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Research Article

Formulation Development And In-Vitro Evaluation Of Metformin Hydrocholoride And Glibenclamide Buccal Patch

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ABSTRACT

In the present study, attempt has been done to develop a novel mucoadhesive drug system in the form of the buccal patches for the release of metformin HCl and glibenclamide in a unidirectional manner, to maintain constant therapeutic levels of the drug for long time. Buccal formulation of metformin HCl and glibenclamide in the form of mucoadhesive patches were developed to a satisfactory level in term of drug release, drug permeation, content uniformity, swelling index, surface pH, thickness and folding endurances. Although all buccal patches exhibited satisfactory results, but best results were obtained from Mf4 and Gf5. the above study concluded that the possibility of the making of mucoadhesive drug delivery system for metformin hcl and glibenclamide which will be more efficacious and acceptable than conventional drug delivery of drug and also having satisfactory controlled release profile which may provide an increased therapeutic efficacy.

INTRODUCTION

Oral drug delivery has been the main route for drug delivery over the decades. For systemic delivery, the oral route was the preferred route of administration for many systemically active drugs. Recent research efforts have focused on positioning a drug delivery system in a particular body region to maximise the availability of biological drugs and minimize dose-dependent side effects. Buccal drug delivery provides an alternative to other conventional methods of systemic drug delivery since the buccal mucosa is relatively permeable with a rich blood supply and serves as an excellent site for drug absorption. This drug delivery system is suitable for drugs that pass through high first-pass metabolism and is used to

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enhance bioavailability by reducing dosing frequency to mouth plasma peak levels, which in turn minimizes adverse side effects. In addition, films have improved patient compliance due to their small size and reduced thickness, compared, for example, to lozenges and tablets. Currently, the buccal film is coming into focus. This dosage form is less friable than most commercialised orally disintegrating tablets, which usually require special packaging. Mucoadhesive buccal film is preferred over adhesive tablets and oral gels –due to flexibility, comfort, and relatively long residences time on oral mucosa. In addition, as mucoadhesion implies attachment to the buccal mucosa, films can be formulated to exhibit systemic or local action. 1, 2 Diabetes mellitus is defined by the American diabetes association expert committee in their 1997 recommendations as "a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secreation, insulin action r both". The chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Thus diabetes covers wide ranges of heterogeneous disease. Several groups of drugs, mostly given by mouth are effective in type2 diabetes mellitus.3 Metformin is Antihyperglycemic agent which improves glucose tolerances in patients with type 2 diabetes mellitus. Its pharmacologic mechanism of action is different from other classes of oral Antihyperglycemic agents. The absolute bioavailability of a metformin HCl under fasting condition is approximately 50 to 60 %.gastrointestinal absorption occurs mainly in the upper intestine and is completed at 6 hours, with peak plasma concentration [Cmax] reached after 2to3hours.the drawback being high dose [1.5-2g/day] low bioavailability [40-60%], short biological half-life [0.9-2.6hrs] requires repeated administration of high doses to maintain effective plasma concentration.4, 5, 6 Glibenclamide is an oral hypoglycemic agent belonging to the second generation of sulfonylurea's used in treatment of type2 non-insulin dependent diabetes. Its hypoglyacemic effect is due to stimulation of insulin release from pancreatic beta cells and sensitization of the peripheral tissue to insulin. Glibenclamide is highly lipophilic [log p=4.7] and poorly soluble in aqueous media.7

MATERIAL AND METHODS

Metformin HCl and glibenclamide was procured from Balaji drugs. Whereas HPMC E-15, HPMC K-4, HPMC K-100 was procured from OZONE internationals, Mumbai. Other excipients used were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Preformulation Study

Preformulation testing is the first step in the development of dosage forms for a drug. Every drug has inherent physical and chemical properties that are considered before formulation development. These physical-chemical properties of drug substances combined with excipients provide basic information related to drug and excipient combinations. Preformulation studies strengthen the scientific basis of the guidelines, provide regulatory relief, save resources in the drug formulation, development, and evaluation processes, boost public health standards, and enhance product quality in dosage form manufacturing. This study will concentrate on the new compound's physicochemical properties that influence the performance of the drug and the effectiveness of the dosage form. The overall concept of preformulation testing is to utilise information useful to the formulator in developing stable and bioavailable dosage forms.

Physical characteristics

Physical characteristics include appearance, colour, odour, taste, and surface nature. The colour of the drug is checked by visual observation, and the odour is checked by taking a smell.

Solubility study

Solubility of a drug defines as the amount of solute that dissolves into the solvent to obtain the saturated solution of solute at constant temperature and pressure. Solubility is the integral parameter of the pre-formulation study. These studies focus on the drug solvent mechanism that occurs during drug delivery. This study gives information about the selection of the best solvent for drug substance and overcomes the challenges that occur in the formulation process.

Descriptive term	Parts of solvent required for part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
soluble	From 10 to 30
Sparingly soluble	From 30 to100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble or insoluble	From 10,000 or more

Melting point determination

The melting point of the drug was determined by the capillary tube method. The observed value was compared with the standard reported value. i.e., by taking a small amount of drug in a closed capillary tube, the capillary was placed in the melting point apparatus. The temperature at which the drug melted was noted as the melting point. From there, the purity of the drug is determined. The reading was recorded in triplicate.

Compatibility study

The compatibility of drugs and polymers under experimental conditions is important data before formulation. Therefore, this study confirms that the drug does not interact with polymers under any chemical or environmental conditions. DSC is the most useful method for the chemical identification of drugs.

FTIR Spectroscopic analysis

The drug-polymer interaction was studied using FTIR spectra. The FTIR spectrum of the moisture-free powdered sample of the drug and the final formulation was recorded on an IR spectrophotometer by the potassium bromide (KBr) pellet method. The spectra were recorded for purity analysis of the drug in the 400 to 4000 cm-1 range. The characteristic peaks of different functional groups were compared with the reported standard peak.

Analytical study

Determination of analytical wavelength

1. **Preparation of standard stock solution of Glibenclamide:**

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of phosphate buffer (pH 6.8) is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with phosphate buffer pH 6.8, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with phosphate buffer ph 6.8 to get a 5 µg/ml concentration.

I. Selection of wavelength

The above 5 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for glibenclamide is determined.

II. Construction of standard calibration curve

From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with phosphate buffer solution pH 6.8 to get a working solution of concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. These series of different concentrations of glibenclamide were scanned at 231 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 5 to 30 µg/ml.

2. **Preparation of standard stock solution of Glibenclamide:**

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of methanol is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with methanol, so as to obtain a concentration of 100 µg/ml. pipette out 0.2 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with methanol to get a 2 µg/ml concentration.

I. Selection of wavelength

The above 2 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for glibenclamide is determined.

II. Construction of standard calibration curve

From the standard stock solution, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with methanol to get a working solution of concentrations 2, 4, 6, 8, 10, and 12 µg/ml respectively. These series of different concentrations of glibenclamide were scanned at 296 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 2 to 12 µg/ml.

Preparation of buccal patch of immediate release glibenclamide

The polymer (HPMC E-15) and plasticizer (PEG-400) were dissolved in a sufficient quantity of distilled water in the separate beaker to prevent excessive air bubble formation. In the second beaker, glibenclamide was added and stirred with the solution for 30 minutes. Then the two solutions were mixed together, and a specified amount of other excipients such as saliva stimulating agents and flavouring agents were added to that mixture, along with a sufficient quantity of remaining water, and stirred for 1 hour. After stirring, keep it for 30 minutes for sonication to remove all air bubbles from the final solution. Then the final solution was cast on a petri plate, and it was dried in the oven at 450C for 12 hours. The film was carefully removed from the petri dish and cut according to the size required for testing (1 cm).

Table -1.1Formulation of immediate release layer

Ingredients Formulation(mg)	GF1	GF2	GF3	GF4	GF5
Glibenclamide (mg)	20	20	20	20	20
HPMC E-15 (mg)	200	250	300	350	400
Citric acid (mg)	50	50	50	50	50
TWEEN (80) (ml)	0.2	0.2	0.2	0.2	0.2
PEG-400 (ml)	0.4	0.4	0.4	0.4	0.4
Ethanol (ml)	10	10	10	10	10
Water (ml)	q.s	q.s	q.s	q.s	q.s

Fig no.1. Formulation Gf1-Gf5

Analytical study

Determination of analytical wavelength

1. **Preparation of standard stock solution of metformin hydrochloride:**

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of phosphate buffer (pH 6.8) is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with phosphate buffer pH 6.8, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with phosphate buffer ph 6.8 to get a 5 µg/ml concentration.

I. Selection of wavelength

The above 5 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for metformin hydrochloride is determined.

II. Construction of standard calibration curve

From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with phosphate buffer solution pH 6.8 to get a working solution of concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. These series of different concentrations of metformin hydrochloride were scanned at 233 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 5 to 30 µg/ml.

2. **Preparation of standard stock solution of metformin hydrochloride:**

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of distilled water is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with distilled water, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with distilled water to get a 6 µg/ml concentration.

I. Selection of wavelength

The above 6 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for metformin hydrochloride is determined.

II. Construction of standard calibration curve

From the standard stock solution, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with distilled water to get a working solution of concentrations 1, 2, 3, 4, 5, and 6 µg/ml, respectively. These series of different concentrations of metformin hydrochloride were scanned at 233 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 1to 6 µg/ml.

Preparation of Buccal Patch of sustained release metformin hydrocholoride

The polymer (HPMC E-15) and plasticizer (PEG-400) were dissolved in a sufficient quantity of distilled water in the separate beaker to prevent

excessive air bubble formation. In the second beaker, metformin HCl was added and stirred with the solution for 30 minutes. Then the two solutions were mixed together, along with a sufficient quantity of remaining water, and stirred for 1 hour. After stirring, keep it for 45 minutes for sonication to remove all air bubbles from the final solution. Then the final solution was cast on a petri plate, and it was dried in the oven at 450C for 12 hours. The film was carefully removed from the petri dish and cut according to the size required for testing (1 cm).

Table -1.2Formulation of sustained release layer

Ingredients Formulation (mg)	MF1	MF2	MF3	MF4	MF5
Metformin HCl (mg)	40	40	40	40	40
HPMC K-4 (mg)	240	440	640	840	1040
PEG-400 (ml)	0.4	0.4	0.4	0.4	0.4
Methanol (ml)	5	5	5	5	5
Water (ml)	q.s	q.s	q.s	q.s	q.s

Fig no.2. Formulation Mf1-Mf5

Evaluation of mucoadhesive buccal patches of metformin hydrochloride and glibenclamide

Physical parameters

a. Thickness
b. Weight of patch
c. Folding endurance
d. Swelling index
e. Measurement of surface Ph

Performance parameters

a. Drug content uniformity
b. In-vitro drug release study
c. Stability study

specified time intervals till to reach saturation. The percent of swelling index (%SI) occurs was determined by following equation.

Physical parameter

1. Thickness

Thickness of patch was measured at 3 different randomly selected spots using screw gauge. The mean and standard were calculated.

2. Folding endurance

Folding endurance of the buccal patches was determined by taking 1cm diameter of patch was repeatedly folding at the same place till it brokes. the no. of times of patch could be folded at the same place without breaking gave the value of the folding endurance. The tests were done3 times and calculate the mean and standard.

3. Weight uniformity of patch

Three different patches were selected randomly and weight of each patch is taken using electronic weighing balance. a mean &standard deviation of all reading is recorded.

4. Swelling index

After determination of the patch weight(W1)the sample were placed on the surface of 2%agar plate kept in an incubator at 37oC±0.2oC.the increase in weight of the swelled patches were noted (W2)at

%SI=W1-W2/W2 *100

Here,

W1 is the measured weight of patch

W2 is the initial weight of non-swollen patch

5. Surface pH

The surface pH of patch was determined to know the possibility of side effects, in vivo as an acidic or alkaline pH may cause irritation to the buccal mucosa. It was our aim to keep the surface pH as close to neutral as possible. The surface pH was determined by taking 3 patches of each formulation and the patch were allowed to swell for 2 hrs on the surface of agar plate. The surface pH was measured by using a pH paper placed on the surface of swollen patch.

