Malaysian Journal of Pharmaceutical Sciences
Vol. 8, No. 2, 45–57 (2010)

COMPARATIVE STUDY OF STAVUDINE MICROSPHERES PREPARED USING ETHYLCELLULOSE ALONE AND IN COMBINATION WITH EUDRAGIT®RS100

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Stavudine microspheres were prepared using ethylcellulose alone and in combination with Eudragit®RS100 by emulsion solvent diffusion method to sustain the release of drug from microspheres. The effect of drug-polymer ratio and stirring speed on percentage yield, encapsulation efficiency, particle size, in vitro release profiles and kinetics of drug release of microspheres were evaluated. The size distribution of the prepared microspheres was done by microscopic method and in vitro dissolution studies were carried out in phosphate buffer (pH 6.8) as the dissolution media to study the drug release profile of the microspheres. Surface topography of microspheres was characterised using scanning electron microscopy (SEM). Fourier transformer infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) were performed to evaluate interaction between drug and polymer(s) which revealed to be compatible. The encapsulation efficiency was found satisfactory and it decreased significantly ($p<0.05$) with increase in drug-polymer concentration. Both the drug-polymer ratio and stirring speed affects the physicochemical properties and release of drug from microspheres. The prepared microspheres satisfactorily released the drug in a sustained and uniform rate following Higuchian kinetics. Sustained release microsphere of stavudine has a potential for oral administration at least once every 12 h.

Keywords: *Stavudine, Ethylcellulose, Eudragit®RS100, Emulsion solvent diffusion method, Microsphere*

INTRODUCTION

Stavudine is a nucleotide reverse transcriptase inhibitor and is primarily used in the treatment of AIDS (Flexner 2007). It has a short biological half-life of 0.8–1.5 h (Martin 1998; Tripathi 2003) and therefore, requires more frequent dosing. To overcome this problem, it is necessary to design sustained release dosage forms of stavudine so as to reduce dosing frequency and thereby improve patient compliance. Microencapsulation has been used as one of the methods to deliver drugs with short half-lives in a controlled fashion. It provides a means to modify and retard drug release. Due to their small particle size, they are widely distributed throughout the gastrointestinal tract (g.i.t.). This potentially improves drug absorption and reduces side effects related to localised build-up of irritating drugs against the g.i.t. mucosa (Khan *et al.* 2010).

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Several methods have been used for microencapsulation of drug. Emulsion solvent diffusion technique is one of such methods which can be used to encapsulate both water soluble and water insoluble drugs (Venkatesan, Manavalan and Valliappan 2009). This method is generally simple, reproducible and economical (Kim, Kim and Oh 1994). Eudragit®RS100 is a hydrophobic polymer and is widely used as a retardant material for the release of drug from the microsphere (Singh *et al.* 2008; Gupta *et al.* 2009). It is a copolymer, which is synthesised from acrylic and methacrylic acid esters with low content of quaternary ammonium groups. Ethylcellulose is a hydrophobic polymer and has been widely used in the preparation of sustained release dosage forms of water-soluble drugs (Alpar and Walters 1981; Palmieri *et al.* 2001; Al-Omran *et al.* 2002; Rao and Murthy 2002).

The objective of this study was to prepare sustained release microsphere of stavudine by emulsion solvent diffusion technique using ethylcellulose alone and in combination with Eudragit®RS100. This study also compared the effects of formulation and process variables on the physicochemical properties of drug.

METHODS

Materials

Stavudine was obtained from Cipla Ltd. (Mumbai) as a gift sample. Ethylcellulose and Eudragit®RS100 were purchased from CDH (P) Ltd. (New Delhi) and Rohn Pharma (Darmstadt, Germany) respectively. Acetone, light liquid paraffin and n-hexane were obtained from Ranbaxy Fine Chemical Ltd. (New Delhi). All chemicals were of analytical grade and used as such.

