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Cellulose Acetate Microspheres as Floating Depot Systems to Increase Gastric Retention of Antidiabetic Drug: Formulation, Characterization and In Vitro–In Vivo Evaluation

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Gastric emptying is a complex process that is highly variable and makes the in vivo performance of drug delivery systems uncertain. In order to avoid this variability, efforts have been made to increase the retention time of the drug delivery systems for more than 12 hours utilizing floating or hydrodynamically controlled drug delivery systems. The objective of this investigation was to develop a floating, depot-forming drug delivery system for an antidiabetic drug based on microparticulate technology to maintain constant plasma drug concentrations over a prolonged period of time for effective control of blood sugar levels. Formulations were optimized using cellulose acetate as the polymer and evaluated in vitro for physicochemical characteristics and drug release in phosphate buffered saline (pH 7.4), and evaluated in vivo in healthy male albino mice. The shape and the surface morphology of the prepared microspheres were characterized by optical microscopy and scanning electron microscopy. In vitro drug release studies were performed and drug release kinetics were calculated using the linear regression method. Effects of stirring rate during preparation and polymer concentration on the size of microspheres and drug release were observed. The prepared microspheres exhibited prolonged drug release (more than 10 hours) and remained buoyant for over 10 hours. Spherical and smooth-surfaced microspheres with encapsulation efficiency ranging from 73% to 98% were obtained. The release rate decreased and the mean particle size increased at higher polymer concentrations. Stirring speed affected the morphology of the microspheres. This investigation revealed that upon administration, the biocompatible depot-forming polymeric microspheres controlled the drug release and plasma sugar levels more efficiently than plain orally given drug. These formulations, with their reduced frequency of administration and better control over drug disposition, may provide an economic benefit to the user compared with products currently available for diabetes control.

Keywords floating microspheres; metformin hydrochloride; cellulose acetate; in vitro release; in vivo

INTRODUCTION

Various approaches have been tried by researchers to prolong the gastric residence time of drugs. Floating drug delivery systems, also known as hydrodynamically balanced systems, have been employed successfully to retain the drug in the stomach (Deshpande, Rhodes, Shah, & Malick, 1996; Moes, 1993; Seth & Tossounian, 1984; Talukdar & Fassihi, 1984; Whitehead, Fell, Collett, Sharma, & Smith, 1998) as single- or multiple-unit systems. Multiple-unit systems are advantageous due to lower chances of dose dumping and less intersubject variability (Rouge, Leroux, Cole, Doelker, & Buri, 1997). Since multiple-unit dosage forms distribute widely they afford the possibility of a longer-lasting and more reliable release of the drug from the dosage form (Sato, Kawashima, Takechi, & Yamamoto, 2003).

Hollow microspheres have been successfully prepared by Kawashima, Niwa, Takechi, Hino, and Itoh (1992) for the drug ibuprofen by emulsion solvent diffusion method using synthetic acrylic polymers. Natural polymers have been used for the preparation of floating microspheres utilizing chitosan, xanthan gum, gelatin, and pectin, among others (Soppimath, Kulkarni, Rudzinski, & Aminanhavi, 2002). Semisynthetic polymers such as methyl cellulose and hydroxyl methyl cellulose have also been utilized for the preparation of floating microspheres utilizing lansoprazole and cimetidine along with other polymers (Muthuswamy, Govindrazan, & Ravi, 2005).

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Metformin hydrochloride was used as the model drug. It is an antidiabetic drug used to treat non–insulin dependent diabetes mellitus (NIDDM-Type II diabetes), alone or in combination with other hypoglycemic agents. This drug in monotherapy is used as an adjunct to diet to lower blood glucose in patients whose hyperglycemia cannot be satisfactorily managed by 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. It has been reported in the physician desk reference electronic library released in 2000 that food decreases the extent and slightly delays the absorption of metformin. Since it needs to be given along with food due to food's antihyperglycemic effect, it is necessary that the simultaneous and prolonged release of the drug takes place throughout the absorption process. The drug shows an absorption window in the stomach and the absorption is confined to the upper part of the intestine (Di Colo, Zambito, Baggiani, Careilli, & Serafini, 2005).

