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FABRICATION OF CEFPODOXIME PROXETIL NANOPARTICLES BY SOLVENT ANTI-SOLVENT PRECIPITATION METHOD FOR ENHANCED DISSOLUTION

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ABSTRACT

The aim of present investigation was to enhance the dissolution rate of a poorly water soluble drug cefpodoxime proxetil by nanosuspension formation using a solvent precipitation method. Cefpodoxime proxetil (CP), a semi-synthetic β- lactum antibiotic of cephalosporin class belonging to BCS class IV with poor solubility and poor permeability is the ideal drug candidate with limited oral bioavailability when orally administered. Selected parameter of nanosuspension such as concentration of drug, solvent-anti solvent volume ratio were varied so as obtain nanoparticle within the size range less than 1µm. Characterization of the cefpodoxime proxetil of nanoparticle was carried out on the basis of scanning electron microscopy (SEM), X-ray diffraction (XRD), FTIR spectrum, particle size, and zeta potential and dissolution tester. Results signify that the combination of lowest concentration of stabilizer HPMC E50 (0.1%w/v) with low stirring speed (800 rpm) tends to achieves mallest particle size 755.6 nm and -22.6 mV, respectively. FTIR spectra shown that no chemical incompatibility between drug and polymer exist. The *in-vitro* drug release (%) cumulative profile of cefpodoxime proxetil nanosuspension was significantly higher (94%) as compared to marketed preparation (54%) in simulated intestinal fluid (pH 6.8).

KEYWORDS

Nanoparticle, Nanosuspension, Anti-solvent and Solubility.

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INTRODUCTION

The design and development of new drug delivery systems with a view to enhance the efficacy of existing drugs is an ongoing process in pharmaceutical research. In drug discovery, about 40% of exciting new molecular entities (NMEs) display low solubility in water leading to poor bioavailability (Subhashis *et al.,* 2011)[1]. It depends

on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism, and susceptibility to efflux mechanisms. Poor bioavailability in oral route has the consequence of more variability and poor controlled plasma concentration and drug effects to the patients. In recent years, much attention has been focused on nanotechnology for delivering of formulations, which is being applied to enhance the solubility bioavailability of lipophilic drugs (Savjani *et al.,* 2012)[2].The nano-sizing of drugs has the potential to increase surface area therefore, enhance solubility, increase rate of dissolution, increase oral bioavailability, more rapid onset of therapeutic action (Shafiq-un-Nabi *et al.,* 2007)[3]. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response. Low aqueous solubility is the major problem encountered during formulation development of new chemical entities as well as generic development as therapeutic efficacy of a drug depends upon the solubility of drug molecules (Alex *et al.,* 2012)[4]. For the effective formulation and to sort out the drug solubility related problem, one should have to be very familiar with the Biopharmaceutical Classification System (BCS). The introduction of the Biopharmaceutical Classification System (BCS) in FDA guidelines represents a major step forward in the regulation of oral drug products. The BCS groups poorly soluble compounds as Class II drugs features poor solubility, high permeability whereas class IV drugs features poor solubility and poor permeability. Class I drugs do not pose any problem in absorption (though its systemic availability may be low due to first pass metabolism) when solubility or permeability are considered, therefore efforts are made to change the properties of Class II, III, IV drugs with respect to dissolution and permeability in order to resemble Class I (Khadka *et al.,* 2014)[5]. Poor bioavailability by the oral route can be due to poor solubility, degradation in GI lumen, poor membrane permeation, pre-mucosal clearance and pre-systemic elimination. The lipid based formulation approach has attracted wide attention in order to enhance drug solubilisation in the gastrointestinal tract (GIT) and to improve the oral bioavailability of poor water soluble drug.

A pharmaceutical nanosuspension is defined as "finely divided biphasic colloidal dispersions of nano size drug particles which are stabilized by surfactants materials".The particle-size distribution of the solid particles in nanosuspension is usually less than 1μm. (Geetha *et al.,* 2014)[6]. The reduction of size of drug particles up to submicron range leads to significant increase in dissolution rate and therefore, enhances bioavailability. Nanosuspension formulation approach is most suitable for the compound with high log P value, high melting point and high dose and for the drugs that are insoluble both in water and in organic media (Shete *et al.,* 2014)[7].

Cefpodoxime proxetil (CP)[8], the drug candidate belonging to class IV category is orally administered, extended spectrum, semi-synthetic β-lactum antibiotic of cephalosporin class. Cefpodoxime proxetil is prodrug; its active metabolite is cefpodoxime. It is used in various conditions such as for the treatment of bacterial infection, like urinary tract infection, gonorrhoea, skin infection and upper and lower tract infection. It is active *in vitro* and *in vivo* again a wide range of Gram - positive and Gram - negative organism, including *Staphylococci, Streptococci, Heamophillus, Influenza, Neisseria gonorrhoeae Escherichia coli, Klebsiella, Pheumoniae.* It is administered as a dry syrup formulation, has been shown to be effective and safe in the treatment of bacterial infection in pediatric patient. Oral cefpodoxime proxetil treats bacterial infection by binding to penicillin- binding proteins thereby causing abnormal bacterial cell wall synthesis and lysis (Borin *et al.,* 1991)[9].

After oral administration, cefpodoxime proxetil is absorbed from the gastrointestinal tract and de-esterifies to active metabolite cefpodoxime. Over the recommended dosing for adult, the usual dose is 100 mg (tablet or suspension) administered orally twice. Dosage must be adjusted according to age and patient symptoms. In cases of severe infection, 200

mg oral dose of the drug should be given twice daily after meal. For treatment of urinary tract infection, respiratory tract infection and uncomplicated gonorrhoea, the usual dose for either the tablet or suspension formulation is 200 mg/day (administered as a single dose). Once daily dosage regimen of cefpodoxime proxetil is currently under investigation for several indications. For children, the usual dose is 5 mg/kg administer twice daily (every 12 hrs for a total daily dose of 10 mg/kg). 50% administered cefpodoxime dose was absorbed systemically. Its action is by binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall; it inhibits the bacterial cell wall synthesis. It is highly stable in the presence of beta-lactamase enzymes. Since it belongs to class IV it has poor solubility and poor permeability in water but can be improved by using novel technology i.e. nanosuspension technology based on particle engineering. The elimination half-life of cefpodoxime proxetil is 2-2.5 hrs and its molecular weight is 557.59 g/mol which make it a suitable drug candidate for administering in the form of nanosuspension.

Nanosuspension had been reported to enhance adsorption and bioavailability it may help to reduce the dose of the convectional oral dosage forms. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster (e.g., oral or intravenous i.e. IV administration of the nanosuspension). This is one of the unique advantages that it has over other approaches for enhancing solubility. The solubility or dissolution rate of a drug is the key-factor in determining the rate and extent of its absorption. Enhancement of the dissolution rate is vital to attain a suitable blood concentration for therapeutic effect, as dissolution rates are typically the rate limiting step for bioavailability (Martindale *et al.,* 2014)[10].

It is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without blockade of the blood capillaries.

Oral route is most preferred route by medical practitioners and manufacturer for the administration of antibiotics due to highest acceptability of patients. So, the aim of the present study was to fabrication of cefpodoxime proxetil nanoparticle for oral delivery system used for the treatments of mild to moderate respiratory tract infection, uncomplicated gonorrhoea and urinary tract infection.

MATERIALS AND METHODS

Materials

Cefpodoxime proxetil was obtained as a gift sample from Uni Speed Pharmaceuticals Pvt. Ltd. Baddi, India. Acetone, DMSO, was purchased from Qualigens Ltd Mumbai, India. Ethanol, Methanol was sourced from Changshu Yangyuan chemical, China and Fisher scientific Pvt, Ltd. Mumbai, India. Hexane, PEG 800, Hydrochloric acid, Potassium Dihydrogen Phosphate was purchased from Qualikems Fine Chem Pvt. Ltd. Oleic acid, Tween 80, Lactic acid were purchased from Molychem, India. PEG 400 was obtained from S.D.Fine Chem. Pvt Ltd. Hydroxy propyl methyl cellulose (E50) was obtained from Colorcon Asia Pvt. Ltd. Sodium hydroxide was obtained from Avarice Laboratories Pvt. Ltd. All chemicals and solvents were of analytical grade. Freshly double distilled water was used in the experiments.

Methods

Determination of organoleptic properties

The physical identification of cefpodoxime proxetil was done by checking its physical appearance i.e. colour, odour, taste and state. Weighed quantity of cefpodoxime proxetil as drug was taken and viewed in well illuminated place.Very less quantity of drug was smelled to get the odour.

Determination of Melting point

Melting point of the drug was determined by using capillary method. Drug was filled into capillary tube by sealing its one end at the height of 3 mm from the closed end. Then, the capillary was introduced into the digital melting point apparatus and the point at which the drug starts melting was noted until the entire sample get melted.

Identification of drug by FTIR and UV-Visible spectroscopy

Fourier transforms infrared spectral spectroscopy (FTIR)

The pure drug was mixed with IR grade potassium bromide in a ratio of (1:100) and pellets were prepared by applying 10 metric ton of pressure in shimadzu hydrophilic press. The pellets were then scanned over range of 4000-400 cm^{-1} in FTIR spectrometer. FTIR spectrum of cefpodoxime proxetil showed the presence of the peaks which complies with the reference spectra.

UV- Visible spectroscopy

100 mg of cefpodoxime proxetil was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 10 ml methanol and sonicated for 5 min. Final volume was made up to the mark with same solvent and strength of 1000 µg/ml was obtained. Further dilution was made with methanol to get 100 µg/ml solutions and scanned under 200 nm to 400 nm in UV-Visible Spectrophotometer (Swamy *et al.,* 2012)[11].

