ELSEVIER

International Journal of Pharmaceutics 137 (1996) 187–197

international journal of pharmaceutics

Preparation of biodegradable microcapsules of zidovudine using solvent evaporation: Effect of the modification of aqueous phase

Tarun K. Mandal[a,*], Srini Tenjarla[b]

[a]*Division of Pharmaceutics, College of Pharmacy, Xavier University of Louisiana, 7325 Palmetto St., New Orleans, LA 70125, USA*

[b]*Department of Pharmaceutical Sciences, Mercer University, Atlanta, GA 30341, USA*

Received 6 November 1995; revised 3 January 1996; accepted 11 March 1996

Abstract

The objective of the present investigation was to improve the efficiency of encapsulation of zidovudine (AZT) in poly(lactide/gycolide) (PLGA 50:50) by modifying the secondary aqueous phase. Surface morphology of the microcapsules was unchanged during the partial saturation of the aqueous phase with calcium chloride. However, partial saturation of the aqueous phase with AZT and a change in the pH of the aqueous phase showed significant effect on the surface morphology. The surface appeared to be wrinkled when the pH of the aqueous phase was adjusted to 10. The particle size was between 8 and 18 μm. The particle size was increased significantly (78–140 μm) when the aqueous phase was partially saturated with AZT (0.25–0.75%). The efficiency of encapsulation did not change when the aqueous phase was partially saturated with calcium chloride (5–30%). The efficiency of encapsulation was pH dependent. The encapsulation increased up to 17% when the aqueous phase was partially saturated with 0.75% AZT. The cumulative drug release from the microcapsules was between 15 and 34% within the first 24 h. A sustained drug release continued up to 60 days.

Keywords: Biodegradable; Poly(lactide/glycolide); Microcapsules; Zidovudine; Solvent evaporation

1. Introduction

Zidovudine (AZT) has been shown to be effective in prolonging the survival of AIDS patients (Brook, 1987; Devita et al., 1987; Fischl et al., 1990). AZT is typically administered orally as a capsule. The bioavailabilty after oral administration is only 60% due to an extensive hepatic first-pass metabolism, which requires the administration of a high dose of the drug (Douglas, 1990). The half-life of the drug is approximately 1 h (Klecker et al., 1987), which requires the frequent administration of the drug. However, patients receiving AZT frequently develop anemia and leukopenia (Richman et al., 1987; Lutton et al., 1990; Chow and Hamburger, 1991). These

* Corresponding author. Tel.: + 504 483 7442; fax: + 504 488 3108.

0378-5173/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0378-5173(96)04520-6

side effects of AZT are dose-dependent and a reduction of the total administered dose reduces the severity of the toxicity (Colson et al., 1991). The development of a sustained-release parenteral formulation of AZT would be beneficial in comparison with the current intermittent dose regimens. Sustained-release formulation would deliver the drug at a continuous rate, by-pass the first-pass metabolism, and would reduce the dose-dependent toxicity by minimizing the fluctuation in plasma concentrations (Shargel and Yu, 1993).

Biodegradable sustained-release microcapsules have been developed for numerous therapeutic agents (Lewis, 1990). The most widely investigated biodegradable polymers for the microencapsulation of therapeutic agents are the aliphatic polyesters based on lactic acid and glycolic acid (PLGA) (Pitt et al., 1992; Cleland and Langer, 1994; Yan et al., 1994). Although several methods are available for the microencapsulation of drugs, the formation of a double water-in-oil-in-water (w/o/w) emulsion followed by the in-water solvent evaporation technique has been widely accepted for the encapsulation of a number of water soluble drugs (Song et al., 1987; Arshady, 1990; Schugens et al., 1994). In the solvent evaporation method, an aqueous solution (primary aqueous phase) or suspension of the drug is emulsified into an organic solution containing the polymer and a suitable emulsifier. This primary emulsion (w/o) is then dispersed in a second aqueous phase containing a suitable emulsifier (secondary aqueous phase), with the formation of a double emulsion (w/o/w). Solid microcapsules are collected following the complete removal of the volatile organic phase (Ogawa et al., 1988; Mac et al., 1989).

The efficiency of encapsulation of therapeutic agent within a polymer microcapsule prepared by the solvent evaporation technique is dependent on the aqueous solubility of the therapeutic agent. We have reported earlier that the efficiency of encapsulation of AZT in PLGA microcapsules prepared by the solvent evaporation technique was only 5% (Mandal et al., 1996a,b). This extremely poor encapsulation of AZT was due to the diffusion of AZT into the aqueous phase during the in-water solvent evaporation. The effect of several formulation and processing factors on the efficiency of encapsulation, surface morphology and drug release profiles was reported earlier (Mandal and Tenjarla, 1995, Mandal et al., 1996a,b). The objective of the present investigation was to improve the efficiency of encapsulation of AZT by modifying the secondary aqueous phase. (1) An inorganic salt (calcium chloride; 0–30%) was added to the secondary aqueous phase to minimize the drug loss. (2) The secondary aqueous phase was partially saturated with AZT (0–0.75%) to minimize the partitioning of the drug during the in-water solvent evaporation. (3) The pH of the secondary aqueous phase was changed from 3 to 10 to minimize the drug loss by changing the ionization behavior of AZT.

