Research Paper

# Microencapsulation of Zidovudine by Double Emulsion Solvent Diffusion Technique using Ethylcellulose

M. K. DAS* AND K. RAMA RAO
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh - 786 004, India.

Zidovudine-ethylcellulose microspheres were prepared by water-in-oil-in-oil double emulsion solvent diffusion method. Spherical free flowing microspheres having an entrapment efficiency of 32-54% were obtained. The effect of polymer-drug ratio, surfactant concentration for secondary emulsification process, volume of processing medium and stirring speed of secondary emulsification process was evaluated with respect to entrapment efficiency and *in vitro* drug release behaviors. Infrared spectroscopy and differential scanning calorimetric analysis confirmed the absence of any drug polymer interaction. The *in vitro* release profile could be altered significantly by changing various processing and formulation parameters to give a controlled release of drug from the microspheres. The *in vitro* release profiles from microspheres of different polymer-drug ratios were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in higuchi model, indicating diffusion-controlled principle. The *n* value varies between 0.23-0.54 obtained from korsemeyer-peppas model confirmed that the mechanism of drug release was diffusion controlled.



Microencapsulation is defined as the application of a thin coating to individual core materials that have an arbitrary particle size range from 5 to 5000 μm[1-2]. It is used to modify and retard drug release[3-4]. Microencapsulation may improve the absorption of a drug and reduce side effects such as irritation of the gastrointestinal mucosa[5]. Zidovudine (azidothymidine, AZT) is widely used for the treatment of Acquired Immuno Deficiency Syndrome (AIDS) and related conditions, either alone or in combination with other antiviral agents. AZT has low oral bioavailability (60%) due to considerable first-pass metabolism and a high clearance, thus necessitating frequent administration of large doses (200 mg every 4-6 h) to maintain therapeutic drug levels[6]. However patients receiving AZT frequently develop anemia and leukopenia[7-9]. The side effects of AZT are dose dependent and a reduction of the total administered dose reduces the severity of the toxicity[10]. Thus the short half-life of 1 h and frequent dosing of large doses due to low oral bioavailability makes the AZT, a good candidate for microencapsulation. Microencapsulation of AZT provides the prolonged release of a single dose, thereby minimizing the frequent administration and hence total dose required to elicit pharmacological activity, thereby reducing the side effects.

Ethylcellulose, a non-biodegradable and biocompatible polymer, is an extensively studied encapsulating material for the controlled release of pharmaceuticals. Several researchers have investigated the utilization of ethylcellulose as a polymer to microencapsulate a drug by coacervation phase separation technique[11-14], emulsion solvent evaporation technique[15-16], spherical crystallization technique[17]. The use of w/o/w double emulsion solvent evaporation method[18] to microencapsulate AZT using poly (lactide/glycolide, PLGA) as the polymer was reported with entrapment efficiency of only 5%. The maximum entrapment of only 17% after modifying the secondary aqueous phase was also reported for AZT[19]. The purpose of the present work was to prepare and evaluate oral controlled release microparticulate drug delivery system of AZT using ethylcellulose by w/o/o double emulsion solvent diffusion method. Various process and formulation parameters such as drug polymer ratio, stirring speed, surfactant concentration, and volume of processing medium were optimized to maximize the entrapment. These microspheres were evaluated for drug content and *in vitro* drug release. Drug-polymer interactions in the solid state were studied by infrared

***For correspondence**
E-mail: du_mkd@yahoo.co.in

spectrophotometry (IR), differential scanning calorimetry (DSC) and ultraviolet spectroscopy (UV). The surface characteristics were evaluated by scanning electron microscopy (SEM).

