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Research Article

# FORMULATION AND EVALUATION OF MUCOADHESIVE GLIPIZIDE MICROSPHERES USING CARBOXY METHYL CELLULOSE

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**ABSTRACT**

The objective of the present investigation was to formulate and evaluate the mucoadhesive glipizide microsphere using carboxy methyl cellulose as synthetic mucoadhesive polymer. 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 short biological half life (0.3+0.7 hours) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day. Microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. Twenty preliminary trail batches F1-F20 of microspheres were prepared by using different volume (10 to70 mL) of glutaraldehyde as cross-linking agent ,cross-linking time 1 to 4 hours and polymer-to-drug ratio 3:1. From these batches the optimized formulation was selected based on the percentage of mucoadhesion and sphericity of microspheres. On the basis of the preliminary trial $3^2$ full factorial design were employed, to study the effect of independent variable X1 (polymer-to-drug ratio 1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500 rpm) on dependent variables percentage mucoadhesion, drug entrapment efficiency, particle size and drug release. The drug polymer compatibility studies were carried out using FTIR. The stability studies were conducted for the optimized formulation. The optimized formulation exhibited a high drug entrapment efficiency of 68%, swelling index 1.57 percentage of mucoadhesive after 1hour 68% and 81.6% of the drug release for 8 hours so it sustained for more than 10 hours. The polymer-to-drug ratio had a more significant effect on the dependent variables.

**KEYWORDS:** Mucoadhesive, microspheres, Glipizide, Carboxy methyl cellulose, 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 [5]. Mucoadhesive microsphere 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. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [6-9]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. 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. It's 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 per day [18,20,21].

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Carboxy methyl cellulose is a synthetic good mucoadhesive and biodegradable synthetic polymer. Thus the development of controlled release dosage forms would clearly be advantageous. Moreover, the site of absorption of sulfonyl urea is in the stomach. Dosage forms that were retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Thus, an attempt was made by using synthetic mucoadhesive polymer (CMC) by using Glipizide as a drug. On the basis of the preliminary trials a $3^2$ full factorial design were employed for all the polymer batches, to study the effect of independent variable X1 (polymer-to- drug ratio1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500rpm) on dependent variables percentage mucoadhesion, drug entrapment efficiency, and particle size. The drug polymer compatibility studies were carried out using FTIR. The stability studies were conducted for the optimized formulation.

## MATERIALS AND METHOD

Glipizide was obtained as gift sample from Madras Pharmaceuticals, Chennai. carboxy methyl cellulose was obtained as gift sample from AET Laboratories, Hyderabad. Span 85(0.5%w/v) was obtained from Loba Chemical Pvt. Ltd, Mumbai. Petroleum ether 80:20 was procured from Willson Lab, Mumbai. Light and heavy Liquid paraffin, Glutaraldehyde of analytical grade are used.

UV Spectrophotometer, Scanning Electron Microscopy, USP XXIV, Basket apparatus (Dissolution), HPLC, Image analyser, Sieve analyser, Optical Microscope, Propeller stirrer (1000 rpm), USP Tablet disintegration apparatus.

## PREPARATION OF MICROSPHERES

Microspheres were prepared by simple emulsification phase separation technique by using carboxy methyl cellulose as polymer and different volume of cross-linking agent (Glutaraldehyde) was added as per method described in Thanoo *et* al [14].

CMC (1.5 gms) was dissolved in 150 ml of 1% v/v aqueous acetic acid solution and the drug (500 mg) was dispersed in the polymer solution. Twenty preliminary trial batches were prepared using the polymer-to-drug ratio 3:1 and stirring was performed using a propeller stirrer at 1000 rpm kept constant. The resultant mixture will be extruded through a syringe (No.20) in 1 lit of liquid paraffin (heavy and light 1:1 ratio). Containing 0.5%w/v Span 85 and stirring was performed using propeller stirrer. After 15 min cross-linking agent glutaraldehyde (25% v/v aqueous solution) was added and stirring was continued. The amount of cross-linking agent and cross-linking time was varied 10 – 70 mL and 1 to 4 hours. In factorial design batches B1-B9, 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 nine batches. Microspheres thus obtained were filtered and washed several time with petroleum ether (80:20) to remove traces of oil. They were finally washed with water to remove excess of cross-linking agent. The microspheres were then dried at room temperature (at $25^0$ 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 (UV/VIS) spectrophotometric method. Aqueous solution of drug was prepared in phosphate buffer (pH 7.4) and absorbance was measured by ultraviolet visible spectrophotometer at 276 nm [22]. The method was validated for linearity, accuracy and precision. The method obeys beer's law in the concentration range of 5- 50 mcg/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.

