

# Utilization of Natural Olibanum Gum Resin as a Rate Controlling Polymer in Design and Evaluation of Microspheres of an Antiretroviral Drug by using a Unique Spray Drying Technique

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## ABSTRACT

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Olibanum gum resin was evaluated as rate controlling agent and to prepare resin-coated microspheres. Zidovudine (AZT), an anti-retroviral drug was selected as novel drug for the experiment. Resin coated microspheres of AZT were prepared by spray drying technique using olibanum gum resin as a rate controlling polymer and the microspheres were evaluated. The resin-coated microspheres were spherical, discrete, free flowing and multinucleate monolithic type. The mean size range was found for formulation D: P1, D: P2, D: P3 in the range of 16.88, 18.51, and 21.72  $\mu\text{m}$  respectively. Entrapment efficiency was in the range of 67.41 to 80.32%. The FTIR and DSC study confirmed that no chemical interaction took place during entrapment process. The X-ray diffraction study indicates the amorphous dispersion of the drug after entrapment into microspheres. The effect of polymeric resin on release profile of drug was calculated. AZT release from the resin coated microspheres was slow over 24 hr and dependant on core: coat ratio, and size of microspheres. Drug release was by non-fickian diffusion mechanism. Good linear relationships were observed between core: coat ratio of the microspheres and release rate. Resin-coated microspheres of AZT exhibited good controlled release characteristics and were found suitable for once a day oral controlled release products.

**Keywords:** Olibanum gum resin, spray drying, non-fickian diffusion, controlled release

## INTRODUCTION

With many drugs, the basic goal of therapy is to achieve a steady-state blood or tissue level that is therapeutically effective and nontoxic for an extended period of time. The design of proper dosage regimen is an important element in accomplishing this goal. This is usually accomplished by maximizing drug availability, i.e., by attempting to attain a maximum rate and extent of drug absorption; however control of drug action through formulation also implies controlling bioavailability to reduce drug absorption rates<sup>1</sup>. Microentrapment is a process whereby relatively thin coating of polymers are applied to small particles of solid or droplets of liquid and dispersions. Microentrapment leads to microspheres or microspheres, which are reservoir type and matrix type respectively. In either case, one or more active ingredient (core) is / are entrapped within matrix, shell or coat which is usually composed of one or more polymers<sup>2</sup>.

AZT (3'-azido-3'-deoxythymidine) is a thymidine analogue in which the 3-hydroxyl group is replaced by an azido (-N<sub>3</sub>) group. It is the first anti-HIV compound approved for clinical

use, is still widely used for treatment of AIDS and AIDS-related complex, either alone or in combination with other antiviral agents. However, the main limitation to the therapeutic effectiveness of AZT is its dose-dependent hematological toxicity. This virustatic drug has a very short half life of 1 h and undergoes considerable first-pass metabolism thus necessitating frequent administration of large doses (200 mg for every 4 h) to maintain therapeutic drug levels<sup>3</sup>. Because the therapeutic index of AZT is low, with a narrow range of plasma concentration of 0.016 to 1.7 mg per liter<sup>4</sup>, side effects occur frequently<sup>5</sup>. After per oral administration, AZT is completely and rapidly absorbed thus leading to very high initial plasma concentrations and consequently high incidence of toxicity. Therefore, to maintain effective plasma concentrations as well as to reduce dose and dose-dependent toxicity, an extended release dosage form of AZT is desirable.

Olibanum is a gum resin obtained from *Boswellia Serrata*, Roxburgh and other species of *Boswellia*. Olibanum consists chiefly an acid resin (56-60%), gum (30-36%) and volatile oil (3-8%). The resin contains mainly a resin acid (boswellic acid) and resene (olibanoresene) in equal proportion<sup>6</sup>. Ether soluble resin extracted from olibanum exhibited excellent release retarding properties in microentrapment for controlled release due to its hydrophobic water repellent

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properties. Preliminary studies indicated that the resin has good film forming property when dried from chloroform solution<sup>7</sup>. In the present work the resin extracted from the olibanum was evaluated as coating material in microentrapment.

