Zidovudine (azidothymidine, AZT), a reverse transcriptase inhibitor, is an effective anti-HIV drug following oral administration. AZT is a bitter white crystalline powder and usually administered as 100–300 mg conventional tablet for the treatment of Acquired Immuno Deficiency Syndrome (1, 2). AZT has a short half life of 60 min and usually is administered three times daily in a dose of 100 mg to maintain therapeutic drug levels (3). AZT has low oral bioavailability (65%) due to considerable first-pass metabolism, and thus necessitating frequent administration of large doses, which in turn leads to high incidence of GI side effects (4, 5). To be able to reduce the number of administrations, it might thus be of interest to develop a controlled release preparation that provides lower but controlled drug concentration.

In this paper, matrix microspheres are considered to achieve the preceding controlled release formulation and also to increase the drug efficiency. Matrix microspheres offer several advantages over other sustained release systems, especially matrix-type tablets, since they can be widely distributed throughout the gastrointestinal tract. They improve drug absorption and minimize side effects due to the localized build up of the drugs at the gastrointestinal mucosa.

One of the popular methods for the entrapment of drugs within water-insoluble polymers is the emulsion solvent evaporation method. The emulsion solvent evaporation technique was fully developed at the end of the 1970s and has been used successfully in the preparation of microspheres made from several biocompatible polymers such as poly(D,L-lactide-co-glycolide) (6, 7), polycaprolactone (8–10), and eudragits (11, 12).

AZT with poly(lactide-co-glycolide) polymers by W/O/W emulsion solvent evaporation method was
reported with entrapment efficiency of only 17% (13). In some recent literature, the maximum entrapment efficiency of AZT reported was 53% with ethylcellulose polymers prepared by W/O/O emulsion solvent evaporation method (14, 15). However, entrapment efficiency of AZT into ammonio methacrylate copolymers by emulsion solvent evaporation method remains to be studied. Recently, there was a study on the effect of the polymer solution viscosity on microsphere dissolution properties prepared by the same method and the results suggested a relationship between apparent viscosity of polymer solutions and the dissolution properties of microspheres (16). However, polymer used in the previous study was of similar composition, but differing only in their molecular weight.

Therefore, the purpose of the present study is to incorporate the anti HIV drug, AZT into matrix microspheres using two polymers of different structural as well as permeability characteristics at different polymer solution concentration, in order to evaluate the entrapment efficiency and microsphere properties, especially their dissolution characteristics.

EXPERIMENTAL

Materials

Zidovudine was obtained as a gift sample from Cipla Ltd., Mumbai, India. The other chemicals were obtained from commercial sources such as: Eudragit RL 100 (poly-(trimethylammonioethyl methacrylate having 5% functional quaternary ammonium groups and Eudragit RS 100 (poly-(trimethylammonioethyl methacrylate having 10% functional quaternary ammonium groups (Rohm Pharma, Darmstadt, Germany); acetone (Lobachem, Mumbai, India); Span 80 (Rankem, Mumbai, India); n-hexane, light liquid paraffin, potassium chloride, sodium chloride, potassium phosphate, monobasic, sodium phosphate dibasic anhydrous ((Ranbaxy Fine Chemicals, New Delhi, India). All other chemicals used were of analytical grade.

Solubility studies or pH solubility profile of pure zidovudine

A saturated solution of zidovudine was prepared by shaking an excess amount in 2 mL of phosphate buffer pH 7.4/distilled water separately at 25 ± 10°C for 24 h. The saturated solution was withdrawn, filtered and analyzed at 266 nm using UV-Vis spectrophotometer (1700, Shimadzu, Japan). Loading efficiencies were calculated as follows:

\[
D.E.E = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100\% 
\]

In vitro drug release studies

In vitro drug release studies were carried out for all products in USP paddle type dissolution test apparatus [Campbell Electronics, Mumbai, India]. The microspheres were evaluated for drug release using 500 mL of phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C and were stirred by paddle
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method at 100 rpm. Because Eudragit RL100 and Eudragit RS100 are independent of the pH, the pH value of the tested dissolution medium was set at 7.4. Two mL aliquots were withdrawn at different time intervals and an equivalent volume of medium prewarmed at 37°C was added to maintain sink condition. Withdrawn samples were analyzed at 266 nm. The data obtained were fitted into various kinetic models to investigate the mechanism of drug release from eudragit microspheres.

Morphology

For morphology and surface characteristics, prepared microspheres were coated with gold in an argon atmosphere using a gold sputter module in a high vacuum evaporator. The surface morphology of the microsphere was then studied by scanning electron microscope (Hitachi S-3600N Scanning Electron Microscope, Japan).

