

PREPARATION AND CHARACTERIZATION OF POLYMETHACRYLATE-BASED MATRIX MICROSPHERES OF CARBAMAZEPINE USING SOLVENT EVAPORATION METHOD

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Abstract

The purpose of the present investigation was to prepare carbamazepine loaded Eudragit[®] microspheres able to modify the release profile of carbamazepine by emulsion-solvent evaporation technique. The effect of polymer, Eudragit[®] RSPO and Eudragit[®] RLPO alone and in combinations of these two acrylate polymers in different concentrations and in different drug polymer ratios on percentage yield, particle size, entrapment efficiency, and *in vitro* drug release behavior had been investigated. A mixed solvent system (MSS) consisting of acetonitrile and dichloromethane in a 1:1 ratio and corn oil was selected as primary and secondary oil phases, respectively. Span 80 was used as the secondary surfactant for stabilizing the external oil phase.

The solid state characterization was carried out by Fourier transform infrared spectroscopy, thermogravimetry, powder x-ray diffractometry, and differential scanning calorimetry. The surface morphology of microspheres was examined by scanning electron microscopy. Microspheres were found spherical and porous in nature. The mean particle size was between 20.74 - 29.33 μm and the encapsulation efficiencies ranged from 52.61-67.39 %. The release profile and encapsulation efficiencies depended mainly on the structure of the polymers used as wall materials. The release rate of the Eudragit[®] RSPO microspheres was much lower than that of Eudragit[®] RLPO microspheres. The release pattern of carbamazepine in distilled water from different microsphere formulations and the commercial product (CP) was found to follow different kinetic models. The formulation (F1) was best explained by Higuchi's equation, formulations F2, F6, F7 and F8 confirmed that the release rates followed Hixson-Crowell cube root law, formulations F3, F4, F9 and commercial product (CP) followed zero order kinetic model of drug release, and the release from formulation F5 was best explained by Korsmeyer-Peppas model. However,

comparison of Akaike information criterion (AIC) values indicated first-order model for most of the formulations as the predominant release kinetics.

Rezumat

Scopul acestui studiu a fost să prezinte prepararea microsferelor de Eudragit® încărcate cu carbamazepină prin tehnica evaporării emulsie – solvent, urmărind astfel modificarea profilului de eliberare a carbamazepinei. Au fost investigate următoarele: efectul de polimer, folosirea Eudragit® RSPO și a Eudragit® RLPO singure sau în combinații ale acestor doi polimeri în concentrații diferite și în raporturi procentuale diferite polimer – substanță activă, dimensiunea particulelor, eficiența blocării și comportamentul substanței active la eliberarea *in vitro*. Fazele uleioase au fost reprezentate de un sistem mixt de solvenți (SMS) constituit din acetoneitril și diclorometan în raport 1 la 1 ca fază primară respectiv ulei de porumb ca fază secundară. Span 80 a fost folosit ca surfactant secundar pentru a stabili faza uleioasă secundară. Caracterizarea stării solide a fost făcută cu ajutorul spectroscopiei infraroșii cu transformată Fourier, termogravimetriei, analizei pulberii prin difractometrie cu raze X și scanare prin calorimetrie diferențială. Morfologia suprafeței microsferelor a fost examinată prin scanare microscopică electronică. Microsferele au prezentat natură sferică și poroasă. Mărimea medie a particulelor a fost între 20,74 și 29,33 μm și eficiența încapsulării a fost cuprinsă între 52,61 și 67,39 %. Profilul eliberării și eficiența încapsulării depind în mare măsură de structura polimerilor folosiți ca materiale pentru peretele microcapsulelor. Rata de eliberare din microsferele pe bază de Eudragit® RSPO a fost mult mai scăzută decât în cazul microsferelor pe bază de Eudragit® RLPO. Modelul eliberării carbamazepinei în apă distilată din diferitele formulări de microsfere și din produsul comercial (PC) a urmat diferite modele cinetice. Formularea (F1) respectă cel mai bine ecuația Higuchi, formulările F2, F6, F7 și F8 au confirmat că eliberarea respectă ecuația (de ordinul 3) Hixson – Crowell; pentru formulările F3, F4, F9 și produsul comercial (PC) eliberarea a urmat un model cinetic de ordinul 0 iar pentru formularea F5 eliberarea a fost cel mai bine reprezentată de modelul Korsmeyer – Peppas. Totuși, compararea prin criteriul informațional Akaike a indicat modelul cinetic de ordinul întâi ca model de eliberare predominant pentru cele mai multe dintre formulări.

Keywords: Carbamazepine, Emulsion solvent evaporation, Eudragit, Microspheres, Mixed solvent system

Introduction

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures, which are associated with characteristic signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain [1, 2]. Epilepsy is a collection of many different types of seizures that vary widely in severity, appearance, cause, consequence and management and is usually controlled, but can not be cured with medication although surgery may be considered in difficult cases [4-6]. Carbamazepine (CBZ) is one of the most important drugs for the treatment of generalized tonic-clonic and both simple and complex partial seizures. It is also the

primary agent for treatment of trigeminal and glossopharyngeal neuralgias [7]. It is 75% bound to plasma protein, primarily to albumin [8]. The drug is characterized by slow and irregular gastrointestinal absorption due its low water solubility [8]. CBZ is also characterized by short half-life on chronic dosing because of the autoinduction of hepatic metabolism [9]. It is therefore both important and advisable to have a drug formulation characterized by prolonged CBZ release [10, 11]. Microencapsulation of CBZ provides the prolonged release of single dose, thereby minimizing the frequent administration and hence total dose required to elicit pharmacological activity, there by reducing the side effects [12].