Spray drying is extensively employed in the pharmaceutical industry to produce raw drugs or excipients or in the microentrapment process. Spray drying technique is based on the drying of the mist of the polymer and drug in air. One of major advantage of spray drying technique is feasibility of operation under aseptic condition, which is rapid, requiring single step operation and suitable for both batch and bulk manufacturing<sup>8</sup>.

There was no work reported on AZT microspheres using olibanum gum resin as a rate controlling polymer. In the present investigation, an attempt has been made to formulate the controlled-release microspheres of AZT by using spray drying technique.

## MATERIALS AND METHODS

AZT was received as a gift sample from Cadila healthcare Ltd., Goa. Olibanum was a gift sample from Girijan Co-op. Corporation Ltd., Tirupati, A.P. The olibanum obtained was dried at 60 °C for 4 hrs, powdered in a blender and the size was reduced to 200 mesh. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

### Extraction of resin fraction from Olibanum gum:

Powdered Olibanum gum (10 gm) was extracted repeatedly with 4×50 ml quantities of solvent ether. The ether extracts were collected in a porcelain dish and concentrated to dryness at 40 °C to obtain the resin fraction. The dry mass was powdered and the size was reduced to 200 mesh.

### Preparation of resin coated AZT microspheres by using spray drying technique:

The drug loaded resin microspheres were prepared in three different core: coat ratios (D: P-10:1, 10:2, 10:3) by dissolving the model drug (AZT) in the resin solution (resin + 95 % ethanol) prior to spray drying. Spray drying was concurrently performed using spray drier (Lab Ultima-222 mini spray dryer, Mumbai, India) with a standard 0.7 mm nozzle. When the liquid was fed to the nozzle with a peristaltic pump,

atomization occurred by the force of the compressed air, disrupting the liquid into small droplets. The droplets, together with hot air, were blown into a chamber where the solvent in the droplets was evaporated and discharged out through an exhaust tube. The dried product was collected from collection bottle.

The solvent quantity (500 ml) kept constant for all over the experiment, and core: coat ratio gets change as shown in the formulation (Table.1). Drug free microspheres were also prepared in the similar manner.

Following conditions were maintained during the process of spray drying for all the formulations:

- Nozzle diameter - 0.7mm
- Atomization pressure - 1.5 kg/cm<sup>2</sup>
- Feed rate – 5 ml/min
- Vacuum in the system - 60 mm/Wc
- Aspirator – 45 %
- Inlet temperature – 70 °C
- Outlet temperature – 40 °C
- Air flow- 30 m<sup>3</sup>/hr

## EVALUATION OF MICROSPHERES

### Percentage yield (% yield)<sup>9</sup>:

The percentage yield was calculated by using the following formula:

$$\text{Percentage yield (\%)} = \frac{\text{Wight of the microspheres recovered from each batch}}{\text{Total weight of the microspheres}} \times 100$$

### Estimation of drug content<sup>10</sup>:

A weighed quantity of microspheres equivalent to 100 mg of drug were crushed into powder and added to 100 ml of simulated intestinal (phosphate buffer pH 7.4) fluid. The resulting mixture was kept stirring at 1000 rpm for 2 hrs and kept it overnight for 24 hrs. Then the solution was filtered through membrane filter (0.45 µm pore size) and analyzed spectrophotometrically (Shimadzu-1700, Shimadzu Corporation, Japan) for AZT content at 260 nm after suitable dilutions. All the experimental units were analyzed in triplicate (n=3).

