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Chitosan loaded mucoadhesive microspheres of Glipizide for treatment of type 2 diabetes mellitus: *In vitro* and *in vivo* evaluation

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ABSTRACT

The objective of the present investigation was to design chitosan loaded mucoadhesive microspheres of glipizide for treatment of type 2 diabetes mellitus: in vitro and in vivo evaluation. Type 2 diabetes mellitus is a heterogeneous disease of polygenic origin and involves both defective insulin secretion and peripheral insulin resistance. Despite the availability of new agents for treatment of type 2 diabetes mellitus, oral sulfonylureas remain a cornerstone of therapy, because they are relatively inexpensive and are well tolerated. Glipizide is a potent, rapid-acting with short duration of action and well tolerated second-generation sulfonylurea effective in reducing postprandial glucose levels. However, risk of postprandial hypoglycemia and post-meal glucose excursions, if always associated with the use of glipizide for treatment of type 2 diabetes mellitus. Since, the site of absorption of glipizide is from stomach thus dosage forms that are retained in stomach by mucoadhesion; would increase absorption, improve drug efficiency and decrease dose requirements. Microsphere carrier systems made by using polymer chitosan having strong mucoadhesive. Microspheres were prepared by simple emulsification phase separation technique. On the basis of the preliminary trials 3^2 full factorial designs were employed, to study the effect of independent variable X_1 polymer-to-drug and the stirring speed X_2 on dependent variables percentage mucoadhesion, drug entrapment efficiency and particle size. The optimized formulation exhibited a high drug entrapment efficiency of 60%, swelling index 0.42, Percentage of mucoadhesive after 1 hour 62% and the drug release was also sustained for more than 10 hours. In vivo testing of the mucoadhesive microspheres to albino Wistar rats demonstrated significant hypoglycemic effect of glipizide.

Keywords: Mucoadhesive, Glipizide, Chitosan, Glutaraldehyde.

INTRODUCTION

A primary object of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the GI tract. Stability problem in the intestinal fluid can be overcome. Therapeutic effect of drugs insoluble in the intestinal fluids can be improved. Mucoadhesive microspheres carrier systems are made from the biodegradable polymers in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems.[1-3] Microspheres form an important part of such novel drug delivery systems. They have carried applications and are prepared using assorted polymers.[1] However, the success of these microspheres is limited owing to their short residence time at the site of absorption.[5] It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes.[6-9] Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.[10-13]

Glipizide is a second-generation oral anti-diabetic drug used in type-2 diabetes (non-insulin dependent diabetes mellitus) that can acutely lower the blood glucose level in humans by stimulation the release of insulin from the pancreas. Its shot biological half life (0.3 - 0.7 hours) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg of per day.[18, 20-21]. Thus, the development of controlled-release dosage forms would clearly be advantageous. Researchers have formulated oral controlled release products of glipizide by various techniques. Moreover, the site of absorption of glipizide is in the stomach. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Thus, an attempt was made in this investigation to use chitosan as a natural mucoadhesive polymer and prepare microspheres. The microspheres were characterized by *in vitro* and *in vivo* tests, and factorial design was used to optimize the variables.

MATERIALS AND METHODS

Glipizide was obtained as gift sample from Madras Pharmaceuticals, Chennai. Chitosan (Purified, Viscosity grade 50) was obtained from Fourt's India Limited, Chennai. Dioctyl sodium sulfosuccinate (DOSS), Heavy and Light liquid paraffin, Glutaraldehyde (25% v/v aqueous solution) and Petroleum ether (80:20) was procured from Will son Lab, Mumbai.

Preparation of microspheres

Microspheres were prepared by simple emulsification phase separation technique by using natural mucoadhesive polymer chitosan. The different volume of cross-linking agent glutaraldehyde was used as per method described in Thanoo *et al.* [14]

Polymer (1.5gms) was dissolved in 150 mL of 1% v/v aqueous acetic acid solution and 500 mg of drug was dispersed in the polymer solution. The resultant mixture will be extruded through a syringe (No. 20) in 1lit of liquid paraffin (heavy and light 1:1 ratio). Containing 0.2% DOSS (dioctyl sodium sulfosuccinate) and stirring was performed using propeller stirrer at different stirring speed. After 15 min cross-linking agent glutaraldehyde was added and stirring was continued. The amount of cross-linking agents (10 to 70 mL) and cross-linking times was varied (1 to 4 hrs). In factorial design batches A1 to A9, the optimized amount of glutaraldehyde was used as a cross-linking agent and cross-linking time. The polymer-to-drug ratio (1:1, 3:1 and 6:1)

and stirring speed (500, 1000 and 1500 rpm) were varied in batches A1 to A9 was showed in Table 1. Microspheres thus obtained were filtered and washed with petroleum ether (80:20) to remove traces of oil. They were finally washed with water to remove excess of glutaraldehyde. The microspheres were then dried at room temperature at 25°C and 60% RH for 24 hours.

Evaluation of microspheres

Drug content

According to literature review the assay for second generation oral-anti diabetic drug glipizide was estimated by ultraviolet visible spectrophotometric method. Aqueous solution of drug was prepared in phosphate buffer (pH 7.4) and absorbance was measured on uv spectrophotometer at 276 nm the method is validated for linearity, accuracy and precision.[22] The method obeys Beer's law in the concentration range of 5 to 50 mµg / mL, a standard drug solution was analyzed repeatedly, the mean error (accuracy) and relative standard deviation (precision) were determined.

