IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2023 Vol.:28, Issue:3
© All rights are reserved by Shivani et al.

Preparation and Evaluation of Cefuroxime Loaded Nanosponge

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Shivani*, Dr. Rajesh Gupta, Pooja Sharma

Sri Sai College of Pharmacy, Badhani, Pathankot, Punjab, India.

Submitted: 21 September 2023
Accepted: 28 September 2023
Published: 30 October 2023

ijppr.humanjournals.com

Keywords: nanosponge; Targeted dug delivery; Solubility improvement; Controlled medicine delivery.

ABSTRACT

Cefuroxime loaded nanosponges were prepared by Emulsion solvent diffusion method using ethyl cellulose, Dichloromethane as a solvent and PVA. The evaluation for percentage yield, co-efficient efficiency, particle size, drug polymer compatibility, scanning electron microscopy and *in vitro* drug release is carried out for the prepared Nanosponges. Their porous structure with number of nanochannels is confirmed by SEM studies. The FTIR spectra showed stable character of Cefuroxime in a mixture of polymers and revealed the absence of drug-polymer interactions. The average particle size of Cefuroxime nanoparticles was found to be in the range of 282.7 nm with PDI 29.7%. The negative zeta potential values were attained to ensure a good stability of nanosponges. The drug release from nanosponges was found to extended upto 2 h. The data obtained in this study suggests that nanosponges of Cefuroxime are promising for increasing the solubility of drug cefuroxime.

INTRODUCTION

The recent advance in nanotechnology has lead to the development of a targeted drug delievery system. It has a long been a problem for medical researchers - how to get them to the right place in the body and how to control the release of the drug to prevent overdoses. The development of new and complex molecules called nanosponges has the potential to solve these problems. Nanosponges are made of microscopic particles with a few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water-soluble molecules[1].

Nanosponges has certainly a new interest in drugs by providing them new life through their therapeutic targets in cancer treatment also. Administration of drug by target-oriented in cancer treatment that improves therapeutic efficacy, reduction in side effect and optimized dosing regimen will be the leading trends in the area of therapeutics. In targeted drug delivery, selective and effective localization of pharmacologically active moiety at a pre-identified target in therapeutic concentration and restricting access to the non-target normal cellular lining and thus decreases toxic effects and increases the therapeutic index of the anti-cancer drug[2].

Nanosponges were developed especially for topical delivery of drugs as they are nonirritating, non-toxic, non-allergic, non-mutagenic. It can deliver drugs that are poorly soluble in water.Nanosponges are tiny spherical particles ranging from 250 nm to 1 micrometer with large porous surface[3].

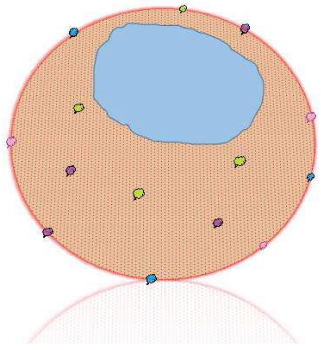


Fig. 1: Structure of a nanosponge showing a cavity for drug.

The nanoparticles can be categorized into three groups based on how they are associated with medicines.

a.)Encapsulating Nanoparticles: Nanosponges and nanocapsules are examples of encapsulating nanoparticles. Alginate Nanosponges, which are sponge-like nanoparticles, have multiple hollows that allow drug molecules to pass through. Nanoparticles are also encapsulated in nanocapsules such as poly (isobutyl cyanoacrylate) (IBCA). Drug molecules can be located in their aqueous core.

b.) Complexion Nanoparticles: This category comprises compound nanoparticles that use electric charges to attract molecules.

c.)Conjugating Nanoparticles: These nanoparticle combinations link or connect to drug substances with covalent bond[4].

MATERIAL AND METHOD

List of material

The following material that was either AR/LR grade or the best possible grade available were used as supplied by the manufacturer. The materials used during the research were Cefuroxime, Ethylcellulose, polyvinyl alcohol, Dichloromethane, Methanol, Acetone, Chloroform, n-octanol, Potassium Dehydrogenate orthophosphate

Table 1: Chemicals used

Sr. No	Materials	Source
1	Cefuroxime	Zeiss Pharma Ltd
2	Poly vinyl alcohol (PVA)	Central Drug House (P) Ltd – CDH
3	Ethyl cellulose	Fisher Scientific India Pvt. Ltd.
4	n-octanol	SD Fine-chem. Ltd, Mumbai
5	Methanol	Fisher Scientific India Pvt. Ltd.
6	Dichloromethane	Fisher Scientific India Pvt. Ltd.
7 9	Potassium Dihydrogen orthophosphate Sodium hydroxide	Thomas Baker Thomas Bakers

Table 2: Equipment used

S. No.	Instruments	Manufacturer
1	UV/VIS Spectrophotometer,	Shimadzu, Japan
2	Digital Weighing balance, (CY220)	Shimadzu, Japan
3	Magnetic stirrer with a hot plate	IKA
4	Ultrasonicator	PCI Analytics, India
5	Vortex mixer	Remi Scientific Instruments, Mumbai
6	Hot air oven	P. L. Tandon & Co, Delhi
7	Dissolution Test Apparatus	Labindia Analytical Instruments Pvt. Ltd, Mumbai
8	pH Meter	Ohaus, USA
9	Melting Point Apparatus	Remi Scientific Instruments, Mumbai
10	Infrared red spectrophotometer (FTIR)	Bruker Alpha, Berlin, Germany
11	Microcentrifuge	Remi Scientific Instruments, Mumbai

Methodology

Preformulation studies

Pre-formulation study is an integral part of the entire development process. It is the study of the physical and chemical properties of the drug prior compounding process. These studies target on the physicochemical properties of the drug that could affect its performance and development of the effective dosage form. For understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. In the simplest case, these preformulation investigations may merely confirm that there are no significant barriers to the development of compound. These study are indispensable protocol for the development of safe, effective and stable dosage forms. The obtained drug sample was identified by various analytical techniques such as IR spectroscopy, UV spectroscopy, melting point etc [5].

Organoleptic characteristics

The drug sample was characterized for the physical characterization like appearance, colour and odour .

Melting point

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under one atmosphere of pressure. It is also called the liquefacation point and both liquid and solid phase coexist in equilibrium. Melting point apparatus is used for the determination of melting point of the drug. A few amounts of the drug were placed in the thin-walled capillary tube 10-15 mm long, about 1mm inside diameter, and closed at one end. The capillary, which contains the sample, was suspended to heat the samples slowly and evenly and a thermometer placed to check the temperature. The temperature range over where the sample is observed to melt it taken as the melting point of the drug [6].

UV spectrum of cefuroxime

UV- visible spectrophotometry is generally used for structural information of various drugs to obtain specific information on the chromophoric part of the molecules in solution when exposed to light in the visible/ultravoilet region of the spectrum absorb light of particular wavelength depending on the type of electron transition associated with the absorption. The UV spectrum is generally recorded as a plot of absorbance versus wavelength.

A double beam UV- visible spectrophotometer was used to know the λ max of drug. A 0.4 to 24 / ml solution of cefuroxime in methanol was scanned in the range of 200-800nm [7].

Estimation of Cefuroxim:

- **Estimation of Cefuroxim by UV – visible spectrophotometer**

The standard stock solution of Cefuroxim (10mg/10ml) was prepared in methanol. This solution was diluted with methanol, to obtain various dilutions from 0.4-24 µg/ ml. absorbance of these solutions was recorded at 276 nm against methanol as blank using UV visible specrophotometer and standard curve was plotted against concentration. From the calibration curve intercept, slop, straight line equation and correlation coefficient were obtained.

1. **Solubility studies**

It is defined as the property of the solute dissolve in a given solvent at a certain temperature.for the quantitative solubility study, surplus amount of drug was taken in a washed test tubes containing 1ml of different solvents (Methanol, Ethanol, Acetone, Chloroform, 0.1N HCL, Water, phosphate buffer saline of pH 6.8 and 7.4) and test tubes were tightly closed. These test tubes were shaken on water bath shaker for 24 hr. at room temperature. After 24 hr each sample was centrifuged 15,000 rpm and the supernatant was withdrawl. After that supernatant was filtered and filtrates was suitably diluted and determined spectrophotometrically [8].

2. **The partition coefficient of drug**

It is unit of measure or concentration ratio between two immiscible aqueous phases. It is also a measure of drug lipophilicity and capacity to cross the cell membrane. Partition coefficient provides a means of characterizing the lipophilic/hydrophillic nature of the drug. Compounds with negative log P values are greater solubility in water than nonpolar organic solvent, compounds with log P values 0 and 0.1 also poorly observe into lipophillic media. If the partition coefficient of the drug is more than 1 it shows more lipophilicity. The partition coefficient is commonly determined using an oil phase of n-octanol and water. In the case n-octanol and water: $p_{o/w}$ = c(n-octanol) / c(water)

The partition coefficient ($P_{o/w}$) therefore is the quotient of two concentrations of drug in n-octanol ($C_{n\text{-}octanol}$) and water (C_{water}) respectively and is usually given in the form of its logarithm to base 10 (log P).

