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Tamarind seed polysaccharide–gellan mucoadhesive beads for controlled release of metformin HCl

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

[a] Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj, Jharpokharia 757086, Odisha, India
[b] Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur 495009, Chhattisgarh, India

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ABSTRACT

The paper describes the development, optimization and evaluation of tamarind seed polysaccharide (TSP)-blended gellan gum (GG) mucoadhesive beads containing metformin HCl through Ca^{2+}-ion cross-linked ionic gelation for oral drug delivery. Effects of GG to TSP ratio and cross-linker ($CaCl_2$) concentration on the drug encapsulation efficiency (DEE, %), and cumulative drug release after 10 h (R_{10h}, %) of TSP–GG mucoadhesive beads containing metformin HCl were optimized by 32 factorial design. The optimized mucoadhesive beads (F-O) showed DEE of 95.73 ± 4.02%, R_{10h} of 61.22 ± 3.44% and mean diameter of 1.70 ± 0.24 mm.These beads were characterized by SEM and FTIR analyses. The *in vitro* drug release from these beads showed controlled-release (zero-order) pattern over a period of 10 h.The optimized TSP–GG 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

Carbohydrate biopolymers have received increased attention for the development of various drug delivery devices for controlled release of small molecular weight drugs (Nayak, Pal, & Das, 2013a; Nayak, Pal, Pradhan, & Hasnain, 2013b; Shukla & Tiwari, 2012). Carbohydrates comprise more than 90% of the dry weight of all biomass and more than 90% of the carbohydrate mass is available in the form of polysaccharides (Mandal, Basu, & Sa, 2010). Polysaccharides are composed of many monosaccharide residues that are joined one to the other by *O*-glycosidic linkages. Traditionally, natural polysaccharides have been widely used as excipients such as suspending agents, emulsifier, jelling agents, disintegrants, binders, film-formers, matrix-formers, mucoadhesive agents, *etc.* in various pharmaceutical formulations (Avachat, Dash, & Shrotriya, 2011; Prajapati, Jani, Moradiya, & Randeria, 2013a). Natural polysaccharides possess some excellent properties, which make them popular and useful for the use in various biomedical applications, are biocompatibility, biodegradability, high swelling capacity, easy availability, low extraction cost, *etc.* (Nayak & Pal, 2012). Besides having many advantages, these materials show some drawbacks such as rapid degradation, low mechanical behavior, chemical instability, *etc.* these drawbacks of natural polysaccharides can be modified through cross-linking, polymer grafting, carboxymethylation, thiolation, esterification, polymer-blending, *etc.* (Ahuja, Singh, & Kumar, 2013; Ghosh & pal, 2013; Jana, Das, Nayak, Sen, & Basu, 2013a; Kaur, Ahuja, Kumar, & Dilbaghi, 2012a; Kaur, Yadav, Ahuja, & Dilbaghi, 2012b; Maiti, Ranjit, Mondol, Ray, & Sa, 2011; Malik, Kumar, & Ahuja, 2012; Nayak & Pal, 2011; Pal & Nayak, 2011; Rana et al., 2011; Sharma & Ahuja, 2011). Among them, polymer-blending is a popular approach to achieve desired functional properties in the pharmaceutical dosage form development (Nayak, Pal, & Das, 2013cPal & Nayak, 2011).

Tamarind seed polysaccharide (TSP) is a natural branched polysaccharide, isolated from the endosperm of 'Indian date' (*Tamarindus indica* L.) seeds, belongs to the family leguminosae (Kaur et al., 2012a,b). It is composed of composed of (1 → 4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and *b*-D-galactopyranosyl (1 → 2)-α-D-xylopyranose linked (1 → 6) to glucose residues (Jana, Saha, Nayak, Sen, & Basu, 2013b). The glucose, xylose and galactose units are present in TSP in the ratio of 2.80:2.25:1.00 (Goyal, Kumar, & Sharma, 2007). Thus, TSP is regarded as a galactoxyloglucan. It is rich in molecular weight of 720–880 kDa (Kaur et al., 2012b). TSP is non-carcinogenic, biocompatible and has excellent stability even in acidic pH range (Jana et al., 2013b). It swells in water and form the mucilaginous solution. TSP is used as binder, thickener, emulsifier, suspending agent, release modifier, *etc.* in various pharmaceutical formulations (Avachat et al., 2011; Deveswaran et al., 2009; Dutta & Bandyopadhyay, 2006; Prajapati, Mashuru, Solanki, & Jani, 2013b). Additionally, it finds use in the development of various types of mucoadhesive drug delivery systems due to its hydrophilic and

* Corresponding author. Tel.: +91 7389263761(mob); fax: +91 591 2360818.
E-mail addresses: drdilip71@gmail.com, drdilip2003@yahoo.co.in (D. Pal).

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mucoadhesive property (Dutta & Bandyopadhyay, 2006; Pal & Nayak, 2012).The previous literature reveals the use of TSP as polymeric-blends with other polymers to modify the drug release in controlled manner for prolonged period from various microspheres and beads (Jana et al., 2013b; Nayak & Pal, 2011; Nayak & Pal, 2013a,b; Pal & Nayak, 2012). However, not a single report is available in the literature regarding the use of TSP as a mucoadhesive blend with gellan gum (GG) for the development of a novel mucoadhesive beads for prolonged drug release.

