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Research Article

EFFECT OF FORMULATION AND PROCESS VARIABLES ON THE CHARACTERISTICS OF MICROSPHERES OF ANTI-VIRAL DRUG (STAVUDINE) PREPARED BY OIL-IN-OIL SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

The aim of this study is to prepare and characterize the microspheres of stavudine (d4T) using ethyl cellulose and eudragit®RS100 as polymeric retardant materials. Microspheres were prepared by oil-in-oil emulsion evaporation technique using ethyl cellulose and eudragit®RS100. The effect of drug-polymer ratio and stirring speed on percentage yield, encapsulation efficiency, particle size, and *in-vitro* release were evaluated. Fourier transforms infrared spectroscopy (FTIR) and differential scanning calorimetric (DSC) revealed no interaction between drug and polymer(s). Scanning electron microscopy (SEM) shows microspheres were spherical. The encapsulation efficiency and particle size decreased significantly ($p<0.05$) with increase in drug-polymer ratio and stirring speed. *In-vitro* release increased with increase in drug-polymer ratio and with decrease in stirring speed. The release of drug was found to be sustained and diffusion controlled. The result suggests that the combination of ethyl cellulose and 1:1 ratio combination with eudrgit®RS100 microsphere may be useful for the control delivery of stavudine.

Key words*:* Stavudine, Ethyl cellulose, Eudragit®rs100, Oil-in-oil emulsion evaporation technique.

INTRODUCTION

Stavudine (d4T) is nucleotide reverse transcriptase inhibitors and used in the treatment of infection by retrovirus, primarily HIV. It is approximately 80% absorbed from the gastrointestinal tract and undergoes considerable first-pass metabolism[1]. As it has short biological half-life 0.8 – 1.5h and low daily dose of 30 mg, d4T should be formulated in a sustained release dosage form to improve patient compliance. Microencapsulation is a process whereby one can formulate controlled/sustained action dose form for drugs having a short half life[2]. Microencapsulation drug absorption and minimize side effects due to the localized build up of drugs against the gastrointestinal mucosa[3].

In the present study, the drug d4T is formulated into microspheres using ethylcellulose and 1:1 ratio combination with eudrgit®RS100 as the retardant by oil-in-oil emulsion solvent evaporation method. Various physicochemical properties like percentage yield, drug entrapment efficiency, particle size distribution and interactions between drug and polymer were studied. *In-vitro* drug releases studied were carried out to determine the effect different formulation and process variables on the release of drug from the prepared microspheres and the kinetic of drug release.

MATERIALS AND METHODS

Materials

Stavudine (Cipla Ltd. Mumbai), ethyl cellulose (CDH (P) Ltd. New Delhi), eudragit®RS100 (Rohn Pharma, Darmstadt, Germany) were used. Acetone and light liquid paraffin were obtained from Ranbaxy Fine Chemical Ltd., New Delhi used as dispersion media. N-Hexane (Ranbaxy Fine Chemical Ltd., New Delhi) was a washing agent. All chemicals received were of analytical grade and were used as such.

Methods

Microspheres were prepared by oil-in-oil emulsion evaporation technique. Ethyl cellulose and ethyl cellulose in a 1:1 ratio combination with eudragit®RS100 was dissolved in 7ml acetone and a given amount of the drug (as shown in table I) were taken in a sample bottle and were dispersed in it to make different drug to polymer ratio 1:1, 1:2, 1:3 shacked for about 5 minutes using cyclomixer. Then the polymer drug dispersion was poured slowly in a thin stream into 40 ml of light liquid paraffin containing 0.5% span – 80, 1% ethyl cellulose (50 mg) and 1% magnesium stearate (50 mg.) were used as a surfactant, saturation and droplet stabilizer in the processing medium respectively. The whole system was then stirred for about 3 h. After 2 h, 10 ml of n-hexane (non solvent) was added in the medium to solidify and stabilizes the microspheres. After stirring process is over the light liquid paraffin was decanted off and microspheres formed were collected by filtration using membrane filter. Microspheres were washed with petroleum ether for several times to completely remove the remaining oil and air dried at room temperature for 12h and collected for further studies.

