

Ionotropically-gelled mucoadhesive beads for oral metformin HCl delivery: Formulation, optimization and antidiabetic evaluation

Amit Kumar Nayak¹ and Dilipkumar Pal^{2*}

¹Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj-757086, Odisha, INDIA

²Institute of Pharmaceutical Sciences, Guru Ghasidash Vishwavidyalya, Koni, Bilashpur-495 009, C.G., INDIA

Received:24 February2012 ; revised :24 July 2012; accepted:31 October 2012

The work investigates the development and optimization of ionotropically-gelled mucoadhesive beads composed of tamarind seed polysaccharide (TSP)-alginate polymer-blend for oral delivery of metformin HCl using 3² factorial design. The optimized mucoadhesive beads exhibited 94.86 ± 3.92 % drug encapsulation efficiency and good mucoadhesivity with the biological membrane in wash-off test, and sustained drug release profile over 10 h. The in vitro drug release from these beads was followed controlled-release (zero-order) pattern ($R^2 = 0.9873$ to 0.9980) with super case-II transport mechanism. The optimized TSP-alginate mucoadhesive beads containing metformin HCl showed significant antidiabetic effect in alloxan-induced diabetic rats over prolonged period after oral administration. These results clearly demonstrated that the developed mucoadhesive beads was found suitable for prolonged systemic absorption of metformin HCl through sustained drug release and mucoadhesive properties after oral administration in the management of non-insulin dependent diabetes mellitus with maintenance of blood glucose level.

Keywords: Mucoadhesion, Ionotropic gelation, Metformin HCl, Antidiabetic, Optimization

Introduction

Metformin is an oral hypoglycemic used to treat non-insulin dependent diabetes mellitus¹. Its oral bioavailability is reported 50-60 % and biological half-life is $1.5-1.6 \text{ h}^{-1-2}$. Therefore, controlled release of metformin could facilitate minimum dosing intervals. It would be profitable to develop a mucoadhesive system, which might ease an intimate contact with the absorbing surfaces of mucous membranes (mucoadhesion)³ and thus, the gastric residence could be prolonged with controlled drug release rate to maximize the therapeutic effect. Among natural polysaccharides, sodium alginate has been investigated widely in drug delivery⁴. It undergoes ionotropic gelation by metal ions like Ca^{2+} , Al^{3+} , etc, and produces hydrogel beads⁴. However, cross-linked alginates are usually fragile⁵. Therefore, to formulate mucoadhesive alginate beads, blends of mucoadhesive polymers are usually employed. A few investigations on alginate-based mucoadhesive microcapsules/beads for oral metformin HCl delivery using blends of natural mucoadhesive materials have been reported in the literature⁶⁻⁸.

Tamarind seed polysaccharide (TSP) is obtained from the seeds of *Tamarindus indica* L⁹. It is used as binder, gelling, emulsifying, suspending and mucoadhesive agent in pharmaceutical formulations¹⁰⁻¹¹. In an investigation, we found that the ionotropically-gelled TSP-alginate beads have the capacity to release drug at a controlled rate over prolonged period⁹. Therefore, in the present investigation, we attempted to develop and optimize metformin HCl-loaded TSP-alginate blend mucoadhesive beads that might deliver metformin HCl in the small intestine at a controlled manner over a longer period.

Materials and Methods

Materials

Metformin HCl (Abhilash Chemicals Pvt. Ltd., India), sodium alginate (Central Drug House, India), and calcium chloride (Merck Ltd., India) were used for this present investigation. TSP was isolated from the seeds of *Tamarindus indica* L. The procedure of TSP isolation has been described by Nayak and Pal⁹. All other chemicals used were of analytical grade.

Preparation of beads containing metformin HCl

Briefly, sodium alginate and TSP aqueous solutions were prepared and mixed together. Afterwards,

*Author for correspondence

E-mail: drdilip2003@yahoo.co.in / drdilip71@gmail.com

metformin HCl was added to the mixture, maintaining polymer to drug ratio as 2 and final mixtures were homogenized for 15 min at 1000 rpm using a homogenizer. The resulting dispersions were dropped into CaCl_2 solution via a 26-gauge needle. After 15 min, beads were collected by decantation, washed repeatedly using distilled water and dried at 37°C for 24 h. The dried beads were stored in a desiccator until used.

Experimental design

A 3^2 factorial design was employed for optimization, which contained two factors namely, sodium alginate to TSP ratio, and CaCl_2 concentration at 3 different levels. The drug encapsulation efficiency (DEE, %), drug release at 2 h (R_{2h} , %) and at 10 h (R_{10h} , %) were selected as responses. The effects of factors were modelled using quadratic mathematical models⁹. One-way ANOVA was applied to estimate the significance of models ($p < 0.05$).

