

Formulation and *In vitro* Evaluation of Eudragit RL 100 Loaded Fexofenadine HCl Microspheres

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Abstract

The present study deals with the formulation and evaluation of Fexofenadine hydrochloride (HCl) loaded sustained release microspheres by emulsion solvent evaporation method with Eudragit RL 100. The effects of percent drug loading on drug encapsulation efficiency, drug content and drug release rate were assessed. *In vitro* dissolution study was performed spectrophotometrically according to USP paddle method using phosphate buffer (pH 6.8) for 10 hours. The release rate of Fexofenadine HCl from the microspheres was significantly increased with the increase of drug loading. The drug release patterns were simulated in different kinetic orders such as zero order release kinetics, first order release kinetics, Higuchi release kinetics, Korsmeyer-Peppas release kinetics and Hixson-Crowell release kinetics to assess the release mechanism and Higuchi release kinetics was found to be the predominant release mechanism. Morphological changes due to different drug loading were assessed by scanning electron microscopic (SEM) technique. Differential scanning calorimetry and fourier transform infra-red (FT-IR) spectroscopy was performed to evaluate compatibility of drug with the polymer. A statistically significant variation in drug encapsulation efficiency and release rate was observed for variation in drug loading.

Key words: Emulsification-solvent evaporation, fexofenadine HCl, eudragit RL 100, microsphere

Introduction

Microencapsulation may be defined as the process of enveloping or coating one substance on or small solid particles, liquid droplets, or gas bubbles with a thin film of shell material, yielding capsules ranging from less than one micron to several hundred microns in size. The inertness is related to the reactivity of the shell with the core material. This technology is mainly used for the purpose of protection, controlled release, and incompatibility of the core materials. Generally the size of the microencapsulated products is considered as larger than 1 micrometer and up to 1000 micrometers in diameter. Commercially available micro particles contained 10-90% w/w core (Yeamin *et al.*, 2010; Gohel and Amin, 1998; Sadat, 2010). Solvent evaporation, coalescence and phase separation,

coacervation, spray drying, interfacial polymerization and ionotropic gelation are the most commonly used techniques for microsphere preparation. Solvent evaporation method simply engages emulsification of a solution containing polymer and drug with an additional medium in which the drug and polymer cannot dissolve. This method has been used to prepare microcapsules of a variety of compounds using several different polymeric materials (Bakan, 1986; Bolourtchian, 2005)

Fexofenadine HCl (FFN) is a second-generation non-sedating histamine H₁ receptor antagonist. As it is included in class III of the biopharmaceutical classification system (BCS), polymers can be used to prolong its duration of action in body, thereby increasing the bioavailability (Gundogdu and Karasulu,

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2011). The aim of the present work is to develop and characterize the Fexofenadine HCl microspheres with biological half-life of 14.4 hrs (Aventis, 2003; Roussel, 1996) and to evaluate the usefulness and feasibility of these microspheres as sustained release dosage form for continuous or long term therapy with high margin of safety. The incorporation of polymer content was kept in a low range for balancing its long half life and low bioavailability. *In vitro* effect of the addition of polymer on the drug content, particle size, morphology of microspheres and, consequently, on the release profile of Fexofenadine HCl microsphere were evaluated to develop formulation which would increase the bioavailability by sustaining the action.

Materials and Methods

Materials

Fexofenadine HCl was received as a generous gift from Incepta Pharmaceuticals Ltd., Eudragit RL 100 (Evonik Industries, Germany), *n*-hexane (MERCK, Germany), Span 80 (sorbitan monooleate) (MERCK,

Germany), ethanol (MERCK, Germany), light liquid paraffin (MERCK, Germany), potassium dihydrogen phosphate (MERCK, Germany) and dipotassium hydrogen phosphate (MERCK, Germany) were used in this study from the indicated sources.

Methods

Preparation of microspheres of Fexofenadine HCl with Eudragit RL 100 polymer by solvent evaporation technique: The microspheres of Fexofenadine HCl were prepared based on the "emulsion-solvent evaporation technique" using Eudragit RL 100. Ethanol was used as solvent and Span80 was used as lipophilic surfactant for Fexofenadine HCl water in oil (W/O) type of emulsions (Odonnell and McGinity, 1997; Ayon, 2014; Jalil and Nixon, 1989).

