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FORMULATION AND EVALUATION OF MICROSPHERES ENCAPSULATING ZIDOVUDINE BY SOLVENT EVAPORATION 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 Eudragit L100 as a polymer for potentially treating HIV and AIDS related conditions. The formulation F1 to F4 which were prepared by solvent evaporation method by varying the concentration of Edragit L100 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, Edragit L100.

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 [1].

Microspheres can be defined as solid, approx. imately spherical particles ranging from 1 to 1000 µm, 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 [2].

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 post poning complications of acquired immune deficiency 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 [3].

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.

Eudragit polymers are series of acrylate and methacrylate polymer available in different ionic forms. Eudragit L 100 is anionic co polymer and is non toxic, biocompatible and biodegradable. With this background, combination of chitosan and zidovudine, Eudragit L 100 and Zidovudine

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were selected as core material for formulation of microspheres to achieve controlled drug release [4].

MATERIALS AND METHODS

Materials

Zidovudine (Strides Arcolabs ltd, Bangalore), Eudragit L100 (Rhomghbh & Co), 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).

Methods

Preparation of microspheres by Solvent evaporation technique

Solvent evaporation technique Zidovudine microspheres were prepared by solvent evaporation method. In this method Eudragit L100 was dissolved in mixture of solvents containing acetone 10 ml and methanol 15 ml in a 100 ml of beaker with the help of magnetic stirrer. Then the solution was added with drug (100 mg). Magnesium stearate (50 mg) as dispersing agent, was dispersed in drug and polymer solution with the help of sonicator. Resulting dispersion was poured in another 250 ml beaker, containing mixture of light liquid paraffin 10ml and span 80 (1% V/V) with continued stirring at 1500 rpm. Stirring was continued for 3 hrs until solvent evaporated completely. After evaporation of solvent, formed microspheres were filtered and residue was washed 4 to 5 times in 50 ml petroleum ether. Microspheres were dried at room temperature for 24 hrs.The composition and the formulation design of these microspheres is given in Table no 1 [5].

EVALUATION OF MICROSPHERES

Particle size analysis

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece. Approximately 300 microspheres were measured [6].

Drug entrapment efficiency

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 [7].

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

Drug content evaluation

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 [8].

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 E5 100 Polaron U.K) in Argonat ambient of 8-10 °C with plasma voltage about 20mA. The sputtering was done for nearly 5 mins to obtain uniform coating on the sample to enable good quality SEM images.

Evaluation of micromeritic properties of microspheres

The microspheres were characterized for their micromeritic properties - angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio [9]

Angle of repose

Angle of repose is determined by employing fixed funnel method. The angle of repose wascalculated 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 transferredinto 50 ml measuring cylinder. It was subjected to tapping for 3times and the volume occupied by the beads was noted. Bulkdensity 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 transferredinto 50 ml measuring cylinder. It was subjected to tapping for 50times and the volume occupied by the beads was noted.

Tappeddensity = Weight of the beads / Tapped volume of the beads.

Hausner 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**

The drug release study was performed using USP dissolution test apparatus paddle type at 37 ± 0.5 ℃ 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 [10].

Stability studies

Whenever a new formulation is developed, it is very essential to establish that the therapeutic activity of the drug has not undergone any change. To conform this, the selected formulations were subjected to stability studies. Generally, the observation of the rate at which the product degrades under normal room temperature requires long time. To avoid this undesirable delay, the principles of the accelerated stability studies are adopted. The International Conference of Harmonization guidelines titled "stability testing for drug substance and product" describes the stability tests requirements for drug registration applications in the European Union, Japan and United States of America.

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 Edragit L100.

Incompatibility studies

Drug-excipients compatibility studies were carried out using FT-IR. The spectra of pure drug (Zidovudine), Edragit L100 and their physical mixture (1:1:1) was obtained from FT -IR spectroscopy studies at wavelength from 4000 to 400 cm^{-1} and the characteristic peaks obtained are shown in figures 1, 2 and 3

Entrapment efficiency and drug content

The entrapment efficiency of F1 to F4 which was prepared by Solvent evaporation method are ranged from 63 % to 81.70 %. An increase in the concentration of Edragit L100 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 method, ranged from 70.67 % to 81.70 % for F1 to F4 as shown in table no 2.

