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FORMULATION AND EVALUATION OF MICROSPHERES OF GLIBENCLAMIDE BY IONOTROPIC GELATION METHOD

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ABSTRACT

Microspheres is novel drug delivery system for improving therapeutic action of drug, increasing prolong action, lowering dose frequency of dosage form and to improve patient complies. Microspheres are reducing oral administration side effect such as gastric irritation in stomach. Glibenclamide microspheres were developed by ionotropic gelation method using sodium alginate and chitosan. Calcium chloride was used as a cross linking agent. Prepared microspheres were evaluated for entrapment efficiency, microsphere size, morphology, FTIR, DSC, *in-vitro* drug release and drug release kinetics. Prepared Glibenclamide microspheres were found discrete, free flowing and spherical. The mean particle size ranged from 349-540 µm and percentage yield ranged between 70 to 98.92%. The size of microsphere was increased by increasing concentration sodium alginate and calcium chloride while the entrapment efficiency was increased with increasing concentration of chitosan. XRD studies confirmed the crystalline nature of Glibenclamide. SEM studies showed that the microspheres are spherical and with rough surface. The *in-vitro* drug release study was carried out in phosphate buffer pH 7.4. Percent drug release was decreased with increase in concentration of sodium alginate and calcium chloride. Decreased drug release rate was obtained in case of F3 formulation containing sodium alginate and chitosan at 3:1 ratio and 5% calcium chloride as a cross-linking agent. The present study conclusively that Glibenclamide microsphere could be prepared successfully and formulation F3 was shows satisfactory result. The prepare Glibenclamide microspheres to maintain an effective of drug concentration in serum for long period of time and reducing gastric irritation.

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INTRODUCTION[1-4]:

Novel drug delivery system means of improving the therapeutic effectiveness of incorporated drugs by providing controlled delivery, targeting and sustained delivery. The drugs in to dosage for with the aim of sustaining drug levels and hence drug action is obtained for as prolong period of time in body. Microspheres are carrier drug delivery system which plays an important role in micro-particulate novel drug delivery system. Microspheres are spherical, free flowing, monolithic matrix type. The main goal of the microspheres drug delivery system is to provide therapeutic amount of drug to the target site in the body. Microspheres are designed to release the drug in sustained and controlled manner, improving bioavailability, entrapment efficiency and lowering dose frequency of drug in the dosage form.

Diabetes mellitus is one of the most common non-communicable diseases in world and it is undoubtedly one of the most challenging health problems in 21st century. Glibenclamide is oral hypoglycemic agent,Anti Arrhythmic agent and BCS class II drug (low solubility and high permeability) are widely used in diabetes treat type II (non insulin dependent diabetes mellitus). It is second generation sulfonylurea's derivatives class of anti diabetic drugs. Its biological half life is 2-4 h.

To study the effect of concentration of polymer and calcium chloride on release profile of glibenclamide The main objective of this research work is to formulate sustained release microspheres of Glibenclamide to reduce dosing frequency and to improve patient compliance.

MATERIALS AND METHODS

Materials

Glibenclamide was obtained as gift sample from Orchid Pharma Ltd, Chennai. Sodium alginates (Loba chem Pvt. Ltd. Mumbai, India), chitosan (yarrow chem. Pvt.ltd), Calcium chloride (Loba chem Pvt. Ltd. Mumbai, India)

Preparation of microspheres

Glibenclamide microspheres were prepared by ionotropic gelation method. Composition of different formulations is shown in Table 1. Two different solutions were prepared separately. First, required amount of chitosan was dispersed in a specified volume of 5% acetic acid solution, allowed to swell for 2 h and transferred in to hot distilled water. In another beaker suitable amount of sodium alginate and drug was mixed well with 100 ml water. Chitosan polymer solution was added into a beaker containing specified concentration of calcium chloride with continuous stirring on magnetic stirrer. Then drug and sodium alginate solution was added drop wise by using 22 G syringe having needle of 0.45 mm inner diameter from a height of about 5 cm into a beaker containing calcium chloride solution with continuous stirring on magnetic stirrer at 400 rpm for 2 h. Prepared microspheres were removed by filtration and washed with distilled water, vacuum dried and stored in well closed container for further use.

Table 1: Composition of Glibenclamide microspheres.