Percentage yield value of microspheres

The prepared microspheres were accurately weighed and the percentage yield was calculated by using the following formula.

$$\text{Percentage yield} = \frac{\text{Amount of microspheres obtained (mg)}}{\text{Theoretical amount}} \times 100 \quad \text{------(1)}$$

Results were based on triplicate determination.

Drug entrapment efficiency

About 50 mg of accurately weighted triturated drug loaded microsphere were added to 50 ml phosphate buffer (pH 6.8). The resulting mixture was shaken by the magnetic stirrer for 4h. The solution was filtered using whatman filter paper and 1ml of this solution was diluted and analyzed spectrophotometrically at 266 nm using U-2001 Hitachi Inc. UV-Visible spectrophotometer.

Drug entrapment efficiency =

$$\frac{\text{Experimental drug Content}}{\text{Theoretical drug Content}} \times 100 \quad \text{..... (2)}$$

Results were based on triplicate determination.

Size distribution of microspheres

Size distribution of microspheres was determined by microscopic method. The ocular micrometer was calibrated using stage micrometer and each division of the ocular micrometer was measured in micrometer. For each batch of the microsphere, 100 particles were counted and done in triplicate.

Drug –polymer interaction studies

FT-IR spectroscopy

The study of drug-polymer interaction FT-IR spectra of pure drug, blank microspheres and drug-loaded microspheres was recorded at room temperature in KBr pellets using JASCOFT-IR (model ho-4200) within the range of 400-4000 cm^{-1}.

Differential scanning calorimetry analysis (DSC)

The DSC analysis of the pure drug, blank microspheres & drug-loaded microspheres were carried out using a Diamond DSC (Perkin Elmer, USA). Samples were sealed in aluminum pans and scanned from 30 to 400^{0}C at a heating rate of 15^{0}C/min in an atmosphere of nitrogen gas.

Scanning electrons microscopy (SEM)

The shape and surface topography of microspheres was conducted using scanning electron microscope (Hitachi, S-3600 N, Japan) for the blank microspheres, drug loaded microspheres before and after dissolution. The samples were fixed on a brass sub using double-sided tape and then gold-coated in vacuum by a sputter coater. The pictures were then taken at an excitation voltage 15 kV.

***In-vitro* drug release study**

The *in-vitro* release studies of drug-loaded microspheres were carried out at 37^{0}C ±1^{0}C at 100 rpm using phosphate buffer pH 6.8 (500 ml) in a USP paddle type dissolution test apparatus. An accurately weighed amount of microspheres (equivalent to 50 mg of drug) were added to dissolution medium and at predetermined interval 2ml of aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium, Aliquots following suitable dilution, were analyzed spectrophotometrically at 266 nm. Dissolution studies were performed for period of 12 hour. The concentration of stavudine in test sample were corrected for sampling effect using following formula –

$$C_n = M_n\left[\frac{V_T}{V_T - V_S}\right] \times \left[\frac{C_{n-1}}{M_{n-1}}\right] \text{ ---------- (3)}$$

Where, C_n and M_n are the corrected and original concentration of the n^{th} sample respectively, V_T and V_S are the volume of dissolution medium and sample withdrawn respectively, C_{n-1} and M_{n-1} are the corrected and original concentration of $(n-1)^{th}$ sample respectively. Results were based on triplicate determination.

Drug release kinetics

Data obtained from in-vitro release studies were fitted to various kinetic equations to find the mechanism of drug release from the ethyl cellulose and in combination with eudragit®RS100 microspheres. The kinetic models used were.

$$M_t = M_o + K_o t \quad \text{(Zero-order equation) ……..… (4)}$$

$$\ln M_t = \ln M_o - K_1 t \quad \text{(First-order equation). ... …… (5)}$$

$$M_t = K.S.t^{1/2} = K_h t^{1/2} \quad \text{(Higuchi equation, Fickian diffusion). . (6)}$$

Where, M_t is the amount of drug release in time t, M_α is the initial amount of drug in the microspheres, S is the surface area of the microspheres and K_0, K_1 and K_h are rate constant of zero order, first order and Higuchi rate equation respectively. In order to define a model, which will represent a better fit for the formulation, dissolution data was further analyzed by Korsemeyer – peppas equation.

$$M_t / M_\alpha = k.t^n \cdot \quad \text{…….. (7)}$$

Where M_t is the amount of drug release at time t and M_α is the amount release at time $t=\alpha$ thus M_t / M_α is the fraction of rug released at time t, k is the kinetic constant, and n is the diffusion exponent that can be used to characterize both mechanism for both solvent penetration and drug release.