- **Shake flask method**

The partition coefficient determination study was performed by using the shake flask method. Excess amount of the drug (Cefuroxim) dissolved in 10ml of two solvents (n-octanol: water) together (1:1) and placed for 24 h. After 24 hr, the two layers were separated and centrifuge for 30 minutes at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective λmax after appropriate dilution [9].

3. FTIR of Cefuroxim and Excipients

FT-IR (Fourier Transform Infrated) spectrum of any compound of drug gives information about the groups present in that particular compound. FT-IR spectroscopy was used for structure analysis. The potassium bromide (KBr) disc technique was employed. KBr has transmitance window of 100% (wave number range 4000-400 1/cm) and thus does not show any absorption in IR spectrum. An FT-IR spectrum of Cefuroxim and drug plus excipients mixture was recorded for the determination of drug interaction with excipients. The KBr disc was prepared using 1mg of Cefuroxime/excipients plus drug in 100mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and Cefuroxim was mixed and subjected to hydraulic pressure to form disc. This disc was placed in an FT-IR chamber. Infrared spectrum was recorded in the 4000-400 cm^{-1} region [10].

4. Drug excipient compatibility study by FTIR:

The compatibility of drug with excipients was ascertained by FT-IR was used as tool to detect any physical and chemical interaction between the drug and excipients. Drug and various excipients were mixed thoroughly in ratio 1:1. Sample were scanned by FTIR under the range of 400-4000cm^{-1}.[11]

Preparation of Cefuroxime loaded NS[12,13]

Cefuroxime nanosponges were prepared by different proportions of ethyl cellulose and polyvinyl alcohol by emulsion solvent diffusion technique. In this method, two phases, namely, continuous phase and disperse phase were used. The disperse phase consists of 250 mg Cefuroxime and specified quantity of ethyl cellulose (Table 5) dissolved in 20 mL of dichloromethane. Then the continuous phase comprised of a definite amount of polyvinyl alcohol (Table 5) in 100 mL water. The organic dispersed phase was added slowly into the aqueous continuous phase at 35°C and the reaction mixture was stirred at 1000 rpm for 3hrs on a magnetic stirrer. The formed Cefuroxime nanosponges were collected by vacuum filtration and dried in an oven at 40ºC for 24 h and stored in a desiccator to ensure the removal of residual solvent.

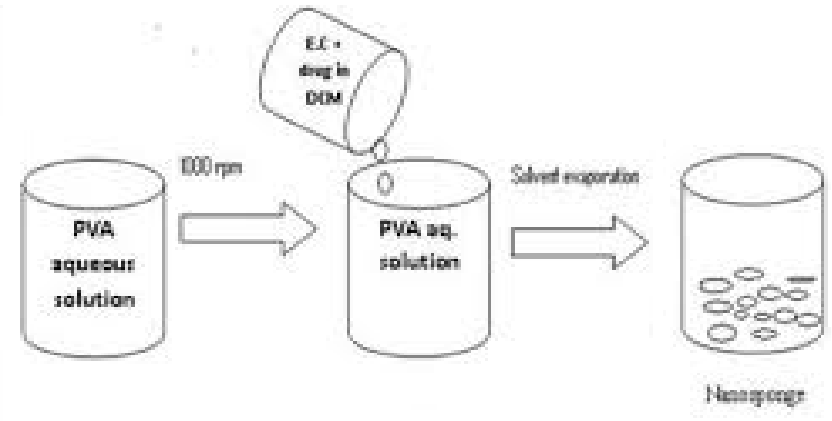


Figure 2: Schematic presentation of the method of preparation of Nanosponge

Table 3: Composition of different nanosponge formulations

Formulation code	F1	F2	F3	F4	F5	F6
Cefuroxime (mg)	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg
Polyvinyl alcohol (mg)	300 mg	250 mg	200 mg	300 mg	250 mg	200 mg
Ethyl cellulose (mg)	200 mg	200 mg	200 mg	300 mg	300 mg	300 mg
Dichloromethane (ml)	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Distilled water (ml)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Characterization of Cefuroxime Nanosponges

1. Entrapment Efficiency & Drug Loading

The entrapment efficiency & drug loading of nanosponges was determined by calculating the amount of entrapped Cefuroxime in then nanosponges. To determine the entrapment efficiency & drug loading of Cefuroxime in nanosponges, calculated amount of nanosponges were adding into 10 ml of methanol. The dispersion was centrifuged for 15 min at 15000 rpm. After centrifugation the supernatant was collected and percentage drug entrapment amount of free Cefuroxime was determined spectrophotometrically (ʎmax= 223 nm). The entrapment efficiency& drug Loading has been determined according to the following equation:

$$EE\ \% = \frac{W_{(Added\ drug)} - W_{(free\ drug)}}{W_{(Added\ drug)}} \times 100 \quad \text{.....(1)}$$

$$.DL\ \% = \frac{W_{(Added\ drug)} - W_{(free\ drug)}}{W_{L(TestedNanosponge)}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, W (added drug) is the amount of drug added during the preparation of nanosponge, W (free drug) is the amount of free drug measured in the lower chamber of the culture tube after centrifugation $W_{L(}$Tested nanosponge) is the amount of total weight of nanosponge tested.

2. In-Vitro Drug Release Study

In-vitro release study of Cefuroxime nanosponges was performed in a USP paddle apparatus (Type II dissolution test apparatus). A sample of nanosponge formulation equivalent to 250 mg of the drug was used for the analysis. The paddle rotation speed was kept at 100 rpm and a temperature of 37 ± 0.5°C was maintained. A release study was carried out in 900 mL of phosphate buffer pH 6.8 (pH of normal skin) as a dissolution medium. Samples (5 mL) were withdrawn from the dissolution apparatus at different time intervals (15, 30, 60,90,120, 180, 240, 360 and 480 min) and replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition. The withdrawal aliquots were filtered and assayed at 223nm. Cumulative percentage of drug release from nanosponges was calculated and compared with that of the pure drug. Percentage cumulative drug determination was carried out in triplicate.

3. Particle size and zeta potential determinations

Particle size analysis and polydispersibility index of nanosponges were performed using dynamic light scattering (DLS) with a scattering angle of 90 at 25 C. The terbinafine nanosponge sample was diluted in distilled water prior to measurement.

4. Morphology study

For morphology, prepared nanosponges were coated with gold–palladium under an argon atmosphere at room temperature and then randomly scanned and photomicrographs were taken at the acceleration voltage of 20 kV. From the resulting image, average particle size was determined.

RESULT AND DISCUSSION

Preformulation studies

Preformulation studies aim to investigate the physical and chemical properties of drug substance. The selected drug was subjected to investigation of physical characterization parameters such as:

Organoleptic properties:

Organoleptic properties of drug cefuroxime found to per I.P monograph. The organoleptic properties of cefuroxime are found to the given Table 4.

Table 4: Organoleptic properties of cefuroxime

Sr. No.	Properties	Inferences
1.	Colour	White
2.	Odour	Odourless
3.	Form	Amorphous
4.	Taste	Bitter

Melting point:

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under one atmosphere of pressure. The melting point determines implies the purity of drug. Melting point of cefuroxime was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in table 5.

