Preparation and Evaluation of Cefixime Nanocrystals

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Abstract

Drug nanocrystals are nanoscopic crystals of the parent compound with dimensions less than 1 µm. A decrease in particle size will lead to an increase in effective surface area in the diffusion layer, which, in turn, increases the drug dissolution rate. Drug nanocrystals are one of the most important strategies to enhance the oral bioavailability of hydrophobic drugs.

Cefixime is the first member of what is generally termed the third generation orally active cephalosporins. These third generation cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against a wide range of gram-negative bacteria.

The aim of this study is to prepare nanocrystals of cefixime as a capsules dosage form in order to increase its oral dissolution rate and bioavailability.

The cefixime nanocrystals were prepared by solvent/antisolvent precipitation method. Certain amount of drug was dissolved in water miscible solvent (methanol used in this study), then this solution was injected (at certain speed) into water containing stabilizer. Upon injection, precipitation of cefixime nanocrystal will occur immediately; this precipitate is sonicated at 37 ˚C for 30 min. then lyophilized. Powder of nanocrystal was obtained and filled into capsules. The physicochemical interaction between drug and addatives was studied using FTIR, DSC,

Results show that the best formula of cefixime nanocrystals prepared by dissolving 20mg/ml of cefixime in methanol, then 5ml was injected at 60ml/hr rate to a 50ml PVP solution as stabilizer in concentration 0.05%, then lyophilized to obtain the cefixime nanocrystal powder. The resulted mean particle size was 9-11 nm and the dissolution rate was significantly higher than that of the raw cefixime powder ($p<0.05$).

Keywords: Cefixime trihydrate, Nanocrystals, Anti-solvent precipitation, PVP, HPMC, Poloxamer 188.

تحضير وتقييم البلورات النانوية للسفكسيم

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الخلاصة

البلورات النانوية هي بلورات نانوية الحجم من المركب الأصلي مع أبعاد أقل من 1 مايكروميتر . ان الانخفاض في حجم الجسيمات يؤدي إلى زيادة في المساحة الفعالة في طبقة الانتشار، والتي بدورها تزيد من معدل انحلال الدواء .البلورات النانوية هي واحدة من أهم الاستراتيجيات لزيادة التوافر الحيوي للأدوية شحيحة الذوبان في الماء عن طريق الفم.

السيفيكسيم هو أول عضو في ما يسمى عموما بالجيل الثالث للسيفالوسبورين الفعالة عن طريق الفم . هذا الجيل من السيفالوسبورين (الجيل الثالث) يختلف عن الاجيال السابقة للمضادات الحيوية بزيادة فعاليته المضادة للبكتيريا السالبة الجرام Grame (negative).

تهدف من هذه الدراسة الى تحضير ا لبلورات النانوية لدواء السيفيكسيم على شكل كبسول من أجل زيادة معدل الذوبان و التوافر الحيوي عن طريق الفم.

حضرت البلورات النانوية للسيفيكسيم باستخدام طريقة الترسيب بواسطة المذيب / المذيب المضاد. حيث تم اذابة كمية معينة من الدواء في محلول قابل للأمتزاج مع الماء (استخدم الميثانول في هذه الدراسة) ، ثم تم حقن هذا المحلول في المياه المحتوي على مثبت . عند الحقن، يحدث ترسب للبلورات النانوية للسفكسيم على الفور ؛ يؤخذ الراسب ويوضع في جهاز الموجات الصوتية (Sonicator) بدرجة حرارة 37 مئوية لمدة 30 دقيقة . ثم يجفف بالتجميد . أخذ المسحوق وعبيء على شكل كبسول.

تظهر النتائج أن أفضل صيغة للبلورات النانوية للسيفيكسيم نتجت عن طريق إذابة 20ملغ/مل من السيفيكسيم في الميثانول، تم حقن 5 مل من هذا المحلول في 50 مل محلول يحتوي على PVP كمثبت بتركيز 0.05 ٪ ، ثم جفف بالتجميد للحصول على مسحوق بلورات السيفيكسيم النانوية . متوسط حجم الجسيمات الناتجة هو 9-11 نانومتر، و معدل الانحلال أعلى بكثير من مسحوق السيفيكسيم الخام ($P<0.05$).

الكلمات المفتاحية: السيفيكسيم ، البلورات النانوية ، الترسيب بواسطة المذيب/ المذيب المضاد ، PVP، HPMC ، بولوكسامير 188 .

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Introduction

Over the last years, the number of poorly soluble drugs has steadily increased. Estimates state that 40% of the drugs in the pipelines have solubility problems [1, 2, and 3]. Progress in high throughput screening methods leads to an even greater amount of newly discovered drugs that have poor water solubility. Other literature states that about 60% of all drugs coming directly from synthesis are nowadays poorly soluble [4]. Poor solubility in water correlates with poor bioavailability. If there is no way to improve drug solubility it will not be able to be absorbed from the gastrointestinal tract into the bloodstream and reach the site of action. There are many ways to solubilize certain poorly soluble drugs. But these methods are limited to drugs with certain properties in regard to their chemistry (eg, solubility in certain organic media) or for example to their molecular size or conformation (eg, molecules to be incorporated into the cyclodextrin (CD) ring structure) [5]. Apart from that, the usage of surfactants or cosolvents is also possible, but sometimes leads to increased side effects and other disadvantages (eg, organic solvent residues). The micronization of drug powders to sizes between 1 and 10 μm in order to increase the surface area, and thus the dissolution velocity, is not sufficient to overcome bioavailability problems of many very poorly soluble drugs of the biopharmaceutical specification class II. A consequent step was to move from micronization to nanonization. [6]. The size reduction leads to an increased surface area and thus according to the Noyes-Whitney equation [7] to an increased dissolution velocity. Therefore nanonizationis a suitable way to successfully enhance the bioavailability of drugs where the dissolution velocity is the rate limiting step. Moreover, below a critical size of 1–2 μm, the saturation solubility is also a function of the particle size. It increases with decreasing particle size below 1000 nm. Therefore, drug nanocrystals possess increased saturation solubility.

