

## Formulation and evaluation of Zidovudine loaded chitosan Microspheres for controlled release

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### Abstract

The aim of this study was to formulate and evaluate zidovudine loaded chitosan microspheres for controlled drug release. Microspheres were prepared by emulsification method using glutaraldehyde as crosslinking agent. The prepared microspheres were characterized for their yield and drug loading, as well by Fourier transform infrared spectroscopy (FTIR), X-ray powder diffractometry and Scanning electron microscopy. The in vitro release studies were performed in pH 7.4, phosphate buffer. The prepared microspheres were free flowing and spherical in shape. The drug-loaded microspheres showed 72-94% of entrapment and release was extended up to 12h. The infrared spectra showed stable character of zidovudine in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. X-ray diffraction patterns showed that there was decrease in crystallinity of the drug. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature. It was found that the drug: polymer ratio, the stirring speed, the concentration of surfactant, and the amount of glutaraldehyde used for crosslinking were the most significant variables which influenced the size of the chitosan microspheres under the applied experimental condition.

### Key words:

zidovudine, chitosan, microspheres, controlled release, glutaraldehyde cross-linking

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### INTRODUCTION

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 3 hrs, thus necessitating frequent administration, low

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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 [1]. Being non toxic, biodegradable and biocompatible, chitosan has been widely used in the formulation of particulate drug delivery systems to achieve controlled drug delivery [2, 3]. Microspheres can be defined as solid, spherical empty particles ranging in size from 1 to 1000  $\mu\text{m}$  [4]. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the controlled release of drug [5,6,7].

With this background, combination of chitosan and zidovudine were selected as core material for formulation of microspheres to achieve controlled drug release [8, 9].

The objective of the present work was to design, formulate, optimize and evaluate zidovudine loaded chitosan microspheres for controlled drug release [6]. Hence different batches of microspheres were prepared according to the working plan [10, 11]. The resultant microspheres were evaluated /characterized for percentage yield, entrapment efficiency, particle size and in vitro drug release [12]. The effect of process variables on microspheres was also studied.

## **MATERIALS AND METHODS**

### **Materials**

Zidovudine was obtained as a gift from m/s Aurobindo Laboratories Ltd, Hyderabad. Chitosan was obtained as a gift sample from Central Institute of Fisheries Technology, Cochin. All other reagents and solvents used were of pharmaceutical or

analytical grade. The equipment used was centrifuge, sonicator, UV spectrophotometer, FTIR, X -RD, Particle size analyzer, Zetasizer and scanning electron microscope.

### **Methods:**

#### **Preparation of microspheres**

The microspheres were prepared by emulsification crosslinking method using gluteraldehyde as crosslinking agent. Accurately weighed quantity of chitosan was dissolved in 1% (v/v) aqueous acetic acid. The drug (zidovudine) was added to the polymer solution and mixed thoroughly. The dispersed phase was then added drop-wise through a disposable syringe (10 ml) to the continuous phase consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing different amounts of surfactant (span 80 ) to form a water in oil (w/o) emulsion. Stirring was continued at different speed using a 3 blade propeller stirrer. After 20 min of stirring, a measured quantity of aqueous gluteraldehyde (25%vol/vol) was added dropwise at regular intervals of 1, 2 and 3 hours respectively and continued for 1 hour after the final addition of gluteraldehyde. The preparation was centrifuged at 3000 rpm, the supernatant was decanted and microspheres obtained as residue were washed 4 times with petroleum ether (60-80°C). After the final wash, microspheres were then air dried at room temperature, collected and stored in desiccators. Blank microsphere was prepared for comparison with the drug loaded microspheres.

#### **Effect of process variables on microsphere properties**

Chitosan microspheres were prepared at different stirring rates, surfactant concentrations (Span 80) in the external phase, crosslinking agent (gluteraldehyde) amount and with various drugs: polymer ratios as stated in Table 1.

