



FORMULATION, DEVELOPMENT AND EVALUATION OF MUCOADHESIVE MICROSPHERE OF STAVUDINE

Raj Karan Pal*¹, Girijesh Kumar Pandey¹, Amit Joshi¹, B.K. Dubey¹, Prabhat Jain²

¹Technocrats Institute of Technology-Pharmacy Education and Research, Bhopal (M.P.)

²Scan Research Laboratories Bhopal (M.P.)

***Correspondence Info:**

Raj Karan Pal,
Department of Pharmaceutics,
Technocrats Institute of
Technology-Pharmacy
Education and Research,
Bhopal, M.P

Email:

rajkaranpal482@gmail.com

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ABSTRACT

Stavudine (D4T, thymidine) is an FDA-approved drug for clinical use in the treatment of HIV infection, AIDS and AIDS-related conditions either alone or in combination with other antiviral agents. The stavudine has a very short half-life (0.8 to 1.5hr) with rapid absorption. The side effects of stavudine are dose dependent and a reduction of the total administered dose reduces the severity of the toxicity. Stavudine is typically administered orally as a capsule and oral solution. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirements. The aim of the present study was to develop stavudine loaded chitosan microspheres by the ionic gelation method using sodium tripolyphosphate (Na-TPP) as the crosslinking agent. The use of ionotropic gelation avoids the possibility of the occurrence of the toxic and undesirable effects associated with the use of glutaraldehyde, a chemical crosslinking agent. The prepared microspheres were evaluated for mean particle size and particle size distribution, drug loading, encapsulation efficiency and in-vitro drug release. FT-IR spectroscopic analysis was performed to ascertain drug polymer interaction. The release profiles showed zero-order release behavior up to 12 hours where the highest drug release was 98.78 % of the stavudine loaded in the chitosan microspheres, indicating a strong crosslinking between chitosan and TPP anions. The surface morphology of the prepared microspheres was studied by SEM. With an increase in the crosslinking density the rate of drug release decreased. From the results of the present investigation it may be concluded that drug loaded chitosan microspheres can be prepared by a simple technique which avoids the use of complex apparatus and special precautions.

Key words: Stavudine, HIV infection, Microspheres, Chitosan, Sodium tripolyphosphate.

Introduction:

Oral route is most suitable and preferable route for drug administration to reach systemic circulation due to its low cost and easy administration. But success of conventional dosage form is limited due to its residence time. Hence mucoadhesive microsphere drug delivery systems are used

to prolong the residence time at the site of application, maintain therapeutically effective plasma drug concentration levels for a longer duration, reducing the dosing frequency and minimize fluctuations in the plasma drug concentration at the steady state in controlled and reproducible manner.

Mucoadhesive microspheres become adhesive on hydration and hence used for localizing the drugs to a particular target site of gastrointestinal tract (GIT) for prolonged period of time. Moreover, it is easy for administration, no patient compliances and flexibility in the formulation. One of the most feasible approaches for achieving a prolonged and predictable drug delivery in gastrointestinal tract (GIT) is to control gastro retentive drug delivery system which will provide important therapeutic options. Mucoadhesive microspheres delivery system is an attractive due to ability of adherence to the mucosal surface and releases the entrapped drug in a sustained release. Bio adhesion phenomenon is associated with biological surface and mucoadhesion associated with i.e. mucin layer of a mucosal tissue. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of the drugs, maximum utilization of drugs and much more intimate contact with intestinal cells, better patient compliance and targeting to specific absorption site (Anil et al., 2011; Bindu et al., 2010; Parmar et al., 2010; Velmurugana and Ali, 2013). Stavudine (2',3'-didehydro-3'-deoxythymidine) is a nucleotide reverse transcriptase inhibitors and primarily used in the treatment of one of the most common chronic disease of the planet, AIDS. It has short biological half-life 0.8–1.5 h and low daily dose of 30 mg is required (Tripathi, 2007; Martin, 1998). The

frequency of dosing is more. To overcome inherent drawbacks associated with conventional dosage forms of stavudine, an attempt is being made to develop an alternative drug delivery system in the form of mucoadhesive microspheres.

