Design and Characterization of Nanocrystal Formulations Containing Ezetimibe

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Ezetimibe is a lipid-lowering compound that selectively inhibits the absorption of cholesterol and related phytosterols from the intestine. As ezetimibe is almost insoluble in water, its bioavailability is too low to be detected. Thus, the objective of this study was to improve the solubility and dissolution rate of ezetimibe by preparing drug nanocrystals utilizing ball milling, high speed homogenization techniques. Pluronic F127 was chosen as a surface modifier to stabilize the nanocrystal formulations. Nanocrystal formulations of ezetimibe were prepared by using ball milling and high speed homogenization techniques. Additionally, the physicochemical characteristics of ezetimibe and nanocrystal formulations were determined by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray analysis and particle size analysis. Tablets were prepared containing ezetimibe nanocrystals formed by high speed homogenization (ultrasonic) and ball milling according to the results of particle size measurements and *in vitro* dissolution rates of the nanocrystal formulations. As a result of these experiments, it was found that the dissolution rate of the nanocrystal formulations increased and although tablet formulations which did not contain any solubilizing agent like sodium lauryl sulfate (SDS), the dissolution profile of these formulations were found similar to the commercial product.

Key words nanocrystal; ezetimibe; dissolution rate; particle size; nanotechnology; bioavailability

Nanotechnology has been improving since the 90's and has a variety of application fields. In pharmaceutics, nanotechnology is used to improve human health at a molecular level. The novel and potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy. The advantages of nanotech drugs compared to conventional counterparts lie on the basis of particle size. Drugs with nano dimension can be used at a lower concentration and can lead to early onset of bioactivity.[1)]

Most of the new chemical compounds developed as a drug have poor solubility in water.[1,2)] Nevertheless, solubility, dissolution rate and therefore, bioavailability of poorly water-soluble drugs can be increased by nanotechnological methods, such as preparation of drug nanocrystals.

There are several advantages of nanocrystal formulations; such as, enhanced oral bioavailability improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration due to decreased particle size range. Moreover, the formulations are aqueous based, and thus, no organic solvents or extreme pH conditions are needed. The process is also useful for moderately soluble drugs when a high concentration of drug in a low volume of fluid is desired.[3)]

Many different techniques can be used to prepare nanocrystal formulations of a drug powder such as, homogenization,[4—12)] co-precipitation, spray drying,[9,13)] milling,[4—8,14)] and precipitation. Nanocrystal dispersions comprise water, active drug substances and a stabilizer. If necessary, other substances such as buffers, salts or sugars can be added. They are physically stable systems due to the presence of the stabilizer that prevents reaggregation of the active drug substances during the preparation process.[3)] Drug nanocrystals are usually produced in liquid dispersion media as in the precipitation and disintegration methods.[15)] In the patents of Elan company numbered US 7101576, WO 2006/074218, WO 09/666539, defined nanodimensions as particle having less than 2000 nm particle size. As the particle size is decreased to nanodimensions, the surface area and thus the surface free energy of the drug particles increase extensively leading to particle aggregation. Therefore, a stabilizer should be included in the formulations to prevent the aggregation of nanosized drug particles.

The model drug ezetimibe ((3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone) is a white crystalline powder and is a Class II type drug. It is the first marketed lipid lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of bile acids, triglycerides, fat-soluble vitamin and is used in the treatment and prophylaxis of atherosclerosis. Poor solubility of the drug is associated with poor dissolution rate and thus low oral bioavailability.[16—18)]

Pluronic F 127 was used as a stabilizer in the formulations. Pluronic F 127 is a FDA (Food and Drug Administration) approved, stable, biologically compatible, biodegradable and non-toxic excipient.[19,20)]

The objective of this study was to improve the solubility and dissolution rate (and therefore bioavailability) of ezetimibe by nanocrystal formulation utilizing several techniques. Also, the physicochemical properties of the nanocrystals were determined by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray and particle size analysis.

