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FORMULATION, DEVELOPMENT AND EVALUATION OF ABACAVIR SUSTAINED RELEASE MICROSPHERES

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ABSTRACT

The purpose of this study was to develop and evaluate Abacavir loaded mucoadhesive microsphere for sustained drug release at the gastric mucosa. In present investigation Chitosan microspheres of Abacavir were prepared by using ionotropic gelation method. Different formulations F1 to F6 were prepared using varying amount of polymers like chitosan and TPP. The prepared and evaluated for Percentage yield, stability of microspheres, drug entrapment mean particle size, zeta potential and *In vitro* drug release study. Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 63.32±0.45–72.23±0.54%. The drug entrapment of different formulations was in range of 65.58±0.45-75.56±0.25% w/w. The maximum percentage yield and entrapment efficiency was found formulation F3 (75.56±0.25). The optimized formulation among other batches subjected to further studies. The stability of all the batches of microspheres was determined by measuring the transmission after the microspheres had been exposed to 0.1N HCl. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equsation, and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Pappas order was maximum i.e 0.978 hence indicating drug release from formulations was found to follow Pappas order release kinetics. Mucoadhesive microspheres can be effectively used for sustained drug release to the gastric mucosa in treatment of upper GIT infection.

Keywords: Floating microsphere, Abacavir, Ionotropic gelation method

INTRODUCTION

Abacavir is an antiretroviral drug which acts by inhibiting Nucleoside Reverse Transcriptase. It is widely used in the treatment of HIV as single drug or in combination with other NRTI's. The oral bioavailability of Abacavir is 83 %, and it is having very short biological half-life of 1.54 ± 0.63 hours,[1] requires frequent dosing. It causes an increased risk of gastrointestinal adverse events, neurological complaints and unique

hypersensitivity syndrome. Consequently, the conventional dosage form has several drawbacks. To overcome the problems associated with Abacavir and to improve its bioavailability, minimize the dosing frequency and adverse reactions it was decided to select this drug for the formulation of the microspheres[2].

MATERIALS AND METHODS

Materials

Abacavir was obtained as gift sample from Pharmaceutical Company, Chitosan and Sodium tripolyphosphate was procured from Himedia chemical Pvt. Ltd. Other solvents and chemicals used in the research were of LR grade. All the studies were carried in distilled water.

Methods

Preparation of chitosan microsphere of Abacavir

Chitosan microsphere was prepared by ionotropic gelation method[3]. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (5% v/v) at room temperature. The drug (25 mg) was dissolved in chitosan solution. 1% Sodium tripolyphosphate solution was prepared in water. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Microsphere were obtained which was air dried for twenty-four hours followed by oven drying for six hours at 40˚C.

Table 1: Formulations of chitosan microsphere prepared

Sr. No	Formulation Code	Abacavir (mg)	Chitosan (mg)	STPP (mg)
1.	**F1**	50	125	500
2.	**F2**	50	250	1000
3.	**F3**	50	125	500
4.	**F4**	50	250	1000
5.	**F5**	50	125	500
6.	**F6**	50	250	1000

Evaluation of microsphere

Percentage Yield

The prepared microsphere F1-F6 were collected and weighed from each formulation. The percentage yield (%) was calculated using formula given below:

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment Efficiency

Amount of Abacavir in each formulation was calculated according to procedure given below[4].Equivalent to 10mg of chitosan microsphere from each batch were accurately weighed. The powder of chitosan microspheres were dissolved in 10 ml 0.1 N HCl and centrifuge at 1000 rpm. This supernatant solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 0.1 N HCl. The supernant was analyzed for drug content by measuring the absorbance at 282nm.

Stability of chitosan microspheres in 0.1 N HCl

The stability of chitosan microspheres in 0.1 N HCl was determined by incubating 0.5% wt/vol suspension of the microspheres in 0.1N HCl for 12 hrs. and measuring the transmission of the samples at 282 nm (Labindia 3000+ spectrophotometer) as reported by Berthold *et al.*,[5]. Chitosan is soluble in acidic pH, therefore, the purpose of carrying out this study was to determine the effect of different cross-linking methods on the solubility of chitosan, which in turn reflects the stability at acidic pH.

Measurement of mean particle size

The mean particle size of the microsphere was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer **(**Malvern particle size analyser**)** at a scattering angle of 90°. A sample (0.5mg) of the microsphere suspended in 5 ml of distilled water was used for the measurement[6].

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate [7].

