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FORMULATION AND CHARACTERIZATION OF CEFIXIME MICROSPHERES

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ABSTRACT

The purpose of this research work was to increase the residence time of drug Cefixime 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 102.5±1.3µm to 110±2.21 µm**,** entrapment efficiency was found to be 75.69±1.91 to 88.35±2.67%, drug content was found to be in the range of 97.46±2.4 to98.95±1.8. Fourier-Transform Infra Red (FT-IR) studies ensured that no drug - polymer interaction in the formulated microspheres and the surface topography revealed a spherical surface for all the formulations and a round cavity enclosed by an outer shell composed of the drug and polymer. In- vitro release profile of microspheres for F6 formulation was found to be 97.87±0.2 at the end of 12 hrs. In release kinetic studies, the F6 formulation followed zero order drug release with non-Fickian diffusion mechanism.

Keywords: Cefixime, FT-IR, SEM, Microspheres.

INTRODUCTION

Microspheres are defined as solid spherical particles containing dispersed drug in either solution or microcrystalline form. They are ranging in size from 1 to 1000 micrometer. Microspheres are in strict sense, spherical solid particles. Microcapsules are small particles that contains an active agent as a core material and coating agent as shell, at present there is no universally accepted size range that particle must have in order to be classified as microcapsules. However, many workers classify capsules smaller than 1 micrometer as nanocapsules and capsules layer more than 1000 micrometer as macroparticles. Commercial microcapsules typically have a diameter between 3-80 micrometer and contain 10-90 weight % cores.

Cefixime, an antibiotic, is a third-generation cephalosporin with antibacterial activity against gram-positive and gram-negative pathogens. The bioavailability of above mention drugs are 40- 50% with a half life of 3-5 hours. To increase the bioavailability of the Cefixime with reducing dosing frequency microspheres were selected as a suitable approach.

MATERIAL AND METHODS

Materials: Cefixime was obtained as a gift sample from Hetero drugs, Hyderabad (India). SCMC, HPMCK4M, EUDRAGIT was obtained from Colorcon india pvt.ltd. Ethanol, DCM,

Tween80,Liquid paraffin were purchased from Colorcon india pvt.ltd All other chemicals and reagents used were of analytical grade.

Preparation of Cefixime Microspheres by non-aqueous solvent evaporation technique:

Microspheres containing Cephalosporin drugs as a core material were prepared by a non- aqueous solvent evaporation method. Drug and different polymer ratio were mixed in the mixture of dichloromethane and ethanol at a 1:1 ratio. The slurry was slowly introduced into 30 ml of liquid paraffin containing 0.01% Tween 80, while stirring at 1200 rpm using a mechanical stirrer equipped with three bladed propellers at room temperature. The solution was stirred for 2 h and the solvent evaporated completely, and filtered by using filter paper. The microspheres obtained were washed repeatedly with petroleum ether (40-60 °C) until free it was from oil. The collected microspheres were dried at room temperature and subsequently stored in desiccators.

Physical characterization of microspheres: [8, 9]

Solubility study:

Excess drug was added carefully using a spatula to 10 ml of the media in a conical flask, while stirring until a heterogeneous system (solid sample and liquid) was obtained. The solution containing excess solid was then capped, and stirred at 150 rpm at room temperature for 24 hours. The solution containing excess solid was filtered using 0.45µm PVDF filter, appropriate dilutions were then made and analyzed using UV spectrophotometer at required nanometer range of drug. The same procedure was fallowed for all selected drugs. (Saturation solubility was carried out at 25^0C using required different buffers).

Determination of absorption maximum (λmax):

The wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region (190-390nm), as they are aromatic or contain double bonds.