Table 5: Meting point of Cefuroxime

Drug	Reference M.P	Observation M.P
Cefuroxime	218-225°C	219.33 °C±0.58

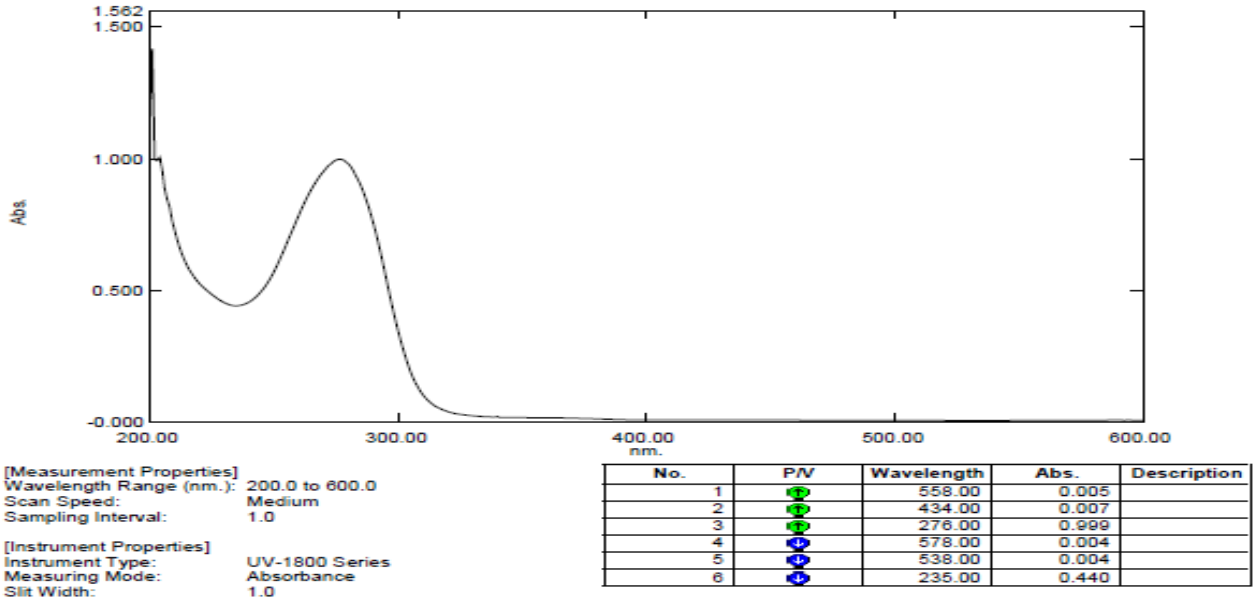
Discussion: The melting point of Cefuroxime was found to be 219.33^0C±0.58 which is in the range of the pure drug. Hence there are no impurities found in the sample.

UV Spectroscopy

Determination of absorption maxima in Methanol

UV-VIS spectroscopy is mainly used for quantitative analysis and serves as a useful auxiliary tools tool for structural elucidation of various drugs to obtain specific information on the chromophoric part of the molecules in solution when exposed to light in the visible/ultraviolet region of the spectrum absorbing light no of particular wavelength depending on the type of electronic transition associated with the absorption. The UV spectrum is generally recorded as a plot of absorbance versus wavelength.

Quantitative analysis of the drug was done was done by using a double-beam UV- visible spectrophotometer. A 12µg/ml solution of Cefuroxime in Methanol was scanned in the range of 200-400nm. The result of the UV spectrum of Cefuroxime is shown in figure 3.



No.	P/V	Wavelength	Abs.	Description
1		558.00	0.005	
2		434.00	0.007	
3		276.00	0.999	
4		578.00	0.004	
5		538.00	0.004	
6		235.00	0.440	

Figure 3: UV Spectrum of Cefuroxime in Methanol

Table 6: Absorption maxima (λ max) of Cefuroxime in Methanol

Name of drug	Absorption maxima (λ max)	
	Observed	Reference
Cefuroxime	276	278

Discussion: The maximum wavelength of Cefuroxime was observed at 276nm.

Preparation of standard curve of Cefuroxime in Methanol

Table 7: Calibration curve of Cefuroxime in Methanol (λ max= 276)

Sr. No.	Concentration (µg/ml)	Absorbance
1.	4	0.178±0.001
2.	8	0.335±0.001
3.	12	0.502±0.002
4.	16	0.646±0.002
5.	20	0.806±0.001
6.	24	0.928±0.001

Mean ± SD, n = 3

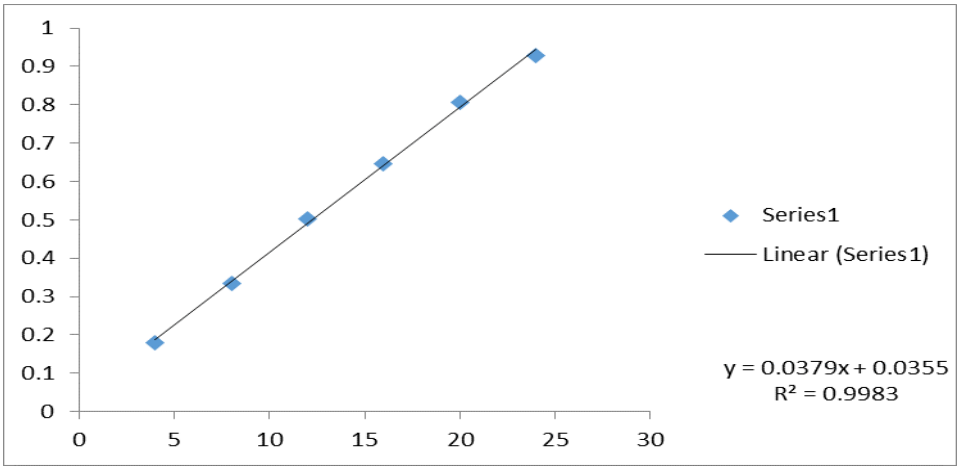


Figure 4: Graph of standard calibration curve of cefuroxime in Methanol.

Table 8: Result of regression analysis of UV method for estimation of Cefuroxime

Statistical parameters	Results
λ max	276
Regression equation ** Y=mx+C	Y=0.037x+0.035
Slope(m)	0.037
Intercept (C)	0.035
Correlation coefficient (R^2)	0.998

Discussion: - The calibration curve for Cefuroxime was obtained by using the 4 to 24 µg/ml concentration of Cefuroxime in methanol. The absorbance was measured at 276 nm. The calibration curve of Cefuroxime as shown in the graph indicated the regression Y=0.037x + 0.035 and R^2 value 0.998, which shows good linearity as shown in **Table 9** and **Figure 6.**

Solubility studies

Solubility of drug in various solvents were carried out to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 276 nm.

Table 9: Solubility studies of Cefuroxime for different solvents

Name of Solvent	Solubility (mg/ml)	Solubility
Water	1.034±0.001	Slightly soluble
Phosphate buffer saline pH 7.4	0.894±0.002	Insoluble
Ethanol	10.262±0.001	Sparingly soluble
Methanol	10.127±0.001	Sparingly soluble
Chloroform	10.846±0.007	Sparingly soluble

* Each value is the mean of three independent determination.

Mean ± SD; n = 3

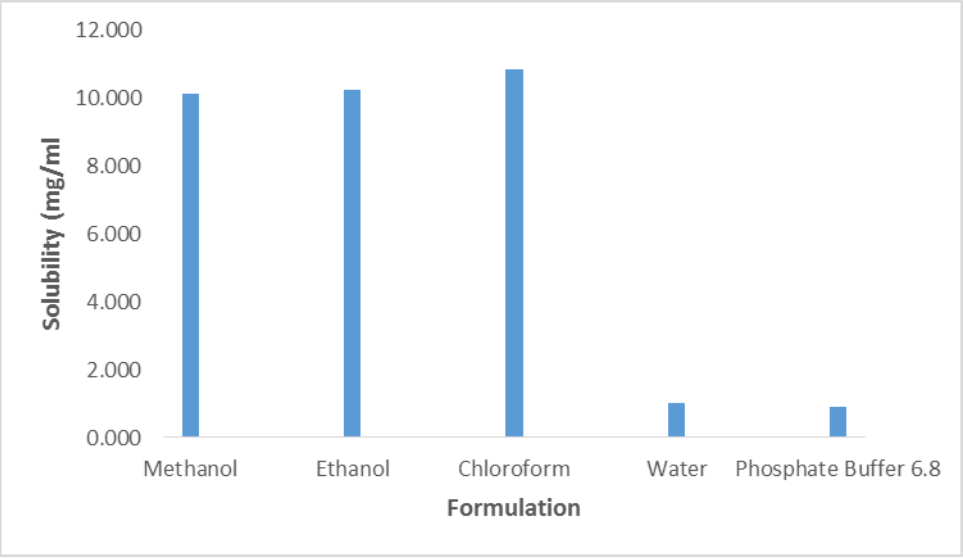


Figure 5: Solubility study of the drug in different solvents

Discussion: From the above data, it is clearly seen that Cefuroxime is highly soluble in Chloroform, ethanol and methanol (**Figure 7 and Table 11**).

Partition coefficient determination

The partition coefficient of the Cefuroxime was determined using n-Octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the hydrophilicity and purity of the drug.

Table 10: Partition coefficient determination of Cefuroxime

The partition coefficient of drug	Solvent system	Reported Log P Values	Observed Log P Values
Cefuroxime	n-octanol:water	0.89	0.80±0.005

Value is expressed as mean ± SD; n = 3

Discussion: The partition coefficient of Cefuroxime in n-Octanol: water was found to be 0.080±0.005 this indicates that the drug is hydrophilic in nature (**Table 12**).