Preparation of standard calibration curve of cefpodoxime proxetil

Preparation of stock solutions of cefpodoxime proxetil in methanol

100 mg of cefpodoxime proxetil was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 100 ml methanol and sonicated for 5 min. Final volume was made up to the mark with same solvent and strength of 1000 µg/ml was obtained. From the above solution 10 ml of solution was transferred in 100 ml volumetric flask and volume was made up to 100 ml with methanol to prepare stock solution of 100µg/ml.

Preparation of serial dilutions

From the standard stock solution, a series of dilutions 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20µg/ml were prepared by taking 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml of solution and was transferred into 10 ml volumetric flasks and volume was made up to 10 ml with methanol and absorbance was taken at 235 nm.

Preparation of stock solutions of cefpodoxime proxetil in phosphate buffer pH 6.8

100mg of cefpodoxime proxetil was weighed and transferred to 100 ml volumetric flask. 100 ml phosphate buffer was added and sonicated for 2 hrs. The volume was made up to the mark with phosphate buffer and the final strength obtained was 1000 µg/ml. From the above solution,10 ml solution was transferred in 100 ml volumetric flask and volume was made up to 100 ml with to phosphate buffer prepare stock solution of 100 µg/ml.

Preparation of serial dilutions

From the standard stock solution, a series of dilutions 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml were prepared by taking 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml of solution and was transferred into 10 ml volumetric flasks and volume was made up to 10 ml with phosphate buffer and absorbance was taken at 235 nm.

Determination of qualitative solubility of Cefpodoxime proxetil in different solvents

Using the standard method, solubility studies were carried out in different solvents and surfactant like purified Water, Methanol, Ethanol, Acetone, Hexane, Tween 80, PEG 400, PEG 800, DMSO, Lactic acid, Oleic acid, 6.8 phosphate buffer and 0.1 N HCl. Excess amount of drug was added to each vial containing 2ml of solvents to saturate the solution. The drug solutions were shaken for 24 hrs in an water bath shaker (Remi, Mumbai, India), maintaining the temperature 37±0.5°C and by providing shaking of 100 agitation/min. Afterwards, solution were centrifuged at 10000 rpm for 15 min and then supernatant was filtered through membrane filter (0.22µm) to remove the remaining drug. The above samples of drug solution were taken and diluted suitably by methanol to observe the absorbance of drug by using UV-Visible spectrophotometer at λmax of 235 nm. The drug concentration was calculated with the help of standard calibration curve of drug in methanol and the graph was plotted between the concentrations vs. absorbance (Patel *et al.,* 2010)[12].

Determination of partition coefficient

Partition coefficient was determined by taking excess amount of cefpodoxime proxetil in 10ml mixture of *n*-octanol and water (1:1) in a separating funnel. This system was shaken immediately for 30 mins and kept undisturbed for overnight to achieve equilibrium. Then the two phases were separated and centrifuge at 10000 rpm for 15 minutes. After centrifugation, the concentration of cefpodoxime proxetil in both phases was determined by measuring the absorbance at 235 nm methanol as blank on UV-Visible spectrophotometer.

The partition coefficient is commonly determined by shake flask method and calculated by formula:

$$P\ (^{O}/_{W}) = \frac{C\ (oil)}{C\ (water)}$$

Where, C (oil) =Conc. of solute in organic phase

C (water) = Conc. of solute in aqueous phase

P ($^{O}/_{W}$) = Partition coefficient

LogP=log ($^{O}/_{W}$).

Determination of drug-excipients compatibility study

Drug and excipient compatibility studies were conducted to determine the compatibility of the excipients with the drug for the preparation of formulation. The FTIR spectrum was recorded by using FTIR after preparing potassium bromide disk. The finely ground drug powder and excipients powder were mixed with powdered potassium bromide and the mixture was pressed with a specific hydraulic compression. The prepared KBr pellet was then observed under Fourier transform infrared spectrometer (FTIR) and the spectrum of drug and excipients was recorded and compared.

Formulation Development

Preparation of Nanosuspension by solvent anti-solvent precipitation method

Weighed amount of drug was taken and dissolved in solvent (ethanol/acetone) at room temperature to form a drug solution. HPMC E50 as stabilizing agent was first dissolved in small quantity of hot anti-solvent *i.e.* deionised water. Drug solution filled in syringe was quickly injected at a fixed flow rate (2-8 ml/min) into anti-solvent kept at a low temperature (4^0C) in an iced water bath. During injection; the mixture was stirred continuously at 1000 rpm for 15 minutes. After precipitation, mixture was kept for 24 hrs for evaporation of volatile solvents. Final cefpodoxime proxetil nanoparticles were collected, filtered and dried at 40^0C in hot air oven (Kakran *et al.,* 2012)[13].

Optimization of cefpodoxime proxetil Nanosuspension

Various formulations were prepared by using solvent anti-solvent precipitation method. Different concentration of drug (mg/ml) and solvent anti-solvent volume ratio was optimized.

Formulation K8 was selected on the basis of percentage yield (% yield) of nanosuspension formation by solvent-antisolvent precipitation method. Percentage yield was calculated on the basis of following formula

$$Percentage\ yeild = \frac{Practical\ yield}{Theoretical\ yield} \times 100$$

Formulation CK2 was selected having same solvent anti-solvent ratio but with different drug concentration on the basis of their saturation solubility in different solvents.

Determination of saturation solubility of cefpodoxime proxetil nanosuspension formulation

2mg of cefpodoxime proxetil loaded nanosuspension was suspended in 2ml of water, 0.1N HCl and pH 6.8 phosphate buffers and shaken at 37°C for 24 hrs. From this, nanosuspension was taken into centrifugation tube and centrifuged at 10,000 rpm for 15 mins. The sample was filtered through 0.22 µm membrane filter and the filtrate was diluted appropriately and was analyzed spectrophotometrically using UV-Visible spectrophotometer at 235nm (Sandhya *et al.,* 2014)[14].

Evaluation of optimized cefpodoxime proxetil nanosuspension formulation

Determination of particle size

Particle size (in nanometers) of cefpodoxime proxetil nanosuspension was determined using a

Beckman coulter instrument (Thadkala *et al.,* 2014)[15].

Determination of Zeta potential

Zeta potential determines the physical stability of nanosuspension. Measurement of zeta potential of the nanosuspension formulation was done by using a Malvern Nano Zeta Sizer instrument (Lakshmi *et al.,* 2010)[16].

Scanning electron microscopy (SEM)

To examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations. The analysis of the particle diameter was determined by the Uthscsa Image Tool program. Five pictures were used to find the average range of particle diameter. The software was used to measure the average diameter of about 80-100 particles in each of the SEM picture (Kakran *et al.,* 2012)[17].

X-ray powdered diffraction analysis

The polymorphic state of drug in the formulation was evaluated by X-ray powdered diffraction analysis.The powder x-ray diffraction (XRD) was performed by Xpert Pro with Spinner PW3064 using Ni filtered, CuKα radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a range of 5°C to 40°C (Arora *et al.,* 2010)[18].

Determination of drug content

The drug content was determined by calibration curve method (Attari *et al.,* 2015)[19].100 mg of nanosuspension was accurately weighed and dissolved into 100 ml of methanol followed by sonication and filtration through whatmann filter paper. The amount of drug was determined by UV-visible spectrophotometer at 235 nm.

***In-vitro* dissolution studies**

The dissolution studies of powder nanosuspension of cefpodoxime proxetil was carried out in dissolution apparatus (USP apparatus II) in 100 ml of phosphate buffer pH 6.8 as a dissolution medium, maintained at 37 ± 0.5°C. The medium stirred at 100 rpm. Aliquots 1 ml of the dissolution medium was withdrawn at 15, 30, 45, 60, 90, 120 and 180 mins time interval and the same amount was added with the fresh medium in order to maintain the sink conditions. Samples were assayed spectrophotometerically at 235 nm.

RESULTS AND DISCUSSION

Cefpodoxime proxetil (drug sample) was observed for organoleptic properties like physical appearance, odor, and melting point. The drug was identified with the help of UV and FTIR and exhibited absorption maxima at 235 nm when methanol was used as solvent as mentioned in literature.

Melting point analysis

The melting range of cefpodoxime proxetil was observed to be 111°C -112°C which complies with reported melting range *i.e.* 111°C -113°C.

An optimum solvent/antisolvent ratio was requisite to obtain the higher percentage yield. Use of a different drug concentration above optimal level increases percentage yield. But while the drug concentration is increased, the nucleation rate too increased owing to higher super saturation solubility in different solvent is decrease. From the Table No.10 it was concluded that formulation CK2 was selected for final formulation.

Particle size

Particle size analysis of selected cefpodoxime proxetil nanosuspension formulation (CK2) was measured by Dynamic Light Scattering phenomenon using a Beckman coulter instrument.

The FTIR of cefpodoxime proxetil has been shown in Figure No.3 intense band at 3326, 3210cm^{-1}, 2986, 2938cm^{-1}, 1761cm^{-1}, 1777cm^{-1}, 1624cm^{-1}, 1453,1375cm^{-1}, and 1275cm^{-1}, 906cm^{-1} corresponding to the functional groups NH_2, C-H, C=O,C=O (amide), C=N, CH_3, C-O, and =C-H. The peaks observed in FTIR of mixture of cefpodoxime proxetil and excipients at 3329cm^{-1}, 2986, 2939cm^{-1}, 1761cm^{-1}1778cm^{-1}, 1620cm^{-1}, 1463, 1375cm^{-1}, 1275cm^{-1}, and 904cm^{-1}. There was no major shifting in the frequencies of above said functional groups of which indicates that there was no chemical interaction between cefpodoxime proxetil and excipients which were used in the formulation.