The administration of drugs in the form of microspheres has received much attention because the microspheres may modify and retard drug release, improve absorption and hence bioavailability of both soluble and insoluble drugs [13]. Emulsion solvent evaporation technique, one of the most popular methods for the encapsulation of drugs within water-insoluble polymers has been used successfully for the manufacture of microspheres and offers several advantages; it is preferable to use other preparation methods such as spray-drying, sonication, and homogenization, etc, because it requires only mild conditions such as ambient temperature and constant stirring [14]. Moreover, this technique makes possible the entrapment of a wide range of drugs having different physical properties and solubility characteristics [15]. Eudragits are biocompatible copolymers synthesized from acrylic and methacrylic acid esters. These polymers are well tolerated by the skin and have been used in the formulation of dosage forms especially matrix tablets for oral sustained release and in tablet coating and have also been used in the microencapsulation of drug [14]. In this work, an attempt has been made to modulate the release profile of carbamazepine by preparing microspheres using solvent evaporation technique with a combination of dual polymer (Eudragit® RSPO and Eudragit® RLPO) at different proportions and corn oil as dispersion medium. Hence, the objective of the present work was to prepare and evaluate a novel microparticulate drug delivery system of CBZ using acrylate copolymers. The prepared microspheres were evaluated for drug content, *in vitro* drug release and their release mechanisms. Particle size of the prepared microspheres was also evaluated by optical microscopic method. Drug polymer interactions in the solid state were studied by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), powder X-ray diffraction (XRD), and differential scanning calorimetry (DSC). The surface characteristics were also evaluated by scanning electron microscopy (SEM).

Materials and Methods

Materials

Carbamazepine and Corn oil were purchased from Yarrow Chem. Products, Mumbai, India. Eudragit[®] RSPO and Eudragit[®] RLPO were received as a gift sample from Evonik Degussa India Pvt. Ltd, Mumbai, India. Span 80 was purchased from Loba Chem Pvt. Ltd, Mumbai, India. Dichloromethane, Acetonitrile, and n-Hexane were purchased from Rankem/RFCL Ltd, Mumbai, India. Tegretal[®] CR 200 (Novartis India Ltd, Mumbai, India) was purchased from a local pharmacy. All the reagents were of analytical grade.

Methods

Preparation of Eudragit[®] microspheres

The preparation of Eudragit[®] microspheres was based on an emulsion solvent evaporation method [16, 17]. The procedures are as follows:

Carbamazepine, Eudragit[®] RSPO and RLPO were dissolved in an acetonitrile/dichloromethane mixture (1:1), and then emulsified into corn oil solution containing 2% Span 80 (w/w) to form the oil₁-in-oil₂ emulsion shown in Table I. The volume ratios of oil₁: oil₂ phases were 6:200. The mixture was mixed with a magnetic stirring bar (400 ± 50 rpm) until the complete evaporation of organic solvent was accomplished (above 20 h). After that the Eudragit microspheres were separated by filtration, the excess of corn oil was eliminated by repeated washing (4 to 5 times) with n-hexane (50 mL) and finally dried over night in desiccators at room temperature to yield free flowing spherical products.

Percentage yield

The percentage yield of the dried microspheres was calculated as per the following formula [18]:

$$\text{Percentage of yield} = \frac{\text{weight of microspheres obtained}}{\text{Weight of (Polymer + drug)}} \times 100$$

Particle Size analysis

The mean particle size of the carbamazepine microspheres was determined by optical microscopy. At least 100 microspheres were analyzed for each preparation and the mean diameter was calculated. All experiments were run in triplicate.

Shape and surface morphology

The morphology of the prepared microspheres was analyzed by scanning electron microscopy (SEM). For scanning electron microscopy samples were prepared by lightly sprinkling microsphere powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then

coated with platinum using a fine coat ion sputter. The microspheres were examined using a scanning electron microscope (JSM-6700F, JEOL, Japan).

Drug Content and Encapsulation Efficiency

The drug content in polymethacrylate microspheres was determined by dispersing accurately weighed 10 mg of formulation in 10 mL of ethanol followed by agitation with a magnetic stirrer for 24 h to dissolve the polymer and extract the drug. The solution was then filtered through Whatman filter paper and the filtrate was assayed for CBZ content by an UV spectrophotometer (Model No.UV-1700, Shimadzu, Japan) at 284 nm after suitable dilution with ethanol. Each determination was made in triplicate.

The drug content was determined by comparing the optical density with that of standard curve of carbamazepine prepared with ethanol. Encapsulation efficiencies were calculated as follows:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

In Vitro Drug Release Studies

The dissolution studies of the microspheres were carried out in a USP dissolution rate test apparatus type I (Model No. TDT-08L, Electrolab, India). Weighed quantities (150 mg) of microspheres were placed in 900 mL of dissolution medium (distilled water) and were stirred at 100 rpm at $37 \pm 0.5^\circ\text{C}$. A 5 mL aliquot was withdrawn from the dissolution medium at pre-determined intervals throughout the 10 h period; at each interval, the withdrawn medium was replaced with an equivalent amount (5mL) of fresh dissolution medium. Collected samples were analyzed by measuring the absorbance through an UV spectrophotometer (Model No.UV-1700, Shimadzu, Japan) at 287 nm after suitable dilution to determine the amount of the carbamazepine released from the microspheres. No interference in the measurement of the drug due to the presence of other ingredients was observed. The percentage of drug release was plotted *versus* time. Each experiment was repeated three times.

Drug Release kinetics

To study the release kinetics data obtained from *in vitro* drug release studies were fitted to various kinetic equations. The kinetic models used zero-order kinetic (Equation 1), first- order (Equation 2), Hixson-Crowell cube root law (Equation 3), and Higuchi kinetic (Equation 4). The zero order rates (Equation 1) describe the systems where the drug release rate is independent of its concentration [19].

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

where K_0 is the zero order rate constant expressed in units of concentration/time, C is the percentage of drug released at time t , in hours.

The first order (Equation 2) which describes the release from systems where the release rate is dependent on concentration [20].

$$\text{Log } C = \text{Log } C_0 - \frac{Kt}{2.303} \quad (2)$$

where C_0 is the initial concentration of drug, K , is the first order constant and t is the time.

Hixson-Crowell cube root law (Equation 3) describes the release from the system where there is a change in the surface area and diameter of the particles with the progressive dissolution of the matrix as a function of time [21].

$$\sqrt[3]{Q_0} - \sqrt[3]{Qt} = K_{HC} t \quad (3)$$

where Qt is the amount of drug released in time t , Q_0 is the initial amount of the drug in the matrix and K_{HC} is the rate constant for the Hixson-Crowell rate equation.

Higuchi's model (Equation 4) describes the release of drugs from an insoluble matrix as a square root of a time dependent process based on Fickian diffusion [22].

$$Qt = K_H t^{1/2} \quad (4)$$

where K_H is the constant reflecting the design variable of the system or Higuchi release rate constant and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Further, to confirm the mechanism of drug release, the release was fitted in Korsmeyer-Peppas model (Equation 5).

$$M_t/M_\infty = Kt^n \quad (5)$$

where M_t/M_∞ is the fractional drug release at time t , K is a kinetic constant characteristic of the drug/polymer system and n is an exponent that characterizes the mechanism of release tracers and is calculated from the slope of the plot of log of fraction of drug released (M_t/M_∞) versus log time [23,24].

