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Research Article

# TO DESIGN MICROSPHERES OF THE ANTIBACTERIAL DRUG CEFACLOR AND OPTIMIZE ITS CHARACTERISTICS

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**ABSTRACT**

**Aim:** This study aims to design microspheres of the antibacterial drug Cefaclor and optimize its characteristics to improve the bioavailability as well as to reduce its side effects. This utilizes statistical software for the optimization of microsphere formulations for the drug Cefaclor. **Method:** Thirteen formulation were prepared and being evaluated. Out of the thirteen formulations the formulation F2 was found to be optimum. Therefore, formulation F2 was selected as an optimized formulation and entrapment efficiency, drug release for this formulation was carried out, and the drug content was found to be optimum in accordance with the official monograph. **Result:** The evaluations parameter of the F2 formulation, i.e. entrapment efficiency, % drug release 83.5%, 71.1% respectively result concluded that all the parameter with in acceptant range. Release kinetic that in-vitro drug release curve fitted under Zero order release, first order release. Out of which the zero order model show R2 value 0.962-0.990 is highest as compared to another model. The drug release was mainly by zero order.

**Keywords:** Microsphere, Optimization, Cesfaclor, Stability study.

## INTRODUCTION

Microspheres are free-flowing solid particle made up of biodegradable and non-biodegradable material, ideally having a particle size in the micron range. Or they may be defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles." Microsphere eases sustained drug release and also reduces or eliminates gastrointestinal tract irritation and used to alter the drug release. Drug absorption and side effects due to irritating drugs against the gastrointestinal mucosa is improved because microsphere is made up of small particle size less than 200 μm, which are widely distributed throughout the gastrointestinal tract. Moreover, the microspheres are of micron size so they can easily fit into various capillary beds which are also having micron size. These offer various advantages which include.

Cefaclor is a second generation, semi-synthetic, broad spectrum, β -lactamase-stable antibiotic cephalosporin. Oral bioavailability of Cefaclor is 70-80%, and it has very short biological half-life 1-2 hours. Cefaclor has higher absorption in the proximal region of the GI tract and poor absorption in the lower part of GIT. When a large amount of drug entered the colon causes antibiotic-associated colitis. Because of this cefaclor is a suitable drug moiety for the gastro-retentive drug delivery system.Current research was planned to develop cefaclor loaded floating microspheres and evaluate the developed microspheres for in vitro and in vivo characteristics

## MATERIALS AND METHODS

**Table 1: Materials used for the formulation of the microsphere**

| S.No. | Item Name | Company Name |
|---|---|---|
| 1 | *Chemicals:* | |
| | Cefaclor pure drug | Ranbaxy PVT. LTD |
| | Ethyl Cellulose | Loba Chem. |
| | Liquid Paraffin | Loba Chem. |
| | Span 80 | Loba Chem. |
| 2 | *Solvents:* | |
| | N-hexane | Loba Chem. |
| | Dichloromethane (DCM) | Loba Chem. |
| | Acetonitrile | Loba Chem. |
| | Distilled Water | |

## FORMULATION AND OPTIMIZATION OF CEFACLOR LOADED MICROSPHERE

The experiments were based on a $3^3$ Box Behnken design consisting of three factors [Stirring speed, Polymer concentration (ethyl cellulose) and Temperature] at three levels (low (-), medium (0) and high (+)) as shown in table no 2 (a). The microspheres were fabricated using a solvent evaporation method. Ethyl cellulose was dissolved in a solvent mixture consisting of DCM: acetonitrile: water (3:3:3). A weighed quantity of drug Cefaclor was dissolved in ethyl cellulose blend. The primary emulsion obtained was then poured into 80 ml liquid paraffin-span 80 solution maintained at a variable temperature and stirred for twoh at variable rpm. Finally, the suspension was filtered and washed with n-hexane for the hardening of microsphere used as such for further analysis.

### Experimental design

From the preliminary trails in the present study, a $3^3$ Box Benkhen design was constructed. The design was employed to study the effect of independent variable i.e.stirring speed, polymer concentration and temperature on dependent variables entrapment efficiency and particle size and drug release. The effect of independent variables was studied at three different levels as shown in table no 2.

## EVALUATION

### Drug entrapment efficiency

For entrapment efficiency, accurately weighed amount of formulation taken along with 10 ml PBS 7.4 and kept for 24hr in a volumetric flask, filtered and analyzed by UV spectrophotometer at λmax. The drug concentration was determined from the regression equation.

### Particle size

Particle size analysis of microsphere was carried out by optical microscopy and scanning electron microscope. About 100 microspheres were selected randomly, and their size was determined using microscopically

**Table 2: Codes for Cefaclor microspheres formulation.**

| S.no | Formulation code | Stirring speed (rpm) | Polymer concentration (mg) | Temperature | Dichloro methane(ml) | Acetonitrile(ml) | Distilled water(ml) |
|---|---|---|---|---|---|---|---|
| 1 | F1 | 700.00 | 375.00 | 30.00 | 3 | 3 | 3 |
| 2 | F2 | 700.00 | 500.00 | 35.00 | 3 | 3 | 4 |
| 3 | F3 | 700.00 | 250.00 | 35.00 | 3 | 3 | 3 |
| 4 | F4 | 700.00 | 375.00 | 40.00 | 3 | 3 | 3 |
| 5 | F5 | 950.00 | 500.00 | 30.00 | 3 | 3 | 3 |
| 6 | F6 | 950.00 | 250.00 | 30.00 | 3 | 3 | 3 |
| 7 | F7 | 950.00 | 375.00 | 35.00 | 3 | 3 | 4 |
| 8 | F8 | 950.00 | 500.00 | 40.00 | 3 | 3 | 3 |
| 9 | F9 | 950.00 | 250.00 | 40.00 | 3 | 3 | 4 |
| 10 | F10 | 1200.00 | 375.00 | 30.00 | 3 | 3 | 3 |
| 11 | F11 | 1200.00 | 500.00 | 35.00 | 3 | 3 | 4 |
| 12 | F12 | 1200.00 | 250.00 | 35.00 | 3 | 3 | 3 |
| 13 | F13 | 1200.00 | 375.00 | 40.00 | 3 | 3 | 3 |

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**Morphological Evaluation**

The microsphere were further evaluated for shape, size, surface morphology and topological properties using scanning electron microscope (FEI Model no- NOVANANO-450) after gold sputtering at a pressure of 5.13E to 4 pascals and the 5KV voltage at $0^0$C were maintained to get the photographs.

Determination of Percentage yield of microspheres: The prepared microspheres were completely dried and then weighed. The percentage yield was calculated by [1-5]

$$\% \ Yield = \frac{hh100}{h}$$

Determination of flow properties of microspheres: The prepared microspheres were evaluated for flow properties including bulk density, tapped density, Carr's index, Hausner ratio and angle of repose. [6-9]

Bulk density: It is the ratio of the total mass of microspheres to the bulk volume of microspheres. It was measured by pouring the weighed microspheres into a measuring cylinder, and the volume was noted. It is expressed in gm/ml and is given by

**Bulk Density= Mass of microspheres/Bulk volume of the microsphere**

**Tapped density**

It is the ratio of the total mass of microspheres to the tapped volume of microspheres. The tapped volume was measured by tapping the microspheres to constant volume. It is expressed in gm/ml and is given by

**Tapped Density= Mass of microspheres/Trapped volume of the microsphere**

**Carr's Index**

It indicates the ease with which a material can be induced to flow. It is expressed in percentage and is given by

**Carr's Index= Tapped density- Bulk Density x100/ Tapped density**

**Hausner Ratio**

It is an indirect index of ease of flow of microspheres. It is measured by

**Hausner ratio= Tapped density/bulk density**

**The angle of Repose (θ)**

The friction forces in a loose powder can be measured by the angle of repose (θ). It is defined as maximum angle possible between the surface of the pile of powder and the horizontal plane.

The microspheres were allowed to flow through a funnel fixed to a stand at a definite height. The angle of repose was then calculated by measuring the height and radius of the heap of microspheres formed. It is measured by

**$\theta = \tan^{-1}$Height/Radius**

**Percentage Drug Entrapment efficiency**

To calculate the % drug entrapment efficiency accurately weighed quantity of microspheres (50 mg) were taken along with 50 ml of phosphate buffer pH 7.4 in a volumetric flask, sonicated and kept for 24 hours. It was then filtered, suitably diluted and then analyzed by UV spectrophotometry at 216 nm.

**% Entrapment Efficiency = Theoretical Entrapment x 100 / Practical Entrapment**

**In vitro release studies of microspheres**

In-vitro release of microspheres was carried out using the diffusion apparatus at 37 ± $0.5^0$C in 150 ml of phosphate buffer pH 7.4. 50 mg formulated microspheres were placed in the apparatus and rpm was set at 100 rpm. A sample of 5 ml was withdrawn at various time intervals and replaced with an equal amount of medium to maintain the sink condition. The withdrawn samples were analyzed by UV spectrophotometer at 264 nm using phosphate buffer 7.4 as a blank solution [10-15].

**Effect of different formulation variables on various evaluation parameters**

The influences of different formulation variables on various evaluation parameters were studied. The effects of polymer concentration (Ethyl cellulose 250-500 mg), temperature and altered stirring speed of mechanical stirrer (700, 900, 1200 rpm) on microspheres characteristics (percentage yield, drug entrapment efficiency, particle size and cumulative drug release) were studied.

