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Development and Evaluation of Novel *Cordia myxa* Fruit Gum based Mucoadhesive Tablets for Gastroretentive Delivery of Losartan Potassium

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Abstract

Aim:
The objective of the present study is to develop novel extended release gastroretentive tablets using *Cordia myxa* fruit gum and its modified form as mucoadhesive polymer in order to overcome issues presented due to short-biological half-life of Losartan Potassium.

Methods:
The mucoadhesive tablets of Losartan potassium was formulated by direct compression technique using different concentrations (10% & 20%, w/w) of naïve and modified *Cordia myxa* gum and compared with well known polymer, carbopol 934. The formulated tablets were evaluated in terms of weight variation, hardness, friability, drug content, *ex vivo* mucoadhesion strength, *ex vivo* mucoadhesion time, *in vitro* drug release and *in vivo* pharmacokinetic study.

Results:
Dissolution of the developed tablets with 20% w/w modified sulfated polysaccharide showed 99.34% cumulative drug release at the end of 24 h. The *in vivo* pharmacokinetic study exhibited 4.4 times higher $AUC_{0-\infty}$ value for tablets formulated with sulfated *Cordia myxa* (587.40 ± 4.8) than the tablets formulated with carbopol 934 (132.56 ± 2.6). The C_{max} of sulfated *Cordia myxa* and carbopol 934 group was 124.13 ± 2.5 µg/mL and 69.88 ± 0.9 µg/mL, and the T_{max} of the sulfated *Cordia myxa* and carbopol 934 group was 6.72 ± 0.2 h and 1.24 ± 0.1 h, respectively.

Conclusion:
The results suggested that sulfated *Cordia myxa* gum as polymer can be successfully employed for formulation of mucoadhesive drug delivery systems to alleviate the drawbacks of the conventional drug therapy of Losartan Potassium for management of hypertension thus improving the patient compliance.

Keywords: *Cordia myxa;* Carbopol; Gastroretentive; Losartan Potassium; Mucoadhesive.

1. INTRODUCTION

Natural polysaccharides have drawn the interest of many researchers in recent years due to their diverse applications in the field of modified drug delivery systems because of their abundant availability, non-toxicity (monomer residues are not hazardous to health), high water solubility or swelling ability, stability to pH variations, economical as well as biodegradable behavior [1]. Recently, the formulation of natural polysaccharide as mucoadhesive polymer in various drug delivery systems like nanoparticles [2,3], microparticles [4], microspheres [5], gels [6], tablets [7], etc., for extended drug release applications has been the focus of drug delivery research.

Cordia myxa, commonly known as '*Lasura*' in Indian language, is an anionic polysaccharide belonging to borage family, Boraginaceae. Polysaccarides obtained from the fruits of *Cordia myxa* has been identified for presence of D-galactouronic acid, xylose, arabinoglucan, galactan, D-glucose and L-arabinose [8,9]. The published research acknowledged the use of *Cordia myxa* as potential non-toxic and safe pharmaceutical excipient with various successful applications [10-16]. Moreover, due to its mucoadhesive properties, it finds its use in the design of oral mucoadhesive drug delivery systems [17].

Cordia myxa contains carboxylic moieties in abundance due to presence of galactouronic acid [18], which can enhance the opportunities for the polysaccharides to form hydrogen bonds with specific glycoprotein based structure of mucin. Additionally, *Cordia myxa* displays rheological properties that are favorable for selection as any mucoadhesive excipient with capability of high viscosity gel formation in water even with low polymeric content [19].

Modification of natural polysaccharides is receiving exceptional consideration because it provides an ineluctable approach for forming new functionalized macromolecules. Modification of polysaccharides by sulfation are known to enhance water solubility and further, swelling index [20,21]. Sulfated polysaccharides obtained by chemical modification have been used as efficient carriers to deliver

therapeutic agents across a mucosal membrane [22]. Sulfation of polysaccharides has been reported for a variety of polysaccharides such as konjac glucomannan [23], *Dendrobium huoshanense* [24], *Agaricus brasiliensis* [25], *Laminaria angustata* [26], *Radix hedysari* [27] and *Phellinus ribisand* [28]. In earlier reports by our research group on Bael fruit gum [29] it was observed that natural polymers like gums containing carboxylic acid moieties are more amenable towards modification and hence, results in higher degree of substitution. This higher degree of substitution, in turn, results in obtaining desired control in case of modified drug delivery products [30-32]. Sulfation of *Cordia myxa* and its mucoadhesive characteristics has not been earlier investigated in development of gastroretentive formulations.

Losartan potassium (LP) is a potent, highly selective, competitive antagonist of angiotensin II receptor and is first line therapy in the management of hypertension [33]. It is readily absorbed from the gastrointestinal tract however bears short half-life of 1.5-2.5 h and a low oral bioavailability of 25-33% [34]. Hence frequent dosing limits its use in managing hypertension and reduces patient compliance considerably. Various researchers have reported extended drug delivery strategy to overcome this limitation of LP [33]. The attempts, therefore, were made to develop mucoadhesive formulations as tablet dosage form of LP. It was envisaged that the gastric residence of the developed formulations could be prolonged to release the LP in a controlled manner over a sustained duration to achieve the maximize therapeutic potential. To meet this objective the present investigation was undertaken to explore the utility of *Cordia myxa* gum as well as with its sulfated modification in providing sustained release of LP and mucoadhesion of its tablet dosage. Central composite design was used for enhancing the output efficiency through the number of experimental runs to be conducted where the impact of three most important process variables on the yield and degree of sulfation was tested.