### Particle size

The particle size of the microspheres was determined by using optical microscopy method[23]. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope.

### Swelling index of microspheres

For estimating the swelling index, the 100 microspheres was suspended in 5 mL of simulated gastric fluid USP (pH 1.2) [24]. The particle size would be monitored by microscopy technique every 1 hour using an optical microscope. The increase in particle size of the microspheres will be noted for up to 8 hours and the swelling index is calculated as per method described by Ibrahim [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 (3inch by 1inch) 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 is 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 performed using USP XXIV basket apparatus22. $37^0$ C+$0.5^0$C at 50 rpm using 900 mL of phosphate buffer (pH7.4) as a dissolution medium. Microspheres equivalent to 10 mg of glipizide were used for the test. 5 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 [27].

**Scanning electron microscopy**

A scanning electron photomicrograph of drug-loaded mucoadhesive microspheres was taken. A small amount of microspheres was spread on glass stub. Afterwards, the stud containing the sample was placed in the scanning electron microscope chamber. The scanning electron photomicrograph is taken at the acceleration voltage of 20kv chamber pressure or 0.6mm Hg, Original magnification X 800 [11].

**Stability testing**

Formulation of glipizide loaded microspheres was tested for stability studies. Both the formulations were divided into 3 sample sets and stored at:
$4 \pm 1^0$ C, 25± $2^o$ C & 60 ± 5% RH and 37± $2^o$ C & 65 ± 5% RH.
After 30 days, the drug release of selected formulations was determined by the method discussed previously in vitro drug release studies and percentage entrapment efficiency was also carried out for the same formulation.

**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 could be involved. The semi empirical equation [28] shown as equation.

$Mt/M\infty = ktn$

Where, $Mt/M\infty$ 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).

**$3^2$ full 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 X1. The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2) show how the response changes when two factors are simultaneously changed. The polynomial terms ($X1^2$ and $X2^2$) 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. drug-to-polymer ratio (X1) and the stirring speed at rpm (X2) on dependent variables % mucoadhesion, drug entrapment efficiency,and particle size.

**FTIR Studies**

FTIR spectroscopic studies were conducted for optimized formulation and glipizide pure drug.

**RESULT AND DISCUSSION**

The mucoadhesive microspheres of an oral anti-diabetic drug glipizide were prepared by simple emulsification phase separation technique. CMC was selected as a synthetic polymer for the preparation because of its biodegradable and mucoadhesive properties. Acetic acid from 1% to 6% v/v was used to prepare polymer solution. But there was no effect in concentration of acetic acid were observed on percentage mucoadhesion or drug entrapment efficiency, therefore 1% v/v of acetic acid was used.

Polymer concentration was important factors, mention in Lee and based on Viscosity of polymers solution. Three different concentrations 0.5%, 1% & 2% v/v were selected. From these 1% concentration show a maximum sphericity were observed so we select 1% w/v of polymer in 1% v/v acetic acid solution and 1:1 heavy and light paraffin was used as dispersion medium and 0.5% w/v of Span 85 was added as anionic surfactant to dispersion medium were found to be essential to minimize aggregation of microspheres.

The volume of cross-linking agent (10-70 mL) and stirring time (1-4 hours). From the twenty batches A1-A20, spherical free flowing shaped microspheres were obtained using 60-70 mL of glutaraldehyde A9-A20, shown in the Table I. Batches F9-F12 was prepared by using 40 mL of glutaraldehyde showed spherical free flowing microspheres and also shows good mucoadhesion and 59% of drug

entrapment efficiency. Batches F13-F16 was showed 70% of drug entrapment efficiency and also showed 84% mucoadhesion. The batches F17-F20 was showed spherical free flowing microspheres and showed 76% of drug entrapment efficiency and decrease in mucoadhesion take place. As the cross linking agent increases, the mucoadhesiveness is decreases and crosslinking time did not show a significant effect on the percentage of drug entrapment efficiency, shown in Table I.

