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Development and *In vivo* evaluation of mucoadhesive tablets of Lafutidine

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Abstract

The aim of the present work was *in vitro* and *in vivo* evaluation of mucoadhesive tablets of lafutidine to prolong the gastric residence time after oral administration. Formulations were prepared using 3^3 full factorial designs to explore the effects of Gum Kondagogu, Gum Olibanum and Guar Gum (as independent variables) on mucoadhesive strength, drug release and *Ex vivo* residence time (as dependent variables) was studied and published in the earlier research paper.

In this investigation the formulated mucoadhesive tablets which was optimized through *in vitro* studies is selected and performed the *in vivo* studies on Human volunteers. The drug-polymer interaction was also studied by conducting FTIR and DSC tests. The *in vitro* release kinetics studies reveal that all formulations fits well with Zero order, followed by Korsmeyer-Peppas, Higuchi and the mechanism of drug release is erosion. After analysis of different evaluation parameters and drug release kinetics, formulation code F22 was selected as a promising formulation for delivery of lafutidine as a mucoadhesive Gastroretentive tablet with best mucoadhesive strength and 99.54% drug release at 12th hour. Radiological evidences suggest that, a formulated tablet was well adhered for >10 h in human stomach. The bioavailability studies of F22 containing lafutidine was carried out which exhibited increased pharmacokinetic parameters of Cmax (268±1.26), Tmax (1.30±1.23 h) and AUC_{0-t} (1048±16.42) as compared to marketed formulations which indicates improved bioavailability of formulations.

Keywords: Lafutidine, Mucoadhesive, Radiographic studies, *In vivo* bioavailability studies.

Introduction

Oral administration is the most convenient, widely utilized, and preferred route of drug delivery for systemic action. However, when administered orally, many therapeutic agents are subjected to extensive presystemic elimination by gastrointestinal degradation and or first pass hepatic metabolism, as a result of which low systemic bioavailability and shorter duration of therapeutic activity. Much attention has been focused, recently on targeting a drug delivery system to a particular region of the body for extended period of drug release, not only for local targeting of drugs but also for the better control of systemic delivery [1]

Naturally occurring polymers, being biocompatible and biodegradable, are currently extensively researched for the development of novel drug delivery systems. There are number of drugs like domperidone, ranitidne, theophylline those have narrow absorption window from upper GIT i.e. stomach. Due to short gastric resident time less than 3 hr these drug reaches the non absorbing distal parts of intestine. Therefore main challenge is to prolong the resident time of drug in stomach. Gastro retentive drug delivery techniques are primarily controlled release drug delivery systems, which gets retained in the stomach for longer period of time, thus helping in absorption of drug for the intended duration of time. It helps to improves bioavailability, reduces drug wastage, improve solubility of drugs that are less soluble at high pH environment (e.g. weakly basic drugs like domperidone, papaverine) [2].

Lafutidine,(±)-2-(furfurylsulfinyl)-N-(4-[4-[piperidinomethyl]-2-pyridyl]oxy-(Z)-2-butenyl) acetamide is a newly developed 2 nd generation histamine H2-receptor antagonist. It is used in the treatment of gastric ulcers, duodenal ulcers, and gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis. It is absorbed in the stomach, reaches gastric cells via the systemic circulation, and rapidly binds to gastric cell histamine H2 receptors, resulting in immediate inhibition of gastric acid secretion. Lafutidine has been shown to increase the gastric mucosal blood flow and gastric mucus secretion also accelerates epithelial restitution in rats. Lafutidine has a receptor binding affinity, which is 2-80 times higher than famotidine, ranitidine and cimetidine [3].

Materials and Methods

Materials

The Lafutidine was obtained as a gift sample from splendid laboratories, Pune. Gum Kondagogu, Gum Olibanum and Guar Gum were obtained from Girijan Co-operative corp. Ltd,

Hyderabad. PVP-K30 was gifted from MSN Labs Ltd, Hyderabad. All other chemicals used were of analytical grade.

Preparation of Mucoadhesive Tablets

Wet Granulation Method

Mucoadhesive tablets of Lafutidine were prepared by wet granulation technique using different concentrations of Gum Kondagagu, Gum olibanum and Guar gum. All the ingredients were passed through sieve no 85# and were mixed uniformly. Granulation was carried out with sufficient quantity of binder solution (PVP K 30 - 5% in isopropyl alcohol). Wet mass was passed through sieve no 12# and dried at 45-55 ^{0}C for 1 hr. Dried granules were sized by sieve no.18#. Add magnesium stearate and talc. Granules obtained were compressed with 9 mm flat punch (Cadmach, Ahmedabad, India) [4].