GG is an anionic exopolysaccharide secreted by the microorganism, *Psedomonus eloda* (Ahuja et al., 2013). 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 (Jana et al., 2013a). According to Food and Drug Administration (FDA), GG may be safely used as a direct food additive for human consumption (Maiti et al., 2011). Due to its ability to form ionically cross-linked beads in presence of di- and/or tri-valent cations, particularly Ca^{2+} ions, GG has been investigated for the controlled release of various kinds of drugs (Babu, Sathigrahi, Kumar, & Pandit, 2010; Maiti et al., 2011; Rajinikanth & Mishra, 2007). The GG beads are stable in low pH; but, swell faster in higher pH. This allows faster and premature releasing of incorporated drugs from Ca^{2+} ion cross-linked GG beads have been investigated by various research groups (Rajinikanth & Mishra, 2007). In the current investigation, we made an attempt to develop TSP-blended GG beads through Ca^{2+}-ion cross-linked ionic gelation technique for oral drug delivery using metformin HCl as a model drug.

Metformin HCl is widely used in the management of non-insulin dependent diabetes mellitus (type-II) as an oral hypoglycemic drug (Maji, Ray, Das, & Nayak,2012). It has been reported that its absolute oral bioavailability is 50–60% (Nayak et al., 2013b). The biological half-life of metformin HCl is 1.5–1.6 h with daily requirement of 1.5–3 g/day and the main absorption site for its absorption is small proximal intestine (Nayak & Pal, 2013b). Therefore, it would be beneficial to develop mucoadhesive beads containing metformin HCl using TSP–GG blend, which might facilitate an intimate adhesion with the absorbing surfaces of mucous membranes (*i.e.*, mucoadhesion) and thus, the gastric residence could be prolonged to release the encapsulated metformin HCl at the absorbing site a controlled manner to maximize the therapeutic benefit. In the current investigation, a 3^2 factorial (2 factors and 3 levels) design-based computer-aided optimization was employed to investigate the effects of two independent factors, *i.e.*, GG to TSP ratio and cross-linker ($CaCl_2$) concentration on the properties of TSP–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. TSP was isolated from the seeds of *Tamarindus indica* L. The procedure of TSP isolation has been described in previously published literatures by our research group (Nayak & Pal, 2011; Pal & Nayak, 2012). All other chemicals and reagents were commercially available and of analytical grade.

2.2. Preparation of TSP–GG beads containing metformin HCl

The TSP–GG beads containing metformin HCl were prepared using calcium chloride ($CaCl_2$) as cross-linking agent through ionic 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, TSP 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 TSP-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 TSP–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 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 TSP–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 TSP–GG beads containing metformin HCl, in which the GG to TSP ratio (X_1, 2 to 4) and concentration of cross-linker ($CaCl_2$) (X_2, 2 to 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} (%) is shown in Table 1. For optimization, the effects of independent variables upon the responses were modelled using quadratic mathematical model (Malakar, Nayak, & Pal, 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 graphs, and contour graphs were analyzed to reveal the effect of independent factors on the measured responses.

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):

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

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 TSP–GG beads containing metformin HCl.

Codes	Factors with normalized levels		Responses[a]		Bead diameter (mm)[b]
	GG to TSP ratio (X_1)	$CaCl_2$ concentration (X_2)	DEE (%)	R_{10h} (%)	
F-1	4 (+1)	5 (+1)	78.87 ± 2.16	70.55 ± 3.12	1.31 ± 0.14
F-2	4 (+1)	3.5 (0)	81.24 ± 2.78	75.33 ± 3.65	1.22 ± 0.18
F-3	4 (+1)	2 (−1)	87.30 ± 3.10	79.16 ± 4.32	1.17 ± 0.09
F-4	3 (0)	5 (+1)	80.79 ± 2.63	76.26 ± 4.22	1.22 ± 0.12
F-5	3 (0)	3.5 (0)	83.04 ± 2.36	71.43 ± 3.03	1.27 ± 0.19
F-6	3 (0)	2 (−1)	90.02 ± 3.22	74.27 ± 4.18	1.35 ± 0.22
F-7	2 (−1)	5 (+1)	83.36 ± 2.74	60.22 ± 2.41	1.32 ± 0.10
F-8	2 (−1)	3.5 (0)	85.95 ± 3.27	67.26 ± 3.05	1.40 ± 0.18
F-9	2 (−1)	2 (−1)	93.13 ± 3.73	67.86 ± 3.17	1.56 ± 0.21
F-O	1.14	1.91	Observed values		
			95.73 ± 4.02	61.22 ± 3.44	1.70 ± 0.24
			Predicted values		
			97.04	59.97	
% Error[c]	1.35	2.08			