**Table1:** Process variables

Process variables	Different Values
stirring rates (rpm)	500 , 1500 ,2500
surfactant concentrations (% w/v)	0.5, 1.0 ,1.5
crosslinking agent amount (ml)	0.5,1.0,1.5
drug: polymer ratios	1:1,1:2,1:3,1:4
Volume of processing medium (ml)	100, 150,200

**Working formula**

**Table 2:** Working formula for preparation of different batches of microspheres

Formulation code	Drug Polymer ratio	Stirring speed (rpm)	Span80 Conc. (%v/v)	Glutaraldehyde Conc.(ml)	Liquid.Paraffin(ml) Heavy + light	
F1	1:1	500	0.5	0.5	50	50
F2	1:2	500	0.5	0.5	50	50
F3	1:3	500	0.5	0.5	50	50
F4	1:4	500	0.5	0.5	50	50
F5	1:2	1500	0.5	0.5	50	50
F6	1:2	1500	1.0	1.0	50	50
F7	1:2	1500	1.0	0.5	50	50
F8	1:2	1500	1.5	0.5	50	50
F9	1:2	1500	1.0	1.5	50	50
F10	1:2	2500	0.5	0.5	50	50
F11	1:2	2500	1.0	0.5	50	50
F12	1:2	2500	1.0	1.0	50	50
F13	1:2	2500	1.0	1.5	50	50
F14	1:2	1500	1.0	1.0	75	75
F15	1:2	1500	1.0	1.0	100	100
Blank Microsphere	0:2	1500	1.0	1.0	50	50

**Evaluation of Microspheres**

**Percentage Yield**

The prepared floating microspheres were collected and weighed. The measured weight was divided by total amount of all non-volatile components, which were used for the preparation of microspheres. The % yield was calculated using following formula

$$\text{Percentage yield} = \frac{\text{Weight of microspheres recovered}}{\text{Weight (drug + polymer)}} \times 100$$

**Drug entrapment efficiency**

Weighed quantity of microspheres were crushed and suspended in ethanol to extract the drug from

During the preparation of different batches of microspheres as given in Table 2, optimization was carried out for different drug polymer ratios, stirring speed, surfactant concentration, crosslinking agent amount and volume of processing medium. The effect of these process variables on yield, encapsulation efficiency and particle size were investigated.

microspheres. After 24 hrs, the filtrate was assayed spectrophotometrically at 267 nm for drug content against ethanol as blank. Corresponding drug concentrations in the samples were calculated from the calibration plot using a regression equation derived from the standard graph. The drug entrapment efficiency (DEE) was calculated by the equation.

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

**Fourier Transform Infrared Spectroscopy (FTIR)**

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug-loaded microspheres using FTIR (Model No. IR Presige-21, Shimadzu). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000  $\text{cm}^{-1}$  and the resolution was 4[1/cm].

#### X-ray powder Diffraction (X-RD)

X-ray powder diffraction was carried out to investigate the effect of microencapsulation process on crystallinity of drug. Powder X-RD patterns were recorded on X-RD (Model no- PW3040 Xpert MPD system) using Philips Analytical X-Ray B.V. The scanning rate employed was  $2^\circ \text{min}^{-1}$ , over the 14 to 88 diffraction angle ( $2\theta$ ) range. The X-RD patterns of drug, polymer, blank microspheres and drug-loaded microspheres were recorded.

#### Particle size measurement

The size of the microspheres was analyzed by laser particle size analyzer (Malvern Instruments, Mastersizer 2000, Malvern, UK) using distilled water. The sample was vortexed for 1 minute before sampling. The samples were then sonicated in a sonicator attached to the instrument throughout the process, and the duration of sonication was kept constant for all samples. The obscuration ranged from 0.08% to 3.62%.

#### In-Vitro Release Studies:

##### In-Vitro Dissolution study of pure drug

This was carried out by taking 100 mg pure drug, using USP basket dissolution apparatus basket type at temperature  $37 \pm 0.5^\circ\text{C}$ , 100 rpm and dissolution medium phosphate buffer 7.4 (500ml). 5 ml samples were withdrawn from the dissolution medium at 1 minute intervals and equivalent volume of fresh dissolution medium was added. After suitable dilution, these samples were analyzed spectrophotometrically by UV visible

spectrophotometer at 267 nm and using standard curve equation.