Particle size analysis

Size distribution was determined by sieving the microparticles using a nest of standard BSS sieves (36, 44, 25) as well as by optical microscopy (Olympus NWF 10X, Japan) fitted with an ocular and stage micrometer. Finally, the approximate sizes of microspheres were confirmed by scanning electron microscopy.

RESULTS AND DISCUSSION

Effect of various processing conditions and variables on the pelletization of zidovudine

The highly water soluble drug zidovudine was successfully incorporated into eudragit polymers using emulsion solvent evaporation method. In this method, drug polymer mixtures were dispersed into an immiscible vehicle to form an emulsion. As the solvent is evaporated, the droplets become gradually concentrated and the nucleation takes place to produce microspheres. It was observed that when the speed of stirrer was below 500 rpm, there was no formation of spherical microspheres. At stirring speeds of 500–700 rpm, the resulting high turbulence caused frothing and adhesion to the container wall, resulting in decreased mean particle size. Flocculation was recognized when no magnesium stearate was added. Especially with 5% magnesium stearate, the microspheres were nearly uniform and free-flowing with good reproducibility. This is because magnesium stearate reduces the interfacial tension and prevents electrification and flocculation during the preparation of microspheres. It is also considered that magnesium stearate cooperates to build a dense surface of the microspheres and prevents the leakage of the drug into the dispersion medium during microsphere preparation resulting in increased entrapment of the drug.

Particle size analysis

The mean particle size of the microspheres ranged from 1750 to 3050 µm (Table 1). The mean size increased with increasing polymer concentrations (4% to 12% w/w), which produces a significant increase in the viscosity, leading to the formation of larger size emulsion droplets and finally a higher microsphere size. The mean size was also influenced by the content and type of eudragit used and its ratio in the formulation. It is observed that microspheres prepared using Eudragit RL100 and RS100 alone at the same concentrations does not show a significant variation in their mean size value. Notably, when the polymer to polymer ratio was 1:1, there was formation of microspheres with larger sizes due to an increase in solution viscosity of the polymers. This synergistic increase in the viscosity could be due to an interaction between the polymers.

Drug entrapment efficiency

The entrapment efficiencies ranged from 57.3 to 82.7%. The entrapment efficiency of zidovudine is dependent upon its solubility in the solvent and continuous phase. An increase in the concentration of polymer (4% to 12% w/v) in a fixed volume of organic solvent resulted in an increase in entrapment efficiency as shown in Table 1. It is also evident that the entrapment efficiency of Eudragit RS100 microspheres was higher than that of the Eudragit RL100 microspheres. This behavior can be explained on the basis of differences of the chemical structures and the % content of quaternary ammonium groups. Eudragit RL100 contains higher amount of quaternary ammonium groups, which facilitates the diffusion of a part of entrapped drug to the surrounding medium during preparation of microspheres.

Eudragit RS100 has thick polymeric surfaces due to the presence of lower amount of quaternary ammonium groups, which restrict the migration of drug particles to the surrounding medium and also helps in the masking of bitter taste of the drug. However, in the case of RL100/RS100 mixture, the encapsulation efficiencies were a little higher than those of microspheres prepared using individual polymers. Notably, the microspheres prepared using 1:1 ratio of both the polymers exhibited the highest encapsulation efficiency, indicating the formation of the
most stable emulsion and the most suitable microsphere structures in the RL/RS mixture ratio of 1:1.

**Scanning electron microscopy (SEM)**

SEM study shows that particles made of Eudragit RL100 and RS100 were spherical and not aggregated. The surface of the drug-loaded microspheres manifested the presence of drug particles, clearly visible from outside at high magnification (Fig. 1a). However, according to the type and concentration of the polymer, there were no significant differences in morphology of the microspheres. The