Experimental

Materials Pluronic F127 was obtained from Uniqema (Belgium) and Ezetimibe was a kind gift from Dr. Reddy's Laboratories (India). 1-Heptane sulfonic acid, sodium hydroxide, acetone, dichloromethane and boric acid were obtained from Merck (Germany). Acetic acid (100%) was obtained from Prolabo (France). Croscarmellose sodium and sodium lauryl sulfate (SDS) were purchased from Sigma Aldrich (Germany), ethanol was obtained from Merko Kimya (Turkey), lactose and Avicel PH 102 were a kind gift from Eczacıbaşı (Turkey) and magnesium stearate and povidone K 30 were a kind gift from Novartis (Turkey). Formulations were prepared by using Millipore ultrapure water. All other chemicals were analytical grade.

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Methods. Preparation of Nanocrystal Formulations High Speed Homogenization (Ultrasonic Probe) Method (UP): A suspension of ezetimibe and pluronic F127 1 : 1 (w/w) was prepared in distilled water to a final concentration of 3% (w/v). To obtain a homogeneous suspension, the suspension was mixed by a magnetic stirrer for half an hour. The suspension was then mixed by an Ultrasonic Probe at 20% power for 1 min. Finally, the dispersion medium was removed by lyophilization for 72 h at −55 °C at 0.01 mmHg pressure.

Ball Milling Method (BM): A suspension of ezetimibe and Pluronic F127 1 : 1 (w/w) was prepared in distilled water, with a final concentration of 3% (w/v). To obtain a homogeneous suspension, the suspension was mixed by a magnetic stirrer for half an hour. The suspension was mixed by 16 agate balls, 20 mm in diameter, for 4 min. The dispersion medium was then removed by lyophilization for 72 h at −55°C at 0.01 mmHg pressure.

Physical Mixture (PM): A physical mixture of ezetimibe and Pluronic F127 at a 1 : 1 ratio (w/w) was prepared manually using a mortar.

Solubility of Ezetimibe The saturation solubility of ezetimibe in the dissolution medium was determined in order to establish sink conditions during dissolution studies. Hence, ezetimibe was dissolved in dissolution medium (0.45% SDS in 50 mM pH 4.5 acetate buffer) and kept in a shaking water bath 37±2 °C with 80 rpm rate for 12 h. The amount of drug dissolved was determined by HPLC at 1 and 12 h.

Preparation of Tablet Formulations When the results of particle size and dissolution rate measurements of powder nanocrystal formulations were considered, tablet formulations were prepared using the nanocrystals that were obtained by ball milling and high speed homogenization (Ultrasonic) methods. As a control group, a tablet formulation of the physical mixture was also prepared. As it is explained below, the formulations, which were prepared by means of wet granulation, were pressed into tablets using an eccentric model tabletting machine.

In the formulations shown in Table 1, instead of ezetimibe, the above mentioned formulations corresponding to 10 mg ezetimibe (UP, BM, and PM with and without SDS) were used. The tablet containing the physical mixture was prepared both with and without SDS in order to make comparisons. The same excipients except SDS were used as in the original market product (Zetia®) in all the tablet formulations.

In order to prepare the ezetimibe tablet formulations was compressed by wet granulation technique. A 1% aqueous solution of Povidone 30 was instilled on the granulated mixture until it became a paste, while homogeneously mixing by a pestle. The paste was passed through a granulator, dried in a 50 °C oven. Finally, tablets of 100 mg weight were compressed utilizing an eccentric model tabletting machine.

Characterization of Formulations FT-IR Analysis of Formulations: Ezetimibe nanocrystals were analyzed using a Fourier transform infrared dpectrometer (Perkin Elmer, U.S.A.). The infrared spectra were detected over the range of 4000—650 cm^{-1}.

X-Ray Analysis of Formulations: X-Ray diffractograms of each formulation were recorded using an Ultima X-Ray Diffractometer. Standard runs using a 40 kV voltage and a scanning rate of 0.02° min^{-1} over a 2θ range of 0—40° were used.