#### In Vitro Dissolution Study of drug-loaded microspheres

In the present study, drug release was studied using a USP dissolution apparatus basket type using phosphate buffer solution (pH 7.4) as dissolution fluids (500 ml) maintained at  $37 \pm 0.5^\circ\text{C}$  at 100 rpm. Accurately weighed samples of microspheres were added to dissolution medium. 5 ml of samples were withdrawn from the dissolution medium at 1 hr intervals. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. These samples were analyzed spectrophotometrically by UV visible spectrophotometer at 267 nm and using standard curve equation.

## RESULTS & DISCUSSION

The microspheres formed were spherical in shape and having smooth or slightly rough in surface. The yield and entrapment efficiency of various formulations was given in Table 3.

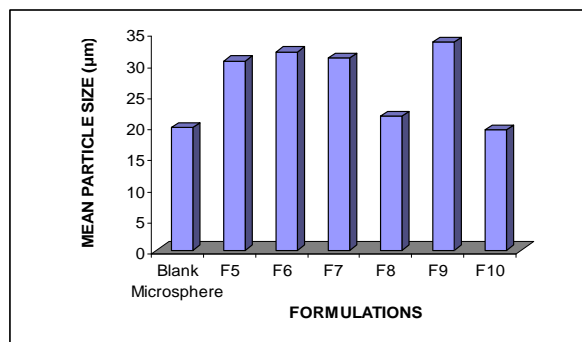
**Table 3:** Percentage Yield and Encapsulation Efficiency of microspheres

Formulation Code	Drug: Polymer Ratio	%Yield	%Drug Entrapment
Blank microspheres	0:2	93	----
F5	1:2	85	92.12
F6	1:2	94	93.54
F7	1:2	92	93.12
F8	1:2	90	91.48
F9	1:2	87	83.25

#### Effect of process variables on Particle Size of selected formulations

It is observed from the Figure 1 that increasing the polymer ratio the mean particle size was increased. When the drug polymer ratio was increased from 1:1 to 1:4, larger particles were formed, because the viscosity of the emulsion medium was increased with

increasing amount of polymer. Due to increase in viscosity, larger emulsion droplets were formed which were difficult to break and hence they are precipitated as such leading to increase in mean particle size.



**Figure1:** Mean Particle Size Distribution in Various Formulations

It was observed from the investigation that when stirring speed was low (500 rpm), larger spherical microspheres were formed. This may be due to inadequate stirring speed which was not able to break the emulsion droplets. Stirring speed above 1500 rpm resulted in formation of spherical small microspheres, which may be due to higher degree of emulsification of the polymeric phase at a higher speed.

Increasing the surfactant concentration the mean particle size had diminished considerably. When medium concentration of span 80 was used, the particle size increases because low amount fail to prevent droplet coalescence in the oil medium. As the concentration increased to 1.5% the mean particle size were reduced.

Further increasing the amount of crosslinking agent (gluteraldehyde) caused a slight increase in the particle size of the formulation. This could be due to adherence of excess crosslinking agents on the surface of the microspheres.

It was observed that the particle size of blank microspheres was lower than the drug loaded microspheres. Hence it confirms that effective drug loading has been taken place in microspheres which increased the particle size of the microspheres.

### Effect of various parameters on entrapment efficiency

The volume of processing medium significantly influences the entrapment efficiency of the formulations. As the volume of processing medium was increased from 100 ml to 150 ml and to 200 ml, the entrapment efficiency significantly decreased.

As the volume of processing medium was increased, the emulsion droplets probably moved freely in the medium, thus reducing the collision induced aggregation which could be the reason of high drug extraction into the processing medium resulting in lower entrapment efficiency.

Effect of crosslinking agent amount on entrapment efficiency was also studied. It was found that on increasing the amount of the crosslinking agent, the drug entrapment efficiency was decreased.