Performance parameter

1. Drug content uniformity

Drug content uniformity was calculated by taking 3 films units of each formulation were taken in separate 100ml volumetric flasks, 100ml of Ph 6.8 phosphate buffer was added and continuously stirred. The solutions were filtered, diluted suitably and analyzed at 233nm in a UV Spectrophotometer. The average of drug content of 3 films was taken as final readings.

2. In-vitro drug release study

A modified Franz cell was used for evaluating the drug release profile diffusion membrane. The receptor compartment was filled with 25 ml of phosphate buffer pH 6.8 and stirred by the use of the Teflon-coated bead on a magnetic stirrer. The upper part of the cell has a pair of Flange between which the cellophane diffusion membrane was placed for release studies. The patch was placed over the diffusion membrane; the flanges held in place were tightened with the screws for the cell setup. The whole assembly was kept on the magnetic stirrer and the temperature was maintained at 370C with the water jacket at 50 rpm speed of the magnetic bead. The withdrawal port was covered with the glass corks which prevent air entrapment. The upper portion of the cell is the donor compartment which was open at the top to maintain the exposure of the system to the ambient conditions. The amount of drug release into receptor solution was determined by removing 1ml of a sample at intervals for 6 hrs. The withdrawn volume was replaced with an equal volume of fresh buffer solution. The drug released was determined by analyzing the sample at 233nm.the result of in-vitro study is represented by the following graphs .The graph was plotted against % drug release versus time.

Fig no.3 Franz Diffusion Cell

3. Moisture content

To determine the physical stability and integrity of the patch, the percentage moisture content is determined.

Procedure-

The prepared patch was weighed individually and kept in desiccators containing calcium chloride at room temperature for 24 h. The patch is weighed

again after a specified interval until it showed a constant weight

The percent moisture content was calculated by using the following formula:

Moisture content =Initial weight –final weight/final weight *100

4. Moisture absorption

The moisture uptake studies give an indication of the relative moisture absorption capacities of polymers and an idea of whether the formulations maintain their integrity after the absorption of moisture. The moisture absorption study gives an idea about the stability of the patch. An increase in the moisture absorption property of the patch indicates that the patch will be less stable. Moisture absorption increases patch weight, decreases drug stability, and increases the disintegration time of the patch.

Procedure:

The patches are weighed accurately and placed in a desiccator containing 100 ml of a saturated solution of aluminum chloride, which maintains 76% and 86% humidity (RH). After 3 days, patches are taken out and weighed. The moisture absorption is calculated using the formula:

Moisture absorption = final weight- initial weight/ initial weight *100

5. Ex vivo permeation study

The ex vivo study of buccal permeation of drug was carried out in an isolated porcine buccal mucosa .The Franz diffusion cell was modified to hold 25 ml of permeation medium and to have inner diameter of 3cm.The goat buccal mucus membrane was fixed in between donor compartment and receptor compartment by facing epithelial layer towards donor compartment. The buccal patch was placed on the mucous membrane to develop mucoadhesion. Both the compartments were tightly clamped to arrest leakage of fluids. In donor compartment phosphate buffer [pH7.4] is added till to touch the rear side of the membrane. The receptor fluid was continuously stirred with a magnetic bead t 50 rpm. the temperature of permeation medium was kept constant at 37± 0.2oc.at every specified time interval,1ml sample was withdrawn and immediately equal volume of fresh medium was replaced. And the sample was analyzed under UV spectrophotometer at 233nm.this study was repeated thrices to obtain average with minimum deviation.

6. Stability studies

Stability of a drug has been defined as the ability of a particulate formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. The purpose of stability testing is to provide evidences on how the quality of a drug substance or drug product varies with time under the influence of a varity of environmental factors such as temperature, humidity and light, and enables recommended storage condition.

ICH specifies the length of study and storage conditions:

Long term testing-25oc/60%±5%RH for 12 months
Accelerated testing-40oc±2oc/75%±5%RH for 6 months

Procedure

Patch obtained from optimized batch i.e., batch Mf4 and Gf5 were subjected to stability testing. The patch was packed in aluminum paper and exposed to 40ºC/75% RH and 30ºC/65% RH in stability chambers (Neutronics) for one months. During the stability storage period the patches were evaluated for physical characteristics, in vitro drug release, and drug content at the end of 30 days of storage period.

The patches were evaluated for various parameters like physical appearance, drug content, and in vitro dissolution study

RESULT AND DISCUSSION

I. Metformin hydrochloride

1. Melting point

The melting point determined by using the capillary tube method. Melting point of metformin

hydrochloride found to be 223oc.this was matching to the literature value 222-226oc indicating the identity and purity of drug sample.

2. Solubility Study

Sr. No	Solvent	Drug solubility
1	Water	Freely soluble
2	Methanol	Slightly soluble
3	Phosphate buffer	Soluble

3. Spectroscopic studies

A. FT-IR study

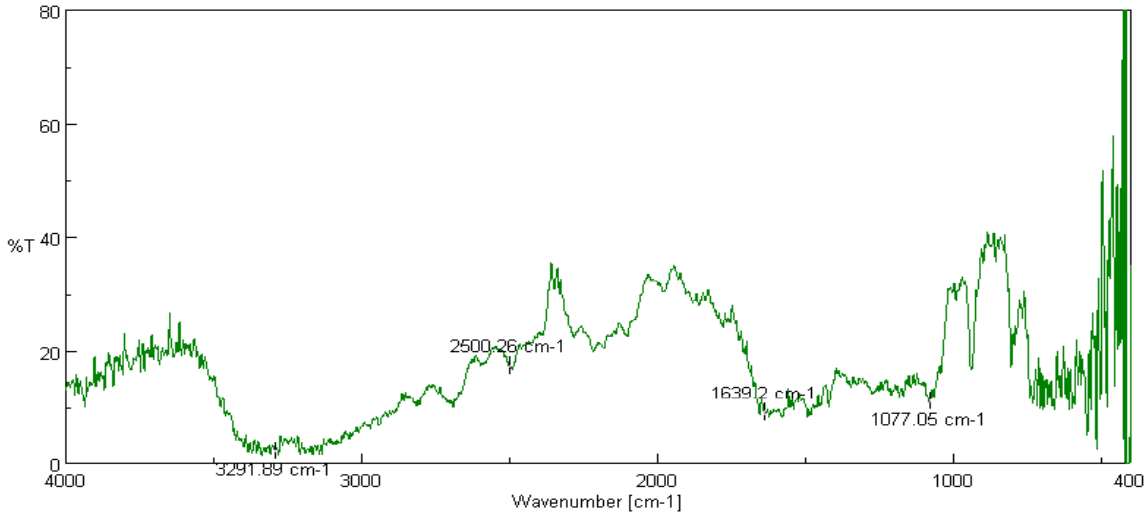
B. UV study

A. FT-IR study:

1. Spectra 1: IR of pure drug
2. Spectra 2: IR of HPMC K-4M
3. Spectra 3: IR of drug and polymer incompatibility

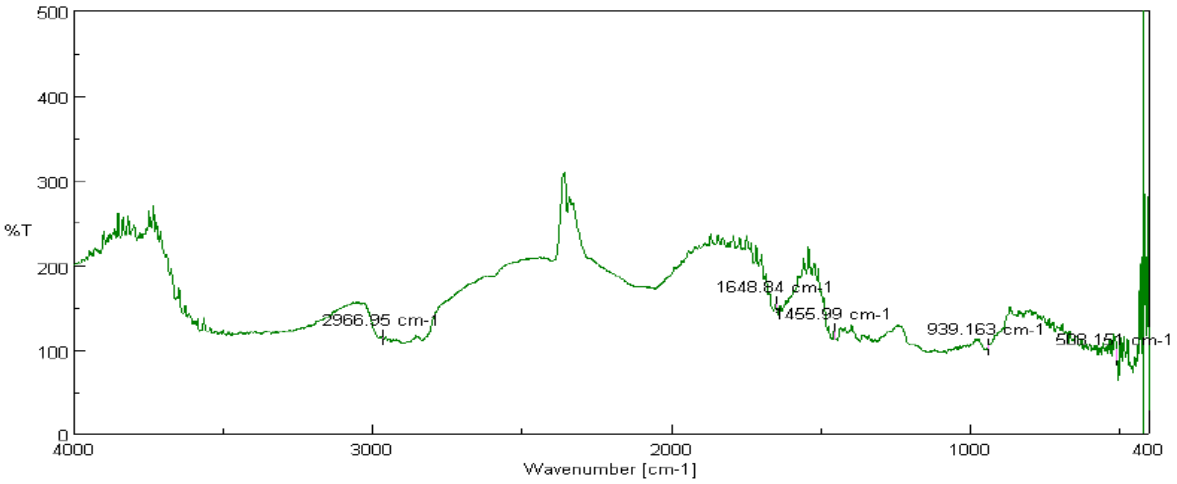
The FT-IR spectrum of metformin hydrochloride is consistent with references spectra given in an analytical profile of drug substance. The drug and polymer were compatible with each other.

Spectra 1: IR of pure drug



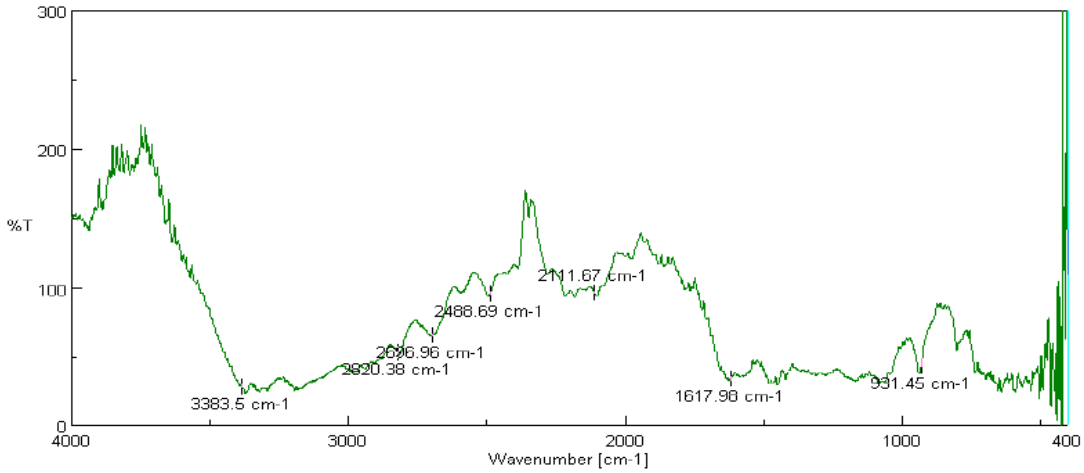
Spectrum 1: FT-IR spectra of pure drug

Spectra 2: IR of HPMC K-4M



Spectrum 2: FT-IR spectra of HPMC K4M

Spectra 3: IR of drug and polymer incompatibility



Spectrum 3: FT-IR spectra of drug + HPMC K4M

B. UV study

1. Preparation of standard stock solution of metformin hydrochloride:

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of phosphate buffer (pH 6.8) is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with phosphate buffer pH 6.8, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with phosphate buffer ph 6.8 to get a 5 µg/ml concentration.

A. Selection of wavelength

The above 5 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for metformin hydrochloride is determined.