Determination of DEE

100 mg of beads were taken and crushed. The crushed powders were placed in a 250 ml volumetric flask. The volume was made up to 250 ml by phosphate buffer, pH 7.4, and kept at $37 \pm 0.5^\circ\text{C}$ for 24 h with occasionally shaking. After the stipulated time, the mixture was stirred at 1000 rpm for 20 min using a magnetic stirrer and then, filtered 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 beads was calculated by the following formula:

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

Bead size measurement

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

In vitro drug release studies

The release of drug from TSP-alginate beads was tested using a basket-type dissolution apparatus USP. Accurately weighed quantities of beads containing metformin HCl equivalent to 100 mg were added to 900 ml of 0.1 N HCl (pH 1.2). The test was carried out for 2 h and then continued in phosphate buffer (pH 7.4) for

next 8 h at 37°C under 50 rpm speed. 5 ml of aliquots was collected at regular time intervals, and the same amount of fresh medium was replaced into vessel to maintain sink condition throughout the experiment. The collected aliquots were measured for drug content using a UV-VIS spectrophotometer (Shimadzu, Japan) at 233 nm against appropriate blank. The *in vitro* drug release data were evaluated kinetically using various mathematical models³⁻⁴.

Mucoadhesion testing

The mucoadhesivity of TSP-alginate beads were evaluated by *ex vivo* wash-off method¹². Freshly excised pieces of goat intestinal mucosa (2 X 2 cm) (collected from slaughterhouse) were mounted on glass slide (7.5 X 2.5 cm) using thread. 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 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°C . After regular time intervals, the machine was stopped and the number of beads still adhering to the tissue was counted.

In vivo antidiabetic evaluation

The antidiabetic evaluation of the optimized beads containing metformin HCl was performed in alloxan-induced diabetic male albino rats³ of either sex (weighing 322-375 gms). 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). The 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, alloxanized rats with fasting blood glucose of ≥ 300 mg/dl were considered diabetic and were employed in the study. The alloxan-induced diabetic rats were divided randomly into 2 groups of 6 rats each and treated as follow: Group A was administered with pure metformin HCl in suspension form and Group B were administered with optimized 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

Table 1—3² full factorial design with observed response values with bead size, for various ionotropically-gelled TSP-alginate beads containing metformin HCl

Code	Factors		Responses (Mean $\pm$ S.D.; n = 3)			Diameter (mm) (Mean $\pm$ S.D.; n = 3)
	A	B	DEE (%)	R _{2h} (%)	R _{10h} (%)	
F-1	1.00 (– 1)	10.00 (+ 1)	94.28 $\pm$ 3.29	14.82 $\pm$ 0.87	69.82 $\pm$ 1.66	1.26 $\pm$ 0.06
F-2	1.00 (– 1)	7.50 (0)	85.70 $\pm$ 2.88	15.45 $\pm$ 0.93	78.46 $\pm$ 1.83	1.36 $\pm$ 0.07
F-3	1.00 (– 1)	5.00 (– 1)	80.43 $\pm$ 2.72	16.83 $\pm$ 1.08	84.78 $\pm$ 2.54	1.52 $\pm$ 0.09
F-4	2.50 (0)	10.00 (+ 1)	88.16 $\pm$ 3.12	12.95 $\pm$ 0.63	76.33 $\pm$ 1.92	1.17 $\pm$ 0.05
F-5	2.50 (0)	7.50 (0)	79.37 $\pm$ 2.53	14.22 $\pm$ 0.94	84.51 $\pm$ 2.45	1.23 $\pm$ 0.06
F-6	2.50 (0)	5.00 (– 1)	74.63 $\pm$ 1.95	15.80 $\pm$ 1.06	91.01 $\pm$ 3.33	1.40 $\pm$ 0.07
F-7	4.00 (+ 1)	10.00 (+ 1)	83.33 $\pm$ 3.07	10.20 $\pm$ 0.44	81.48 $\pm$ 2.28	1.05 $\pm$ 0.03
F-8	4.00 (+ 1)	7.50 (0)	75.34 $\pm$ 2.12	11.31 $\pm$ 0.65	89.92 $\pm$ 3.51	1.18 $\pm$ 0.04
F-9	4.00 (+ 1)	5.00 (– 1)	71.86 $\pm$ 1.88	13.72 $\pm$ 0.95	95.84 $\pm$ 4.06	1.22 $\pm$ 0.03
F-O	1.20	10.75				
	Observed values (Mean $\pm$ S.D.; n = 3)		94.86 $\pm$ 3.92	15.18 $\pm$ 0.86	69.48 $\pm$ 2.52	
	Predicted values		97.02	14.60	67.74	1.24 $\pm$ 0.07
	% Error		- 2.23	3.97	2.57	

(+1) = high, (0) = medium, and (-1) = lower values;
A = Sodium alginate to TSP ratio;
B = CaCl₂ concentration;
DEE (%) = drug encapsulation efficiency (%);
R_{2h} (%) = cumulative drug release after 2 h;
R_{10h} (%) = cumulative drug release after 10 h;
% error = (observed value – predicted value) / predicted value X 100

(0.1 ml) from tail-tip of each rat at regular time intervals under mild ether anaesthesia, and were analyzed for blood glucose by oxidase-peroxidase method using commercial glucose kit.