DSC Analysis of Formulations: Thermal properties of the powder samples were investigated using a DSC Q 100 (TA Instruments, U.S.A.). Approximately, a sample of 5—20 mg was weighed in an aluminum pan and a heating rate of 10 °C/min was employed in the range of 10—250 °C. Analyses were performed under a nitrogen purge (50 ml·min^{-1}). Empty aluminum pans were used as a reference. The change of heat of the samples was monitored with respect to change in temperature.

Analysis of Particle Size Distribution: In order to determine the particle size distribution of the prepared formulations, a Malvern Zeta Sizer (Nano Series Nano ZS) was utilized. A preweighed (10—30 mg) amount of the nanocrystal formulations prepared with different methods (UP, BM) was dispersed in water up to a volume of 5 ml in a volumetric flask. These aqueous dispersions were then vortexed for 3 min and particle size analysis was carried out by 3 consecutive measurements for each sample.

Dissolution Studies *In vitro* dissolution studies were performed using a SOTAX dissolution apparatus. A 5 ml sample was withdrawn from the dissolution medium at 10, 20, 30 and 45 min, and then the same amount of fresh medium was added to the dissolution medium. For each formulation and control group, the experiment was repeated 6 times. For each formulation and control group, % dissolved ezetimibe values were plotted with respect to time. The dissolution medium used in this work was 500 ml 0.45% SDS in 50 mM pH 4.5 acetate buffer which has been reported in the "Dissolution Methods for Drug Products" guide of FDA. The dissolution studies were carried out utilizing the USP apparatus II (pedal) method, stirred at 50 rpm at 37±0.5 °C. The % dissolved ezetimibe amount was calculated using the amount of ezetimibe in 20 mg nanocrystal formulations. Quantitative analysis of ezetimibe was performed using a validated reversed-phase high-pressure liquid chromatography (RP-HPLC) method.[21]

Statistical Analysis The reported data represent the mean value±S.D. Significance of difference between particles sizes of formulations were evaluated using Kruskal–Wallis at the probability level of 0.05.

Table 1. The Composition of the Tablet Formulations Containing Ezetimibe Nanocrystals Prepared by UP, BM, PM−SDS, PM+SDS

Tablet content	mg/tablet			
	UP[a]	BM[b]	PM−SDS[c]	PM+SDS[d]
Ezetimibe nanocrystal	20	20	20	20
Lactose	50	50	50	48
Avicel PH 102	20	20	20	20
Povidone K 30	1	1	1	1
Croscarmellose sodium	8	8	8	8
Sodium lauryl sulfate	0	0	0	2
Magnesium stearate	1	1	1	1
Total	100	100	100	100

a) High speed homogenization method (ultrasonic), *b*) ball milling, *c*) physical mixture without SDS, *d*) physical mixture with SDS.

Results and Discussion

Solubility of Ezetimibe In order to be able to carry out the dissolution studies of ezetimibe, sink conditions should be established. The solubility of ezetimibe in the dissolution medium (50 mM pH 4.5 Acetate Buffer/0.45% SDS) was determined to be 0.17±1.7 mg/ml in 1 h and 0.20±0.4 mg/ml in 12 h. Thus, it could be said that sink conditions were established in the dissolution studies.

Characterization of Formulations. FT-IR Analysis of Formulations As it is shown in Fig. 1, there are some characteristic peaks that belong to ezetimibe, which are also seen in the FT-IR spectra of the formulations. In other words, there is no difference between the FT-IR spectra of ezetimibe in the formulations and the FT-IR spectrum of ezetimibe itself. This result indicates that ezetimibe structure is preserved in the nanocrystal formulations. The results showed