The formulations are made by using design of experiment method (factorial designs)

Study type: Response surface
Design type: Central Composite
Design mode: Quadratic

Table No: 1 Design Summary Of Formulation By Natural Polymers

F.NO	LAFUTIDINE (mg)	GK (mg)	GO (mg)	GG (mg)	MCC (mg)	PVP K-30 (mg)	TALC (mg)	MAGNESIUM STEARATE (mg)	TOTAL WEIGHT (mg)
F1	10	10	10	10	140	12	4	4	200
F2	10	30	10	10	120	12	4	4	200
F3	10	10	30	10	130	12	4	4	200
F4	10	30	30	10	100	12	4	4	200
F5	10	10	20	10	130	12	4	4	200
F6	10	30	20	10	110	12	4	4	200
F7	10	20	10	10	130	12	4	4	200
F8	10	20	30	10	110	12	4	4	200
F9	10	20	20	10	120	12	4	4	200
F10	10	10	10	40	110	12	4	4	200
F11	10	30	10	40	90	12	4	4	200
F12	10	10	30	40	90	12	4	4	200
F13	10	30	30	40	70	12	4	4	200
F14	10	10	20	40	100	12	4	4	200
F15	10	30	20	40	80	12	4	4	200
F16	10	20	10	40	100	12	4	4	200
F17	10	20	30	40	80	12	4	4	200
F18	10	20	20	40	90	12	4	4	200
F19	10	10	10	60	90	12	4	4	200
F20	10	30	10	60	70	12	4	4	200
F21	10	10	30	60	70	12	4	4	200
F22	**10**	**30**	**30**	**60**	**50**	**12**	**4**	**4**	**200**
F23	10	10	20	60	80	12	4	4	200
F24	10	30	20	60	60	12	4	4	200
F25	10	20	10	60	80	12	4	4	200
F26	10	20	30	60	60	12	4	4	200
F27	10	20	20	60	70	12	4	4	200

GK: Gum Kondagogu **GO: Gum Olibanum** **GG: Guar Gum.**

Mcc: Micro Crystalline Cellulose **PVP K-30: Polyvinyl Pyrolidone K-30.**

In-vitro dissolution studies

The USP dissolution test apparatus (apparatus II paddle type) was used to study the drug release from the tablets. The dissolution medium was 900 ml of 0.1N HCl buffer pH 1.2. The release was

performed at 37 ± 0.5 C, with a rotation speed of 100 rpm. 5ml samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through whatmann filter paper and analyzed after appropriate dilution by UV spectrophotometer at 220 nm and drug release was determined from standard curve [5]

Dissolution Parameters

Dissolution medium: 900 ml of 0.1 N HCl buffer with pH 1.2
RPM: 100
Temp: 37 ± 0.5 c
Sample volume withdrawn: 5ml sample
λ *max* : 220 nm
Time interval: 0, 1, 2, 3, 4, 6, 8, 10 & 12h.

Drug Excipient Compatibility Studies

The drug excipient compatibility studies were carried out by Fourier transform infrared spectroscopy (FTIR), DSC & SEM.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminium pans at a rate of 10 C/ min between 25 and 350 C temperature rang under nitrogen atmosphere. Empty aluminium pan was used as a reference.[6]

SEM studies

The surface and shape characteristics of Tablets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized tablets were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The tablets were characterized for hardness, mucoadhesive strength and cumulative % drug released during the stability study period.[6]

In-vivo bioavailability studies

In vivo study protocol

Twelve healthy male subjects with a mean age of 28.83±3.60 years (ranging from 24 to 34 years), mean weight 69.33±7.61Kg (ranging from 61 to 79 Kg) and a mean height of 173.17 ± 10.46cm (ranging from 157 to 182cm) participated in this study. Informed and signed consent and approval of the Human Ethical Committee were obtained. The volunteers were judged healthy on the basis of their previous medical history, physical examination and routine laboratory tests. None of the subjects used alcohol or tobacco. All subjects were free from drugs 15 days before and during the study. They were randomly divided into 2 groups of 6 subjects each. The subjects were fasted over night at least 10h prior to dose. After collecting the zero hour blood sample (blank). A standardized high fat-breakfast approximately 900KCal was given in the morning half-an-hour before administration. Group A received Formulated lafutidine mucoadhesive tablets and group B received commercial formulation with 200ml of water. All the subjects were given a glass of water for every 2h (approximately 200ml). Standardized lunch, snacks and dinner was provided to all the subjects respectively at 4, 8 and 12h after the administration of formulations. Blood samples (2ml) were collected by the intravenous route using heparinized disposable syringes at the following times: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 20 and 24h. The blood samples were collected in vacutainers containing EDTA as anticoagulant and immediately centrifuged at 3000rpm for 15min. The separated plasma samples were stored at -200 C until analyzed [7] .