2. MATERIALS AND METHODS

2.1. Materials

Fruits of *Cordia myxa*, partially ripe were procured locally from Punjab (India) and were authenticated by taxonomists of Herbarium, Punjabi University, Patiala, Punjab, India (Authentication voucher No: 59109). Losartan Potassium was received from Yarrow Chem, Mumbai. All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Method of Extraction of *Cordia myxa* gum

Cordia myxa gum (CMG) was extracted from the fruits by modifying the method reported earlier [35]. Briefly, the fruits, washed under running water, were wiped dry and stored in plastic zipped bags at -20˚C until used. The outer thin skin (pulp) was removed manually and rest of the gummy part was mashed in glacial acetic acid solution (2% v/v). The slurry was stirred using mechanical stirrer for 30 min with heating and was kept aside overnight. To remove debris, the slurry was filtered through muslin cloth and added to acetone to precipitate the gum. Finally, the precipitates were dried in an oven at temperature not exceeding 50˚C. The dried gum was grounded to obtain fine powder. The gum was dialyzed and freeze dried to obtain pure gum.

2.2.2. Sulfated Modification of CMG by Ultrasonication

Ultrasonication of CMG was done to prepare sulfated *Cordia myxa* gum (SCMG) by modifying the method reported earlier [29]. CMG (200mg) was added in sulphuric acid (5M, 10M or 15M) before being subjected to ultrasonication for 2h, 4h or 6h at 0°C, 15°C or 30°C. The samples were subsequently neutralized using 0.1N NaOH, dialyzed (Himedia-60 LA390-5MT) and further lyophilized.

2.2.3. Optimization of Reaction Conditions by Experimental Design

The effect of three factors, the concentration of sulphuric acid, reaction temperature and reaction time on yield and degree of sulfation (DS) were investigated. Three levels (-1, 0 and +1) per factor were employed using 5M, 10M or 15M concentration of sulphuric acid, reaction temperature of 0°C, 15°C or 30°C and reaction time of 2 h, 4 h or 6 h, respectively. Twenty experimental runs were designed according to central composite design (Table 1).

Central composite statistical design of response surface methodology (RSM) is used to statistically optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the yield and DS of SCMG. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables (concentration of sulphuric acid, reaction temperature and reaction time) and dependent variables (yield and DS of SCMG). Analysis of the experimental design was carried out using Design Expert software (Version 9.0.3.1, Stat-Ease, Inc. Minneapolis, MN). Analysis of variance were performed by ANOVA procedure.

2.2.4. Characterization of Sulfated CMG

2.2.4.1. Elemental analysis of SCMG

Lyophilized SCMG samples were analyzed for sulfur content (% S) by elemental analysis using Elementar Vario Micro (Elementar, Analysensysteme, Germany). DS was calculated according to the following equation [36]:

$$DS = \frac{0.162 \times (\%S / 32)}{100 - [(80/32) \times \%S}$$

where, % S is the sulfur content (%).

2.2.4.2. Swelling Index

The swelling index of CMG and SCMG was carried out in buffer pH 1.2, pH 2.0 and distilled water. The samples (100 mg) individually were soaked in different media (100 ml) for 24 h. The swollen material was carefully removed, superficial dried using a blotting paper and weighed [29]. The swelling index was calculated as:

$$\text{Swelling Index} = \frac{W_f - W_i}{W_i}$$

where, w_f is the weight of swollen material and w_i is the initial weight of the dry material.

Table 1. Experimental plan of central composite design using levels and coded symbol of factors with observed responses studied for sulfation of *Cordia myxa* gum

Experimental Runs	Factors with normalized levels			Responses	
	A Sulphuric Acid (M)	B Temperature (ºC)	C Time (h)	Yield (mg)	DS
1	15	30	2	18	0.10
2	5	0	6	73	0.58
3	10	0	2	80	0.75
4	5	0	2	77	0.61
5	10	15	4	91	0.97
6	5	30	2	53	0.33
7	10	15	6	95	1.08
8	5	30	6	56	0.43
9	10	15	4	84	0.8
10	15	0	6	120	1.42
11	10	15	4	82	0.77
12	15	0	4	115	1.28
13	10	15	4	85	0.83
14	10	15	4	92	1.01
15	10	15	2	54	0.39
16	15	15	2	160	1.84
17	15	30	6	20	0.18
18	5	15	4	46	0.27
19	10	30	4	62	0.52
20	10	15	4	89	0.92

Factors	Coded Symbol	Levels		
		-1	0	+1
Concentration of Sulphuric Acid (M)	A	5	10	15
Reaction Temperature (ºC)	B	0	15	30
Reaction Time (h)	C	2	4	6

Table 2. Composition of Formulation Batches of Losartan Potassium Mucoadhesive Tablets using Different Polymers.