Statistical analysis

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

Results and Discussion

Optimization by factorial design

For 3² factorial design, a total 9 trial formulations were prepared for two factors (sodium alginate to TSP ratio and CaCl₂ concentration), which were varied at three different levels. The DEE (%), R_{2h} (%), and R_{8h} (%) were evaluated as responses. According to trial proposal, various ionotropically-gelled TSP-alginate beads containing metformin HCl was prepared. Overview of the experimental plan and observed responses values were presented in Table 1. The values of investigated responses measured were fitted into the 3² factorial

design to get model equations for responses analyzed in this investigation. The results of the ANOVA indicated that these models were significant for all response parameters (Table 2).

The influence of factors on investigated responses was further elucidated by response surface methodology (RSM). RSM is a widely proficient approach in the development and optimization of drug delivery devices⁹. In the RSM, the three-dimensional response surface graph is very useful in learning about the main and interaction effects of the factors, whereas two-dimensional contour plot gives a visual representation of the response values¹³. The three-dimensional response surface plot and corresponding contour plot relating DEE indicates the increment with the decreasing of sodium alginate to TSP ratio (A), and increasing of CaCl₂ concentration (B) (Figure 1a). A decrease in R_{2h} values was observed with the increasing sodium alginate to TSP ratio (A), and increasing of CaCl₂ concentration (B) (Figure 2a). However, a decrease in R_{10h} values with the decreasing sodium alginate to TSP ratio (A), and increasing of CaCl₂ concentration (B) is indicated by the three-dimensional response surface plot relating R_{10h} (Figure 3a). All the two-dimensional contour plots (Figure 1b, 2b and 3b) were found to be nonlinear indicating nonlinear relationships between sodium alginate

Table 2—Summary of ANOVA for the response parameters in 3^2 factorial design

Source	Sum of Squares	d. f.	Mean Square	F value	p-value Prob > F
(a) For DEE (%)					
Model	412.03	5	82.13	660.17	< 0.0001 (S)
A	148.80	1	148.80	1192.08	< 0.0001 (S)
B	251.55	1	251.55	2015.24	< 0.0001 (S)
AB	1.42	1	1.42	11.34	0.0435 (S)
A ²	2.43	1	2.43	19.50	0.0215 (S)
B ²	7.83	1	7.83	62.71	0.0042 (S)
(b) For R _{2h} (%)					
Model	36.79	5	7.36	261.48	0.0004 (S)
A	23.48	1	23.48	834.51	< 0.0001 (S)
B	11.70	1	11.70	415.93	0.0003 (S)
AB	0.57	1	0.57	20.29	0.0205 (S)
A ²	0.72	1	0.72	5.73	0.0148 (S)
B ²	0.31	1	0.31	11.00	0.0452 (S)
(a) For R _{10h} (%)					
Model	520.4	5	104.09	4855.83	< 0.0001 (S)
A	194.71	1	194.71	9082.97	< 0.0001 (S)
B	322.67	1	322.67	15051.83	< 0.0001 (S)
AB	0.09	1	0.09	4.20	0.1329 (NS)
A ²	0.64	1	0.64	29.96	0.0120 (S)
B ²	2.36	1	2.36	101.17	0.0018 (S)

^a d. f. indicates degree of freedom

X₁ and X₂ represent the main effects (factors); X₁² and X₂² are the quadratic effect; X₁X₂ is the interaction effect. S and NS indicate significant and not significant, respectively.