Determination of lafutidine in Human plasma by HPLC method

Determination of lafutidine using internal standard Domperidone by high performance liquid chromatography with a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm 4.6 mm i.d) as stationary phase and the mobile phase consist of 0.02M Potassium dihydrogen phosphate: Acetonitrile: Methanol in a ratio of 50:35:15v/v/v at the flow rate of 1ml/min. and the wavelength detection was done at 285nm. The retention time for Lafutidine and Domperidone were found to be 4.3 and 5.6 min, respectively [7].

Preparation of Plasma Samples for HPLC Analysis

Human plasma (0.5ml) was prepared for chromatography by precipitating proteins with 2.5ml of ice-cold absolute ethanol for each 0.5ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol

and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200µ1 of 50% of acetonitrile and 50% 0.1% ortho phosphoric acid was injected for HPLC analysis.

Pharmacokinetic Analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity. The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in ng/ml at the last time point and K is the elimination rate constant. Various pharmacokinetic parameters like area under the curve [AUC], elimination half life ($t_{½}$). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic program. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant [8].

In-Vivo radiographic studies

The bio-study protocol for radiographic studies was approved by Institutional Human Ethics Committee, No: IHEC/VGOPC/053/2015. From the formulations 25mg and 40mg of the drug was changed with barium sulfate to make them x-ray opaque. The subjects were given these tablets with breakfast. The volunteers were given 200 mL of water at zero time, to ensure the absence of radio-opaque material in the stomach. X-ray images were taken using (Genesis 50, Josef Bets chart AG, Brunnen, Switzerland) in standing position after 0.5, 2, 4 and 10 hrs post-administration of tablets. From the X-ray films gastric residence and position was interpreted.

Results & Discussion

Physico-chemical parameters of lafutidine mucoadhesive tablets

The prepared tablets were evaluated for different physico-chemical properties and the results are found to be within the pharmacopoeial limits.

Kinetic modeling of drug release

To explore the mechanism of drug release from Mucoadhesive tablets, various kinetic models like zero order, first order, Higuchi and Korsmeyer-Peppas equations were applied to the different formulations. The release kinetics of best formulation (F22) was shown in Table 4. From the data it was concluded that the

Table: 2 Release kinetics of optimized formulation of Lafutidine mucoadhesive tablets:

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
F22	0.994	8.020	0.842	0.119	0.946	29.51	0.628	2.155

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.994 indicates that the drug release follows a zero order mechanism Table No:2. This data indicates a lesser amount of linearity when plotted by the first order equation. Hence it can be concluded that the major mechanism of drug release follows zero order kinetics.

Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer-Peppas plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value close to one i.e. 0.946 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer-Peppas plots i.e. 0.628 suggest that the drug release from tablets was anomalous Non fickian diffusion.

