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RESEARCH ARTICLE

DEVELOPMENT AND CHARACTERIZATION OF FACTORIALLY DESIGNED 5-FLUOROURACIL MICROSPHERES

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Microspheres of 5-fluorouracil were prepared for prolonged or controlled drug delivery, to improve bioavailability/stability and to target drug to specific sites. 5-Fluorouracil was encapsulated with eudragit RL 100 and ethyl cellulose using an *o/o* emulsion solvent evaporation method. Factorial design was used to study the effect of stirring speed, stirring time and phase ratio on cumulative percent of drug release. It was found that cumulative percent of drug release increases at the high level of stirring speed, stirring time and phase ratio. The effect was highest in case of stirring speed and lowest in case of phase ratio. Microspheres (batch MA-5) were characterized by spherical shape, absence of aggregates, a mean diameter of 107.92±1.12 μm, a recovery of 78.82±1.26% (*w/w*) and an encapsulation efficiency of 76.78±1.19% (*w/w*). ANOVA was applied on cumulative percent of drug release to study the fitting and significance of model. The estimated model may be further utilized as response surface for cumulative percent of drug release of 5-FU microspheres.

Key words: Eudragit RL 100, 5-Fluorouracil, Ethyl cellulose, Solvent evaporation method, Microspheres.

INTRODUCTION

Controlled drug delivery occurs when a polymer/drug system is designed to release the drug in a predetermined manner. The main purpose of these release systems is to achieve a more effective therapy *i.e.* a delivery profile that would yield a high blood level of the drug over a long period of time, avoiding the large fluctuations in drug concentration and reducing the need of several administrations (Duarte *et al* 2007). Microspheres are one of the multi-particulate delivery systems and are used for prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance (Davis and Illum, 1988; Ritschel, 1989). One of the popular methods for the encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method. The technique of emulsion solvent evaporation is preferred to other preparation methods like spray-drying, sonication and homogenization, because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the core materials. The emulsion solvent evaporation method has been utilized successfully by various researchers for the preparation of microspheres using various biocompatible polymers such as ethyl cellulose, poly(D-L-lactide-coglycolide) (PLGA) (Murakami *et al* 2000; Choi *et al* 2002), poly(ε-caprolactone) (PCL) and Eudragit(s) (Lamprecht *et al* 2000; Arshady, 1990; Esposito *et al* 1996; 2005; Lorenzo-Lamosa *et al* 1998; Kim *et al* 2002; Yang *et al* 2001). In the present study, factorially designed microspheres of 5-fluorouracil (5-FU)

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were prepared using eudragit RL 100 and ethyl cellulose by an *o/o* emulsion solvent evaporation method. The aim behind the study was to investigate the effect of stirring speed, stirring time and phase ratio on cumulative percent of drug release.

MATERIALS AND METHODS

Sample of 5-FU was obtained as gift sample from Fresenius Kabi Oncology, Ghaziabad, India. Eudragit RL 100 was gifted by Rohm GmbH Degussa, Germany. Ethyl cellulose was obtained from Loba Chemicals, India. All other chemical reagents were of analytical grade and were used as received. De-ionized water was used for all the experiments.

Experimental design

Microspheres were formulated according to the factorial design to study the effect of independent variables on the release pattern of 5-FU. To evaluate three factors at two levels, the factorial design consisted of eight batches (MA-1 to MA-8).

