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Trigonella foenum-graecum L. seed mucilage-gellan mucoadhesive beads for controlled release of metformin HCl

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) seed mucilage (FSM)-gellan gum (GG) mucoadhesive beads containing metformin HCl for oral use were developed through ionotropic-gelation technique. Effects of GG to FSM ratio and cross-linker ($CaCl_2$) concentration on the drug encapsulation efficiency (DEE, %), and cumulative drug release after 10 h (R_{10h}, %) of ionotropically-gelled FSM-GG mucoadhesive beads containing metformin HCl were optimized by 3^2 factorial design. The optimized mucoadhesive beads showed DEE of 92.53 ± 3.85% and R_{10h} of 55.28 ± 1.58% and mean diameter of 1.62 ± 0.22 mm. The *in vitro* metformin HCl release from these ionotropically-gelled FSM-GG beads was prolonged over 10 h and followed zero-order model with super case-II transport mechanism. The optimized mucoadhesive beads also exhibited pH-dependent swelling, good mucoadhesivity with biological mucosal membrane and significant hypoglycemic effect in alloxan-induced diabetic rats over prolonged period after oral administration.

1. Introduction

Mucilages are naturally occurring, high molecular weight polyuroides consisting of sugars and uronic acid units (Singh, Kumar, Longyan, & Ahuja, 2009). They are normal physiological products formed within the cell/deposited on it in layers (Prajapati, Jani, Moradiya, & Randeria, 2013a). Plant-derived mucilages are important natural polysaccharides with wide range of pharmaceutical applications such as suspending agents, thickeners, emulsifying agents, binders, disintegrants, film-formers, matrix-formers, mucoadhesive agents, *etc.* (Kulkarni, Gowthmarajan, Rao, & Suresh, 2002; Prajapati, Prajapati, & Acharya, 2006; Edwin, Edwin, Dosi, Raj, & Gupta, 2007; Nayak, Pal, Pany, & Mohanty, 2010; Pal, Nayak, & Kalia, 2010; Saeedi, Morteza-Semnani, Ansoroudi, Fallah, & Amin, 2010; Avachat, Dash, & Shrotriya, 2011; Nerkar & Gattani, 2011; Prajapati et al., 2013a; Nayak, Pal, & Santra, 2013c). Recently, plant-derived mucilages have been used to develop various mucoadhesive beads as potential drug carriers for controlled drug delivery (Nayak, Pal, & Das, 2013a; Nayak, Pal, Pradhan, & Hasnain, 2013b; Nayak et al., 2013c; Nerkar and Gattani, 2011). However, most of these mucoadhesive beads were prepared through physical cross-linking by ionotropic-gelation technique (Nayak et al., 2013a,b,c). In these ionotropically-gelled mucoadhesive beads, plant-derived mucilages were blended with ionic polymers, mainly.

Fenugreek (*Trigonella foenum-graecum* L.) seed mucilage (FSM) is already investigated as suspending agent (Nayak, Das, & Maji, 2012), binder (Sabale, Patel, Paranjape, & Sabale, 2009), mucoadhesive gelling agent (Dutta & Bandyopadhyay, 2005) and disintegrating agent (Kumar, Patil, Patil, Patil, & Paschapur, 2009). It has antidiabetic property, too (Kumar, Shetty, Sambaiah, & Salimath, 2005). In previous reports, our research group has already reported the utility of FSM as mucoadhesive polymer-blends with two anionic polymers, namely sodium alginate and low methoxy pectin in the development of mucoadhesive beads through ionotropic-gelation technique for the use in oral drug delivery (Nayak et al., 2013a,b). However, no report is available in the literature on the development of mucoadhesive beads made of FSM-gellan gum (GG) blends for controlled drug release.

GG is an anionic, linear, exopolysaccharide, produced as a fermentation product by pure culture of *Psedomonus eloda* (aerobic, gram negative non-pathogenic bacterium) (Vijan, Kaity, Biswas, Isaac, & Ghosh, 2012). It is composed of the tetrasaccharide, (1 → 4)-L-rhamnose-α (1 → 3)-D-glucose-β-(1 → 4)-D-glucorunic acid-β-(1 → 4)-D-glucose as a repeating unit in the molar ratio of 2:1:1 (Emeje, Franklin-Ude, & Odoefule, 2010; Jana, Das, Nayak,

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Sen, & Basu, 2013). Its deacetylated form has excellent gelling ability with the influence of divalent or trivalent metal cations particularly Ca^{2+} and Al^{3+} ions (Babu, Sathigrahi, Kumar, & Pandit, 2010; Maiti, Ranjit, Mondol, Ray, & Sa, 2011). This gelation of deacetylated GG with the influence of divalent or trivalent metal cations is called ionotropic-gelation of GG (Agnihotri, Jawalkar, & Aminabhai, 2006). In view of this characteristic property of metal cation-induced ionotropic-gelation, it has been widely used in formulation of multiple-unit sustained drug release GG beads (Rajinikanth & Mishra, 2007; Babu et al., 2010; Narker, Sher, & Pawar, 2010; Maiti et al., 2011). These GG beads are stable in low pH; but, unstable in higher pH as these swell in rapid rate in higher pH than low pH (Babu et al., 2010). This phenomenon produces premature releasing of incorporated drugs from various ionotropically-gelled cross-linked GG beads in intestinal pH. Blending of other polymers with GG has been researched as a popular approach to limit the above discussed drawbacks (Ahuja, Yadav, & Kumar, 2010; Prajapati, Mashuru, Solanki, & Jani, 2013b). In the current investigation, we attempted to develop FSM-GG blend beads through ionotropic-gelation using metformin HCl as a model drug.