FTIR Studies

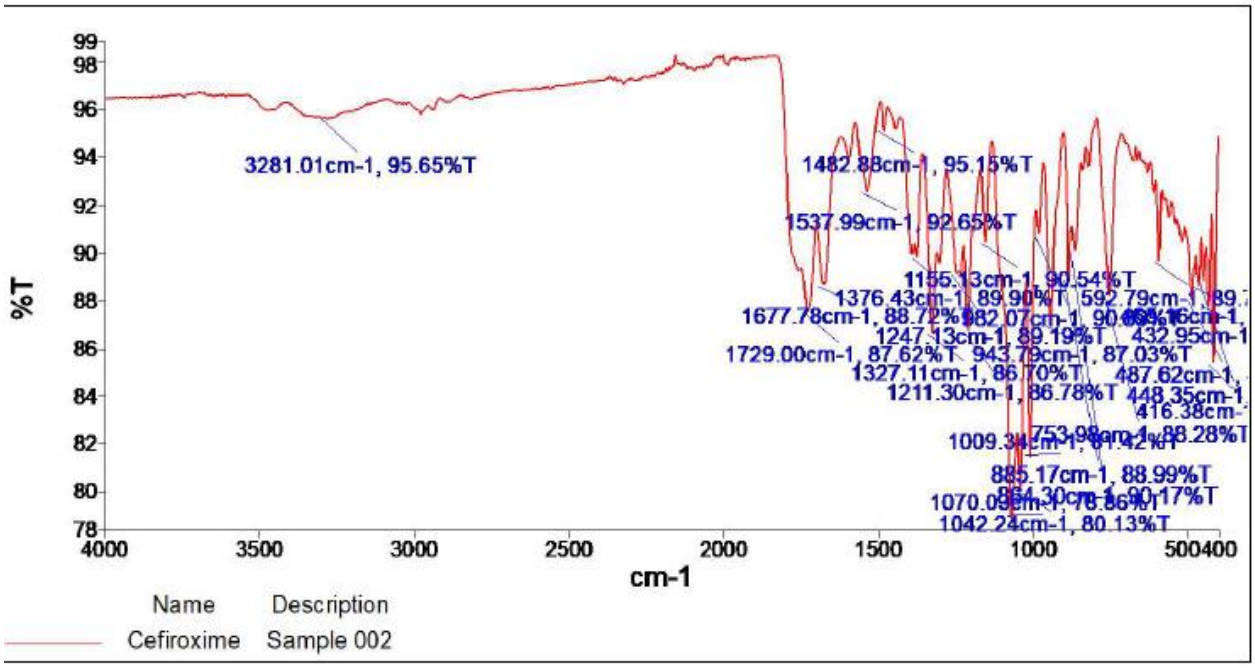


Figure 6: **FTIR study of Cefuroxime**

Table 11: FTIR interpretation of cefuroxime

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
C-N stretching	1677.68cm^{-1}	1677.78cm^{-1}
C-C stretching	1390cm^{-1}	1376.43cm^{-1}
C-S stretching	943cm^{-1}	943.79cm^{-1}
C-O stretching	1460.6 cm^{-1}	1482.88 cm^{-1}

Discussion: The FTIR spectra of Cefuroxime are shown in **Figure 8**; **Table 13**. The IR absorption peaks of Cefuroxime shown at 1677.78 cm^{-1} (C-N stretching), (C=C Stretching), at 1376.43 cm^{-1}(C-S Stretching), at 943.79 cm^{-1}(C-O Stretching), at 1482.88 cm^{-1} were all observed in the spectra of Cefuroxime. This observation confirmed the purity and authenticity of the Cefuroxime.

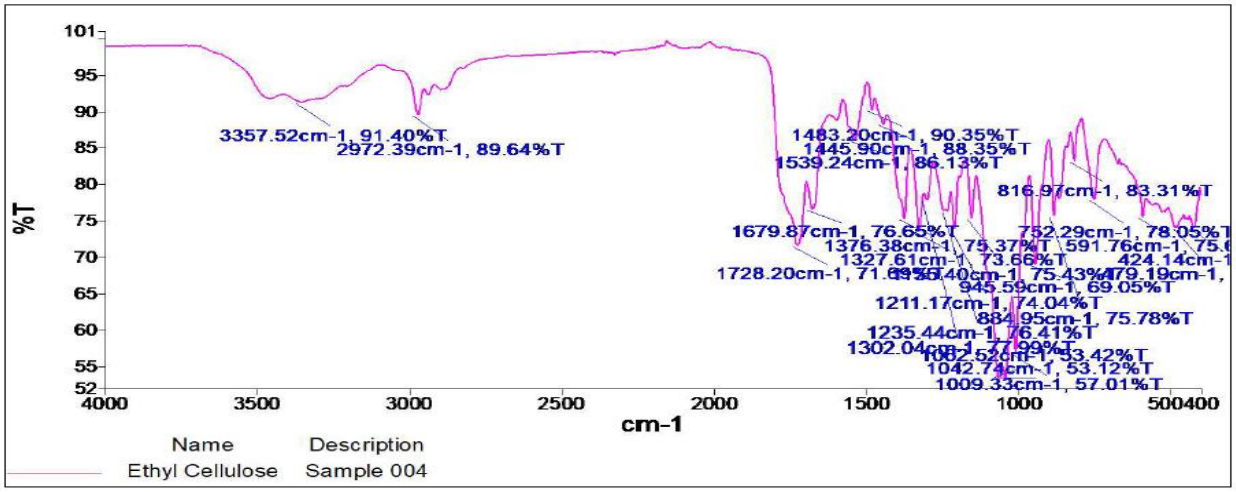


Figure 7: FTIR Spectrum of Ethylcellulose

Table 12: Interpretation of FTIR spectrum of Ethylcellulose

Reported peak (cm^{-1})	Observed peak (cm^{-1})	Characteristics Peaks
2974cm	2972.39cm^{-1}	C-H stretching
3485cm	3357.52 cm^{-1}	-OH stretching
1091 cm	1062cm^{-1}	C-O-C stretching
1373cm	1376 cm^{-1}	C-H Bending

Discussion: The FTIR spectra of ethyl cellulose are shown in figure 9 and Table 14. The principal IR absorption peaks of ethyl cellulose were observed at 2972 cm^{-1} (C-H) stretching, 3357.52 cm^{-1} (O-H bonding), 1062 cm^{-1} (C-O-C stretching), 1376 cm^{-1} (C-H) Bending. These observed principal peaks confirmed the purity and authenticity of the Ethyl cellulose.

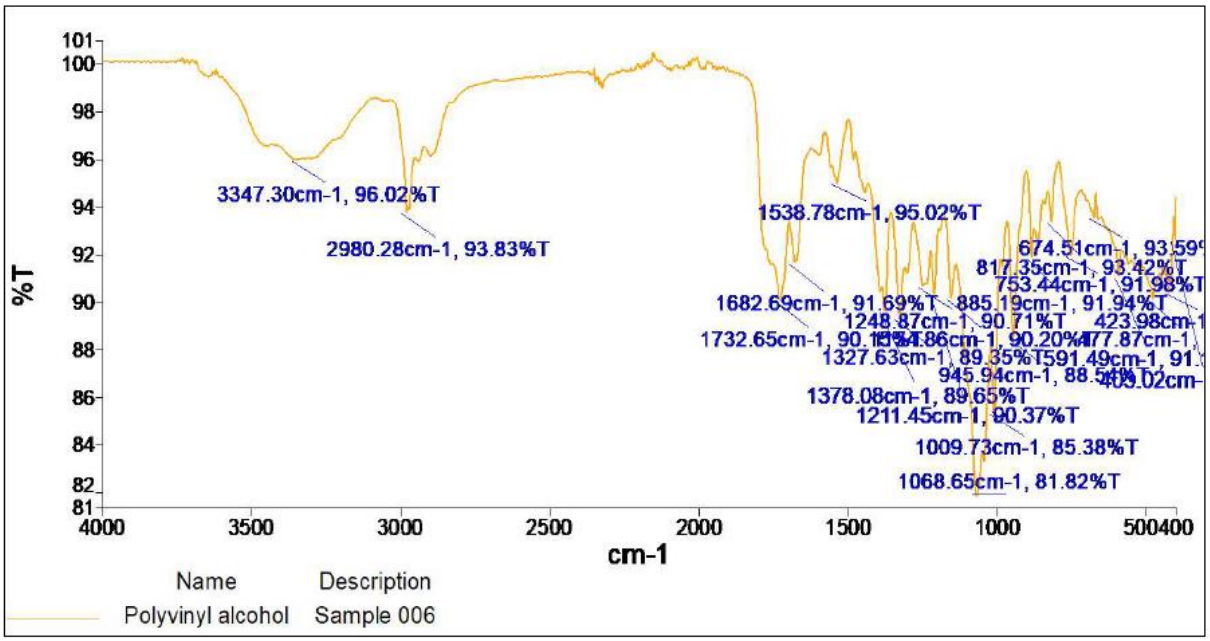


Figure 8: FTIR Spectrum of Polyvinyl alcohol

Table 13: Interpretation of FTIR spectrum of polyvinyl alcohol

Characteristics Peaks	**Reported (cm^{-1})**	**Observed(cm^{-1})**
O-H streching	3550-3200cm^{-1}	3347.30cm^{-1}
C-H stretching	2840-3000cm^{-1}	2980.28cm^{-1}
C=O stretching	1750-1735cm^{-1}	1732.65cm^{-1}

Discussion: The FTIR spectra of polyvinyl alcohol are shown in Figure 10; table 15. The principal IR absorption peaks of polyvinyl alcohol were observed at 3347.30cm^{-1} (O-H) stretching, 2980.28cm^{-1} (C-H bonding), and 1732.65cm^{-1} (C=O stretching). These observed principal peaks confirmed the purity and authenticity of the Polyvinyl alcohol.