2. Materials and methods

2.1. Materials

The copolymer poly(DL-lactic/glycolic acid) (Molecular weight 105 680; lactic acid/glycolic acid 50/50, Resomer RG 506, inherent viscosity 0.8) was obtained from Boehringer Ingelheim, Ingelheim, Germany. The surfactant L-α phosphatidylcholine was obtained from Avanti Polar-lipids, Inc., Albaster, U.S.A. AZT was obtained from Burroughs Wellcome Co., U.S.A. Potassium biphthalate, potassium phosphate monobasic, boric acid, sodium hydroxide, hydrochloric acid, Polyvinyl alcohol (PVA), chloroform, methanol and dichloromethane were obtained from Sigma Chemical Co., St. Louis, U.S.A.

2.2. Experimental methods

2.2.1. Preparation of biodegradable microcapsules

Sustained-release biodegradable microcapsules of AZT were prepared using copolymers of poly(DL-lactic/glycolic) acid by the solvent evaporation technique (Alonso et al., 1993). A specific amount (50 mg) of AZT powder was dissolved in 2 ml of deionized water (primary aqueous phase) and then emulsified in 10 ml of dichloromethane/methanol mixture (8 ml dichloromethane and 2 ml methanol) containing 1 g of PLGA. The selec-

Table 1
Description of formulations and processing conditions

Formulation	Amount of AZT (mg)	Amount of PLGA (g)	Aqueous phase (containing 0.3% PVA)
Control	50	1	—
Batch A	50	1	5% calcium chloride
Batch B	50	1	10% calcium chloride
Batch C	50	1	15% calcium chloride
Batch D	50	1	20% calcium chloride
Batch E	50	1	30% calcium chloride
Batch F	50	1	0.25% AZT
Batch G	50	1	0.50% AZT
Batch H	50	1	0.75% AZT
Batch I	50	1	pH 3.0[a]
Batch J	50	1	pH 5.0[b]
Batch K	50	1	pH 7.0[c]
Batch L	50	1	pH 10.0[d]

[a]Potassium bithalate and hydrochloric acid (0.2 M).
[b]Potassium bithalate and sodium hydroxide (0.2 M).
[c]Potassium phosphate monobasic and sodium hydroxide (0.2 M).
[d]Boric acid, potassium chloride, and sodium hydroxide (0.2 M).

tion of the organic solvents and their proportion was based on the earlier experiments (Mandal and Tenjarla, 1995). It was reported earlier that the use of dichloromethane alone resulted in microcapsules of porous surface. The use of methanol along with dichloromethane improved the surface morphology by eliminating the pores. The PLGA solution was previously mixed with 0.5 ml of lipophilic surfactant L-α-phosphatidylcholine in chloroform (8 mg/ml). The emulsification was carried out by sonication at output 4 (50 W) for 30 s (ultrasonic probe, Sonic and Materials Inc., CT). The resulting emulsion was further emulsified in a specific volume (25 ml) of an aqueous solution of PVA (1%) by vortexing for 15 s and then diluted in 100 ml of modified aqueous phase (as listed in Table 1) containing PVA (0.3%) (secondary aqueous phase). The resultant emulsion (w/o/w) following the addition of 1% PVA solution was immediately diluted with 0.3% PVA to stabilize the system by reducing the osmotic pressure difference. Development of an excessive osmotic pressure difference may destabilize the system by excessive water flux or rupture of the oil layer. The use of lower concentration of PVA solution during the in-water solvent evaporation also minimizes the transfer of active ingredients from the microcapsules to the external aqueous solutions. The system was stirred magnetically for 16 h to allow complete evaporation of the solvent.

AZT microcapsules were finally collected by centrifugation at 3000 rpm and washed four times with deionized water to remove any residual PVA on the surface of the microcapsules. The microcapsules were carefully freeze dried to remove any residual water. The microcapsules were dried completely to prevent any degradation of PLGA during storage. Since the major degradation route for PLGA is hydrolysis, the presence of any water in the microcapsules will initiate the degradation process. The microcapsules were collected as free-flowing powder and stored in a desiccator.

2.2.2. Determination of total content

For each formulation, a 20 mg sample was dissolved in 1 ml of dichloromethane. Four ml of methanol was added to the solution followed by ultracentrifugation (35 000 rpm at 15°C) to completely separate the precipitated copolymer. The amount of AZT in each sample was determined by measuring the absorbance of clear supernatant in a spectrophotometer (DU 640, Beckman, OH) at 267 nm. Each experiment was performed in triplicate.

2.2.3. In vitro dissolution studies

For each formulation, a 40-mg sample was placed in a 10-ml tube and incubated in 5 ml of pH 7.4 phosphate buffer with constant shaking (20 rpm) at 37°C. Samples (600 μl) were collected at scheduled times using a filter pipette and centrifuged for 10 min at 10 000 rpm. The sample was spectrophotometrically analyzed for AZT content. Fresh phosphate buffer was added to the incubated sample (600 μl) to maintain sink conditions. Dissolution studies were performed independently in triplicate.

