PREPARATION AND CHARACTERIZATION OF MICROSPHERES UTILIZING RATE-CONTROLLING MEMBRANES FOR THE MANAGEMENT OF DIABETES MELLITUS

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ABSTRACT

The present research work aimed at the formulation of film-coated microspheres incorporating glibenclamide drug and their evaluation for the management of diabetes mellitus (DM). Microspheres were prepared by solvent evaporation methodology by the usage of ethyl cellulose as polymer, ethanol and dichloromethane as solvents and Tween 80® as a non-ionic surfactant. The film-coated membrane was prepared by pan coating method, incorporating ethyl cellulose, isopropyl alcohol, diethyl phthalate and sodium lauryl sulfate. This film membrane was coated on microspheres with the help of a spray gun. The efficiency of entrapment of the film coated microspheres of F5* batch, among different formulations, is highest and comes out to be in the range of 76.65±0.58. The percentage yield was observed to be 73.32±0.14. Morphological studies conducted by scanning electron microscope show spherical microspheres of uniform size. *In vitro* drug release study conducted of the coated microspheres of glibenclamide shows the highest amount of release of 97.44% in the F5*batch. The best-fit model was determined by the highest R^2 value. Further, the developed formulation helps in reduction in dose dumping, with better patient compliance, and also masks the bitter taste of the drug.

Keywords: Microspheres, diabetes mellitus, glibenclamide, film-coated membrane

INTRODUCTION

Innovative drug delivery systems by modulation of membrane characteristics and controlled release of active molecules have become significant areas of interest during the past few decades. It has also enabled the development of a new generation of drugs[1,2]. This type of drug delivery system has characteristic features such as improved bioavailability, minimizing dosing frequency, and reducing peril of local irritation and systemic toxicity[3].

Diabetes mellitus (DM) encompasses group of metabolic disorders, mainly of carbohydrate metabolism alongwith protein and fat metabolism, and is characterized by hyperglycemia, which arises because of relative or absolute deficit of insulin secretion, resistance to the action of insulin, or both. The main reasons for DM are lifestyle features, food habits, and increased stress levels. DM is divided into two categories, namely Type I diabetes mellitus, and Type II diabetes mellitus. Among them, Type II DM is most common, usually due to resistance to insulin action (a provision in which the human body is unable to use insulin properly), along with the deficiency of insulin in the body. Glibenclamide is an oral anti-diabetic drug, belonging to the class of sulphonyl ureas. This drug is utilized to regulate high blood sugar in adults with Type II DM[4,5]. The biological half-life of glibenclamide is only 4-6 h, and it is quickly excreted from the body. The mechanism of action (MOA) of glibenclamide involves the ATP-sensitive K^+ channel inhibition causing the depolarization of cell membrane, finally leading to stimulation of insulin release. In this research investigation,

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microspheres of glibenclamide were formulated and studies were performed on the formed microspheres by using ethyl cellulose polymer to develop a film for controlled release because glibenclamide requires a controlled release formulation for the better management of hypoglycemia and to improve its therapeutic potential and patient compliance[6,7].

Microspheres are very small spherical particles. The term "monolithic spheres" is used to describe the form of microspheres[8]. It is a round, hollow, and solid object. Microspheres are nano sized particles that are dispersed throughout the matrix with a polymer coating[9]. Ethyl cellulose microspheres can be produced via solvent evaporation methodology by using ethyl cellulose as a polymer[10]. They are composed of biodegradable proteins or synthetic polymers[11]. Several of polymers have been developed for their characteristic feature in the formulation of microspheres for the concept of controlled drug delivery[12]. The advantages of microspheres also include masking the bitter taste, odor and reducing dose-dumping[13].

A rate controlling membrane is a special kind of membrane that has pores on its surface. The pores have diameters ranging from less than 1 nm to more than 100 nm. The size and distribution of the pores determine the properties of the porous material[14,15]. Membranes are usually made from a solid matrix with defined pores on the surface, which can be used for filtration, separation and absorption processes. The pore's diameter can be lowered to less than 1nm, which is one of the main advantages of this technique for drug delivery. Membranes are made from substances that dissolve easily in the body, such as lipids or proteins, and can be tolerated to release drugs at a specific rate over time[16,17]. Thus, it was observed during this research that the film-coated microspheres using the glibenclamide drug can be utilized for the management of diabetes mellitus.

