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FORMULATION DEVELOPMENT AND *IN-VITRO* CHARACTERIZATION OF SITAGLIPTIN MICROSPHERES

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ABSTRACT

The present work is aimed to develop a sustain release microspheres for sitagliptin, a antihyperglycemic drug by employing polymers such as albumin and chitosan. These polymers have been taken in various proportions and the effect of these polymers on the drug release pattern is studied. Total of nine formulations have been developed and were evaluated for various parameters to determine the nature of the prepared formulations. All the formulations have shown good micromeritic properties and it has revealed that the microspheres has good flow properties by which it can be easily filled into capsules if desired. The other properties of microspheres such as entrapment efficiency, percentage yield, drug content, particle size and swelling index values were optimum. From the in-vitro drug release studies it is found that albumin and chitosan in the ratio of 3:1 and drug: polymer ratio of 1:2 has shown maximum drug release of 83.91% at the end of 12 hours and is thus optimized and the drug release from this optimized formulation was by diffusion of drug molecules from the polymer matrix.

KEYWORDS: Sitagliptin; Microspheres; Antihyperglycemic; Albumin; Chitosan.

INTRODUCTION

In order to achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day.[1] This results in fluctuations in drug level and consequently undesirable toxicity and poor efficiency and other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems.[2-3] The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major advance towards solving the problem associated with the existing drug delivery system.[4-5] The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue.[6]

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery to achieve spatial arrangement and temporal delivery of drug. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems.[7,8]

Sitagliptan is an oral antihyperglycemic agent that works by competitively inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4) that breaks down the incretins GLP-1(glucagon-like peptide-1) and GIP (gastric inhibitory peptide), gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal. It has the bioavailability of 87% and halflife of 8-14 hours.[9] Thus it is formulated into microspheres in order to sustain the release of drug and to make it available for absorption process by maintaining desired amount of drug in the blood circulation thereby improving its bioavailability.

MATERIALS AND METHODS

Materials: Sitagliptan was obtained from Chandra labs, kukatpally, Hyderabad. Albumin, Chitosan, Gelatin, Calcium carbonate, Liquid paraffin, Span 80 and Petroleum ether were supplied by S. D. Fine Chemicals Ltd., Mumbai.

Methods

A) Preparation of Sitagliptan Microspheres

Sitagliptan microspheres were prepared by Heat stabilization technique. Sitagliptan is dispersed in mixture of 5ml of 1%w/v albumin solution, 5ml of 2%w/v chitosan in 2% acetic acid and poured into 5ml of 15% w/v gelatin solution (water) containing 1.5% w/v $CaCO_3$ (calcium carbonate) and then the above solution is transferred with the help of syringe in to 25ml of liquid paraffin containing 0.5% w/v span 80 and stirred gently for 10min at 60-70°c and 1000rpm (w/o emulsion is formed) then it is cooled at 50°c for 30min, washed with petroleum ether and dried at 45°c. The same procedure is repeated for other ratios of drug and polymer.[10]

Table 1: Formulation of Sitagliptan Microspheres

Formulation code	Drug: Polymer ratio	Polymer ratio (albumin: chitosan)
F_1	1:1	1:1
F_2	1:1.5	1:2
F_3	1:2	1:3
F_4	1:1.5	2:1
F_5	1:2	1:1
F_6	1:2.5	2:3
F_7	1:2	3:1
F_8	1:2.5	3:2
F_9	1:3	1:1

B) Characterization of Microspheres

Micromeritic properties[11, 12]

The microspheres were characterized by micromeritic properties such as particle size, bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose.

Bulk density

It is the ratio of powder to bulk volume. The bulk density depends on particle size distribution, shape and cohesiveness of particles. Accurately weighed quantity of microspheres were carefully poured into graduated measuring cylinder through large funnel and volume was measured which is called bulk volume. Bulk density is expressed in gm/ml and is given by,

$$Bulk\ density = \frac{Mass\ of\ microspheres}{bulk\ volume}$$

Tapped density

In this method microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density microspheres.

$$Tapped\ density = \frac{Mass\ of\ microspheres}{tapped\ volume}$$

Compressibility index or Carr's Index

Compressibility index is an important measure that can be obtained from bulk and tapped densities. In theory, the less compressible a material is the more flow able it is.

Percent Compressibility index was determined by using the formula,

$$\text{Carr's Index} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

Hausner's ratio

Hausner's ratio of microspheres was determined by comparing tapped density to bulk density using the equation

$$\text{Hausners ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and angle of repose was calculated using the following equation.

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

Here, θ - Angle of repose; h - Height of granules above the flat surface; r - Radius of the circle formed by the microspheres heap.

Percentage yield

The percentage production yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated from the following formula[13],

$$\%\text{Yield} = \frac{\text{Practical mass(microspheres)}}{\text{Theoretical mass(Polymer + Drug)}}$$

Drug entrapment efficiency

Microspheres equivalent to 100mg of the drug sitagliptin were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using 0.1N HCl. After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 288nm.[14,15] The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\text{Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Particle size analysis

Samples of the microspheres were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 microspheres sizes were calculated under 45 x magnifications. The average particle size was determined by using the Edmondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where, n – Number of microspheres observed; d – Mean size range.

