

EVALUATION OF ZIDOVUDINE ENCAPSULATED ETHYLCELLULOSE MICROSPHERES PREPARED BY WATER-IN-OIL-IN-OIL (W/O/O) DOUBLE EMULSION SOLVENT DIFFUSION TECHNIQUE

MALAY KUMAR DAS* and KALAKUNTALA RAMA RAO

Department of Pharmaceutical Sciences, Dibrugarh University,
Dibrugarh-786004, Assam, India

Abstract: The preparation of zidovudine-loaded ethylcellulose microspheres by w/o/o double emulsion solvent diffusion method with high entrapment capacity and sustained release is described. A mixed solvent system (MSS) consisting of acetonitrile and dichloromethane in a 1:1 ratio and light liquid paraffin was selected as primary and secondary oil phases, respectively. Span 80 was used as the secondary surfactant for stabilizing the external oil phase. Spherical free flowing microspheres were obtained. The prepared microspheres were characterized by entrapment efficiency, *in vitro* release behavior, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). The drug-loaded microspheres showed 32 – 55% entrapment capacity. The *in vitro* release profile could be altered significantly by changing various processing and formulation parameters to give sustained release of drug from the microspheres. The DSC thermograms confirmed the absence of any drug-polymer interaction. SEM studies showed that the microspheres were spherical and porous in nature. The *in vitro* release profiles from microspheres of different polymer-drug ratios were best fitted to Higuchi model with high correlation coefficient and the *n* value obtained from Korsmeyer-Peppas model was ranged between 0.23 – 0.54. The drug release was found to be diffusion controlled mechanism.

Keywords: Zidovudine, ethylcellulose, microspheres, w/o/o double emulsion, Higuchi model, diffusion-controlled mechanism

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 nm (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 Syndromes (AIDS) and related conditions, either alone or in combination with other antiviral agents. This virustatic drug has low oral bioavailability (60%) due to considerable first-pass metabolism, thus necessitating frequent administration of large doses (200 mg every 4 – 6 h) to maintain therapeutic drug level (6). However, patients receiving AZT frequently develop anemia and leucopenia (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 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, one of the extensively studied encapsulating material for the controlled release of pharmaceuticals, was selected as the retardant material for AZT. 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) and 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 with high entrapment capacity and extended release. Various process

* Corresponding author: e-mail: du_mkd@yahoo.co.inmail.com

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 differential scanning calorimetry (DSC). The surface characteristics were evaluated by scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

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 used. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

Methods

Preparation of microspheres

All microspheres were prepared by the w/o/o double emulsion solvent diffusion method (20). The effect of various formulation and processing factors on microspheres characteristics were investigated by changing polymer-drug ratio, surfactant concentration, stirring speed and the volume of external oil phase. Weighed amounts of ethylcellulose and AZT (different ethylcellulose:AZT ratios were 1:0.25, 1:0.5, 1:0.75 and 1:1) 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 deionized 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 (variable volume of 50 mL, 100 mL and 200 mL) containing Span 80 (variable concentrations of 0.5%, 1% and 2% w/v) as a surfactant with constant stirring (speed variation 500, 1000 and 1500 rpm) 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 decantation, freed from liquid paraffin by repeated washing with n-hexane (3 × 50 mL) and finally air dried over a period of 12 h.

Physical characterization of microspheres

Drug entrapment efficiency: 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 mm 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:

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Microscopy studies: 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) to render them electrically conductive.

Differential scanning calorimetry (DSC)

Drug-polymer interactions were studied by DSC analysis. The DSC analysis of pure AZT, blank microspheres and drug-loaded microspheres were carried out in the heating range of 25 to 250°C at a rate of 10°C min⁻¹ using Universal V 2.5 H Differential scanning calorimeter.

In vitro release studies

The United States Pharmacopoeia basket-type dissolution rate test apparatus was used for all the *in vitro* release studies. A weighed quantity of the microspheres (355 mm 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±1°C). At preset time intervals 5 mL aliquots were withdrawn and replaced by an equal volume of fresh prewarmed dissolution medium maintaining sink condition throughout the experiment. After suitable dilution, the samples were analyzed for drug quantification at 266 nm using Hitachi U-2001 UV-VIS spectrophotometer. The concentrations of AZT in samples were calculated using regression equation (Absorbance = -0.0006 + 0.0383 × concentration, R² = 0.9998) of the calibration curve of AZT in phosphate buffer of pH 7.4 and 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 (equation 1), first-order kinetic (equation 2) and Higuchi kinetic (equation 3).

$$Q_t = K_0 t \quad (1)$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad (2)$$

$$Q_t = K_h t^{1/2} \quad (3)$$

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 amount of drug present in the microspheres and K_0 , K_1 and K_h are the constants of the equations.

