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### FORMULATION AND EVALUATION OF MICROSPHERES ENCAPSULATING ZIDOVUDINE BY IONIC GELATION TECHNIQUES

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#### ABSTRACT

The aim of the present study is an attempt to formulate and evaluate microspheres drug delivery of Zidovudine by using Chitosan as a polymer for potentially treating HIV and AIDS related conditions. The formulation F1 to F4 which were prepared by ionic gelation method by varying the concentration of Chitosan polymer ratios which were significantly affected the *in vitro* drug release from the prepared formulations. The *in vitro* drug release studies were carried out by using phosphate buffer pH 7.4. Drug and physical mixture were characterized by FTIR. The formed microspheres showed prolonged *in vitro* drug release. It might contribute better patient compliance while reduce frequency of dosing and by acceptable sustained-release dosage form Zidovudine microspheres promote a fast and effective against the AIDS related conditions.

#### KEYWORDS

Zidovudine, Microspheres, HIV and AIDS related conditions, Controlled drug delivery, Sustained release and Chitosan.

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#### INTRODUCTION

Novel drug delivery systems present an opportunity for formulation scientists to overcome many challenges associate with anti retroviral (ARV) drug therapy. Most of these drugs bear some significant draw backs such as relatively short half life, low bioavailability, poor permeability and undesirable side effects. So the efforts have been made to design drug delivery systems for anti retroviral therapy as reducing dosing frequency, increase

bioavailability, decrease degradation/metabolism in GIT, improve CNS penetration and inhibit CNS efflux, deliver them to target cells and selectively minimal side effects<sup>1</sup>.

Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000  $\mu\text{m}$ , containing dispersed drug in either solution (or) microcrystalline form. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microspheres are characteristically free-flowing powders consisting of proteins/synthetic polymers that are biodegradable in nature. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release<sup>2</sup>.

Anti-retroviral drugs are active against human immunodeficiency virus (HIV) which is a retrovirus. They are useful in prolonging and improving the quality of life and postponing complications of acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC), but do not cure the infection. The first antiretroviral (ARV) drug Zidovudine was developed in 1987. Over the past 20 years >20 drugs belonging to 3 classes have been introduced and a large number of others are under development<sup>3</sup>.

In the present era, Zidovudine an antiretroviral drug belonging to non- nucleosides reverse transcriptase inhibitor has gained immense popularity in the treatment of HIV AIDS and AIDS related conditions. However, Zidovudine has a half life of only 0.5 to 3hrs, thus necessitating frequent administration, low oral bioavailability and administration of Zidovudine exhibits many dose dependant side effects. Hence a properly designed and optimized dosage form is needed which will not only provide control release of Zidovudine but also will minimize the risk of side effects thus making Zidovudine treatment more patient friendly.

Chitosan, a natural compound obtained by alkaline deacetylation of chitin, is a unique cationic polymer, Being non toxic, biodegradable and biocompatible; chitosan has been widely used in the formulation of

particulate drug delivery systems to achieve controlled drug delivery. With this background, combination of chitosan and zidovudine were selected as core material for formulation of microspheres to achieve controlled drug release<sup>4</sup>.

## MATERIAL AND METHODS

### MATERIAL

Zidovudine (Strides Arcolabs Ltd, Bangalore), Chitosan (Central marine fisheries research institute, Chochin, India), Methanol (SD fine chemical ltd, Mumbai, India), acetone (SD fine chemical ltd, Mumbai, India), span 80 (SD fine chemical ltd, Mumbai, India), liquid paraffin (SD fine chemical ltd, Mumbai, India).