Antibacterial Study: Firstly, the agar plate was prepared taking nutrient agar as the source in Petri dish. The pure culture was then added to it and incubated for three days at a temperature of 37±0.5°C. Well was constructed and the formulations in the form of suspensions were added to it, and again allowed for incubation at the optimum requirements of temperature and pressure. The selected micro-organisms were E. coli and S. aureus. The zone of inhabitation was measured after the incubation period.

**Physical Stability of microsphere Samples**

The physical stability parameters like appearance, color of resulting microsphere were studied under different storage condition. The physical stability includes appearances, the color of resulting microsphere under different storage conditions. The sample shows a change in certain parameter after the storage condition of one month under different storage condition.

**Release Kinetic Models**

To analyse the mechanism for the drug release and drug release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix, Krosmeyers-Peppas and Hixson Crowell model. In this by comparing the R-values obtained, the best-fit model was selected.

## RESULTS AND DISCUSSION

### Preformulation Studies

Determination of λ max by UV spectroscopy

λ max of Cefaclor was found to be 264 nm when scanned between 400-200 nm using UV-Vis spectrophotometer.

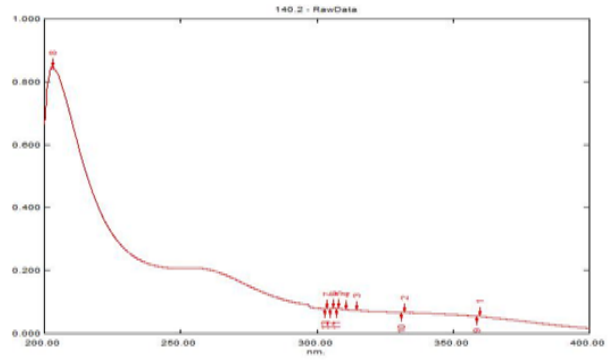


**Fig.1: Absorption maxima of the drug Cefaclor**

### HPLC

The HPLC of Cefaclor was performed with a suitable mobile phase consisting of potassium dihydrogen phosphate (buffer): Methanol (solvent) in a ratio of 80:20 and adjusted to pH 2.30 suitably. The flow rate was settled at 1.00 ml/min and the internal standard used was caffeine. The chromatogram was obtained with the retention time of 7.59 min of cefaclor.

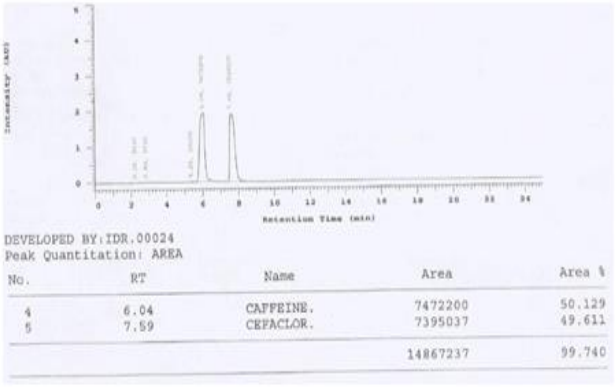


**Fig.2: Chromatogram of the drug with standard internal caffeine**

### Calibration Curves in different solvents

#### a. Calibration curve in 0.1N HCl

The absorbance of standard solutions was measured at 264 nm

**Table 3: Calibration curve of Cefaclor in 0.1N HCl at 264 nm.**

| S.No. | Concentration(µg/ml) | Absorbance(nm) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 5 | 0.125 |
| 3 | 10 | 0.213 |
| 4 | 15 | 0.312 |
| 5 | 20 | 0.414 |
| 6 | 25 | 0.515 |
| 7 | 30 | 0.621 |
| 8 | 35 | 0.754 |
| 9 | 40 | 0.914 |

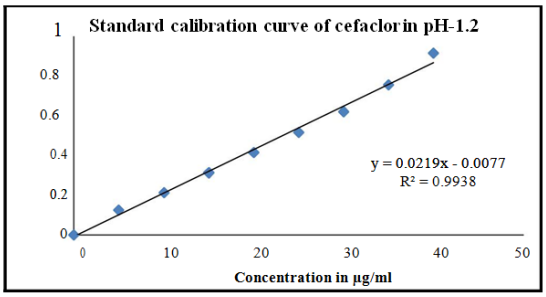


**Fig. 3: Calibration curve of Cefaclor in 0.1N HCl at λ max 264nm**

#### b. Calibration curve in Phosphate buffer 7.4

The absorbance of standard solutions was measured at λ max of 264nm.

**Table 4: Calibration curve of Cefaclor in Phosphate buffer 7.4 at λ max of 264nm**

| S.No. | Concentration(µg/ml) | Absorbance(nm) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 5 | 0.178 |
| 3 | 10 | 0.262 |
| 4 | 15 | 0.371 |
| 5 | 20 | 0.48 |
| 6 | 25 | 0.597 |
| 7 | 30 | 0.732 |
| 8 | 35 | 0.818 |
| 9 | 40 | 0.966 |

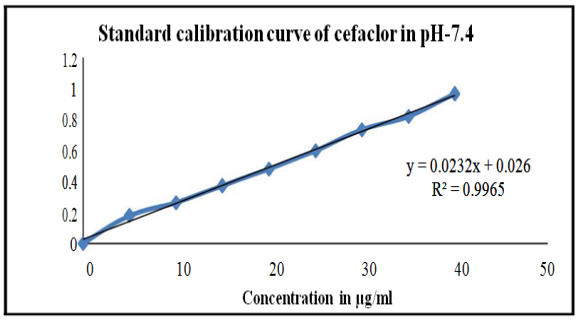


**Fig. 4: Calibration curve of Cefaclor Phosphate buffer 7.4 at λ max 264nm**

Calibration of Cefaclor was prepared in phosphate buffer by making suitable dilutions and was found to be linear. Thus it follows Lambert-Beers law and the R2 value was found to be 0.994.

#### c. Calibration curve in Phosphate buffer 4

The absorbance of standard solutions was measured at λ max of 264nm.

**Table 5: Calibration curve of cefaclor in phosphate buffer four at λ max of 264nm**

| S.No. | Concentration(µg/ml) | Absorbance(nm) |
|---|---|---|
| 1. | 0 | 0 |
| 2. | 5 | 0.124 |
| 3. | 10 | 0.226 |
| 4. | 15 | 0.341 |
| 5. | 20 | 0.43 |
| 6. | 25 | 0.559 |
| 7. | 30 | 0.662 |
| 8. | 35 | 0.75 |
| 9. | 40 | 0.904 |

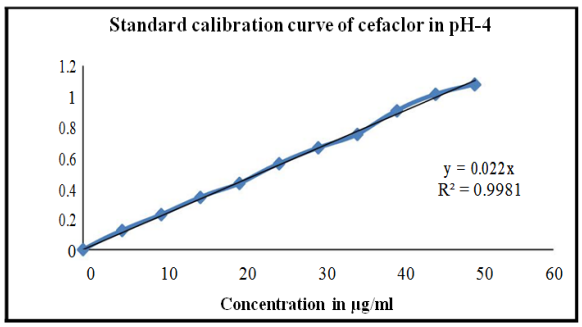


**Fig. 5: Calibration curve of Cefaclor in phosphate buffer four at λ max 264nm**

Calibration of Cefaclor was prepared in Phosphate buffer 4 by making suitable dilutions and was found to be linear. Thus it follows Lambert-Beers law and the $R^2$ value was found to be 0.998.

**Melting point determination**

The Melting point of Cefaclor was determined using the open capillary method and was found to be 327.3 ± 0.67 $^0$C

**Table 6: Melting point determination**

| S. No. | Melting Point | Average* |
|---|---|---|
| 1 | 328 | |
| 2 | 327 | 327.3±0.67 ºC |
| 3 | 327 | |

Melting point in ºC, Values are expressed in Mean ± SD, n=3

**Solubility determination**

**Table 7: Solubility of Cefaclor in various solvents**

| S.No. | Components | Solubility(mg/ml) |
|---|---|---|
| 1. | Distilled Water | Freely soluble |
| 2. | Phosphate buffer pH-4 | Soluble |
| 3. | Phosphate buffer pH-1.2 | Soluble |
| 4. | Phosphate buffer pH-7.4 | Freely Soluble |

The order of solubility of Cefaclor the above 4 solvents were found to be Distilled water> Phosphate buffer pH-7.4> Phosphate buffer pH-4> Phosphate buffer pH-1.2.

**Fourier Transform Infrared Studies (FTIR)**

FTIR spectrum of pure drugs and combination with the excipients were shown in Fig.s. There is no considerable change in drug characterization peaks and the results obtained with drug – excipients showed good compatibility.

