International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

FORMULATION AND CHARACTERIZATION OF CEFUROXIME AXETIL FLOATING MICROSPHERES

Marimuthu S*, Nantheeswaran S, Shanmugarathinam A and Purachikody A

Department of Pharmaceutical Technology, Anna University, Tiruchirappalli, Tiruchirappalli – 620024, Tamilnadu, India

***Corresponding author e-mail:** marimpharm@gmail.com

ABSTRACT

The purpose of this research work was to increase the residence time of drug Cefuroxime axetil by formulating as floating microspheres and to study the effect of formulation variables on microsphere characteristics. Microspheres were prepared by solvent evaporation method in which ethyl cellulose used as a release retardant polymer. Nine different formulations were prepared by changing drug to polymer ratio, volume of internal phase, volume of external phase and stirring time. The prepared microspheres were characterized for drug - polymer compatibility by IR, percentage yield, particle size analysis, drug entrapment efficiency, surface morphology by SEM, bulk density, percentage buoyancy, in-vitro release and release kinetic studies. Results of these evaluations showed that particle size in the range of 58.52 to 77.36µm**,** encapsulation efficiency was found to be 60.7 to 75.7%, Percentage buoyancy of all formulations were in the range of 62.12 to 81.23%. Fourier-Transform Infra Red (FT-IR) studies ensured that no drug - polymer interaction in the formulated microspheres and the Scanning Electron Microscopy (SEM) photograph revealed that microspheres were spherical in nature with rough surface. In- vitro release profile of microspheres were in the range of 73.47 % to 89.78%, at the end of 12 hrs. In release kinetic studies, most of the formulation followed hiquchi release kinetics and follows anomalous transport (non- fickian) mechanism. This entire evaluation confirmed that drug: polymer ratio has significant effect on microsphere characteristics than the other variables used, and also the in-vivo bioavailability of the drug will increase because the buoyancy of microspheres in simulated gastric fluid was satisfactory.

Keywords: Cefuroxime axetil, Ethyl cellulose, Floating microspheres, Gastric residence time

INTRODUCTION

The Floating drug delivery system (FDDS) is one of the Gastro retentive technique becomes most promising drug delivery to improve the bioavailabity of drug which is unstable in the intestinal environment and also FDDS provides prolonged drug release by increasing the residence time of the drug in GIT due to its buoyancy capacity in stomach fluid. [1, 2] To achieve the buoyancy of dosage form in stomach fluid, the dosage form should have less density than the density of stomach fluid which is approximately 1.004 g/cc. This low density dosage forms can be prepared by using low density polymers and by hollow microspheres. The drugs which are unstable in intestine and the drug with short biological half life are more suitable for the floating drug delivery system. [3, 4] Cefuroxime axetil is a Second-generation cephalosporin used in the treatment of infections of upper and lower respiratory tract, otitis media, urinary tract infections, sepsis and uncomplicated gonorrhea.

Cefuroxime axetil is a prodrug developed to increase the oral absorption of the drug by attaching the ester group (axetil) with cefuroxime to increase the lipophilicity of drug. Eventhough the drug cefuroxime axetil has low oral bioavailabilty (37-52%) due to intestinal enzyme esterase, which hydrolyzes the ester group axetil. [5] So the absorption efficiency of the drug get decreased which resulting reduced oral bioavailability. In order to increase the

bioavailability of this prodrug, floating microspheres was formulated which avoid the entry of drug in to the intestine where mainly enzyme esterase present. And also the drug has very short half life (1-2 hrs), so it's prescribed as twice or thrice daily. So this drug formulated as a sustained release microspheres in which ethyl cellulose used as a release retardant polymer. [6]

MATERIAL AND METHODS

Materials: Cefuroxime axetil was obtained as a gift sample from Madras Pharmaceuticals, Chennai (India). Ethylcellulose was procured from Signet chemical corporation, Mumbai. Dichloromethane, Ethanol and Tween-80 purchased from S.D fine chemicals, Mumbai. All other chemicals and reagents used were of analytical grade.

