Formulation and Evaluation of Ezetimibe Nanoparticles

Yasser A.Ali[*] and *Shaimaa* N. Abd-Alhammid [*, 1]

[*]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq,

Abstract

The aim of this study is to formulate and evaluate ezetimibe nanoparticles using solvent antisolvent technology. Ezetimibe is a practically water-insoluble drug which acts as a lipid lowering drug that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. Ezetimibe prepared as nano particles in order to improve its solubility and dissolution rate.

Thirty formulas were prepared and different stabilizing agents were used with different concentrations such as poly vinyl pyrrolidone (PVPK-30), poly vinyl alcohol (PVA), hydroxy propyl methyl cellulose E5 (HPMC), and poloxamer. The ratios of drug to stabilizers used to prepare the nanoparticles were 1: 2, 1:3 and 1:4.

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-F30 was ranged from 85% ± 1 to 98 % ± 1. On the other hand dissolution rate increasing as the particle surface area is increase due to reduction of particle size to the nano range.

The results showed that poly vinyl pyrrolidone (PVPK-30) was found to be the best stabilizer.

Keywords: Ezetimibe, Nanoparticles, Particle Size, poly vinyl alcohol.

تصييغ وتقييم حبيبات الازيتيميب بواسطة الجسيمات النانوية

ياسر عبد الصاحب علي[*] و شيماء نزارعبد الحميد[*،١]

[*]فرع الصيدلانيات، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

أن الهدف من هذه الدراسة هو لصياغة وتقييم جسيمات نانوية لعقار الازيتمايب باستخدام تكنولوجيا الترسيب من مضاد المذيب. ازيتمايب هو دواء غير ذائب في الماء وهو دواء يعمل على خفض الدهون التي تثبط بشكل انتقائي امتصاص الكولسترول من الأمعاء و المواد الدهنية ذات الصلة. ازيتمايب أعد كجسيمات نانوية بغية تحسين القابلية للذوبان ومعدل الامتصاص.

تم إعداد ثلاثين صيغة باستخدام بوليمرات استقرار مختلفة استخدمت بتراكيز مختلفة مثل الفاينيل بايروليدون المتعدد (PVP)، وبولي الفينيل الكحول (PVA)، هيدروكسي بروبيل الميثيل السليلوز(HPMC E5) ، وبولوكسامير. وكانت نسب الدواء إلى المثبتات المستخدمة في إعداد الجسيمات النانوية هي ١:٢ و ١:٣ و ١:٤.

وقيمت "جسيمات نانوية " من حيث الحجم الحبيبي للجسيمات وكفاءة انحباس الدواء، ودراسة الشكل البياني للتحرر الدوائي ، وكذلك دراسة التوافق (مطيافية الأشعة تحت الحمراء ، وقياس المسح التفاضلي) ومجهر القوة الذرية.النسبة المئوية لكفاءة انحباس الدواء للصيغ الدوائية من الصيغة الاولى الى الصيغة ٣٠ هي من ٨٥% الى ٩٨%.من ناحية اخرى يزداد تحرر الدواء كلما صغر حجم الجسيمات النانوية لزيادة المساحة السطحية للجسيم. وأظهرت النتائج أن بولي الفينيل بايروليدون (PVP K-٣٠) هو أفضل بوليمر استقرار للجسيمات النانوية.

الكلمات المفتاحية :- إزتيميب، الجسيمات النانوية، الحجم الحبيبي، بولي الفينيل الكحول.

Introduction

Solubility is of the most important parameters to achieve the desired concentration of a drug in the systemic circulation for pharmacological response to be shown. A number of methodologies can be adapted to improve solubilization of poor water soluble drug and further to improve its bioavailability include chemical modification , pH adjustment, solid dispersion, complexation, co-solvency, and micronization [(1)].

One of these methods is the nanosuspension which is colloidal dispersions of nano-sized drug particles that are produced by a proper method and stabilized by a suitable stabilizer [(19)]. The particle size distribution of the solid particles in nano suspensions is usually less than one micron with an average particle size ranging from 200 and 1000 nm [(2)]. Ezetimibe is a member of new class of lipid - lowering compounds that selectively inhibit the intestinal absorption of cholesterol and decrease cholesterol absorption [(3)].

Ezetimibe is categorized as a class II agent (poorly water soluble and highly permeable with a relative

[1]Corresponding author E-mail: shaimaa-alsamariai@yahoo.com.

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Bioavailability range from 35-65 % [4].

The aim of this study is to formulate and evaluate ezetimibe nanoparticles using solvent antisolvent technology.

Materials and Methods

Materials

Ezetimibe powder, was purchased from (Provizer Pharma, Gujarat, India). Poly vinyl pyrrolidone PVP K-30 (BDH chemicals LTD, Liverpool, England). Poly vinyl alcohol (Riedal De Haen Ag Seelze, Hannover, Germany), HPMC (Provizer Pharma, Gujarat, India). Poloxamer 188 (BDH chemicals LTD, Liverpool, England). Methanol (GCC Analytical reagent, UK). brij35 (Riedal De Haen Ag Seelze, Hannover, Germany). All other chemicals were of analytical grade.

