

Formulation and *In Vitro* Characterisation of Floating Microspheres of Glipizide

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Abstract:

In the present work, microspheres of Glipizide using Sodium alginate along with Carbopol 940, HPMC K4M, HPMCK100M, Ethyl Cellulose as copolymers were formulated to deliver Glipizide via oral route. The results of this investigation indicate that ionic cross linking technique of Ionotropic gelation method can be successfully employed to fabricate Glipizide microspheres. FT-IR spectra the physical mixture revealed that the drug is compatible with the polymers and copolymers used. Micromeritic studies revealed that microspheres have good flow properties. The mean particle size of the prepared microspheres was in the size range of 512-903 μ m and are suitable for bioadhesive microspheres for oral administration. Various parameters such as drug entrapment efficiency, % yield, swelling nature, in-vitro mucoadhesion strength were determined for the prepared formulations.

Keywords: Glipizide, Carbopol, Microspheres, Sodium alginate, Ionotropic gelation.

INTRODUCTION:

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μ m. They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin. The synthetic polymers include polylactic acid and polyglycolic acid. Microspheres are small and have large surface to volume ratios¹. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity².

Microspheres have varied applications and are prepared using assorted polymers. However the success of these microspheres is limited owing to their short residence time at the site of absorption³. So, various attempt have been made to increase the bioavailability as well as prolong the gastric residence time of dosage form in the stomach resulted in development of bio adhesive drug delivery system which will provide an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive 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 site⁴. Mucoadhesive drug delivery system are the systems which utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time.

Gastric mucoadhesive drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of

gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability⁵.

MATERIALS AND METHODS:

Materials:

Glipizide was procured from Hetero Drugs pvt.ltd., Hyderabad . The polymers like HPMCK4M, HPMCK100M, Ethyl cellulose, Carbopol940, Calcium chloride were procured from Loba chemicals Hyderabad.

Methodology:

Construction of Calibration curve:

Accurately weighed 100 mg of glipizide pure drug and dissolved in 100 ml of ethanol stock. From the above solution was subsequently diluted with 0.1N HCl to obtain series of dilutions containing 2, 4, 6, 8,10,12,14,16,18,20 μ g/ml of glipizide per ml solution. The absorbance of the above solution was measured at 277 nm by using UV spectrophotometer taking 0.1N HCl as blank. Then a graph was plotted and shown in figure 1.

Fourier Transform-Infrared Spectroscopy(FTIR):⁶

The freshly prepared drug and excipients mixtures spectra were recorded by using FT-IR. Then the mixtures were examining for their comparing the recorded spectra.

Preparation of Microspheres:

Microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer (as mentioned in table-1) were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. Glipizide (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added to calcium chloride (4% w/v) solution using a

26G needle. The addition was done with continuous stirring at 300rpm. The added droplets were retained in the calcium chloride for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation and the separated microspheres were washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried⁶.

CHARACTERIZATION OF MICROSPHERES:

Micrometric properties⁷:

The microspheres were characterized by micrometric properties such as particle size, bulk density, tapped density, compressibility index, Hausners ratio etc.

1. Bulk density:

In this method microspheres are transferred to a measuring cylinder and are tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{bulk volume}} \times 100$$

2. Tapped density:

In this method microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density of microspheres. Percent Compressibility index was determined by using the formula

$$\text{Carr's Index} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

3. Hausners ratio:

Hausners ratio of microspheres was determined by comparing tapped density to bulk density using the equation

$$\text{Hausners ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

4. Angle of repose:

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in

such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and angle of repose was calculated using the following equation $\theta = \tan^{-1} h/r$

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$$\% \text{yield} = \frac{\text{Practical Mass (microspheres)}}{\text{Theoretical Mass (polymer+ drug)}}$$

5. Drug entrapment efficiency⁸:

Microspheres equivalent to 15 mg of the drug Glipizide were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using simulated gastric fluid pH 1.2. After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 277 nm the amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{Drug entrapment efficiency} = \frac{\text{Experimental Drug content}}{\text{Theoretical content}} \times 100$$

6. Particle size⁹:

Samples of the microspheres were analysed for particle size by optical microscope. The instrument was calibrated and found that 1 unit of eyepiece micrometer was equal to 12.5 μ m. Nearly about 100 micro particles sizes were calculated under 45 x magnifications. The average particle size was determined by using the Edmondson's equation

$$D_{\text{mean}} = \frac{\sum nd}{\sum n}$$

Where, n – Number of microspheres observed; d – Mean size range.