Formulation Code	Drug (mg)	Sodium Alginate (g)	Chitosan (g)	Calcium Chloride (g)
F1	100	3	1	4
F2	100	3	2	4
F3	100	3	1	5
F4	100	2	2	4
F5	100	3	2	5
F6	100	2	1	5
F7	100	2	2	5
F8	100	2	1	4

Evaluation of microspheres[5-8]:

Particle size analysis:

Size of Glibenclamide microspheres was measured by optical microscopy method. A standard stage micrometer was used to calibrate the eye piece micrometer. Size of 100 microspheres from each batch was measured and average particle size was calculated.

Percent swelling index:

Swelling index (pH dependent equilibrium water uptake) of Glibenclamide microspheres was measured by placing 10 mg in to 100 ml phosphate buffer pH 7.4 and pH 0.1 N HCl respectively and allowed to swell for 24 h at 37˚C. Swelling index was calculated by formula –

Swelling index (%) = (Final weight of microspheres- Initial weight of microspheres)/Initial weight of microspheres ×100

Percentage yield:
Percentage yield of all prepared formulations of Glibenclamide microspheres were calculated by formula-

Percentage yield =Weight of dried microspheres/ Weight of total drug and polymer ×100.

Drug entrapment efficiency

Formulated Glibenclamide microspheres (10 mg) were crushed using motor and pestle, suspended in 100 ml phosphate buffer pH 7.4 in to 100 ml volumetric flask with continues starring by magnetic stirrer. On next day, it was filtered, 1ml from filtrate was diluted by 10 ml medium and absorbance was measured on UV spectrophotometer at 226 nm. Drug entrapment efficiency was calculated by formula-

Percent drug entrapment efficiency = Actual drug content / Theoretical drug content ×100.

Fourier transform infrared (FTIR) spectroscopy

The drug, physical mixture of drug and polymers and optimized formulation were subjected for FTIR analysis. Samples were scanned over a range of 4000-400cm^{-1} on JASCO FTIR 460 spectrophotometer using KBr powder.

Different scanning calorimeters (DSC) study

The drug, physical mixture of drug and polymers and optimized formulation were subjected to differential scanning calorimeter (Perkin Elmer 4000). Indium/ zinc standards were used to calibrate the temperature and enthalpy scale. 1 mg of each sample was sealed in aluminum pans and heated at a constant rate 20˚C/min. over a temperature range of 40-300˚C and inert atmospheres was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

Scanning electron microscopy (SEM)

Morphology and surface characterization was done by SEM. Samples for the SEM analysis were prepared by sprinkling the microspheres on one side of the double adhesive stub. The stub was then coated with fine gold dust. The microspheres were then observed with the scanning electron microscope at 10 KV.

X-ray diffraction (X-RD) Studies:

The X-ray diffraction patterns was obtained for Glibanclamide and formulation of microsphere using a X-ray diffractometer (BRUERD8 Advanced) with a Cu as anode material and graphite as monochromatic, operated voltage of 35 KV and current 20 mA. Samples were analyzed in 2θ angle range of 5˚-70˚ and the process parameters were set as scan step size of 0.02˚ (2θ) and scan time was 0.5 to 1 second.

***In-vitro* drug release studies:**

In-vitro dissolution studies were carried out in 900 ml of phosphate buffer pH 7.4, maintained at 37 ± 0.5^{o}C at 50 rpm using USP type I dissolution test apparatus **(Electrolab TDT 08L)** under sink conditions. At predetermined time intervals, 1 ml of each sample was withdrawn and replaced by equal volume of fresh medium kept at same temperature. Samples were filtered through Whatman filter paper no. 41, diluted and analyzed on UV-visible spectrophotometer (Shimadzu, UV 1700) at 226 nm.

Drug release mechanism:

In-vitro drug release data was fitted to Zero-order, First-order, Higuchi (matrix) and Korsemeyer-peppas model. Drug release kinetic was analyzed by plotting cumulative drug release Vs time by fitting to an exponential equation-

$$M_t/M_a = Kt^n$$

Where, M_t/M_a is the fraction of the drug release at time t and K is the rate constant and n is the release exponent.