Flow property determination of the microspheres

Bulk density: Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. Accurately weighed amount of granules taken in a 50 ml capacity measuring cylinder was tapped for 100 times on a plane hard wooden surface and estimated the LBD and TBD, calculated by using following formulas.

$$\textbf{LBD (Loose bulk density)} = \frac{\text{Mass of powder}}{\text{Volume of Packing}}$$

$$\textbf{TBD (Tapped bulk density)} = \frac{\text{Mass of powder}}{\text{Tapped Volume of Packing}}$$

Compressibility index: Percent compressibility of powder mix was determined by Carr's compressibility index, calculated by using following formula: -

$$\textbf{Carr's Index} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \text{X}100$$

Hausners ratio: It is determined by comparing tapped density to the bulk density by using following equation: -

$$\textbf{Housner's ratio} = \frac{\text{Tapped bulk density}}{\text{Loose Bulk density}}$$

***In-vitro* release studies**

***In vitro* drug release in gastrointestinal fluids**

The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows: A weighed quantity of formulation (equivalent to 10mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media 0.1 N HCl (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 282 nm for percent of release Abacavir microsphere using UV visible spectrophotometer. The release of Abacavir microsphere was calculated with the help of Standard curve of Abacavir[8].

Drug release kinetic data analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

To study the release kinetics of Abacavir from the mucoadhasive microspheres the release data was fitted to these three equations

Zero order equation: When a graph of the cumulative percentage of the drug released from the matrix against time is plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration.

$Q_t = k_0.t$ …… (1)

Where $Q_{t\ is}$ the percentage of drug released at time t and k_0 is the release rate constant;

First order equation: -

In $(100-Q_t)$ = In 100- $k_I.t$ …….. (2)

Where k_I is the release rate constant;

7.2.8.2.3 Higuchi's equation:-

$Q_t = k_H.t^{1/2}$ …….. (3)

Where K_H is the Higuchi release rate constant[8]

Korsemeyer-Peppas:-

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation[9-10]

$$Q_t/Q_\infty = k_{KP}.t^n$$

Where Q_t/ Q_∞ is the fraction of drug released at time t, k_{KP}a constant compromising the structural and geometric characteristics of the device and n is the release exponent.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when $0.5 < n < 1$, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when $n > 1.0$ super case II transport is apparent. 'n' is the slope value of log M_t/M_∞ versus log time curve.

Stability studies for optimized formulation

Stability study data was revealed that the optimized microsphere formulation (F3) stable after 3 month of storage at 4ºC while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % EE, average particle size and physical appearance.

RESULTS AND DISCUSSIONS

In present investigation Chitosan microspheres of Abacavir were prepared by using ionotropic gelation method. Different formulations F1 to F6 were prepared using varying amount of polymers like chitosan and TPP. The prepared and evaluated for Percentage yield, stability of microspheres, drug entrapment mean particle size, zeta potential and *In vitro* drug release study. Percentage yield of different formulation

was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 63.32±0.45–72.23±0.54% table 2.

The drug entrapment of different formulations was in range of 65.58±0.45-75.56±0.25% w/w table 2. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Abacavir microspheres. The maximum percentage yield and entrapment efficiency was found formulation F3 (75.56±0.25). The optimized formulation among other batches subjected to further studies. The stability of all the batches of microspheres was determined by measuring the transmission after the microspheres had been exposed to 0.1N HCl. In the present investigation, the acid instability of the chitosan microspheres led to the dissolution of the microspheres, and the sample became more transparent. Since the decrease in turbidity is directly dependent on the disintegration of the microspheres, transmission is a measure of the concentration of non disintegrated microspheres. A low transmission indicates high stability, and a high transmission implies that the microspheres dissolved in HCl table 3. The results of measurement of mean particle size of optimized formulation F3 microspheres were found 220.36nm figure 1. The average particle size of microspheres increased with increasing polymer concentration, since at higher concentrations the polymer solution dispersed into larger droplets due to increasing the viscosity of polymer solution and it was the reason behind the enhancement of average particle size of microsphere. Mean particle size and size distribution were studied to observe the effect of drug concentration.

All the samples were measured in water at 25°C in triplicate. Results of zeta potential of optimized formulation F3 microspheres were found to be -34.5 mV figure 2. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equsation, and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Pappas order was maximum i.e 0.978 hence indicating drug release from formulations was found to follow Pappas order release kinetics table 4-5 .

The average particle size of microspheres was found 220.12, 225.65 and 232.14 nm after 1, 2 and 3 month of storage at 4.0 ±0.2°C while at 25-28±2°C the average particle size was found 285.65, 312.25 and 374.45nm after 1, 2 and 3 month of storage at 4.0 ±0.2°C. % EE in microspheres formulation was 65.58, 55.56 and 49.98 after 1, 2 and 3 month of storage at 25-28±2°C while there were no significant changes in % EE and physical appearance in microspheres formulation was observed after 3 month of storage at 4ºC table 6.