Ingredients (mg)	Formulation Code					
	MF1	MF2	MF3	MF4	MF5	MF6
Drug	50	50	50	50	50	50
CMG	20	40	-	-	-	-
SCMG	-	-	20	40	-	-
Carbopol 934	-	-	-	-	20	40
Avicel pH 101	50	50	50	50	50	50
Magnesium Stearate	2	2	2	2	2	2
Talc	4	4	4	4	4	4
Lactose	74	54	74	54	74	54
Total weight	200	200	200	200	200	200

2.2.4.3. Spectrophotometric Analysis

The fourier transformation infrared spectroscopy of CMG and SCMG was performed using FTIR spectrophotometer (Shimadzu, Japan). Measurements were obtained in the frequency range of 4000–500 cm^{-1}.

Nuclear magnetic resonance analysis was performed using a NMR spectrometer (Bruker Avance II 400 NMR spectrometer). ^1H NMR spectras were recorded using D_2O as solvent for CMG and SCMG at temperature 50°C and frequency of 400 MHz [13].

2.2.5. Formulation of Mucoadhesive Tablets of LP

Various formulations (MF1-MF4) containing LP were prepared using CMG and SCMG as mucoadhesive polymers in different concentrations (10% and 20% w/w). All the excipients, except magnesium stearate were sifted (Table 2) through # 40 mesh sieve. Presifted excipients were loaded into the bed of stainless steel octagonal blender and were blended for 30 min at slow speed. LP was sifted through # 30 mesh sieve and loaded into the bowl of octagonal blender and mixed for 30 min. Magnesium stearate was sifted through # 60 mesh sieve and was loaded

into the bowl of octagonal blender along with premixed powder blend and tumbled for 5 min at fast speed. Lubricated powder was compressed using an 8-mm diameter concave punch in a tablet punching machine (AK Industries, Nakodar, Punjab, India) to form tablets weighing 200 mg.

2.2.6. Comparative Study

To evaluate the mucoadhesive potential of SCMG, comparative study was performed by formulating a batch with carbopol 934 as known mucoadhesive polymer. Two formulation batches of mucoadhesive tablets (MF5 and MF6) containing different concentrations (10% and 20% w/w) of carbopol 934 were prepared (Table 2).

2.2.7. Evaluation of Directly Compressible Powder

The mixture prepared for preparation of tablets, prior to compression was evaluated for parameters like bulk density, tapped density, angle of repose, carr's index and hausner's ratio.

2.2.8. Evaluation of Mucoadhesive Tablets

The tablets from each formulation batch (MF1 to MF6) were evaluated for weight variation, hardness, friability, drug content, *ex vivo* mucoadhesive strength, *ex vivo* mucoadhesion time, *in vitro* drug release study and *in vivo* pharmacokinetic study.

2.2.8.1. Weight Variation

Formulated tablets (20 tablets) were weighed collectively and individually. Percentage weight variation was calculated from collective weight.

2.2.8.2. Hardness and Friability

The formulated tablets were evaluated for hardness and friability parameters using Monsanto hardness tester (Perfit, India) and Roche friabilator (Model 902, EI, India) respectively [17].

2.2.8.3. Drug Content

A powdered quantity equivalent to 50 mg of LP was shaken with 0.1 N HCl (100 ml) for 15 min. The drug content in samples was analyzed at 250 nm using a UV/VIS double beam spectrophotometer (Systronics, India). The samples were earlier prepared by dilution and filtration through a 0.45 µm membrane filter (Milipore, US) [37].

2.2.8.4. *Ex vivo* Mucoadhesive Strength

The mucoadhesive strength of formulated tablets was determined using texture analyzer by measuring the force required to detach the tablet from a freshly sized goat's gastric mucosa tissue [38]. In brief, goat's gastric mucosa tissues were obtained from the local slaughter house and used immediately. The tablet was attached to the probe (stainless steel cylindrical probe with 10 mm diameter) using cyanoacrylate adhesive. The probe was lowered at a speed of 0.5 mm/s so that the tablet made contact with mucosal tissue. A constant force of 1 N was applied for 60 s, after which the probe was withdrawn at a speed of 0.5 mm/s to the distance of 15 mm. The mucoadhesive strength, i.e. the maximum force required for separating the tablet from the mucosal surface was obtained [39]. Each measurement was repeated three times.

2.2.8.5. *Ex vivo* Mucoadhesion Time

The *ex vivo* mucoadhesion time was examined after adhering the formulated tablet on goat's gastric mucosa. Gastric mucosa was pasted on a glass slide using a cyanoacrylate tape, and the tablet was wetted with a drop of 0.1N HCL and adhered to mucosal tissue. The glass slide was then placed in a beaker, which was filled with 200 ml of 0.1N HCL and kept at 37±0.5ºC. After 2 min, a slow stirring rate (50 rpm) was applied to simulate the gastric environment, and time of tablet's detachment from goat's gastric mucosal tissue was recorded as mucoadhesion time [40].

2.2.8.6. *In vitro* Dissolution Study

The *in vitro* dissolution rate of mucoadhesive tablets containing LP was estimated using USP paddle type apparatus rotating at a speed of 50 rpm at 37±0.5°C using 900 ml of 0.1 N HCl solution. At various time intervals of 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h, sample was withdrawn (5 ml) and replaced with same amount of buffer (0.1N HCl). The samples were filtered through 0.45 µm membrane filter and analyzed using UV spectrophotometer (Systronics, India) at wavelength of 250 nm. Cumulative percent drug release was calculated using an equation obtained from the calibration curve and plotted against time to determine the release profile. Release data was evaluated according to zero order, first order, Higuchi and Korsmeyer- Peppas models [41,42].