MATERIALS AND METHODS

Materials

Stavudine was obtained as free gift sample from Hetero labs limited, Baddi. Excipients used were Chitosan from Qualigens, Mumbai. Glacial acetic acids were purchased from Merck Specialities pvt. Ltd., Mumbai, Sodium tripolyphosphate was purchased from Loba Chem. Pvt. Ltd (Mumbai, India). All other chemicals and reagent used were of analytical grade. Ultrapure water was used throughout the study.

Preparation of Mucoadhesive Microspheres

Chitosan solution of was prepared in 1% acetic acid using homogenizer (Remi motors, Mumbai) at 5000rpm for about 30 minutes, then drug was added to chitosan solution. Microspheres were formed by dropping the bubble-free dispersion of chitosan –drug solution through a disposable syringe (10 ml) onto a gently stirrer (100 rpm) at room temperature in 1% TPP (Sodium tripolyphosphate) solution. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water and then they were vacuum dried (Kalyankar and Nalanda, 2010). The composition of formulations given in table 1.

Table 1 Formulations of the mucoadhesive microspheres

Sr. No	Formulation Code	Stavudine (mg)	Chitosan (mg)
1.	F1	10	25
2.	F2	10	50
3.	F3	10	75
4	F4	10	100
5	F5	10	125
6	F6	10	150

Analytical method development

Determination of absorption maxima

A solution of containing the concentration 10 µg/ ml was prepared in 0.1N HCl. UV spectrum was taken using Double beam UV/VIS spectrophotometer (Labindia-3000+). The solution was scanned in the range of 200-400nm.

Preparation calibration curve

Accurately weighed 10 mg of drug was dissolved in 10 ml of 0.1N HCl solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 0.1N HCl solution. Prepare suitable dilution to make it to a concentration range of 10-50 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). Linearity of standard curve was assessed from the square of correlation coefficient (r²) which determined by least-square linear regression analysis.

Fourier Transform Infrared (FTIR) spectroscopy

The physical properties of the physical assortment were comparing with those of stavudine pure drug. Samples was assorted comprehensively through 100mg potassium bromide IR powder as well as compacted under vacuum at a pressure of concerning 12 psi for 3 minutes. The ensuing disc was mounted in an appropriate holder in Brukers Alpha IR spectrophotometer and the IR spectrum was recorded from 3500 cm to 500 cm. The resultant spectrum was compared for any spectrum changes.

Evaluation of microspheres

Percentage yield

The prepared microspheres with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for

the preparation of the microspheres (Venkatesh et al., 2012).

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

Drug entrapment

The various formulations of the mucoadhesive microspheres were subjected for drug content. 10 mg of mucoadhesive microspheres from all batches were accurately weighed and crushed. The powder of 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 percentage drug entrapment was calculated using calibration curve method.

Measurement of mean particle size

The mean size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement.

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Horiba Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate (Nagahara et al., 1998).

Shape and Surface characterization of microspheres by scanning electron microscopy (SEM)

From the formulated batches of microspheres, formulations (F5) which showed a suitable balance among the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was practical in a high

vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on dissimilar magnification and higher magnification (200X) was used for surface morphology.

In-vitro release studies

The drug release rate from mucoadhesive microspheres was passed out using the USP type II (Electro Lab.) dissolution paddle instrument. A weighed amount of mucoadhesive microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCl (pH=1.2) maintained at $37 \pm 0.5^{\circ}\text{C}$ and stirred at 55rpm. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples analyzed spectrophotometrically at 255 nm to determine the concentration of drug present in the dissolution medium (Brahmankar and Jaiswal, 1988).

Stability studies for optimized formulation

Accelerated Testing, are the studies designed to amplify the rate of chemical degradation or physical change of a drug substance or drug product by exaggerated storage conditions as part of the formal stability studies. The optimized formulation F5 was taken and

accelerated stability study was performed by taking suitable quantity of microspheres. The microspheres were placed in air-tight glass container at $40 \pm 2^{\circ}\text{C}/75 \pm 5\%$ RH. At suitable sampling interval the samples were withdrawn and evaluated for various parameters.

Results and Discussion

Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 65.56–85.45%. The drug entrapment efficacies of different formulations were in range of 62.32- 75.65% w/w. 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 sofosbuvir microspheres Table 2. On the basis of the maximum percentage yield and drug entrapment was found to be formulation F5 in mucoadhesive microspheres so formulation F5 was further studies. The results of measurement of mean particle size of optimized formulation F5 of mucoadhesive microsphere was found 178.5 nm Fig 1.

