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Development and statistical optimization of mucoadhesive drug delivery system of famotidine using *hibiscus esculentus* polysaccharide.

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Abstract

The present study was aimed to formulate and evaluate oral mucoadhesive drug delivery system of purified *Hibiscus esculentus* L polysaccharide (HEP) using famotidine as model drug.

A central composite design for 2 factors at 3 levels each was employed to evaluate the effect of critical variables i.e. concentration of HEP and PVP K30 on drug release and mucoadhesive properties of the formulated tablets. FT-IR spectroscopy and Differential Scanning Calorimetry was carried out to evaluate drug polymer interaction. Formulated tablets were evaluated for physical properties, drug release characteristic and physical stability. *Ex-vivo* mucoadhesion study using goat gastric mucosa was carried out to ascertain the mucoadhesion potential of formulated tablets.

The response surface analysis clearly indicated the dominating effect of HEP on mucoadhesive strength, mucoadhesion time and dissolution half life, while PVP K30 has an additive effect on all afore mentioned responses. The drug release from the matrix tablets was highly affected by the concentration of release retardants polysaccharide. The kinetics of drug release was found to be first order in low concentration but with increase in polymer concentration the release pattern shifted towards zero order and is governed by both Higuchi and Hixson-Crowel equation indicating a coupling effect of diffusion and erosion.

The result of the study suggests that, HEP can be optimistically explored as excellent mucoadhesive agent with controlled release characteristics.

Keywords: *Hibiscus esculentus* polysaccharide, Mucoadhesive drug delivery system, Mucoadhesive strength, central composite design (CCD), Texture analysis.

Introduction

The real challenge in the development of an oral controlled-release drug delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form within the gastrointestinal tract (GIT) until all the drug is completely released at the desired period of time. In recent years gastro-retentive drug delivery systems has received enormous attention by the formulators especially for the drugs with limited absorption window. Several gastro-retentive drug delivery approaches being designed and developed, including high density or sinking systems that is retained in the bottom of the stomach [1], low density or floating systems that causes buoyancy in gastric fluid [2], mucoadhesive systems that causes bioadhesion to stomach mucosa [3], unfoldable, extendible, or swellable systems which limits emptying of the dosage forms through the pyloric sphincter of stomach [4], super-porous hydrogel systems [5], magnetic systems [6] etc. The advantages associated with the mucoadhesive drug delivery systems include increased dosage form residence time, improved drug bioavailability, reduced administration frequency and simplified administration of a dosage formand termination of a therapy as well as the possibility of targeting particular body sites and tissues [7]. Excellent mucoadhesive performance is typically observed for polymers possessing charged groups or non-ionic functional groups capable of forming hydrogen bonds with mucosal surfaces [7].

Optimization with factorial designs is a powerful, efficient and systemic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development work [8, 9]. The response surface method has been applied to dosage form design for various kinds of drugs by many researchers [10]. In the development of an extended release dosage form an important issue is to design an optimized formulation with minimum number of trials in short time. For this a computerized optimization technique, based on response surface methodology (RSM) utilizing a polynomial equation has been widely used. RSM can be defines as a statistical method that uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations [11]. Many statistical experimental designs have been recognized as useful techniques to optimize process variables. RSM is widely used when only a few

significant factors are involved in optimization. Various types of RSM designs include 3^2 full factorial designs [12, 13], Central Composite Design [14] and Box-Behnken design [15].

Famotidine is histamine H2 receptor antagonist. It is widely prescribed in active duodenal ulcers, gastric ulcers, gastro esophageal reflux disease and erosive esophagitis [16]. Famotidine is having ashort biological half-life of 2.5 - 3.5 hrs and 40 –45% oral bioavailability [17]. Since the drug is sparingly soluble in water (0.1%at 20ºC), has a short elimination half-life and narrow absorption window in proximal GI tract, several attempts have been made to improve its physiological availability [18].

Material and Methods

Materials

Famotidine was obtained as a gift sample from Torrent Pharmaceutical Limited., Indrad, India. *Hibiscus esculentus* fruits were purchased from local market of Guwahati, Assam, India and the polysaccharide was extracted and purified in laboratory as per the method describe by Dash S. et al [19]. Polyvinyl Pyrrolidine K-30 (PVP K-30) was obtained from S.D Fine Chemicals Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

Drug-polysaccharide compatibility by FTIR and DSC study

The study of drug–excipient compatibility is an important process in the development of a stable solid dosage form [20]. Incompatibility between drugs and polymers can alter the stability and bioavailability of drugs, thereby affecting its safety and/or efficacy.

FTIR spectra of famotidine, HEP and 1:1 w/w physical mixture of famotidine-HEP, stored 24hours in a glass dessicator, were recorded after on a FTIR spectrophotometer (Bruker Alpha-E, Bruker®, Germany) in the range of 400–4000 cm^{-1} using an ATR attachment equipped with zinc selenium optical assembly. The spectrum was a mean of sixteen consecutive scans of the same sample. Processing of the FTIR data was performed using OPUS software.

A differential scanning calorimeter (DSC-60, Shimadzu, Japan) was used for analysis of thermal stress on HEP famotidine and their mixture. Individual samples (famotidine and HEP) as well as 1:1 w/w physical mixtures of drug and excipients were weighed to about 5 mg in the DSC aluminum pan and scanned in the temperature range of 25–300ºC in nitrogen environment. A heating rate of 20ºC per minute was used, and the thermograms were reviewed for evidence of any interaction.

Experimental Design

A 3^2 full factorial design was constructed where the amounts of HE polysaccharide (X_1) and PVP K30 (X_2) were selected as two independent variables. It is suitable for investigating the quadratic response and for constructing a second-order polynomial model, thus enabling optimization. The levels of two factors were selected on the basis of literatures available and preliminary investigations carried out before implementing the experimental design. Optimization of formulation of mucoadhesive matrix tablet was done by Design Expert® Software (Version 8.2.0, Stat-Ease Inc.) All the formulations were prepared and evaluated for various pre-compression & post-compression parameters and effect of the polymers was studied on the in-vitro performances. The data obtained was interpreted in the software and polynomial equation was obtained. The responses (dependent variables) studied for this investigation was mucoadhesion time (Y_1), dissolution half-life (Y_2) and release exponent (Y_3). The polynomial equations required for the purpose of ANOVA are obtained from the Factorial designs. The equation is shown as below. Table 1 summarizes the levels of independent variables.

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \text{..... Eq. 1}$$

Table – 1: Coded variables with respective levels.

Factors (Independent variable)	Actual values (% $^w/_w$)			Response (independent variable)
	□ 1	0	+ 1	
X1	20	30	40	Y1 = Mucoadhesion force Y2 = Dissolution half-life Y3 = Release exponent
X2	5	7.5	10	

Table – 2: Composition of formulations for various batches.