The objective of the present investigation was to develop floating microspheres of metformin hydrochloride in order to achieve an extended release in the upper gastrointestinal tract (GIT), which may result in an enhanced absorption and thereby improved bioavailability. Microspheres have been prepared for antidiabetic drugs utilizing alginate for glipizide, and mucoadhesive microspheres of metformin utilizing ethyl cellulose as polymer, but no attempts have been reported yet to develop floating microspheres for enhancing the bioavailability of metformin, which is predominantly absorbed from the stomach and upper GIT. Cellulose acetate was used as the polymer due to its water insolubility, which will help in sustaining the drug release for a longer time without increasing the polymer concentration in the formulations. Also, cellulose acetate has used successfully to produce floating microspheres by various investigators as Soppimath (2001). The prepared microspheres were evaluated for size, in vitro drug release, buoyancy, and incorporation efficiency. The effect of various formulation variables on the size and drug release was investigated. The in vivo evaluation was done in healthy male albino mice.

EXPERIMENTAL

Materials

Metformin hydrochloride was a gift from Sun Pharmaceuticals, Baroda; cellulose acetate was obtained from CDH, New Delhi; light mineral oil from Thomas Baker, Mumbai; chloroform from S D Fine chemicals, Boisar; and acetone from Qualigens, Mumbai. All other chemicals and reagents used were of analytical grade.

Preparation of Microspheres

Microspheres were prepared by emulsion solvent evaporation technique (Obeidat & Price, 2004). Polymer was dissolved in acetone; drug was dispersed and mixed thoroughly. This was then added slowly to liquid paraffin containing span 80 (1%). After 20 minutes of stirring 2 ml of n-hexane was added as the nonsolvent. The mixture was then stirred continuously at 650 rpm until the solvent evaporated completely. The formed microspheres were then filtered using Whatmann filter paper no. 41, washed with n-hexane, air dried, and stored in amber-colored bottles until analyzed.

Characterization of Microspheres

Size and Shape of Microspheres

The size of the microspheres was determined using an optical microscope fitted with a micrometer. An average of five determinations was taken.

Scanning Electron Microscopy (SEM)

The morphology of microspheres was analyzed to characterize the surface of formed microspheres using a scanning electron microscope (Leica Cambridge, United Kingdom) operating at 12 KV. The dried microspheres were mounted directly onto the sample stub and coated with gold dust under reduced pressure in a gold coating unit prior to observation.

In Vitro Buoyancy Studies

The in vitro buoyancy studies were carried out by time lag method (Patel, Patel, Patel, Bharadia, & Patel, 2006). Fixed quantities of prepared microspheres were placed in a 100-ml beaker containing 0.1 N HCl. The time taken by the microspheres to rise to the surface and float was determined as floating lag time.

Buoyancy Percentage

Microspheres (0.1 g) were spread over the surface of a USP XXIV dissolution apparatus (type II) filled with 900 ml 0.1 mol l^{-1} HCl containing 0.02% Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 hours. The floating and settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remain floating and the total mass of the microspheres.

Incorporation Efficiency

To determine the incorporation efficiency, microspheres were thoroughly crushed by trituration and suspended in a minimum amount of acetone. The suspension was suitably diluted with water and filtered to remove the shell fragments. Drug content was assayed spectrophotometrically (Systronics UV 2101) at 233 nm using a calibration curve. Each batch was examined for drug content in a triplicate manner. The entrapment efficiency of the microspheres was calculated by dividing the actual drug content by the theoretical drug content of microspheres.