Methods

Preparation of ezetimibe nanosuspension

Nanosuspensions were prepared by the solvent evaporation technique which is also called solvent antisolvet technique [5], as shown in table (1), (2), (3) that the ezetimibe was dissolved in 10 ml methanol and poured into 100 ml water containing different types of stabilizers (alone and in combination) maintained at a temperature of 50°C and subsequently stirred at agitation speed of 500 revolution per minute (rpm) on magnetic stirrer for 1 hour to allow the volatile solvent to evaporate. The organic solvents which contain 10 mg of ezetimibe were added by means of a syringe drop by drop positioned with the needle directly into stabilizers containing water. The ratios of drug to stabilizers used to prepare the nanosuspension were 1: 2, 1:3 and 1:4. Then centrifuge to obtain the nanoparticles.

Table (1): Composition of ezetimibe nanosuspension using different types of stabilizers at drug: stabilizer ratio 1:2.

Formula no. / Materials	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Ezetimibe(mg)	10	10	10	10	10	10	10	10	10	10
PVP(mg)	20				10	10	10			
PVA(mg)		20			10			10	10	
HPMC(mg)			20			10		10		10
Poloxamer188 (mg)				20			10		10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10	10
Water (ml) QS	100	100	100	100	100	100	100	100	100	100

Table (2): Composition of ezetimibe nanosuspension using different types of stabilizers at drug: stabilizer ratio 1:3.

Formula no. / Materials	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
Ezetimibe(mg)	10	10	10	10	10	10	10	10	10	10
PVP(mg)	30				15	15	15			
PVA(mg)		30			15			15	15	
HPMC(mg)			30			15		15		15
Poloxamer188				30			15		15	15
Methanol (ml)	10	10	10	10	10	10	10	10	10	10
Water (ml) QS	100	100	100	100	100	100	100	100	100	100

Table (3): Composition of ezetimibe nanosuspension using different types of stabilizers at drug: stabilizer ratio 1:4

Formula no. / Materials	F21	F22	F23	F24	F25	F26	F27	F28	F29	F30
Ezetimibe(mg)	10	10	10	10	10	10	10	10	10	10
PVP(mg)	40				20	20	20			
PVA(mg)		40			20			20	20	
HPMC(mg)			40			20		20		20
Poloxamer 188				40			20		20	20
Methanol (ml)	10	10	10	10	10	10	10	10	10	10
Water (ml) QS	100	100	100	100	100	100	100	100	100	100

Evaluation of the prepared nanosuspension

Particle size determination was done using ABT-9000 nano laser particle size analyzer at 25ºC without dilution of the samples. The average particle size (D) was measured for all the prepared formulas at ratios of 1: 2 (F1-F10), 1: 3 (F11-F20) and 1: 4(F21- F30). Each sample was sonicated for 20 minute before measuring and each sample was measured in triplicate.

Determination of drug entrapment efficiency (EE) of nanosuspension

The freshly prepared nanosuspension of ezitimibe: stabilizer ratio 1:2, 1:3 and 1:4 was centrifuged at 20,000 rpm for 20 minutes using ultracentrifuge. The amount of non incorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 232 nm using UV-visible spectrophotometer. The entrapment efficiency (EE %) was calculated by subtracting the amount of the 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 [6, 7].

The Percentage of drug entrapment efficiency (% EE) could be achieved by the following equation :

entrapment efficiency = (Weight initial drug- Weight free drug) / Weight initial drug

Freeze drying of nanosuspension

In order to retrieve 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 [8].

In-vitro dissolution profile of nanosuspension

In vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using lyophilized powder in 500 ml of 0.1N HCL (pH 1.2) and phosphate buffer solution (pH 6.8) as dissolution mediums containing 2% brij 35 and maintained at 37 °C and 50 rpm for ezetimibe lyophilized powder formulas. The freshly prepared formula F1-F4, F11-F14 and F20-F24 where freeze dried to get the nanoparticles then immersed in dissolution medium. Samples (5ml) were withdrawn at regular intervals of 5 minutes for 120 minutes and replaced with fresh dissolution medium to keep sink conditions. Samples were filtered through filter paper and assayed spectro photometrically on UV-Visible spectrophotometer at 232 nm wave length [9].

Fourier transform infrared spectroscopy (FTIR)

The fourier transform infrared spectroscopy (FTIR) spectra were obtained using FTIR spectroscope. Samples which studied are: Pure ezetimibe powder, PVP K-30, Physical mixture of ezetimibe, and PVP K-30 at ratio (1:4); respectively. Lyophilized powder (nanoparticles) of the selected formula (F21), Microcrystalline cellulose PH 102 (Avicel) ® , lactose ,Magnesium Sulphate.

All these samples were grounded and mixed thoroughly with potassium bromide. The

spectrum obtained was in between the wave number of 4000-400 cm^{-1} [10].

Differential scanning calorimetry (DSC)

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 nanoparticles.

