Research Article

Invitro-Invivo Design, Development and Evaluation of Sustained Release Glibenclamide Microspheres by Ion Gelation Technique

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ABSTRACT
The purpose of this research was to formulate and evaluate alginate beads of micro particles. Oral administration of Glibenclamide 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. Gastro intestinal absorption of Glibenclamide in man is uniform, rapid and essentially complete having peak plasma cone. 1-3 hours after single oral dose and half life of eliminating 2 hours in normal subjects. The aim of the study is to design, characterize and evaluate sodium alginate micro spheres of Glibenclamide both in-vitro and in-vivo methods. The prepared micro spheres were free flowing and spherical in shape and characterized for drug loading, flow properties, moisture content, drug content, DSC, X-ray diffraction study and scanning electron microscopy(SEM). The X-ray diffraction study & DSC obtained from various formulations of spray dried micro spheres showed no interaction within these formulations. The in-vitro release studies were performed using pH 7.4 phosphate buffer for 12 hours from which the different drug polymer ratios are followed Higuchi model. SEM studies showed that the micro spheres are spherical and porous in nature. In-vivo study of the micro spheres in healthy rabbits showed a glycemic control for a period of 8-10 hours.

Keywords: Microsphere, Differential Scanning Calorimetry, X-ray diffraction study.

INTRODUCTION

Diabetes mellitus is a debilitating and often life- threatening disorder with increasing incidence of its complications such as diabetic nephropathy, diabetic cardinomyopathy which prevail as a result of hyperglycemia. Glibenclamide is a sulfonyl urea group of drug for better management of hyperglycemia.

The objective of this investigation was the enhancement of dissolution sustaining for a prolonged period of time and consequently rapid hyperglycemic control by preparing Glibenclamide micro spheres using sodium alginate. Also the objective is justifying the formulation a sustained release one both by in-vitro and in-vivo methods having zero drug polymer interaction[1].

EXPERIMENTAL METHODS

1. Materials

Glibenclamide was obtained as gift sample from Dr. Reddy's lab, Hyderabad. Sodium alginate (LOBA CHEMIE, Art. 5760) was obtained from Emami Limited, Kolkata. Calcium chloride dehydrates GR was collected from S.D. Fine Chemicals, Mumbai. Other chemicals were of analytical Reagent grade**.**

2. Method of Preparation of Micro spheres

1%, 1.5 %, 2% & 2.5% w/v aqueous solution of sodium alginate was by a REMI stirrer of speed 500rpm to form a homogeneous polymer solution. The drug sample was dispersed in an appropriate proportion i.e. 1:1, 1:2 & 1:3 ratios and stirring was continued for one to two hours to allow complete dispersion. The dispersion was drop from a glass van syringe having 18-G hypodermic needle to the magnetically stirred calcium chloride water solution at a rate of 1ml per minute at stirring speed of 800 rpm. The beads are collected followed by washing and drying at 25ºc and relative humidity 30% [2].

Table 1: Formulation Design of Micro particles

S.No.	%w/v of formulation	Ratio (Drug: Sod. Alginate)	Distilled Water in ml.	Sodium Alginate (gm)	Drug(gm)
1	1	1:1	25	0.125	0.125
2	1	1:2	25	0.083	0.167
3	1	1:3	25	0.062	0.187
4	1.5	1:1	25	0.187	0.187
5	1.5	1:2	25	0.125	0.250
6	1.5	1:3	25	0.093	0.281
7	2	1:1	25	0.25	0.25
8	2	1:2	25	0.166	0.334
9	2	1:3	25	0.125	0.375
10	2.5	1:1	25	0.312	0.312
11	2.5	1:2	25	0.208	0.417
12	2.5	1:3	25	0.156	0.469