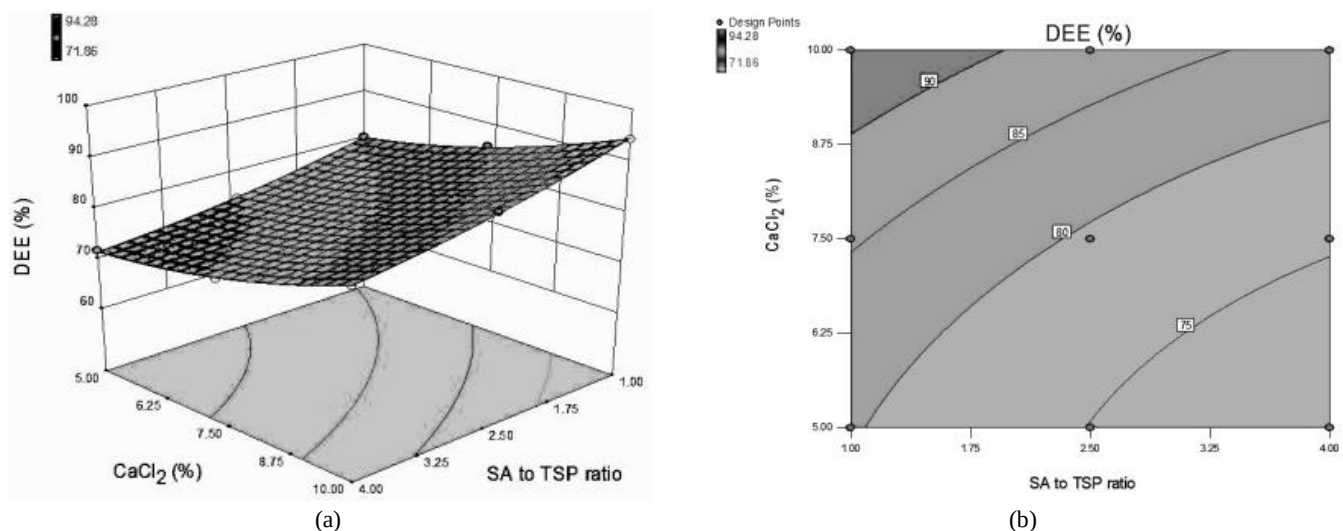


Fig. 1—Effect of factors (sodium alginate to TSP ratio, and CaCl_2 concentration on DEE (%), presented by response surface plot (a), and contour plot (b)

to TSP ratio and concentration of CaCl_2 on all measured responses investigated.

A numerical optimization technique was employed to develop new formulations with desired response (optimum quality). The desirable ranges of factors were restricted to $1.00 \leq A \leq 1.25$, and $9.00 \leq B \leq 11.00$ %;

whereas responses were restricted to $95.00 \leq \text{DEE} \leq 100.00$ %, $14.50 \leq R_{2h} \leq 15.00$ and $65.00 \leq R_{10h} \leq 70.00$ %. 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. In order to evaluate optimization capability of models generated

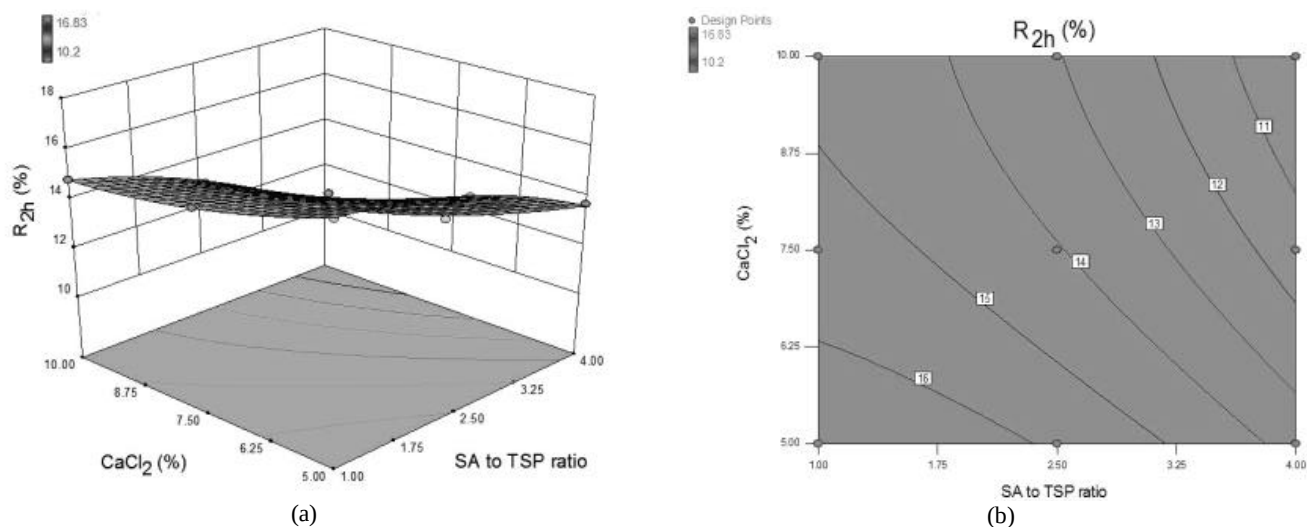


Fig. 2—Effect of factors (sodium alginate to TSP ratio, and CaCl₂ concentration on R_{2h} (%), presented by response surface plot (a), and contour plot (b)