Thermal characteristics of the same samples that are studied by FTIR were determined also by an automatic thermal analyzer system. Therefore accurately weighed samples (5mg) were placed in non hermetically aluminum pans and heated at the rate of 10 ºC/minute against an empty aluminum pan as a reference covering a temperature range of 40 ºC to 300 ºC [11].

Atomic force microscopy

Atomic force microscopy **(**AFM) is capable of scanning the surfaces in controlled environmental conditions, also can measure the particle size of the nanoparticles accurately. The size and surface morphology of ezetimibe nanoparticles in F21 were confirmed by atomic force microscopy after drying of the formula. The optimized formula F21 were lyophilized and dried 15 minutes in desiccators. Particle size, 3D-dimension graph and histogram of particle size distribution were obtained [12, 13].

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 formulas F1-F4 at drug: polymer ratio 1:2 was ranged from 95.4 -956.5 nm measured by particle size analyzer, while for F11-F14 at drug: polymer ratio 1:3 the particle size ranged from 66.52-899.1 nm. On the other hand F21-F24 at drug :polymer ratio the particle size of these formula range from 35.3- 901.2 using PVP K-30 , PVA, HPMC and poloxamer 188 as primary stabilizers .

The formulations containing PVP K-30, PVA , and HPMC as stabilizers had small significant particle size in comparison with the formulation containing poloxamer 188 that gave larger particle size ($p<0.05$). Poloxamer188 (pluronic F68)® is a block co-polymer, responsible for the hydrophobic interaction with the drug molecule ,the crystal growth inhibition is mainly due to the hydrophobic polypropylene oxide group (PPO) in the pluronic polymer , while the hydrophilic polyethylene oxide (PEO) chains provide steric hindrance upon aggregation[14]. Poloxamer 188 can form a valuable mechanical and thermodynamic barrier at the interface that hinders the approach and coalescence of individual emulsion droplets at their optimum level. Although this mechanism of poloxamer 188, but it gave larger particle size in all three ratios 1:2, 1:3 and 1:4 drug: polymer in formulas F4, F14 and F24; respectively.

High particle size of F4, F14 and F24 that contain poloxamer188 as a stabilizer may be attributed to the insufficient affinity, of poloxamer188 to ezetimibe, and possess a slow diffusion rate and ineffective adsorption onto the drug particle surface in the water–methanol mixture. However, if there is no affinity between the particle surface and the polymer, the attractive forces between two particles become dominant due to depletion of polymer from the gap of two particles (depletion force)[15].

Poly dispersity index values of F1, F2 and F3 ranged from 0.002 -0.005 indicate that these formulas are mono disperse standard. While for F4 that contain poloxamer 188 PDI value was 0.439 which indicate mid range poly dispersity system. The surface area values of the particles in F1, F2 and F3 ranged from 39.2 - 57.53 (m^2/g) while for F4 that contain poloxamer 188 particle surface area value was 3.89 (m^2/g) which has the smallest particle surface area ($p<0.05$) in comparison with other formulas because it has largest particle size [16].

For drug :stabilizer ratio 1:3, polydispersity index values of F11, F12 and F13 ranged from 0.002 - 0.033 indicate that these formulas are monodisperse standard, while for F14 that contain poloxamer 188 PDI value was 0.557 which indicates mid range polydispersity system .

Specific surface area values of the particles in F11, F12 and F13 ranged from 66.21 – 99.23 (m^2/g) while for F14 that contain poloxamer 188 the SSA value was 4.25 (m^2/g) which has the smallest SSA ($p<0.05$) in comparison with other formulas because it has largest particle size.

On other hand for drug : polymer ratio 1:4 the poly dispersity index values of formulas F21, F22 and F23 range from 0.002-0.031 which indicate that these formulas are mono disperse system , while for F24 that contain poloxamer188 PDI value was 0.811 which indicates mid range poly dispersity system Specific surface area values of the particles in F23, F22 and F21 ranged from 122.21 – 557.23 (m^2/g) while for F24 that contain poloxamer188 the SSA value was 4.25 (m^2/g) which has the smallest SSA ($p<0.05$) in comparison with other formulas because it has largest particle size.

This difference in values of PDI could be attributed to the efficiency of stabilizers, which cover the organic/aqueous interface of the nano droplets and prevent them from coalescing to each other. From the obtained results, one can conclude that the poloxamer188 is not suitable as a primary stabilizer for nanoparticles because of poor adsorption and poor affinity of poloxamer188 to the ezetimibe molecules.

Particle size ranged from 35.57 nm for PVPK30 to 999 nm for poloxamer188, which appeared to be affected by relative viscosity of the polymeric dispersion in the presence of stabilizers and followed the trend: PVP > PVA > HPMC > poloxamer188. Nanosuspension with PVP K-30 as stabilizers possessed the smallest particle size while that containing poloxamer188 had the largest particle size [(17)].

Effect of polymer concentration on the size of ezetimibe nanoparticles

The effect of the polymer concentration on the particle size of ezetimibe nanosuspention have been investigated by depending on three ratios of drug : polymer concentration (1:2) in the preparation of F1-F4 and in 1:3 of drug : polymer ratio in the formulation of F11-F14. And in the ratio of 1:4 in the formulas F21- F24. Polymer concentration affecting on the adsorption affinity of the stabilizers to the particle surface.