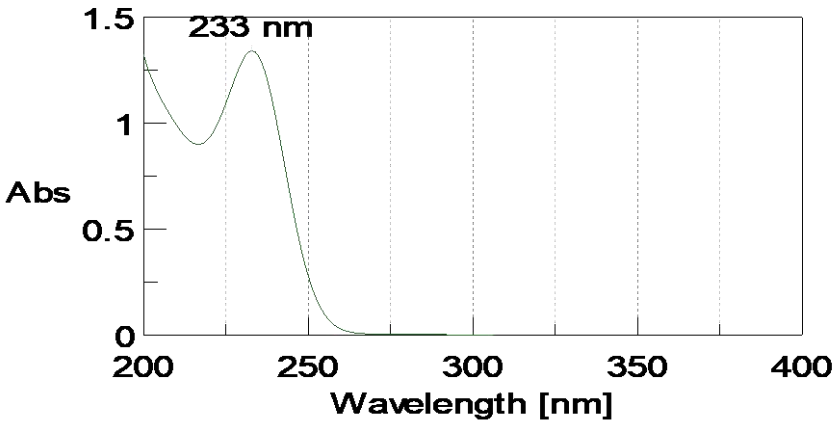


Fig. no. 4 -UV spectrum of metformin hydrochloride in phosphate buffe

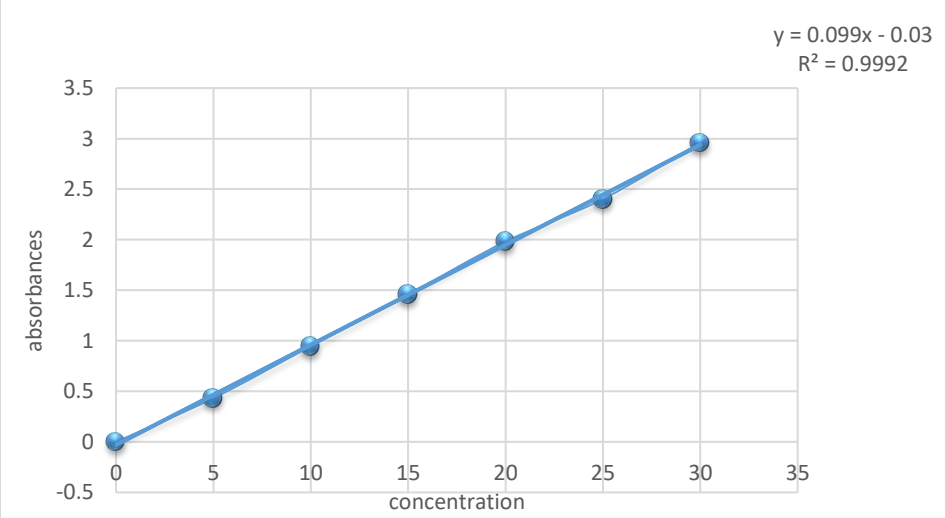
B. Construction of standard calibration curve

From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with phosphate buffer solution pH 6.8 to get a working solution of concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. These series of different concentrations of metformin hydrochloride were scanned at 233 nm using a UV spectrophotometer (Jasco V-630), and the

absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 5 to 30 µg/ml.

Table-1 Standard curve of metformin hydrochloride in phosphate buffer

Sr. No	Concentration	Absorbances
1	5	0.4327
2	10	0.9461
3	15	1.4589
4	20	1.9814
5	25	2.4024
6	30	2.9602



Graph no 1: Standard calibration curve of metformin hydrochloride in phosphate buffer

UV parameters for calibration curve in pH6.8 phosphate buffer solution

Parameter	Observation
Wavelength (λ max)	233 nm
Correlation coefficient (R2)	0.9992
Slope	0.099
Y-intercept	0.03
Solvent	Phosphate buffer

2. Preparation of standard stock solution of metformin hydrochloride:

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of distilled water is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with distilled water, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with distilled water to get a 6 µg/ml concentration.

A. Selection of wavelength

The above 6 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for metformin hydrochloride is determined.

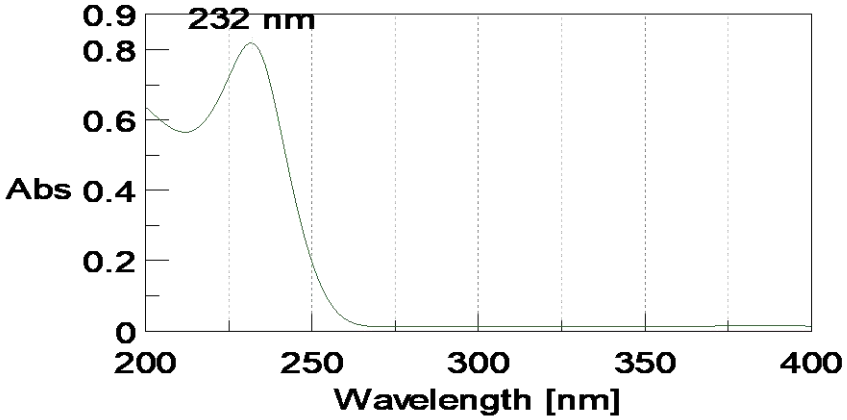


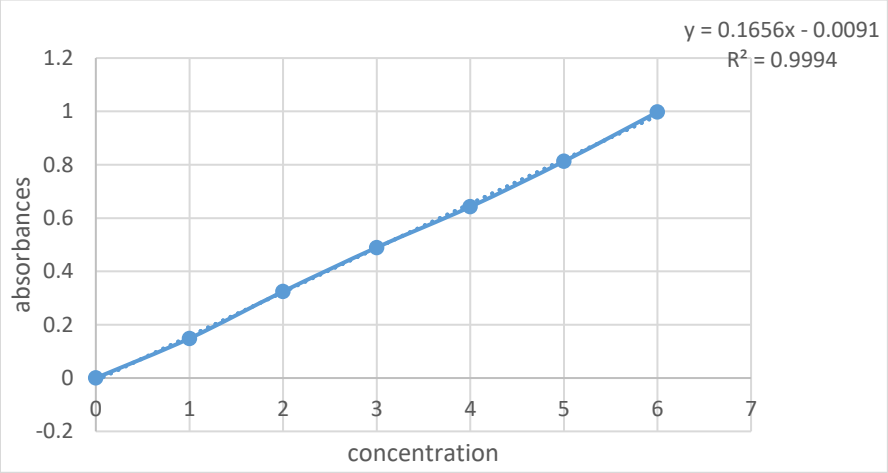
Fig. no. 5 UV spectrum of metformin hydrochloride in distilled water

B. Construction of standard calibration curve

From the standard stock solution, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with distilled water to get a working solution of concentrations 1, 2, 3, 4, 5, and 6 µg/ml, respectively. These series of different concentrations of metformin hydrochloride were scanned at 233 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 1to 6 µg/ml.

Table no -2 Standard curve of metformin hydrochloride in distilled water

Sr. No	Concentration	Absorbances
1	1	0.1485
2	2	0.3245
3	3	0.4896
4	4	0.6421
5	5	0.8125
6	6	0.9974



Graph no 2: Standard calibration curve of metformin hydrochloride in distilled water

UV parameters for calibration curve in distilled water

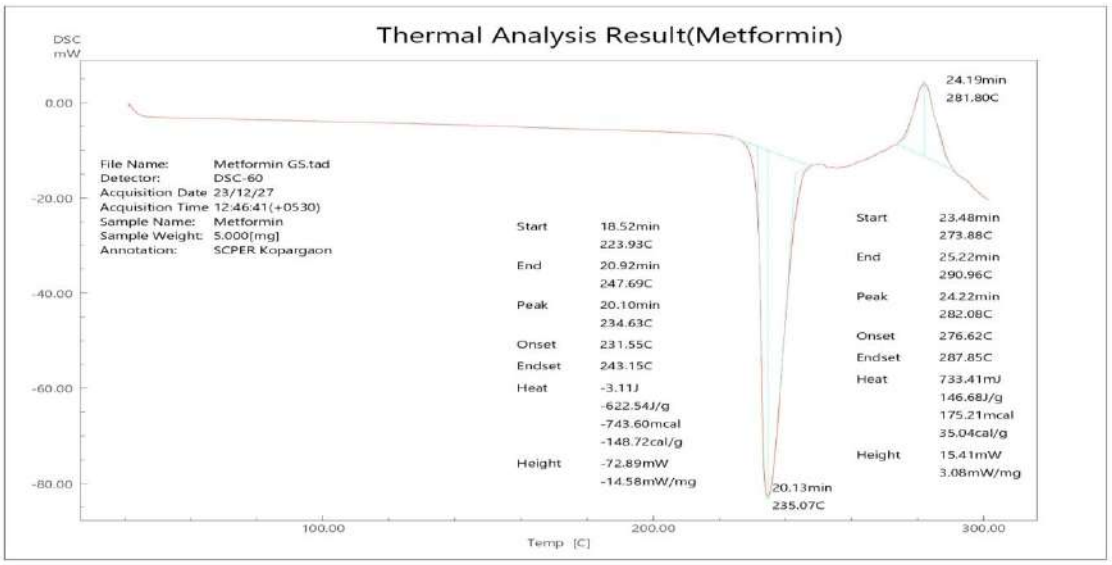
Parameter	Observation
Wavelength (λ max)	232 nm
Correlation coefficient (R2)	0.9994
Slope	0.165
Y-intercept	0.0091

3. Thermal analysis study

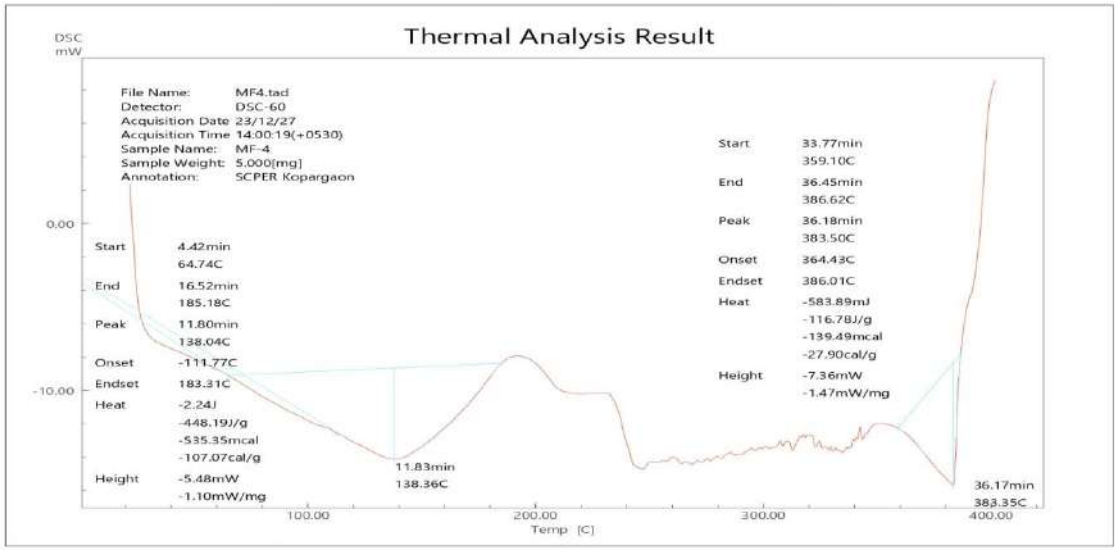
DSC is a thermal analytical technique used for analyzing thermal transitions involving thermal energy with great sensitivity. The thermal behavior of the pure drug (metformin HCl) and the

optimized formulation was characterized by using the Shimadzu DSC TA60 instrument graph shown figure below. These changes may be recorded appearances, shift or disappearance of characteristic endothermic or exothermic peaks and changes in shape and enthalpy value of peaks. These changes are indication of possible chemical incompatibility.

The thermogram of metformin HCl pure drug fig. display a sharp endothermic peak at 235.07C corresponding to its melting temperature. The DSC thermogram of metformin HCl and polymer mixture thermogram 2 recorded at temperature 138.36 C exhibits melting endotherm of metformin HCl with slight shifting towards lower temperature. The board peak with slight change in melting temperature was recorded.