Determining the correlation coefficient assessed fitness of the data into various kinetic models. The rate constants or respective models were also calculated from slope.

Statistical analysis of data

All the means are presented with their standard deviation (mean±S.D). An unpaired student's t-test was used to compare the effect off different parameters on the mean particle size, yield, entrapment efficiency and percentage release of drug. A p value of <0.05 was considered significant.

RESULT AND DISCUSSION

Yield and drug entrapment efficiency

The influence of different preparation conditions on stavudine content (as shown in Table II) was evaluated and yield of microspheres were found from the range of 80-95%.

The encapsulation efficiency (as shown in Table II) was found more in ethyl cellulose microspheres than in combination microspheres. When drug to polymer ratio increased from 1:1 to 1:3, the entrapment efficiency was decreased significantly. ($P < 0.05$, student's t-test).

Particle size of microspheres

The mean particle sizes (as shown in Table II) of the formulations were found to be in the range of 208-422 µm. It was found to be affected by preparation conditions.

Effects of drug to polymer ratio on particle size

The effects of drug to polymer ratio on the mean particle size of microspheres were determined and as shown in the Fig. I. The amount of stavudine was kept constant and the amount of polymer added was varied. The particle size decreases significantly ($P < 0.05$ student's t-test) with increase in the drug to polymer ratio. It might be due to increase in viscosity of the internal phase[4-6].

Effects of stirring speed on particle size

The stirring speed also found to influence the particle size as shown in Fig II. When the stirring speed of all formulation increased from 800rpm to 1200rpm a significant decrease ($P< 0.05$ students t-test) in particle size was observed for ethyl cellulose and ethyl cellulose in combination with eudragit®RS100 microspheres. Increase in high shear results decrease in size of microdroplets of the emulsion, resulting formulation of smaller size microspheres[7-9]. A more uniform particle size was seen at 100 rpm compared to 800 and 1200 rpm.

Table 1: Formulation of stavudine microspheres containing ethyl-cellulose (EC) and 1:1 ratio combination with eudragit®RS100

Form code	D:P[a]	Amount of drug (mg)	Amount of EC (mg)	Amount of eudragit®-RS100 (mg)	Amount of Mg-stearate (mg)	EC in processing medium (mg)	Stirring speed (rpm)
FA1	1:1	300	300	-	-	-	800
FA2	1:2	200	400	-	-	-	800
FA3	1:3	150	450	-	-	-	800
FA4	1:1	300	300	-	-	-	1000
FA5	1:2	200	400	-	-	-	1000
FA6	1:3	150	450	-	-	-	1000
FA7	1:1	300	300	-	-	-	1200
FA8	1:2	200	400	-	-	-	1200
FA9	1:3	150	450	-	-	-	1200
FA10	1:1	300	150	150	50	50	800
FA11	1:2	200	100	300	50	50	800
FA12	1:3	150	337.5	112.5	50	50	800
FA13	1:1	300	150	150	50	50	1000
FA14	1:2	200	100	300	50	50	1000
FA15	1:3	150	337.5	112.5	50	50	1000
FA16	1:1	300	150	150	50	50	1200
FA17	1:2	200	100	300	50	50	1200
FA18	1:3	150	337.5	112.5	50	50	1200

*0.5% Span 80 was used in every case. a : Drug- polymer ratio.

Table 2: Percentage yield, entrapment efficiency and particle size of stavudine microspheres

Form. code	% yield (mean ± SD, n = 3)	Entrapment efficiency (%) (mean ± SD, n = 3)	Mean particle size (µm) (mean ± SD, n = 3)
FA1	87.5+3.2	80.0+5.33	310±1.54
FA2	90.6+4.6	78.0+3.14	298+2.63
FA3	80.0+ 2.3	76.2+4.87	291+1.60
FA4	95.2+3.5	85.1+2.77	251+6.54
FA5	78.2+4.4	76.0+2.89	242+2.50
FA6	81.5+4.6	65.4+4.25	238+1.12
FA7	78.7+2.3	70.0+ 5.51	230+2.60
FA8	87.6+4.8	72.0+2.47	218+4.10
FA9	89.4+3.8	60.0+3.23	213+3.80
FA10	88.3+1.2	72.2+1.02	422±5.62
FA11	93.5+2.3	83.4+4.02	395±3.55
FA12	83.0+2.8	73.6+2.31	375±4.32
FA13	86.4+1.0	72.3+4.11	351±3.08
FA14	90.2+3.2	74.1+1.04	336±4.76
FA15	93.1+4.0	74.2+3.27	331±2.21
FA16	79.3+3.4	73.3+5.03	288±1.33
FA17	89.2+4.6	85.8+1.05	273±3.87
FA18	85.4+1.0	77.4+3.22	255±5.01