In general as the concentration of polymers increase the particle size decrease at fixed drug concentration except for poloxamer188, which indicated that the drug particle surface has been sufficiently covered well by the stabilizer molecules [(18)].

It has been noticed that the particle sizes of F1, F2 and F3 using PVP K-30, PVA and HPMC; respectively as stabilizer were decreased when the concentration of polymer increased in the formulas F11, F12 and F13 and they were further decreased in particle size when the concentration of the polymer increased as shown in the formulas F21, F22 and F23 using the same stabilizers, while F4 , F14 and F24 containing poloxamer 188, as the only stabilizer, have maintained within the same value of particle size and not increased sufficiently even when the concentration was increased. It can be interpreted as the fact that poloxamer 188, by itself have poor adsorption properties and affinity to the molecules of ezetimibe that prevent particle agglomeration and this finding did not agree with the reported one[(19)].

Polymer concentration plays a great role in the stabilization of nanoparticles because of too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Ostwald's ripening[(20)].The decrease in the particle size is accompanied by a rapid, highly increase in the surface area. Thus, the process of primary coating of the newer surfaces competes with the agglomeration of the uncoated surfaces. Hence, an increase in the surfactant concentration in the primary dispersion results in rapid coverage of the newly formed particle surfaces [(21)].

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

The particle size of F5, containing PVA and PVP K-30 as stabilizers combination at drug: stabilizer ratio 1:2 was decreased significantly ($p<0.05$) from 140 nm to 40.23 nm in F25 at drug: stabilizer ratio 1:4 ratio when the stabilizer concentration increased.

Poloxamer 188, when used singly was not so effective in reducing the particle size as a stabilizer, rather flocculation was observed. This could be due to its high hydrophilicity (HLB = 29) due to which it may not be undergoing preferential adsorption on the nanoparticle surface. However, poloxamer188 in combination with PVP k-30, PVA and HPMC , it worked synergistically therefore the particle size of ezetimibe nanosuspension was drastically reduced.

It has been found that the combination of PVPK-30 and poloxamer188 have reduced particle size this mainly due to

that PVP K-30 is reported to be a protective colloid which is indicative of its greater adsorption potential for the nanoparticles [22]. This is expected as the stabilizers used for preparing the ezetimibe nanosuspensions are either hydrophilic polymers or non-ionic surfactants which stabilize the particles by steric stabilization [22, 23].

Determination of drug entrapment efficiency of nanosuspension

The percentage of drug entrapment efficiency of F1-F30 was ranged from 85% ± 1 to 98 % ± 1 as shown in figure (11). It is clear that the increase in stabilizer concentration increased the drug entrapment efficiency, but the study revealed that the concentration of stabilizers at ratio 1:2 drug: stabilizer was sufficient to give the optimized entrapment efficiency. F4 containing poloxamer188 as stabilizer had the lowest entrapment efficiency, while F21 containing PVP-k-30 as the only stabilizer had the higher entrapment efficiency. This may be due to the presence of optimum stabilizer and optimum stabilizer concentration [24].

In vitro dissolution study

The dissolution profile was done for F1-F4, F11-F14 and F21-24 of drug: stabilizer ratio 1:2, 1:3 and 1:4 respectively. The dissolution of the prepared formulas was carried in 0.1N HCL solution (pH1.2) and phosphate buffer solution (pH6.8) in the presence of 2% brij-35 to get the selected formula that can increase the dissolution rate in these buffers which are simulated to gastric and intestinal fluids. In the dissolution study one notice enhancement of dissolution rate, according to Noyes-Whitney equation the dissolution rate increasing as the particle surface area is increase due to reduction of particle size to the nano range.

Superior dissolution of ezetimibe nanoparticles may potentially improve bioavailability and other drug performances. PVPk-30 containing ezetimibe nanoparticles at drug - polymer ratio 1:4 with particle size of 35.57 nm showed the highest drug release rate as 100% of drug dissolved in 10 minutes whereas, poloxamer 188 containing ezetimibe nanoparticles at drug - polymer ratio 1:2 with particle size of 999 nm showed about 33.9% of drug dissolved within 10 minute of dissolution test in both 0.1 N HCL (pH 1.2) and phosphate buffer (pH 6.8)[25, 26].

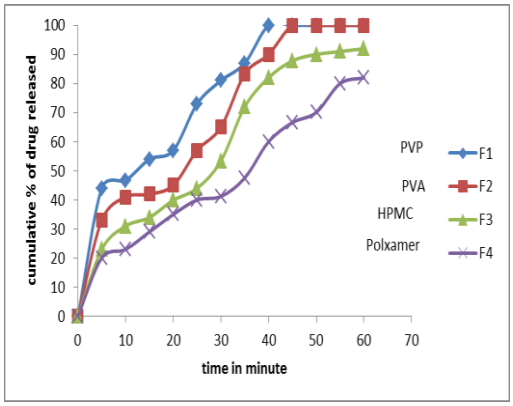

Figure (1): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F1, F2, F3, and F4 in 0.1 N HCl solution (pH 1.2) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature .