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

TSP–GG beads containing metformin HCl were gold coated by mounted on a brass stub using double-sided adhesive tape and under vacuum in an ion sputter with a thin layer of gold (3–5 nm) for 75 s and at 20 kV to make them electrically conductive and their morphology was examined by scanning electron microscope (ZEISS EVO 40, 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 (Perkin Elmer Spectrum RX I, USA). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 3500, and 500 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 TSP–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 TSP–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. A volume of 5 ml 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 TSP–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 TSP–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). A quantity of 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 TSP–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 327–360 g). The acclimatized rats were kept fasting for 24 h with water *ad libitum*. All experiments were performed between 8 AM and 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 two groups of six 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 TSP–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 TSP–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). 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. 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 TSP–GG beads containing metformin HCl

Recent years, the ionic gelation of GG offers new opportunities in the field of drug encapsulation and sustained–controlled drug release applications (Babu et al., 2010; Emeje, Franklin-Ude, & Odoefule, 2010). GG is a water soluble polysaccharide and it has excellent gelling ability with the influence of divalent Ca^{2+} ions is well known for the preparation of various drug-loaded beads (Babu et al., 2010). Chemically, the gelation and aggregation occurs *via* ionic cross-linking between divalent Ca^{2+} ions and two carboxylate groups belonging to glucuronic acid molecules in the gellan chains (Maiti et al., 2011). The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexation with divalent Ca^{2+}-ions and hydrogen bonding with water. In the literature, several Ca^{2+}-ion cross-linked GG beads as oral sustained drug release carriers are reported (Babu et al., 2010; Rajinikanth & Mishra, 2007). However, most of the Ca^{2+}-ion cross-linked GG beads have experienced rapid and premature drug release of encapsulated drugs in intestinal (alkaline) pH.

Currently, blending of polymers has been employed to improve various functional properties such as swelling, stability, gel-strength and desired sustained drug release (Banerjee, Singh, Bhattacharya, & Chattopadhyay, 2013; Malakar, Nayak, & Das, 2013a; Malakar, Nayak, Pal, & Jana, 2013b; Nayak & Pal, 2011; Nayak et al., 2013a,b,c; Pal & Nayak, 2011; Prajapati et al., 2013b; Soares, de Castro, Cury, & Evangelista, 2013). Additionally, the changes in polymer ratio can result in wide range of physicochemical properties that should provide different drug release patterns (Nayak & Pal, 2011; Pal & Nayak, 2011). Again, blending with mucoadhesive polymers can also improve mucoadhesivity of various systems for the use in mucoadhesive drug delivery (Nayak et al., 2013a,b; Nayak & Pal, 2013a; Nayak & Pal, 2013b; Pal & Nayak, 2011; Pal & Nayak, 2012). In the current study, Ca^{2+}-ion cross-linked TSP–GG beads were investigated to develop multiple-unit mucoadhesive systems of metformin HCl for oral use.

3.2. Optimization

Currently, optimization by means of computer-aided statistical experimental design methodologies have successfully applied in the development of various pharmaceutical dosage forms (Guru, Nayak, & Sahu, 2013; Malakar et al., 2012, 2013a,b). The statistical optimization technique encompasses designing a set of experiment that will reliably measure the response variables, fitting mathematical models to the data, conducting appropriate statistical tests to select best possible model and determining the values of independent formulation variables to produce optimum response (Nayak & Pal, 2013b). Among various statistical designs, factorial designs are popular and effective experimental design, which have been employed in the formulation optimization in the development of various drug deliveries (Malakar & Nayak, 2012; Nayak & Pal, 2011; Nayak et al., 2013a,b; Nayak & Pal, 2013a,b). Factorial designs are experimental design techniques by which factors involved in the formulation process can be identified to analyze their relative importance. In addition this technique is able to identify the interactions, if any, between the factor(s) chosen (Nayak & Pal, 2013b).

According to the trial plan of the 3^2 factorial design, nine trial formulations of TSP–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 TSP 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

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	173.16	5	34.63	929.21	<0.0001 (S)
X_1	1.59	1	1.59	42.60	0.0073 (S)
X_2	3.22	1	3.22	86.49	0.0026 (S)
X_1X_2	0.45	1	0.45	12.04	0.0403 (S)
X_1^2	0.26	1	0.26	6.89	0.0787 (NS)
X_2^2	9.40	1	9.40	252.30	0.0005 (S)
(b) For R_{10h} (%)					
Model	263.64	5	52.73	289.38	0.0003 (S)
X_1	14.41	1	14.41	79.13	0.0030 (S)
X_2	4.12	1	4.12	22.63	0.0176 (S)
X_1X_2	0.24	1	0.24	1.29	0.3384 (NS)
X_1^2	3.27	1	3.27	17.94	0.0241 (S)
X_2^2	1.11	1	1.11	6.07	0.0906 (NS)

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

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. These models were evaluated statistically by applying one-way ANOVA ($p < 0.05$). The result of the ANOVA is shown in Table 2.