Formulation of 5-fluorouracil microspheres

o/o Solvent evaporation method was used to prepare 5-FU microspheres. Initial studies were performed using factorial design to optimize the amount of polymers used *i.e.* ethyl cellulose and eudragit RL 100. Preliminary studies revealed that low amount of eudragit RL 100 (1 g) and high amount of ethyl cellulose (5 g) resulted in highest mean encapsulation efficiency of 74.11± 0.97% and mean recovery of 81.12±1.26% and mean diameter of 92.56±1.72 μm. Therefore, low amount of eudragit RL 100 (1 g) and high amount of ethyl cellulose (5 g) was further utilized for the formulation of microspheres. Further, the effect of three independent factors *viz.* stirring speed (X_1), stirring time (X_2), and internal/external phase ratio (X_3) was further investigated on the cumulative percent of drug release using factorial design. All of the microspheres were prepared by the *o/o* solvent evaporation technique. Liquid paraffin and acetone systems were used for the preparation of microspheres. Magnesium stearate was used as a droplet stabilizer to prevent droplet coalescence in the oil medium and *n*-hexane was added as a non-solvent to the processing medium to solidify the microspheres (Sahoo *et al* 2005). Ethyl cellulose and eudragit RL 100 were accurately weighed and dissolved in acetone. 5-FU (1 g) was added into the polymer solution and the dispersion was stirred for 15 min using a magnetic stirrer. The drug-polymer dispersion was slowly delivered into the external phase, liquid paraffin, containing 0.1% glyceryl monostearate (emulsifier) and stirred at 500 rpm for 2 h. The microspheres were filtered and washed with *n*-hexane. The prepared microspheres were dried at 50°C for 4 h, and stored in the desiccator for further evaluation. Eight formulations of 5-FU loaded microspheres were prepared based on a 2^3 factorial design, which are summarized in **Table 1**.

Characterization of 5-fluorouracil microspheres

Particle size analysis

The particle size and size distribution of the prepared microspheres were measured by laser diffraction in a particle size analyzer (Mastersizer, Malvern Instruments, UK). The dried powder samples were suspended in de-ionized water and sonicated for 1 min with an ultra-sound probe before measurement. The obtained homogeneous suspension was determined for the equivalent volume diameter and measurements were made in triplicate for each batch of microspheres.

Determination of encapsulation efficiency

Accurately weighed 10 mg of 5-FU loaded microspheres were dissolved in 100 ml of phosphate buffer solution (pH 7.2) by shaking with magnetic stirrer for 24 h. The solution was filtered and aliquots were assayed spectrophotometrically at 266 nm. The encapsulation efficiency was calculated using the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Calculated drug concentartion}}{\text{Theoretical drug concentartion}} \times 100 \qquad \text{(Eq. 1)}$$

Microspheres recovery

Microspheres recovery efficiency was calculated as percentage of weight of the obtained microspheres, using as reference the total amount of polymer used for the preparation. The percentage of recovery does not take into account the residual water and oil contents in the particles, these parameters have been disregarded because microspheres appear very dry and non-greasy.

Differential scanning calorimetric studies

Differential scanning calorimetric (DSC) measurements were carried out with a type of DSC V-9.0 Build 275 instrument, Waters Ltd.

The instrument was calibrated using indium as standard. Samples (2 mg) were placed in sealed aluminium pans and heated from 27°C to 300°C at a rate of 10°C/min under nitrogen atmosphere (100 ml/min), with empty pan as reference.

IR Spetrophotometry

Fourier transform infrared spectra were recorded on FTIR- Perkin Elmer, USA. 1 mg of the sample was triturated with 300 mg of finely powdered and dried KBr was used to prepare the pellet. These quantities are usually sufficient to give a disc of 13 mm diameter and a spectrum of suitable intensity. A small amount of triturated sample was taken into a pellet maker and was compressed at 10 kg/cm^2. The pellet was kept onto the sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1}.

X-ray powder diffractometry studies

The powder X-ray diffraction study was carried out to characterize the polymorphic forms of 5-FU and 5-FU loaded Eudragit RL 100 microspheres. A Philips X-pert PW 3050/60 (Almelo, Netherlands) was used as X-ray generator for Cu Kα; radiation (λ =1.54178 A°). Data was collected in the continuous scan mode using step size of 0.01° 2θ. The scanned range was 5-50°C.