Particle size analysis

The mean particle size of formulation F1 to F4 prepared by Solvent evaporation method was found to be in the range of 65 to 80.5 µm shown in. The result showed that as the polymer concentration increases, the particle size also increases which was showed in fig 4.

Surface morphology

Scanning Electron photomicrographs for the optimised formulation F1 and F4 was shown in figure. The SEM photomicrographs indicated that the microspheres were spherical in shape having particle size of 1 µm for F4 and Surface of the microsphere appear to be rough, may be due to the presence of drug. Fig 4. 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 -30.30 Mv, which indicates that the microspheres prepared by ionic gelation method was more stable. All the formulations showed angle of repose in the range of 25 to 32, Carr's index, was between 12 to 15 and Haunser's ratio < 1.2, all the parameters indicating good flow property. Of particular note are formulations F1 to F4 prepared by Solvent evaporation method which showed the best flow properties as shown in table no 3.

***In vitro* drug release**

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 Solvent evaporation method shows F1-70.54%, F2- 69.45%, F3-68.34% and F4-67.69% were shown in Fig 6. The increase in Edragit L100 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, KorsemayerPeppas. The regression coefficient values for all these models are shown in Table 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 (67.69 %) 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 partiicle size, entrapment efficiency and drug release. This revealed that the formulation stable on storage at 30±2°C and 65±5% RH.

Table 1. Composition of Zidovudine microspheres formulations

Formulation Code	Drug (mg)	Eudragit L100 (mg)	Acetone (ml)	Methanol (ml)	Liquid Paraffin (ml)	Span 80 (ml)
F1	100	100	10	15	10	10
F2	100	200	10	15	10	10
F3	100	300	10	15	10	10
F4	100	400	10	15	10	10

Table 2. Entrapment efficiency and drug content

Formulation code	% Drug content	% Entrapment efficiency
F1	70.67	63.0
F2	75.87	65.8
F3	80.56	76.8
F4	84.45	81.70

Table 3. Some micromeritic properties of microspheres

Formulation Code	Angle of repose	Tapped density (g/cm^3)	Bulk density (g/cm^3)	Carr's index (%)	Haunser's ratio
F1	$26°42^1$	0.583	0.486	12.98	1.15
F2	$27°42^1$	0.566	0.548	12.68	1.12
F3	$29°26^1$	0.581	0.563	14.13	1.15
F4	$31°12^1$	0.647	0.588	14.89	1.17

Table 4. Results of model fitting for Zidovudine microspheres

Formulation code	Zero order	First order	Higuchi/matrix	Peppas plot	
				r^2 values	'n' values
F1	0.8577	0.9604	0.9891	0.9315	0.3632
F2	0.8339	0.9338	0.9709	0.9516	0.3860
F3	0.8822	0.9670	0.9025	0.9087	0.3168
F4	0.8927	0.9631	0.9606	0.9661	0.4173

