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# Formulation and evaluation of ethyl cellulose microspheres prepared by the multiple emulsion technique

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The aim of this study was to formulate and evaluate microencapsulated controlled release preparations of metformin hydrochloride using ethyl cellulose as the retardant material with high entrapment efficiency and extended release. Microspheres were prepared by the double emulsion solvent diffusion method. A mixed solvent system consisting of acetonitrile and dichloromethane in 1 : 1 ratio and light liquid paraffin were chosen as the primary and secondary oil phases, respectively. Span® 80 was used as the surfactant for stabilizing the secondary oil phase. The prepared microspheres were characterized by drug loading, optical microscopy and scanning electron microscopy (SEM). The *in vitro* release studies were performed in a series of buffer solutions with variable pH. The drug loaded microspheres showed 55–85% of entrapment and the release was extended for up to 12 h. SEM studies revealed that the microspheres were spherical and porous in nature. Data obtained from *in vitro* release studies were fitted to various kinetic models and high correlation was obtained with the Higuchi model. The drug release was found to be diffusion controlled. Oral administration of the microspheres to the albino mice provided decreased plasma glucose for more than 10 h.

## 1. Introduction

Metformin hydrochloride is an antidiabetic drug used to treat NIDDM (non insulin dependent diabetes mellitus) and is indicated as an adjunct to diet to lower blood glucose in cases where hyperglycemia cannot be controlled satisfactorily on diet alone. The recommended dosing schedule for the drug involves dose escalation with each dose given with meals. This allows metformin to be better tolerated, as gastrointestinal symptoms usually associated with therapy may be minimized (US patent). It has been reported in the physician desk reference electronic library release 2000 that food decreases the extent and slightly delays the absorption of metformin. The drug has a short half-life of about 2–4 h. It is available as conventional and sustained release tablet for oral use and is absorbed well from the stomach. Metformin was selected as the model drug due to its frequent administration, short half life and very high water solubility. As food delays the absorption and leads to improper bioavailability, formulation of an encapsulated dosage form will be a better option that the conventionally available plain and sustained released dosage forms. Most of the microencapsulation techniques are used with lipophilic drugs and are not very successful with hydrophilic drugs. The method utilized here is a multiple emulsion method using a mixed solvent system of acetonitrile and dichloromethane. This method has been utilized successfully to prepare microspheres of zidovudine using poly(lactide glycolide) (PLGA) with an encapsulation efficiency of 5% (Mandal et al. 1996), using ethyl cellulose with an encapsulation efficiency up to 55% (Rama Rao et al. 2005).

The objective of the present investigation was to prepare controlled release microspheres of a very highly water-soluble antidiabetic drug by improving encapsulation efficiency. Utilization of double emulsion solvent diffusion method and mixed solvent method (MSS) consisting of acetonitrile and dichloromethane in 1 : 1 ratio was chosen as the primary oil phase and light liquid paraffin was used as a processing medium. The presence of internal water phase helps in stabilization of emulsion and hardening of the microspheres (Badri et al. 1999). Span ®80 was used as the surfactant to stabilize the secondary oil phase; n-hexane was added as a non-solvent towards the end of the process to further solidify the microspheres.

## 2. Investigations, results and discussion

Water in oil in oil (w/o/o) double emulsion solvent diffusion method was used for the formulation of the microspheres. The preparation of microspheres was carried out by emulsifying an aqueous solution into a solution of drug and polymer in mixed solvent system comprising of acetonitrile and dichloromethane in equal ratio, followed by emulsification of this primary emulsion (w/o) into an external oil phase to form water in oil in oil (w/o/o) emulsion. The solvents of the system were removed by a combination of extraction and evaporation. Acetonitrile is

**Table 1: Processing and formulation parameters**

| Variable parameters | Experimental constants |
|---|---|
| Polymer concentration | Drug concentration |
| Polymer to drug ratio (1 : 0.5, 1 : 1, 1 : 1.5, 1 : 2) | Volume of the mixed solvent system |
| Stirring speed of the emulsification process (450 rpm and 1000 rpm) | Volume of the aqueous phase |
| | Volume of the non solvent |

a polar, water miscible and oil immiscible organic solvent whereas dichloromethane is non polar and oil miscible (Badri et al. 1990) in nature. During the formation of microspheres, dichloromethane was extracted by liquid paraffin and acetonitrile was evaporated during stirring. After introduction of o/w primary emulsion into liquid paraffin, stirring stabilized the droplets. No surfactant was required as ethyl cellulose has the additional property of stabilizing o/w emulsion (Melzar et al. 2003). For the stabilization of the secondary phase Span 80 was used, which has got a HLB value of 4.3 and it thus helps in reducing the surface tension at the interface. The process formulation parameters are given in Table 1.