An optimum solvent/antisolvent ratio was requisite to obtain the higher percentage yield. Use of a different drug concentration above optimal level increases percentage yield. But while the drug concentration is increased, the nucleation rate too increased owing to higher super saturation solubility in different solvent is decrease. From the Table No.10 it was concluded that formulation CK2 was selected for final formulation.

Table No.1: Optimization of Nanosuspension Formulations

S.No	Formulation Code	Solvent	Anti-solvent	Drug (mg)	Solvent anti-solvent volume ratio (ml)	Stabilizing agent (HPMC E50)%(w/v)
1	K1	Acetone	Water	40	1:10	0.1
2	K2	Acetone	Water	40	1:20	0.1
3	K3	Acetone	Water	40	1:30	0.1
4	K4	Acetone	Water	40	1:40	0.1
5	K5	Acetone	Water	40	1:50	0.1
6	K6	Acetone	Water	40	1:60	0.1
7	K7	Ethanol	Water	40	1:10	0.1
8	K8	Ethanol	Water	40	1:20	0.1
9	K9	Ethanol	Water	40	1:30	0.1
10	K10	Ethanol	Water	40	1:40	0.1
11	K11	Ethanol	Water	40	1:50	0.1
12	K12	Ethanol	Water	40	1:60	0.1

Table No.2: Optimization of drug loaded Nanosuspension on the basis of different drug concentration

S.No	Formulation code	Solvent	Anti-solvent	Drug(mg)	Solvent anti-solvent volume ratio
1	CK1	Ethanol	water	40	1:20
2	CK2	Ethanol	water	50	1:20
3	CK3	Ethanol	water	60	1:20
4	CK4	Ethanol	water	90	1:20

Formulation CK2 was selected having same solvent anti-solvent ratio but with different drug concentration on the basis of their saturation solubility in different solvents.

Table No.3: Interpretation of Infrared Spectrum bands of Cefpodoxime proxetil

S.No	Functional groups stretching and bending	Observed value(cm^{-1})
1	NH_2	3326, 3210
2	C-H	2986, 2938
3	C=O (ester)	1761
4	C=O (amide)	1677
5	C=N	1624
6	CH_3 bending	1453, 1375
7	C-O	1275
8	=C-H	906

The IR spectrum of drug sample has shown identical peaks as reported into reference sample of cefpodoxime proxetil.

Table No.4: Standard Calibration curve of Cefpodoxime proxetil in methanol

S.No	Concentration (µg/ml)	Absorbance				± SD	% RSD
		Set 1	Set 2	Set 3	Mean		
1	2	0.095	0.091	0.093	0.093	0.001	1.075269
2	4	0.168	0.172	0.170	0.170	0.002	1.176471
3	6	0.221	0.224	0.227	0.224	0.003	1.139286
4	8	0.285	0.286	0.287	0.286	0.001	0.34965
5	10	0.358	0.362	0.360	0.360	0.002	0.555556
6	12	0.448	0.443	0.438	0.443	0.005	1.128668
7	14	0.516	0.520	0.524	0.520	0.004	0.769231
8	16	0.624	0.623	0.624	0.624	0.001	0.160256
9	18	0.685	0.679	0.691	0.685	0.006	0.875912
10	20	0.710	0.720	0.715	0.715	0.005	0.699301

Table No.5: Standard Curve of Cefpodoxime proxetil in pH 6.8 phosphate buffer (PB)

S.No	Concentration (µg/ml)	Absorbance				± SD	% RSD
		Set 1	Set 2	Set 3	Mean		
1	2	0.039	0.038	0.037	0.037	0.001	2.040816
2	4	0.081	0.080	0.079	0.080	0.001	1.369863
3	6	0.123	0.121	0.125	0.123	0.002	1.626016
4	8	0.171	0.166	0.175	0.170	0.001	0.588235
5	10	0.226	0.228	0.227	0.227	0.001	0.440529
6	12	0.263	0.273	0.268	0.268	0.005	1.865672
7	14	0.318	0.316	0.314	0.316	0.002	0.632911
8	16	0.356	0.358	0.357	0.357	0.001	0.280112
9	18	0.400	0.398	0.399	0.399	0.001	0.250627
10	20	0.458	0.466	0.462	0.462	0.004	0.865801

Determination of partition coefficient

Table No.6: Partition coefficient of Cefpodoxime proxetil in water and n-octanol

S.No	Solvent	Absorbance	Conc. µg/ml	DF µg/ml	Conc. mg	Conc. mg/ml	partition co-efficient	Log P
1	Water	0.35	9.3888	938.888	0.9388	9.38888	18.284	1.2620
2	*n*-Octanol	0.63	17.166	17166.6	17.1666	171.666		

The partition co-efficient of Cefpodoxime proxetil between *n*-octanol and water was found to be 18.284 and log P value of 1.2 show that drug is lipophilic in nature and complies with reported log P value 0.99 *i.e.* (Drugbank.com, 2013).

Solubility studies

Table No.7: Solubility studies of Cefpodoxime proxetil in water and different solvents

S.No	Solvents used	Solubility (mg/ml)	Solubility profile
1	Tween 80	95833	Very soluble
2	Oleic acid	2241.66	Very soluble
3	Lactic acid	1930.55	Very soluble
4	DMSO	408.33	Freely soluble
5	Methanol	372.22	Freely soluble
6	Acetone	228.88	Freely soluble
7	PEG 400	228.88	Freely soluble
8	PEG 200	216.11	Freely soluble
9	0.1N HCl	27.7777	Sparingly soluble
10	6.8 phosphate buffer	12.277	Sparingly soluble
11	Ethanol	2.777	Sparingly soluble
12	Water	1.3944	Sparingly soluble
13	Hexane	0.19722	Practically insoluble

Table No.8: Interpretation of infrared spectrum bands of HPMC E50

S.No	Functional groups stretching and bending	Observed value(cm^{-1})
1	O-H	3464
2	C-H	2934, 2837
3	CH_3	1459, 1377
4	C-O	1072

Formulation Development

Effect of solvent anti-solvent volume ratio

Table No.9: Percentage yield of different Cefpodoxime proxetil nanosuspension formulations

S.No	Formulation code	Theoretical yield	Practical yield	Percentage yield= practical yield/ Theoretical yield×100
1	K1	0.04	0.025	62.5
2	K2	0.04	0.030	75.0
3	K3	0.04	0.023	57.5
4	K4	0.04	0.019	47.5
5	K5	0.04	0.017	42.5
6	K6	0.04	0.012	30.0
7	K7	0.04	0.032	80.0
8	K8	0.04	0.035	87.0
9	K9	0.04	0.028	70.0
10	K10	0.04	0.026	65.0
11	K11	0.04	0.021	52.5
12	K12	0.04	0.014	35.0

Effect of drug concentration

Table No.10: Effect of drug concentration on solubility and percentage yield in nanosuspension formulation

S.No	Formulation code	SAS volume ratio	Drug (mg)	Solubility study in methanol (mg/ml)			Solubility study in 6.8 phosphate buffer (mg/ml)			%age Yield
				Water	0.1 N HCl	6.8 buffer	Water	0.1 N HCl	6.8 buffer	
1	CK1	1:20	40	5	47	22	13	91	37	87
2	CK2	1:20	50	19	72	24	20	95	38	95
3	CK3	1:20	60	22	21	22	13	79	35	78
4	CK4	1:20	90	21	24	26	12	51	27	73

Table No.11: Particle size of Cefpodoxime proxetil nanosuspension formulation by Beckman coulter Instrument

S.No	Formulation code	Particle size(nm)	Polydispersity index	Diffusion constant(cm^2/sec)
1	CK2	755.6	0.327	6.510e-009

Table No.12: Calculation for drug content of Cefpodoxime proxetil nanosuspension in formulation

S.No	Solvent	Absorbance	Conc. (µg/ml)	DF (x100) (µg/ml)	Conc. (mg)	% drug content= (initial-final)/initial×100
1	Methanol	0.257	6.805555556	680.555556	0.68055556	99.31944444

Table No.13: *In-vitro* dissolution profile data of formulation (CK2)

Time (min)	Abs.	Conc. (µg/ml)	DF (x50) (µg/ml)	Conc. (mg)	Conc. in 100 ml	Cumulative Drug release	% Cumulative Drug release	% Drug Release
15	0.245	11.08695652	554.3478261	0.554347826	55.43478261	55.43478261	55.43478	55.4347
30	0.286	12.86956522	643.4782609	0.643478261	64.34782609	64.90217391	64.90217	64.3478
45	0.324	14.52173913	726.0869565	0.726086957	72.60869565	73.80652174	73.80652	72.6086
60	0.364	16.26086957	813.0434783	0.813043478	81.30434783	83.22826087	83.22826	81.3043
90	0.382	17.04347826	852.173913	0.852173913	85.2173913	87.95434783	87.95435	85.2173
120	0.397	17.69565217	884.7826087	0.884782609	88.47826087	92.0673913	92.06739	88.4782
180	0.411	18.30434783	915.2173913	0.915217391	91.52173913	95.99565217	95.99565	91.5217

Table No.14: *In-vitro* dissolution profile of marketed formulation (Tablet)