Table.no: 1 Composition of microspheres:

| Composition | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Glipizide | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Sodium alginate(mg) | 350 | 350 | 350 | 350 | 350 | 350 | 350 | 350 |
| HPMC K4M(mg) | 100 | -- | 50 | -- | -- | -- | -- | -- |
| HPMCK100M(mg) | -- | 100 | -- | 50 | -- | -- | -- | -- |
| Carbopol(mg) | -- | -- | -- | -- | 50 | 100 | -- | -- |
| Ethyl cellulose(mg) | -- | -- | -- | -- | -- | -- | 50 | 100 |
| Calcium chloride(g) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

7. Swelling study¹⁰:

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2. The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{W_t - W_0}{W_0} \times 100$$

Where W_0 & W_t are initial weight and Final weights of microspheres.

8. Scanning electron microscopy(SEM)¹¹:

The SEM analysis of the microspheres was carried out by using Jeol JSM 5300, Japan. The surface topography of the uncoated and coated (optimized) microsphere and cross section of optimized microsphere were examined under a FEI-Philips XL-30 Analytical Electron microscope. The samples were loaded on copper sample holder and sputter coated with carbon followed by Gold.

9. In-vitro drug release¹²:

The dissolution studies were performed in a fully calibrated eight basket dissolution test apparatus

(37 ± 0.50 C, 50 rpm) using the USP type-I rotating basket method in simulated gastric fluid pH 1.2 (900ml). A quantity of accurately weighed microspheres equivalent to 15mg Glipizide each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 269nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment

RESULTS AND DISCUSSION:

FT-IR Studies: The pure glipizide showed the prominent peaks at 3424 cm^{-1} due to N-H stretching of NH_2 , 2955 cm^{-1} due to C-H₂ aliphatic, 1658 cm^{-1} C=N aliphatic, 1554 cm^{-1} C-H aliphatic and C=O stretching, 1404 cm^{-1} C=C stretching of aromatic ring, 1171 cm^{-1} C-N stretching vibrations of amines. The studies revealed that there is no drug excipient interactions and stable nature of formulation.