Drug excipient compatibility studies

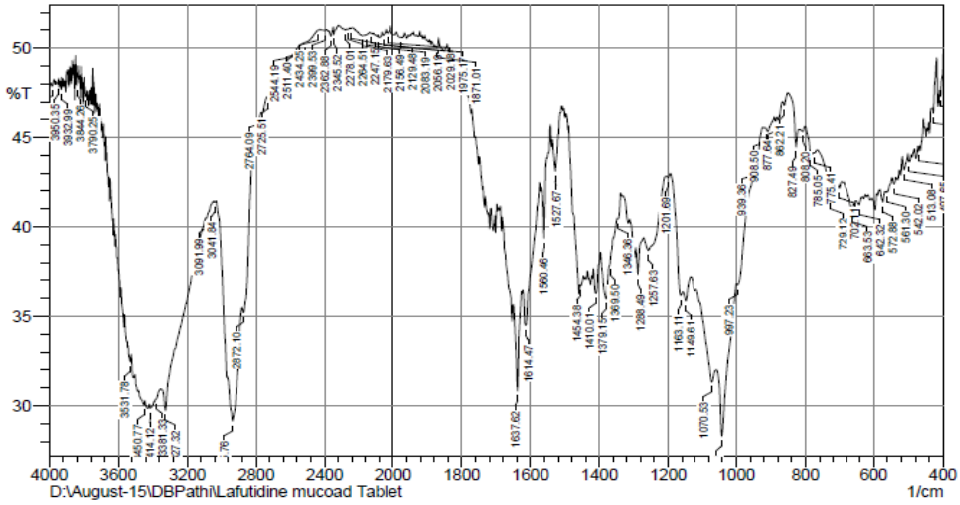

Figure: 2 FT-IR spectrum of pure drug Lafutidine

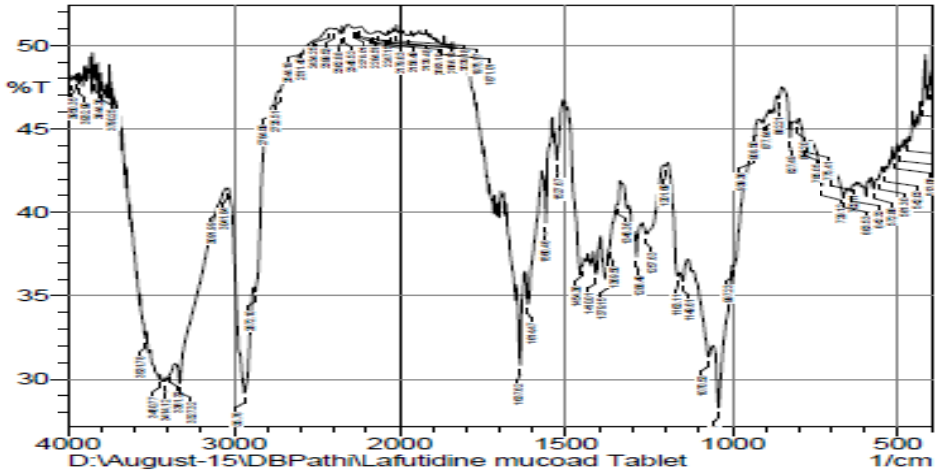

Figure: 3 FT-IR spectrum of optimized formulation F22

Possible interactions between drug and polymer in formulations were investigated by FTIR. FTIR spectra of LAFT and optimized formulation were examined. FTIR spectrums are properly labelled and shown in (Fig.). FTIR of pure LAFT characteristic sharp peaks of alkene stretching (═C–H and CH2) vibration at 3324.32–3016.48 cm 1 and alkane stretching (–CH3, –CH2 and –CH) vibration at 2853.73 cm 1. Also exhibited C ═O stretch at 1738.2 cm 1 due to saturated ketone and C ═O–NH stretching at 1635.90 cm 1. A selective stretching vibration at 1561.57 cm 1 and 1525.80 cm 1 for primary and secondary amine was also observed. For functional groups like S ═O stretch and –C–S stretch showed vibrations at 1041.78 cm 1 and 729.57 cm 1respectively.

Overall there was no alteration in peaks of Lafutidine pure drug and optimized formulation, suggesting that there was no interaction between drug & excipients. There is additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug , indicating absence of any interaction.