RESULTS AND DISCUSSION

The purpose of research work was to prepare sodium alginate microspheres coated with chitosan as drug release modifiers in a sustained release system. We had prepared microspheres containing Glibenclamide by ionotropic gelation method and examined the effects of various factors like concentration of sodium alginate, chitosan and calcium chloride on drug release rate.

Particle size analysis

Mean particle size analysis of different formulations of Glibenclamide microspheres was carried out using optical microscopy technique. Results of mean particle size with standard deviation are shown in Table 2. From results it was observed that, by increasing the concentration of sodium alginate and calcium chloride, the mean particle size of microspheres was increased.

Entrapment efficiency

The actual amount of Glibenclamide present in the different formulations of microspheres was determined by measuring entrapment efficiency. The percent entrapment efficiency showed a dependence on the ratio of sodium alginate: chitosan and extent of crosslinking. By ratio of sodium alginate and chitosan (3:1), increase in percent entrapment efficiency was observed. The effect of cross-linking on percent entrapment efficiency showed a significant effect. As the concentration of cross linking agent was increased, an increase in percent entrapment efficiency was observed. Results of % entrapment efficiency were included in the Table 2.

Swelling index

The swelling study for all formulations was carried out in acidic medium (pH 1.2) and alkaline medium (pH 7.4). Results are given in Table 2. The data indicates that, as the amount of crosslinker in the matrices increases, the percent equilibrium water uptake significantly increases. This is due to increased calcium chloride cross-linking agent to higher cross linked to sodium alginate hence density and pore volume of the polymeric network with increasing matrix formulation.

Table 2: Results of % yield, particle size, % entrapment efficiency and swelling index.

FC	% yield	Practical size (µm)	Entapment efficency %	Swelling index (%) Medium↓ Acidic	Alkaline
F1	95.23	532 ±	74.75 ±	60.18	78.25
F2	79.78	510 ±	91.14 ±	22.38	38.14
F3	98.92	540 ±	70.16 ±	70.16	87.85
F4	70	500 ±	85.77 ±	10.12	30.58
F5	81.13	512 ±	92.68 ±	52.68	50.08
F6	97.03	403 ±	68.48 ±	60.48	69.99
F7	84.28	506 ±	95.18 ±	15.85	35.18
F8	96.37	349 ±	77.85 ±	57.85	60.38

FC- Formulation code, (Mean ± SD, n=3)

Fourier transform infrared (FTIR) spectroscopy

FTIR absorption spectrum of Glibenclamide was recorded by potassium bromide dispersion technique using JASCO (4600) FT-IR spectrophotometer as shown in Fig. 1. Characteristic peak at C=O Stretching-1596.23cm^{-1}, OH Stretching-3273.27 cm^{-1}, C-H Stretching-2923.41 cm^{-1}, SO2-1245.83 cm^{-1}, C-O-C stretching- 1049 cm^{-1}, C-Cl stretching -685.57 cm^{-1}.

FTIR spectrum of physical mixture of Glibenclamide along with sodium alginate and chitosan (Fig. 2) showed characteristics peaks at 3316.19 cm^{-1}, 2929.06 cm^{-1}, 1714.41cm^{-1}, 685.57cm^{-1}, 104941cm^{-1}, 1156.12 cm $^{-1}$ typical of N-H symmetrical stretching vibration, C-H stretching, C=O stretching, C-Cl stretching, C-O-C stretching and CO stretching vibration respectively. These peaks are the characteristic peaks of Glibenclamide and were not affected and prominently observed in FTIR spectrum of physical mixture. These spectra indicated no interaction between drug and polymers.

Differential scanning calorimeter (DSC):

DSC studies were performed for drug and drug-loaded microspheres. DSC thermogram of drug showed a sharp melting endotherm at 176.9 ^{o}C as shown in Fig. 3. DSC thermogram of microspheres (Fig. 4) showed a shallow and broad peak at 78.1 ^{o}C which indicates drug is molecularly dispersed in the polymers.

Scanning electron microscopy (SEM)

The morphological evaluation of microspheres was done by scanning electron microscopy as shown in Fig. 5 and Fig. 6. SEM study revealed that the microspheres were spherical in shape with rough outer surface. Some drug particles may be deposited on the outer surface of the microspheres.