### *2.1. Scanning electron microscopy (SEM) analysis*

As seen from the SEM, the microspheres are spherical and porous (Fig. 1). The addition of n-hexane as the non solvent causes for quick precipitation of the polymer, leaving the surface porous. The surface is rough and shows the presence of pores along with drug particles. This presence of drug particles on the surface might be involved in the initial burst effect.

### *2.2. Effect of formulation variables on particle size and size distribution*

The polymer to drug ratio was varied to find out the effect of polymer on the size and surface characteristics. It was found that with the increase in polymer concentration, larger microspheres were produced because of the increase in the viscosity of the phase. Due to this increased viscosity and continuous stirring, the smaller droplets coalesce to form larger droplets. It was seen that with the increase in percentage of Span 80 used, the mean particle size decreased (Fig. 2).

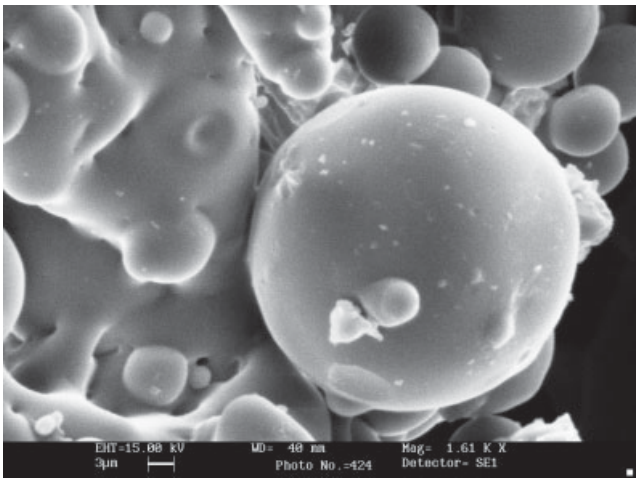


Fig. 1: Electron micrograph of ethyl cellulose microspheres

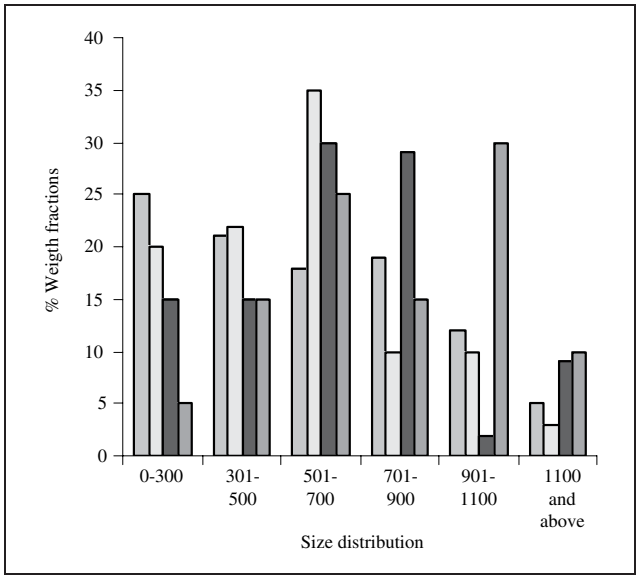


Fig. 2: Effect of Polymer to drug ratio on particle size (n = 5), 1 : 0.5 (A), 1 : 1 (B), and 1 : 1.5 (C), 1 : 2 (D); □ % weight fractions MA1; □ % weight fractions MA2; ■ % weight fractions MA3; ▨ % weight fractions MA4

Altering the stirring speed during the emulsification process greatly affected the size of the microspheres and its release pattern was also changed. Particle size increased with decrease in the stirring speed. It was found that a constant speed of 450 rpm gave uniformly sized microspheres in the range of 200 ± 20 µm.

### *2.3. Yield and percentage entrapment efficiency*

The yield of the microspheres was found to be about 75%. The entrapment efficiency was in the range of 62 ± 3%. To increase percentage entrapment, stirring speed was decreased and the non-solvent was added towards the end of the stirring process. The non-solvent causes quick precipitation of the polymer on the drug surface leading to higher drug entrapment.

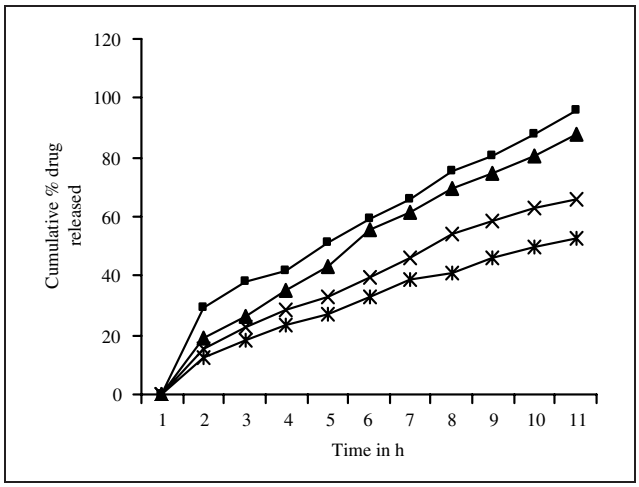


Fig. 3: Cumulative percentage release of metformin hydrochloride (N = 5) from ethyl cellulose microspheres with different drug to polymer ratio; —■— Cumulative % drug released A; —▲— Cumulative % drug released B; —×—; Cumulative % drug released C; —✳— Cumulative % drug released D