Cefixime is the first member of what is generally termed the third generation orally active cephalosporins. These third generation cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against a wide range of gram-negative bacteria. [8] Cefixime is freely soluble in methanol but has low aqueous which affects its rate of absorption, resulting in a low and variable oral bioavailability of about solubility and poor dissolution in gastric fluid 40-50 % [9]. It has been classified as biopharmaceutical classification system class II drug [10].Therefore we aimed to prepare Cefixime nanocrystals as a capsule dosage form in order to increase its oral dissolution rate and bioavailability.

Material and Method

Materials

Cefixime was supplied by "Samaraa Drug Industry, Iraq".Poloxamer 188 was purchused from "HiMedia Laboratories, India", Polyvinylpyrrolidone (PVP) from "Riedel De Haen AG Seelze, Honnover, Germany", HPMC from "HiMedia Laboratories, India", Methanol from "GCC Analytical reagent, UK", Magensium stearate from "Barlocher,GMBH, Germany", Hydrochloric acid and Potassium dihydrogen phosphate from "BDH Chemical LTD, England" and Sodium hydroxide from "Carlo Erba Reagents,(Spain)".

Method

Preparation of cefixime nanocrystals

Cefixime nanocrystals were prepared by the precipitation technique which is also called antisolvent precepitation method . Cefixime was dissolved in a methanol (5 ml) at room temperature, this was poured into 50 ml of water containing different types of surfactants and subsequently stirred at agitation speed of 250 round per minute (rpm) on magnetic stirrer for 1 hour to allow the volatile solvent to evaporate. Addition of organic solvents at certain rate by means of a syringe positioned with the needle directly into surfactant containing water. Variable drug to surfactant ratio was used. [11]

Twenty four formulas (F1-F24) were prepared by this technique demonstrated in table (1) with their composition.

Characterization of nanoparticles

Particle size analysis

Particle size determination of the prepared formulas (F1-F24) was done by using ABT-9000 nano laser particle size analyzer at scattering angle 90º. The average particle size reflects the size of those particles which constitute the bulk of the sample volume was measured after performing the experiment in triplicates . The polydispersity index (PDI) of each formula was also determined as a measurment for the width of the size distribution , it is a parameter to define the particle size distribution of nanoparticles obtained from a particle analyzer. PDI is an index of width or spread or variation within the particle size distribution. The analyzer also determines the specific surface area for each sample.

Table 1: Components of various formulas.

Formula	Cefixime Conc. Mg/Ml	Volume Injected Ml	Poloxamer 188 Conc%	PVP Conc %	HPMC Conc %	Solution Vol Ml	Injection Speed Ml/Hr
C*	20	5				50	60
1	20	5	0.025			50	60
2	20	5	0.05			50	60
3	20	5	0.1			50	60
4	20	5	0.2			50	60
5	20	5		0.025		50	60
6	20	5		0.05		50	60
7	20	5		0.1		50	60
8	20	5		0.2		50	60
9	20	5			0.025	50	60
10	20	5			0.05	50	60
11	20	5			0.1	50	60
12	20	5			0.2	50	60
13	20	2.5	0.025			50	60
14	20	2.5		0.025		50	60
15	20	2.5			0.025	50	60
16	20	10	0.1			50	60
17	20	10		0.1		50	60
18	20	10			0.1	50	60
19	10	5	0.025			50	60
20	10	5		0.025		50	60
21	10	5			0.025	50	60
22	5	5	0.0125			50	
23	5	5		0.0125		50	60
24	5	5			0.0125	50	60

- Control

Freeze drying of the prepared nanocrystals

After the evaluation of the prepared formulas (F1-F24), the formula with lowest particle size was selected and lyophilized using vacuum freeze dryer at a controlled temperature of −44 ˚C and the pump operating at a pressure of 2.5 × 10 pascal over a period of 48–72 hour. The yielded powder was used for further studies and also it is used to prepare the capsules.

Determination of present yield and entrapment efficiency:

Nanocrystals after being dried were weighed and the yield was calculated as a percentage of the total weights of starting material (polymer and drug) introduced into the system (this represents the theoretical weight of nanocrystals), and the actual weight of nanocrystals obtained. The percent yield was calculated using equation 1 [12]:

$$\%\text{Yield} = \frac{\text{Actual weight of nanocrystals gained}}{\text{Theoretical weight of nanocrystals}} \times 100 \quad \text{eq.(1)}$$

The entrapment efficiency of nanocrystals was determined from the theoretical and actual drug contents. The percent entrapment efficiency was calculated using equation 2 [12].

$$\%\ \text{Entrapment Efficiency (EE)} = \frac{\text{Actual drug content}}{\text{Theoretical drug}} \times 100 \quad \text{eq.(2)}$$

Preformulation studies of the prepared nanocrystals powder

The flowability of a powder is of critical importance in the production of pharmaceutical dosage forms in order to get a uniform feed as well as reproducible filling of capsules, otherwise high dose variations will occur.