### Table 1. Effect of polymer type and concentration on the encapsulation efficiency and the mean size (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Polymer concentration (%)</th>
<th>Drug to polymer ratio</th>
<th>Encapsulation efficiency (%)</th>
<th>Mean size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Eudragit RL 100 4%</td>
<td>1:4</td>
<td>57.4</td>
<td>1271 ± 2.1</td>
</tr>
<tr>
<td>A2</td>
<td>Eudragit RS 100 8%</td>
<td>1:8</td>
<td>65.1</td>
<td>1750 ± 3.1</td>
</tr>
<tr>
<td>A3</td>
<td>Eudragit RL 100 12%</td>
<td>1:12</td>
<td>73.7</td>
<td>2881 ± 4.5</td>
</tr>
<tr>
<td>B1</td>
<td>Eudragit RL 100 4%</td>
<td>1:4</td>
<td>57.9</td>
<td>1350 ± 1.8</td>
</tr>
<tr>
<td>B2</td>
<td>Eudragit RS 100 8%</td>
<td>1:8</td>
<td>67.5</td>
<td>1800 ± 3.2</td>
</tr>
<tr>
<td>B3</td>
<td>Eudragit RL 100 12%</td>
<td>1:12</td>
<td>87.1</td>
<td>3000 ± 3.7</td>
</tr>
<tr>
<td>A1B3</td>
<td>RL:RS = 1:3 12%</td>
<td>1:12</td>
<td>65.8</td>
<td>2981 ± 4.1</td>
</tr>
<tr>
<td>A1B1</td>
<td>RL:RS = 1:1 12%</td>
<td>1:12</td>
<td>78.2</td>
<td>3051 ± 3.5</td>
</tr>
<tr>
<td>A3B1</td>
<td>RL:RS = 3:1 12%</td>
<td>1:12</td>
<td>73.1</td>
<td>2781 ± 1.2</td>
</tr>
</tbody>
</table>

### Table 2. Kinetic evaluation of drug release data for microsphere formulation.

<table>
<thead>
<tr>
<th>Polymer concentration (total 12% w/w, D.P 1:12)</th>
<th>Kinetic models</th>
<th>R²</th>
<th>K₀</th>
<th>R²</th>
<th>K₁</th>
<th>R²</th>
<th>Kh</th>
<th>R²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL 100</td>
<td>Zero-order</td>
<td>0.997</td>
<td>0.643</td>
<td>0.664</td>
<td>0.740</td>
<td>0.967</td>
<td>9.4</td>
<td>0.986</td>
<td>0.78</td>
</tr>
<tr>
<td>RS 100</td>
<td>First-order</td>
<td>0.820</td>
<td>0.418</td>
<td>0.924</td>
<td>0.342</td>
<td>0.960</td>
<td>5.12</td>
<td>0.976</td>
<td>0.384</td>
</tr>
<tr>
<td>RL:RS (1:3)</td>
<td>Higuchi</td>
<td>0.995</td>
<td>0.633</td>
<td>0.650</td>
<td>0.696</td>
<td>0.956</td>
<td>9.26</td>
<td>0.709</td>
<td>0.599</td>
</tr>
<tr>
<td>RL:RS (1:1)</td>
<td>Korsmeyer -Peppas</td>
<td>0.954</td>
<td>0.518</td>
<td>0.803</td>
<td>0.495</td>
<td>0.991</td>
<td>7.02</td>
<td>0.994</td>
<td>0.542</td>
</tr>
<tr>
<td>RL:RS (3:1)</td>
<td></td>
<td>0.898</td>
<td>0.447</td>
<td>0.938</td>
<td>0.363</td>
<td>0.989</td>
<td>5.84</td>
<td>0.987</td>
<td>0.472</td>
</tr>
</tbody>
</table>

Figure 1.(a) Scanning electron microscopic photographs of drug loaded microspheres made of 1:1 ratio of Eudragit RL 100 and RS 100 polymers before dissolution.

Figure 1.(b) Scanning electron microscopic photographs of drug loaded microspheres made of 1:1 ratio of Eudragit RL 100 and RS 100 polymers after dissolution.
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Figure 2. Dissolution profile of Zidovudine loaded Eudragit RL 100 microspheres prepared by the emulsion solvent evaporation method. Drug/polymer ratio was 1:4 (●), 1:8 (▲) and 1:12 (■) (mean ±SD, n = 3).

Figure 3. Dissolution profile of zidovudine loaded Eudragit RS 100 microspheres prepared by the emulsion solvent evaporation method. Drug/polymer ratio was 1:4 (●), 1:8 (▲) and 1:12 (■) (the mean ±SD, n = 3).

Figure 4. Effect of different proportion of RL/RS 100 mixture on the dissolution profile of zidovudine at a polymer concentration of 12% w/w. RL/RS ratio was 3:1 (○), 1:1 (□), 1:3 (▲). (the mean ± SD, n = 3).
presence of pores were detected on the microspheres surface, which increased in size and number after dissolution indicating leaching of the drug through these channels (Fig. 1b).

**Solubility study in different media**

The solubility of zidovudine in water and phosphate buffer (pH 7.4) was found to be 1.57 ± 0.41 mg/mL and 1.917 ± 0.45 mg/mL, respectively. It implies that drug will be better absorbed from the alkaline environment of the intestine. Hence, the dissolution study was carried out in phosphate buffer at pH 7.4.