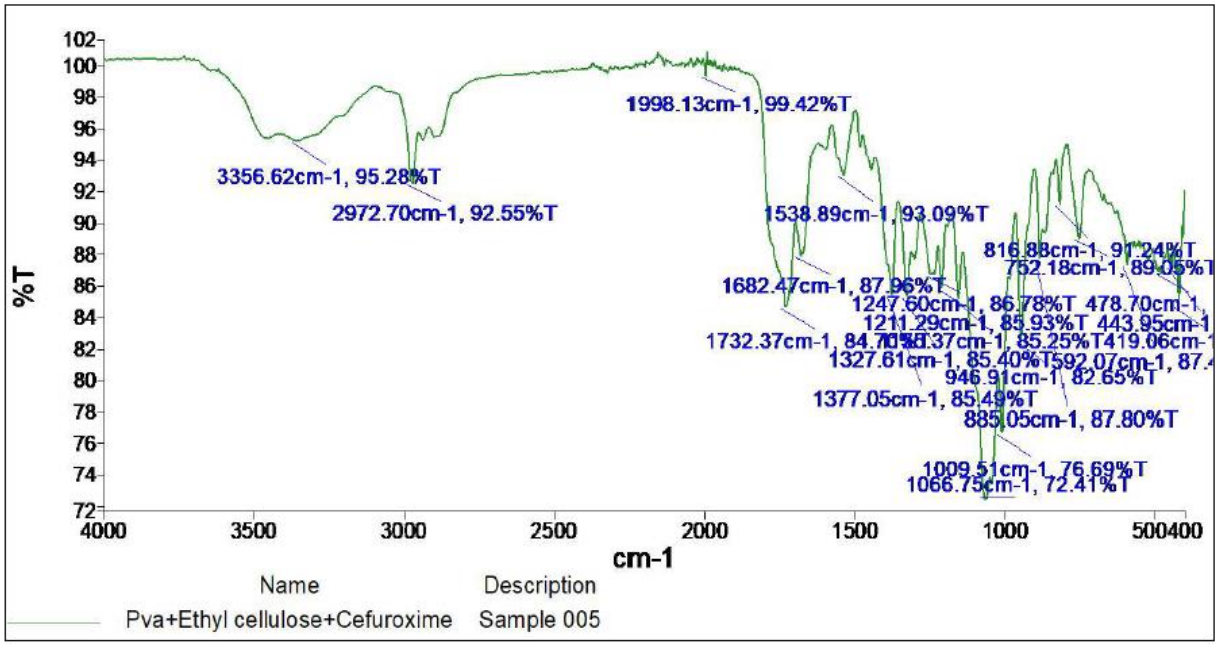


Figure 9: FTIR Spectrum of Physical mixture

Table 14: Interpretation of FTIR spectrum of Physical mixture

Reported peak (cm^{-1})	Observed peak (cm^{-1})	Characteristics peaks
1677.78cm^{-1}	1682.47cm	C-N stretching
1376.43cm^{-1}	1377.05cm	C-C stretching
943.79cm^{-1}	946.91cm	C-S stretching
1482.88cm^{-1}	1538.89cm	C-O stretching
3357.52cm^{-1}	3356.62cm	-OH stretching
1376cm^{-1}	1377.05cm	C-H Bending
2980.28cm^{-1}	2972.70cm	C-H stretching

Discussion: FTIR of physical mixture studies (figure: 11 table: 16) were carried out to eliminate the possibility of interaction between drug and excipients used with the analytical method of drug estimation. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipient peaks. Hence no interaction was observed in this mixture.

Characterization of Polymer for nanosponge preparation

For the preparation of Cefuroxime loaded Nanosponge, the carriers (polyvinyl alcohol and ethyl cellulose) were subjected to screening for the carriers to be utilized for successful formation of nanosponge.

Preparation of nanosponge of cefuroxime

Nanosponge was prepared by using the mind technique. Carriers that are used for the preparation of Nanosponge are mentioned in table 15.

Table 15: Composition of Nanosponge of Cefuroxime

S.No	Formulation code	Molar Ratio	Drug (mg)	Polyvinyl alcohol	Ethylcellulose	Dichloromethane (ml)
1	F1		250	300	200	20
2	F2		250	250	200	20
3	F3		250	200	200	20
4	F4		250	300	300	20
5	F5		250	250	300	20
6	F6		250	200	300	20

Evaluation of nanosponge of Cefuroxime

Entrapment Efficiency & Drug Loading

Percentage Drug Entrapment and loading of all formulations was given in table 18.

Table 16: Percentage Entrapment Efficiency & Drug Loading of Nanosponge formulations containing Cefuroxime.

S.No.	FormulationCode	(%)Entrapment efficiency	Drug loading (%)
1	F_1	74.769±0.161	33.02599±0.0272
2	F2	88.506±1.225	26.21055±0.0355
3	F3	84.051±0.235	27.59297±0.0293
4	F4	78.133±0.093	30.9478±0.0600
5	F5	77.342±0.539	29.18702±0.0343
6	F6	69.718±1.516	28.40724±0.0496

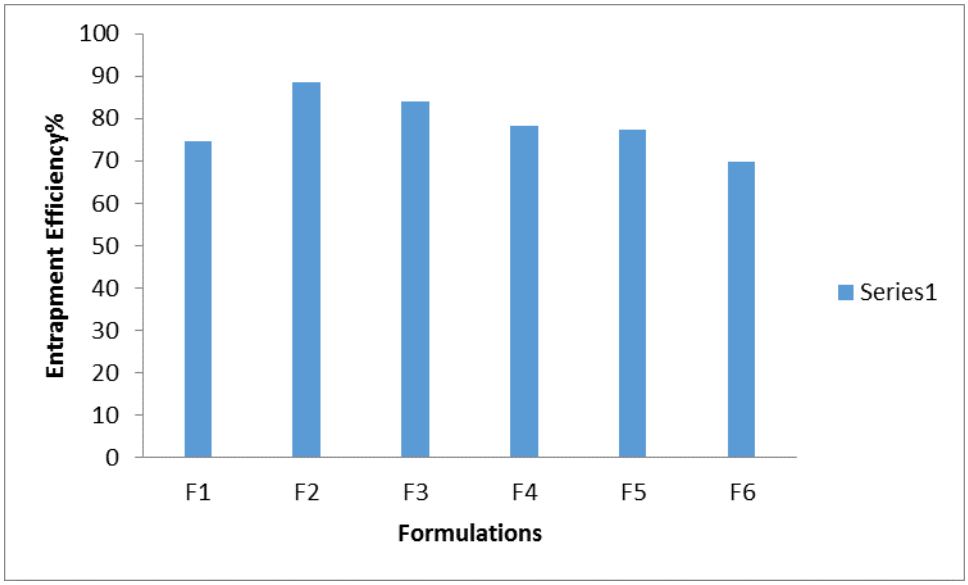


Figure 10: Percentage of drug entrapment of Nanosponge formulations containing Cefuroxime