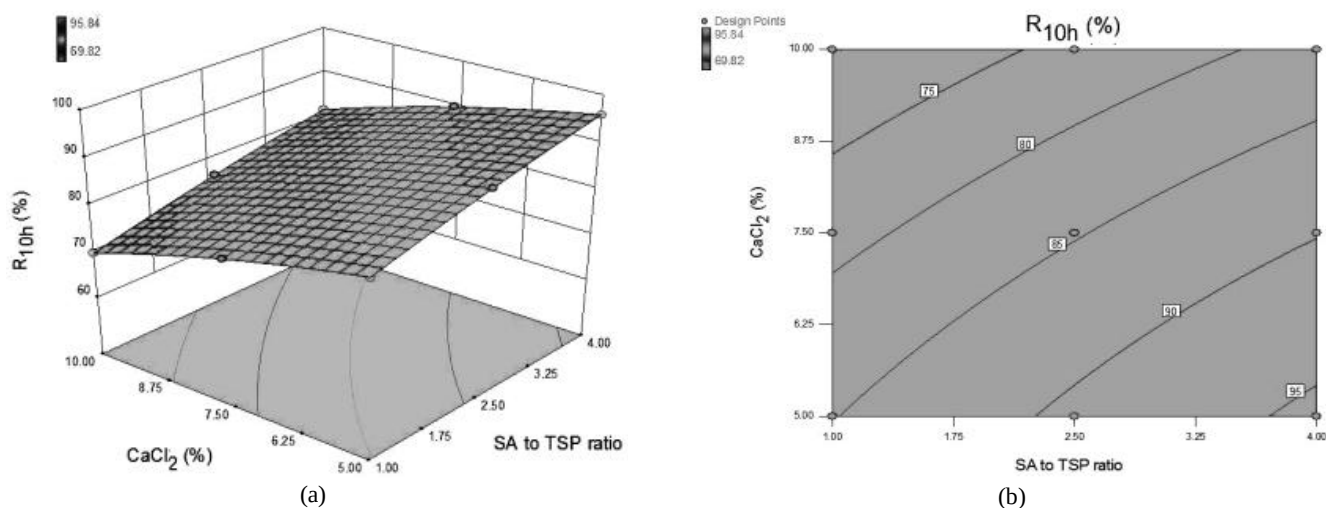


Fig. 3—Effect of factors (sodium alginate to TSP ratio, and CaCl₂ concentration on R_{10h} (%), presented by response surface plot (a), and contour plot (b)

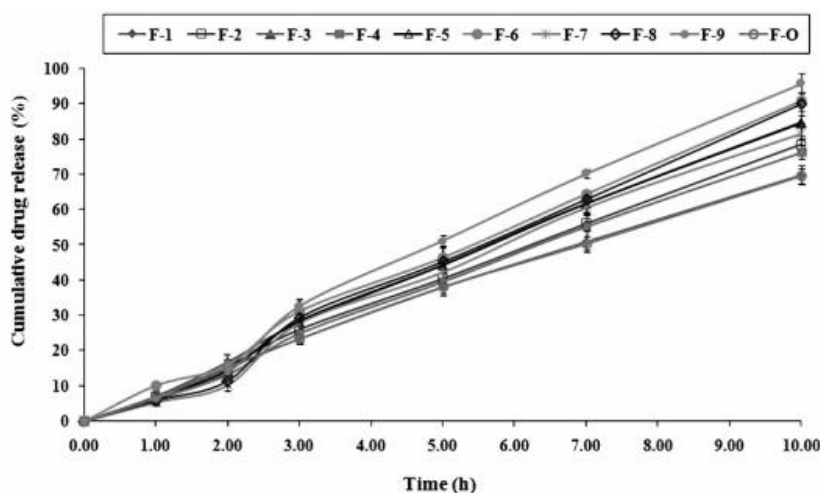
by the 3² factorial design, one optimal setting was chosen to formulate TSP-alginate beads containing metformin HCl. The optimized TSP-alginate beads containing metformin HCl (F-O) was evaluated for DEE (%), R_{2h} (%), and R_{10h} (%). Table 1 lists the results of experiments done with predicted responses by mathematical models and those actually observed. The optimized TSP-alginate beads containing metformin HCl (F-O) showed DEE of 94.86 ± 3.92 %, R_{2h} of 15.18 ± 0.86 % and R_{10h} of 69.48 ± 2.52 % within small error-values (- 2.23, 3.97 and 2.57, respectively), indicating that mathematical models achieved from the 3² factorial design were well fitted.

