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Preparation, in vitro and ex-vivo Evaluation of Mirtazapine Nanosuspension and Nanoparticles Incorporated in Orodispersible Tablets

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Abstract

The objective of the present investigation was to enhance the solubility of practically insoluble mirtazapine by preparing nanosuspension, prepared by using solvent anti solvent technology. Mirtazapine is practically insoluble in water which act as antidepressant .It was prepared as nano particles in order to improve its solubility and dissolution rate. Twenty formulas were prepared and different stabilizing agents were used with different concentrations such as poly vinyl pyrrolidone (PVPK-90), poly vinyl alcohol (PVA), poloxamer 188 and poloxamer 407. The ratios of drug to stabilizers used to prepare the nanoparticles were 1: 1 and 1:2. The prepared nanoparticles were evaluated for particle size, entrapment efficiency, dissolution study, Fourier transform infrared spectroscopy, differential scanning calorimetry, and atomic force microscopy. The percentage of drug entrapment efficiency of F1-F20 was ranged from 78.2% ± 1 to 95.9 % ± 1. The release rate and extent of mirtazapine nanoparticles were inversely proportional to the particle size of the drug i.e. it decreased when particle size increase. It is concluded that the nanoprecipitation have potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of mirtazapine. The prepared nanosuspension showed enhanced dissolution which may lead to enhanced solubility of mirtazapine.

Keywords: Mirtazapine, Nanoparticles, Particle Size, Poloxamer.

تحضير وتقييم في المختبر وخارج الجسم الحي لمعلق الميرتازابين النانوي و الجسيمات النانوية مدمجة في اقراص قابلة للتشتت

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الخلاصة

ان الهدف من هذه الدراسة هو تعزيز قابليه الذوبان لعقارالميرتازابين غير القابلة للذوبان عمليا من خلال اعداد تعليق نانوي , تم اعداده باستخدام باستخدام تكنلوجيا المذيبات المضادة للالمذيبات. ميرتازابين هو دواء غير ذائب بالماء وهو دواء يستخدم لعلاج الاكتئاب الشديد. ميرتازابين اعد كجسيمات نانوية بغيه تحسين القابلية للذوبان ومعدل الامتصاص.

تم اعداد عشرين صيغه باستخدام بوليمرات استقرار مختلفة استخدمت بتراكيز مختلفة مثل مثل الفاينيل بايرولدون المتعدد PVP, وبولي الفنيل الكحول PVA, بولوكسامير ١٨٨و بولوكسامير ٤٠٧. وكانت نسب الدواء الى المثبتات المستخدمة في اعداد الجسيمات النانويه هي ١:١, ١:٢.

وقيمت جسيمات نانوية من حيث الحجم الحبيبي للجسيمات وكفاءه انحباس الدواء، ودراسة الشكل البياني للتحرر الدوائي، وكذلك دراسة التوافق (مطيافيه الأشعة تحت الحمراء، وقياس المسح التفاضلي) ومجهر القوة الذرية. النسبة المئوية لكفاءة انحباس الدواء للصيغ الدوائية من الصيغة الاولى الى الصيغة عشرين هي من ٧٨,٢% الى ٩٥,٩%. من ناحية اخرى يزداد تحرر الدواء كلما صغر حجم الجسيمات النانوية لزيادة المساحة السطحية للجسيم. ويمكن استنتاج ان الجسيمات النانوية لديها القدرة على صياغة تعليق متجانس مع جسيمات نانوية مستقرة موحدة الحجم من الميرتازابين. وأظهرت ان تعزيز الانحلال يؤدي إلى تعزيز الذوبان من الميرتازابين.

الكلمات المفتاحية: ميرتازابين، الجسيمات النانوية، الحجم الحبيبين، بولوكسامير.

Introduction

Solubility is of the most important parameter to achieve the desired concentration of drug in systemic circulation for pharmacological response to be shown. Poorly water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. Low water solubility is the major problem encountered with formulation development of new chemical entities[(1)]. Several formulation techniques exist for the manufacturing of nanosuspension, precipitation has been applied to prepare submicron particles, especially for the poorly soluble drugs. Rapid addition of a drug solution to the anti-solvent leads to sudden super saturation of drug and formation of ultrafine crystalline or amorphous drug solids[(2)]. Mirtazapine is an antidepressant drug used for the treatment of moderate to severe depression, molecular formula: $C_{17}H_{19}N_3$[(3)]. The drug has bioavailability of 50 % due to first-pass metabolism, high protein binding (80 %) and very high half-life (20 – 40 h)[(4)]. The aim of this study is to formulate and evaluate mirtazapine nanoparticles using solvent anti solvent method.

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Materials and Method

Materials

Mirtazapine powder was purchased from (Hyper-chem, China), PVP K-90(Hangzhou Sunflower ,China),PVA(JP&SB Converting Services, Spain), poloxamer 188 and poloxamer 407(HIMEDIA (Mumbai, India), methanol (Scharlau Chemie, S.A. Spain). All other chemicals were of analytical grade.

Method

Preparation of mirtazapine nanosuspension

Nanosuspensions of mirtazapine were prepared by the solvent evaporation technique, which is also termed as anti-solvent precipitation method. Mirtazapine powder was dissolved in methanol (4 ml) at room temperature. This was poured into 20 ml of water containing different types of stabilizer (alone and in combination) maintained at 50^0C and subsequently stirred at agitation speed of 500 revolution per minute (rpm) on magnetic stirrer for 60 min.to allow the volatile solvent to evaporated [(5)]. The resultant organic solution of drug (organic phase) was added drop by drop by means of a plastic syringe positioned with the needle directly into aqueous solution of stabilizer. The ratios of drug to stabilizer used to prepare the nanosuspension were 1:1 and 1:2, as shown in table (1,2).

Table1. Composition of mirtazapine nanosuspension using different stabilizers at drug: stabilizer ratio 1:1.

Formula	Mirtazapine (mg)	Poloxamer 188 (mg)	Poloxamer407 (mg)	PVP-k90 (mg)	PVA (mg)	Methanol (ml)	Water (ml)
F1	**15**	**15**				**4**	**20**
F2	**15**		**15**			**4**	**20**
F3	**15**			**15**		**4**	**20**
F4	**15**				**15**	**4**	**20**
F5	**15**	**7.5**	**7.5**			**4**	**20**
F6	**15**	**7.5**		**7.5**		**4**	**20**
F7	**15**	**7.5**			**7.5**	**4**	**20**
F8	**15**		**7.5**	**7.5**		**4**	**20**
F9	**15**		**7.5**		**7.5**	**4**	**20**
F10	**15**			**7.5**	**7.5**	**4**	**20**

Table2. Composition of mirtazapine nanosuspension using different stabilizers at drug: stabilizer ratio 1:2

Formula	Mirtazapine (mg)	Poloxamer 188 (mg)	Poloxamer407 (mg)	PVPk-90 (mg)	PVA (mg)	Methanol (ml)	Water (ml)
F11	15	30				4	20
F12	15		30			4	20
F13	15			30		4	20
F14	15				30	4	20
F15	15	15	15			4	20
F16	15	15		15		4	20
F17	15	15			15	4	20
F18	15		15	15		4	20
F19	15		15		15	4	20
F20	15			15	15	4	20

Evaluation of the prepared nanosuspension

Particle size and size distribution

Particle size determination was done by using Angstrom Advanced Inc. ABT-9000 USA particle size analyzer which is a dynamic light scattering works by measuring the intensity of light scattered by the molecules in the sample as a function of time, at scattering angle 90° and a constant temperature of 25 °C. The polydispersity index (PDI) which is a measure of the width of the size distribution of each formula of mirtazapine nanosuspension was measure of the distribution of particle size of nanoparticles obtained from a particle analyzer, PDI is an index of spread or variation or width within the particle size distribution. Also, the analyzer determines the specific surface area of each sample[6] .