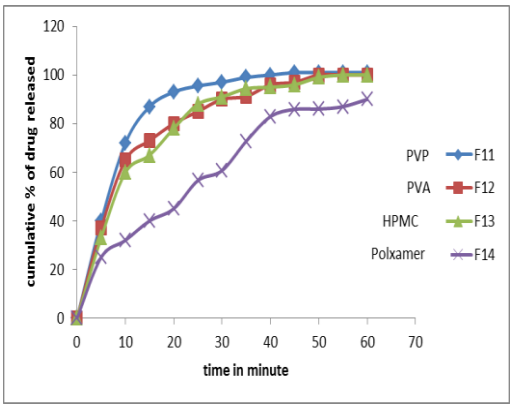

Figure (2): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F11, F12, F13, and F14 in 0.1 N HCL solutions (pH 1.2) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature.

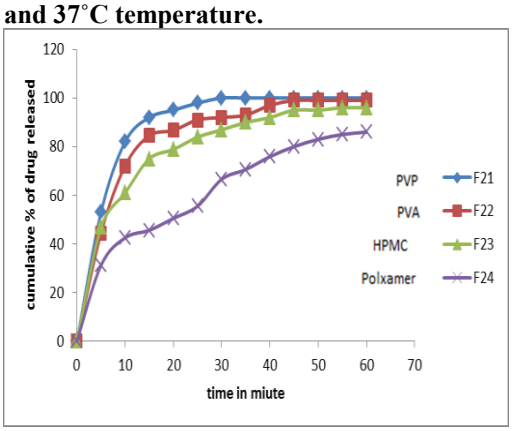

Figure (3): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F21, F22, F23, and F24 in 0.1 N HCL solutions (pH 1.2) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature.

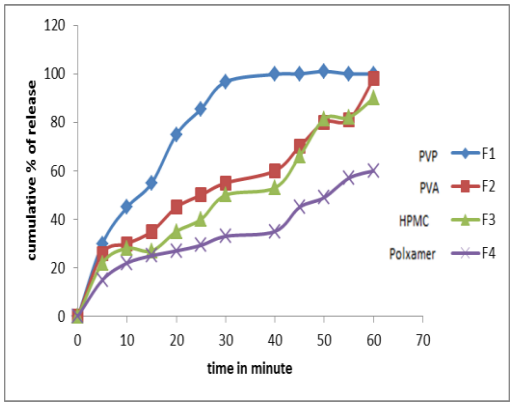

Figure (4): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F1, F2, F3, and F4 in phosphate buffer (pH 6.8) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature.

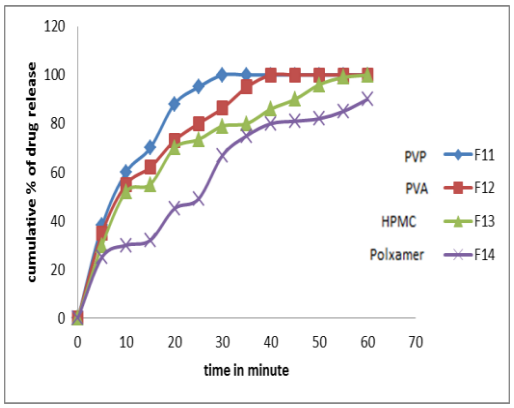

Figure (5): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F11, F12, F13, and F14 in phosphate buffer (pH 6.8) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature.

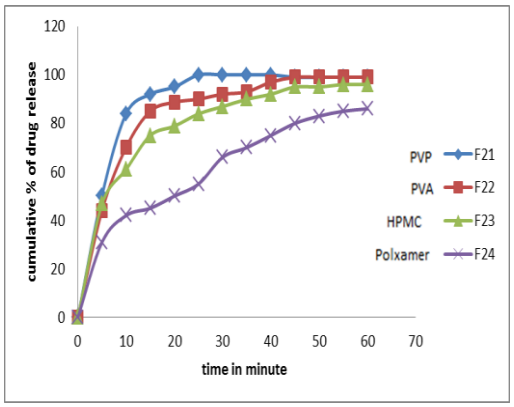

Figure (6): Effect of polymer type on the dissolution profile of ezetimibe nanoparticles from F21, F22, F23, and F24 in phosphate buffer (pH 6.8) containing 2% brij-35 w/v at 50 r.p.m and 37˚C temperature.

Drug content in lyophilized powder

The drug content result showed that 25 mg of lyophilized powder of the selected formula (F 21) contain 5 mg ±0.1 of ezetimibe when determined by UV-visible spectrophotometer at λmax 232 nm.

Fourier transforms infrared spectroscopy

FTIR is one of the most widely reported spectroscopic techniques for solid-state characterization. The characteristics absorption bands of ezetimibe are:

1. O–H stretching band at 3650—2700 cm^{-1}
2. C-O stretching bands of the lactam ring at 1725—1714 cm^{-1}
3. C=C stretching band of the aromatic ring at 1600—1500 cm^{-1}
4. C–F stretching band at 1000—1200 cm^{-1}
5. C–O stretching band at 1300—1000 cm^{-1}

FTIR spectra of ezetimibe nanosuspention and tablet show no change in shifting of the position of the major functional groups indicating no major interaction between the drug and the stabilizer PVP K-30 and other excipients. FTIR spectra of physical mixture also showed peaks at similar position. Hence, it can be conclude that there was no possible interaction between the drug, the stabilizer and the used excipients [27, 28].