DEE

The DEE (%) of TSP-alginate beads containing metformin HCl were within the range between 71.86 ± 1.88 to 94.86 ± 3.92 % w/w (Table 1). It was observed that DEE (%) was increased with decreasing of sodium alginate to TSP ratio in polymer-blend, which was used to prepare TSP-alginate beads containing metformin HCl. The increased DEE (%) with decreasing sodium alginate to TSP ratio could be due to the viscosity increment of the polymeric solution by the TSP addition, so that, it might have been prevented drug leaching to the cross-linking solution. Again, the DEE (%) of TSP-alginate beads was increased with increasing CaCl₂ concentration due to the high degree of cross-linking by the interaction

Table 3 Results of curve fitting of the *in vitro* metformin HCl release data from different ionotropically-gelled TSP-alginate mucoadhesive beads

Formulation codes	Correlation coefficient (R^2)				Release exponent (n)
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
F-1	0.9959	0.8465	0.6152	0.9916	1.05
F-2	0.9980	0.8651	0.6042	0.9954	1.05
F-3	0.9971	0.8555	0.5959	0.9936	1.07
F-4	0.9964	0.8649	0.5617	0.9932	1.10
F-5	0.9944	0.8513	0.5553	0.9899	1.12
F-6	0.9952	0.8478	0.5608	0.9889	1.11
F-7	0.9975	0.8600	0.6009	0.9942	1.06
F-8	0.9873	0.8325	0.4663	0.9739	1.23
F-9	0.9895	0.8325	0.4981	0.9810	1.21
F-O	0.9965	0.8638	0.6477	0.9958	1.00

Fig. 4—*In vitro* drug release from various ionotropically-gelled TSP-alginate beads containing metformin HCl (F-1 to F-O) [Mean $\pm$ S.D., n = 3]

between sodium alginate in the polymer-blend, and Ca^{2+} present in the cross-linking solution. The TSP-alginate beads prepared using lower CaCl_2 concentration might have larger pores due to insufficient cross-linking and drug leaching may occur through the pores that may result in lower drug encapsulation⁶.

Bead size

The sizes of various ionotropically-gelled TSP-alginate beads containing metformin HCl was measured by optical microscopic method, and they were within the range of 1.05 ± 0.03 to 1.52 ± 0.09 mm (Table 1). Increasing the bead size was found with the increasing incorporation of TSP in polymer-blend. This could be explained due to the increase in viscosity of the polymer solution with the incorporation of TSP in increasing ratio

that in turn increased the droplet size during addition of the polymer blend solution to the cross-linking solution. On the other hand, with the increasing amount of TSP, the number free sites available for cross-linking could be less so that the size of these beads prepared with decreasing sodium alginate to TSP ratio was increased. Again, the decrease in particle size of TSP-alginate beads was observed, when concentrated CaCl_2 solution was used as cross-linking solution. This may due to shrinkage of polymeric gel by higher degree of cross-linking with the high concentration of cross-linker.

In vitro drug release

The *in vitro* drug release of TSP-alginate beads containing metformin HCl evaluated in the 0.1 N HCl (pH, 1.2) for first 2 h and then, in phosphate buffer

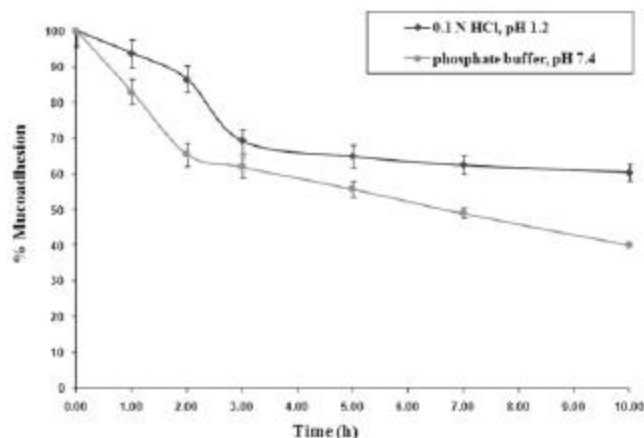


Fig 5—Result of *ex vivo* wash-off test to assess mucoadhesive properties of ionotropically-gelled TSP-alginate beads containing metformin HCl in 0.1 N HCl, pH 1.2, and in phosphate buffer, pH 7.4 [Mean $\pm$ SD, n=3]