Drug-Polymer Compatibility of drug polymer concentration (Drug: Ethyl cellulose) and their interpretations.

a. IR spectra of pure drug

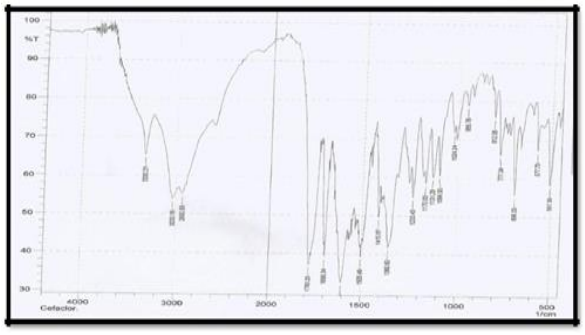

**Fig. 6: IR spectra of pure drug**

b. IR spectra of drug: Polymer (100:250) physical blend

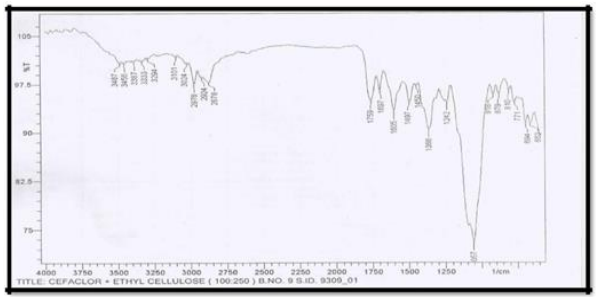

**Fig. 7: IR spectra of drug:polymer (100:250) physical blend**

c. IR spectra of drug:polymer (100:500) physical blend

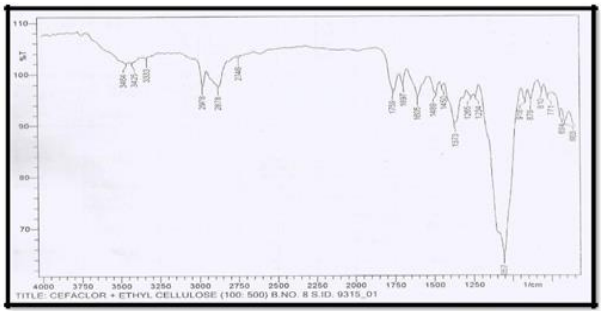

**Fig. 8: IR spectra of drug: polymer (100:500) physical blend**

**Determination of percentage yield of microspheres**

**Table 8: Percentage yield (%) of microspheres**

| S.no | Formulation code | % Yield |
|---|---|---|
| 1 | F1 | 74.10% |
| 2 | F2 | 88.13% |
| 3 | F3 | 61.40% |
| 4 | F4 | 57.60% |
| 5 | F5 | 86.60% |
| 6 | F6 | 96.60% |
| 7 | F7 | 79.20% |
| 8 | F8 | 82.40% |
| 9 | F9 | 72.40% |
| 10 | F10 | 87.40% |
| 11 | F11 | 85.30% |
| 12 | F12 | 68.40% |
| 13 | F13 | 66.70% |

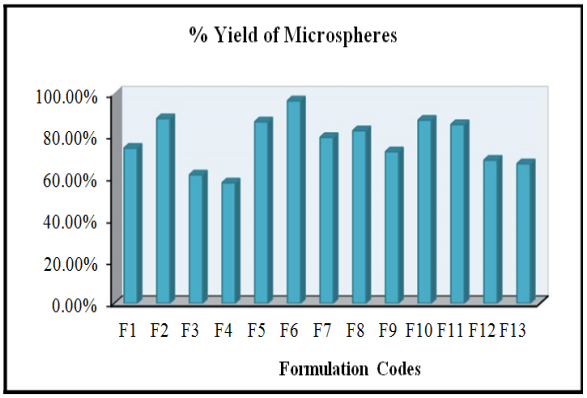


**Fig. 9: Percentage yield (%) of microspheres**

**The percentage yields for all formulations were determined. The values varied from 57.60% to 96.60%**

**Determination of flow properties of microspheres**

**Table 9: Flow properties of cefaclor microspheres**

| Formulation Codes | Bulk Density (gm/cm$^3$) | Tapped Density (gm/cm$^3$) | Carr's Index | Hausner Ratio | Angle of Repose (θ ) |
|---|---|---|---|---|---|
| F1 | 0.66 | 0.76 | 13.15 | 1.15 | 20.21±0.56 |
| F2 | 0.67 | 0.78 | 14.10 | 1.16 | 22.47±0.12 |
| F3 | 0.66 | 0.77 | 14.28 | 1.16 | 19.63±0.23 |
| F4 | 0.64 | 0.73 | 12.32 | 1.14 | 21.06±0.12 |
| F5 | 0.65 | 0.73 | 10.95 | 1.07 | 20.13±0.67 |
| F6 | 0.67 | 0.76 | 11.84 | 1.13 | 16.69±0.11 |
| F7 | 0.68 | 0.79 | 13.92 | 1.13 | 20.51±0.09 |
| F8 | 0.66 | 0.75 | 12.00 | 1.15 | 21.13±0.84 |
| F9 | 0.66 | 0.76 | 13.15 | 1.15 | 18.30±0.42 |
| F10 | 0.66 | 0.76 | 13.15 | 1.15 | 17.26±0.28 |
| F11 | 0.65 | 0.75 | 13.33 | 1.15 | 18.14±0.34 |
| F12 | 0.67 | 0.77 | 12.98 | 1.14 | 16.49±0.63 |
| F13 | 0.64 | 0.75 | 14.66 | 1.17 | 18.33±0.47 |

**Table 10: % Entrapment efficiency of microspheres**

| S.no. | Formulation code | %Entrapment efficiency |
|---|---|---|
| 1 | F1 | 67.1 |
| 2 | F2 | 83.5 |
| 3 | F3 | 68.2 |
| 4 | F4 | 62.3 |
| 5 | F5 | 58.4 |
| 6 | F6 | 61.2 |
| 7 | F7 | 58.3 |
| 8 | F8 | 52.4 |
| 9 | F9 | 65.2 |
| 10 | F10 | 63.1 |
| 11 | F11 | 74.5 |
| 12 | F12 | 47.2 |
| 13 | F13 | 58.4 |

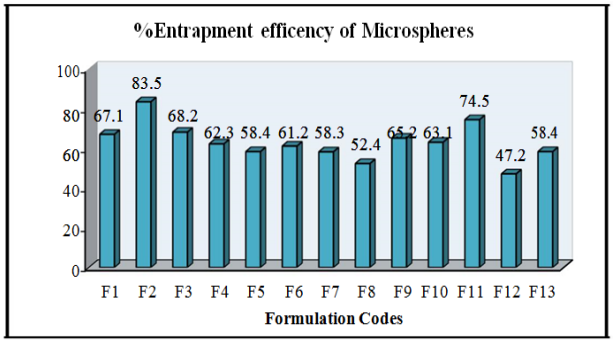


**Fig.10: Percentage entrapment efficiency of microspheres**

The EE was calculated and % EE of each formulation was shown in the table no 11 F2 formulation has the highest EE of 83.5%.

**Particle size analysis**

**Table 11:Size distributions of microspheres for different formulations**

| FC | 0-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-40 | 41-50 |
|---|---|---|---|---|---|---|---|---|
| F1 | 1 | 28 | 15 | 6 | 11 | 21 | 15 | 3 |
| F2 | 6 | 18 | 25 | 19 | 8 | 7 | 10 | 5 |
| F3 | 2 | 15 | 21 | 18 | 12 | 8 | 7 | 6 |
| F4 | 0 | 8 | 26 | 19 | 10 | 14 | 12 | 9 |
| F5 | 0 | 7 | 30 | 21 | 22 | 16 | 4 | 0 |
| F6 | 0 | 18 | 36 | 15 | 13 | 17 | 1 | 0 |
| F7 | 0 | 9 | 21 | 21 | 18 | 22 | 5 | 3 |
| F8 | 0 | 11 | 20 | 15 | 25 | 16 | 12 | 1 |
| F9 | 6 | 22 | 31 | 24 | 8 | 7 | 1 | 0 |
| F10 | 0 | 6 | 29 | 15 | 17 | 16 | 6 | 8 |
| F11 | 0 | 5 | 22 | 24 | 18 | 18 | 8 | 5 |
| F12 | 0 | 19 | 26 | 17 | 7 | 15 | 16 | 0 |
| F13 | 0 | 3 | 15 | 11 | 21 | 27 | 10 | 8 |

**Zeta sizer**

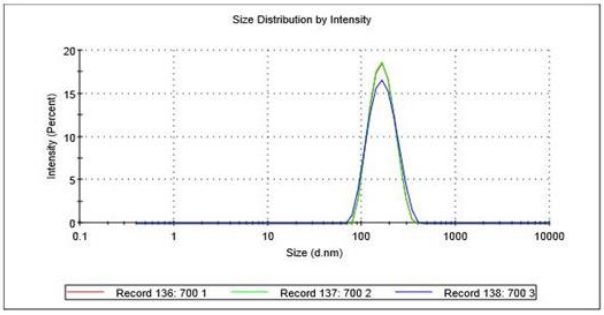


**Fig. 11: Size distribution analysis using zeta sizer**

**Surface morphology**

The morphology of the formulations prepared by emulsification solvent evaporation method was investigated by SEM. It was observed by SEM analysis that the formulations are uniformly spherical. The spongy and porous nature of the formulations can be seen in the Fig..