Preparation of microspheres: [7, 8]
Microspheres were prepared by solvent evaporation method. Nine formulations were prepared by changing drug to polymer ratio, volume of internal phase, volume of external phase and stirring time. The compositions for the various formulations are shown in Table no 1. Weighed amount of Cefuroxime axetil and ethyl cellulose was dissolved in a mixture of dichloromethane and ethanol in the ratio of 1:1. This organic phase was added drop wise to the water containing 0.01% Tween 80 as a surfactant, at the same time the water was stirred at speed of 1300 rpm. After particular time interval the resulting microspheres were separated by filtration and it air dried then stored in a desiccators until further use.

Physical characterization of microspheres: [8, 9]
Particle size analysis*:* Particle size of microspheres was determined in terms of average diameter by optical microscopic method using stage and eye piece micrometer.

Drug entrapment efficiency [DEE]: To determine the drug entrapment efficiency, weighed amount of microspheres were thoroughly crushed and dissolved in ethanol then it filtered. The filtered solution was analyzed for drug content after suitable dilution by UV spectrophotometry at 277nm.

Bulk density: Bulk density is the ratio of the weight of powder to the volume it occupies. It express in g/cc.

Floating behavior of microspheres: To assess the floating behavior, weighed amount of microspheres were spread over the surface of a USP XXIV dissolution apparatus type-2 filled with 900ml of 0.1 N Hydrochloric acid containing 0.02% Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 hrs. The floated and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed.

Morphological study using SEM *(Hitachi-S3400N, Japan)****:*** Scanning electron microscopy (SEM) was performed to characterize the surface morphology of optimized microspheres of Cefuroxime axetil. Dried microspheres were coated with gold foil under an argon atmosphere in a gold coating unit and it focused under SEM.

In-vitro Drug release: Weighed amount of cefuroxime axetil loaded microspheres was placed in USP paddle apparatus using 900ml of 0.1N hydrochloric acid containing 0.02% Tween 80 is added to dissolution medium to mimic the stomach containing natural surfactant like bile salts and phospholipids. Samples were withdrawn at a predetermined interval. The withdrawn samples were suitably diluted and analyzed by UV spectrometry at 277 nm.

In-vitro release kinetics: To describe the kinetics of the drug release from the controlled release formulation, various mathematical equations like zero order, first order, Higuchi and Hixson- crowell equations were used. Dissolution data was further analyzed using Peppas and Korsemeyer equation.

Drug and polymer interaction studies by Fourier Transform Infrared spectroscopy (FT-IR): FT-IR spectra of cefuroxime axetil, ethyl cellulose and cefuroxime axetil loaded ethylcellulose microspheres were taken by Fourier Transform Infrared spectroscopy (Shimadzu, model 8400S) at moderate scanning speed.

RESULTS AND DISCUSSION

Preparation of microspheres: Microspheres were prepared by solvent evaporation method. Many of the researchers employed with solvent evaporation method due to its simplicity and reproducibility.

The Cefuroxime axetil is a slightly water soluble drug, so aqueous solution used as a continuous phase in the preparation of microspheres which reduces the partition of drug in to the continuous phase. Tween 80 used as emulsifying agent which has the HLB value of 15 and is expected to reduce the interfacial tension between the two immiscible phases.

Solvent combination: Selection of solvent is very important for microspheres preparation. A mixture of ethanol and dichloromethane used for this microspheres preparation as solvent. Because when non- polar solvent dichloromethane used alone the polymer get precipitated rapidly at the time of mixing with water. So to reduce the non- polarity of the dichloromethane, ethanol was added to that solvent. During microspheres formation ethanol gets diffused in to the water and dichloromethane was evaporated.

Effect of continuous phase volume (aqueous phase): In microspheres preparation, when the continuous phase volume was increased from 50 ml to 100 ml and 150 ml, the percentage yield and entrapment efficiency of drug was decreased gradually. This may due to increasing the partition of drug in to the continuous phase when the continuous phase volume was increased. The mean particle size of microspheres formulations were compared in Figure 1. When continuous phase volume increased the particle size decreased and buoyancy of microspheres increased. In- vitro drug release from the microspheres was slightly increased with increasing the continuous phase volume in microspheres preparation process. This may due to the more porous nature of the microspheres while increasing continuous phase volume as mentioned earlier. The effect of continuous phase volume on microspheres characteristics was compared in Table 2.

Effect of internal phase volume (organic phase): In microspheres preparation, when the volume of internal phase increased from 5ml, 10ml and 15ml the yield was increased because when less amount of internal phase solvent employed that evaporated rapidly before mixing with continuous phase so it formed as a fibers and aggregates which reduce the yield of microspheres.