### METHOD

#### Preparation of microspheres by Ionic gelation technique<sup>5</sup>

Chitosan microspheres were prepared by ionic cross linkage of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid 0.25% V/V at various concentration, 1.0, 2.0, 3.0, 4.0 mg/ml under magnetic stirring at room temperature, 25ml of 0.84% w/v TPP aqueous solution was added drop wise to 100 ml of chitosan solution containing 100 mg of Zidovudine. The stirring was continued for about 20 min and pH was adjusted by 0.1 N NaOH. Microspheres formed immediately and were left into the original solution for 1 hr to ensure internal gellification. Then they were filtered, washed with alcohol and dried at room temperature. The composition and the formulation design of this microsphere is given in Table No.1.

#### Evaluation of microspheres

##### Particle size analysis<sup>6</sup>

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece. Approximately 300 microspheres were measured.

##### Drug entrapment efficiency<sup>7</sup>

Microspheres equivalent to 10 mg of pure drug were crushed and then dissolved in distilled water with the help of ultrasonic stirrer for 3 hrs, and filtered and analyzed spectrophotometrically at 267 nm

using UV spectrophotometer. Entrapment efficiency was calculated as follows.

**Entrapment efficiency = actual drug content / theoretical drug content × 100**

#### **Drug content evaluation<sup>8</sup>**

Drug content in the microspheres was estimated by an UV spectrophotometrically based on the measurement of absorbance at 267 nm in phosphate buffer of pH 7.4. Estimated percent drug content was determined from the analysis of 50mg microspheres and the theoretical percent drug content was calculated.

#### **Scanning electron microscope (SEM) study**

Surface morphology of the microsphere will be determined by using a scanning electron microscope.

#### **Procedure**

The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the samples putter coating unit (Model E5100Polaron U.K) in Argon at ambient of 8-10°C with plasma voltage about 20mA. The sputtering was done for nearly 5mins to obtain uniform coating on the sample to enable good quality SEM images.

#### **Evaluation of micromeritic properties of microspheres<sup>9</sup>**

The microspheres were characterized for their micromeritic properties - angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio.

#### **Angle of repose**

Angle of repose is determined by employing fixed funnel method. The angle of repose was calculated by using the following formula.

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

Where h = height of the pile, r = radius of the base of the pile.

#### **Bulk density**

Accurately weighed amount of the beads and transferred into 50 ml measuring cylinder. It was subjected to tapping for 3times and the volume

occupied by the beads was noted. Bulk density was estimated by using the following formula.

Bulk density = Weight of the beads / Bulk volume of the beads

#### **Tapped density**

Accurately weighed amount of the beads and transferred into 50 ml measuring cylinder. It was subjected to tapping for 50times and the volume occupied by the beads was noted.

Tapped density = Weight of the beads / Tapped volume of the beads

#### **Hausner's ratio**

It can be calculated by using the formula:

Hausner's ratio = Tapped density / Bulk density

#### **Carr's index**

It can be calculated by using the following formula

Carr's index (%) = Tapped density – Bulk density / Tapped density X 100

#### **True density**

It was done by using Liquid displacement method by using Specific gravity bottle. This method is possible if the microsphere were non-porous. For this solvent is selected in such way a loaded beads were insoluble in it.

**True density = weight of sample/ weight of liquid displaced by solids**

#### **In vitro drug release study<sup>10</sup>**

The drug release study was performed using USP dissolution test apparatus paddle type at  $37 \pm 0.5$  °C and at 100 rpm using 500 ml of phosphate buffer pH 7.4, as dissolution medium for 12 hrs. Microspheres equivalent to 10 mg of drug were used for the test. Five ml of sample solution was withdrawn at different time intervals and equal volume of medium was added to maintain the sink condition. Withdrawn sample were analyzed at 267 nm by using UV spectrophotometer. The data obtained were fitted in to various kinetic modelsto investigate the mechanism of drug release from microspheres.

## **RESULTS AND DISCUSSION**

In the present study an attempt was made to formulate zidovudine as microparticulate drug delivery system in order to localize drug at the absorption site, enhance its bioavailability, reduce

dose, thereby improving patient compliance. Microparticulate system of zidovudine was formulated using Chitosan.