In Vitro Dissolution Study

A diffusion setup was established with the help of glass chamber utilizing a membrane to act as the barrier. This setup was introduced into the dissolution media containing 250 ml 0.1 N HCl maintained at 37 ± 0.5°C with stirring using a thermal controlled magnetic stirrer at 100 rpm. Samples (5 ml) were withdrawn at regular intervals and replenished with the same amount of the fresh prewarmed dissolution fluid each time to maintain the sink conditions. The samples were then analyzed spectrophotometrically at 233 nm. Linear regression was used to characterize the in vitro release mechanism.

Statistical Analysis

Experimental results were expressed as mean ± *SD*. Differences were considered statistically significant at $p < .05$ for the in vivo results. The release kinetics were evaluated considering three different models including zero order, first order, and Higuchi equation, and the selection was based on the comparison of the relevant correlation coefficients and linearity tests.

In Vivo Evaluation

In vivo evaluation studies were conducted on animals with pure drug and formulation. Normal, healthy male albino mice were selected with average weight of about 35 g. The plasma glucose levels were measured following oral administration of the microspheres equivalent to dose of the drug, 60 mg/kg body weight (Lord, Atkins, & Bailey, 1983). The experiments were conducted as per a crossover randomized block design ($n = 5$). Three sets of animals were used as control, pure drug treated, and formulation treated. For the control the fasting was done overnight and water ad libitum was allowed. No drug or formulation was administered. The products were administered orally in the morning following overnight fasting. No food or liquid other than water (ad libitum) was allowed during the experimental period. Once the zero-hour blood sample was collected, the selected formulation was administered orally after suspending in 2 ml of 0.1% sodium carboxy methyl cellulose solution. Blood samples (0.1 ml) were withdrawn from the tail vein of the mice up to 12 hours at 30-minute intervals. Plasma glucose levels were determined using Prestige I Q, Blood Glucose Monitoring System, Home Diagnostics Inc., Florida. Plasma glucose levels and reduction in plasma glucose levels were calculated.

TABLE 1
Processing and Formulation Parameters

Variable Parameters	Experimental Constants
1. Polymer concentration	1. Drug concentration
2. Polymer-to-drug ratio (1:1, 1:2, 1:3, 1:4)	2. Volume of the solvent system
3. Stirring speed of the emulsification process (650 rpm, 450 rpm, 400 rpm, 800 rpm)	3. Volume of the aqueous phase
	4. Volume of the nonsolvent

TABLE 2
Batch Specifications of the Prepared Microspheres

Batch Code	Drug:Polymer Concentration	Temperature (°C)	Amount of Nonsolvent	Stirring Speed
FM1	1:1	30 ± 5	2 ml	650 rpm
FM2	1:2	30 ± 5	2 ml	650 rpm
FM3	1:3	30 ± 5	2 ml	650 rpm
FM4	1:4	30 ± 5	2 ml	650 rpm
FM5	1:5	30 ± 5	2 ml	650 rpm
FM6	1:1	30 ± 5	2 ml	450 rpm
FM7	1:1	30 ± 5	2 ml	400 rpm
FM8	1:1	30 ± 5	2 ml	800 rpm

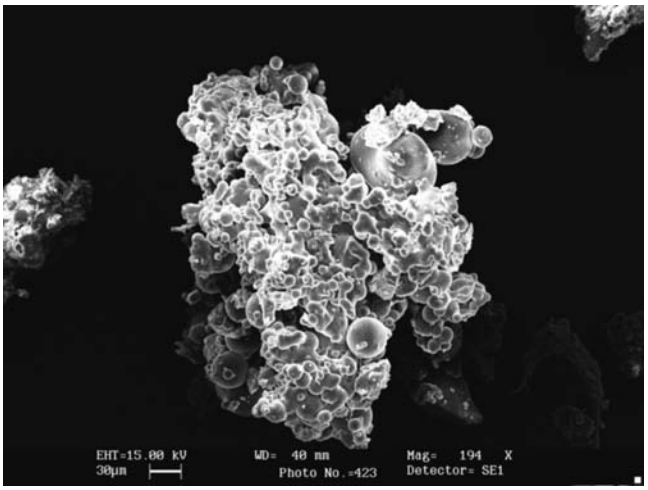

FIGURE 1. SEM of the formulations.