The powder flowability of prepared cefixime nanocrystals were characterized by angle of repose [13].

In vitro dissolution profile of cefixime nanocrystals capsules

Dissolution studies of capsules containing cefixime nanocrystal were performed in dissolution apparatus using the paddle method according to USP 30.

Capsules containing cefixime nanocrystal (equivalent to 200 mg cefixime) were dispersed on 900 mL of dissolution media (0.1 N HCl pH of 1.2 and phosphate buffer pH 7.2) at 37˚C ± 0.5 and rotated at 100 rpm. Five milliliter samples were withdrawn for analysis periodically and replaced by the same volume of fresh media maintained at 37˚C ± 0.5. The withdrawn samples were filtered. The amount of cefixime released in the medium was diluted and determined spectrophotometrically at λ_{max} of 285 nm. The concentration of cefixime released at different time intervals was determined using the equation obtained from the calibration curve. Each in vitro release study was performed in triplicate. After each time interval , the average value of absorbance was computed and finally the percent of mean drug release was plotted against time to obtain dissolution profiles[11, 14].

Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy (FT-IR) spectrum were studied to detect any sign of interaction or complexation may occur between cefixime and stabilizers used in the preparation of the nanocrystals and with the excipients used in the preparation of capsules. The spectrum was obtained using FT-IR Shimadzu 8300 Japan . Samples which studied were pure drug , PVP and lyophilized powder of selected formula (F6). All these samples were grounded and mixed thoroughly with potassium bromide, at 1:5 (sample : potassium bromide) weight ratio. The spectrum obtained was in between the wave number of 4000-400 cm^{-1}[15, 16].

Differential scanning calorimetry (DSC) study

DSC can be used to determine the compatibility between the drug and excipients and also used to evaluate the crystalline state of drug especially when converted to nanocrystals. Thermal characteristics of the same materials that examined in FTIR study were determined by an automatic thermal analyzer system (Shimadzu, DSC– 60, Japan). Accurately weighed samples (5mg) were placed in nonhermetically aluminium pans and heated at the rate of 20 ºC/minute against an empty aluminium pan as a reference covering a temperature range of 50 ºC to 300 ºC [17, 18].

Powder X-ray diffraction analysis (PXRD)

X-ray diffraction is used to study the atomic and molecular structure of crystalline substances such as drugs and excipients. X-rays diffraction patterns (diffractograms) can be used to confirm the crystalline nature of a sample. Therefore, this information is used to verify whether the substances are crystalline or amorphous .PXRD diffractograms of the pure drug and lyophilized powder of F6 were recorded using Shimadzu diffractometer 6000 (Shimadzu , Japan) with input voltage at 220V/50Hz .

Surface morphology studies

Scanning electron microscopy (SEM)

The morphology of raw drug and cefixime nanocrystals were examined by scanning electron microscope (VEGA3 Tescan Czech republic) operated with a secondary detector at an acceleration voltage of 10 kv and at 100x magnification for raw drug and 15 kv and 10 kx for F13 . The morphology of raw drug was done by direct deposition of powder on double-sided carbon tape and coated with gold. While for liquid nanocrystals sample was prepared by the droplet evaporation technique. A droplet of liquid was deposited on double-sided carbon tape and dried at room temperature for the evaporation of water and then coated with gold[19].

Atomic force microscopy (AFM)

AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging and also can measure the particle size of the nanoparticles accurately . Droplets of selected formulas were deposited on freshly cleaved mica and dried 15 minutes in oven. Particle size,histogram of particle size distribution and 3D surface morphology of each formulawere obtained [19].

Results and Discussion

Particle size results of the prepared cefixime nanocrystal

Most of the prepared cefixime nanocrystal formulas showed a particle size result within nano range except formula 16 wide variations from 9nm to 651nm. Only one formula occur out of nanorange (F16) which gave a particle size range of 3539 - 4456nm. as shown in table (2).

It has been shown that formula 6 score the lowest average particle size, so this formula selected for further evaluation. Also it has been seen that PVP provided the lowest particle size when used as stabilizer. This could be due to PVP high affinity for both hydrophilic and hydrophobic surfaces [20]. And this means that PVP has a higher affinity to adsorb cefixime than Poloxamer 188 and HPMC.

Table (2): Particle size, specific surface area and polydispersity index results of various formulas.

FORMULA	Average particle size (nm)	Specific surface area (SSA) (m^2/g)	Polydispersity index (PDI)
C	250-315	7.64	0.263
1	397-629	4.94	0.018
2	28-31	71.45	0.337
3	140-223	13.44	0.018
4	140-199	13.91	0.012
5	199-281	9.13	0.009
6	9-11	201.18	0.585
7	88-125	21.28	0.015
8	88-125	22.09	0.011
9	70-88	26.20	0.429
10	39-44	50.38	0.439
11	281-500	6.43	0.021
12	315-500	5.86	0.028
13	397-500	4.98	0.013
14	199-281	9.68	0.012
15	281-500	6.80	0.031
16	3539-4456	0.52	0.000
17	177-250	10.58	0.013
18	445-561	4.47	0.009
19	353-500	5.36	0.011
20	39-50	48.48	0.292
21	111-140	17.88	0.120
22	353-561	29.65	0.173
23	62-79	29.65	0.173
24	315-445	6.15	0.011

Percent yield and entrapment efficiency of cefixime nanocrystals:

The percentage yield of cefixime nanocrystals of the selected formula (F6) was 87.6% and entrapment efficiency was 88%.

