Carbohydrate Polymers 107 (2014) 41–50

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Ispaghula mucilage-gellan mucoadhesive beads of metformin HCl: Development by response surface methodology

Amit Kumar Nayak[a,*], Dilipkumar Pal[b], Kousik Santra[a]

[a] Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj 757086, Odisha, India
[b] Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur 495 009, C.G., India

ARTICLE INFO

Article history:
Received 26 December 2013
Received in revised form 28 January 2014
Accepted 5 February 2014
Available online 15 February 2014

Keywords:
Ispaghula husk
Mucilage
Gellan gum
Mucoadhesion
Controlled drug release
Response surface methodology

ABSTRACT

Response surface methodology based on 3^2 factorial design was used to develop ispaghula (*Plantago ovata* F.) husk mucilage (IHM)-gellan gum (GG) mucoadhesive beads containing metformin HCl through Ca^{2+}-ion cross-linked ionotropic-gelation technique for the use in oral drug delivery. GG to IHM ratio and cross-linker ($CaCl_2$) concentration were investigated as independent variables. Drug encapsulation efficiency (DEE, %) and cumulative drug release after 10 h (R_{10h}, %) were analyzed as dependent variables. The optimized mucoadhesive beads (F-O) showed DEE of 94.24 ± 4.18%, R_{10h} of 59.13 ± 2.27%. These beads were also characterized by SEM and FTIR analyses. The *in vitro* drug release from these beads showed controlled-release (zero-order) pattern with super case-II transport mechanism over 10 h. The optimized beads showed pH-dependent swelling and good mucoadhesivity with the goat intestinal mucosa. The optimized IHM-GG mucoadhesive beads containing metformin HCl exhibited significant antidiabetic effect in alloxan-induced diabetic rats over 10 h.

1. Introduction

Gellan gum (GG) is an anionic polysaccharide, produced as fermentation product by pure culture of *Psedomonus eloda* (Vijan, Kaity, Biswas, Isaac, & Ghosh, 2012; Ahuja et al., 2010). 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, Sen, & Basu, 2013). It undergoes ionotropic-gelation in the presence of di- or tri-valent metal cations. 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). They have been used in the formulation of multiple-unit particulates like microspheres, beads, *etc.* (Babu, Sathigrahi, Kumar, & Pandit, 2010; Narker, Sher, & Pawar, 2010; Maiti, Ranjit, Mondol, Ray, & Sa, 2011). They are found stable in the stomach pH (acidic) than intestinal pH (alkaline) due to comparatively rapid swelling in alkaline pH than acidic pH (Babu et al., 2010). In the intestinal environment, where the concentration of monovalent ions (*e.g.*, Na^+, K^+) is higher and exceeds the concentration of multivalent metal cations (*e.g.*, Ca^{2+}, Al^{3+}). Therefore, these ionotropic-gels tend to lose their stability over the term due to diffusion leading to exchange of multivalent metal cations for monovalent ones, which results faster and premature release of encapsulated drugs from ionotropically-gelled GG particulates in intestinal pH. Moreover, the drug encapsulation in these systems is low. To overcome these limitations, modifications of ionotropically-gelled GG-based particulates have been investigated (Maiti et al., 2011; Kulkarni, Mangod, Mutalik, & Sa, 2011; Kulkarni, Nagathan, Biradar, & Naikawadi, 2013). Blending of other natural polymers with GG in the development of ionotropically-gelled GG-based particulates for drug delivery was also reported (Ahuja, Yadav, & Kumar, 2010; Prajapati, Mashuru, Solanki, & Jani, 2013; Nayak, Pal, & Santra, 2014b). However, development of ionotropically-gelled GG-based beads made of GG and ispaghula (*Plantago ovata* F.) husk mucilage (IHM) blends for controlled drug release is not reported till date.

IHM is hydrophilic in nature, which contains a high amount of highly branched neutral arabinoxylan and non-reducing terminal sugar residues (Nayak, Pal, & Santra, 2013c). It is widely used as excipients in various drug delivery applications (Prajapati, Prajapati, & Acharya, 2006; Singh, 2007; Shirsand, Suresh, Para,

* Corresponding author at: Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj 757086, Odisha, India. Tel.: +91 9583131603.
E-mail address: amitkrnayak@yahoo.co.in (A.K. Nayak).

http://dx.doi.org/10.1016/j.carbpol.2014.02.022
0144-8617/

Swamy, & Kumar, 2009; Deveswaran et al., 2010; Nayak et al., 2013c). Our research group has already reported the utility of isolated IHM as mucoadhesive polymer-blend with sodium alginate in the development of mucoadhesive beads through ionotropic-gelation technique (Nayak et al., 2013c). In the current study, we made an attempt to develop metformin HCl-loaded mucoadhesive beads for oral use using IHM-GG polymer-blend through ionotropic-gelation.

Metformin HCl is an orally administered biguanide, which is widely used in the management of non-insulin dependent diabetes mellitus (type-II) (Basak, Kumar, & Ramalingam, 2008; Boldhane & Kuchekar, 2009). Its biological half-life is 1.5–1.6 h (Nayak, Pal, Pradhan, & Hasnain, 2013b). It shows dose-dependent and saturable transport with absorption window in the upper intestine (Kumar & Ahuja, 2012). Its bioavailability is reported to be improved by gastric-retention (Sharma & Bhattyacharya, 2008). Therefore, it would be valuable to develop mucoadhesive beads of metformin HCl using IHM-GG blend, which might facilitate mucoadhesion on the mucous membrane. As a result of this, the gastric residence of the developed beads could be prolonged to release the encapsulated metformin HCl at a controlled manner over a longer period to maximize the therapeutic benefit.

