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Performance Evaluation of Mucoadhesive Potential of Sodium Alginate on Microspheres Containing an Anti-Diabetic Drug: Glipizide

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ABSTRACT
The objective of the present investigation was to design mucoadhesive microspheres to achieve a substantial increase in length of stay of the drug in the GI tract of glipizide for treatment of type 2 diabetes mellitus. Glipizide is a second-generation sulfonylurea derivative used for the treatment of type II diabetes. Its short biological half-life (0.3 ± 0.7 h) necessitates the need to be administered in two or three doses of 2.5-10 mg per day. Mucoadhesive microsphere exhibit a prolonged residence time at the site of application and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved or better therapeutic performance of drug. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. In the present study, alginate based mucoadhesive microspheres of were prepared by ionotropic external gelation technique utilizing calcium chloride ($CaCl_2$) as a cross linking agent, to take the advantage of swelling and mucoadhesive property of alginate beads for improving the oral delivery of glipizide. Interaction studies performed using FTIR spectroscopy and DSC revealed that there was no drug to polymer interactions. The prepared microspheres are discrete, spherical and free flowing which was characterized by entrapment efficiency, particle size, micromeritic properties, *in-vitro* release behavior, scanning electron microscopy (SEM), *in-vitro* wash off test etc. Depending upon the variability in the concentration of sodium alginate, time of cross linking agent, the factors like particle size, and incorporation efficiency and release rate and mucoadhesion properties of microspheres varies. It was observed that increasing the polymer concentration along with the cross-linking time given the better affect on microspheres characteristic and percentage release of drug. Formulation F8 containing 5% w/v sodium alginate was selected as best formulation by considering its better % drug entrapment [84.31%] and flow properties [Carr's index (8.204), angle of repose (31.15)].

Keywords: Glipizide, mucoadhesive microspheres and ionotropic external gelation technique.

INTRODUCTION

The GI tract is the most preferred and commonly used route for the delivery of drugs. For oral drug delivery, the drug absorption is limited by the GI transit time of dosage forms. Since many drugs are absorbed only from upper small intestine, localizing oral drug delivery systems [1] in the stomach or in the duodenum would significantly improve the extent of drug absorption. Bioadhesion is defined as the attachment of synthetic or biological macromolecules to the biological surface. The biological surface can be epithelial tissue or the mucus coat on the surface of tissue. When applied to a mucus coat, bioadhesive interactions [2] occur primarily with the mucus layer and this phenomenon is referred to as 'mucoadhesion'. Since most of the bioadhesive materials interact with mucus before they reach the mucosa, most bioadhesive materials presently available are actually mucoadhesives.

Mucoadhesive drug delivery systems [2] have three distinct advantages when compared to conventional dosage forms. Firstly, the mucoadhesive systems, which are readily localized in the region applied to, can improve and enhance the bioavailability of drugs. Secondly, these dosage forms can facilitate the intimate contact with underlying absorption surface resulting in a better absorption. Lastly, they can prolong residence time at the site of application to permit once or twice a day dosing.

Alginates, which are naturally occurring substances, found in brown algae have received much attention as a vehicle for controlled drug delivery. [3] Alginates can be considered as

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block polymers which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks. Dropwise addition of aqueous alginate solution to the aqueous solution containing calcium ions and or any other di and polyvalent cations cause spherical gel formation Alginate is known to be nontoxic when taken orally and also has a protective effect on the mucous membranes of the upper gastrointestinal tract. The dried alginate beads have the property of reswelling and thus they can act as controlled release system. This property is susceptible to pH, which protects the acid-sensitive drug from gastric juice. Sodium alginate has been used to prepare buccal adhesive formulations. [4] Because sodium alginate, with a greater portion of hydroxyl groups than the other polymers, could bind more strongly with the oligosaccharide chains.

Glipizide is a potent, rapid-acting with short duration of action and well tolerated second-generation sulfonylurea effective in reducing postprandial glucose levels. The primary mode of action of glipizide in experimental animals appears to be the stimulation of insulin secretion from the beta cells of pancreatic islet tissue and is thus dependent on functioning beta cells in the pancreatic islets. In human's glipizide appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. [5] Sulfonylurea's likely bind to ATP-sensitive potassium-channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin. 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. [6] 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.

Numerous approaches have been investigated for formulation of controlled release dosage forms of different therapeutic agents including proteins, peptides, and even cells. Microencapsulation has become a common technique in the production of controlled release dosage forms. The polymeric gel beads are prepared by using number of natural, biodegradable polymers. Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogels. [7] Bivalent alkaline earth metals like Ca^{2+} undergoes ionic interaction with COOH moiety of sodium alginate and results in cross linking of sodium alginate. [8] Since, the use of alginates, gellan gum, chitosan, and carboxymethyl cellulose for the encapsulation of drug and even cells, ionotropic gelation technique has been widely used for this purpose.

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.

MATERIALS AND METHODS

Materials

Glipizide was obtained as a gift sample from Natco Pharma Ltd, Hyderabad, India and Sodium alginate, Calcium chloride, Methanol were purchased from Merck Specialities Pvt. Ltd., Mumbai, India and other reagents were of analytical grade.

Methods

Method of Preparation of Microspheres [8]

The alginate microspheres were prepared by ionotropic-external gelation technique. In this method, weighed quantity of the drug Glipizide was added to 50 ml of phosphate buffer solution (pH-7.4) containing the sodium alginate and thoroughly mixed with a stirrer at 400 rpm. For the formation of microspheres, 50 ml of this solution was extruded drop wise from a needle of 22 G in diameter from a height of about 6 cm into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. Then the solution containing the gel formed microspheres was filtered by using Whatman filter paper no-1. The microspheres were allowed to dry at about 30-40°C and stored in well closed container for further use.

Process Variables [9]

The following process variables were investigated (Bore diameter of the needle; concentration of calcium chloride and sodium alginate; height of dropping; cross-linking time; drying time and temperature) and the different batches thus produced were analyzed for size, shape, drug content and drug release.