Drug entrapment efficiency

50 mg of microspheres were crushed in a glass mortar and pestle, and the powdered microspheres was suspend in 10 mL of phosphate buffer solution (pH 7.4). After 24 hours, the solution filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula;

Practical drug content / Theoretical drug content x 100.

Surface morphology

A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron photomicrograph chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kv chamber pressure or 0.6 mmHg, original magnification X 800.[11]

Particle size

The particle size of the microspheres was determined by using optical microscopy method.[23] Approximately 50 microspheres were counted for particle size using a calibrated optical microscope.

Swelling index

The swelling ability of microspheres in physiological media was determined by optical microscopy method. The 100 microspheres were suspended in 5 mL of stimulated gastric fluid USP (pH 1.2). The particle size was monitored by microscopy technique every 1 hour up to 8 hours using an optical microscope.[25]

***In vitro* wash-off test for microspheres**

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test reported by Lehr *et al*.[26] A 1cm by 1cm piece of rat stomach mucosa was tied onto a glass slide (3 inch by 1 inch) using thread. Microspheres were spread onto the wet rinsed tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus were operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted.

Drug release study

The drug release study will perform using USP XXIV basket apparatus at 37 °C ± 0.5°C and 50 rpm using 900 mL of phosphate buffer (pH7.4) as dissolution medium. Microspheres equivalent to 10 mg of glipizide were used for the test. Five mL of sample was withdrawn at predetermined time intervals and filtered through a 0.45 micron membrane filter, diluted suitably and analyzed. Spectrophotometrically an equal amount of fresh medium was replaced immediately after withdrawn of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lamberts-Beer's law equation. The t80 was calculated using the weibull equation.[27]

Release kinetics and mechanism

To know the release mechanism and kinetics of glipizide, optimized formulation was attempted to fit in to mathematical models and n, r^2 values for zero order, first order, higuchi and peppas models. The peppas model is widely used, when the release mechanism is not well known or more than one type of release would be involved.

$Mt / M\infty = ktn$

Where, *M*t / *M*∞ is fraction of drug released at time't', k represents a constant, and n is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-fickian release, the value of n falls between 0.5 and 1.0; while in case of fickian diffusion, n = 0.5; for zero-order release (case II transport), n = 1; and for supercase II transport, n > 1. Observation of all the r^2 values indicated that the highest r^2 (0.9756) value was found for zero order release. According to 'n' value it is one, so it follows non-fickian diffusion with zero order release (case II transport).

Factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, Y is the dependent variable, b0 is the arithmetic mean response of the nine runs, and bi is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1 X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables i.e. polymer-to-drug ratio (X_1) and the stirring speed at rpm (X_2) on dependent variables % mucoadhesion, drug entrapment efficiency and particle size.

***In vivo* anti-diabetic study**

In vivo evaluation studies for glipizide mucoadhesive microspheres were performed on normal healthy wistar rats weighing 250 to 300 g each. The approval of the Institutional Animal Ethics Committee was obtained before starting of the study. The study was conducted in accordance with standard institutional guidelines. Two groups of Wistar rats (5 in each group) that were fasted with water at least 12 hours before the experiments were used for the study. Before drug administration, a blood sample as a control was taken for each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes. The blood glucose level for the

control and test sample was determined using the glucose measuring instrument Medisence (Abbott Laboratories, Bedfort, MA). The instrument was self calibrated, and the samples were allowed to dry before the results were read to avoid contamination of the lens. Pure glipizide and mucoadhesive microspheres of glipizide were administered orally to each group using stomach intubations. A dose of 800 µg/kg of glipizide was administered in suspension form for each rat. Blood samples were collected at predetermined time at 1 hour intervals up to 24 hours, and the blood glucose level was performed as per method described earlier. The percentage reduction in blood glucose level was measured.[31-32]

Stability testing

Optimized formulations of microspheres were tested for stability studies. Both the formulations were divided into 3 sample sets and stored at 4 ± 1, 25°C ± 2°C and 60 ± 5% RH and 37 ± 2°C and 65 ± 5% RH. After 30 days, *in vitro* drug release studies and percentage entrapment efficiency were determined.

RESULTS AND DISCUSSION

The glipizide mucoadhesive microspheres were prepared by simple emulsification phase separation technique using chitosan as natural polymer. Acetic acid from 1% to 8% v/v was used to prepare polymer solution. But there was no effect in concentration of acetic acid was observed on percentage mucoadhesion or drug entrapment efficiency, therefore 1% v/v of acetic acid was used. Polymer concentration was an important factor, mentioned in Lee *et al* based on viscosity of polymers solution. Three different concentrations 0.5, 1 and 2% v/v were selected for trial batches, from this 1% concentration showed a maximum sphericity was observed so we select 1% w/v of polymer in 1% v/v acetic acid was found to be the optimum concentration and 1:1 heavy and light paraffin was used as dispersion medium and 0.2% DOSS (dioctyl sodium sulfosuccinate) surfactant to dispersion medium was found to be essential to minimize aggregation of microspheres. Cross-linking agent 25% v/v aqueous solution of glutaraldehyde was selected due to its high rate of cross-linking and increased in glutaraldehyde concentration caused highly cross-linked spheres and become dense by hardening process. The long term exposure to 100 ppb glutaraldehyde vapour cause respiratory tract lesions including hyperplasia of squamous epithelium etc, therefore, it is important to remove excess of glutaraldehyde from the microspheres to avoid any toxic reactions. The chitosan microspheres are a useful tool to improve the uptake of hydrophilic substance across epithelial layer. The glutaraldehyde was deposited on the surface of microspheres so easy removal of the unreacted free glutaraldehyde as reported by Sahin *et al.*[33]