Thermogram 1: DSC Thermogram of metformin HCl



Thermogram 2: DSC Thermogram of formulation

Evaluation of metformin hydrochloride patch

Physical properties

1. Weight of patch

Weight of patch containing metformin hydrochloride is given in table no 3.the values to be found in range of 20-28mg

2. Thickness of patch

The thickness of the prepared buccal patches of each formulation was determined within the range of 0.88-0.97mm.is given in table no 3.

3. Folding endurances

The folding endurance of each formulation was determined within ranges of 246 to 284.is given in table no3.

4. Measurement of surface pH

Table no.3 shows the result of surface pH values for each formulation. These values represent the mean of three replicate determinations. They were found to be within the range of 6.5to 7.1 for all formulations and were almost within range of salivary Ph i.e.., 6.2 to 7.4.it represents the better patient acceptability.

Table no – 3 Evaluation of physical parameter of different buccal patch of metformin hydrochloride

Formulation	Weight of patch (mg±S.D)	Thickness (mm±S.D)	Folding endurances (mean±S.D)	Surface pH
Mf1	22±1.02	0.92±0.76	254±0.91	7.1
Mf2	24±0.92	0.94±0.87	246±0.87	6.5
Mf3	26±0.81	0.97±0.98	261±0.76	6.7
Mf4	28±0.64	0.88±0.69	284±0.62	6.8
Mf5	30±0.90	0.96±0.78	273±0.79	6.9

Mean ± SD (n=3)

Performances parameter

1. Drug uniformity

This test involves the assay of individual units of a specified number of dosage forms to determine homogeneity in their content. From the result, it can be concluded that the patch does not show a significant deviation from the average value. The drug content uniformity was determined for all formulations by spectrophotometric method. The uniformity of drug content indicates the uniform distribution of drugs in all the patches. The drug content varies between 90.69 ±1.03 to 98.99 ± 0.21. It showed that levels of the drug in the buccal patch fulfill the limit of content uniformity.

Table no – 4 Drug content

Sr. No.	Formulation	Observation
1	Mf1	94.06 ±0.54
2	Mf2	93.24 ±0.94
3	Mf3	97.64 ±0.46
4	Mf4	98.99 ±0.21
5	Mf5	90.69 ±1.03

Mean ± SD (n=3)

Moisture content and moisture absorption

Moisture content gives an idea about the hygroscopic nature of polymers, excipients and drugs. The moisture content was found to be in the range of 2.81±0.42 to 4.79±0.66 % given in Table. It was found that there is a negligible amount of moisture present in all patches It is observed that, when there is an increase in polymer concentration, the moisture absorption capacity of the patch also increased. It may be due to the hydrophilic nature of the polymer The Values of content and moisture absorption are given in the below table no 5

Table no -5 Moisture content and moisture absorption

Sr. No.	Formulation	Moisture	Moisture Absorption
1	Mf1	4.53±0.95	0.546±0.98
2	Mf2	3.55±0.75	0.434±0.61
3	Mf3	3.42±0.59	0.505±0.75
4	Mf4	2.81±0.42	0.424±0.43
5	Mf5	4.79±0.66	0.563±0.64

3. Swelling index

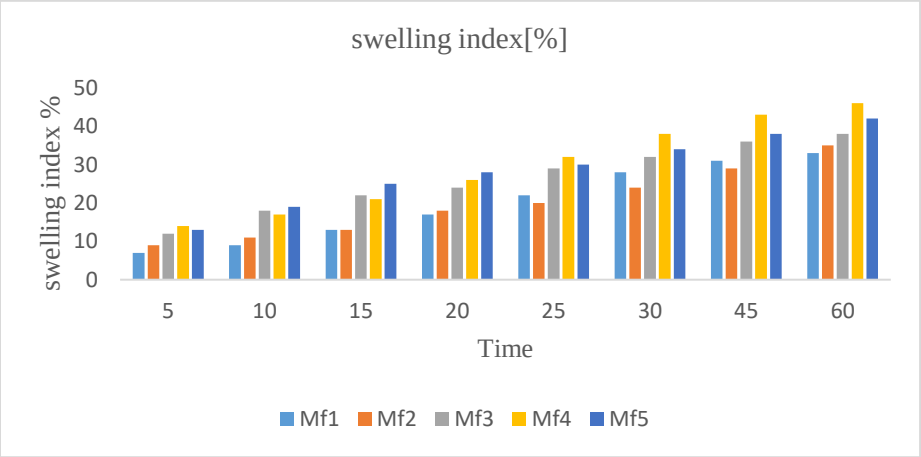
The swelling behaviour is directly related to relative moisture uptake nature of polymer and thereby provides information related to

formulation and their integrity after absorption of moisture. The percentage of swelling was found to within range of 33±0.79-46±0.53

Table no- 6 Swelling index %

Time	Swelling Index(%)				
	Mf1	Mf2	Mf3	Mf4	Mf5
0	0	0	0	0	0
5	7±0.82	9±0.98	12±0.87	14±0.76	13±0.92
10	9±0.98	11±0.65	18±0.48	17±0.84	19±0.49
15	13±0.94	13±0.71	22±0.64	21±0.63	25±0.83
20	17±0.42	18±0.86	24±0.98	26±0.87	28±0.94
25	22±0.84	20±0.56	29±0.95	32±0.59	30±0.67
30	28±0.76	24±0.47	32±0.49	38±0.91	34±0.59
45	31±0.54	29±0.63	36±0.71	43±0.55	38±0.63
60	33±0.79	35±0.71	38±0.64	46±0.53	42±0.69

Mean ± SD (n=3)

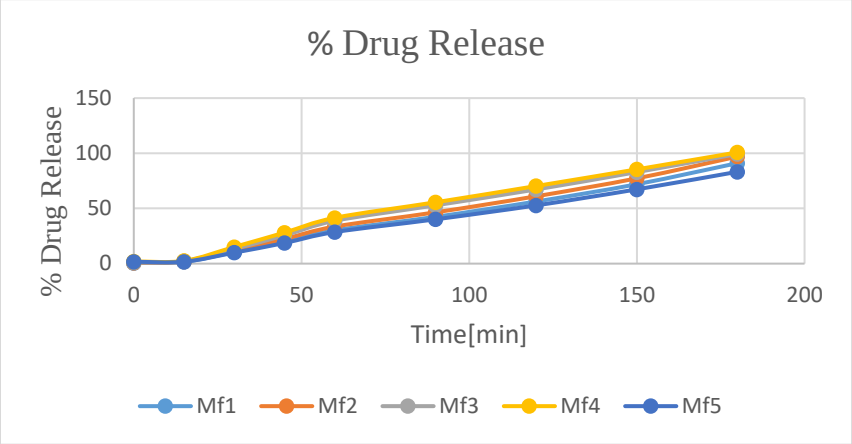


In-vitro release study

Drug release is usually controlled by diffusion mechanism due to the swelling of polymers after hydration which releases the drug. Among the five formulations, F4 showed a maximum drug release of 100.81% in 3 hrs. due to the high wet ability of the polymer HPMCk4m. HPMC k4 patches showed a fairly fast drug release; in 3 hrs. all the amount of incorporated drug was released.

Table no 7- In-vitro release %

Time (min)	In-vitro release (%)				
	Mf1	Mf2	Mf3	Mf4	Mf5
0	0	0	0	0	0
15	1.65	1.95	2.32	2.46	1.72
30	10.5	11.84	13.32	15.12	9.95
45	20.06	22.42	25.67	28.17	18.72
60	30.21	33.58	38.82	41.53	28.60
90	42.41	46.48	52.84	55.59	40.13
120	56.31	61.05	67.23	70.32	52.77
150	71.95	77.29	82.94	85.43	67.29
180	91.07	96.99	99.35	100.81	83.22

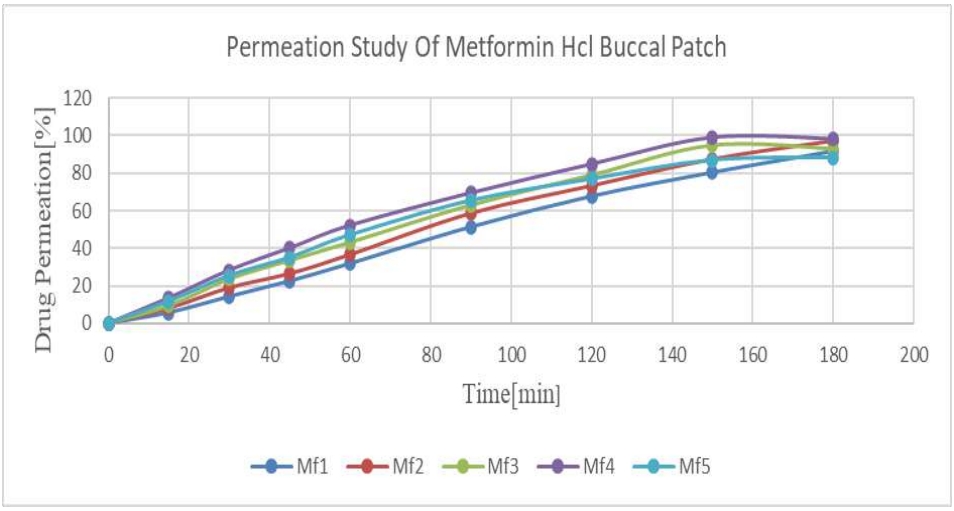


5. Ex vivo permeation study

The permeation character of metformin HCl buccal patches is shown in table no 17.the percentage of drug permeated across buccal mucosa was found to be maximum of 98.84% for the formulation Mf4 at 150 min of study. Subsequently formulation Mf3 exhibited maximum release at 150 min; formulate Mf1, Mf2 and Mf5 at 180min of study.

Table no 8- ex-vivo release study of buccal patch of metformin HCl

Time (min)	Ex vivo release (%)				
0	**Mf1**	**Mf2**	**Mf3**	**Mf4**	**Mf5**
15	0	0	0	0	0
30	5.65	7.95	9.32	13.46	11.72
45	14.21	18.84	23.58	28.24	25.36
60	22.45	26.34	33.47	39.98	35.04
90	31.78	36.47	43.14	52.06	47.01
120	51.26	58.48	63.01	69.35	65.38
150	67.58	73.14	79.05	84.68	76.87
180	80.25	87.29	94.94	98.84	86.91



Graph 4 -% Ex vivo permeation study

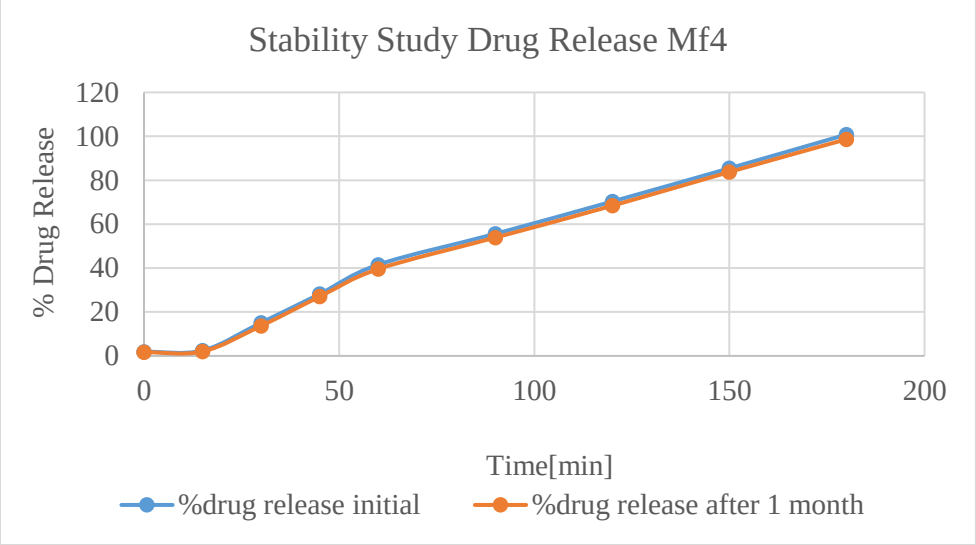
6.Accelerated Stability study

The stability of selected formulation Mf4 was assessed at accelerated condition of temperature

and humidity of 40± 2oc and 75± 5%as per ICH guideline. The evaluated parameters after exposure to such accelerated condition were given in table no .After 1 month of stability study the subjected patch exhibited slightly variation of drug content, weight variation, folding endurance and in-vitro release were within the satisfactory level.