The model equation relating DEE (%) became:

$$\text{DEE (\%)} = 118.73 - 5.44X_1 - 10.46X_2 + 0.22X_1X_2 + 0.36X_1^2 + 0.96X_2^2 \quad [R^2 = 0.9994; \text{ adjusted } R^2 = 0.9983; \quad \text{predicted } R^2 = 0.9932; \quad F = 929.21; \quad p < 0.05].$$

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

$$R_{10h}\ (\%) = 47.36 + 13.54X_1 + 0.21X_2 - 0.16X_1X_2 - 1.28X_1^2 - 0.33X_2^2 \quad [R^2 = 0.9979; \quad \text{adjusted } R^2 = 0.9945; \text{ predicted } R^2 = 0.9749; \quad F = 289.38; \quad p < 0.05].$$

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

$$\text{DEE (\%)} = 118.73 - 5.44X_1 - 10.46X_2 + 0.22X_1X_2 + 0.96X_2^2$$

$$R_{10h}\ (\%) = 47.36 + 13.54X_1 + 0.21X_2 - 1.28X_1^2$$

The influences of main effects (factors) on responses (here, DEE, and R_{10h}) were further elucidated by response surface methodology (RSM). RSM is a widely used approach in the development and optimization of drug delivery formulations (Ahuja, Yadav, & Kumar, 2010; Lee, Chung, & Lee, 2008; Madgulkar, Kadam, & Pokharkar, 2008; Nayak & Pal, 2011). The purpose of the RSM is to understand a model as fully as possible the effect of factors and their levels, over the whole of the experimental domain, and to predict the response inside the domain (Nayak et al., 2013a). The RSM usually gives 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 variables (factors) and the two-dimensional contour graphs gives 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 TSP 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 TSP 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 d).

To develop new formulations with desired response (optimum quality), numerical optimization technique based on the desirability approach was employed. The desirable ranges of the factors were restricted to $1 \leq X_1 \leq 2$, and $1.5 \leq X_2 \leq 2\%$ w/v; whereas the desirable ranges of responses were restricted to $95 \leq \text{DEE} \leq 100\%$, and $55 \leq R_{10h} \leq 60\%$. The optimal values of responses were obtained by numerical analysis using the Design-Expert® Version 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. 2a and b, respectively. In order to evaluate the optimization capability of the mathematical models generated according to the results of 3^2 factorial design, optimized TSP–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 $X_1 = 1.14$ and $X_2 = 1.91\%$ w/v. Optimized TSP–GG beads containing metformin HCl (F-O) were evaluated for DEE (%) and R_{10h} (%). They showed DEE of $95.73 \pm 4.02\%$ and R_{10h} of $61.22 \pm 3.44\%$ with small percentage error-values (1.35 and 2.08, respectively) (Table 1). The calculated small percentage error values indicate that mathematical models obtained from the full 3^2 factorial design were well fitted.

3.3. DEE

The DEE (%) of all these TSP–GG beads containing metformin HCl ranged 78.87 ± 2.16 to $95.73 \pm 4.02\%$ (Table 1). The investigated factors, GG to TSP 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 factors, GG to TSP ratio and concentration of $CaCl_2$ as cross-linker. This phenomenon could be due to the increase in viscosity of the polymeric solution by the increasing of TSP addition in the polymer-blend solution. And it might have been prevented drug leaching to the cross-linking solution. The decreasing drug encapsulation in these beads containing metformin HCl with the increment of the concentration of $CaCl_2$ as cross-linker in the used cross-linking solution may be attributed by the fact, where water might be expelled as the gelation proceeds due to ionic gelation by Ca^{2+}-ions. The expulsion of water might cause convective loss of drug molecules from the TSP–GG beads during preparation.

3.4. Bead size

Particle size of TSP–GG beads containing metformin HCl was within the range of 1.17 ± 0.09 to 1.70 ± 0.24 mm (Table 1). Increasing size of beads was found with the decreasing of GG to TSP ratio and concentration of $CaCl_2$ as cross-linker. This phenomenon could be explained due to the increase in viscosity of the TSP–GG polymer-blend solutions with the incorporation of TSP in increasing ratio, which might increase the droplet size during addition of the polymer-blend solution to the cross-linking solution. On the other hand, the number free sites available for cross-linking could be less with the increasing amount of TSP in the

