
Ethyl Cellulose Microspheres of Glipizide: Characterization, *In Vitro* and *In Vivo* Evaluation

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Ethyl cellulose microspheres of glipizide were prepared by an industrially feasible emulsion-solvent evaporation technique and the microspheres were investigated. The microspheres are spherical, discrete and free-flowing. Encapsulation efficiency was in the range of 81-91%. Glipizide release from the microspheres was slow and diffusion controlled and extended over a period of 10 d and depended on core:coat ratio, wall thickness and size of the microspheres. Good linear relationships were observed between percent coat, wall thickness and release rate of the microspheres. In the *in vivo*, the microspheres produced a sustained hypoglycemic effect over 6 d in normal rabbits. These microspheres were found suitable for parenteral controlled release.

Microspheres have been widely accepted as a means to achieve oral and parenteral controlled release¹. The microspheres require a polymeric substance as a coat material or carrier. A number of different substances both biodegradable as well as non-biodegradable have been investigated² for the preparation of microspheres. Ethyl cellulose (EC) is a non-biodegradable, biocompatible, non-toxic, cellulose polymer having good film forming properties³. Though it has been extensively used in coating^{4,5} and microencapsulation⁶⁻⁹, its application in microspheres for controlled release has not been reported. This study describes the preparation and evaluation of ethyl cellulose microspheres of glipizide with an objective of achieving parenteral controlled release over longer periods of time. Glipizide is an effective widely used antidiabetic drug. It has a short biological half-life⁹ of 3.4 ± 0.7 h and is rapidly eliminated. Because of its short half-life, gastrointestinal disturbances and chronic usage, attempts have been made to develop parenteral controlled release microspheres of glipizide. Glipizide was incorporated in to ethyl cellulose microspheres and the microspheres were investigated by *in vitro* and *in vivo* methods.

MATERIALS AND METHODS

Glipizide was a gift sample from M/s Micro Labs Ltd., Pondicherry. EC (BDH, having an ethoxyl content of 47.5% by weight and a viscosity of 22 cps in a 5% concentration by weight, in a 80:20 toluene-ethanol solution at 25°), chloroform GR (Merck) and sodium carboxymethylcellulose (sodium CMC with a viscosity of 1500-3000 cps of a 1% w/v solution at 25°, Loba-Chemie) were procured from commercial sources. All other reagents used were of analytical grade.

Preparation of microspheres:

EC polymer (2 g) was dissolved in chloroform (100 ml) to form a homogenous polymer solution. Core material, glipizide (0.8 g) was added to the polymer solution (10 ml) and mixed thoroughly. The resulting mixture was then added in a thin stream to 200 ml of an aqueous mucilage of sodium CMC (0.5%) contained in a 450 ml beaker, while stirring at 1000 rpm to emulsify the added dispersion as fine droplets. A Remi make medium duty stirrer with speed meter (Model RQT 124) was used for stirring. The solvent, chloroform was then removed by continuous stirring at room temperature (28°) for 3 h to produce spherical microspheres. The microspheres were collected by vacuum filtration and washed repeatedly with water. The product was then air dried to obtain discrete microspheres. Different proportions of core to coat materials namely 9:1 (MC1), 8:2 (MC2), and 7:3

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(MC3) were used to prepare microspheres with varying thickness.

Estimation of glipizide:

Glipizide content in the microspheres was estimated by an UV spectrophotometric method¹⁰ based on the measurement of absorbance at 223 nm in phosphate buffer of pH 7.4. The method was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range 0-10 µg/ml. When a standard drug solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 1.2%, respectively.

Characterization of microspheres:

For size distribution analysis, different sizes in a batch were separated by sieving using a range of standard sieves. The amounts retained on different sieves were weighed.

Encapsulation efficiency was calculated using the equation, encapsulation efficiency=(estimated percent drug content/theoretical percent drug content)×100. Theoretical mean wall thickness of the microspheres was determined by the method of Luu *et al.*¹¹ using the equation, $h = \frac{\bar{r}}{r} (1-p) d_{1/3}$ [$pd_2 + (1-p)d_1$], where h is the wall thickness, \bar{r} is the arithmetic mean radius of the microspheres, d_1 is the density of core material, d_2 is the density of the coat material and p is the proportion of the medicament in the microspheres. The microspheres were observed under a scanning electron microscope (SEM-Leica, S430, UK). For SEM, the microspheres were mounted directly onto the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr).