Methods

Microspheres of ethylcellulose were prepared by emulsion solvent diffusion technique, using ethylcellulose alone and in different ratio combinations with Eudragit®RS100 (Table 1). Drug and polymer were dissolved in a solvent system consisting of acetonitrile and dichloromethane (1:1 ratio). The resultant solution was extruded through a syringe (No. 20) in aqueous medium (2 mL) under stirring at 500 rpm using a mechanical stirrer (Remi Motors, Mumbai, India) for 5 min, to form primary emulsion (w/o). The w/o primary emulsion was slowly added to 50 mL of light liquid paraffin containing 0.5% Span 80, 1% ethylcellulose (50 mg), 1% w/v magnesium stearate (50 mg) as a tensioactive agent, saturation and droplet stabiliser in the processing medium, respectively to form w/o/o multiple emulsion. The whole system was stirred for about 3 h. After the stirring process was over, the light liquid paraffin was decanted off and microspheres formed were collected by filtration using ordinary filter paper (pore size 25 μm). They were washed with petroleum ether (40°C–60°C) for several times to completely remove the oil. The microspheres were then air dried at room temperature for 12 h and collected for further studies.

Percentage of Yield of Microspheres

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

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

Drug Entrapment Efficiency

Microspheres equivalent to 50 mg stavudine were accurately weighed and triturated using mortar and pestle. The powdered microsphere was added to 50 mL phosphate buffer (pH 6.8). The resulting mixture was shaken using magnetic stirrer for 4 h. The solution was filtered through Whatman filter paper and 1 mL of this solution was suitably diluted and analysed spectrophotometrically at 266 nm using UV-Visible spectrophotometer (Hitachi U-2001, Japan). The drug entrapment efficiency was calculated as per the following equation:

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100 \quad (2)$$

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 μm. For each batch of the microsphere, 100 particles were counted and done in triplicate (Shashikant *et al*. 2009).

Drug-polymer Interaction Studies

Fourier transformer infrared spectroscopy (FTIR)

To study the drug-polymer interaction, FTIR spectra of pure drug, blank microspheres and drug-loaded microspheres were recorded at room temperature in KBr pellets using JASCOFT-IR (model no-4200, Jasco Global, Mumbai) within the range of 400–4000 cm^{-1}.

Differential scanning calorimetry (DSC)

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

Table 1: Formulation of stavudine microspheres containing ethylcellulose and in combination with Eudragit®RS100.

Form. Code	D:P[a]	Stavudine (mg)	Ethylcelluolse (mg)	Eudragit®RS100 (mg)	Stirring speed (rpm)
FB1	1:1	300	300	–	800
FB2	1:2	200	400	–	800
FB3	1:3	150	450	–	800
FB4	1:1	300	300	–	1000
FB5	1:2	200	400	–	1000
FB6	1:3	150	450	–	1000
FB7	1:1	300	300	–	1200
FB8	1:2	200	400	–	1200
FB9	1:3	150	450	–	1200
FB10	1:1	300	150	150	800
FB11	1:2	200	100	300	800
FB12	1:3	150	337.5	112.5	800
FB13	1:1	300	150	150	1000
FB14	1:2	200	100	300	1000
FB15	1:3	150	337.5	112.5	1000
FB16	1:1	300	150	150	1200
FB17	1:2	200	100	300	1200
FB18	1:3	150	337.5	112.5	1200

Notes: *0.5% Span 80 was used in every case; a: drug-polymer ratio; Form: Formulation.

Scanning Electron Microscopy (SEM)

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

In vitro Release Study

The in vitro release studies of microspheres were carried out in a United States Pharmacopoeia (USP) paddle type dissolution test apparatus (Pharmatest PTW II, Pharmatest Apparatus, Hainburg, Germany) using 900 mL phosphate buffer (pH 6.8). The dissolution medium was stirred at 100 rpm with the temperature maintained at 37C±1°C (Ghosh, Nayak and Roy 2007). Microspheres equivalent to 50 mg of stavudine were added to the dissolution medium. At predetermined intervals, 2 mL of aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. Aliquots were suitably

diluted and drug content was measured using UV-VIS spectrophotometer (Hitachi U-2001, Japan) at 266 nm.

Release Kinetics

Data obtained from in vitro release studies were fitted to various kinetic equations to find the mechanism of drug release from the microspheres. The kinetic models used were:

$M_t = M_o + K_o t$ (zero-order equation) ……...…….....(3)

$\ln M_t = \ln M_o - K_1 t$ (first-order equation)…….(4)

$M_t = K.S.t^{1/2} = K_h t^{1/2}$ (Higuchi equation based on Fickian diffusion)…….....(5)

Where, M_t is the amount of drug released in time t, M_o is the initial amount of drug in the microspheres, S is the surface area of the microspheres and K_o, 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 analysed by Korsemeyer-Peppas equation.