Table no 9 Stability study results

Sampling time interval	Weight variation	Folding endurance	Drug content
Initial	28±0.64	284±0.62	98.99 ±0.21
1month	27.9±0.72	283 ±0.89	98.70±0.32



Graph 5-Stability study drug release of Mf4

II. Glibenclamide

1. Melting point

The melting point determined by using the capillary tube method. Melting point of metformin hydrochloride found to be 170oc.this was matching to the literature value 169-170oc indicating the identity and purity of drug sample.

2. Solubility study

Sr. No	Solvent	Drug solubility
1	Methanol	Slightly soluble
2	Phosphate buffer	Soluble

3. Spectroscopic studies

A. FT-IR study

B. UV study

A. FT-IR study:

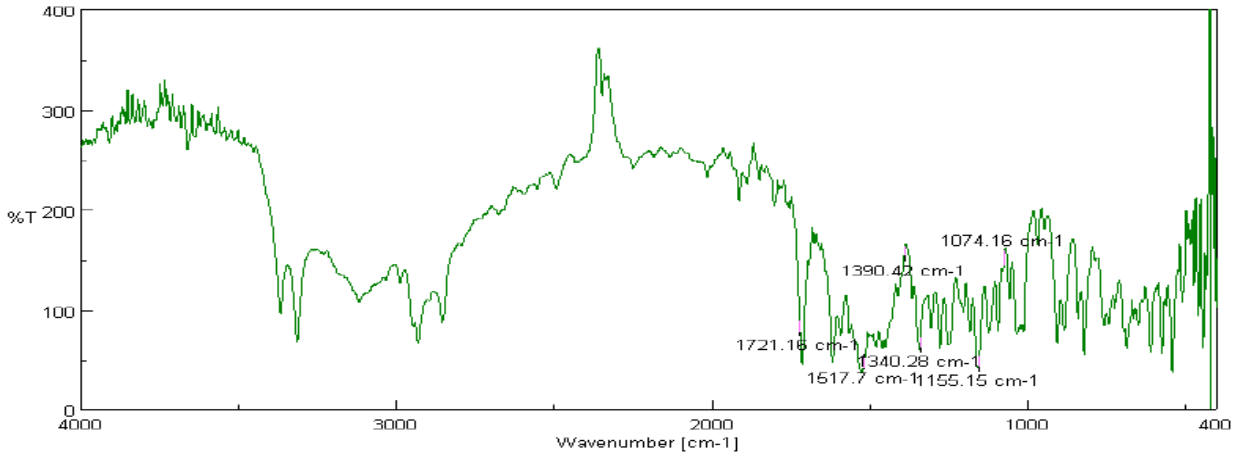
1. Spectra 1: IR of pure drug

2. Spectra 2: IR of HPMC E-15

3. Spectra 3: IR of drug and polymer incompatibility

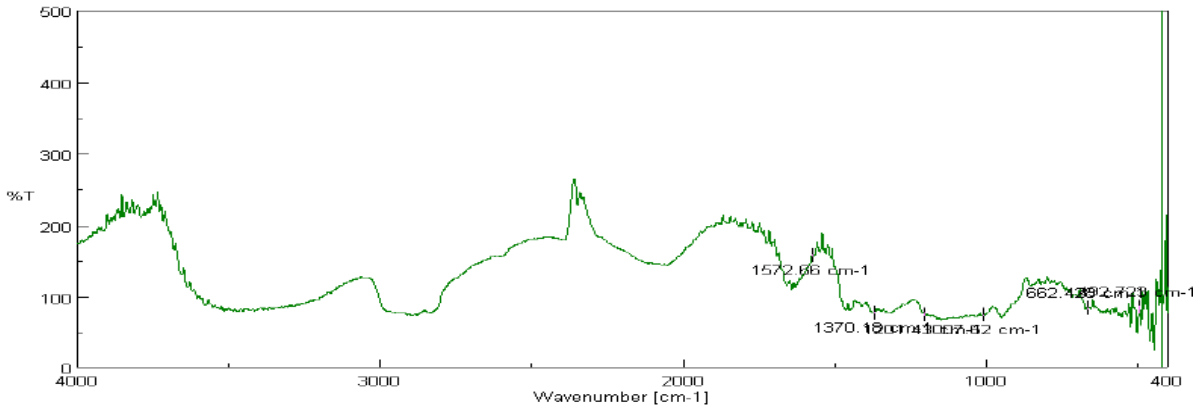
Spectra 1: IR of pure drug

The FT-IR spectrum of Glibenclamide is consistent with references spectra given in an analytical profile of drug substance. The drug and polymer were compatible with each other.



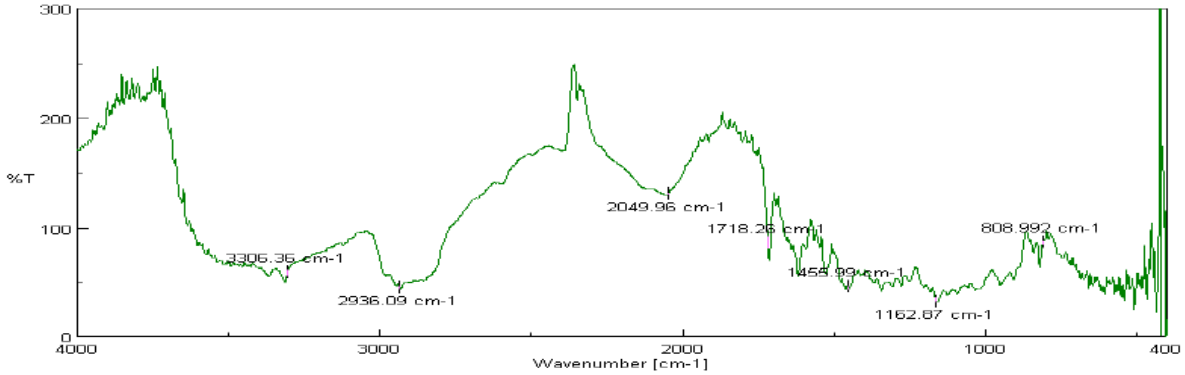
Spectrum 1: FT-IR spectra of pure drug

Spectra 2: IR of HPMC E-15



Spectrum 2: FT-IR spectra of HPMC E-15

Spectra 3: IR of drug and polymer incompatibility



Spectrum 3: FT-IR spectra of drug +HPMC E-15

B. UV study

1. Preparation of standard stock solution of Glibenclamide:

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of phosphate buffer (pH 6.8) is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with phosphate buffer pH 6.8, so as to obtain a concentration of 100 µg/ml. pipette out 0.5 ml

from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with phosphate buffer ph 6.8 to get a 5 µg/ml concentration.

I. Selection of wavelength

The above 30 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for glibenclamide is determined.

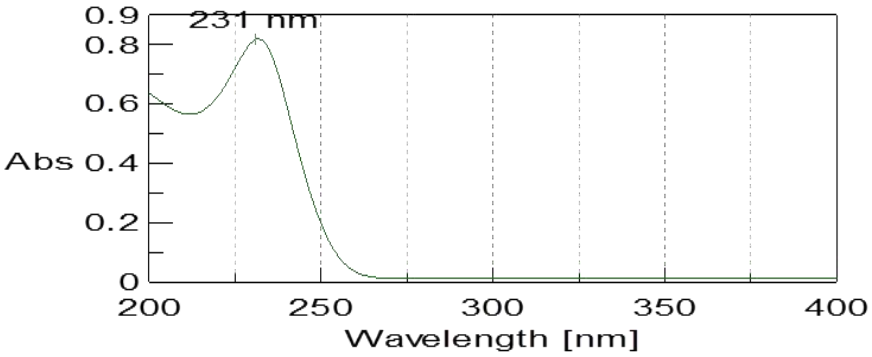


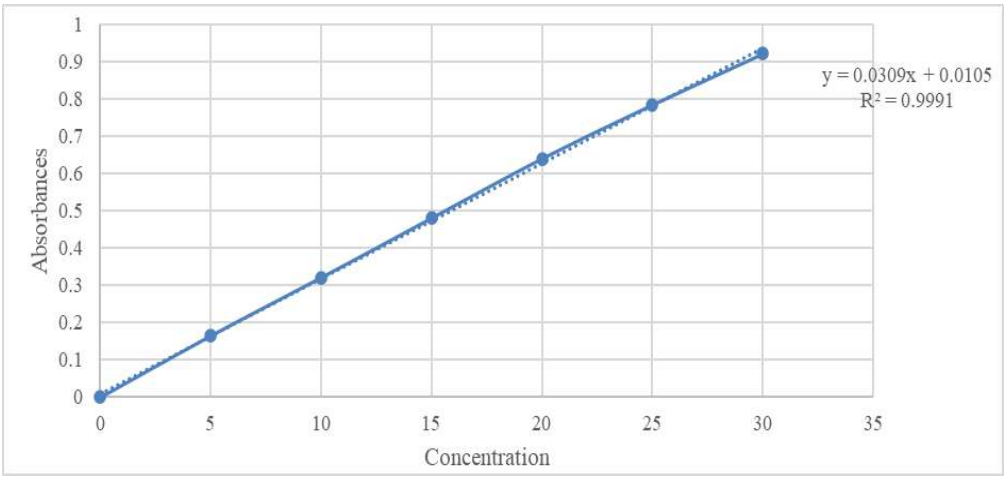
Fig no. 6. UV spectrum of metformin hydrochloride in phosphate buffer

II. Construction of standard calibration curve

From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with phosphate buffer solution pH 6.8 to get a working solution of concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. These series of different concentrations of glibenclamide were scanned at 231 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 5 to 30 µg/ml.

Table no 10- Standard curve of glibenclamide in phosphate buffer

Sr. No	Concentration	Absorbances
1	5	0.1654
2	10	0.3214
3	15	0.4812
4	20	0.6405
5	25	0.7845
6	30	0.9214



Graph no6- Standard calibration curve glibenclamide in phosphate buffer

Parameter	Observation
Wavelength (λ max)	231 nm
Correlation coefficient (R2)	0.9991
Slope	0.0309
Y-intercept	0.0105
Solvent	Phosphate buffer

UV parameters for calibration curve in pH6.8 phosphate buffer solution

2. Preparation of standard stock solution of Glibenclamide:

10 mg of the drug was weighed accurately and transferred into 100 ml of a volumetric flask. A sufficient quantity of methanol is added to the flask to dissolve the drug. The solution is sonicated and then diluted up to 100 ml with methanol, so as to obtain a concentration of 100 µg/ml. pipette out 0.2 ml from the above 100 µg/ml stock solution, added to a 10 ml volumetric flask, and diluted up to 10 ml with methanol to get a 2 µg/ml concentration.

I. Selection of wavelength

The above 8 µg/ml solution was scanned in the UV range 400–200 nm in a quartz cell against a blank solution separately using a UV visible spectrophotometer. From the obtained spectra, λmax for glibenclamide is determined.