Preliminary trail batches of microspheres were prepared by using chitosan as polymers, the volume of cross-linking agent 10 to 70 mL and stirring speed were varied from 500, 1000 and 1500 rpm. From these batches, 60 mL of cross-linking agent and 1 hour cross-linking time was the optimum amount and time used for the preparation of mucoadhesive microspheres. Increase in the cross-linking time (1 to 4 hours) was inversely affected the percentage mucoadhesion. The cross-linking chitosan mucoadhesive polymer probably becomes more rigid and thus mucoadhesiveness decreases. The cross-linking time did not have a significant effect on the percentage drug entrapment efficiency.

On the basis of the preliminary trials 3^2 full factorial design were employed, to study the effect of independent variable X_1 (polymer-to- drug ratio 1:1, 3:1 and 6:1) and the stirring speed X_2 (500, 1000 and 1500 rpm) on dependent variables percentage mucoadhesion, drug entrapment efficiency and particle size. The results depicted in Table 1 clearly indicate that all the dependent

variables are strongly dependent on the selected independent variable as they show a wide variation among the nine batches.

Factorial equation for drug entrapment efficiency and particle size

The drug entrapment efficiency was an important variable for assessing the drug loading capacity of microspheres and their drug release profile, thus suggesting the amount of drug availability at site. The following polynomial equation was derived by multiple regression analyses of the data.

$$Y = 69.11 + 10.01\ X1 - 2.91\ X2 - 0.484\ X1^2 - 7.03\ X2^2 - 0.38\ X1\ X2$$

The drug entrapment efficiency of chitosan loaded mucoadhesive microspheres varied from 49% to 54%, 66% to 72%, and 70% to 77% at lower, medium and higher levels of polymer-to-drug ratio respectively, have shown good correlation coefficient 0.9984. Results of the equation indicate that the effect of (X_1) polymer-to-drug ratio is more significant than (X_2) stirring speed. However stirring speed has a negative effect on drug entrapment efficiency, hence the stirring speed increased, the particle size decreased, and the drug entrapment efficiency has also decreased were tabulated in Table 1.

Table 1 Formulations of Chitosan Loaded Glipizide Mucoadhesive Microspheres by 3^2 Full Factorial Design Layout

Batch Code	Variable levels in coded from		% Mucoadhesion After1 hours	Drug Entrapment Efficiency (%)	Swelling Index	Particle Size
	X1	X2				
A1	-1	-1	52	54.25	0.888	60.6
A2	-1	0	46	52.68	0.824	58.2
A3	-1	1	43	49.12	0.812	50.2
A4	0	-1	78	72.00	1.182	67.1
A5	0	0	69	70.84	1.123	64.0
A6	0	1	62	66.96	1.082	60.8
A7	1	-1	80	77.12	1.412	98.0
A8	1	0	73	73.54	1.298	89.8
A9	1	1	67	70.67	1.242	74.4

Note: All batches were prepared using 60 mL of glutaraldehyde and cross-linking time of 1 hours

Translation of coded levels in actual units			
Variables level	**Low (-1)**	**Medium (0)**	**High (+1)**
Polymer-to-drug ratio (X1)	1:1	3:1	6:1
Stirring speed (X2) rpm	500	1000	1500

Table 2 *In vitro* Release Profile of Glipizide Mucoadhesive Microspheres Loaded Chitosan (A4)

Time	Root Time	Log time	Abs	CDR	% CDR	Log % CDR	% Drug Retained	Log % Drug Retained	(% Retained)
1	1	0	0.0294	5.094	25.47	0.707	74.53	1.872	4.208
2	1.414	0.3010	0.0342	6.502	32.51	0.813	67.49	1.829	4.071
3	1.752	0.4771	0.0387	7.886	39.43	0.896	60.57	1.782	3.927
4	2	0.6020	0.0433	9.334	46.67	0.970	53.33	1.726	3.764
5	2.236	0.6989	0.0493	11.164	55.82	1.047	44.18	1.645	3.535
6	2.441	0.7781	0.055	12.988	64.94	1.113	35.06	1.544	3.272
7	2.645	0.8450	0.0621	15.23	76.15	1.182	23.85	1.377	2.878
8	2.828	0.9030	0.0693	17.572	87.86	1.244	12.14	1.084	2.298

Table 3 Model Fitting for the Release Profile of Optimized Glipizide Mucoadhesive Microspheres

Formulation Code	Zero Order	First Order	Higuchi Matrix	Korsmeyer-Peppas		Hixon-Crowell	Best Fit Model
	R	R	R	R	N	R	
Chitosan	0.989 0	0.881	0.944	0.955	0.592	0.930	Zero

***R** = correlation coefficient; **N**= slope (≤ 0.5 – fickian diffusion; 0.5 < n < 1 – non fickian diffusion; 1 – Case – II transport; > 1 – super case –II transport)*