From these preliminary trial batches the best optimized formula was selected. On the basis of the preliminary trials $3^2$ full factorial design were employed, to study the effect of independent variable X1 (polymer-to-drug ratio 1:1, 3:1 and 6:1) and the stirring speed X2 (500, 1000 and 1500 rpm) on dependent variables percentage mucoadhesion, drug entrapment efficiency, and particle size. B1-B9 was prepared by using 60 mL of glutaraldehyde and 2 hours cross-linking time shown in Table II. Batches B1-B3 was prepared by using 1:1 polymer-to- drug ratio with different stirring speed. The % mucoadhesion after 1 hour is 55%, and drug entrapment efficiency was found to be 51%. If the stirring speed increased means the % of mucoadhesion and the drug entrapment is decreased, shown in Table II. Batches B4-B6 was prepared by using 3:1 polymer-to-Drug ratio with different stirring speed. The % mucodhesion after 1hour is 68% and drug entrapment efficiency was found to be 68%. Batches B6-B9 were prepared using 6:1 polymer-to-Drug ratio with different stirring speed, if the stirring speed increased means the % of mucoadhesion and drug entrapment efficiency is decreased. Shown in Table II from the nine formulation Batches B5 is the optimized formulation and they are spherical free flowing shown in Fig 1. The percentage mucoadhesion, drug entrapment efficiency, and particle size showed good correlation coefficient $r^2$ 0.9811, 0.9973 and 0.9871. In vitro drug release studies were carried out the percentage drug dissolved at different time interval was calculated using the Lambert's-Beer's equation, 81.6% of drug release for eight hours so, we conclude that the microsphere of glipizide could sustain the release of the drug for more than 10 hours. The stability studies were carried out by storing the optimized formulations at $4^0 \pm 1^0$ C, 25 $\pm 2^0$ C & 60 $\pm$ 5% RH and $37 \pm 2^0$ C & $65 \pm 5$% for one month. Two parameters namely percentage entrapment efficiency and in vitro release studies were carried out. The drug release at $4 \pm 1^0$ C. showed 89.23% and percentage entrapment efficiency 70.1%, the drug release at 25 $\pm 2^0$ C & 60 $\pm$ 5% RH showed 93.50% and percentage entrapment efficiency 68.20% and the drug release at 37 $\pm 2^0$ C & 65 $\pm$ 5% RH showed 93% and percentage entrapment efficiency 65.76%.

The FTIR spectroscopy indicates there was no interaction took place between drug and the polymer. In vitro drug release of the optimized formula B5 is given in Table III and Model fitting for the release profile of formulations were shown in table no. IV. The sphericity of optimized batch was shown in Fig.1 and 2.

**Table. I Preliminary Trial Batches of glipizide mucoadhesive microsphere using carboxy methyl cellulose**

| Batchcode | Vol. of glutaraldehyde (ml) | Cross linking time(h) | % Mucoadhesion after 1 hr. | Drug Entrapment Efficiency (%) | Sphericity of microsphere |
|---|---|---|---|---|---|
| F1 | 10 | 1 | 89 | 35 | Very Irregular |
| F2 | 10 | 2 | 83 | 37 | |
| F3 | 10 | 3 | 78 | 39 | |
| F4 | 10 | 4 | 76 | 41 | |
| F5 | 20 | 1 | 85 | 48 | Slightly Irregular |
| F6 | 20 | 2 | 79 | 52 | |
| F7 | 20 | 3 | 72 | 54 | |
| F8 | 20 | 4 | 66 | 57 | |
| F9 | 40 | 1 | 76 | 54 | Spherical from following |
| F10 | 40 | 2 | 70 | 56 | |
| F11 | 40 | 3 | 63 | 58 | |
| F12 | 40 | 4 | 62 | 59 | |
| F13 | 60 | 1 | 86 | 66 | |
| F14 | 60 | 2 | 84 | 70 | |
| F15 | 60 | 3 | 72 | 71 | |
| F16 | 60 | 4 | 64 | 71 | |
| F17 | 70 | 1 | 62 | 67 | |
| F18 | 70 | 2 | 55 | 69 | |
| F19 | 70 | 3 | 48 | 72 | |
| F20 | 70 | 4 | 42 | 76 | |

**Note: All batches were prepared by polymer-to-drug ratio of 3:1 at 1000 rpm speed**

**Table. 11 Formulation of carboxy methyl cellulose loaded glipizide mucoadhesive microsphere by using $3^2$ full Factorial design layout**

| Batch Code | Variable levels in coded from | | % Mucoadhesion After1h | Drug Entrapment Efficiency (%) | Swelling Index | Particle Size |
|---|---|---|---|---|---|---|
| | X1 | X2 | | | | |
| B1 | -1 | -1 | 55 | 51.42 | 0.743 | 57.0 |
| B2 | -1 | 0 | 49 | 49.35 | 0.679 | 55.2 |
| B3 | -1 | 1 | 46 | 46.12 | 0.667 | 47.2 |
| B4 | 0 | -1 | 73 | 69.83 | 1.637 | 64.1 |
| **B5** | **0** | **0** | **68** | **68.64** | **1.170** | **61.2** |
| B6 | 0 | 1 | 65 | 63.85 | 0.937 | 57.8 |
| B7 | 1 | -1 | 83 | 74.22 | 1.297 | 95.0 |
| B8 | 1 | 0 | 75 | 70.86 | 1.153 | 86.8 |
| B9 | 1 | 1 | 70 | 67.45 | 1.097 | 71.4 |