$M_t/M_\alpha = k.t^n$ ……...…(6)

M_t/M_α is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusion exponent that can be used to characterise both mechanism for both solvent penetration and drug release.

Statistical Analysis of Data

All the means are presented with their standard deviation (mean±SD). An unpaired student's t-test and one-way analysis of variance (ANOVA) were used to compare the effect of different parameters on the mean particle size, yield, entrapment efficiency and in vitro release of drug. A p value of <0.05 was considered significant.

RESULTS

In trial batch, the in vitro release of stavudine significantly (p<0.05) decreased with increase in volume of light liquid paraffin from 20 mL to 50 mL. On the other hand, a decrease in the in vitro drug release when light liquid paraffin was increased from 50 mL to 60 mL was not significant (p<0.05). Span80 in the concentration of 0.5% was found to be suitable to prevent aggregation of the microspheres.

All the microspheres were fine and free flowing. The influence of formulation and process variables on percentage yield was evaluated. The percentage yield of microspheres prepared by ethylcellulose alone and in combination with Eudragit®RS100 was found to be in the range of 56%–79% and 69%–87%, respectively (Table 2).

The entrapment efficiency of stavudine in microspheres prepared by ethylcellulose alone and in combination with Eudragit®RS100 is presented in Table 2. The entrapment efficiency of stavudine prepared by ethylcellulose alone and in combination with Eudragit®RS100 was found to be in the range of 47%−70% and 56%−72%, respectively. The entrapment efficiency decreased significantly (p<0.05) with increased drug-polymer ratio.

The mean particle sizes (Table 2) were found in the range of 206−290 µm and 201−413 µm for microspheres prepared with ethylcellulose alone and in combination with Eudragit®RS100. A reduction in microspheres size was observed with increase in the drug-polymer ratio but was not statistically significant (p<0.05).

The stirring speed was also found to have a profound effect on the particle size of microspheres. When the stirring speed of all formulation was increased from 800 rpm to 1200 rpm, a significant (p<0.05) reduction of mean particle size was observed. It affected in same way for both types of microspheres, prepared by ethylcellulose alone and in combination with Eudragit®RS100. The FTIR spectra of pure stavudine [Fig. 1(a)] showed sharp peak at 1689 cm^{-1} (C=O stretching of aromatic structure), 3168 cm^{-1} (-NH stretching) and 2882 cm^{-1} (C-H stretching of CH^3 group). The identical peaks were also present in drug loaded ethylcellulose/Eudragit®RS100, mixed polymeric microsphere [Fig. 1(d)]. The thermogram of stavudine that corresponds to its melting point are 161°C to 167°C (Sahoo *et al.* 2005). The less sharp endotherm was also observed at 160°C for ethylcellulose and Eudragit®RS100 mixed polymeric microspheres.

The SEM study of drug-loaded ethylcellulose microspheres revealed spherical, non-aggregated and porous surface of microspheres [Fig. 2(a)]. On the other hand, rough and rugged surface [Fig. 2(b)] of microspheres was observed in microspheres prepared using ethylcellulose and Eudragit®RS100 combination. The surface topography study of microspheres after release study [Fig. 2(c) and 2(d)] showed the presence of pore on both types of microspheres.

The in vitro release of stavudine from microspheres was found to be triphasic for both types of microspheres. The variation of release was observed from the microspheres prepared by different retardant materials (Fig. 3 and 4). The in vitro release of drug from ethylcellulose microspheres is more sustained as compared to ethylcellulose and Eudragit®RS100 combination microspheres. The effect of drug to polymer ratio on in vitro release was studied. Figures 3(a) and 3(b) depict the release of stavudine from microspheres. The rate of drug release decreased significantly (p<0.05) with increasing amount of polymer. The release profiles [Fig. 4(a) and 4(b)] of the microspheres showed that the release of stavudine from microspheres was also affected by stirring speed. Release curve indicates that with an increase in stirring speed, the release of drug from the microspheres increased significantly (p<0.05). The release mechanisms of stavudine from different microspheres (Table 3) were determined by comparing their respective correlation coefficient.

DISCUSSION

The stavudine microspheres were prepared by emulsion solvent diffusion technique using ethylcellulose alone and in combination with Eudragit®RS100 as retardant material. Various trial batches were taken to select process parameters such as volume of secondary oil phase and stirring speed. The volume of secondary oil phase is an important factor

related to the formulation of microspheres. The volume of light liquid paraffin was selected on the basis of the in vitro release study. Different volume of light liquid paraffin (20 to 60 mL) was used in the trial batch. Concentration of Span80 plays an important role in the formulation of microspheres prepared by emulsion solvent diffusion technique. Therefore, concentration of Span80 was selected by taking into account their aggregation phenomenon.