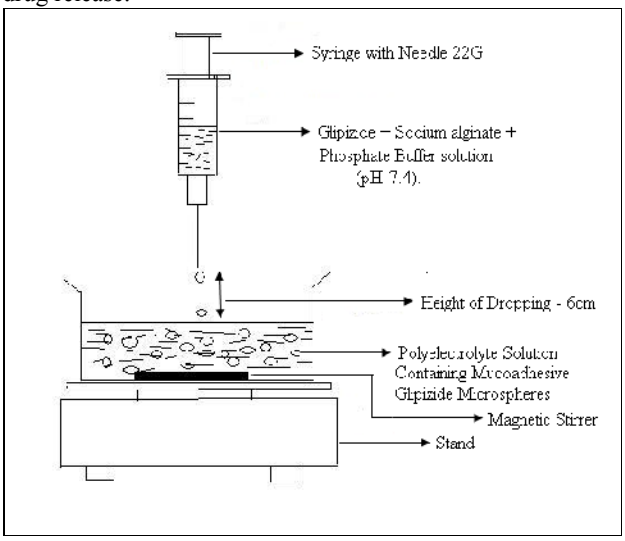

Fig. 1: Method of preparation of Glipizide Microspheres

Fourier Transform Infrared Spectroscopy (FTIR) Studies [10]

The infrared (IR) spectra were recorded using an FTIR spectrophotometer (Perkin Elmer Spectrum GX) by the KBr pellet method in the wavelength region between 400 and 4000 cm^{-1}. The spectra obtained for glipizide and physical mixtures of glipizide with polymers were compared to check compatibility of drug with polymers.

Differential Scanning Calorimeter (DSC) Studies [10]

Thermograms of the samples were obtained by a Perkin-Elmer differential scanning calorimeter (Pyris 6 DSC, software Pyris manager, Perkin-Elmer Schweiz AG, Hunenberg, Switzerland). Samples of 3 mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10°C/min over a temperature range of 50 to 350°C.

Drug Entrapment Efficiency, Drug Loading and Yield Percentage [11]

The amount of Glipizide present in the sodium alginate microspheres was determined by taking the known amount of microspheres in which 10 mg of drug should be present theoretically. Then the microspheres ware crushed and the powdered microspheres was taken and dissolved in 10 ml of phosphate buffer (pH-7.4) solution and stirred for 15 minutes with an interval of 5 minutes and allowed to keep for 24 hours. Then the solution was filtered through Whatman No.1 filter paper. 0.1ml of this solution was diluted upto 10ml with phosphate buffer (pH-7.4) solution and the absorbance was measured spectrophotometrically at 275 nm against phosphate buffer (pH-7.4) solution as blank with the help of UV double beam spectrophotometer and concentrations were determined by employing simultaneous equation:

$$Y = mx+c$$

$$\text{DEE (\%)} = [\text{Experimental drug Content / Initial Drug Content into the Formulation}] \times 100$$

$$\text{Drug Loading (\%)} = [Q_m / Wm] \times 100$$

Where, W_m = weight of the microspheres; Q_m = quantity of the drug present in the microspheres.

Particle Size Analysis [10]

Particles having size range between 50 and 1500µm are estimated by sieving method. In this method the size is expressed as $d_{sieve,}$ which describes the diameter of a sphere that passes through the sieve aperture as the asymmetric particle. The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula**:**

$$\text{Mean particle size} = \sum(\text{mean particle size of the fraction} \times \text{weight fraction}) / \sum(\text{weight fraction})$$

Angle of Repose [12]

The flow characteristics are measured by angle of repose. Improper flow is due to frictional forces between the particles. These forces are quantified by angle of repose. It can calculate by, $\tan\theta = h / r$ or $\theta = \tan^{-1}(h / r)$ where, h = height of pile, r = radius of the base of the pile and θ = angle of repose.

Bulk Density and Tapped Density [12]

Bulk density and tapped density were measured by using 50 ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times and then tapped volume was noted down. Bulk density and tapped density were calculated.

Carr's Index [12]

Compressibility index (Ci) or Carr's index value of microparticles was computed according to the following equation:

$$\text{Carr's index (\%)} = [(\text{Tapped density} - \text{Bulk Density}) / \text{Tapped Density}] \times 100.$$

Table 1: Formula and Composition with Process Variables

Formulation code	Amount of glipizide (% w/v)	Concentration of sodium alginate (% w/v)	Concentration of calcium chloride (% w/v)	Cross-linking time (min)
F1	1	2.0	6.0	10
F2	1	3.0	6.0	10
F3	1	4.0	6.0	10
F4	1	5.0	6.0	10
F5	1	2.0	6.0	15
F6	1	3.0	6.0	15
F7	1	4.0	6.0	15
F8	1	5.0	6.0	15
F9	1	2.0	6.0	20
F10	1	3.0	6.0	20
F11	1	4.0	6.0	20
F12	1	5.0	6.0	20

Table 2: Process Variables Optimized Data

S. No.	Process Variables	Optimized Data
1.	Bore diameter of the needle	22 G.
2.	Height of dropping	6 cm from the level of $CaCl_2$ Solution.
3.	Drying time and temperature	30º to 40ºC for 2-3 hrs.
4.	Sodium alginate concentration	5%w/v dispersion.
5.	Calcium chloride concentration	6% w/v solution.
6.	Cross-linking time	15 minutes.

Table 3: Flow Properties (Mean ± SD, Where n = 5)

Formulation code	Angle of Repose	Bulk Density	Tapped Density	Carr's Index	Hausner's Ratio
F1	26.58 ± 2.67	0.663 ± 0.014	0.723 ± 0.006	8.238 ± 2.46	1.090 ± 0.028
F2	30.01 ± 0.44	0.709 ± 0.019	0.767 ± 0.039	7.404 ± 4.37	1.082 ± 0.050
F3	27.96 ± 1.77	0.724 ± 0.011	0.797 ± 0.013	9.101 ±1.45	1.099 ± 0.09
F4	29.14 ± 1.26	0.725 ± 0.019	0.752 ± 0.03	3.532 ±2.08	1.036 ± 0.02
F5	27.66 ± 2.37	0.673 ± 0.008	0.726 ± 0.015	7.264 ±1.89	1..078 ± 0.02
F6	27.16 ± 1.50	0.690 ± 0.010	0.747 ± 0.010	7.621 ± 0.758	1.082 ± 0.08
F7	29.47 ± 2.13	0.707 ± 0.009	0.771 ± 0.011	8.291 ± 0.089	1.090 ± 0.001
F8	31.15 ± 1.99	0.682 ± 0.009	0.743 ± 0.011	8.204 ± 0.098	1.089 ± 0.001
F9	28.23 ± 1.39	0.632 ± 0.004	0.681 ± 0.009	7.001 ±0.723	1.075 ± 0.008
F10	28.69 ± 1.67	0.627 ± 0.005	0.679 ± 0.006	7.608 ± 0.075	1.082 ± 0.005
F11	30.13 ± 2.09	0.653 ± 0.015	0.702 ± 0.006	5.975 ± 1.981	1.063 ± 0.022
F12	31.39 ± 0.60	0.696 ± 0.026	0.742 ± 0.017	6.215 ± 2.019	1.066 ± 0.023