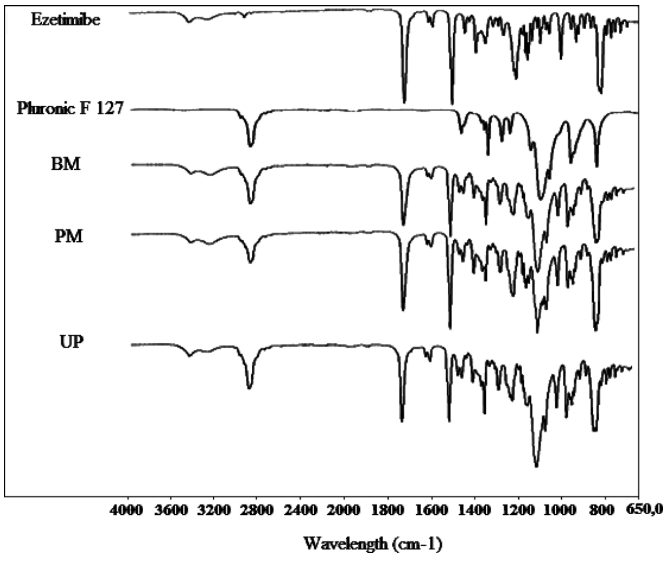

Fig. 1. FT-IR Spectra of the Nanocrystal Formulations, Ezetimibe, Physical Mixture and the Excipient

that in nanocrystal formulations of ezetimibe there were not chemical or morphological changes. In addition, the lack of change in functional groups showed that preparation method or combination of nanocrystal formulation had no effect on the chemical stability of ezetimibe.

The characteristic peaks related to ezetimibe structure are as follows:

C=O tension bands of the carbonyl group at 1760—1690 cm^{-1},

C=C resonance double bond tension bands that belong to the aromatic chain at 1600—1500 cm^{-1},

Strong C–O tension bands of the ester group at 1300—1000 cm^{-1},

Strong aliphatic tension bands at 3000—2850 cm^{-1},

Expanded O–H tension bands of the carboxylic acid group at 3650—2700 cm^{-1},

C–F tension bands at 1000—1200 cm^{-1},

p-disubstituted benzene tension bands at 800—1000 cm^{-1}.

X-Ray Analysis of Formulations The X-ray diffractograms of the nanocrystal formulations, Pluronic F 127, ezetimibe, physical mixture and nanocrystal tablet formulations (described in 'Preparation of Tablet Formulations') are