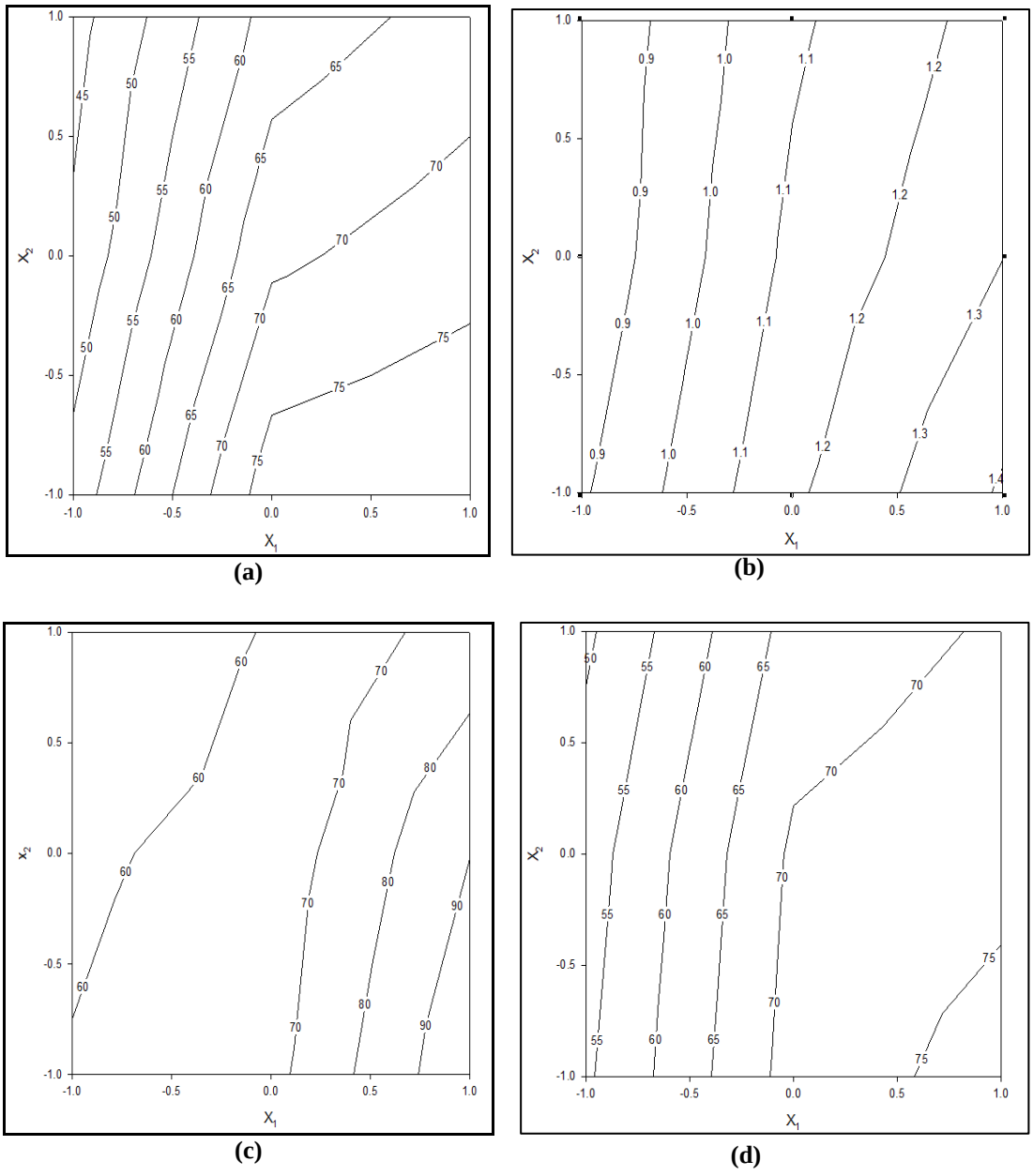
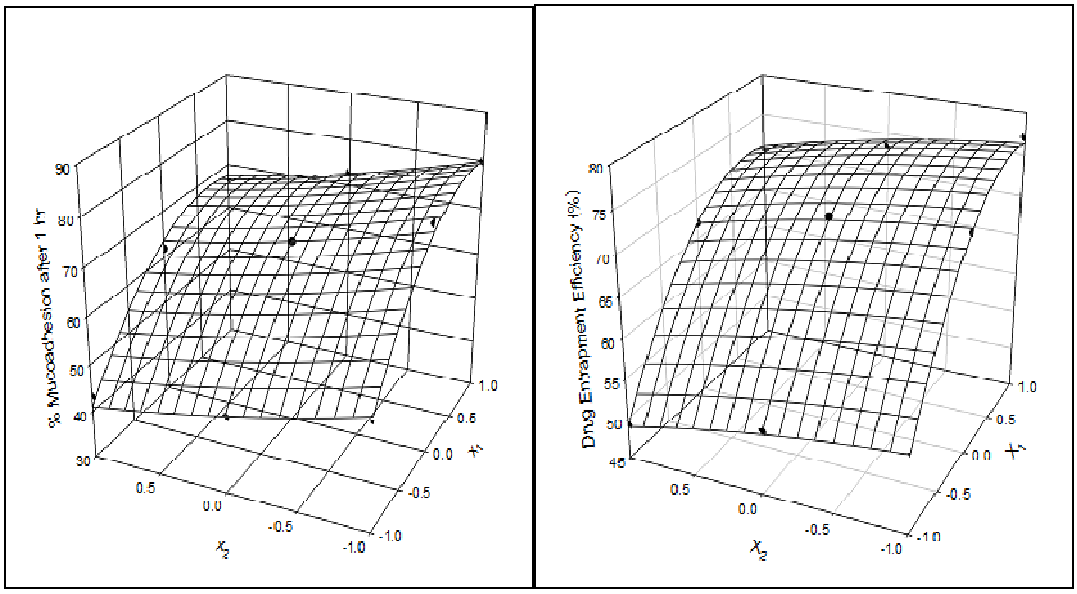
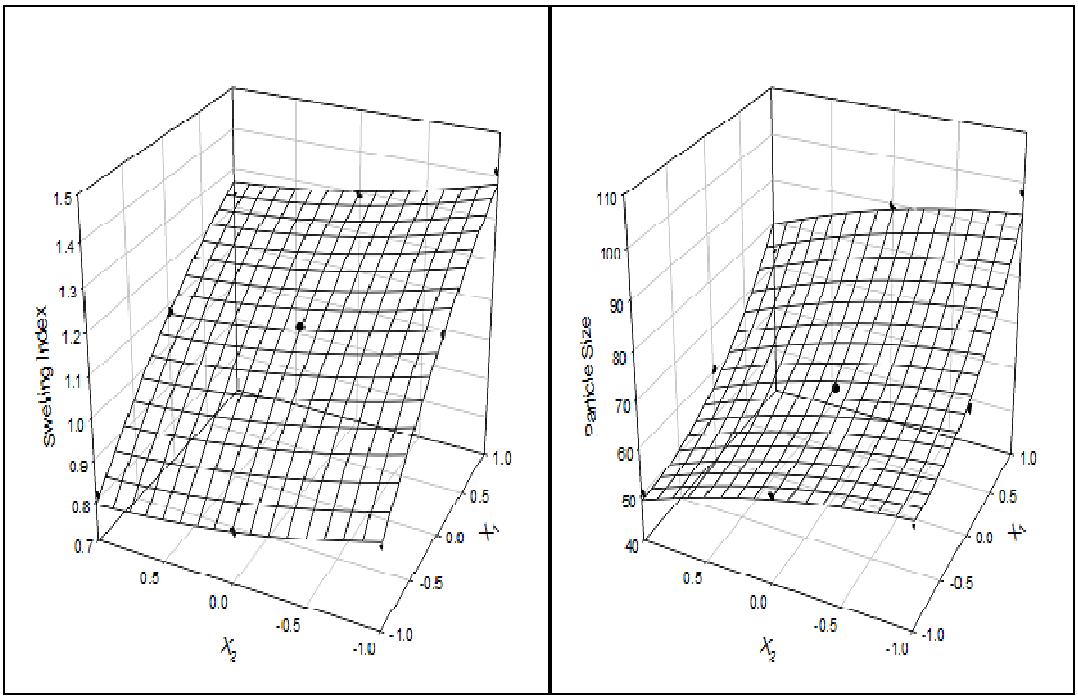