In vitro drug release study, USP Paddle method:

Release of glipizide from the microspheres of size 20/35 and 35/60 was studied in phosphate buffer of pH 7.4 (900 ml) using an USP XXIII three-station dissolution rate test apparatus (model DR-3, M/s Campbell Electronics) with rotating paddle at 50 rpm and 37±1° as prescribed for glipizide tablets in USP XXIV. A sample of microspheres equivalent to 10 mg of glipizide was used in each test. Samples were withdrawn through a filter (0.45 µ) at different time intervals over a period of 24 h and were assayed at 223 nm for glipizide using a Shimadzu UV-150 double beam spectrophotometer. The drug release experiments were conducted in triplicate (n=3).

In vitro drug release study, shaking flask method:

Microspheres equivalent to 10 mg of glipizide were taken into a 250 ml conical flask containing 150 ml of phosphate buffer of pH 7.4. The conical flask was kept on a rotary flask shaker and was subjected to continuous shaking under moderate speed. At each sampling time (6, 12, 24 h and later every 24 h until complete release was obtained) the entire volume of dissolution fluid (150 ml) was collected retaining the microspheres in the flask and replaced with fresh quantity of dissolution fluid. The samples collected were assayed for glipizide content at 223 nm. Each drug release experiment was repeated for three times (n=3).

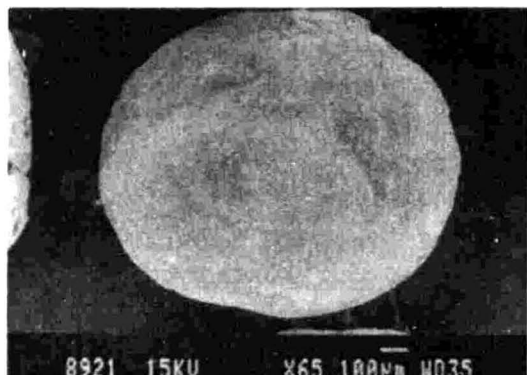
In vivo evaluation:

In vivo evaluation of the microspheres was performed using New Zealand strain normal healthy rabbits procured from Mahaveera Enterprises, Hyderabad. Serum glucose levels of the rabbits were measured following administration of microspheres subcutaneously, using protocols that were approved by the Institutional Animal Ethics Committee (Reg. No. 516/01/a/CPCSEA). The *in vivo* studies were carried out on glipizide at dose of 400 µg/kg and microspheres MC1, MC2 and MC3 of size 35/60 at a dose equivalent to 800 µg/kg of glipizide. After collecting zero hour blood samples, the products were administered subcutaneously as a suspension in sterile water for injection to the rabbits through a syringe with needle No. 22. The animals were given food and water *ad libitum*. Blood samples (0.5 ml) were collected at different intervals of time (1, 2, 3, 4, 5, 6 h and later every 6 h upto 10 d or until the serum glucose level reaches to normal) after administration. The serum glucose concentrations were determined by a known oxidase-peroxidase method¹² employing Glucose Kit supplied by Dr. Reddy's Laboratory, Diagnostic Division, Hyderabad. Blood samples collected were allowed to clot without any anticoagulant and were centrifuged immediately at 5000 rpm for 20 min to separate the serum. To the serum (0.02 ml) and standard (0.02 ml) in separate clean dry test tubes, enzyme reagent (2 ml) was added, mixed well and incubated at 37° for 10 min. The solutions were diluted to 5 ml with distilled water and the absorbance of the pink coloured solutions were measured in a Shimadzu UV-150 double beam spectrophotometer at 505 nm using a reagent blank.