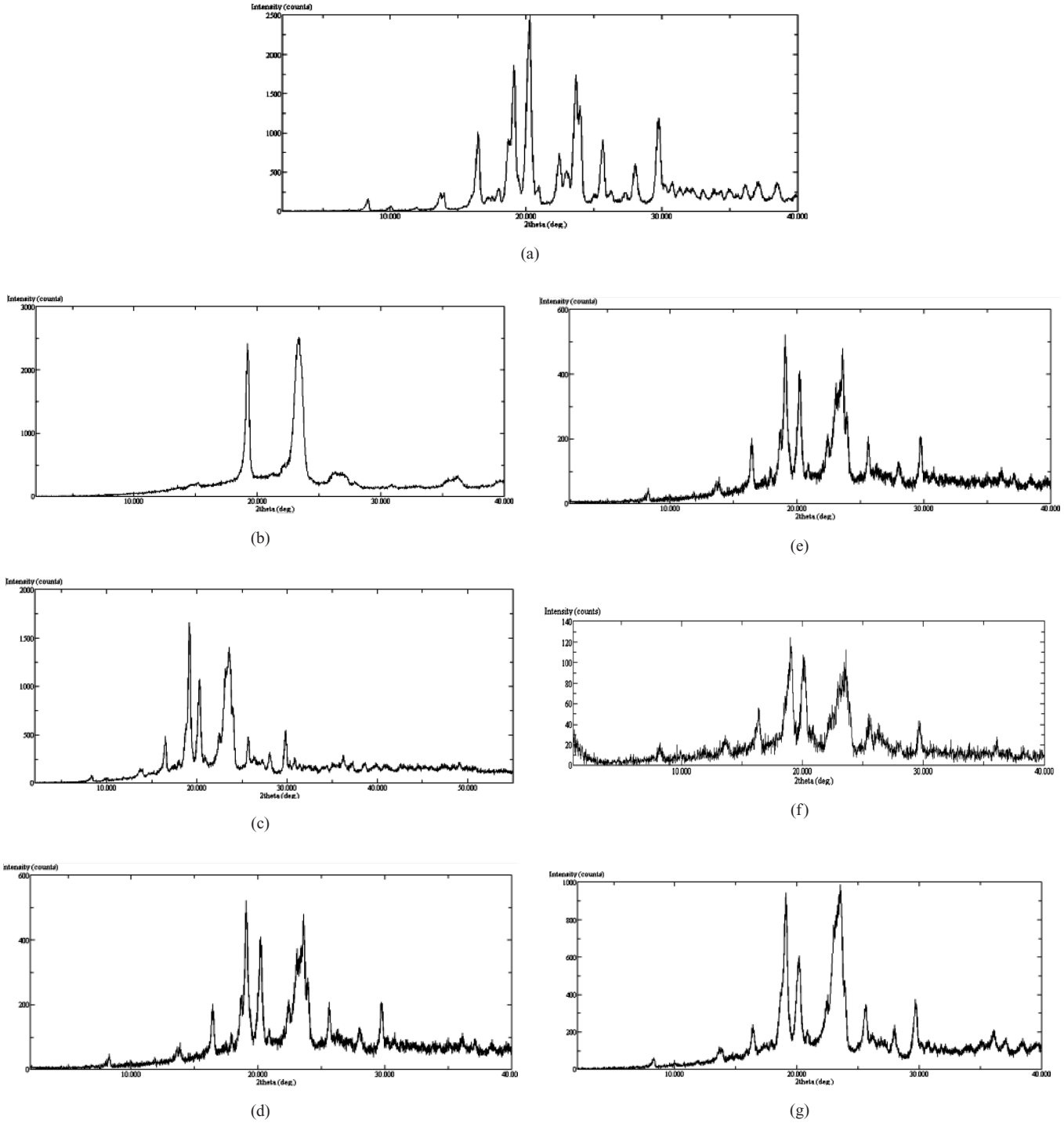

Fig. 2. X-Ray Diffractogram of a) Ezetimibe, b) Pluronic F 127, c) the Physical Mixture, d) the Nanocrystal Formulation (Ball Milling), e) the Nanocrystal Formulation (Ultrasonic Probe), f) the Tablet Formulation (Ball Milling), g) the Tablet Formulation (Ultrasonic Probe)

given in Figs. 2a—g. Peaks in the X-ray diffractograms indicate the presence of crystal structure while no peaks are observed in the presence of amorphous structure. X-Ray diffraction analysis of the nanocrystal formulations was performed to determine the crystalline form of the active drug substance and reduction in the amount of crystalline form of the active drug substance. The crystal structure of ezetimibe is clearly seen in the X-ray diffractogram (Fig. 2a). When the results are examined, only ezetimibe and Pluronic F 127 have a crystal structure and there were no differences among the formulations regarding the crystal structure, and ezetimibe crystal structure was preserved in all formulations. The X-ray diffractogram of the tablet formulation indicated that the crystal morphology of ezetimibe was preserved since the characteristic peaks were sustained. Thus, it can be inferred that the process of wet granulation probably did not have any negative effect on the crystal structure of the drug. The interaction of ezetimibe with Pluronic F 127 was confirmed by the change of intensity of the drug and excipient peaks when incorporated into the nanocrystal formulations. Moreover, depending on the method of preparation, the degree of interaction between the drug and excipient varied leading to changes in the intensity of the peaks. The preservation of the crystal structure of the drug in the formulation is crucial for the sustained stability of the drug during its shelf-life. On the other hand, the drug in the amorphous state has better dissolution properties compared to the crystal form. Thus, decreasing the drug particle size to nanodimensions while preserving the crystal morphology, leads to improved dissolution profile while keeping the drug intact (*i.e.* sustained chemical stability). In the nanocrystal formulations prepared, both the crystal state and stability of ezetimibe were preserved while improving the dissolution properties, as discussed in the following sections.[22)]

DSC Analysis of Formulations Differential scanning calorimetric analysis were carried out to examine the thermal behavior of the components used in the nanocrystal formulations. The DSC thermograms of the nanocrystal formulations, Pluronic F 127, ezetimibe and physical mixture are given in Fig. 3. Molecules in the crystal structure have a melting endotherm while molecules in the amorphous state do not exhibit a melting endotherm.[23)] As seen in Fig. 3, the sharp melting peak of ezetimibe (164.65 °C) disappeared completely in all nanocrystal formulations, implying that ezetimibe was covered completely with Pluronic F 127 in all nanocrystal formulations.