(pH, 7.4) for the next 8 h. Metformin HCl release from these beads in the acidic medium was slow (less than 17 % after 2 h) (Figure 4). A decrease in drug release after 2 h was observed in the bead formulations containing higher ratio of alginate than TSP due to the shrinkage of alginate at acidic pH (as alginate is pH sensitive). The trace amount of initial drug release could probably be due to the surface adhered drug. After that, the drug release was observed faster in phosphate buffer (pH, 7.4) comparatively, due to the higher swelling rate of these beads in phosphate buffer. The percentage metformin HCl released from TSP-alginate beads in 10 h (R_{10h} , %) was within the range of 69.48 ± 2.52 % to 95.84 ± 4.06 % and sustained over 10 h (Figure 4). This was also found to be higher with the decreasing sodium alginate to TSP ratio as polymer-blend and increasing Ca^{2+} concentration in cross-linking solution. TSP, present in the bead as polymer-blend could bind better with water to form a viscous gel-structure due to its hydrophilic nature, which might blockade the pores on the surface of beads and sustain the drug release profile. Again, the high degree of cross-linking by higher $CaCl_2$ (cross-linker) concentration could slower the drug release from highly cross-linked TSP-alginate beads containing metformin HCl.

The *in vitro* metformin HCl release from TSP-alginate beads was followed the zero-order kinetics model ($R^2 = 0.9873$ to 0.9980) as the best-fit model over a period of 10 h (Table 3) indicating controlled drug release behavior. The values of release exponent (n) were ranged within 1.00 to 1.23, indicating super case-II transport mechanism controlled by swelling and relaxation of polymeric-blend matrix.

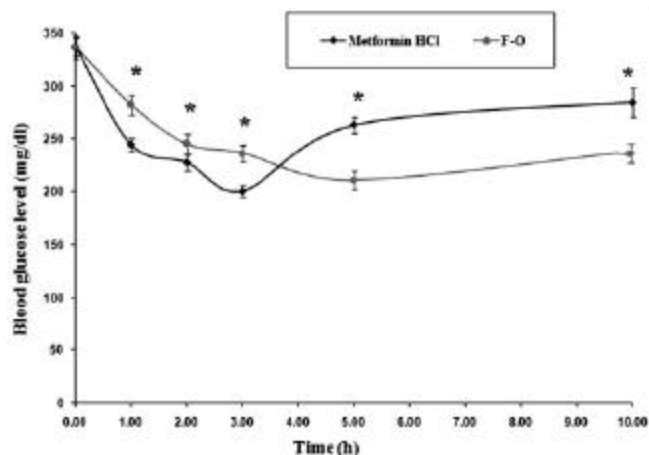


Fig 6—Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and ionotropically-gelled mucoadhesive beads containing metformin HCl (F-O) [Mean $\pm$ SD, n= 6]. The data were analyzed for significant differences ($p < 0.05$) by paired samples t-test and those points are marked by “*”. The statistical analysis was conducted using MedCalc software version 11.6.1.0.

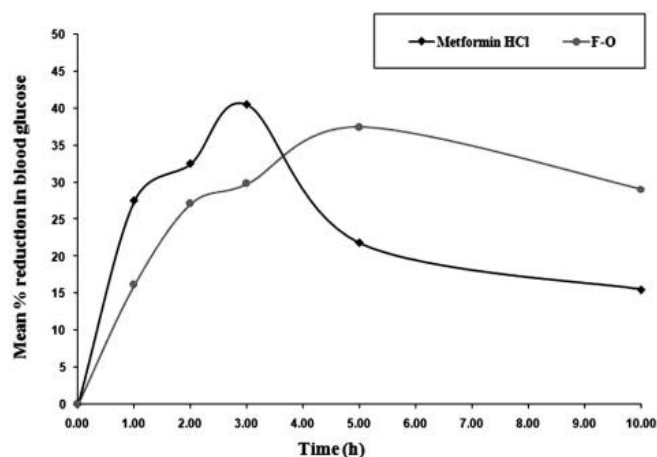


Fig 7—Comparative *in vivo* mean percentage reduction in blood glucose level in alloxan-induced diabetic rats after oral administration of pure metformin HCl and ionotropically-gelled mucoadhesive beads containing metformin HCl (F-O)

Mucoadhesion

The *ex vivo* wash off was faster in intestinal pH than at gastric pH (Figure 5). The decreased mucoadhesion of TSP-alginate beads containing metformin HCl in phosphate buffer may be resulted due to the erosion of calcium ion⁴. These results indicated that the developed beads containing metformin HCl had good mucoadhesivity.