In previous work by Sahoo *et al.* (2005), the entrapment efficiency of stavudine microspheres prepared by w/o/o double emulsion solvent diffusion method using ethylcellulose and polyvinyl pyrrolidine as retardant material was reported to be in the range of 41%–65%. In our present research work, the entrapment efficiency of the microspheres was higher than the earlier study.

The reduction of the microspheres size was observed with increase in drug-polymer ratio, which might be due to a decrease in viscosity of the internal phase (Amperiadou and Georgarakis 1995; Bolourtchian, Karimi and Aboofazeli 2005; Jelvehgari

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

Form. code	Yield*(%)	Entrapment efficiency* (%)	Mean particle size*(µm)
FB1	66.2±2.3	64.15±2.87	290±5.06
FB2	79.0±3.5	55.05±2.56	277±6.44
FB3	74.3±1.2	49.33±1.92	269±4.18
FB4	62.0±2.5	70.00±1.12	251±2.78
FB5	80.5±1.8	67.11±3.01	245±3.67
FB6	69.6±3.2	57.00±1.72	234±4.22
FB7	56.0±4.8	64.41±2.61	221±4.98
FB8	71.0±2.4	59.20±2.32	212±5.11
FB9	70.3±3.7	47.25±1.25	206±4.04
FB10	69.2±1.2	70.10±3.44	413±2.11
FB11	77.9±3.1	65.50±2.09	389±5.54
FB12	72.6±4.2	64.5.1±4.11	380±2.98
FB13	79.4±3.2	59.43±1.98	276±3.23
FB14	87.1±2.4	72.10±3.04	267±1.09
FB15	73.2±5.3	56.10±2.32	264±6.38
FB16	75.7±3.3	69.32±2.13	209±5.03
FB17	81.2±3.1	63.16±5.01	201±2.65
FB18	69.6±5.1	63.21±4.30	198±1.65

Note: *mean±SD, n=3; form: formulation.

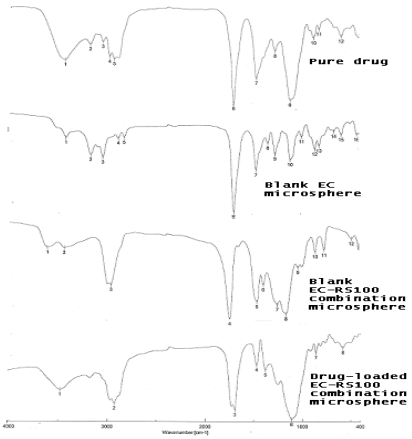
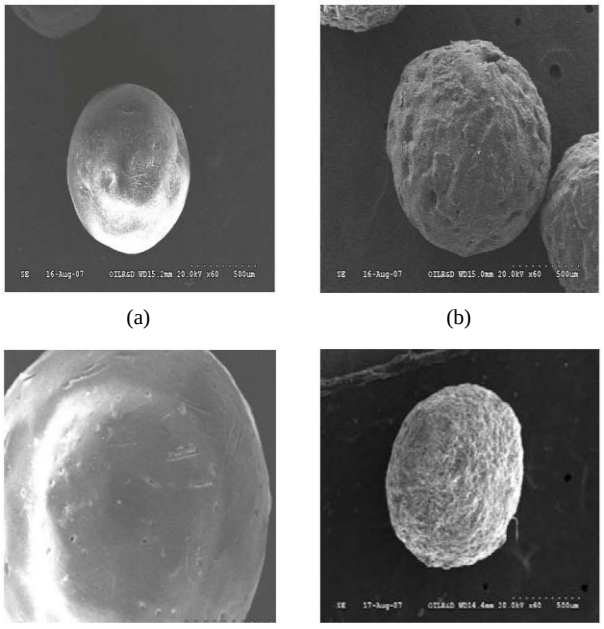


Fig. 1: FTIR spectrum of (a) stavudine, (b) blank ethylcellulose microspheres, (c) blank ethylcellulose-Eudragit®RS100 combination microspheres, (d) drug-loaded ethylcellulose-Eudragit®RS100 combination microspheres.