Further, to confirm the mechanism of drug release, the first 60 % of drug release was fitted in Korsmeyer-Peppas model (equation 4):

$$M_t / M_\infty = K_p t^n \quad (4)$$

where M_t / M_∞ is the fraction of the drug release at time t , 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_∞) vs. log of time (t).

RESULTS AND DISCUSSIONS

Preparation of microspheres

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 should 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.

Differential scanning calorimetry (DSC)

The compatibility of AZT in ethylcellulose microspheres was evaluated through DSC analysis. The DSC curves of pure AZT, AZT-loaded ethylcellulose microspheres and blank ethylcellulose microspheres are presented in Figure 1. It was evident from the DSC profile (Figure 1a) that AZT exhibited a sharp endothermic peak at 124°C, which corresponds to the reported melting temperature of the drug. The same DSC profile (Figure 1b) of the drug appeared at the temperature corresponding to its melting point in the AZT-loaded ethylcellulose microspheres but with the loss of its sharp appearance. It appears that there is a significant reduction of drug crystallinity in the microspheres. The DSC

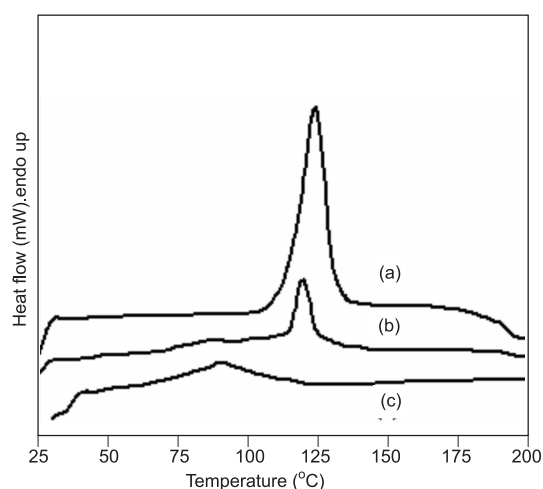


Figure 1. DSC thermograms: (a) pure zidovudine, (b) zidovudine loaded ethylcellulose microspheres and (c) ethylcellulose blank microspheres.