Determination of drug entrapment efficiency (EE) of nanosuspension

The freshly prepared nanosuspension of drug: stabilizer ratio 1:1, and 1:2 was centrifuged at 20,000 rpm for 20 minutes using ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml with water at 290 nm using UV-visible spectrophotometer. It was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. For each formulation the experiment was repeated in triplicate and the average was calculated [7].

In vitro dissolution profile of nanosuspension

The in vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using mirtazapine nanosuspension in 900 ml of 0.1N HCL (pH 1.2) maintained at 37 ± 0.5°C , 50 rpm and samples (5ml) were withdrawn at regular intervals of 5 minutes for 120 minutes and replaced with fresh dissolution medium. Samples were filtered through filter paper and assayed spectrophotometrically on UV-Visible spectrophotometer at 315 nm wave length [8].

Freeze drying of nanosuspension

In order to make nanoparticles in dried-powder state from the nanosuspensions, water-removal was conducted through freeze-drying, so that each formula was lyophilized using vacuum freeze dryer at a controlled temperature of (- 45) °C and the pump operating at a pressure of 2.5 × 10 pascal over a period of 48–72 hour. The yielded powders were used for further studies and also it is used to prepare the tablets [9].

Formation of mirtazapine nanoparticles tablet

Mirtazapine formulated in to orodispersible tablets by direct compression method containing drug equivalent to 15mg mirtazapine. All ingredients were properly mixed to gather then compressed in to tablets prepared by after freeze drying of formula (F15) that gave the best in vitro dissolution profile in minute in comparison with other nanoparticle formulas and pure drug, show as in table (3) [10].

The orodispersible tablets were prepared using Avicel PH102 (MCC), crospovidone and, magnesium stearate as a diluent, disintegrente ,and lubricant at different concentration and tested to obtain the optimum formula that show the accepted hardness and the best in vitro dissolution profile[11].

Table 3. Composition of mirtazapine tablets

Materials	Quantity per tablet (mg)	
	F 15 a	F 15 b
Lyophilized Powder	45	45
Avicel PH 102 (MCC)	82	92
Crospovidone	20	10
Magnesium Stearate	3	3
Tablet Weight (mg)	150	150

Precompression studies of the prepared nanoparticle 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 tablet dies otherwise high dose variations will occur. The powder flowability of prepared mirtazapine tablets were characterized by angle of repose, Hausner's ratio and Carr's index [12].

Evaluation of Mirtazapine Orodispersible Tablets

Tablets were evaluated for hardness test, friability test, content uniformity test and weight variation tests [13], and dissolution study.

In vitro dissolution profile of mirtazapine tablets

An in vitro dissolution test was conducted in a dissolution apparatus according to the USP paddle method. The temperature was maintained at 37 ± 0.5°C, and the stirring rate was at 50 rpm.

The commercial mirtazapine tablet accurately weighed bulk drug and were dispersed in 900 ml of dissolution medium (0.1 N HCl). 5 ml samples were drawn, and the same volume of fresh dissolution medium was added at 5, 10, 15, 20, 30, 45, 60, 70, 90, 105, 120 minutes.. Then, the samples were filtered through a 0.1-µm syringe filter immediately before dilution, when necessary. Drug content was determined with a UV spectrophotometer at **315** nm for 0.1 N HCl (pH 1.2) [14].

Fourier transform infrared spectroscopy (FTIR)

The (FTIR) spectra were recorded for pure drug and optimized formulation using KBr pellet technique.The pellets were prepared using KBr hydraulic press under hydraulic pressure of 150 kg/cm2 . The spectra were scanned over 3600-400 cm−1 at ambient temperature with a resolution of 4 cm−1, using FT-IR 2500 apparatus [15].

Differential scanning calorimetry (DSC)

DSC investigations were performed using DSC apparatus model DSC-6. Samples of about 5 mg of pure drug powder and selected formula are placed in an aluminum pan and the experiment was carried out under nitrogen atmosphere at a flow rate of 40 mL/min and scanning rate of 10°C/min in the range of 15-300°C[16].

High performance liquid chromatographic (HPLC)

RP-HPLC system was used for this study and the specifications are given below. A Waters HPLC equipped with SPA- 20A detector, an isocratic chromatographic separation technique was conducted utilizing Symmetry® ODS-C18 (250 × 4.6mm; 5µm) column and Breeze software.

Chromatographic conditions:

- Mobile phase: HPLC grade of Methanol: 0.1M ammonium formate solution in a ratio of 77:23 percent (v/v) was filtered through (0.45µm) Millipore filter.
- Flow rate: it was maintained at 1.0 ml/min of the mobile phase.
- Detection was carried out by UV- detector; at a wavelength of 315 nm and the running time was 10 min. One hundred milligrams of mirtazapine was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in 50 ml HPLC grade methanol and sonication for about 10 minutes and then made up to the volume with HPLC grade methanol. From this stock solution (1mg/ml) eight serial dilutions (1.66, 2.5, 5, 10, 20, 30, 40, and 50 µg/ml) were prepared. Twenty microliters of each dilution were injected into the column and the corresponding chromatograms were obtained [17].

Atomic force microscopy (AFM)

The AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging. The size and surface morphology of mirtazapine nanoparticle were confirmed by atomic force microscopy of the formula. Samples were determined in tapping mode, exerting pyramidal cantilevers with Pt probes. All results were recorded under ambient laboratory condition and scanning frequency of 2Hz. Resonance frequency was 79.491 kHz and a constant force in the range 2.5-10Nm^{-1}, driving amplitude 334.6mv. silicon chip was newly operated by peeling off its upper layer to Form the sample. Particle size, 3D-dimension graph and histogram of particle size distribution were obtained[18].

Statistical Analysis

The results of the experiments were given as a mean of triplicate samples ± standard deviation and were analyzed according to the paired T test and one way analysis of variance (Single Factor ANOVA) at the level of ($P < 0.05$).