2.2.4. Particle size and morphology

Size, morphology and surface appearance of microcapsules were examined by scanning electron microscopy (SEM) (Amray AMR 1000A, USA). Samples for SEM were mounted on metal stubs and coated with gold to a thickness of 200–500 Angstrom. Pictures were taken and the microcapsules sizes were determined according to a reference scale.

2.2.5. Statistical analysis

The efficiency of encapsulation of AZT and the amount of drug released from the different formulations of microcapsules during the in vitro study was compared using SAS software package. A P value of < 0.05 was considered as evidence of a significant difference.

3. Results and discussion

AZT is a water-soluble drug and a significant amount of the drug was diffused into the secondary aqueous phase during the in-water solvent evaporation (Mandal et al., 1996a). In an attempt to minimize the drug loss during the solvent evaporation, the secondary aqueous phase was modified. The microcapsules prepared using different conditions are described in Table 1.

3.1. Effect of partial saturation of the aqueous phase with calcium chloride

An inorganic salt (calcium chloride; 0%, 5%, 10%, 15%, 20%, and 30%) was added to the secondary aqueous phase to minimize the drug loss by lowering the solubility of AZT due to the salting-out effect. The microcapsules used for this study were all prepared by the initial formation of a w/o/w emulsion. The six batches of microcapsules (Control, Batch A, Batch B, Batch C, Batch D, and Batch E) prepared for this study were experiencing the same process up to the formation of the w/o/w emulsion. During the final stage, the in-water solvent evaporation, the secondary aqueous phase containing 0.3% PVA was partially saturated with different concentrations of calcium chloride as listed in table 1. These microcapsules were evaluated and the results are listed in Table 2. A comparison of particle size reveals a similar average particle size in all six batches. The particles are all less than 30 μm with a range of average sizes from 8 μm to 18 μm in diameter. This observation shows that when the secondary aqueous phase was partially saturated with calcium chloride, the particle size of the final microcapsules did not change significantly. The efficiency of encapsulation of AZT was determined by measuring the total amount of AZT present in each 20 mg sample of the microcapsules, i.e. core-loading experimental, and comparing this value with the expected amount of AZT in each of the samples based on the drug loading during the preparation, i.e. core-loading theoretical. The partial saturation of the secondary

Table 2
Characteristics of microcapsules: Effect of partial saturation of the secondary aqueous phase with calcium chloride

Batch	Particle size (Range) (μm)	Efficiency of encapsulation[a] (%)	
		Mean (n = 3)	Results of ANOVA
Control	18 (2–28)	7.46	NS[b]
A	10 (3–24)	7.17	
B	10 (1–16)	7.54	
C	8 (3–14)	7.65	
D	13 (3–26)	7.60	
E	9 (3–16)	7.90	

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.
[b]NS = Not significant.

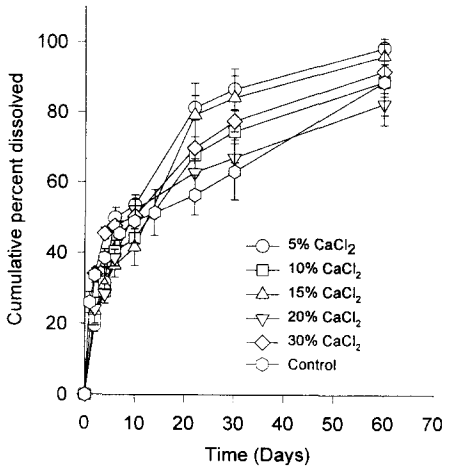

Fig. 1. Effect of partial saturation of the secondary aqueous phase with calcium chloride: Dissolution profiles of microcapsules, Control (0%), Batch A (5%), Batch B (10%), Batch C (15%), Batch D (20%) and Batch E (30%).

aqueous phase with calcium chloride, irrespective of the concentration, did not change the efficiency of encapsulation. All six batches of the microcapsules encapsulated a statistically similar amount of the drug.

The dissolution of AZT was compared by calculating the cumulative percentage of the drug released at a specific sampling time. Fig. 1 shows the dissolution profiles of the batches A–E and control. The amount of drug released within the first 24 h was ranged from 19 to 34%. The microcapsules prepared by partial saturation of the secondary aqueous phase with 30% calcium chloride showed a significantly higher initial 'burst effect' than the other batches of the microcapsules. Following the initial release, a sustained-drug release continued up to 60 days. However, no particular rank-order correlation was observed among the cumulative percent dissolved and the amount of calcium chloride at any other sampling period.

The scanning electron micrographs of the microcapsules of batches A–E and control reveal that the modification of the secondary aqueous phase with calcium chloride did not change the surface morphology. All six batches produced very smooth and spherical microcapsules (Fig. 2).

