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Calcium pectinate-fenugreek seed mucilage mucoadhesive beads for controlled delivery of metformin HCl

Amit Kumar Nayak[a], Dilipkumar Pal[b,*], Sabinesh Das[a]

[a] Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj 757086, Odisha, India
[b] Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur 495009, C.G., India

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ABSTRACT

Calcium pectinate-fenugreek seed mucilage (FSM) mucoadhesive beads containing metformin HCl was developed through ionic-gelation. Effects of pectin and FSM amounts on drug encapsulation efficiency (DEE) and cumulative drug release at 10 h ($R_{10\,h}$) were optimized using 3^2 factorial design. The DEE (%) was within the range of 63.16 ± 2.88 to 96.03 ± 4.67% (w/w). The *in vitro* drug release from these beads (56.64 ± 1.47 to 93.63 ± 4.52% over 10 h) was followed controlled-release (zero-order) pattern ($R^2 = 0.993$ to 0.997) with super case-II transport mechanism. The average size of beads was within 1.47 ± 0.14 to 2.08 ± 0.18 mm. The beads were also characterized by SEM and FTIR. The swelling and mucoadhesivity of these beads were influenced by pH of the medium. The optimized beads also exhibited good mucoadhesivity and significant hypoglycemic effect in alloxan-induced diabetic rats over prolonged period after oral administration.

1. Introduction

Natural excipients are attractive alternatives to the synthetic excipients, because of their easy availability, biocompatibility, biodegradability, low toxicity, environmental friendliness and low price (Avachat, Dash, & Shrotriya, 2011; Maiti et al., 2010; Satturwar, Fulzele, & Dorle, 2003). The ability to produce a wide range of excipients based on their properties and molecular weight, natural excipients have became a thrust area in majority of investigations related with the development of drug delivery systems (Edwin, Edwin, Dosi, Raj, & Gupta, 2007).

Mucilages are naturally occurring, high molecular weight polyuroides consisting of sugars and uronic acid units (Singh, Kumar, Longyan, & Ahuja, 2009). They are normal physiological metabolism products formed within the cell/deposited on it in layers. Plant mucilages serve as food reserve, membrane thickeners and seed germination (Singh et al., 2009). Plant mucilages are important polysaccharides with wide range of pharmaceutical applications such as thickeners, binding agents, water retention agents, emulsion stabilizers, suspending agents, disintegrating agents, film formers, matrix formers, *etc.* (Avachat et al., 2011; Edwin et al., 2007; Kulkarni, Gowthmarajan, Rao, & Suresh, 2002; Malviya, Srivastava, & Kulkarni, 2011; Nayak, Pal, Pany, & Mohanty, 2010; Nerkar & Gattani, 2011; Pal, Nayak, & Kalia, 2010; Singh et al., 2009).

Recent years, a great deal of attention has been paid to the development of mucoadhesive hydrogel beads using plant-derived mucilages for controlled release drug delivery (Nayak, Pal, Pradhan, & Hasnain, 2013; Nerkar & Gattani, 2011; Sharma & Bhattacharya, 2008). Most of these hydrogel beads for mucoadhesive drug delivery were prepared through physical cross-linking by ionic gelation technique. Through the ionotropic gelation technique, the drug encapsulation within these beads is achieved in an eco-friendly environment avoiding the possible toxicity of reagents used in chemical cross-linking (Nayak, Hasnain, Beg, & Alam, 2010; Nayak et al., 2013; Sharma & Bhattacharya, 2008). In these formulations, plant mucilages are mainly blended with various ionic polymers before their cross-linking.

Fenugreek seed mucilages (FSM), isolated from methi (*Trigonella foenum-graecum* L.) seeds, is already investigated as mucoadhesive gelling agent (Dutta & Bandyopadhyay, 2005), binding agent (Sabale, Patel, Paranjape, & Sabale, 2009) and disintegrating agent (Kumar, Patil, Patil, Patil, & Paschapur, 2009). It is also reported that the FSM has laxative and antidiabetic properties (Kumar, Shetty, Sambaiah, & Salimath, 2005; Riad & El-Baradie, 1959). In an investigation by our research group, we have already investigate the utility of FSM, as a mucoadhesive polymer-blend with sodium alginate for the development of mucoadhesive beads by

* Corresponding author at: Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur 495009, C.G., India. Tel.: +91 7389263761.
E-mail addresses: drdilip2003@yahoo.co.in, drdilip71@gmail.com (D. Pal).

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ionic gelation technique for the use in oral delivery of metformin HCl (Nayak et al., 2013). To the best of our knowledge, no report is available in the previous literature on the development of mucoadhesive beads composed of FSM and pectin for controlled drug release.

Pectin is an acidic water soluble, inexpensive, non-toxic, heterogenous polysaccharide (Atyabi, Inanloo, & Dinarvand, 2005). It consists mainly of linearly connected (1–4) linked α-D-galacturonic acid residues interrupted by some rhamnogalacturonic acid residues and α-L-rhamnopyranose by α-(1–2) linkage (Sharma & Ahuja, 2011). Pectin can be classified as low methoxyl (LM, with a 25–50% degree of methylation) and high methoxyl (HM, with a 50–80% degree of methoxylation) pectins (Lee, Kim, Chung, & Lee, 2009). LM pectin can form a gel structure, a so-called 'egg-box model' by ionic gelation with various divalent cations like Ca^{2+}, Zn^{2+}, *etc.* (Das, Ng, & Ho, 2010; Sriamornsak, Nunthanid, Cheewatanakornkool, & Manchun, 2010). During last few decades', various ionically gelled pectinate beads has been widely investigated in the design of oral drug delivery carriers (Lee, Chung, & Lee, 2008; Sriamornsak et al., 2010; Das et al., 2010). Unfortunately, these ionically gelled calcium pectinate beads experienced with low drug entrapment efficiency and premature drug release of incorporated small molecular drugs (Lee et al., 2008). To overcome these drawbacks, several modifications of calcium pectinate gel beads have been investigated by various research groups (Atyabi, Majzoob, Iman, Salehi, & Dorkoosh, 2005; Chakraborty et al., 2010; Desai, 2005; Sharma & Ahuja, 2011). In the present study, we made an attempt to investigate the development of ionically gelled mucoadhesive calcium pectinate-FSM beads for controlled drug release.

