Dissolution Enhancement of Danazol Nanoparticles prepared by Nanoprecipitation Method

Luma Safa eldin. Al-hassnaui[1], Firas A. Rahi[2], Mohammed S. Al-lami[3]

[1] Pharmacist, AL-Diwanyia teaching hospital, Iraq, ph.luma86@gmail.com

[2] Faculty of Pharmacy, **Alturath University College. firas.aziz @turath.edu.iq

[3]College of Pharmacy, University of Basra, Al Basrah, Iraq .hmohsabbar@gmail.com

Keywords: Danazol; solvent/antisolvent precipitation; polymers; Polyvinyl pyrrolidone; nanoparticles; Characterizaion; dissolution rate; release.

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Abstract

Danazol is a weak androgenic steroid taking by mouth and it's practically insoluble in water. This study was accomplished to prepare danazol nanosuspension by nanoprecipitation method at a diverse (polymer to drug) ratio of 0.5:1, 1:1, 2:1 and 3:1 utilizing Hydroxypropyl methylcellulose (HPMC) -A4C, HPMC-E50 and Polyvinylpyrrolidone (PVP) K-15 as stabilizers. The particle size of the organized formulas was in the nano-sized and the finest formula was F12 at a polymer to drug ratio of 0.5:1 which gave the littlest particle size of (33nm). The investigations of drug–stabilizer compatibility were studied by Fourier transform infrared (FTIR) spectroscopy and Differential scanning calorimetry (DSC), crystalline state by XRD, size, and figure/ shape of nanoparticles by Field Emission Scanning Electron Microscopy (FESEM) and the results revealed that there was no interaction between the danazol and stabilizer and there was the partial translation of danazol from crystalline to an amorphous state . The loading efficiency was (91.2% ± 0.4) in the (F12). The simple capsule was set of F12 and the filler was lactose and the in vitro dissolution study was accompanied consuming 0.1N HCl (pH 1.2) with 2% w/v Brij-35, phosphate buffer solution (pH 6.8) with 2% w/v Brij-35 as dissolution media. 100% of the danazol was released from the nanoparticle capsule in both dissolution media within 30 minutes, Whereas , the raw and physical blend capsules as controls were nearly complete in 120 minutes. In conclusion , nanoprecipitation method is an easy, effective method to make danazol nanoparticles with a more rapidly in vitro dissolution rate than raw drug and its physical mixture with stabilizer.

Keywords: Danazol; solvent/antisolvent precipitation; polymers; Polyvinyl pyrrolidone; nanoparticles; Characterizaion; dissolution rate; release.

دانازول هو ستيرويد منشط الذكورة ضعيف يؤخذ عن طريق الفم وهو عملياً غير قابل للذوبان في الماء. أُنجزت هذه الدراسة لتحضير تعليق نانوي لدانازول بطريقة الترسيب النانوي بنسبة متنوعة (بوليمر إلى عقار) 0.5: 1 ، 1: 1 ، 2: 1 و 3: 1 باستخدام هيدروكسي بروبيل ميثيل سلولوز (HPMC) -A4C و HPMC-E50 و Polyvinylpyrrolidone (PVP) K- 15 كمثبتات. كان حجم الجسيمات للصيغ المنظمة في الحجم النانوي وكانت أفضل صيغة هي F12 بنسبة بوليمر إلى عقار 0.5: 1 والتي أعطت أصغر حجم جسيم (33 نانومتر). تمت دراسة تحقيقات توافق الأدوية مع المثبت بواسطة التحليل الطيفي للأشعة تحت الحمراء (FTIR) والقياس الحراري للمسح التفاضلي (DSC) ، والحالة البلورية بواسطة XRD ، وحجم وشكل / شكل الجسيمات النانوية عن طريق الفحص المجهري الإلكتروني لمسح الانبعاث الميداني (FESEM) والنتائج كشفت أنه لا يوجد تفاعل بين دانازول والمثبت وكان هناك تحول جزئي للدانازول من حالة البلورية إلى حالة غير متبلورة. بلغت كفاءة التحميل (91.2٪ ± 0.4) في (F12). تم تعيين الكبسولة البسيطة من F12 والحشو كان اللاكتوز ودراسة الذوبان في المختبر مصحوبة باستهلاك 1.20.1 pH) N HCl) مع 2٪ وزن / حجم Brij-35 ، محلول فوسفات منظم (6.8 pH) مع 2٪ وزن ضد Brij-35 كوسيلة حل. تم إطلاق 100 ٪ من الدانازول من كبسولة الجسيمات النانوية في كل من وسطا الذوبان في غضون 30 دقيقة ، في حين أن كبسولات المزيج الخام والفيزيائية كعناصر تحكم اكتملت تقريبًا في 120 دقيقة. طريقة الترسيب النانوي هي طريقة سهلة وفعالة لصنع جسيمات دانازول النانوية بمعدل ذوبان أسرع في المختبر من العقار الخام وخلطه الفيزيائي مع المثبت.