RESULTS AND DISCUSSION

The prepared glibenclamide micro spheres by ion gelation technique were discrete, spherical and free flowing having a good percentage yield. Scanning electron microscopy images demonstrated spherical shaped micro particles and presence of pores which gives the relevant idea of better drug absorption. Thermal behavior of glibenclamide micro spheres with sodium alginate by dsc shows no peak indicating no drug polymer interaction. The x-ray diffraction pattern of the pure drug shows peaks that are sharp and intense signifying its crystalline nature[10]. But its mixture with sodium alginate reduce the number of peaks and peak heights which suggest that the crystallinity converted to amorphous form and it is in good agreement with enhanced solubility. The in-vitro release data were plotted graphically by taking cumulative percent drug release versus time and the plots were found to obey kinetics of higuchi model[5]. The in-vivo study carried out in rabbits where the author did not damage the β cells by alloxan or streptozotacine as usually done. By taking the base line fasting sugar line and on the administration of the formulation, the percentage reduction in blood glucose level gave a very good bench marking anti diabetic formulation of sustained release action[4].

1. Evaluation of Physical Properties

Glibenclamide micro spheres of different ratios were evaluated for its Percentage yield, melting point, flow properties, drug content and moisture by auto Karl Fischer titration[14].

Table 2: Determination of % yield of Glibenclamide

S. No.	Formulations	% w/v	Ratio (D:P)	% yield	Melting point in ºC	% incorporation	% Moisture content
1	Pure Gb	-	-	-	171	-	1.3
2	Gb:Sod.alg	1	1:1	68.8	152	8.2	2.3
3	Gb:Sod.alg	1	1:2	73.2	149	7.1	2.6
4	Gb:Sod.alg	1	1:3	80.4	155	6.3	2.5
5	Gb:Sod.alg	1.5	1:1	82.133	156	7.1	2.3
6	Gb:Sod.alg	1.5	1:2	83.466	163	6.5	3.4
7	Gb:Sod.alg	1.5	1:3	87.2	165	5.8	3.1
8	Gb:Sod.alg	2	1:1	91.8	167	6.7	1.9
9	Gb:Sod.alg	2	1:2	94.4	163	5.7	2.3
10	Gb:Sod.alg	2	1:3	97.8	162	4.9	2.2
11	Gb:Sod.alg	2.5	1:1	94.88	164	5.9	2.5
12	Gb:Sod.alg	2.5	1:2	96.16	167	3.4	2.6
13	Gb:Sod.alg	2.5	1:3	97.28	165	3.6	2.4

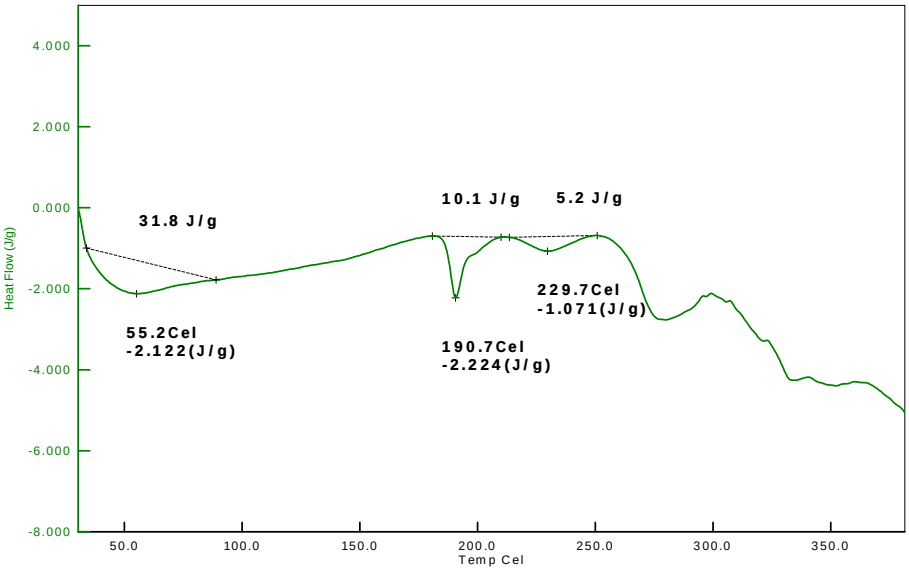
Table 3: Determination of flow properties of Glibenclamide