Flowability study of the prepared nanocrystal powder (Angle of Repose)

Angle of repose was measured for the selected nanocrystal formula (F6), to observe the flow properties of powder. Results show that the formula had poor flowability (angle of repose 50.19˚). So magnesium stearate (Mg.St.) 0.5% was added to improve the flow properties of the formula to insure efficient filling into capsules during the manufacturing process [21].

In vitro release study

In vitro drug release studies of the capsules containing selected formula, raw drug and marketed cefixime capsules (Cefix®) were carried out in 0.1N HCl containing 0.1% methanol and phosphate buffer pH (7.2) to simulate in vivo release in both stomach and intestine. Methanol was added to the 0.1N HCl solution to increase cefixime solubility and hence reach to sink condition. Results show significant increase ($P<0.05$) in dissolution rate of all the selected formula than the raw drug and the marketed one (figure 1). These results expected according to Noyes– Whitney equation where the solid dissolution rate is directly proportional to its surface area exposed to the dissolution medium. Also these results agreed to that of Mansouri *et al.* where they prepared ibuprofen nanoparticles by using solvent/antisolvent precipitation technique and found that the prepared ibuprofen nanoparticles had a dissolution rate 2.33 times more than that of raw drug [22].

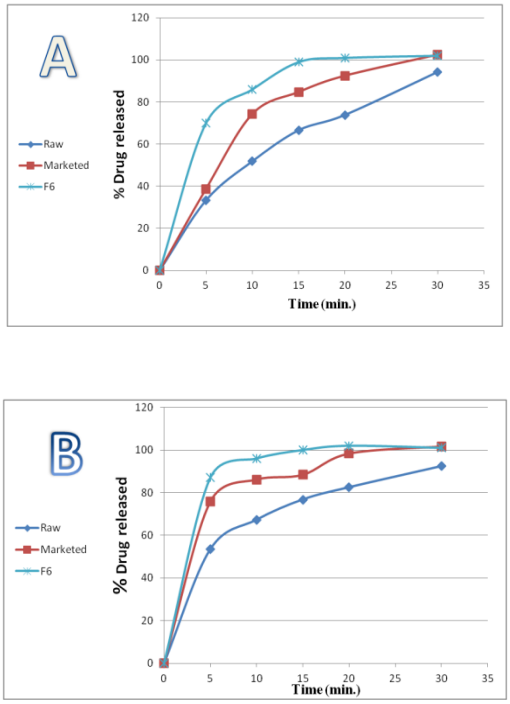

Figure 1: The dissolution profile of the selected formula (F6) at 37ºC ± 0.5 ºC and 50 rpm. A: in 0.1N HCl +0.1% methanol media.B: In phosphate buffer pH(7.2)

Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of raw cefixime, PVP, magnesium stearate and cefixime nanocrystals selected formulas (F6) were studied by FTIR spectroscopy using KBR disc (Fig. 2-5). The FT-IR spectra of pure cefixime (figure 2) powder display a characteristic –NH2 absorption peak at 3296 cm^{-1}, which is a normal range of absorption of primary amines. It exhibits a strong band for C=O stretching of the nonconjugated carboxylic acid at 1770 cm^{-1} whereas the second band which is expected to shift to lower frequency (owing to conjugation) appears as an overlapping band. The carbonyl of cyclic as well as acyclic amide appears at 1670 cm^{-1}. These peaks are agreed with the reported one which indicates the purity of the drug [23].

The results indicate that there was no chemical incompatibility between drug and polymer as all the characteristic IR peaks related to pure drug were also appear in the IR spectrum of the formulas.

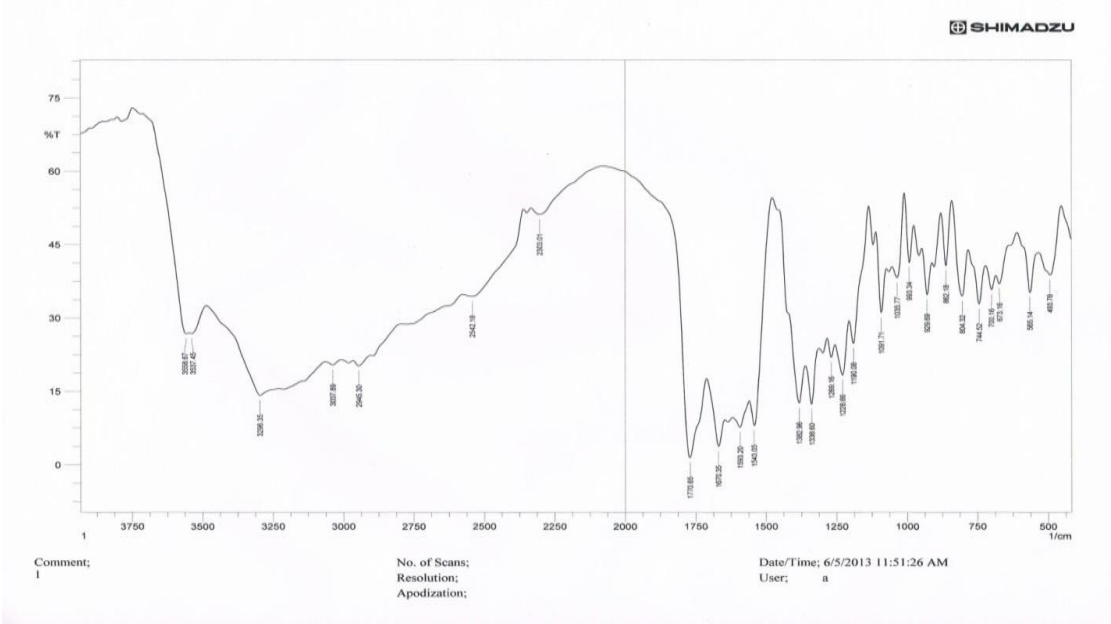

Figure 2: FT-IR spectrum of pure cefixime .