Figure 1 Counter Plot showing the Effect of Polymer-to-Drug Ratio (X1) and Stirring Speed (X2) on: % Mucoadhesion (a), Swelling Index (b), Drug Entrapment Efficiency (c), Particle Size (d) for Optimized Polymer Chitosan.



(1) **(2)**



(3) **(4)**

Figure 2 Surface Graphs Showing Effect of Variables on (1) % Mucoadhesion (2) Drug Entrapment Efficiency (3) Swelling Index (4) Particle Size for Optimized Polymer (Chitosan).

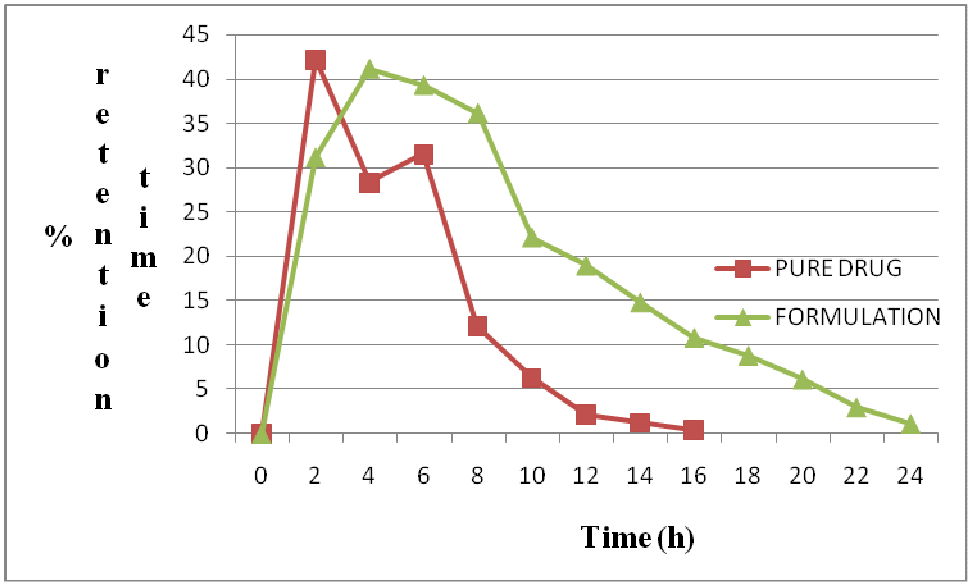


Figure 3 Percentage Reduction in Blood Glucose Levels following Oral Administration of Pure Drug and Formulation in Normal Rats (n = 5)