Ingredients	Weight of Ingredients mg/tablet								
	MT 1	MT 2	MT 3	MT 4	MT 5	MT 6	MT 7	MT 8	MT 9
Famotidine	40	40	40	40	40	40	40	40	40
Hibiscus esculentus polysaccharide	32	32	32	48	48	48	64	64	64
Polyvinyl pyrollidone (PVP K30)	8	12	16	8	12	16	8	12	16
Microcrystalline cellulose	75.2	71.2	67.2	59.2	55.2	51.2	43.2	39.2	35.2
Magnesium stearate	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Talc	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Total Tablet weight	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**

Preparation of Mucoadhesive matrix tablets of Famotidine

Table – 2 enlist the composition of different trial formulations prepared using *Hibiscus esculentus* polysaccharide (HEP) as mucoadhesive material and polyvinyl pyrrolidone (PVP) K30 as binder. Amount of talc and magnesium stearate was kept unchanged for all the formulations. Microcrystalline cellulose (MCC) was used as diluent. Powdered HEP and MCC was mixed with famotidine in a V-blender for 10 mins. The powder blend was granulated with PVP K30 solution and screened through a sieve # 12, wet granules were dried at 50ºC for 6 hours. Dried granules were screened through sieve # 16 and magnesium stearate and talc was mixed for 5 minutes in a double-cone blender (VJ Instruments Pvt. Ltd. India) for 5mins at 20 rpm. Tablets were compressed on a 8-station Mini Press-I rotary tablet compression machine (Shakti Pharmatech. India) fitted with 8-mm flat-faced round punches using sufficient compression force to obtain a hardness of 4 to 5 Kg/cm containing 40 mg of famotidine per tablet.

Tablet thickness

A vernier caliper was used to determine thickness of 10 randomly selected tablets. Results are expressed as mean values ± SD.

Hardness and tensile strength

Five tablets were randomly selected from each formulation and crushing strength of each tablet was measured using Monsanto hardness tester. The mean hardness of five tablets was determined and expressed in Kg/cm^2.

Friability

The friability test was carried out in Roche Friabilator [20]. 20 tablets were randomly selected from each batch and initial weight (W_o) was determined after dedusting and placed in the rotating drum of friabilator. They were subjected to 100 falls of 6 inches height (25rpm for 4min). After completion of 100 rotations, the tablets were removed, dedusted by using soft-bristle brush and weighed (W_1) accurately. The test was repeated for three times. The percent loss in weight (or friability) was calculated by the formula given below:

$$\% \text{ Friability} = \left(1 - \frac{W_1}{W_0}\right) 100 \quad \text{..... Eq. 2}$$

Drug content uniformity

Twenty tablets were weighed and powdered. An amount of the powder equivalent to 20mg of famotidine was dissolved in 100ml of 0.1N hydrochloric acid, followed by stirring for 30 minutes. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan) at 265nm using 0.1 N hydrochloric acid as blank.

Swelling Index

The swelling of tablet involves the absorption of a liquid resulting in an increase in weight and volume. Liquid uptake by the polymers results to saturation of capillary spaces within the polymer chain or hydration of macromolecule [23]. To determine the extent of matrix swelling, three tablets from each batch were weighed and placed in a petri-dish containing 25 ml of 0.1N hydrochloric acid. After each 2 hrs interval the tablets were removed from media, excess of media was wiped off by using filter paper and weighed again up to 12 hrs [24]. The swelling index was calculated using following formula.

Swelling Index (S.I.)= $\frac{W_t - W_0}{W_0}$ 100 ..... Eq. 3

In vitro drug release study

In vitro release of famotidine from the prepared mucoadhesive tablets was studied using USP XXIV dissolution rate test apparatus (DS-8000, Labindia Analytical Instruments Pvt. Ltd., Mumbai, India) employing the paddle (Apparatus-II). 900mL of 0.1N hydrochloric acid was used as dissolution medium maintained at a temperature of 37±0.5ºC and the paddle was rotated at 50 rpm. 5mL of samples were withdrawn with a syringe fitted with a pre-filter at predetermined time intervals and immediately replaced with 5mL of fresh medium maintained at 37±0.5ºC. The samples were suitably diluted and the absorbance was measured at 265nm using UV-Visible spectrophotometer (UV-1800Shimadzu, Kyoto, Japan). The in vitro release study was performed in triplicate for each formulation. Cumulative percentage drug release was calculated using the equation obtained from the standard curve.

Drug release kinetic study

Mathematical modeling of drug delivery and predictability of drug release mechanism is a field of steadily increasing industrial importance. The accuracy of a mathematical theory generally increases with increasing model complexity. There is no general mathematical theory that can be applied to all types of drug delivery systems. To investigate the kinetics of drug release from formulated famotidine mucoadhesive tablets, the data of in-vitro drug release study were fitted to different mathematical models. The order of drug release from matrix systems was described by using zero order [25] or first order kinetics [26, 27]. The mechanism of drug release from matrix systems was studied by using Higuchi diffusion model [28] and Hixson-Crowell erosion model [29]. Korsemeyer-Peppas support the drug release mechanism for further judgment [30, 31]. The respective equations for these models are shown below:

Zero Order Model : Q_0 $Q_t = K_0 t$ ..... Eq. 4

First Order Model : $\log C = \frac{\log C_0 - Kt}{2.303}$ ..... Eq. 5

Higuchi Model : $f_t = Q = A\sqrt{D(2C-C_S)C_S t}$ ..... Eq. 6

Hixson-Crowell Model : $W_0^{1/3} - W_t^{1/3} = \kappa t$ ..... Eq. 7

Korsemeyer – Peppas Model : $M_t / M = Kt^n$ ..... Eq. 8

Mucoadhesion study

In vitro/ex vivo tests are important in the development of controlled release mucoadhesive drug delivery systems because these tests contribute to studies of mechanical and physical stability, superficial interaction between formulation and mucous membrane, duration of adhesion and strength of the bioadhesive bond.

Determination of *ex-vivo* mucoadhesion strength

Mucoadhesion testing of the sample tablets was carried out using a texture analyzer (TAXT plus, Stable Micro Systems, UK) with 50N load cell equipped with mucoadhesive holder [32]. A tablet was attached to the cylindrical probe (10mm in diameter) by double sided adhesive tape. Goat gastric mucosa was utilized as the model membrane for mucoadhesive strength determination of various formulations. The tissue (about 20X20mm) was equilibrated for 15min at 37.0±0.5ºC before placing onto the holder stage of mucoadhesive holder. The probe with the tablet attached was lowered at a rate of 0.5mm/sec. until a contact with the membrane was made. A contact force of 1N was maintained for 60 second, and the probe was subsequently withdrawn at a 0.5mm/sec. to the distance of 15mm. By using the texture analyzer, the maximum force required to separate the tablet from the tissue (i.e. maximum detachment force; F_{max}) was measured using Texture Exponent 32 software.

Determination of *ex-vivo* mucoadhesion time

The *ex-vivo* mucoadhesive time was determined using a modified USP disintegration test apparatus. The goat gastric mucosa was collected from local slaughter house and used within 2 hours of sacrificing the animal. The mucous membrane was separated by removing the underlining fatty layer and loose tissues. 900ml 0.1N hydrochloric acid was used as disintegration medium and maintained at 37±2 C throughout the experiment [33]. The segment of goat gastric mucosa (3 3cm) was glued with cyanoacrylate glue to the surface of glass slab, which was then vertically attached to the apparatus. Three tablets of each formulation were hydrated on one surface with 0.1N hydrochloric acid and the hydrated surface was brought into contact with the mucosal membrane and allowed the apparatus to move up and down. The time required for complete detachment of the tablets from surface was recorded. The results were analyzed for mean and standard deviation.