### 2.4. In vitro release studies

The *in vitro* release of the drug metformin hydrochloride was uniform after an initial burst effect. This initial burst effect was probably due to the presence of the drug particles on the surface, as seen from the SEM. This effect needs not to be avoided as *in vivo* it may be associated with giving the initial plasma concentration, which may be maintained by the later releases. The release profile is given in Fig. 3. As the concentration of ethyl cellulose increased from D : P 1 : 0.5 to 1 : 2, the release from the microspheres decreased which might be associated with increased thickness of the polymer matrix. The *in vitro* release kinetics were determined out to find the release mechanism. The release regression values were best fit with Higuchi plot rather than first order and zero order. The drug release of metformin hydrochloride was proportional to the square root of time, thus indicating a diffusion controlled release mechanism (Fig. 4).

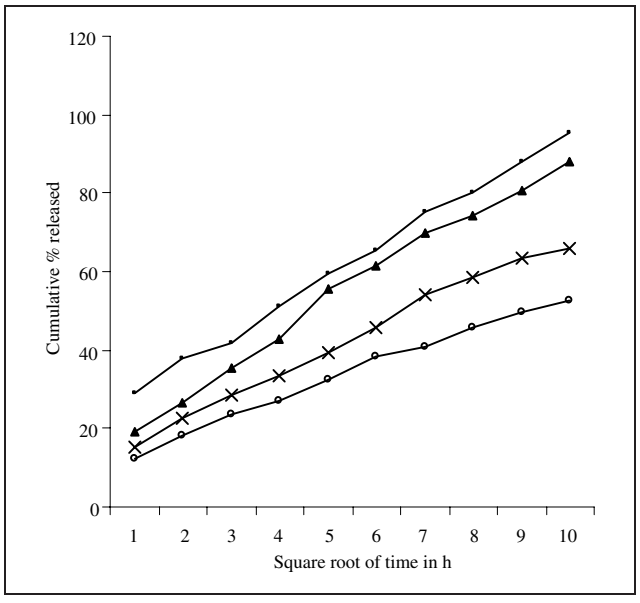


Fig. 4: Higuchi Plot of metformin hydrochloride release from ethyl cellulose microspheres of various drug to polymer ratio; —■— Cumulative % released A; —▲— Cumulative % released B; —×— Cumulative % released C; —○— Cumulative % released D

### 2.5. In vivo evaluation

*In vivo* evaluation of the microspheres (Fig. 5) was carried out in healthy male albino mice by measuring the hypoglycemic effect produced after their oral administration at a dose equivalent to 60 mg per kg body weight of metformin hydrochloride, in comparison to pure drug at the same dose. When pure drug was administered, the plasma glucose level declined rapidly within 30 min and then it was restored after 2 h. In case of microspheres, the reduction in glucose level was slower; it reached maximum reduction 4 h after administration, and the reductions in glucose levels were sustained for prolonged duration. A 25% reduction in glucose is considered a significant hypoglycemic effect (Kahn et al. 1991). The hypoglycemic effect was maintained during the period of 0.5 h to 2 h when pure drug was given, but the hypoglycemia was maintained for over 10 h with the formulation. The sustained hypoglycemic effect observed for a longer period of time in case of microspheres is due to the slow release and absorption of metformin hydrochloride. The hypoglycemic effect of metformin hydrochloride thus could be sustained for over 10 h with microspheres prepared by the multiple emulsion method.

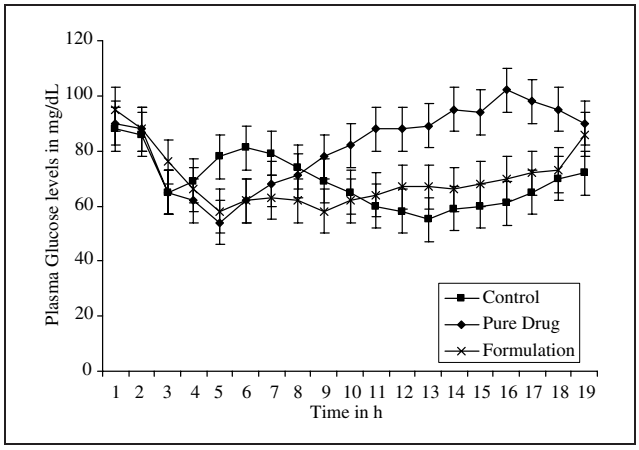


Fig. 5: *In vivo* reduction of plasma glucose level in normal albino mice (n = 3) following oral administration of pure drug and ethyl cellulose microspheres

## 3. Experimental

### 3.1. Materials

Metformin hydrochloride was donated by Themis laboratories, India; ethyl cellulose was purchased from Central Drug House, India. All other chemicals and reagents used were of analytical grade.