Formulation design: Total 5 batches (each of 900mg) of microspheres of Fexofenadine HCl (Table 1) were prepared and designated as F1 to F5 using Eudragit RL 100.

Table 1. Formulation protocol for the microspheres prepared by using Eudragit RL 100 with different drug loading.

Formulation	Drug : Polymer	Drug (mg)	Eudragit RL 100 (mg)
F1	2:1	600	300
F2	1.5:1	540	360
F3	1:1	450	450
F4	1:1.5	360	540
F5	1:2	300	600

In vitro characterization of polymeric microspheres of Fexofenadine HCl:

Production yield: The yield of production was calculated as the amount of microspheres obtained with respect to the theoretical content of microsphere. The calculation of percentage yield was done by using the following formula (Yadav and Jain, 2011):

$$\text{Yield (\%)} = \frac{\text{Amount of microspheres obtained in grams}}{\text{Wt. of total amount of materials added in grams}} \times 100$$

Determination of drug entrapment efficiency: 10 mg of sample from each batch of microspheres was

accurately weighed and crushed in a glass mortar-pestle and the powdered microspheres were suspended in 6.8 phosphate buffer solution. After 24 hrs the solution was filtered and the filtrate was analyzed for drug content. The drug entrapment was calculated using the formula (Patel, 2007) –

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

Micromeritics study: Bulk density and tapped density were determined using a volumetric cylinder. Accurately weighed microspheres were gently poured

using a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped density were noted using tapping method using measuring cylinder of 10 ml (Garud, 2015). Flow properties were studied by determining the Carr's compressibility index and Hausner ratio. Particles having excellent flow properties will have value of Carr's Compressibility Index, Hausner Ratio and Angle of Repose in the range of 5-10, 1.00-1.11 and 25-30, respectively.

Study of surface morphology by scanning electron microscopy (SEM): Scanning electron microscopy was used to study the shape and surface morphology of the microspheres and the effects of polymer concentration on the microsphere shape, integrity and their drug release pattern. (Ramachandran, 2011). A small amount of micro-spheres was spread on aluminum stub. Afterwards, the stub containing the sample was placed in the scanning electron microscopy (SEM) chamber. A scanning electron photomicrograph was taken at the acceleration voltage of 30 KV, chamber pressure of 0.6 mm Hg.

In vitro dissolution study of microspheres containing Fexofenadine HCl: The paddle type (Type II) dissolution apparatus was used to study in-vitro drug release from microspheres. A weighed amount of microspheres (60 mg) was used for the study. 900 ml of dissolution medium (pH 6.8 phosphate buffer) was maintained at a temperature of 37 ± 0.5 °C and rotation speed of 100 rpm was used for the study. The in-vitro release studies were performed for 10 hrs. The dissolution process was carried out for 10 hours and 10 ml dissolution sample from each dissolution media was withdrawn at a predetermined intervals of 30 min, 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour, 8th hour, 9th hour and 10th hour and the release was measured by UV spectrophotometric method at 259nm. The initial volume of dissolution medium was maintained by adding 10 ml of fresh dissolution medium after each withdrawal (Costa, 2001; Peppas, 1985).

Data analysis: To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. The zero order rate Eq. (1) describes the systems where the drug release rate is independent of

its concentration. The first order Eq. (2) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from microspheres as a square root of time dependent process based on Fickian diffusion Eq. (3). The Hixson-Crowell cube root law Eq. (4) describes the release from systems where there is a change in surface area and diameter of particles or tablets.

$$C = k_0 t \quad (1)$$

Where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$\text{Log}C_0 - \text{Log}C = kt / 2.303 \quad (2)$$

Where, C_0 is the initial concentration of drug and K is first order constant.

$$Q = Kt^{1/2} \quad (3)$$

Where, K is the constant reflecting the design variables of the system.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HCT} \quad (4)$$

Where, Q_t is the amount of drug released in time t , Q_0 is the initial amount of the drug in tablet and K_{HCT} is the rate constant for Hixson-Crowell rate equation.

Successive fractional dissolution time: To characterize the drug release rate in different experimental conditions, $T_{25\%}$, $T_{50\%}$, $T_{80\%}$ and MDT were calculated from dissolution data.