(a) (b)

(c) (d)

Fig. 2: SEM photograph (×60) of drug loaded ethylcellulose microsphere (a) before and (c) after dissolution; drug loaded ethylcellulose and Eudragit®RS100 microsphere (b) before and (d) after dissolution.

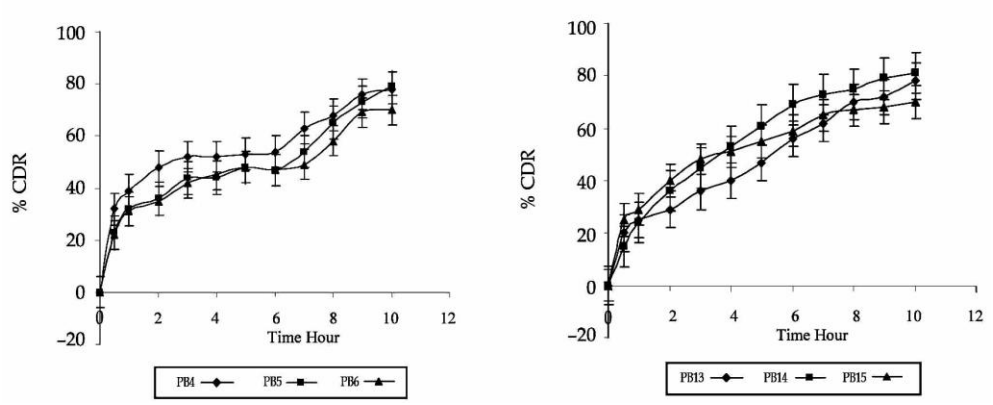


Fig. 3: Effect of different drug: polymer ratio on cumulative percent release of drug from (a) ethylcellulose and (b) ethylcellulose-Eudragit®RS100 combination microspheres (mean±SD, n=3).

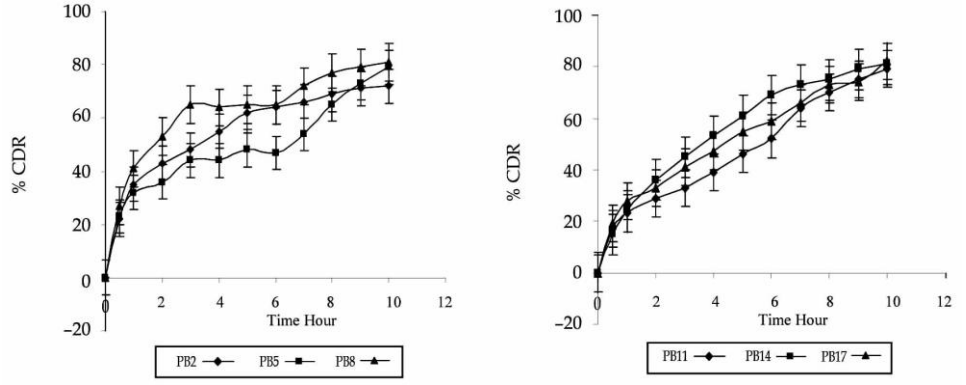


Fig. 4: Effect of stirring speed on cumulative percent release of drug from (a) ethylcellulose and (b) ethylcellulose-Eudragit®RS100 combination microspheres (mean±SD, n=3).

et al. 2010). The decrease in particle size with increased stirring speed might be due to an increase in shear rate which in turn decreases the sized of microdroplets of the emulsion, resulting in the formation of smaller sized microspheres (Babay, Hoffman and Benita 1988; Kawashima *et al.* 1989; Lee, Park and Choi 2000).

FTIR study revealed that there was no interaction between drug and polymer and this was confirmed by the DSC study. The drug may have been dispersed in crystalline or amorphous form or dissolved in the polymeric matrix during formation of the microspheres. Any abrupt or drastic change in the thermal behaviour of either drug or polymer may indicate a possible drug-polymer interaction. SEM study of microspheres after the release study showed the presence of pores which supports the prediction that the mechanism of drug release from the microsphere is through diffusion controll.