2.2.8.7. *In vivo* Pharmacokinetic Study

Male Wistar rats weighing 200-250g, maintained on standard laboratory diet (Ashirwad Feed Industry, Chandigarh, India) and having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to 12 h cycle of light and dark. The experimental protocol was approved by institutional animal ethics committee and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forest, Government of India (Maharishi Markendeshwar College of Pharmacy Animal Facility). The protocol for this study was approved by the IAEC of MMCP, MMU, Mullana, Ambala, India. The tablet was made to be swallowed by administration of 1 ml water.

Group I: Oral administration of LP with naive *Cordia myxa* (MF2)

Group II: Oral administration of LP with sulfated *Cordia myxa* (MF4)

Group III: Oral administration of LP with Carbopol 934 (MF6)

0.5 ml of blood samples was collected through the orbital plexus from rat at various time intervals (0, 1, 2, 4, 8, 16, 20, 24 h). Plasma was immediately separated from the blood cells by centrifugation at 6000 rpm for 10 min and stored frozen at - 20°C till HPLC analysis [43].

Pharmacokinetic parameters like C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were calculated using plasma concentration vs. time profile (Actual time of sample collection) of both investigational products using WinNonlin Professional Software Version 5.0.1 (Pharsight Corporation, USA).

2.2.9. Statistical Analysis

Data obtained from the study was statistically analyzed using one-way ANOVA followed by Tukey's multiple range test as post-hoc analysis. A value of $p<0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Optimization of Reaction Conditions by Central Composite Design

Sulfation of CMG was attempted by carrying a total of 20 experimental runs under different conditions for optimizing the three independent parameters that were obtained by varying the reaction parameters in order to obtain the maximum yield and DS. The reaction parameters selected were concentration of sulphuric acid, temperature and time (Table 1). The yield of SCMG was found to be in between 18 to 160 mg and the value of DS ranged from 0.1 to 1.84. The responses involved (yield and DS of SCMG) at different experimental combination for coded variables are given in Table 1. Using multiple regression analysis on experimental data polynomial equation of second-order was generated, in which the yield and DS were the responses and A, B and C were the coded values for the three different factors selected (Equation 1 and 2).

Mathematical Equations

$$DS = 0.86+0.13A-0.42B+0.020C-0.27AB-0.012AC+0.062BC-0.56A^2+0.20B^2+0.14\ C^2 \quad \text{Eqn (1)}$$

$$Yield = 86.31+3.60A-33.75B-1.08C-21.24AB-1.45AC+4.48BC-39.48\ A^2+15.27\ B^2+6.95\ C^2 \quad \text{Eqn (2)}$$

The ANOVA showed that the regression coefficient, R^2 was found out to be 0.9812 and 0.9786 for yield and DS respectively. The relationship between independent and dependent variables was depicted in 3D response surface graphs generated by Design Expert software. It provided a visual interpretation of relationship between responses and factors at each level [44]. The response surface plots are shown in Fig. 1. The results reported that controlling the reagent amount has more pronounced effect than controlling the reaction time and reaction temperature to obtain SCMG with high DS [28]. This was in accordance with the previous studies that higher amount of reagent was essential and has more pronounced effect to obtain high yield and DS [45].

It was concluded from the mathematical equations [Eqn (1) and (2)] and 3D graphs (Fig. 1) that there was a decrease in the DS with increase in the reaction temperature (B) whereas, increase in the DS occurred when there was increase in molar ratio of sulphuric acid (A) and reaction time (C). The yield value decreased with increase in the reaction time (C) and temperature (B) while the yield increased with increase in molar ratio of sulphuric acid (A). This suggested that the reaction temperature (B) had a negative impact on the DS of SCMG whereas the reaction time (C) and reaction temperature (B) both had a negative effect on the yield of SCMG. The reason can be the hydrolysis or degradation of polysaccharides resulting from the strong acidity of sulfating agent [46]. The generated mathematical equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries. The positive sign in the polynomial equations is an indicative of direct correlation between responses (like yield and DS) and independent variables (A, B and C). However, the negative sign shows antagonistic effect of a variable. Analysis by central composite design indicated that the most predominant influence on DS and yield of SCMG was exerted by variable A (molar ratio of sulphuric acid).

The extent of the impact of variables on DS and yield followed the order: variable B (reaction temperature) < C (reaction time) < A (molar ratio of sulphuric acid). It was observed that maximum yield and DS was obtained using 15 M of sulphuric acid with a reaction time of 2 h at 15°C. To validate the results of the model, an experiment was carried out under the optimal conditions decided by the experimental design. The validation result revealed that the experimental values were in good agreement with the predicted ones, suggesting that there was no significant difference between experimental and predicted values. It was concluded that the response model was adequate for reflecting the expected optimization.

3.2. Characterization of Sulfated CMG

3.2.1. Spectrophotometric Analysis

The FTIR spectra of CMG and SCMG samples are shown in Fig. 2. In the spectra of CMG (Fig. 2A), a broad absorption band at 3380 cm^{-1} was observed, which, can be attributed to –OH stretching. A peak appearing at 1380 cm^{-1} was due to C-O stretching, while peaks at 1190 cm^{-1} and 1100 cm^{-1} can be ascribed to C-O-C stretch of ether. The absorption bands at 1620 cm^{-1} and 1435 cm^{-1} could be ascribed to asymmetrical and symmetrical COO− stretching vibration, respectively. In the spectra of SCMG (Fig. 2B) two new bands at 1739 cm^{-1} and 1630 cm^{-1} appeared suggesting C=O stretching vibration and unsaturated bond formed in the sulfation process respectively. Two additional bands each at 1252 cm^{-1} and 850 cm^{-1} could be ascribed to asymmetrical S=O stretching vibration and symmetrical C−O−S vibration possibly associated to a $C-O-SO_3$ group, respectively.