3.2. Effect of partial saturation of the aqueous phase with AZT

In an attempt to minimize the drug loss during the in-water solvent evaporation, the secondary aqueous phase was partially saturated with AZT (0%, 0.25%, 0.50%, and 0.75%). The four batches of microcapsules (control, Batch F, Batch G, and Batch H) were experiencing the same process up to the formation of the w/o/w emulsion. During the final stage, the secondary aqueous phase containing 0.3% PVA was changed from batch to batch as listed in Table 1. The results obtained from the evaluation of these four batches of microcapsules are listed in Table 3. A comparison of the particle size reveals that the average size of the microcapsules increased significantly compared with the control batch when the secondary aqueous phase was partially saturated with the core material i.e. AZT. The microcapsules of the control batch were 30 μm or smaller with an average size of 18 μm in diameter. Whereas, partial saturation of the secondary aqueous phase with 0.25% AZT increased the average size up to 79 (20–150) μm. The average size of the microcapsules increased further, 140 (47–253) μm, when the secondary aqueous phase was partially saturated with 0.50% AZT. However, there was no further significant size increase when the secondary aqueous phase was partially saturated with 0.75% AZT. The efficiency of encapsulation increased significantly when the secondary aqueous phase was partially saturated with AZT. There was a positive rank order correlation between the efficiency of encapsulation and the amount of AZT in the secondary aqueous phase. The efficiency of encapsulation increased up to 17% when the secondary aqueous phase was partially saturated with 0.75% AZT. This observation shows that partial saturation of the secondary aqueous phase with AZT significantly increases the efficiency of encapsulation by minimizing the drug loss through diffusion during the in-water solvent evaporation.

Fig. 3 shows the dissolution profiles of the batches F–H and control. Microcapsules of all four batches showed similar dissolution characteristics up to 10 days. However, the microcapsules

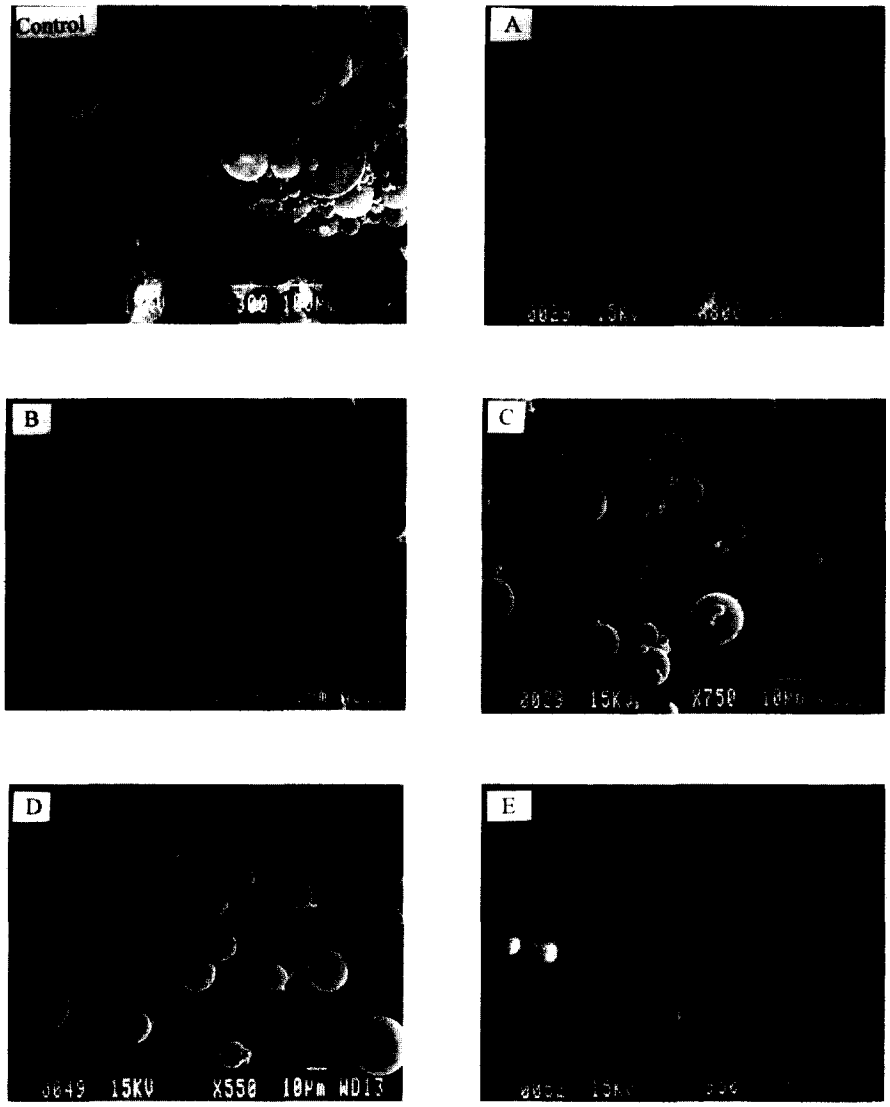

Fig. 2. Effect of partial saturation of the secondary aqueous phase with calcium chloride: SEM photographs of microcapsules, Control (0%), Batch A (5%), Batch B (10%), Batch C (15%), Batch D (20%) and Batch E (30%).

prepared by partial saturation of the secondary aqueous phase with AZT (Batches F–H), irrespective of the degree of saturation, showed significantly higher dissolution compared with the control, during 10–45 days sampling. This observation shows that partial saturation of the secondary aqueous phase with AZT significantly influenced the dissolution characteristics of the microcapsules. The presence of AZT in the secondary aqueous phase during the in-water solvent evaporation produced microcapsules with higher dissolution rates. This higher dissolution was due to the presence of higher amount of encapsulated drug in the microcapsules.