Fig 1. FTIR of pure Zidovudine

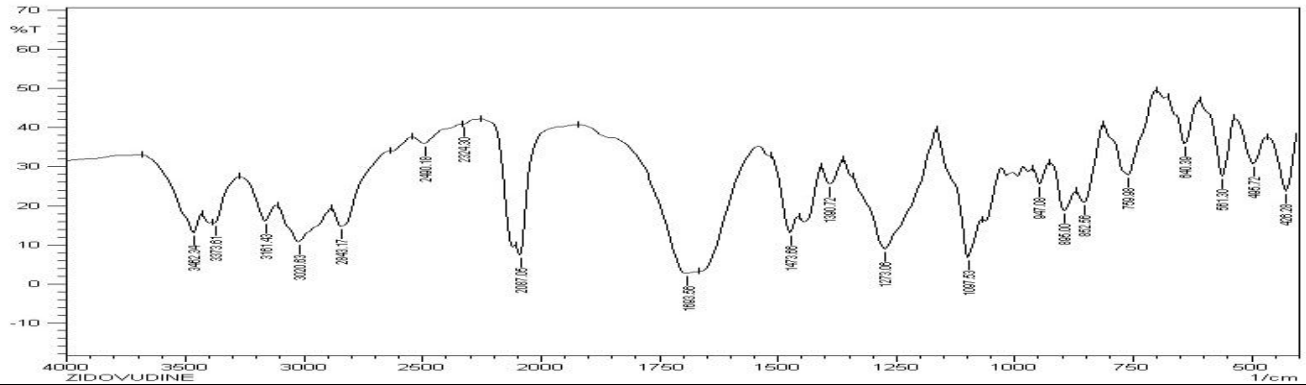


Fig 2. FTIR of Edragit L100

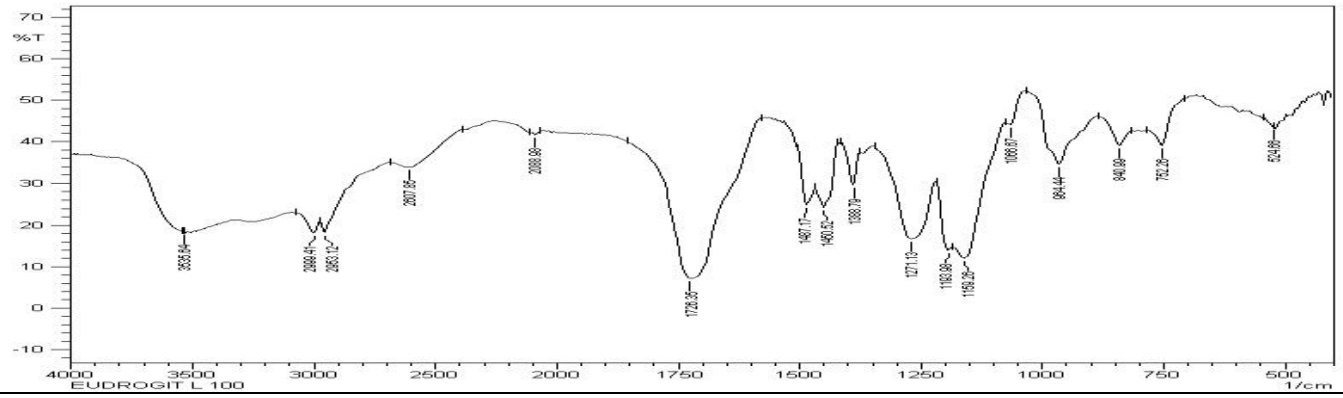


Fig 3. FTIR of Zidovudine+Edragit L100

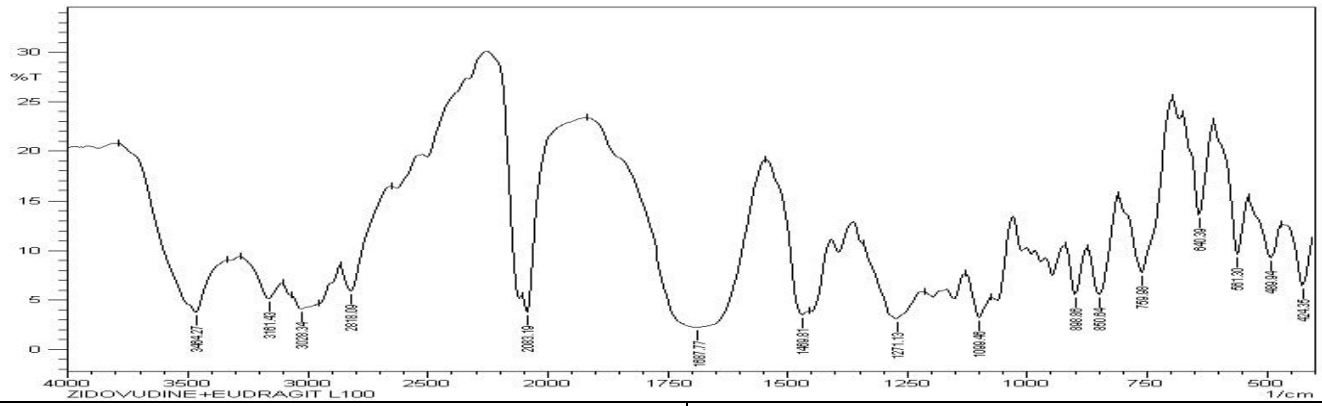


Fig 4. Microphotograph of Zidovudine microspheres

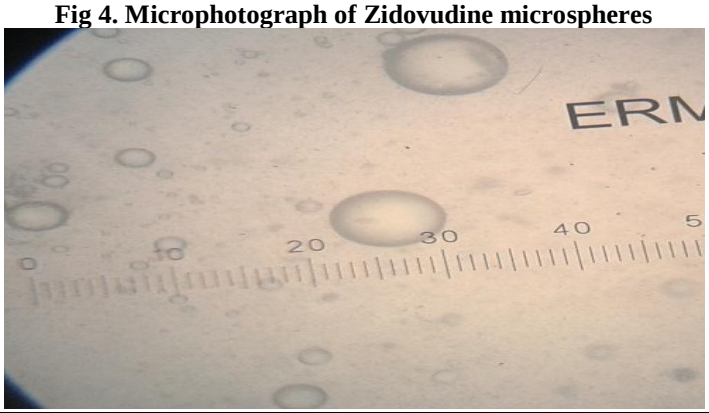


Fig 5. SEM image of Zidovudine microspheres

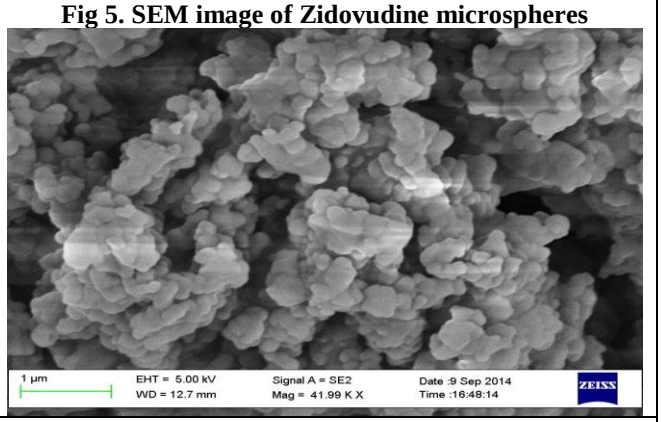