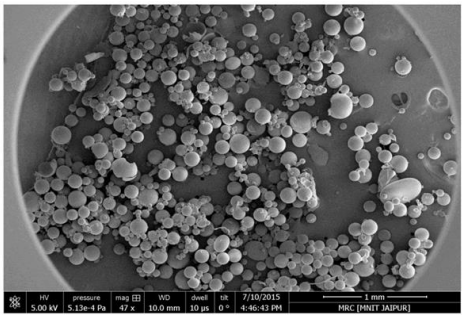

**Fig. 12: Scanning electron microscopy (SEM) of formulations**

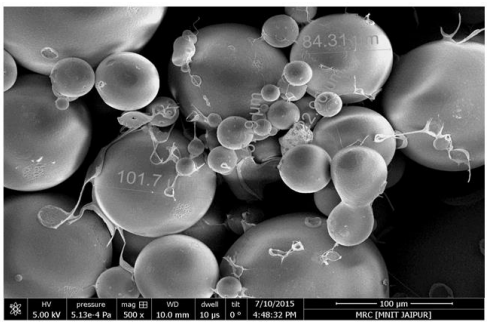

**Fig. 13: Scanning electron microscopy (SEM) of formulations**

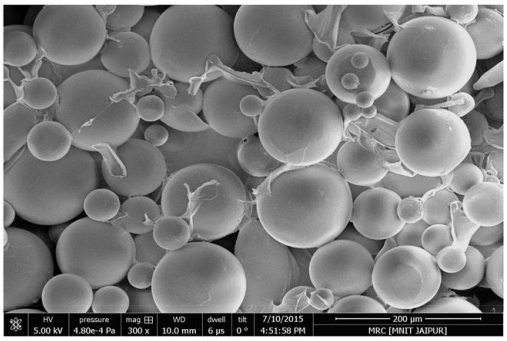

**Fig. 14: scanning electron microscopy (SEM) of formulations**

**Effect of different formulation variables on various evaluation parameters**

**a. Effect of polymer on different factors**

**Table 12: Effect of polymer on different factors**

| FC | Polymer Concentration (mg) | Entrapment Efficiency (%) | Drug Release (%) | Particle size(mm) |
|---|---|---|---|---|
| F1 | 375 | 67.1 | 60.2 | 8 |
| F2 | 500 | 83.5 | 71.1 | 8 |
| F3 | 250 | 68.2 | 70.3 | 23 |
| F4 | 375 | 62.3 | 56.2 | 28 |
| F5 | 500 | 58.4 | 42.2 | 8 |
| F6 | 250 | 61.2 | 54.3 | 18 |
| F7 | 375 | 58.3 | 62.7 | 23 |
| F8 | 500 | 52.4 | 48.2 | 28 |
| F9 | 250 | 65.2 | 53.6 | 13 |
| F10 | 375 | 63.1 | 64.4 | 13 |
| F11 | 500 | 74.5 | 67.5 | 28 |
| F12 | 250 | 47.2 | 56.1 | 8 |
| F13 | 375 | 58.4 | 62.5 | 23 |

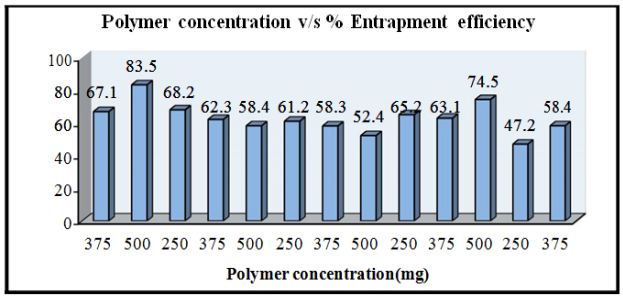


**Fig.15: Effect of polymer concentration on % entrapment efficiency**

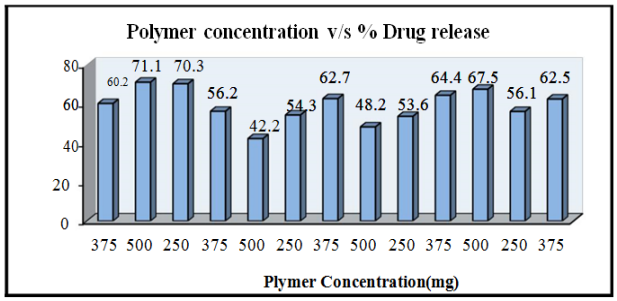


**Fig.16: Effect of polymer concentration on % drug release**

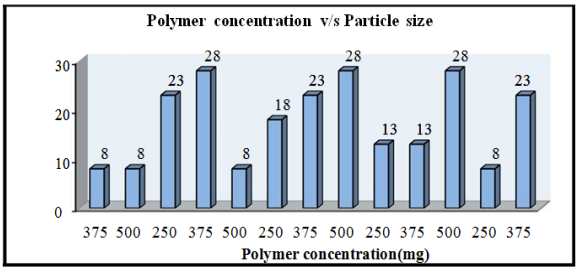


**Fig. 17: Effect of polymer concentration on particle size**

The effect of on drug release, entrapment efficiency, particle size varies with varying in polymer concentration.

**b. Effect of stirring speed on different factors**

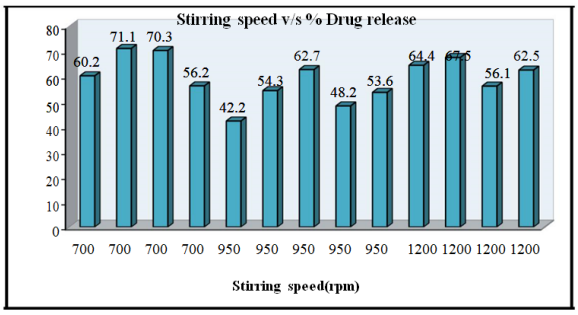


**Fig. 18: Effect of stirring speed on % drug release**

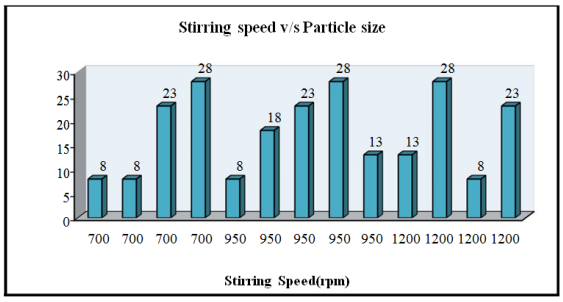


**Fig. 19: Effect of stirring speed on particle size**

**The effect of on drug release, entrapment efficiency, particle size varies with varying in Stirring speed.**

**C. Effect of temperature on different factors**

**Table13: Effect of temperature on various parameters**

| FC | Temperature(°C) | %Entrapment efficiency | %Drug release | Particle size(mm) |
|---|---|---|---|---|
| F1 | 30 | 67.1 | 60.2 | 8 |
| F2 | 35 | 83.5 | 71.1 | 8 |
| F3 | 35 | 68.2 | 70.3 | 23 |
| F4 | 40 | 62.3 | 56.2 | 28 |
| F5 | 30 | 58.4 | 42.2 | 8 |
| F6 | 30 | 61.2 | 54.3 | 18 |
| F7 | 35 | 58.3 | 62.7 | 23 |
| F8 | 40 | 52.4 | 48.2 | 28 |
| F9 | 40 | 65.2 | 53.6 | 13 |
| F10 | 30 | 63.1 | 64.4 | 13 |
| F11 | 35 | 74.5 | 67.5 | 28 |
| F12 | 35 | 47.2 | 56.1 | 8 |
| F13 | 40 | 58.4 | 62.5 | 23 |

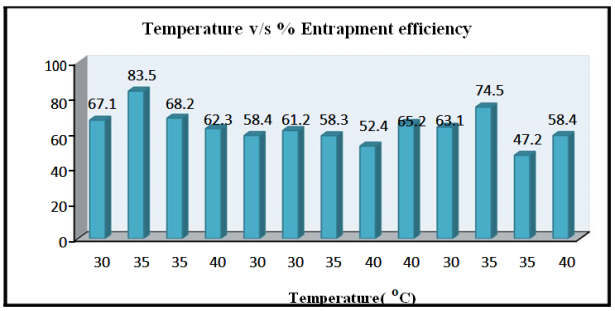


**Fig.20: Effect of temperature on %entrapment efficiency**

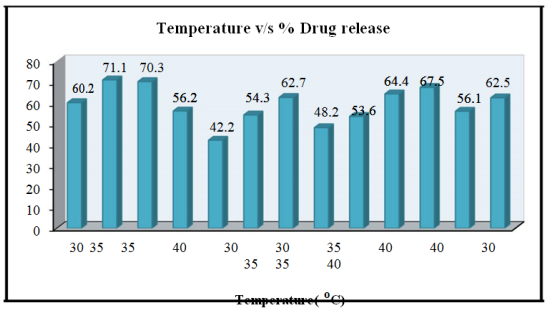


**Fig. 21: Effect of temperature on % drug release**

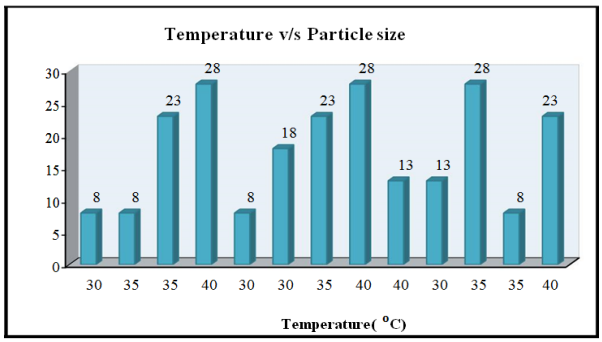


**Fig. 22: Effect of temperature on particle size**

The effect of on drug release, entrapment efficiency, particle size varies with varying in temperature.