To test the applicability of the release kinetics models, they were compared using the statistical parameters, coefficient of determination (r^2) and Akaike information criterion (AIC) [25]. Whereas, r^2 is calculated simply from correlation coefficient (r), AIC measures the goodness of fit when comparing several models and is based on maximum likelihood. It is a standard statistical parameter for dissolution comparison. The minimum

value of AIC indicates the best fit model and is calculated by the following equation –

$$AIC = n \cdot \ln(WSSR) + 2 \cdot p \quad (6)$$

where, n = number of dissolution data points, p = number of parameters in each model, and

WSSR = weighed sum squares of residues calculated as-

$$WSSR = \sum_{i=1}^n [w_i (y_i - \hat{y}_i)^2] \quad (7)$$

where, w_i = a weighing factor (optional), y_i = dissolution data points and $\hat{y}_i$ = predicted values of y_i .

Solid-state studies

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Fourier Transform Infrared, (FT-IR) Spectroscopy (Spectrum-100, Perkin Elmer, USA) was performed using the KBr disc method. Samples were mixed with KBr powder and compressed into 12 mm disc using a hydraulic press at a pressure of 10 tons for 30 s. The scanning range was 400-4000 cm^{-1} .

X-Ray Diffraction (XRD) Analysis

The x-ray diffraction patterns of the samples were obtained using an x-ray diffractometer (Rich Siefert, XRD-3000P) at 40 kV, 15 mA over a 2θ range of 0-80°, using Cu K α radiation wavelength of 1.5405 Å.

Thermo gravimetric Analysis (TGA)

The TGA analyses were performed with TA instruments (SDTQ 600). The TGA analyses of pure carbamazepine, eudragit RLPO, RSPO, physical mixture of drug and polymer and drug loaded microspheres were conducted at a heating rate of 10°C min^{-1} from 30-700°C with the use of dry nitrogen as the effluent gas.

Differential Scanning Calorimetry (DSC)

Thermal analyses of the samples were performed using DSC-TA system (Perkin Elmer Diamond DSC, USA). All samples were sealed in a crimped aluminium pan by application of the minimum possible pressure and heated at a rate of 10°C min^{-1} from 30-200°C in an atmosphere of dry nitrogen as effluent gas by passing at a flow rate of 30 mL min^{-1} . An empty aluminium pan was utilized as the reference pan. Differential scanning calorimetry was conducted first with samples with the pure polymers, Eudragit® RLPO, RSPO, pure CBZ, and physical mixture of drug and polymer, then the drug loaded microspheres were analyzed and compared for possible drug-polymer interactions.

Results and Discussion

Preparation of Eudragit[®] Microspheres

The preparations of dual polymer microspheres of carbamazepine were successfully achieved using the emulsion-solvent evaporation technique. This process uses two solvents (termed as mixed solvent system) as dispersed medium and a suitable non-aqueous processing medium to enable formation of oil₁-in-oil₂ emulsion [26]. Components of the mixed solvent system can be selected from any of the commonly used organic solvents such as acetonitrile, dichloromethane, methanol, acetone, ethanol etc [27, 28]. In this work acetonitrile and dichloromethane have been selected as solvent system. Acetonitrile is a unique organic solvent which is polar, water miscible and oil-immiscible, but with oil as a processing medium did not ensure formation of a stable emulsion and a non-polar solvent such as dichloromethane was included to decrease polarity of the acetonitrile solution. The optimal proportion of acetonitrile and dichloromethane was found to be 1:1, which enabled emulsion formation and yielded good microspheres [16].

Table I
Formulation code and composition of various CBZ loaded Eudragit[®] microspheres.

Formulation Code	Drug-Polymer ratio	Drug (mg)	Eudragit RSPO (mg)	Eudragit RLPO (mg)
F1	1:4	60	240	-
F2	1:8	60	480	-
F3	1:12	60	720	-
F4	1:4	60	-	240
F5	1:8	60	-	480
F6	1:12	60	-	720
F7	1:8	60	360	120
F8	1:8	60	240	240
F9	1:8	60	120	360

Percentage Yield and Particle Size Analysis

The results showed (Table II) that the yield was satisfactory and varied from 70.27-85.81%. The mean particle size of the carbamazepine loaded microspheres ranged from 20.74 to 29.33 μm (Table II). It was seen

that the mean size was influenced by the content and type of polymer and its ratio in the formulation. The mean size increased with increasing polymer concentration. This may be due to the fact that, increased polymer concentration produced a significant increase in viscosity [29], thus leading to an increase in emulsion droplet size and finally formation of microspheres of larger size [27, 28].