Time (min.)	Abs.	Conc. (µg/ml)	DF (x50) (µg/ml)	Conc. (mg)	Conc. in 100 ml (mg)	Cumulative Drug release	%Cumulative Drug release	% Drug Release
15	0.211	9.608695652	480.4347826	0.480434783	48.04347826	48.04347826	48.04348	48.0434
30	0.233	10.56521739	528.2608696	0.52826087	52.82608696	53.30652174	53.30652	52.8260
45	0.252	11.39130435	569.5652174	0.569565217	56.95652174	57.96521739	57.96522	56.9565
60	0.271	12.2173913	610.8695652	0.610869565	61.08695652	62.66521739	62.66522	61.0869
90	0.286	12.86956522	643.4782609	0.643478261	64.34782609	64.34782609	64.34783	64.3478
120	0.292	13.13043478	656.5217391	0.656521739	65.65217391	68.48478261	68.48478	65.6521
180	0.318	14.26086957	713.0434783	0.713043478	71.30434783	71.30434783	71.30435	71.3043

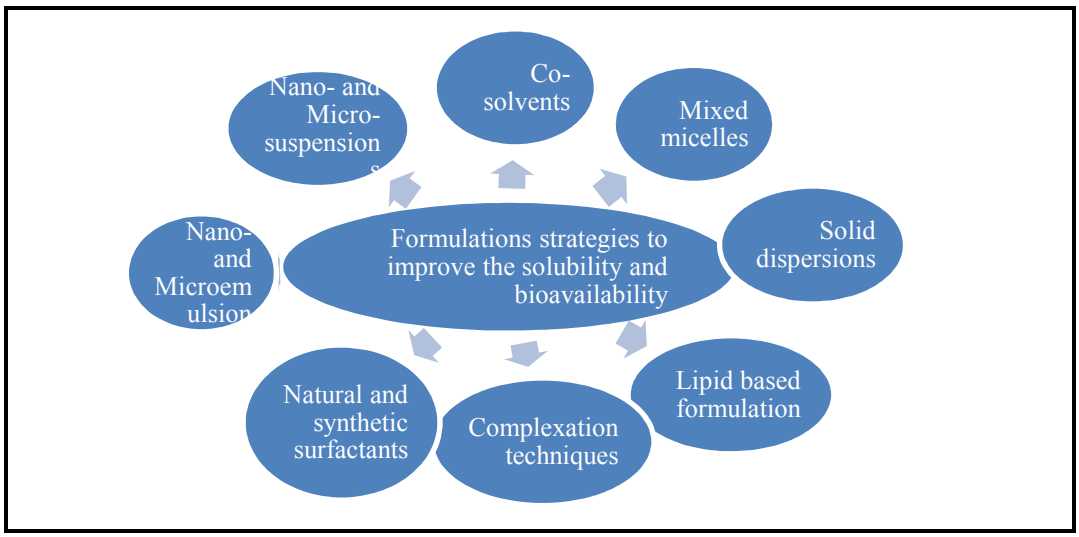


Figure No.1: Formulation strategies to improve solubility and bioavailability of the drug

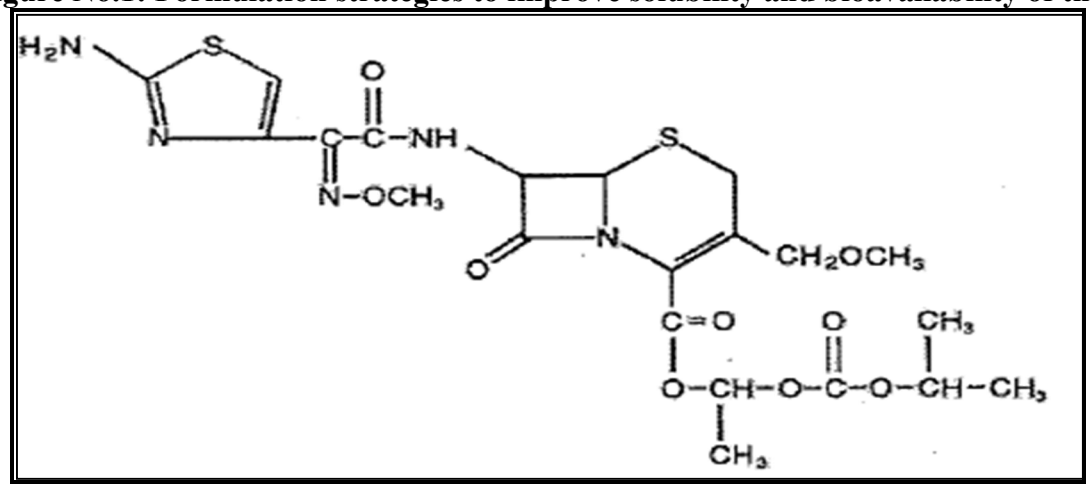


Figure No.2: Structure of Cefpodoxime proxetil (Drugs.com)

Identification of drug by Fourier transforms infrared spectral spectroscopy

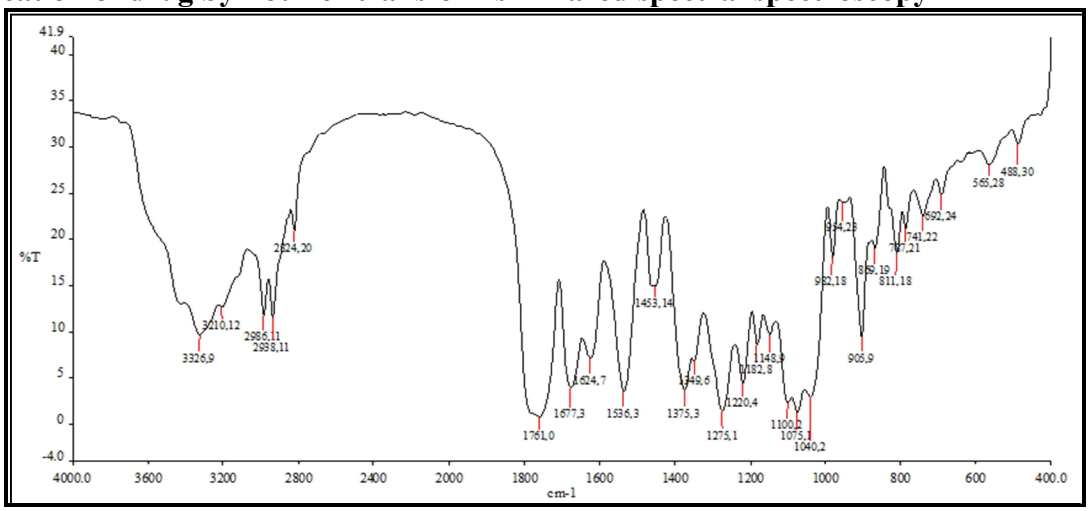
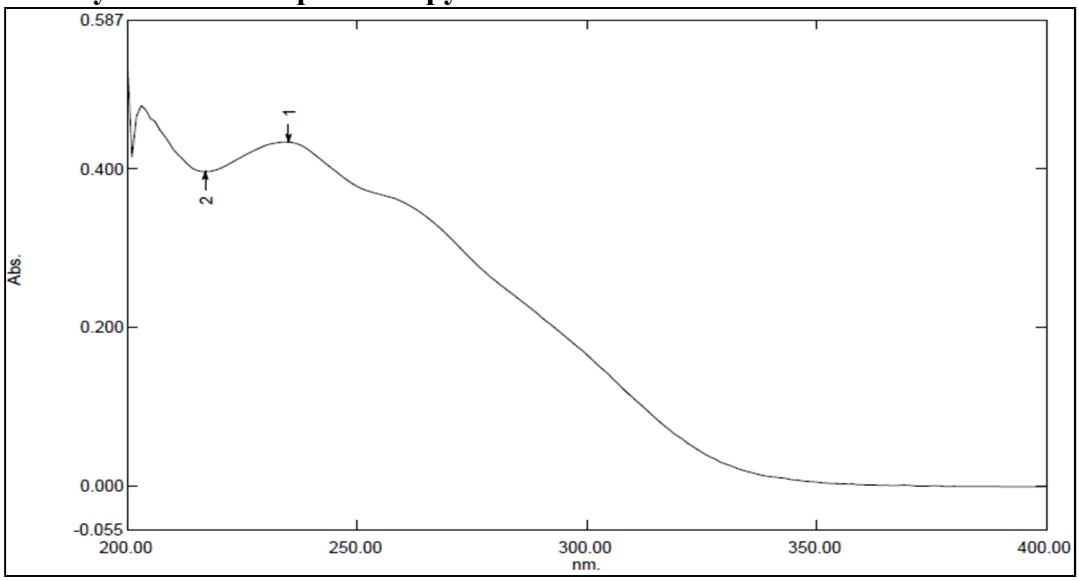