### 3.2. Preparation of microspheres

For the preparation of microspheres the double emulsion method was used as suggested by Rama Rao et al. (2005) with slight modifications. The polymer was dissolved in a mixed solvent system (MSS) of acetonitrile and dichloromethane. Drug was added and mixed. Then 3 ml of distilled water were added and this primary emulsion was stirred at 450 rpm for 15 min. Then, this w/o emulsion was poured into liquid paraffin containing Span® 80 as the surfactant. This was stirred using a mechanical stirrer for 3 h, for the complete evaporation of the solvent. 10 ml of n-hexane was added as the non solvent after 2 h of the stirring process (Table 1).

### 3.3. Entrapment efficiency

The prepared microspheres were dissolved in warm toluene and then the drug was back extracted by adding distilled water to this system and thoroughly shaken. The drug solution obtained was analyzed spectrophotometrically at 233 nm after suitable dilutions. The entrapment values are given in Table 2.

**Table 2: Effect of formulation parameters on properties of microspheres**

| Batch | D : P | % Entrapment efficiency | % Yield | % Drug released at the end of the study |
|---|---|---|---|---|
| A | 1 : 0.5 | 45 ± 3 | 69 ± 2 | 95 |
| B | 1 : 1 | 62 ± 4 | 75 ± 1.5 | 87 |
| C | 1 : 1.5 | 51 ± 2 | 65 ± 3 | 65 |
| D | 1 : 2 | 35 ± 5 | 66 ± 5 | 49 |

### 3.4. Size distribution and size analysis

Microspheres were separated into different size fractions by sieving for 10 min using a mechanical shaker containing standard sieves according to the Indian Pharmacopoeia. The particle size distribution was determined and mean particle size of microspheres was calculated by the formula

$$\text{Mean particle size} = \frac{\Sigma\,(\text{Mean particle size of the fraction x weight fraction})}{\Sigma\,(\text{Weight fraction})} \quad (1)$$

### 3.5. Scanning electron microscopy (SEM)

The 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 (Leica Electron Optics, Cambridge, USA) at 15 kv.

### 3.6. In vitro release studies

The in vitro release studies of the drug incorporated microspheres were carried out at $37 \pm 5$ °C and at 100 rpm using phosphate buffer pH 7.4 (200 ml) in sink conditions using a diffusion cell. Accurately weighed samples of microspheres were added to the donor cell after suspending in 5 ml of the buffer and at pre-set intervals; 5 ml of aliquots are withdrawn and replaced by an equal volume of fresh dissolution medium. The aliquots were analysed spectrophotometrically at 233 nm after proper dilution if required.

### 3.7. Release kinetics

Data obtained from *in vitro* analysis were fitted to various kinetic equations (Costa and Lobo 2001) to find out the mechanism of drug release from microspheres. The kinetic equations used were zero order equation, first order equation and Higuchi model. The following plots were made $Q_t$ vs. t (zero order model), log $(Q_0 - Q_t)$ vs. t (first order model) and $Q_t$ vs. $\sqrt{t}$ (Higuchi model), where $Q_{t\ is}$ the drug released at time t and $Q_0$ is the initial amount of the drug present in the microspheres. The rate constants were calculated for the respective models.

### 3.8. In vivo evaluation

*In vivo* evaluation studies were conducted on animals with pure drug and formulation. Normal, healthy male albino mice with an average weight of about 35 g were selected. The plasma glucose levels were measured following the oral administration of the microspheres equivalent to dose 60 mg per kg body weight (Chowdhary et al. 2003). The approval of an animal ethics committee was obtained before starting the study. The experiments were conducted in a crossover randomized block design ($n = 5$). The products were administered orally in the morning following overnight fasting. No food or liquid other than water was allowed during the experimental period. After the zero hour blood sample was collected, the product in the study was administered orally after suspending in 2 ml of 0.1% sodium carboxy methyl cellulose solution. Blood samples (0.5 ml) were withdrawn from the tail vein of the mice at 30 min up to 12 h. Plasma glucose levels were determined using a Prestige I Q, Blood Glucose Monitoring System, Home Diagnostics Inc. Florida, USA. Plasma glucose levels and percentage reduction in plasma glucose levels were calculated.

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