Accurately weighed 100mg of drug was dissolved in pH 6.8 buffer taken in a clean 100 ml volumetric flask. The volume was made up to 100ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 5ml was pipette out in 50ml volumetric flask. The volume was made up to 50ml using pH 6.8 buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using pH 6.8 buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

PREPARATION OF CALIBRATION CURVE

Procedure for standard curve in pH 6.8:

10 mg of drug was dissolved in 10 ml of pH 6.8 by slight shaking (1000 mcg/ml). 1 ml of this solution was taken and made up to 20 ml with pH 6.8, which gives 20 mcg/ ml concentration (stock solution). From the stock solution, concentrations of 5, 10, 15, 20 and 25 µg/ml in pH 6.8 were prepared. The absorbance of diluted solutions were measured at particular nanometer and a standard plot was drawn using the data obtained. The correlation coefficient was calculated.

FTIR analysis:

The drug-polymer interactions were studied by FTIR spectrometer, Shimadzu 8400 S. 2% (w/w) of the sample, with respect to a potassium bromide (KBr; SD Fine Chem. Ltd., Mumbai, India) was mixed with dry KBr. The mixture was ground into a fine powder using mortar and then compressed into a KBr discs in a hydraulic press at a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of 2 cm–1 using Happ-Genzel apodization. The characteristic peaks were recorded

MICROMERETIC PARAMETERS:

Bulk Density: Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. It is determined by pouring pre-sieved blend into a graduated cylinder via a large funnel and measure the volume and weight as is given by

Bulk density= weight of blend/Bulk volume

Tapped density: Tapped density is determined by placing a graduated cylinder containing known mass of blends on a mechanical tapped apparatus, which is operated for a fixed number of taps until the powder bed volume has reached a minimum volume. Using the weight of the drug in the cylinder and this minimum volume, the tapped density may be computed.

Tapped density=weight of blend/tapped volume of blends

Compressibility Index: The compressibility index of the granules was determined by Carr's compressibility index.

Carr's index (%) = [(TBD – LBD) × 100]/TBD

Hausner's ratio: Hausner's ratio was determined as the ratio between the tapped density to that of the bulk density.

H.R = Tap Density / Bulk Density

Angle of repose: The manner in which stresses are transmitted through a bed and beds response to applied stress is reflected in the various angles of friction and response. The most commonly used of these is angle of repose, which may be determined experimentally by a number of methods. The method used to find the angle of repose is to pour the powder in a conical heap on a level flat surface and measure the inclined angled with the horizontal pile.

$\theta = \tan^{-1}(h/r)$

Particle Size

It is possible to use ordinary microscope for particle size determination in the range of 0.2 to above100 µm to measure particle size of individual microsphere.[55]All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. Ocular micrometer was calibrated with the stage micrometer. Slides of dilute suspensions of microspheres in liquid paraffin were prepared and slides were placed on mechanical stage of microscope. The diameter of 100 microspheres was measured randomly by optical microscope and average particle size was determined.

Scanning electron microscopy (SEM)

In the pharmaceutical industry, SEM may be used as a qualitative tool for the analysis of drug substance and drug product in order to obtain information on the shape and surface structure of the material. SEM plays an important role in the characterization of nanoscale and sub-micron particles. It has been used to determine surface topography, texture and to examine the morphology of fractured or sectioned surfaces. The examination of the surface of polymeric drug delivery systems can provide important information about the porosity and microstructure of device.

Actual drug content and entrapment efficiency

10 mg of microspheres were accurately weighted and transferred in a 50 ml volumetric flask. Volume was adjusted with 1% SLS and microspheres were dissolved by ultra-sonication for 3 h at25 °C. The samples were filtered through 0.2 µm membrane filter. 5 ml from the sample solution was transferred to 50 ml volumetric flask and volume was adjusted to 50 ml with same medium and absorbance of samples was measured at 288 nm using UV-spectrophotometer. Actual drug content (AC)and encapsulation efficiency (EE) were calculated using following equations. All analyses were carried out in triplicate.

$$AC(\%) = \frac{Cact}{Cms} \times 100$$

$$EE(\%) = \frac{Cact}{Cthe} \times 100$$

Where,

M_{act}= Actual Cefixime content in microspheres

M_{ms}= Weighed quantity of microspheres

M_{the}= Theoretical quantity of Cefixime in microspheres calculated from the quantity added in the process.