RESULTS AND DISCUSSION

Floating microspheres were prepared by the solvent evaporation method using cellulose acetate as the polymer (Tables 1 and 2). The SEM photographs showed that the fabricated microspheres were spherical and exhibited a range of sizes within each batch (Figures 1 and 2). The microspheres floated for a prolonged period over the surface of dissolution medium without any aggregation. Buoyancy percentage of the microspheres was in the range of 56% ± 4.2% (Batch FM2) to 88% ± 3.2% (Batch FM4). Large variation in the buoyancy percentage was observed in the formulations (Table 3).

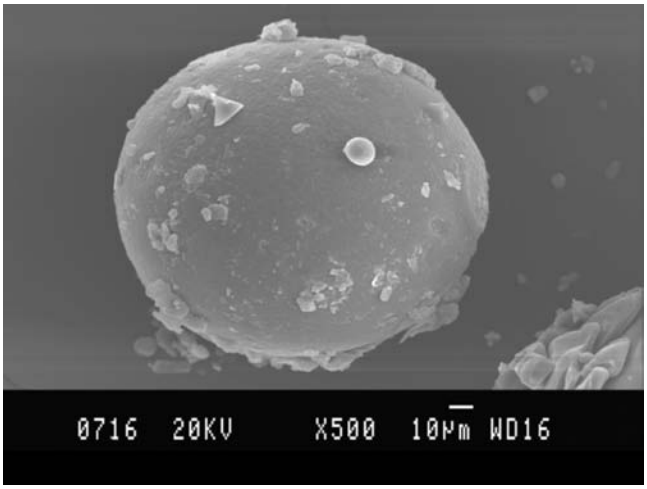

FIGURE 2. SEM of the microspheres.

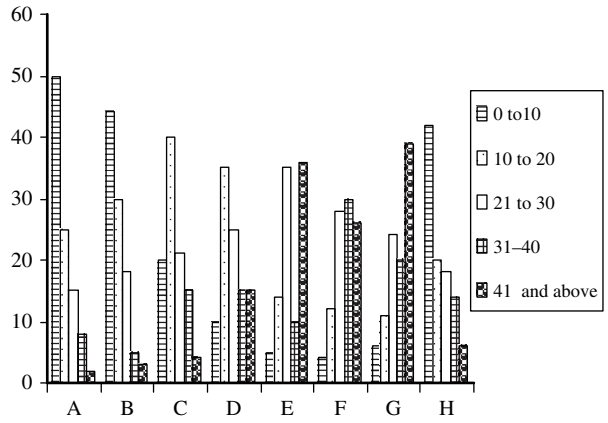

FIGURE 3. Effect of polymer-to-drug ratio on particle size (n = 5); 1:1 (A); 1:2 (B); 1:3 (C); 1:4 (D); 1:5 (E); 1:1 (F); 1:1 (G); 1:1 (H).

TABLE 3
Physical Parameters for Microspheres

Batch Code	Mean Particle Size in µ[a]	Incorporation efficiency[b] (%)	Buoyancy[c] (%)
FM1	4.5	85	88
FM2	7	87	56
FM3	15	90	92
FM4	18	89	88
FM5	23	78	89
FM6	30	88	78
FM7	42	90	75
FM8	3	75	70

[a]Mean ± *SD*, n = 10.
[b]Mean ± *SD*, n = 3.
[c]Mean ± *SD*, n = 3.

Microspheres were prepared by using a gradually increasing cellulose acetate concentration in comparison with a fixed concentration of the drug to asses the effect of polymer concentration on the size of the microspheres. The mean particle size of the microspheres significantly increased with increase in the polymer concentration ($p < .05$) and was in the range of 2 to 30 µm. The viscosity of the medium increases with higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities (Srivastava, Ridhurkar, & Wadhwa, 2005), resulting in the formation of larger particles. An increase in average size of the microspheres was also seen when the stirring speed was decreased. This could be attributed to the incapability of the stirrer at low speed to break up the bulk of the polymer into finer droplets (Figure 3).