X-ray diffraction (X-RD) Studies

X ray diffractogram for Glibanclamide and formulation are presented in Fig. 7 and 8 respectively. Glibanclamide showed characteristics peaks between 15 and 35^{0} (2θ) due to its crystalline nature. XRD pattern of drug loaded microspheres showed characteristics peak but at less intensity to pure drug.

***In vitro* drug release study**

The *In-Vitro* drug release studies of all formulations were observed in the range of 75.24-90.09 % shown in Fig. 9. This indicates that for all formulations, release rate is depend up on the concentration of sodium alginate chitosan and calcium chloride polymer. Model fitting and Kinetic assessment of drug release from microspheres shown in Table No. 3 and 4.

Table 3 : % cumulative drug release of F1-F8 batch.

Time in (min)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
30	7.08	4.13	5.92	7.08	10.03	18.89	4.13	12.98
60	12.99	15.64	10.03	12.99	18.90	21.86	15.94	24.80
120	21.86	30.71	18.90	21.86	33.67	36.64	30.71	39.58
180	36.64	42.55	27.77	36.64	39.61	42.58	42.55	51.43
240	45.53	54.40	36.55	45.53	45.56	51.48	54.40	57.39
300	57.38	66.26	45.55	57.38	57.41	63.34	66.26	63.35
360	63.35	72.24	54.45	75.15	60.42	75.21	75.19	75.23
420	78.17	86.78	63.36	89.99	83.51	78.25	88.55	81.21
480	78.26	87.17	75.24	90.09	83.60	80.69	88.65	81.30

Table 4: Release kinetics data for different formulations.

Formulation Code	n	Best Fit Model
F1	0.8605	Peppas
F2	0.9858	Zero
F3	0.7902	Zero
F4	0.9128	Peppas
F5	0.7155	Peppas
F6	0.5582	First
F7	0.9985	Zero
F8	0.6334	Peppas

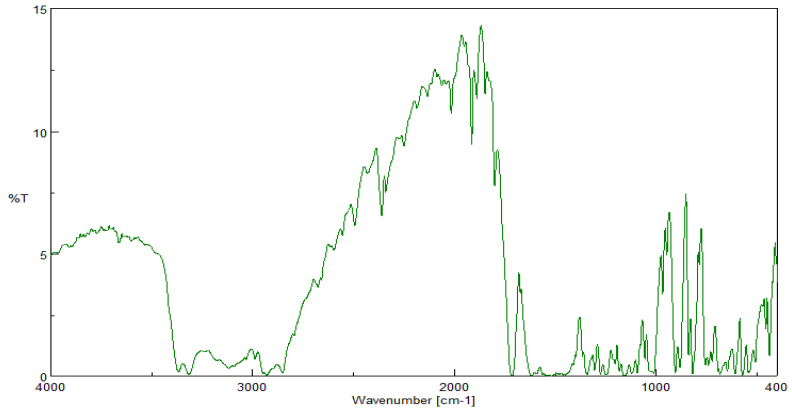

Fig. 1: FTIR spectrum of Glibenclamide.

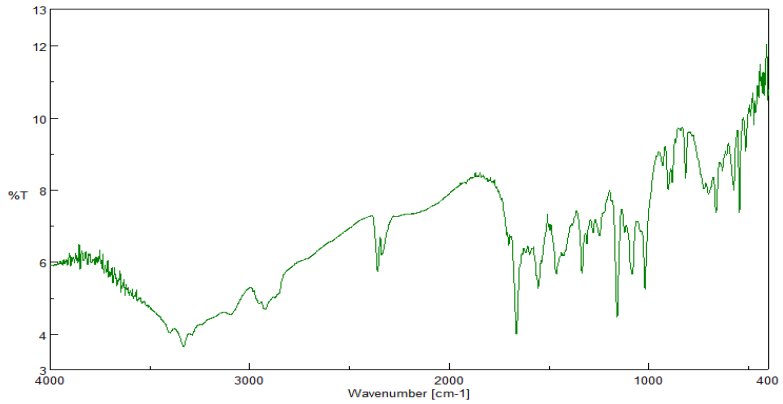

Fig. 2: FTIR spectrum of physical mixture of drug, sodium alginate and chitosan.