Invitro Dissolution Studies:

The dissolution test measures the amount of time required for certain percentage of the drug substance in a tablet to go into solution under a specified set of conditions. It describes a step towards physiological availability of the drug substance, but it is not designed to measure the safety or efficacy of the formulation being tested.

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.

Stability studies:

Stability studies were conducted for the optimized formulation confirmed from the in vitro dissolution data, for Particle size,% Yield, Entrapmnent efficiency, &% Drug content at at 40°C /75%RH for a period of 3 months.

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 solubility of Cefixime is very poor in water (0.13 mg/ml) and in 0.1N HCl (0.081mg/ml). The solubility of Cefixime increased with increase in pH6.8 of the buffer from 0.81 to 2.15 mg/ml.

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.

Determination of absorption maxima (λ_{max}) of Cefixime:

The maximum absorbance of the Cefixime in pH 6.8 was found to be 286nm as shown in Fig. Hence, the wavelength of 286nm was selected for analysis of drug in dissolution media.

Standard curve of Cefixime:

A linear relationship was observed between concentration of drug solution in pH 6.8and absorbance, over the concentration range of 5-25µg/mL. The coefficient of correlation ($R^{2)}$ was found to be 0.9990, indicating that the drug solution obeys Beer's- Lambert law in the concentration range of 5-25µg/ml. Hence it was concluded that dissolution samples can be analyzed in 0.1N HCl by measuring absorbance at 286nm using UV-Visible Spectrophotometer.

FTIR Studies:

The Cefixime and Excipients interaction was studied by comparing the FTIR spectrum of the optimized blend with that of Cefixime pure drug as shown in Table and Fig. The comparison study demonstrates that there was no interaction between the drug and other ingredients of the formulation including Excipients such as HPMC, Eudragit and SCMC as shown in Table and Fig, thus revealing compatibility of the selected drug with the excipients.

MICROMERETIC PARAMETERS:

The flow properties like bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio was found to be 0.212 g/cc, 0.285 g/cc, 24^{o}, 29.82 and 1.425 respectively, which indicates that flow of API is poor as per I.P limits.

Particle Size

The particle size of the formulations F-I to F-9 were found to be in the ranges from 102.5±1.3µm to 110±2.21 µm.

Scanning electron microscopy analysis (SEM)

The optimized formulation was evaluated for its surface morphology by using Scanning electron microscopy. The outer surface of the microspheres was found to be smooth. The surface topography revealed a spherical surface for all the formulations and a round cavity enclosed by an outer shell composed of the drug and polymer. The particle size was found to be 100µm.

Actual drug content and entrapment efficiency

The particle size of the formulations F-I to F-9 were found to be in the ranges from 75.69±1.91 to 88.35±2.67% and 97.46±2.4 to98.95±1.8respectively.

Invitro Dissolution Studies:

The formulations F1- F3 prepared with (ratios range 1:1, 1:1.5, 1:2) concentration of polymer like SCMS and drug release as shown in Table. As the polymer concentration was decreases the drug release was increases. This might be due to insufficient entrapment of the drug formulations contain low concentration of hydrophilic polymer (SCMC).

The formulations F1 showed burst effect and released 98.09±0.23% at the end of 4hrs. The formulations F2 and F3 drug release was 99.84±0.6%, 99.85±0.7 at the end of 6 and 10 hrs respectively, due to increase the polymer concentration, further increases the concentration of polymer (F3) drug release was decreased.

The formulations F4, F5, releases 98.81±0.78, 95.41±0.07 at the end of 10hrs. Compared to low concentration of polymer (HPMC) in formulations, F-6 where it was found to be 97.87±0.22 at the end of 12 hrs shown Fig. The HPMC (high viscosity and high molecular weight) upon contact with dissolution medium swelling occur due to the disruption of hydrogen bonding among the polymeric chains and form a thick gel layer on the surface, which gets eroded over period of time. Thus, this parameter was responsible for sustained/controlled drug release rate.