Metformin HCl is an orally administered biguanide, which is widely used in the management of non insulin dependent diabetes mellitus (NIDDM or Type II diabetes mellitus) alone or in combination with other hypoglycemic (Patel, Ray, & Thakur, 2006; Basak, Kumar, & Ramalingam, 2008; Boldhane & Kuchekar, 2009). It has a short biological half-life of 1.5–1.6 h and the daily requirement of it is 1.5–3 g/day (Nath, Nath, Mazumdar, Sharma, & Sarkar, 2009; Maji, Ray, Das, & Nayak, 2012). It is reported to be absorbed mainly in small proximal intestine at the upper part of gastrointestinal tract (GIT) (Nayak & Pal, 2013a; Nayak & Pal, 2013b). Therefore, it would be beneficial to develop mucoadhesive beads containing metformin HCl using FSM-GG blend, which might retain in the GIT for prolonged duration to facilitate an intimate adhesion with the absorbing surfaces of mucous membranes (*i.e.*, mucoadhesion). Thus, the gastric residence could be prolonged to release the encapsulated metformin HCl at the absorbing site a controlled manner to achieve maximum absorption and better bioavailability.

Optimization of a formulation using conventional approach involves studying the influence of the independent formulation factors by changing single factors at a time, while keeping others as constant (Malakar, Nayak, & Pal, 2012). This method is laborious, time consuming and incapable of effective optimization as it does not consider the interactive effects of all factors of interest (Nayak et al., 2012a,b). Therefore, it is important to understand the influence of factors on the formulation quality with a minimal number of experimental trials and subsequent selection of factors to develop optimized formulation using established statistical tools such as factorial design (Malakar et al., 2012). Factorial experimental designs are considered the most effective statistical optimization tools in estimating the effects of various formulation variables with minimum experimentation and time, where all factors are studied in all possible combinations by analyzing the influence of individual variables and their interactions using minimum experiments (Malakar & Nayak, 2012). Based on the factorial designs, the optimization technique encompasses the generation of model equations for investigated responses over the experimental design to determine the settings of factor values to achieve optimum formulation(s) (Guru, Nayak, & Sahu, 2013). In the current investigation, a 3^2 factorial (2 independent factors in different 3 levels) design-based computer-aided optimization was employed to investigate the effects of two independent factors, *i.e.*, GG to FSM ratio and cross-linker ($CaCl_2$) concentration on the properties of FSM-GG mucoadhesive beads containing metformin HCl relating to drug encapsulation and drug release.

2. Experimental

2.1. Materials

Metformin HCl (Abhilash Chemicals Pvt. Ltd., India), deacetylated gellan gum (GG) (SRL India Ltd., India), calcium chloride (Merck Ltd., India) were used. FSM was isolated from the fenugreek (*Trigonella foenum-graecum* L.) seeds, which were collected from Jharpokharia market in the month of September 2011. The procedure of FSM isolation has been described in previous report (Nayak et al., 2013b). All other chemicals and reagents were commercially available and of analytical grade.

2.2. Preparation of FSM-GG beads containing metformin HCl

The FSM-GG beads containing metformin HCl were prepared using calcium chloride ($CaCl_2$) as cross-linking agent through ionotropic-gelation. GG aqueous dispersions were prepared separately using distilled water by heating at 60 °C using magnetic stirrer (Remi Motors, India). On the other hand, FSM aqueous dispersions were prepared separately using distilled water at room temperature using magnetic stirrer. Both the dispersions were well mixed together with stirring for 10 min at 1000 rpm using magnetic stirrer to prepare FSM-GG dispersion mixtures maintaining 2% w/v polymer concentration in all formulations. Afterwards, metformin HCl was added to the dispersion mixture maintaining the ratio of drug to polymer 1:2 in all formulations. The final polymer-blend dispersion mixture of FSM-GG containing metformin HCl was homogenized for 20 min at 1000 rpm using a homogenizer (Remi Motors, India) and ultrasonicated for 5 min for debubbling. The resulting dispersion was then added via18-gauge needle. The added droplets were retained in the $CaCl_2$ solution for 15 min to complete the curing reaction and to produce rigid beads. The wet beads were collected by decantation, and washed two times with distilled water and dried at 40 °C for 24 h. The prepared dried FSM-GG beads containing metformin HCl were stored in a desiccator until used.

2.3. Experimental design for optimization

A two-factor, three-level factorial design (3^2) was employed for formulation optimization of FSM-GG beads containing metformin HCl, in which the GG to FSM ratio (X_1, 2–4) and concentration of cross-linker ($CaCl_2$) (X_2, 2–5% w/v) were selected as independent variables (factors) varied at three different levels: low (−1), medium (0), and high (+1) levels. The drug encapsulation efficiency (DEE, %), and cumulative drug release after 10 h (R_{10h}, %) 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_{10h} (%) are shown in Table 1. For optimization, the effects of independent variables upon the responses were modeled using quadratic mathematical model (Nayak, Pal, & Santra, 2014):

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

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 ($p < 0.05$) of the models, individual factors and interactions of individual factors. The surface response plots, and contour plots were analyzed to reveal the effect of independent factors on the measured responses.

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 FSM-GG beads containing metformin HCl.