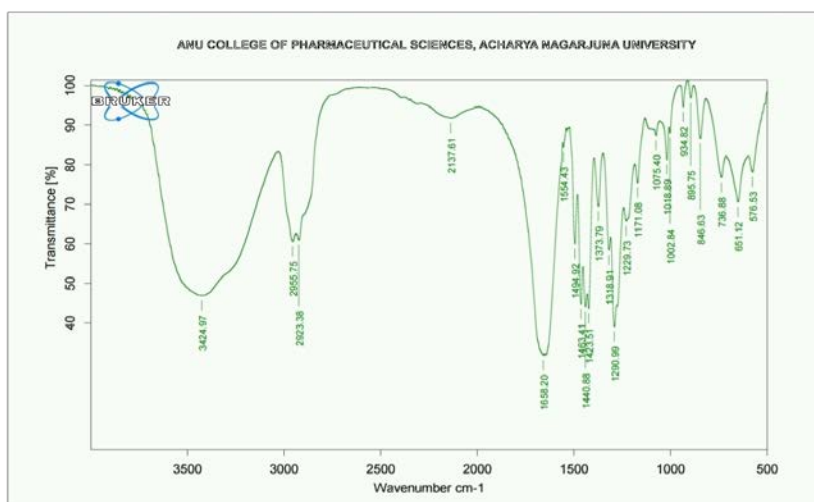


Figure no .1 .FTIR Spectra of pure Glipizide

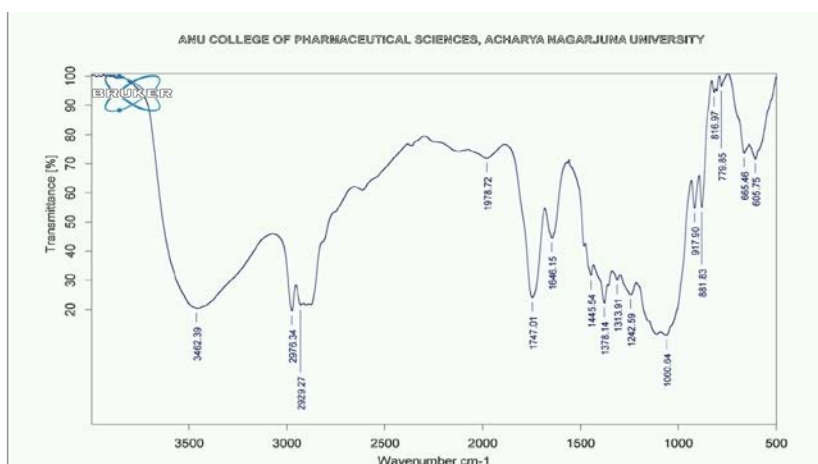


Figure no .2 . FTIR Spectra of Ethyl cellulose

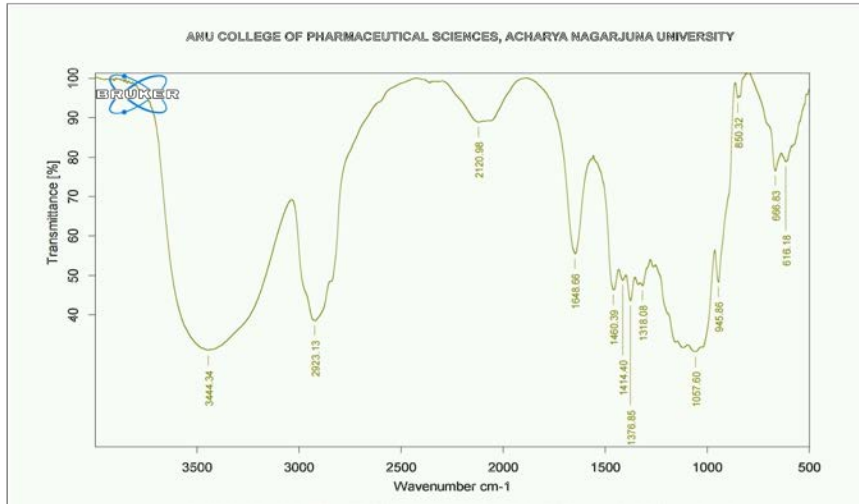


Figure no .3.FTIR Spectra of HPMCK4M

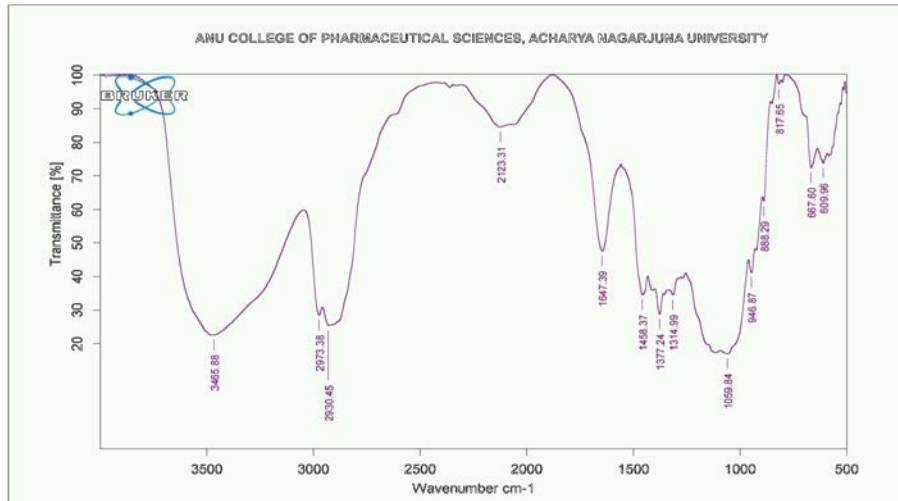


Figure no .4.FTIR Spectra of HPMCK100M

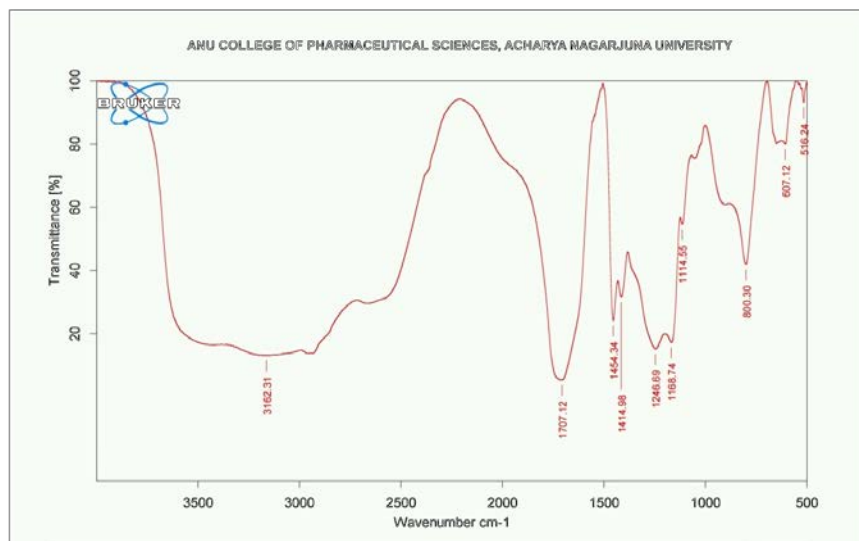


Figure no .5. FTIR Spectra of Carbopol940

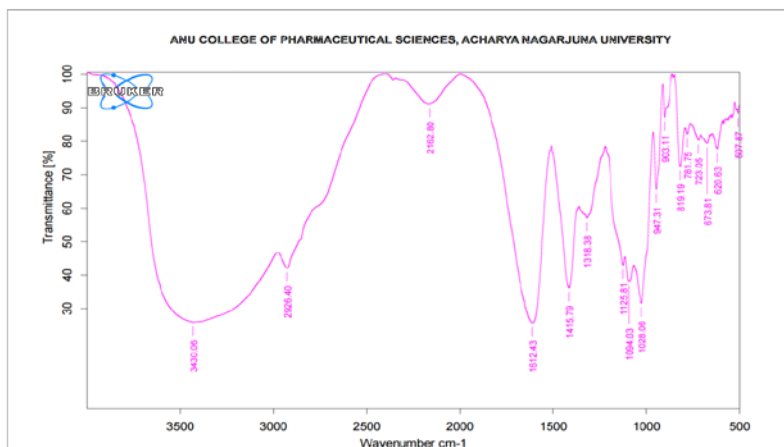


Figure no .9.FTIR Spectra of Glipizide+HPMCK100M

EVALUATION OF PHYSICAL MIXTURE:

Table 02: Micrometric properties of microspheres

| Formulation | Bulk density(gm/cc) | Tapped density(gm/cc) | Carr's index (%) | Hausners ratio | Angle of repose(θ) |
|-------------|---------------------|-----------------------|------------------|----------------|-----------------------------|