Figure No.3: FTIR Spectrum of Cefpodoxime proxetil as pure drug

Identification by UV- Visible spectroscopy



No.	P/V	Wavelength	Abs.	Description
1		235.00	0.433	
2		217.00	0.396	

Figure No.4: UV-Visible Spectroscopy of Cefpodoxime proxetil at 235nm

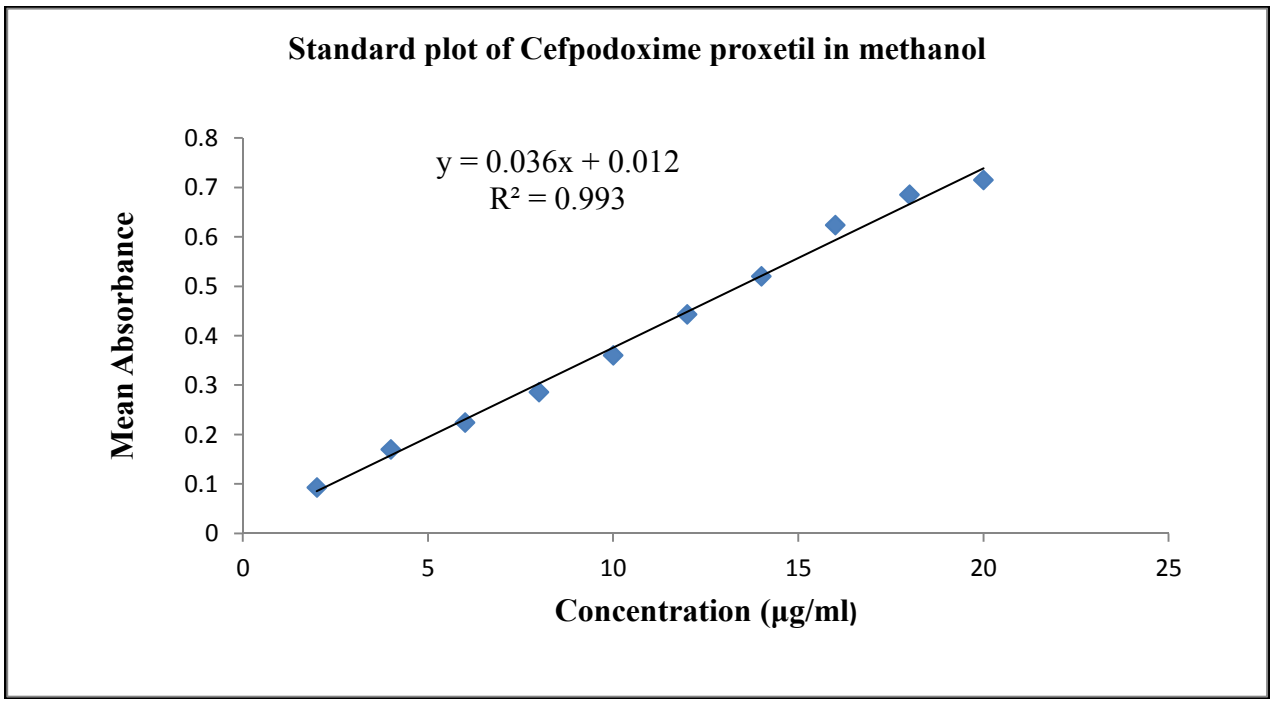


Figure No.5: Standard plot Curve of Cefpodoxime proxetil in methanol at 235 nm

The standard curve of cefpodoxime proxetil as shown in graph specify the regression equation Y = 0.036x+ 0.012 and R^2 value is 0.993, which show good linearity.

Preparation of standard curve of Cefpodoxime proxetil in pH 6.8 phosphate buffer (PB)

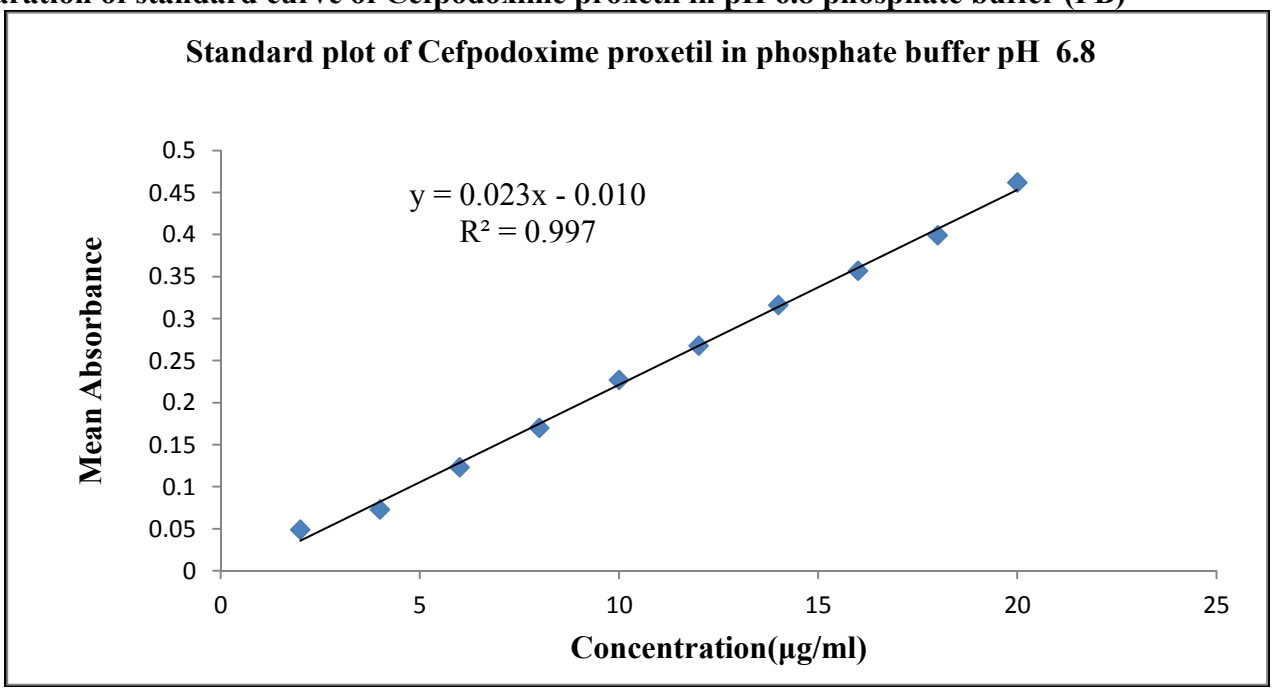


Figure No.6: Standard Curve of Cefpodoxime proxetil in pH 6.8 phosphate buffer at 235 nm

The standard curve of cefpodoxime proxetil as shown in graph indicates the regression equation Y =0.023 xs - 0.010 and R^2 value is 0.997, which showed that there is proportional increase in the absorbance along with concentration and good linearity.

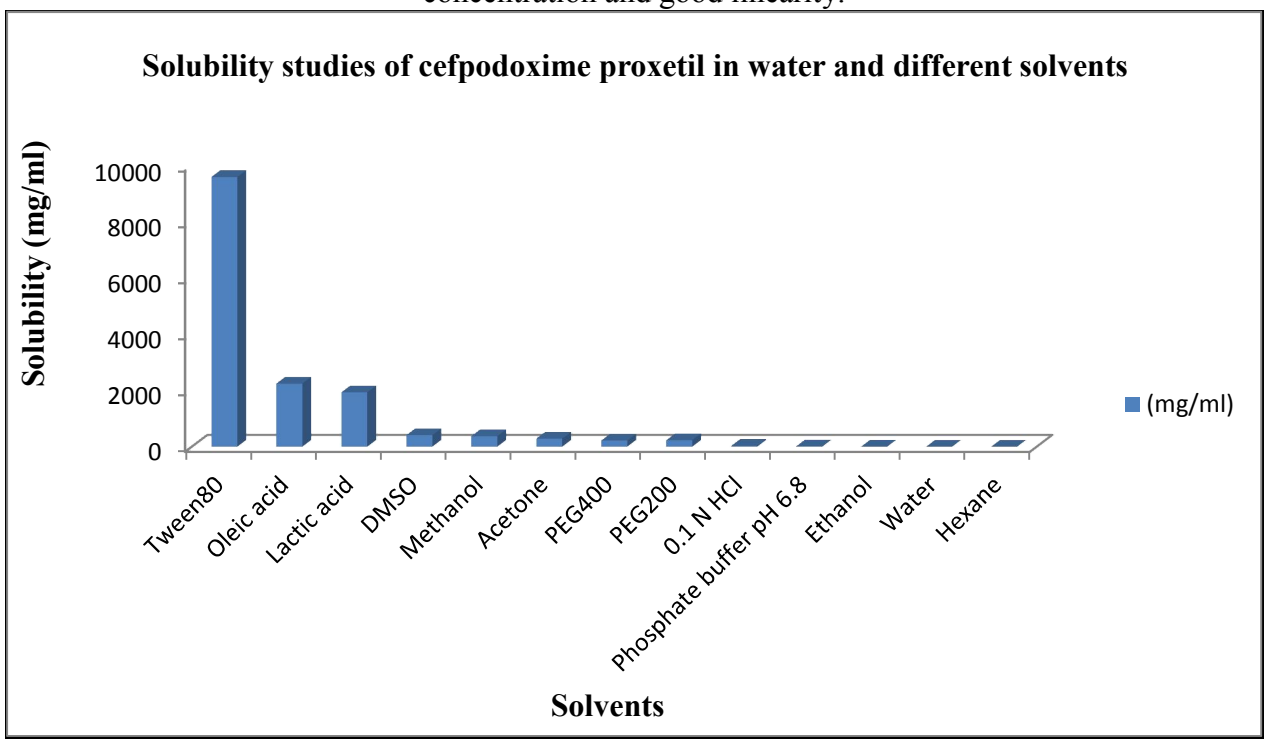


Figure No.7: Solubility studies of Cefpodoxime proxetil in water and different solvents

