818	27.005	30.183	30.818	30.818	36.537
120	32.222	31.497	32.295	35.780	40.164	39.582	35.780	35.780	53.032
180	38.470	43.807	46.804	50.029	53.535	50.400	50.029	50.029	66.296
240	54.028	58.74	64.724	64.506	69.659	69.013	64.506	64.506	86.757
300	82.860	74.864	84.2	87.472	84.448	90.782	87.472	87.472	99.916
360	98.815	96.961	99.535	100.001	99.1485	99.550	100.001	100.001	

the wafer forming polymers used, having combined properties of gelation and fast melt (as shown in Table 4; Figure 11 c). Thus, the permeation studies carried out on the PMT wafers showed that the correct combination of polymers having properties which allow the wafers to melt away fast enough while allowing the formation of a gel mass to allow the complete release is the most acceptable formulation(GX2)

3.6.11. In vitro Drug Release Studies

The *in vitro* drug release studies were carried out on all the sets of formulated PMT wafers. The drug release data of the first set G_1, G_2 and G3, showed a drug release of 98.815%, 96.961% and 99.535%.respectively at the end of 360 sec of study period (as shown in Table 5; Figure 12a). The second set; X1, X2, and X3 showed a drug release of 100.00%, 99.148% and 99.55% respectively at the end of 360 sec of study period (as shown in Table 5; Figure 12b). The drug release increased substantially when used in combination. This was clearly evident from the release studies carried out with the third set of wafers GX1, GX2 and GX3. They gave a drug permeation of 100.00% at the end of 360 sec, 99.572% at the end of 240 sec and 99.916% at the end of 300 sec respectively of study period. This faster release of the drug can be accounted to the optimum ratio of the wafer forming polymers used having both properties of gelation and fast melt (as shown in Table 5; Figure 12c). The drug release were found to be much more faster than that of the permeation for the same formulations due to the fact that a much larger sink condition was maintained during the drug release studies which lead to a much faster release of the drug into the media.

3.7. Stability Studies

The accelerated stability studies of Promethazine HCl wafers and film were performed as per the ICH guidelines to

investigate whether the wafers and film are affected during storage conditions. The sample batch wafers (GX2) and film were kept at $40^0C \pm 2^0C/75\% \pm 5\%$ RH for a period of 3 months. The physical appearance, moisture uptake, disintegration time, drug content permeation rate and dissolution rate were measured for selected formulations at the end of 1, 2 and 3 months. The results showed that there was no significant difference between the initial and aged Promethazine HCl wafers during the initial two months but substantial amount of changes were observed at the end of the third month. As the selected formulations were physically and chemically stable for 2 months at accelerated stability conditions of 40°C and 75 % RH, the shelf life can be predicted to be more than 12 weeks as per ICH guidelines when storage conditions are not exceeding 30^0C.

4. Conclusion

Promethazine HCl is an anti emetic drug with 25% of systemic oral bioavailability. The main objective of the studies described was to develop fast dissolving sublingual wafers of Promethazine HCl, with enhanced oral bioavailability for the treatment of motion sickness. As the drug is very bitter in taste, taste masking was attempted by adopting various techniques among which an inclusion complex of drug with β -Cyclodextrin was excellently taste masked. This was confirmed by the taste analysis done using an e-tongue. The analytical studies like FTIR, DSC, XRD, NMR and Mass spectroscopy proved the formation of Promethazine - β Cyclodextrin inclusion complex. Fast dissolving wafers of Promethazine HCL were designed using water soluble semi-synthetic and natural polymers by lyophilization method. Gelatin, Xanthan Gum and Methyl cellulose were selected as suitable polymers. The formulated wafers were evaluated for physical appearance, surface texture, weight uniformity, thickness uniformity, surface pH, drug content uniformity, *in vitro* disintegration time, *ex* vivo drug permeation and *in vitro* drug release. On the basis of the results obtained, formulation GX2 was found to be the most acceptable. Stability studies for best wafer formulation was performed in accelerated condition as per the ICH guidelines and observed that there was no appreciable changes in the drug content, disintegration time, *ex* vivo drug permeation and *in vitro* drug release for the first two months followed by drastic degradation in the wafer formulation in the third month. The shelf-life of the Promethazine HCl wafer was predicted to be 12 weeks when stored at temperature below 30^oC. Thus a fast releasing lyophilized sublingual wafers of Promethazine HCL for the rapid and effective drug delivery with improved bio availability was successfully developed.

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