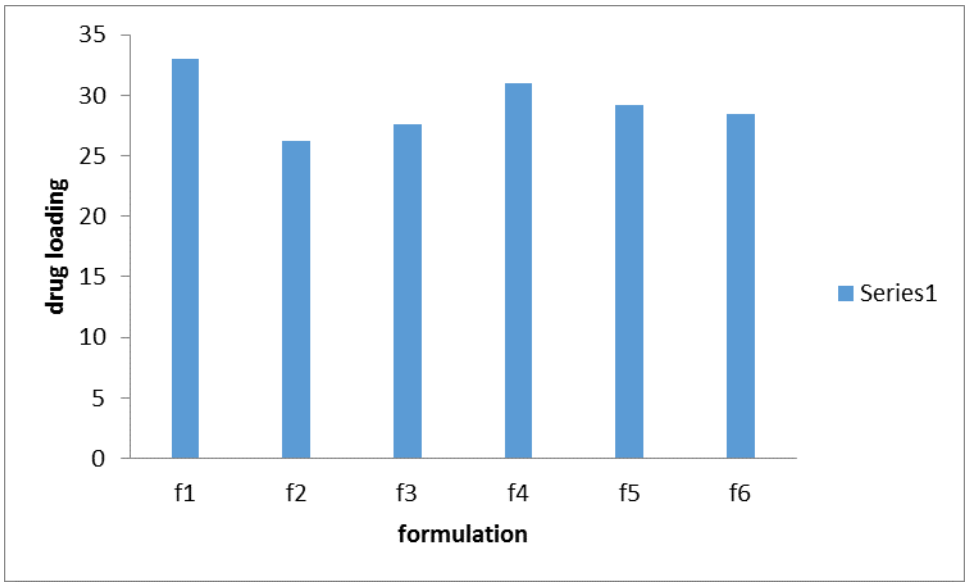


Figure 11: Percentage drug loading of Nanosponge formulations containing Cefuroxime

From **Table 12 and 13**, it was found that the Percentage drug entrapment of all formulation was found to be in a range 74.769±0.161 to 69.718±1.516 & drug loading of all formulation was found to be in the range 33.02599±0.0272 to 28.40724±0.0496.

FTIR spectra of the final formulation

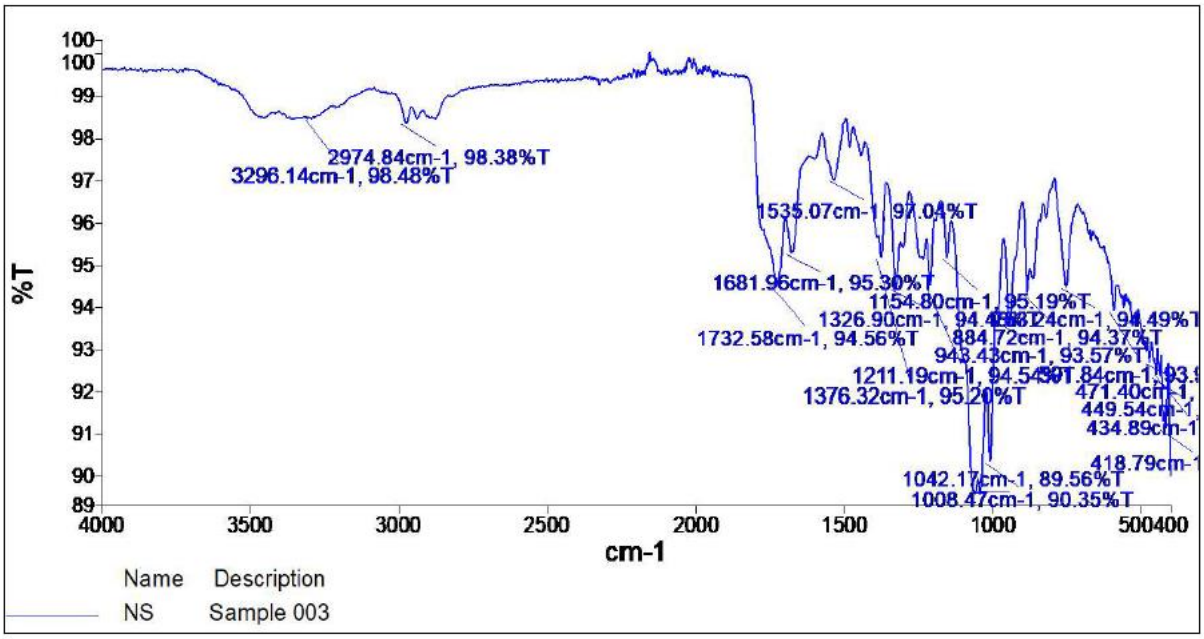


Figure 12: FTIR spectra of Formulation F2

Table 17: FTIR interpretation of FTIR spectra of F2 formulation batch

Characteristics Peaks	**Reported (cm^{-1})**	**Observed(cm^{-1})**
C–O stretching band	1141.9 & 1076.32	1178.55
Carbonyl group (C=O) band	1759.14	1745.64
C–H stretching vibration peak	2974.33 & 2870.17	2976.26
–OH stretching vibration peak	3489.81	3506.7
C–H bending	1373.36	1371.43

As seen **Figure 13 demonstrates** spectrum of formulation (F2), peaks were obtained at 1178.55cm^{-1} (C–O stretching band),1745.64 cm^{-1} (Carbonyl group (C=O) band), 2976.26 cm^{-1} (C–H stretching vibration peak), 3506.7cm^{-1} (–OH stretching vibration peak)and 1371.43cm^{-1} (C–H bending). The FT-IR spectra of the final formulation Terbinafine-loaded nanosponge (F2) maintained some of the Terbinafine peaks with slight shifting.

Particle Size of F2 Formulation

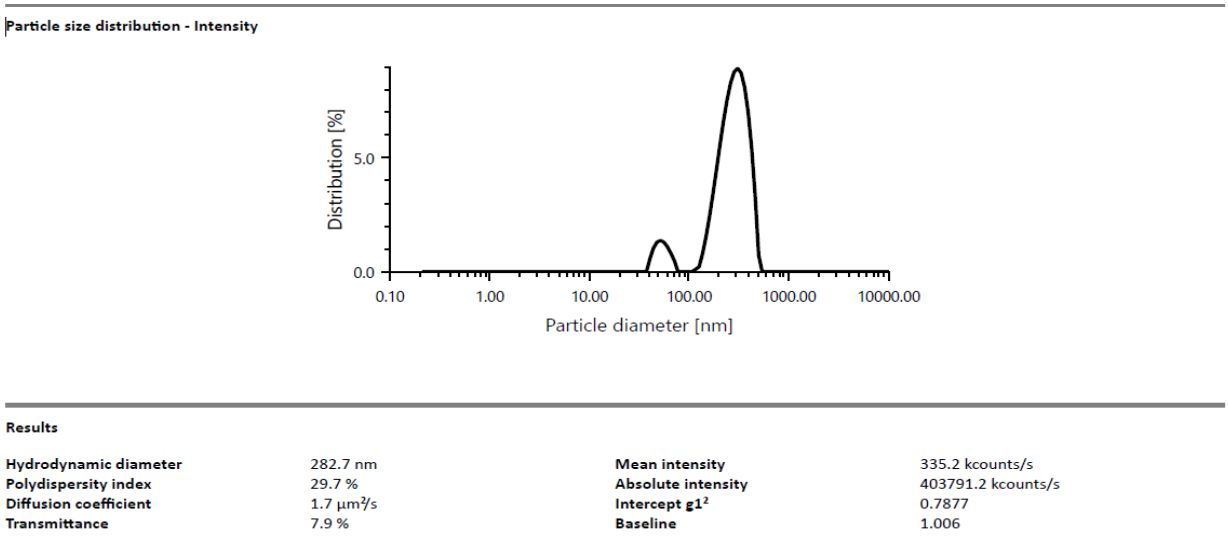


Figure 13: Particle size peak of F2 formulation

Figure 13 demonstrated particle size of the F2 formulation was 282.7 nm with PDI 29.7%.

Zeta Potential of F2 Formulation

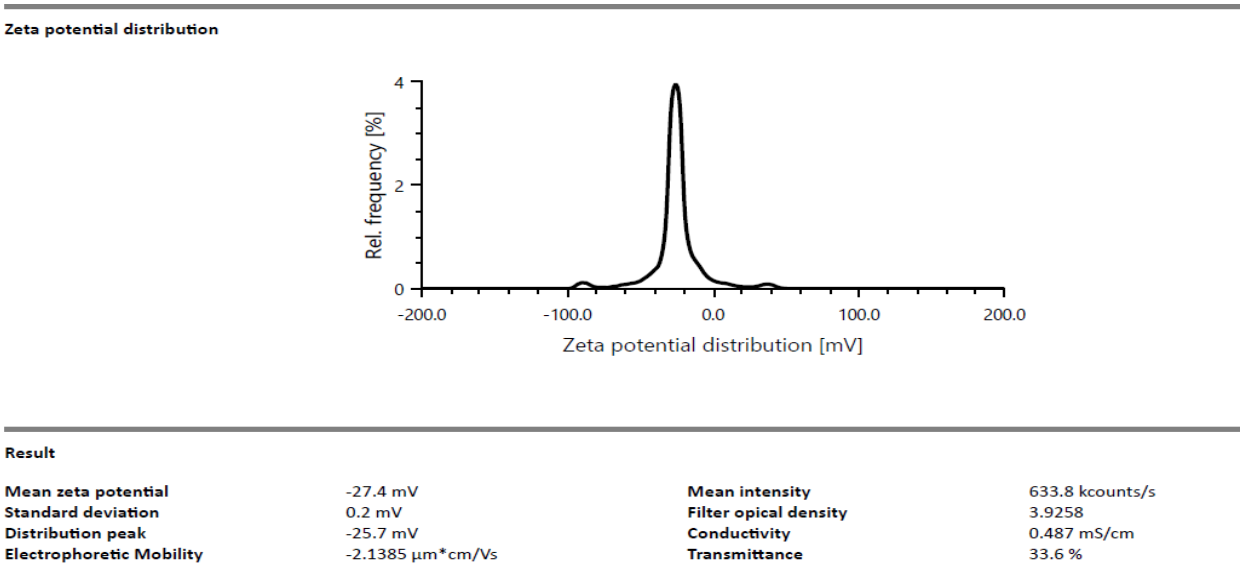


Figure 14: Zeta potential graph of F2 formulation

Discussion: Figure 16 demonstrated zeta potential of F2 formulation was -27.4 mV represents the stability of the formulation.