### Fourier Transform Infrared Spectroscopy (FTIR)

The study of IR spectra of zidovudine loaded chitosan microspheres as per shown in Figure 2, demonstrated that the characteristic peaks for C–Br stretch alkyl halides, C–N stretch aliphatic amines, C–N stretch aromatic amines, C=O stretch carbonyls, O–H stretch, H–bonded alcohols, phenols were appeared at  $563\text{ cm}^{-1}$ ,  $1101\text{ cm}^{-1}$ ,  $1269\text{ cm}^{-1}$ ,  $1672\text{ cm}^{-1}$  and  $3419\text{ cm}^{-1}$  respectively. The absorption peaks were almost similar to those obtained from the pure drug and reported values. Hence FTIR study confirms that drug was compatible with the polymer and there is no drug polymer interaction.

### X-ray powder Diffraction (X-RD)

The X-ray powder diffraction patterns of pure drug, polymer, blank microspheres and drug loaded microspheres as stated in Figure 3 reveals that the intensity of the peaks for the pure drug was sharp. But when it was incorporated into the polymer matrix, the drug peaks showed a loss of sharpness probably due to decreased crystallinity of the drug.

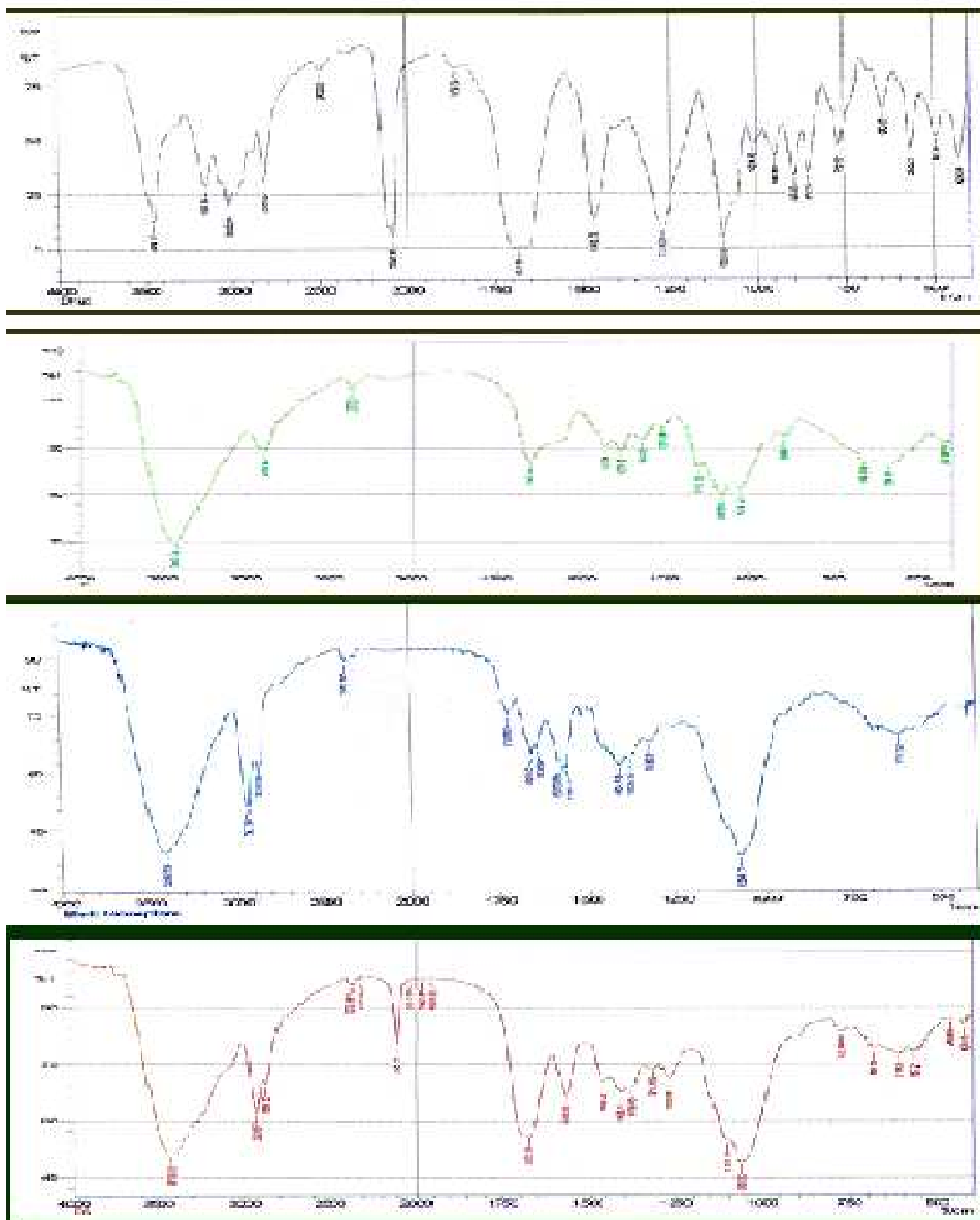


Figure 2: IR spectra of a) pure drug b) polymer c) blank microspheres d) drug loaded microspheres



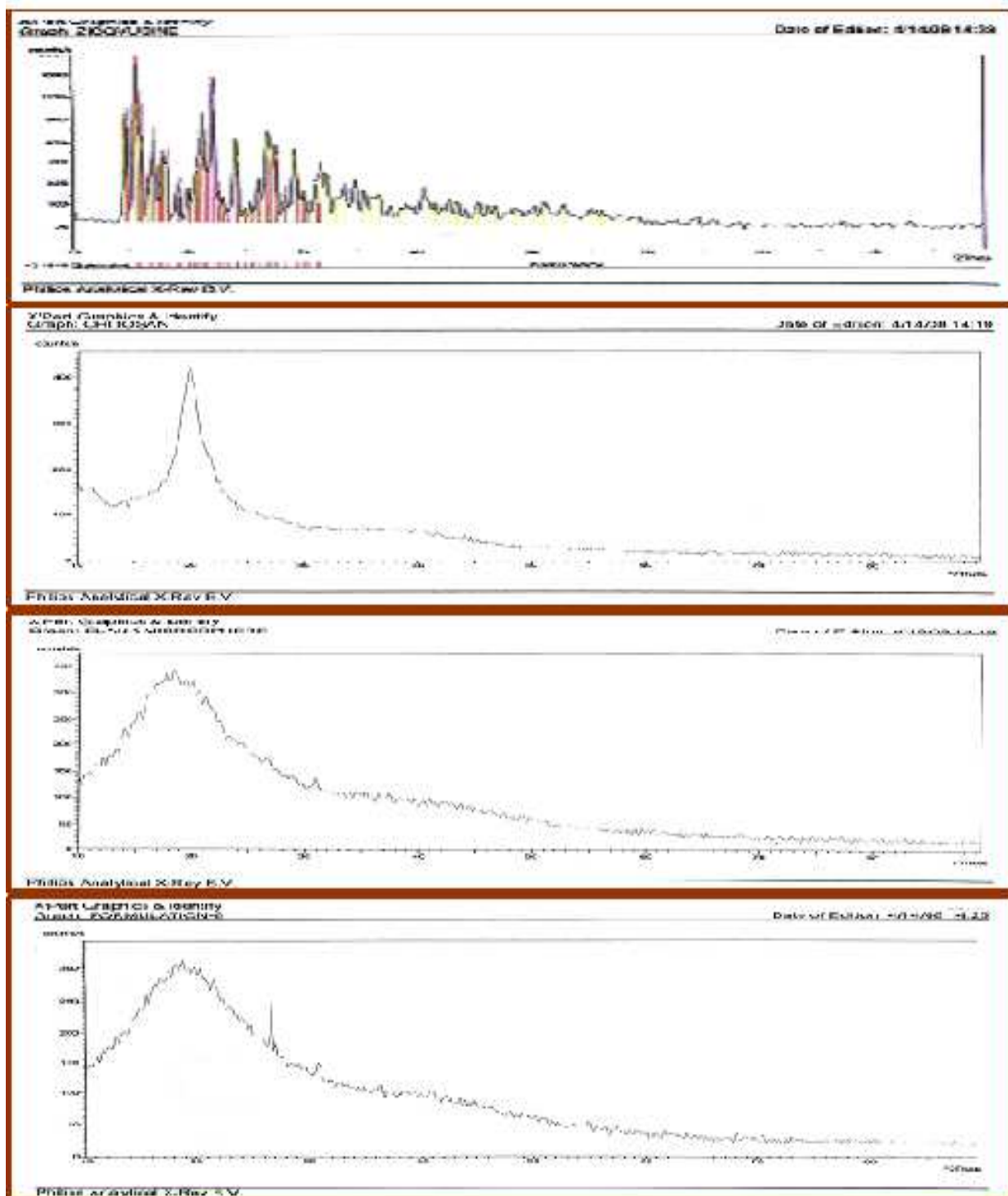
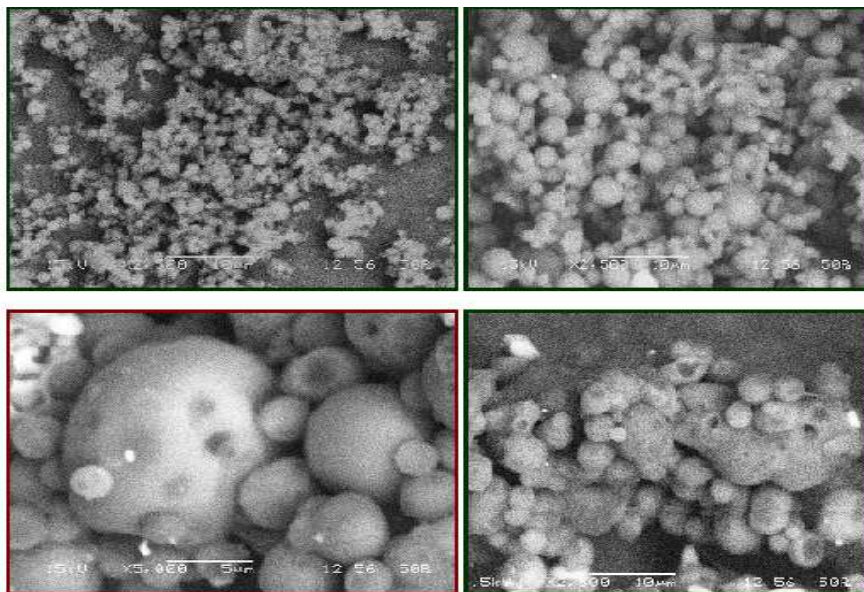


