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Development and Evaluation of Sustained Release Microspheres of Repaglinide for Management of Type 2 Diabetes Mellitus

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Abstract

Sustained release dosage form is essential for diabetic patients which is marked by continuous therapy along with high margin of safety, patient compliance and fulfill economical features. Repaglinide is a class of meglitinide, a drug of choice to formulate microspheres by utilizing sodium alginate, olibanum gum and pectin in different ratios by using ionic-gelation method. Excellent results were found in rheological behavior and release studies. Microspheres size and percentage yield was found in the range of 694 µm to 727 µm and 73% to 75% respectively. SEM revealed that microspheres were discrete, spherical and free flowing. Entrapment efficiency was variable, ranges from 55% to 75%. Uniform drug release was observed in drug release kinetics, followed Higuchi model with non-fickian release. These microspheres proved to be suitable for oral sustained release of repaglinide.

Keywords: Repaglinide, Microsphere, Ionic-gelation method, Olibanum gum, Pectin

1. Introduction

Modern era has emphasized on modified release dosage forms to achieve and maintain therapeutic amount of drug in the blood or tissue to improve pharmacokinetic of drug and increase patient compliance as well as reducing side effects for a prolong period of time [1,2]. Many therapeutic benefits gained by incorporating drug into coat material to form microspheres by using ionic gelation method [3]. Microencapsulation is the application of a thin coating to individual core material that has an arbitrary particle size range from 5-5000 µm [4]. Diabetes mellitus is a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and an increased risk of complication from the vascular disease [5]. Repaglinide has a great utility as a therapeutic agent in the treatment of diabetes mellitus.

Repaglinide, S(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)butyl) amino)-2-oxoethyl) benzoic acid (Fig.1), a fast and short-acting meglitinide analog was chosen as the drug candidate because of very short biological half-life of 1hour, low bioavailability (50%) and poor absorption in the upper intestinal tract, since it is indicated for the development of a dosage form with increased gastric residence time [6,7].

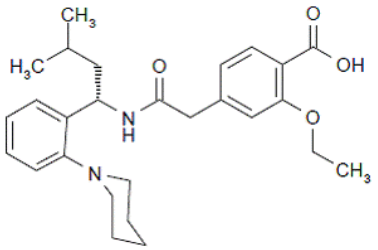

Fig.1. Chemical structure of Repaglinide

This study is marked to evaluate different polymers to improve drug stability for the patients suffering from long term non-insulin dependent diabetes mellitus [8]. The objective of present study is to formulate a once-daily sustained release microencapsulated dosage form of Repaglinide which can provide continuous therapy and high margin of safety. This can be achieved by controlling release factors and to determine drug release parameters as per different release kinetic models [9].

2. Materials and Methods

2.1 Materials

Repaglinide was obtained as a gift sample from Wilshire Laboratories (Pvt) LTD. Lahore, Pakistan. Polymers such as Sodium alginate, Olibanum gum and Pectin were purchased from (China), (BDH Laboratories) and (Merck) respectively. Chemicals such as Sodium hydroxide (Merck), Calcium Chloride (Merk), Mono Basic Potassium Phosphate (Riedel de Haen), Chloroform (Riedel de Haen) and all other chemicals used were of analytical grade.

2.2 Method for Preparation of Microspheres

Microspheres of Repaglinide were prepared by ionic gelation method [10]. Repaglinide in different ratios of polymers, sodium alginate, pectin, and olibanum gum were prepared as shown in Table 1.

Polymer was dissolved in distilled water in a reagent bottle by using magnetic stirrer to form homogenous mixture. Repaglinide was dissolved in100 ml of chloroform in a well-closed volumetric flask. Solution of drug was added to polymer solution and then mixed with magnetic stirrer at speed of 1000 rpm in order to form a homogenous blend. 15% Solution of calcium chloride was prepared, then drug polymer solution was dropped manually from a hypodermic syringe through needle size number 26G into solution of calcium chloride, resultant microspheres called calcium alginate microspheres.

They were allowed to harden in gelling bath for 30 minutes and were filtered with Whatmann filter paper (No.4), washed with distilled water, allowed to dry in air at room temperature for 30 minutes and then were transferred to petri dishes and dried in oven at 37C° until a constant weight was obtained.