Codes	Factors with normalized levels		Responses[a]		Bead diameter (mm)[b]
	GG to FSM ratio (X_1)	$CaCl_2$ concentration (X_2)	DEE (%)	R_{10h} (%)	
F-1	4 (+1)	5 (+1)	69.36 ± 2.27	69.55 ± 3.47	1.20 ± 0.11
F-2	4 (+1)	3.5 (0)	81.12 ± 2.43	75.33 ± 3.15	1.14 ± 0.14
F-3	4 (+1)	2 (−1)	85.96 ± 3.23	80.06 ± 3.36	1.09 ± 0.10
F-4	3 (0)	5 (+1)	76.23 ± 2.27	67.89 ± 2.31	1.15 ± 0.15
F-5	3 (0)	3.5 (0)	84.88 ± 2.54	72.22 ± 2.18	1.20 ± 0.15
F-6	3 (0)	2 (−1)	89.13 ± 3.42	75.32 ± 2.34	1.27 ± 0.20
F-7	2 (−1)	5 (+1)	80.17 ± 2.40	59.45 ± 1.97	1.24 ± 0.13
F-8	2 (−1)	3.5 (0)	88.82 ± 3.11	64.94 ± 2.36	1.32 ± 0.20
F-9	2 (−1)	2 (−1)	92.14 ± 3.56	66.62 ± 2.88	1.49 ± 0.23
F-O	1.04	1.96	Observed values		1.62 ± 0.22
			92.53 ± 3.85	55.28 ± 1.58	
			Predicted values		
			93.31	53.51	
% Error[c]	0.85	3.31			

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

2.4. 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 & Pal, 2013a):

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

2.5. 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.

2.6. Scanning electron microscopy (SEM) analyses

Samples 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 (JEOL, JSM-6360, Japan).

2.7. Fourier transform-infrared (FTIR) spectroscopy analyses

Samples were reduced to powder and analyzed as KBr pellets by using a Fourier transform-infrared (FTIR) spectroscope (PerkinElmer Spectrum RX I, USA). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 3800 and 600 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 1 cm/s.

2.8. In vitro drug release studies

The release of metformin HCl from various FSM-GG 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 FSM-GG 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 initial 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.

2.9. Analysis of in vitro drug release kinetics and mechanism

In order to predict and correlate the *in vitro* drug release behavior from formulated various FSM-GG beads containing metformin HCl, it is necessary to fit into a suitable mathematical model. The *in vitro* drug release data were evaluated kinetically using various important mathematical models like zero-order, first-order, Hixson-Crowell, Higuchi, and Korsmeyer–Peppas models (Nayak & Pal, 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: $Q = Q_0\ e^{kt}$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Hixson-Crowell model: $Q^{1/3} = kt + Q_0{}^{1/3}$; 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). 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 is $\geq$0.85, it is case-II transport (Nayak, Khatua, Hasnain, & Sen, 2011; Pal & Nayak, 2011).

2.10. Evaluation of swelling behavior

Swelling behavior evaluation of optimized FSM-GG 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., 2013a):

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

2.11. Ex vivo mucoadhesion testing

The mucoadhesive property of optimized FSM-GG beads containing metformin HCl were evaluated by *ex vivo* wash-off method (Pal & Nayak, 2012). Freshly excised pieces of goat intestinal mucosa (2 × 2 cm) (collected from slaughterhouse) were mounted on glass slide (7.5 × 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.

2.12. Pharmacodynamic evaluation

In vivo pharmacodynamic studies were performed in alloxan-induced diabetic male albino rats of either sex (weighing 332–363 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, alloxan-induced 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 FSM-GG 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 FSM-GG beads containing metformin HCl were evaluated.

2.13. 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. Preparation of FSM-GG beads containing metformin HCl

The FSM-GG beads containing metformin HCl was prepared using $CaCl_2$ as cross-linker. The gelation of polymer-blends in presence of Ca^{2+} ions is based on multivalent metal cation-induced ionotropic-gelation property of GG (Ahuja et al., 2010). The strategy employed for ensuring gelation of GG is similar to the beads preparation using anionic polysaccharides like sodium alginate, pectin, *etc.* The mechanism of ionotropic-gelation property of GG in presence of multivalent metal cations involves the formation of double helical junction zones followed by aggregation of double helical segments to form three-dimensional network by complexation with multivalent metal cations and hydrogen bonding with water (Hamcerencu, Desbrieres, Khoukh, Popa, & Riess, 2008). In the current investigation, we attempted to develop mucoadhesive beads based on ionotropic-gelation of GG for controlled release of metformin HCl. In the formulation of these ionotropically-gelled FSM-GG mucoadhesive beads, FSM was used as mucoadhesive polymer-blend with GG at various ratios. When aqueous dispersions of FSM-GG blends in various ratios containing metformin HCl were dropped into various concentrations of $CaCl_2$ solutions, ionotropically-gelled FSM-GG beads containing metformin HCl were formed instantaneously.

3.2. Optimization

According to 3^2 factorial design, 9 trial formulations of FSM-GG beads containing metformin HCl were prepared through Ca^{2+}-ion induced ionotropic-gelation technique. Overview of matrix of the design including two independent factors (*i.e.*, GG to FSM ratio, X_1 and concentration of $CaCl_2$ as cross-linker, X_2) investigated responses (*i.e.*, DEE and R_{10h}) is presented in Table 1. The values of investigated responses measured for all trial formulations were fitted in the 3^2 factorial design using Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA) to get model equations for responses analyzed in this investigation.

The model equation relating DEE (%) became:

$$\text{DEE}(\%) = 86.16 + 1.50X_1 + 6.34X_2 - 0.77X_1X_2 - 0.49{X_1}^2 - 1.23{X_2}^2 [R^2 = 0.9971;\ p < 0.05]$$

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

$$R_{10h}(\%) = 32.21 + 22.22X_1 + 1.85X_2 - 0.52X_1X_2 - 2.45X_1^2 - 0.44X_2^2 [R^2 = 0.9954;\ p < 0.05]$$