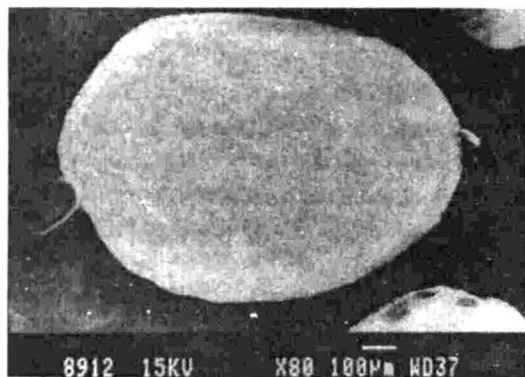
RESULTS AND DISCUSSION

Ethyl cellulose microspheres of glipizide could be prepared by an emulsion-solvent evaporation method employ-

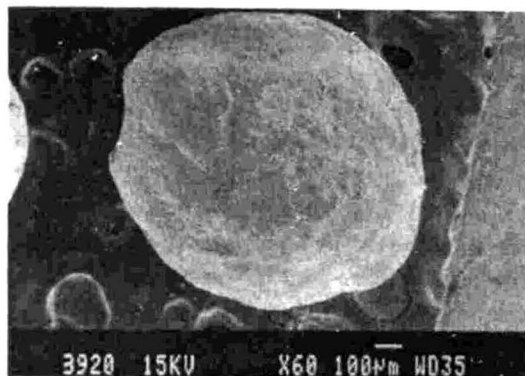
ing chloroform as a solvent for the polymer. The microspheres were found to be discrete, spherical, and free flowing. SEM (fig. 1) indicated that the microspheres are spherical with smooth surface. The nature of the method indicates that the microspheres were multi-nucleated, monolithic type. The sizes could be separated and a more uniform size range of microspheres could readily be obtained.



a)



b)



c)

Fig. 1: SEM Photographs of EC Microspheres.

MC1 (a), MC2 (b) and MC3 (c). The microspheres are spherical with smooth surface.

The size analysis of different batches of microspheres showed that about 66% were in the size range of $-35+60$ ($375 \mu\text{m}$) mesh size. The size distribution of the microspheres was found to be normal in all the batches. The mean size of the microspheres was increased as the proportion of coat in the microspheres was increased. The mean size of the microspheres was 369.9 ± 9.1 , 638.1 ± 10.5 and $683.4 \pm 16.3 \mu\text{m}$, respectively in the batches of microspheres prepared employing core:coat ratio of 9:1, 8:2 and 7:3.

Low C.V. ($< 1.2\%$) in percent drug content indicated uniformity of drug content in each batch of microspheres (Table 1). The encapsulation efficiency was in range 81-91% with various products. Drug content of the microspheres was found to be nearly the same in different sieve fractions. As the microspheres are spherical, the theoretical mean thickness of the wall that surrounds the core particles in the microspheres was calculated as per Luu *et al*¹¹. Microspheres prepared employing various ratios of core:coat were found to have different wall thickness.

Glipizide release from the microspheres of size 20/35 and 35/60 was studied in phosphate buffer of pH 7.4 for a period of 24 h employing USP paddle dissolution rate test apparatus. Glipizide release from all the microspheres was slow and spread over extended period of time. The release in 24 h was 34% to 70% depending on the size and core:coat ratio. The release depended on core:coat ratio, wall thickness and size of the microspheres.

As glipizide release from the microspheres was very slow in the USP paddle method, these microspheres were further evaluated for drug release over longer periods of time, until complete release was observed, by shaking flask method. Glipizide release from all the microspheres was slow and spread over 10 d. The release of glipizide from 0-10 d followed zero order kinetics with 'r' greater than 0.945 and the corresponding release rates (K_0) are given in Table 1. The release rate depended on the proportion of coat, wall thickness and size of the microspheres. As the proportion of coat was increased glipizide release rate was decreased. The release rate was increased as the size of the microspheres was decreased. Good linear relationships were observed between percent coat, wall thickness and release rate (K_0) in both the sizes studied (fig. 2). The drug release mechanism from these microspheres was diffusion controlled as plots of amount released Vs square root of time were found to be linear ($r > 0.945$).