Physical stability study

Short-term physical stability studies were carried out according to the International Conference on Harmonization (ICH) guidelines [34]. The optimized formulation of Famotidine mucoadhesive tablets (M_{Opt}) were enclosed in a polyethylene bottle using a screw cap and placed in a stability test chamber (Remi Environment Test Chamber, Remi Laboratory Instruments, India). The chamber environmental condition was set at 40ºC temperature and75% relative humidity and maintained for three months. At specified time intervals, the tablets were examined for any statistical difference in their hardness values, matrix integrity, *in*-vitro dissolution profile mucoadhesion strength and mucoadhesion time using a paired Student's t-test [35]. Differences were considered to be significant at $p < 0.05$.

Statistical Analysis

All experiments were repeated at least three times. Results are expressed as means μ standard deviation (SD). Statistical analysis was carried out employing one-way ANOVA followed by studentized range test using the Design Expert® Software (Version 8.2.0, Stat-Ease Inc.). A p-value less than 0.05 were considered statistically significant.

Result and discussion

Drug-excipient compatibility study

To assess the compatibility between HEP and famotidine, the spectra of their physical mixture was compared with spectrum of individual components (Figure – 1). The FTIR spectra of HEP shows peaks at 3233.69 cm^{-1} (—OH), 719.23 cm^{-1} (—C O), 1612.69 cm^{-1} (—COO—), 1411.18 cm^{-1} (—COO—), 1243.84 cm^{-1} (—C—O). The peaks at 2924.16 cm^{-1} is characteristic of methyl C-H stretching associated with aromatic rings. Peak at 3233.69 cm^{-1} is due to hydrogen bonded hydroxyl groups that contribute to the complex vibrational stretches. The FTIR spectra of famotidine shows mejor peaks at 3505.56 (N—H), 3103.39 (C—H), 1373.17 (—SO_2), 1181.28 (—SO_2), 904.37 (S—N). The FTIR spectrum of physical mixture shows all characteristic peaks with minor shift indicating absence of interactions. This spectrum showed, alcoholic –OH stretch at 3500.93 cm^{-1}, –NH2 & -NH stretch at 3394 & 3256.63 cm^{-1} respectively, C=N stretch at 1597.82 cm^{-1}, C-S stretch at 716.18 cm^{-1}, $S(=O)_2$ asymmetric and symmetric stretching at 1326.10 cm^{-1} and 1140.21 cm^{-1} respectively. All the peaks of HEP and famotidine remains unchanged in the physical mixture.

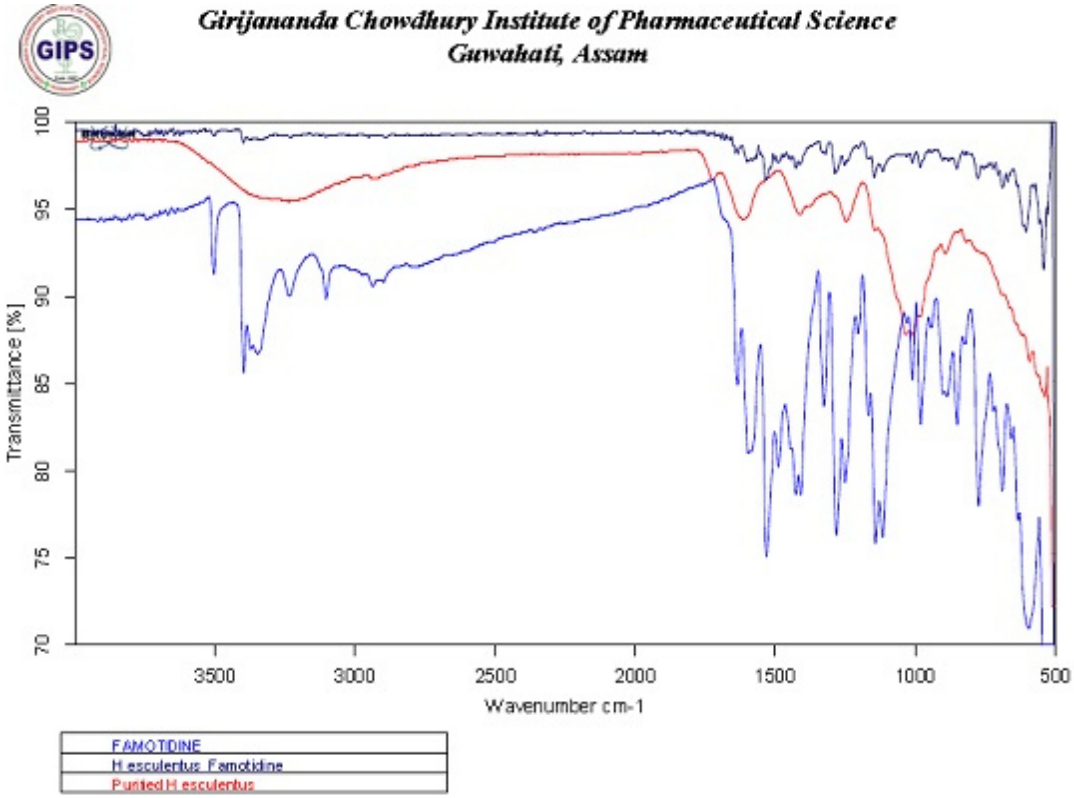

Figure – 1: FTIR spectra of famotidine, HEP and their physical mixture.

DSC thermograms of famotidine, HEP and their physical mixture is shown in figure – 2. DSC thermogram of famotidine shows a sharp endothermic peak at 165.96 ºC which is characteristic melting peak of pure famotidine. DSC thermogram of HEP shows its melting endothermic peak at 146.04ºC. Thermogram of mixture of famotidine with HEP showed a wider melting endotherm at 158.28ºC due to melting of famotidine and polymer in mixture. The onset of the endotherm is 142.17ºC and ends at 169.04ºC which indicating the overlapping of both melting peaks of famotidine and HEP. There was neither any other endothermic peak nor any sharp exothermic peaks within the scanning range indicating there is not significant chemical and physical interaction between famotidine and HEP.