Table 2 Percentage yield for different formulation

S. No.	Formulation	Percentage Yield* (Mean ± S.D)	% Entrapment Efficiency* (Mean ± S.D)
1	F1	63.32±0.45	65.58±0.45
2	F2	65.58±0.63	69.98±0.32
3	F3	72.23±0.54	75.56±0.25
4	F4	69.98±0.32	68.87±0.14
5	F5	65.56±0.25	63.32±0.65
6	F6	68.78±0.45	65.54±0.74

***Average of three determinations (n=3)**

Table 3 Stability of prepared microspheres formulation in 0.1N HCl

S. No.	Formulation code	% Transmittance		
		2 hrs	8 hrs	12 hrs
1	F1	74.45	58.89	22.25
2	F2	78.45	65.58	33.32
3	F3	85.56	45.56	12.98
4	F4	75.56	69.98	24.45
5	F5	69.98	55.58	23.56
6	F6	72.23	48.89	26.65

Table 4 Result of flow properties of different microspheres formulation

Formulation code	Parameters			
	Loose Bulk density(gm/ml)	Tapped bulk density(gm/ml)	Carr's Index (%)	Hausner's Ratio
F1	0.895	1.125	20.44	1.2569832
F2	0.854	1.325	35.55	1.5515222
F3	0.789	1.108	28.79	1.4043093
F4	0.965	1.112	13.22	1.1523316
F5	0.857	1.121	23.55	1.3080513
F6	0.749	1.115	32.83	1.4886515

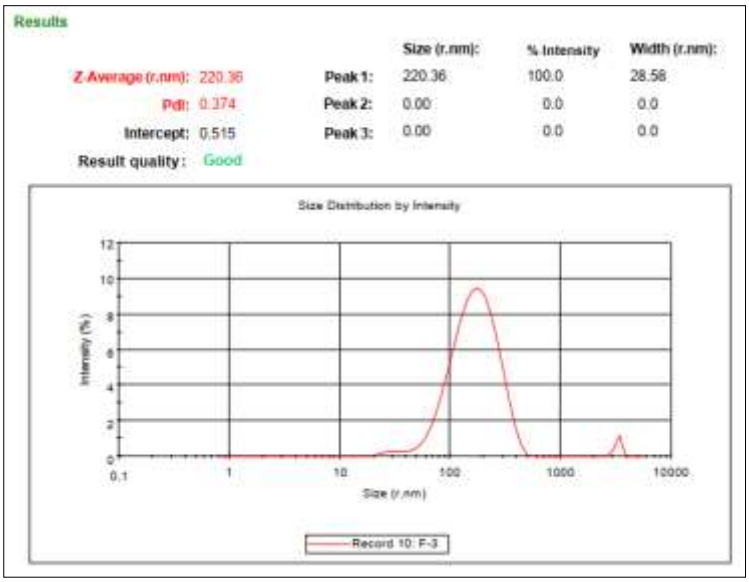

Figure 1 Particle size data of optimized microsphere formulation F3

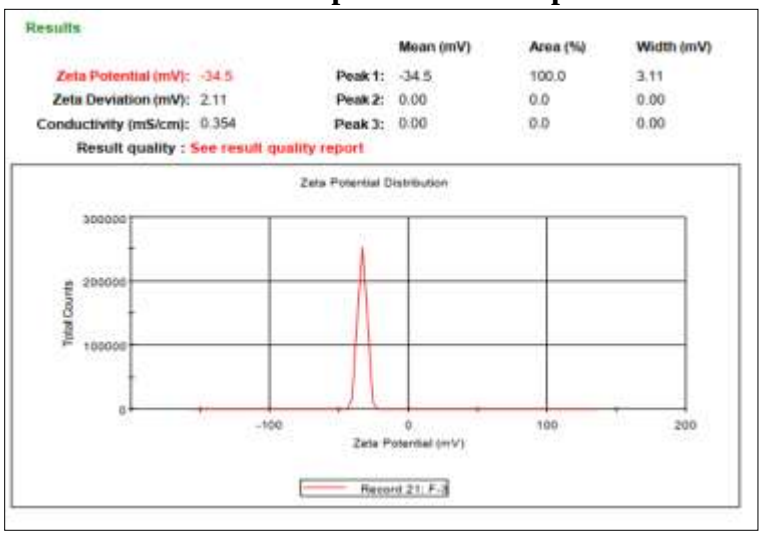

Figure 2 Zeta potential data of floating microsphere F3

Table 5 Cumulative % drug release of Abacavirfrom microspheres

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release
1		1	11.25
2		2	23.32
3		3	32.25
4		4	45.56
5		5	52.23
6	SGF (pH 1.2)	6	59.98
7		7	63.32
8		8	69.74
9		9	73.32
10		10	85.56
11		12	96.65

Table 6 Regression analysis data of microspheres Formulation

Formulation	Zero order	First order	Pappas plot
F3	R^2 = 0.978	R^2 = 0.843	R^2 = 0.988

Table 7 Characterization of stability study of optimized formulation of microspheres F3

Characteristic	Time (Month) 1 Month		2 Month		3 Month	
Temperature	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Average particle size (nm)	220.12	285.65	225.65	312.25	232.14	374.45
% EE	75.25	65.58	73.32	55.56	72.23	49.98
Physical Appearance	Normal	Normal	Normal	Normal	Normal	Normal

CONCLUSION

In this study, Abacavir loaded mucoadhesive microsphere was developed by ionic gelation method for drug delivery to the mucous layer in upper GIT. Microspheres were successfully developed with the synthetic polymers. Formulation F3 provided good mucoadhesion, and steady drug release implying its potential for the treatment of upper GIT infections

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