Scanning electron microscopic analysis

The shape and surface characteristics of microspheres were analyzed by scanning electron microscopy (SEM). Samples were dusted on a double-sided adhesive tape applied previously to an aluminium stub. Excess samples were removed and stub sputter coated (Polaron Sputter 7040) with 30 nm layer of gold-palladium. Samples were then observed with scanning electron microscope (Leo 0430, Leica Cambridge Ltd., Cambridge, UK)

In vitro release studies

In vitro dissolution studies were carried out at 37°C (±0.5°C) at 100 rpm with USP Dissolution Apparatus II (DS 8000, Labindia, India). An accurately weighed sample of microsphere was suspended in the dissolution media consisting of 500 ml of 0.1 N (pH 1.2) hydrochloric acid and dissolution was performed for 2 h. At the end of the 2 h, 400 ml of 0.1 M tribasic sodium phosphate was added to all dissolution vessels, the pH was adjusted to 7.2 (±0.2) and the dissolution was continued for 12 h. Aliquots of dissolution fluid were withdrawn at specified time intervals to assay the released drug spectrophotometrically at 266 nm. Corrections were made for the removal of samples by replacing the equal amount of buffer solution.

Drug release pattern from microspheres

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equations like zero order (% release *vs* t), first order (log% release *vs* t) and Higuchi model (Mt/M∞ *vs* t). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation, ($Mt/M\infty = kt^n$, where Mt is the amount of drug released at time t and M∞ is the amount released at time ∞, thus the Mt/M∞ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent), a measure of the primary mechanism of drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

RESULT AND DISCUSSION

Microencapsulation by the solvent evaporation method is, in principal, quite simple and involves two major steps, the formation of stable droplets of the drug-containing polymer solution and the subsequent removal of solvent from the droplets. In practice, however, the reproducible manufacturing of microspheres with the desired properties (good encapsulation efficiency, suitable release profile and particle distribution, acceptable solvent residuals), can be difficult due to the large number of factor influencing the outcome, such as solvent composition, total volume and phase volume ratio, polymer concentration, stirring speed, stirring time etc. The effect of each of these parameters has to be determined empirically, predictions and scale up remain a problem.

Encapsulation efficiency, recovery and particle diameter

Eight formulations of 5-FU were prepared using factorial design (**Table 1**). These batches were evaluated for mean encapsulation efficiency, mean recovery and mean particle diameter. The mean encapsulation efficiency, mean recovery and mean particle diameter of MA-1 to MA-8 are shown in **Table 2**. From the above prepared batches, batch MA-5 had been selected as the optimized batch because of its highest

mean encapsulation efficiency of 76.78±1.19% and the well optimized and controlled mean diameter of 107.92±1.12 μm.

Differential scanning calorimetric studies

DSC thermograms of pure drug and drug loaded microspheres were taken. A single endothermic peak in case of 5-FU at 282.60°C corresponds to its melting point (**Figure 1**). 5-FU loaded microspheres showed a broad small peak at 281.73°C (**Figure 2**), indicating the presence of drug in crystalline form. The reduction of height and sharpness of the endothermic peak is due to the presence of polymer in the microspheres.

Table 1. Formulation of microspheres

Batch	X_1 (rpm)	X_2 (h)	X_3 (% *v/v*)
MA-1	+1 (1000)	+1 (4)	+1 (1:10)
MA-2	-1 (500)	-1 (2)	-1 (1:5)
MA-3	+1 (1000)	-1 (2)	+1 (1:10)
MA-4	+1 (1000)	+1 (4)	-1 (1:5)
MA-5	-1 (500)	-1 (2)	+1 (1:10)
MA-6	-1 (500)	+1 (4)	-1 (1:5)
MA-7	-1 (500)	+1 (4)	+1 (1:10)
MA-8	+1 (1000)	-1 (2)	-1 (1:5)

X_1, stirring speed; X_2, stirring time; X_3, phase ratio; The values in brackets represent real values of X_1, X_2 and X_3.