Formulation	% w/v	Ratio (D:P)	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index	Packing factor
Pure Gb	-	-	0.4313	0.5505	21.65	1.276
Gb:Sod.alg	1	1:1	0.4218	0.6605	36.13	1.565
Gb:Sod.alg	1	1:2	0.4315	0.6609	34.71	1.531
Gb:Sod.alg	1	1:3	0.4228	0.6507	35.02	1.539
Gb:Sod.alg	1.5	1:1	0.4197	0.6009	30.15	1.431
Gb:Sod.alg	1.5	1:2	0.3998	0.5012	20.23	1.253
Gb:Sod.alg	1.5	1:3	0.4718	0.6210	24.02	1. 316
Gb:Sod.alg	2	1:1	0.4278	0.5890	27.36	1.376

Gb:Sod.alg	2	1:2	0.4107	0.5995	31.49	1.459
Gb:Sod.alg	2	1:3	0.5001	0.6987	28.42	1.397
Gb:Sod.alg	2.5	1:1	0.4813	0.6019	20.03	1.250
Gb:Sod.alg	2.5	1:2	0.4513	0.6089	25.88	1.349
Gb:Sod.alg	2.5	1:3	0.4116	0.5993	31.31	1.456

2. Differential Scanning Calorimeter (DSC) Studies

10mg of the pure glibenclamide powder, sodium alginate and glibenclamide sodium alginate mixture at appropriate ratios were subjected to DSC studies using Perkin Elmer DSC 7 model having scanning rate 10º per min[7].



3. Scanning Electron Microscopy

Scanning electron photo micro graphs of glibenclamide micro spheres were taken. A small amount of micro spheres were spread on glass stub[6]. After wards the stub containing the sample was placed in the in scanning electron microscope JSM 5610 LV SEM, JEOL, Datum Ltd (Japan) Chamber at accelerated voltage of 20kv, chamber pressure 0.6mm hg at different magnification[17].