In the current study, metformin HCl was investigated as model drug. Metformin HCl is a biguanide antihyperglycemic agent, which is widely used in the management of non-insulin dependent diabetes mellitus (NIDDM, Type-II) (Basak, Kumar, & Ramalingam, 2008; Maji, Ray, Das, & Nayak, 2012; Nayak & Pal, 2013). It has biological half-life of 1.5–1.6 h and daily requirement of it is 1.5–3 g/day (Nath, Nath, Mazumdar, Sharma, & Sarkar, 2009). The main absorption site of metformin HCl is proximal small intestine (Nagarwal, Srinatha, & Pandit, 2009). Hence, this would be beneficial to develop a mucoadhesive polymeric beads containing metformin HCl using pectinate-FSM blend through ionic gelation technique. This type of ionically gelled mucoadhesive beads might facilitate an intimate contact with the absorbing surfaces of mucous membranes (*i.e.*, mucoadhesion) and thus, the gastric residence could be prolonged to release the encapsulated drug at the drug absorbing site at a controlled rate to maximize the therapeutic effect. A computer-aided optimization technique using response surface methodology based on 3^2 (two factors and three levels) factorial design was employed to investigate the composition of polymer-blend on the properties of calcium pectinate-FSM mucoadhesive beads containing metformin HCl relating to drug encapsulation and drug release.

2. Experimental

2.1. Materials

Metformin HCl (Abhilash Chemicals Pvt. Ltd., India), low methoxy pectin (MW ~30,000–100,000, Loba-chemie, India), calcium chloride (Merck Ltd., India) were used. FSM was isolated from the fenugreek (*T. foenum-graecum* L.) seeds, which were collected from Jharpokharia market in the month of September 2011. All other chemicals and reagents were commercially available and of analytical grade.

2.2. Isolation of FSM from fenugreek seeds

FSM was isolated from fenugreek seeds according to the previously reported literature (Nayak et al., 2013). Fenugreek seeds (200 g) were soaked in distilled water (1.5 l) at room temperature for 12 h and boiled using water bath until the preparation of slurry. After cooling, the slurry was cooled and kept in refrigerator overnight to settle out undissolved materials. The upper clear solution was decanted and concentrated at 60 °C by water bath to 1/3rd of its original volume. The solution was cooled at room temperature and poured into thrice the volume of acetone with continuous stirring. The precipitate was washed repeatedly with acetone and dried at room temperature for 24 h. The isolated material was passed though sieve number 80 and stored in desiccators until further use.

2.3. Preparation of calcium pectinate-FSM beads containing metformin HCl

Calcium pectinate-FSM beads containing metformin HCl were prepared using calcium chloride ($CaCl_2$) as cross-linking agent by ionic gelation method. Briefly, required amounts of LM pectin and FSM were dissolved in deionized water (20 ml) using magnetic stirring for 30 min. Afterwards, metformin HCl was added to the mixture solutions of pectin-FSM for each formulation maintaining polymer to drug ration 2:1 and mixed thoroughly using a homogenizer (Remi Motors, India). The final pectin-FSM mixture solutions containing metformin HCl were ultra-sonicated for 5 min for debubbling. The resulting dispersion was then added *via* a 21-gauge needle drop wise into 100 ml of 5% (w/v) $CaCl_2$ solution. Added droplets were retained in the $CaCl_2$ solution for 15 min to complete the curing reaction. The wet beads were collected by decantation. These wet beads were washed two times with distilled water and dried at 37 °C for overnight. The dried beads were stored in a desiccator until used.

2.4. Experimental design for optimization

To reduce the number of trials necessary to attain maximum numbers of information on product properties, the screening was performed applying a 3^2 factorial design. The amount of pectin (X_1, 650–750 mg) and FSM (X_2, 100–200 mg) as polymeric blend were defined as the selected independent variables, which were varied at three levels, low level (−1), medium level (0) and high level (+1). The drug encapsulation efficiency (DEE, %) and cumulative drug release after 10 h ($R_{10\,h}$, %) were used as dependent variables (responses). Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA) was used for the generation and evaluation of the statistical experimental design. The matrix of the design including investigated responses *i.e.*, DEE (%) and $R_{10\,h}$ (%) are shown in Table 1.

For optimization, the effects of independent variables upon the responses were modeled using quadratic mathematical model generated by 3^2 factorial design (Guru, Nayak, & Sahu, 2013; Malakar, Nayak, & Pal, 2012):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2$$

where, Y is the response; b_0 is the intercept and b_1, b_2, b_3, b_4, b_5 are regression coefficients. X_1 and X_2 are individual effects; X_1^2 and X_2^2 are quadratic effects; X_1X_2 is the interaction effect. One-way ANOVA was applied to estimate the significance of the model ($p < 0.05$).

Table 1
Experimental plan of 3^2 full factorial design (coded values in bracket) with observed response values with bead size for different formulations of calcium pectinate-FSM beads containing metformin HCl.

Codes	Factors with normalized levels		Responses[a]		Bead diameter (mm)[b]
	Pectin (mg, X_1)	FSM (mg, X_2)	DEE (%)	R_{10h} (%)	
F-1	650.00 (−1)	100.00 (−1)	63.16 ± 2.88	93.63 ± 4.52	1.47 ± 0.14
F-2	650.00 (−1)	150.00 (0)	66.22 ± 2.85	87.94 ± 3.26	1.54 ± 0.14
F-3	650.00 (−1)	200.00 (+1)	71.97 ± 2.23	82.07 ± 2.86	1.77 ± 0.20
F-4	700.00 (0)	100.00 (−1)	65.88 ± 2.73	86.44 ± 3.30	1.52 ± 0.12
F-5	700.00 (0)	150.00 (0)	68.19 ± 2.51	80.20 ± 2.08	1.65 ± 0.14
F-6	700.00 (0)	200.00 (+1)	72.48 ± 2.02	73.93 ± 2.47	1.82 ± 0.19
F-7	750.00 (+1)	100.00 (−1)	69.07 ± 1.92	79.58 ± 2.68	1.68 ± 0.15
F-8	750.00 (+1)	150.00 (0)	71.08 ± 1.99	73.28 ± 2.34	1.77 ± 0.17
F-9	750.00 (+1)	200.00 (+1)	74.77 ± 3.04	66.70 ± 1.88	1.92 ± 0.19
			Observed values		
F-O	698.31	348.72	96.03 ± 4.67	56.64 ± 1.47	2.08 ± 0.18
			Predicted values		
			98.87	54.77	
% Error[c]			−2.87	3.41	

[a] Mean ± S.D., $n = 3$.
[b] Mean ± S.D., $n = 20$.
[c] Error (%) = (observed value − predicted value)/predicted value × 100.