Table 2. Particle size, recovery and encapsulation efficiency of 5-fluorouracil loaded microspheres

Batch	Mean encapsulation efficiency (%) ± S.D.	Mean recovery (%) ± S.D.	Mean diameter (μm) ± S.D.
MA-1	61.68±1.13	58.17±1.65	58.12±1.96
MA-2	62.01±1.28	61.23±1.54	146.78±1.76
MA-3	69.34±1.32	62.13±1.23	87.12±1.54
MA-4	60.11±1.88	59.24±1.48	52.17±1.23
MA-5	76.78±1.19	78.82±1.26	107.92±112
MA-6	70.24±1.47	71.34±1.48	83.71±1.36
MA-7	69.91±1.62	72.12±1.56	89.13±1.86
MA-8	68.72±1.87	68.14±1.78	81.71±1.73

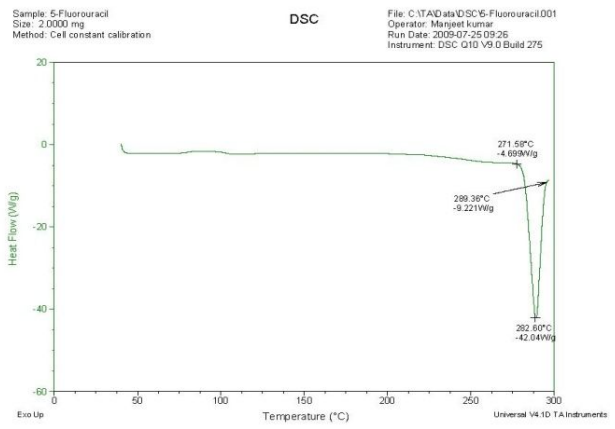

Fig. 1. DSC thermogram of pure drug (5-FU)

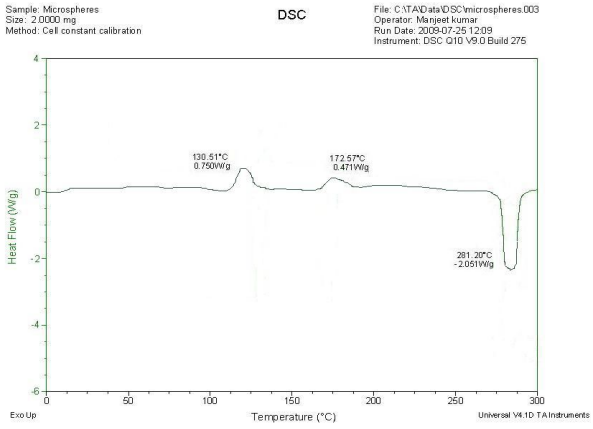

Fig. 2. DSC thermogram of microspheres (MA-5)

IR Spetrophotometry

The IR spectrum of pure drug showed the characteristics peaks at 3122.8 cm^{-1} for NH stretching, 1243.4 cm^{-1} for the C-H in-plane deformation and 812.1 cm^{-1} for the C-H out-of-plane deformation, 1720.9 cm^{-1} for C=O stretch and at 1655.4 cm^{-1} for C=N stretch. There was no significant difference in the IR spectra of pure 5-FU and drug loaded microspheres as observed in **Figure 3, 4**. The results suggested drug stability during the encapsulation process.