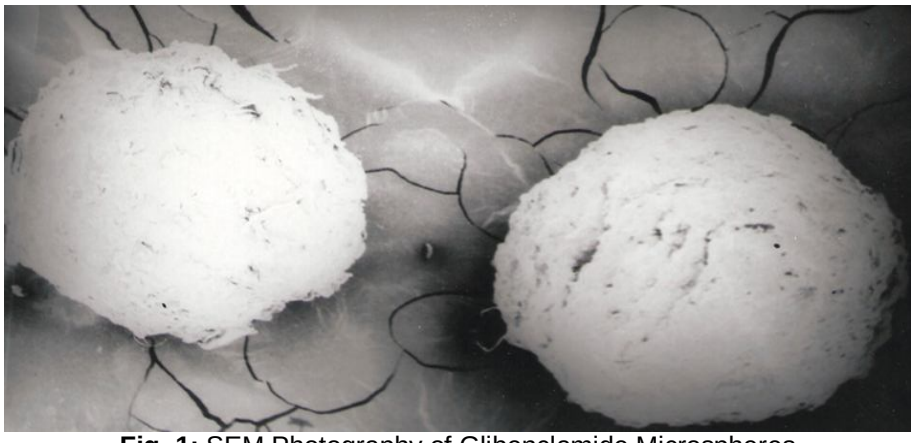
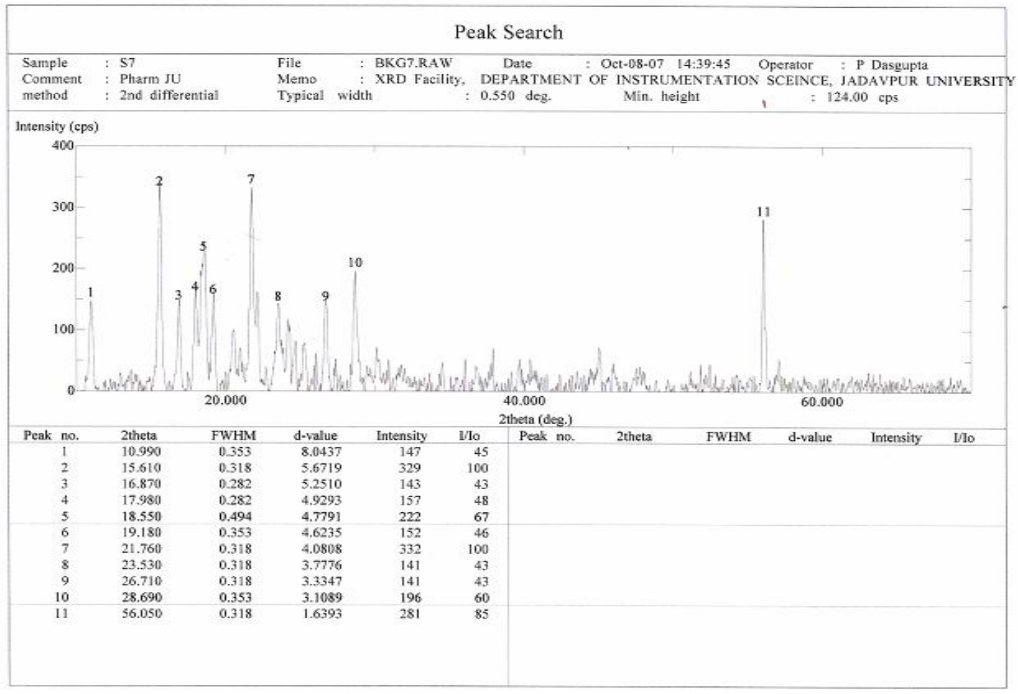


Fig. 1: SEM Photography of Glibenclamide Microspheres

4. X-Ray Diffraction Study

X-ray diffraction of the pure drug, polymer and sample mixture at different proportions were performed using high power X-ray diffract meter XRD-6000, from shimadzu Corporation, Japan.



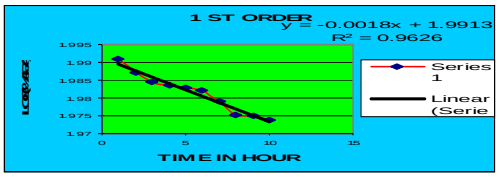
Peak no.	2theta	FWHM	d-value	Intensity	I/Io	Peak no.	2theta	FWHM	d-value	Intensity	I/Io
1	10.990	0.353	8.0437	147	45						
2	15.610	0.318	5.6719	329	100						
3	16.870	0.282	5.2510	143	43						
4	17.980	0.282	4.9293	157	48						
5	18.550	0.494	4.7791	222	67						
6	19.180	0.353	4.6235	152	46						
7	21.760	0.318	4.0808	332	100						
8	23.530	0.318	3.7776	141	43						
9	26.710	0.318	3.3347	141	43						
10	28.690	0.353	3.1089	196	60						
11	56.050	0.318	1.6393	281	85						

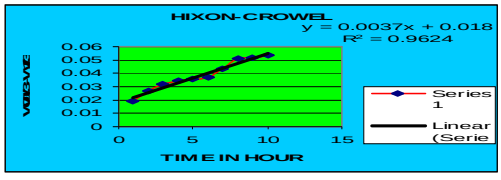
5. In-Vitro Dissolution Rate Studies

In-vitro dissolution rate studies of the micro spheres were performed using USP XX type-2 (electro lab TDP- 06T) apparatus[21]. Drug release was studied in 900ml of 7.2 pH phosphate buffer 37± 0.5ºC at 100 rpm. 1ml sample was withdrawn at regular intervals and the same quantity of pre warmed fresh dissolution medium was replaced. The samples withdrawn were assayed spectro photo metrically at 203nm using shimadzu 1700 UV visible spectrophotometer.