Table 4: *In-vitro* Release Kinetics Parameters for Glipizide Microspheres

Formulation code	Zero Order Model		First-Order Model		H-M Model		Korsmeyer-Peppas Model	
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	n
F1	0.988	10.72	0.943	0.263	0.989	41.82	0.988	0.885
F2	0.991	10.69	0.944	0.258	0.992	41.70	0.991	0.906
F3	0.990	10.70	0.941	0.249	0.992	41.78	0.986	0.960
F4	0.990	10.72	0.950	0.237	0.990	41.81	0.985	0.999
F5	0.989	10.75	0.943	0.263	0.988	41.90	0.988	0.914
F6	0.990	10.75	0.944	0.258	0.990	41.94	0.988	0.939
F7	0.990	10.79	0.941	0.249	0.991	42.10	0.982	0.998
F8	0.990	10.65	0.950	0.237	0.989	41.51	0.986	0.999
F9	0.989	10.88	0.939	0.260	0.987	42.40	0.985	0.965
F10	0.989	10.68	0.948	0.242	0.990	41.66	0.982	0.990
F11	0.991	10.55	0.947	0.233	0.989	41.09	0.982	0.994
F12	0.993	10.60	0.947	0.226	0.990	41.25	0.979	1.023

Hausner's Ratio [12]

Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation: Hausner's Ratio = Tapped density / Bulk Density.

Surface Topography [10]

The samples for the scanning electron microscope (SEM) analysis were prepared by sprinkling the microspheres on one side of an adhesive stub. Then the microspheres were coated with gold before microscopy. Finally the morphology and size of the microspheres were observed with the scanning electron microscope (FEI Quanta-200 MK2, Netherlands).

***In-vitro* Drug Release Studies** [13]

Release of Glipizide from the microspheres was studied in phosphate buffer of pH 7.4 (900 ml) using a Dissolution Rate Test Apparatus with a rotating paddle stirrer at 50 rpm and 37 ± 1°C as prescribed for glipizide tablets in USP XXIV. A sample of microspheres equivalent to 10 mg of glipizide was used in each test. Samples of dissolution fluid were withdrawn at different time intervals and were assayed at 275 nm for glipizide content using a Shimadzu UV- 1700 double-beam spectrophotometer (Shimadzu Corporation, Japan). Three trials were carried out for all formulations. From this percentage drug release was calculated and plotted against the function of time to study the pattern of the drug release.

***In-vitro* Drug Release Kinetics** [14]

Drug release data were fitted to kinetic model including the zero order, first order, Higuchi matrix, Korsmeyer-Peppas release equations to find the equation with the best fit.

Swelling Study [10]

Microspheres (100 mg) were placed in little excess of PBS (pH 7.4) and allowed to swell to constant weight. The microspheres were removed, blotted with filter paper and their changes in weight were measured at an interval period of 10 minutes and recorded. The degree of swelling (a) was then calculated from the formula:

$$SR= (W_g - W_o) / W_o$$

Where, Wg is final weight, Wo is initial weight of formulation.

***In-vitro* Wash off Test for Microspheres** [13]

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test. A 1-cm by 1-cm 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 was 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.

RESULTS AND DISCUSSION

In this project attempts have been made to prepare the alginate microspheres bearing glipizide by ionotropic external gelation technique demonstrated in Fig. 1. On characterizing the microspheres it was observed that the sodium alginate concentration as well as cross-linking time affects the microspheres characteristics and the percentage release of drug. Better results were found by increasing the polymer concentration along with the cross-linking time. The process variables were investigated and the different batches thus produced were analyzed for size, shape, drug content and drug release. Optimized process variables are described by Table 2.

The FTIR spectra analysis of glipizide and the physical mixtures shows that there was no significant interaction between drug and polymers as shown in Fig. 2 to 4. In the present investigation, DSC thermograms of pure drug, pure sodium alginate, drug and polymer physical mixture and formulation (F8) as shown in Fig. 5 to 9, prominent melting endotherms of pure glipizide and a physical mixture of drug and polymer were found at 218.66°C and 205.06°C. Drug-loaded Alginate microspheres showed a broad small peak at 204.79°C, indicating the presence of drug in crystalline form. The reduction of height and sharpness of the endotherm peak is due to the presence of polymers in the microspheres.

The results of drug loading increased from 15.22 ± 0.004% to 28.28 ± 0.29% of microsphere with increasing the amount of polymer as well as cross linking time. The percent encapsulation efficiency was increased up to 84.31 ± 0.91% with increasing polymer concentration sodium alginate and cross-linking time up to 15 minutes. The percent encapsulation efficiency was described by Fig. 10. The mean diameter of alginate microspheres increased from 608 ± 0.45µm to 714 ±0.72µm. The average particle size of microspheres increased with increasing polymer concentration and decreases with increasing the cross-linking time, which can described by Fig. 11.

The value of Angle of repose of formulation within the range of 31°, indicating good flow properties for the microspheres. The tapped density values ranged between 0.679 ± 0.006 to 0.797 ± 0.013 g/cm^3 and bulk density range between 0.627 ± 0.005 to 0.725 ± 0.019g/ cm.^{3} The result of Carr's Index range from 3.532 ±2.08 to 9.101 ±1.45%, suggests excellent flow characteristics of the microspheres. Hausner's Ratio range from 1.036 ± 0.02 to 1.099 ± 0.09% which indicates good flow property of microspheres as shown in Table 3.

Scanning electron microscopy (FEI Quanta-200 MK2, Netherlands) was used to observe the surface morphology of alginate microspheres with drug before and after dissolution study described by Fig. 14 to 16. The alginate microspheres were subjected to *in-vitro* drug release rate by dissolution profiles of glipizide is shown in Fig. 17 to 20. The initial drug release of glipizide microsphere at 1hr is 10.01% and then found 92.23% at the end of 8 h.

It was found that drug release rate decreased as the concentration of sodium alginate increased and also with increased cross-linking time. The results of dissolution data from dissolution profile fitted to various drug release kinetic equations of zero order, first order, Higuchi and korsemeyer-peppas having r, n and k. r is value of correlation coefficient, k is a release rate constant and n is the diffusional release exponent. The drug release kinetic of different formulation can be described by Table 4.