#### **Incompatibility studies**

Drug-excipients compatibility studies were carried out using FT-IR. The spectra of pure drug (Zidovudine), Chitosan and their physical mixture (1:1:1) was obtained from FT-IR spectroscopy studies at wavelength from 4000 to 5000  $\text{cm}^{-1}$  and the characteristic peaks obtained are shown in Figures No.1, 2 and 3.

#### **Entrapment efficiency and drug content**

The entrapment efficiency of F1 to F4 which was prepared by ionic gelation method is ranged from 67 % to 84 %. An increase in the concentration of Chitosan in a fixed volume of solvent resulting increase entrapment as shown in Table No.2. Higher values of drug content was observed for all the formulation prepared by ionic gelation method, ranged from 75.32 % to 87.60 % for F1 to F4 as shown in Table No.2.

#### **Particle size analysis**

The mean particle size of formulation F1 to F4 prepared by ionic gelation method was found to be in the range of 67.5 to 95  $\mu\text{m}$  shown in. The result showed in Figure No.4 that as the polymer concentration increases, the particle size also increases.

#### **Surface morphology**

The SEM photomicrographs indicated that the microspheres were spherical in shape having particle size of 20 $\mu\text{m}$  for F4 and Surface of the microsphere appear to be rough, may be due to the presence of drug. Figure No.5. Reveals that the mean microspheres size as observed by optical microscope is significantly higher than that observed under scanning electron microscope. It might be explained by the fact that the incompletely dried microspheres (remaining at swollen state) were observed under optical microscope, whereas the microsphere particles were fully dried when SEM study was performed.

#### **Zeta potential**

The zeta potential was measured for the optimized F4 formulation of Zidovudine microspheres

prepared by ionic gelation method, which was found to be -35.19Mv, which indicates that the microspheres prepared by ionic gelation method was more stable.

#### **Micromeritic properties**

All the formulations prepared by ionic gelation methods showed angle of repose in the range of 25 to 32, Carr's index, was between 12 to 15 and Hausner's ratio < 1.2, all the parameters indicating good flow property. Of particular note are formulations F1 to F4 prepared by ionic gelation method which showed the best flow properties as shown in Table No.3.

#### **In vitro drug release study**

*In vitro* release study of Zidovudine from various formulations was conducted for 12 hrs by using USP paddle type dissolution test apparatus. Cumulative % drug release was plotted against time. All the formulation showed more than 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of microspheres which released faster showing slight dose dumping. It has been found that from the microspheres of formulation F1-F4 prepared by ionic gelation method shows F1-75.47 %, F2- 68.70 %, F3-67.46 % and F4-65.76 % were shown in Figure No.6. The increase in Chitosan ratio from F1 to F4 causes decrease in the drug release.

#### **Release kinetics**

To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using BCP dissolution software. The models selected were Zero order, Higuchi Matrix, Korsmeyer Peppas. The regression coefficient values for all these models are shown in Table No.3. In all the cases the best fit model was found to be Higuchi with 'n' value below 0.5 suggesting the fickian release mechanism for the drug i.e., diffusion controlled. The results of model fitting were shown in Table No.4.

The study of drug release kinetics showed that majority of the formulations governed by Higuchi model. The curve was obtained after plotting the cumulative amount of drug released from each formulation against time. Formulation F4 (65.76 %)

showed proper controlled release while other formulation showed more amount of drug release in 24hrs. Formulation F4 has correlation coefficient (r =0.9939) value and follows drug release by Higuchi model.

#### Stability studies

The intermediate stability study for F4 was performed for 6 months according to the ICH guide

lines. Drug entrapment, particle size and drug release were fixed as physical parameters for stability testing and stability studies of selected formulation F4 showed that negligible changes in particle size, entrapment efficiency and drug release. This revealed that the formulation stable on storage at 30±2°C and 65±5% RH.