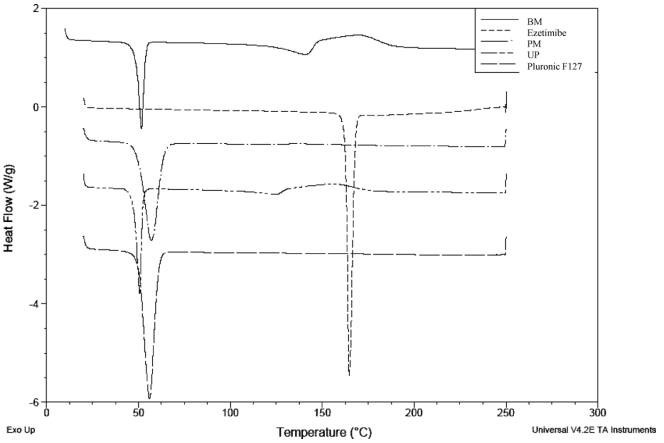

Fig. 3. Differential Scanning Calorimeter Thermograms of the Nanocrystal Formulations, Ezetimibe, Physical Mixture and the Excipient

Analysis of Particle Size Distribution A Malvern Zeta Sizer (Nano Series Nano ZS) was utilized in order to determine the particle size distribution of the formulations. The average particle size values regarding these formulations are given in Table 2. According to these data, the average particle size of the nanocrystal formulations decreased to a significant extent as compared to ezetimibe and physical mixture, and the difference in particle size could be considered as statistically significant. As it is depicted in Table 2, while the average particle size of ezetimibe was 6432±1024 nm and of the physical mixture was 6302±670 nm in terms of intensity, an 80% and 73% decrease in particle size was achieved with the BM and UP methods, respectively. It can be clearly seen from Table 2 that the method of preparation had a significant impact of the final particle size distribution of the nanocrystal formulations. In all formulations, incorporation of a stabilizer, Pluronic F 127, prevented aggregation of newly formed nanosized drug particles. The particle size of the drug could be decreased to the desired dimensions by setting different parameters in each method. For instance, in the BM method, ezetimibe suspension was milled using the appropriate size and number of agate balls, and the mixing speed was adjusted to give a final average particle size of 1259±62 nm. In the UP method, ezetimibe suspension was exposed to ultrasonic sound waves and particle size was decreased to an average value of 1736±88 nm by setting the probe study time and % strength.

Dissolution Studies The results of the dissolution studies are presented in Tables 3 and 4. By calculating the similarity factor (f_2) value with model-free techniques, the difference between the commercial product and the tablets containing the nanocrystal formulations regarding the dissolution rate profiles was determined.[24)] The similarity test is

Table 2. Average Particle Size of Ezetimibe and Nanocrystal Formulations (n=3)

Formulation	Average particle size ±S.D. (nm)
Ezetimibe	6432±1024
PM	6302±670
UP	1736±88
BM	1259±62

Table 3. The % Amount of Ezetimibe Nanocrystal Dissolved from Different Tablet Formulations in 500 ml 0.45% SDS in 50 mM pH 4.5 Acetate Buffer (n=6) (BM, PM−SDS, PM+SDS, UP, Commercial Product)

Time (min)		BM[a)]	PM−SDS[b)]	PM+SDS[c)]	UP[d)]	Commercial product
10	X[e)]	75.94	55.18	57.84	71.39	63.82
	% VC[f)]	1.32	1.70	1.35	1.43	3.27
20	X	88.72	57.06	59.21	75.46	87.09
	% VC	0.93	1.65	2.58	1.29	1.38
30	X	95.13	59.15	71.54	95.81	95.81
	% VC	0.78	1.43	8.09	1.04	0.96
45	X	99.34	65.26	72.36	97.25	95.11
	% VC	1.12	2.91	8.53	0.90	0.78

a) Ball milling, *b*) physical mixture without SDS, *c*) physical mixture with SDS, *d*) high speed homogenization method (ultrasonic), *e*) average, *f*) coefficient of variation.