In vitro release studies of metformin hydrochloride were performed in 0.1 mol L^{-1} HCl for 12 hours. The cumulative percentage release of the drug significantly decreased with the increase in the polymer concentration. The increased density of the polymer matrix at higher polymer concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Also, smaller particles are produced at lower polymer concentrations and have a larger surface area exposed to dissolution medium, giving rise to faster drug release. Drug release was higher in the case of the microspheres prepared by high agitation speed, which is mainly due to smaller microspheres produced and also poor formation of film over the core (Table 4, Figure 4).

The data obtained for in vitro release were fitted in to equations for the zero order, first order. and Higuchi release models. The interpretation of data was based on the value of the resulting regression coefficients (Table 5). The in vitro drug release showed the highest regression coefficient values for zero order, indicating non-Fickian diffusion to be predominant mechanism of drug release (Figure 5).

TABLE 4
Effect of Formulation Parameters on Properties of Microspheres

Batch	D:P	% Yield	% Drug Released at the End of the Study
FM1	1:1	69 ± 2.0	95
FM2	1:2	75 ± 1.5	87
FM3	1:3	65 ± 3.0	65
FM4	1:4	66 ± 3.5	49
FM5	1:5	70 ± 2.0	52
FM6	1:1	70 ± 3.0	85
FM7	1:1	65 ± 2.0	88
FM8	1:1	45 ± 2.0	98

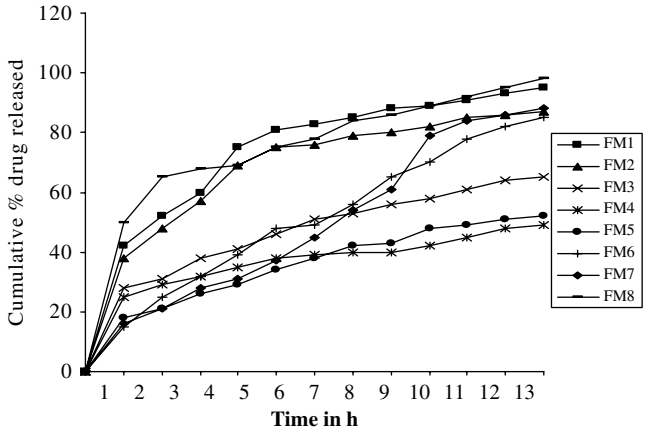

FIGURE 4. In vitro release profile of metformin hydrochloride (n = 5) from cellulose acetate microspheres with different drug-to-polymer ratios.

TABLE 5
Kinetic Equation Parameters of the Formulations

	R^2 Value		
B. No.	Zero Order	First Order	Higuchi
FM1	0.9417	0.9306	0.9392
FM2	0.9847	0.8939	0.9421
FM3	0.9730	0.9922	0.9900
FM4	0.9609	0.9407	0.9771
FM5	0.9745	0.9642	0.9833
FM6	0.9842	0.8741	0.9952
FM7	0.9914	0.8893	0.9142
FM8	0.9549	0.9014	0.9746

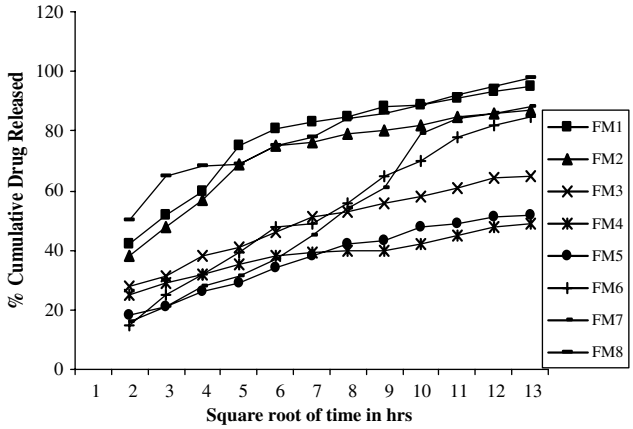

FIGURE 5. Higuchi plot of metformin hydrochloride release from cellulose acetate microspheres containing various drug-to-polymer ratios.