The successful development of pharmaceutical formulations requires careful consideration of a number of formulation parameters influencing the performance of the formulation. Many experiments fail their purpose because they are not properly thought out or designed and even the best data analysis cannot compensate for a lack of planning (Nayak et al., 2013c). The statistical experimental design allows simultaneous investigation of the effects of several process variables, as well as their actual significance on the considered response and possible inter-relationship among them, giving maximum information with the fewest number of trial experiments (Shah & Pathak, 2010; Malakar & Nayak, 2012; Guru, Nayak, & Sahu, 2013). For this purpose, optimization by response surface methodology (RSM) utilizing polynomial equations has been widely used (Ahuja et al., 2010; Nayak & Pal, 2011). The RSM is used 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 (Nayak, Pal, & Das, 2013a). This methodology requires minimum experimentation and time, thus providing to be far more effective and also cost-effective than the conventional methods of optimization (Malakar, Nayak, & Pal, 2012). Therefore, a computer-aided optimization method using RSM based on 3^2 factorial (2 factors and 3 levels) design was employed to investigate the effects of two independent variables (factors), *i.e.*, polymer-blend ratio (GG to IHM ratio) and cross-linker ($CaCl_2$) concentration on the properties of ionotropically-gelled IHM-GG mucoadhesive beads containing metformin HCl relating to drug encapsulation and drug release. Swelling and mucoadhesion behaviors of the optimized beads in different physiological pH were studied and analyzed. Furthermore, the pharmacodynamic activity of the optimized beads was assessed in alloxan-induced diabetic rats.

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. IHM was isolated from ispaghula (*Plantago ovata* F.) husk (Shree Baidyanath Ayurved Bhawan Pvt. Ltd., India). The procedure of IHM isolation has been described in previously published paper by our research group (Nayak et al., 2013c). All other chemicals and reagents were commercially available and of analytical grade.

2.2. Preparation of ionotropically-gelled IHM-GG beads containing metformin HCl

The ionotropically-gelled IHM-GG beads containing metformin HCl were prepared using calcium chloride ($CaCl_2$) as cross-linker. Aqueous dispersions of GG were prepared separately using distilled water by heating at 60 °C using magnetic stirrer (Remi Motors, India). On the other hand, IHM 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 IHM-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 IHM-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 *via* an18-gauge needle. The added droplets were retained in the $CaCl_2$ solution for 15 min to complete the curing reaction and to form 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 IHM-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 ionotropically-gelled IHM-GG beads containing metformin HCl, in which the GG to IHM ratio (X_1, 2 to 4) and concentration of cross-linker ($CaCl_2$) (X_2, 2 to 4% 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). The design matrix consisted 9 experimental points. The matrix of the design including values and levels of investigated factors and responses is shown in Table 1. For optimization, the effects of independent factors upon the dependable responses were modeled using quadratic mathematical model (Malakar et al., 2012):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4{X_1}^2 + b_5{X_2}^2$$

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 ($p < 0.05$). Individual response parameters were evaluated using the *F*-test. The surface response plots and contour plots were analyzed to reveal the effect of independent factors on the measured 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.

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 IHM-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 IHM-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 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 IHM-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 & Pal, 2011).

2.10. Evaluation of swelling behavior

Swelling behavior evaluation of optimized IHM-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 IHM-GG beads containing metformin HCl were evaluated by *ex vivo* wash-off method (Nayak & Pal, 2013a). 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. In vivo antidiabetic evaluation

In vivo studies were performed in alloxan-induced diabetic male albino rats of either sex (weighing 326–365 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 (i.p.) 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, rats with fasting blood glucose of 300 mg/dl or more were considered diabetic and were employed

Table 1
Experimental plan of 3^2 factorial design (coded values in bracket) with observed response values with bead size for different formulations of various ionotropically-gelled IHM-GG beads containing metformin HCl.

Codes	Factors with normalized levels		Responses[a]		Bead diameter (mm)[b]
	GG to IHM ratio (X_1)	$CaCl_2$ concentration (X_2)	DEE (%)	R_{10h} (%)	
F-1	4 (+1)	4 (+1)	69.49 ± 3.62	70.07 ± 2.18	1.40 ± 0.12
F-2	4 (+1)	3 (0)	82.72 ± 4.03	76.92 ± 3.18	1.30 ± 0.10
F-3	4 (+1)	2 (−1)	86.94 ± 4.11	82.22 ± 3.42	1.28 ± 0.12
F-4	3 (0)	4 (+1)	75.53 ± 3.70	66.05 ± 2.38	1.32 ± 0.13
F-5	3 (0)	3 (0)	86.07 ± 3.26	72.28 ± 3.30	1.37 ± 0.15
F-6	3 (0)	2 (−1)	90.11 ± 4.52	77.08 ± 3.27	1.43 ± 0.17
F-7	2 (−1)	4 (+1)	78.84 ± 3.03	58.34 ± 2.04	1.40 ± 0.12
F-8	2 (−1)	3 (0)	88.98 ± 3.72	63.18 ± 2.23	1.54 ± 0.18
F-9	2 (−1)	2 (−1)	92.48 ± 4.62	65.62 ± 2.72	1.61 ± 0.17
F-O	1.39	1.98	Observed values		
			94.24 ± 4.18	59.13 ± 2.27	1.72 ± 0.19
			Predicted values		
			92.68	57.06	
% Error[c]			1.68	3.63	

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

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 IHM-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.

2.13. Statistical analysis

Statistical optimization was performed using Design-Expert® Version 8.0.6.1 software (Stat-Ease Inc., USA). All measured data are expressed as mean ± standard deviation (S.D.). The *in vivo* data was tested for significant differences ($p < 0.05$) by paired samples t-test. The statistical analyses were conducted using MedCalc software, version 11.6.1.0.

3. Results and discussion

3.1. Optimization of ionotropically-gelled IHM-GG beads containing metformin HCl

According to the trial plan of the 3^2 factorial design, 9 trial formulations of IHM-GG beads containing metformin HCl were prepared through Ca^{2+}-ion induced ionic gelation technique. Overview of matrix of the design including two independent variables (factors) (*i.e.*, GG to IHM 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 to get model equations for responses analyzed in this investigation. These models were evaluated statistically by applying one-way ANOVA ($p < 0.05$) (Table 2).