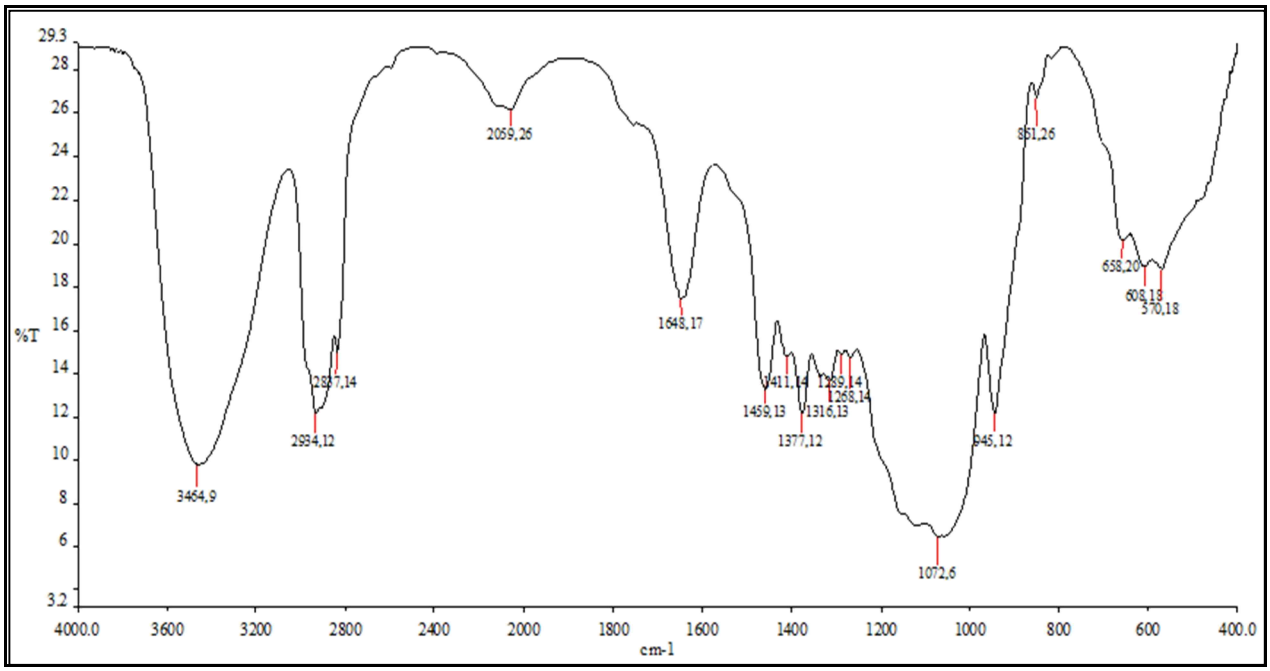


Figure No.8: FTIR spectrum of HPMC E50

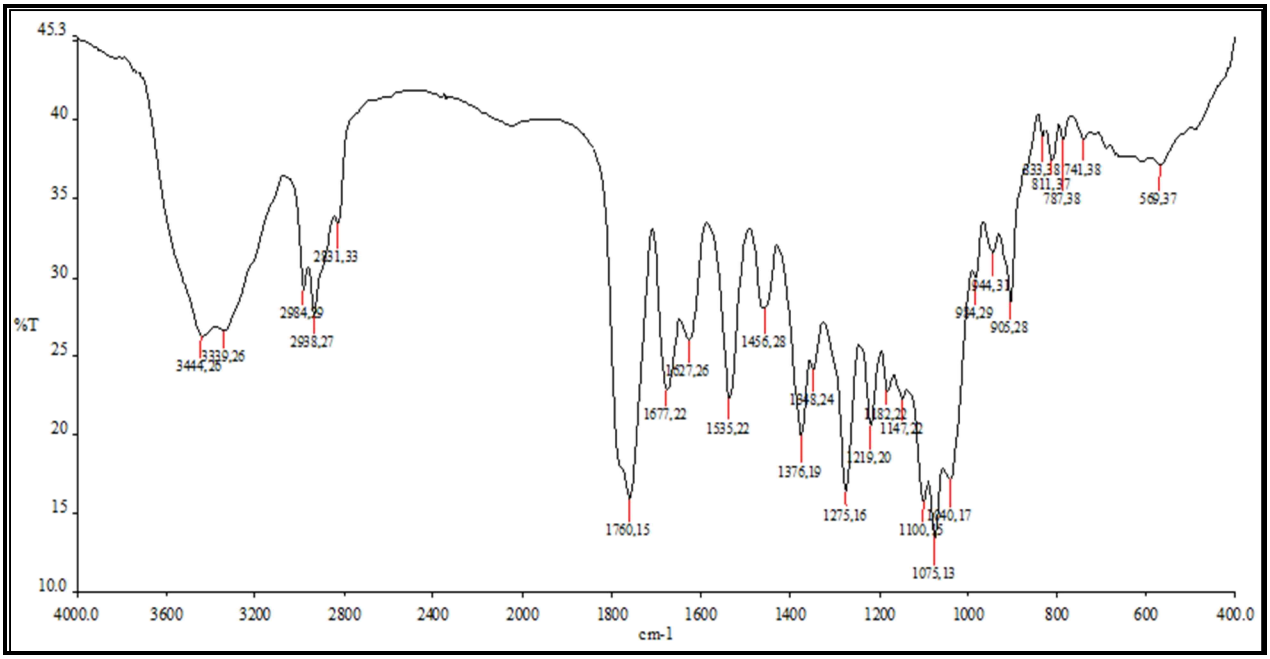


Figure No.9: FTIR spectrum of Cefpodoxime proxetil and HPMC E50 (Mixture sample)

Drug polymer interaction studies are carried out to eliminate the possibility of interaction between drug and polymer used with analytical method of drug estimation. The FTIR **s**pectra of pure drug and pure polymer are shown in Figure No.3, Figure No.8 and physical mixtures of drug with excipient (HPMC E50) are shown in Figure No.9 respectively. The peaks observed in FTIR of mixture of cefpodoxime proxetil and excipients at 3339 cm^{-1}, 2884, 2938 cm^{-1}, 1760 cm^{-1}1777 cm^{-1}, 1627 cm^{-1}, 1456, 1376 cm^{-1}, and 1275 cm^{-1}, 905 cm^{-1}. Drug shows characteristic peak and there be no significant changes in the position of the characteristic peak of drug when mixed with excipients which indicate compatibility of drug with polymer.

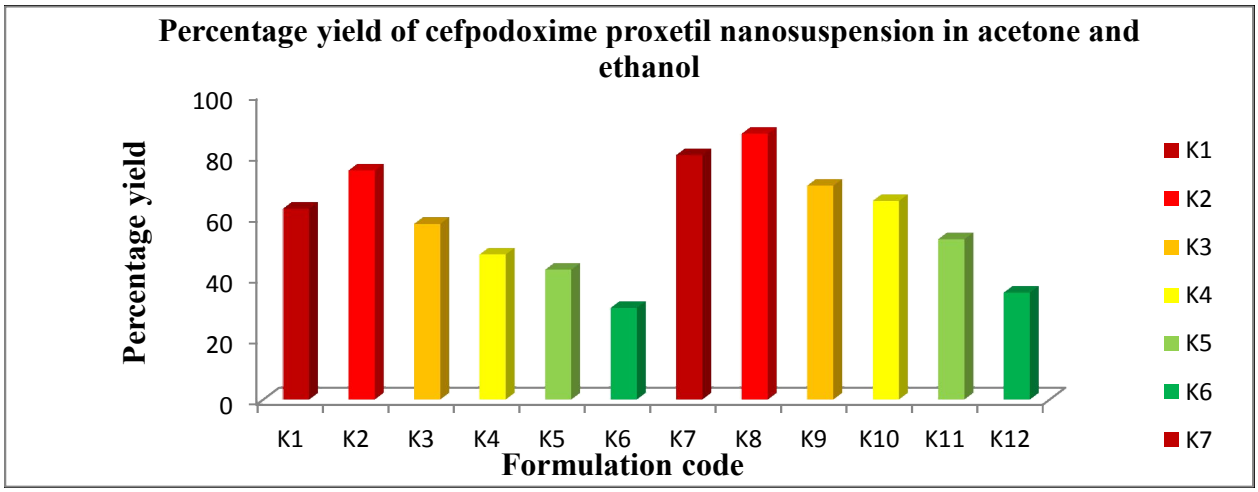


Figure No.10: Percentage yield of different Cefpodoxime proxetil nanosuspension formulations

Formulation K7, K8, and K1 showed highest percentage yield as compared to other. The maximum percentage yield of nanosuspension formulation was found to be 87% for K8 formulation containing solvent anti-solvent in the ratio of 1:20. So, it was concluded that ethanol produce more precipitation as compared to acetone. Simultaneously on increasing solvent anti-solvent ratio percentage yield was reduced.