The scanning electron micrographs of the microcapsules of batches F–H and control (Fig. 4)

Table 3
Characteristics of microcapsules: Effect of partial saturation of the secondary aqueous phase with AZT

Batch	Particle size (Range) (μm)	Efficiency of encapsulation[a] (%)	
		Mean (n = 3)	Results of SNK[b]
Control	23 (2–30)	7.46	Control < F < G < H
F	79 (20–150)	8.55	
G	140 (47–253)	12.64	
H	135 (46–277)	17.06	

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) x 100.
[b]SNK = Student Newman Keul's Multiple range test.

reveal that partial saturation of the secondary aqueous phase with AZT had a detrimental effect on the surface morphology. The batch prepared by partial saturation of the secondary aqueous phase with 0.25% AZT (Batch F) show few microcapsules with collapse structure along with a high proportion of smooth and spherical microcapsules. The relative proportion of collapse microcapsules increased significantly with the increase concentration of AZT in the secondary aqueous phase (Batches G and H). This observation shows that partial saturation of the secondary aqueous phase with AZT reduces the film adhesion forces; and the higher tensile stress associated with this film formation resulted in the formation of hollow pockets that partially collapsed during the solvent evaporation. The presence of hollow pockets inside the collapsed microcapsules allowed easy penetration of the dissolution medium within the matrix and resulted in higher drug release.

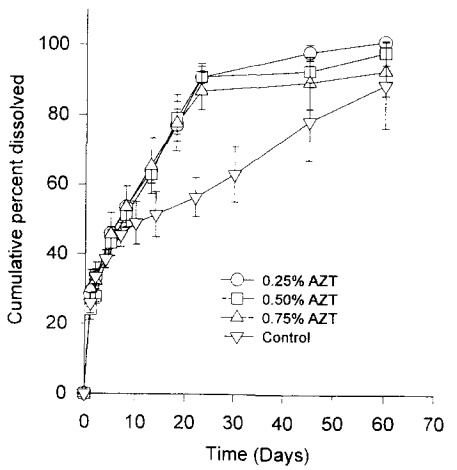

Fig. 3. Effect of partial saturation of the secondary aqueous phase with AZT: Dissolution profiles of microcapsules, Control (0%), Batch F (0.25%), Batch G (0.50%) and Batch H (0.75%).

3.3. Effect of the pH of the aqueous phase

The pH of the secondary aqueous phase was changed from 3 to 10 to minimize the drug loss by changing the ionization behavior of AZT. The effect of the pH was studied at four levels (3, 5, 7 and 10). The microcapsules used for this study were all prepared by the initial formation of a w/o/w emulsion. The four batches of microcapsules (Batch I, Batch J, Batch K, and Batch L) prepared for this study were experiencing the same process up to the formation of the w/o/w emulsion. During the final stage, the pH of the secondary aqueous phase containing 0.3% PVA was changed from batch to batch as listed in Table 1. The results obtained from the evaluation of these four batches of microcapsules are listed in Table 4. A comparison of the particle size reveals a similar average particle size in all four batches. The particles are all less than 30 μm with a range of average sizes from 9 μm to 15 μm in diameter. This observation shows that when the pH of the secondary aqueous phase was changed from 3 to 10, the particle size of the final microcapsules did not change significantly. However, a comparison of the efficiency of encapsulation shows that the amount of AZT encapsulated was pH dependent. The amount encapsulated at pH 5 was significantly higher than the amount encapsulated at pH