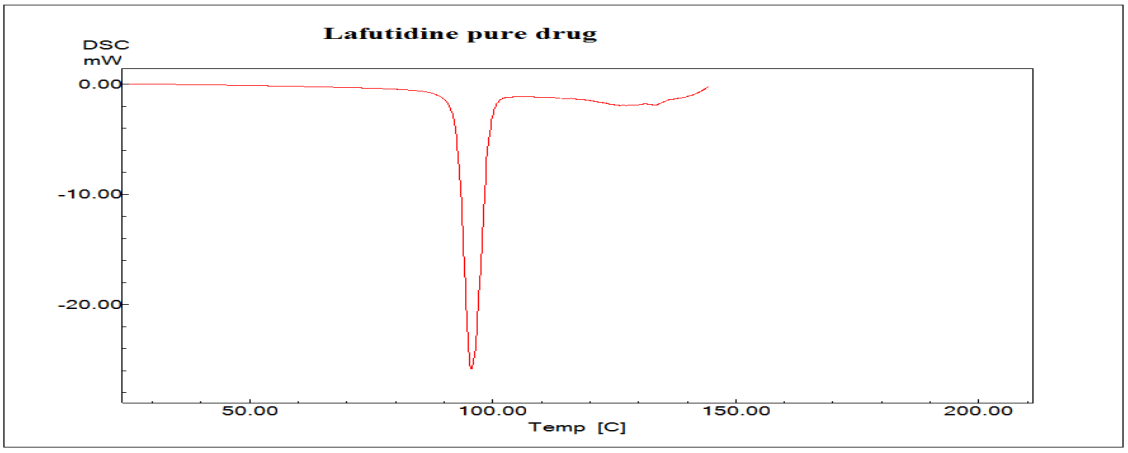

Figure: 4 DSC thermogram of lafutidine

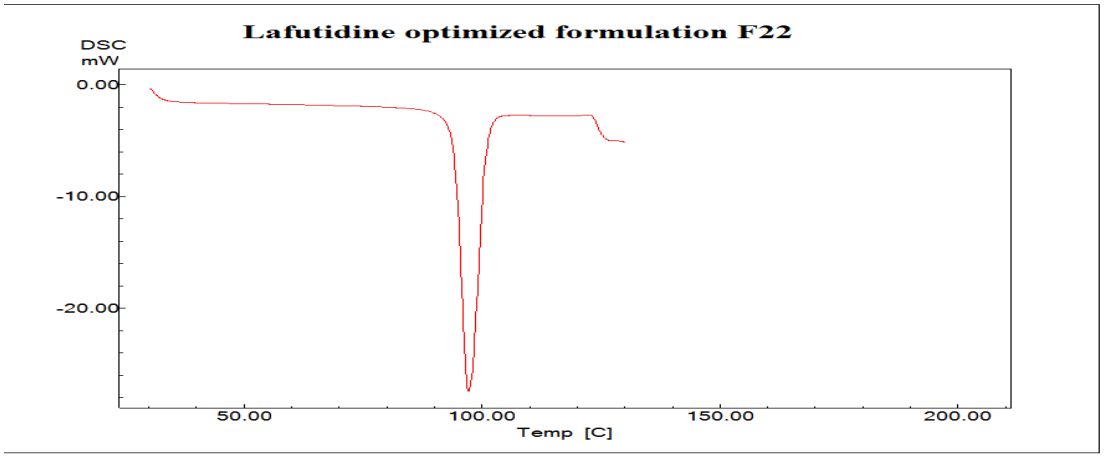

Figure: 5 DSC Thermogram of optimized tablets F22

DSC was used to detect interaction between Lafutidine and excipients. The thermogram of Lafutidine exhibited a sharp endotherm melting point at 96 ^{0}C. The thermogram of microsphere loaded with Lafutidine exhibited a sharp endotherm melting point at 99 ^{0}C DSC results of formulated Lafutidine is slightly higher that is 96 ^{0}C .The DSC thermogram of microsphere loaded with Lafutidine retained properties of Lafutidine, as well as polymer properties. There is no considerable change observed in melting endotherm of drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.