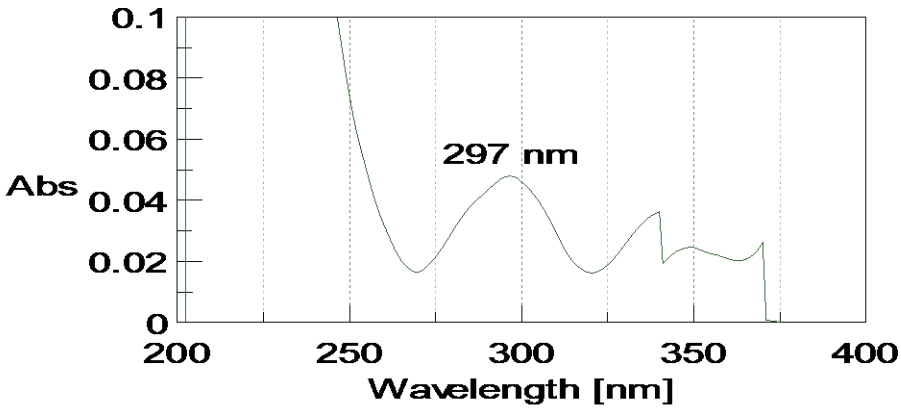


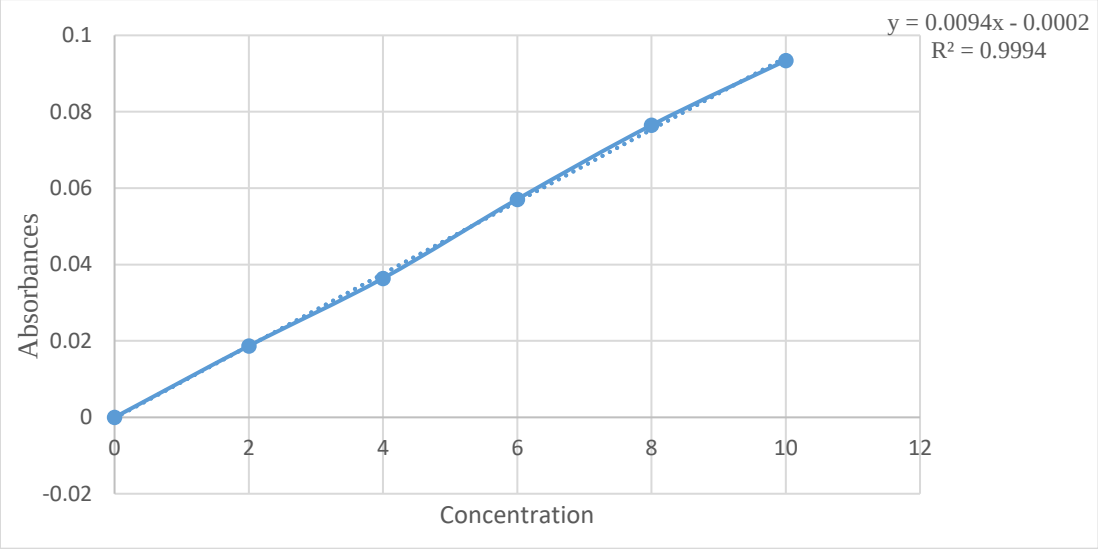
Fig no. 7. UV spectrum of glibenclamide in methanol

II. Construction of standard calibration curve

From the standard stock solution, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml were pipetted out in 10 ml of a volumetric flask and diluted up to 10 ml with methanol to get a working solution of concentrations 2, 4, 6, 8, 10, and 12 µg/ml respectively. These series of different concentrations of glibenclamide were scanned at 296 nm using a UV spectrophotometer (Jasco V-630), and the absorbance of all dilutions was recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 2 to 12 µg/ml.

Table no 11 Standard curve of glibenclamide in methanol

Sr. No	Concentration	Absorbances
1	2	0.0187
2	4	0.0364
3	6	0.0571
4	8	0.0765
5	10	0.0934



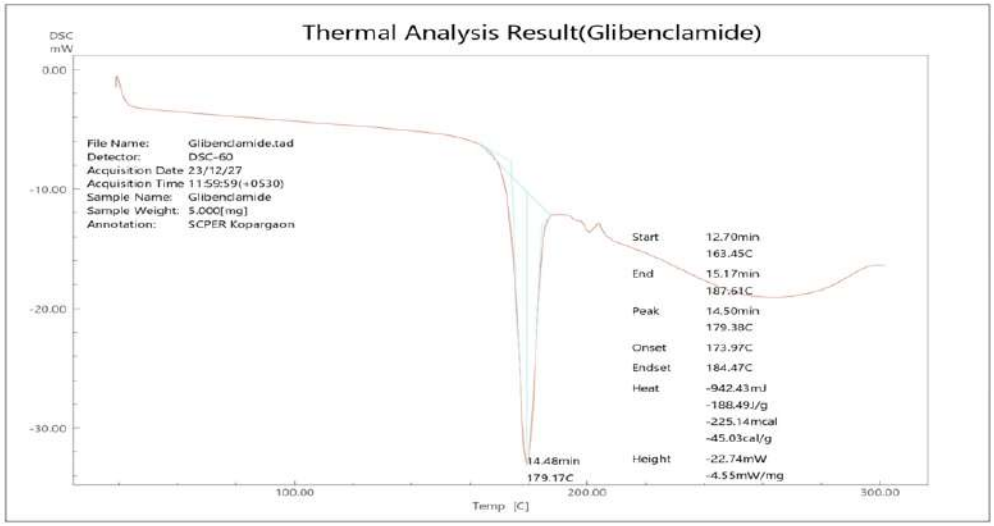
Graph Standard calibration curve of glibenclamide in methanol

Parameter	Observation
Wavelength (λ max)	296 nm
Correlation coefficient (R2)	0.9994
Slope	0.0094
Y-intercept	0.0002
Solvent	Methanol
Parameter	Observation
Wavelength (λ max)	296 nm

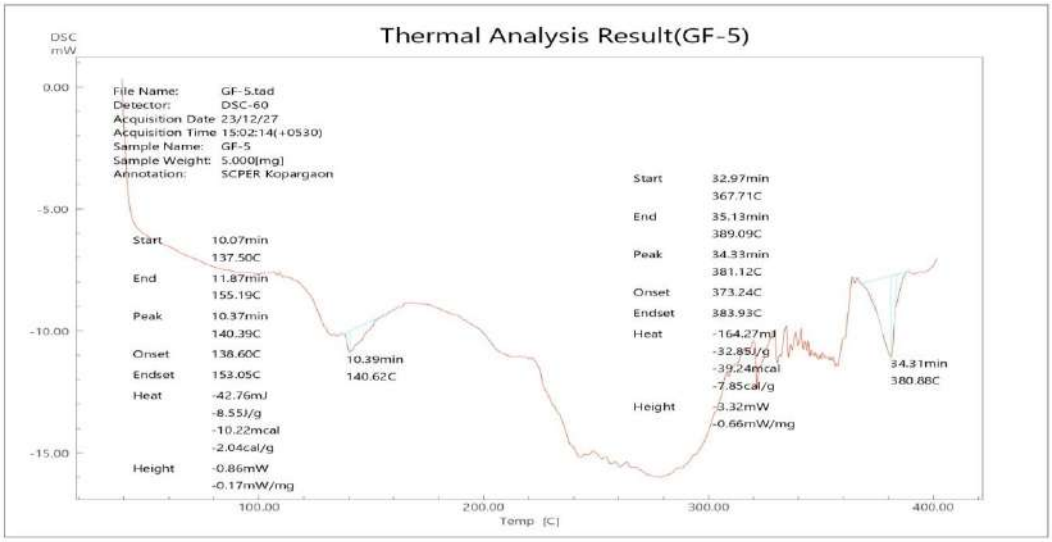
UV parameters for calibration curve in methanol

4. Thermal analysis study

DSC is a thermal analytical technique used for analyzing thermal transitions involving thermal energy with great sensitivity. The thermal behaviour of the pure drug (glibenclamide) and the optimized formulation was characterized by using the Shimadzu DSC TA60 instrument graph shown figure below. These changes may be recorded appearances, shift or disappearance of characteristic endothermic or exothermic peaks and changes in shape and enthalpy value of peaks. These changes are indication of possible chemical incompatibility. The thermograms of glibenclamide pure drug fig. display a sharp endothermic peak at 179.17 C corresponding to its melting temperature. The DSC thermogram of glibenclamide and polymer mixture thermogram 2 recorded at temperature 140.62 C exhibits melting endotherm of glibenclamide with slight shifting towards lower temperature. The board peak with slight change in melting temperature was recorded.



Thermogram 1: DSC Thermogram of glibenclamide



Thermogram 2: DSC Thermogram of formulation

Evaluation of Glibenclamide patch

Physical properties

1. Weight of patch

Weight of patch containing metformin hydrochloride is given in table no 12 .the values to be found in range of 23-31mg

2. Thickness of patch

The thickness of the prepared buccal patches of each formulation was determined within the range of 0.73-0.96mm.is given in table no 12.

3. Folding endurances

The folding endurance of each formulation was determined within ranges of 247to 281.is given in table no 12.

4. Measurement of surface pH

Table no.12 shows the result of surface pH values for each formulation. These values represent the mean of three replicate determinations. They were found to be within the range of 6.5to 7.1 for all formulations and were almost within range of salivary Ph i.e.., 6.2 to 7.4.it represents the better patient acceptability.

Table no 12 Evaluation of physical parameter of different buccal patch of glibenclamide

Formulation	Weight of patch (mg±S.D)	Thickness (mm±S.D)	Folding endurances (mean±S.D)	Surface pH
Gf1	23±1.06	0.96±0.79	252±0.82	6.7
Gf2	25±0.94	0.82±0.58	247±0.71	6.9
Gf3	27±0.86	0.74±0.64	279±0.96	7.1
Gf4	29±0.67	0.89±0.88	266±0.87	6.5
Gf5	31±0.48	0.73±0.54	281±0.65	6.8

Mean ± SD (n=3)

Performances parameter

1. Drug uniformity

This test involves the assay of individual units of a specified number of dosage forms to determine homogeneity in their content. From the result, it can be concluded that the patch does not show a significant deviation from the average value. The drug content uniformity was determined for all formulations by spectrophotometric method. The uniformity of drug content indicates the uniform distribution of drugs in all the patches. The drug content varies between 86.98±0.74 to 98.71 ± 0.65. It showed that levels of the drug in the buccal patch fulfill the limit of content uniformity.