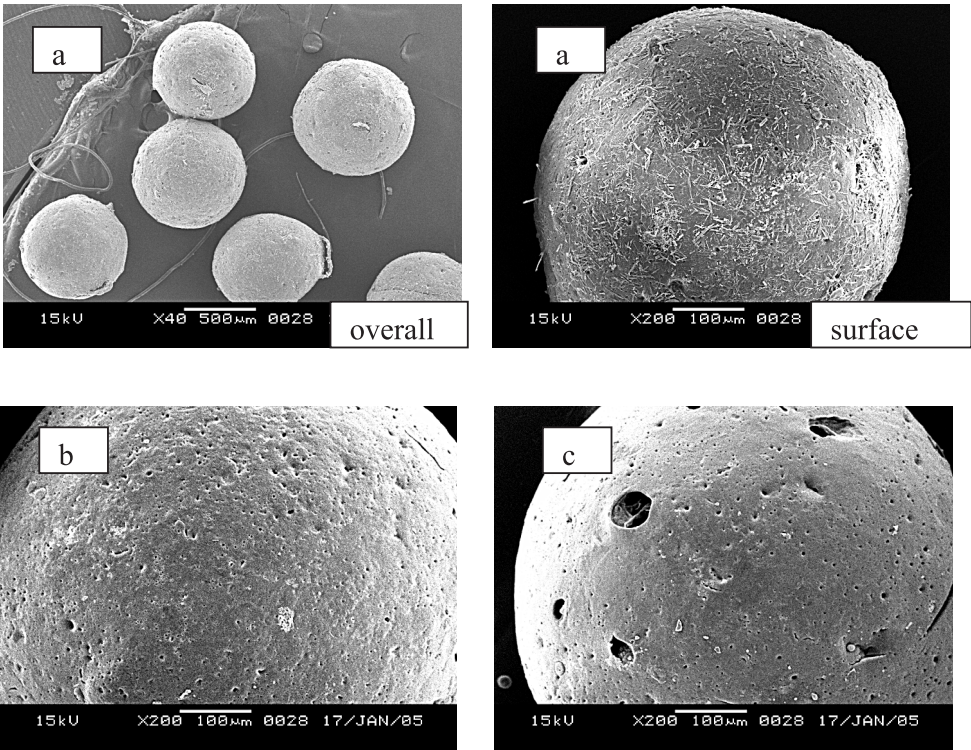


Figure 2. Scanning electron micrographs of (a) zidovudine loaded microspheres, (b) blank microsphere and (c) microspheres collected after release study.

Table 1. Effect of various parameters on drug entrapment efficiency.

Processing and formulation parameters	Theoretical drug content (%)	Experimental drug content (%)	Entrapment efficiency (%) (mean* ± SD)
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