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

$$\mathrm{DEE}(\%) = 86.16 + 1.50X_1 + 6.34X_2 - 0.77X_1X_2 - 1.23{X_2}^2$$

$$R_{10h}(\%) = 32.21 + 22.22X_1 + 1.85X_2 - 2.45{X_1}^2$$

The influences of independent formulation factors on responses (here, DEE, and R_{10h}) were further explained by response surface methodology. Response surface methodology is a widely used and popular approach in the development and optimization of various drug delivery formulations in pharmaceutical field, which has been utilized by the formulators to investigate the combined effects (interactions) of investigated independent factors on the desired responses (Ahuja et al., 2010; Nayak and Pal, 2011). The mutual interactive behavior of factors has been analyzed and presented by three-dimensional response surface graphs and two-dimensional contour graphs relating measured responses. The three-dimensional response surface graphs are very useful in learning about the main effects and interaction effects of the independent factors and the two-dimensional contour graphs give a visual representation of values of the response (Malakar et al., 2012). The three-dimensional response surface graph and two-dimensional contour graph relating DEE (Fig. 1a and b) indicate the increment of DEE with the decreasing of both GG to FSM ratio (X_1) and concentration of $CaCl_2$ as cross-linker (X_2). However, a decrease in R_{10h} values with the decreasing of both GG to FSM ratio (X_1) and the increasing concentration of $CaCl_2$ as cross-linker (X_2) is indicated by the three-dimensional response surface graph and two-dimensional contour graph relating R_{10h} (Fig. 1c and 1d).

To develop new formulations with optimum response, numerical optimization technique based on the desirability approach was followed. The desirable ranges of the factors were restricted to $1 \leq X_1 \leq 2$, and $1.5 \leq X_2 \leq 2\%$; whereas the desirable ranges of responses were restricted to $93 \leq \mathrm{DEE} \leq 100\%$, and $50 \leq R_{10h} \leq 60\%$. 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 validate the optimization capability of the mathematical models generated according to the results of 3^2 factorial design, optimized FSM-GG 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 $X_1 = 1.04$ and $X_2 = 1.96\%$ w/v. Optimized FSM-GG beads containing metformin HCl (F-O) were evaluated for DEE (%) and R_{10h} (%). They showed DEE of $92.53 \pm 3.85\%$ and R_{10h} of $55.28 \pm 1.58\%$ with small percentage error-values (0.85 and 3.31, respectively) (Table 1). Calculated small percentage error values indicate that the predicted response values given by the mathematical models and observed response values are in good agreement. This also indicates the good-fitting of the models obtained from the 3^2 factorial design.

3.3. Drug encapsulation

The DEE (%) of all these ionotropically-gelled FSM-GG beads containing metformin HCl ranged 69.36 ± 2.27 to $92.53 \pm 3.85\%$ (Table 1). The investigated factors, GG to FSM ratio (X_1) and concentration of $CaCl_2$ as cross-linker (X_2) significantly influenced DEE (%) ($p < 0.05$). It was also observed that the drug encapsulation in these beads containing metformin HCl was increased with the decreasing of both the GG to FSM ratio and concentration of $CaCl_2$. Increase in drug encapsulation in these beads with the decreasing of GG to FSM ratio could be due to the increase in viscosity of the polymer-blend solution by the increasing of FSM addition, which might have been prevented drug leaching to the cross-linking solution. The decreasing drug encapsulation in the iontropically-gelled FSM-GG beads containing metformin HCl with the increment of the concentration cross-linker ($CaCl_2$) may be attributed by the fact, where water might be expelled as the gelation proceeds rapidly from the surface of the beads to its center due to ionotropic-gelation by Ca^{2+}-ions. The expulsion of water might cause convective loss of drug molecules from the beads during preparation due to higher degrees of cross-linking. Similar results were observed earlier (Maity, Dey, Banik, Sa, Ray, & Kaity, 2010; Kulkarni, Mangod, Mutalik, & Sa, 2011).

3.4. Bead size

The size of ionotropically-gelled FSM-GG beads containing metformin HCl was within the range of 1.09 ± 0.10 to 1.62 ± 0.22 mm (Table 1). Increasing size of beads was found with the decreasing of GG to FSM ratio and concentration of $CaCl_2$ as cross-linker. Increasing of bead size with increasing addition of FSM in polymer-blend solution could be explained based on hydrodynamic viscosity concept. The viscosity increment of the polymer-blend solution with the addition of FSM in increasing ratio might form larger droplets of polymer-blend solutions during passing through the needle to the cross-linking solution containing Ca^{2+} ions. Again, the number free sites available for cross-linking could be less with the increasing amount of FSM in the FSM-GG polymer-blend. So, the size of these beads prepared with decreasing GG to FSM ratio might be increased. On the other hand, a decrease in size of FSM-GG beads was observed, when concentrated $CaCl_2$ solution was used as cross-linking solution. This could be due to the formation of more rigid polymeric network by the high degree of cross-linking. This is in agreement with the earlier reports (Kulkarni, Nagathan, Biradar, & Naikawadi, 2013).

3.5. SEM analyses

The morphological analysis of the optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O) was visualized by SEM and is presented in Fig. 2. The SEM photograph of these beads showed spherical shaped beads of irregular rough surface. The surface of these beads exhibited very rough surface with characteristic large wrinkles, which might be caused by partly collapsing the polymeric gel network during drying (Nayak et al., 2013a). However, few polymeric derbies and drug crystals were seen on the bead surface. Presence of polymeric derbies on the bead surface could be due to the simultaneous gel bead preparation and formation of the polymer-blend matrix; whereas the presence of drug crystals on the bead surface might be formed as a result of their migration along with water to the surface during drying (Nayak et al., 2013b).