FT-IR analysis

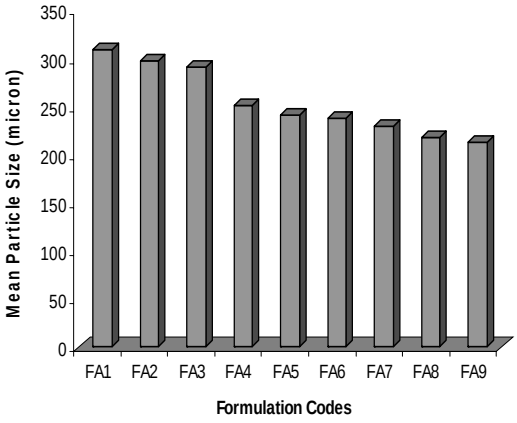
The FT–IR spectra of pure stavudine (Fig. IIIa) showed sharp peak at 1689 cm^{-1} C=O stretching of aromatic structure), 3168 cm^{-1} (-NH stretching) and at 2882 cm^{-1} for C-H stretching of CH_3 group. The identical peaks were also present in drug loaded ethyl cellulose and eudragit®RS100 mixed polymeric microsphere (Fig IIId). It was expected that there was no interaction between drug and polymer.

Differential scanning calorimetry analysis (DSC)

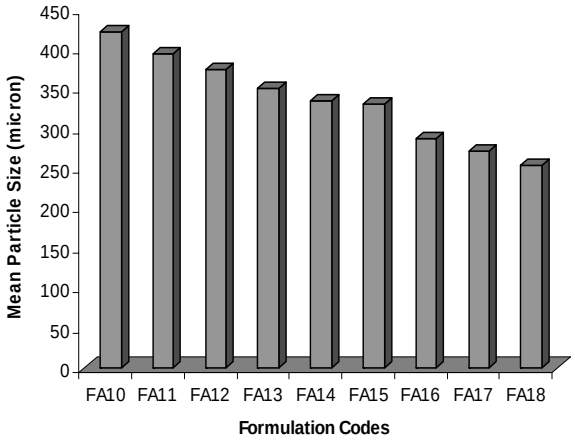
The drug may have been dispersed in crystalline or amorphous from or dissolved in the polymeric matrix during formation of the microspheres. Any abrupt of drastic change in the thermal behavior of either drug or polymer may indicate a possible drug-polymer interaction. The thermogram of stavudine corresponds to its melting point 161^0C to 167^0C[10]. This endotherm was also observed for ethyl cellulose and eudragit®RS100 mixed polymeric microspheres at 160^0C where it was less sharp and this suggests that there is a significant reduction in drug crystalinity in the polymer matrix.

Table 3: Correlation coefficient (R^2) and constant (k) for drug to polymer ratios 1:1, 1:2 and 1:3, after fitting of dissolution data to the different kinetic models

Polymer type	D:P	Kinetic models							
		Zero-order		First-order		Higuchi Model		Korsemeyer-Peppas Model	
		R^2	K_0	R^2	K_1	R^2	K_h	R^2	n
EC	1:1	0.535	41.695	0.872	1.649	0.767	25.475	0.9024	0.258
	1:2	0.608	37.833	0.896	1.702	0.827	21.558	0.9347	0.296
	1:3	0.638	32.897	0.852	1.743	0.853	17.739	0.9309	0.358
EC+ Eudragit®RS100	1:1	0.820	25.686	0.966	0.841	0.969	0.5213	0.9869	0.520
	1:2	0.947	15.403	0.976	0.963	0.992	0.5049	0.9910	0.589
	1:3	0.762	26.857	0.971	0.829	0.935	0.5628	0.9887	0.558