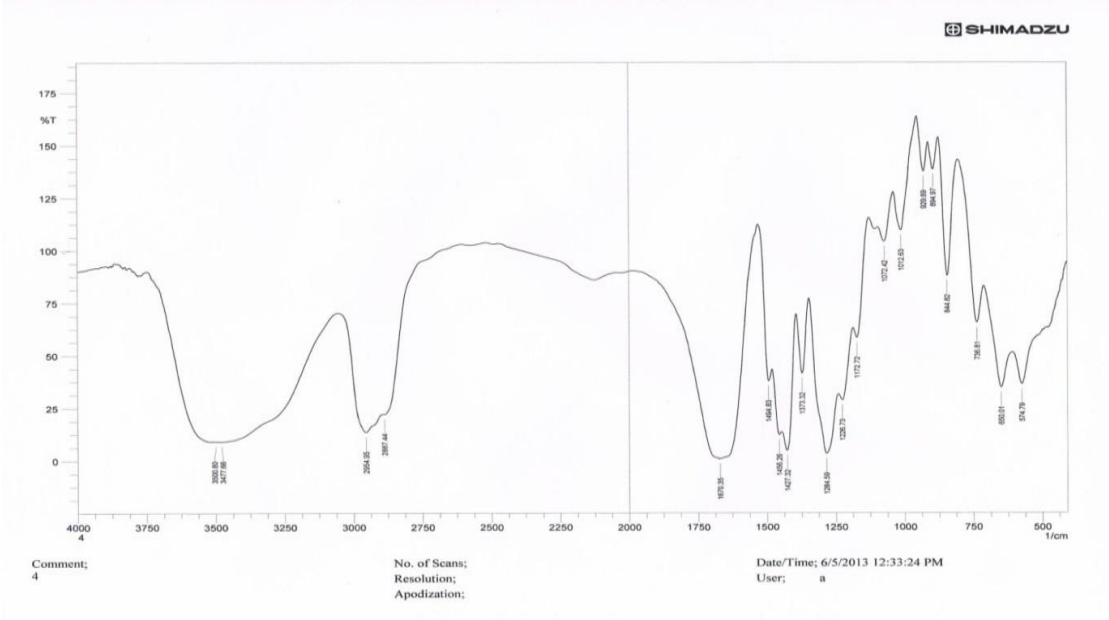

Figure 3: FTIR spectra of PVP.

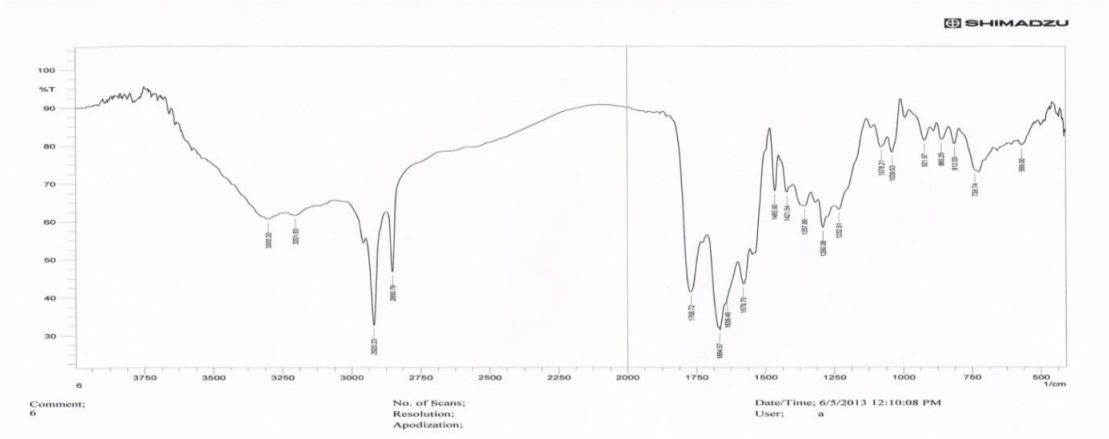

Figure 4: FTIR spectra of cefixime nanocrystals using PVP as stabilizer + Mg.St. (F6).