**Table No.1: Composition of microspheres formulations prepared ionic gelation technique**

S.No	Formulation Code	Drug (mg)	Chitosan (mg)	Acetic acid %v/v	TPP %w/v (ml)
1	F1	100	100	0.25	25
2	F2	100	200	0.25	25
3	F3	100	300	0.25	25
4	F4	100	400	0.25	25

**Table No.2: Entrapment efficiency and drug content**

S.No	Formulation code	% Entrapment efficiency	% Drug Content
1	F1	67	75.32
2	F2	70.5	78.45
3	F3	75.0	80.78
4	F4	84.0	87.60

**Table No.3: Some micromeritic properties of microspheres**

S.No	Formulation code	Angle of repose	Tapped density (g/cm <sup>3</sup> )	Bulk density (g/cm <sup>3</sup> )	Carr's index (%)	Hausner's ratio
1	F1	25°43 <sup>1</sup>	0.608	0.625	13.19	1.12
2	F2	27°34 <sup>1</sup>	0.659	0.568	12.22	1.14
3	F3	29°18 <sup>1</sup>	0.663	0.586	13.81	1.13
4	F4	32°12 <sup>1</sup>	0.720	0.645	15.46	1.18

**Table No.4: In vitro release kinetics**

S.No	Formulation code	Zero order	First order	Higuchi/matrix	Peppas plot	
					r <sup>2</sup> values	'n' values
1	F1	0.8514	0.9433	0.9858	0.9856	0.4857
2	F2	0.8499	0.9502	0.9852	0.9852	0.4847
3	F3	0.8676	0.9666	0.9839	0.9986	0.4945
4	F4	0.8711	0.9581	0.9911	0.9868	0.4853