**Table.1: Formulation details of AZT microspheres by using spray drying technique**

Sr. No.	Formulation code	Drug (Zidovudine)	Polymer (Resin)	D:P ration	Solvent (95% ethanol)	Total D:P weight
1.	D:P1	5 gm	500 mg	10:1	500 ml	5.5 gm
2.	D:P2	5 gm	1000 mg	10:2	500 ml	6.0 gm
3.	D:P2	5 gm	1500 mg	10:3	500 ml	6.5 gm

### Drug entrapment efficiency:

Drug entrapment efficiency was calculated using the following formula:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

### Particle size analysis:

The microspheres were evaluated for particle size and shape using stereomicroscope (115 X). A suspension of the microsphere was prepared on a slide using Nujol and cover to form a specimen. This slide was observed under the microscope. Size of around 250 particles was measured for each batch on the different portions of the slide. From the size distribution, the average particle size was calculated from each batch of microspheres.

### Scanning electron microscopy (SEM):

The shape and surface morphologies of the blank microspheres and drug-loaded microspheres were investigated using scanning electron microscopy (XL 30 ESEM Philips). The dried microspheres were coated with gold foil ( $100\text{ }\text{\AA}$ ) under an argon atmosphere in a gold coating unit and SEM in both higher and lower resolutions were observed.

### Differential scanning calorimetry (DSC):

The DSC analysis of pure drug, drug-loaded microspheres, and drug free microspheres were carried out using Shimadzu DSC 60 to evaluate any possible drug-polymer interaction. The analysis was performed at a rate  $10.0^{\circ}\text{C min}^{-1}$  from  $20^{\circ}\text{C}$  to  $300^{\circ}\text{C}$  temperature range under nitrogen flow of  $25\text{ ml min}^{-1}$ .

### FTIR analysis:

The drug-polymer interactions were studied by FTIR spectrometer, Shimadzu 8400 S. 2% (w/w) of the sample, with respect to a potassium bromide (KBr; SD Fine Chem. Ltd., Mumbai, India) was mixed with dry KBr. The mixture was ground into a fine powder using mortar and then compressed into a KBr discs in a hydraulic press at a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of  $2\text{ cm}^{-1}$  using Happ-Genzel apodization. The characteristic peaks were recorded.

### X-Ray diffraction studies (XRD):

The spectra were recorded using a Philips, PW-171, x-ray diffractometer with Cu-NF filtered  $\text{CuK}\bar{\alpha}$  radiation. Quartz was used as an internal standard for calibration. The powder x-ray diffractometer was attached to a digital graphical assembly and computer with Cu-NF 25 KV/20 mA tube as a  $\text{CuK}\bar{\alpha}$  radiation source in the  $2\bar{\alpha}$  range  $0-50^{\circ}$ .

### In-vitro drug release studies:

The *in-vitro* release rate of AZT from resin-coated microspheres were carried out for 24 hours using basket type dissolution apparatus (USP-XXIII Electrolab, Mumbai) containing 900 ml of simulated gastric fluid maintained at  $37\pm0.5^{\circ}\text{C}$  and speed of agitation at 50 rpm and dissolution was carried out for 2 hours. Then the dissolution medium was changed to simulated intestinal (phosphate buffer pH 7.4) fluid, and the process was further continued for up to 24 hours.

After suitable dilutions, the samples were analyzed spectrophotometrically at 260 nm. The release data obtained were fitted into various mathematical models like zero order, first order, Higuchi, Korsmeyer-Peppas.

## RESULTS AND DISCUSSION

### Particle size and entrapment efficiency

The mean size range was found for formulation D: P1, D: P2, D: P3 in the range of  $16.88$ ,  $18.51$ , and  $21.72\text{ }\mu\text{m}$  respectively. Entrapment efficiency was found to be in the range of 67.41 to 80.32 % and increases with increase in core: coat ratio.

### SEM

Prepared microspheres were spherical and completely covered with polymer coat. The surface of the drug-loaded resin microspheres was rough manifested the presence of drug particles (fig:2), while the microphotographs of drug free (dummy) microspheres shows smooth porous surface (fig.3). All the microspheres had small pores on their surfaces, which will be responsible for control drug release.