**In vitro dissolution studies**

Figures 2 and 3 show that the release of zidovudine from microspheres made of Eudragit RL100 and RS100 polymers depended on the polymer concentration used. This indicates that the drug release rate decreases with increasing amount of the polymer. This can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with higher polymer concentration. It was also observed that the release rate of drug from Eudragit RL100 microspheres was a little higher than that of Eudragit RS 100 microspheres because Eudragit RL100 contains higher amount of quaternary ammonium groups, which renders it more permeable and accelerates the drug release as reported by Obeidat and Price (16).

These observations could be attributed to the fact that RS100 microspheres have thicker polymeric surface as compared to RL100 microspheres. The thick polymeric barrier slows the entry of surrounding dissolution medium in to the microspheres and hence less quantity of drug leaches out from the polymer matrices of the microspheres exhibiting slow release with a lag time of 2 h. (Fig. 3). However, initial burst release was observed for RS 100 microspheres in the first hour, which is probably due to the rapid dissolution of the drug adhered on the surface of the microspheres. The desired prolonged release was observed for microspheres prepared with 8% w/w and 12% w/w concentration with a percentage drug release of > 80% in 8 and 10 h, respectively.

Figure 4 reveals the effect of two polymers, Eudragit RL 100/RS 100 mixture on the release profiles of Eudragit RS 100 microspheres at a polymer concentration of 12 % w/w. The microspheres represented in Figure 4 were formed by constantly keeping the drug : polymer ratio at 1:12. The results confirm that Eudragit RL 100 could be added to Eudragit RS 100 microspheres to control the rate of drug release and more pronounced release can be obtained after 8 h with less bursting effect, because, the addition of Eudragit RL100 increases the porosity of the RS100 membrane to the surrounding dissolution medium and hence, more controlled rate of drug release is observed (Fig. 4).

In order to describe the kinetics of the release process of drugs from microsphere preparation, the data were fitted with different kinetics models. From the kinetic data (Table 2), it can be observed that the release of zidovudine from Eudragit RL100 microspheres and microspheres prepared using a combination of both the polymers exhibit diffusional characteristics and is highly correlated with Higuchi spherical matrix release, followed by zero order (23).

These findings indicated that the internal structure of the Eudragit RL 100 microspheres was a polymeric matrix containing dispersed drug. The Eudragit RL 100 microspheres immediately released almost all the drug upon dispersing, exhibiting diffusion controlled release mechanism from the matrix. On the other hand, Eudragit RS 100 microspheres showed a three phases composition. First, an initial release due to the drug desorption from the particle surface; secondly, a lag time for a certain period, resulting from the diffusion of the drug into microsphere surface; and thirdly, a constant sustained release of the drug resulting from the diffusion through the polymer wall as well as its erosion. This suggested that the release of zidovudine from Eudragit RS 100 microsphere exhibits diffusional characteristics, closely following Higuchi model and is highly correlated with first order release kinetics.

This differences in drug release behavior suggested structural differences of the wall materials, and it is dependent on the content of the quaternary ammonium groups. As we know, the content of the quaternary ammonium group of the Eudragit RL 100 microsphere (10%) is higher than that of the Eudragit RS 100 microsphere (5%). Thus, in the Eudragit RL 100 microspheres, the drug might be dispersed evenly in the matrix of the polymer and the surface would be loose, due to the high charge density. On the other hand, in the case of Eudragit RS 100 microsphere, lower charge density produces more packed structures than those of Eudragit RL 100 microspheres.

**CONCLUSION**

In this study, zidovudine was successfully encapsulated into two structurally different eudragit
polymers and their combinations. By using a optimal proportion of magnesium stearate as droplet stabilizer, uniform and reproducible microspheres could be prepared. The surface structure of the microsphere was spherical and smooth. The encapsulation efficiencies were successfully increased with eudragit polymers which range 56.4–87.1% and the mean size was between 1000–3000 µm. The release rate of Eudragit RS100 microspheres exhibit a lag time at the initial release and the best release was observed with formulation B2. On the other hand, Eudragit RL 100/RS100 mixture microspheres give the increased rate of drug release at a controlled rate and the best release was observed with formulation A1B1. The release pattern of the Eudragit RL 100 microspheres followed the Higuchi equation indicating Fickian diffusion, whereas Eudragit RS 100 microspheres exhibited triphasic drug release profile, with an initial burst, an lag period and then diffusion of drug through the wall material.

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