| F1 | 0.45 | 0.55 | 18.18 | 1.22 | 27.91 |
| F2 | 0.47 | 0.58 | 14.54 | 1.17 | 28.23 |
| F3 | 0.50 | 0.55 | 13.79 | 1.16 | 29.34 |
| F4 | 0.46 | 0.50 | 14.54 | 1.19 | 26.71 |
| F5 | 0.50 | 0.50 | 13.79 | 1.16 | 29.34 |
| F6 | 0.47 | 0.58 | 18 | 1.17 | 28.23 |
| F7 | 0.41 | 0.56 | 14.0 | 1.21 | 26.78 |
| F8 | 0.47 | 0.54 | 18.11 | 1.22 | 26.71 |

Table 03: Characterization of microspheres

| Formulation | %yield | Particle size (nm) | %DEE | % Swelling |
|-------------|--------|--------------------|-------|------------|
| F1 | 80 | 512 | 82.66 | 28 |
| F2 | 83 | 617 | 84.4 | 42 |
| F3 | 85 | 711 | 84.66 | 62 |
| F4 | 62.2 | 826 | 88.66 | 85 |
| F5 | 87 | 517 | 53.2 | 24 |
| F6 | 80 | 642 | 55 | 39 |
| F7 | 86 | 792 | 68.86 | 55 |
| F8 | 86.66 | 834 | 76.66 | 64 |

SEM Analysis:

The studies revealed that the microspheres are appeared spherical and rough but there is no aggregation.