Results and Discussion

Evaluation of nanosuspension

Particle size analysis

The particle size of F1-F4 at drug : stabilizer ratio 1:1 was ranged from 429 - 691 nm measured by particle size analyzer (as shown in table 4) while for F11-F14 at drug : stabilizer ratio 1:2 the particle size ranged from 379 - 572 nm as in table (4) using poloxamer 188 , poloxamer 407 , PVP- K90 and PVA as primary stabilizers. PVP K-90 , poloxamer and PVA are stabilizers for nanosuspension. Vinyl groups of PVA (Polyvinyl alcohol), due to their hydrophobic nature tend to adsorb onto the hydrophobic part of mirtazapine nanoparticles while –OH extend themselves outside into the aqueous environment and thus providing stabilization to the nanoparticles and preventing agglomeration. –OH bonds of PVA makes hydrogen bonding with water molecules and thus viscosity of it increases [19].

Polydispersity index is a parameter used to define the particle size distribution obtained from the particle size analyzer. Polydispersity index gives degree of particle size distribution at range from 0.021 to 0.420 depending on formulation variables. The formula F10 showed lowest PDI (0.029) at drug : stabilizer ratio 1:1 and 0.114 at drug : stabilizer ratio 1:2, as seen in table (4) ; that indicate good uniformity of nanoparticle size. Uniformity of particle size is determined by polydispersity index values in which the low value means the best uniformity[20].

The range of PDI values (0-0.05) means (monodisperse system), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid-range polydispersity), and >0.7 (very polydispersity). From the obtained results, one can conclude that the poloxamer 188 and poloxamer 407 are suitable as a primary stabilizer for nanoparticles because of poor adsorption and affinity of poloxamer to the drug molecules.

Effect of polymer concentration on the size of Mirtazapine nanoparticles

The effect of the stabilizer concentration on the particle size was investigated by depending on two ratios 1:1 of drug : stabilizer in the preparation of F1-F10 and 1:2 of drug : stabilizer in the preparation of F11-F20. Not only the type of stabilizer affects the particle size, but also the concentration of the stabilizers used. Stabilizer concentration also influences on the adsorption affinity of non-ionic stabilizers to particle surface.

In general, as the concentration of stabilizer increase the particle size decrease at fixed drug concentration, the concentration of stabilizer may give negative effect (decrease particle size) or positive effect of on particle size (increase particle size). It can also influence on the adsorption affinity of non-ionic stabilizers to particle surface. In general, as the concentration of stabilizer increases the particle size decreases at fixed drug concentration.[21].

It was observed that with an increase in surfactant concentration in the nanosuspension from the particle size of the nanosuspension decreases. This was due to the decrease in relative viscosity, which led to decrease in particle size. It means that hydrodynamic diameter of particle decreased with increase in the concentration of the surfactant.The concentration of surfactant affected on particle size because too little concentration of stabilizer induces agglomeration or aggregation of particles [22]. As shown in tables (4,5) the size range of particles is decrease in the sequence of F1 (429nm) > F11 (383 nm), F2 (444nm) > F12 (401nm) that correspond to 1:1, 1:2 of drug: stabilizer (poloxamer 188, poloxamer 407) ratio, respectively. These results indicated that mean size of particles showed a regular decrease with increasing the concentration of poloxamer. These effects due to a process of a primary covering of the newer surfaces competing with the aggregation of the uncovered surfaces. Hence, an elevation in ratio of surfactant in the primary dispersion results in rapid enclosing of the newly formed particle surfaces. There was an optimum concentration of surfactant, above which the increase in concentration did not result in a decrease in particle size due to saturation point; these results are in agreement when poloxamer was used as stabilizer at different ratio [23].

Poloxamer is a block co-polymer, can act as a surfactant, is responsible for the hydrophobic association with the molecules of drug.

The inhibition of the crystal growth is mainly related to the hydrophobic part (polypropylene oxide group PPO) in the pluronic polymer, while the second chain which is (the hydrophilic oxide) (PEO) can provide steric hindrance against particles aggregation[24]. The size range of particles is also decreased in the sequence F3 (460) > F13 (379nm), F4 (691nm) > F14(572nm) that correspond to 1:1, 1:2 of drug: stabilizer PVA, PVP k-90 ratio, respectively.

On the other hand, the adsorption of surfactant makes the particles less hydrophobic and thereby reduces the hydrophobic forces of attractions (van der Waals interactions) and that reduced particle growth and aggregation[25].

Effect of combination of two polymers on the size of mirtazapine nanoparticles

The particle size of (F5-F10) of drug : stabilizer ratio 1:1 was ranged from 310-610 nm (table 4) , (F15- F20) of drug : stabilizer ratio 1:2 was ranged from 146-544 nm (Table 5). At ratio1:2 drug : stabilizer large particle size show in combination of poloxamer 188 and PVP k-90 gave higher size than alone that show in F16 (544nm). In F11that contain poloxamer 188 alone get particle size 383 nm and in F13 that contain PVP k-90 alone get particle size 379 nm that mean PVP has a higher affinity to adsorb mirtazapine than Poloxamer 188, these results due to that the combination lead to increase viscosity of the disperse media, so it is ineffective combination and cannot stabilize the nanoparticulate system [26]. Nanoparticles formulation generally requires addition of appropriate stabilizers to lower the free surface energy of the nanoparticles and prevent particle aggregation and/or particle growth. The high surface free energy of nanoparticles is readily lowered by lowering the solid–liquid interfacial tension upon addition of surfactants[24].

The formula F15 showed lowest PDI (0.021), as seen in table 5 at drug : stabilizer ratio 1:2, that indicate good uniformity of nanoparticle size. Uniformity of particle size is determined by polydispersity index values in which the low value means the best uniformity when used two stabilizer (poloxamer 188 + poloxamer 407).

Table 4. Particle Size , PDI and EE% of formulas at drug: stabilizer ratio 1:1.

Formula	Stabilizers	Particles size	PDI	EE%
F1	Poloxamer 188	429	0.171	83.9
F2	Poloxamer407	444	0.214	83.3
F3	PVP-k90	460	0.187	78.2
F4	PVA	691	0.293	85.3
F5	Poloxamer188+Poloxamer407	342	0.178	89.3
F6	Poloxamer188+PVP-k90	610	0.420	78.7
F7	Poloxamer188+PVA	492	0.123	88
F8	Poloxamer407+PVP-k90	488	0.331	86.5
F9	Poloxamer407+PVA	325	0.142	89.6
F10	PVP-k90+PVA	310	0.029	89.6

Table 5. Particle size, PDI and EE% of formulas at drug: stabilizer ratio 1:2.