## MATERIALS AND METHODS

Zidovudine was received as a gift sample from Cipla Ltd., Mumbai. Ethylcellulose (14 cps viscosity grade, Central Drug House, Mumbai), dichloromethane (Ranbaxy Fine Chemicals, New Delhi), acetonitrile (Lobachem, Mumbai), light liquid paraffin (Thomas Baker, Mumbai) and n-hexane (BDH, Mumbai) were also used in the study. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

**Preparation of microspheres:**

All microspheres were prepared by the w/o/o double emulsion solvent diffusion method[20]. Weighed amounts of ethylcellulose and AZT were dissolved in 5 ml of a mixture of acetonitrile and dichloromethane (1:1). The initial w/o emulsion was formed by adding 2 ml of deionised water to the drug-polymer solution with constant stirring at 500 rpm for 5 min. The w/o primary emulsion was then slowly added to light liquid paraffin containing span 80 as a surfactant with constant stirring for 2 h. The n-hexane (10 ml) was added to harden the formed microspheres and the stirring was further continued for 1 h. The resulting microspheres were separated by filtration, freed from liquid paraffin by repeated washing with n-hexane (50 ml) and finally air dried over a period of 12 h.

**Variation of formulation factors:**

Different ethylcellulose:AZT ratios (1:0.25, 1:0.5, 1:0.75 and 1:1) were used in order to investigate the effect of polymer:drug ratio on release and the physical characterization of microspheres. The various concentrations of span 80 (0.5%, 1% and 2%) were used to investigate the influence of surfactant concentrations on release and the physical characterization of microspheres. The effect of stirring rate (500, 1000 and 1500 rpm) and the volume of processing medium (light liquid paraffin, 50, 100 and 200 ml) on microspheres characteristics were investigated.

**Physical characterization of microspheres:**

A weighed quantity of microspheres were crushed into powder and added to 100 ml of phosphate buffer of pH 7.4. The resulting mixture was kept stirring at 1000 rpm for 2 h. Then the solution was filtered through membrane filter (0.45 μm pore size) and 1 ml of this solution was diluted using phosphate buffer of pH 7.4 and analyzed spectrophotometrically for AZT content at 266 nm. The drug entrapment efficiency was determined using the relationship: Drug entrapment efficiency= experimental drug content x100/theoretical drug content.

The shape and surface morphologies of the blank microspheres, drug-loaded microspheres and microspheres collected after release study were investigated using scanning electron microscope (JEOL, JSM-6360) at 15 kV. Prior to examination samples were gold coated under vacuum (Fine coat, Ion Sputter, JFC-1100) render them electrically conductive.

**Interaction studies:**

Drug-polymer interactions were studied by UV spectroscopy, IR spectroscopy and DSC analysis. The UV spectra of a solution of pure AZT in phosphate buffer of pH 7.4 and the solution prepared for the determination of drug entrapment efficiency of drug-loaded microspheres were recorded in the range of 200 to 400 nm using Hitachi U-2001 UV-Vis spectrophotometer. The IR spectra were recorded for pure AZT, blank microspheres and AZT-loaded microspheres using Perkin Elmer-883 IR spectrophotometer. The scanning range was 400 to 4000 $cm^{-1}$. The DSC analysis of pure AZT, blank microspheres and drug-loaded microspheres were carried out in the heating range of 25 to $250^0$ at a rate of $10^0$ $min^{-1}$ using Universal V 2.5 H differential scanning calorimeter.

***In vitro* release studies:**

The USP basket-type dissolution rate test apparatus was used for all the *in vitro* release studies. A weighed quantity of the microspheres (355 μm size fraction) was suspended in 500 ml of phosphate buffer of pH 7.4. The dissolution medium was stirred at 100 rpm and maintained at constant temperature ($37\pm1^0$). At preset time intervals 5 ml aliquots were withdrawn and replaced by an equal volume of fresh prewarmed dissolution medium. After suitable dilution, the samples were analyzed at 266 nm using Hitachi U-2001 UV-Vis spectrophotometer. The concentrations of AZT in samples were corrected to compensate the loss due to sample withdrawal, using the equation proposed by Hayton and Chen[21].