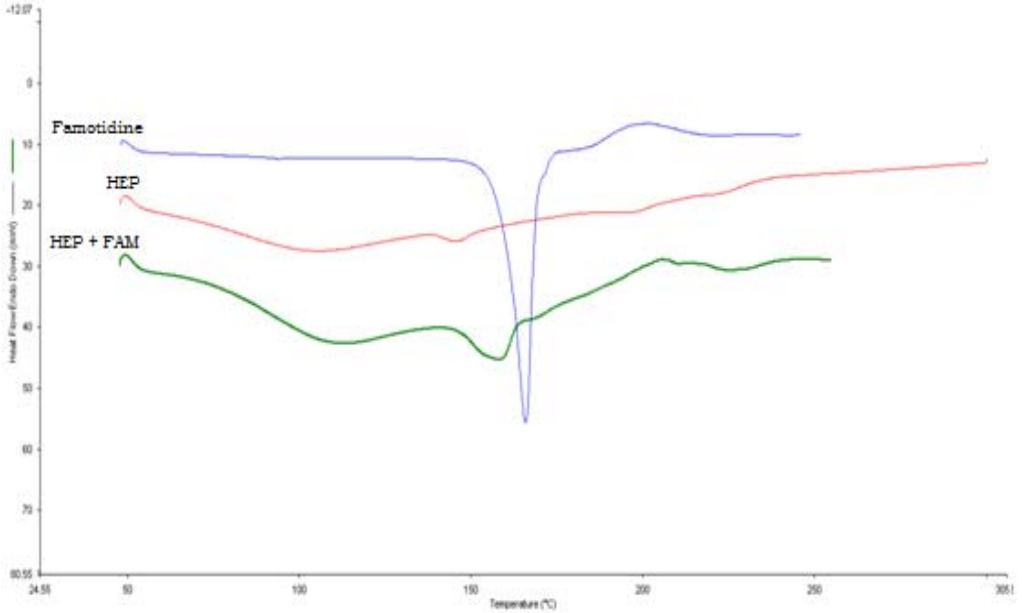

Figure – 2: DSC thermograms of Famotidine, HEP and their physical mixture.

Post-compression tablet evaluation studies

The assessment results of thickness, hardness, friability and drug content are presented in Table 3. The tablet thickness of the various formulations was found to be in the range of 2.61 ± 0.042 to 2.74 ± 0.075. The hardness of all tablet were in the range of 5.1 ± 0.6 to 5.6 ± 0.6 kg/cm^2. Hardness increased as the amount of concentration of HEP increased. This indicates the binding potentiality of the polysaccharide. Since tablet hardness is not a perfect index to evaluate the strength of the tablets, friability percentage was also used to test the hardness of tablets. The friability values of all the prepared tablets were less than 1% which indicated that the test was compiled with the official compendial tests for tablets as per IP.

Table – 3: Post-compression characteristics of the famotidine mucoadhesive tablets.

Parameters	Formulations Batches								
	MT 1	MT 2	MT 3	MT 4	MT 5	MT 6	MT 7	MT 8	MT 9
Thickness (mm.) [a]	2.62 ± 0.065	2.61 ± 0.042	2.62 ± 0.039	2.68 ± 0.071	2.70 ± 0.041	2.66 ± 0.081	2.72 ± 0.027	2.74 ± 0.075	2.73 ± 0.031
Hardness (Kg/cm^2) [a]	5.1 ± 0.6	5.3 ± 0.8	5.4 ± 0.3	5.4 ± 0.6	5.4 ± 0.3	5.2 ± 0.4	5.5 ± 0.5	5.5 ± 0.4	5.6 ± 0.6
Friability (% w/w) [b]	0.64 ± 0.020	0.66 ± 0.016	0.64 ± 0.021	0.43 ± 0.019	0.42 ± 0.011	0.40 ± 0.015	0.34 ± 0.014	0.32 ± 0.017	0.32 ± 0.010
Uniformity of Content (% w/w) [b]	98.23 ± 0.18	98.71 ± 0.29	97.08 ± 0.09	98.62 ± 0.07	97.66 ± 0.15	98.25 ± 0.08	99.04 ± 0.23	97.37 ± 0.07	98.02 ± 0.18
Uniformity of Weight (mg) [b]	161.8 ± 3.27	159.7 ± 2.46	162.0 ± 3.05	161.4 ± 2.11	160.9 ± 1.28	158.8 ± 2.51	159.3 ± 2.33	161.3 ± 1.95	161.5 ± 2.83

a: mean ± SD, n= 6 ; b: mean ± SD, n=20

In-vitro drug release study

The *in vitro* drug release data of HEP matrix tablets containing famotidine is presented in Table – 4 and the drug release profiles is shown in Figure 3. Clearly identifiable differences were observed in the release behaviour of all famotidine formulations. In the formulations, release of famotidine in the first hour varies between 49.69 in MT1 and 27.31 in MT9 and duration of drug release extended from 8 hour in MT1 to more than 12 hour in MT9 in the matrix tablet. The result reveals the effect of polymer concentration on release of famotidine from the HEP matrix tablet. With increase concentration of HEP retardation in drug release takes place, which clearly indicate the release rate controlling behaviour of HEP.

When matrices containing swellable polymers are exposed to dissolution medium, tablet surface becomes wet and hydrated to form a gel layer. The initial release of drug from these matrices occurs by the drug dissolution in the water penetrated into the matrix. The overall drug release from these matrices is governed by hydration, gel layer formation and drug diffusion into gel layer and to the dissolution media [36]. As the concentration of HEP, it causes an increase in viscosity of the swollen gel matrix, which decreases the water diffusion in to the core layer. Decrease in hydration of matrix contributes more hindrance for drug diffusion and consequently decrease in release rate [37]. Polymer erosion also plays a major role in releasing drug from these matrices [38]. These considerations indicate that HEP have the potential to sustain the release of the drug from matrix tablets.

Table – 4: Cumulative *in-vitro* drug release data of all batches.

Time (Hrs)	Cumulative % drug release								
	MT 1	MT 2	MT 3	MT 4	MT 5	MT 6	MT 7	MT 8	MT 9
0.5	38.81 ± 1. 68	36.09 ± 1.65	34.74 ± 1.98	26.61 ± 1.56	23.17 ± 1.86	22.16 ± 1.67	17.29 ± 1.89	16.73 ± 1.96	15.86 ± 1.89
1	49.96 ± 1.72	47.06 ± 1.45	41.65 ± 1.76	38.15 ± 1.67	36.61 ± 1.78	33.76 ± 1.79	29.14 ± 1.85	27.48 ± 2.01	27.31 ± 1.87
2	58.82 ± 1.39	56.17 ± 1.86	51.07 ± 1.64	49.85 ± 1.86	47.04 ± 1.53	42.87 ± 1.78	38.07 ± 1.73	35.05 ± 2.21	35.62 ± 1.67
3	69.15 ± 1.51	67.33 ± 2.23	59.39 ± 1.76	58.54 ± 1.75	57.02 ± 1.58	52.41 ± 1.79	48.72 ± 1.85	44.50 ± 1.56	44.07 ± 1.62
4	78.37 ± 1.74	74.92 ± 1.76	68.94 ± 1.54	69.22 ± 1.53	68.29 ± 1.75	65.31 ± 1.79	59.73 ± 1.93	56.71 ± 1.84	56.49 ± 1.63
5	85.23 ± 1.89	84.01 ± 1.87	79.59 ± 1.96	78.31 ± 1.68	75.13 ± 1.86	72.89 ± 1.94	66.37 ± 1.84	62.36 ± 1.67	62.33 ± 1.89
6	95.03 ± 1.95	91.22 ± 1.54	88.61 ± 1.65	86.94 ± 1.68	81.72 ± 1.56	79.65 ± 1.94	74.52 ± 1.93	70.15 ± 1.73	70.30 ± 1.85
8	99.33 ± 1.54	96.04 ± 1.52	94.96 ± 1.73	93.73 ± 1.59	93.41 ± 1.95	88.50 ± 1.78	82.38 ± 1.76	78.84 ± 1.68	78.63 ± 1.84
10		99.87 ± 1.65	99.27 ± 1.78	99.18 ± 1.89	99.82 ± 1.79	92.57 ± 1.63	89.02 ± 1.74	88.27 ± 1.69	89.25 ± 1.67
12						95.84 ± 1.87	94.33 ± 1.69	92.42 ± 1.89	99.10 ± 1.95
14						99.60 ± 1.69	99.94 ± 1.57	99.98 ± 1.23	