Figure (9) demonstrate the DSC thermogram of ezetimibe that showed sharp characteristic endothermic peak at 165.30ºC and this agrees with the references. This gives an indication that the drug has crystalline nature with high purity. The DSC thermograms of the ezetimibe of tablet of the selected formula F21, lyophilized powder and physical mixture are shown in figure (10) that show that the drug in the crystal structure have a melting endotherm while ezetimibe molecules in the amorphous state do not exhibit a melting endotherm. As seen in Figures (9), and (10) the sharp melting peak of ezetimibe (165.30 °C) is completely disappeared in these figures that's mean the stabilizer PVP K-30 completely converted ezetimibe particles into amorphous state [29].

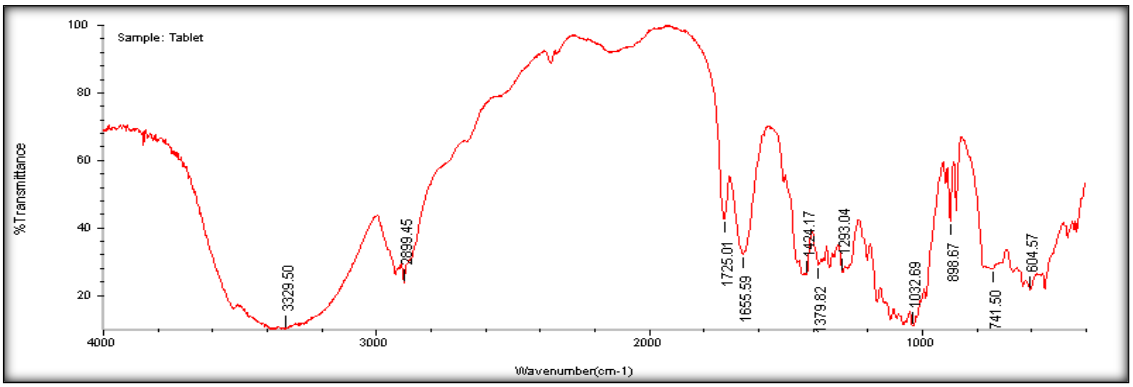

Figure (7): FTIR spectrum of eztimibe

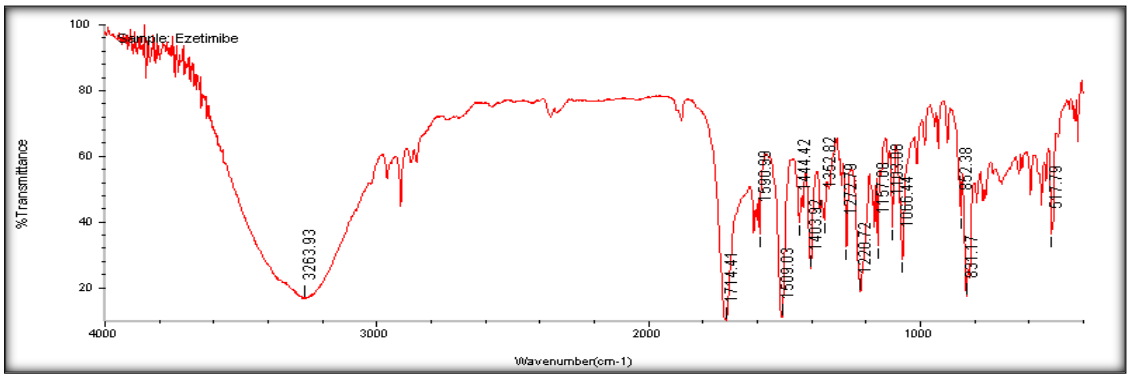

Figure (8): FTIR spectrum of ezetimibe nanosuspension Differential Scanning Calorimetry