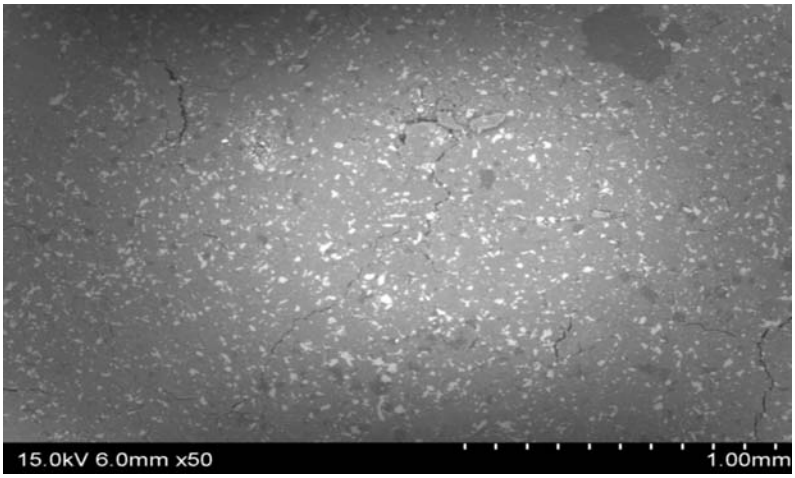

Figure: 6 Scanning Electron Microscopy of lafutidine mucoadhesive

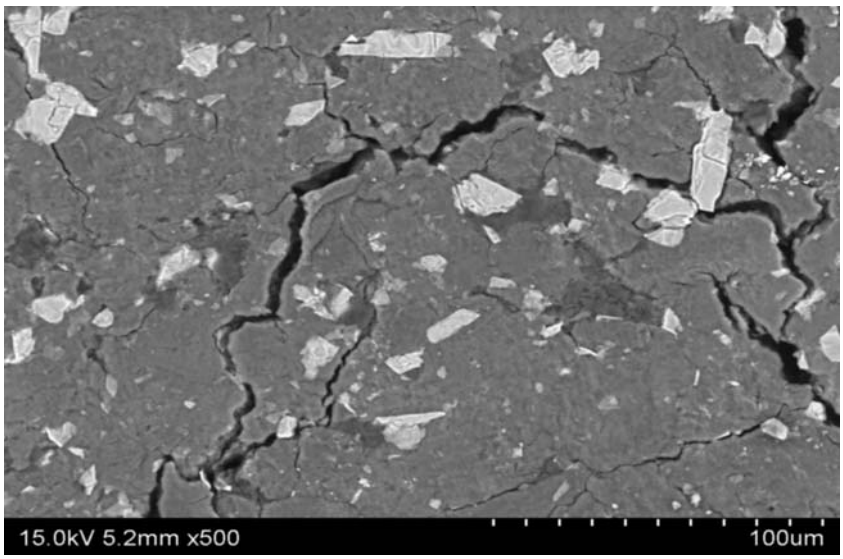

Figure: 7 Scanning Electron Microscopy of lafutidine mucoadhesive

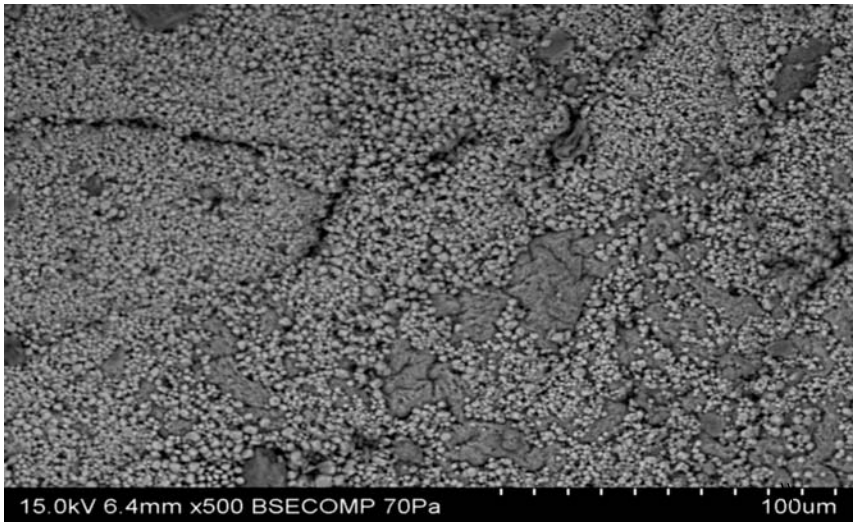

Figure: 8 Scanning Electron Microscopy of lafutidine mucoadhesive tablets

Radiographic studies

Intragastric behavior of lafutidine mucoadhesive tablets

The radiographic images were taken at different periods post administration of the barium sulfate-loaded tablet in three human volunteers. It is clear that the tablet appears more or less at the same position for the initial 4 h. This could be related to its floating ability. Later on, the tablet was slightly moved downwards, yet, remained within the stomach till the end of 10 h. The increased gastric residence time favours increase in the bioavailability of drugs.