Table no 13 Drug content

Sr. No.	Formulation	Observation
1	Gf1	86.98±0.74
2	Gf2	95.74±0.67
3	Gf3	90.45±0.80
4	Gf4	96.63±0.79
5	Gf5	98.71±0.65

Mean ± SD (n=3)

2. Moisture content and moisture absorption

Moisture content gives an idea about the hygroscopic nature of polymers, excipients and drugs. The moisture content was found to be in the range of 2.53±0.55 to 4.23±0.87 % given in Table. It was found that there is a negligible amount of moisture present in all patches It is observed that, when there is an increase in polymer concentration, the moisture absorption capacity of the patch also increased. It may be due to the hydrophilic nature of the polymer. The values of moisture content and moisture absorption are given in the below table

Table no 14 Moisture content and moisture absorption

Sr. No.	Formulation	Moisture Content	Moisture Absorption
1	Gf1	4.23±0.87	0.646±0.88
2	Gf2	2.95±0.92	0.434±0.61
3	Gf3	3.72±0.65	0.565±0.55
4	Gf4	3.41±0.71	0.694±0.73
5	Gf5	2.53±0.55	0.423±0.48

Mean ± SD (n=3)

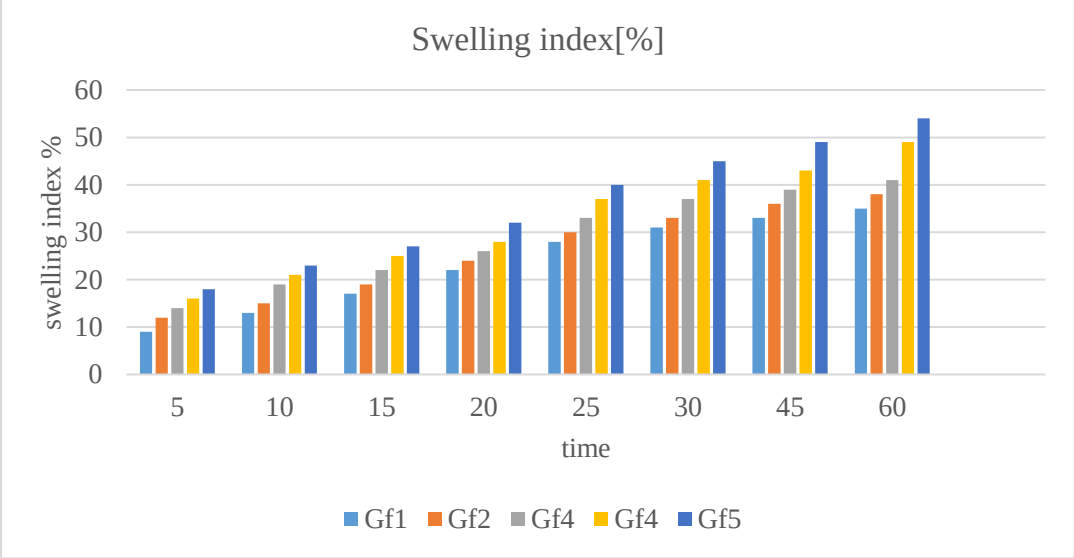
3. Swelling index

The swelling behaviour is directly related to relative moisture uptake nature of polymer and thereby provides information related to formulation and their integrity after absorption of

moisture. The percentage of swelling was found to within range of 35±0.78-54±0.54

Table no 15 Swelling index %

Time (min)	Swelling index (%)				
	Gf1	Gf2	Gf3	Gf4	Gf5
0	0	0	0	0	0
5	9±0.98	12±0.85	14±0.81	16±0.96	18±0.61
10	13±0.84	15±0.83	19±0.96	21±0.71	23±0.71
15	17±0.65	19±0.74	22±0.92	25±0.67	27±0.79
20	22±0.75	24±0.68	26±0.56	28±0.59	32±0.57
25	28±0.54	30±0.54	33±0.67	37±0.84	40±0.67
30	31±0.97	33±0.98	37±0.87	41±0.63	45±0.87
45	33±0.51	36±0.52	39±0.75	43±0.52	49±0.68
60	35±0.78	38±0.73	41±0.64	49±0.74	54±0.54



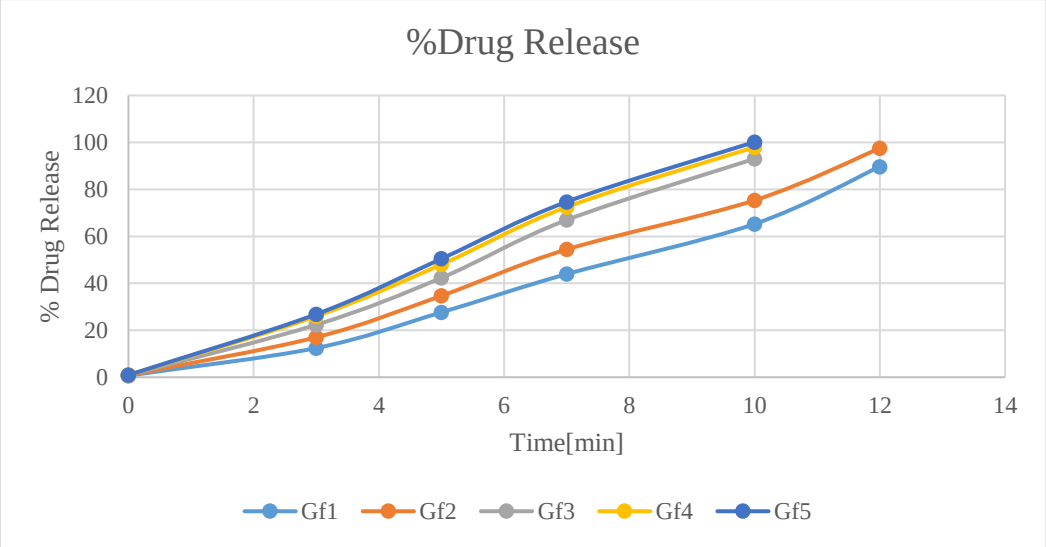
Graph -swelling index %

4. In-vitro release study

Drug release is usually controlled by diffusion mechanism due to the swelling of polymers after hydration which releases the drug. Among the five formulations Gf5 showed a maximum drug release of 100.17% in 10min due to the high wet ability of the polymer HPMC E-15. HPMC E-15 patches showed a fairly fast drug release; in 10min all the amount of incorporated drug was released.

Table no 16: In-vitro release study

Time (min)	In-vitro release (%)				
0	Gf1	Gf2	Gf3	Gf4	Gf5
3	0.709	0.84	0.96	0.96	0.96
5	12.45	17.02	22.35	25.98	26.85
7	27.66	34.70	42.35	48.05	50.48
10	43.95	54.44	66.99	72.49	74.72
12	65.29	75.35	93.02	97.97	100.17



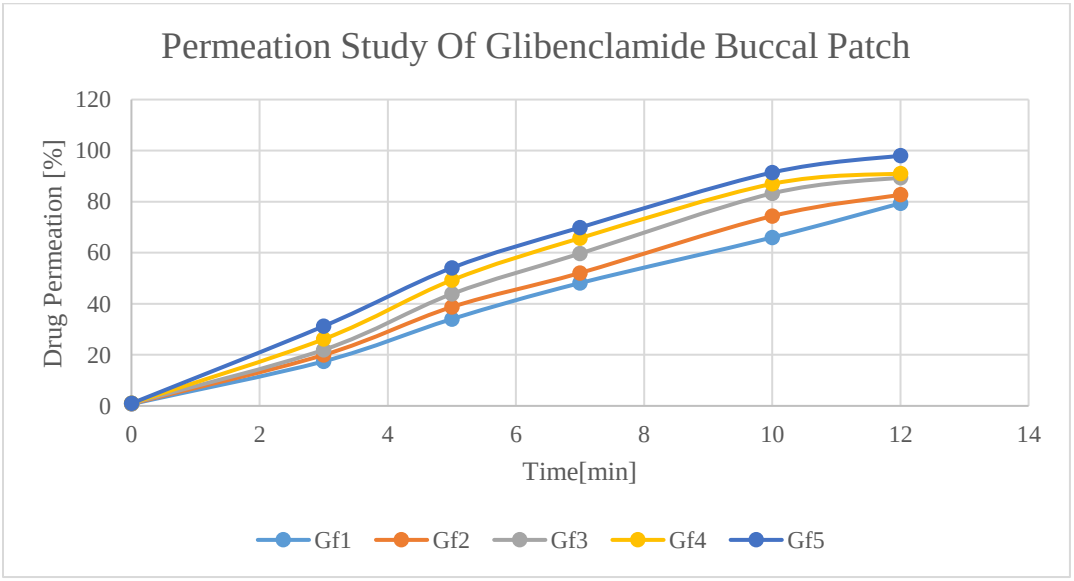
Graph-%drug release of glibenclamide [Gf1-Gf5]

5. Ex vivo permeation study

The permeation character of glibenclamide buccal patches is shown in table no 17.the percentage of drug permeated across buccal mucosa was found to be maximum of 98.02% for the formulation Gf5 at 12 min of study.

Table no 17 ex-vivo release of buccal patch of glibenclamide

Time (min)	Ex vivo release (%)				
0	**Gf1**	**Gf2**	**Gf3**	**Gf4**	**Gf5**
3	0.709	0.84	0.96	0.96	0.96
5	17.45	19.96	21.98	26.14	31.24
7	34.02	38.74	43.84	49.25	54.04
10	48.12	52.07	59.67	65.71	69.87
12	65.98	74.35	83.25	86.98	91.43



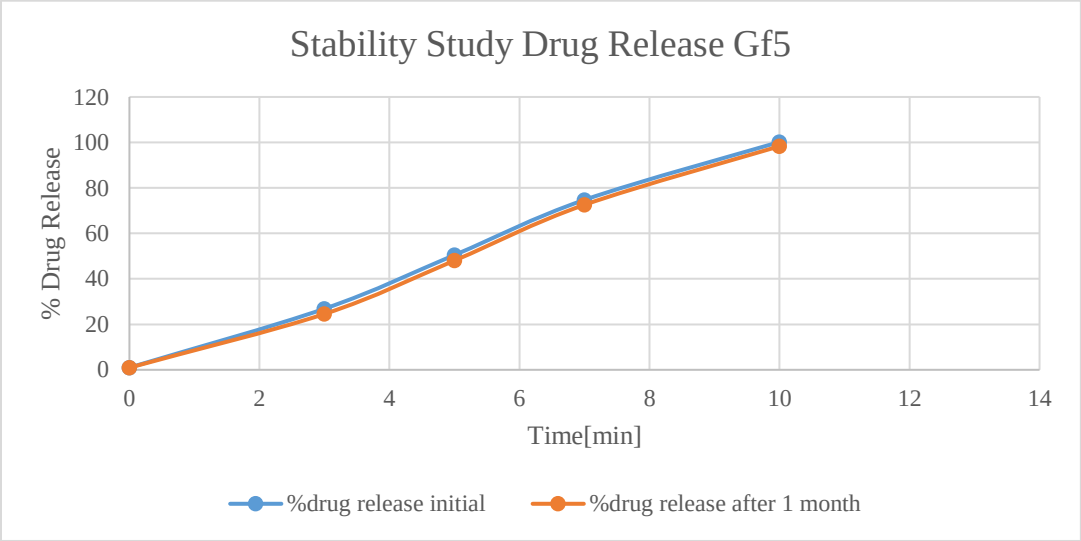
Graph -% Ex vivo permeation study

4. Accelerated Stability study

The stability of selected formulation Gf5 was assessed at accelerated condition of temperature and humidity of 40± 2oc and 75± 5%as per ICH guideline. The evaluated parameters after exposure to such accelerated condition were given in table no 18. After 1month of stability study the subjected patch exhibited slightly variation of drug content, weight variation, folding endurance and in-vitro release were within the satisfactory level.

Table no 18 Stability study results

Sampling time interval	Weight variation	Folding endurance	Drug content
Initial	31±0.48	281±0.65	98.71 ±0.63
1 month	30±0.72	280±0.69	97.90±0.42



Graph-Stability study drug release of Gf5

DISCUSSION

Buccal route is most valuable route of administration for systemic drug delivery and it leads direct access to the systemic circulation and bypasses drugs from hepatic first pass metabolism and enzymatic degradation in GI provide high bioavailability. Metformin hydrocholoride (Biguanides) and glibenclamide (sulfonyl urea) is used in treatment of diabetes mellitus. The conventional doses release the entire drug in just few minutes and therefore the therapeutic concentrations are maintained for a short period of time generating a need for administration of another dose. Whereas BDDS persists the residences time of the dosage form at the absorption site, hence raises the bioavailability. It provides fast absorption because of huge blood supply and good perfusion rates. Therefore, formulation of buccal patch of metformin and glibenclamide will be beneficial. In the present work effort have been made to develop the controlled release mucoadhesive buccal patch of metformin HCl and glibenclamide prepared by solvent casting technique using HPMC K-4 and HPMC E-15in different ratio to formulate buccal patch

Preformulation study

1. Metformin HCl

Determination of lamda max of metformin HCl

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. From the scanning of drug, it was concluded that the drug had lamda max of 234nm, which was nearly equal to 233nm

as reported. Also, IR spectrum was concordant with the reference spectrum of metformin HCl.