Table 1: Composition of formulations of microspheres

Formulation code	Drug	Sodium Alginate	Pectin	Olibanum Gum
	---------- (W/V %) ----------			
RPG 1	1	1	--	--
RPG 2	1	1	0.5	--
RPG 3	1	1	0.75	--
RPG 4	1	1	1	--
RPG 5	1	1	--	0.5
RPG 6	1	1	--	0.75
RPG 7	1	1	--	1

2.3 *Characterization and Evaluation of Microspheres*

2.3.1 Percentage yield (w/w)

The dried microspheres were weighed and their percentage yield (w/w) was calculated from the weight of dried microspheres (W_1) and the sum of dry weight of initial materials i.e. drug and polymers (W_2) as the following formula [11].

$$\text{Percentage yield} = W_1 / W_2 \times 100$$

2.3.2 Particle size analysis

Particle size analysis of drug loaded microspheres was determined by optical microscopic method using a compound microscope [12, 13]. At least 100 microspheres were analyzed for each preparation and the mean particle size was determined by using Edmondson's equation

$$D \text{ mean} = \Sigma nd/\Sigma n,$$

Where n= number of microspheres observed and d= mean size range

2.3.3 Flow properties of microspheres

2.3.4 Angle of repose

Weighed quantities of microspheres were passed through a funnel fixed on a stand at a specific height upon graph paper. A static heap of powder with only gravity acting upon it was tending to form a conical mound. The height of the heap (h) and radius (r) of lower part of cone were measured. The angle of repose was calculated using formula [14].

$$\theta = \tan^{-1} h/r$$

2.3.5 Carr's index

The simple test was evaluated for the flow ability of a powder by comparing the poured density and tapped density of a powder. It was determined by taking small quantity of microsphere samples in 10 ml measuring cylinder. The height of the sample was measured before and after tapping indicates the poured and tapped density.

Carr's index was calculated as:

$$I = \rho t - \rho d / \rho t \times 100$$

Where ρt is tapped density and ρd is bulk density.

Carr's index < 15% gives good flow characteristics and above 25% indicates poor flow characteristics [14].

2.3.6 Hausner's ratio

Hausner ratio was calculated using formula [14].

$$\text{Hausner's ratio} = \rho t / \rho d$$

A value <1.2 is preferable for free flow; however a Hausner's ratio close to 1 indicates good flow properties.

2.3.7 Percentage Encapsulation Efficiency

Microspheres were evaluated to estimate percentage of drug entrapped in polymer matrix by crushing 100mg of microspheres in pestle and mortar and then dissolving these powdered microspheres in 100 ml ethanol. Vortexed solution for 5 minutes and then filtered it with Whatmann filter paper (No.4) in order to remove polymeric material. The filtered sample was suitably diluted and analyzed spectrophotometrically at 243 nm wave length in phosphate buffer pH 6.8 [15]. The measured absorbance was then converted to the amount of Repaglinide by using standard calibration curve using (2-20 µg/ml). Percentage entrapment was calculated by using formula.

EE % = Entrapped drug per gm in microsphere / Theoretical amount of drug per gm microsphere x 100

2.3.8 Shape and surface morphology

The external morphology of microspheres was analyzed by scanning electron microscopy (SEM). In scanning electron microscopy samples were prepared by lightly sprinkling microsphere powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of (150–200 Å) using a fine coat ion sputter [16]. The microspheres were examined under scanning electron microscope

2.3.9 In Vitro Drug Release studies

Dissolution studies of Repaglinide microspheres were carried out by enclosing microspheres in hard gelatin capsule in a quantity equivalent to 120mg dose of drug. For dissolution studies, paddle apparatus is used in order to study the release behavior of microspheres. The dissolution study was carried out for 2 hours in 900ml of 0.1 N HCl solution at 37˚C ±0.5˚ C and rotation speed was 100 rpm.

5ml of aliquots of dissolution medium was withdrawn at definite interval and analyzed by U.V spectrophotometer for Repaglinide contents at λ max 243-nm, the dissolution medium was kept constant by adding the same volume of fresh dissolution medium after each withdrawal. After 2 hours these microspheres were shifted to pH 6.8 buffer medium under similar conditions as described above.