2.5. Determination of DEE

100 mg of beads were taken and were crushed using pestle and mortar. The crushed powders of drug containing beads were placed in a 250 ml volumetric flask and the volume was made up to 250 ml by phosphate buffer, pH 7.4 and kept for 24 h with occasionally shaking at 37 ± 0.5 °C. After the stipulated time, the mixture was stirred at 500 rpm for 20 min using a magnetic stirrer (Remi Motors, India). The polymer debris formed after disintegration of bead was removed filtering through Whatman® filter paper (No. 40). The drug content in the filtrate was determined using a UV-VIS spectrophotometer (Shimadzu, Japan) at 233 nm against appropriate blank. The DEE (%) of these prepared beads was calculated by the following formula (Nayak, Das, & Maji, 2012):

$$\text{DEE}\,(\%) = \frac{\text{Actual drug content in beads}}{\text{Theroretical drug content in beads}} \times 100$$

2.6. Bead size measurement

Particle size of 100 dried beads from each batch was measured by optical microscopic method for average particle size using an optical microscope (Olympus). The ocular micrometer was previously calibrated by stage micrometer.

2.7. Scanning electron microscopy (SEM) analyses

FSM-alginate beads containing metformin HCl were gold coated by mounted on a brass stub using double-sided adhesive tape and under vacuum in an ion sputter with a thin layer of gold (3–5 nm) for 75 s and at 20 kV to make them electrically conductive and their morphology was examined by scanning electron microscope (ZEOL, JSM-5800, Japan).

2.8. Fourier transform-infrared (FTIR) spectroscopy analyses

Samples were reduced to powder and analyzed as KBr pellets by using a Fourier transform-infrared (FTIR) spectroscope (Perkin Elmer Spectrum RX I, USA). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 3600 cm^{-1} and 500 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 1 cm/s.

2.9. In vitro drug release studies

The release of metformin HCl from various calcium pectinate-FSM beads was tested using dissolution apparatus USP (Campbell Electronics, India). The baskets were covered with 100-mesh nylon cloth to prevent the escape of the beads. The dissolution rates were measured at 37 ± 1 °C under 50 rpm speed. Accurately weighed quantities of beads containing metformin HCl equivalent to 100 mg were added to 900 ml of 0.1 N HCl (pH 1.2). The test was carried out for 2 h and then continued in phosphate buffer (pH 7.4) for next 8 h. 5 ml of aliquots was collected at regular time intervals, and the same amount of fresh dissolution medium was replaced into dissolution vessel to maintain the sink condition throughout the experiment. The collected aliquots were filtered and suitably diluted to determine the absorbance using a UV–vis spectrophotometer (Shimadzu, Japan) at 233 nm against appropriate blank.

2.10. Analysis of in vitro drug release kinetics and mechanism

In order to predict and correlate the *in vitro* release behavior of metformin HCl from formulated calcium pectinate-FSM beads, it is necessary to fit into a suitable mathematical model. The *in vitro* drug release data were evaluated kinetically in important mathematical models (Nayak & Pal, 2011; Nayak, Khatua, Hasnain, & Sen, 2011; Pal & Nayak, 2011):

Zero-order model: $Q = kt + Q_0$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.
First-order model: $Q = Q_0\, e^{k \cdot t}$, where Q represents the drug released amount in time t and Q_0 is the start value of Q; k is the rate constant.
Higuchi model: $Q = kt^{0.5}$, where Q represents the drug released amount in time t and k is the rate constant.
Korsmeyer–Peppas model: $Q = kt^n$, where Q represents the drug released amount in time t, k is the rate constant and n is the release exponent, indicative of drug release mechanism.

The accuracy and prediction ability of these models were compared by calculation of squared correlation coefficient (R^2) (Nayak & Pal, 2011; Pal & Nayak, 2011). Again, the Korsmeyer–Peppas model was employed in the *in vitro* drug release behavior analysis of these formulations to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport) and case-II transport

(relaxation-controlled release). When n is $\leq$0.43, it is Fickian release. The n value between 0.43 and 0.85 is defined as non-Fickian release. When, n value is $\geq$0.85, it is case-II transport (Pal & Nayak, 2011).

2.11. Evaluation of swelling behavior

Swelling behavior evaluation of optimized calcium pectinate-FSM beads containing metformin HCl were carried out in two different aqueous media: 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4). 100 mg beads were placed in vessels of dissolution apparatus (Campbell Electronics, India) containing 500 ml respective media. The experiment was carried out at 37 ± 1 °C under 50 rpm paddle speed. The swelled beads were removed at predetermined time interval and weighed after drying the surface by using tissue paper. Swelling index was determined using the following formula (Nayak et al., 2012):

$$\text{Swelling index}\,(\%) = \frac{\text{Weight of beads after swelling} - \text{Dry weight of beads}}{\text{Dry weight of beads}} \times 100$$

2.12. Ex vivo mucoadhesion testing

The mucoadhesive property of optimized calcium pectinate-FSM beads containing metformin HCl were evaluated by *ex vivo* wash-off method (Pal & Nayak, 2011). Freshly excised pieces of goat intestinal mucosa (2 cm × 2 cm) (collected from slaughter-house) were mounted on glass slide (7.5 cm × 2.5 cm) using thread. About 50 beads were spread onto the wet tissue specimen, and the prepared slide was hung onto a groove of disintegration test apparatus. The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4), separately, at 37 ± 0.5 °C. After regular time intervals, the machine was stopped and the number of beads still adhering to the tissue was counted.