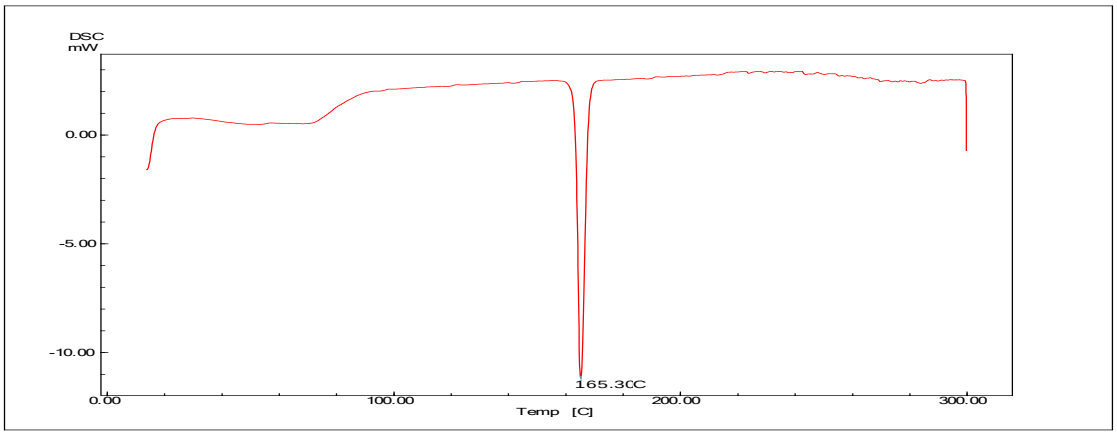

Figure (9): DSC thermogram of ezetimibe powder

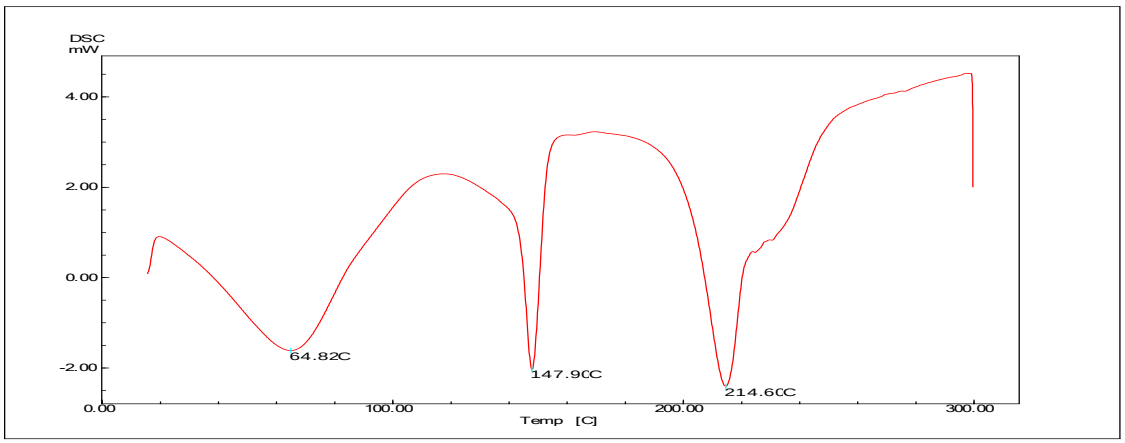

Figure (10): DSC thermogram of ezetimibe tablet of formula F21

Evaluation of surface morphology

Atomic force microscopy study

The morphological analysis and particle size of F21 performed by AFM showing irregular to spherical shaped nanoparticles with a size of 31 nm as seen in figure (11) as it approved by the histogram of particle size distribution in figure (12) also figure (13) shows histogram of particle size distribution of F21 by atomic force microscopy.

The formulation was found to be stable and no aggregation of particles could be observed. The particle size of F21 obtained by AFM was comparable to or equal to that measured by ABT-9000 nano laser particle size analyzer (35.57 nm) and this in agreement with particle size measurements provide the good size distribution and the stability of ezetimibe nanparticles [31].