* n = 3

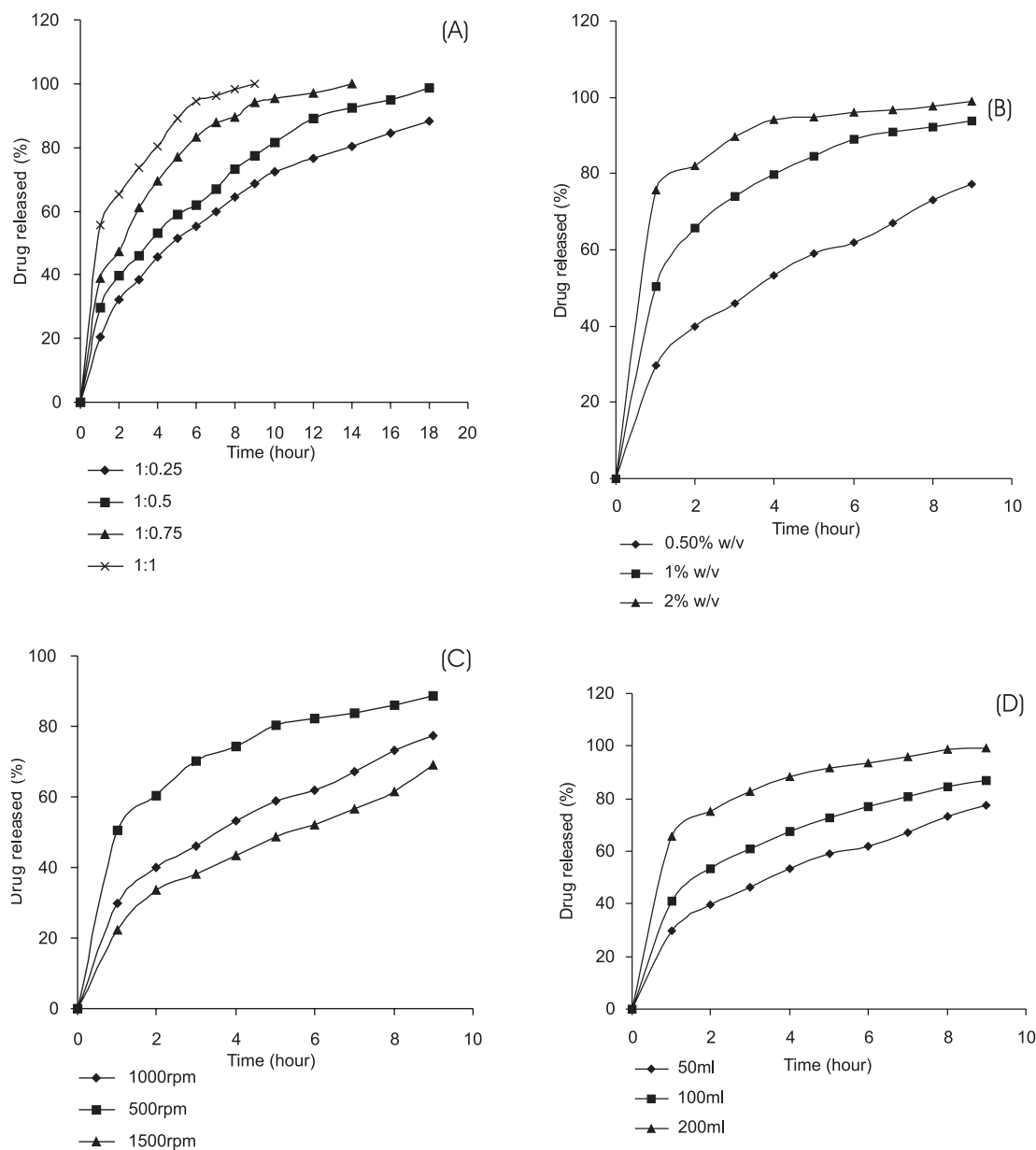


Figure 3 (a): Effect of polymer-drug ratio on *in vitro* zidovudine release profile. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5 % w/v; volume of processing medium, 50 mL; stirring speed, 1000 rpm were kept constant; (b): Effect of variable concentration of Span 80 on *in vitro* AZT release from microspheres. Encapsulation conditions: volume of processing medium, 50 mL; stirring speed, 1000 rpm and polymer-drug ratio, 1:0.5 were kept constant; (c): Effect of stirring speed of secondary emulsification process on *in vitro* AZT release from microspheres. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5 % w/v; volume of processing medium, 50 mL and polymer-drug ratio, 1:0.5 were kept constant; (d): Effect of volume of processing medium on *in vitro* AZT release from microspheres. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5 % w/v; stirring speed, 1000 rpm and polymer-drug ratio, 1:0.5 were kept constant.

profile of the blank microspheres (Figure 1c) did not exhibit endothermic peak at 124°C. The DSC study apparently revealed that the drug was compatible with the polymer and neither drug decomposition nor drug-polymer interactions occurred in the freshly prepared microspheres.

Scanning electron microscopy (SEM) analysis

The surface topography of the microspheres was investigated by SEM. As seen in Figure 2, they were spherical in shape and exhibited porous surfaces. The SEM of drug-loaded microspheres in Figure 2a had rough surface due to higher concen-

tration of drug in the microspheres as compared to the blank microspheres (Figure 2b). Surface study of the microspheres after release study showed bigger pores (Figure 2c) suggesting that the drug was released through pores and the mechanism of drug release was diffusion controlled.

Entrapment efficiency

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 significant decrease 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. And also the viscosity of the polymer solution at higher drug loading was very high and is 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 significant 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 proportionally 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 from 54% to 44% and 32%, respectively. When the volume of processing medium was increased, the emulsion droplets can be moved freely in the medium, and they had very less chance to collide with each other, thereby yielding small and uniform microspheres. It may be the 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

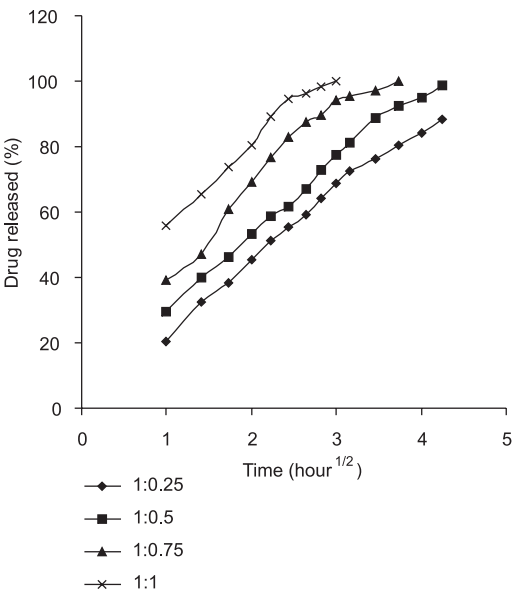


Figure 4. Higuchi plot of AZT release from ethylcellulose microspheres carrying different polymer-drug ratio. Encapsulation conditions: Surfactant (Span 80) concentration, 0.5% w/v; volume of processing medium, 50 mL; stirring speed, 1000 rpm were kept constant.

Table 2. *In vitro* release kinetic parameters of AZT-loaded ethylcellulose microspheres

Polymer-drug ratio	Kinetic models							
	Zero-order		First-order		Higuchi model		Korsmeyer-Peppas model	
	R ²	K ₀ (%/h)	R ²	K ₁ (h ⁻¹)	R ²	K _h (%/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

R² is the coefficient of correlation; K₀, K₁ and K_h are the release rate constants for zero-order, first-order and Higuchi model, respectively and n is the release exponent of Korsmeyer-Peppas model.