Formula	Stabilizers	Particles size	PDI	EE%
F11	Poloxamer188	383	0.079	88.2
F12	Poloxamer407	401	0.192	93.7
F13	PVP-k90	379	0.113	89.2
F14	PVA	572	0.051	87.8
F15	Poloxamer188+Poloxamer407	146	0.021	95.9
F16	Poloxamer188+PVP-k90	544	0.341	79.6
F17	Poloxamer188+PVA	381	0.132	87.8
F18	Poloxamer407+PVP-k90	338	0.038	87.2
F19	Poloxamer407+PVA	239	0.188	88.7
F20	PVP-k90+PVA	208	0.114	89.7

Determination of drug entrapment efficiency of nanosuspension (EE%)

The EE% of the formulations from 78.2% – 95.9 % (Table 4,5) The drug entrapment efficiency of F15was high when compared to other formulations. In present work, a relatively high %EE (95.9) in F15 was obtained for most of the prepared mirtazapine nanosuspension formulas which may be attributed higher affinity towards the lipid matrix due to its lipophilic partition coefficient [27]. They represent integral parameters in the formulation due to their influence on drug release characteristics and therefore its bioavailability to the biological system. Hydrophobic drug molecules are easier to be incorporated in nanoparticles with higher efficiency relative to hydrophilic drugs due to the later tendency to partition into the aqueous phase-out of the lipid phase during homogenization[28].

In-vitro drug release study of mirtazapine nanosuspension

In vitro dissolution study was performed for all formulas using USP dissolution test apparatus-II. In 0.1N HCl and in phosphate buffer solution (pH 6.8) media showed the F15 that contain poloxamer 188 and poloxamer 407 stabilizers gave the best release when comparison with other formulas and the formula shows a maximum cumulative percentage drug release of 99.9% within 20 min. The release of F15 in media of 0.1N HCl and in phosphate buffer pH6.8, the maximum cumulative percentage drug release reach to 99.9 % within 20 minutes and in phosphate buffer solution (pH 6.8) release of F15 reach 90.2 % in 40 minutes[29] (Figure1).

The release of F15 was compared with the pure drug in media of 0.1N HCl (Figures 2) the maximum cumulative percentage drug release of F15 was 99.9 % within 20 minutes, whereas the pure drug having a release of 93.2 % within 60 minutes. The obtained results are in good accordance with Noyes–Whitney equation which states that the increase in saturation solubility and the decrease in particle size lead to an increased dissolution rate[30].

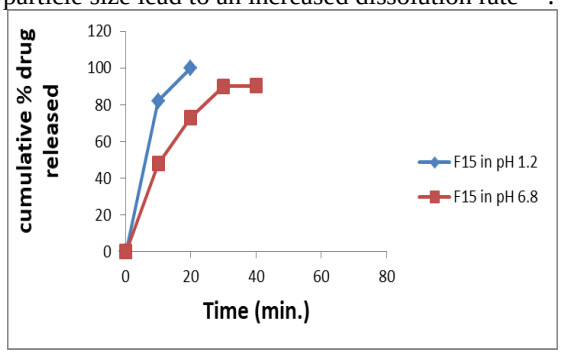

Figure 1. Dissolution profile of mirtazapine (F15) nanosuspension in 0.1 N HCl (pH 1.2) and in phosphate buffer (pH 6.8) at 37°C .

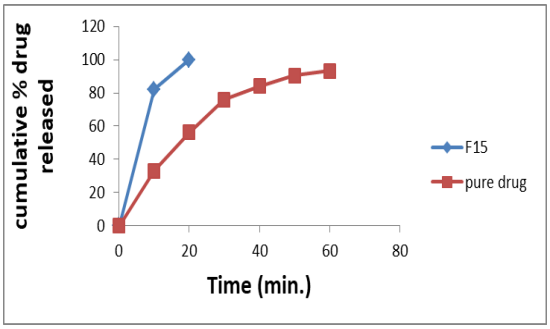

Figure 2. Dissolution profile of mirtazapine (F15) nanosuspension and pure drug in 0.1N HCl (pH 1.2) at 37°C

Drug content in lyophilized powder

The drug content result of lyophilized powder of the selected formula (F 15) 97.64% of mirtazapine when determined by UV-visible spectrophotometer at λmax 315 nm**.**

Evaluation of mirtazapine nanoparticles powder

Powder flowability

Angle of repose and compressibility index of the powder of the formulas (F15a and F15b) were reported in Table (6).

Table 6. Flow properties of prepared blends of mirtazapine incorporating drug nanoparticles.

Formula	Angle of repose	Carr's index	Hausner ratio	Physical property	
				Angle of repose	Carr's index
F15a	13.6	11.4	1.16	Excellent	good
F15b	21	27.5	1.34	good	poor

Evaluation of mirtazapine tablets

The mechanical properties of pharmaceutical tablets are quantifiable by the friability, hardness or crushing strength. The hardness of all the formulas as shown in **Table** (7) had an acceptable values 7, 6.5 kg/cm^2. The hardness of F15a containing MCC (Avicel)® 82 mg was 7kg /cm2 larger than F15b .

During compression, MCC (Avicel)® PH 102 is believed to undergo stress relief deformation by several mechanisms, this might be attributed to the hydrogen bonds formed among the hydroxyl groups of the adjacent cellulose particles of (Avicel)®, which are brought closely together by plastic deformation during compression, so that it produces hard tablets at low compression forces[31].

The loss in total weight of the tablets due to friability was found in all formulation, which indicated to be less than 1% for friability and which confirms the mechanical stability of tablets[32]. Physical properties of the prepared tablets, weight variation and drug content, demonstrated in **Table** (7). The weight variation of F15a, F15b was within the pharmacopoeia limits which is ± 7.5% of the average weight. Weight variation of the prepared tablets was within the limit (149.2 mg, 147.9 mg) and this indicates that there is no deviation from the limit of 7.5% of USP pharmacopoeia limits[33]. The content uniformity of the prepared formulas was within the accepted pharmacopeia limits (85% - 115%) and this mean that all the formulations revealed good uniformity and had yielded results from 101% , 98.7 respectively. Disintegration time of prepared tablets about 10 sec. and 13 sec.

Table 7. Mechanical strength and physical properties of the prepared mirtazapine incorporating drug nanoparticles

Formula	Hardness (kg/cm2)	Friability %	Weight variation (mg)	Drug content (%)	Disintegration time (sec.)
F15a	7	0.45	149.2	101	10
F15b	6.5	0.67	147.9	98.7	13

In Vitro dissolution study of tablet

The release profiles of the prepared mirtazapine tablets incorporating drug nanoparticles (F15a, F15b) and tablet marketing of mirtazapine as a reference were tested in 0.1N HCl (pH 1.2) and phosphate buffer solution (pH 6.8) as shown in figures (3) and (4), F15a was faster compared with F15b and the marketed tablet of mirtazapine.

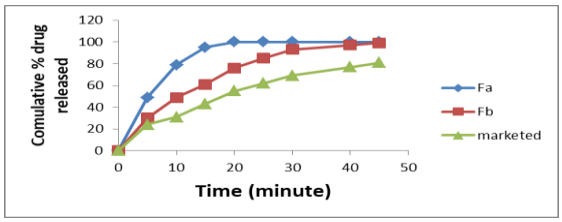

Figure 3. Dissolution profile of prepared tablets and mirtazapine marketed in buffer (pH 1.2) at 50 r.p.m and 37ºC .