In vivo evaluation of the microspheres was carried out in healthy male albino mice by measuring the hypoglycemic effect produced after oral administration at a dose equivalent to 60 mg/kg body weight of metformin hydrochloride, in comparison with administration of pure drug at the same dose. When pure drug was administered, the plasma glucose level declined rapidly within 30 minutes, and then it was restored after 2 hours. In case of microspheres, the reduction in glucose level was slower; it reached maximum reduction at 3.5 hours after administration, and the reductions in glucose levels were sustained for prolonged duration (Figures 6 and 7).

A 25% reduction in glucose is considered a significant hypoglycemic effect (Kahn & Shechter, 1991). The hypoglycemic effect was maintained during the period of 0.5 hours to 2 hours when pure drug was given, but the hypoglycemia was maintained for over 10 hours with the formulation. The sustained hypoglycemic effect observed for longer period of time in case

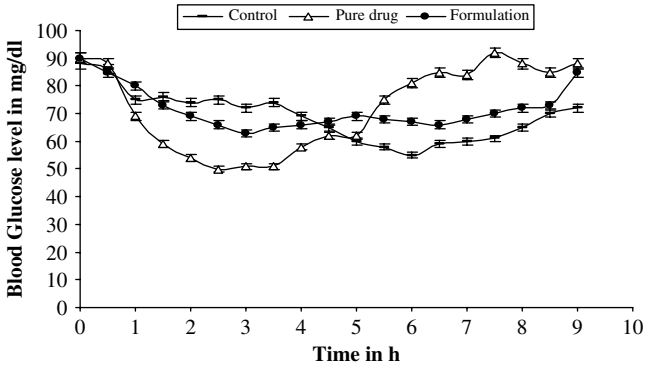

FIGURE 6. In vivo reduction of plasma glucose level in normal albino mice (n = 3) following oral administration of pure drug and cellulose acetate microspheres.

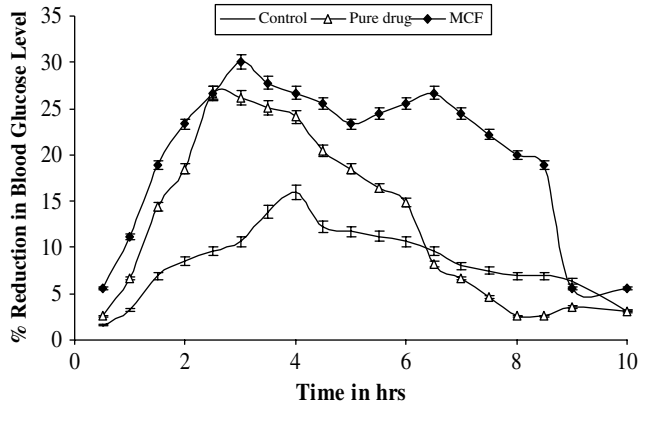

FIGURE 7. In vivo percent reduction of plasma glucose level in normal albino mice (n = 3) following oral administration of pure drug and cellulose acetate microspheres.

of microspheres is due to the slow release and absorption of metformin hydrochloride over an extended period of time. The hypoglycemic effect of metformin hydrochloride thus could be sustained for over 10 hours with floating microspheres prepared by solvent evaporation technique utilizing cellulose acetate as the polymer.

CONCLUSIONS

The incorporation of the highly water-soluble antidiabetic drug metformin hydrochloride was done using cellulose acetate as the polymer. The formulations exhibited sufficient floating properties and it was seen that with the increase in the stirring speed, the size of the formulation decreased, as well as the entrapment efficiency. Percentage drug released at the end of the study was affected by the polymer concentration.

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