MATERIALS AND METHODS

Materials

Glibenclamide was procured from Prudence Pharmaceuticals Ltd., Ankhleshwar, Gujarat, India. Ethylcellulose, isopropyl alcohol, dichloromethane, sodium lauryl sulfate and Tween 80® were purchased from Loba Chemie, Mumbai (India). All the chemical reagents and catalysts employed in this study were of analytical grade.

Methods

Preparation of film-coated microspheres

The microspheres were produced via solvent evaporation methodology with slight modifications. The microspheres of glibenclamide were prepared by weighing the proper ratio of drug and polymer, and dissolving them in ethanol and dichloromethane. Then, this mixture was added to Tween 80® solution (0.5%) at a slow rate with constant stirring for about 2 h. The resultant microspheres were passed through the filtration process and washed with distilled water. Then, the microspheres were dried for 12 h and kept in desiccators[18]. The membrane was prepared by the drying method using a spray gun, as shown in Fig. 1. A weighed amount of ethyl cellulose was dissolved in isopropyl alcohol and diethyl phthalate. Sodium lauryl sulfate was added to the above solution. This solution was cast on a petri dish to observe the drying properties of the membrane[19]. The membrane solution was sprayed (coated) on the prepared microsphere by spray gun method. Finally, film-coated microspheres were obtained, as shown in Fig. 2. The description of the formulation chart of glibenclamide microspheres is depicted in Table I and formulation chart of coating membrane is depicted in Table II.

Table I: Formulation chart of glibenclamide microspheres

Sr. No.	Formulation code	Drug (mg)	Polymer (g)	Ethanol (mL)	Dichloro-methane (mL)	Tween 80® (%)	Distilled water (mL)	Stirrer speed RPM
1.	F1	100	1	25	25	0.5	100	700
2.	F2	100	2	25	25	0.5	100	800
3.	F3	100	3	25	25	0.5	100	1200
4.	F4	100	2	25	25	0.5	100	1200
5.	F5*	100	1	25	25	0.5	100	1300
6.	F6	100	3	25	25	0.5	100	1300

Fig. 1: Spray gun

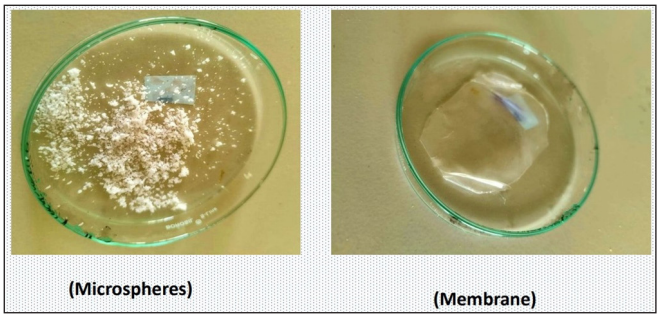

Fig. 2: Glibenclamide microspheres and membrane

Evaluation of film-coated microspheres

Glibenclamide loaded microspheres were evaluated for external morphology and shape, entrapment efficiency, percentage yield and *in vitro* drug release.

Scanning electron microscopy

A scanning electron microscope (SEM) was employed to examine the external morphology and shape of the formed batches of sample formulations. Fine gold was sputtered in a high vacuum evaporator and the sample was fixed on carbon tape. The acceleration voltage was set to 10kV throughout the scanning, and higher magnification microphotographs were recorded at dissimilar magnifications and used to ascertain the surface morphology[20, 21].

Entrapment efficiency

Glibenclamide loaded microspheres (10mg) were powdered and suspended in 100mL methanolic:water (5:95 V/V) mixture. The dispersion was stirred thoroughly for about 20 minutes on a magnetic stirrer and then filtered through a Whatman filter paper grade 1 (size 10 mm). Drug content was determined at 234 nm spectrophotometrically via a regression equation, which is consequent to the standard graph[22,23].