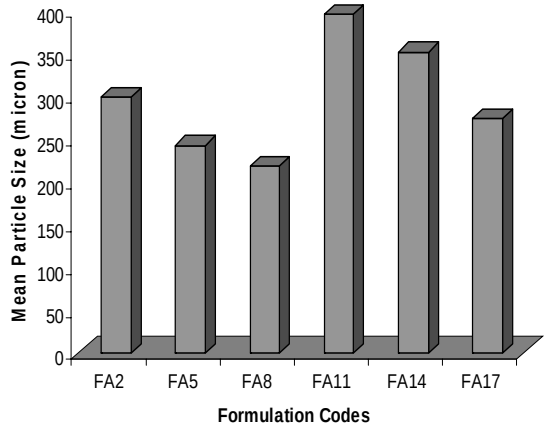


(a)



(b)

Fig. 1: Effect of drug to polymer ratio on microspheres particle size of drug loaded ethylcellulose microspheres (a) and ethylcellulose in combination with eudragit®RS100 microspheres (b)



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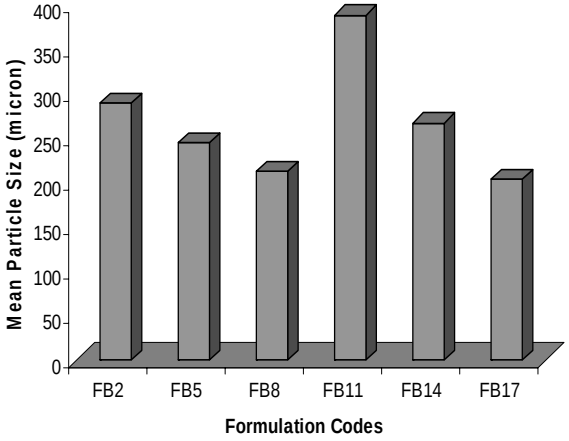


(b)

Figure 2: Effect of stirring speed on mean particle size of drug loaded ethylcellulose (a), ethylcellulose and in combination with eudragit®RS100 microspheres (b)

Scanning electron microscopy analysis

The scanning electron microscopy of drug-loaded ethyl cellulose microspheres revealed that the microspheres posses spherical, nom-aggregated and porous surface (Fig. IVb) but ethyl cellulose in combination with eudragit®RS100 microspheres revealed that microsphere posses a rough and rugged surface (Fig IVe). The surface of the blank microspheres of ethyl cellulose in combination with eudragit®RS100 (Fig. IVd) are more smother than that of drug-loaded microspheres (Fig. IVe) and that might be due to crystalline nature of encapsulated drug which was present on the surface of the microspheres. The surface study of microspheres after release study (Fig. 4c and 4f) showed the presence of pore, which helps to predict the mechanism of drug release from the microsphere, was diffusion controlled.

***In-vitro* release study**

The effect of drug to polymer concentration on *in-vitro* release was studied and as shown in figures Va and Vb. The release of d4T from ethyl cellulose and in combination with eudragit®RS100 microspheres illustrated the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices, which indicates that the release rate decreases significantly (P<0.05 student's t-test) with increasing the amount of polymer. It might be due to increase in coat thickness surrounding the drug particles thereby increasing the distance traveled by the drug throughout coat[11-13].

The effect of stirring speed on *in-vitro* release of d4T from for ethyl cellulose and in combination with eudragit®RS100 microspheres are shown in Fig. VIc and VId. Release curve indicates that with the increase of stirring speed, the d4T release increase significantly (P< 0.05 student's t-test) for elthylcellulose or in combination with eudragit®RS100 microspheres. At 10 hours, maximum 80% and 85% release observed for ethyl cellulose and polymer mixture microspheres respectively. When the stirring speed decreases a high initial burst release of around 38.45% to 50.25% within 2 hours was observed for all the formulation. This initial burst release can be attributed as desired effect, which ensures the quick initial plasma therapeutic concentration of drug. This might be due to the drug migration was high for low stirring speed and more amount of drug remain in all microsphere surface and when stirring speed increased, drug migration was less due to collision of emulsion droplets[14].