3.6. FTIR analyses

FTIR spectra analyses of GG, FSM, optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O) and metformin HCl are shown in Fig. 3. The FTIR spectrum of GG showed characteristic band around 3627 cm^{-1} due to stretching of —OH, band at 3300–2930 cm^{-1} due to —C—H stretching, 1660 cm^{-1} indicating C=O stretching, 1405 cm^{-1} for methyl —C—H bonding and 891 cm^{-1} for —C—O stretching of alkyl ether. In the FTIR spectrum of isolated FSM showed characteristic peaks between 3510–3155 cm^{-1} due to —OH, at

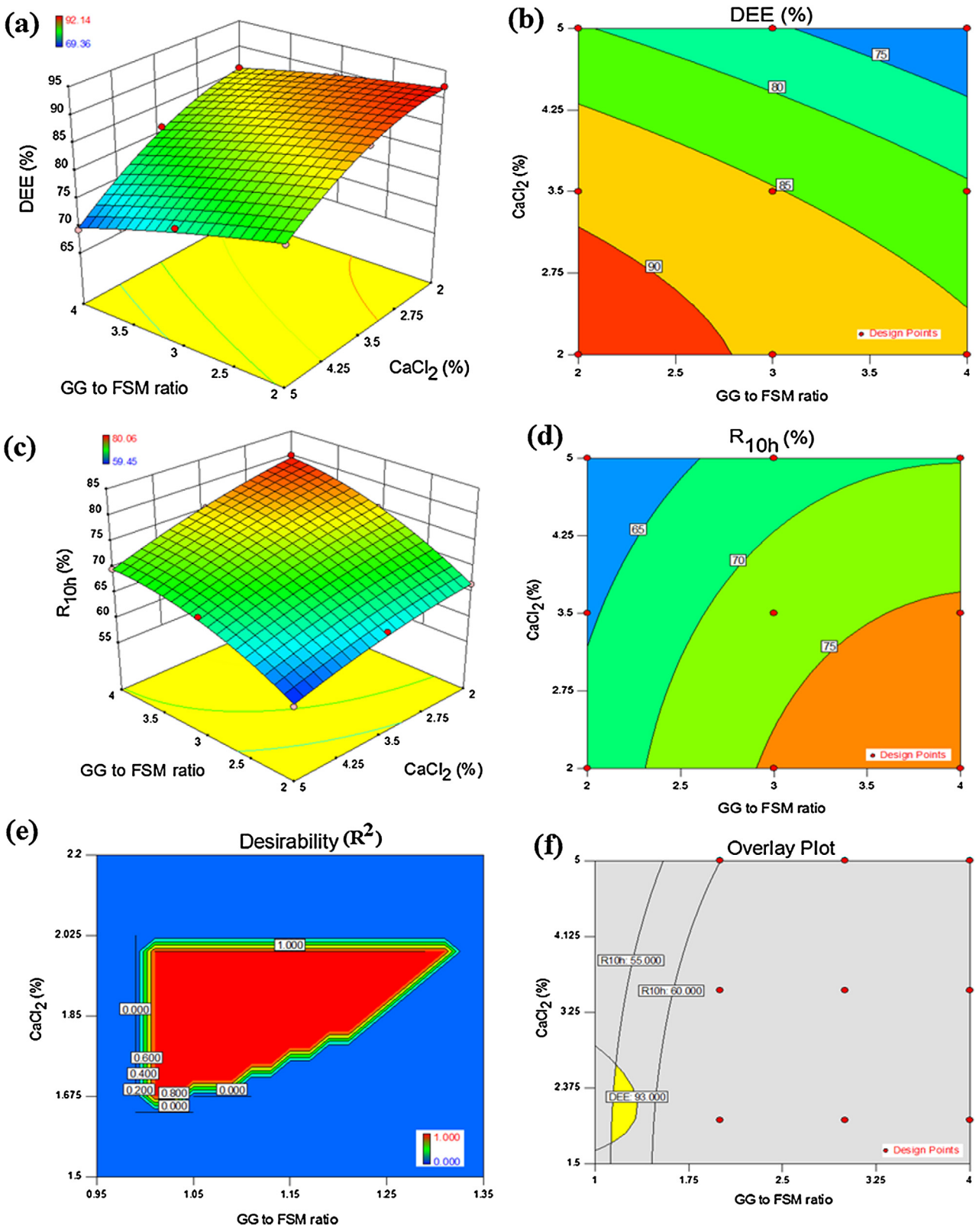

Fig. 1. (a) Three-dimensional response surface graph showing the effects of GG to FSM ratio and $CaCl_2$ concentration on DEE (%); (b) two-dimensional corresponding contour graph showing the effects of GG to FSM ratio and $CaCl_2$ concentration on DEE (%); (c) three-dimensional response surface graph showing the effects of GG to FSM ratio and $CaCl_2$ concentration on R_{10h} (%); (d) two-dimensional corresponding contour graph showing the effects of GG to FSM ratio and $CaCl_2$ concentration on R_{10h} (%); (e) the desirability plot indicating desirable regression ranges; (f) The overlay plot indicating the region of optimal process variable settings.

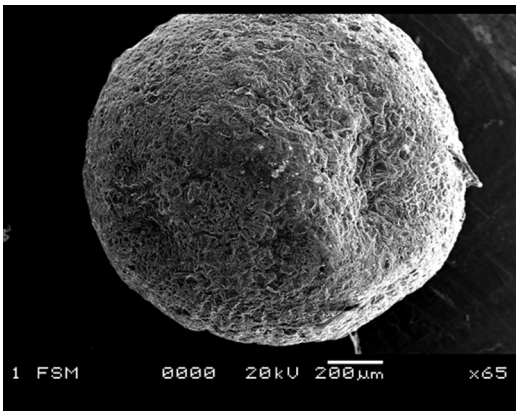

Fig. 2. SEM photograph of ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O).