The model equation relating DEE (%) became:

$$\text{DEE}(\%) = 72.02 + 3.30X_1 + 17.40X_2 - 0.95X_1X_2 - 0.66{X_1}^2 - 3.69{X_2}^2$$
$$[R^2 = 0.9979;\ \text{Adjusted}\ R^2 = 0.9943;\ \text{Predicted}\ R^2 = 0.9759;\ F = 279.76;\ p < 0.05]$$

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

$$R_{10h}(\%) = 25.86 + 25.13X_1 + 3.96X_2 - 1.22X_1X_2 - 2.41{X_1}^2 - 0.90{X_2}^2$$
$$[R^2 = 0.9986;\ \text{Adjusted}\ R^2 = 0.9962;\ \text{Predicted}\ R^2 = 0.9829;\ F = 420.05;\ p < 0.05]$$

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

$$\text{DEE}(\%) = 72.02 + 3.30X_1 + 17.40X_2 - 0.95X_1X_2 - 3.69{X_2}^2$$

$$R_{10h}(\%) = 25.86 + 25.13X_1 + 3.96X_2 - 1.22X_1X_2 - 2.41{X_1}^2$$

The influences of independable factors on dependable responses investigated (here, DEE, and R_{10h}) were further elucidated and analyzed by response surface methodology. The response surface methodology usually indicates the mutual interactive behavior of factors through 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 gives a visual representation of values of the response (Malakar et al., 2012). The response surface graph (Fig. 1a) and corresponding contour graph relating DEE (Fig. 1b) indicate the increment of DEE with the decreasing of both GG to IHM 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 IHM ratio (X_1) and the increasing concentration of $CaCl_2$ as cross-linker (X_2) is indicated by the response surface graph (Fig. 1c) and corresponding contour graph relating R_{10h} (Fig. 1d).

The optimized IHM-GG beads containing metformin HCl (F-O) were formulated using selected process variable settings by numerical analysis according to the 3^2 factorial design and also evaluated for DEE (%) and R_{10h} (%) (Table 1). The desirable ranges of the factors were restricted to $1 \le X_1 \le 2$, and $1.5 \le X_2 \le 2$% w/v; whereas the desirable ranges of responses were restricted to $92 \le \text{DEE} \le 100$%, and $50 \le R_{10h} \le 60$%. 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

Table 2
Summary of ANOVA for quadratic models.

Source	Sum of squares	d.f.*	Mean square	*F* value	*p*-value prob > *F*
(a) For DEE (%)					
Model	453.94	5	90.79	279.76	0.0003 (S)
X_1	8.92	1	8.92	27.49	0.0135 (S)
X_2	40.00	1	40.00	123.24	0.0016 (S)
X_1X_2	3.63	1	3.63	11.18	0.0443 (S)
X_1^2	0.88	1	0.88	2.70	0.1990 (NS)
X_2^2	27.26	1	27.26	83.99	0.0027 (S)
(b) For R_{10h} (%)					
Model	468.79	5	93.76	420.05	0.0002 (S)
X_1	2.72	1	2.72	12.17	0.0398 (S)
X_2	3.86	1	3.86	17.29	0.0253 (S)
X_1X_2	5.93	1	5.93	26.56	0.0142 (S)
X_1^2	11.63	1	11.63	52.11	0.0055 (S)
X_2^2	1.61	1	1.61	7.20	0.0748 (NS)

d.f.* indicates degree of freedom.
S and NS indicate significant and not significant, respectively.