### DSC

AZT exhibited a sharp endothermic peak at  $127.49^{\circ}\text{C}$ , which corresponds to its melting point. The peak of the drug in the formulation has shifted slightly to  $123.62^{\circ}\text{C}$ , alongwith one more small endothermic peak at  $140.74^{\circ}\text{C}$  has appeared may be due to some impurities in the formulation. The exothermic peak in the thermogram of both pure drug and formulation was appeared, this may be due to the presence of moisture (fig:4).

### FTIR

Drug polymer interaction was also studied by FTIR analysis (fig.5) shows the IR spectra of pure AZT. The characteristic C-N(amine) stretching, NH stretching, C=O stretching and azide group stretching of pure drug was observed at  $1144\text{ cm}^{-1}$ ,  $3350\text{ cm}^{-1}$ ,  $1667\text{ cm}^{-1}$  and  $2012\text{ cm}^{-1}$  respectively. The characteristic peaks confirmed the structure of AZT. The same peaks were also reported in drug loaded microspheres, whereas, absent in blank (dummy) polymeric microspheres prepared using resin fraction. There was no change or shifting of characteristic peaks of AZT in drug loaded microspheres

suggested that there was no significant drug polymer interaction which indicates the stable nature of drug in formulations.

#### PXRD

PXRD technique was used to define the nature of drug in the microparticles. The X-ray diffraction studies are useful to investigate the crystallinity of the drugs after entrapment into the dosage forms. AZT has shown characteristic intense peaks between the  $2\theta$  of  $18^\circ$  and  $42^\circ$  due to its crystalline nature (fig.6). Whereas, in case of drug free and drug loaded microspheres, no intense peaks of drug were noticed between the  $2\theta$  of  $18^\circ$  and  $42^\circ$ . This indicates the amorphous dispersion of the drug after entrapment into microspheres.

#### In-vitro release

The cumulative *in-vitro* release studies of different formulations of AZT were performed in pH 1.2 buffer for first 2 hrs. Then buffer was replaced with pH 7.4 after 2 hrs and release was studied for further 24 hrs. The cumulative release of AZT significantly decreased with increasing polymer (resin fraction) concentration (Table.2). The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix.

Among all the formulations, microspheres prepared using

Sr. No.	Time (hrs)	Formulations (% CPR)		
		D:P 1	D:P 2	D:P 3
1	0	0	0	0
2	0.5	5.82	4.62	3.77
3	1	8.31	7.20	6.17
4	2	13.45	12.43	10.97
5	4	21.68	20.91	18.94
6	6	30.94	28.80	27.09
7	8	43.63	42.69	40.89
8	10	55.21	53.66	52.12
9	12	70.55	68.92	67.38
10	15	82.12	79.55	77.50
11	18	87.27	86.67	86.07
12	24	98.41	95.84	92.41

drug: polymer ratio (10:3) showed good results and showed control drug release for more than 24 hrs (92.41%).

The release data were fitted into various kinetic models such as, zero order, Higuchi<sup>11</sup>, and Korsmeyer-Peppas<sup>12</sup>, in order to find out the mechanism of drug release from polymeric spheres. The correlation coefficient, rate constant and diffusion coefficient were calculated (Table.3).

Table.3: Correlation coefficient ( $r^2$ ), Constant (K) and Diffusion exponent (n) after fitting of dissolution data into various release kinetic models

Formulatio	ZERO ORDER		HIGUCHI		KORSMEYER-PEPPAS	
	$r^2$	K <sub>0</sub>	$r^2$	k <sub>h</sub>	$r^2$	N
D:P1	0.946	0.082	0.884	2.290	0.981	0.803
D:P2	0.950	0.080	0.873	2.221	0.984	0.853
D:P3	0.950	0.077	0.858	2.147	0.985	0.904

Analysis of the release data as per zero order kinetic model is best suited to describe release rate of drug from the microspheres. When the release data was analyzed as per peppas equation, the release exponent 'n' was in the range of 0.803 to 0.904 with all the microspheres indicating non-fickian diffusion as the release mechanism. Plots of percent release Vs square root of time (Higuchi's plots) were found to be linear ( $R^2 > 0.884$ ) indicating that the dug release from the microspheres was by non-fickian diffusion mechanism.