Table II

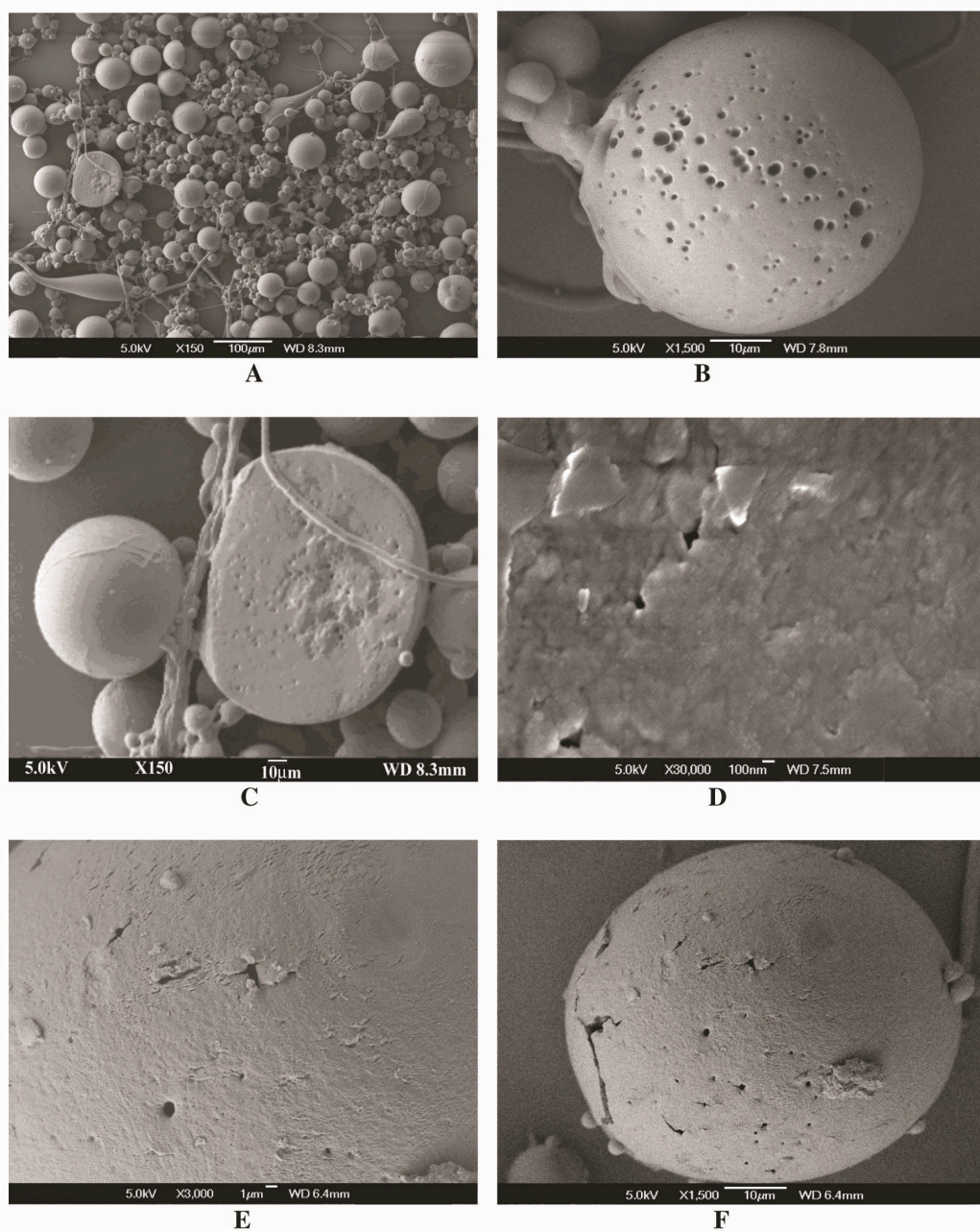
Percentage yield, mean particle size, theoretical drug content, actual drug content, and encapsulation efficiency of different formulations (n = 3).

Formulation Code	Percentage Yield $\pm$ SD	Mean particle Size ($\mu\text{m} \pm$ SD)	Theoretical drug content (%)	Actual drug content ($\% \pm$ SD)	Encapsulation efficiency ($\% \pm$ SD)
F1	73.98 $\pm$ 2.52	21.33 $\pm$ 2.05	20	10.72 $\pm$ 0.15	53.62 $\pm$ 0.73
F2	80.75 $\pm$ 0.70	23.63 $\pm$ 0.53	11.11	7.06 $\pm$ 0.16	63.53 $\pm$ 1.42
F3	85.81 $\pm$ 0.48	28.00 $\pm$ 2.34	7.69	5.18 $\pm$ 0.16	67.39 $\pm$ 2.14
F4	70.27 $\pm$ 1.54	20.74 $\pm$ 1.30	20	10.52 $\pm$ 0.15	52.61 $\pm$ 0.90
F5	77.82 $\pm$ 1.36	22.52 $\pm$ 1.78	11.11	6.79 $\pm$ 0.09	61.11 $\pm$ 0.81
F6	84.13 $\pm$ 0.72	29.33 $\pm$ 1.45	7.69	5.01 $\pm$ 0.11	65.16 $\pm$ 1.46
F7	78.83 $\pm$ 1.78	23.74 $\pm$ 2.40	11.11	6.52 $\pm$ 0.12	58.68 $\pm$ 1.07
F8	82.00 $\pm$ 1.50	24.33 $\pm$ 2.33	11.11	7.35 $\pm$ 0.22	66.17 $\pm$ 1.99
F9	78.55 $\pm$ 1.40	24.15 $\pm$ 1.76	11.11	6.29 $\pm$ 0.10	56.66 $\pm$ 0.93

Increase in viscosity resulting in increased particle size may be due to a high degree of collisions between the droplets leading to a fusion of semi formed particles and producing an overall increase in the size of the microspheres [30, 31].

Scanning Electron Microscopy (SEM)

SEM photographs of the microspheres, (Figure 1) illustrates that the drug loaded microspheres were spherical and had a smooth surface without visible drug crystals, which suggested that carbamazepine was molecularly entrapped (dissolved) in the polymeric matrix [32].

**Figure 1**

SEM photographs of CBZ microspheres (F8): (A) and (B) surface morphology before dissolution; (C) cross-sectional view of cleaved microsphere; (D) external surface of the microsphere, (E) and (F) surface of the microsphere after dissolution.

The surface morphology evaluation also revealed that the size of the microspheres was not uniform. The presence of some small pores on the surface of the microsphere possibly due to evaporation of the solvent during the process and the porous nature of the microsphere could be useful to increase the surface area and dissolution [33].

Drug Content and Encapsulation Efficiency

The drug content of carbamazepine in the microspheres was determined. It was found that the theoretical drug content varied between 7.69%-20% and the actual drug content in the microsphere formulation (F1-F9) ranged from 5.01%-10.72% (Table II).

The encapsulation efficiency of carbamazepine in the prepared microspheres formulation (F1-F9) varied from 52.61% to 67.39% (Table II). The encapsulation efficiency of carbamazepine obtained in the experimental condition was lower than the result described by Dong *et al.*, (2007) [32] but almost similar to those reported by Kim *et al.* (2002) [16]. The encapsulation efficiency of the drug depends on the solubility of the drug in the solvent and continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency [34]. Generally, the efficiencies of the Eudragit[®] RSPO microspheres were higher than those of the Eudragit[®] RLPO microspheres, which can be attributed to the structural differences, i.e. the differences of the content of the quaternary ammonium group. High content of the ammonium group facilitates the diffusion of a part of entrapped drug to the surrounding medium during preparation of microspheres.