*In Vitro* Dissolution Studies of Cefaclor from microspheres

**Table 14: % Cumulative drug release of F1, F2, F3, F4**

| S.No. | Time(min.) | %CDR | | | |
|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 10 | 4.23 | 26.4 | 6.42 | 4.23 |
| 3 | 20 | 10.5 | 34.8 | 11.3 | 10.2 |
| 4 | 30 | 16.9 | 44.5 | 15.6 | 12.03 |
| 5 | 60 | 23.9 | 49.8 | 18.5 | 13.55 |
| 6 | 90 | 38.4 | 51.4 | 20.4 | 18 |
| 7 | 120 | 40.1 | 53.7 | 25.4 | 20.33 |
| 8 | 150 | 44.2 | 57.6 | 28.6 | 21.6 |
| 9 | 180 | 47.6 | 59.4 | 32.4 | 28.6 |
| 10 | 210 | 49.8 | 61.2 | 36.8 | 34.7 |
| 11 | 240 | 52.4 | 62.3 | 39.4 | 37.1 |
| 12 | 270 | 56.5 | 65.4 | 46.5 | 41.2 |
| 13 | 300 | 58.2 | 71.5 | 49.2 | 46.5 |
| 14 | 330 | 59.4 | 72.1 | 52.1 | 48.7 |
| 15 | 360 | 61.2 | 74.3 | 54.3 | 54.2 |
| 16 | 390 | 63.7 | 76.4 | 57.6 | 56.3 |
| 17 | 420 | 64.8 | 81.6 | 61.5 | 59.4 |
| 18 | 450 | 65.2 | 82.3 | 66.4 | 61.4 |
| 19 | 480 | 67.1 | 83.5 | 68.2 | 62.3 |

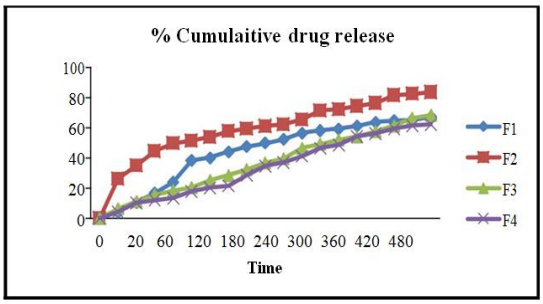


**Fig. 23: % Cumulative drug release of F1, F2, F3, F4**

**Table 15: Percent Cumulative drug release of F5, F6, F7, F8**

| S. No. | Time(min.) | %CDR | | | |
|---|---|---|---|---|---|
| | | F5 | F6 | F7 | F8 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 10 | 3.2 | 5.3 | 4.1 | 3.4 |
| 3 | 20 | 7.8 | 8.4 | 6.8 | 7 |
| 4 | 30 | 15.23 | 11.4 | 9.4 | 11.9 |
| 5 | 60 | 18.4 | 13.5 | 12.4 | 13.4 |
| 6 | 90 | 22.3 | 17.6 | 14.9 | 15.3 |
| 7 | 120 | 25.6 | 21.9 | 17.4 | 18.2 |
| 8 | 150 | 28.4 | 26.4 | 23.4 | 21.4 |
| 9 | 180 | 31.6 | 29.7 | 26.9 | 23.3 |
| 10 | 210 | 35.1 | 32.1 | 29.4 | 25.1 |
| 11 | 240 | 36.8 | 36.4 | 32.5 | 28.9 |
| 12 | 270 | 39.4 | 40.1 | 34.5 | 30.4 |
| 13 | 300 | 43.1 | 47.1 | 38.6 | 33.8 |
| 14 | 330 | 46.4 | 49.3 | 42.1 | 37.1 |
| 15 | 360 | 52.4 | 55.9 | 45.6 | 41.3 |
| 16 | 390 | 53.7 | 58.4 | 49.7 | 43.7 |
| 17 | 420 | 55.9 | 60.7 | 53.1 | 47.8 |
| 18 | 450 | 56.7 | 61 | 56.4 | 50.9 |
| 19 | 480 | 58.4 | 61.2 | 58.3 | 52.4 |

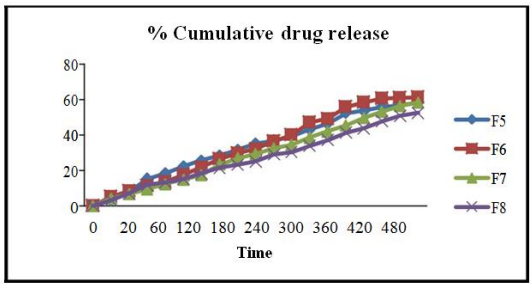


**Fig. 24: % Cumulative drug release of F5, F6, F7, F8**

**Table 16: % Cumulative drug release of F9,F10,F11,F12,F13**

| S.No. | Time(min.) | | | %CDR | |
|---|---|---|---|---|---|
| | | F9 | F10 | F11 | F12 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 10 | 5.9 | 5.1 | 8.6 | 2.9 |
| 3 | 20 | 8.2 | 7.9 | 11.2 | 4.8 |
| 4 | 30 | 12.6 | 11.2 | 14.6 | 5.4 |
| 5 | 60 | 15.4 | 13.4 | 18.7 | 7.1 |
| 6 | 90 | 18 | 17.9 | 20.9 | 8.7 |
| 7 | 120 | 21.3 | 20.1 | 22.3 | 12.5 |
| 8 | 150 | 25.4 | 25.6 | 24.1 | 18.4 |
| 9 | 180 | 29.4 | 28.6 | 27.4 | 22.4 |
| 10 | 210 | 31.4 | 32.7 | 29.4 | 26.7 |
| 11 | 240 | 34.9 | 33.1 | 32.5 | 28.4 |
| 12 | 270 | 38.2 | 38 | 39.4 | 30.1 |
| 13 | 300 | 41.3 | 40.6 | 44.1 | 32.4 |
| 14 | 330 | 47.8 | 45.3 | 49.4 | 33.8 |
| 15 | 360 | 51.9 | 49.7 | 56.7 | 36.2 |
| 16 | 390 | 55 | 53.9 | 62.1 | 39.7 |
| 17 | 420 | 58.1 | 57.1 | 68.4 | 40.1 |
| 18 | 450 | 62.4 | 61.4 | 72.2 | 42.4 |
| 19 | 480 | 65.2 | 63.1 | 74.5 | 47.2 |

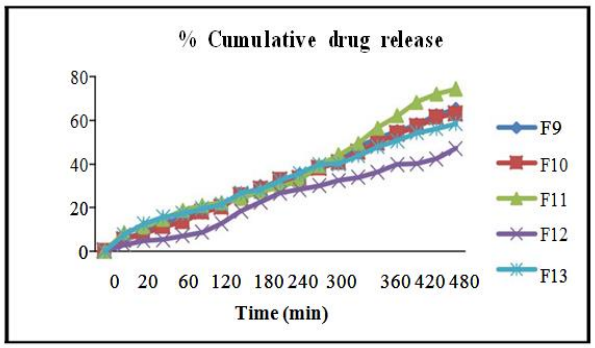


**Fig. 25: % Cumulative drug release of F9,F10,F11,F12,F13**

**Kinetic modeling of drug release**

**Table 17: Kinetics of drug release**

| Formulation Code | Zero Order | | First Order | | Higuchi | |
|---|---|---|---|---|---|---|
| | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope |
| F1 | 0.902 | 3.696 | 0.966 | -0.027 | 0.936 | 26.08 |
| F2 | 0.864 | 3.499 | 0.969 | -0.037 | 0.953 | 26.96 |
| F3 | 0.996 | 3.714 | 0.982 | -0.026 | 0.971 | 27.77 |
| F4 | 0.991 | 3.649 | 0.980 | -0.028 | 0.967 | 26.07 |
| F5 | 0.984 | 3.275 | 0.980 | -0.021 | 0.914 | 23.41 |
| F6 | 0.990 | 3.671 | 0.979 | -0.017 | 0.949 | 26.40 |
| F7 | 0.997 | 3.289 | 0.970 | -0.024 | 0.942 | 30.73 |
| F8 | 0.995 | 2.851 | 0.973 | -0.017 | 0.959 | 29.42 |
| F9 | 0.997 | 3.570 | 0.970 | -0.024 | 0.971 | 29.22 |
| F10 | 0.997 | 3.510 | 0.973 | -0.023 | 0.954 | 29.25 |
| F11 | 0.962 | 3.657 | 0.902 | -0.030 | 0.952 | 24.37 |
| F12 | 0.969 | 2.397 | 0.984 | -0.015 | 0.960 | 29.90 |
| F13 | 0.992 | 3.175 | 0.987 | -0.020 | 0.988 | 30.94 |

**a. Hixson Crowell plots**

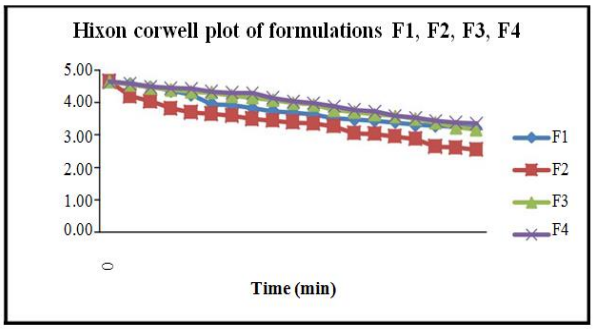


**Fig. 26: Hixson Crowell plots of F1, F2, F3, F4**

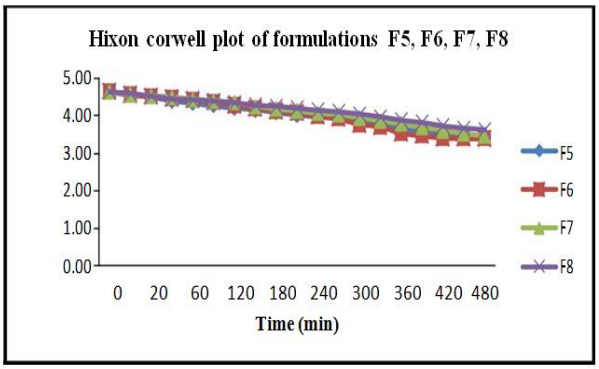


**Fig. 27: Hixson Crowell plots of F5, F6, F7, F8**

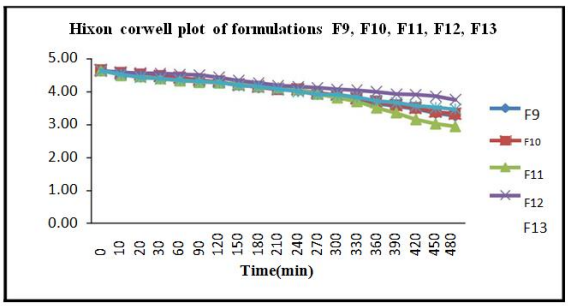


**Fig. 28: Hixson Crowell plots of F9, F10, F11, F12, F13**

Mixed kinetics of drug release was obtained when mathematical models were applied to the data obtained from the in-vitro dissolution of the three formulations exhibited zero-order kinetics(F1, F2, F3 ) while other formulations exhibited first order release kinetics. This may be due to the increase in the size of the microsphere produced and leading to zero order kinetics and excessive compact nature of the formulation regarding the release. The rest of the formulation exhibited first order release as expected because of the water-soluble nature of the drug.