In vivo evaluation of the microspheres of glipizide was

TABLE 1: GLIPIZIDE CONTENT, ENCAPSULATION EFFICIENCY, WALL THICKNESS AND RELEASE CHARACTERISTICS OF EC MICROSPHERES

Microspheres (Size)	Glipizide content (%)	Encapsulation Efficiency (%)	Wall thickness (μ)	T ₉₀ (Days)	K ₀ (mg/day)
MC1 (20/35)	80.32 (0.6)	89.2	47.4	8.0	0.927
MC1 (35/60)	81.76 (1.2)	90.8	24.6	6.0	0.952
MC2 (20/35)	69.12 (0.8)	86.4	72.9	9.6	0.848
MC2 (35/60)	69.28 (1.4)	86.6	36.9	8.5	0.916
MC3 (20/35)	57.16 (1.2)	81.6	100.2	>10.0	0.776
MC3 (35/60)	59.26 (0.6)	84.6	56.1	9.0	0.883

K₀ is zero order release rate and T₉₀ is time for 90% release. *Figures in parentheses are coefficient of variation (CV) values.

carried out in normal rabbits by measuring the hypoglycemic effect produced after their subcutaneous administration. When glipizide pure drug was administered a rapid reduction in serum glucose levels was observed and a maximum reduction of 42.9% was observed at 2 h after administration and glucose levels were also recovered rapidly to the normal level within 6-7 h. Whereas, in the case of microspheres the maximum reduction observed was lower than that of pure drug and the reduced glucose levels were sustained over longer periods of time (fig. 3). A 25% reduction in glucose levels is considered¹³ as a significant hypoglycemic effect.

The hypoglycemic effect was maintained during the period from 0.5 h to 4 h following the administration of glipizide pure drug. Whereas in the case of microspheres, the hypoglycemic effect was maintained during the period from 2 h to 36 h in the case of MC1 and from 2 h to 6 d in case of MC2. The percent reduction observed with MC3 was in the range 20-25% during the period from 4 h to 6 d. As such the product MC3 has not produced a significant hypoglycemic effect. This may be due to the very slow release of glipizide from these microspheres (MC3) and the release rate was not adequate to produce significant hypoglycemic effect. The

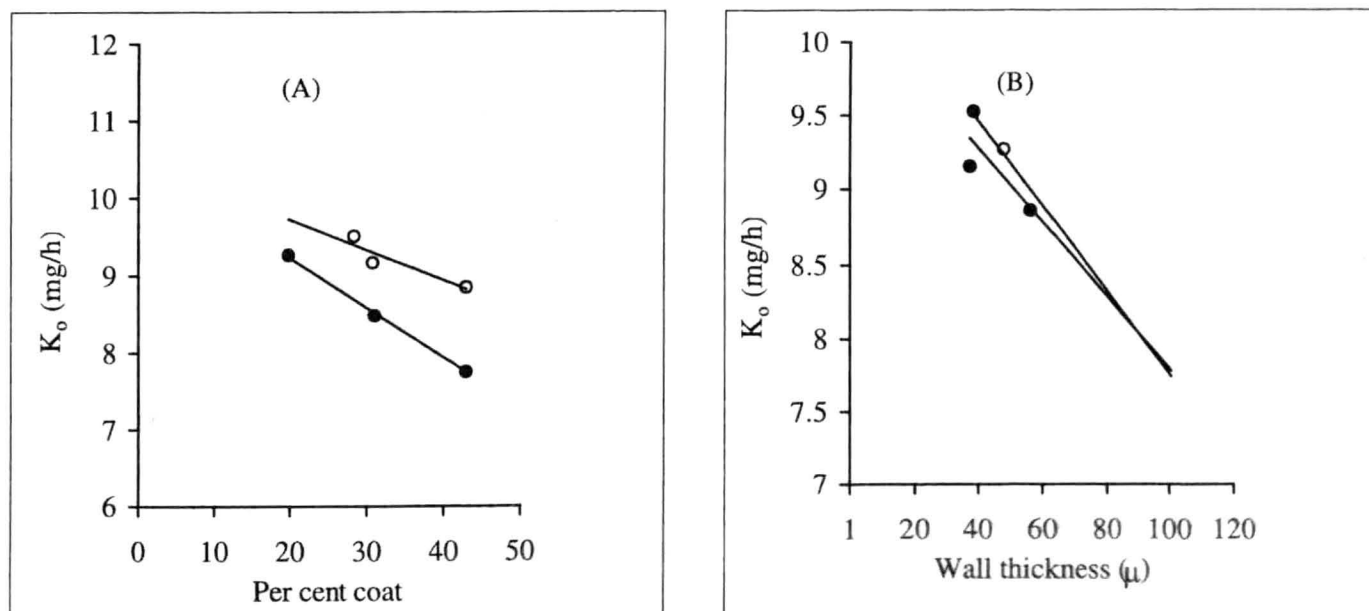


Fig. 2: Relationship between percent coat, wall thickness and release rate of microspheres.