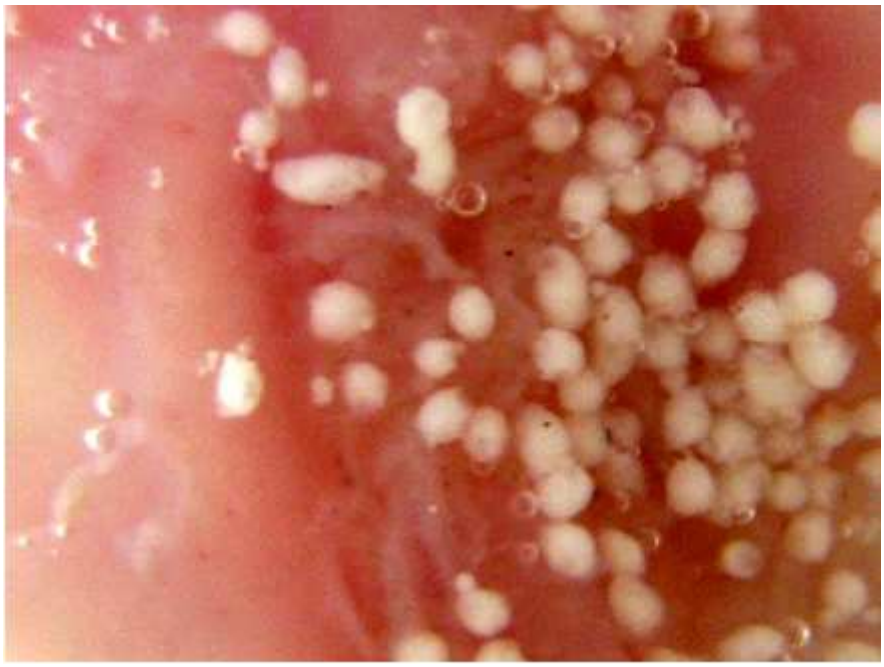


Figure 4 *In vitro* Wash-Off Test Carried out on Glipizide Loaded Chitosan Mucoadhesive Microspheres (batch A4), using Rat Stomach.