Figure 3. XRD of a) pure drug b) polymer c) blank microsphere d) drug loaded microspheres

### Scanning Electron Microscopy (SEM)

The surface topography of the microspheres was investigated by SEM as per given in Figure 4. SEM analysis suggests that the microspheres were found to be spherical. Drug-loaded microspheres had rough surfaces due to higher concentration of drug in the microspheres as compared to the blank microspheres.



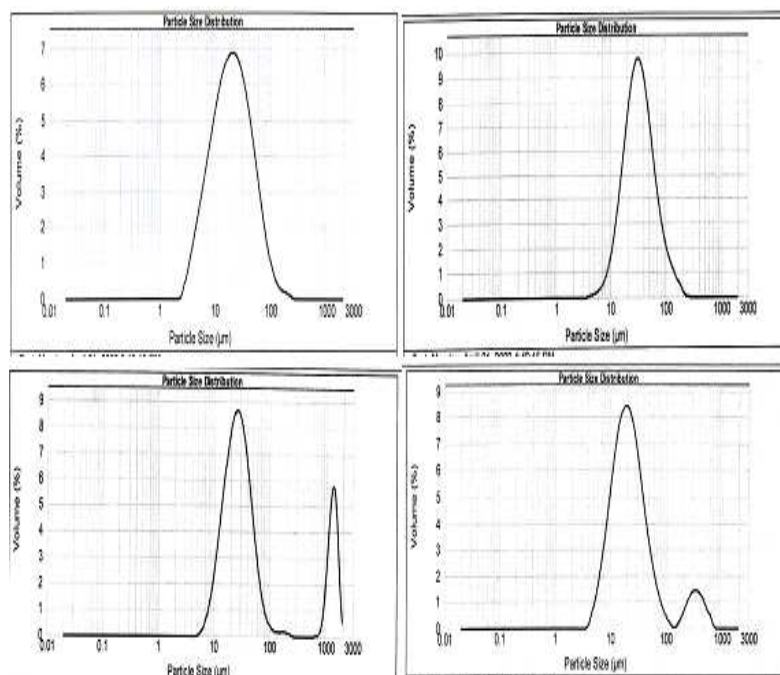
**Figure 4:** Scanning electron photomicrograph of a) blank microspheres b) drug loaded microspheres c) Microspheres collected after 6hrs of release study d) microspheres collected after 12hrs of release study