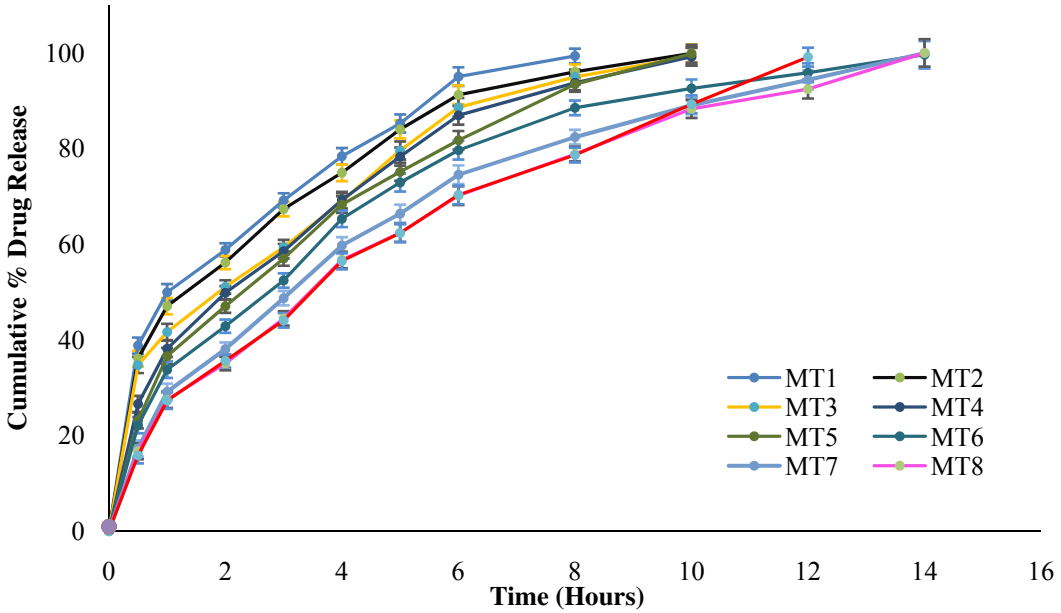

Figure – 3: *In-vitro* drug release profile for mucoadhesive Famotidine tablets

Kinetic study of drug release

The mechanism of drug release from matrix type drug delivery systems is a complex phenomenon. The R^2 values and model constants of corresponding mathematical models are presented in Table – 5. From the zero order and first order plots, it can be easily understood that increase in polymer concentration results in retardation of drug release. The best linearity was observed with Higuchi equation, indication drug release by diffusion through swellable matrix. A high linearity of Hixson cube-root model also been observed. Therefore it may be stated that, drug release from HEP matrix tablets follows diffusion coupled with erosion. The 'n' value of Korsemeyer-Peppas model indicated the magnitude of drug diffusion from swellable matrix. The 'n' values of the prepared formulations were found to be between 0.347 and 0.562. This implies that release may be fickian or non fickian (anamolous) depending upon polymer concentration. Higher concentration of natural polymer shifts the release pattern from fickian to non fickian. This indicates that at low polymer concentration only diffusion is dominating mechanism of release shifting to combination of diffusion and erosion based drug release mechanism when polymer concentration is increased.

Table – 5: Kinetic analysis of *in-vitro* drug release mechanism.

Model		Formulation Code								
		MT 1	MT 2	MT 3	MT 4	MT 5	MT 6	MT 7	MT 8	MT 9
Zero Order	R	0.449	0.346	0.504	0.607	0.676	0.511	0.659	0.751	0.801
	K_0	16.096	13.373	12.917	12.766	12.529	9.356	9.001	9.571	9.790
First Order	R	0.935	0.980	0.967	0.982	0.972	0.997	0.993	0.993	0.985
	K_1	-0.1802	-0.1629	-0.1485	-0.1411	-0.1503	-0.1119	-0.0977	-0.0897	-0.0891
Higuchi	R	0.977	0.937	0.966	0.986	0.995	0.969	0.990	0.994	0.993
	K_H	35.991	35.879	34.347	33.775	32.985	29.576	28.171	27.283	27.917
Hixon-Crowel	R	0.974	0.982	0.985	0.990	0.973	0.985	0.962	0.991	0.964
	K_{HC}	-0.4239	-0.3691	-0.3430	-0.3415	-0.3606	-0.2524	-0.2548	-0.2159	-0.2646
Korsemayer - Peppas	R	0.988	0.992	0.980	0.994	0.993	0.984	0.988	0.992	0.993
	K_{KP}	48.176	46.463	41.220	37.709	34.968	35.075	29.854	27.004	25.468
	n	0.347	0.352	0.375	0.448	0.482	0.459	0.521	0.534	0.562

Statistical optimization of mucoadhesive tablets

In order to optimize the formulation of mucoadhesive tablet of famotidine, the effect of selected variables viz. amount of HEP and amount of PVP K30 was studied on the nature and the performance of the drug delivery device. The most influential responses those represent the nature and the overall performance of the formulated device are mucoadhesive strength (R1), mucoadhesion time (R2) and dissolution half-life (R3). According to the central composite design, nine formulations were prepared by varying the amount of independent variables. The individual and interactive effects of the independent variables on the selected responses have been studied and presented in a tabular form in Table – 6. The data obtained in the study was statistically fitted to linear, interactive and quadratic models.

Table – 6: Response parameters of various formulations prepared as per the experimental design.

Formulation code	Factor 1	Factor 2	Response B_1	Response B_2	Response B_3
	HEP	PVP K30	Mucoadhesion Strength	Mucoadhesion Time	T50
	% w/w	% w/w	N	Min	Min
MT 1	20 (-1)	5 (-1)	11.1 ± 0.59	74 ± 1.89	99.6 ± 3.32
MT 2	20 (-1)	7.5 (0)	11.4 ± 0.78	73 ± 2.54	109.8 ± 3.86
MT 3	20 (-1)	10 (+1)	12.1 ± 0.65	76 ± 1.87	131.4 ± 2.89
MT 4	30 (0)	5 (-1)	41.8 ± 0.79	157 ± 2.13	138.0 ± 2.96
MT 5	30 (0)	7.5 (0)	42.1 ± 1.03	180 ± 1.76	148.8 ± 3.54
MT 6	30 (0)	10 (+1)	43.1 ± 1.12	196 ± 1.85	166.2 ± 3.21
MT 7	40 (+1)	5 (-1)	58.5 ± 0.98	381 ± 1.74	196.2 ± 2.95
MT 8	40 (+1)	7.5 (0)	59.9 ± 1.16	497 ± 1.98	216.6 ± 2.76
MT 9	40 (+1)	10 (+1)	52.9 ± 1.03	472 ± 2.13	213.6 ± 3.31