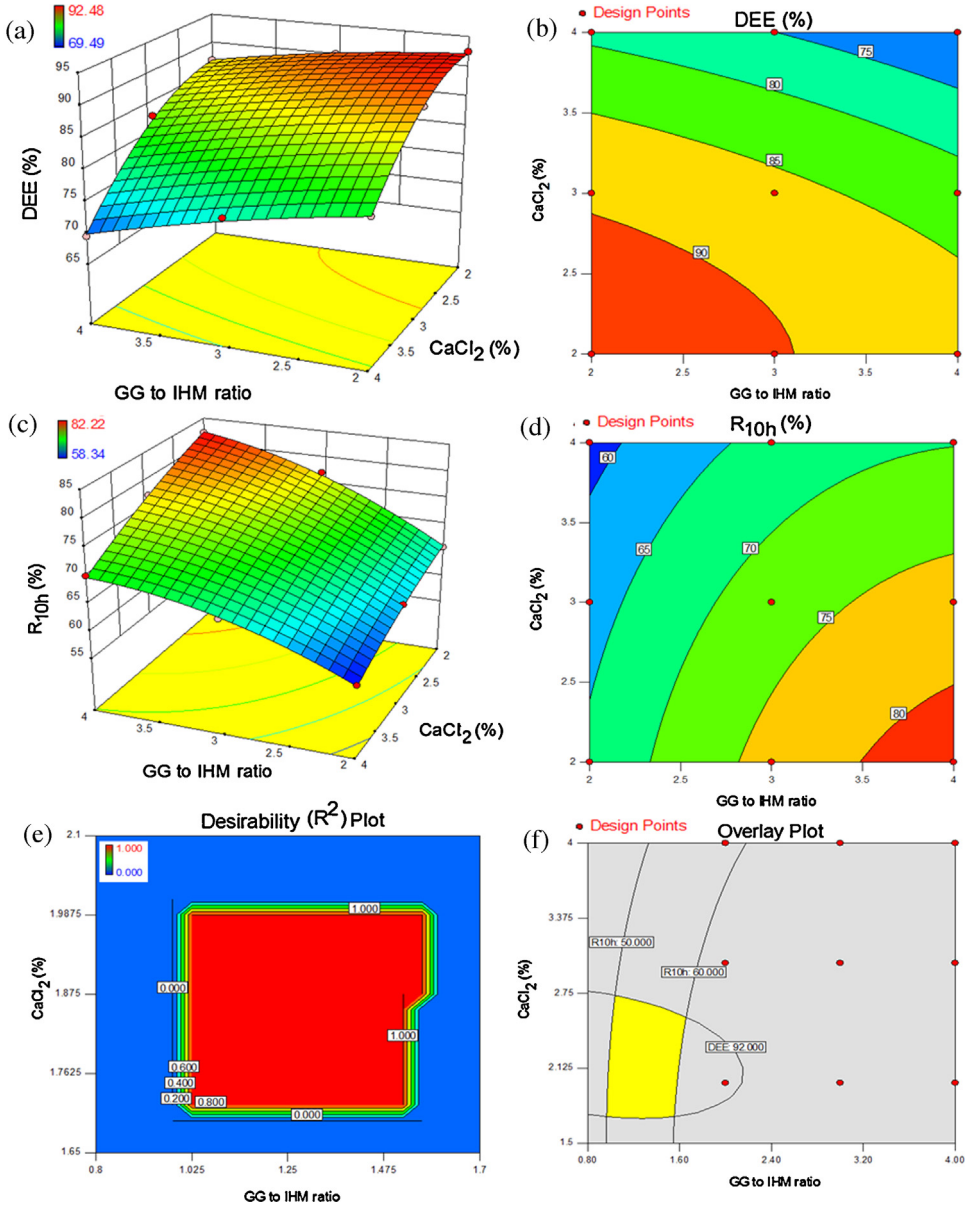

Fig. 1. (a) Three-dimensional response surface graph showing the effects of GG to IHM ratio and $CaCl_2$ concentration on DEE (%); (b) two-dimensional corresponding contour graph showing the effects of GG to IHM ratio and $CaCl_2$ concentration on DEE (%); (c) three-dimensional response surface graph showing the effects of GG to IHM ratio and $CaCl_2$ concentration on R_{10h} (%); (d) two-dimensional corresponding contour graph showing the effects of GG to IHM 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 (yellow area). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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 IHM-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 $X_1 = 1.39$ and $X_2 = 1.98\%$ w/v. Optimized FSM-GG beads containing metformin HCl (F-O) were evaluated for DEE (%) and R_{10h} (%). They showed DEE of $94.24 \pm 4.18\%$ and R_{10h} of $59.13 \pm 2.27\%$ with small percentage error-values (1.68 and 3.63, 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.

3.2. DEE

All these ionotropically-gelled IHM-GG beads containing metformin HCl showed DEE (%) of ranged 69.49 ± 3.62 to $94.24 \pm 4.18\%$ (Table 1). GG to IHM ratio (X_1) and concentration of $CaCl_2$ as cross-linker (X_2) was found to influence DEE (%) significantly ($p < 0.05$). It was also observed that the drug encapsulation in these beads was increased with the decreasing of GG to IHM ratio and concentration of $CaCl_2$, which could be due to the increase in viscosity of the polymeric solution by the increasing IHM addition in the polymer-blend solution. This might have been prevented drug leaching to the cross-linking solution during ionotropically-gelled bead preparation. Again, the IHM contains the xylan backbone with $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ β-D linkages, which are highly substituted with arabinose or aldobiouronic acid residue. It seems that aldobiouronic acid might participate in cross-linking with $CaCl_2$ and consequently further improving the DEE (Sharma and Bhattyacharya, 2008). On the other hand, the decreasing drug encapsulation with increasing concentration of $CaCl_2$ may be attributed by the fact, where water might be expelled as the ionotropic-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 these ionotropically-gelled IHM-GG beads during preparation in case of higher degrees of cross-linking. Almost similar results were observed in earlier reports by our research group (Nayak et al., 2014b).

3.3. Bead size

The sizes of ionotropically-gelled IHM-GG beads containing metformin HCl was within the range of 1.28 ± 0.12 to 1.72 ± 0.19 mm (Table 1). Increasing the size of these beads was found with the increasing incorporation of IHM in polymer-blend solution, which could be explained based on hydrodynamic viscosity concept. The viscosity increment of the polymer-blend solution with the addition of IHM 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. On the other hand, the number free sites available for cross-linking could be less with the increasing amount of IHM in the polymer-blend, so that the size of these beads prepared with decreasing GG to IHM ratio was increased. Again, the decrease in bead size was observed, when concentrated $CaCl_2$ solution was used. This could be due to the formation of more rigid polymeric network by the high degree of cross-linking by the high concentration of cross-linker (*i.e.* $CaCl_2$). This is in agreement with the earlier report on the development of ionotropically-gelled beads of metformin HCl made of tamarind seed polysaccharide-GG blends (Nayak et al., 2014b).

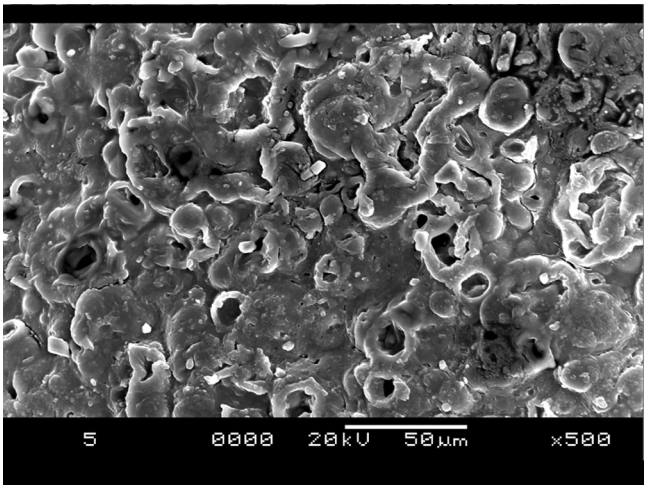

Fig. 2. Surface morphology of the optimized ionotropically-gelled IHM-GG beads containing metformin HCl (F-O) visualized by SEM photograph.