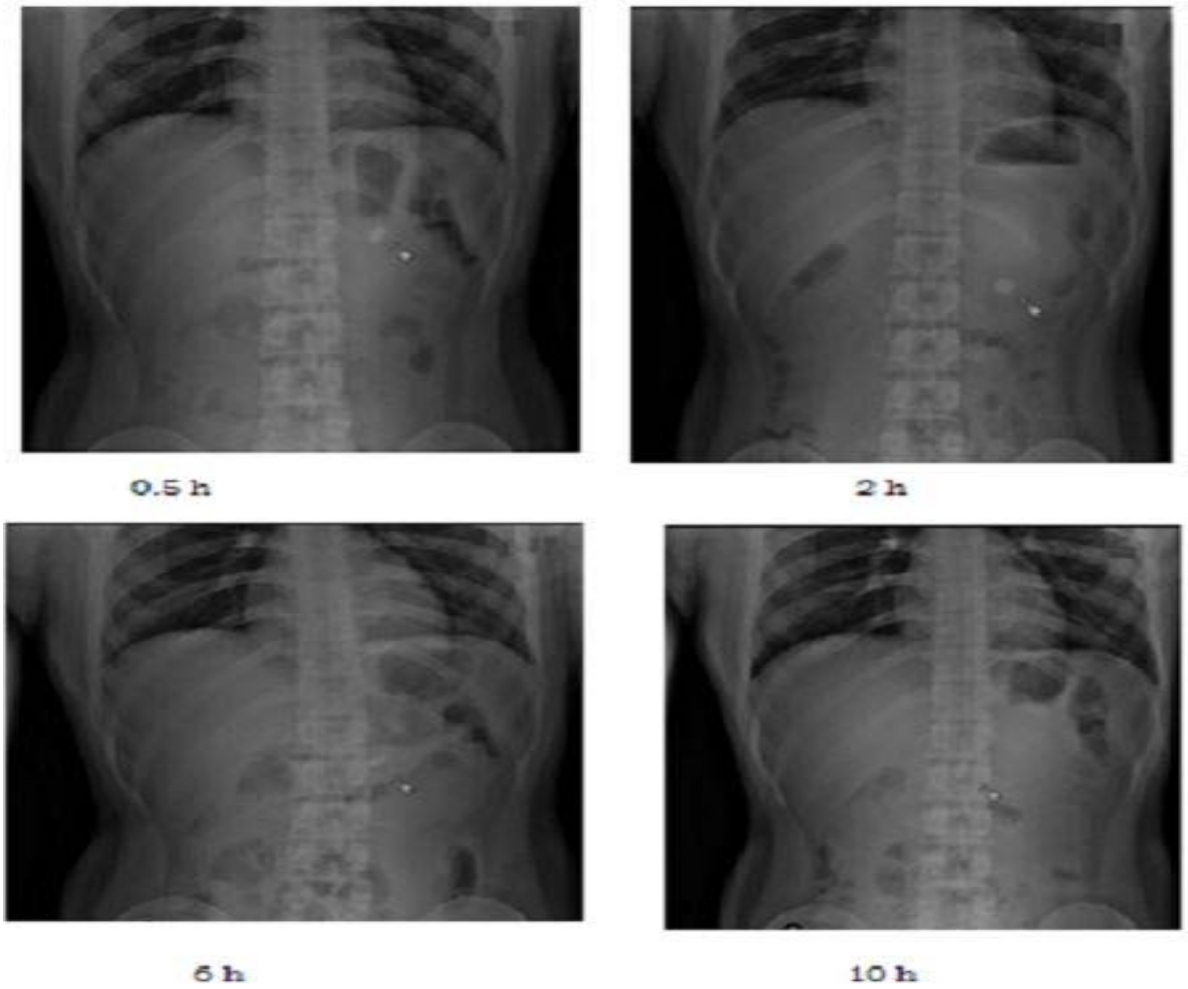

Figure: 9 Radiographic Images of a $BaSo_4$ loaded Lafutidine mucoadhesive tablet (F 22) in the stomach