Swelling study

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2. The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$Swelling\ ratio = \frac{W_t - W_0}{W_0} \times 100$$

Where W0 & Wt are initial weight and final weight of microspheres respectively.

***In-vitro* drug release study**

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^{0}C, 100 rpm) using the USP type – I rotating basket method in 0.1N HCL

(900ml). A quantity of accurately weighed microspheres equivalent to 100 mg sitagliptin from each formulation was employed in dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 288nm.[14, 15] At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed 0.1N HCL maintaining sink conditions throughout the experiment.

***In-vitro* drug release kinetics**

To analyze the *in-vitro* drug release mechanism from the developed formulations, various kinetic models were used. The dissolution profile of the formulations were fitted to zero order, first order, Higuchi and Korsmeyer equation / Peppa's model to ascertain the kinetic modeling of the drug release.[16, 17]

$C = K_0 t$

Where, K_0 = Zero-order rate constant and t = Time

$LogC = LogC_0 - Kt / 2.303$

Where, C_0 = initial concentration of drug and K = First order constant.

$Q = K\ t1/2$

Where, K = Constant and t = Time.

$M_t / M_\alpha = Kt^n$

$Log\ M_t / M_\alpha = Log\ K + n\ Log\ t$

Where, $M_t / M\alpha$ = the fraction of drug released at time't', K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system and n = Diffusion exponent related to the mechanism of the release.

Table 2: Mechanism of drug release

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<n<1.0	Anomalous transport or non-Fickian	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

RESULTS AND DISCUSSIONS

Evaluation of micromeritic properties of Sitagliptin Microspheres

Sitagliptin Microspheres were prepared and observed for micro particle analysis. All the formulations were evaluated for bulk density, tapped density, % compressibility, hausner's ratio and angle of repose. The results of % compressibility, hausner's ratio and angle of repose were found to be <16, <1.25 and <30 respectively. These results show that the formulations have excellent flow properties.

Table 3: Micromeritic properties of Sitagliptin Microspheres (F1-F9)

Formulation code	Bulk density (g/cc)	Tapped density (g/cc)	Carr's Index	Hausner's Ratio	Angle of repose(θ)
F1	0.45±0.045	0.52 ± 0.09	15.60±0.2	1.15±0.02	28.06± 0.31
F2	0.45±0.045	0.50 ± 0.07	12.23±0.6	1.11±0.04	27.58± 0.15
F3	0.44±0.044	0.50 ± 0.09	12.58±0.8	1.13±0.08	28.44± 0.11
F4	0.45±0.045	0.52 ± 0.04	15.19±0.1	1.15±0.06	28.36± 0.13
F5	0.44±0.044	0.52± 0.01	15.48±0.6	1.18±0.08	28.52± 0.19
F6	0.45±0.045	0.51 ± 0.04	13.48±0.8	1.13±0.09	29.32± 0.19
F7	0.51±0.045	0.59 ± 0.04	14.48±0.8	1.15±0.09	29.69± 0.19
F8	0.45±0.045	0.52±0.04	15.19±0.1	1.15±0.05	27.36±0.23
F9	0.44±0.04	0.50±0.1	12.58±0.8	1.13±0.09	29.33±0.16

Percentage Yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increased. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug-polymer solution, adhesion of polymer solution to the magnetic bead and lost during the washing process. The percentage yield was found to be in the range of 80 to 88% and is recorded in Table-4.

Drug Entrapment Efficiency

Percentage drug entrapment efficiency of Sitagliptin ranged from 62.66 to 88.66%. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared beads is displayed in Table-4.

Table 4: Evaluation of the prepared Microspheres

Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency	Swelling index (%)
F_1	80	79.40	62.66	30.32
F_2	83.33	78.66	64.4	33.66
F_3	85	78.70	66.66	39.91
F_4	86	79.5	70	42.33
F_5	82.22	71.07	73.2	33.11
F_6	80	72.25	75	35.18
F_7	88	85.29	88.66	43.55
F_8	87	83.5	86.66	48.65
F_9	80	83.01	83.73	42.75

Particle size analysis

The developed formulations were subjected to particle size analysis by using optical microscopy method and the results are shown in the table-5. The average particle size of the microspheres was found to be in the range of 435.6-621.3µm.

Swelling index

Swelling index was found to range from 30% to 45% within two hours time period, which shows that the formulations swell to a certain degree after coming in contact with the simulated gastric medium. The % swelling index of the prepared beads is displayed in Table-5.