Statistical Data Analysis

The statistical analysis of the data obtained from trial batches was performed by multiple linear regression analysis using Design Expert® 8.0.7 software (Stat-Ease Inc. USA). The data clearly indicates that the values of three dependent variable viz. mucoadhesion strength, mucoadhesion time and dissolution half-life strongly depends on independent variables viz. amount of HEP and PVP K30. Table 6 shows the results of analysis of variance (ANOVA), which was performed to identify significant and insignificant factors. The model F-values for the responses i.e. mucoadhesive strength, mucoadhesive time and T_{50} were found to be 147.81, 63.53 and 113.88 respectively. This implies that the models were significant. The values of prob > F (Less than 0.05) for all the responses indicated the significance of the model.The polynomial equations relating the responses to the factors have been generated by multiple linear regression analysis as expressed below (eq. 9-11) –

$$\text{Mucoadhesion strength} = 43.14 + 22.78X_1 - 0.55X_2 - 1.65X_1X_2 - 8.02X_1^2 - 1.22X_2^2 \quad \text{Eq. 9}$$

$$\text{Mucoadhesion time} = 193.67 + 187.83X_1 + 22.00X_2 + 22.25X_1X_2 + 84.50X_1^2 - 24.00X_2^2 \quad \text{Eq. 10}$$

$$\text{Dissolution half-life } (T_{50}) = 157.80 + 47.60X_1 + 12.19X_2 \quad \text{Eq. 11}$$

Where, X_1 and X_2 are coded values of the test variables i.e. amount of HEP and PVP K30 in $\%^{w}/_{w}$. The equations can be used to draw the conclusion after considering the magnitude of the coefficient and the mathematical sign it carries.

Effect on Mucoadhesive strength

The polynomial equation for mucoadhesive strength (Eq. 9) indicates the positive effect of HEP and negative effect of PVP K30 on mucoadhesion strength. As observed in the experimental set up, the mucoadhesion strength increased from 11.1 to 58.5 and from 12.1 to 52.9 at low and high level of PVP K30 respectively, as the concentration of HEP was increased, which clearly point towards the mucoadhesive potential of HEP. Whereas influence of PVP K30 is negligible as compare to natural polysaccharide (HEP). It is also observed that, at higher level, PVP K30 adversely affect the bioadhesion potential of the isolated polysaccharide. This is may be due to impaired swelling of the natural polysaccharide (HEP) matrix owing to polymeric interaction. Figure 4A and Figure 4B represents the contour plot and 3D response surface plot clearly shows the influence of each polymer on the mucoadhesive strength of formulated tablets. From the plots a sharp augmentation of mucoadhesion is observed with increase in HEP concentration from 20% w/w to 30% w/w, this may be due to availability of more adhesive sites and polymer chains for interpenetration with the mucin as discussed earlier.

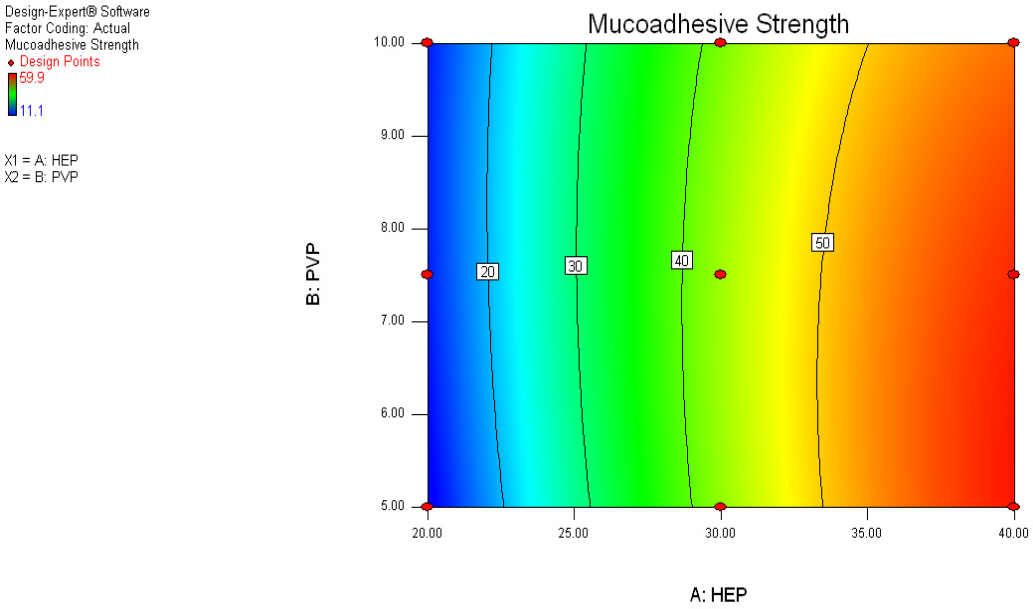

Figure – 4A: Contour plot showing the influence of HEP and PVP K30 on the mucoadhesion strength.

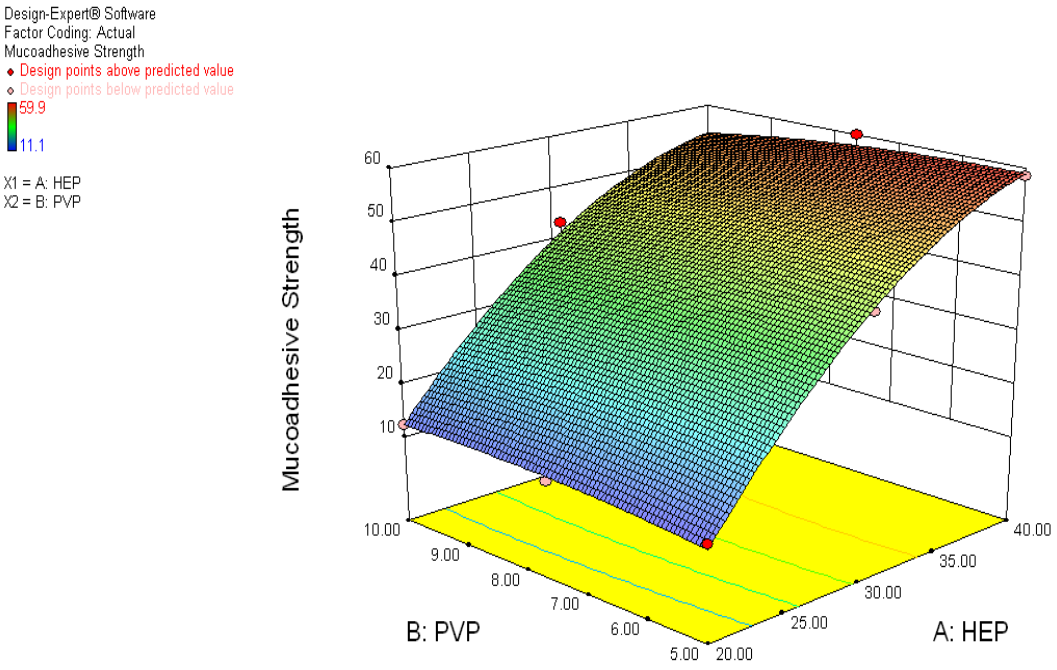

Figure – 4B: 3D Response Surface graph showing the influence of HEP and PVP K30 on the mucoadhesion strength.