The formulations (F7, F8 and F9) were tried with Eudragit (ratios range 1:1, 1:1.5, 1:2) as retardant being insoluble in gastric pH. The formulations F7 was found to be 100.14±0.49 at the end of 10hrs due to low polymer concentration effect. F8 and F9 showed better control on drug release than other formulations and also exhibited incomplete drug release which might be due to hydrophobic polymer (Table and Fig).

The formulation F6 was made with the HPMC in the drug polymer ratio of 1:1.5 and drug release was found to be 97.87±0.22% at the end of 12hrs with better drug release pattern. The reason might be to this fact is formation of thick gel layer by matrices around the surface that delays diffusion and release of drug, thus F6 was considered as optimized formulation

RELEASE KINETIC MODELS:

The optimized formulation F6 has coefficient of determination (R^2) values of Zero order, First order, Higuchi and Korsmeyer Peppas of 0.9560, 0.7870, 0.9820 and 0.9920 respectively. A good linearity was observed with the zero order. The slope of the

regression line from the Higuchi plot indicates the rate of drug release through mode of diffusion, and further confirms the diffusion mechanism. The data fitted into the Korsmeyer Peppas equation which showed linearity with slope n value of 0.598 for optimized formulation F6. This n value indicates the coupling of (swelling, polymer relaxation) diffusion and erosion mechanism. This type of drug release is called as anomalous diffusion. Thus, it indicates the drug release from the tablet follows non-Fickian diffusion mechanism. The presence of swelling and crosslinked polymers within the matrix structure might be responsible for the drug release controlled by more than one process. Thus, with regarded to release kinetics, the optimized batch F6 follows best fitted into peppas model and showed zero order drug release with non-Fickian diffusion mechanism.

Stability studies of optimized formulation (F6):

Stability studies were conducted for Particle size,% Yield, Entrapmnent efficiency, &% Drug content and confirmed that there was no significant change in the parameters of optimized formulation at storage condition of 40°C ± 2°C / 75 ± 5 %RH after 6 months.

CONCLUSION

In this research work attempt was made to increase the bioavailability of the Cefixime with reducing dosing frequency microspheres. Formulation was successfully made and in –vitro evaluation of 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 provides sustained release of drug over more than 12 hours. (iv) Drug release from microspheres follows zero order drug release with non-Fickian diffusion mechanism. (v) 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: Formulation design of Microspheres:

Sr.no	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	CEFIXIME	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	SCMC(gm)	1	1.5	2	-----	------	------	------	------	------
3	HPMCK4M	------	------	------	1	1.5	2	------	------	------
4	EUDRAGIT(gm)	------	------	------	------	------	------	1	1.5	2
5	Ethanol (ml)	6	10	12	15	20	23	10	15	20
6	DCM (ml)	6	10	12	25	20	23	10	15	20
7	Tween (ml)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
8	Liquid paraffin(ml)	90	90	90	90	90	90	90	90	90

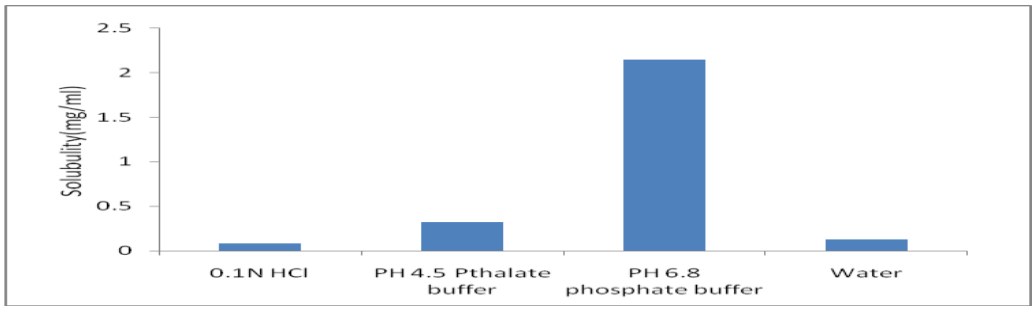

Fig. 1. Saturation solubility of CEFIXIME:

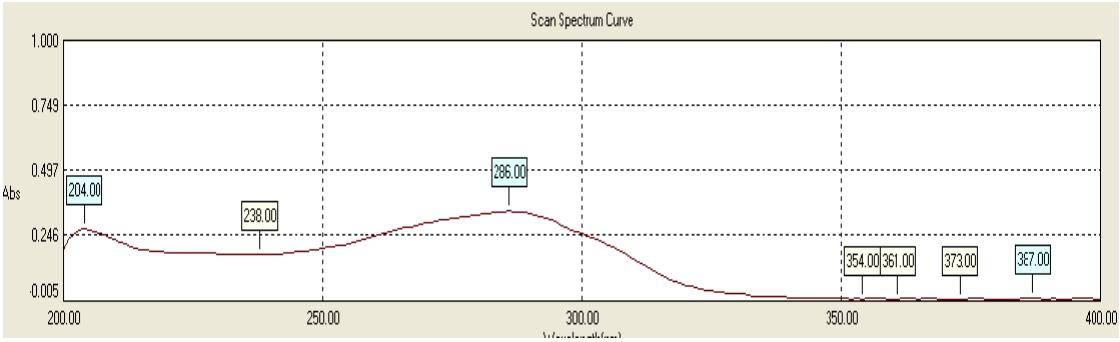

Fig 2. Determination of absorption maxima (λ_{max}) of CEFIXIME:

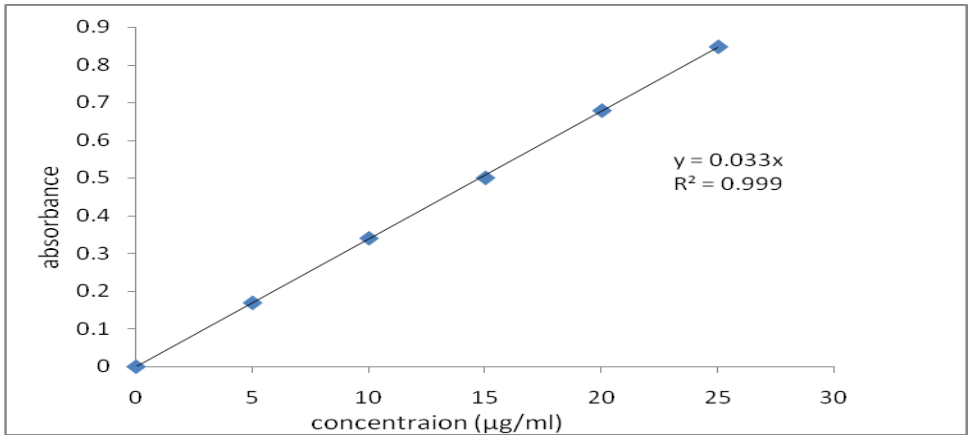

Fig.3 : Standard curve of CEFIXIME in pH 6.8 (λ_{max} 286)

Table 2. : Characterization of Cefixime microspheres

Parameters	Bulk density (gm/cc)	Tapped density (gm/cc)	Hausner's ratio	Compressibility index
F1	0.454±0.12	0.526±0.14	1.55±0.02	13.64±0.01
F2	0.411±0.05	0.524±0.32	1.27±0.09	21.40±0.21
F3	0.397±0.12	0.497±0.14	1.25±0.07	20.04±0.21
F4	0.416±0.32	0.495±0.5	1.18±0.19	11.5±0.31
F5	0.429±0.09	0.542±0.21	1.27±0.12	20.97±0.09
F6	0.49±0.08	0.64±0.21	1.30±0.04	23.4±0.08
F7	0.409±0.10	0.552±0.09	1.34±0.12	25.84±0.10
F8	0.54±0.024	0.67±0.10	1.24±0.10	19.4±0.11
F9	0.384±0.31	0.50±0.12	1.13±0.09	23.08±0.09