In vivo antidiabetic 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 beads containing metformin HCl is presented in Figure 6 and Figure 7, respectively. In case of the group treated with pure metformin HCl, a rapid reduction in blood glucose level was observed up to 3 h, and after that, the blood glucose level recovered rapidly towards the normal level. In the group treated with optimized mucoadhesive beads containing metformin HCl, the reduction in blood glucose level was slower than that of the group treated with pure metformin HCl up to 3 h and attained more than 25 % in between 1 to 2 h, which was maintained over 10 h. A 25 % reduction in glucose level is considered a significant hypoglycemic effect¹⁴. There were significant differences ($p < 0.05$) between the blood glucose level after oral administration of pure metformin HCl and optimized mucoadhesive beads containing metformin HCl (F-O). Therefore, the significant hypoglycemic effect by the optimized mucoadhesive beads containing metformin HCl (F-O) was observed over 10 h.

Conclusion

Ionotropically-gelled mucoadhesive beads composed of TSP-alginate polymer-blend for oral delivery of metformin HCl was successfully/ developed using 3² factorial design. The method for the preparation of these mucoadhesive beads was found to be simple, easily controllable, economical and consistent. In addition, the excipients used for the formulation of ionotropically-gelled mucoadhesive beads were cheap and easily available. These developed optimized mucoadhesive beads demonstrated high drug encapsulation, good mucoadhesivity with the biological membrane, sustained drug release profile at a controlled rate and significant antidiabetic activity in alloxan-induced diabetic rats over prolonged period after oral administration. Therefore, these mucoadhesive beads was found suitable for prolonged systemic absorption of metformin HCl through sustained drug release and mucoadhesive properties after oral administration maintaining tight blood glucose level and improved patient compliance in the management of non-insulin dependent diabetes mellitus.

References

- 1 Choudhary PK & Kar M. Controlled release metformin hydrochloride microspheres of ethyl cellulose prepared by different methods and study on the polymer affected parameters, *J Microencapsul*, **26** (2009) 46-53.
- 2 Ali J, Arora S, Ahuja A, Babbar AK, Sharma RK, Khar RK & Baboota S. Formulation and development of hydrodynamically balanced system for metformin: *In vitro* and *in vivo* evaluation, *Eur J Pharm Biopharm*, **67** (2007) 196-201.
- 3 Pal D & Nayak AK. Development, optimization, and anti-diabetic activity of gliclazide-loaded alginate-methyl cellulose mucoadhesive microcapsules. *AAPS Pharm Sci Tech*, **12** (2011) 1431-1441.
- 4 Nayak AK, Khatua S, Hasnain MS & Sen KK. Development of diclofenac sodium-loaded alginate-PVP K 30 microbeads using central composite design, *DARU, J* **19** (2011) 356-366.
- 5 Kroll E, Winnik FM & Ziolo RF, *In situ* preparation of nanocrystalline γ -Fe₂O₃ in iron (II) cross-linked alginate gels, *Chem Mater*, **8** (1996) 1594-1596.
- 6 Sharma VK & Bhattacharya A, Release of metformin hydrochloride from ispaghula-sodium alginate beads adhered on cock intestinal mucosam, *Indian J Pharm Educ Res*, **42** (2008) 365-372.
- 7 Maurya DP, Sultana Y, Aquil M, Kumar D, Chuttani K, Ali A & Mishra AK. Formulation and optimization of alkaline extracted ispaghula husk microparticles of isoniazid – *in vitro* and *in vivo* assessment, *J Microencapsul*, **28** (2011) 472-482.
- 8 Sachan NK & Bhattacharya A. Evaluation of Assam Bora rice starch as a possible natural mucoadhesive polymer in formulation of microparticulate controlled drug delivery systems, *J Assam Sci Soc*, **47** (2006) 34-41.
- 9 Nayak AK & Pal D. Development of pH-sensitive tamarind seed polysaccharide-alginate composite beads for controlled diclofenac sodium delivery using response surface methodology, *Int J Biol Macromol*, **49** (2011) 784-793.
- 10 Jani GK, Shah DP, Prajapati VD & Jain VC. Gums and mucilages: versatile excipients for pharmaceutical formulations, *Asian J Pharm Sci*, **4** (2009) 309-323.
- 11 Dutta R & Bandyopadhyay AK. A nasal drug delivery system for diazepam using natural mucoadhesive polysaccharide obtained from tamarind seeds, *Saudi Pharm J*, **14** (2006) 115-119.
- 12 Nayak AK, Hasnain MS, Beg S & Alam MI. Mucoadhesive beads of gliclazide: Design, development, and evaluation, *Sci Asia*, **36** (2010) 319-325.
- 13 Malakar J, Nayak AK & Pal D. Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion-gelation method, *Int J Biol Macromol*, **50** (2012) 138-147.
- 14 Maiti S, Dey P, Banik A, Sa B, Ray S & Kaity S. Tailoring of locust bean gum and development of hydrogel beads for controlled oral delivery of glipizide, *Drug Deliv*, **17** (2010) 288-300.