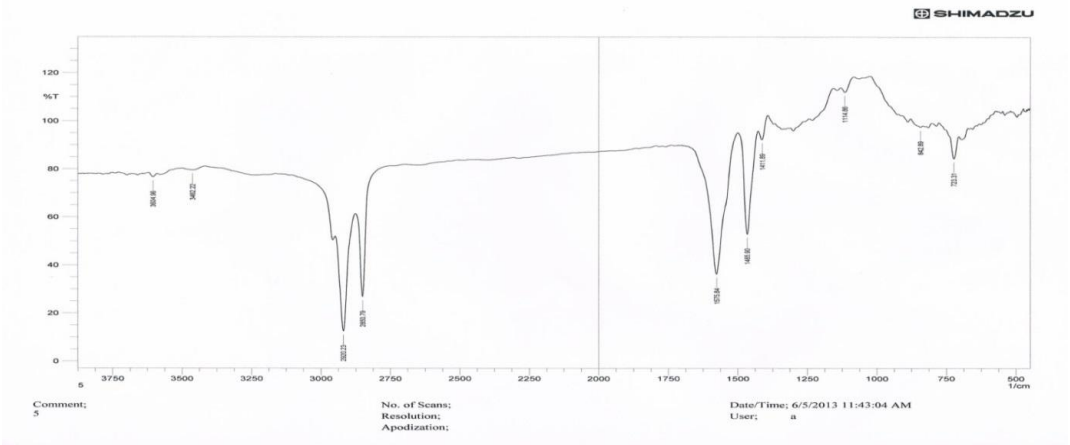

Figure 5: FTIR spectrum of Mg. St.

Differential scanning calorimetry (DSC)

DSC thermogram (figuer 6) of cefixime trihydrate shows broad endothermic peak at 217^{0}C, which is its melting point as cefixime melt with decomposition over the range 218-225ºC. Before melting point, DSC shows board endothermic peak at 125^{0}C which represent the removal of hydrates from Cefixime trihydrate[24].

The DSC thermogram of the selected formulas (F6) (figure 9) shows absence of the melting point peak, this result can be attributed to the change in the crystilanity state of the prepared formulas to the amorphous state (which also confirmed by XRD) and not to chemical interaction as FTIR study showed that there was no chemical interaction between the drug and the excipients. More over the board endothermic peak suggests that these formulas are product of physical mixture of the drug and the polymer used to prepare them, if it is a reaction product which might have formed during the formulation, it has given rise to short range of melting process with 2 to 3^{0}C, which has not happened in this case, it confirms the drug used in the formulation is in the free state rather than in the chemically reacted form. Drug is freely available to the system whenever administered [25].

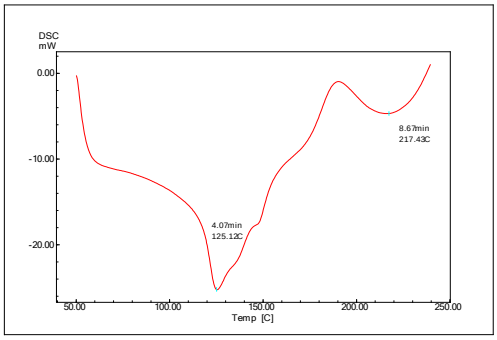

Figure 6: DSC curve of cefixime trihydrate.

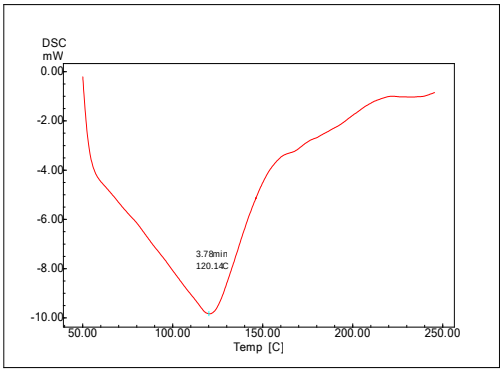

Figure 7: DSC curve of PVP.

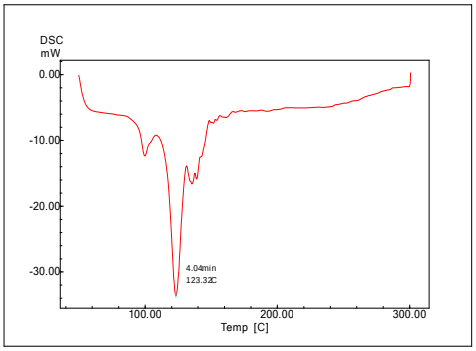

Figure 8: DSC curve of Mg.St.

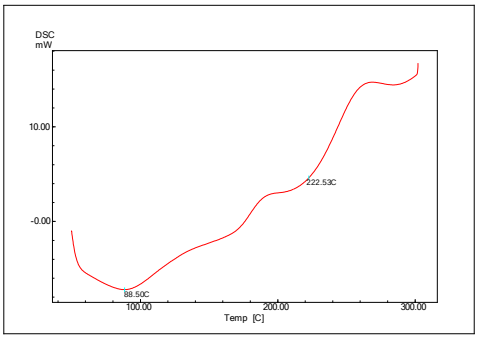

Figure 9: DSC curve of cefixime nano-crystals formula using PVP as stabilizer + Mg.St. (F6) .

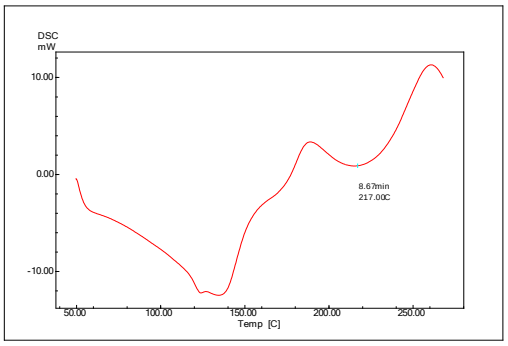

Figure 10: DSC curve cefixime and PVP (1:1) physical mixture.