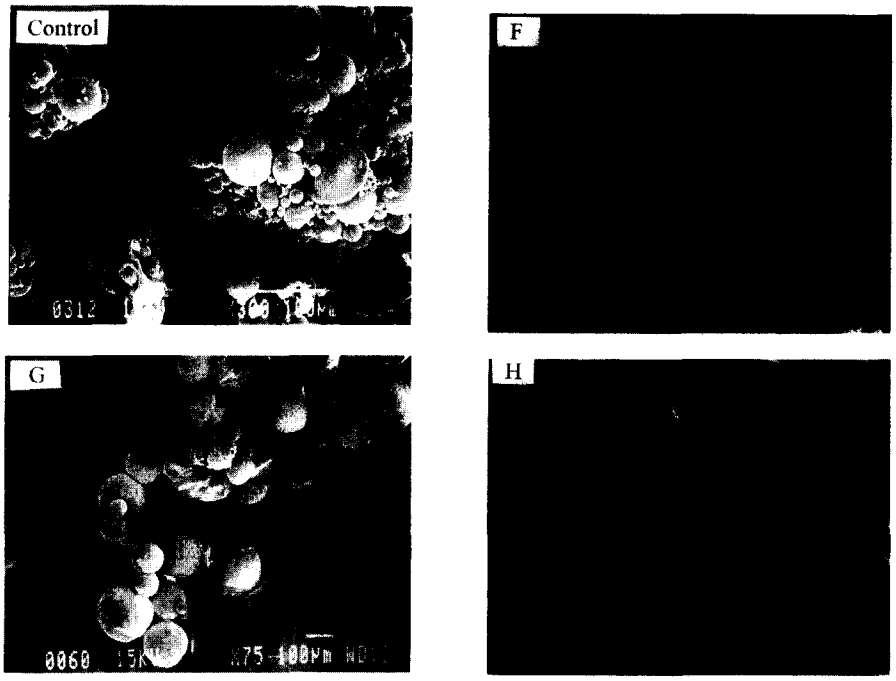

Fig. 4. Effect of partial saturation of the secondary aqueous phase with AZT: SEM photographs of microcapsules, Control (0%), Batch F (0.25%), Batch G (0.50%) and Batch H (0.75%).

3 but the efficiency of encapsulation decreased as the pH was further increased up to 10. As the pH of the secondary aqueous phase was increased from 3 to 10, AZT (pKa = 9.68), a weakly acidic drug, changes from a less ionized form (less water-soluble) to a more ionized form (more water-soluble) (ratio of ionized:unionized at pH 3, $2.09:10^7$; at pH 5, $2.09:10^5$; at pH 7, $2.09:10^3$, at pH 10, 2.09:1). This was reflected in the efficiency of encapsulation that was significantly decreased due to the increase in pH. At pH 10, AZT was predominantly in the ionized form, which increased its aqueous solubility by several fold. This increased aqueous solubility was responsible for the higher drug diffusion and drug loss to the secondary aqueous phase at this pH during the in-water solvent evaporation. Interestingly, the efficiency of encapsulation at pH 3 was significantly lower than the encapsulation amount at pH 5. This contradiction may be due to the effect of this acidic pH on the precipitation behavior of PLGA. A change in the rate of precipitation of polymer at the droplet interface may change the total amount of the encapsulated drug. The drug partitioned into the aqueous phase as long as the polymer was in the liquid state. As soon as the polymer at the droplet surface precipitated, no further drug diffused to the aqueous phase. Fig. 5

Table 4
Characteristics of microcapsules: Effect of the pH of the secondary aqueous phase

Batch	Particle size (Range) (μm)	Efficiency of encapsulation[a] (%)	
		Mean (n = 3)	Results of SNK[b]
I	10 (3–16)	3.75	I < J > K > L
J	15 (5–29)	5.17	
K	9 (3–10)	4.15	
L	13 (4–26)	2.93	

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) x 100.
[b]SNK = Student Newman Keul's Multiple range test.

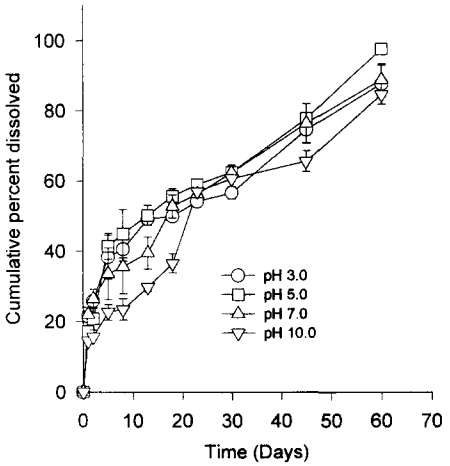

Fig. 5. Effect of the pH of the secondary aqueous phase: Dissolution profiles of microcapsules, Batch I (pH 3.0), Batch J (pH 5.0), Batch K (pH 7.0) and Batch L (pH 10.0).

shows the dissolution profiles of batches I–L. The pH of the secondary aqueous phase during the in-water solvent evaporation did not affect the dissolution of AZT from the microcapsules in general. However, the microcapsules of batch L showed a significantly slower dissolution than the other batches, up to 18 days followed by a significant 'burst effect'. This slower dissolution was due to the presence of a smaller amount of AZT in these microcapsules compared with other batches. As a result, the relative proportion of PLGA was higher in this batch L that reduced the diffusion of AZT through the polymer. The drug release from all four batches was continued up to 60 days.

The scanning electron micrographs of the microcapsules of batches I–L show that batches I and J produced very smooth and spherical microcapsules (Fig. 6). Whereas, pictures taken at higher magnification (not shown here) reveal that batch K produced spherical microcapsules but the surface was appeared to be slightly dented. Microcapsules of batch L lost their smooth and spherical appearance completely. These microcapsules are elongated in shape and the surface was appeared to be wrinkled. These observations reveal that the pH of the secondary aqueous phase significantly influenced the surface morphology. The acidic pH (3 and 5) produced very smooth and spherical microcapsules but the alkaline pH (10) has a detrimental effect on the surface morphology.