DISSOLUTION PROFILE OF GLIBENCLAMIDE (1:2)

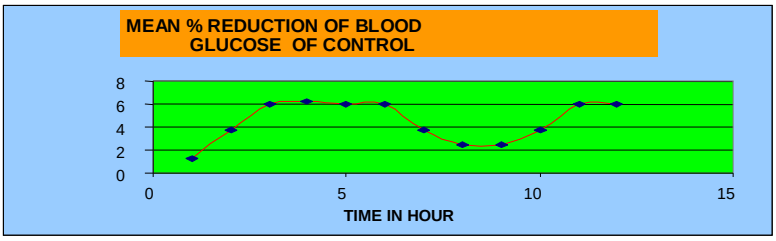
Time in hr	SQRT	CR	%MR	ARA	%ARA	Log(%ARA)	W01/3-W1/3
0.25	0.5	0.327	1.635	19.673	98.365	1.9928406	0.01487494
0.5	0.707107	0.327	1.635	19.673	98.365	1.9928406	0.01487494
1	1	0.417	2.085	19.583	97.915	1.9908492	0.01899786
2	1.414214	0.582	2.91	19.418	97.09	1.9871745	0.02658946
3	1.732051	0.702	3.51	19.298	96.49	1.9844823	0.03213768
4	2	0.747	3.735	19.253	96.265	1.9834684	0.03422419
5	2.236068	0.777	3.885	19.223	96.115	1.9827912	0.03561701
6	2.44949	0.807	4.035	19.193	95.965	1.9821129	0.03701127
7	2.645751	0.942	4.71	19.058	95.29	1.9790473	0.04330351
8	2.828427	1.107	5.535	18.893	94.465	1.9752709	0.05103451
9	3	1.122	5.61	18.878	94.39	1.974926	0.05173955
10	3.162278	1.167	5.835	18.833	94.165	1.9738895	0.05385693
11	3.316625	1.452	7.26	18.548	92.74	1.9672671	0.06734597

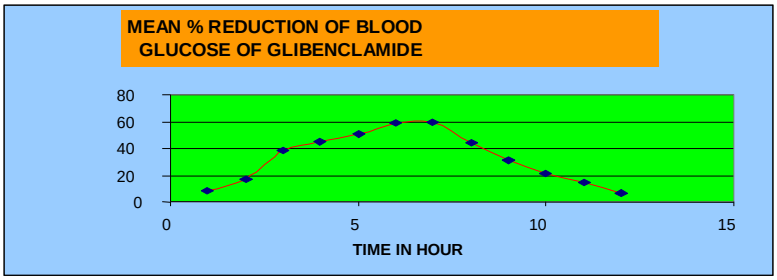




6. In-vivo study

Five groups of rabbits (one control and four tests) were fasted (with free water) at least 12 hours before the experiment. Each group consists of five mice weighing 20-25 grams each. Before drug administration a blood control samples from the tail vein using a syringe. The blood glucose level was determined using the glucose measuring instrument (SURE STER 'LIFENSCAN, Inc', Johnson-Johnson Company, Milpitas, California USA). The instrument was self calibrated and the appropriate ratio of formulation (drug; polymer:: 1:2) was administering it through oral route in the form of suspensions at 1mg/kg body weight. The experiment was carried out for 12 hours at every 1 hour interval.





CONCLUSION

The design and development method of sustained release glibenclamide micro spheres by ion gelation technique is simple. The spherical shape and surface topography by SEM gives demarcation for better absorption. The in-vitro dissolution studies and in-vivo studies in rabbits showing an onset of action for 8-10 hours proving the anti diabetic formulation a sustained release drug delivery system.

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