It was observed that, under similar conditions the drug loaded microspheres were bigger in size compared to the blank microspheres which again confirms the data obtained during particle size analysis that effective drug loading has been taken place in microspheres which increased the particle size of the drug loaded microspheres.

Further, SEM analysis of the microspheres collected after release study showed bigger pores suggesting that the drug was released through pores.

#### Particle Size Analysis

In the present study, the particle size of selected formulations was determined using a Malvern Mastersizer.



**Figure 5:** Particle Size Distribution of A) Blank Microspheres; B) Formulation F6; C) Formulation F7; D) Formulation F8



As per given in figure 5 the prepared microspheres revealed a unimodal size distribution. Blank microspheres showed the mean particle size distribution of 19.71  $\mu\text{m}$ . The mean particle size of formulation F6 was 31  $\mu\text{m}$ . Particle size distribution

curve for different formulations is presented in Figure 4. The reduced particle size will help in achieving our goal by enhancing the controlled delivery of zidovudine.

#### In-Vitro Drug Release

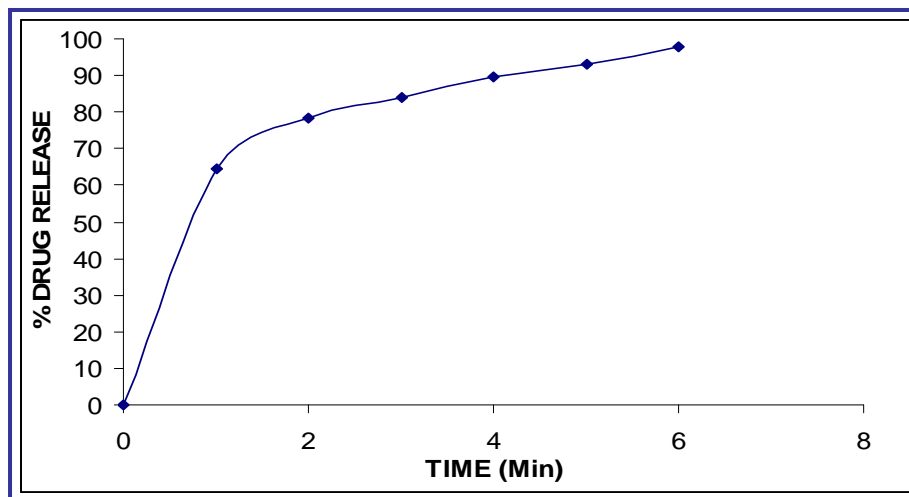


Figure 6: In vitro release Profile of Pure Drug

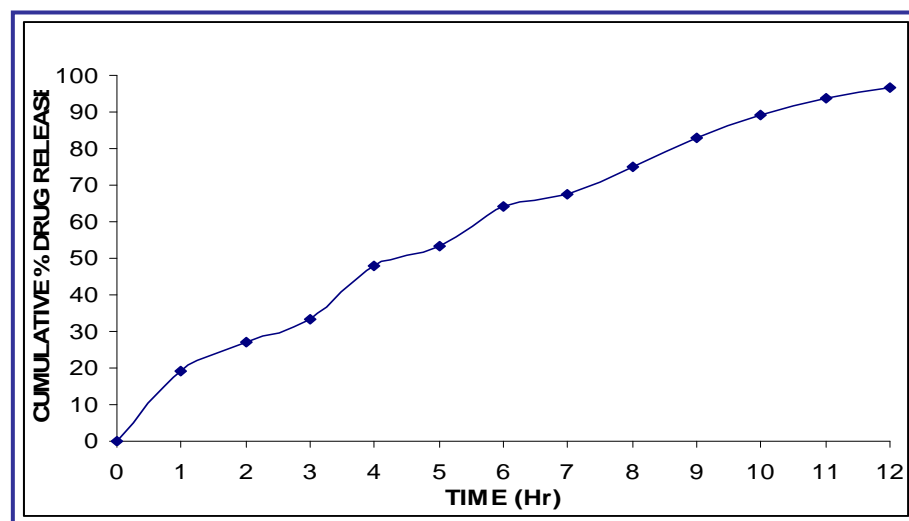


Figure 7: In vitro release Profile of Optimized Formulation

From the in vitro dissolution study of pure drug (Figure 6) and optimized formulation (F6) as shown in Figure 7, it was observed that pure drug (zidovudine) was released at faster rate. About 97% drug was released within 6 minutes but when encapsulated in chitosan microspheres, 96% drug was released from the formulation in 12 hrs

indicating controlled release of the drug from the optimized formulation.

#### CONCLUSION

Formulation and evaluation of zidovudine loaded chitosan microspheres for controlled release was found to be potential and effective in terms yield, encapsulation efficiency, particle size distribution,

and in vitro release characteristics. Even though the zeta potential value was still lower, compared to the other reported formulations, further studies are required to find out the exact cause and to improve the zeta potential. The investigation of optimum formulation showed controlled drug release and could, therefore, produce some benefits such as reduction in total dose, frequency of administration, and dose related systemic side effects, in HIV / AIDS patients.