**Note: All batches were prepared by using 60ml glutraldehyde and crosslinking time 2h**

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

**Table. III In vitro release profile Glipizide mucoadhesive microsphere formulation CMC (B5)**

| Time | Root Time | Log time | Abs | CDR | % CDR | Log % CDR | % Drug Retained | Log % Drug Retained | (%Retained)$^{1/3}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 0.0278 | 4.698 | 23.49 | 1.370 | 76.51 | 1.883 | 4.245 |
| 2 | 1.414 | 0.3010 | 0.0335 | 6.306 | 31.53 | 1.498 | 68.47 | 1.835 | 4.091 |
| 3 | 1.752 | 0.4771 | 0.0398 | 8.128 | 40.64 | 1.608 | 59.36 | 1.773 | 3.900 |
| 4 | 2 | 0.6020 | 0.045 | 9.736 | 48.68 | 1.687 | 51.32 | 1.710 | 3.716 |
| 5 | 2.236 | 0.6989 | 0.0501 | 11.366 | 56.83 | 1.754 | 43.17 | 1.635 | 3.508 |
| 6 | 2.441 | 0.7781 | 0.0557 | 13.18 | 65.9 | 1.818 | 34.1 | 1.532 | 3.242 |
| 7 | 2.645 | 0.8450 | 0.0596 | 14.638 | 73.19 | 1.864 | 26.81 | 1.428 | 2.992 |
| 8 | 2.828 | 0.9030 | 0.0644 | 16.322 | 81.61 | 1.911 | 18.39 | 1.264 | 2.639 |

**Table. IV Model Fitting for the Release Profile of Glipizide mucoadhesive microsphere formulation CMC (B5)**

| Formulation Code | Zero Order | First Order | Higuchi Matrix | Korsmeyer-Peppas | | Hixon-Crowell | Best Fit Model |
|---|---|---|---|---|---|---|---|
| | R | R | R | R | N | R | |
| **CMC** | 0.999 | 0.963 | 0.983 | 0.987 | 0.609 | 0.984 | Zero |

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

**Fig No.01 scanning electron microphotograph of glipizide loaded CMC mucoadhesive microspheres**

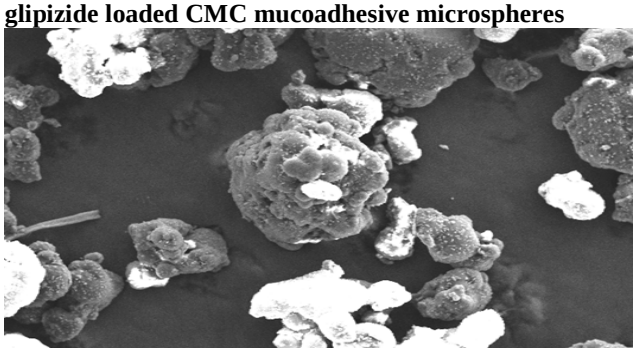
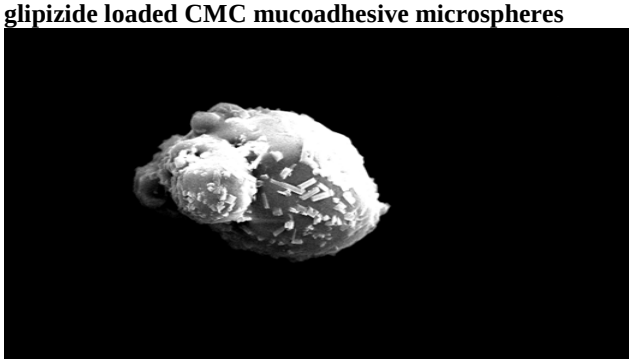


**Fig No.02 scanning electron microphotograph of glipizide loaded CMC mucoadhesive microspheres**



**CONCLUSION**

The results of 32 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.The microspheres of the best batch exhibited a high percentage mucoadhesion of 68% after 1 hour, 68% drug entrapment efficiency and 81.6% of drug release for eight hours. The microsphere of glipizide could sustain the release of the drug for more than 10 hours.

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