2.13. Pharmacodynamic evaluation

In vivo pharmacodynamic studies were performed in alloxan-induced diabetic male albino rats of either sex (weighing 313–355 g). The acclimatized rats were kept fasting for 24 h with water *ad libitum*. All experiments were performed between 8 AM to 12 PM to minimize circadian influences. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared before starting. The experimental animals were handled as per guidelines of committee for the purpose of control and supervision on experimental animals (CPCSEA). All efforts were made to minimize both the suffering and number of animals used.

The male albino rats were made diabetic by intraperitoneal administration of freshly prepared alloxan solution at a dose of 150 mg/kg dissolved in 2 mM citrate buffer (pH 3.0). After one week of alloxan administration, alloxanized rats with fasting blood glucose of 300 mg/dl or more were considered diabetic and were employed in the study for 10 h. After initial collection of blood samples from the alloxan-induced diabetic rats, they were divided randomly into 2 groups of 6 rats each and treated as follow: Group A was administered with pure metformin HCl (100 mg/kg body weight) in suspension form and Group B were administered with optimized calcium pectinate-FSM beads containing metformin HCl, both at a dose equivalent to 100 mg metformin HCl/kg body weight using oral feeding needle. Blood samples were withdrawn (0.1 ml) from tail tip of each rat at regular time intervals under mild ether anesthesia, and were analyzed for blood glucose by oxidase-peroxidase method using commercial glucose kit. Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized calcium pectinate-FSM beads containing metformin HCl were evaluated.

2.14. Statistical analysis

Statistical optimization was performed using Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA). The *in vivo* data was tested for significant differences ($p < 0.05$) by paired samples t-test. All other data was analyzed with simple statistics. The simple statistical analysis and paired samples t-test were conducted using MedCalc software version 11.6.1.0.

3. Results and discussion

3.1. Optimization

The main challenge in pharmaceutical product development is to find the appropriate combination of variables that will yield the product with optimal quality. The conventional method of pharmaceutical optimization based on changing one variable at a time while keeping others fixed is laborious and time consuming (Nayak et al., 2012). This method is also requires a complete series of experiments for every variables (factors) of interest. Moreover, such a method does not provide means of observing possible interactions between variables. In contrast, factorial experimental designs offer a number of potential advantages. For instance, formulators could easily analyze the influence of individual variables with minimum experimentation and time, where all variables are studied in all possible combinations (Malakar & Nayak, 2012). Based on the factorial design experiment, the optimization technique encompasses factorial design experiment that will reliably measure responses, generating mathematical model equations using experimental data, conducting appropriate statistical tests to select best possible model and determining the values of independent variables to produce optimum responses (Guru et al., 2013).

For the 3^2 factorial design, 9 trial formulations were suggested by Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA) for two independent variables: amount of pectin (X_1, 650–750 mg) and FSM (X_2, 100–200 mg) as polymeric blend, which were varied at three levels: low level (−1), medium level (0) and high level (+1). The DEE (%) and $R_{10\,h}$ (%) were evaluated as dependent variables (responses). Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA) was used for the generation and evaluation of the statistical experimental design. According to this trial plan, calcium pectinate-FSM beads containing metformin HCl were prepared by ionic gelation technique. Overview of matrix of the design including investigated responses (*i.e.*, DEE and $R_{10\,h}$) is presented in Table 1. The values of investigated responses measured for all trial formulations were fitted in the 3^2 factorial design to get model equations for responses analyzed in this investigation.

The model equation relating DEE (%) became:

$$\text{DEE}\,(\%) = 106.35 - 0.20X_1 + 0.16X_2 - 3.11 \times 10^{-4}X_1X_2 + 2.11 \times 10^{-4}X_1^2 + 4.23 \times 10^{-4}X_2^2$$

[$R^2 = 0.998$; $F = 284.11$; $p < 0.05$]

The model equation relating $R_{10\,h}$ (%) became:

$$R_{10\,h}(\%) = 254.027 - 0.32X_1 - 0.02X_2 - 1.32 \times 10^{-4}X_1X_2 + 1.37 \times 10^{-4}X_1^2 - 3.27 \times 10^{-5}X_2^2$$

[$R^2 = 0.999$; $F = 9944.00$; $p < 0.05$]

Model simplification was carried out by eliminating non-significant terms ($p > 0.05$) in model equations resulting from the multiple regression analysis (Nayak & Pal, 2011), giving:

$$\text{DEE}(\%) = 106.35 - 0.20X_1 + 0.16X_2 - 3.11 \times 10^{-4}X_1X_2 + 4.23 \times 10^{-4}X_2^2$$

$$R_{10\,h}(\%) = 254.027 - 0.32X_1 - 0.02X_2 - 1.32 \times 10^{-4}X_1X_2 + 1.37 \times 10^{-4}X_1^2$$

The influences of main effects (factors) on responses (here, DEE and $R_{10\,h}$) were further elucidated by response surface methodology. Response surface methodology is a widely proficient approach in the development and optimization of drug delivery devices (Lee et al., 2008; Nayak & Pal, 2011; Shivkumar, Patel, Desai, Ahsok, & Arulmozhi, 2007). The purpose of the response surface methodology is to understand a model as fully as possible the effect of factors and their levels, over the whole of the experimental domain, and to predict the response inside the domain. Moreover, it can be used for optimizing a formula (*i.e.* maximizing one or more of the responses, keeping the formulation variable setting within a satisfactory range), carrying out simulations with the model equations and plotting the responses. The three-dimensional response surface graph is very useful in learning about the main and interaction effects of the independent variables (factors), whereas two-dimensional contour plot gives a visual representation of values of the response (Malakar et al., 2012). The three-dimensional response surface plots (Fig. 1a and b) and corresponding contour plots (Fig. 1c and d) were presented to estimate the effects of the independent variables (factors) on each response investigated. The three-dimensional response surface graph relating DEE (Fig. 1a) indicates the increment in both the values with the increasing of pectin amount (X_1) and FSM amount (X_2) in the formulated calcium pectinate-FSM beads containing metformin HCl prepared by ionotropic gelation technique. However, a decrease in $R_{10\,h}$ values with the increasing pectin amount (X_1) and FSM amount (X_2) is indicated by the three-dimensional response surface graph relating $R_{10\,h}$ (Fig. 1b). All the contour plots relating measured responses (Fig. 2a and b) indicate nonlinear relationships between two independable variables (here, pectin amount, X_1 and FSM amount, X_2) on all measured responses, investigated in this study.