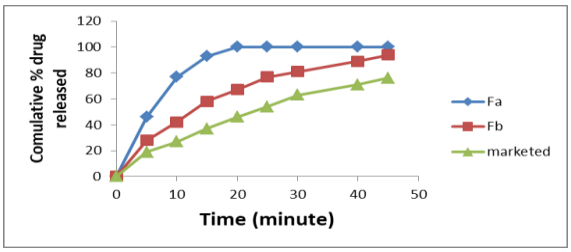

Figure 4. Dissolution profile of prepared tablets and mirtazapin marketed in buffer (pH 6.8) at 50 r.p.m and 37ºC.

Fourier transform infrared spectroscopy (FTIR)

FTIR is one of the most widely reported spectroscopic techniques for solid-state characterization. IR spectroscopy of mirtazapine (Figure 5), N-H stretching 3245 cm^{-1}, Methyl group attached to a N2 atom gives rise to a band at 2854 cm^{-1} , Bands for C-C stretching of the phenyl group appeared at 1585 cm^{-1} and 1444 cm^{-1}. The primary aromatic amines with N directly on the ring give bands at 1336-1200 cm^{-1}.

The benzene ring C-H appears in the range of 1359-1074 cm^{-1} and 788-636 cm^{-1} for the in plane and out of plane bending vibrations respectively [34, 35]. The characteristic bands of mirtazapine as lyophilized powder, blend powder of best formula (F15a) show The benzene ring C-H appears 1084 cm^{-1}, C-H stretching vibrations band of methyl group at 3101.94 cm^{-1}, Bands for C-C stretching of the phenyl group appeared at 1640 cm^{-1}, N-H stretching peak show between (3101-3369) cm^{-1}.

It was observed that there were no changes in these main peaks in the FTIR spectra of a mixture of drug and excipients. The FTIR study demonstrate that no physical or chemical interactions of Mirtazapine with other excipients. These are the main characteristic absorption band show in figure (6,7).