Scanning Electron Microscopy

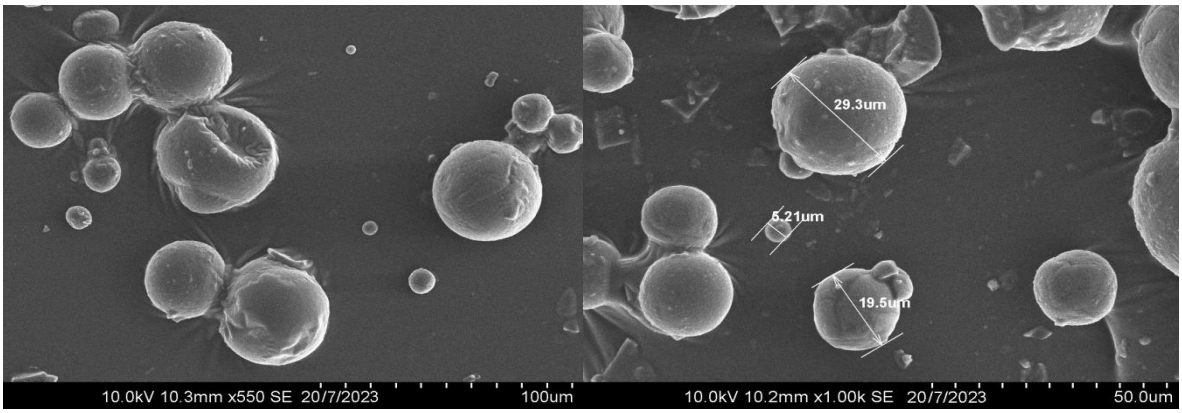


Figure 15: SEM Image of Formulation F2

Discussion: The SEM study was done to check the surface morphology of the drug particles. The SEM of Nanosponge was of irregular shape and size. Figure 17 clearly demonstrates crystal shape of Cefuroxime was completely changed in Nanosponge showing embedded Cefuroxime crystals in the matrix.

In-vitro Drug release study

The in-vitro drug release of Formulation F2 and Pure drug was given in table 20.

Table 18: Percentage of drug release of Formulation F2 and Pure drug

Sr.No.	Time (hr)	Pure Drug release (%)	Drug Release of Formulation (%)
0	0	0	0
1	0.083	4.505±0.826	14.957±2.564
2	0.250	15.135±0.541	25.214±0.740
3	0.417	25.405±0.541	30.051±2.669
4	0.583	32.293±2.672	39.744±1.282
5	0.750	34.054±0.541	55.983±1.282
6	0.917	36.216±1.081	62.821±4.121
7	1.083	39.820±0.624	64.530±1.480
8	1.250	44.144±2.437	73.415±1.282
9	1.417	58.378±2.162	83.368±2.669
11	1.583	66.486±0.541	87.607±2.564
12	1.750	77.117±0.826	91.880±1.958
13	2.000	84.405±0.826	98.632±1.480

Value is expressed as mean ± SD; n = 3.

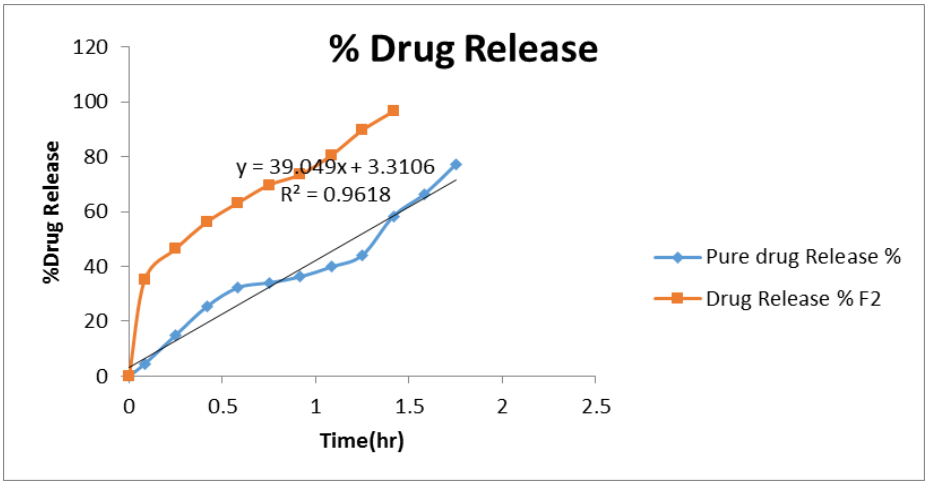


Figure 16: In-Vitro Drug Release of Nanosponge of Cefuroxime and Pure Drug.

Discussion: The in-vitro release of drug from the Nanosponge of Cefuroxime was found to be higher as compared to a pure drug that showed the effect of the carrier in drug release property. Table 20 indicated that in vitro release of Nanosponge showed 98.632±1.480 released within 2 hours. The release profiles of Nanosponge of Cefuroxime employed yielded an immediate Cefuroxime release. From the in-vitro drug release study it was found that F2 formulation showed higher drug release as compared to pure drug.

In-vitro drug release kinetic

To understand the mechanism by which the drug was released from the Nanosponge of ethyl cellulose and Cefuroxime, F2 formulation, various release kinetics models including zero order, first order, Higuchi and Korsmeyer - Peppas model were applied as shown in Figure 19 to 22.