Table 2 Percentage Yield for Different Formulation

Formulation	Percentage Yield	Drug entrapment (% w/w) of prepared microsphere
F ₁	68.98±0.25	68.89±0.25
F ₂	65.56±0.12	65.45±0.56
F ₃	73.25±0.36	62.32±0.47
F ₄	76.54±0.14	67.89±0.58
F ₅	85.45±0.25	75.65±0.65
F ₆	72.15±0.14	69.98±0.84

Shape and surface characteristic of sofosbuvir microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation

examines at two different magnifications 55X which illustrate the smooth surface of microspheres Fig 3.

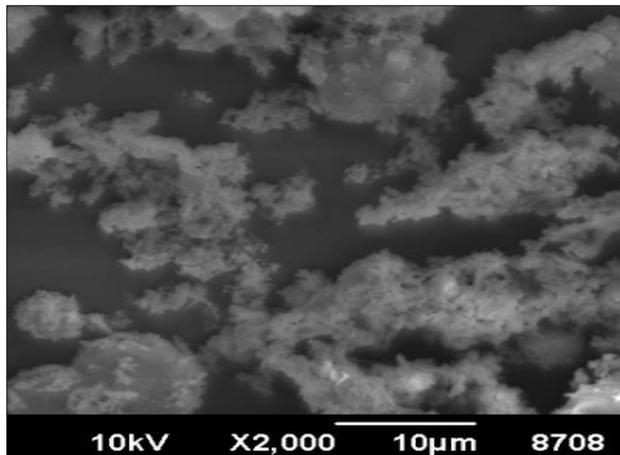


Fig. 3 SEM image of optimized mucoadhesive formulation F5

type II (Electro Lab.) dissolution paddle instrument. A weighed amount of mucoadhesive microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCl (pH=1.2) maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 55rpm. From the graphs (Fig 4) and data (Table 3) of in vitro drug release study, it was observed that formulation F 1 has low drug release and maximum amount of drug release found in formulation F5. The kinetics of drug release from the microspheres was studied by mathematical modeling the drug release to zero order, first order kinetics Table 4 and Fig.5 & 6.

The drug release rate from mucoadhesive microspheres was passed out using the USP

Table 3 Release study data of formulation F1-F6

Time (hr)	% of Drug Release					
	F1	F2	F3	F4	F5	F6
0.5	45.65	42.56	39.98	30.25	22.56	14.56
1	55.65	53.65	50.45	45.56	35.56	22.45
2	89.98	85.45	78.98	65.45	42.12	33.41
4	98.48	93.12	90.12	79.98	55.65	45.65
6	-	98.78	95.65	95.45	65.58	56.65
8	-	-	98.78	99.98	77.89	68.98
10	-	-	-	-	89.98	70.23
12	-	-	-	-	98.78	76.65

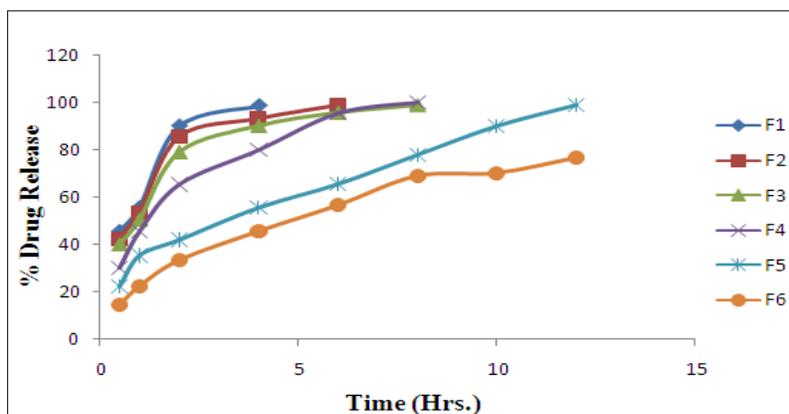


Fig.4 Graph of release study of formulation F1-F6

Table 4 Release kinetics of optimized formulation of microsphere F5

Time (h)	Square Root of Time(h) ^{1/2}	Log Time	Cumulative% Drug Release	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	22.56	1.353	77.44	1.889
1	1	0	35.56	1.551	64.44	1.809
2	1.414	0.301	42.12	1.624	57.88	1.763
4	2	0.602	55.65	1.745	44.35	1.647
6	2.449	0.778	65.58	1.817	34.42	1.537
8	2.828	0.903	77.89	1.891	22.11	1.345
10	3.162	1	89.98	1.954	10.02	1.001
12	3.464	1.079	98.78	1.995	1.22	0.086