Alginate microspheres swell in phosphate buffer 7.4, the result of swellability index can be described by Fig. 12. Degree of swelling was found to be increased with polymer concentration and cross-linking time. The study of *in vitro* bioadhesion revealed that all the batches of prepared microspheres had good bioadhesive property ranging from 36 to 58%. On increasing the polymer (Sodium- Alginate) concentration, the bioadhesive property of the microspheres also increased as shown in Fig. 13.

The microspheres were meant to deliver through oral route as capsules. Microspheres were prepared using polymer sodium

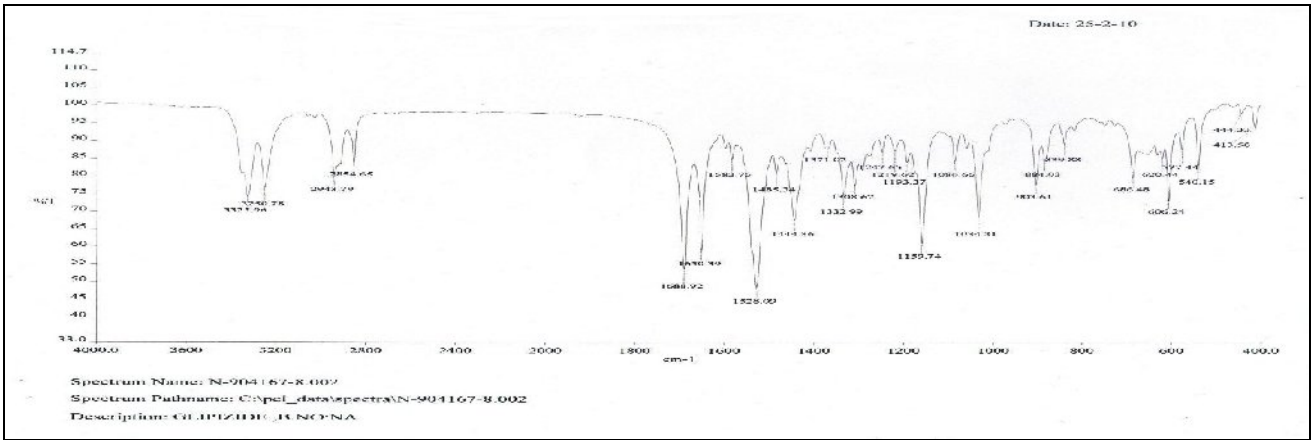

Fig. 2: FTIR Spectra of Glipizide

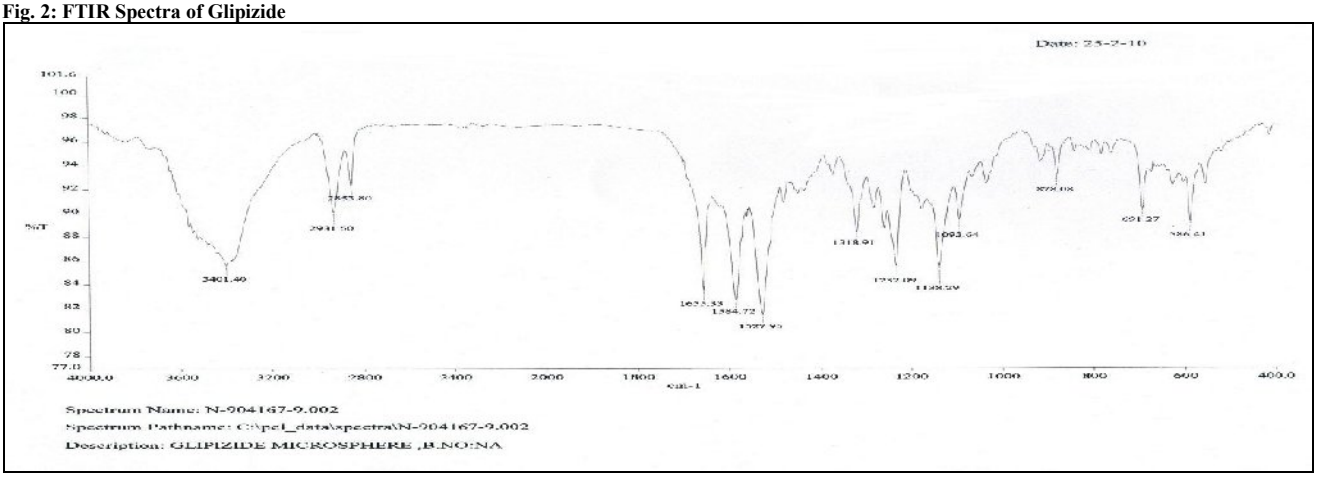

Fig. 3: FTIR Spectra of Sodium alginate

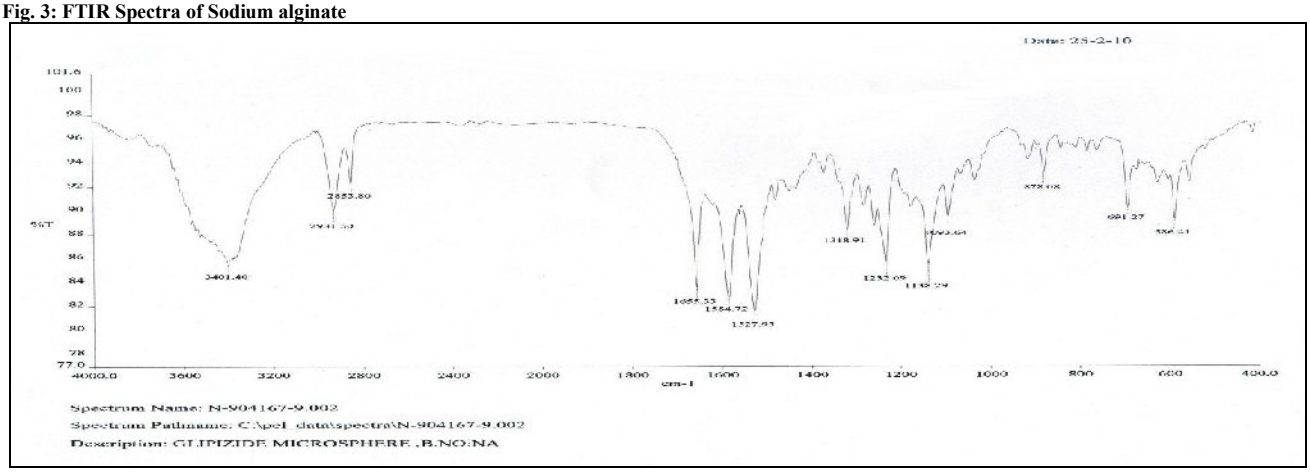

Fig. 4:FTIR Spectra of Glipizide microspheres with Sodium Alginate

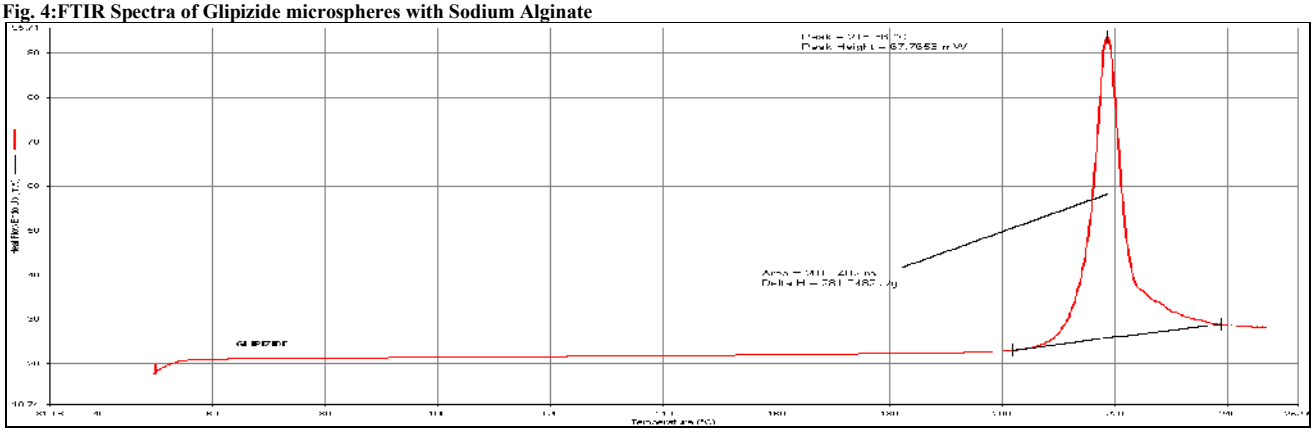

Fig. 5: DSC Thermogram of Glipizide

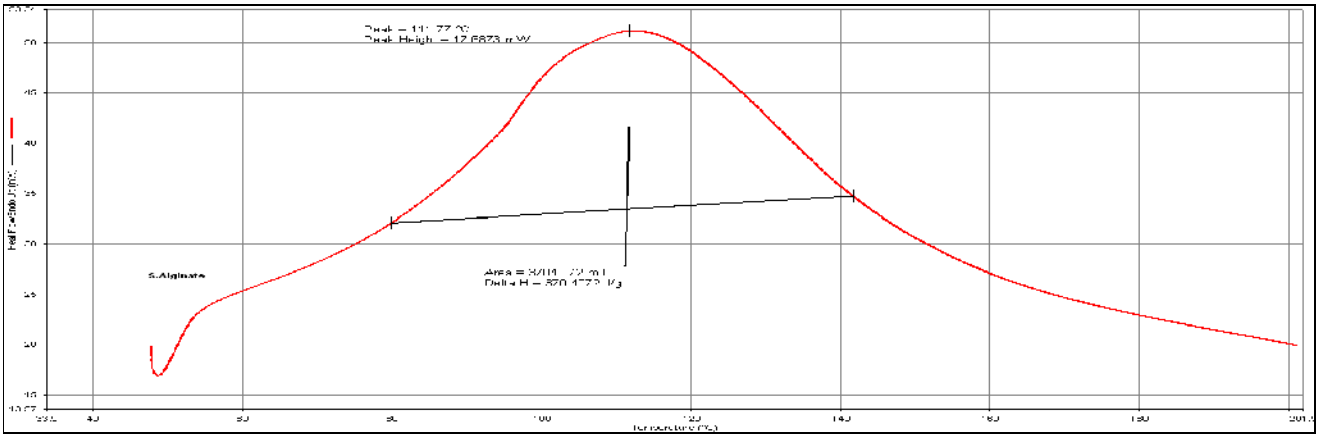

Fig. 6: DSC Thermogram of Sodium Alginate

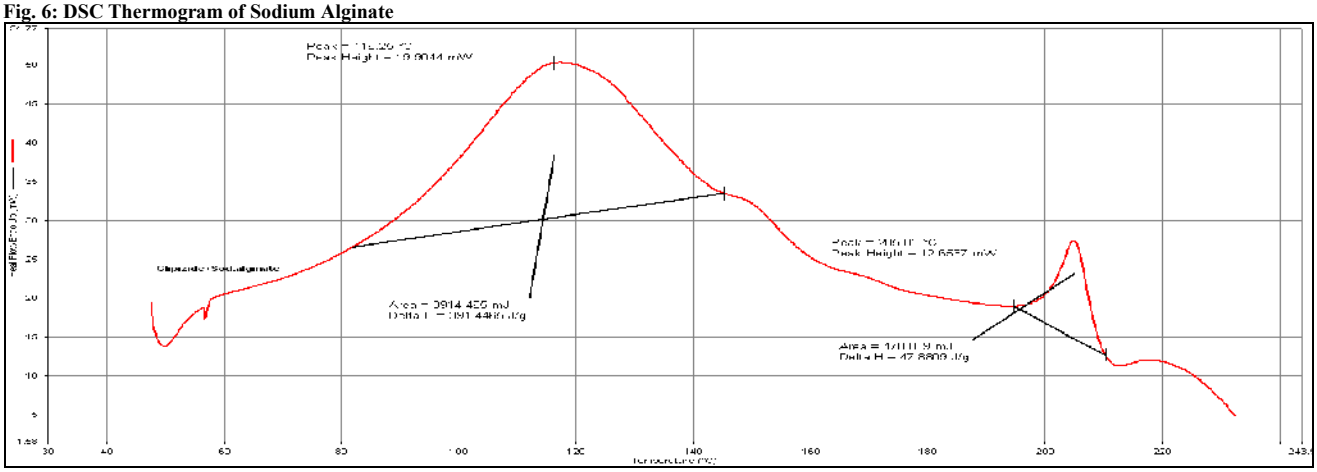

Fig.7: DSC Thermogram of Physical mixture

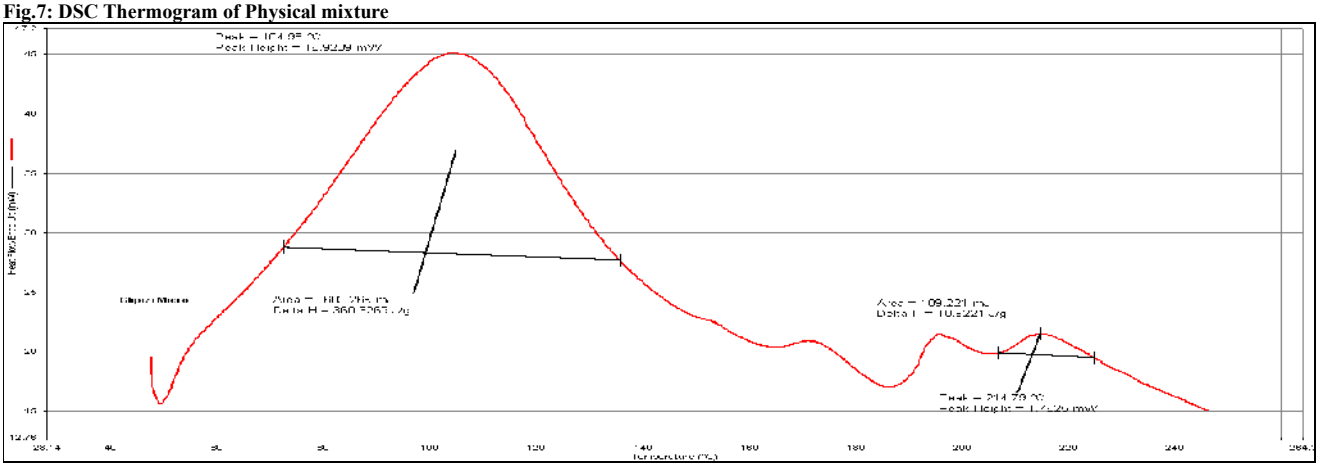

Fig. 8: DSC Thermogram of Formulation (F8)