Statistical analysis: Statistical analysis of the results was performed by using one-way analysis of variance (ANOVA) followed by Dennett's t-test for comparisons. The limit of significance was set at $p < 0.05$.

Compatibility studies of drug and polymer with FexofenadineHCl microspheres:

Fourier transforms infrared spectrophotometry (FTIR): The Fourier transform infrared (FT-IR) spectra of pure drug, pure polymer, physical mixture of drug and polymer and optimized microsphere formulations were recorded with FT-IR 8400S Shimadzu spectrophotometer in the range 400-4000 cm^{-1} . Appropriate quantity of KBr and sample (in the ratio of 100: 0.1) were mixed by grinding in an agate mortar. Pellets were made with about 100 mg mixture. The IR spectrum was obtained to evaluate the chemical integrity and compatibility of the drug with the polymers in the microspheres (Balpande, 2013).

Differential Scanning Calorimetry (DSC): DSC study was carried out to evaluate the interaction between the drug and the polymers in the microspheres by using a Differential Scanning Calorimeter (DSC 60, Shimadzu). The specific heat and enthalpies of transition were determined.

Results and Discussion

Production yield (%) of microspheres: For microspheres prepared with Eudragit RL 100 there is a moderate variation in the yield (%) value, the lowest value being 96.97% for F1 (66.66 % drug loading) and the highest one being 102.61% for F5 (33.33% drug loading). Figure 1 shows that yield did not change in any particular pattern with drug loading.

Drug entrapment efficiency (%) of microspheres: UV spectrophotometric method was employed to determine the entrapment efficiency of the drug in the microsphere prepared. The entrapment efficiency was determined by measuring the absorbance at 259 nm using pH 6.8 Phosphate buffer solutions. The maximum drug entrapment efficiency is 90.94 % for F5 (33.33% drug loading) and the minimum drug entrapment efficiency is 82.53% for F1 (66.66% drug loading). So, the bar diagram reveals that drug entrapment efficiency increase with the increase of polymer content.

Micromeritics study of microspheres prepared with Eudragit RL 100: The microspheres prepared with Eudragit RL 100 exhibits excellent to good flow properties. F3 showed best flowability among all the formulations (Table 2).

Observation of particle morphology by scanning electron microscope (SEM): Figure 2 reflects the effect of Eudragit RL100 on the shape and surface morphology of the microsphere. Batch F1 (66.67% drug loading) is the microsphere of Fexofenadine HCl with a rough surface. The shape of the microspheres of Batch F5 (33.33% drug loading) is almost spherical and the surface of the particles is smoother. No significant fusion among particles was observed. Presence of pores or cracks may cause quick release since these facilitate the penetration of dissolution medium into the microsphere. Nature of the surface influences the stability and dissolution characteristics of the microspheres. Surface morphology also revealed presence of porous microparticles. If surface is rough, there are more chances of wetting and contact of water with the microsphere than the smoother one.

In vitro dissolution and kinetic studies of Fexofenadine HCl loaded polymeric microsphere: Microspheres of five different drug loading (33.33%, 40%, 50%, 60%, 66.66%) using Eudragit RL 100 individually were examined for dissolution pattern. The obtained data were subjected to various kinetics treatments for investigating their release pattern (Figure 3). The batches described in this segment are F1, F2, F3, F4 and F5. All batches from F1 to F5 were examined for dissolution pattern (Table 3). F5 showed to have best release retardant property as having the highest polymer content. After dissolution of microsphere, the percent release of drug after 1 hour, 5 hour and 10 hour were 35.06%, 62.44% and 96.38% respectively.

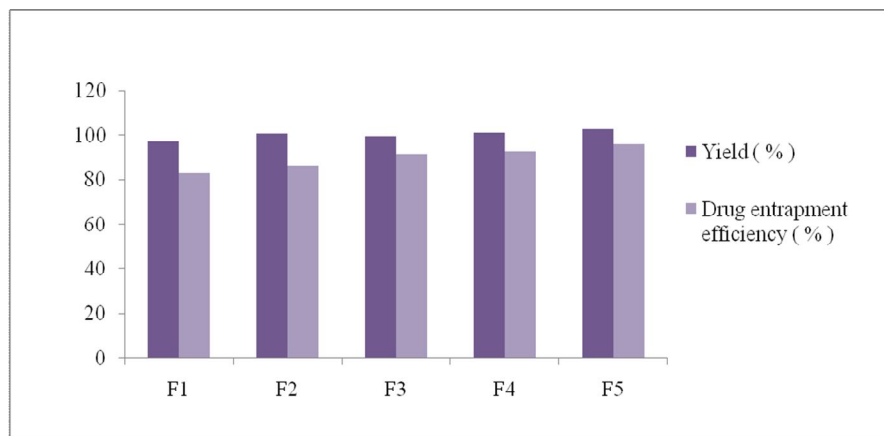


Figure 1. Production yield (%) and drug entrapment efficiency of microspheres.