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_O	R^2	K^1	R^2	K_h	R^2	N
EC	1:1	0.794	25.757	0.963	1.835	0.919	11.676	0.980	0.412
	1:2	0.887	17.721	0.875	1.925	0.939	4.301	0.918	0.536
	1:3	0.858	18.483	0.901	1.899	0.946	5.848	0.980	0.412
EC	1:1	0.960	12.939	0.973	0.964	0.975	0.523	0.950	0.617
+	1:2	0.918	15.819	0.994	0.943	0.993	0.505	0.993	0.625
Eudragit® RS100	1:3	0.840	21.718	0.977	0.867	0.976	0.533	0.992	0.507

The triphasic release of the microspheres might be due to the following: first, an initial burst release due to the drug desorption from the particle surface; secondly, a plateau for a certain period, resulting from the diffusion of the drug into the microspheres surface; and thirdly, a constant sustained release of the drug resulting from the diffusion through the polymer wall as well as its erosion (Kawashima *et al.* 1989).

The variation of release from the microspheres prepared by different retardant materials is due to the different functional group present in the polymers. Ethylcellulose and Eudragit®RS100 contain ethoxy and quaternary ammonium group, respectively. The release of drug from ethylcellulose microspheres was more sustained as compared with the microspheres prepared by using the combination of ethylcellulose Eudragit®RS100. This is due to gel forming nature of the ethylcellulose which thereby increases the tortuosity of travel of the drug through polymers. In the mixed polymeric microspheres, the drug might be dispersed evenly in the matrix of the polymer and the surface of the microspheres would be loose, due to high charge density of Eudragit®RS100. On the other hand, in the case of ethylcellulose microspheres, lower charge density produces more packed structure than those of mixed polymeric microspheres (Singh and Agarwal 2002).

The significant (p<0.05) decrease in release rate with increasing amount of polymer might be due to an increase in coat thickness surrounding the drug particles thereby increasing the tortuosity to travel the drug through the polymer matrices (Jalsenjak, Nicolaidou and Nixon 1976; Mortada 1982; Kim, Kim and Oh 1994).

The release of drug from microspheres increased significantly with increasing stirring speed. This might be due to the fact that as the stirring speed is increased the particle size of microspheres is decreased, resulting in increased surface area in the dissolution medium and the drug travels the lesser pathway in between the polymer matrices. The mechanism of drug release from both types of microspheres was found to be diffusion controlled.

CONCLUSION

Stavudine microspheres were prepared successfully using emulsion solvent diffusion technique using ethylcellulose alone and in combination with Eudragit®RS100. It was found that the drug-polymer ratio and stirring speed affects the physicochemical properties like percentage yield, encapsulation efficiency, particle size and in vitro release of drug from both types of microspheres. It can be concluded from this study that stavudine could be made into a sustained release drug delivery system using ethylcellulose alone or in combination with Eudragit®RS100. However, the microsphere prepared by combination of ethylcellulose and Eudragit®RS100 is more suitable in view of percentage yield, entrapment efficiency and sustained release phenomenon.

ACKNOWLEDGEMENT

The authors are grateful to the All India Council for Technical Education (AICTE), New Delhi for granting our scholarship and for providing other financial supports for this study. The authors are also thankful to Cipla Ltd., Mumbai, India for providing the drug sample.

REFERENCES

AL-OMRAN, M. F., AL-SUWAYEH, S. A., EL-HELW, A. M. & SALEH, S. I. (2002) Taste masking of diclofenac sodium using microencapsulation, *Journal of Microencapsulation*, 19: 45–52.

ALPAR, H. O. & WALTERS, V. (1981) The prolongation of the in vitro dissolution of a soluble drug (phenethicillin potassium) by microencapsulation with ethylcellulose, *The Journal of Pharmacy and Pharmacology*, 33: 419–422.

AMPERIADOU, A. & GEORGARAKIS, M. (1995) Controlled release salbutamol sulphate microcapsules prepared by emulsion solvent-evaporation technique and study on release affected parameters, *International Journal of Pharmaceutics*, 115: 1–8.

BABAY, D., HOFFMAN, A. & BENITA, S. (1988) Design and release kinetic pattern evaluation of indomethacin microspheres intended for oral administration, *Biomaterials*, 9: 482–488.

BOLOURTCHIAN, N., KARIMI, K. & ABOOFAZELI, R. (2005) Preparation and characterization of ibuprofen microspheres, *Journal of Microencapsulation*, 22: 529–538.

FLEXNER, C. (2007) HIV drug development: The next 25 years, *Nature Reviews Drug Discovery*, 6: 959–966.

GHOSH, A., NAYAK, U. K. & ROY, P. (2007) Development, evaluation and method selection for the preparation of lamuvidine microspheres, *International Journal of Pharmacy*, 6: 52–57.