4. Conclusion

The overall results presented in this study provided evidence that a modification of the secondary aqueous phase during the in-water solvent evaporation changed the characteristics of the microcapsules. Particle size of the microcapsules was sensitive to the partial saturation of the secondary aqueous phase with AZT. An increase in the concentration of AZT in the secondary aqueous phase resulted in a larger microcapsule. Whereas, the partial saturation of the secondary aqueous phase with calcium chloride did not change the size of the microcapsules. The size of the microcapsules was also remained unchanged when the pH of the secondary aqueous phase was changed. The efficiency of encapsulation was maximum when the secondary aqueous phase was partially saturated with 0.75% AZT. However, partial saturation of the secondary aqueous phase with AZT resulted in collapse microcapsules. The efficiency of encapsulation did not change when the secondary aqueous phase was partially saturated with calcium chloride, irrespective of the concentrations. This observation reveals that the presence of calcium chloride during the in-water solvent evaporation did not minimize the drug loss through diffusion. However, the drug loss through diffusion changed due to the change in pH of the secondary aqueous phase. The efficiency of encapsulation reduced drastically when the pH of the secondary aqueous phase was adjusted to 10. AZT is a weakly acidic drug and become more soluble at high pH. An increase in solubility was responsible for higher drug loss during the in-water solvent evaporation. This alkaline pH also had a detrimental effect on the surface morphology of the microcapsules. The surface of the microcapsules was appeared to be wrinkled when the pH of the secondary aqueous phase was adjusted to 10. The drug-release characteristic of the microcapsules was not affected due to the modification of the secondary aqueous

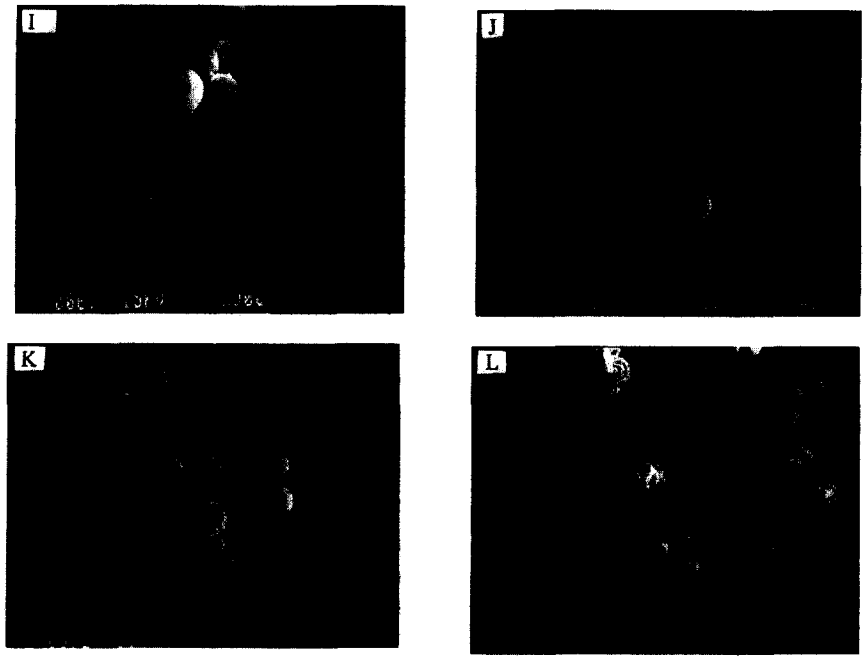

Fig. 6. Effect of the pH of the secondary aqueous phase: SEM photographs of microcapsules, Batch I (pH 3.0), Batch J (pH 5.0), Batch K (pH 7.0) and Batch L (pH 10.0).

phase, except when AZT was used for partial saturation. These later microcapsules released a significantly higher amount of AZT during the second half of the dissolution experiments. Finally, the overall dissolution profiles showed a very high standard deviation due to a wide range of particles within a particular batch. Since the batch sizes were very small, it was not possible to compare the dissolution profiles using replicate samples of identical size range.

Acknowledgements

We thank Burroughs Wellcome Co. for the generous supply of AZT. This work was funded in part by the National Institute on Drug Abuse grant # DA07970 and the NIH/AMHPS AIDS Research Consortium.

References

Alonso, M.J., Cohen, S., Park, T.G., Gupta, R.K., Siber, G.R. and Langer, R., Determinants of release rate of tetanus vaccine from polyester microspheres. *Pharm. Res.*, 10 (1993) 945–953.

Arshady, R., Microspheres and microcapsules, a survey of manufacturing techniques: part III: solvent evaporation. *Polym. Eng. Sci.*, 30 (1990) 915–924.

Brook, I., Approval of zidovudine (AZT) for acquired immunodeficiency syndrome. *J. Am. Med. Assoc.*, 258 (1987) 1517.

Chow, F-P.R. and Hamburger, A.W., In vivo evaluation of the anemia induced by azidothymidine (AZT) in a murine model of AIDS. *Eur. J. Haematol.*, 47 (1991) 91–97.