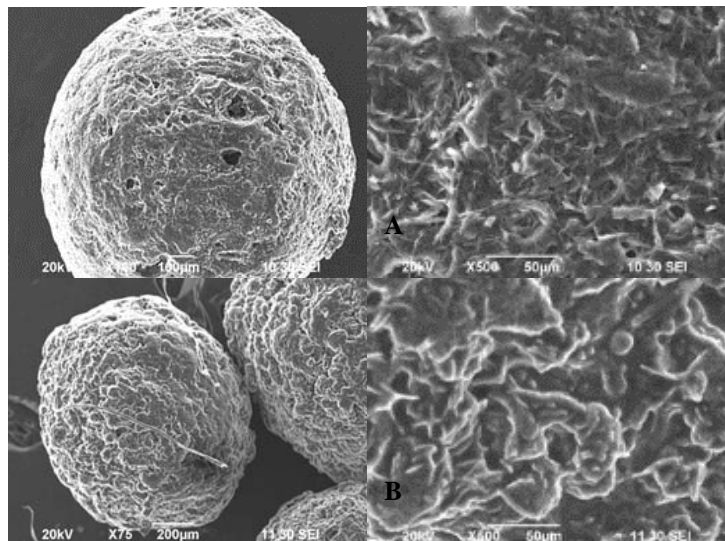


Figure 2. Scanning electron microscopic view of microspheres A. Formulation F1 (66.67% drug loading) and B. Formulation F5 (33.33% drug loading).

Table 2. Results of micromeritics study of microspheres.

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's compressibility index	Hausner ratio
F1	0.12	0.14	14.29	1.17
F2	0.11	0.12	8.89	1.10
F3	0.10	0.11	8.33	1.09
F4	0.10	0.12	14.29	1.17
F5	0.11	0.13	15.56	1.18

Table 3. Release rate constants and R² values for different release kinetics of five formulations (F1, F2, F3, F4 and F5) of Fexofenadine HCl microspheres.

Formulation	Zero order		First order		Higuchi		Korsmeyer- Peppas			Hixson- Crowell	
	K ₀	R ²	K ₁	R ²	K _H	R ²	n	K _{KP}	R ²	K _{HC}	R ²
F1	8.047	0.907	-0.382	0.771	28.92	0.979	0.357	0.403	0.952	0.308	0.931
F2	8.06	0.915	-0.348	0.802	28.87	0.981	0.369	0.389	0.954	0.296	0.936
F3	8.008	0.918	-0.309	0.856	28.62	0.979	0.371	0.382	0.949	0.281	0.948
F4	7.96	0.920	-0.283	0.891	28.42	0.981	0.376	0.374	0.951	0.268	0.957
F5	7.98	0.928	-0.269	0.903	28.39	0.981	0.386	0.361	0.944	0.262	0.961

Best fitted model for these formulations was Higuchi (R²=0.981). The release mechanism of this formulation followed Fickian transport (Table 4). F6 showed least release retardant property. After dissolution of microsphere the percent release of drug

after 1 hour, 5 hour and 10 hour were 40.88%, 67.13% and 99.56% respectively. Best fitted model for this formulation was Higuchi (R²=0.979). The release mechanism of this formulation followed Fickian transport (Table 4).