**Release kinetics[22]:**

In order to investigate the mechanism of AZT release from microspheres of different EC:AZT (1:0.25, 1:0.5, 1:0.75 and 1:1) ratios, the release data were analyzed with the

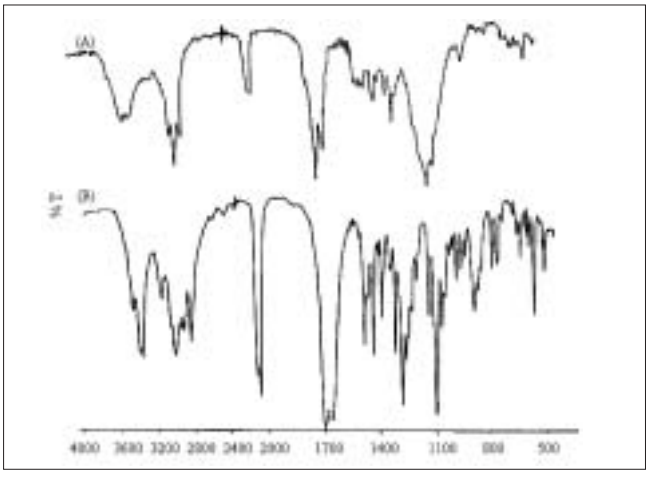
following mathematical models, zero-order kinetic (Eqn.1), first-order kinetic (Eqn. 2) and Higuchi kinetic (Eqn. 3); $Q_t = K_0\ t$-(1), $\ln Q_t = \ln Q_0 - K_1\ t$-(2) and $Q_t = K_h\ t^{1/2}$-(3). The following plots were made, $Q_t$ vs. t (zero-order kinetic model), $\ln (Q_0 - Q_t)$ vs. t (first-order kinetic model) and $Q_t$ vs. $t^{1/2}$ (Higuchi model), where $Q_t$ is the percent of drug released at time t, $Q_0$ is the initial amoumt of drug present in the microspheres and $K_0$, $K_1$ and $K_h$ are the constant of the equations.

Further to confirm the mechanism of drug release, the first 60% of drug release was fitted in Korsmeyer-Peppas model (Eqn. 4), which is, $M_t/M_á = K_p t^n$-(4), where $M_t/M_á$ is the fraction of the drug release at time t and $K_p$ is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released ($M_t/M_á$) vs. log of time (t).

## RESULTS AND DISCUSSION

AZT, due to its hydrophilicity, is likely to preferentially partition out into the aqueous medium, leading to low entrapment efficiency, when encapsulated using aqueous phase as the processing medium[23]. Depending on the processing conditions as much as 80% of the AZT can partition out into the outer processing medium[19]. In our study, attempt was made to encapsulate AZT with sufficiently high entrapment efficiency by w/o/o double emulsion solvent diffusion method using a non-aqueous processing medium. The primary requirement of this method to obtain microspheres is that the selected solvent system for polymer be immiscible with non-aqueous processing medium[24]. Acetonitrile is a unique organic solvent, which is polar, water miscible and oil immiscible. All other polar solvents like methanol, ethanol, ethyl acetate, acetone and dimethyl sulfoxide are oil miscible and will not form emulsions of the polymer solution in oil. With oil as the processing medium, use of acetonitrile alone as a solvent did not ensure formation of primary emulsion of the aqueous phase in the polymer solution. Immediately on mixing, the water miscibility of the acetonitrile brought about the precipitation of the polymer (ethylcellulose). Hence, a non-polar solvent, namely dichloromethane was included with acetonitrile to decrease the polarity of the polymer solution. Additionally, it was also desirable that the second solvent be oil miscible, so that solvent removal is facilitated through extraction by processing medium leaving behind a viscous polymer solution. The optimal proportion of acetonitrile and dichloromethane was found to be 1:1, which enabled emulsion formation and yielded good microspheres. No surfactant was used for stabilizing primary emulsion, since ethylcellulose has the additional property of stabilizing w/o emulsion[25]. Span 80, a representative of the nonionic dispersing agent, was used to stabilize the secondary emulsification process. It has the HLB value of 4.3 and is expected to have a high disparity for the present emulsion system by reducing the surface tension at the interface.