3.4. SEM analyses

The surface morphological analysis of the optimized ionotropically-gelled IHM-GG beads containing metformin HCl (F-O) was visualized by SEM and is presented in Fig. 2. 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). In addition, few polymeric derbies were seen on the bead surface, which could be due to the simultaneous gel bead preparation and formation of the polymer-blend matrix (Nayak et al., 2013b).

3.5. FTIR analyses

FTIR spectra analyses of GG, IHM, optimized ionotropically-gelled IHM-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,

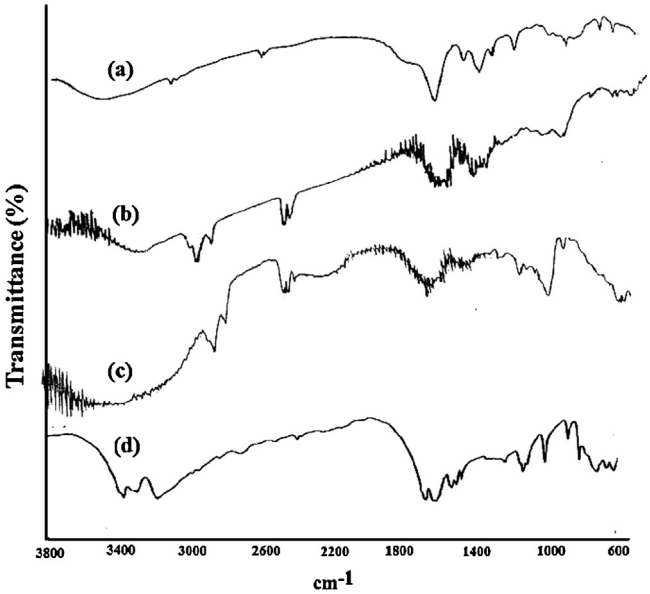

Fig. 3. FTIR spectra of (a) GG, (b) IHM, (c) ionotropically-gelled optimized IHM-GG beads containing metformin HCl (F-O) and (d) pure metformin HCl.

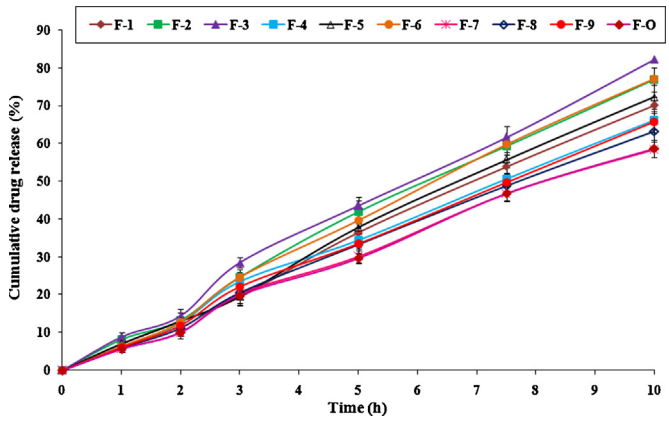

Fig. 4. *In vitro* drug release from various ionotropically-gelled IHM-GG beads containing metformin HCl [Mean ± S.D., $n = 3$].

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 IHM, a strong absorption band at 3455 cm^{-1} was observed due to —OH stretching along with some complex bands in the region 1200–1030 cm^{-1} due to —C—O and C—O—C stretching vibrations, which are the characteristic of the natural polysaccharides. In addition, the absorption bands in the region 930–820 cm^{-1} and 785–730 cm^{-1} due to vibrational modes of pyranose rings of polysaccharides was observed. The FTIR spectrum of optimized ionotropically-gelled IHM-GG beads containing metformin HCl (F-O) confirmed all characteristic peaks of both GG and IHM without any significant alteration or shifting. 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. These characteristic peaks were also appeared in the optimized beads containing metformin HCl without any significant alteration or shifting. This indicated that the drug (here metformin HCl) maintained its identity even after the bead formation using IHM-GG polymeric-blends through ionotropic gelation.

3.6. *In vitro* drug release

The *in vitro* metformin HCl release evaluation of all these newly developed ionotropically-gelled beads showed prolonged metformin HCl release over 10 h (Fig. 4). Metformin HCl release from these IHM-GG beads in the acidic dissolution medium (0.1 N HCl; pH, 1.2) was found comparatively slower than that of alkaline dissolution medium (phosphate buffer; pH, 7.4). 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. After that, comparatively faster metformin HCl release was observed in alkaline dissolution medium (pH, 7.4) than that of acidic dissolution medium (pH, 1.2). This trend could be due to the fact that these beads swelled rapidly in alkaline dissolution medium than in acidic medium, which led to comparative increased drug release 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 IHM-GG beads containing metformin HCl after 10 h (R_{10h}, %) was within the range of 58.34 ± 2.04–82.22 ± 3.42, and this was found lowering with the decreasing GG to IHM ratio and increasing $CaCl_2$. In case of the IHM-GG beads prepared with polymer-blends of decreasing GG to IHM ratio, these beads could bind better with water to form viscous gel-structure due to increased hydrophilic property of polymer-blends with increasing IHM addition. The flexible non cross-linked polymeric ionized xylan chains of IHM might be responsible to produce the more viscous gel, which may blockade the pores on the surface of beads and thus, sustain the drug release profile. Again, the drug release from TSP-GG beads containing metformin HCl prepared using higher with higher $CaCl_2$ concentration was comparatively sustained than the beads formulated with that of lower concentration. The higher concentration of $CaCl_2$ (*i.e.*, cross-linker) can produce high degree of cross-linking and thereby slower the drug release from highly cross-linked IHM-GG beads containing metformin HCl (Nayak et al., 2013c). Furthermore, the release of cationic drug, metformin HCl could be retarded due to the electrostatic interaction between the negative charge of ionized carboxyl groups present in polymer-blends (IHM-GG) and positive charge of the cationic drug.