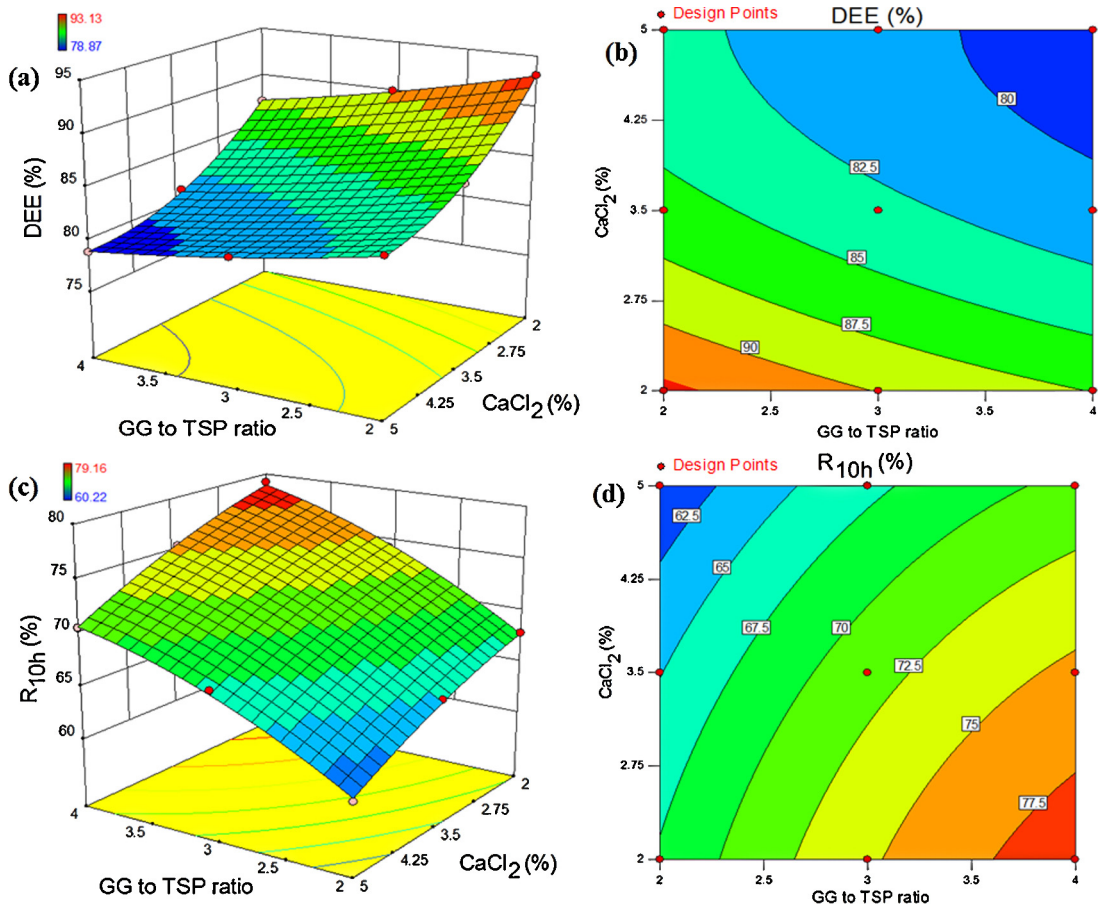

Fig. 1. (a) Three-dimensional response surface graph showing the effects of GG to TSP ratio and $CaCl_2$ concentration on DEE (%); (b) Two-dimensional corresponding contour graph showing the effects of GG to TSP ratio and $CaCl_2$ concentration on DEE (%); (c) Three-dimensional response surface graph showing the effects of GG to TSP ratio and $CaCl_2$ concentration on R_{10h} (%); (d) Two-dimensional corresponding contour graph showing the effects of GG to TSP ratio and $CaCl_2$ concentration on R_{10h} (%).

polymer-blend, so that the size of these beads prepared with decreasing GG to TSP ratio was increased. Again, the decrease in particle size of TSP–GG beads was observed, when concentrated $CaCl_2$ solution was used as cross-linking solution. This could be due to shrinkage of polymeric gel by higher degree of cross-linking with the high concentration of cross-linker (*i.e.*, $CaCl_2$) (Nayak et al., 2013c).

3.5. SEM analyses

The SEM photograph of optimized TSP–GG beads containing metformin HCl (F-O) (Fig. 3) showed spherical but discrete with irregular rough surface due to leaching of drug in cross-linking solution forming large perturbations. The surface of these beads exhibited very rough surface with characteristic large wrinkles and

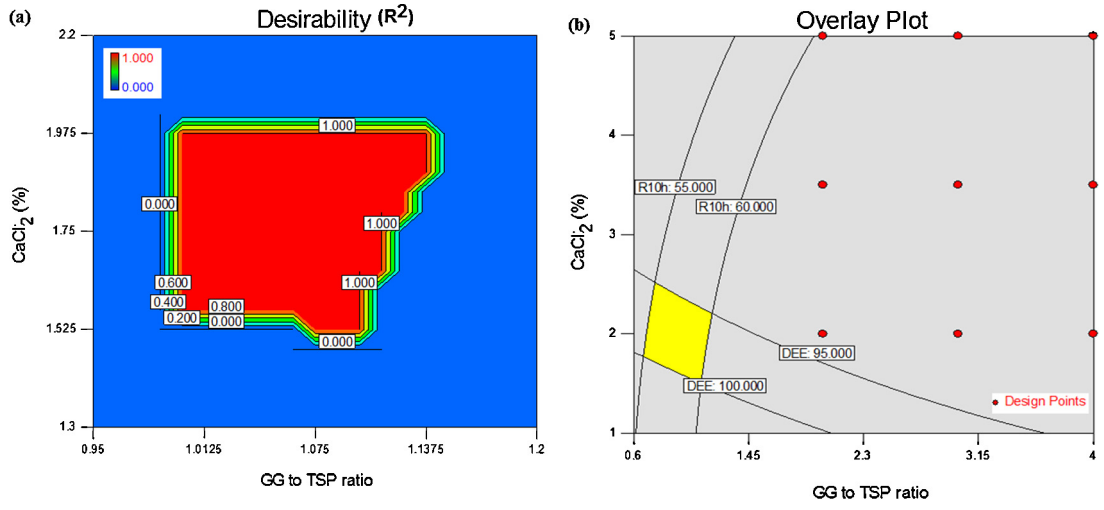

Fig. 2. (a) The desirability plot indicating desirable regression ranges and (b) The overlay plot indicating the region of optimal process variable settings.