Preparation of standard calibration curve of metformin HCl

From the standard curve of metformin HCl (table no, graph), it was observed that the drug obeys beer's law in concentration range of 5-30µg/ml in phosphate buffer. The linear regression equation generated was used for the calculation of amount of drug.

Determination of IR spectrum of metformin HCl

Physical mixture of drug and polymer was characterized by FT-IR Spectral analysis for any physical as well as chemical alteration of the drug characteristic. From the results, it was concluded that there was no interference in the functional group as the principal peak of metformin were found to be unaltered in the drug–polymer physical mixture, indicating they were compatible chemically.

Drug excipients compatibility studies

Drug-excipients compatibility studies from an important part of pre-formulation studies for the determination of interaction between drug and excipients. It is determined after storage of specific time period by using suitable analytical technique such as uv-visible spectroscopy, FT-IR study, DSC study and the results are indicating that there is no interaction between drug and excipients.

2. Glibenclamide

Determination of lamda max of glibenclamide

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. From the scanning of drug, it was concluded that the drug had lamda max of 220-350nm, which was nearly equal to 231nm as reported. Also, IR spectrum was concordant with the reference spectrum of glibenclamide.

Preparation of standard calibration curve of glibenclamide

From the standard curve of glibenclamide (table no, graph), it was observed that the drug obeys beer's law in concentration range of 5-30µg/ml in phosphate buffer. The linear regression equation generated was used for the calculation of amount of drug.

Determination of IR spectrum of glibenclamide

Physical mixture of drug and polymer was characterized by FT-IR Spectral analysis for any physical as well as chemical alteration of the drug characteristic. From the results, it was concluded that there was no interference in the functional group as the principal peak of metformin were found to be unaltered in the drug–polymer physical mixture, indicating they were compatible chemically.

Drug excipients compatibility studies

Drug-excipients compatibility studies from an important part of pre-formulation studies for the determination of interaction between drug and excipients. It is determined after storage of specific time period by using suitable analytical technique such as uv-visible spectroscopy, FT-IR study, DSC study and the results are indicating that there is no interaction between drug and excipients.

Formulation design

1. Metformin hydrochloride

Formulation of mucoadhesive buccal patch of metformin HCl

Total 5 formulations of muco-adhesive buccal patch were prepared with different ratio and proportion of HPMC K-4 by solvent casting technique. The prepared muco-adhesive patches were then evaluated for various physico-chemical tests like thickness, folding endurances, weight variation, swelling index, drug uniformity, moisture content, moisture absorption, surface pH, In-vitro release, stability study.

2. Glibenclamide

Formulation of mucoadhesive buccal patch of metformin HCl

Total 5 formulations of muco-adhesive buccal patch were prepared with different ratio and proportion of HPMC E-15 by solvent casting technique. The prepared muco-adhesive patches were then evaluated for various physico-chemical tests like thickness, folding endurances, weight variation, swelling index, drug uniformity, moisture content, moisture absorption, surface pH, In-vitro release, stability study.

Evaluation parameter

1. Metformin HCl

Physical properties

Thickness of patch

The thickness of the prepared buccal patches of each formulation was determined within range of 0.88-097mm.

Weight of patch

The weight of prepared buccal patch of each formulation was determined within the range of 22-30mg.

Folding endurance

The folding endurance of each formulation was determined within the range of 246-284.it revealed that good flexibility of patch.

Measurement of surface pH

They were found to be within the range of 6.5-7.1 for all formulation and were almost within range of salivary pH i.e.,6.2 to 7.4. there was no considerable difference in surface pH of patches. It represents the better patient acceptability.

2. Glibenclamide

Physical properties

Thickness of patch

The thickness of the prepared buccal patches of each formulation was determined within range of 0.88-097mm.

Weight of patch

The weight of prepared buccal patch of each formulation was determined within the range of 22-30mg.

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Performance parameter

1. Metformin HCl

Content uniformity

Table no 1 shows the result of drug content uniformity in each formulation. Three replicates of each test were carried out. The mean drug content was found to be in the range of 90.69-98.99% for [each patch size 1cm] the prepared buccal patch formulations. It is indicating the uniform distribution of drug in polymer matrix.

Swelling index

Swelling index [%] of all buccal patches containing metformin HCl is given in table no 1.the swelling of patch was changes with respect to polymer ratios. The values were found to within range of 33±0.79-46±0.53it was maximum for Mf4 i.e., 46±0.53.it is revealed that swelling nature of polymer.

Moisture content and moisture absorption

Moisture content gives an idea about the hygroscopic nature of polymers, excipients and drugs. The moisture content was found to be in the range of 2.81±0.42 to 4.79±0.66 % given in Table. It was found that there is a negligible amount of moisture present in all patches

In vitro release study

The in-vitro drug release was studied in phosphate buffer pH 6.8.the in-vitro drug release studies were carried out the result is shown in table no 1.the maximum release was observed in Mf4 formulation, it was upto 180min.the release is due to the uniform and proper mixing of drug and

polymers which enables the drug to release in steady state manner.

Ex-vivo drug permeation study

The result of ex-vivo permeation study through goat buccal mucosa indicated that permeation of drug of all tested formulation was increased significantly in presences of permeation enhancer poly ethylene glycol. Formulation Mf4 shows greater drug permeation and is selected for further study.

2. Glibenclamide

Content uniformity

Table no 1 shows the result of drug content uniformity in each formulation. Three replicates of each test were carried out. The mean drug content was found to be in the range of 86.98-98.71% for [each patch size 1cm] the prepared buccal patch formulations. It is indicating the uniform distribution of drug in polymer matrix.

Swelling index

Swelling index [%] of all buccal patches containing glibenclamide is given in table no 1.the swelling of patch was changes with respect to polymer ratios. The values were found to within range of 35±0.78-54±0.5.it was maximum for Gf5i.e; 54±0.5.it is revealed that swelling nature of polymer.

Moisture content and moisture absorption

Moisture content gives an idea about the hygroscopic nature of polymers, excipients and drugs. The moisture content was found to be in the range of 2.53±0.55 to 4.23±0.87 % given in Table. It was found that there is a negligible amount of moisture present in all patches

In vitro release study

The in-vitro drug release was studied in phosphate buffer pH 6.8.the in-vitro drug release studies were carried out the result is shown in table no 1.the maximum release was observed in Gf5 formulation, it was upto 10 min. the release is due to the uniform and proper mixing of drug and

polymers which enables the drug to release in steady state manner.

Ex-vivo drug permeation study

The result of ex-vivo permeation study through goat buccal mucosa indicated that permeation of drug of all tested formulation was increased significantly in presences of permeation enhancer poly ethylene glycol. Formulation Gf4 shows greater drug permeation and is selected for further study.

Criteria for optimization

1. Metformin HCl

The formulation Mf4 is optimized on the basics of in-vitro release, swelling index, folding endurances and greater permeation. The ex-vivo release studies were performed by using 7.4 pH phosphate buffer for formulation Mf4 by using goat mucosa as a model membrane and it was shown that good permeability across the membrane above 90mins.

2. Glibenclamide

The formulation Gf5 is optimized on the basics of in-vitro release, swelling index, folding endurances and greater permeation. The ex-vivo release studies were performed by using 7.4 pH phosphate buffers for formulation Gf5 by using goat mucosa as a model membrane and it was shown that good permeability across the membrane above 7 mins.

Stability study

1. Metformin HCl

The stability of selected formulation Mf4 was assessed at accelerated condition of temperature and humidity of 40± 2oc and 75± 5%as per ICH guideline over 1 month. Stability studies were carried out to predict the degradation that may occur over prolonged period of storage at various temperature and humidity for formulation Mf4 over a period of 1 month. The result of stability studies, which were conducted for 1 month, as shown in table no 1.the result obtained showed a

slight decrease in, In-vitro release of formulation Mf4 as compared to the fresh formulation Mf4.

2. Glibenclamide

The stability of selected formulation Gf5 was assessed at accelerated condition of temperature and humidity of 40± 2oc and 75± 5%as per ICH guideline over1 month. Stability studies were carried out to predict the degradation that may occur over prolonged period of storage at various temperature and humidity for formulation Gf5 over a period of 1 month. The result of stability studies, which were conducted for 1 month, as shown in table no 1.the result obtained showed a slight decrease in, In-vitro release of formulation Gf5 as compared to the fresh formulation Gf5.

SUMMARY

The present study mainly focused to formulate a buccal mucoadhesive drug delivery system or the delivery of metformin HCl and glibenclamide. During this study an attempt was made to formulate mucoadhesive buccal patches of metformin HCl and glibenclamide by solvent casting technique using various combinations of mucoadhesive polymers. The polymers used for the study were selected based on their mucoadhesive property as it was reported in previous studies. Mucoadhesive polymers hydroxypropyl methyl cellulose was used in various proportions. Effect of permeation enhancers i.e., polyethylene glycol was studied.

Pre-formulation studies were performed to standardize the method of estimation of metformin HCl and glibenclamide by spectrophotometric method. The drug polymer interaction study was done by FTIR, DSC analysis of physical mixtures of drug and polymers. This study confirmed that there was no drug polymer interaction in the physical mixture sample stored at 40± 2oc and 75% ±5%RH for 1 month. Total 5 formulations were made using polymers [HPMC] with different ratio. All formulation showed uniform surface, thickness and mass. Among 5 formulations Mf4 and Gf5 showed optimum folding endurances, swelling index, drug content, in vitro drug release and ex vivo drug permeation. The stability of formulations was confirmed by accelerated stability studies as per ICH x guidelines. Formulation Mf4 showed maximum percentage of swelling 46±0.53, it is revealed that swelling nature of polymer. Mf4 show drug content 98.99% for [each patch size 1cm], it is indicating the uniform distribution of drug in polymer matrix. The maximum release was observed in Mf4 formulation, it was 100.81% upto 180min.the release is due to the uniform and proper mixing of drug and polymers which enables the drug to release in steady state manner. And drug permeation of the formulation Mf4 is found to be 98.84% at 150 min of study. Hence Mf4 is considered to be the optimized formulation. Stability studies were carried out for Mf4 formulation they have showed good stability when stored at accelerated stability state as per the ICH guideline and the value were within a permissible limit. Formulation Gf5 showed maximum percentage of swelling 54±0.54, it is revealed that swelling nature of polymer. Gf5 show drug content 98.71 ± 0.65for [each patch size 1cm], it is indicating the uniform distribution of drug in polymer matrix. The maximum release was observed in Gf5 formulation, it was 100.17% up to 10min.the release is due to the uniform and proper mixing of drug and polymers which enables the drug to release in steady state manner. And drug permeation of the formulation Gf5 is found to be98.02 % at 12 min of study. Hence Gf5 is considered to be the optimized formulation. Stability studies were carried out for Gf5 formulation they have showed good stability when stored at accelerated stability state as per the ICH guideline and the value were within a permissible limit.

CONCLUSION

In the present study, attempt has been done to develop a novel mucoadhesive drug system in the form of the buccal patches for the release of metformin HCl and glibenclamide in a unidirectional manner, to maintain constant therapeutic levels of the drug for long time. Buccal formulation of metformin HCl and glibenclamide in the form of mucoadhesive patches were developed to a satisfactory level in term of drug release, drug permeation, content uniformity, swelling index, surface pH, thickness and folding endurances. Although all buccal patches exhibited satisfactory results, but best results were obtained from Mf4 and Gf5. the above study concluded that the possibility of the making of mucoadhesive drug delivery system for metformin hcl and glibenclamide which will be more efficacious and acceptable than conventional drug delivery of drug and also having satisfactory controlled release profile which may provide an increased therapeutic efficacy. These novel unidirectional buccal patches can be considered as alternative to oral route of administration of metformin and glibenclamide. This route may also provide additional advantages of bypassing the first pass metabolism and providing greater therapeutic index as a result of increased bioavailability in patients suffering from diabetes mellitus.

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