The result of the curve fitting into various mathematical models like zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer–Peppas models is given in Table 3. When the respective squared correlation coefficients (R^2) of these ionotropically-gelled IHM-GG beads containing metformin HCl were compared using various mathematical models, it was found that the drug release was followed the zero-order model ($R^2 = 0.9934–0.9980$) over a period of 10 h of *in vitro* drug release. Also, the Korsmeyer–Peppas model ($R^2 = 0.9891–0.9973$) was also observed to be closest to the best-fit zero-order model. The best-fit of zero-order model indicated that the drug release from various IHM-GG beads containing metformin HCl followed controlled-release pattern. The values of release exponent (n) determined from Korsmeyer–Peppas model ranged from 0.99 to 1.10, indicating the drug release from these

Table 3
Results of curve fitting of the *in vitro* metformin HCl release data from various ionotropically-gelled IHM-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	0.9980	0.9160	0.5868	0.9621	0.9970	1.02
F-2	0.9951	0.8977	0.6062	0.9468	0.9898	1.01
F-3	0.9945	0.8900	0.6249	0.9426	0.9873	0.99
F-4	0.9950	0.8663	0.6148	0.9319	0.9915	1.03
F-5	0.9973	0.9148	0.5739	0.9610	0.9963	1.04
F-6	0.9969	0.8778	0.5515	0.9404	0.9936	1.10
F-7	0.9934	0.8804	0.5913	0.9375	0.9891	1.04
F-8	0.9970	0.8837	0.5923	0.9425	0.9950	1.05
F-9	0.9969	0.8789	0.5977	0.9410	0.9937	1.04
F-O	0.9940	0.8845	0.5869	0.9414	0.9909	1.05

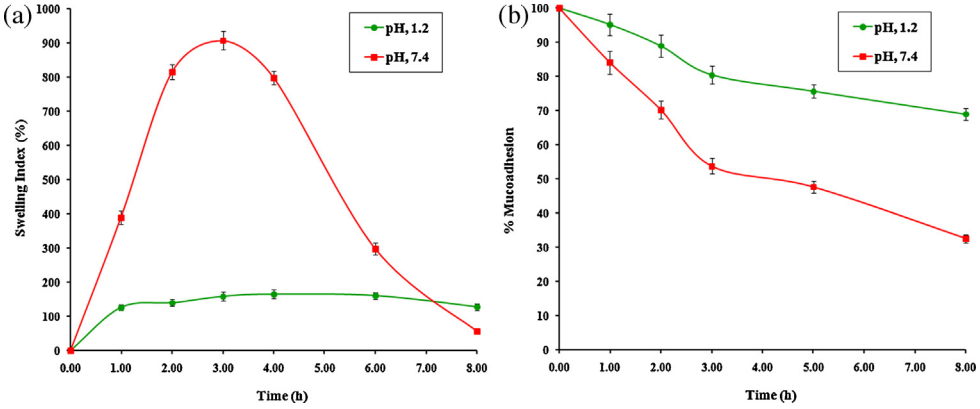

Fig. 5. (a) Swelling behavior of ionotropically-gelled optimized IHM-GG beads containing metformin HCl (F-O) in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4) [Mean ± S.D., $n = 3$]; (b) mucoadhesive behavior of ionotropically-gelled optimized IHM-GG beads containing metformin HCl (F-O) in 0.1 N HCl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

IHM-GG beads containing metformin HCl followed the super case-II transport mechanism controlled by swelling and relaxation of polymeric-blend matrix, which could be attributed due to polymer dissolution and polymeric chain swelling or relaxation (Nayak et al., 2013a).

3.7. Swelling behavior

The swelling behavior of optimized IHM-GG beads containing metformin HCl (F-O) was studied in acidic gastric pH (0.1 N HCl, pH 1.2) and alkaline intestinal pH (phosphate buffer, pH 7.4). The swelling of optimized IHM-GG beads was found dependent on the pH of the test medium. The swelling index of the optimized beads 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 optimized beads was noticed at 2–3 h in alkaline pH (pH 7.4) and after which, erosion and dissolution of took place. It was already reported in the previous literature 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 IHM-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 IHM-GG beads and the Na^{+} ions present in phosphate buffer due to the influence of Ca^{2+}-sequestrant phosphate ions. This might result in disaggregation of the ionotropically-gelled IHM-GG matrix structure leading to matrix erosion and dissolution of the swollen beads in alkaline pH (phosphate buffer, pH 7.4), which could increase the drug release rate.

3.8. Mucoadhesivity

The mucoadhesivity of optimized IHM-GG beads containing metformin HCl (F-O) was analyzed by *ex vivo* wash-off test using goat intestinal mucosa in acidic gastric pH (0.1 N HCl, pH 1.2) and alkaline intestinal pH (phosphate buffer, pH 7.4). The *ex vivo* wash off of these newly developed IHM-GG beads was found faster in alkaline pH than at acidic pH (Fig. 5b). The rapid *ex vivo* wash-off observed at alkaline pH could be due to ionization of carboxyl and other functional groups of the IHM-GG matrix structure, which could increase their solubility with reduced adhesive strength. The results of the wash off test confirmed good mucoadhesive potential of optimized IHM-GG beads containing metformin HCl, which could lead higher bioavailability of the encapsulated drug due to increased gastric residence time and a closer contact between the absorptive membrane and these beads. This could also allow drug release over sustained periods of time, reducing the need of readministration of dosage form. 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 IHM also have ability to form non-covalent bonds like van der Waals forces or ionic interactions, resulting mucoadhesion.