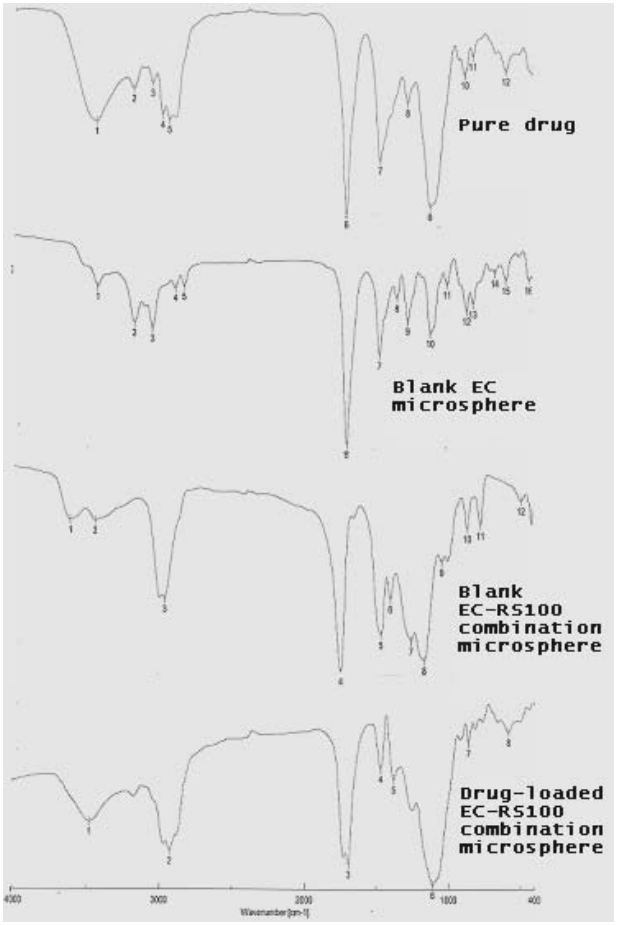
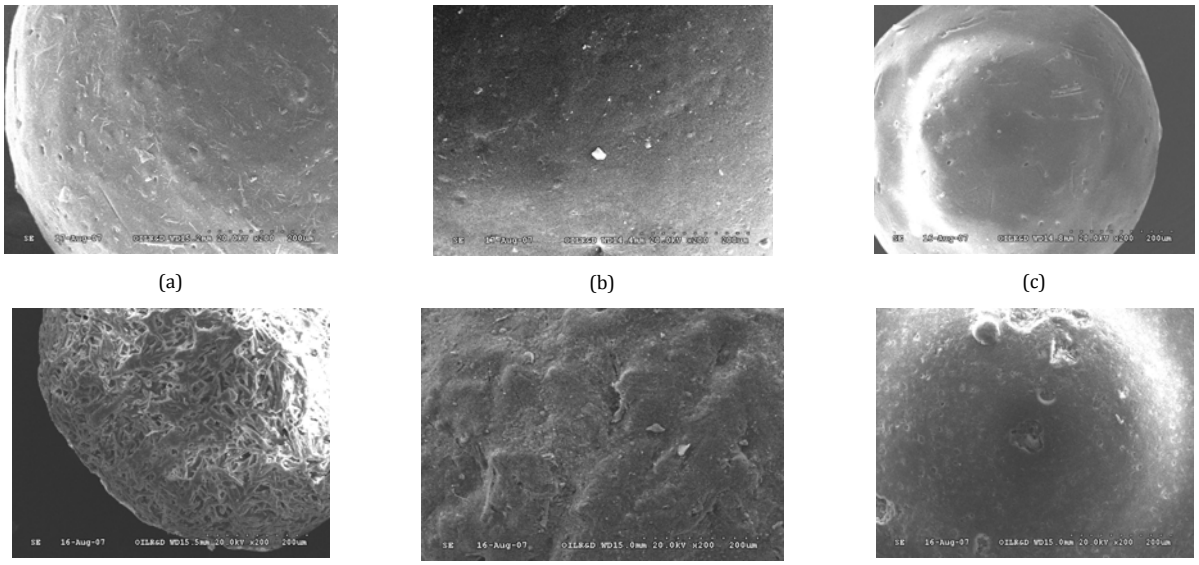