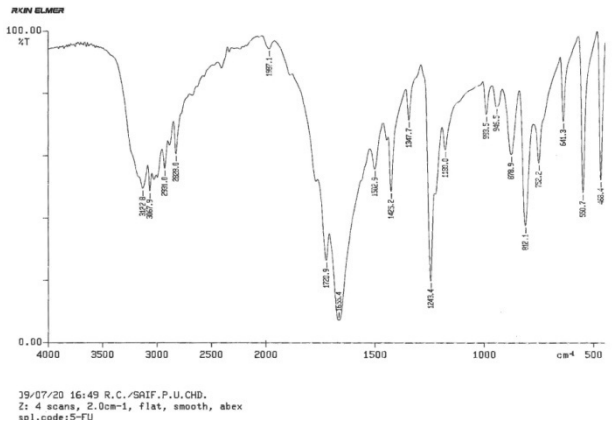

Figure 3. FTIR spectra of 5-Fluorouracil

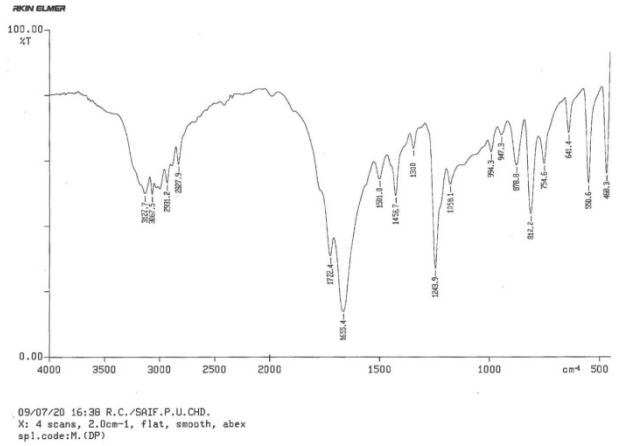

Fig. 4. FTIR spectra of microspheres (MA-5)

X-ray powder diffractometry studies

The X-ray powder diffraction patterns of pure 5-FU and 5-FU loaded microspheres were shown in the **Figure 5, 6** respectively. The sharp peaks of drug were also present in the microspheres. The sharpness of the peaks in the 5-FU loaded microspheres also confirmed the presence of the drug in the crystalline form.

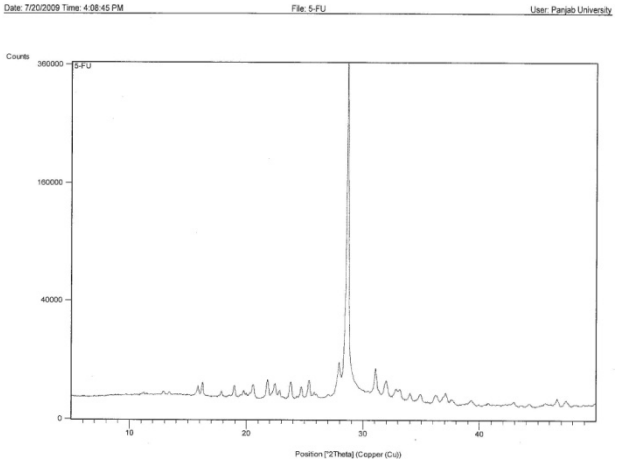

Fig. 5. X-ray powder diffraction pattern of pure 5-FU

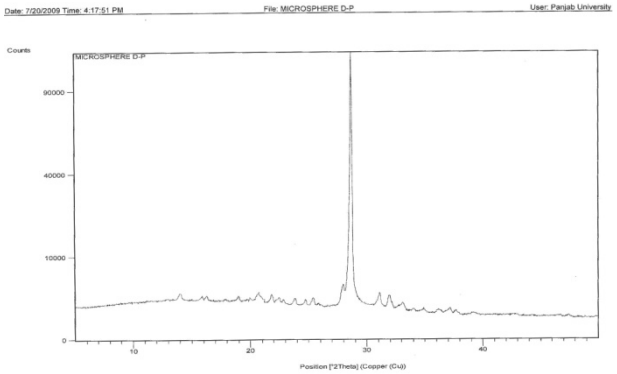

Fig. 6. X-ray powder diffraction pattern of microspheres (MA-5)

Scanning electron microscopic studies

Surface morphology of the microspheres was examined by SEM. As shown in **Figure 7, 8**, microspheres are spherical in nature without agglomerations. The surface morphology of 5-FU incorporated blend microspheres was evaluated after the *in vitro* release experiments. SEM photographs of the neat microspheres have the characteristic porous structure on the surface. An increase in the pore size was found after the drug release (**Figure 8**).