The ^1H NMR spectrum of CMG (Fig. 3A) revealed two singlets at high field (δ 3.30 ppm (s), δ 3.31 ppm (s). This could be related to the environments of methyl groups of rhamnose and the protons linked to C-6 (δ 3.65, δ 3.70 ppm) and C-4 of galactose (δ 3.98, 4.28 ppm), respectively and suggesting the existence of two different galactose derivatives. The anomeric protons could be assigned to β-sugar residues (δ 4.72–4.76 ppm) and the α-sugar residue (δ 5.1–5.3 ppm), as reported earlier [47]. The H-1 resonance for methyl β-L-Ara p (δ 5.00 ppm) and methyl α-L-Ara p (δ 4.52 ppm) were also observed as reported previously for other gums [48]. The anomeric region contains the proton signals (δ 5.18, 5.28 ppm) attributed to α-L-rhamnose [47]. The three closely neighbored signals observed in the ^1H NMR spectrum (δ 4.15, δ 4.02 and δ 3.84 ppm) can be assigned to H-1 of α-glucose. The ^1H NMR spectrum of CMG showed signals corresponding to β-D-galactopyranose, α-L-arabinofuranose, α-L-rhamnose, β-D-glucuronic acid and 4-O-methyl-α-D-glucuronic acid as represented in Fig. 3A. A complete assignment of the 1H signals of the 4-O-methyl β-D-glucuronic acid was

established. The H-1 (δ 4.32 ppm) signals proved β-configuration of 4-O-methyl-β-D-glucuronic acid. The O-methyl group could be assigned to 1H at δ 3.5 ppm.

The ^{1}H NMR spectra and chemical shifts of SCMG (Fig. 3B) shows that the signals from the α anomeric proton at δ 5.13 ppm and δ 5.28 ppm were assigned to 3,6 α-L-anhydrogalactose and α-L-galactose-6-sulfate, respectively. The H-1 of β-D-galactose was linked to α-L-galactose 6-sulfate and that of β-D-galactose was linked to 3,6 α-L-anhydrogalactose, at δ 4.33 and 4.54, respectively [47].