Powder x-ray diffraction (XRD)

The results obtained from DSC reasonably agreed with the results obtained by PXRD. The change in the crystalline state of cefixime nanocrystals was further confirmed by X-ray diffraction. The X-ray patterns of the pure cefixime(figures 11)displayed the presence of numerous narrow and symmetrical characteristic diffractionpeaks with high intensity this indicated the crystalline structure of the drug , while XRD of prepared formula (figure 12) showed no sharp peak and less intensity of the diffraction peak when compared to that of raw drug indicating that the crystalline structure of cefixime was lost and converted into amourphous form. This results agreed with previous researches where Junghanns *et al.* stated that depending on the production technology, processing of drug

microcrystals to drug nanoparticles can lead to an either crystalline or to an amorphous product, especially when applying precipitation. In the strictest sense, such an amorphous drug nanoparticle should not be called nanocrystal. However, often one refers to "nanocrystals in the amorphous state" [6].

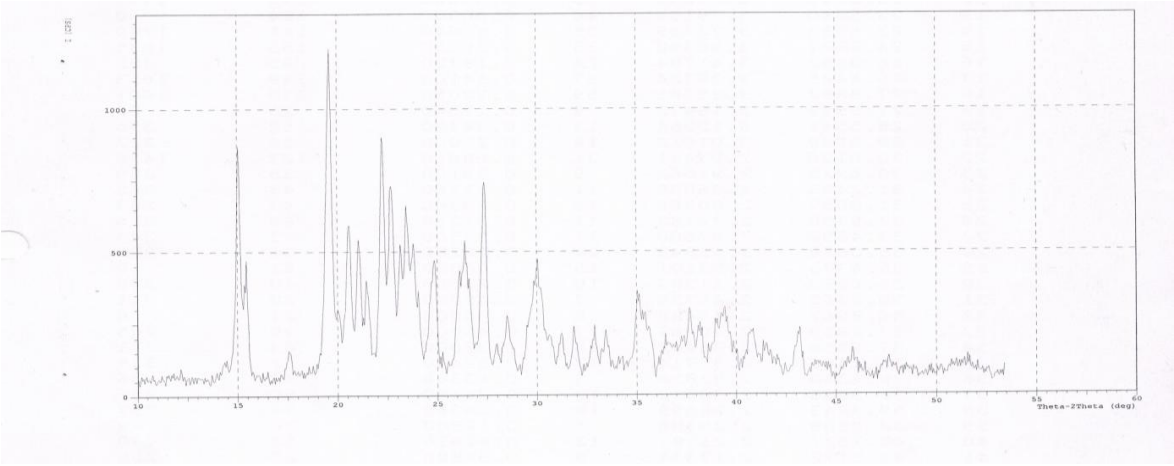

Figure 11: XRD diffractogram of cefixime trihydrate.

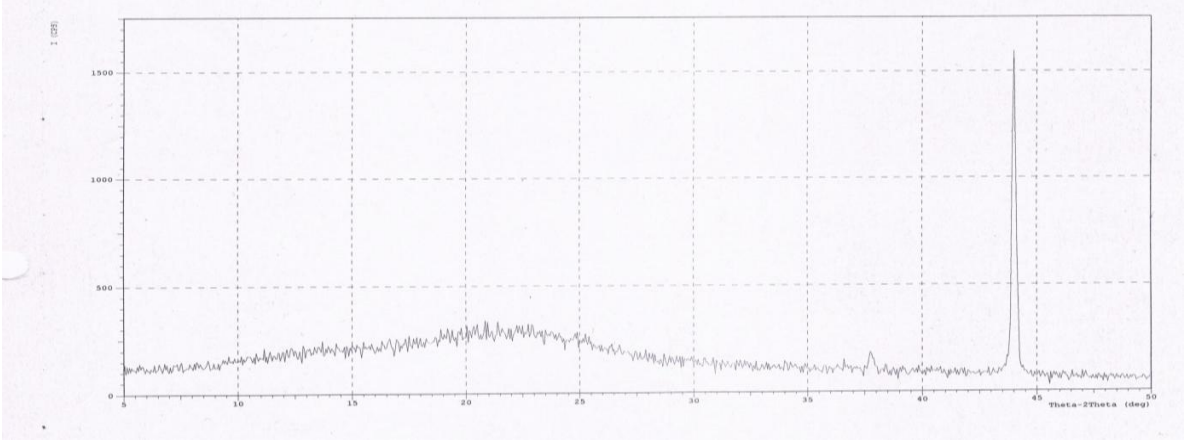

Figure 12: XRD diffractogram of formula 6.

Surface morphology studies

Scanning electron microscope (SEM)

The shape of nanocrystals obtained from the selected formula (F6) visualized by scanning electron microscope. Formula 6 magnified at 5 kx, 10 kx and 50 kx. (figure 13). It has been noticed from SEM images that the formula gave uniform submicron sized particles and this results will confirmed by AFM.