Table 3: Particle size, Drug Entrapment Efficiency of Cefixime microspheres

Formulation Code	Particle Size (µm)	% Yield	Entrapment Efficiency	Drug Content
F1	106.5±2.3	93.70±1.28	87.04±1.92	98.56±0.63
F2	110±2.21	87.82±2.01	78.68±2.1	98.48±0.91
F3	103.4±1.42	92.70±1.19	85.04±1.87	97.59±1.97
F4	102.5±1.3	85.95±1.98	76.87±1.91	98.64±2.01
F5	103.2±0.9	94.82±2.16	88.35±2.67	98.46±3.22
F6	103±2.8	86.90±3.05	86.98±2.08	98.78±1.4
F7	108.6±1.7	93.25±1.37	75.69±1.91	99.11±2.1
F8	106±2.35	85.82±2.01	76.68±2.1	97.46±2.4
F9	103.8±1.8	93.70±1.28	87.04±1.92	98.95±1.8

Table 4: Dissolution profile of CEFIXIME formulations (Mean±SD; n=6)

Time (hr)	Percentage of Cumulative drug release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	42.45±0.47	35.35±0.89	28.45±0.36	32.84±0.15	29.98±0.44	20.25±0.77	20.23±0.17	14.45±0.58	11.52±0.41
2	72.45±0.54	68.85±0.87	48.92±0.54	44.85±0.43	39.45±0.51	33.46±0.15	29.85±0.55	20.89±0.70	15.23±0.30
4	98.09±0.23	82.85±0.56	62.45±0.67	59.98±0.26	47.42±0.78	45.55±0.09	50.25±0.33	29.85±0.21	25.32±0.55
6		99.84±0.6	78.58±0.59	70.23±0.75	62.45±0.30	58.88±0.48	78.89±0.60	48.88±0.56	30.51±0.21
8			89.23±0.65	86.55±0.10	79.98±0.19	69.89±0.70	89.95±0.74	54.85±0.61	38.54±0.02
10			99.85±0.7	98.81±0.78	95.41±0.07	79.54±0.36	100.14±0.49	62.85±0.31	45.23±0.09
12	--	--	--			97.87±0.22		69.85±0.05	51.21±0.10

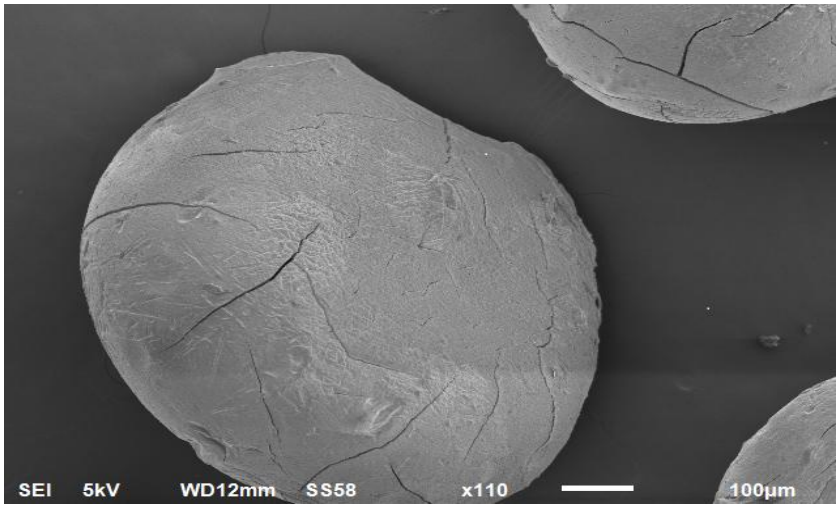

Fig.4. Scanning electron microscopy analysis (SEM)