In Vitro Drug Release Studies

Table III represents the release of carbamazepine from Eudragit[®] microspheres (RSPO and RLPO) in different drug: polymer ratio. The rate of drug release from the microspheres depended on the polymer concentration and the type of polymer used and indicated that the release rate decreased with the increasing amount of polymer. The decrease in release rate with increasing content of the polymer 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 the increasing of polymer concentration [35]. Rahman *et al.* (2011) [36] suggested that increased polymer concentration affects the drug leaching and diffusion process from the microspheres, by making it less porous and slower drug release rate occurs. As compare to Eudragit[®] RSPO, the Eudragit[®] RLPO microspheres show a little higher drug release rate, which is attributed to the difference in the content of quaternary ammonium groups. The content of the quaternary

ammonium groups of the Eudragit[®] RLPO microspheres (10%) is higher than that of the Eudragit[®] RSPO microspheres (5%).

Table III

In vitro drug release profile of various CBZ loaded Eudragit microspheres (F1- F9) and commercial product (CP) (n = 3).

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9	CP
0	0	0	0	0	0	0	0	0	0	0
0.25	8.09	6.95	3.56	18.15	9.93	7.99	7.24	8.87	9.21	22.27
0.5	11.27	9.18	5.99	25.21	15.42	12.29	11.65	13.26	13.2	33.32
0.75	13.45	12.04	8.76	36.93	22.29	16.95	14.96	18.88	19.19	36.55
1	15.46	13.56	10.84	43.94	29.17	20.17	17.17	24.01	33.16	42.48
2	18.3	15.6	13.26	50.94	46.62	23.4	20.75	26.94	45.42	52.86
3	23.16	17.04	15.69	64.94	60.37	27.1	24.61	31.1	54.83	59.32
4	26.18	20.43	17.31	76.38	73.86	30.69	27.09	34.27	67.38	63.23
5	28.69	21.7	18.46	85.6	83.11	32.84	31.22	36.47	78.5	67.94
6	31.21	23.23	20.53	94.6	88.67	34.45	33.7	40.38	86.77	72.39
7	32.71	27.05	23.31			43.83	40.31	47.47		81.15
8	35.05	30.86	25.39			47.94	48.85	52.35		86.31
9	38.57	33.16	26.77			54.57	51.88	58.71		92.06
10	43.76	35.7	28.5			59.23	56.62	69.21		94.75

Thus, in the Eudragit[®] RLPO microspheres, the drug might be dispersed evenly in the matrix of the polymer and the surface would be loose due to high charge density. On the other hand, in case of Eudragit[®] RSPO microsphere, lower charge in density produces more packed structures than those of Eudragit[®] RLPO microspheres [16]. Though the release rate of CBZ from the Eudragit[®] RSPO microspheres was slow, it gradually increased with the addition of Eudragit[®] RLPO. The microspheres prepared with Eudragit[®] RSPO together with RLPO at 1:1 ratio (F8) was found to show better release rate as compared to other formulations.

Drug Release Kinetics

For all formulations (F1-F9) and the commercial product (Tegritol[®] CR tablet) (Figure 6) the release rate constant was calculated from the slope of the appropriate plots and the regression coefficient (r^2) was determined. The release rate constants and regression coefficients (r^2) for all microsphere formulations including commercial product using different kinetic equations are listed in Table IV. It was observed that *in vitro* drug release of formulation (F1) was best explained by Higuchi's equation as the plots showed the highest linearity [37]. The correlation values of Higuchi's plot of formulations F1 was found to be 0.9890. The cube root of the percentage of drug remaining in the matrix *versus* time curve showed straight line for

the formulations (F2, F6, F7 and F8) and confirmed that the release rates followed Hixson-Crowell cube root law, indicating that the drug releases by dissolution and with the changes in surface area and diameter of the particles or tablets [38]. The correlation values of Hixson-Crowell cube root plots of formulations F2, F6, F7 and F8 were found to be 0.9936, 0.9701, 0.9923 and 0.9870 respectively.

Table IV
Fit for various CBZ loaded Eudragit® microspheres and commercial product (CP) using different kinetic equations for describing release kinetics.

Formulation Code	Zero Order		First Order		Hixson-Crowell		Higuchi		Korsmeyer-Peppas		n
	K_0 (h ⁻¹)	r ²	K_1 (h ⁻¹)	r ²	K_{HC} (h ^{1/3})	r ²	K_H (h ^{1/2})	r ²	K_{KP} (h ⁻ⁿ)	r ²	
F1	2.8978	0.9845	-0.0179	0.9803	-0.1053	0.9694	12.3560	0.9890	0.1438	0.9771	0.4411
F2	2.5010	0.9857	-0.0144	0.9775	-0.1021	0.9936	10.0798	0.9726	0.1183	0.9239	0.4317
F3	1.9520	0.9951	-0.0106	0.9950	-0.0910	0.9841	8.7302	0.9903	0.1006	0.9727	0.4263
F4	10.5348	0.9934	-0.1957	0.9207	-0.2132	0.9855	38.0170	0.9926	0.4109	0.9647	0.4447
F5	12.0131	0.9718	-0.1617	0.9925	-0.2706	0.9330	39.5815	0.9864	0.2973	0.9960	0.6337
F6	4.3175	0.9502	-0.0316	0.9206	-0.0971	0.9701	16.7875	0.9517	0.1735	0.8830	0.4651
F7	4.4624	0.9782	-0.0313	0.9536	-0.1412	0.9923	16.5631	0.9604	0.1474	0.9283	0.5294
F8	4.7209	0.9582	-0.0387	0.8937	-0.1298	0.9870	18.8475	0.9566	0.2048	0.8881	0.4416
F9	10.8502	0.9971	-0.1392	0.9645	-0.2424	0.9841	37.5747	0.9815	0.3198	0.9899	0.5421
CP	5.6847	0.9893	-0.1095	0.9261	-0.1132	0.9743	27.1643	0.9793	0.4093	0.9770	0.3479

The release profile of carbamazepine from the formulations F3, F4, F9 and commercial product (CP) with correlation coefficient 0.9951, 0.9934, 0.9971 and 0.9893 respectively displayed very fairly good fitting with zero order kinetic model of drug release, confers that the release is independent of the concentration [39]. Log cumulative percentage of drug release *versus* log time curve showed straight line for the formulation F5 and confirmed that the release rate followed Korsmeyer-Peppas model [37]. However, comparison of AIC values (Table V) indicated First-order model (values underlined in table) as the predominant release kinetics. This is in agreement with the release exponent (n) obtained from Korsmeyer-Peppas model (Table IV) which 'n' values indicated Fickian release.