AZT release from the resin-coated microspheres was slow and extended over longer period of time and dependant on core: coat ratio, and size of the microspheres. It was found that the drug release from microspheres was more controlled when drug: polymer ratio was high (fig.1).

Fig. 1: *In-vitro* drug release profile of AZT loaded microspheres

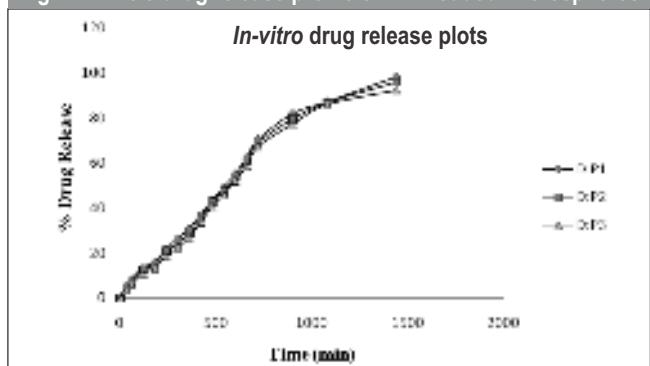


Fig.2: SEM image of resin containing microsphere of AZT

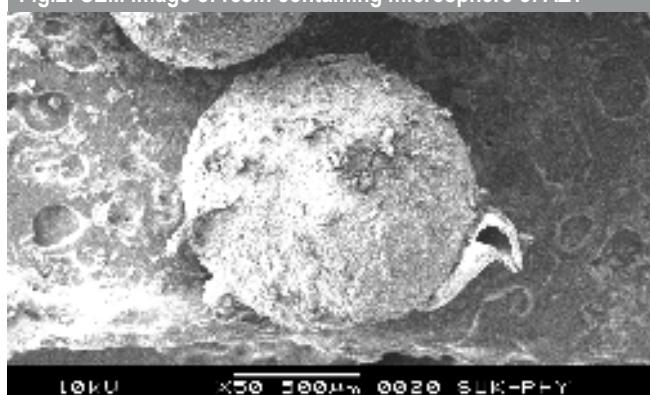


Fig.3: SEM image of drug free (dummy) microsphere

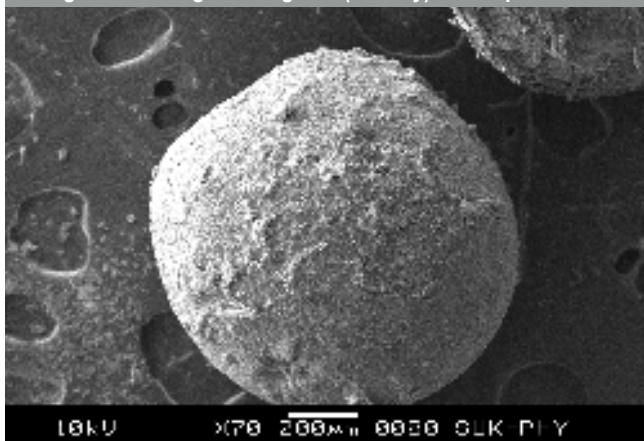


Fig.4: DSC curves of AZT, resin and drug loaded resin microspheres

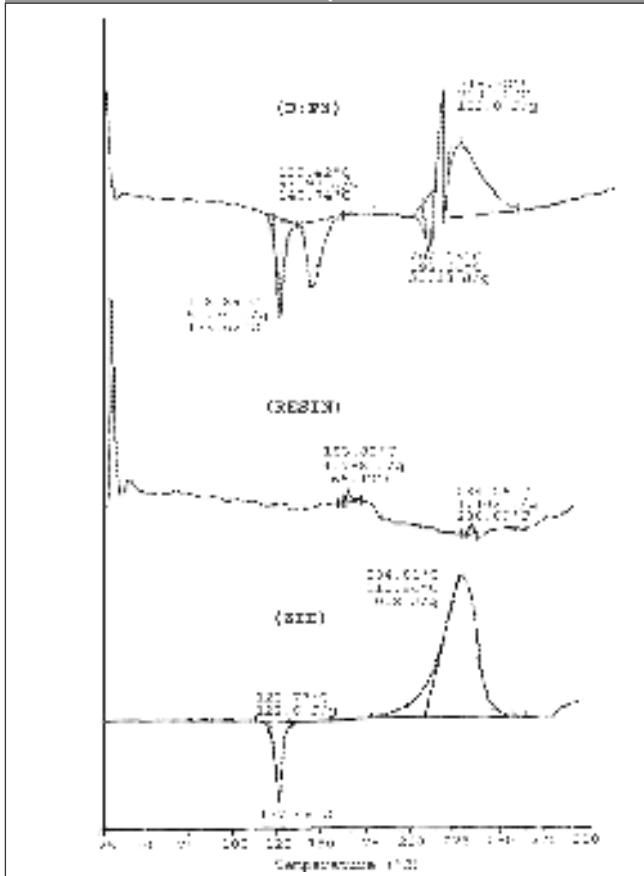


Fig.5: FTIR spectrum of AZT, resin and drug loaded resin microspheres

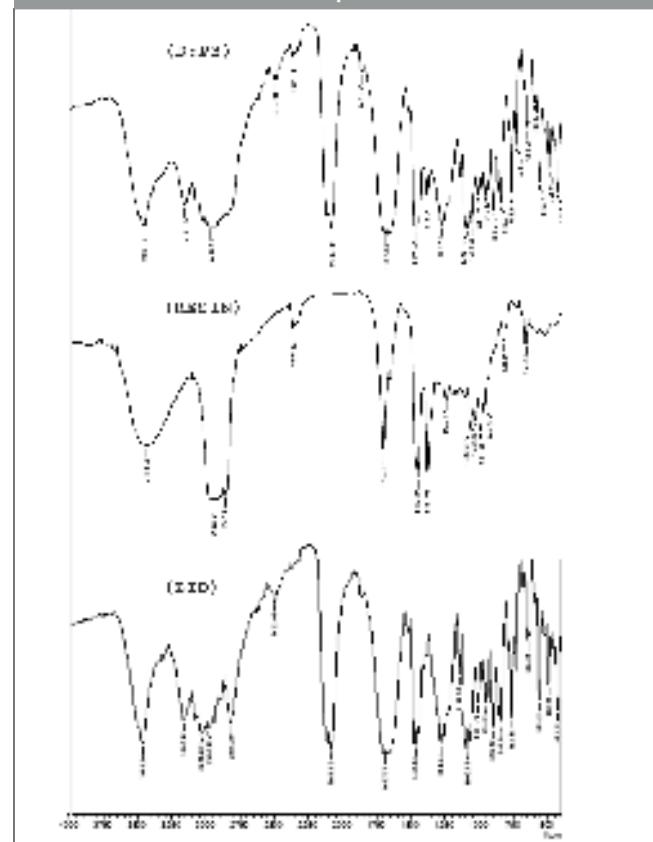
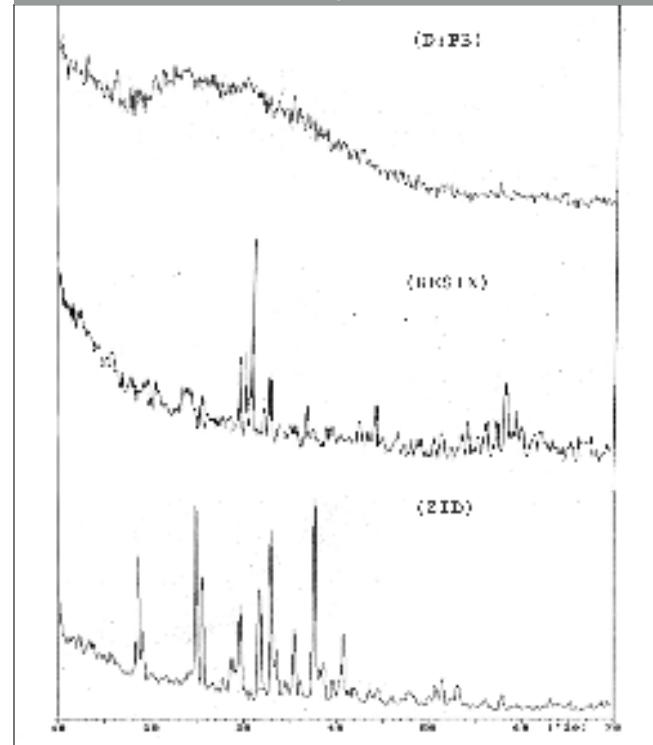