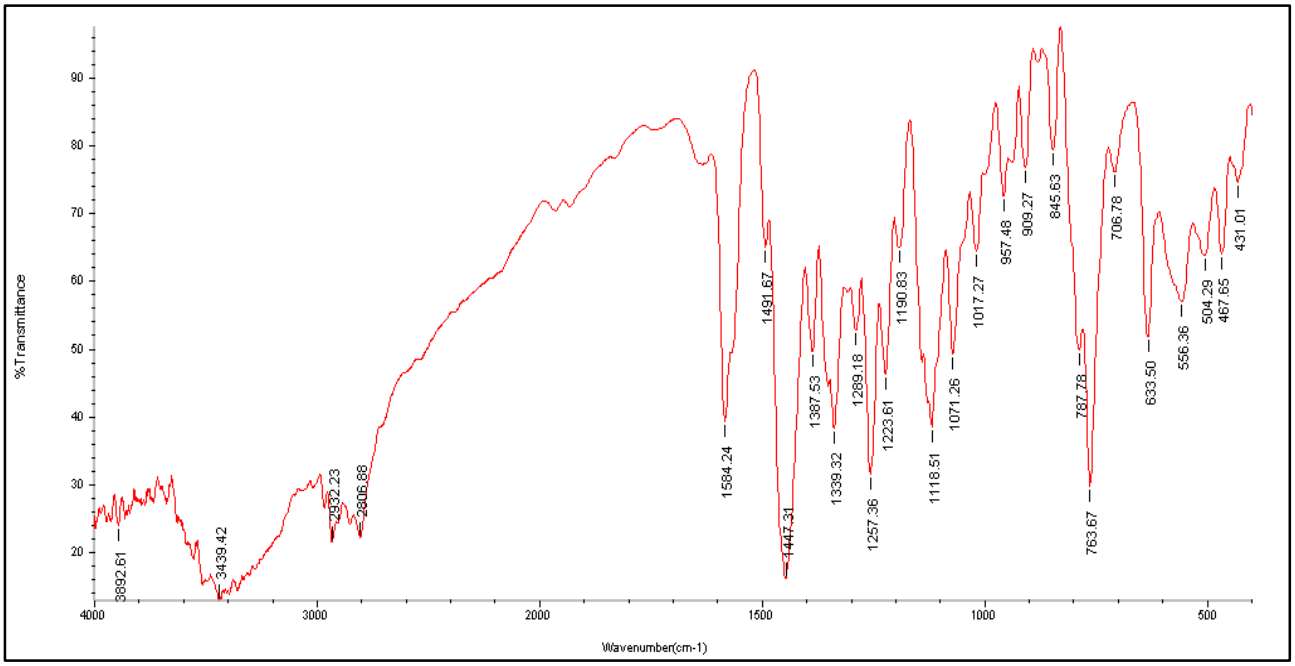

Figure 5.FT–IR spectra of mirtazapine pure powder

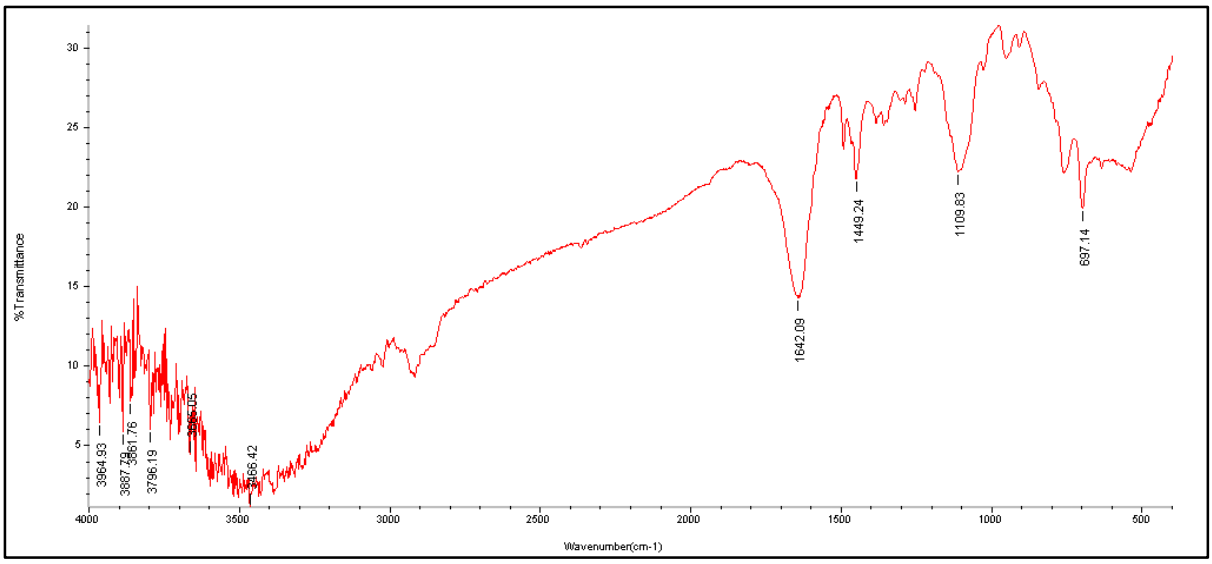

Figure 6. FTIR spectrum of lyophilized powder

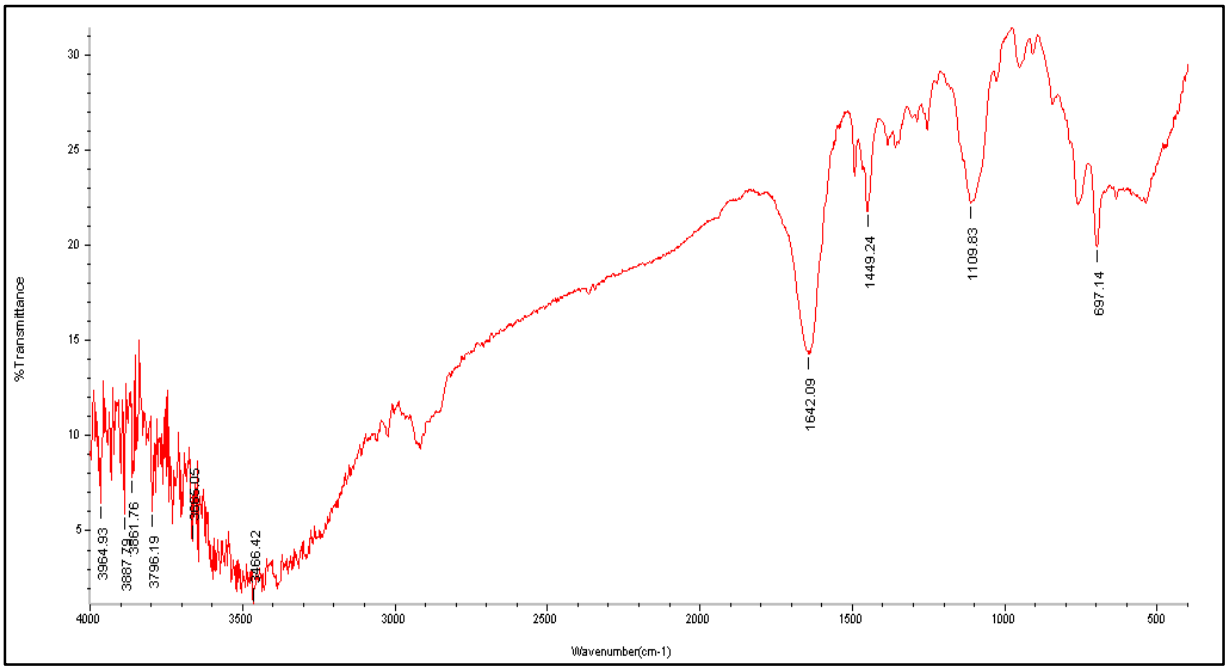

Figure 7. FTIR spectrum of blend powder best formula(F15a)

Differential Scanning Calorimetry

Figure (8) demonstrate DSC of mirtazapine showed sharp characteristic endothermic peak at 117ºC and this agrees with published results. This gives an indication that the drug has crystalline nature with high purity. For lyophilized powder, the melting point of mirtazapine disappeared as in figure (9) giving a strong indication that the drug lost the crystallinity state and converted to an amorphous form[36] .