Particle size of the microspheres also decreased with increasing internal phase volume. This can be explained as in less amount of solvent the polymer solution was more viscous which produce larger droplet when poured in to the continuous phase, so particle size was increased. Entrapment efficiency of drug in microspheres was decreased with increasing the internal phase volume. This may due to the movement of drug particle from internal phase to continuous phase was increased because of decreasing the viscosity of drug-polymer solution. So the entrapment efficiency was decreased. Internal phase volume does not have any significant effect on floating behavior and in- vitro release of microspheres. The effect of internal phase volume on microspheres characteristics was compared in Table 3.

Effect of stirring time: In microsphere preparation, when stirring time was increased from 30min to 60 and 90 min, the yield and entrapment efficiency was decreased due to increasing the partition of drug to the continuous medium with increasing stirring time. It has no effect on particle size, floatability and in-vitro drug release. . The effect of stirring time on microspheres characteristics was compared in Table 4.

Effect of drug: polymer ratio: In microspheres preparation, when the drug: polymer ratio increased from 1:1 to 1:1.5 and 1:2, the yield was gradually decreased by increasing drug: polymer ratio due to increasing the drug: polymer ratio increase the viscosity of the solution in which solvent get evaporated rapidly before mixing with continuous phase so it formed as a fibers and aggregates which reduce the yield. Entrapment efficiency of the drug in microspheres was increased with increasing drug: polymer ratio because increased polymer amount provides more binding site for the drug molecules. Particle size of the microspheres was increased with increasing drug: polymer ratio. This can be explained as when the drug: polymer ratio was increased the polymer solution was more viscous which produce larger droplet when poured in to the continuous phase, so particle size was increased. In- vitro drug release was decreased with increasing drug: polymer ratio due to increasing the diffusional path length of drug molecules. . The effect of drug: polymer ratio on microspheres characteristics was compared in Table 5.

Bulk density: Bulk densities of formulations were in the range of 0.2610 to 0.5217 gm/ml. It confirms the buoyancy of microspheres in stomach fluid because the densities of formulations are less than the density of stomach fluid (1.004 gm/ml)**.** Bulk densities of the formulations are given in Table 6.

In- vitro release kinetics: In- vitro dissolution data of all formulations were applied to various kinetic models to find the mechanism of drug release. Most of the formulations follows Hiquchi release kinetics which indicated that the release was diffusion controlled. According to the n- value obtained from korsemeyer peppas model, formulation F1follows non- fickian case II mechanism which indicated the dominant mechanism for drug transport is due to polymer matrix relaxation (i.e., the transition from glassy to rubbery state). formulation F3 follows super case II transport mechanism which indicated in

addition to diffusion, other release mechanism including matrix erosion and polymer relaxation might be involved, and formulations F2, F4, F5, F6, F7, F8, F9 follows anomalous transport (non- fickian) which indicated the coupling of Fickian diffusion and polymer matrix relaxation. Regression coefficient value and n-values of the formulations were given in Table 7 and 8 respectively.

In- vitro dissolution: Among the all of the formulations, F8 shows highest drug release of 89.78% and formulation F9 shows least drug release of 73.47%. This may be due to the changes in the drug: polymer concentration (F8- 1:1, F9- 1:2). Only changes in the drug: polymer ratio has significant effect on the in- vitro drug release. All other variables has not such effect on the in –vitro drug release. In-vitro release profile of microspheres formulation was compared in Figure 2.

Surface morphology by SEM: The surface of the microspheres was evaluated by SEM. It shows the microspheres were spherical and uneven surfaces. This tendency of the microspheres surface was most probably resulted from the mechanism of solvent evaporation. The image of the microspheres has shown in Figure 3 and 3a.

Drug and polymer interaction studies by FT-IR: The IR spectra of cefuroxime axetil pure drug containing bands in the region of 3469.2, 1677.68, 1390, 943, 1071.34 Cm^{-1}, due to the presence of NH-Amide, C=O, C-N, C-C and C-S linkage respectively. These bands also presented in the spectra of drug loaded ethylcellulose microspheres with very slight shift in the region which confirms there was no drug and polymer interaction. FT-IR spectra of cefuroxime axetil, ethylcellulose and drug loaded ethylcellulose microspheres are shown in Figure no.4, 5 and 6 respectively. Functional group region in the FT-IR spectra compared in Table 9.