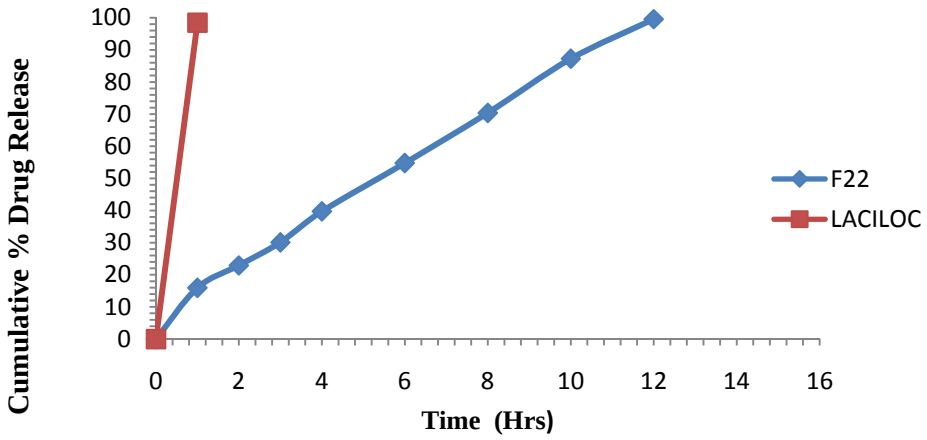

Figure: 10 Percentage drug release of Lafutidine formulations F22 & Innovator

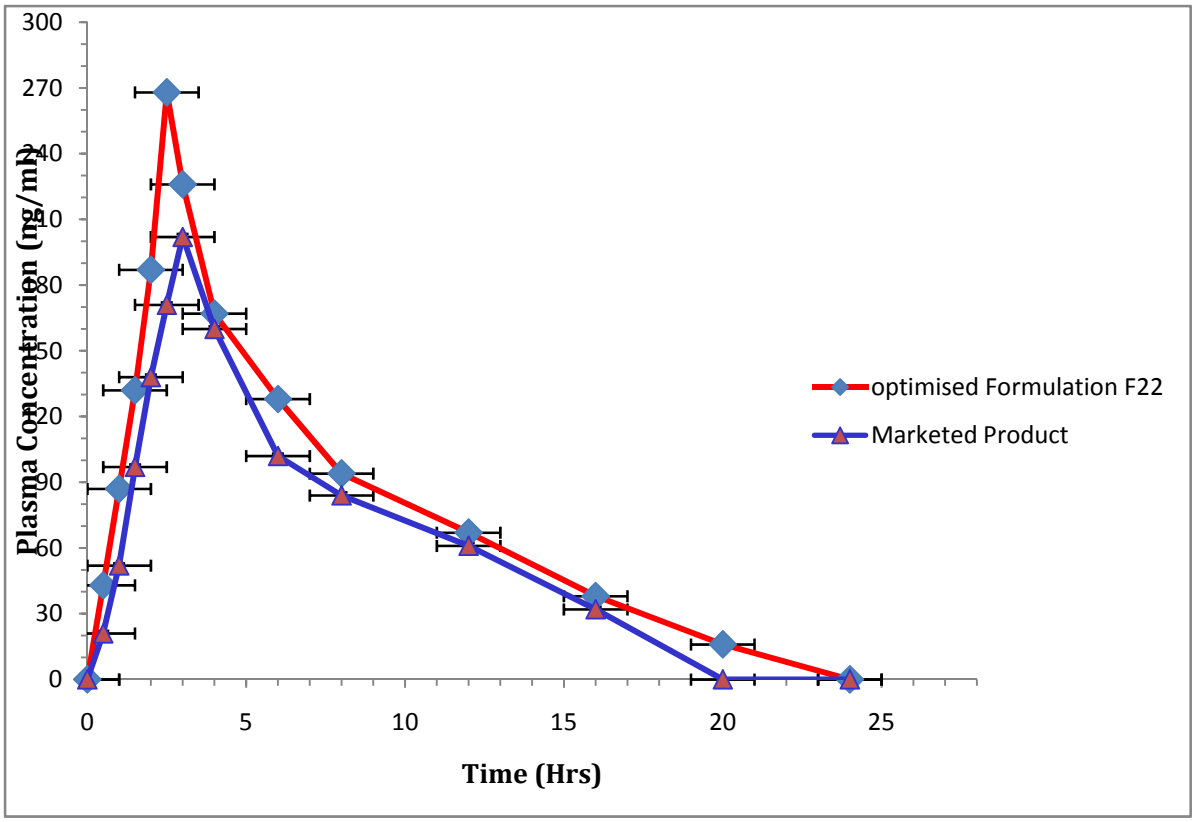

Figure: 12 Plasma concentrations at different time intervals for lafutidine optimized formulation and Marketed Product

Table: 4 Comparison of pharmacokinetic parameters of lafutidine optimized formulation and Marketed Product

Parameters	Lafutidine Optimized formulation (F22)	Marketed Product
C_{max}(ng/ml)	268±31.26	206±29.52
AUC_{0-t}(ng. h/ml)	1048±16.42	862±24.26
AUC_{0-} (ng. h/ml)	1225±38.54	1004±35.14
T_{max} (h)	2.30±1.23	3.78±0.29
$t_{1/2}$ (h)	2.21 ± 0.91	2.96 ± 0.88
K_{el} (h^{-1})	2.807 ± 0.11	2.189 ± 0.33

Bioavailability parameters

Mean plasma concentration profiles of prepared lafutidine optimized formulation and marketed product are presented in Figure.10 Lafutidine optimized formulation exhibited as sustained release *in vivo* when compared with marketed tablet. All the pharmacokinetics parameters displayed in Table.4 in this study in human subjects, prolonged drug absorption was achieved with the test formulation. The average peak concentration of the test formulation was significantly higher than that of the reference

(268±31.26 ng/ml for the test formulation versus 206±29.52 ng/ml for the reference). In order to estimate the amount of drug absorbed from the test formulation, the relative bioavailability was calculated from the AUC of the reference and test formulations (1004±35.14 ng.h/ml for the reference product versus 1225±38.54 ng.h/ml for the test formulation). The results indicated that the test formulation could increase the bioavailability of Lafutidine in humans effectively. In this study, the Lafutidine mucoadhesive tablet produce higher bioavailability than that of a marketed product, this overall increase in bioavailability and increased gastric residence time due to mucoadhesion of tablet in the stomach region for 10 h [9].

Conclusion

Lafutidine mucoadhesive oral tablets could be formulated using the drug, Gum Kondagogu, Gum Olibanum and Guar Gum with different proportions using 3^3 full factorial designs. It can be seen that there is a synergistic effect when polymers are used in combinations. The *in vitro* release kinetics studies reveal that all formulations fits well with Zero order, followed by Korsmeyer-Peppas, Higuchi and the mechanism of drug release is erosion.

From the formulations F1-F27 the formulation F 22 was selected as optimized formulation because it showed maximum release and the other properties such as swelling index was also low, mucoadhesion force shown good and the Post and pre compression parameters were found to be within the Pharmacopeial limits.

Radiological evidences suggest that, a formulated tablet was well adhered for >10 h in human stomach. The bioavailability studies of F 22 containing lafutidine was carried out which exhibited increased pharmacokinetic parameters of Cmax, Tmax and AUC as compared to marketed formulations which indicates improved bioavailability of formulations.

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