Percentage yield of microspheres

The formulated microspheres were dried, collected and weighed to determine drug percentage yield. The accurate weight of the produced microspheres is divided by the total amount of all excipients and the drug employed in the microsphere's preparation, resulting in the total percentage yield of microspheres[24,25].

In vitro drug release study

Microsphere drug dissolution studies were executed by using a USP dissolution apparatus in 900 mL of phosphate buffer (pH 6.8). The coated microspheres filled capsule of size 00 were weighed and added to the dissolving basket and run at 100 rpm with thermostatically controlled rotation regulated at 37°C. Throughout the dissolution tests, the ideal sink environment was maintained. The test solution was removed at an appropriate interval from the dissolution vessel and examined at 234 nm spectrophotometrically[26,27].

Drug release kinetic study

To investigate the mechanism of the glibenclamide drug released from film-coated microspheres, release

Table II: Formulation chart of coating membrane

S. No.	Formulation code	Polymer (EC) (g)	Isopropyl alcohol (mL)	Diethyl phthalate (mL)	Sodium lauryl sulphate (mg)
1.	P1	4	50	4	500
2.	P2	4	50	3	400
3.	P3	4	50	2	300
4.	P4	4	50	3	500
5.	P5*	4	50	2	400
6.	P6	4	50	3	300

kinetics were determined according to several kinetics models such as zero-order kinetics, first-order kinetics, Hixson Crowell model, Korsemeyer-Peppas model, and Higuchi's model. The obtained results for *in vitro* studies were fitted in several models of the data treatment as follows:

- Cumulative percent release drug vs. time(zero-order)
- Log cumulative percent retained drug vs. time (first order)
- Log cumulative percent release drug vs. square root of time (equation of Higuchi's classical diffusion)
- Log of cumulative % release vs. log time (Korsemeyer-Peppas exponential equation)[3]

RESULTS

Scanning electron microscopy (SEM)

SEM analysis was employed to examine the shape and surface characteristics of the coated microspheres and membranes. The surface morphology of the formulation, evaluated at 100X magnification, revealed the smooth surface of membranes and microspheres. Shape and surface characterization of optimized batch P5* membrane and film-coated microspheres is shown in Figs. 3 and 4.

Entrapment efficiency

Out of all the formulations, formulation no F5* was found to have the highest level of entrapment efficiency.

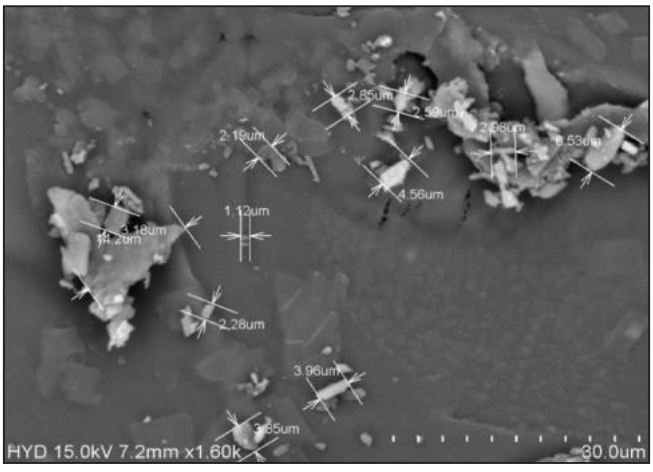

Fig. 3: Scanning electron microscopy of pores

The entrapment efficiency of film-coated microspheres is shown in Table III.

Table III: Drug entrapment efficiency for different formulations

Formulation	Drug entrapment of prepared microspheres (%w/w)
F1	67.08±0.25
F2	66.45±0.36
F3	63.32±0.45
F4	68.89±0.74
F5*	**76.65±0.58**
F6	68.98±0.65

Percentage yield of microspheres

The percentage yields of the formulations are in the range of 62.23-73.32%. In the F5* formulation, the highest percentage yield is observed at 73.32±0.14. The SEM image of drug loaded microsphere is shown in Fig. 4. The percentage yield of different formulations is mentioned in Table IV.