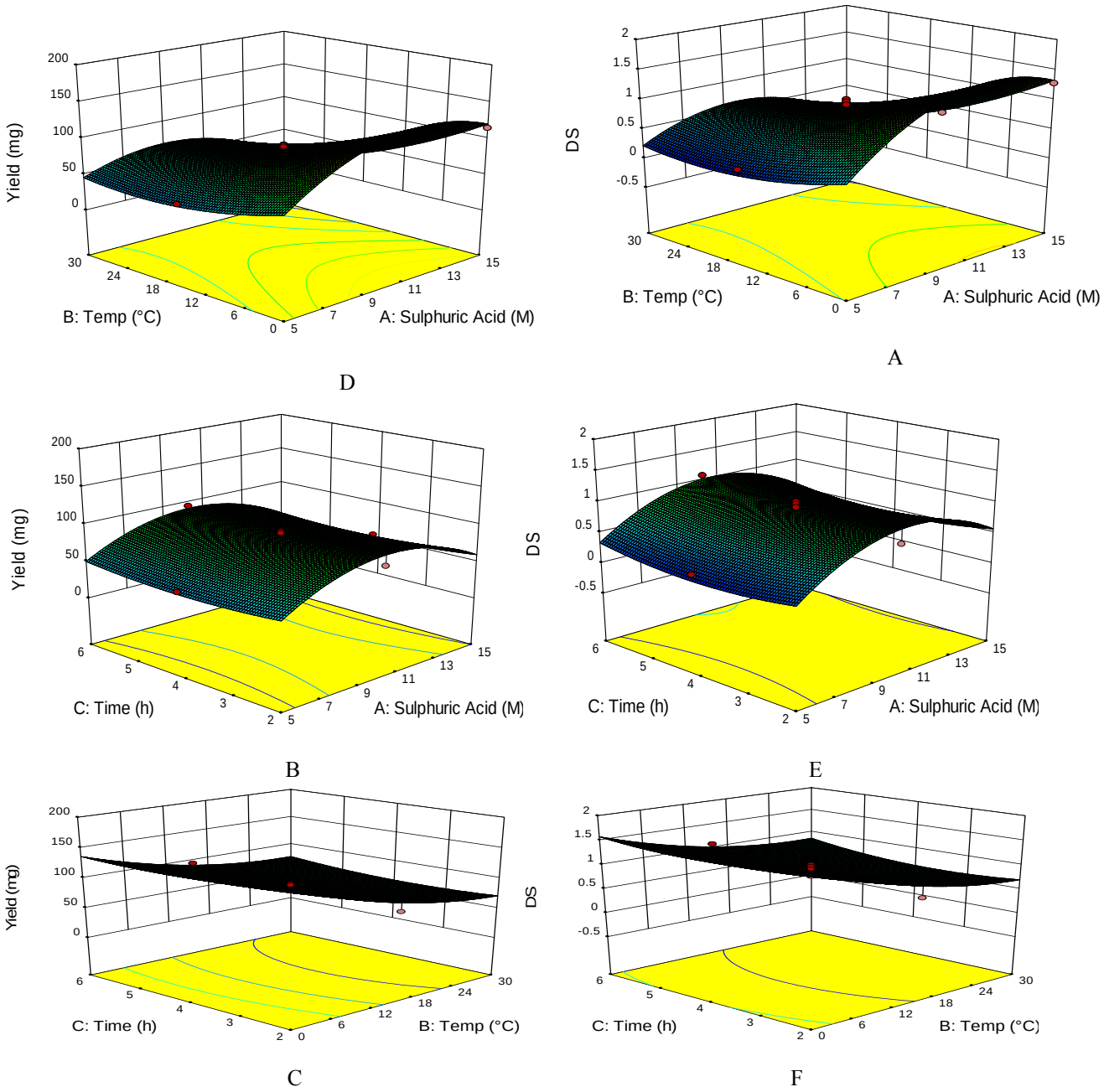

Figure 1. Response surface graphs showing the effects of concentration of sulphuric acid, temperature and time on yield (A, B & C) and DS of SCMG (D, E & F).

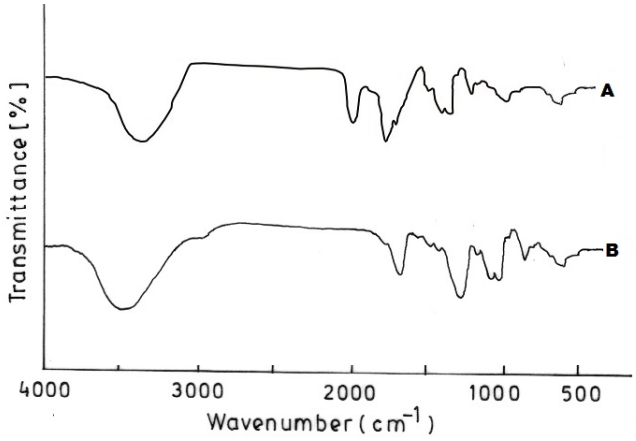

Figure 2. FT-IR spectra of **(A)** CMG and **(B)** SCMG.

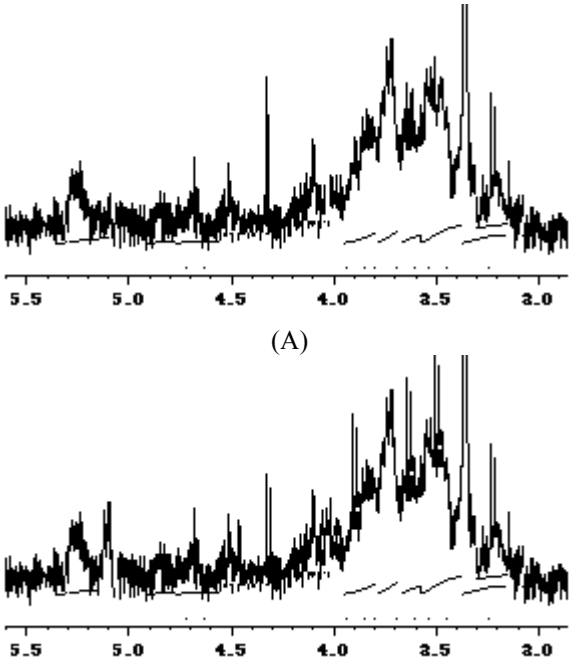

Figure 3. Solid state ^{1}H NMR spectra of **(A)** *Cordia myxa* gum (CMG) and **(B)** Sulfated *Cordia myxa* gum (SCMG).

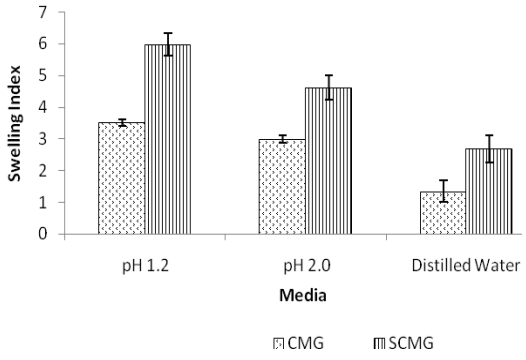

Figure 4. Swelling index of CMG and SCMG in various pH media.

3.2.2. Swelling Index

The swelling index of CMG and SCMG were studied in buffer pH 1.2, pH 2.0 and distilled water (Fig. 4). The swelling index of CMG and SCMG in different media was observed to follow the order: pH 1.2 > pH 2.0 > distilled water. The swelling index of SCMG was found to be higher than that of CMG in all the medias used. High swelling index would be useful for sustaining drug release for prolonged period of time. Therefore, higher magnitude of swelling of SCMG compared to CMG can be expected to be useful for modulating the drug release from dosage forms.

3.3. Evaluation of Directly Compressible Blend

The carr's index, hausner's ratio and angle of repose for the batch MF4 was found to be, 16.66 ± 0.43, 1.20 ± 0.02 and 28.9 ± 1.76 respectively (Table 3) indicating good flow characteristics of SCMG particles that would ensure least segregation during compression process.