b. Higuchi Plots of formulations

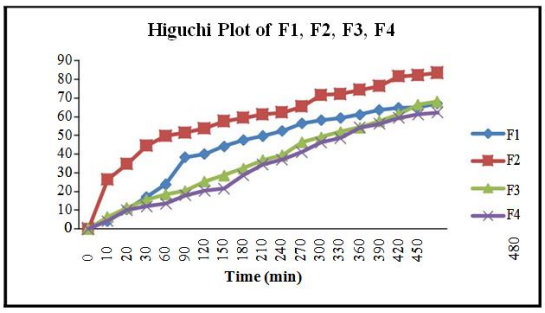


**Fig. 29: Higuchi plot of F1, F2, F3, F4**

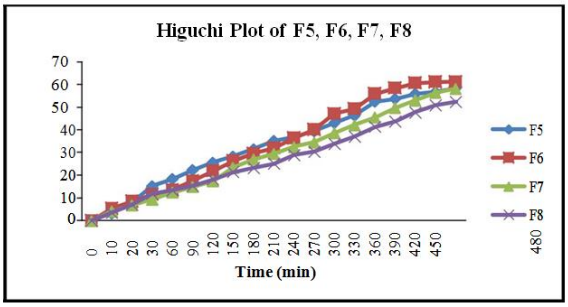


**Fig. 30: Higuchi plot of F5, F6, F7, F8**

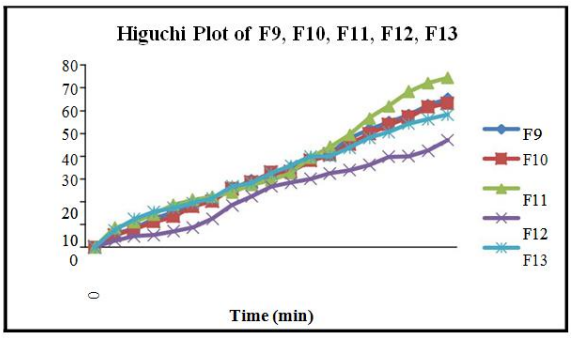


**Fig. 31: Higuchi plot of F9, F10, F11, F12, F13**

c. First order kinetics Plots

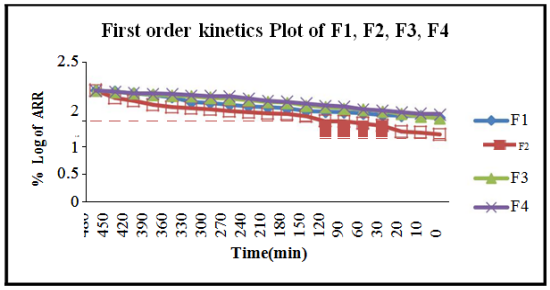


**Fig. 32: First order kinetics Plots of F1, F2, F3, F4**

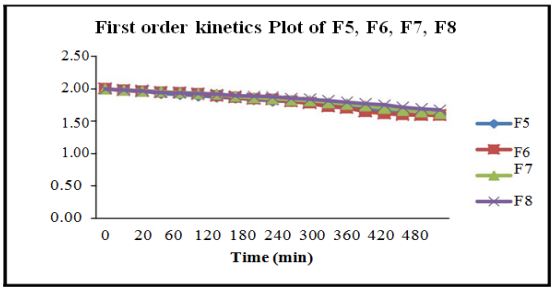


**Fig. 33: First order kinetics plots of F5, F6, F7, F8**

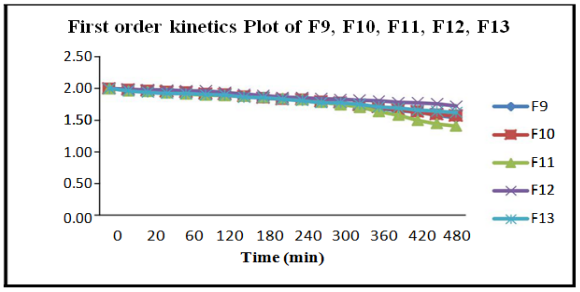


**Fig. 34: First order kinetics plots of F9, F10, F11, F12, F13**

d. Korsmeyer plots

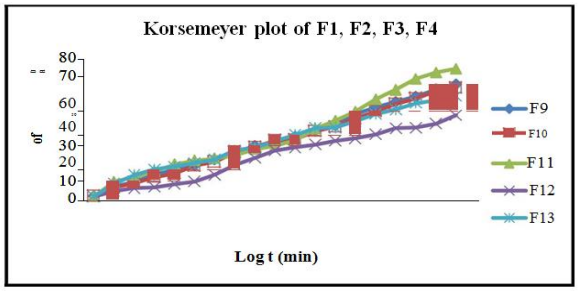


**Fig. 35: Korsmeyer plot of F1, F2, F3, F4**

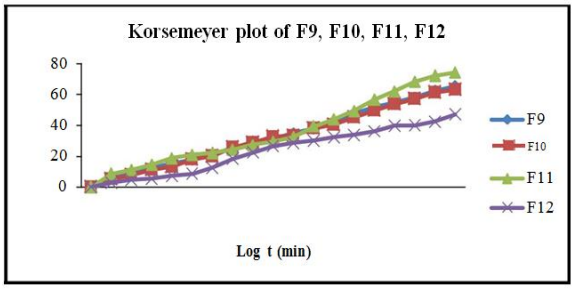


**Fig. 36: Korsmeyer plot of F5,F6, F7, F8**

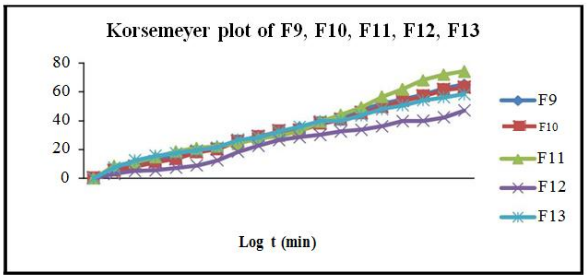


**Fig. 37: Korsmeyer plot of F9, F10, F11, F12, F13**

Ethyl cellulose was used as the polymer for the formulation of microsphere for the drug of Cefaclor which is semipermeable in nature, and this may have resulted in diffusion controlled release of drug from the microsphere as evident from the R2 values of higuchi kinetics which are in the range 0.914 to 0.988

Optimization of Formulations

The Box- Behnken method is used to optimize the main effect, interaction effect and quadratic effect.

To study the effects, i.e.- about the combination of factors which should be included in the analysis, all the effects are selected. The risk level was optimised and the ANOVA data obtained.

The results show that the effects are non-significant and the effect of stirring speed and temperature are main factors that will affect the entrapment efficiency from the formulations as exhibited by the F-values of less than 0.05. No significant model terms are present.

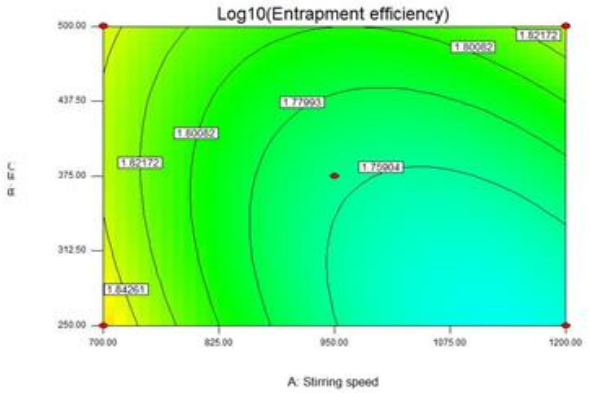


**Fig. 38 : Optimized graph between particle size, %entrapment efficiency, %drug release**

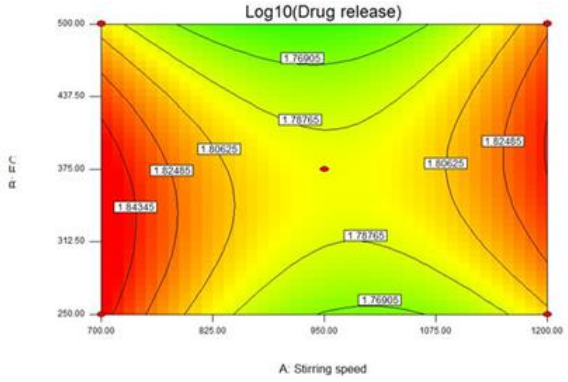


**Fig. 39: Optimized graph between particle size, %entrapment efficiency, % drug release**

The result shows that the effect are non-significant and the effect of polymer concentration and stirring speed are main factors that will effect the particle size from the formulations as exhibited but the F-values of less than 0.0500

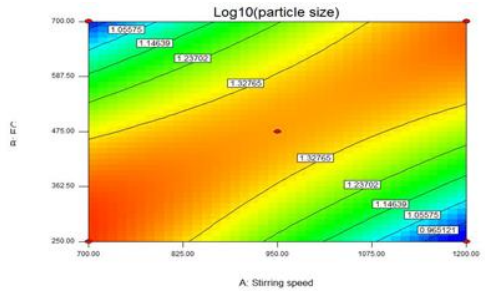


**Fig. 40: Optimized graph between particle size, %entrapment efficiency, %drug release**

The result shows that the effect is non significant and the effect of temperature and stirring speed are main factors that will effect the drug release from the formulations as exhibited but the F-values of less than 0.0500.