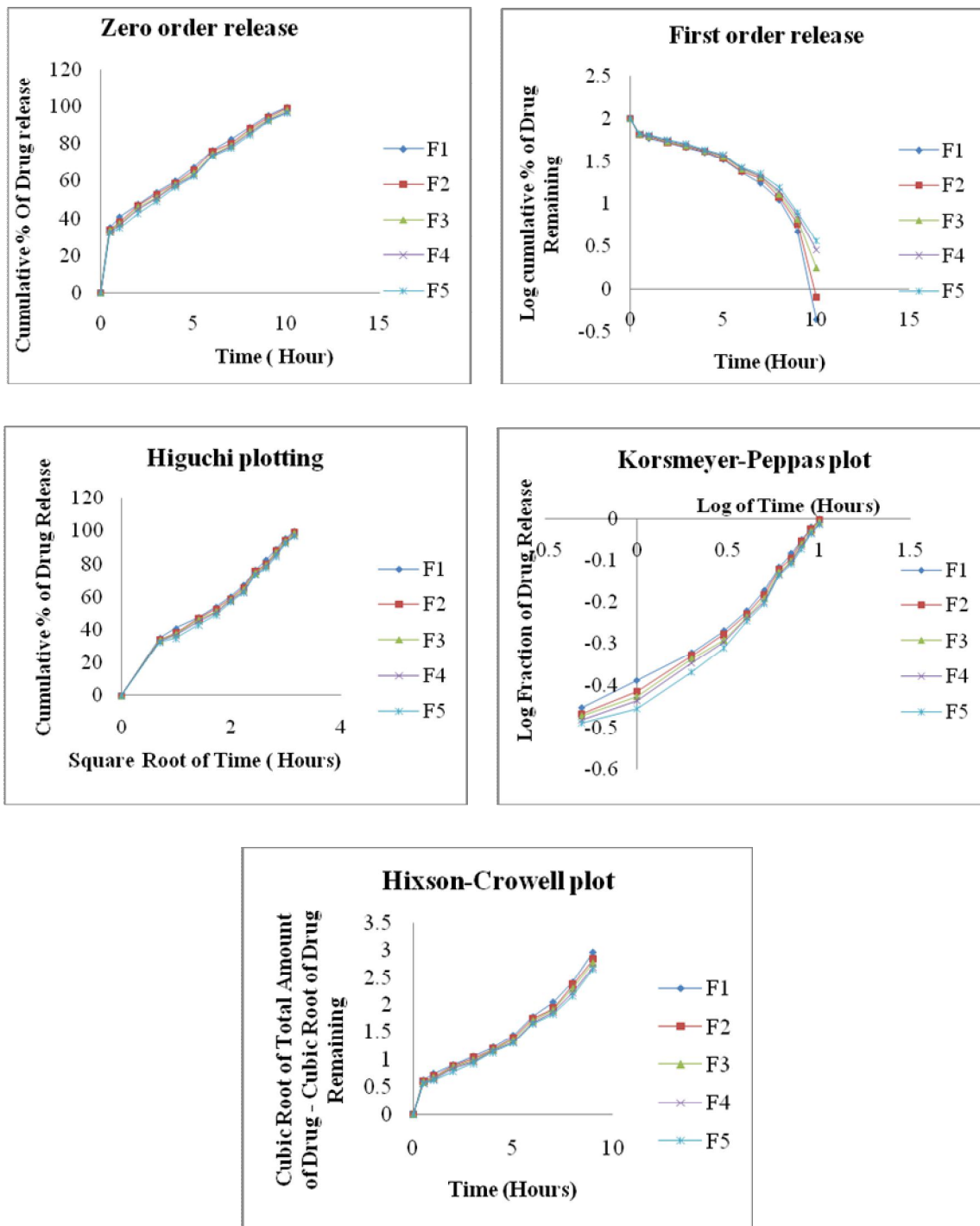


Figure 3. Release kinetics of five formulations (F1-F5) of Fexofenadine HCl microspheres prepared with Eudragit RL100. A. Zero order release. B. First order release C. Higuchi plot D. Korsmeyer-Peppas plot E. Hixson-Crowell plot.