3.4. Characterization of Mucoadhesive Tablets

3.4.1. Weight Variation

The average weight of 20 tablets along with standard deviation of entire formulations has been presented in Table 4. The percentage of weight variation of individual tablets from the average weight was found to be within ± 5% w/w which proved that the entire tablets have passed the USP weight variation test.

3.4.2. Hardness

The hardness of mucoadhesive tablets of losartan potassium was observed to be in range of 6.1 ± 0.5 kg/cm^2 to 6.4 ± 0.6 kg/cm^2 as shown in Table 4. At this hardness tablets passed friability tests, where all tablet batches showed friability less than 1% w/w. This ensures acceptable mechanical strength of tablets.

3.4.3. Drug Content

The drug content for MF1 to MF6 was found to be in the range of 98.35 ± 1.6% to 103.53 ± 2.2% as shown in Table 4. The results indicated that tablets of entire batches complied with official specifications.

3.4.4. *Ex vivo* Mucoadhesive Strength

It was observed that mucoadhesive strength of the tablets increased with sulfation of CMG. It has been proposed that the interaction between the mucus and SCMG is a result of physical entanglement and secondary bonding, mainly H-bonding and vander Waals attraction. Polymer characteristics that are necessary for mucoadhesion indicate: strong H-bonding groups, strong anionic charges, high molecular weight, sufficient chain flexibility, and surface energy properties favoring spreading onto mucus [39,49]. CMG express ionic groups (-COO$^-$) that have the ability to interact with functional groups present in mucin. Due to modification of CMG, the anionic sulfur groups increases on SCMG, which promotes SCMG interaction with cationic charges of mucin. Thus, increasing the mucoadhesive strength of formulated tablets. The mucoadhesive strength of all formulation batches are evident in Table 4.

3.4.5. *Ex vivo* Mucoadhesion Time

The mucoadhesion time for the various batches is depicted in Table 4. The formulations containing SCMG (batch MF4) took more time (24 h) for complete detachment when compared to the tablets containing CMG i.e. MF2 batch (6 h) and carbopol 934 i.e. MF6 batch (8 h) ($P<0.05$). The reason behind this can be the fact that the SCMG had anionic sulfur groups due to the sulfation modification and the mucosal mucin protein surface has $–NH_3^+$ groups, both carrying opposite electrical charges. When the polymer contacts with the mucosal surface, electron transfer occurs in an attempt to balance Fermi levels, causing the formation of a double layer of electrical charge at the polymer–mucin interface, which enhances the time of contact of SCMG with mucosal surface [50,38].

3.4.6. *In vitro* Drug Release Study

The *in vitro* dissolution profile of the formulated batches of mucoadhesive tablets of losartan potassium is represented in Fig. 5. When cumulative % drug release versus time graph was plotted it was observed that, for three of the polymers used, the drug release rate from SCMG formulations (MF3 to MF4) was found to be higher ($P<0.001$) as compared to formulation containing CMG (MF1 to MF2) and Carbopol 934 (MF5 to MF6). A controlled release pattern of drug and mucoadhesion was observed from the batch MF4 throughout the 24 h of study. The formulation batch was subjected to various release models. The r^2 value of the Higuchi matrix was close to 1, the drug release follows matrix diffusion and erosion kinetics.

3.4.7. *In vivo* Pharmacokinetic Study

From the results obtained from *in vivo* pharmacokinetic study it was observed that MF4 exhibited AUC_{0-t} of 479.91±3.9 µg.hr/ml, which was four folds higher than MF6, AUC_{0-t} 120.69±2.3 µg.hr/ml. As shown in Table 5, MF4 attained higher concentration (C_{max}) of 124.13±2.5 µg/ml as compared to MF6 showing C_{max} of 69.88 ±0.9 µg/ml. The AUC for MF4 was significantly higher ($P<0.001$) than that of MF6, indicating improved bioavailability for LP. Thus, it can be concluded on the basis of pharmacokinetic study that SCMG (MF4) could be a promising mucoadhesive polymer that can be employed successfully to formulate modified drug delivery system of LP for better management and control of hypertension.

Table 3. Precompression Parameters of Powder Blend

Formulation Code	Bulk Density (g/cc)	Tapped Density	Hausner's Ratio	Carr's Index	Angle of Repose(°)
MF1	0.42 ± 0.01	0.51 ± 0.02	1.21 ± 0.02	17.64 ± 0.23	26.3 ± 2.32
MF2	0.46 ± 0.03	0.58 ± 0.03	1.26 ± 0.02	20.68 ± 0.17	29.5 ± 2.56
MF3	0.43 ± 0.02	0.50 ± 0.01	1.16 ± 0.01	14 ± 0.13	23.2 ± 0.26
MF4	0.45 ± 0.02	0.54 ± 0.03	1.20 ± 0.02	16.66 ± 0.43	28.9 ± 1.76
MF5	0.49 ± 0.02	0.52 ± 0.02	1.06 ± 0.02	5.76 ± 0.35	24.8 ± 1.23
MF6	0.48 ± 0.02	0.56 ± 0.02	1.16 ± 0.02	14.28 ± 0.27	26.5 ± 1.41

*All the values are expressed as mean ± SD, n=3. SD: Standard Deviation

Table 4. Physicochemical Evaluation Parameters of Losartan Potassium Mucoadhesive Tablets