A numerical optimization technique using the desirability approach was employed to develop new formulations with desired response (optimum quality). The desirable ranges of the undependable variables (factors) were restricted to $650 \leq X_1 \leq 700$ mg and $250 \leq X_2 \leq 350$ mg; whereas the desirable ranges of responses were restricted to $95 \leq \text{DEE} \leq 100\%$ and $50 \leq R_{10\,h} \leq 550\%$. The optimal values of responses were obtained by numerical analysis using the Design-Expert 8.0.6.1 software based on the criterion of desirability. The desirability plot indicating desirable regression ranges for optimal process variable settings and overlay plot indicating the region of optimal process variable settings was presented in Fig. 1e and f, respectively.

In order to evaluate the optimization capability of the mathematical models generated according to the results of 3^2 factorial design, optimized calcium pectinate-FSM beads containing metformin HCl were prepared using one of the optimal process variable settings proposed by the design ($R^2 = 1$). The selected optimal process variable setting used for the formulation of optimized formulation was $X_1 = 698.31$ mg and $X_2 = 348.72$ mg. Optimized calcium pectinate-FSM beads containing metformin HCl (F-O) were evaluated for DEE (%) and $R_{10\,h}$ (%). The optimized beads containing metformin HCl (F-O) showed DEE of $96.03 \pm 4.67\%$ and $R_{10\,h}$ of $56.64 \pm 1.47\%$ with small percentage error-values (−2.87 and 3.41, respectively) (Table 1). Percentage error evaluation is helpful in establishing the validity of generated model equations to describe the domain of applicability of optimization model. The percentage error values indicate that mathematical models obtained from the full 3^2 factorial design were well fitted.

3.2. DEE

The DEE (%) of all these calcium pectinate-FSM beads containing metformin HCl ranged 63.16 ± 2.88 to $96.03 \pm 4.67\%$ (w/w) (Table 1). It was observed that the drug encapsulation in these beads containing aceclofenac was increased with the increasing of both pectin and FSM amount as polymer-blend. The increased DEE with the increasing amount of both pectin and FSM in these newly developed beads could be due to the increase in viscosity of the polymer-blend solutions with the increasing amount of polymer addition. This might have been prevented drug leaching from the prepared beads to the cross-linking solution. In addition, the increasing amount of pectin in polymer-blend solution might have been elevated the cross-linking by $CaCl_2$ through availing more numbers anionic of sites of pectin molecules for ionic cross-linking by calcium ions.

3.3. Bead size

The average size of these formulated dried beads containing metformin HCl ranged from 1.47 ± 0.14 to 2.08 ± 0.18 mm (Table 1). Increasing the bead size was found with the increasing amount of the polymers, pectin and FSM into bead formulations containing metformin HCl. This might be attributed due to the increase in viscosity of the polymer-blend (pectin and FSM) solutions with incorporation of both the polymers in increasing ratio that in turn increased the droplet size during addition of the polymer blend solution to the cross-linking solution.

3.4. SEM analyses

The morphological analysis of the optimized calcium pectinate-FSM beads containing metformin HCl (F-O) was visualized by SEM and is presented in Fig. 2. The SEM photograph of these beads showed spherical shape with a rough surface. The surface of these beads exhibited very rough surface with characteristic large wrinkles and cracks. These cracks and wrinkles may be caused by partly collapsing the polymeric gel network during drying (Nayak et al., 2013). However, polymeric derbies were seen on the bead surface, which could be due to the method of preparation (*i.e.*, simultaneous gel bead preparation and formation of the polymer-blend matrix).

3.5. FTIR spectroscopy analyses

The Fourier transform-infrared spectra (FTIR) of pectin, FSM, calcium pectinate-FSM blank beads, calcium pectinate-FSM beads containing metformin HCl, and metformin HCl are shown in Fig. 3. The FTIR spectra of pectin showed principal absorption peaks at 3400.50 cm^{-1} and 2933.00 cm^{-1} that was due to —OH and —CH stretching vibration peaks. The peaks at 1459.27 cm^{-1} and 1356.45 cm^{-1} could be assigned to CH_2 and —OH bending vibration peaks respectively. In the FTIR spectrum of isolated FSM showed characteristic peaks of —OH between 3511.6 cm^{-1} and 3154.3 cm^{-1}, —CH_3 at 2925.48 cm^{-1}, —CH stretching between 2920.0 cm^{-1} and 2852.4 cm^{-1}, ether linkage at 1455–1400 cm^{-1} and —CO stretching at 1017.7 cm^{-1}. The FTIR spectrum of calcium pectinate-FSM blank beads showed all characteristic peaks of both pectin and FSM without any significant interaction. In the FTIR spectrum of metformin HCl, the principal absorption peaks appeared at 3169 cm^{-1} due to the N—H stretching of the primary amine group