Fig.6: XRD spectrum of AZT, resin and drug loaded resin microspheres



## CONCLUSION

Olibanum resin was found suitable as microencapsulating agent and the resin-coated microspheres exhibited good controlled release characteristics and were found suitable for oral controlled release products. The spray drying technique found to be an excellent approach in the design of controlled release microspheres of AZT.

## REFERENCES

1. Nicholas GL, sustained release dosage forms, in Leon Lachman, The theory and practice of industrial pharmacy, 3<sup>rd</sup> edition, page no: 230.
2. Longer MA, Robinson JR. 1990. In Remington Pharmaceutical Science, Eighteenth Ed<sup>n</sup>. Mack Publishing Company, Eastern Pennsylvania: 18042, 1676-86.
3. Kieburtz KD, Seidlin M, Lambert JS, Dolin R, Reichman R. Extended follow-up of peripheral neuropathy in patients with AIDS and AIDS-related complex treated with dideoxyinosine, J. Acquir. Immune Defic. Syndr. 1992; 5: 60-4.
4. Escalante S, France M. A reduced dose of zidovudine in patients with AIDS. N Engl J Med. 1991; 324: 995.
5. Richman DD, Fischl MA, Grieco MH. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. 1987; 297: 192-7.
6. Patil JS, Kadam DV, Marapur S.C., Kamalapur M.V. Pharmaceutical and medicinal applications of Olibanum gum and its constituents : A review. J. Pharm Res. 2010; 3(3): 587-89.
7. Chowdary KPR, Mohapatra P. Evaluation of Olibanum and its resin as rate controlling matrix for controlled release of diclofenac. Indian J. Pharm. Sci., 2006, 68 (4): 497-500.
8. Shinde ML, Virag A, Ghodke DS. Impact of formulation and process variable on the preparation of acyclovir microspheres by spray drying using factorial design. Int. J. Res. Pharm. Sci. 2010; 1(3): 353-7.
9. Das MK, Rao RK. Microentrapment of Zidovudine by double emulsion solvent diffusion technique using ethylcellulose. Ind J. Pharm Sci. 2007; 69(2): 244-50.
10. Gowda DV. Preparation and evaluation of waxes/fat microspheres loaded with lithium carbonate for controlled release. Ind. J. Pharm. Sci. 2007; 69(2): 251-6.
11. Costa P, Lobo JM. Review – modeling and comparison of diffusion profiles. Eur. J. Pharm. Sci. 2001; 13: 123-33.
12. Peppas NA. Analysis of Fickian and non fickian drug release from polymers. Pharma Acta. Helv. 1985; 60: 110-11.

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