Table 5: Evaluation of the prepared Microspheres

Formulation code	Average Particle size (µm)	Swelling index (%)
F_1	512.4	30.32
F_2	531.1	33.66
F_3	621.3	39.91
F_4	598.6	42.33
F_5	492.1	33.11
F_6	509.1	35.18
F_7	435.6	43.55
F_8	483.7	48.65
F_9	498.1	42.75

***In-vitro* drug release studies**

The release of drug from all the developed formulations was studied and the results are mentioned in the tables- 6, 7, 8 and represented in the figure 1. It is shown that increase in the polymer ratio led to increase in sustainability to certain extent and then led to decrease in the

release, which indicates the saturation of the drug at higher concentrations of polymer. Among all the formulations F7 shows more sustainability after 9 hour where as all the other formulations have shown optimum sustainability. Highest drug release was observed at 12hr in formulation F7 ie., 83.9% when compared to other formulations. Therefore F7 was considered as optimized formulation after considering all the parameters.

Table 6: Dissolution Data of Sitagliptin Microspheres (F1-F3)

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F1	F2	F3
0	0	0	0
2	35.42 ±3.2	34.16 ±1.25	34.39±1.2
4	45.55 ±1.21	47.01 ±1.34	45.65±7.34
6	53.61 ±1.65	53.89 ±1.9	51.95 ±8.7
8	62.16 ±3.47	62.50±1.30	61.21 ±2.8
10	68.31 ±2.02	68.91 ±0.93	66.45±1.34
12	76.45±1.25	78.89±1.56	76.59±1.65

Table 7: Dissolution Data of Sitagliptin Microspheres

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F4	F5	F6
0	0	0	0
2	34.21±1.8	32.62±2.2	34.21±0.9
4	45.21±5.52	45.87±0.67	45.21±5.8
6	51.45±5.3	52.03±7.17	53.07±3.06
8	61.52±1.22	61.82±7.65	60.85±1.92
10	66.75±1.48	67.22±4.19	66.59±1.35
12	76.02±1.88	75.29±4.84	78.56±4.3

Table 8: Dissolution Data of Sitagliptin Microspheres

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F7	F8	F9
0	0	0	0
2	36.32±0.18	33.52±2.52	33.25±0.61
4	51.62±0.63	48.23±4.51	47.31±0.62
6	55.31±1.25	53.61±7.02	52.72±2.22
8	64.52±0.15	65.25±7.59	64.32±3.83
10	68.91±13	69.32±7.81	69.85±0.67
12	83.91±3.16	76.23±1.89	74.45±3.21

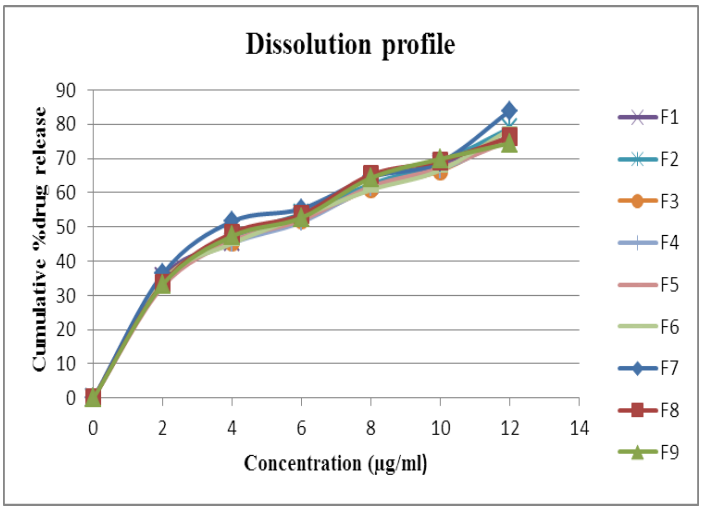

Figure 1: Dissolution profile of Sitagliptin Microspheres

Release Kinetic Studies

In-vitro drug release data of optimized formulation (F7) was subjected to kinetic study by linear regression analysis according to zero order and first order kinetic equations, Higuchi and Korsmeyer-Peppas models and the mechanism of drug release was found to be by Higuchi's model i.e., the drug release is by diffusion. The values are given in the table-9. The F7 formulation follows first order kinetics with R^2 value of 0.9317 and also follows Higuchi model with an R^2 value of 0.9844 and also follows non fickanian model of drug release.

Table 9: Regression coefficient values for kinetic models

	ZERO	FIRST	HIGUCHI	PEPPAS
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	5.889464286	-0.05603176	22.65506214	1.450970956
Intercept	16.17607143	1.963053391	1.935458668	0.545848097
Correlation	0.937478829	-0.96822924	0.992152896	0.845158123
R^2	0.878866555	0.937467862	0.98436737	0.714292252

CONCLUSIONS

In the present study satisfactory attempt has been done to develop sitagliptin microspheres by using the polymers such as albumin and chitosan in various ratios and the results have shown that the developed formulations have satisfactory micromeritic properties and therefore they could be easily filled into capsules and administered as solid dosage form with sustained drug action. The formulation F7 has shown good drug release properties along with various evaluation parameters and is thus optimized. Thus, the formulated microspheres seem to be a potential candidate as an oral controlled drug delivery system in prolonging the drug release and increasing the bioavailability of drug.

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