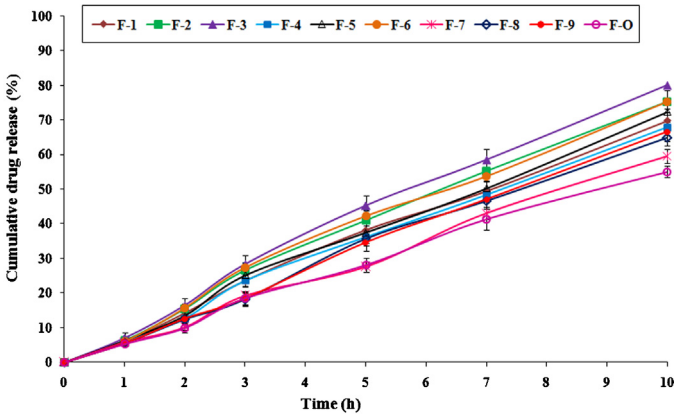

Fig. 4. *In vitro* drug release from various ionotropically-gelled FSM-GG beads containing metformin HCl [Mean ± S.D., $n = 3$].

2925.48 cm^{-1} $-CH_3$, 2920–2855 cm^{-1} due to —C—H stretching, between 1455–1400 cm^{-1} due to ether linkage and at 1018 cm^{-1} due to —C—O stretching. In the FTIR spectrum of metformin HCl, the principal absorption peaks were appeared at 3169 cm^{-1} due to the N—H stretching of the primary amine group ($-NH_2$) 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 optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O), various characteristic peaks of GG, FSM and metformin HCl were appeared without any significant shifting or deviation. Therefore, it can be said that the optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O) had significant characters of metformin HCl, suggesting absence of any interaction between metformin HCl and the polymers used as blends in the bead preparation (*i.e.*, GG and FSM).

3.7. *In vitro drug release*

The *in vitro* metformin HCl release evaluation of all these newly developed ionotropically-gelled FSM-GG beads containing metformin HCl was carried out in the 0.1 N HCl (pH, 1.2) for first 2 h and then, in phosphate buffer (pH, 7.4) for next 8 h. The *in vitro* metformin HCl release from ionotropically-gelled FSM-GG beads showed prolonged release of drug over 10 h (Fig. 4). Metformin HCl release from these FSM-GG beads in the acidic dissolution medium (0.1 N HCl; pH, 1.2) was slow (less than 16.30% after 2 h) and after that, faster metformin HCl release was observed in alkaline dissolution medium (phosphate buffer; pH, 7.4), comparatively. The trace amount of drug release from these beads at the initial stage of the *in vitro* drug release study could probably be due to the surface adhered drug. From the results of *in vitro* drug release study, it is clear that these beads swelled rapidly in alkaline dissolution medium than in acidic medium, which led to comparative increased drug release in alkaline dissolution medium. In alkaline dissolution medium, probably a large swelling force was created by electrostatic repulsion between the ionized carboxylic acid groups of GG-backbone. Actually, the gel-structure might become loose and soluble when exposed to in phosphate buffer (pH, 7.4), because the Ca^{2+} ions involved in the ionotropically-gelled GG-based network could not only be displaced by the Na^+ ions but also be sequestered by phosphate ions present in phosphate buffer (pH, 7.4). The cumulative drug release from these beads containing metformin HCl after 10 h (R_{10h}, %) was within the range of 55.28 ± 1.58–80.06 ± 3.36. The R_{10h}, % was found lowering significantly ($p < 0.05$) with the decreasing GG to FSM ratio in polymer-blends and increasing $CaCl_2$ concentration in cross-linking solution used in the preparation of these ionotropically-gelled beads (according to the ANOVA results of 3^2 factorial design and response surface methodology). Due to increment of hydrophilic property of FSM-GG beads prepared with decreasing GG to FSM ratio could bind better with water to form viscous gel on the bead surface, which might blockade the pores on the surface of these beads and thus, sustain the drug release profile. The slow release of drug from these beads prepared with higher concentration of $CaCl_2$ (*i.e.*, cross-linker) could be due to the fact that free volume of matrix decreases and the hindering movements of the solute through the bead matrix at higher degree of cross-linking.

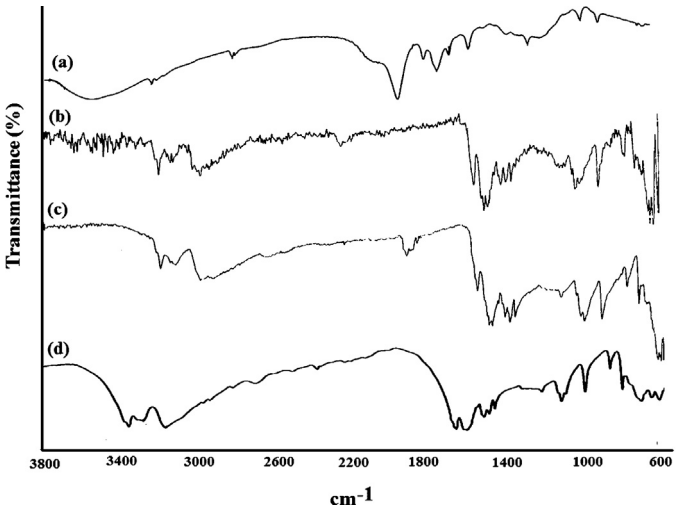

Fig. 3. FTIR spectra of (a) GG, (b) FSM, (c) ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O) and (d) pure metformin HCl.