CONCLUSION

In this research work attempt was made to increase the gastric residence of cefuroxime axetil by formulating as floating microspheres. Formulation was successfully made and in –vitro evaluation of floating microspheres shows encouraging results. By these evaluations following statement can be concluded (i) No interaction between the drug and polymer was confirmed. (ii) The desired yield and entrapment efficiency was obtained. (iii) It shows good buoyancy over 12 hrs in the acidic medium. (iv) It provides sustained release of drug over more than 12 hours. (v) Drug release from microspheres follows coupling mechanism of fickian diffusion and polymer matrix relaxation. (vi) The drug: polymer ratio has significant effect on the all characteristics of microspheres but other variables have effect on only few characteristics of the microspheres.

Table 1: Variables used in the microspheres formulations

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug: polymer ratio	1: 1.5	1: 1.5	1: 1.5	1: 1.5	1: 1.5	1: 1.5	1: 1.5	1: 1	1: 2
Organic phase volume (ml)	10	10	10	16	5	10	10	10	10
Aqueous phase volume (ml)	50	100	150	100	100	100	100	100	100
Stirring time (min)	30	30	30	30	30	60	90	30	30

Table 2: Effect of continuous phase volume on microspheres properties

Formulation code	Continuous phase volume (ml)	Yield (%)	Entrapment efficiency (%)	Particle size (µm)	Percentage of drug release	Buoyancy (%)
F1	50	77.5	68.2	70.26	78.99	67.9
F2	100	73.5	62.1	65.36	82.60	72.65
F3	150	71.75	60.7	58.52	84.65	75.0

Table 3: Effect of internal phase volume on microspheres properties

Formulation code	Internal phase volume	Yield (%)	Entrapment efficiency (%)	Particle size (µm)	Buoyancy (%)	Percentage of drug release
F4	5	67.0	75.7	77.36	69.0	76.60
F1	10	77.5	68.2	70.26	75.0	78.99
F5	15	78.0	60.9	69.18	62.0	81.39

Table 4: Effect of stirring time on microspheres properties

Formulation code	Stirring time (min)	Yield (%)	Entrapment efficiency (%)	Particle size (µm)	Buoyancy (%)	Percentage of drug release
F1	30	77.5	68.2	70.26	75.0	78.99
F6	60	71.75	64.43	72.56	70	82.64
F7	90	68.8	61.76	69.89	71	82.56

Table 5: Effect of drug: polymer ratio on microspheres properties

Formulation code	Drug: polymer ratio	Yield (%)	Entrapment efficiency (%)	Particle size (µm)	Buoyancy (%)	Percentage of drug release
F8	1:1	79.2	62.8	63.94	72.36	89.78
F1	1:1.5	77.5	68.2	70.26	75.0	78.99
F9	1:2	73.2	74.2	75.68	81.23	73.47

Table 6: Bulk densities of the microspheres formulations

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Bulk density (gm/ml)	0.30	0.47	0.39	0.52	0.49	0.32	0.33	0.31	0.26

Table 7: Regression co-efficient (r^2)

Kinetic models	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order	0.991	0.954	**0.995**	0.801	0801	0.757	0.776	0.798	0.861
First order	0.978	**0.988**	0.929	0.981	0.984	0.978	0.99	0.978	0.983
Hiquchi	0.941	0.979	0.927	**0.993**	**0.992**	**0.997**	**0.996**	**0.995**	0.984
Hixson-crowell	**0.992**	0.987	0.943	0.982	0.984	0.981	0.983	0.972	**0.985**

Table 8: n values from Korsemeyer Peppas model

Formulation code	n- values	Mechanism of release
F1	0.853	Case II or Zero order release, (non- fickian) it refers the dominant mechanism for drug transport is due to polymer matrix relaxation
F2	0.621	Anomalous transport (non- fickian), refers to the coupling of Fickian diffusion and polymer matrix relaxation.
F3	0.879	Super case II transport. It refers in addition to diffusion, other release mechanism including matrix erosion and polymer relaxation might be involved
F4	0.514	Anomalous transport (non- fickian), refers to the coupling of Fickian diffusion and polymer matrix relaxation
F5	0.556	
F6	0.498	
F7	0.537	
F8	0.574	
F9	0.534	