Formulation Batches	Weight Variation (mg)	Hardness (kg/cm^2)	Friability	Drug Content (%)	*Ex vivo* Mucoadhesion Strength (g)	*Ex vivo* Mucoadhesion Time (h)
MF1	203 ± 2.2	6.3 ± 0.6	0.37 ± 0.04	99.15 ± 1.4	16.98 ± 1.2	4 ± 0.42
MF2	202 ± 1.6	6.1 ± 0.5	0.39 ± 0.06	103.53 ± 2.2	24.76 ± 1.6	6 ± 0.36
MF3	201 ± 1.8	6.4 ± 0.5	0.44 ± 0.04	98.72 ± 1.4	34.62 ± 2.2	10 ± 0.69
MF4	203 ± 2.4	6.2 ± 0.4	0.36 ± 0.02	99.66 ± 1.8	62.51 ± 2.6	24 ± 0.75
MF5	202 ± 1.6	6.4 ± 0.6	0.47 ± 0.06	101.88 ± 2.0	19.56 ± 1.2	6 ± 0.45
MF6	198 ± 2.1	6.3 ± 0.4	0.35 ± 0.02	98.35 ± 1.6	32.18 ± 1.6	8 ± 0.61

Table 5. Pharmacokinetic parameters of LP from MF 4 and MF 6 after single oral administration in rats

Formulation	Cmax (µg/mL)	Tmax (h)	AUC_{0-t} (µg.hr/ml)	$AUC_{0-\infty}$
MF 4	124.13 ± 2.5	6.72 ± 0.2	479.91 ± 3.9	587.40 ± 4.8
MF 6	69.88 ± 0.9	1.24 ± 0.1	120.69 ± 2.3	132.56 ± 2.6

Data represented as mean ± SD (n=3).

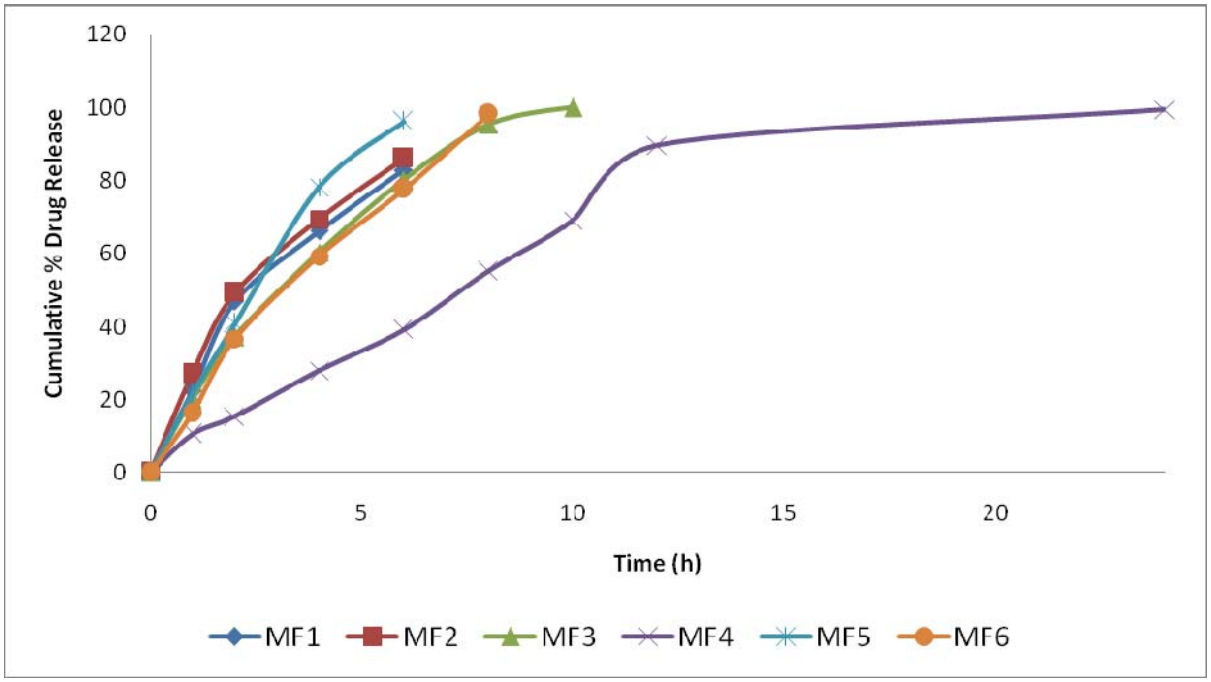

Figure 5. *In vitro* drug release data of various formulation batches (MF1-MF6) prepared using different concentrations of CMG, SCMG & Carbopol 934.

4. CONCLUSION

In the present study, central composite experimental design proved to be useful for the optimization of the reaction conditions for sulfation of CMG. Molarity of sulphuric acid, temperature and time of reaction played a vital role in influencing the DS. The prepared mucoadhesive tablets facilitated an intimate contact with the absorbing surfaces of mucous membrane and thus the gastric residence could be prolonged to deliver LP at a controlled rate at the absorption site to produce optimal therapeutic effect, minimizing the dosing frequency and enhancing the patient compliance. This gives an insight that the sulfated *Cordia myxa* (MF4) gum could be successfully employed for stomach specific mucoadhesion and controlled drug release profile of LP.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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