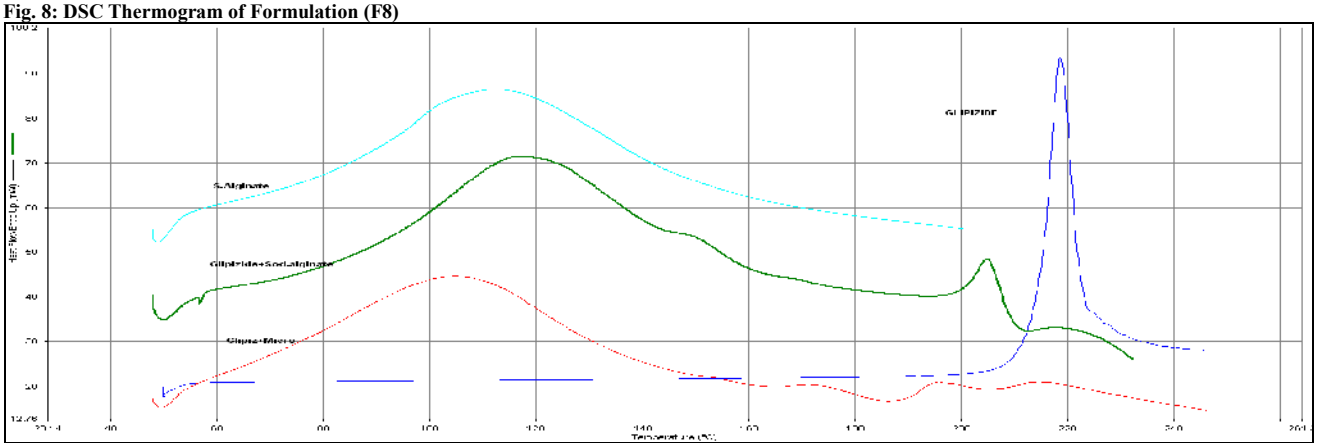

Fig. 9: DSC Thermogram of Drug, Polymer, Physical mixture and Formulation

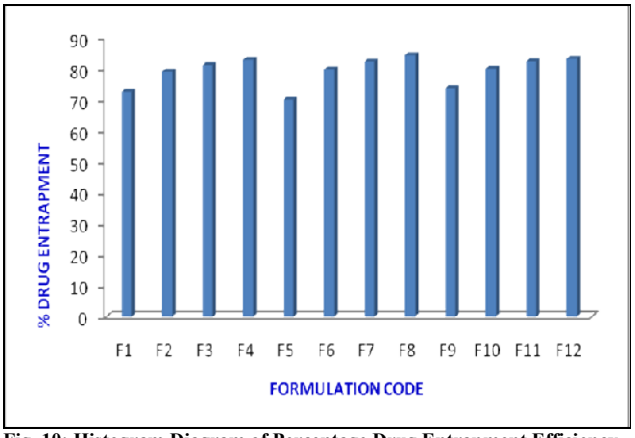

Fig. 10: Histogram Diagram of Percentage Drug Entrapment Efficiency

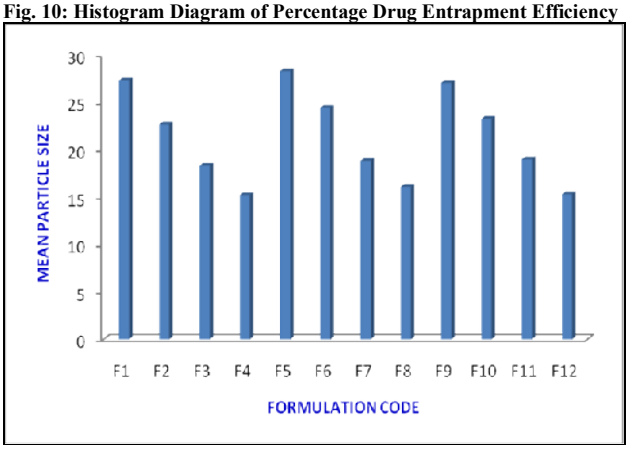

Fig. 11: Histogram Diagram of Mean Particle Size of F1 to F12

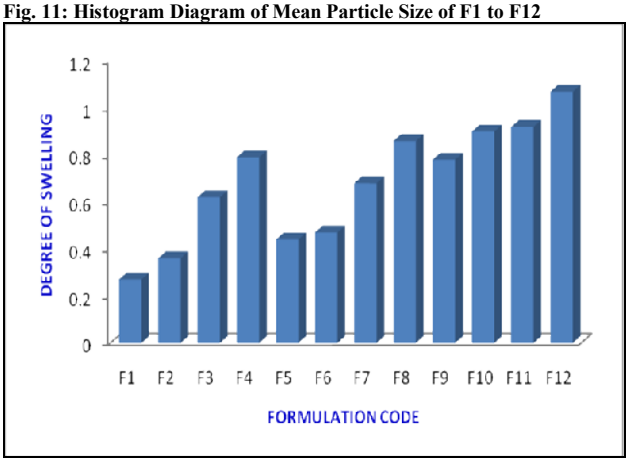

Fig. 12: Histogram Diagram of Degree of Swelling Of Microspheres

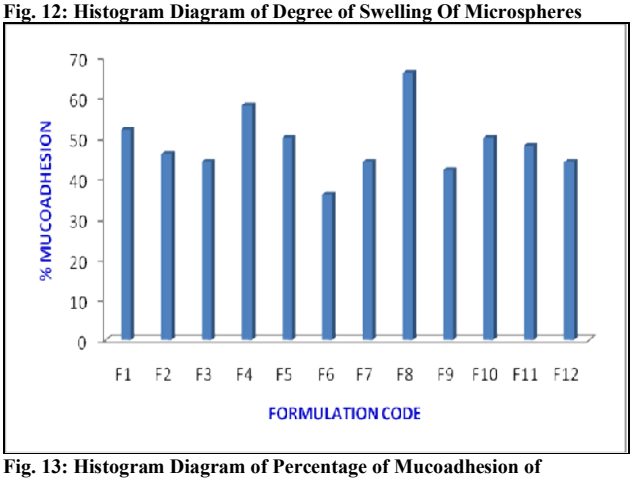

Fig. 13: Histogram Diagram of Percentage of Mucoadhesion of Microspheres