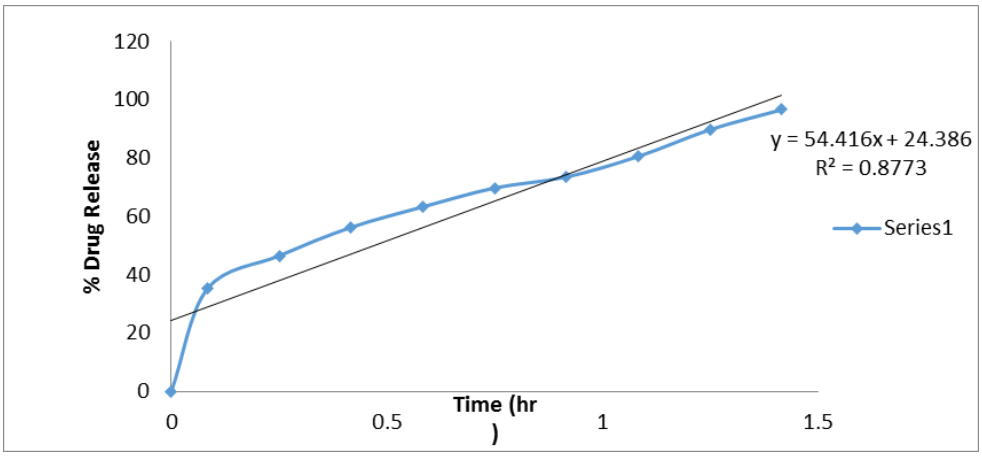


Figure 17: Zero order release kinetics of optimized F2 formulation

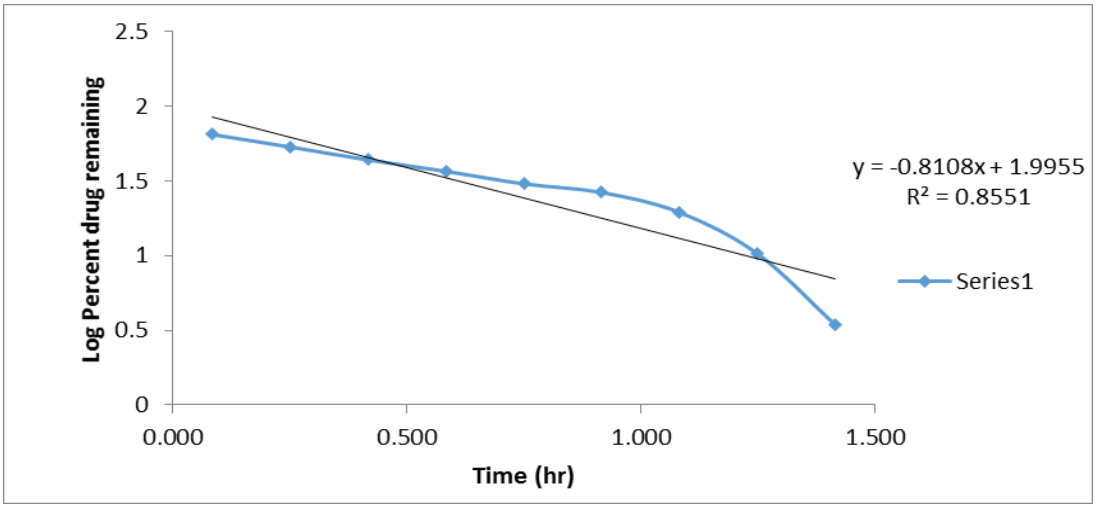


Figure 18: First-order release kinetics of optimized F2 formulation

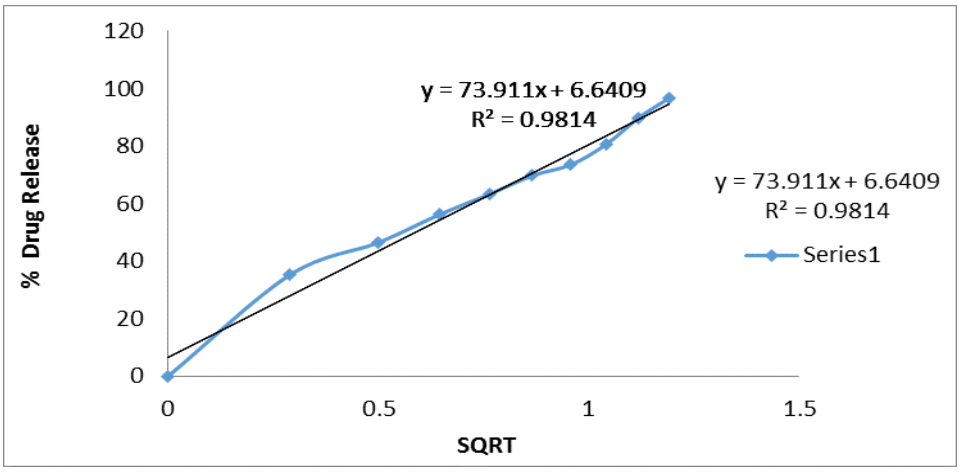


Figure 19: Higuchi order release kinetics of optimized F2 formulation

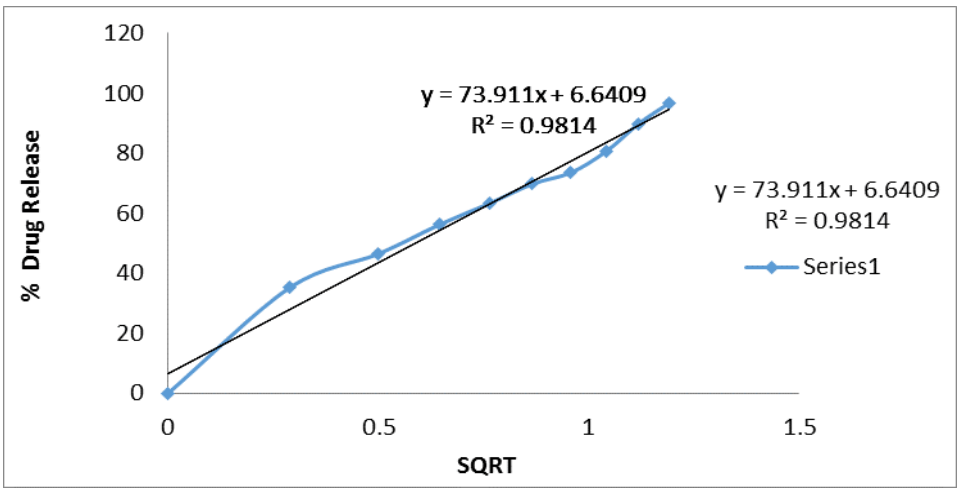


Figure 20: Korsmeyer Peppas release kinetics of optimized F2 formulation

Mathematical models are commonly used to predict the release mechanism and compare the release profile. For all the optimized formulations, the % drug release vs time (zero order), log percent drug remaining vs time (first order), log percent drug release vs. square root of time (Higuchi plot), log of log % drug release vs. log time (Korsmeyer and Peppas Exponential Equation) and cube root of initial drug concentration - cube root of a fraction of drug remaining vs time (Hixson Crowell plot) were plotted.

Table 19: Kinetic equation parameter of formulation F2

Formulation Code	Zero order		First order		Higuchi		K. Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F2	54.410	0.877	0.810	0.855	73.910	0.981	0.007	0.976

In each case, R^2 value was calculated from the graph and reported in Table 19 and Figure 19 to figure 22. Considering the determination coefficients, First Order model was found (R^2=0.981) to best fit the release data. This demonstrates that Cefuroxime molecules was loaded in the Nanosponge and the drug was released from the Nanosponge by an immediate mechanism.

CONCLUSION:

It is well known that 1/3rd of drug population is water-insoluble. Hence there is a need for enhancement of solubility and dissolution of such drugs. It emerges out of the analysis of most of the approaches that these are based on generating drug dispersion at nanoscale level. To maintain faster solubility and dissolution kinetic of nanosponge, it is essential to keep integrity of such active pharmaceutical ingredients. From all aspects, I concluded that in vitro data obtained for nanosponge of cefuroxime showed increase solubility in phosphate buffer having pH=6,8 and the method of preparation of cefuroxime found to be simple, reproducible, provide good solubility and drug content. Also, it is obtained that there was an increase in solubility when polyvinyl alcohol used as a carrier in 1:1. Thus formulation prepared slowed immediate release behavior.

REFERENCES

1) A.R. Thakre, Y. N. Gholse, R. H. Kasliwal. “Nanosponges: A novel approach of drug delivery system” Journal of Medical Pharmaceutical and Allied Sciences, 2016; 23:78-92.

2) Tukaram S. Patil, Nishigandha A. Nalawade, Vidya K. Kakade, Sumedha N. Kale. "Nanosponges A novel targeted drug delivery for cancer treatment" international journal of Advance Research and Development, 2017; Vol. 2(4):55-62 .
3) Priyanka D, Sindhu S, Saba Maanvizhi "Design Development and Evaluation of Ibuprofen Loaded Nanosponges for Topical Application" International Journal of ChemTech Research, 2018;11, :217-227 .
4) Shaikh Bilal J, Patil Abhishek S, Bhosale Ankush S, Raut Indrayani D, Nitalikar Manojkumar M. "Nanosponges: an evolutionary trend for targeted drug delivery" Int. Journal of Pharmaceutical Sciences and Medicine,2021; .6(2), 1-14.
5) Ali J,Khar R and Ahuja A, A textbook of dosage form design. Birla Publications Pvt Ltd, Delhi. 2008, Page no: 100-107.
6) Pandey A and Rath B. Pharmaceutical Preformulation Studies with Special Emphasis on Excipients Compatibility Chem Inform.2012; 43(23) : page no: 20-25.
7) Casay G, Quattrocchi O, Hauck W, Hernandez-Cardoso A and Belsky J.USP melting point reference standards: Evaluation of parameter that affect the melting point, USP Pharmacopeial Forum, PF39(4).
8) Pavia DL, Lampmam GM and Kriz GS. Introduction to Spectroscopy. 3RD Harcourt college publishers, Orlando, Florida, USA. 2001.
9) Baka E, Comer JE and Takacs-Noovak K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as a model compound. Journal of Pharmaceutical and Biomedical Analysis 2008; 46(2): 335-341.
10)Xia XR, Baynes RE, Monteiro-Riviere JE. Determination of the partition coefficients and absorption kinetic parameters of chemicals in a lipophilic membrane/water system by using a membrane-coated fiber technique. European Journal of Pharmaceutical Sciences 2005 :;24(1): 15-23.
11)Chatwal GR and Anand SK. Instrumental methods of chemical analysis. 5TH Himalya publishing house, Delhi. 2002.
12)Badwan AA, Rashid I, AI Omari MM, Darras FH, Chitin and chitosan as direct compression excipients in pharmaceutical applications. Marine drugs. 2015; 13(3): 1519-1547.