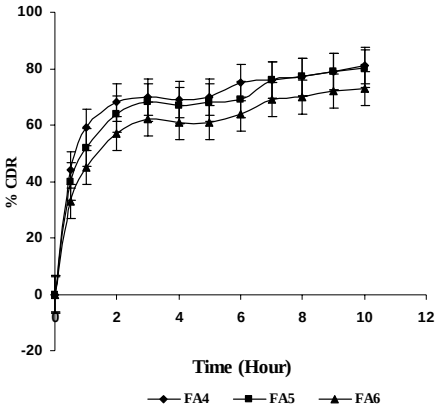
Fig. 3: FT-IR spectrum of (a) stavudine pure drug, (b) blank ethylcellulose microspheres, (c) Blank ethylcellulose-eudragit®RS100 combination microspheres, (d) Drug-loaded ethylcellulose-eudragit®RS100 combination microspheres



(a) (b) (c)

(d) (e) (f)

Fig. 4: SEM photograph (×200) of (a) blank ethyl cellulose microsphere (b) drug loaded ethyl cellulose microsphere before dissolution (c) drug loaded ethyl cellulose microsphere after dissolution (d) blank ethyl cellulose and eudragit®RS100 combination microsphere (e) drug loaded ethyl cellulose and eudragit®RS100 combination microsphere before dissolution (f) drug loaded ethyl cellulose and eudragit®RS100 combination microsphere after dissolution.



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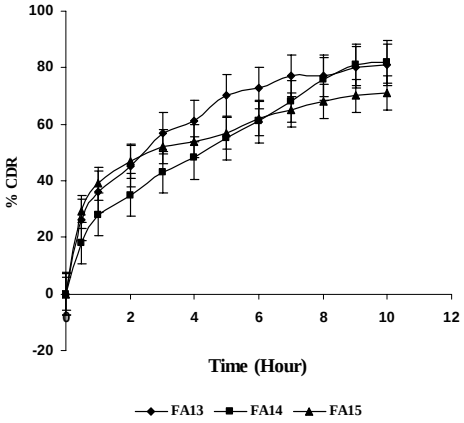
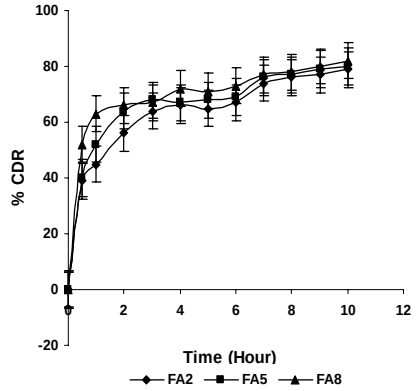
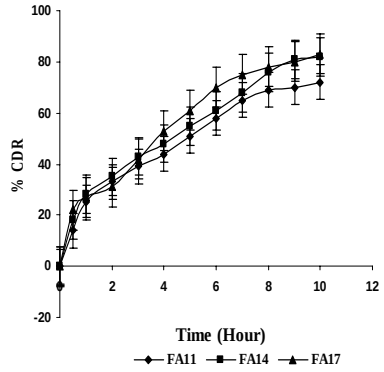


(b)

Fig. 5: Cumulative percent release of drug (% CDR) from (a) ethylcellulose and (b) ethylcellulose- eudragit®RS100 combination microspheres with different drug: polymer ratio (mean ± SD, n = 3)



(c)



(d)

Fig. 6: Cumulative percent release of drug (% CDR) from (a) ethylcellulose and (b) ethylcellulose- eudragit®RS100 combination microspheres with different stirring speed (mean ± SD, n = 3).

Release kinetics

The release mechanisms of stavudine from various formulations (as shown in Table III) were determined by comparing their respective correlation coefficient. It would appear that the mechanism of drug release from microspheres was diffusion controlled[14].

CONCLUSION

Stavudine microspheres were prepared successfully using oil-in-oil emulsion evaporation method. Drug and polymer ratio and stirring speed influenced the yield, entrapment efficiency and particle size of the microspheres. Yield and encapsulation efficiency were high for all formulations. It was observed that with increase in the polymer concentration and stirring speed the mean particle size of microsphere decreases. The assessment of released kinetics revealed that the drug release from microsphere was diffusion-controlled process. Therefore, stavudine controlled release microsphere formulation using ethyl cellulose and in combination of eudragit®RS100 as retardant material, may reduce dose frequency and as well as improve patient compliance.

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