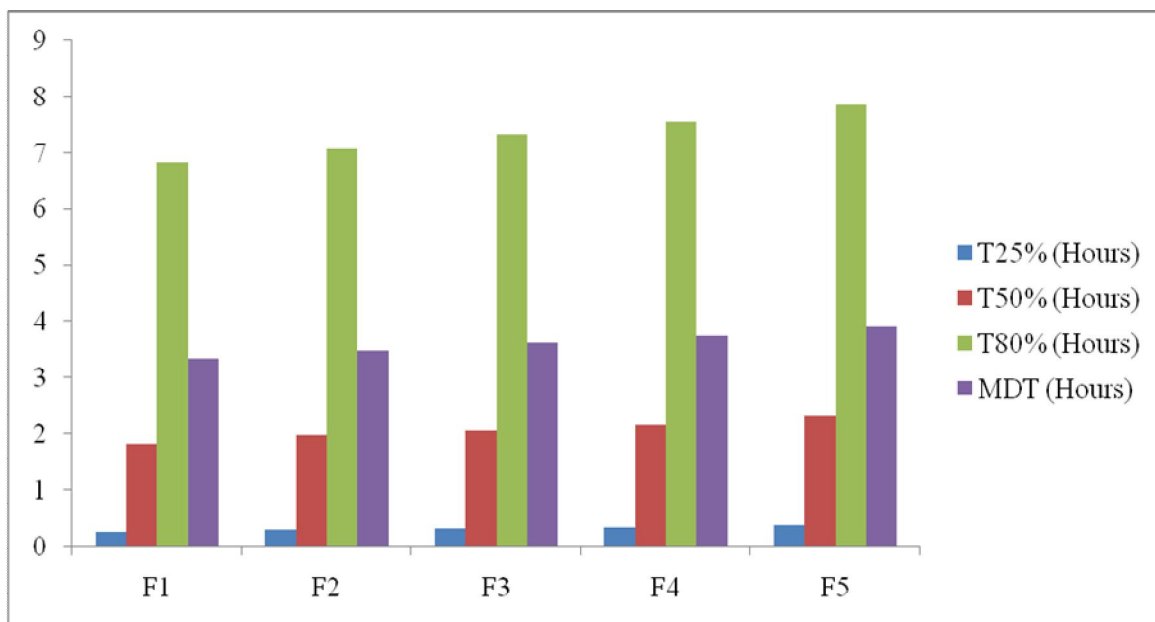


Figure 4. Bar diagram representing successive fractional dissolution time of five formulations F1, F2, F3, F4 and F5.

Table 4. The best fitted model and mechanism of drug release from F1, F2, F3, F4 and F5.

Formulation	Best fitted model	n value	Release mechanism
F1	Higuchi	0.357	Fickian transport
F2	Higuchi	0.369	Fickian transport
F3	Higuchi	0.371	Fickian transport
F4	Higuchi	0.376	Fickian transport
F5	Higuchi	0.386	Fickian transport

Table 5. Summary of impact analysis of % drug loading on different formulation parameters of formulations F1- F5 using simple linear regression model (Level of significance, 0.05).

Formulation parameters	Regression coefficient	P-value	Result	Comment
Yield (%)	0.014662	0.0617	P-value > 0.05	Statistically insignificant
Drug Entrapment efficiency (%)	0.043044	0.00354	P-value < 0.05	Statistically significant
Time required for 50% drug release (T _{50%})	0.0014960	0.00239	P-value < 0.05	Statistically significant

Successive fractional dissolution time: Successive fractional dissolution time was observed to be highest for F5 and lowest for F1. Figure 4 shows an opposite relationship between successive fractional dissolution time and drug loading. T_{50%} for formulations F1-F5 was not too high which indicates moderate release retarding capability of the polymer.

Analysis of impact of % drug loading on different formulation: Table 5 reveals that, for microspheres (F1-F5) prepared with Eudragit RL 100, decrease in drug loading has no significant effect on % yield but has significant increasing effect on % drug entrapment efficiency. The time required for 50% of drug release (T_{50%}) increases with increase in the total amount of

Eudragit RL 100 (decrease in drug loading). Statistically it shows that, with increase in the total amount of Eudragit RL 100 by 1 mg, $T_{50\%}$ increases by 0.0014960 hours. Considering the level of significance 0.05, P-value has been found to be 0.00239 for

formulations F1-F5. This indicates that, P value is < 0.05. So it can be concluded that, the effect of the change in the amount of Eudragit RL 100 on $T_{50\%}$ is statistically significant.