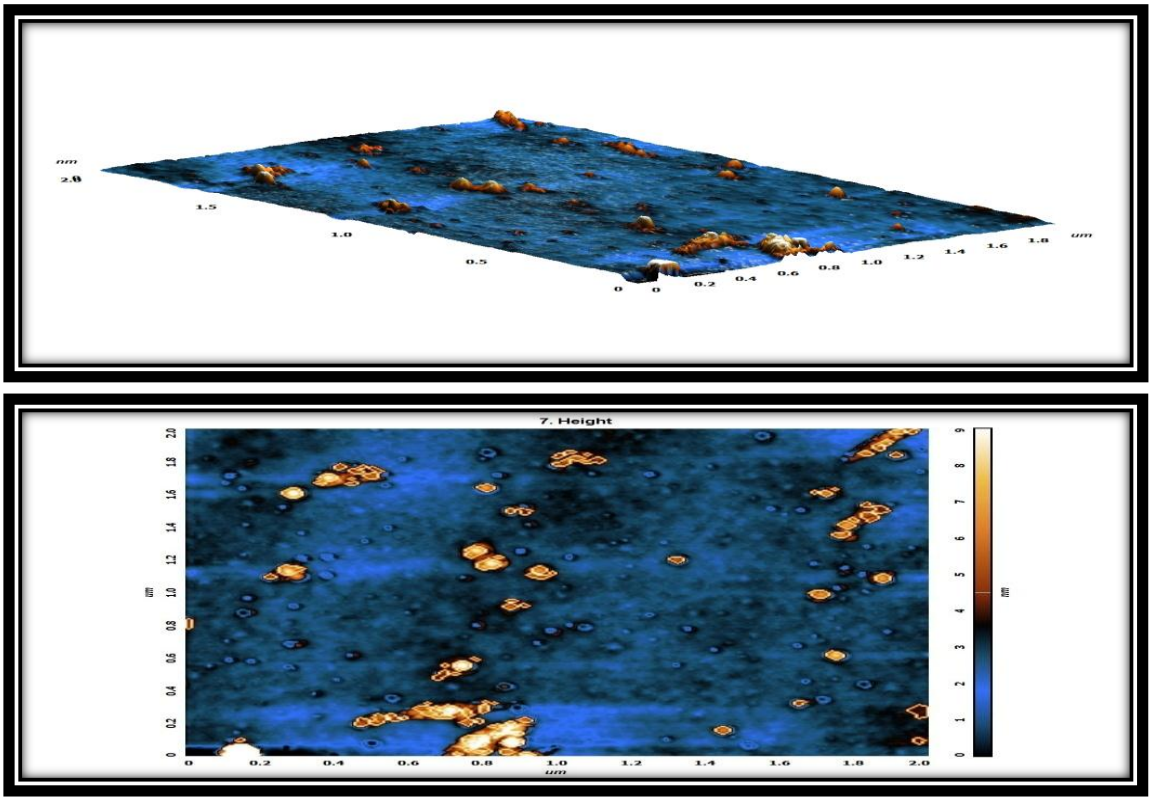

Figure (11): Atomic force microscopy of formula F21 showing cross section and long tudinal section of the nanoparticles surface.

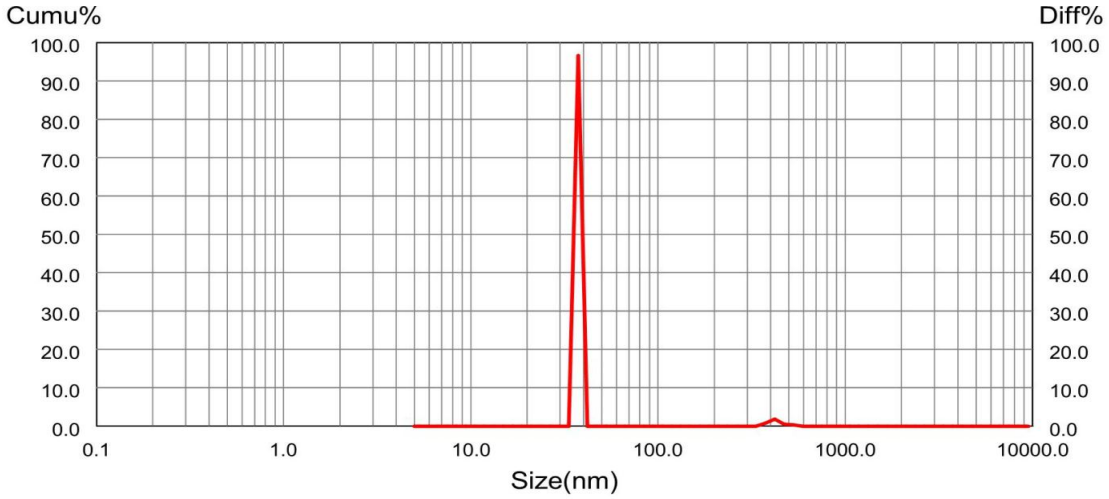

Figure (12): Particle size distribution of F21 by particle size analyzer ABT-9000

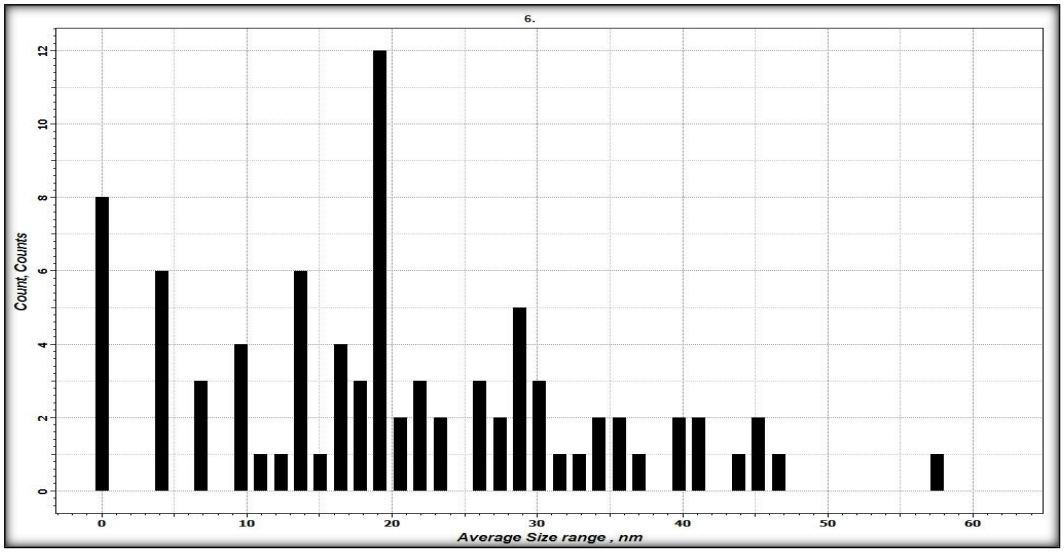

Figure (13): Histogram of particle size distribution of F21 by AFM

Conclusion

Nano particulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs.

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