GUPTA, B. K., PAL, R., CHAKRABORTY, M. & DEBNATH, R. (2009) Design, evaluation and optimization of microcapsules of leflunomide with Eudragit®RS100 and Eudragit®RL100 by solvent evaporation technique, *Asian Journal of Pharmaceutics*, 3: 309–313.

JALSENJAK, I., NICOLAIDOU, C. F. & NIXON, J. R. (1976) The in vitro dissolution of phenobarbitone sodium from ethyl cellulose microcapsules, *The Journal of Pharmacy and Pharmacology*, 28: 912–914.

JELVEHGARI, M., NOKHODCHI, A., REZAPOUR, M. & VALIZADEH, H. (2010) Effect of formulation and processing variables on the characteristics of tolmetin microspheres prepared by double emulsion solvent diffusion method, *Indian Journal of Pharmaceutical Sciences*, 72: 72–78.

KAWASHIMA, Y., NIWA, T., HANDA, T., TAKEUCHI, H., IWAMOTO, T. & ITOH, Y. (1989) Preparation of prolonged-release spherical micromatrix of ibuprofen with acrylic polymer by emulsion-solvent diffusion method for improving bioavailability, *Chemical & Pharmaceutical Bulletin*, 37: 425–429.

KHAN, S. A., AHMAD, M., MURTAZA, G., AAMIR, M. N., AKHTAR, N. & KOUSAR, R. (2010) Formulation of two-drug controlled release non-biodegradable microparticles for potential treatment of muscles pain and spasm and their simultaneous spectrophotometeric estimation, *Acta Poloniae Pharmaceutica*, 67: 299–306.

KIM, C. K., KIM, M. J. & OH, K. H. (1994) Preparation and evaluation of sustained release microspheres of terbutaline sulphate, *International Journal of Pharmaceutics*, 106: 213–219.

LEE, J. H., PARK, T. G. & CHOI, H. K. (2000) Effect of formulation and processing variables on the characteristics of micro sphere for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method, *International Journal of Pharmacy*, 196: 75–83.

MARTIN, A. R. (1998) *Antiviral agents: Textbook of organic medicinal and pharmaceutical chemistry*, 327–337 (New York: Lippincot-Raven).

MORTADA, S. M. (1982) Preparation of ethyl cellulose microcapsules using the complex emulsion method, *Die Pharmazie*, 37: 427–429.

PALMIERI, G. F., BONACUCINA, G., DI MARTINO, P. & MARTELLI, S. (2001) Spray-drying as a method for micro particulate controlled release systems preparation: advantages and limits, I, water-soluble drugs, *Drug Development and Industrial Pharmacy*, 27: 195–204.

RAO, B. S. & MURTHY, K. V. (2002) Studies on rifampicin release from ethyl cellulose coated nonpareil beads, *International Journal of Pharmaceutics*, 231: 97–106.

SAHOO, S. K., MALLICK, A. A., BARIK, B. B. & SENAPATI, P. C. (2005) Formulation and in vitro evaluation of Eudragit®RS100 microsphere of stavudine, *Tropical Journal of Pharmaceutical Research*, 4: 369-375.

SHASHIKANT, D. B., YOGESH, S. R., RUPESH, M. S., KAPIL, R. P. & RAHULKUMAR, D. R. (2009) Formulation and evaluation of floating microspheres of ketorolac trometamol, *International Journal of Pharmaceutical Research and Development*, 1: 1–8.

SINGH, B. & AGARWAL, R. (2002) Design development and optimization of controlled release microcapsules of dilitiazem hydrochloride, *Indian Journal of Pharmaceutical Sciences*, 64: 378–385.

SINGH, D., SARAF, S., DIXIT, V. K. & SARAF, S. (2008) Formulation optimization of gentamicin loaded eudragit RS100 microspheres using factorial design study, *Biological & Pharmaceutical Bulletin*, 31: 662–667.

TRIPATHI, K. D. (2003) *Essentials of medical pharmacology*, 725–734 (New Delhi: Jaypee Brothers' Medical Publishers [P] Ltd.).

VENKATESAN, P., MANAVALAN, R. & VALLIAPPAN, K. (2009) Microencapsulation: A vital technique in novel drug delivery system, *Journal of Pharmaceutical Sciences & Research*, 1: 26-35.