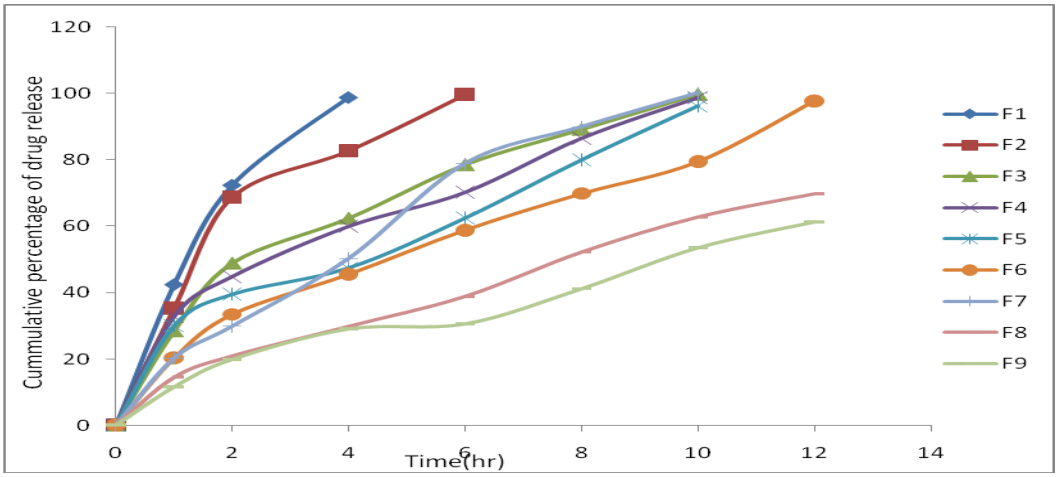

Fig 5: *Invitro* dissolution profile of CEFIXIME formulations

Table 5: Stability data of optimized formulation (F6) physico-chemical parameters

Parameter	**Initial**	**After 3 months at 40°C /75%RH**	**After 6 months at 40°C /75%RH**
Particle size	103± 2.8	102.47 ± 2.2	102.89 ± 2.55
% Yield	86.90±3.05	86.81±2.89	86.92±3.11
Entrapmnent efficiency	86.98±2.08	86.87±1.87	86.94±2.01
% Drug content	98.78±1.4	98.70±1.05	98.76±1.33

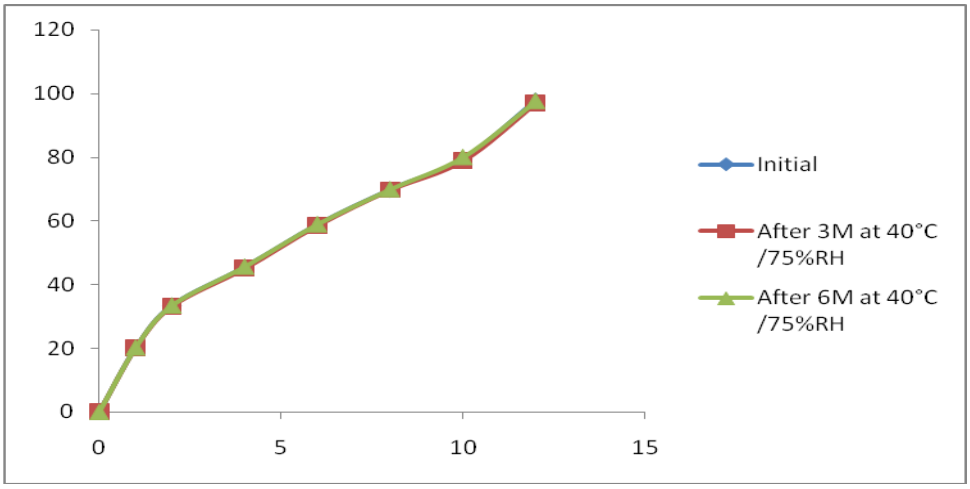

Fig 6: Optimized formulation of CEFIXIME (F6) *invitro* dissolution at 40°C /75%RH

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