The *in vitro* metformin HCl release data from various ionotropically-gelled FSM-GG beads containing metformin HCl was evaluated kinetically using various mathematical models. The result of the curve fitting into various mathematical models is given in Table 2. From the curve-fitting results indicates the best-fitting of the zero-order model over a period of 10 h of drug release. Also, the Korsmeyer–Peppas model ($R^2 = 0.9887–0.9972$) was also observed to be closest to the best-fit zero-order model ($R^2 = 0.9903–0.9985$). The best fit of zero-order model indicated that the drug release from various ionotropically-gelled FSM-GG beads containing metformin HCl followed controlled-release pattern. The values of release exponent (n) determined from Korsmeyer–Peppas model ranged from 1.03 to 1.09, indicating the drug release from these

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

Formulation code	Correlation coefficient (R^2)					Release exponent (n)
	Zero-order	First-order	Higuchi	Hixson-Crowell	Korsmeyer–Peppas	
F-1	0.9967	0.8669	0.6048	0.9383	0.9930	1.03
F-2	0.9937	0.8354	0.6091	0.9139	0.9879	1.06
F-3	0.9914	0.8361	0.6170	0.9114	0.9887	1.06
F-4	0.9947	0.8571	0.6015	0.9260	0.9914	1.05
F-5	0.9950	0.8557	0.5954	0.9272	0.9906	1.06
F-6	0.9903	0.8194	0.6082	0.9022	0.9824	1.08
F-7	0.9948	0.8982	0.5819	0.9528	0.9922	1.03
F-8	0.9952	0.8751	0.5643	0.9385	0.9932	1.09
F-9	0.9985	0.8829	0.5612	0.9467	0.9972	1.08
F-O	0.9948	0.8790	0.5985	0.9386	0.9939	1.04

FSM-GG beads containing metformin HCl followed the super case-II transport mechanism controlled by swelling and relaxation of polymeric-blend matrix. This could be attributed due to polymer dissolution and polymeric chain enlargement or relaxation (Nayak et al., 2014).

3.8. Swelling behavior

The swelling behavior of optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O) was found dependent on the pH of the medium. The swelling index of the of optimized beads containing metformin HCl (F-O) was found lower in gastric pH (0.1 N HCl, pH 1.2) in comparison with that of in intestinal pH (phosphate buffer, pH 7.4) (Fig. 5a). Maximum swelling of these beads was noticed at 2–3 h in phosphate buffer (pH 7.4) and after which, erosion and dissolution of these beads took place. It was reported that pH affects the conformation of anionic GG chains in acidic medium by increasing the shielding effect of repulsion of carboxyl groups, which is also enhanced by the addition of cross-linking cations and change of anionic nature of GG determined by the dissociation of carboxyl group (Yamamoto & Cunha, 2007), whereas in alkaline medium GG has high swelling properties (Narker et al., 2010). The swelling behavior of Ca^{2+}-ion cross-linked GG in alkaline pH (pH 7.4) could be explained by the ion exchanging between Ca^{2+} ions of the Ca^{2+}-ion cross-liked GG and the Na^{+} ions present in phosphate buffer with the influence of Ca^{2+}-sequestrant phosphate ions. This could result in disaggregation of the ionotropically-gelled FSM-GG matrix structure leading to matrix erosion and dissolution of the swollen beads in alkaline pH (phosphate buffer, pH 7.4), which might increase the drug release rate.

3.9. Mucoadhesivity

The results of *ex vivo* wash-off test of optimized ionotropically-gelled FSM-GG beads containing metformin HCl (F-O) 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 FSM-GG beads was found faster in intestinal pH than at gastric pH. The rapid *ex vivo* wash-off observed at alkaline pH could be due to ionization of carboxyl and other functional groups of the ionotropically-gelled FSM-GG matrix structure, which increased their solubility with reduced adhesive strength. Therefore, the results of the wash off test confirmed good mucoadhesive potential of these developed ionotropically-gelled FSM-GG containing metformin HCl in gastrointestinal tract (G.I.T). The mucoadhesive property of these beads could be attributed to the presence of hydroxyl groups of both the hydrophilic polymers used as polymer-blend, which have the ability to form hydrogen bonds with the mucous membranes. However, hydrophilic polymers like GG and FSM also have ability to form non-covalent

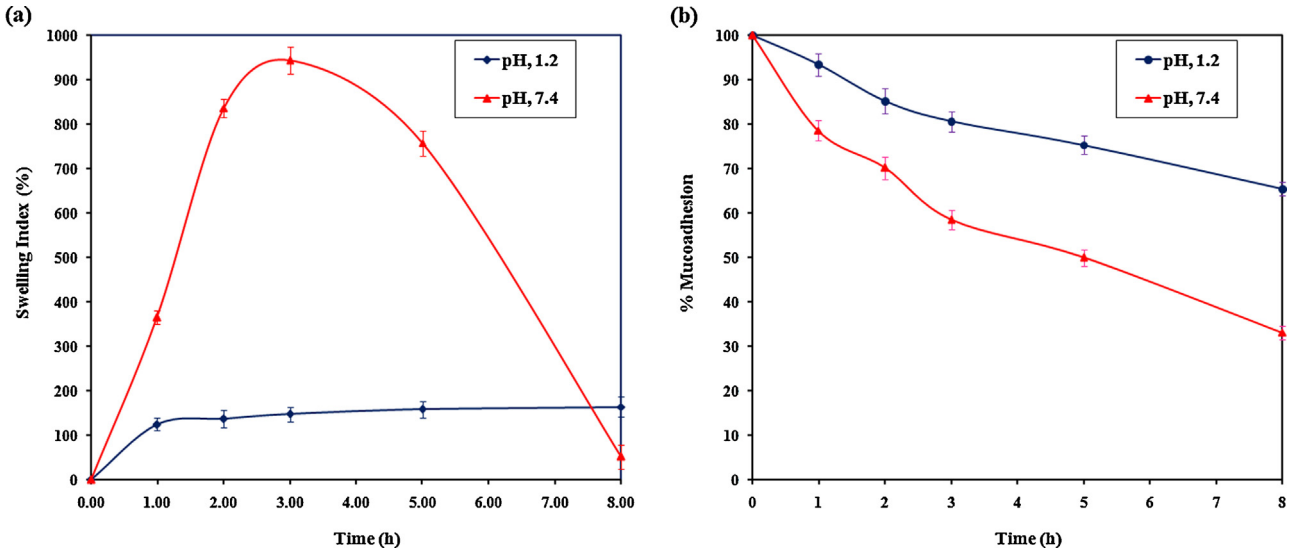

Fig. 5. (a) Swelling behavior of ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O) in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4) [Mean ± SD, $n = 3$]; (b) Mucoadhesive behavior of ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O) in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4) [Mean ± SD, $n = 3$].