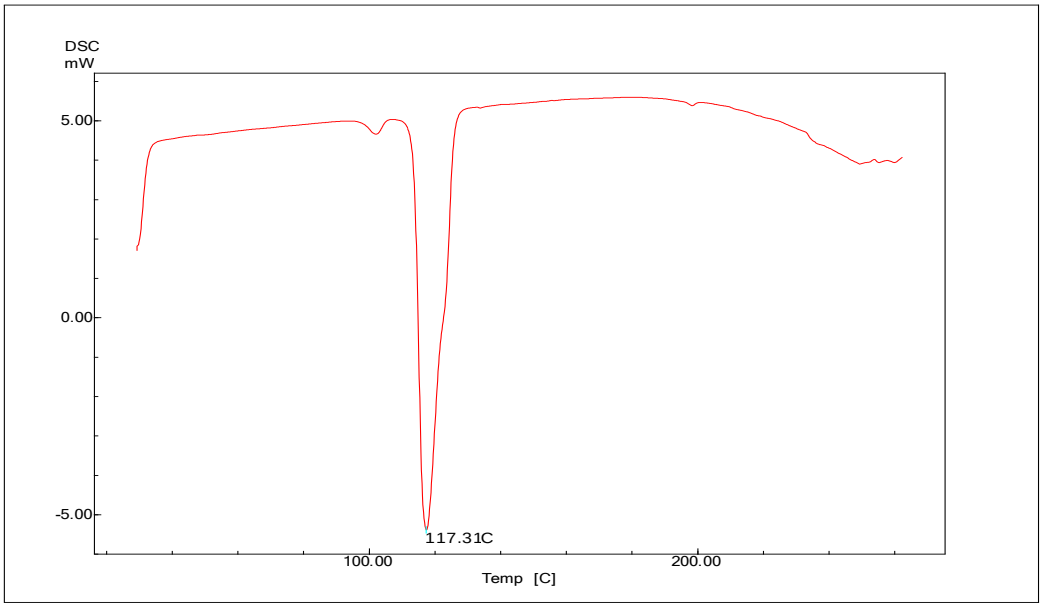

Figure 8. DSC thermogram of mirtazapine pure powder

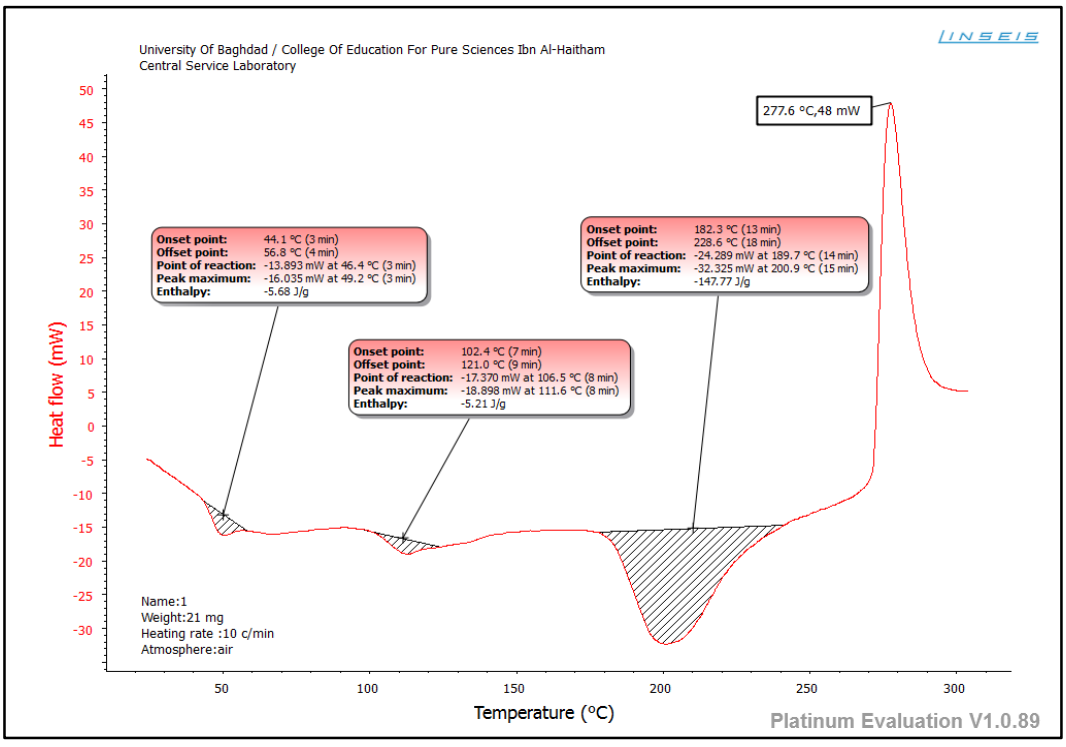

Figure9. DSC thermogram of lyophilized powder

Analytical RP-HPLC Method

Assay for mirtazapine was determined using HPLC technology to be compared with the UV spectroscopy. Figure (10) shows the HPLC chromatogram of mirtazapine as pure powder in the mobile phase. [The retention time of mirtazapine in the HPLC chromatogram was 7.141 minutes , for lyophilized powder of mirtazapine nanosuspension for best formula (F15) the retention time in the HPLC chromatogragram was 7.129 minutes, as shown in figure (11)].

From the results it was found that no significant difference between the two methods for the assay of mirtazapine pure powder and mirtazapine lyophilized powder.

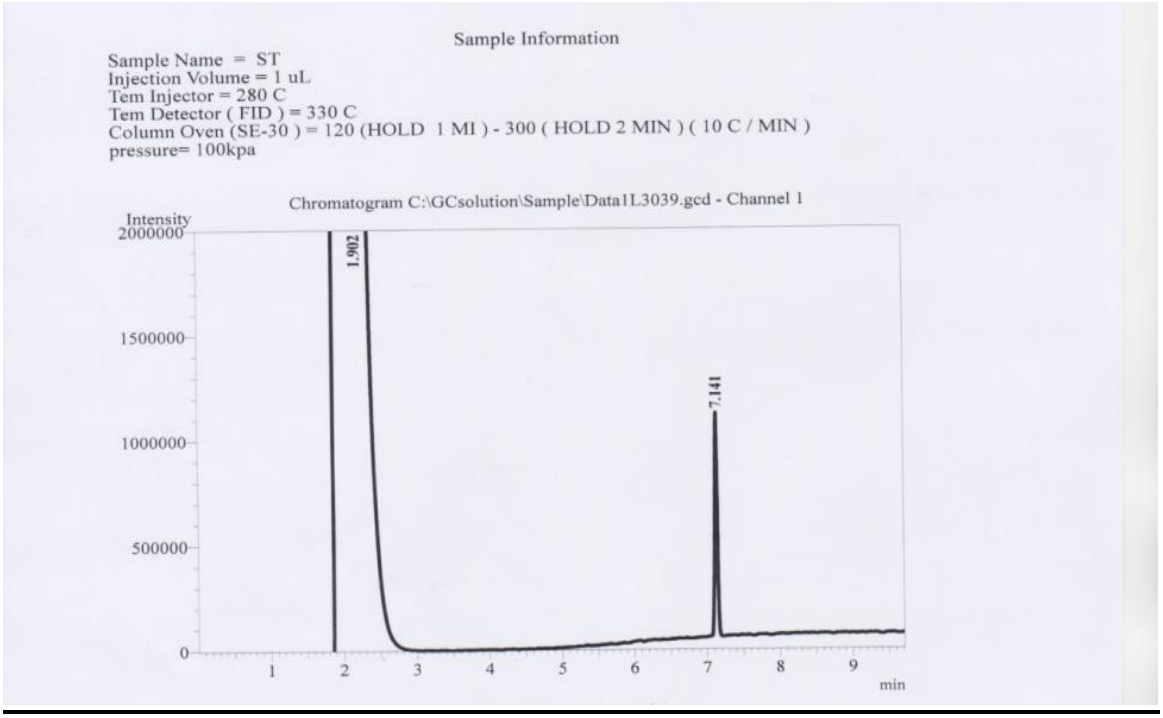

Figure 10. HPLC chromatogram of mirtazapine pure powder

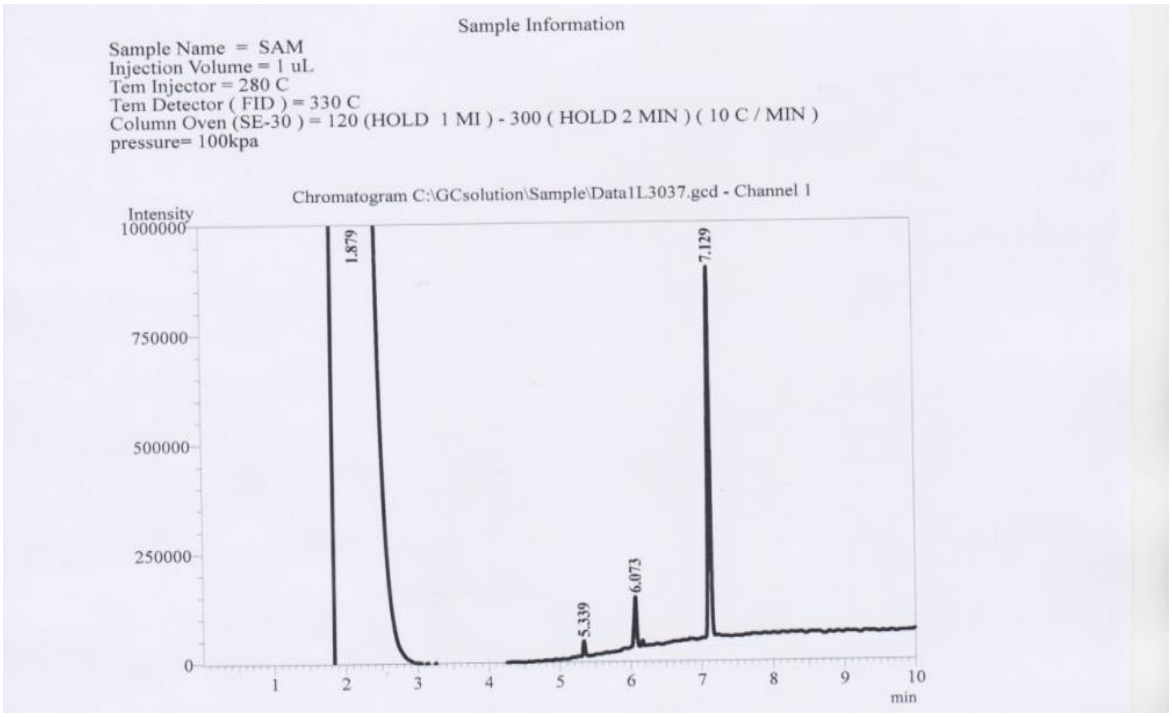

Figure 11. HPLC chromatogram of lyophilized powder for mirtazapine in the selected formula, f15