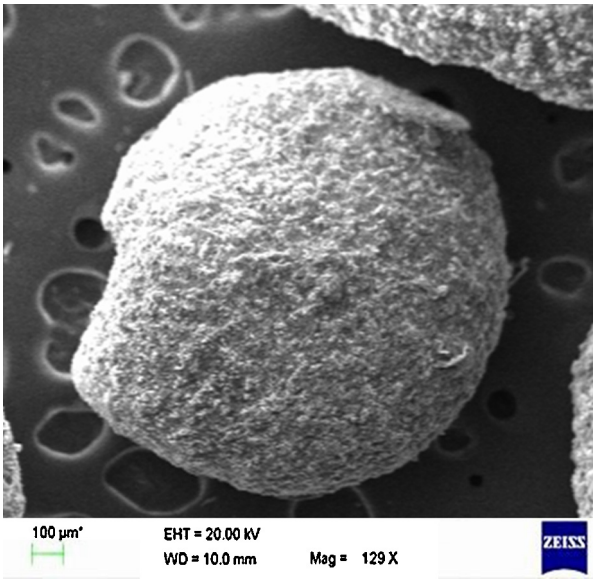

Fig. 3. SEM photograph of optimized TSP–GG beads containing metformin HCl (F-O).

cracks, which might be caused by partly collapsing the polymeric gel network during drying (Malakar et al., 2013b). 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, TSP, TSP–GG beads without metformin HCl, optimized TSP–GG beads containing metformin HCl (F-O) and metformin HCl are shown in Fig. 4. 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 TSP, the characteristic principal peaks at 1043.06, 1650.37, 2925.55 and 3403.48 cm^{-1} for –HC=O stretching vibration, –CH–OH stretching vibration, aliphatic C–H stretching, and for the

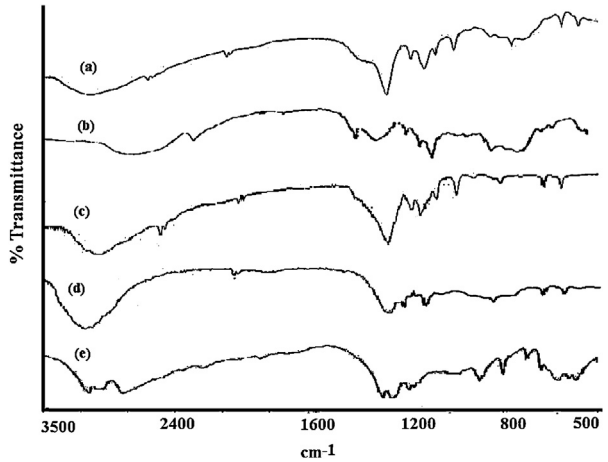

Fig. 4. FTIR spectra of (a) GG, (b) TSP, (c) TSP–GG beads without drug, (d) optimized TSP–GG beads containing metformin HCl (F-O) and (e) pure metformin HCl.

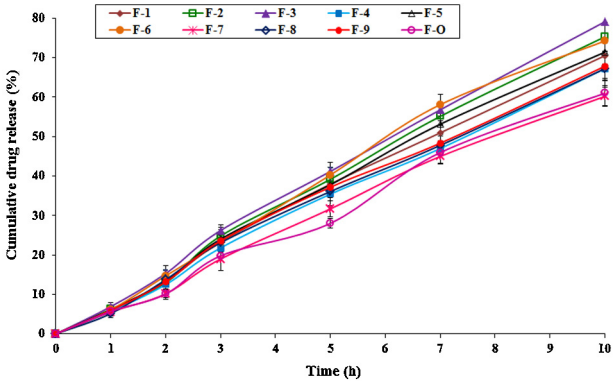

Fig. 5. *In vitro* drug release from various TSP–GG beads containing metformin HCl [Mean ± S.D., $n = 3$].

OH groups, respectively were found. The FTIR spectrum of optimized TSP–GG beads without metformin HCl (F-O) confirmed all characteristic peaks of both GG and TSP without any significant deviation. 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 ionically-gelled optimized TSP–GG beads containing metformin HCl (F-O), various characteristic peaks of GG, TSP and metformin HCl were appeared without any significant shifting. Therefore, it can be said that the ionically-gelled optimized TSP–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 beads preparation (*i.e.*, GG and TSP).