Fig. 14: SEM of Glipizide Microspheres

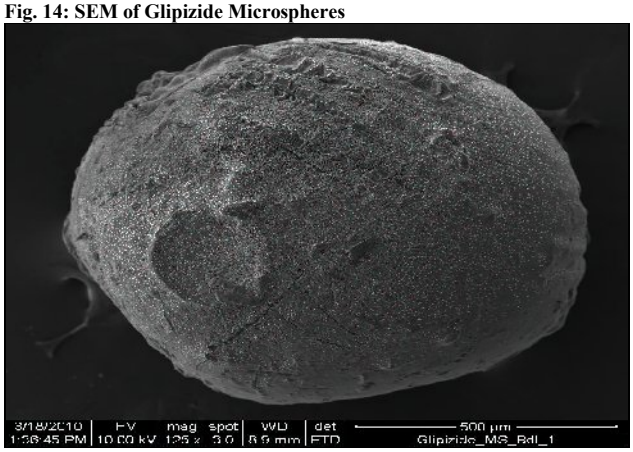

Fig. 15: SEM of Glipizide Microspheres Before Dissolution

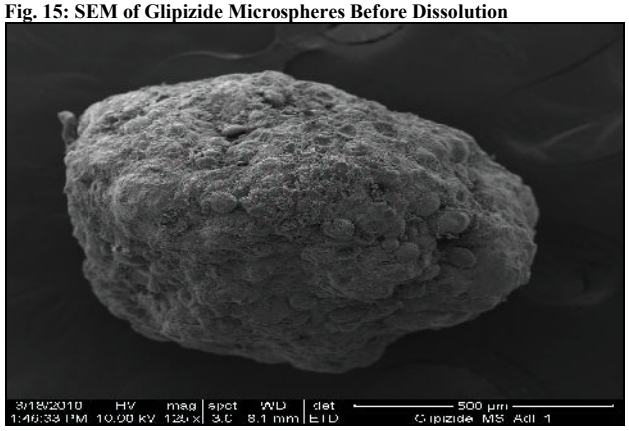

Fig. 16: SEM of Glipizide Microspheres after Dissolution

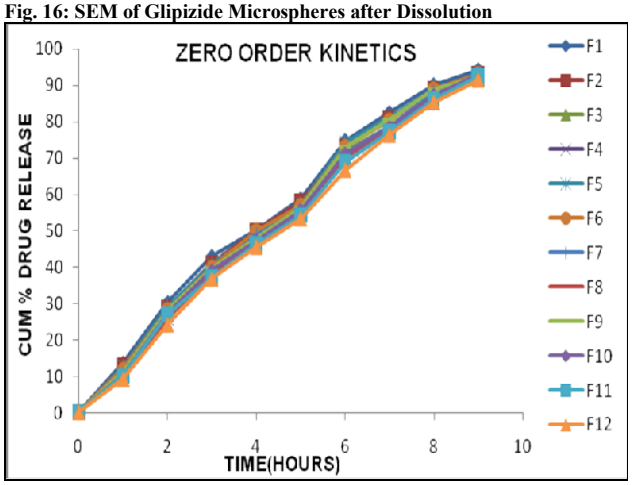

Fig. 17: Release data of GPZ fitted in Zero- Order Kinetics for formulations from F1 to F12

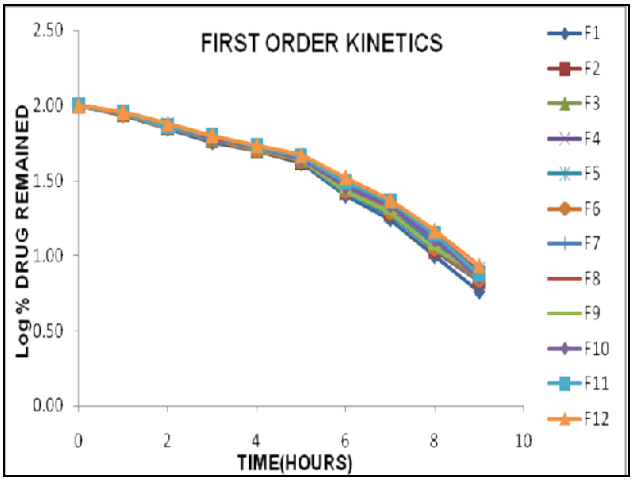

Fig. 18: Release data of GPZ fitted in First- Order Kinetics for formulations From F1 to F12