The compatibility of AZT in ethylcellulose microspheres was evaluated through UV spectroscopy, IR spectroscopy and DSC analysis. The UV spectra of pure drug solution and the dissolution medium after drug release study were identical and the characteristics $\lambda_{max}$ of pure AZT was appeared at 266 nm on the UV spectra of the dissolution medium after drug release study. It indicates no drug-polymer interactions. This was further confirmed by IR spectroscopy and DSC analysis. The study of IR spectra of AZT-loaded ethylcellulose microspheres (fig. 1a) demonstrated that the characteristics absorption peaks for carbonyl group stretching vibration and azide group stretching vibration were appeared at 1694 $cm^{-1}$ and 2102 $cm^{-1}$, respectively. The absorption peaks were almost similar to those obtained from the pure drug (fig. 1b) and to the reported values[26]. The DSC curves of pure AZT, AZT-loaded ethylcellulose microspheres and blank ethylcellulose microspheres are presented in fig. 2. It was evident from the DSC profile (fig. 2a) that AZT exhibited a sharp endothermic peak at $124^0$ which corresponds to the reported melting temperature of the drug. The same DSC profile (fig. 2b) of the drug appeared at the temperature corresponding to its melting point in the AZT-loaded ethylcellulose microspheres but with the loss




**Wavenumber ($CM^{-1}$)**

**Fig. 1: IR Spectra: (A) zidovudine-loaded microspheres and (B) pure zidovudine.**

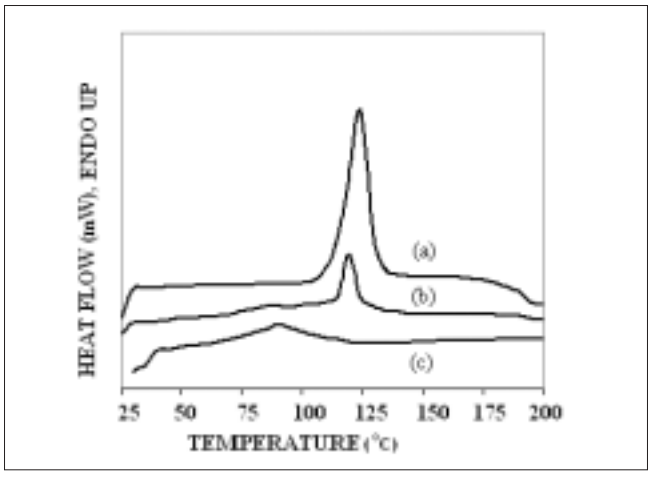



**Fig. 2: DSC thermograms**
**DSC thermograms of (a) pure zidovudine, (b) zidovudine-loaded ethylcellulose microspheres and (c) ethylcellulose blank microspheres.**

of its sharp appearance. It appears that there is a significant reduction of drug crystallinity in the microspheres. The DSC profile of the blank microspheres (fig. 2c) did not exhibit endothermic peak at $124^0$. These studies apparently revealed that the drug was compatible with the polymer and neither drug decomposition nor drug-polymer interactions occurred in the freshly prepared microspheres.

The surface topography of the microspheres was investigated by SEM. As seen in fig. 3, they were spherical in shape and exhibited porous surfaces. The SEM of drug-loaded microspheres in fig. 3a had rough surface due to higher concentration of drug in the microspheres as compared to the blank microspheres (fig. 3a, 3b). Surface study of the microspheres after release study showed bigger pores (fig. 3c) suggesting that the drug was released through pores and the mechanism of drug release was diffusion controlled.

The effects of various process and formulation parameters on the drug entrapment efficiency of microspheres are shown in Table 1. The highest (54%) entrapment efficiency was achieved by increasing polymer-drug ratio from 1:0.25 to 1:0.50. With further increase in polymer-drug ratio from 1:0.50 to 1:1, a statistically significant decrease ($P<0.05$, Student's t-test) was observed on encapsulation efficiency of AZT. The higher drug loading typically results in lower encapsulation efficiency due to higher concentration gradients resulting the drug to diffuse out of the polymer/solvent droplets to the external processing medium. The viscosity of the polymer solution at higher drug loading was very high and was responsible for the formation of larger polymer/solvent droplets. It caused a decrease rate of entrapment of drug due to slower hardening of the larger particles, allowing time for drug diffusion out of the particles, which tends to decrease encapsulation efficiency. Among the different polymer-drug ratios investigated, 1:0.50 polymer-drug ratio had the optimum capacity for drug encapsulation. Keeping the drug-polymer ratio constant, there was a statistically significant ($P<0.05$, student's t-test) decrease in encapsulation efficiency of AZT with increasing the concentration of surfactant for secondary emulsification. This may be due to the fact that the increase in surfactant concentration proportionately increases miscibility of acetonitrile with light liquid paraffin (processing medium), which may increase the extraction of AZT into the processing medium. The volume of processing medium significantly influences the entrapment efficiency of AZT microspheres (Table 1). As the volume of the processing medium was increased from 50 ml to 100 ml and to 200 ml, the entrapment efficiency significantly decreased ($P<0.05$, Student's t-test) from 54% to 44% and 32%, respectively. As the volume of processing medium was increased, the emulsion droplets probably moved freely in the medium, thus reducing collision induced aggregation and yielding small and uniform microspheres. This could also be the