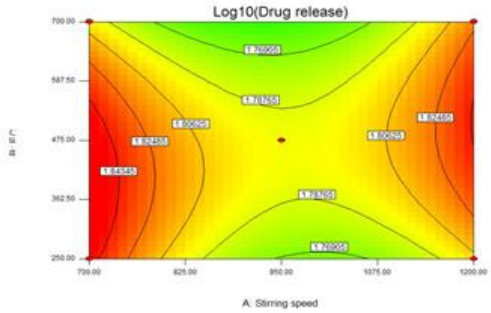


**Fig. 41: Optimized graph between particle size, %entrapment efficiency, %drug release**

The result shows that the effect are non-significant and the effect of temperature and polymer concentration are main factors that will affect the entrapment efficiency from the formulations as exhibited but the F-values of less than 0.0500.

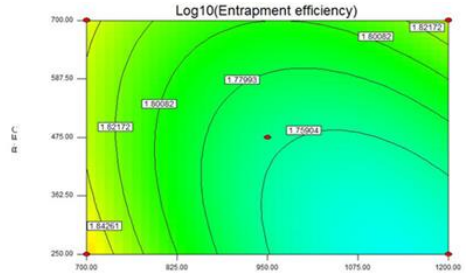


**Fig. 42: Optimized graph between particle size, %entrapment efficiency, %drug release.**

The result shows that the effect is non-significant and the effect of particle size and polymer concentration are main factors that will affect the particle size from the formulations as exhibited but the F-values of less than 0.0500

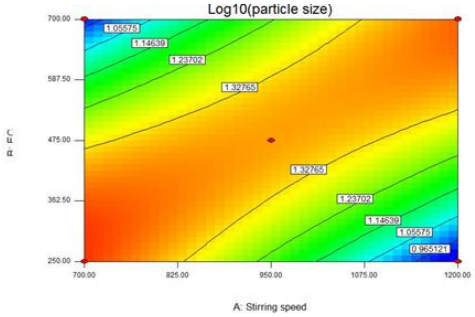


**Fig. 43: Optimized graph between particle size, %entrapment efficiency, %drug release**

**Antimicrobial study**

The antimicrobial analysis was done using the agar well diffusion method and MIC value determined. The MIS value of the optimized formulation was found to be equivalent to the marketed formulation

**Table 18: Antimicrobial activity (zone of inhibition)**

| S. No | Name of Bacteria | The diameter of Zone of Inhibition in (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Formulation concentration (µg/ml) | | | Standard | | Control |
| | | F2 | F5 | F17 | Cefaclor (S1) | Marketed Preparation (S2) | Sodium CMC |
| 1 | S. aureus | 18 mm | 21.2 mm | 22.9 mm | 24.6mm | 22.5mm | No activity |
| 2 | E. Coli | 13 mm | 14.3 mm | 15.1 mm | 17.3mm | 16.4mm | No activity |

**Stability study**

The physical Stability, including appearance, color, and pH of the resulting formulation was studied under various conditions. Formulation showed no change in colour or appearance during under all storage conditions for the duration 15days.

**Table 19: Physical stability of Cefaclor**

| Formulation code | Time | Colour | | Clarity | |
|---|---|---|---|---|---|
| | | RT | 40ºC | RT | 40ºC |
| F2 | 15 days | Yellowish White | Yellowish White | Yellowish White | Yellowish White |

**CONCLUSION**

The aim of the present study is to design microspheres of Cefaclor and optimize their characteristics to improve the bioavailability as well as potentially to reduce its side effects.

To enhance bioavailability and permeability of Cefaclor loaded microspheres.

Cefaclor is marketed in the form of conventional tablet and suspension only, but both oral dosage forms have high solubility and dissolution profile, i.e., approx. 55%. Thus there remains a need and opportunity for an improved oral Cefaclor formulation ,i.e., microspheres that deliver the drug in both solubilize form and in a predictable manner which is independent of pH in GIT; that also reduces intra and inter-subject variability. In general, the microspheres have better in vitro dissolution rate as compared to other water-soluble drugs and existing dosage forms, therefore, can significantly enhance the solubility and thus the bioavailability of Cefaclor; due to which we can reduce the amount of drug. Usually, Cefaclor is micronized to obtain better dissolution profile.

A preformulation study of Cefaclor drug was performed. The result revealed are as follows:-

Cefaclor drug was found to be white to off-white in color, amorphous in nature, almost odorless with slightly bitter taste.

The solubility studies were performed,and it was found to be slightly soluble in 0.1 N HCl; very slightly soluble in phosphate buffer pH 4; freely soluble in water, phosphate buffer pH 7.4.

Melting point test was carried out by employing the capillary method and using melting point test apparatus, and the melting point was found to be in a range 327.30C.

FTIR a spectroscopy study was carried out with help Elmer FTIR spectrometer modified version 10.01.00' and the sample spectra were compared with standard spectra, and it showed no compatibility issues. Identification and authentication of drug sample were done by high-performance liquid chromatography,

and it was scanned at YL9120 UV Detector A at 265 nm,and it was according to the official monograph,and that data obtained to comply with the parameter peak height and retention time.

Identification and authentication of drug sample were done by ultraviolet spectroscopy, and it was scanned in the range of 200-400 nm. Drug absorption maximum λ max was found to be 264 nm. Absorption maximum showed that drug sample was authentication.

The calibration curve of Cefaclor was prepared in various solvent and buffers,and it was determined in methanol and buffer pH 4, 7.4 and 0.1N HCl the respective regression coefficient was found to be 0.998, 0.996, 0.993.

The preformulation study was carried out with a vision to determine the authenticity and purity of the drug sample. The physical characterization, melting point, FT-IR and HPLC studies were performed for the identification of drug solubility analysis of drug was done in different solvents. Quantitative estimation of the drug was carried out by standard calibration curve preparation in different solvents. Bypreformulation studies physical characterization, solubility studies, melting point, partition coefficient, FT-IR study and HPLC; it was concluded that the drug sample was found to be pure and authentic and there was no variation found in drug sample. The drug found to be suitable for further preparation of microsphere and evaluations studies.

But both the drug as well as the finished product should be kept at temperature < 300C to achieve better shelf life and maintain its potency for a longerperiod.

Angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio and drug content was calculated and were found to be in the range of 16.69-22.47o, 0.64-0.68 gm/cm3, 10.95 - 14.66gm/cm3, 37.55%, 1.07-1.17 and entrapment efficiency was 83.5% respectively, from the evaluations studies of the Cefaclor microspheres.

Out of the thirteen formulations F1-F13 based on the parameter, the formulation F2 was found to be optimum. Therefore, formulation F2 was selected as an optimized formulation and entrapment efficiency, drug release for this formulation was carried out, and the drug content was found to be optimum in accordance with the official monograph.

The evaluations parameter of the F2 formulation,i.e., entrapment efficiency, % drug release 83.5%, 71.1% respectively results concluded that all the parameter with in acceptant range.

Release kinetic that in-vitro drug release curve fitted under Zero order release, first order release. Out of which the zero order model show R2 value 0.962-0.990 is highest as compared to another model. The drug release was mainly by zero order.

Stability study was carried out at 400C and the room temperature for the optimized formulation for one month there were slightly acceptable changes observed in physical and chemical parameters. The result was concluded that formulation F2 was stable under room temperature and higher temperature condition..