Table V
Comparison of AIC values for the dissolution kinetics models

Formulation Code	Zero-Order	First-Order	Hixson-Crowell	Higuchi	Korsmeyer-Peppas
F1	12.3757	-30.7367	-13.4020	20.9978	14.1232
F2	10.7226	-32.0232	-20.5914	24.5783	13.6053
F3	3.8711	-41.3651	-17.5725	16.0372	13.1083
F4	8.6573	-5.4326	-9.6024	19.9531	9.6323
F5	13.1821	-12.7745	-4.2180	22.9995	9.5135
F6	21.0562	-19.4879	-11.6982	33.9830	14.5643
F7	17.6255	-22.0632	-16.9546	32.5625	14.1906
F8	21.0366	-16.3281	-15.3916	34.7118	14.8873
F9	6.5793	-9.4280	-8.6843	23.8992	9.5543
CP	16.5922	-9.0260	-13.5489	34.4922	15.6042

Mechanism of Drug Release

In order to better characterize the drug release mechanisms for the polymeric systems studied, the data were plotted in Korsmeyer et al's equation (Equation 5) as log cumulative percentage of drug released vs. log time and the exponent n was calculated through the slope of the straight line. Log cumulative percent drug release *versus* time curves of all formulations (F1-F9) including commercial product indicated a good linearity, the release exponent n obtained from K-P model shows that the majority of the formulations followed anomalous Fickian release ($n > 0.43$ for spherical geometry) since the calculated ' n ' values were very near to the theoretical ' n ' values [40]. Two formulations (F2 and F3) and commercial product have ' n ' value ≤ 0.43 indicating Fickian diffusion. The formulation F8 is releasing drug through anomalous diffusion which is further supported by the fact that the r^2 value for the Hixson-Crowell kinetic have been found to be the highest amongst the kinetic models investigated, indicating that the drug releases are controlled by more than one process, i.e. coupling the diffusion and erosion mechanism-so-called anomalous diffusion [37, 40].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrophotometric analysis of pure drug, polymers, drug-polymer physical mixture and drug loaded microspheres were recorded and depicted in Figure 2. In FTIR spectra of pure CBZ powder (Figure 2A) the characteristic bands at 3466.20 cm^{-1} ($-\text{NH}$ valence vibration), 1676.13 cm^{-1} ($-\text{CO}-\text{R}$ -vibration), 1604.83 and 1595.18 cm^{-1} (range of $-\text{C}=\text{C}-$ and $-\text{C}=\text{O}$ vibration and $-\text{NH}$ deformation), 1384.94 and 1019 cm^{-1} were seen. The band at 1271 cm^{-1} ($-\text{C}=\text{N}$ bond) is less intense than that of 1246.06 cm^{-1} and the band at 852.26 cm^{-1} is weak [41]. The FTIR spectra of Eudragit[®] RSPO (Figure 2B) and RLPO (Figure 2C) show the characteristic bands of the ester groups at 1150 - 1190 cm^{-1} and 1240 cm^{-1} as well as the $\text{C}=\text{O}$ ester vibration at 1730 cm^{-1} . In the IR spectra of the physical mixture (Figure 2D) as well as those of CBZ loaded Eudragit microspheres (F8) (Figure 2E), the principal peaks for CBZ in the microspheres appeared at 3466.20 cm^{-1} indicates the presence of NH group and range of $-\text{C}=\text{C}-$ and $-\text{C}=\text{O}$ vibration and $-\text{NH}$ deformation at 1676.13 , 1604.83 and 1595.18 cm^{-1} as well as $-\text{C}=\text{N}$ bond at 1271 cm^{-1} .

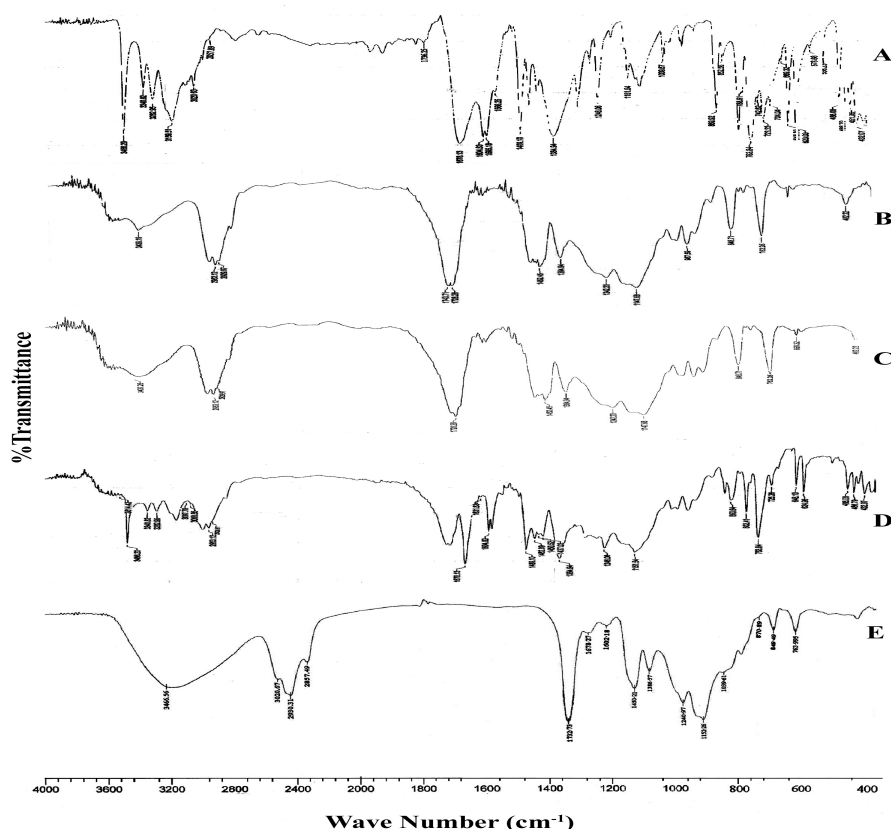


Figure 2

FTIR spectra of (A) carbamazepine, (B) Eudragit[®] RSPO, (C) Eudragit[®] RLPO, (D) physical mixtures of CBZ, Eudragit[®] RSPO, and Eudragit[®] RLPO (1:4:4), and (E) carbamazepine loaded Eudragit[®] microspheres (F8).

These results indicated that no chemical interaction or changes took place during preparation and the drug was found to be stable in the formulation.