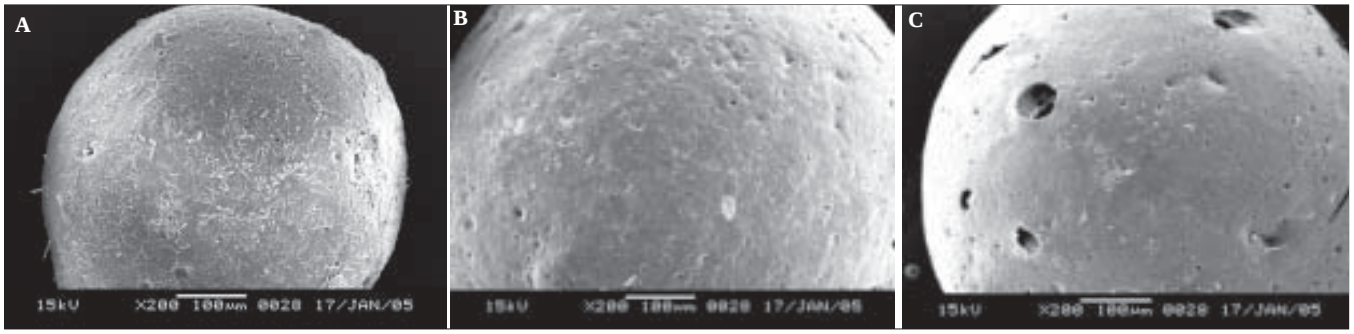



**Fig. 3: Scanning electron micrographs**
**Scanning electron micrographs of a) zidovudine loaded microspheres; b): blank microspheres and c): microspheres collected after release study.**



A

**TABLE 1: EFFECT OF VARIOUS PARAMETERS ON ENTRAPMENT EFFICIENCY**

| Processing and formulation parameters | Theoretical drug content (%) | Experimental drug content (%) | Entrapment efficiency (%) |
|---|---|---|---|
| EC: AZT ratio | | | |
| 1 : 0.25 | 20 | 8.60 | 43.00±1.87 |
| 1 : 0.50 | 33.33 | 17.99 | 53.98±0.58 |
| 1 : 0.75 | 42.86 | 18 | 41.99±1.31 |
| 1 : 1 | 50 | 22.50 | 45.00±0.77 |
| Surfactant (Span 80) | | | |
| Concentration (% w/v) | | | |
| 0.5 | 33.33 | 17.99 | 53.98±0.58 |
| 1.0 | 33.33 | 14.67 | 44.01±0.98 |
| 2.0 | 33.33 | 10.67 | 32.01±0.67 |
| Volume of processing medium (ml) | | | |
| 50 | 33.33 | 17.99 | 53.98±0.58 |
| 100 | 33.33 | 14.67 | 44.01±0.78 |
| 200 | 33.33 | 10.67 | 32.01±1.67 |
| Stirring speed of secondary emulsification (rpm) | | | |
| 500 | 33.33 | 12.99 | 38.97±1.22 |
| 1000 | 33.33 | 17.99 | 53.98±0.58 |
| 1500 | 33.33 | 12.99 | 38.97±0.96 |