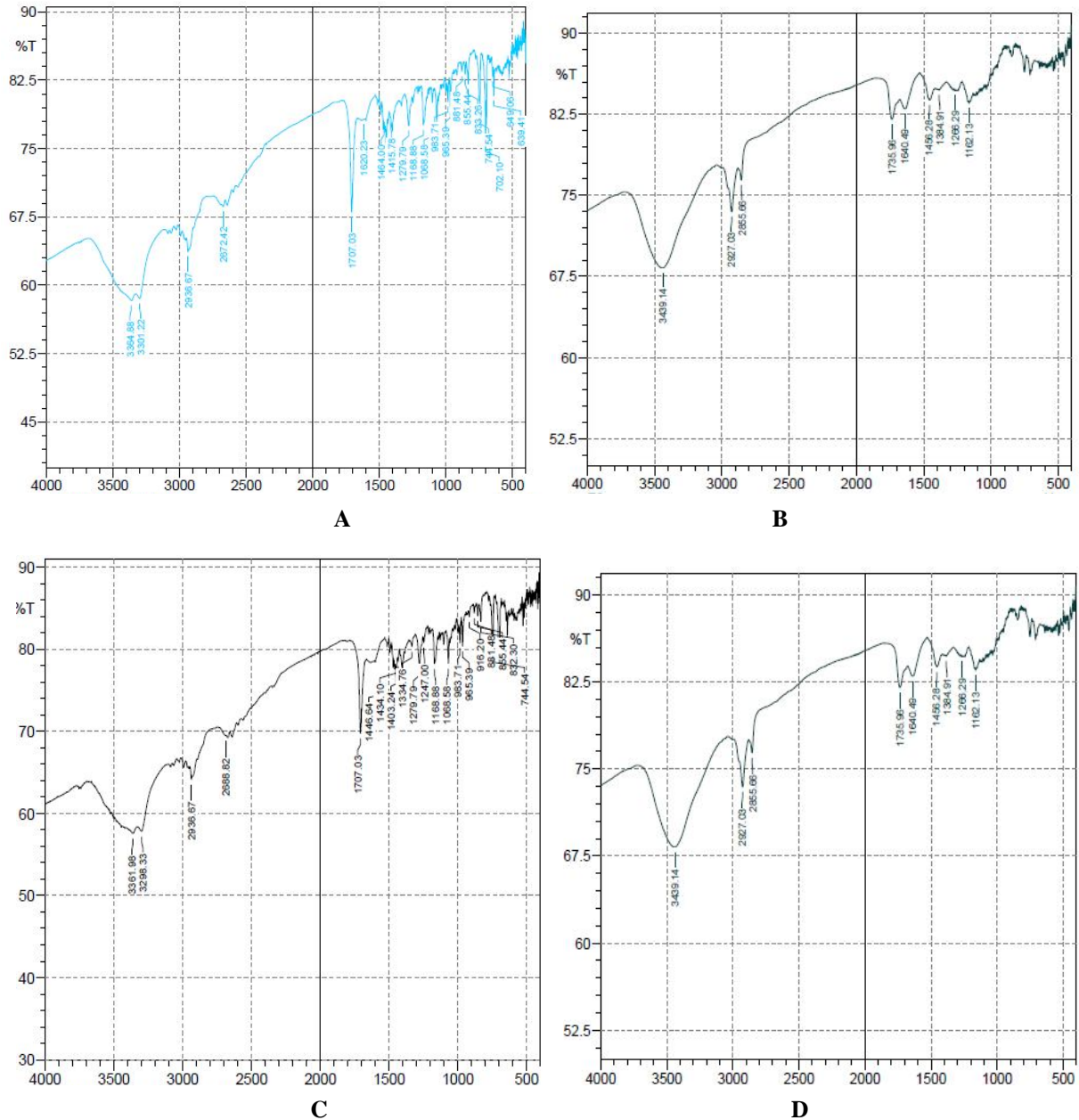


Figure 5. FTIR Spectra of A. Pure Fexofenadine HCl (S6), B. Eudragit RL100 (S1), C. Physical mixture of Fexofenadine HCl and (S2) Eudragit RL100 and D. F3.