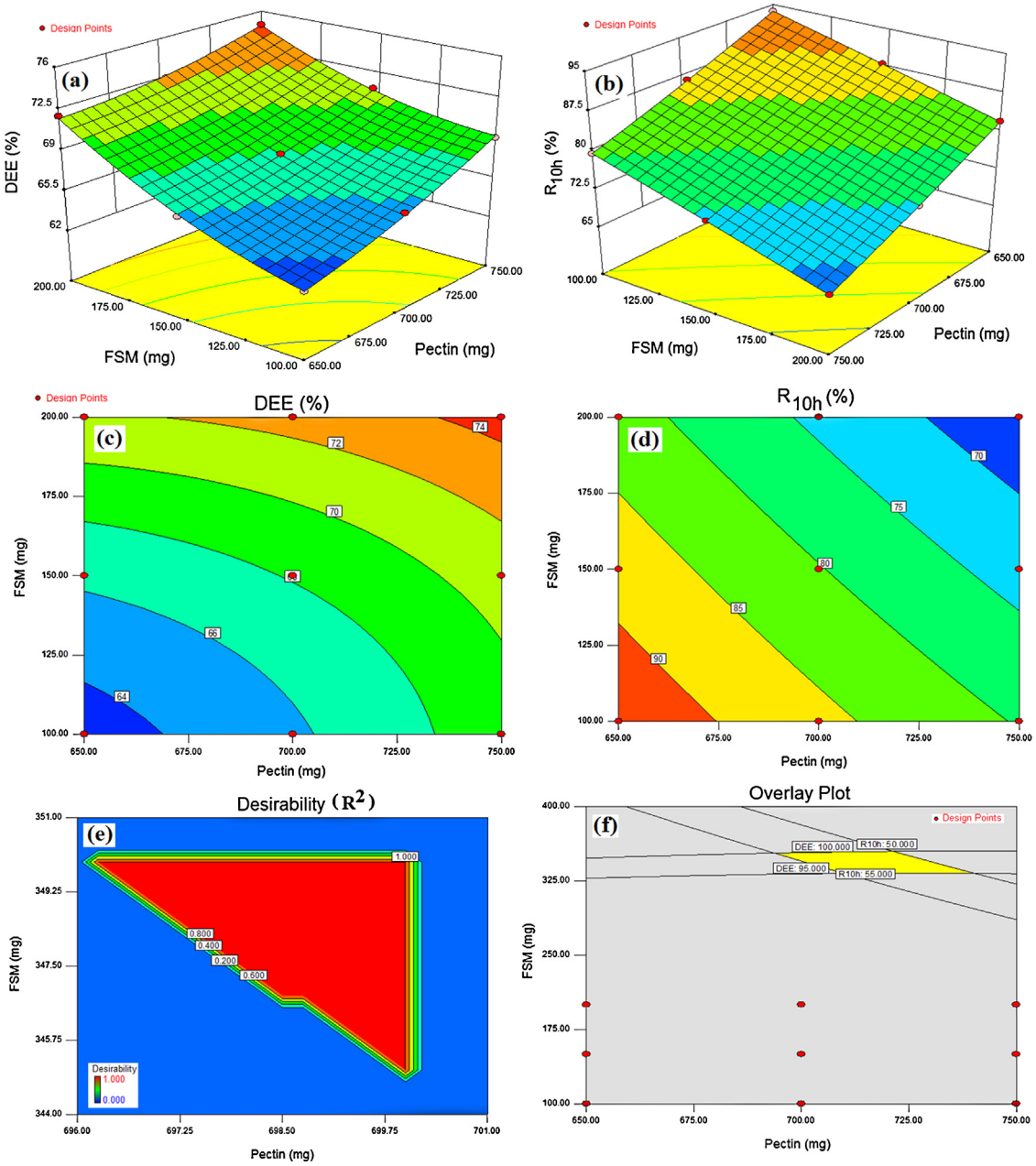

Fig. 1. Three-dimensional response surface plots showing the effects of amount of pectin (mg) and FSM (mg) on (a) DEE (%) and (b) R_{10h} (%); two-dimensional corresponding contour plots showing the effects of amount of pectin (mg) and FSM (mg) (c) DEE (%) and (d) R_{6h} (%); the desirability plot (c) indicating desirable regression ranges and the overlay plot (d) indicating the region of optimal process variable settings.

($-NH_2$) and at 1063 cm^{-1} due to C—N stretching and a peak at 1584 cm^{-1} occurs due to N—H bending vibrations of the primary amine group. In the FTIR spectrum of calcium pectinate-FSM beads containing metformin HCl, various characteristic peaks of pectin, FSM and metformin HCl were appeared without any significant shifting. In short, the calcium pectinate-FSM beads containing metformin HCl had significant characters of metformin HCl in the FTIR spectrum, suggesting, there were no interaction between the drug, metformin HCl and the polymers used (pectin and FSM) in the bead formulation.

3.6. *In vitro* drug release

The *in vitro* drug release behavior of these newly developed ionically gelled calcium pectinate-FSM beads containing metformin HCl showed prolonged metformin HCl release over 10 h (Fig. 4). Metformin HCl release from these beads in the acidic medium was slow (less than 15% after 2 h) due to the shrinkage of pectinate gel at acidic pH. The trace amount of drug release could probably be due to the presence of drug crystals onto bead surface. After that, faster drug release was observed in phosphate buffer (pH, 7.4) comparatively, probably due to the higher swelling rate of these beads in phosphate buffer. The cumulative percentage drug released from calcium pectinate-FSM beads containing metformin HCl after 10 h (R_{10h}, %) was within the range of 56.64 ± 1.47 to 93.63 ± 4.52%. However, the drug release from these beads was also found to be delayed with the increasing of polymer amount. In case of beads containing higher polymer contents, the more hydrophilic property of the polymers could probably binds better with water to form viscous gel structure, which might blockade the pores on the surface of beads and

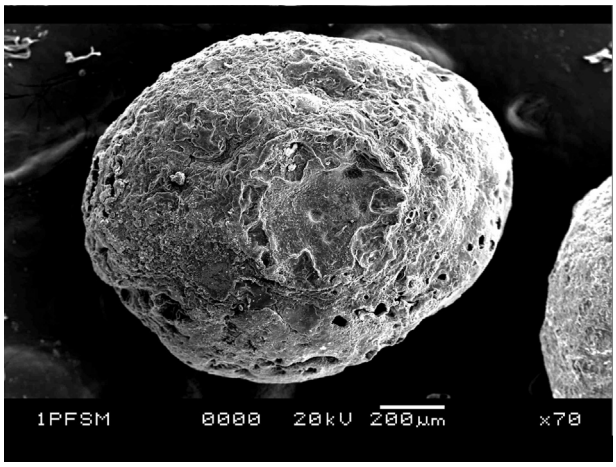

Fig. 2. Scanning electron microphotograph of the optimized calcium pectinate-FSM beads containing metformin HCl prepared through ionotropic gelation (F-O).