3.9. In vivo antidiabetic evaluation

The comparative *in vivo* blood glucose levels in alloxan-induced diabetic rats and the mean percentage reduction in blood glucose level after oral administration of pure metformin HCl and optimized IHM-GG mucoadhesive beads containing metformin HCl (F-O) are presented in Fig. 6(a and b, respectively). A rapid reduction (46.18%) 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 IHM-GG 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 (30.82%). Significant differences ($p < 0.05$) were found between the blood glucose level after administration of pure metformin HCl and optimized beads containing metformin HCl 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 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, 2011). Therefore, the significant hypoglycemic effect by the optimized mucoadhesive beads containing metformin HCl (F-O) was observed over a prolonged period of 10 h. The results of the *in vivo* antidiabetic evaluation in alloxan-induced diabetic rats clearly demonstrated the potential of the optimized ionotropically-gelled IHM-GG mucoadhesive beads containing metformin HCl (F-O) for maintaining tight blood glucose level over a prolonged time.

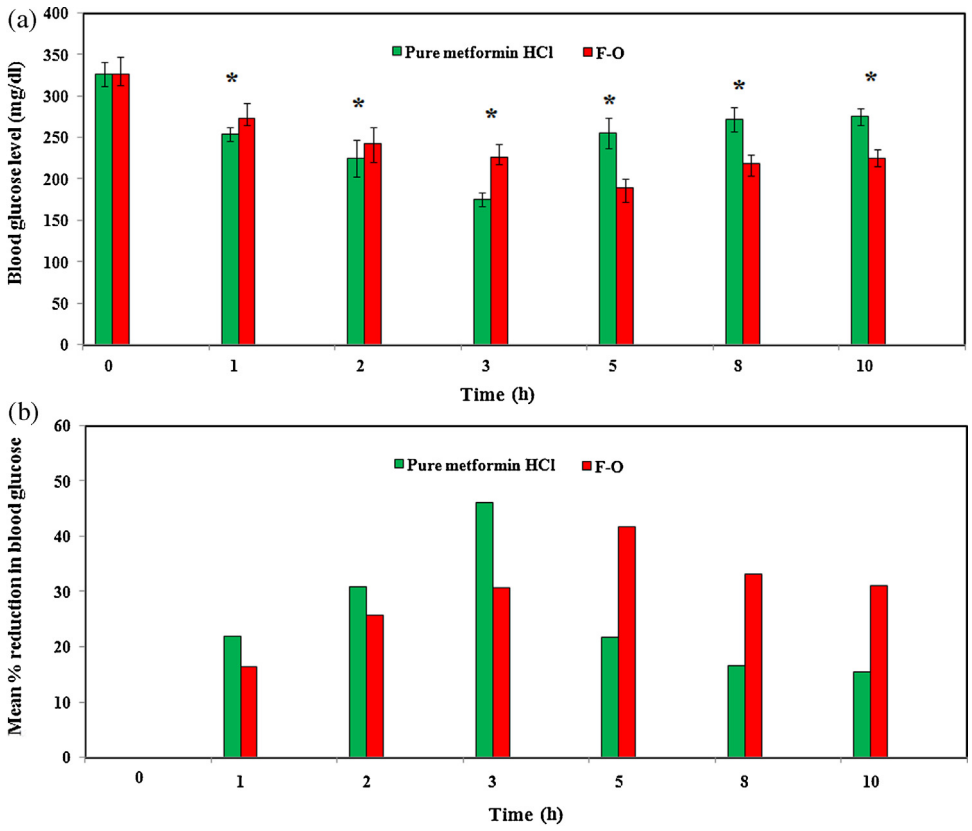

Fig. 6. (a) Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl (standard) and ionotropically-gelled optimized IHM-GG mucoadhesive beads containing metformin HCl (F-O) [Mean ± S.D., $n = 6$]. The data were analyzed for significant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

4. Conclusion

IHM-GG mucoadhesive beads containing metformin HCl for oral drug delivery was developed through Ca^{2+}-ion cross-linked ionotropic-gelation technique and optimized by response surface methodology based on 3^2 factorial design. The optimized ionotropically-gelled IHM-GG beads containing metformin HCl demonstrated high drug encapsulation efficiency, good mucoadhesivity with the goat intestinal mucosal membrane, pH-dependent swelling and suitable controlled drug release pattern over prolonged period. The optimized IHM-GG mucoadhesive beads containing metformin HCl exhibited significant *in vivo* antidiabetic potential in alloxan-induced diabetic rats over prolonged period after oral administration, indicating the suitability to maintain tight blood glucose level and improved patient compliance.

References

Ahuja, M., Yadav, M., & Kumar, S. (2010). Application of response surface methodology to formulation of ionotropically gelled gum cordial/gellan beads. *Carbohydrate Polymers*, *80*, 161–167.

Babu, R. J., Sathigrahi, S., Kumar, M. T., & Pandit, J. K. (2010). Formulation of controlled release gellan gum macro beads of amoxicillin. *Current Drug Delivery*, *7*, 36–43.

Basak, S. C., Kumar, K. S., & Ramalingam, M. (2008). Design and release characteristics of sustained release tablet containing metformin HCl. *Brazilian Journal of Pharmaceutical Sciences*, *44*, 477–483.

Boldhane, S. P., & Kuchekar, B. S. (2009). Gastroretentive drug delivery of metformin hydrochloride: Formulation and *in vitro* evaluation using 3^2 full factorial design. *Current Drug Delivery*, *7*, 36–43.

Deveswaran, R. S., Furtado, S., Bharath, S., Abraham, S., Basavaraj, B. V., & Madhavan, V. (2010). Evaluation of disintegrants properties of *Plantago ovata* mucilage in comparison with other super disintegrants. *Archives of Pharmaceutical Sciences and Research*, *2*, 230–235.

Emeje, M. O., Franklin-Ude, P. I. F., & Odoefule, S. I. (2010). Evaluation of fluid uptake kinetics and drug release from gellan gum tablets containing metronidazole. *International Journal of Biological Macromolecules*, *47*, 158–163.