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#### REFERENCES

- 1) Paul W. and Sharma C.P., Chitosan, a drug carrier for the 21st century: a review. *STP Pharma Sciences*. 2000: 5-22.
- 2) Sinha V.R, Singla A.K, Wadhawan S, et al. Chitosan microspheres as a potential carrier for drugs. *Int. J. Pharm.* 2004: 1-33.
- 3) Kas H.S. Chitosan: properties, preparation and application to microparticulate systems. *J Microencapsulation*. 1997: 689-711.
- 4) Burgess D. J., Hickey A.J. Microsphere technology and applications. In: Swarbrick J., Boylan, J.C. ed. *Encyclopedia of pharmaceutical technology*, 1<sup>st</sup> ed. New York: Marcel Dekker; 1995. 1-29.
- 5) Raymond C R., Sheskey P. J. and Owen S.C. *Hand book of pharmaceutical excipients*. 5<sup>th</sup> ed. pharmaceutical press (PhP), APhA, 159-161
- 6) Sahoo S.K., Mallick A. A., Barik B. B. and Senapati P. C. Formulation and in vitro evaluation of eudragit microspheres of stavudine. *Tropical*

*Journal of Pharmaceutical Research*. 2005; 4 (1): 369-375.

- 7) Behera B.C., Sahoo S.K., Dhal S, Barik B.B, Gupta B.K. Characterization of glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method. *Tropical Journal of Pharmaceutical Research*. 2008; 7(1): 879-885.
- 8) Ghosh A., Nayak U. K., Roy P. Development, evaluation and method selection for the preparation of lamivudine microspheres <http://www.priory.com/pharmacy/Lamivudine.htm> [accessed on 30/09/11]
- 9) Chithambara Thanoo, B., Sunny M.C. and Jayakrishnan A. Crosslinked chitosan microsphere: preparation and evaluation as a matrix for the controlled release of pharmaceuticals. *Journal of Pharmacy and Pharmacology*. 1992;44(4) :283-286.
- 10) Ko. J.A., Park H.J., Hwang S.J., Park J.B. and Lee J.S. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. *International Journal of Pharmaceutics* 2002; 249:165-174.
- 11) Dubey R. R., Parikh R. H. Two stage optimization process for formulations of chitosan microspheres. <http://www.aapspharmstech.org/view.asp?art=pt050105&pdf=yes> [accessed on 30/09/2011]
- 12) Das M. K, Rao K. R. Micro encapsulation of zidovudine by double emulsion solvent diffusion technique using ethyl cellulose. *Indian Journal of Pharm. Sc.* 2007; 69(2): 244-250.