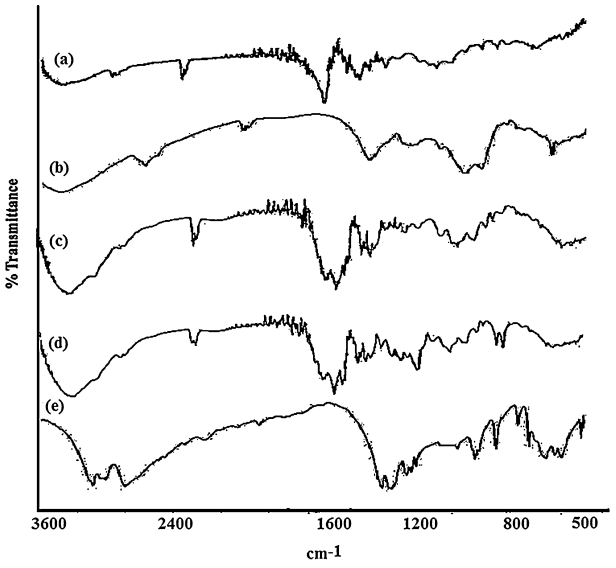

Fig. 3. FTIR spectra of (a) pectin, (b) isolated FSM, (c) calcium pectinate-FSM blank bead, (d) optimized calcium pectinate-FSM beads containing metformin HCl (F-O) and (e) pure metformin HCl.

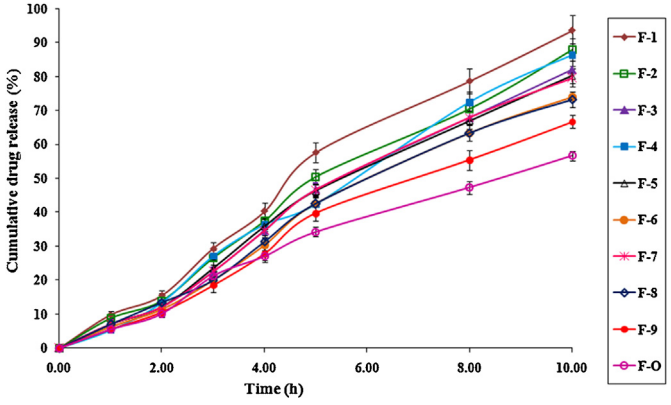

Fig. 4. *In vitro* drug release from various calcium pectinate-FSM beads containing metformin HCl prepared through ionotropic gelation [mean ± S.D., $n = 3$].

could delayed the drug release from these formulated beads. The another reasonable explanation of the delayed drug release can be attributed to increasing coating efficiency of the drug particles with the increasing polymer content employed in the formulation.

The *in vitro* drug release data from various calcium pectinate-FSM beads containing metformin HCl were evaluated kinetically using various mathematical models like zero order, first order, Higuchi and Korsmeyer–Peppas model. The results of the curve fitting into these above-mentioned mathematical models are given in Table 2. When respective correlation coefficients of these beads were compared, the metformin HCl release from these beads was found to follow zero order model ($R^2 = 0.993–0.997$) over a period of 10 h. In addition, Korsmeyer–Peppas model ($R^2 = 0.988–0.994$) was found to be closer to the best-fit zero order model. The best fit of zero order model indicated that the drug release from these beads followed controlled-release pattern. The values of release exponent (n) determined from *in vitro* drug release data of various calcium pectinate-FSM beads containing metformin HCl ranged from 1.03 to 1.14, indicating the drug release from these mucoadhesive beads followed the super case-II transport mechanism controlled by swelling and relaxation of polymeric-blend (calcium pectinate-FSM) matrix. This could be attributed due to polymer dissolution and enlargement or relaxation of polymeric chain.

3.7. Swelling behavior

The swelling index of optimized calcium pectinate-FSM beads containing metformin HCl was found lower in 0.1 N HCl, pH 1.2 in comparison with that of in phosphate buffer, pH 7.4, initially (Fig. 5a). This was occurred due to shrinkage of pectinate at acidic pH. Maximum swelling of these beads was noticed at 2–3 h in phosphate buffer, pH 7.4 and after which, erosion and dissolution took place. The swelling behavior of these beads containing metformin HCl in phosphate buffer, pH 7.4 could be explained by the ion exchanging between Ca^{2+} ions of the ionically cross-liked beads and the Na^{+} ions present in phosphate buffer with the influence of Ca^{2+}-sequestrant phosphate ions. This could result in disaggregation in the calcium pectinate-FSM matrix structure leading to matrix erosion and dissolution of the swollen beads.

3.8. Mucoadhesivity

The results of *ex vivo* wash-off test of optimized calcium pectinate-FSM beads containing metformin HCl using goat intestinal mucosa in gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4) are presented in Fig. 5b. The *ex vivo* wash off of these newly developed beads was found faster in intestinal pH than at gastric pH. In 0.1 N HCl. The percentage of beads adhering to the goat intestinal mucosal tissue varied from 65.88 ± 4.56% over 8 h, whereas, this was 33.35 ± 2.33% in phosphate buffer. The rapid wash-off observed at intestinal pH could be due to ionization of carboxyl and other functional groups of polymers, which increased their solubility with reduced adhesive strength. Therefore, the results of the wash off test confirmed that the developed optimized calcium pectinate-FSM beads containing metformin HCl had good mucoadhesivity.

3.9. Pharmacodynamic evaluation

The comparative *in vivo* blood glucose level and the mean percentage reduction in blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized calcium pectinate-FSM mucoadhesive beads containing metformin HCl is presented in Fig. 6(a and b, respectively). In case of the group treated with pure metformin HCl (Group A), a

Table 2
Results of curve fitting of the *in vitro* metformin HCl release data from various calcium pectinate-FSM beads containing metformin HCl.