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 pores and drug particles on the surface of the microspheres as shown in Figure 2.

In vitro drug release

The *in vitro* release of AZT from ethylcellulose 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 Figure 3a – 3d. 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 the 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 drugs are released from the microspheres, more channels are 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, the 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 for 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 due to free movement of emulsion droplets with increasing volume of processing medium. The change of stirring speed of the secondary emulsification process also influenced the drug release profile as shown in Fig. 3c.

Kinetic modeling of release data

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 (Figure 4) 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 Korsmeyer-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 ranged between 0.23 – 0.54, indicating that 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 high 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.

Acknowledgements

The authors are grateful to the All India Council for Technical Education, New Delhi for granting scholarship and contingency to K. Rama Rao in relation to this study.

REFERENCES

1. Onofrio G.P.D., Oppenheim R.C., Batman N.E.: *Int. J. Pharm.*, 2, 91 (1979).
2. Bakan J.A.: in *The Theory and Practice of Industrial Pharmacy*, Lieberman H.A., Lachman L. and Kanig J.L. Eds., p. 412, Lea and Febiger, Philadelphia, PA 1986.
3. Ertan G.: *J. Microencapsulation*, 14, 379 (1997).
4. Ozyazici M., Sevgi F., Ertan G.: *Int. J. Pharm.*, 138, 25 (1996).
5. Li S.P.: *Drug Dev. Ind. Pharm.*, 14, 353 (1988).
6. Klecker R.W., Collins J.M., Yarchan R., Thomas R., Jenkins J.F., Border S., Myers C.S.: *Clin. Pharmacol. Ther.*, 41, 407 (1987).
7. Richman D.D., Fischl M.A., Grieco M.H., Gottlieb M.S., Voberding P.A., Laskin O.L., Leedom J.M., Groopman J.E., Mildvan D., Hirsch M.S., Jackson G.G., Durack D.T.: *N. Engl. J. Med.*, 317, 192 (1987).

8. Lutton J.D., Mathew A., Levere R.D., Abraham N.G.: *Amer. J. Hematol.*, 35, 1 (1990).
9. Chow F.P.R. and Hamburger A.W.: *Eur. J. Hematol.*, 47, 91 (1991).
10. Colson E.R., Horwitz R.I., Bia F.J., Viscoli C.M.: *Arch. Intern. Med.*, 151, 709 (1991).
11. Sajeev C., Vinay G., Archana R., Saha R.N.: *J. Microencapsulation*, 19, 753 (2002).
12. Wu J.C., Su S.G., Shyu S.S., Chen H.: *J. Microencapsulation*, 11, 297 (1994).
13. Tsai Y.L., Jong C.C., Chen H.: *J. Microencapsulation*, 18, 701 (2001).
14. Singh J., Robinson D.H.: *Drug Dev. Ind. Pharm.*, 14, 545 (1988).
15. Saravanam G., Bhasker K., Srinivasa R.G., Dhanaraju M.D.: *J. Microencapsulation*, 20, 289 (2003).
16. Zinutti C., Kedzierewicz F., Hoffman M., Maincent P.: *J. Microencapsulation*, 11, 555 (1994).
17. Akbuga J.: *Int. J. Pharm.*, 76, 193 (1991).
18. Mandol T.K., Shekleton M., Onyebueke E., Washington L., Penson T.: *J. Microencapsulation*, 13 (5), 545 (1996).
19. Mandol T.K., Tenjarla S.: *Int. J. Pharm.*, 137, 187 (1996).
20. Lee J.H., Park T.G., Choi H.K.: *Int. J. Pharm.*, 196, 75 (2000).
21. Hayton W.L., Chen T.: *J. Pharm. Sci.*, 71, 820 (1982).
22. Costa P. and Lobo J.M.S.: *Eur. J. Pharm. Sci.*, 13, 123 (2001).
23. Mandol T.K., Lopaez-Anaya A., Onyebueke E., Shekleton M.: *J. Microencapsulation*, 13 (3), 257 (1996).
24. Badri V.N., Thomas P.A., Pandit J.K., Kulkarni M.G., Mashelkar R.A.: *J. Control. Release*, 58, 9 (1999).
25. Melzer E., Kreuter J., Daniels R.: *Eur. J. Pharm. Biopharm.*, 56, 23 (2003).

Received: 7.02.2006