Table 4. Comparison of the Tablet Formulations Containing Ezetimibe Nanocrystals with the Commercial Tablet Formulation Using the f_2 Similarity Factor

Dissolution medium	f_2 factor	
	BM-T	UP-T
500 ml 0.45% SDS in 50 mM pH 4.5 acetate buffer	61.855	62.889

BM-T: ball milling tablet; commercial product. UP-T: high speed homogenization (ultrasonic) tablet; commercial product.

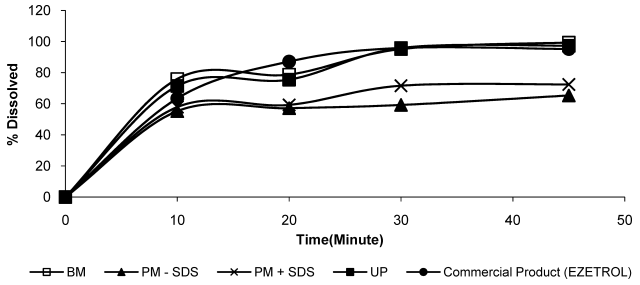

Fig. 4. Dissolution Profile of Ezetimibe in 500 ml pH 4.5 50 mM Acetate Buffer/0.45% SDS from the Tablets Containing the Nanocrystal Formulations ($n=6$)

BM: ball milling, PM−SDS: physical mixture without SDS, PM+SDS: physical mixture with SDS, UP: high speed homogenization method (ultrasonic). Ezetimibe nanocrystals were prepared to improve the solubility and dissolution rate of the drug utilizing ball milling and high speed homogenization techniques. Tablet formulations were prepared using the nanocrystals. As a control group, a tablet formulation of the physical mixture was prepared both with SDS (sodium lauryl sulfate) and without SDS. Also, dissolution profiles were compared to the commercial product, which contains SDS as a solubilizing agent. Although nanocrystal tablet formulations did not contain any solubilizing agents like SDS, the dissolution profiles were found similar to the commercial product ($f_2>50$). As a result, the improvement of the dissolution rate of ezetimibe could be attributed to the preparation of drug nanocrystals.

made up for comparing the outcomes of the *in vitro* dissolution rate studies. If the value of the similarity factor is equal to or above 50, then it refers to the presence of similarity among the *in vitro* dissolution profiles of the formulations. It appears from the results that the tablets which were prepared by both BM and UP, are similar to the commercial product when the dissolution studies are carried out in the dissolution medium containing 500 ml pH 4.5 50 mM acetate buffer/0.45% SDS. It is also worth nothing that the presence of a surface active agent (SDS) in the dissolution medium did not seem to have an impact on the increase of the drug dissolution rate (Fig. 4, Table 3). In other words, the improvement of the dissolution rate of ezetimibe could be attributed to the preparation of drug nanocrystals.

In conclusion, nanocrystal formulations of ezetimibe were prepared and characterization of these formulations and dissolution studies were performed. The formulations, prepared by BM and UP which had low particle size (1259±62, 1736±88 nm) and high dissolution rate (97.25, 99.34%) were chosen and their tablet formulations were prepared. Unlike the commercial product, the tablet formulation of ezetimibe nanocrystals did not contain any solubilizing agent, such as sodium lauryl sulfate. Although these formulations did not contain SDS, the dissolution rate of these formulations was found similar to the commercial product which contains SDS in its formulation. The findings of the present study indicate that a method for the preparation of stable nanocrystal formulations of ezetimibe with an average particle size of lower than 2000 nm utilizing High Speed Homogenization (ultrasonic) and ball milling could be successfully developed. Moreover, the dissolution rate of this poorly water-soluble drug could be improved significantly by using drug nanocrystals in the tablet formulations. Preparation of nanocrystal formulations is simple and reproducible, and thus, could be used to improve the dissolution profiles of other poorly water-soluble active drug substances.

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