3.7. In vitro drug release

The *in vitro* drug (metformin HCl) release evaluation of all these newly developed ionically-gelled TSP–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. All these beads showed prolonged metformin HCl release over 10 h (Fig. 5). Metformin HCl release from these TSP–GG beads in the acidic dissolution medium (0.1 N HCl; pH, 1.2) was slow (less than 15.30% after 2 h) and after that, faster metformin HCl release was observed in alkaline dissolution medium (phosphate buffer; pH, 7.4), comparatively. This phenomenon could be due to the fact that these beads swelled rapidly in alkaline dissolution medium than in acidic medium, and this 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. The trace amount of drug release at the initial stage of the dissolution study could probably be due to the surface adhered drug. The cumulative drug release from TSP–GG beads containing metformin HCl after 10 h (R_{10h}, %) was within the range of 61.22 ± 3.44 to 79.16 ± 4.32, and this was found lowering with the decreasing GG to TSP ratio in polymer-blends and increasing $CaCl_2$ concentration in cross-linking solution used in the preparation of these ionically-gelled beads. In case of the TSP–GG beads prepared with polymer-blends of decreasing GG to TSP ratio, these beads could bind better with water to form viscous gel-structure due to increased hydrophilic property of polymer-blends with increasing TSP addition, 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

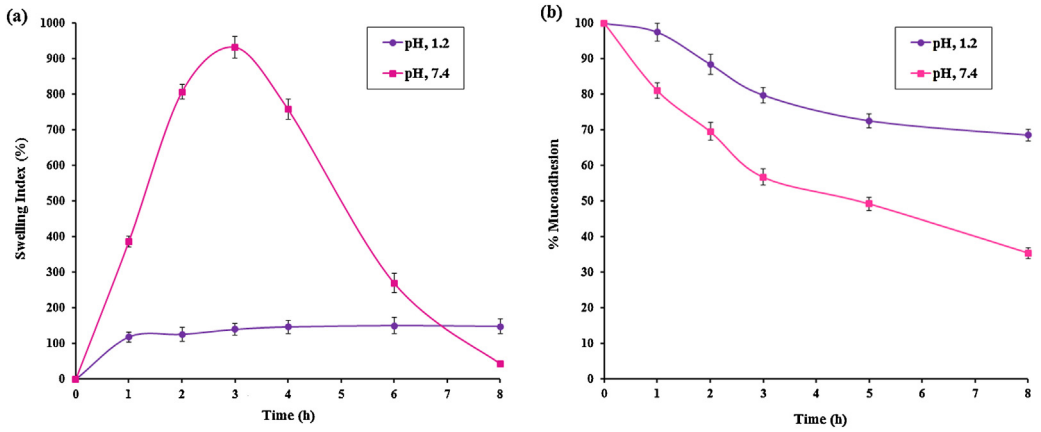

Fig. 6. (a) Swelling behavior of optimized TSP–GG beads containing metformin HCl (F-O) in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4) [Mean ± SD, $n = 3$]; (b) Mucoadhesive behavior of optimized TSP–GG beads containing metformin HCl (F-O) in 0.1 N HCl (pH 1.2), and phosphate buffer (pH 7.4) [Mean ± SD, $n = 3$].

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 TSP–GG beads containing metformin HCl (Nayak et al., 2013c).

The *in vitro* drug release data from various ionically-gelled TSP–GG beads containing metformin HCl was evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas models. The accuracy and prediction ability of these models were determined using regression analysis. The result of the curve fitting into various mathematical models is given in Table 3. When the respective squared correlation coefficients (R^2) of these beads containing metformin HCl were compared, it was found that the drug release was followed the zero-order model ($R^2 = 0.9907$ to 0.9975) over a period of 10 h. Also, the Korsmeyer–Peppas model ($R^2 = 0.9855$to 0.9950) 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 TSP–GG beads containing metformin HCl followed controlled-release pattern. The values of release exponent (n) determined from Korsmeyer–Peppas model ranged from 1.05 to 1.09, indicating the drug release from these TSP–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., 2013a).

3.8. Swelling behavior

The swelling of optimized ionically-gelled TSP–GG beads containing metformin HCl (F-O) was found lower in acidic gastric pH (0.1 N HCl, pH 1.2) in comparison with that of in alkaline intestinal pH (phosphate buffer, pH 7.4) (Fig. 6a). Maximum swelling of these TSP–GG beads containing metformin HCl was noticed at 2–3 h in alkaline pH (pH 7.4) and after which, erosion and dissolution of these beads took place. GG being an anionic polysaccharide, pH affects the conformation of 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, Sher, & Pawar, 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 ionically-gelled TSP–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. The swelling result suggests that these TSP–GG beads will swell in the stomach after oral administration. As they are subsequently transferred to upper part of intestine, the particles will begin to swell more and behave as matrices for controlled release of incorporated drug.

3.9. Mucoadhesivity

The results of *ex vivo* wash-off test of optimized ionically-gelled TSP–GG beads containing metformin HCl (F-O) using goat intestinal mucosa in acidic gastric pH (0.1 N HCl, pH 1.2) and alkaline intestinal pH (phosphate buffer, pH 7.4) are presented in Fig. 6b. The *ex vivo* wash off of these newly developed TSP–GG beads was found faster in alkaline pH than at acidic pH. The rapid *ex vivo*