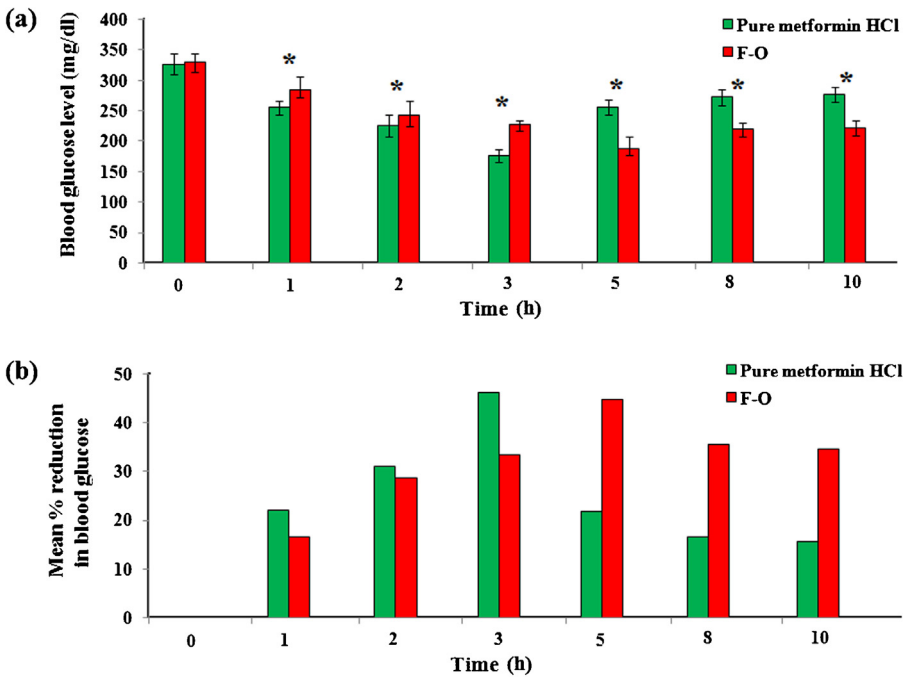

Fig. 6. (a) Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl (standard) and ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O) [Mean ± S.D., $n = 6$]. The data were analyzed for significant differences (*$p < 0.05$) 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 (standard) and ionotropically-gelled optimized FSM-GG beads containing metformin HCl (F-O).

bonds like van der Waals forces or ionic interactions, resulting mucoadhesion. Achieving mucoadhesion of a formulation in the G.I.T. could lead higher bioavailability of the encapsulated drug due to increased gastric residence time and a closer contact between the absorptive mucous membrane and the formulation (Hagesaether, Bye, & Sande, 2008). This could also allow drug release over sustained periods of time, reducing the need of readministration of dosage form.

3.10. Pharmacodynamic evaluation

The pharmacodynamic performance of optimized ionotropically-gelled FSM-GG mucoadhesive beads containing metformin HCl (F-O) after oral administration was evaluated in alloxan-induced diabetic rats. The comparative *in vivo* blood glucose level and the mean percentage reduction in blood glucose level after oral administration of pure metformin HCl and optimized FSM-GG mucoadhesive beads containing metformin HCl (F-O) are presented in Fig. 6(a and b, respectively). A rapid reduction in blood glucose level was observed within 3 h of oral administration in case of the group (Group A) treated with pure metformin HCl. After that, the blood glucose level recovered rapidly toward the normal level. In case of the group (Group B) treated with optimized FSM-GG mucoadhesive beads containing metformin HCl (F-O), 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 FSM-GG mucoadhesive beads containing metformin HCl (F-O) at each time-point measured. However, the reduction in glucose level was increased gradually with the increment of time in case of Group B (treated with optimized FSM-GG mucoadhesive beads containing metformin HCl) and was sustained over 10 h. A 25% reduction in glucose level is considered a significant hypoglycemic effect (Pal and Nayak, 2012). Therefore, the significant hypoglycemic effect by the optimized FSM-GG mucoadhesive beads containing metformin HCl (F-O) was observed over 10 h. The results of the pharmacodynamic evaluation in alloxan-induced diabetic rats clearly demonstrated that the ability of the developed ionotropically-gelled mucoadhesive beads containing metformin HCl to maintain tight blood glucose level and improved patient compliance by enhancing, controlling and prolonging systemic absorption of metformin HCl.

4. Conclusion

Ca^{2+}-ion cross-linked ionotropically-gelled mucoadhesive beads containing metformin HCl made of FSM-GG polymer-blends for oral drug delivery was developed and optimized by 3^2 factorial design. These ionotropically-gelled FSM-GG beads containing metformin HCl were of spherical in shape and within the range of 1.09 ± 0.10–1.62 ± 0.22 mm. DEE in these beads containing metformin HCl were ranged 69.36 ± 2.27–92.53 ± 3.85%. The *in vitro* metformin HCl release from these ionotropically-gelled FSM-GG beads was prolonged over 10 h and followed zero-order model with super case-II transport mechanism. The optimized ionotropically-gelled FSM-GG mucoadhesive beads containing metformin HCl (F-O) showed DEE of 92.53 ± 3.85% and R_{10h} of 55.28 ± 1.58%. The optimized beads containing metformin HCl (F-O) also displayed pH-dependent swelling, good mucoadhesivity with the goat intestinal mucosa in different pHs and significant hypoglycemic activity in alloxan-induced diabetic rats over prolonged period after oral administration.

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