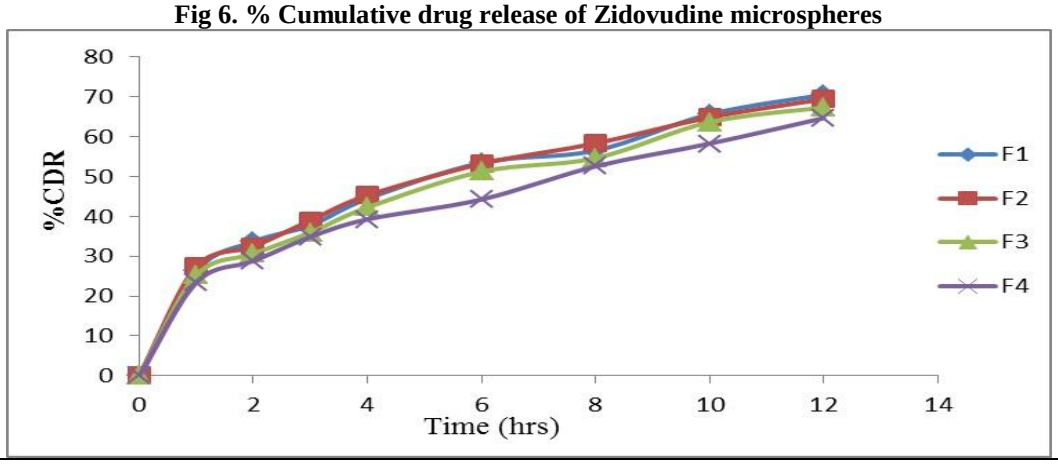


Fig 6. % Cumulative drug release of Zidovudine microspheres



CONCLUSION

The present study demonstrated the successful preparation of Zidovudine microspheres and their evaluation. Formulation F4 showed high entrapment efficiency (81.70 %), particle size (1 μm) 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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