Table 3
Results of curve fitting of the *in vitro* metformin HCl release data from various TSP–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	0.9960	0.8575	0.5907	0.9277	0.9939	1.07
F-2	0.9956	0.8627	0.5673	0.9303	0.9928	1.09
F-3	0.9975	0.8639	0.6005	0.9334	0.9950	1.05
F-4	0.9972	0.8640	0.5877	0.9335	0.9948	1.07
F-5	0.9949	0.8591	0.5911	0.9277	0.9936	1.07
F-6	0.9957	0.8686	0.5837	0.9282	0.9948	1.08
F-7	0.9951	0.8821	0.5753	0.9396	0.9938	1.07
F-8	0.9948	0.8290	0.5931	0.9126	0.9855	1.09
F-9	0.9939	0.8506	0.6123	0.9217	0.9914	1.05
F-O	0.9907	0.8933	0.5651	0.9477	0.9898	1.05

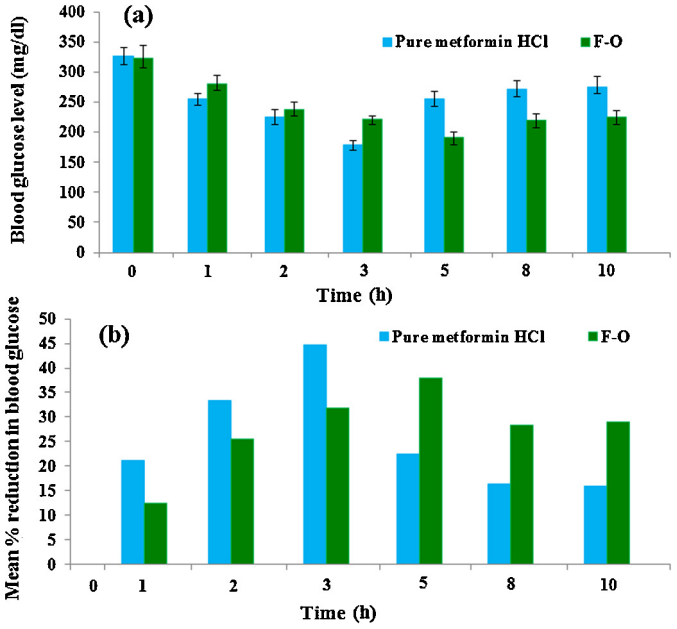

Fig. 7. (a) Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl (standard) and optimized TSP–GG beads containing metformin HCl (F-O) [Mean ± S.D., $n = 6$]. The data were analyzed for significant differences ($^*p < 0.05$) by paired 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 optimized TSP–GG beads containing metformin HCl (F-O).

wash-off observed at alkaline pH could be due to ionization of carboxyl and other functional groups of the ionically-gelled TSP–GG matrix structure, which increased their solubility with reduced adhesive strength. Therefore, the results of the wash off test confirmed that the developed optimized ionically-gelled TSP–GG beads containing metformin HCl had good mucoadhesivity. 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 various non-covalent bonds (like hydrogen bonds, van der Waal's forces and ionic interactions) with mucus molecules.

3.10. Pharmacodynamic evaluation

The comparative *in vivo* blood glucose level and the mean percentage reduction in blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized TSP–GG mucoadhesive beads containing metformin HCl (F-O)is presented in Fig. 7 (a and b, respectively). In case of the group treated with pure metformin HCl (Group A), a rapid reduction in blood glucose level was observed within 3 h of administration, and after that, the blood glucose level recovered rapidly toward the normal level. In case of the group (Group B) treated with optimized TSP–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 TSP–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 TSP–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 & Nayak, 2012). Therefore, the significant hypoglycemic effect by the optimized TSP–GG mucoadhesive beads containing metformin HCl (F-O) was observed over 10 h.

4. Conclusion

Novel mucoadhesive beads containing metformin HCl made of GG–TSP polymer-blends for oral drug delivery was developed through Ca^{2+}-ion cross-linked ionically gelation technique. Effects of GG to TSP ratio and cross-linker ($CaCl_2$) concentration on DEE and R_{10h} of TSP–GG mucoadhesive beads containing metformin HCl were optimized by 3^2 factorial design and analysed by RSM. An increment of DEE with the decreasing of both GG to TSP ratio and concentration of $CaCl_2$ as cross-linker was observed. However, a decrease in R_{10h} values with the decreasing of both GG to TSP ratio and the increasing concentration of $CaCl_2$ as cross-linker was also detected by RSM. The optimized mucoadhesive beads (F-O) showed DEE of 95.73 ± 4.02% and R_{10h} of 61.22 ± 3.44% with small percentage error-values (1.35 and 2.08, respectively). The *in vitro* drug release from these beads was followed controlled-release (zero-order) pattern ($R^2 = 0.9907$ to 0.9975) with super case-II transport mechanism ($n = 1.05$ to 1.09) over a period of 10 h. The optimized TSP–GG mucoadhesive beads containing metformin HCl (F-O) also displayed pH-dependent swelling, good mucoadhesivity with the biological mucous membrane and significant hypoglycemic activity in alloxan-induced diabetic rats over prolonged period after oral administration. Thus, these newly developed GG–TSP mucoadhesive beads could possibly be lucrative in terms of prolonged systemic absorption of metformin HCl maintaining tight blood glucose level and advanced patient compliance. This type of mucoadhesive beads can also be formulated to load other drugs demanding controlled drug release over a longer period to improve their bioavailability and therapeutic efficacy.

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