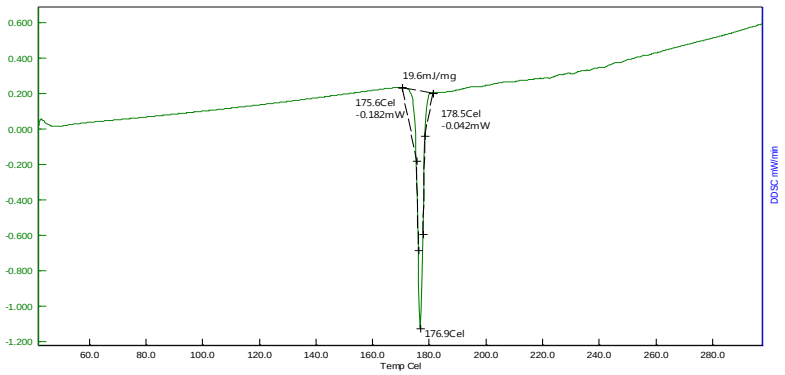

Fig. 3: DSC thermogram for Glibenclamide.

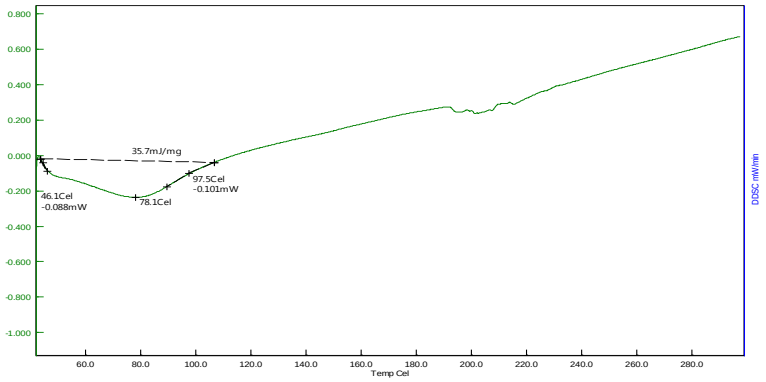

Fig. 4: DSC thermogram for Glibanclamide microsphere.

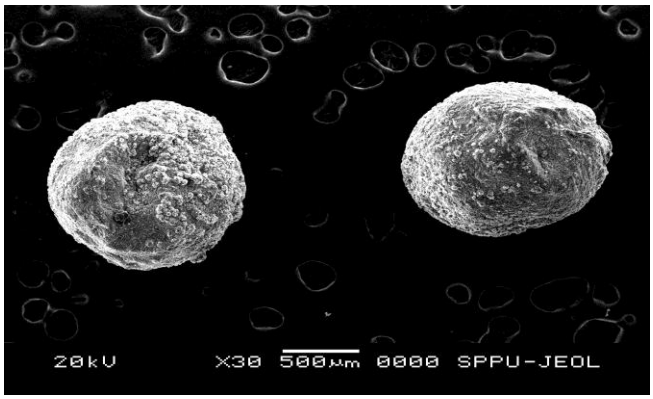

Fig. 5: Scanning electron micrograph of Glibanclamide microspheres.

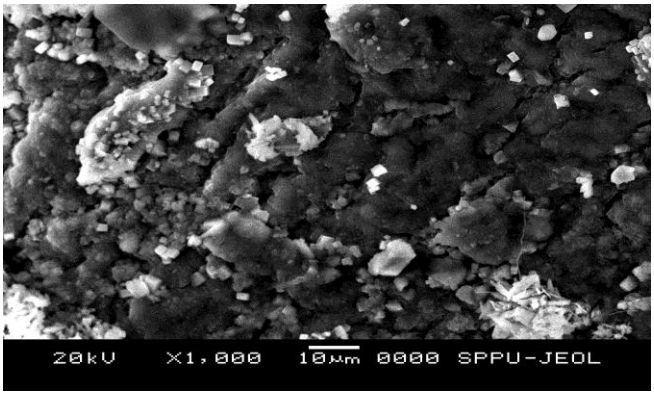

Fig. 6: Topography and magnification of Glibanclamide microsphere.