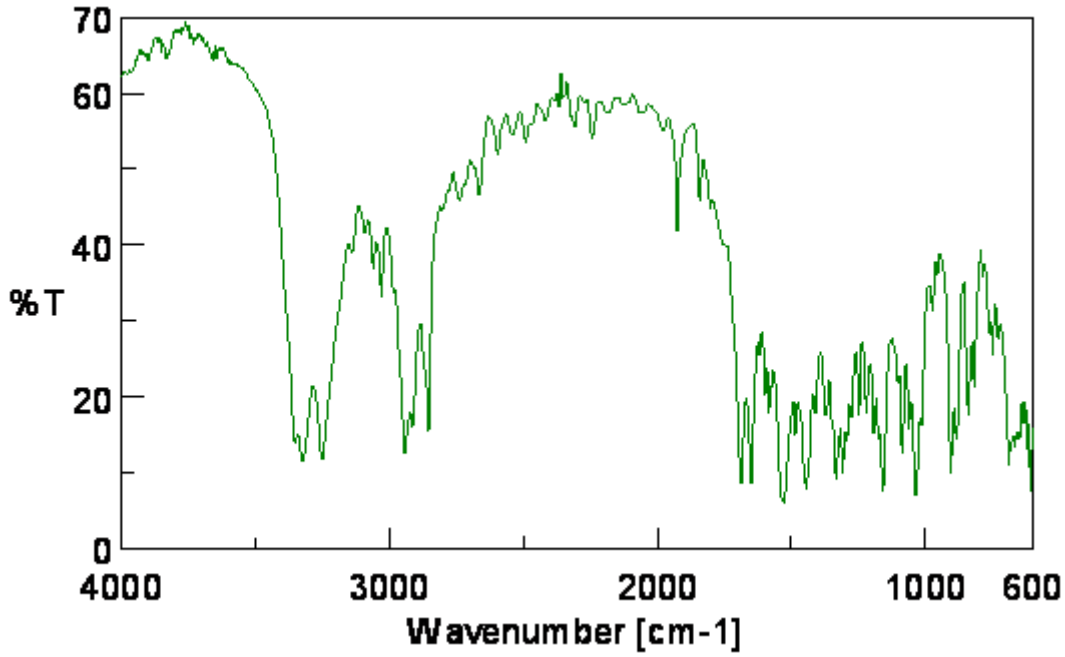


Figure 5 FTIR Spectra for Glipizide Pure Drug

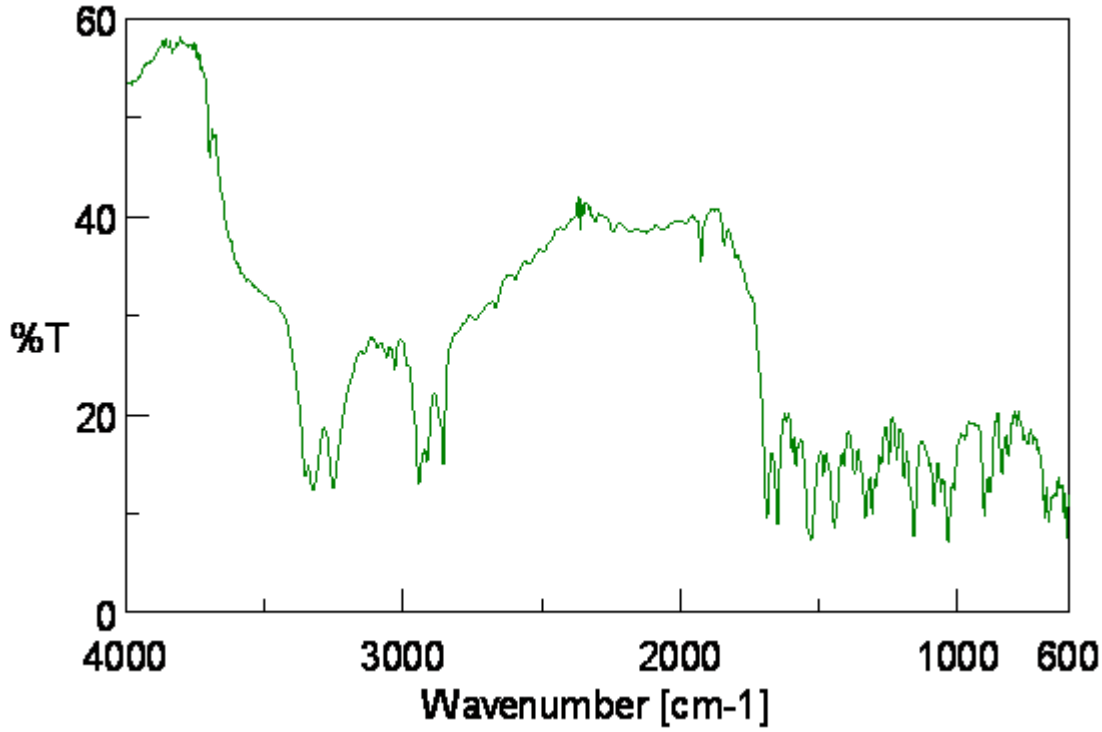


Figure 6 FTIR Spectra for Glipizide and Chitosan

The particle size of the mucoadhesive microspheres of chitosan varied from 50 to 98 µm and has shown good correlation coefficient 0.9878. However stirring speed has a negative effect on particle size, thus the stirring speed increased, the particle sizes decreased.

$Y = 67.1 + 4.69\ X1 - 4.47\ X2 - 0.98\ X_2^2 - 0.98\ X1\ X2$

Factorial equation for percentage mucoadhesion and swelling index

The *in vitro* wash-off test for percentage mucoadhesion of chitosan loaded mucoadhesive microspheres after 1 hour varied from 43% to 52%, 62% to 78% and 67% to 80% at lower, medium and higher levels of polymer-to-drug ratio and has shown good correlation coefficient 0.9803. However stirring speed has a negative effect on percentage mucoadhesion. As the polymer-to-drug ratio increases, the percentage mucoadhesion also increases; because more amounts of polymer results in higher amount of free-COOH groups, which are responsible for binding with sialic acid groups in mucus membrane and thus results in increase in mucoadhesive properties of microspheres. *In vitro* mucoadhesive test has shown that glipizide mucoadhesive microspheres adhered more strongly to gastric mucous layer and would retain in gastrointestinal tract for an extended period of time were showen in Figure 4.

$$Y = 77.81 + 15.78\,X1 - 6.91\,X2 - 9.98\,X2^2 - 2.1\,X1\,X2$$

The microspheres (100) were suspended in 5 mL of simulated gastric fluid USP (pH 1.2). The particle size would be monitored by microscopy technique every 1 hour using an optical microscope. The increase in particle size of the microspheres would be noted up to 8 hours. The swelling index of chitosan varied from 0.812 to 1.412. Surface graphs showing effect of variables on % mucoadhesion, drug entrapment efficiency, swelling index and particle size for optimized polymer (chitosan) were shown in Figure 2. Chitosan could be covalently cross-linked with glutaraldehyde through its amino groups. The aldehyde groups of the glutaraldehyde formed covalent imine bonds with the amino groups of chitosan, due to the resonance established with the adjacent double ethylenic bonds via a Schiff reaction. It is reflects that polymeric chains during cross-linking procedure, the extent of the swelling index depends on the cross-linking. Therefore, the denser the cross-linking bridges between the chitosan molecules, the more packed is the structure. Such structure can be characterized by lower and slower penetration of the solvent through the chain structure of the polymer, suggesting that the swelling ratio and hence the release characteristics of the microspheres can be controlled by varying the content of the cross-linking agent used during the manufacturing process. Since glutaraldehyde is responsible for the formation of cross-links, increasing the amount of glutaraldehyde and cross-linking time will increase the polymer density, resulting in reduction of the macromolecular chain mobility, and formation of more stable and rigid spheres that shows a lower tendency to swell. The finding of this investigation is in agreement with an earlier study performed by many group of researchers report. The plots of cumulative percentage drug release vs. time, cumulative percent drug retained vs. time, log cumulative percent drug retained vs. time and cumulative percent drug release in (mg) vs. time and result of curve fitting of best batch were drawn and represented graphically.