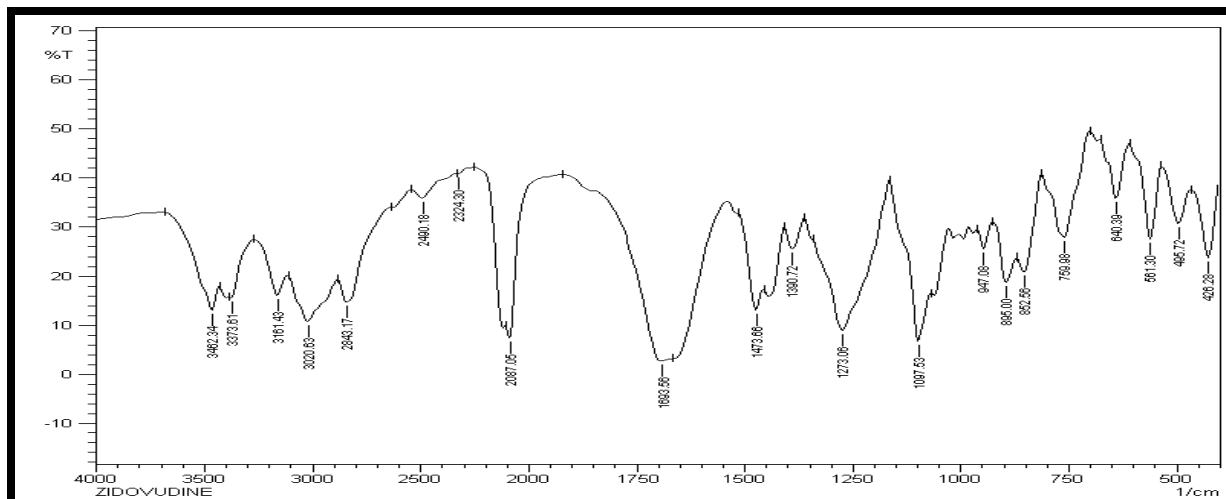


Figure No.1: FTIR of pure Zidovudine

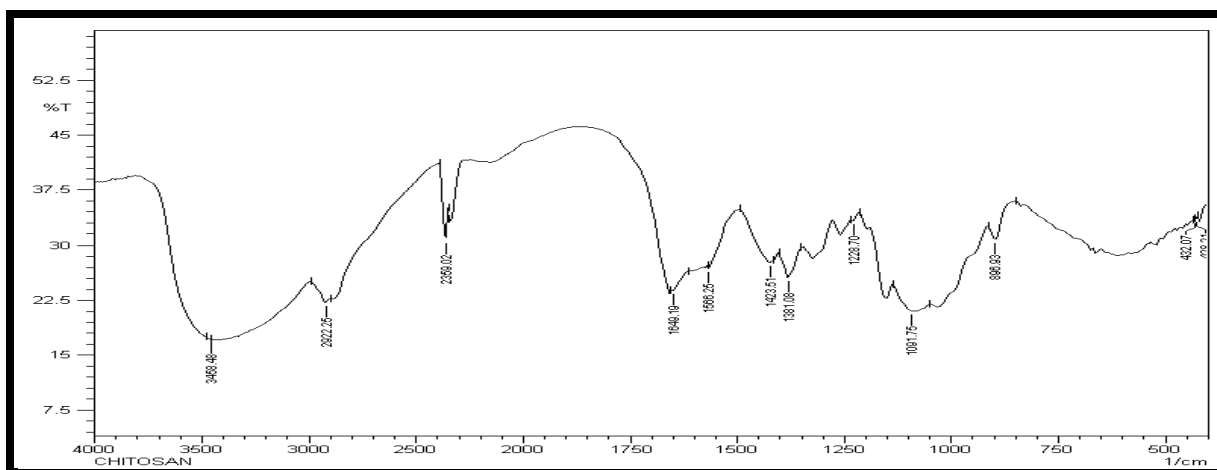


Figure No.2: FTIR of Chitosan

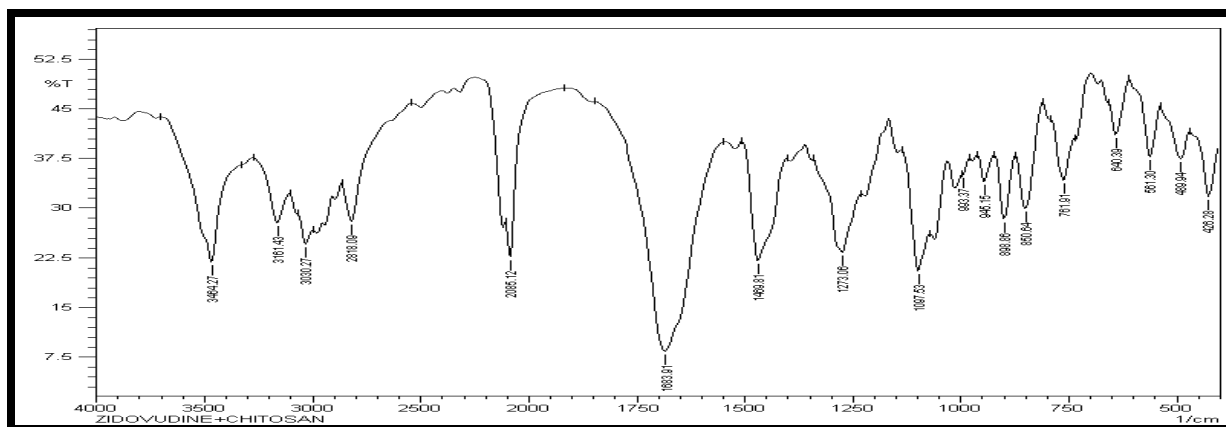


Figure No.3: FTIR of Zidovudine+Chitosan

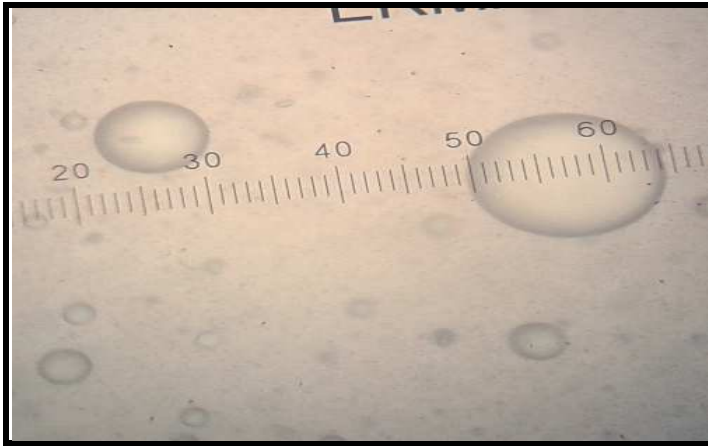


Figure No.4: Microphotograph of Zidovudine microspheres

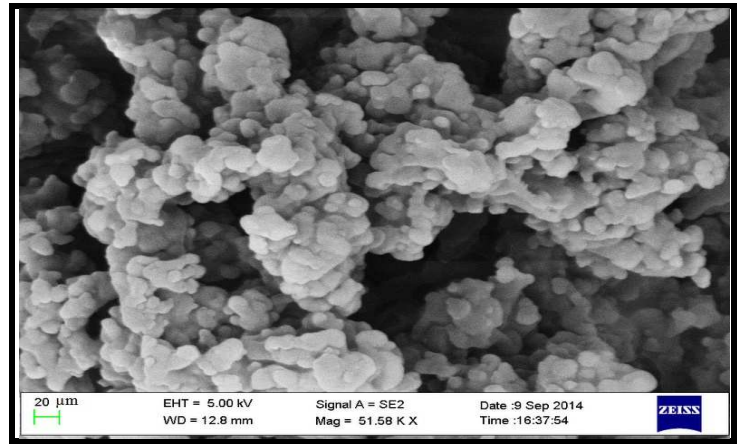


Figure No.5: SEM image of Zidovudine microspheres

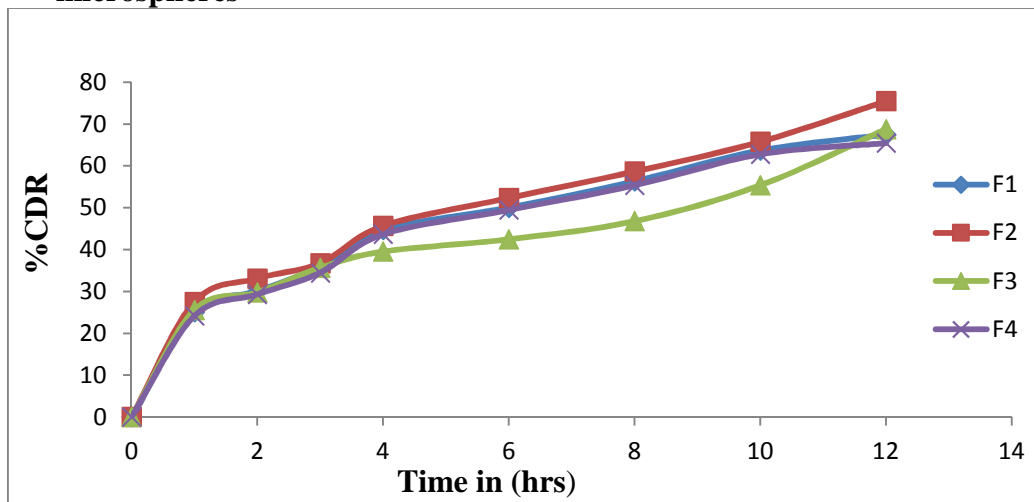


Figure No.6: % Cumulative drug release of Zidovudine microspheres

## CONCLUSION

The present study demonstrated the successful preparation of Zidovudine microspheres and their evaluation. Formulation F4 showed high entrapment efficiency (87 %), particle size (95  $\mu\text{m}$ ) and drug release (67.69 %) over 24 hrs. Hence it was considered to be good microsphere formulation with greater bioavailability and lesser side effects.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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