Release kinetics was obtained by using zero order [17], first order [18], Higuchi model [19], Korsmeyer Peppas model [20] and Hixson-Crowell model [21].

3. Results and Discussion

The present investigation was carried out on the formulation and evaluation of oral controlled release microspheres of Repaglinide, which is meant for treatment of Type II diabetes mellitus. Repaglinide microspheres were developed by ionic gelation method employing pectin and Olibanum gum as polymers.

The particle size of microspheres is within range of 694-767 µm, whereas percentage yield varies with in range of 73- 75% in different formulations as shown in table 2. Results of angle of repose for microspheres, that is below 30^{o} and Carr's index values, which are below 10% and Hausner's ratio, which is less than 1.11 shows that microspheres have excellent flow behaviour.

Table 2. Physical characteristics of microspheres

Formulation code	MPS	Y	AR	CI	HR	E
RPG 1	694 ±1.64	73.42 ±0.47	28.84 ±0.35	8.43 ±0.41	1.04	55.35 ±0.37
RPG 2	732 ±1.01	68.17 ±0.21	26.56 ±0.94	8.13±0.11	1.06	63.04 ±0.48
RPG 3	742 ±2.61	69.22 ±0.26	26.14 ±0.21	7.27±0.59	1.04	63.42 ±0.19
RPG 4	767 ±1.73	71.45 ±0.51	25.93 ±0.47	6.87 ±0.29	1.02	66.35 ±0.20
RPG 5	694 ±2.04	73.05 ±0.17	26.72 ±0.71	5.74 ±0.88	1.03	73.97 ±0.75
RPG 6	702 ±2.04	74.62 ±0.64	24.32 ±0.01	6.39±0.71	1.02	74.32 ±0.55
RPG 7	727 ±2.11	75.49 ±0.26	23.21 ±0.32	8.52 ±0.63	1.05	75.61 ±0.36

MPS = Mean particle size(µm), Y= % Age Yield, AR = Angle of Repose

CI = Carr's Index, HR = Hausner's Ratio %, E = Age Entrapment

3.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy of Repaglinide loaded microspheres are presented in Fig. 2. The microspheres prepared with varying polymers showed nearly spherical shape with some cracks in formulation containing sodium alginate which results in the fast release of drug from microspheres. SEM photographs revealed the absence of crystals of drug on the surface of the microspheres made with polymers indicating uniform distribution of drug within these microspheres.

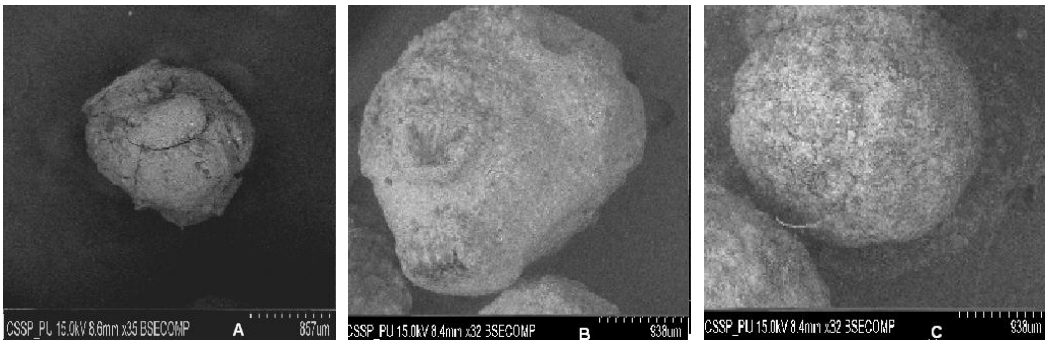

Fig. 2 Scanning Electron Micrographs of ; (A) Repaglinide microspheres prepared with sodium alginate (B) Repaglinide microspheres prepared with sodium alginate and pectin and (C) Repaglinide microspheres prepared with sodium alginate and olibanum gum

3.2 In Vitro drug release studies

Dissolution studies of microspheres were carried out in order to study drug release behaviour of polymer matrix. The release was studied in 0.1N HCl and phosphate buffer pH 6.8 for 12 hours.