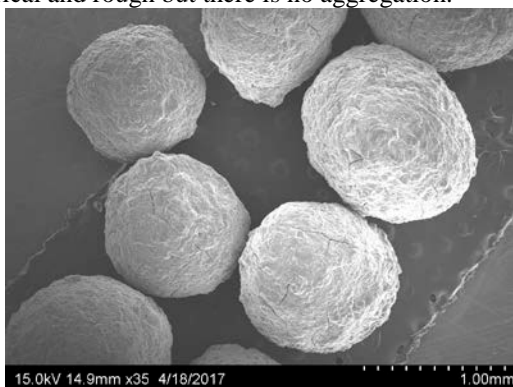


Figure no.10. The SEM Image of microspheres examined under a FEI-Philips XL-30 Analytical Electron microscope

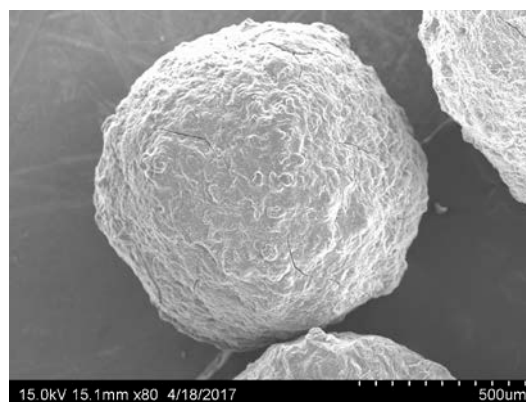


Figure no .11. Figure no.10. The SEM Image of microspheres examined under a FEI-Philips XL-30 Analytical Electron microscope zoom in

Invitro Dissolution rate studies:**Table 04: Comparative profile of %Drug dissolved IN Formulation F1-F8**

| S.No | Time(hr) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|------|----------|-------|------|------|------|------|-------|-------|-------|
| 1. | 1 | 12.5 | 8.16 | 0.70 | 0.42 | 9.9 | 3.3 | 14.3 | 18.2 |
| 2. | 2 | 13.5 | 12.3 | 9.5 | 12.5 | 12.1 | 16.0 | 16.7 | 13.7 |
| 3. | 3 | 18.7 | 20.4 | 12.8 | 22.8 | 17.8 | 30.1 | 30.2 | 22.8 |
| 4. | 4 | 34.4 | 42.0 | 25.6 | 30.2 | 39.1 | 52.0 | 45.8 | 27.8 |
| 5. | 5 | 51.8 | 50.1 | 30.4 | 56.0 | 53.4 | 61.9 | 60.3 | 39.1 |
| 6. | 6 | 63.6 | 70.0 | 51.6 | 65.0 | 66.0 | 68.2 | 78.4 | 42.9 |
| 7. | 7 | 82.6 | 71.6 | 63.6 | 71.2 | 72.4 | 70.6 | 83.4 | 65.8 |
| 8. | 8 | 85.4 | 86.5 | 95.5 | 84.6 | 85.5 | 86.5 | 85.5 | 77.9 |
| 9. | 9 | 97.9 | 98.6 | 98.8 | 96.5 | 85.5 | 101.3 | 97.9 | 86.9 |
| 10. | 10 | 100.6 | 99.7 | 99.9 | 98.5 | 99.9 | 113.4 | 105.5 | 100.2 |

CONCLUSION:

The results of this investigation indicate that ionic cross linking technique of inotropic gelation method can be successfully employed to fabricate Glipizide microspheres. Micrometrics studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-903 μ m and are suitable for bio adhesive microspheres for oral administration. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion. The in-vitro mucoadhesive study demonstrated that microspheres of Glipizide using sodium alginate along with Carbopol as copolymer adhered to the mucus to a greater extent than the microspheres of Glipizide using sodium alginate along with Carbopol and HPMC K4M as copolymers. The invitro drug release decreased with increase in the polymer and copolymer concentration. Out of all the formulations prepared formulation T4 has shown maximum drug release at the end of hour. The drug release is found to follow non-fickian diffusion. From the results it is concluded that the formulation T4 containing Na alginate and Carbopol in ratio have shown to be promising one with desired properties for the delivery of Glipizide via oral route.

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