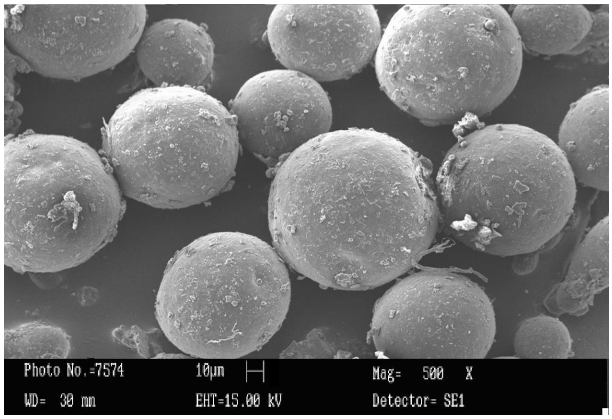

Fig. 7. SEM photograph of 5-FU loaded microspheres (MA-5) before release

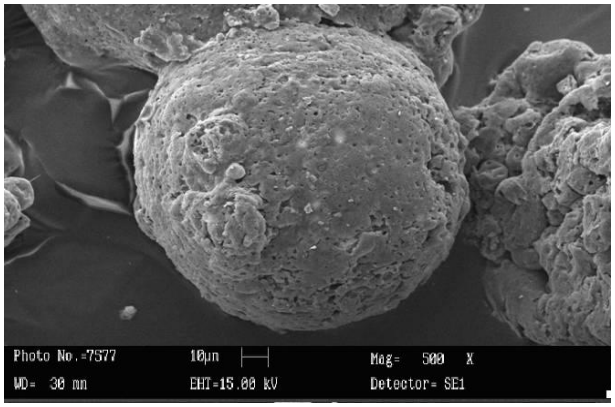

Fig. 8. SEM photograph of 5-FU loaded microspheres after release (MA-5)

In vitro release

In vitro release profiles give important information on the efficiency of the delivery systems for the controlled release of drugs. *In vitro* 5-FU release studies from microspheres were performed in pH 1.2 buffer (first 2 h) and phosphate buffer, pH 7.2 (after 2 h) at 37 ± 0.5°C. The release pattern of 5-FU from microspheres is illustrated in **Figure 9**, indicating the sustained release pattern over 12 h. At the initial stage, the burst effect related to the drug entrapped near the surface of the microspheres was remarkably small. Such a small initial burst is probably due to the low permeability of water in eudragit RL 100 and ethyl cellulose. In other words, the hydrophobic property of eudragit RL 100 and ethyl cellulose causes the delay of water penetration, thus the diffusion of the drug through the amorphous region into the release medium was retarded, ultimately leads to small burst effect.

Kinetics of drug release

In order to investigate the release mechanism of present drug delivery system, the data obtained from *in vitro* release of final optimized batch (MA-5) were fitted into equations for the zero-order, first-order, Higuchi release model and Peppas equation. The *in vitro* drug release showed the regression coefficient values for Higuchi's model (**Figure 10**) (R^2 = 0.9802) and Peppas model (**Figure 11**) (R^2 = 0.9633).

Experimental design

Cumulative percent of drug release increases at the high level of stirring speed, stirring time and phase ratio. The effect was highest in case of stirring speed and lowest in case of phase ratio. The model, developed from multiple linear regression, (**Table 3**) to estimate effect (Y) *i.e.* cumulative percent of drug release can be represented mathematically as:

$$Y = 78.965 + 4.772\ X_1 + 3.094\ X_2 + 1.24\ X_3$$

where Y = Cumulative percent of drug release; X_1 = stirring speed; X_2 = stirring time; X_3 = phase ratio.

ANOVA was applied on cumulative percent of drug release to study the fitting and significance of model (**Table 4**). F-test was carried out to compare the regression mean square with residual mean square. The ratio F = 66.818 shows regression to be significant. The estimated model, therefore, may be utilized to predict cumulative percent of drug release of 5-FU from microspheres.

Table 3. Multiple linear regression for cumulative percent of drug release

Batch	X_1	X_2	X_3	Cumulative percent of drug release
MA-1	+1	+1	+1	87.746
MA-2	-1	-1	-1	68.45
MA-3	+1	-1	+1	81.23
MA-4	+1	+1	-1	85.16
MA-5	-1	-1	+1	72.993
MA-6	-1	+1	-1	76.477
MA-7	-1	+1	+1	78.851
MA-8	+1	-1	-1	80.813
X-Coff.	4.772	3.094	1.24	78.965 (const.)