X-Ray Diffraction (XRD) Analysis

The X-ray diffraction patterns of pure CBZ, Eudragit[®] RSPO, RLPO, physical mixture of the formulation ingredients and CBZ loaded Eudragit microspheres are presented in Figure 3. The diffractograms of pure CBZ (Figure 3A) showed the presence of characteristic high intensity diffraction peaks at $2\theta = 14.95^\circ, 15.05^\circ, 19.5^\circ, 27.4^\circ, 27.45^\circ, 32.058^\circ, 36.09^\circ, 40.9^\circ$ and 46.55° and for the physical mixture of CBZ and polymers (Figure 3D) peaks were detected at $2\theta = 14.95^\circ, 15.0^\circ, 20.55^\circ, 27.45^\circ$ and 27.5° [42].

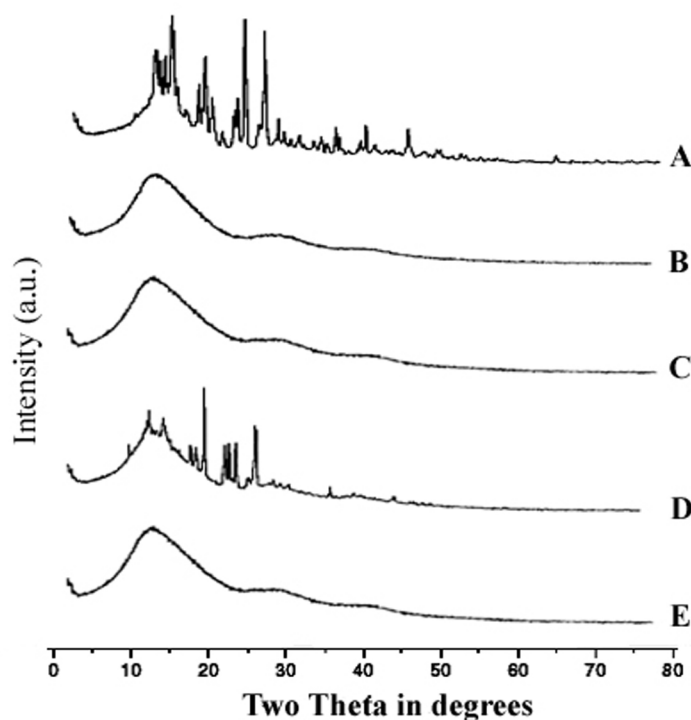


Figure 3

XRD spectra of (A) carbamazepine, (B) physical mixtures of CBZ, Eudragit[®] RSPO, and Eudragit[®] RLPO (1:4:4), (C) Eudragit[®] RSPO, (D) Eudragit[®] RLPO, and (E) CBZ loaded Eudragit[®] microspheres (F8).

The XRD patterns of pure Eudragit[®] RSPO (Figure 3B) and RLPO (Figure 3C) were typical of amorphous substances, without any intense peak in their diffractograms. The absence of characteristic crystal peaks of CBZ in the diffractograms of formulation (F8) (Figure 3E) indicated that CBZ was either dissolved in the Eudragit[®] RSPO/RLPO or (less likely) dispersed in amorphous form [32].

Thermogravimetric Analysis (TGA)

TGA measures the amount and rate of change in the mass of a sample as a function of temperature in controlled atmosphere. TGA spectra of the pure CBZ, Eudragit[®] RSPO, RLPO, physical mixture of the formulation ingredients and CBZ loaded Eudragit microspheres are presented in Figure 4. From the TGA trace of pure CBZ (Figure 4A) it appeared that the melting point of carbamazepine (189°C) corresponds to the Form II CBZ polymorph. Although not the lowest energy polymorph, it appears that Form II polymorph of CBZ is a common occurrence due to

hydrogen bond mediated thermodynamic stability afforded to the drug crystals. The TGA trace for the physical mixture of drug and polymers (Figure 4D) retained the same melting peak indicating that the Form II was present. In both cases, there is a slight weight loss as revealed by the TGA trace at the melting point indicative of solvate loss at that temperature [43].