Formulation code	Correlation coefficient (R^2)				Release exponent (n)
	Zero order	First order	Higuchi	Korsmeyer–Peppas	
F-1	0.996	0.902	0.607	0.991	1.03
F-2	0.996	0.912	0.527	0.992	1.09
F-3	0.997	0.900	0.553	0.992	1.10
F-4	0.995	0.868	0.550	0.988	1.09
F-5	0.996	0.899	0.548	0.991	1.10
F-6	0.993	0.915	0.532	0.994	1.10
F-7	0.996	0.908	0.544	0.992	1.11
F-8	0.995	0.915	0.534	0.994	1.11
F-9	0.996	0.912	0.527	0.992	1.12
F-O	0.994	0.916	0.522	0.993	1.14

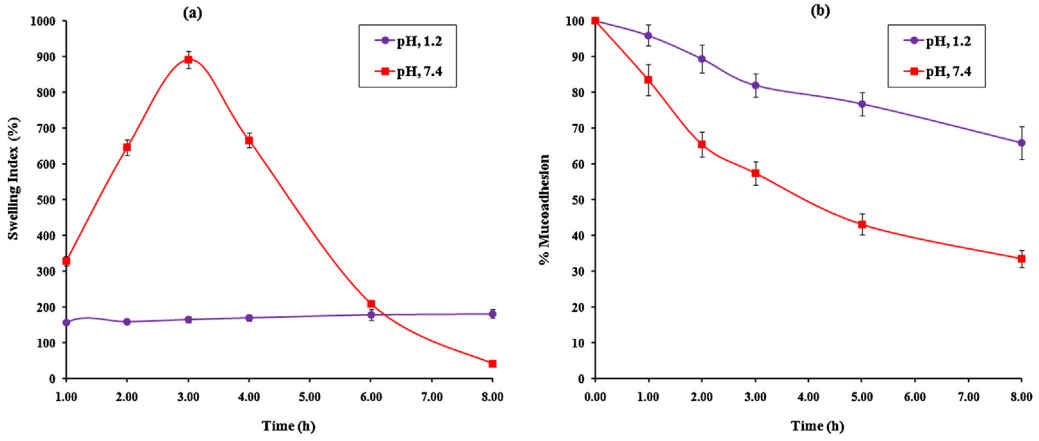

Fig. 5. (a) Swelling behavior of optimized calcium pectinate-FSM beads containing metformin HCl in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) [mean ± SD, $n = 3$]; (b) mucoadhesive behavior of optimized calcium pectinate-FSM beads containing metformin HCl in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) [mean ± SD, $n = 3$].

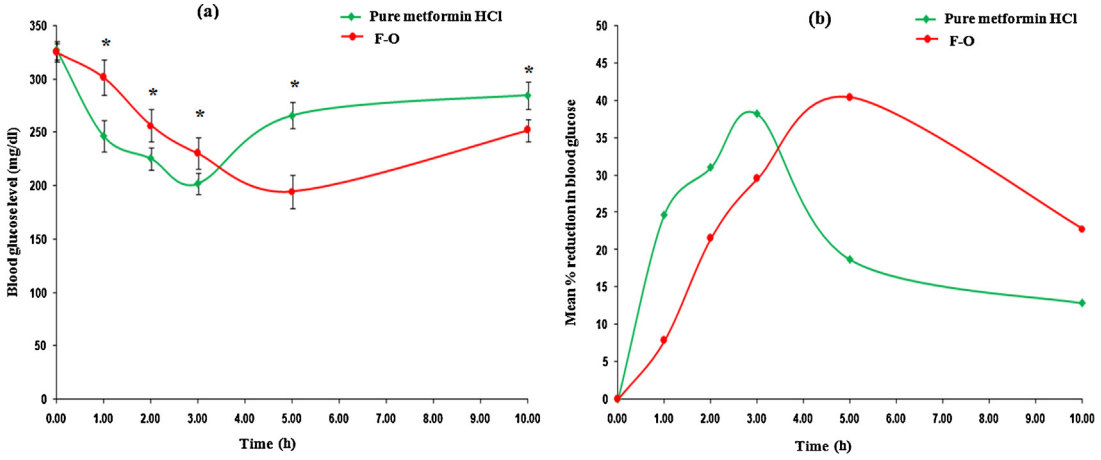

Fig. 6. (a) Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized calcium pectinate-FSM beads containing metformin HCl (F-O) [Mean ± S.D., $n = 6$]. The data were analyzed for significant differences (*$p < 0.05$) by paired samples t-test. The statistical analysis was conducted using MedCalc software version 11.6.1.0; (b) comparative *in vivo* mean percentage reduction in blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized calcium pectinate-FSM beads containing metformin HCl (F-O).

rapid reduction in blood glucose level was observed within 3 h of administration and after that, the blood glucose level recovered rapidly toward the normal level. In case of the group (Group B) treated with optimized mucoadhesive beads containing metformin HCl, the reduction in blood glucose level was found slower than that of the group treated with pure metformin HCl (Group A) up to 3 h. Significant differences ($p < 0.05$) were found between the blood glucose level after administration of pure metformin HCl and optimized JFSS-alginate mucoadhesive beads containing metformin HCl (F-O) at each time-point measured. However, the reductions in glucose level were increased gradually with the increment of time in case of Group B (treated with optimized calcium pectinate-FSM mucoadhesive beads containing metformin HCl) and were sustained over 10 h. A 25% reduction in glucose level is considered a significant hypoglycemic effect (Pal & Nayak, 2012). Therefore, the significant hypoglycemic effect by the calcium pectinate-FSM mucoadhesive beads containing metformin HCl (F-O) was observed over 10 h. In addition, the combined effect of metformin HCl and

FSM for assessing hypoglycemic effect in alloxan-induced diabetic rats could not be ruled out, as FSM possess antidiabetic property (Nayak et al., 2013).

4. Conclusion

These newly developed ionically gelled calcium pectinate-FSM mucoadhesive beads containing metformin HCl displayed high drug encapsulation, good mucoadhesivity with the biological membrane, suitable controlled *in vitro* drug release pattern and also significant hypoglycemic activity in alloxan-induced diabetic rats over prolonged period after oral administration, which could possibly be profitable in terms of prolonged systemic absorption of metformin HCl maintaining tight blood glucose level and advanced patient compliance. The ionic gelation technique for the preparation of these calcium pectinate-FSM mucoadhesive beads was found to be simple, reproducible, easily controllable, economical and consistent process. Additionally, the excipients used for the formulation of calcium pectinate-FSM mucoadhesive beads were cheap and easily available. This type of mucoadhesive beads can also be formulated to load other drugs demanding sustained release in controlled manner over a longer period to improve their bioavailability and therapeutic efficacy.

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