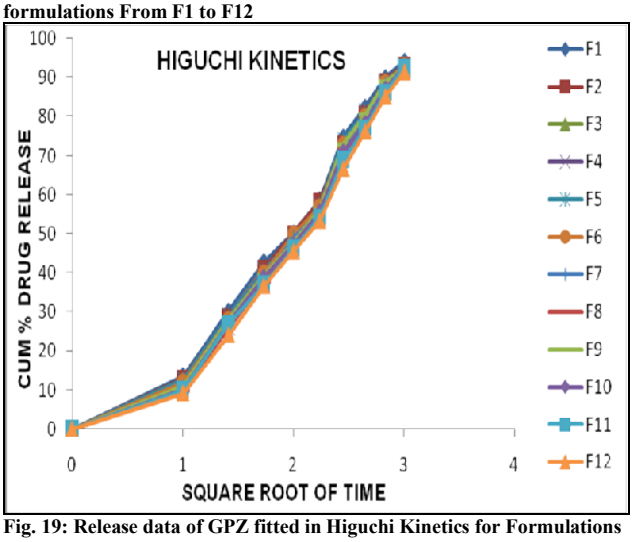

Fig. 19: Release data of GPZ fitted in Higuchi Kinetics for Formulations From F1 to F12

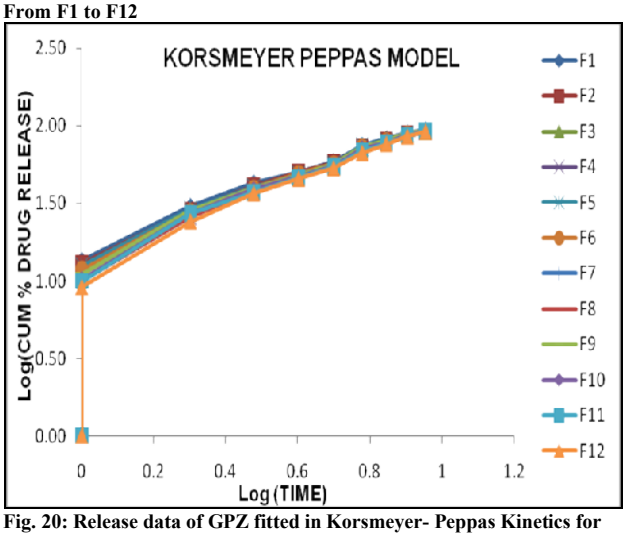

Fig. 20: Release data of GPZ fitted in Korsmeyer- Peppas Kinetics for Formulations F1 to F12

alginate, out of which 5%w/v sodium alginate microspheres were found to be satisfactory. Among the different formulations of F1 to F12, the formulation F8 containing 5% w/v sodium alginate was selected as best formulation; considering its better % drug entrapment [84.31%] and flow properties [Carr's index (8.204), angle of repose (31.15), tapped density (0.743), bulk density (0.682)]. Drug release was sustained up to 8 hrs. The drug dissolution profile was also found to follow Higuchi Matrix kinetics. The microspheres were spherical, discrete and compact, free flowing and size distribution was between 608 µm to 714 µm. SEM of the formulation F8 showed the formation of pores on the surface after *in vitro* dissolution studies. The drug–polymer interaction results suggested no interaction between drug and polymers was observed. Based on the *in vitro* characterization, it was concluded that Glipizide could be administered orally as microspheres as a sustained release dosage form.

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REFERENCES

1. Chowdary KPR, Srinivas L. Mucoadhesive drug delivery systems: A review of current status. Indian Drugs 2000; 37(9): 400-406.
2. Ahuja A, Khar RK, Ali J. Mucoadhesive drug delivery systems. Drug. Dev. Ind. Pharm. 1997; 23(5): 489-515.
3. Gavini E, Rassu G, Sanna V, Cossu M, Giunchedi P. Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: *in vitro*/*ex-vivo* studies. J Pharm Pharmacol. 2005; 57(3): 287-294.
4. El-Gindy GA. Formulation development and *in-vivo* evaluation of buccoadhesive tablets of verapamil hydrochloride. Bull Pharm Sci. 2004; 27: 293-306.
5. Foster RH, Plosker GL. Glipizide: a review of the pharmacoeconomic implications of the extended-release formulation in type 2 diabetes mellitus. Pharmacoeconomics 2000; 18: 289-306.
6. Chowdary KPR, Balatripura G. Design and *in vitro* evaluation of mucoadhesive controlled release oral tablets of glipizide. Ind J Pharm Sci. 2003; 65: 591-594.
7. Bhattacharya A, Maitra P, Mukherjee A. Alginate-based nanocapsular antineoplastic drug delivery system by pneumatic nebulization. Indian J. Pharm. Sci. 2003; 65(5): 477-481.
8. Belgamwar V, Shah V, Surana SJ. Formulation and evaluation of oral mucoadhesive multiparticulate system containing metoprolol tartarate: an *in vitro-ex vivo* characterization. Current Drug Delivery 2009; 6: 113-121.
9. Patil DA, Patil GB, Deshmukh PK, Belgamwar VS, Fursule RA. Chitosan coated mucoadhesive multiparticulate drug delivery system for gliclazide. Asian Journal of Pharmaceutical and Clinical Research 2009; 2(2): 62-68.
10. Mazumder R, Nath LK, Haque A, Maity T, Choudhury PK, Shrestha B, Chakraborty M, Pal RN. Formulation and *in vitro* evaluation of natural polymers based microspheres for colonic drug delivery. International J Pharm Pharmaceut Sci. 2010; 2 (1): 211-219.
11. Senthil A, Thakkar Hardik R, Sivakumar T, Jain DA. Formulation and evaluation of mucoadhesive glipizide microspheres using carboxy methyl cellulose. International Journal of Preclinical and Pharmaceutical Research 2011; 2(1): 45-51.
12. Michael EA, Pharmaceutics the Design and Manufacture of Medicines, 3rd ed., Churchill Livingstone Elsevier, New York, USA 2007.
13. Chowdary KPR, Srinivasa YR. Mucoadhesive microcapsules of glipizide: *in vitro* and *in vivo* ealuation. Ind J Pharm Sci. 2003; 65(3): 279-284.
14. Anandrao RK, Kumaresh SS, Tejraj MA, Walter ER. *In-vitro* Release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. Eur. J. Pharm. Biopharm. 2001; 51: 127-33.