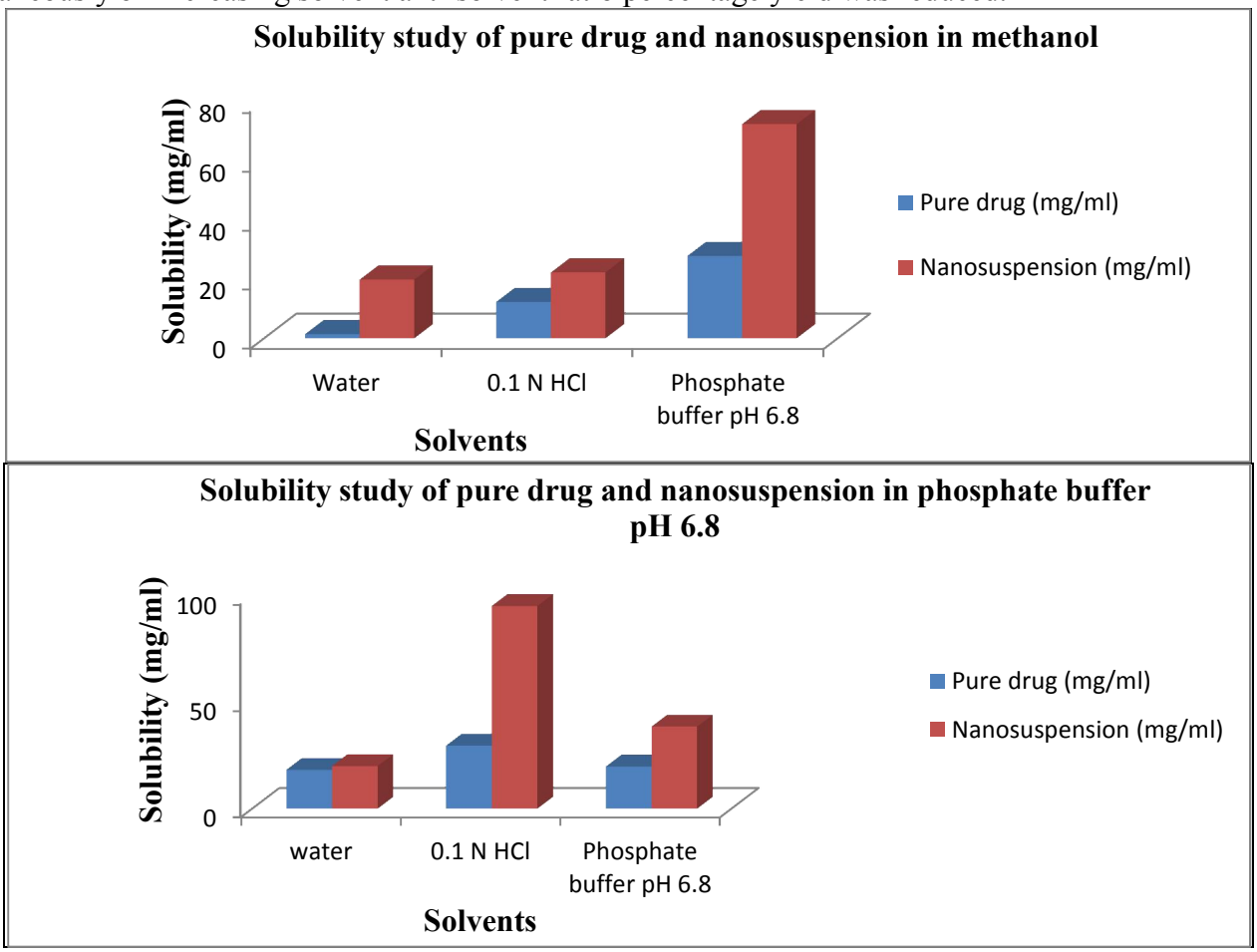


Figure No.11: Solubility of Cefpodoxime proxetil loaded nanosuspension in methanol and 6.8 Phosphate buffer

Particle size

Particle size analysis of selected Cefpodoxime proxetil nanosuspension formulation (CK2) was measured by Dynamic Light Scattering phenomenon using a Beckman coulter instrument.

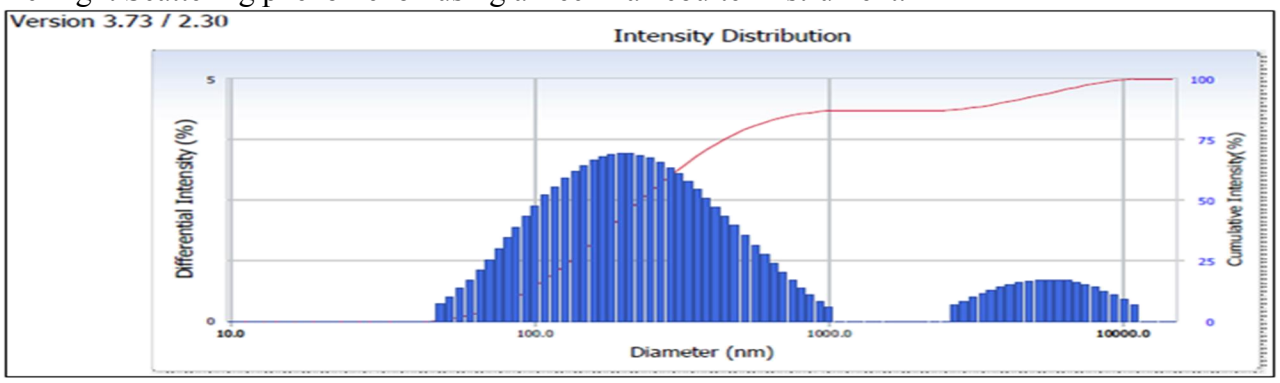


Figure No.12: Particle size analysis of Cefpodoxime proxetil nanosuspension formulation

Zeta potential of nanosuspension formulation

Zeta potential of sonicated cefpodoxime proxetil nanosuspension was determined by Malvern nano zetasizer instrument**.**

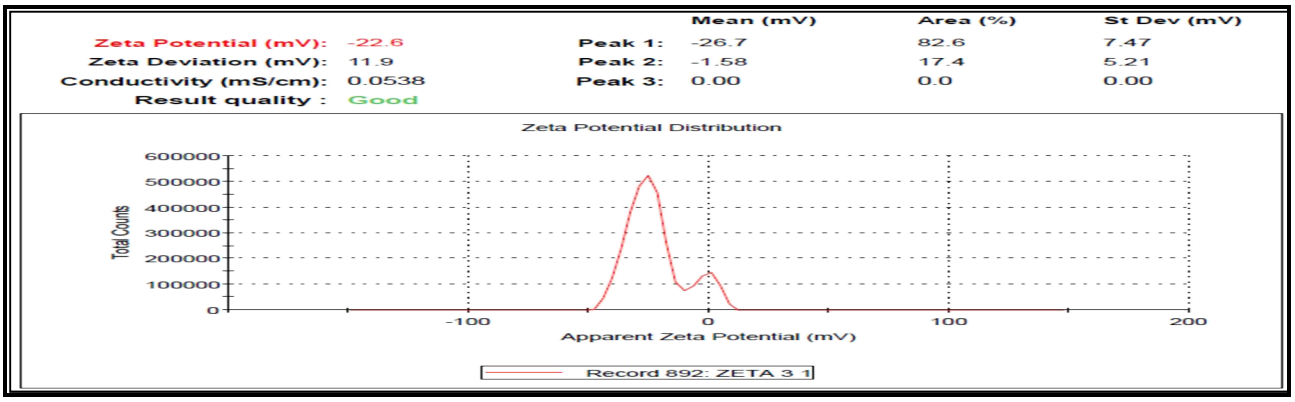


Figure No.13: Zeta potential analysis of nanosuspension

Particle size analysis and size distribution of the selected nanosuspension formulation (CK2) was measured by Dynamic Light Scattering phenomenon using a Malvern zeta sizer instrument.

Fourier transforms infrared spectral analysis

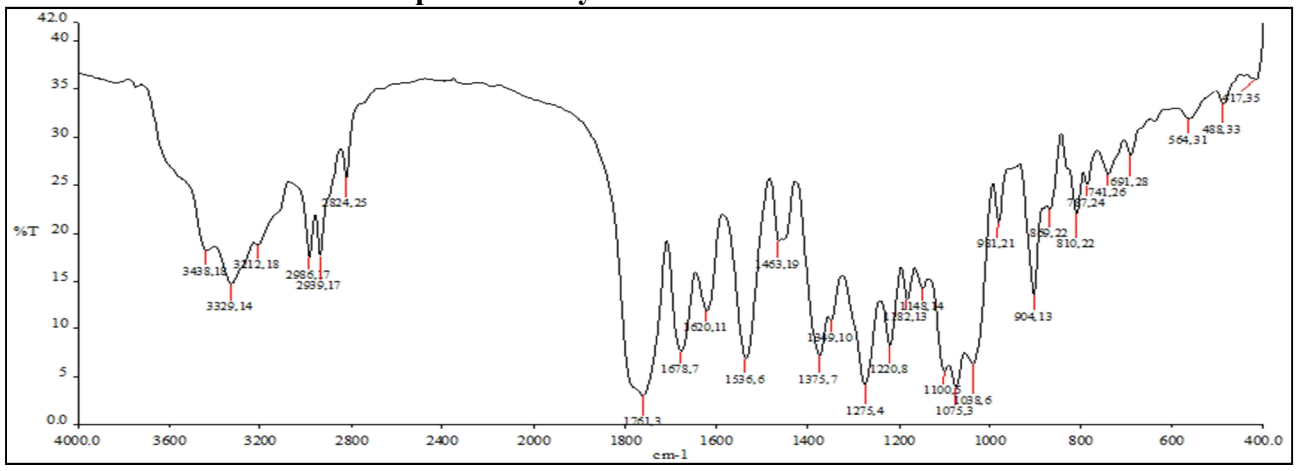


Figure No.14: FTIR spectrum of formulation containing Cefpodoxime proxetil

The FTIR of cefpodoxime proxetil has been shown in Figure No.3 intense band at 3326, 3210cm^{-1}, 2986, 2938cm^{-1}, 1761cm^{-1}, 1777cm^{-1}, 1624cm^{-1}, 1453,1375cm^{-1}, and 1275cm^{-1}, 906cm^{-1} corresponding to the functional groups NH_2, C-H, C=O,C=O (amide),C=N, CH_3, C-O, and =C-H. The peaks observed in FTIR of mixture of cefpodoxime proxetil and excipients at 3329cm^{-1}, 2986, 2939cm^{-1}, 1761cm^{-1}1778cm^{-1}, 1620cm^{-1}, 1463, 1375cm^{-1}, 1275cm^{-1}, and 904cm^{-1}. There was no major shifting in the frequencies of above said functional groups of which indicates that there was no chemical interaction between cefpodoxime proxetil and excipients which were used in the formulation.