**TABLE 2: *IN VITRO* RELEASE KINETIC PARAMETERS OF AZT-LOADED ETHYLCELLULOSE MICROSPHERES**

| Polymer-drug ratio | Kinetic models | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Zero-order | | First-order | | Higuchi model | | Korsemeyer-peppas model | |
| | $R^2$ | $K_0$ (% mg/h) | $R^2$ | $K_1$ ($h^{-1}$) | $R^2$ | $K_h$(% $mg/h^{1/2}$) | $R^2$ | n |
| 1 : 0.25 | 0.9611 | 3.73 | 0.9942 | 0.0469 | 0.9982 | 20.90 | 0.9957 | 0.55 |
| 1 : 0.5 | 0.9689 | 3.97 | 0.9692 | 0.0892 | 0.9955 | 22.09 | 0.9977 | 0.42 |
| 1 : 0.75 | 0.9222 | 4.63 | 0.9681 | 0.1251 | 0.9716 | 23.61 | 0.9859 | 0.40 |
| 1 : 1 | 0.9644 | 5.60 | 0.9727 | 0.2057 | 0.9883 | 23.59 | 0.9934 | 0.23 |

$R^2$ is the coefficient of correlation; $K_0$, $K_1$ and $K_h$ are the release rate constants for zero-order, first-order and higuchi model, respectively and n is the release exponent of korsemeyer-peppas model.



reason for higher drug extraction into the processing medium resulting in lower entrapment efficiency. The encapsulation efficiency was also influenced with changing the stirring speed of the second emulsification process. The highest entrapment efficiency was observed with the stirring speed of 1000 rpm. The change of stirring speed from 1000 rpm to 500 and 1500 rpm significantly decrease the entrapment efficiency due to the formation of larger and smaller emulsion droplets, respectively, ensuring drug diffusion out of the microspheres before they harden. The assumption of drug diffusion to the processing medium was supported by SEM analysis, which showed the presence of drug particles on the surface of the microspheres (fig. 3).

The *in vitro* release of AZT from ethyl cellulose microspheres exhibited initial burst effect, which was due to the presence of drug particles on the surface of the microspheres. The initial burst effect may be attributed as a desired effect to ensure initial therapeutic plasma concentrations of drug. The release profiles are illustrated in Fig. 4a-4d. Factors such as polymer-drug ratio, surfactant concentration for secondary emulsification, volume of processing medium and stirring speed of secondary emulsification govern the drug release from microspheres. In order to keep the total surface area of the microspheres constant and thus to get comparable results, the release studies were carried out using same size fractions of microspheres containing equivalent amount of AZT from different batches. Drug release rates increased with increasing amounts of AZT in the formulation. Higher level of AZT corresponding to lower level of the polymer in the formulation resulted in an increase in the drug release rate. As more drug is released from the microspheres, more channels are probably produced, contributing to faster drug release rates. In addition, higher drug levels in the microsphere formulation produced a higher drug concentration gradient between the microspheres and dissolution medium, thus drug release rate was increased. As the concentration of Span 80 increased a faster drug release was observed. This may be attributed to the presence of more free drug on the surface of the microspheres with increasing the concentration of Span 80 used for