Guru, P. R., Nayak, A. K., & Sahu, R. K. (2013). Oil-entrapped sterculia gum-alginate buoyant systems of aceclofenac: development and *in vitro* evaluation. *Colliods Surfaces B: Biointerfaces*, *104*, 268–275.

Hamcerencu, M., Desbrieres, J., Khoukh, A., Popa, M., & Riess, G. (2008). Synthesis and characterization of new unsaturated esters of gellan gum. *Carbohydrate Polymers*, *71*, 92–100.

Jana, S., Das, A., Nayak, A. K., Sen, K. K., & Basu, S. K. (2013). Aceclofenac-loaded unsaturated esterified alginate-gellan gum microspheres: *in vitro* and *in vivo* assessment. *International Journal of Biological Macromolecules*, *57*, 129–137.

Kulkarni, R. V., Mangod, B. S., Mutalik, S., & Sa, B. (2011). Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem–resin complex for controlled release application. *Carbohydrate Polymers*, *83*, 1001–1007.

Kulkarni, R. V., Nagathan, V. V., Biradar, P. R., & Naikawadi, A. A. (2013). Simvastatin loaded composite polyspheres of gellan gum and carrageenan: *in vitro* and *in vivo* evaluation. *International Journal of Biological Macromolecules*, *57*, 238–244.

Kumar, A., & Ahuja, M. (2012). Carboxymethyl gum kondagogu: synthesis, characterization and evaluation as mucoadhesive polymer. *Carbohydrate Polymers*, *90*, 637–643.

Maiti, S., Ranjit, S., Mondol, R., Ray, S., & Sa, B. (2011). Al^{3+} ion cross-linked and acetalated gellan hydrogel network beads for prolonged release of glipizide. *Carbohydrate Polymers*, *85*, 164–172.

Malakar, J., & Nayak, A. K. (2012). Formulation and statistical optimization of multiple-unit ibuprofen-loaded buoyant system using 2^3-factorial design. *Chemical Engineering Research and Design*, *9*, 1834–1846.

Malakar, J., Nayak, A. K., & Pal, D. (2012). Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion-gelation method. *International Journal of Biological Macromolecules*, *50*, 138–147.

Narker, M., Sher, P., & Pawar, A. (2010). Stomach-specific controlled release gellan beads of acid-soluble drug prepared by ionotropic gelation method. *AAPS PharmSciTech*, *11*, 267–277.

Nayak, A. K., & Pal, D. (2011). Development of pH-sensitive tamarind seed polysaccharide-alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *International Journal of Biological Macromolecules*, *49*, 784–793.

Nayak, A. K., & Pal, D. (2013). Ionotropically-gelled mucoadhesive beads for oral metformin HCl delivery: Formulation, optimization and antidiabetic evaluation. *Journal of Scientific and Industrial Research*, *72*, 15–22.

Nayak, A. K., Pal, D., & Das, S. (2013). Calcium pectinate-fenugreek seed mucilage mucoadhesive beads for controlled delivery of metformin HCl. *Carbohydrate Polymers*, *96*, 349–357.

Nayak, A. K., Pal, D., Pradhan, J., & Hasnain, M. S. (2013). Fenugreek seed mucilage-alginate mucoadhesive beads of metformin HCl: design, optimization and evaluation. *International Journal of Biological Macromolecules*, *54*, 144–154.

Nayak, A. K., Pal, D., & Santra, K. (2013). *Plantago ovata* F. mucilage-alginate mucoadhesive beads for controlled release of glibenclamide: development, optimization and *in vitro–in vivo* evaluation. *Journal of Pharmaceutics*,. Article ID 151035.

Nayak, A. K., Pal, D., & Santra, K. (2014). Tamarind seed polysaccharide-gellan mucoadhesive beads for controlled release of metformin HCl. *Carbohydrate Polymers*, *103*, 154–163.

Pal, D., & Nayak, A. K. (2011). Development, optimization, and anti-diabetic activity of gliclazide-loaded alginate-methyl cellulose mucoadhesive microcapsules. *AAPS PharmSciTech*, *12*, 1431–1441.

Prajapati, S. T., Prajapati, V. D., & Acharya, S. R. (2006). Characterization of disintegration properties of *Plantago ovata* mucilage in formulation of dispersible tablets. *Indian Journal of Pharmaceutical Education and Research*, *40*, 208–211.

Prajapati, V. D., Mashuru, K. H., Solanki, H. K., & Jani, G. K. (2013). Development of modified release gliclazide biological macromolecules using natural biodegradable polymers. *International Journal of Biological Macromolecules*, *55*, 6–14.

Shah, M., & Pathak, K. (2010). Development and statistical optimization of solid lipid nanoparticles of simvastatin by using 2^3 full-factorial design. *AAPS PharmSciTech*, *11*, 489–496.

Sharma, V. K., & Bhattyacharya, A. (2008). Release of metformin hydrochloride from ispaghula-sodium alginate beads adhered on cock intestinal mucosa. *Indian Journal of Pharmaceutical Education and Research*, *42*, 365–372.

Shirsand, S. B., Suresh, S., Para, M. S., Swamy, P. V., & Kumar, D. N. (2009). *Plantago ovata* mucilage in the design of fast disintegrating tablets. *International Journal of Pharmaceutical Sciences*, *1*, 41–45.

Singh, B. (2007). Psyllium as therapeutic and drug delivery agent. *International Journal of Pharmaceutics*, *334*, 1–14.

Vijan, V., Kaity, S., Biswas, S., Isaac, J., & Ghosh, A. (2012). Microwave-assisted synthesis and characterization of acrylamide grafted gellan, application in drug delivery. *Carbohydrate Polymers*, *90*, 496–506.

Yamamoto, F., & Cunha, R. L. (2007). Acid gelation of gellan: effect of final pH and heat treatment conditions. *Carbohydrate Polymers*, *68*, 517–527.