Effects on Mucoadhesion time

The contour and response surface plot (Figure 5A and Figure 5B) illustrate that the value of mucoadhesion time increased from 74minto 381min and from 76min to 472min at low and high level of PVP K30 respectively, as the concentration of HEP was increased while the value of mucoadhesion time increased from 74min to 76min and from 381min to 472min at low and high levels of HEP respectively, as the concentration of PVP K30 was increased. Polynomial equation for mucoadhesion time (Eq. 10) illustrated that

both the independent variables viz. HEP and PVP K30 have additive effect on mucoadhesion time. It is also clear that the natural polysaccharide has a greater influence in the response variable as compare to PVP K30.

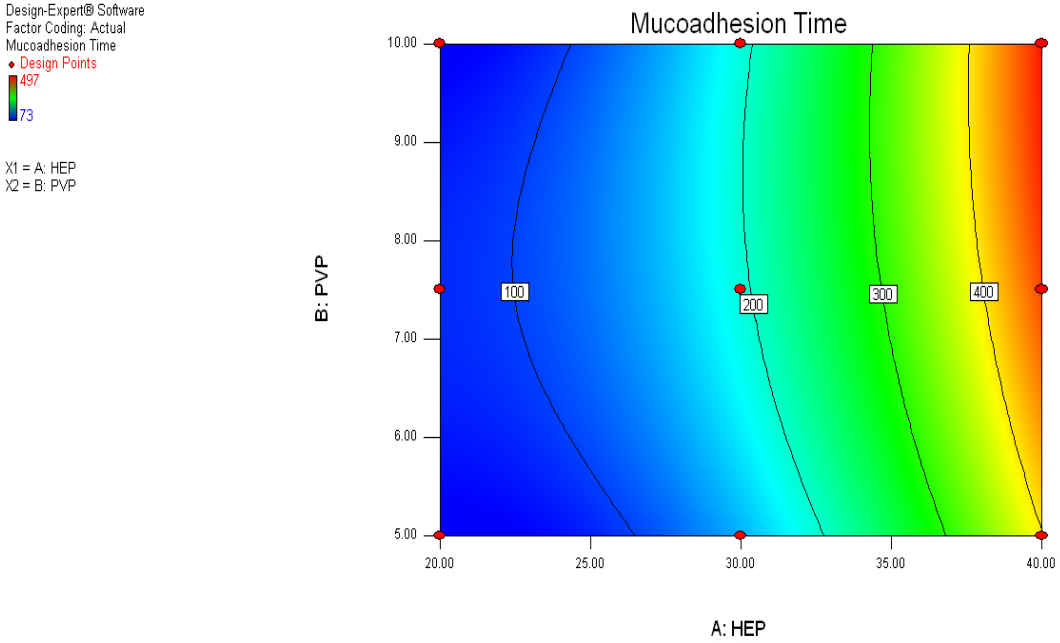

Figure – 5A: Contour plot showing the influence of HEP and PVP K30 on the mucoadhesion time.

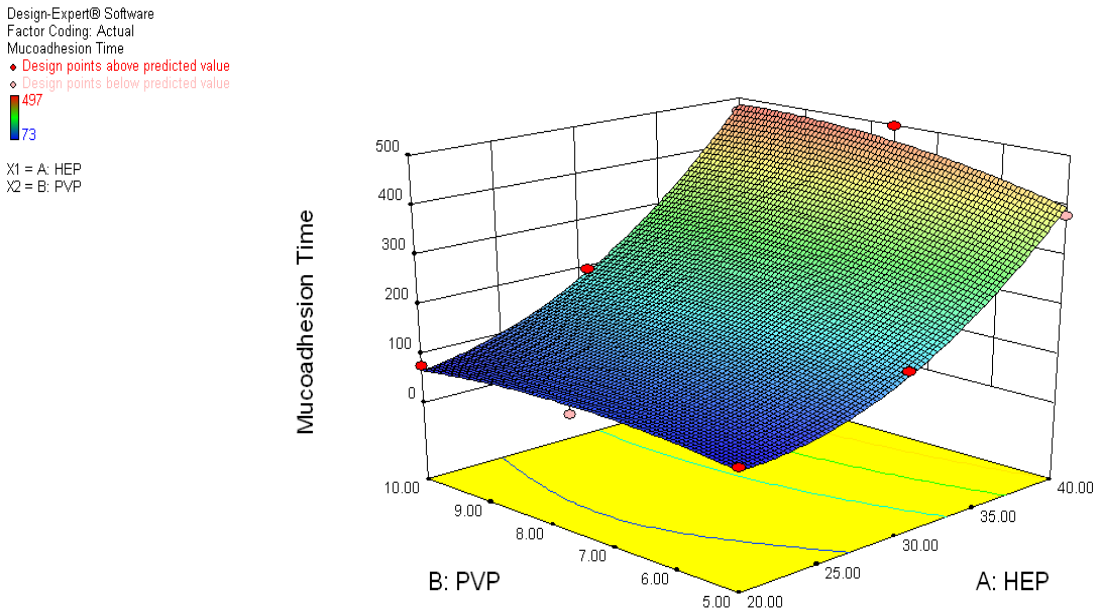

Figure – 5B: 3D Surface Response graph showing the influence of HEP and PVP K30 on the mucoadhesion time.

Effect on Dissolution half-life (T_{50})

The polynomial equation for mucoadhesion time (Eq. 11) illustrates that both the variables viz. HEP and PVP K30 was found to have additive effect on T_{50}. Figure 6A and Figure 6B represent the contour plot and three dimensional analysis for the studied response properties of time to 50% of famotidine release. From the contour plot it can be concluded that the T50 increases with

augmentation of both variables. The response changes the variables in a linear and ascending manner. But the contour plot shows that HEP has a completely greater influence on the response variables than the PVP K30. From the contour plot it is evident that the inclining trend was obtained with ascending order of HEP. The enhancement of T50 with increase in natural polymer concentration may be ascribed to increase in polymer chain density leading to pronounced chain entanglements and/or interpenetrations, thereby hindering the transport of drug molecules through the matrix. These findings point towards release retardant potential of HEP in formulation of matrix tablets.