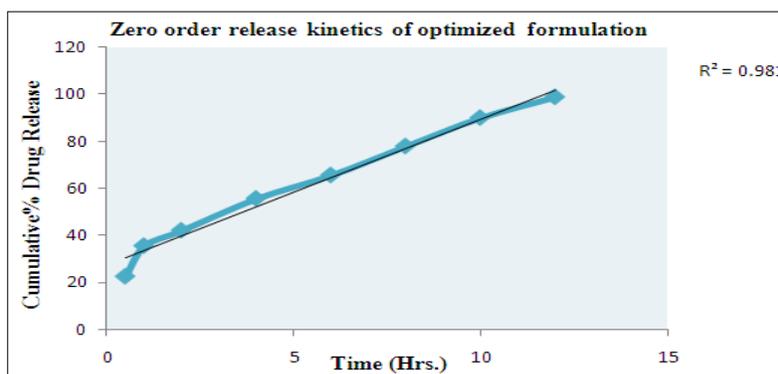


Fig 5 Zero order release kinetics graph of optimized formulations

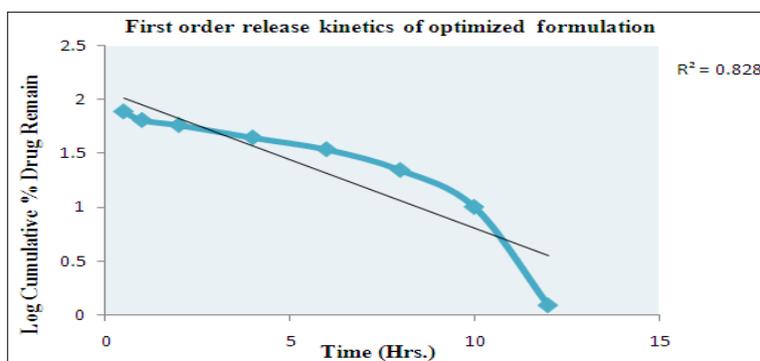


Fig. 6 First order release kinetics graph of optimized formulations

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, first order kinetic equation, in order to determine the mechanism of drug release. When the

regression coefficient values were compared, it was observed that ‘r’ values of microsphere was maximum zero order i.e 0.981 hence indicating drug release from formulations was follow zero order Table 5.

Table 5 Comparative study of regression coefficient for selection of optimized Formulation F5

Release Kinetics		Zero order	First order
R ²	Mucoadhesive Microsphere	0.981	0.828

According to ICH guidelines, 3 months accelerated stability study at 40±2°C and 75±5% RH optimized formulations (F5) was carried out. It showed negligible change over time for parameters like appearance, drug content, dissolution and assay etc., No significant difference observed in the drug content between initial and formulations stored at 40±2°C & 75±5% RH for 3 months.

Conclusion

There are a myriad techniques available for entrapment and controlled release of important drugs, but only a few pass all the requirements laid down for safe, effective and targeted drug delivery to humans. Chitosan is a very attractive alternative for use in human system because it has long been used as food additive. Controlled drug delivery systems aim to ensure sustained release of drugs in their therapeutic range and chitosan based microspheres are being increasingly used. In case of chitosan/TPP based controlled drug release preparations, chitosan and TPP concentration, pH of TPP and drug concentration are very important parameters for formation of non-fragile, spherical microspheres with good drug encapsulation and tunable in vitro drug release behavior. The stavudine loaded chitosan microspheres were prepared by ionic gelation using Na-TPP as the crosslinking agent. The in vitro dissolution study results indicated formulation F5 found to be the best formulation with controlled release of the drug over a period of 12 hours. The drug release from the microspheres followed zero order kinetics. The results of all studies have been shown to be satisfactory. Thus, Chitosan -TPP based release formulations are an attractive alternative to traditional drug delivery systems.

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