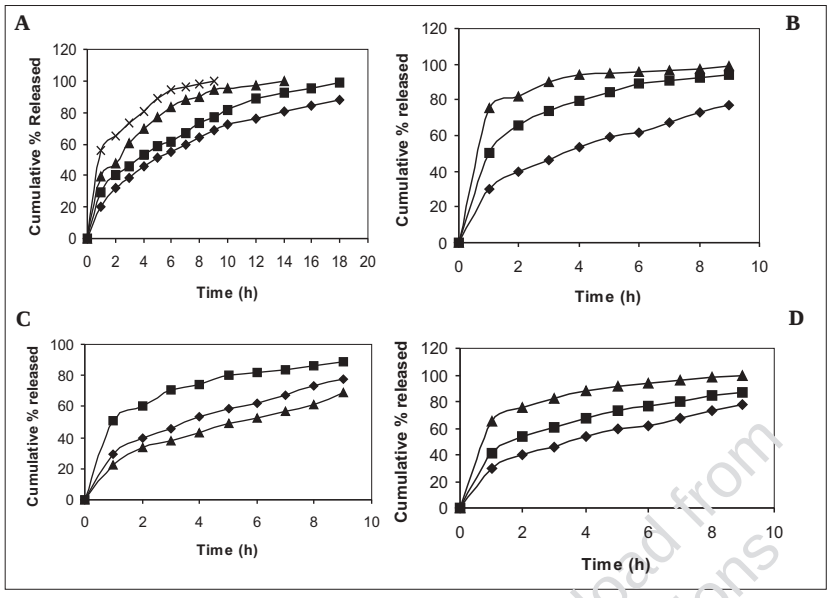



**Fig. 4: Effect of various conditions on *in vitro* drug release**
**a) Effect of polymer-drug ratio on *in vitro* drug release profile. Cumulative % AZT release from various microspheres carrying different polymer-drug ratios, 1:0.25 (-♦-), 1:0.5 (-■-), 1:0.75 (-▲-) and 1:1 (-x-). Encapsulation conditions were surfactant (Span 80) concentration- 0.5% w/v; volume of processing medium- 50 ml; stirring speed- 1000 rpm. b) Effect of surfactant (Span 80) on *in vitro* AZT release from microspheres carrying polymer-drug ratios of 1:0.5: (-♦-) 0.5% w/v, (-■-) 1% w/v and (-▲-) 2% w/v. Encapsulation conditions were volume of processing medium- 50 ml and stirring speed-1000 rpm. c) Effect of stirring speed of secondary emulsification process on *in vitro* AZT release from microspheres carrying polymer-drug ratio of 1:0.5: (-♦-) 1000 rpm, (-■-) 500 rpm and (-▲-) 1500 rpm. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5% w/v; volume of processing medium, 50 ml. d): Effect of volume of processing medium on *in vitro* AZT release from microspheres carrying polymer-drug ratio of 1:0.5: (-♦-) 50 ml, (-■-) 100 ml and (-▲-) 200 ml. Encapsulation conditions were surfactant (Span 80) concentration- 0.5% w/v and stirring speed-1000 rpm.**

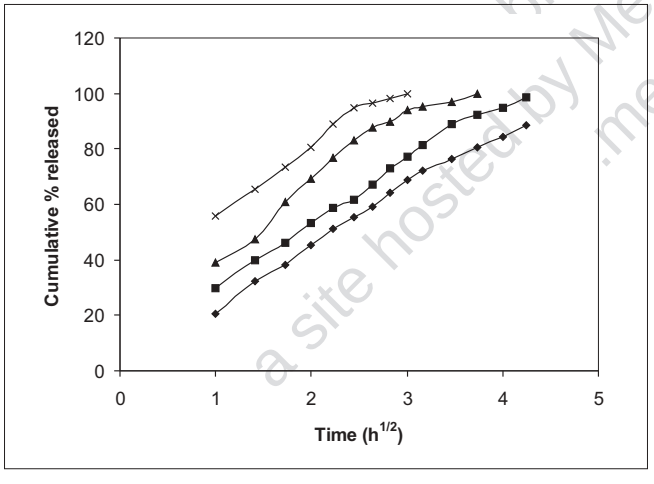



**Fig. 5: Higuchi plot of AZT release from ethylcellulose microspheres.**
**Cumulative % AZT release from various microspheres carrying different polymer-drug ratio: (♦) 1:0.25, (■) 1:0.5, (▲) 1:0.75 and (x) 1:1. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5% w/v; volume of processing medium, 50 ml; stirring speed, 1000 rpm.**

secondary emulsification process. The faster drug release was observed from microspheres prepared using large volume of processing medium. It may be due to the higher migration of drug to the surface of the microspheres during solvent evaporation from the freely moved emulsion droplets in large volume of processing medium. The change of stirring speed of the secondary emulsification process also influenced the drug release profile as shown in fig. 4c.

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in higuchi, first-order and followed by zero-order equations as given in Table 2. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the Higuchi plot (fig. 5) rather than first-order and zero-order models. The drug release was proportional to square root of time, indicating that the drug release from ethyl cellulose microspheres was diffusion controlled. The data obtained were also put in Korsemeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microspheres of different drug to polymer ratio was between 0.23-0.54, indicating the mechanism of the drug release was diffusion controlled. The release also showed higher correlation with the Korsmeyer-Peppas model, as shown in Table 2.

In conclusion, the attempt to prepare controlled release microspheres of zidovudine with increased entrapment efficiency was successful, even though the entrapment efficiency was still lower compared to the same process reported for other hydrophilic drugs. Further studies are required to find out the exact cause for the difference and to improve the entrapment efficiency.

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