The maximum incorporation efficiency was 78.73% for chitosan and the *in vitro* drug release for eight hours was 87.86%. Among these chitosan (A4) batch has shown the good percentage of mucoadhesion 78%, 87.86% of drug release for eight hours, and 72% of drug entrapment efficiency while comparing with all the polymers and they were spherical in shape and the drug remained dispersed in the polymer matrix in amorphous state. Chitosan (A4) batches seem a promising candidate for achieving drug release up to 10 hours. The drug release mechanism from the mucoadhesive microspheres was found to be controlled release because plots of percentage cumulative drug release vs. square root of time were found to be linear with the regression coefficient (r). The release profile fitted to higuchi-matrix equation, 'r' correlation coefficient value was found to be 0.9440 for the best batch (A4). The release profile fitted to korsmeyer-peppas equation, the 'r' value was found to be 0.9550 and 'n' value was 0.5920 for the best batch

(A4), where n = slope (n ≤ 0.5 - fickian diffusion; 0.5 < n <1 – non fickian diffusion; 1- case-II transport; > 1- super case- II transport). The release profile fitted to hixon-crowell models equation, the 'r' value was found to be 0.9300 for the best batch (A4). The release profile fitted to zero order and first order equation, the 'r' value was found to be 0.9890 and 0.8890 for the best batch (A4). The curve fitting, simulation and plotting was performed in Excel (Microsoft Software Inc., USA) and Sigma plot® version 10.0 (Sigma plot soft ware, Jangel Scientific Software, San Rafael, CA). The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables so as to obtain the desired response. An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plot showing the effect of polymer-to-drug ratio (X_1) and stirring speed (X_2) on: % mucoadhesion, entrapment efficiency, swelling index and particle size were shown in Figure 1.

***In vivo* anti-diabetic study**

Based on the results of other associated parameters present formulations A4 were chosen for evaluation of *in vivo* anti-diabetic study in standard animal models. The drug glypizide was administered at a dose equivalent to 800 µg/kg. Glipizide pure drug was administered in a suspension form at the same dose. Glipizide pure drug was administered, a rapid reduction of 42.83% was observed within 2 hours after oral administration. Blood glucose levels were recovered rapidly to the normal level within 8 hours. In the chitosan loaded glipizide mucoadhesive microspheres, the reduction in blood glucose levels was slow and reached maximum reductions within 4 hours after oral administration were shown in Figure 3. This reduction in blood glucose levels was sustained over a longer periods of time 10 hours. Kahn and Shechter have suggested that a 25% reduction in blood glucose level is considered a significant hypoglycemic effect.[34] 25% of hypoglycemic effect was maintained for a period of 0.5 to 2 hours period after oral administration of pure glipizide. In the case of glipizide mucoadhesive microspheres shows significant hypoglycemic effects was maintained for a period of 1 to 9 hours. The sustained hypoglycemic effect observed over long period of time because of the mucoadhesive microspheres is due to slow release of drug and absorption of glipizide over longer periods of time. Glipizide sustained release formulation is significantly more effective than the immediate release formulation of glipizide in reducing fasting plasma glucose levels and side effects as per Berelowitz *et al*.[35] The optimized batch A4 was studied its potential and associated to control blood glucose level in animal. In this study sustained release mucoadhesive microspheres of glipizide exhibited significant important in diabetic parameters like glucose as compared to immediate release formulation of sustained drug glipizide. It may be the other polysaccharides such as starch and other additive also contain the precursors of glucose in the formulation of oral dosage forms administrated available in market. The result reflects that mucoadhesive microspheres were sustain regimen maintain the *vivo* significant effect in animal models.

Stability studies revealed that, there was a reduction in entrapment efficiency after storage for one month at 4 ± 1°C, 25 ± 2°C with 60 ± 5% RH and 37 ± 2°C with 65±5% RH. It was also revealed that formulations stored at 4±1°C have shown maximum entrapment followed by the storage at 25 ± 2°C at 60 ± 5% RH and 37 ± 2°C at 65 ± 5% RH conditions. Chitosan (A4) stored at 4 ± 1°C has shown 91.12 % drug release, at 25 ± 2°C with 60 ± 5% RH has shown 95.32% and at 37 ± 2°C with 65 ± 5% RH has shown 98.76%, and the percentage of drug entrapment

efficiency at 4 ± 1°C, 25 ± 2°C with 60 ± 5% RH and 37 ± 2°C with 65 ± 5% RH for one month was found to be 72%, 72% and 69%.

The individual IR spectra of the pure drug glipizide as well as the combination spectra of the drug and polymers were shown in Figures 5 to 6. In all the combinations the wave numbers related to aliphatic secondary amine, carbonyl, sulphur-oxy groups were nearly same, which indicates no interaction between glipizide and polymers.

CONCLUSION

The results of a 3^2 full factorial design revealed that the polymer-to-drug ratio and stirring speed significantly affected the dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and swelling index. As the concentration of glutaraldehyde increases, the mucoadhesiveness decreases and there was no significant effect in time. Stirring speed has negetive effect on drug release. Chitosan microspheres (A4) exhibited a high percentage mucoadhesion of 78%, drug entrapment efficiency of 72%, mean particles size of 67.1 µm, swelling index of 1.18 and 87.86% of drug release for eight hours indicates the mucoadhesive microspheres of glipzide could sustain the release of the drug for more than 10 hours. Biodegradable microspheres are one of the most useful devices to deliver materials in an effective, prolonged and safe manner. The *in vivo* study demonstrated significant hypoglycemic activity of the mucoadhesive microspheres of glipizide from chitosan shown significant activity.

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