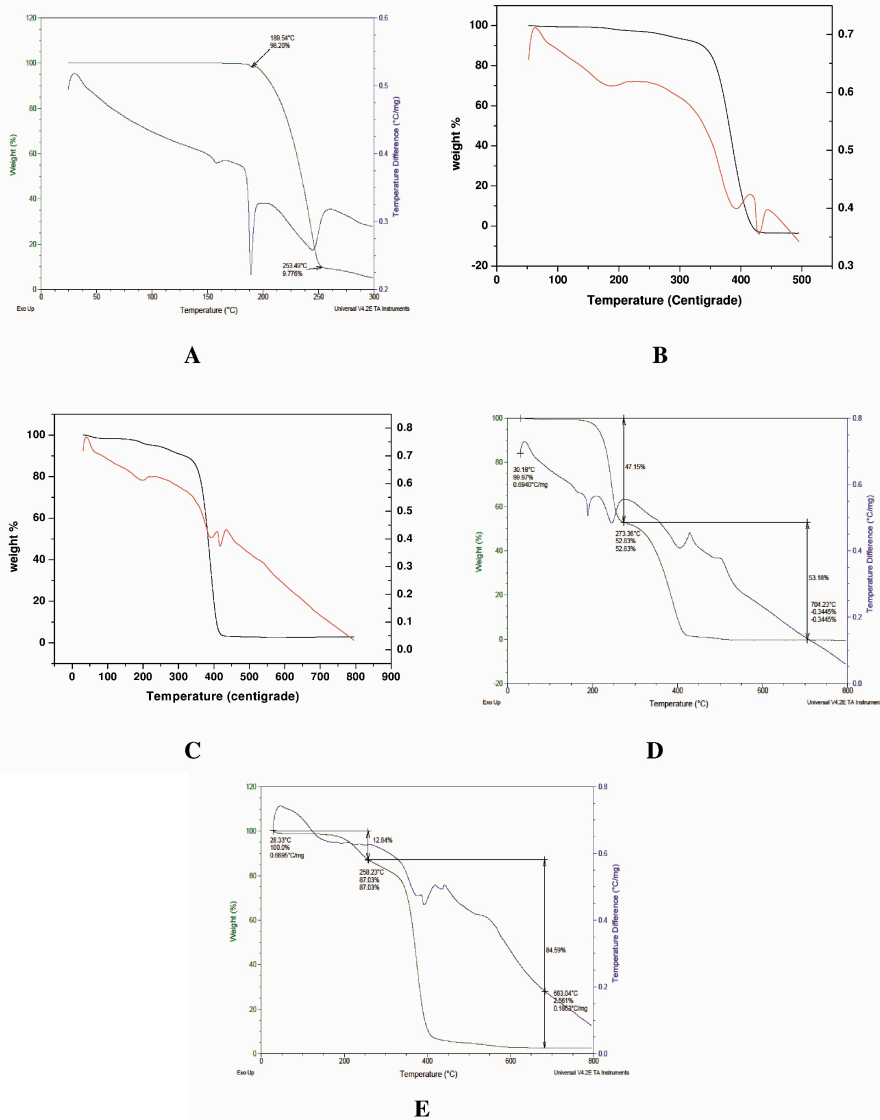


Figure 4

TGA spectra of (A) carbamazepine, (B) Eudragit® RSPO, (C) Eudragit® RLPO, (D) physical mixtures of CBZ, Eudragit® RSPO, and Eudragit® RLPO (1:4:4), and (E) CBZ loaded Eudragit® microspheres (F8).

At still higher temperature (250°C), a peak corresponding to presumably the CBZ evaporation occurs. Overall, the TGA trace for the physical mixture was a combination of the drug and polymer TGA traces. This indicates no detectable interaction among the drug and polymers used. However, no melting peak for the CBZ was observed in the TGA trace of the CBZ loaded Eudragit microspheres (F8) (Figure 4E). Due to the fact that the CBZ peak was retained in the physical mixture, but was absent in the microspheres (F8), we can conclude that during processing (i.e., solution of the drug in the volatile solvent and subsequent solidification on solvent evaporation), the Form II crystalline form of CBZ has changed into its amorphous form, presumably to the hindrance in formation of suitable intermolecular hydrogen bonds as a consequence of the presence of Eudragit polymers. This conversion affords intimate dispersion of the drug in polymer matrix and a hugely increased solvent accessible surface area for the drug molecule that is expected to lead to higher dissolution rates from the formulation affording complete delivery of the drug from the dosage form [13].

Differential Scanning Calorimetry (DSC)

DSC analysis provides information about the state of the drug along with the physical stability of the formulation ingredients after process technology [13]. Figure 5 shows the DSC thermograms of pure CBZ, polymers, drug-polymer physical mixture, and CBZ loaded Eudragit microspheres. The DSC trace of pure CBZ (Figure 5A) showed two melting endotherm at 166.66°C and 189.54°C [41, 44, 45] and the thermograms of Eudragit[®] RSPO (Figure 5B) and RLPO (Figure 5C) exhibited almost similar fusion peaks at 61°C and 200°C. The drug-polymer physical mixture (Figure 5D) had shown two endothermic peaks at 163.66°C and 186.97°C that indicated the melting of CBZ. The DSC thermogram of drug loaded Eudragit[®] microspheres (Figure 5E) did not show any endothermic peak corresponding to the fusion of CBZ which may be explained either by low drug content or complete dissolution of carbamazepine crystals in the Eudragit microspheres (i.e. the presence of carbamazepine in an amorphous state) [9,46].

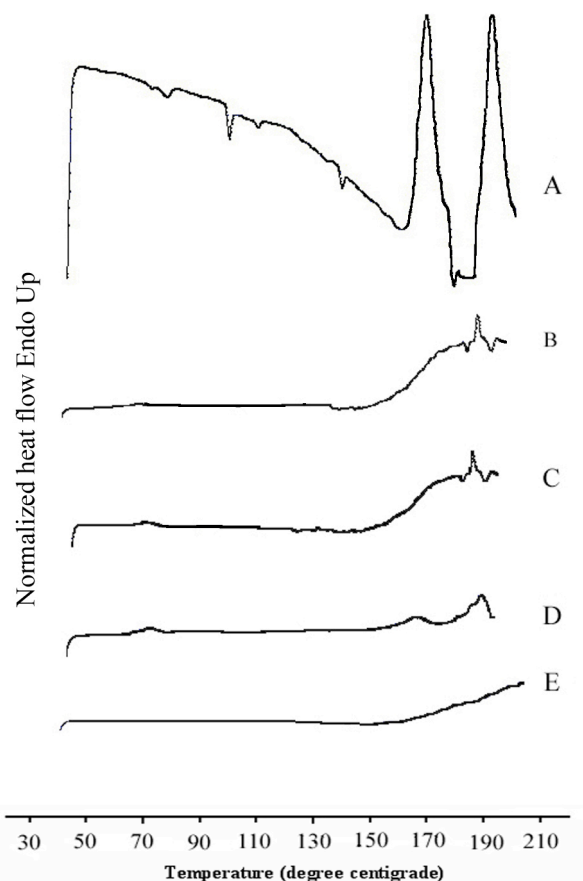


Figure 5. DSC curves of (A) Carbamazepine, (B) Eudragit® RSPO, (C) Eudragit® RLPO, (D) physical mixtures of carbamazepine, Eudragit® RSPO, and Eudragit® RLPO (1:4:4), and (E) carbamazepine loaded Eudragit® microspheres (F8).

Conclusions

In this study, well shaped spherical microspheres, able to promote a sustained release of carbamazepine were produced by emulsion-solvent evaporation technique, using Eudragit® RSPO and Eudragit® RLPO as wall materials. The drug entrapment efficiency, as well as the carbamazepine release profile, was influenced by the polymeric composition and drug-polymer ratios of the prepared microspheres. The best entrapment efficiency was obtained when Eudragit® RSPO and RLPO were used at 1:1 ratio for the microencapsulation. The Eudragit® RSPO microspheres showed the slowest carbamazepine release profile; where as rapid carbamazepine release was found in the Eudragit® RLPO microspheres. So the Eudragit®

RLPO could be added to Eudragit® RSPO microspheres to control the rate of drug release. The differences in drug release behaviour suggested structural differences of the wall materials, dependent on the content of the quaternary ammonium group. The absence of drug polymer interaction was revealed by the physicochemical characterization. Finally, in the carbamazepine-Eudragit® RSPO-Eudragit® RLPO systems at the ratio of 1:1 (representing a sum of the characteristics of the binary systems from both the physicochemical and dissolution point of view), showed better carbamazepine release rate as compared to formulations prepared by Eudragit® RSPO and Eudragit® RLPO separately.

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