**REFERENCES**


1. Drug bank- www.drugbank.ca/drug/DB00833.
2. Box-Behnken-http://support.minitab.com/en-us/minitab/17/topic-library/modeling-statistics/doe/response-surface-designs/what-is-a-box-behnken-design/
3. Box- Behnken Table- http://www.itl.nist.gov/ div898/handbook /pri/section3/pri3363.htm
4. http://www.merckmanuals.com/professional/infectious_diseases/bacteria_and_antibacterial_drugs/overview_of_antibacterial_drugs.html
5. Swathi Chilukala*, Vijaya Kumar Bontha, Rajeswara Rao Pragada; “In-Vitro And In-Vivo Characterization Of Cefaclor Loaded Floating Microspheres”; Indo American Journal of Pharmaceutical Research, 2016,5277-5281
6. Lachman Leon, Lieberman HA. The Theory and Practice of Industrial Pharmacy, Varghese Publishing House, 1987; 3: 607-625.
7. Gupta NV, Shirodker N, Getyala A, Bhat RS. Bioadhesive vaginal tablets containing spray-dried microspheres loaded with clotrimazole for treatment of vaginal candidiasis. Acta Pharma 2013:63:359-372.
8. Kataria S, Middha A, Sandhu P, Bilandi A, Kapoor B. Microsphere: a review, Int J Res Pharm Chem. 2011:1:1184-1198.
9. Alagusndaram M, MadhuSudanaChetty C, Umashankari. K, Badrinath A V, Lavanya C, Ramakanth S. Microsphere as a novel drug delivery System- A review, Int J Chem tech Res. 2009:1:3:526-534.
10. Shaikh A, Sakarkar DM, Misal RB, Mohare Y, Hattiambier K. Bioadhesive microspheres as a controlled drug delivery system: a review. American J Pharmatech Res. 2013:3:170-189.
11. Sharma RN, Pancholi SS. Optimization techniques in the pharmaceutical industry: a review. J Curr Pharm Res. 2011:7 (1): 21-28.
12. Parida P, Mishra SC, Sahoo SS, Behera A, Nayaka BP. Development and characterization of ethylcellulose based microsphere for sustained release of nifedipine. J Pharm Ana. 2014 (In press, corrected proof).
13. Deshmukh R K , Naik J B. Aceclofenac microspheres: quality by design approach. Mat Sci and Eng, 2014:36:320-328.
14. Sun L, Wan S, Yu L, Wang L. Optimization and modeling of preparation conditions of TiO2 nanoparticles coated on hollow glass microspheres using response surface methodology. Sep and Purification Tech. 2014:125:156-162.
15. Raval JP, Naik DR, Amin KA, Patel PS. Controlled-release and antibacterial studies of doxycycline-loaded poly(e-caprolactone) microspheres. J Saudi Chem Soc. 2014:18:566– 573.
16. Ali MS, PanditV, Jain M, DharKL. Mucoadhesivemicroparticulate drug delivery system of curcumin against Helicobacter pylori infection: Design, development and optimization. J Adv Pharm Technol Res. 2014:5:48–56.
17. Kar M, Priyanka GP, and Jain DK. Design and development of solid lipid nanoparticles of thiocolchicoside by box-behnken design. Indo American J Pharm Res. 2014:4:1187-1196.
18. Sultana S, Talegaonkar S, Singh D, Ahmad R, Manukonda V, F.J. A, Bhatnagar A. An approach for lacidipine loaded gastroretentive formulation prepared by different methods for gastroparesis in diabetic patients. Saudi Pharm Journal. 2013:21:293–304.
19. Shah J, Jan M, Shah S, and Khan MN. Development and validation of hplc method for simultaneous determination of ceftriaxone and cefaclor in commercial formulations and biological sample. J MexChemSoc 2013:57: 314-320.
20. Marais E, Hamman J, Plessis L, Lemmer R and Steenekamp J. Eudragit® L100/N-trimethylchitosan chloride microspheres for oral insulin delivery, Saudi Pharm Journal. 2013:18:6734-6747.
21. Gaur PK, Mishra S, Kumar A& Panda BP. Development and optimization of gastroretentivemucoadhesive microspheres of gabapentin by Box– Behnkendesign.Artif Cell Nano. Bio. Tech. 2013:85:55-62.
22. Bhavsar MD., Tiwari SB., Amiji MM. Formulation optimization for the nanoparticles-in-microsphere hybrid oral delivery system using factorial design. J Control Rel. 2006:110:422-430.
23. Yuta N, Yasuyoshi A, Yasunori I, Shigeru I. A novel mathematical model considering change of diffusion coefficient for predicting dissolution behavior of acetaminophen from wax matrix dosage form. Int J Pharm. 2012:428: 82-90.
24. Nitanai Y, Agata Y, Iwao Y, Itai S. A novel mathematical model considering change of diffusion coefficient for predicting dissolution behavior of acetaminophen from wax matrix dosage form. Int J Pharm.2012:428:82-90.
25. Wasnik S, Parmar P, Singh D, Ram A. Preparation and characterization of floating drug delivery system of

azithromycin. ActaPoloniae Pharm Drug Res. 2012:69:515-522.
26. Cortesi R, Ravani L, Menegatti E, Esposito E, Ronconi F, Eudragit® microparticles for the release of budesonide: A comparative study. Ind J Pharm Sci. 2012:74:415-421.
27. Patel MI, Patel RR, Patel JR. Preparation , optimization and characterization of multiple unit microsphere containing Kollidone. J Pharm Bio. All. Sic. 2012:4:85-95.
28. Devrim B, Bozkir A, Canefe K. Formulation and evaluation of reconstitutable suspensions containing ibuprofen-loaded eudragit microspheres. ActaPoloniae Pharm Drug Res, 2011:68:593-599.
29. Beck B, Betz T, Schmel P, Kleimnn M, Bakocosy T. Impact of caprolectants for the stabilization of biodegradable nanoparticles on the performance of air jet ultrasonic and vibrating mesh nebulizer. J Pharma. Biopharm. 2012:82:272.
30. Oh TO, Kim JY, JM Ha, SC C, Yun-Seok R, Chun WP, Eun SP. Preparation of highly porous gastroretentive metformin tablets using a sublimation method. Eur J Pharm and Biopharm. 2013:83:460–467.
31. Mennin N, Furlanetto S, Cirri M, Mura P. Quality by design approach for developing chitosan-Ca-alginate microspheres for colon delivery of celecoxib-hydroxypropyl-b-cyclodextrin-PVP complex. Eur J Pharm and Biopharm 2012:80:67-75.
32. Ahmed SM, Elbashir AA, Enein HY. New spectrophotometric method for determination of cephalosporins in pharmaceutical formulations. Arabian J Chem. 2011:5:48-53.
33. Fernandesa M, Goncalvesa IC, Nardecchiaa S, Amarala IF, Barbosa Mário A, Martinsa M, Cristina L. Modulation of stability and mucoadhesive properties of chitosan microspheres for therapeutic gastric application. Int J Pharm. 2013:454:116-124.
34. Sipos P, Rajko R, Pintye HK, Eros I and Szabo RP. Formulation optimization of sustained-release ammonio methacrylate copolymer microspheres. Effects of log p and concentration of polar cosolvents, and Role of the Drug/Copolymer Ratio. Pharmaceutics. 2011:3:830-847.
35. Dey S, Pramanik S, and Malgope A. Formulation and optimization of sustained release stavudine microspheres using response surface methodology. Inter. Sch. Res. Net. Pharma. 2011:10:627-632
36. Awasthi R, Kulkarni GT, Pawar VK, and Garg G. Optimization studies on gastroretentive floating system using response surface methodology. AAPS Pharm Sci Tech. 2011:13: 85-93.
37. Shazalia SM, Elbashir AA, Enein HY. New spectrophotometric method for determination of cephalosporins in pharmaceutical formulations. Arabian J Chem. 2011.
38. Rao PV, Sushanta M. Development and validation of new RP-HPLC method for the determination of cefaclor in pharmaceutical dosage forms and in human plasma. Res J Chem Sci. 2011:1:36-39.
39. Cun D, Jensen DK, Maltesen MJ, Bunker M, Whiteside P , Scurr D, Foged C, Nielsen HM. High loading efficiency and sustained release of siRNA encapsulated in PLGA nanoparticles: Quality by design optimization and characterization. Eur J Pharm and Biopharm. 2011:77:26-35.
40. Nayak AK, Das B, Gastroretentivehydrodynamically balanced systems of ofloxacin: In vitro evaluation. Saudi Pharm Journal. 21:2013:113–117.
41. Mostafavia A, Emamia J, Varshosaza J, Daviesb NM, Rezazadeh M. Development of a prolonged-release gastroretentive tablet formulation of ciprofloxacin hydrochloride:
42. Pharmacokinetic characterization in healthy human volunteers. Int J Pharm. 2011:409:128–136.
43. Kotagale NR, Parkhe AP, Jumde AB, Khandelwal HM, Umekar MJ, Ranitidine hydrochloride-loaded ethyl cellulose and eudragitRs 100 buoyant microspheres: Effect of pH modifiers. Ind J Pharm Sci. 2011:73:626-633.
44. Nath B, Kantanath L, Kumar P. Preparation and in vitro dissolution profile of zidovudine loaded microspheres made of Eudragit RS 100, RL 100 and their combinations. ActaPoloniae Pharm Drug Res. 2011:68:409-415.
45. Saravanan M, Boddapati A, Development and evaluation of ethylcellulose floating microsphere loaded carbohydrate with ranitidine hydrochloride by novel solvent evaporation matrix erosion method. Carbohydrate Poymer. 2011:85:592-598.
46. Guan J, Cheng P, Huang SJ, Wu JM, Li ZH, You XD, Hao LM, Guo Y, Li RX, Zhang H. Optimized preparation of levofloxacin-loaded chitosan nanoparticles by ionotropic gelation, Physics Procedia . 2011:22:163-169.
47. HamedelnielEI, BajdikJ, SoványT, Pintye K. Delayed release matrix pellet preparation containing an alkalizing pore-former agent. Chemical Engineering Research and Design. 2011:89:1006-1010
48. Buranachai T, Praphairaksit N, and Muangsin N. Chitosan/Polyethylene Glycol Beads Crosslinked with Tripolyphosphate and Glutaraldehyde for Gastrointestinal Drug Delivery. AAPS Pharm Sci Tech. 2010:11:1128-1137.
49. Berchanea NS, Jebrail FF, Andrewsb MJ. Optimization of PLG microspheres for tailored drug release. Int J Pharm. 2010:383:81-88.