Relationship between percent coat and release rate (A) and wall thickness and release rate (B) of EC microspheres of size 20/35 (●) and 35/60 (○).

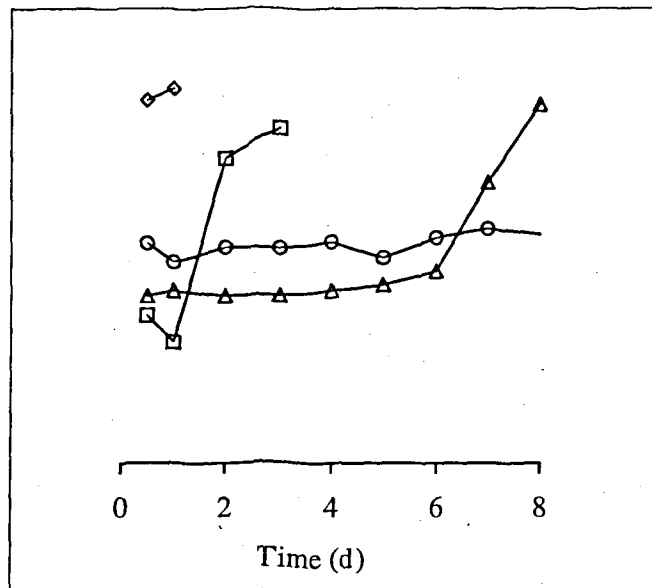
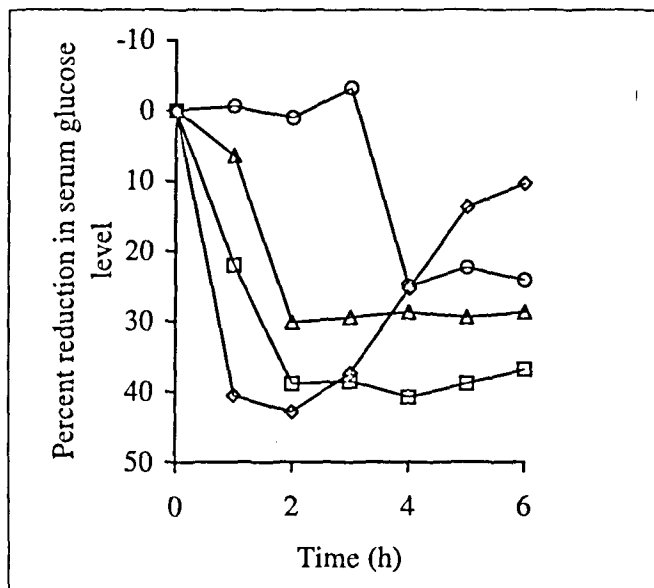


Fig. 3: Percent reduction in serum glucose following subcutaneous administration of microspheres of glipizide. Percent reduction in serum glucose following the subcutaneous administration of glipizide (◇) and its microspheres MC1 (□), MC2 (△) and MC3 (○).

sustained hypoglycemic effect observed over longer periods of time in the case of microspheres MC1 and MC2 is due to the slow release and absorption of glipizide from these microspheres administered subcutaneously. The hypoglycemic effect of glipizide could be sustained over a period of 6 d with microspheres MC2.

Thus spherical microspheres of ethyl cellulose containing glipizide could be prepared by an emulsion-solvent evaporation method employing chloroform as a solvent. The method is industrially feasible as it involves emulsification and removal of solvent, which can be controlled precisely. Encapsulation efficiency was in the range 81-91%. Glipizide release from the microspheres was slow and extended over a period of 10 d and depended on core: coat ratio, wall thickness and size of the microspheres. Drug release was diffusion controlled and followed zero order kinetics. In the *in vivo*, the hypoglycemic effect of glipizide was sustained over a period of 6 d with microspheres MC2 administered subcutaneously. Thus, the ethyl cellulose microspheres were found suitable for parenteral controlled release.

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