Evaluation of surface morphology

Atomic Force Microscopy Study (AFM)

AFM is akind of scanning probe microscopes (SPM). It is an instrument 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.

The morphological analysis of mirtazapine pure powder performed by AFM showing spherical shaped nanoparticles Figure (12) . It was found to be

stable and no aggregation of particles could be observed [37].

The formulation was found to be stable and no aggregation of particles could be observed. The particle size of F15 obtained by AFM was comparable to or equal to that measured by ABT-9000 nano laser and this agreement in particle size measurements provide the good size distribution and the stability of mirtazapine nanoparticles[38], as show in figure (13).

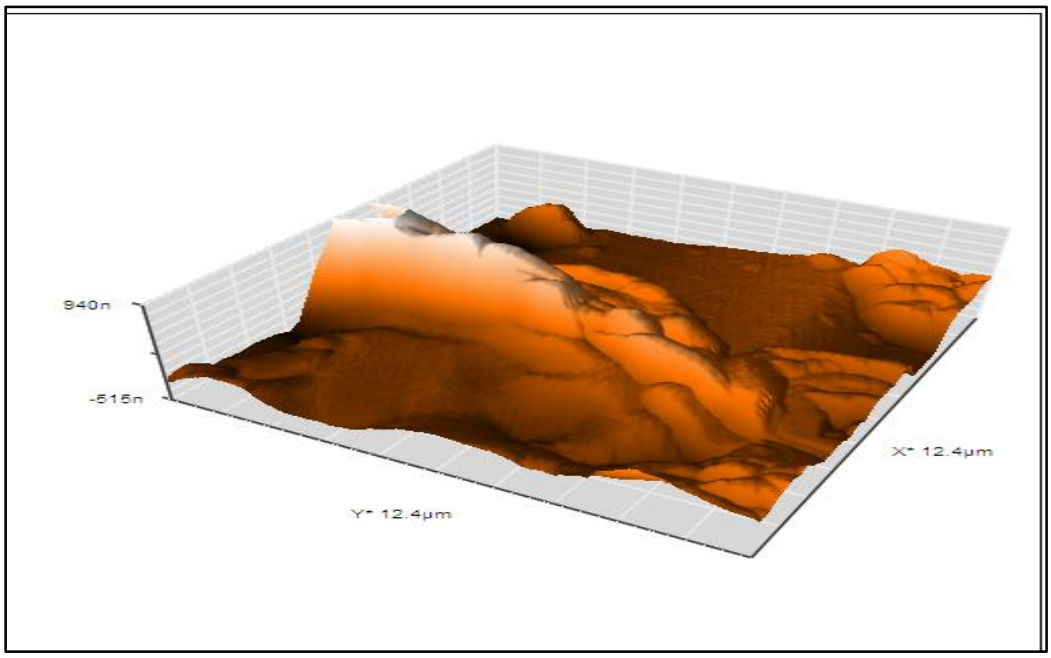

Figure12. AFM of mirtazapine pure powder

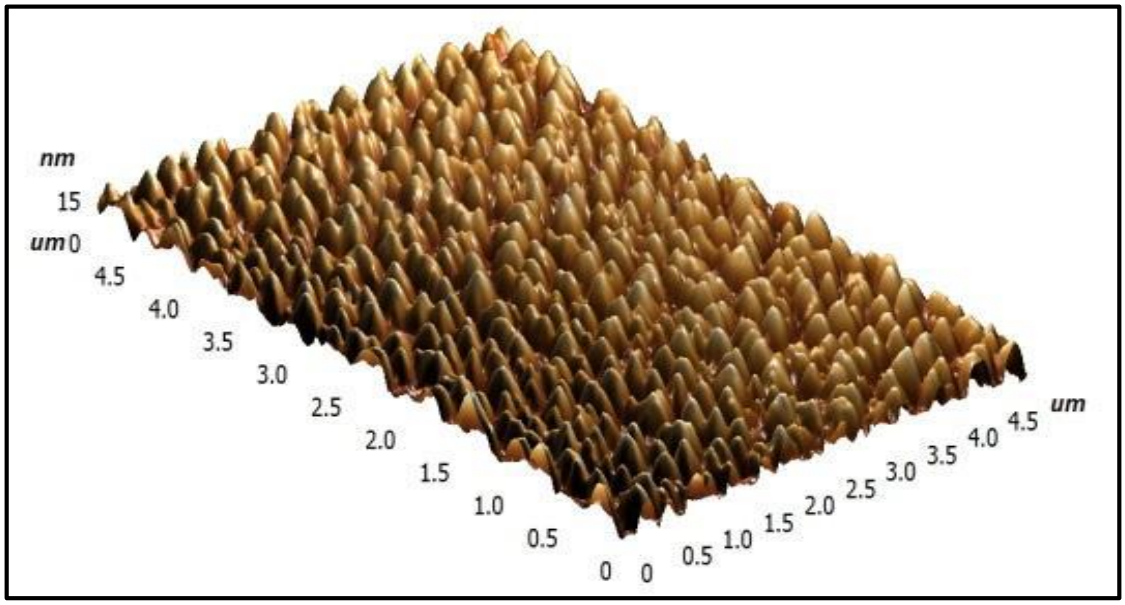

Figure 13. AFM of F15

Conclusion

Nano particulate systems such as anti-solvent precipitation have a great potential method, being able to convert poorly soluble mirtazapine. Mirtazapine nanoparticles were successfully prepared using different types of stabilizers alone and combination of stabilizers at drug : stabilizer ratios 1:1 and 1:2. Drug : stabilizer ratio 1:1 was effective to stabilize mirtazapine nanoparticles and the particle size was decrease as the stabilizer concentration increase. The selected formula F15, containing poloxamer 188 and poloxamer 407 as stabilizers combination, showed good entrapment efficiency of 93 % and faster dissolution rate than other formulas and pure drug. Selected formula

F15a was prepared as an orodispersible tablet by direct compression method and characterized by acceptable hardness, low friability and produced higher dissolution rate in comparison with the marketed tablet.

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