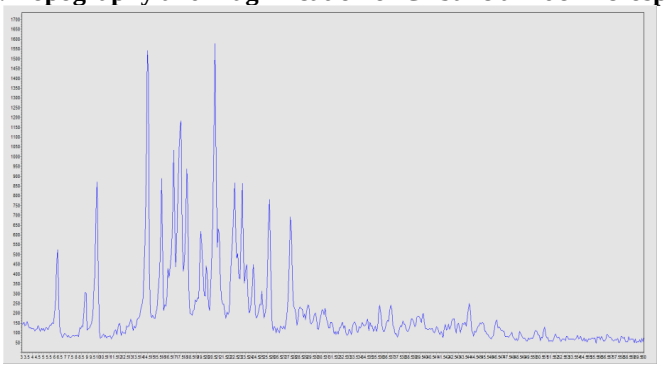

Fig. 7: XRD for Glibanclamide.

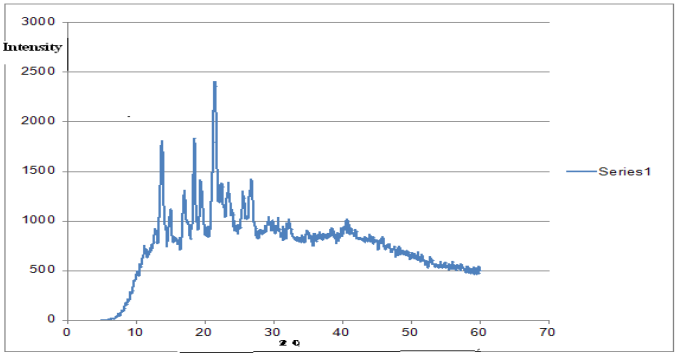

Fig. 8: XRD for Glibanclamide microsphere.

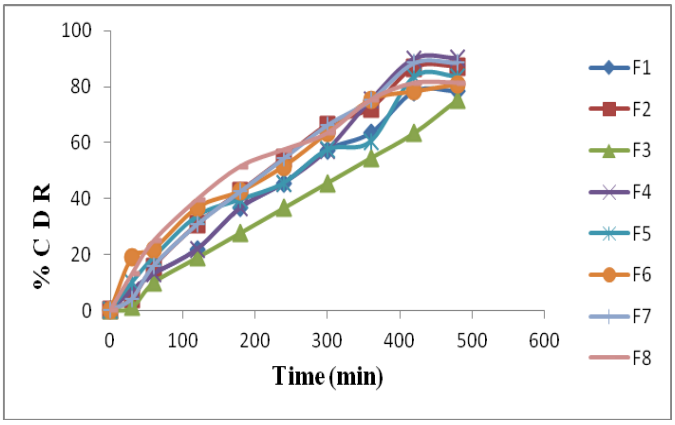

Fig. 9: Zero order drug release from microspheres.

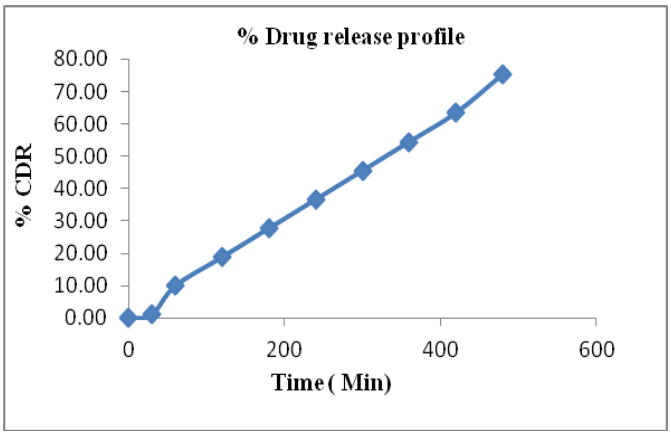

Figure 10: Plot of cumulative % drug release Vs time (min) for obtained batch F3.

CONCLUSION

Ionotropic gelation method was used for preparation of Glibenclamide microspheres using calcium chloride as a crosslinking agent. SEM micrograph showed spherical shape with rough surface of microspheres. FTIR spectrum of physical mixture showed no interaction between drug and polymers. DSC thermogram of microsphere has confirmed that the drug is molecularly distributed in polymers. The conclusion that F3 batch are give best result as compare to other batches prepared successfully by (3:1) concentration of sodium alginate and chitosan polymer .Thus Glibenclamide microspheres are promising pharmaceutical dosage form which can provide sustained release of the drug. The entire process is feasible in an industrial scale and demands pilot study.

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