Table 9: Comparison of functional group region in the IR- spectrum

Functional group of drug	Corresponding region in the spectrum of pure drug (cm^{-1})	Corresponding region in the spectrum of drug and polymer mixer (cm^{-1})	Limit (cm^{-1})
C – S (stretch)	592.1	584.78	705 – 570
C = O (carbonyl)	1677.681	1677.66	1760 - 1670
C – N(stretch)	1071.34	1070.83	1090 - 1020
NH - amide	3469.20	3472.16	3500 - 3300
C - C	943.12	946.26	1300 – 700

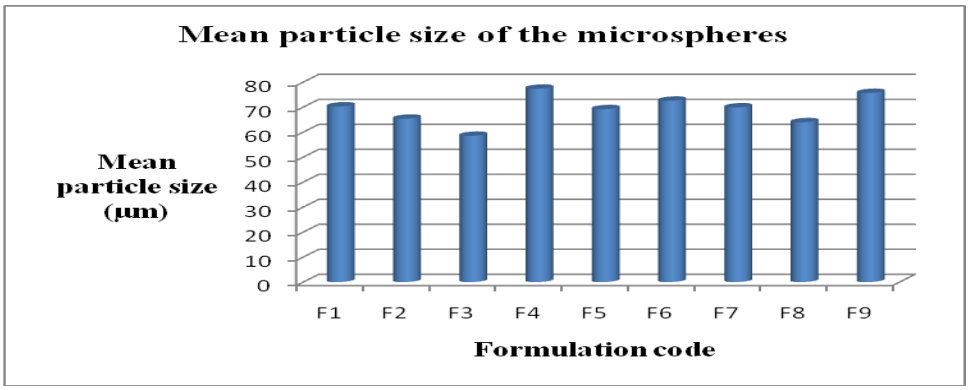

Figure 1: Mean particle size of microspheres formulations

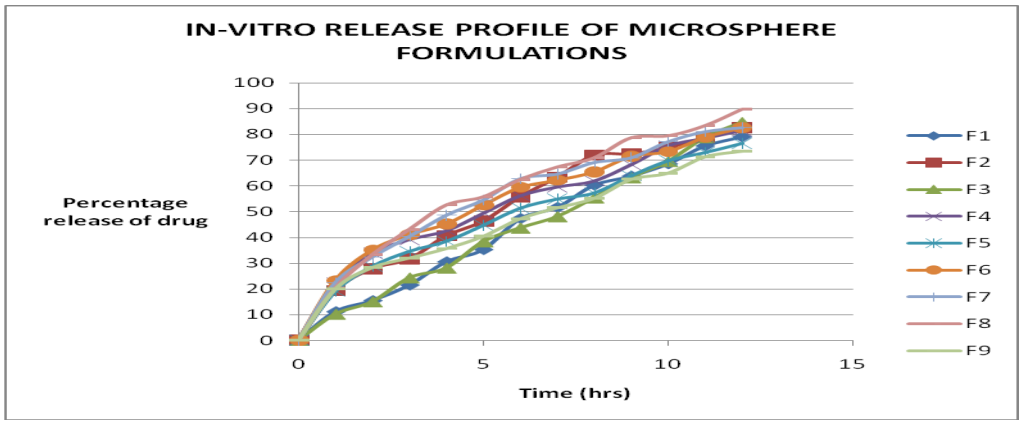