Increase the drug polymer ratio correspondingly decreases the drug release. Repaglinide release from the microspheres was found slow which controlled over extended period and release was found to be dependent on the nature of the polymer used. The drug release from the microspheres was sustained over a prolonged period of time at pH 6.8 with greater retardation in microspheres containing olibanum gum and this proved to be the best formulation.

Dissolution data obtained from dissolution studies of different formulations was fitted in Zero order, first order, Higuchi Model, Korsmeyer-Peppas Model and Hixson–Crowell Model. The values of correlation coefficient obtained by fitting dissolution data of formulations RPG 1to RPG 7 in these kinetic models are given in table 3. In most of the formulated microspheres the drug release followed Higuchi Model. In all trials, drug release mechanism was studied by applying Korsmeyer–Peppas model, n values range between 0.818 and 0.889, indicating non-fickian release.

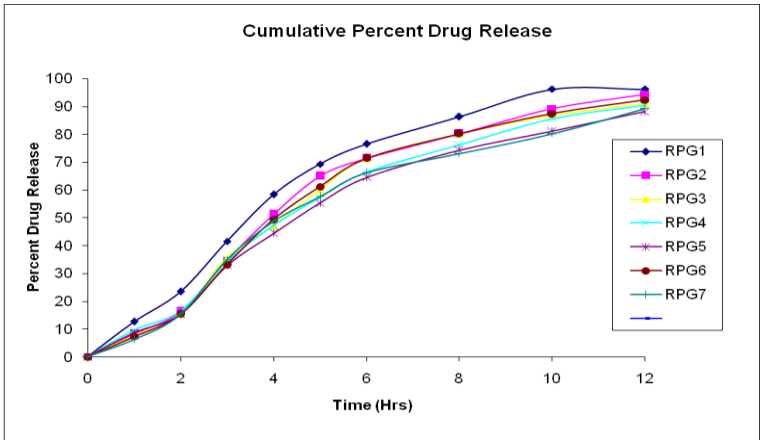

Fig 3 Repaglinide released from various formulations of microspheres

Table 3: Values of correlation coefficient for the fit of various kinetic models

Formul-ation	Zero order Model		First order Model		Higuchi Model		Korsmeyer-Peppas Model			Hixson-Crowell Model	
	k_o	R^2	K_1	R^2	k_{HC}	R^2	k_p	n	R^2	k_H	R^2
RPG 1	9.436	0.924	0.335	0.984	41.05	0.977	0.134	0.852	0.967	0.237	0.889
RPG 2	7.960	0.893	0.254	0.992	37.72	0.961	0.127	0.818	0.942	0.212	0.921
RPG 3	7.807	0.894	0.223	0.994	36.98	0.961	0.128	0.841	0.942	0.212	0.892
RPG 4	7.544	0.921	0.209	0.996	35.47	0.976	0.105	0.865	0.958	0.202	0.879
RPG 5	7.349	0.921	0.188	0.997	34.67	0.976	0.110	0.878	0.956	0.204	0.894
RPG 6	7.931	0.894	0.233	0.996	37.56	0.961	0.108	0.889	0.943	0.216	0.927
RPG 7	7.341	0.898	0.180	0.987	33.52	0.973	0.120	0.881	0.930	0.208	0.931

4. Conclusion

Microspheres of Repaglinide coated with Olibanum gum to form once daily dosage form is an effective system for sustained release of drug. Varying combinations of drug-polymer were helpful in designing these formulations. Among these, RPG 5, which contains Repaglinide-olibanum gum was considered best of all due to efficient release of drug from the microspheres. Entrapment efficiency, percentage release, mean particle size and drug release behaviour varies with increased drug polymer ratio. Microspheres were discrete, spherical and free flowing cleared in SEM studies. Drug release was both diffusion and dissolution controlled which followed Higuchi Model. An increase in polymer ratio decreased drug release profile of drug hence provide better patient compliance, cost effective and increased bioavailability for prolonged period of time.

5. Conflict-Of-Interest Policy

Authors do not have any commercial affiliations, or potential conflicts of interest associated with this work submitted for publication.

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