Scanning electron microscopy

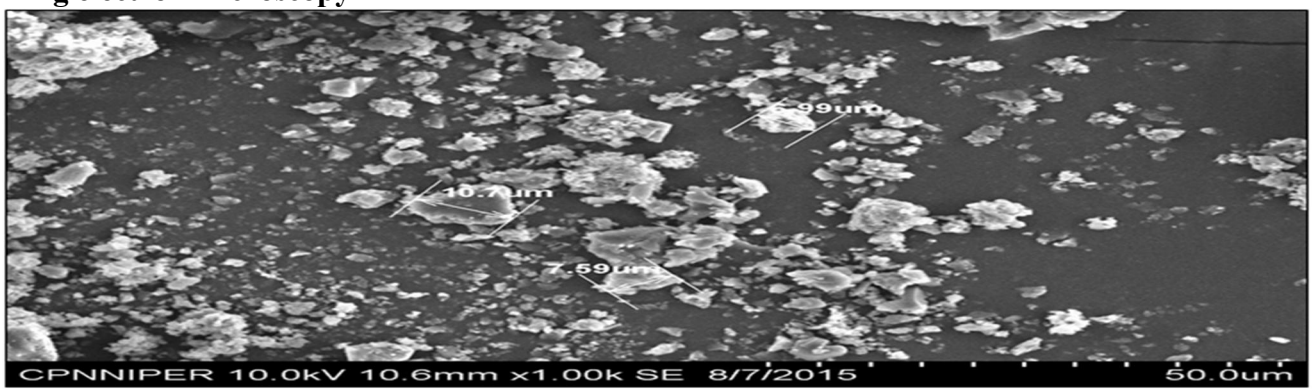


Figure No.15: SEM photomicrographs of nanosuspension formulation

X-ray powdered diffraction analysis

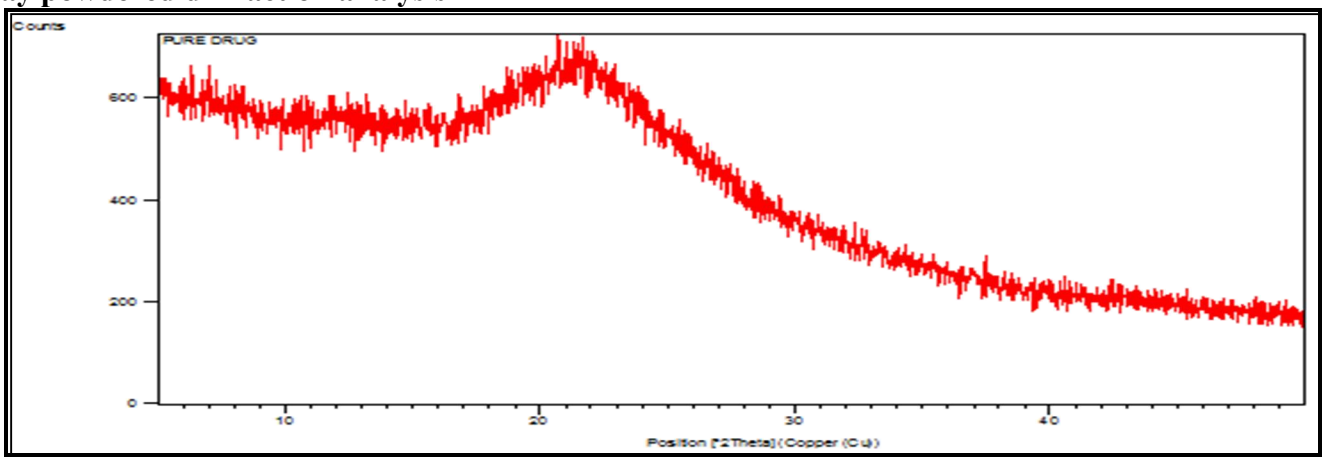


Figure No.16: XRD data for pure Cefpodoxime proxetil

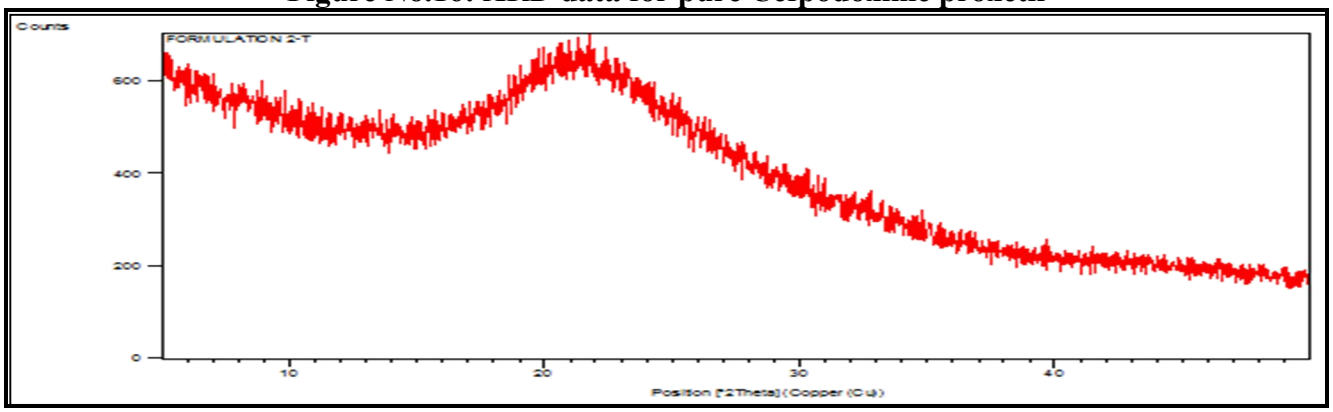


Figure No.17: XRD data for nanosuspension loaded Cefpodoxime proxetil

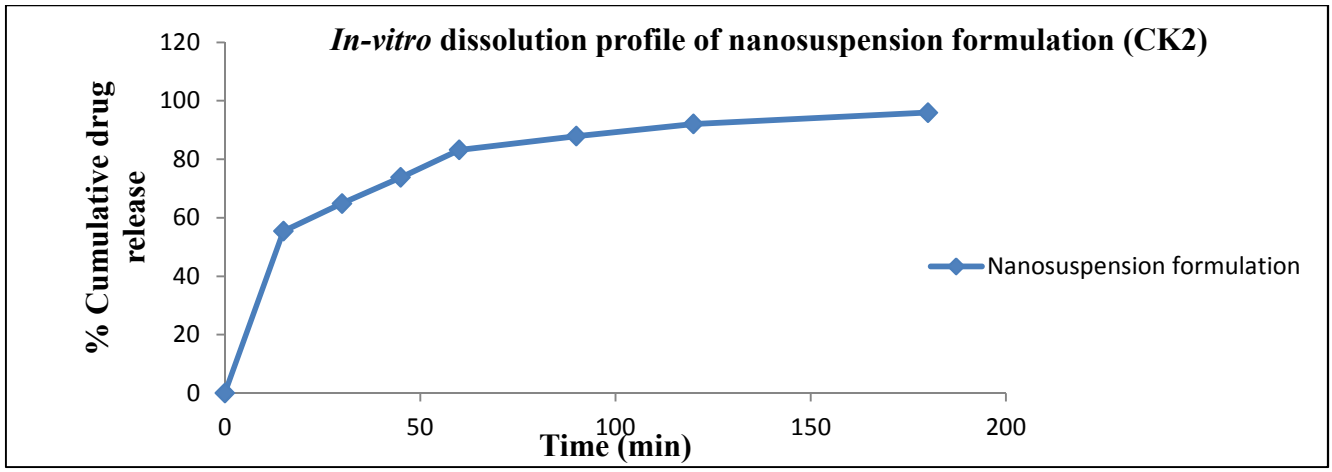


Figure No 18: *In-vitro* dissolution profile of nanosuspension formulation (CK2)

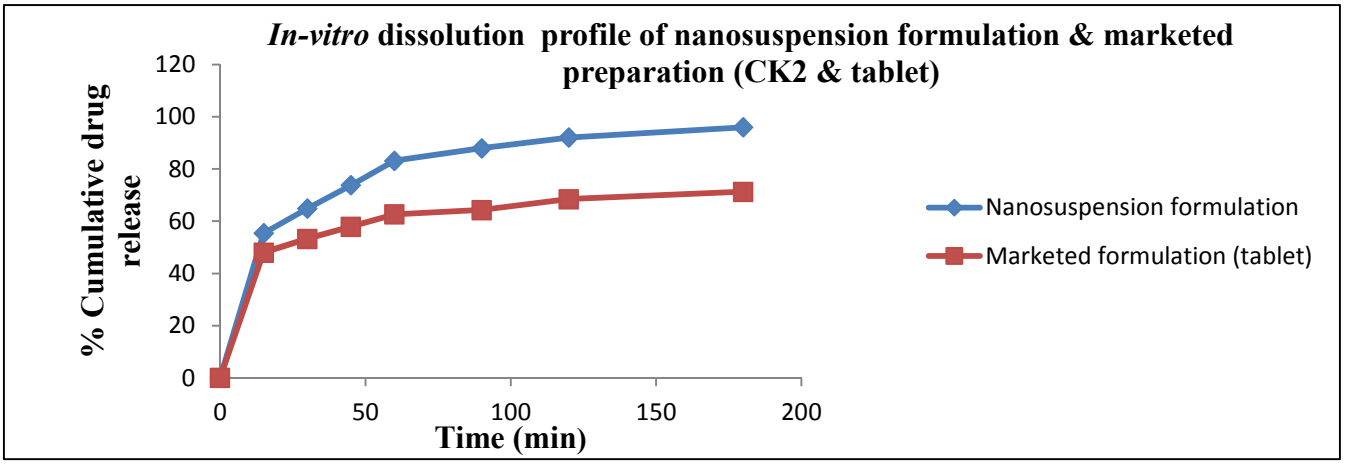


Figure No 19: Comparison of *in-vitro* dissolution profile of CK2 and tablet

CONCLUSION

Nanosuspension is chiefly seen as vehicles for administering poorly water soluble drug has been largely solved the dissolution problems to improve drug absorption and bioavailability. Production techniques such as media milling and high-pressure homogenization have been successfully employed for large-scale production of nanosuspension. The applications of nanosuspension in parentral and oral routes have been very well investigated.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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