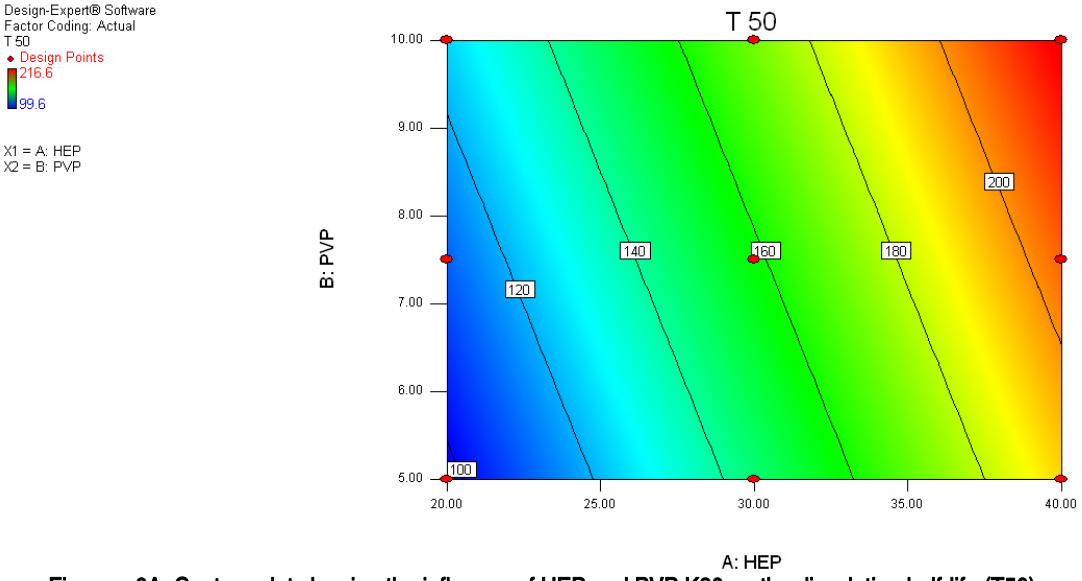

Figure – 6A: Contour plot showing the influence of HEP and PVP K30 on the dissolution half-life (T50).

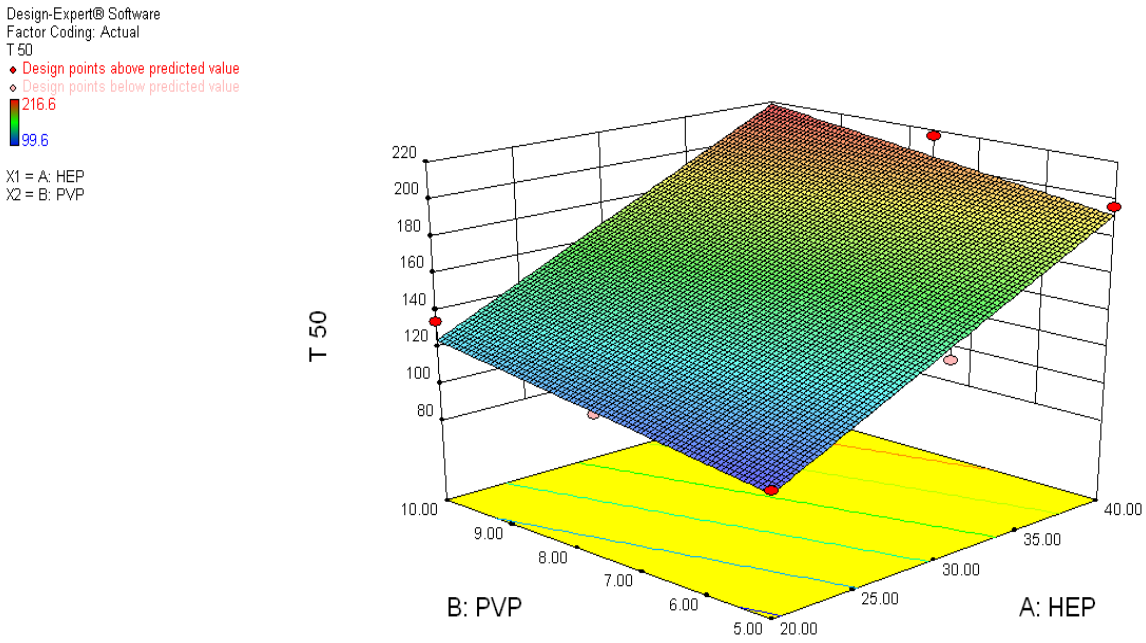

Figure – 6B: 3D Surface Response graph showing the influence of HEP and PVP K30 on the dissolution half-life (T50).

Search for optimum formulation

This was the most important part of response surface methodology. Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material [39]. A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. Upon comprehensive evaluation of the feasibility search and subsequently exhaustive grid searches, the formulation composition with HEP concentration of 40% and the amount of PVP K 30 was 9.63%, fulfilled maximum requirements of an optimum formulation, desirability 0.958. The higher desirability value indicates the more suitability of the formulation in terms of maximized mucoadhesion and better regulation of drug release rate. The optimized formulation was evaluated for various dependent variables. The response values were calculated and compared to the corresponding predicted values. Table 7 lists the values of the observed responses and those predicted by mathematical models along with the percentage prediction errors. The prediction error for the response parameters ranged between 1.43 and 5.14%.

Table – 7: Comparison of experimentally observed responses of the optimized HEP mucoadhesive matrix tablet with predicted responses.

Response parameter	Observed value	Predicted value	Error (%)
Mucoadhesive Strength (N)	52.325	55.161	5.14
Mucoadhesive time (min)	479.194	486.166	1.43
Dissolution half-life T50 (Min)	208.543	216.583	3.17

Physical stability study

Statistical analysis of the results, before and after conducting the stability studies for 3 months, was carried out using paired Student's t-test. No significant difference ($p > 0.05$) was observed in the tablet appearance, hardness or thickness. The similarity factor (f_2) was calculated for comparison of dissolution profile before and after stability studies. The f_2 values were found more than 50 (96.46 and 88.02 respectively after one and three months) that indicate a good similarity between both the dissolution profiles. Similarly, no significant difference was observed in the drug content, swelling percent, mucoadhesion strength and mucoadhesion time. The periodic data of stability study is presented in Table 8. The results of stability studies indicate that the developed formulation has good stability.

Table – 8: Results of short term physical stability study.

Parameters	Study period (Months)		
	0	1	3
Appearance	Off-white coloured round shaped tablets	Off-white coloured round shaped tablets	Off-white coloured round shaped tablets
Hardness (Kg/m^2)	5.5 ± 0.07	5.6 ± 0.04	5.6 ± 0.08
Thickness (mm)	2.73 ± 0.02	2.73 ± 0.02	2.73 ± 0.01
Drug content (% w/w)	98.62 ± 0.17	98.74 ± 0.23	98.58 ± 0.13
Dissolution profile similarity factor (f_2 %)	97	94	96
Mucoadhesion strength (N)	53.72 ± 0.19	52.16 ± 0.26	52.85 ± 0.22
Mucoadhesion time (Minutes)	471 ± 3.83	494 ± 5.04	483 ± 3.29

Conclusion

The experimental findings of the present study clearly pointed towards concentration dependant mucoadhesion and release retardant potential of purified *Hibiscus esculentus* L polysaccharide in the formulation of mucoadhesive matrix tablet of famotidine. Kinetic analysis reveals that the drug release from the formulation governs by chiefly diffusion coupled with surface erosion. A high degree of prognosis observed in the response surface analysis indicates efficiency and fitness of the selected model in optimization of formulation. The dependant variables, namely mucoadhesive strength, mucoadhesion time and dissolution half-life found to be modulated by formulation variables viz. concentration of HEP and concentration of PVP K30. Though, the effect of HEP is much prominent as compare to that of PVP K30. This result indicates that, the purified polysaccharide of *Hibiscus esculentus* can be optimistically explored for potential mucoadhesive release retardant in various pharmaceutical formulations.

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