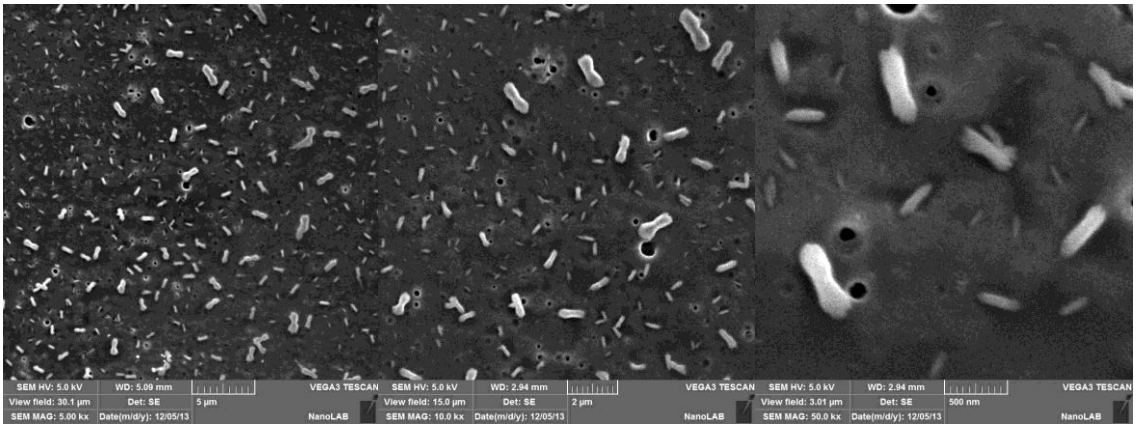

Figure 13: SEM images of (F6).

Atomic force microscope (AFM)

The atomic force microscope (AFM) is one kind of scanning probe microscopes (SPM) which are instruments that measure the properties of surfaces. AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging. With the high precision of the AFM, in principle it is possible to determine the dimensions of nanoparticles with high accuracy. AFM allows the visualization of samples with resolution in three dimensions x-, y- and z-directions in atmospheric or submerged conditions [26, 27].

The morphological analysis and particle size of formula (F6) performed by AFM (figure 14).

Results show spherical shaped nanoparticles with a size of 50-150 nm as it approved by the histogram of particle size distribution (figure 15). The formulation was found to be stable and no aggregation of particles could be observed. The particle size obtained by AFM was larger than that measured by ABT-9000 nano laser particle size analyzer, this variation because particle size analyzer is only capable of giving information about the volumetric mean diameter of a great number of particles, but results on the real size distribution are rather difficult to obtain. For a precise determination of single particle dimensions, size and distribution microscopic techniques are required [26].

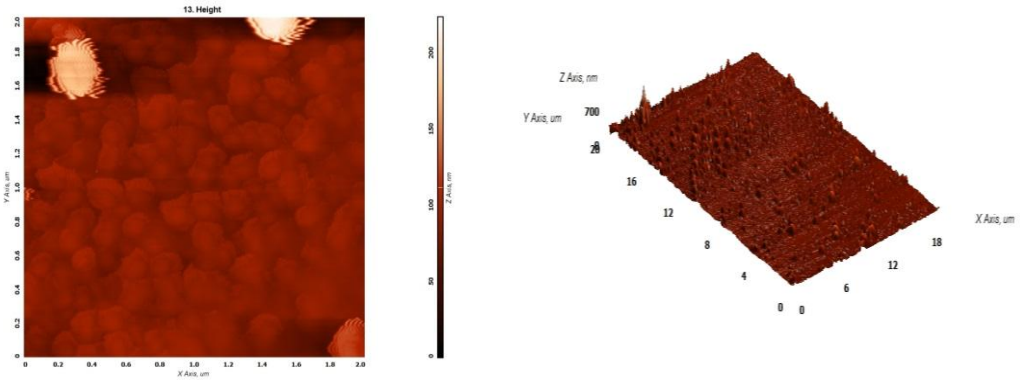

Figure 14: AFM image (F6).

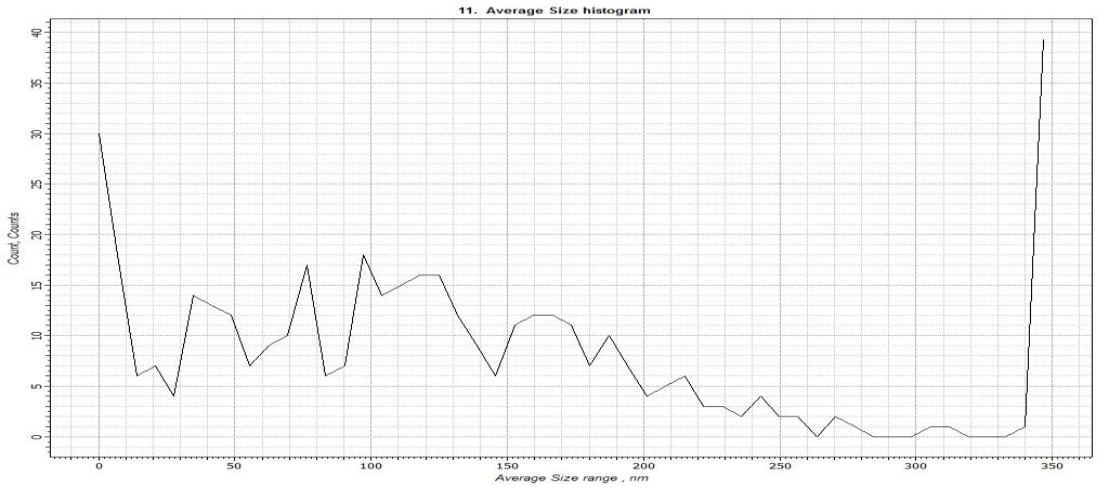

Figure 15: AFM average size histogram of (F6).

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