Figure 2: In- vitro release profile of microspheres formulations

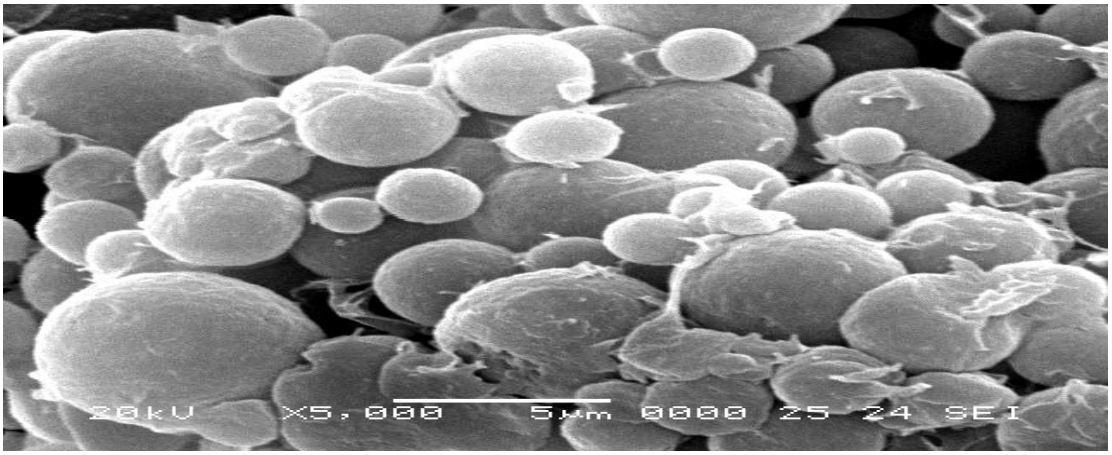

Figure No.3: SEM photograph of Formulation F9 at 5000 magnification level

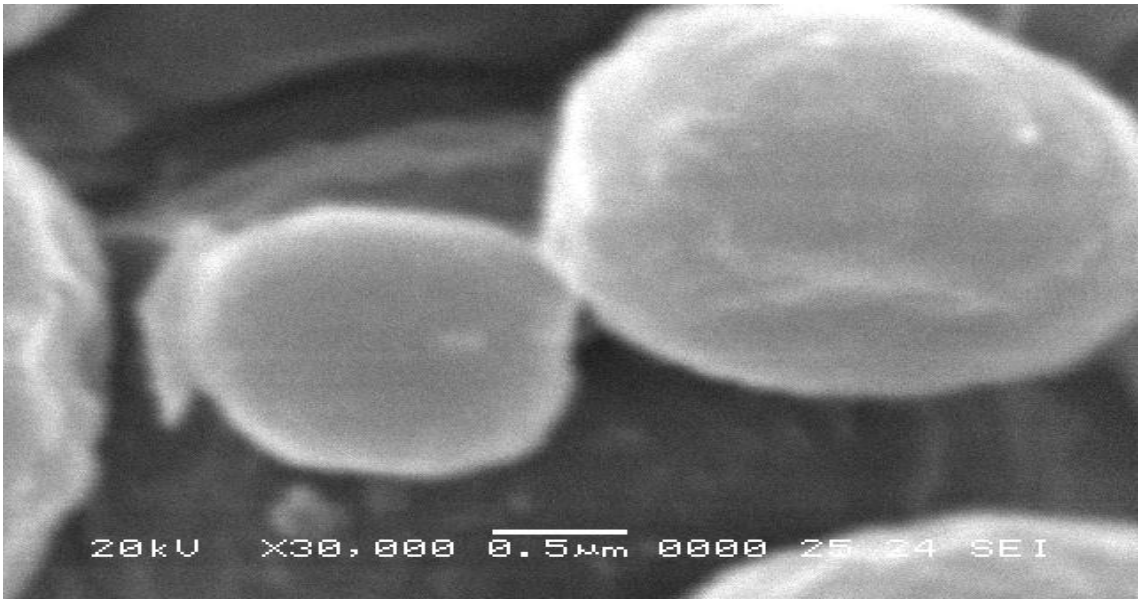

Figure 3(a): SEM photograph of Formulation F9 at 30,000 magnification level

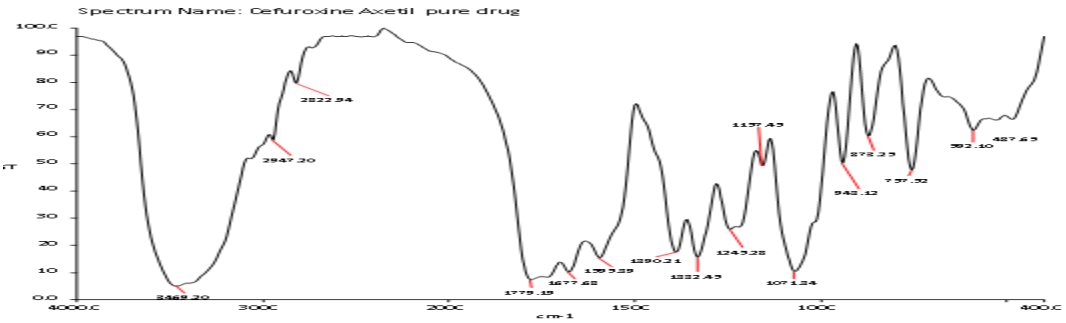

Figure 4: FT-IR spectra of drug cefuroxime axetil

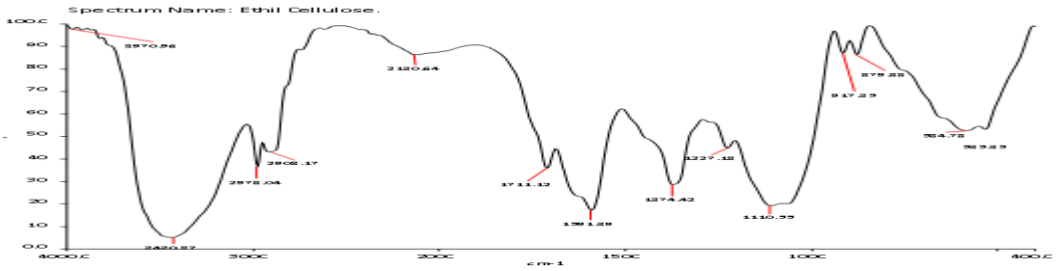

Fig 5: FT-IR spectra for Ethyl cellulose polymer

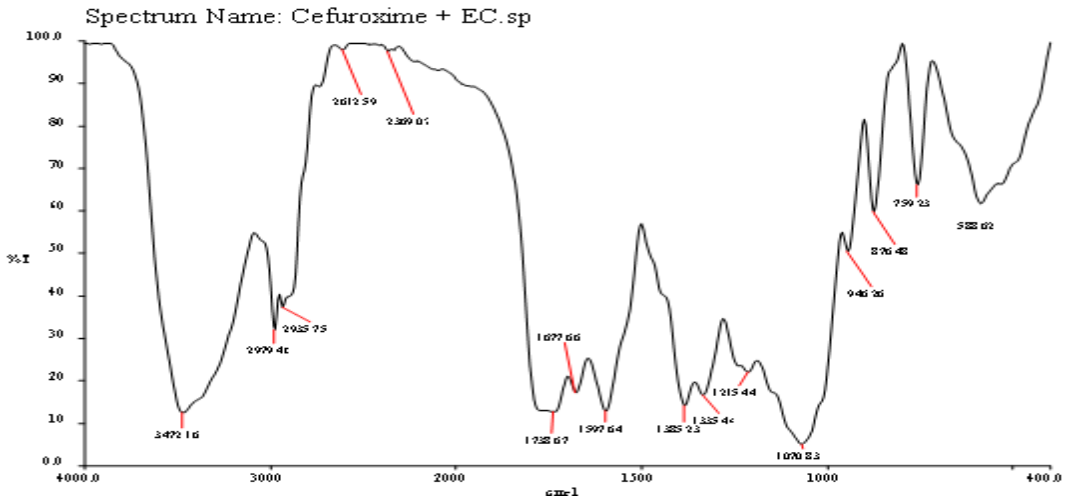

Fig 6: FT-IR spectra of Cefuroxime axetil loaded ethyl cellulose microspheres

REFERENCES

1. Alagusundaram M, Maadhusudan Chetty. Int J Chem Tech Res, 2009; 1(3): 526-34.
2. Vyas, RK Khar. Targeted and controlled drug delivery, 2007; Edition.418.
3. Whitehead L, J T Fell, J H Collett. Eur J Pharma Sci, 1996; 4 (1): 182.
4. Mojaverian P, Vlasses P H, Kellner P M. Pharm Res, 1988; 10: 639–44.
5. Deepa M K, Karthikeyan M. Iranian J Pharm Sci, 2009; 5(2): 69-72.
6. Amol V. Pande, et al. Int J Pharm Biomed Res, 2010; 1(4): 122-8.
7. Tatjana mateovic. Chem PharmBull, 2005; 53(1): 143-6.
8. Patrick B, Donnell. 1997; 28:25-42.
9. Amperiadou, et al. Int J Pharm, 1995; 115: 1-8.
10. Srivastava. Acta Pharm, 2005; 55: 277-85.