Table 4. ANOVA of the regression (cumulative percent of drug release)

	Degree of freedom	Sum of squares	Mean square	F	F-significance
Total	7	276.463	-	-	-
Regression	3	271.054	90.351	66.818	0.000713*
Residual	4	5.409	1.352	-	-

*P < 0.05

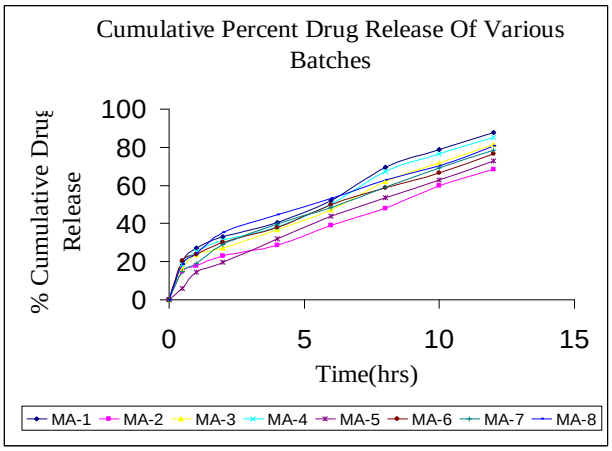

Fig. 9. Drug release pattern from microspheres (MA-1 to MA-8)

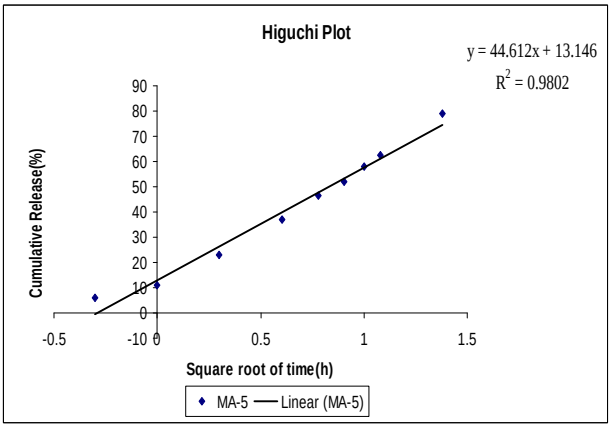

Fig. 10. Higuchi plot of the optimized batch (MA-5)

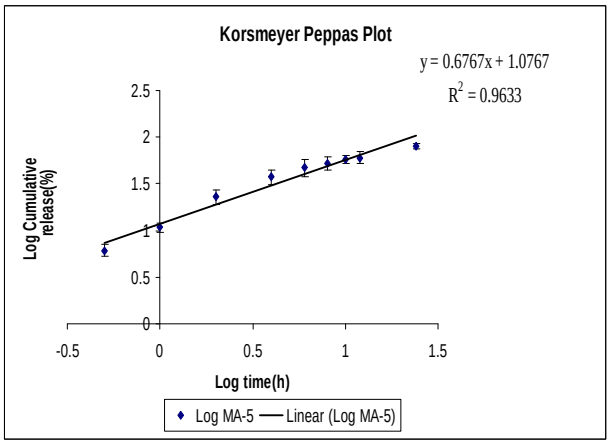

Fig. 11. Korsmeyer Peppas plot of optimized batch (MA-5)

CONCLUSION

The present study aimed to produce eudragit RL 100 and ethyl cellulose microspheres by a solvent evaporation method. This investigation has provided an understanding of the effects of some process parameters on particle size and shape, recovery and encapsulation efficiency. Selection of the appropriate experimental conditions resulted in the production of eudragit RL 100 and ethyl cellulose based microspheres characterized by spherical shape, absence of aggregates, a mean diameter of 107.92±112 μm, a recovery of 78.82±1.26% (*w/w*) and an encapsulation efficiency of 76.78±1.19% (*w/w*). Controlled release without initial peak levels achieved with these microspheres formulations can reduce dosing frequency, decrease side effects and improve patient compliance.

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