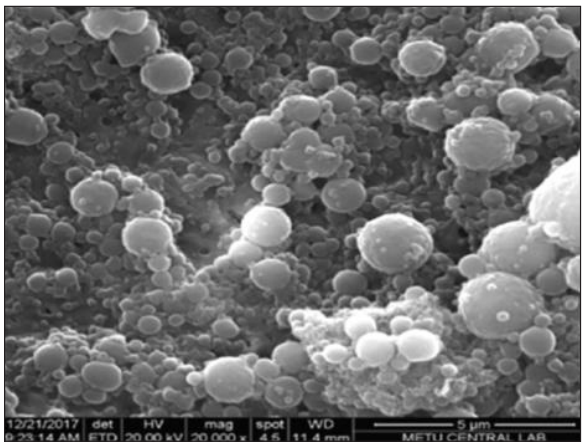

Fig. 4: Scanning electron microscopy image of microspheres

Table IV: Percentage yield of different formulations

Formulation	Percentage yield
F1	62.23±0.45
F2	65.58±0.32
F3	69.95±0.25
F4	68.85±0.36
F5*	**73.32±0.14**
F6	64.25±0.25

In vitro drug release of coated microspheres

In vitro drug release analysis was done to assure that the coated microspheres of glibenclamide are delivered to the target area and to elucidate the release kinetic for the developed formulation. It shows the drug release from its different formulation as compared to the plain microspheres. The amount of drug release of F5* is maximum, showing 97.44 % as compared to other formulations. The amount of drug release from porous microspheres is comparatively high than plain microspheres, as shown in Fig. 5. The drug release kinetic data of film-coated microspheres F5* formulation is depicted in Table V.

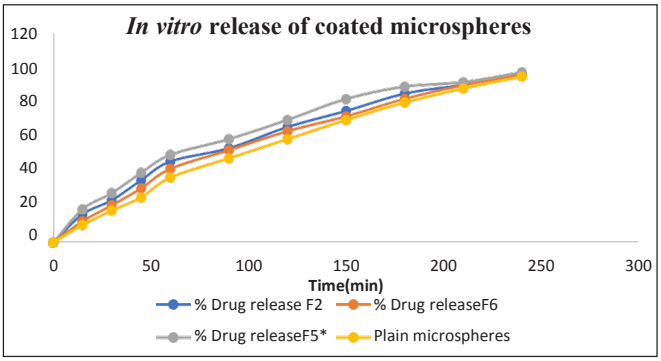

Fig. 5: Drug release of coated microspheres

Drug release kinetic studies

The best-fit model was determined by the highest R^2 value, as shown in Table V. The highest regression value was calculated for 0.9909, which means the formulation F5* fits in zero-order kinetics.

Table V: Drug release kinetic data of film-coated microspheres F5* formulation

Formulation	Zero-order	First order equation	Higuchi equation	Korsmeyer-Pappas equation
F5*	0.9909	0.9871	0.996	0.9866

DISCUSSION

The microspheres of glibenclamide were successfully formulated by using polymers such as ethyl cellulose via the solvent evaporation method. The membrane was prepared by spray drying method using ethyl cellulose as film-forming material.

Glibenclamide is a very effective oral anti-diabetic drug that improves glycemic control and regulates the levels of circulating insulin. Film-coated microspheres have a low density and a very large surface area. Their superior absorption capability distinguishes them from traditional microspheres. Membrane-coated microspheres were also evaluated by scanning electron microscopy, as well as for entrapment efficiency, besides yield of microspheres, drug release kinetics, and *in vitro* drug release studies. Scanning electron microscopy image showed F5* formulation of film-coated microspheres to be spherical in shape. The percentage yield of the different formulations was in the range of 62.23-73.32%. In F5 *formulation, the highest percentage yield was observed, which was found to be 73.32±0.14. The entrapment efficiency of formulation F5* was found to be 76.65%. *In vitro* drug release of F5*formulation was found to be 97.44 ±0.89% up to 6 h i.e., in a controlled manner.

CONCLUSION

This project was conducted to explore the possibility of film coated microspheres for the management of diabetes mellitus. Glibenclamide-loaded microspheres were formulated successfully and optimized via emulsion solvent evaporation methodology by using ethylcellulose as a film forming agent.

The carrier used in this study has higher solubility and porosity, which provides higher bioavailability. These types of microspheres improve patient compliance, reduce dosing frequency and are cost-effective.

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