Cleland, J.L. and Langer, R., Formulation and delivery of proteins and peptides: design and development strategies. In Cleland, J.L. and Langer, R. (Eds.), *Formulation and Delivery of Proteins and Peptides*, American Chemical Society, Washington, DC, 1994, pp. 1–19.

Colson, E.R., Horwitz, R.I., Bia, F.J. and Viscoli, C.M., Zidovudine (AZT) for the treatment of patients infected with human immunodeficiency virus type 1. An evaluation

of effectiveness in clinical practice. *Arch. Intern. Med.*, 151 (1991) 709–713.

Devita, V.T., Broder, S., Fauci, A.S., Kovacs, J.A. and Chabner, B.A., Developmental therapeutics and the acquired immunodeficiency syndrome. *Ann. Inter. Med.*, 106 (1987) 568–581.

Douglas, R.D., Antimicrobial agents. In Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P. (Eds.), *The pharmacological basis of therapeutics*, Pergaman press, New York, 1990, pp. 1182–1184.

Fischl, M.A., Richman, D.D., Hansen, N., Collier, A.C., Carey, J.T., Para, M.F., Hardy, W.D., Powderly, W.G., Allan, J.D., Wong, B., Merigan, T.C., Dolin, R., McAuliffe, V.J., Hyslop, N.E., Rhame, F.S., Balfour, H.H., Spector, S.A., Volberding, P., Pettinelli, C., Anderson, J. and the AIDS Clinincal Trials Group, The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection. *Ann. Inter. Med.*, 112 (1990) 727–737.

Klecker, R., Collins, J., Yarchoan, R., Thomas, R., Jenkins, J.F., Broder, S. and Myers, C.E., Plasma and cerebrospinal fluid pharmacokinetics of 3-azido-3-deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. *Clin. Pharmacol. Ther.*, 41 (1987) 407–412.

Lewis, D.H., Biodegradable polymers as drug delivery systems. In Chasin, M. and Langer, R. (Eds.), *Drugs and Pharmaceutical Sciences*. Vol. 45., Marcel Dekker, New York, 1990, pp. 1–42.

Lutton, J.D., Mathew, A., Levere, R.D. and Abraham, N.G., Role of heme metabolism in AZT-induced bone marrow toxicity. *Am. J. Hematol.*, 35 (1990) 1–5.

Mac, A., Negi, D. and Friend, D. Preparation and characterization of poly (styrene) microcapsules containing corrosion inhibitors. *J. Microencapsulation.*, 6 (1989) 361–367.

Mandal, T.K. and Tenjarla, S., Biodegradable microcapsules: Optimization of formulation using factorial design. *Pharm. Sci.*, 1 (1995) 367–370.

Mandal, T.K., Lopez-Anaya, A., Onyebueke, E. and Shekleton, M., Preparation of biodegradable microcapsules containing zidovudine (AZT) using solvent evaporation technique. *J. Microencapsulation*, (1996a) in press.

Mandal, T.K., Shekleton, M., Onyebueke, E., Washington, L. and Penson, T., Effect of formulation and processing factors on the characteristics of biodegradable microcapsules of zidovudine. *J. Microencapsulation*, (1996b) in press.

Ogawa, Y., Yamamoto, M., Takada, S., Okada, H. and Shimamoto, T., Controlled release of leuprolide acetate from polylactic acid or copoly (lactic/glycolic) acid microcapsules: influence of molecular weight and copolymer ratio of polymer. *Chem. Pharm. Bull.*, 36 (1988) 1502–1507.

Pitt, C.G., Cha, Y., Shah, S.S. and Zhu, K.J., Blends of PVA and PGLA: control of the permeability and degrability of hydrogels by blending. *J. Control. Rel.*, 19 (1992) 189–200.

Richman, D.D., Fischl, M.A., Grieco, M.H., Gottliab, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Hirsch, M.S., Jackson, G.G., Durack, D.T., Nusinoff-Lehrman, S. and the AZT Collaborative Working Group, The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.*, 317 (1987) 192–197.

Schugens, Ch., Laruelle, N., Nihant, N., Grandfils, Ch., Jerome, R. and Teyssie, Ph., Effect of emulsion stability on the morphology and porosity of semicrystalline poly l-lactide microparticles prepared by w/o/w double emulsion-evaporation. *J. Control. Rel.*, 32 (1994) 161–176.

Shargel, L. and Yu, A.B.C., *Applied Biopharmaceutics and Pharmacokinetics*, 3rd Edn, Appleton and Lange, Connecticut, 1993, pp. 232–233.

Song, C.X., Sun, H.F. and Feng, X.D., Microspheres of biodegradable block copolymer for long-acting controlled delivery of contraceptives. *Polymer J.*, 19 (1987) 485–491.

Yan, C., Resau, J.H., Hewetson, J., West, M., Rill, W.L. and Kende, M., Characterization and morphological analysis of protein-loaded poly(lactide-co-glycolide) microparticles prepared by water-in-oil-water emulsion technique. *J. Control. Rel.*, 32 (1994) 231–241.