Compatibility studies of Fexofenadine HCl microspheres:

Fourier transforms infrared spectroscopy (FT-IR): FTIR spectra of pure Fexofenadine HCl (S6), physical mixture of Fexofenadine HCl and Eudragit RL 100 (S1) and Formulation F3, Fexofenadine HCl microspheres prepared with Eudragit RL 100 (indicated as F8) were observed (Figure 5). The FTIR spectra of pure Fexofenadine HCl depict characteristic absorption band at 3364.88 cm^{-1} , 1707.03 cm^{-1} , 1464 cm^{-1} , 1279.79 cm^{-1} and 1168.88 cm^{-1} which represent the presence of broad, O-H stretching vibrations, carbonyl (C=O) stretching of Carboxylic acid, aromatic C=C stretching and C-O stretching vibration of tertiary alcohol respectively. The FTIR spectra of F3 Fexofenadine HCl loaded microsphere (as indicated by F8) shows characteristic absorption band at 3439.14 cm^{-1} , 1735.96 cm^{-1} , 1456.28 cm^{-1} , 1266.29 cm^{-1} and 1162.13 cm^{-1} which represent the presence of broad, O-H stretching

vibrations, carbonyl (C=O) stretching of Carboxylic acid, aromatic C=C stretching and C-O stretching vibration of tertiary alcohol, respectively. It indicates no change of functional groups.

Differential scanning calorimetric (DSC) study: DSC studies were conducted for pure Fexofenadine HCl, Eudragit RL 100 and their physical mixtures and F3 (microspheres of drug prepared with Eudragit RL 100).

The data obtained from all of these samples are viewed in Figure 6 as combined thermo gram of drug, polymer and microspheres prepared with Eudragit RL 100. No drastic change occurred to the melting point of the microspheres in comparison with pure Fexofenadine HCl. So it can be said there is no such incompatibilities in the physical mixture or in the microspheres prepared with Eudragit RL 100 in comparison to pure Fexofenadine HCl.

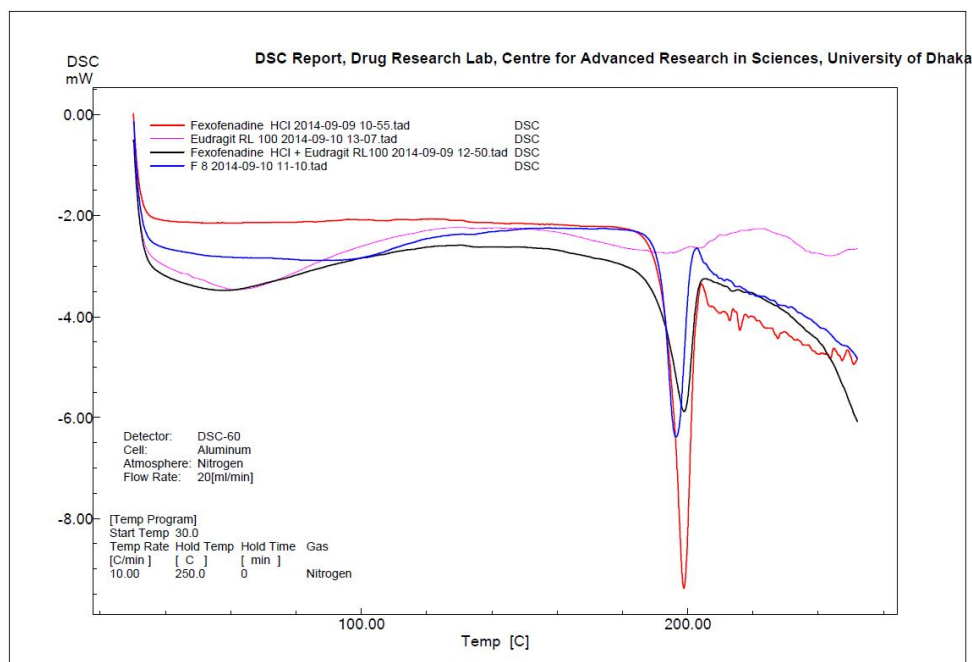


Figure 6. Combined DSC thermogram of pure Fexofenadine HCl, Eudragit RL100 and their physical mixtures and F8, Fexofenadine HCl microspheres prepared with Eudragit RL100 (representing F3).

Conclusion

Polymeric microspheres of Fexofenadine HCl were prepared successfully by emulsification-solvent

evaporation technique using Eudragit RL100. In this study, it has been demonstrated that Eudragit RL 100 polymer sustained the release of Fexofenadine HCl from

the microspheres in a moderate manner. It retarded the release of the drug in an increasing manner with the increase of the polymer content. So Eudragit RL100 can be used for moderate retarding effect on drug release.

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