Introduction

The poor solubility of many new chemical entities has become the main challenge in the drug development practice. Drugs with this property are hard to formulate by conventional methods and frequently displayed poor bioavailability **(1).** The enhancement of drug solubility can be achieved by modifications such as the formation of the co-crystals **(2)**, pro-drug **(3)**, or solid dispersion **(4)** etc. Micronization of the drugs rises their rate of dissolution and absorption by increasing their surface area, but micronization has failed to increase saturation solubility and bioavailability of the compounds with very low aqueous-solubility; For that reason, the additional step of reducing the particle size to the nanometer size range was made up , the development of danazol nanosuspensions is a useful approach to increase bioavailability of (poorly water-soluble drugs) , and can be conditioned reformulate current drugs for improving bioavailability**(5)**. Nanoparticles can be well-defined as the colloidal particles possessing a size ranging from 10 to 1000 nm **(6)**. Danazol is a gonadotropin inhibitor, mostly used in therapy of endometriosis and hereditary angioneurotic edema, in addition to earlier use as a contraceptive **(7)**. It is 17 *a* -Pregna-2,4-dien-20-yno[2,3-d] isoxazol-17b-ol, with the molecular weight (M.wt) is (337.5); Log P (octanol/water), is 4.21.The chemical structure of danazol given in (Figure 1).Have melting point (m.p) of 224.4_ 226.8° C with decomposition . It is practically insoluble in water, freely soluble in chloroform, soluble in acetone**;** sparingly soluble in ethanol **(8)**. Danazol is neutral drug and the subclassification of BCS Class II, c: for neutral drugs with No pKa or (p*K*a < 0 or ~ >8) **(9).** It is absorbed from small and large intestines independent of pH of the environment in GIT **(9)**. Owing to its poor aqueous solubility, the absolute bioavailability of danazol reported by **Sunesen** and colleagues, "the absolute bioavailability was 11 ± 5.2% when danazol was administered in the fasted state with 200 ml water " **(10)**

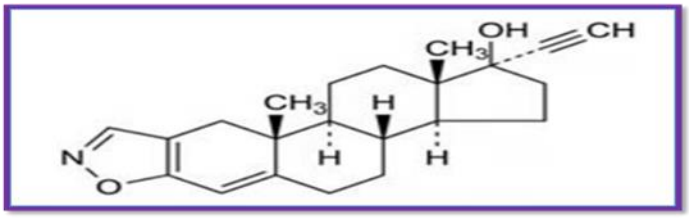

Fig.1 Chemical structure of Danazol [11]

The aim of present study is to the formulation of danazol nanoparticle as a capsule dosage form in order to improve its *in vitro* dissolution rate and show how is the application of solvent/anti-solvent technique for hydrophobic drug is easy and effective.

Materials and method

Materials

Danazol (99 % pure) was purchased from Micro Orgo- Chem,India. Hydroxypropyl methylcellulose (HPMC) grades: HPMC-A4C and HPMC-E50 was purchased from Gromax chemical, USA. Poly vinyl pyrrolidone K-15(PVP K-15)was provided by Lupi.Medico, India. Hydrochloric acid from BDH Laboratory Co and Potassium di-hydrogen orthophosphate from Sd Fine-chem. All other chemicals and solvents were of analytical reagent grade, and distilled water also was used in this study.

Methods

Preparation of danazol nanoparticles

The danazol nanoparticles formulas as shown in Table (1) were prepared by nanoprecipitation using a modified method of solvent / anti-solvent precipitation technique [11]. Solutions of different concentrations of drug of 6.66, 10 and 20mg/mL in acetone were prepared, and 5mL of each solution was inoculated at 1mL/minute by means of syringe situated with needle directly into 50mL water containing a stabilizer with constant stirring 500rpm on magnetic stirrer at 10° C. The polymer of HPMC-A4C, HPMC-E50 and PVP K-15 used as a stabilizer. The ratios of polymer to the drug used to prepare the nanoparticles were 0.5:1, 1:1,2:1and 3:1. The finished products were freeze-dried and preserved in tightly closed containers for characterization and additional experimental works.

Particle size and poly dispersity index measurement

The particle size of freshly prepared nanosuspensions was measured by "ABT9000 Nano Laser Particle Size Analyzer" (Angstrom, USA). Particle size distribution curves were originated.

Additionally, the average particle size, "polydispersity index " (PDI), and the specific surface area (SSA) for each formula were documented [13].

Different formulation variables affecting the properties of prepared of composition shown in table 1. The composition and variable conditions of preparation nanoparticles were studied by utilizing the prepared different fifteen formulas are listed in table (1).

Formula No.	Polymer Type	Polymer:Drug ratio	Solvent:Antisolvent (SAS)ratio (Acetone :Water)	Drug concentration mg/mL
F1	HPMC-A4C	1:1	1:10	10
F2	HPMC-E50	1:1	1:10	10
F3	PVP-K15	1:1	1:10	10
F4	HPMC-A4C	0.5:1	1:20	20
F5	HPMC-A4C	1:1	1:20	20
F6	HPMC-A4C	2:1	1:20	10
F7	HPMC-A4C	3:1	1:20	6.66
F8	HPMC-E50	0.5:1	1:20	20
F9	HPMC-E50	1:1	1:20	20
F10	HPMC-E50	2:1	1:20	10
F11	HPMC-E50	3:1	1:20	6.66
F12	PVP-K15	0.5:1	1:20	20
F13	PVP-K15	1:1	1:20	20
F14	PVP-K15	2:1	1:20	10
F15	PVP-K15	3:1	1:20	6.66

Table (1): Composition of danazol formulas

Characterization of lyophilized danazol nanoparticles

Determination of drug content and Entrapment efficiency

A modified method of E-Gendy's team [14] was used in the determination of actual danazol content. Danazol content in dried powder was measured by dispersing 5mg of the lyophilized powder in 10mL of ethanol. The dispersion was sonicated in a sonicator for 15min. Then the mixture was centrifuged at ~15,000rpm for 30min to remove insoluble components and the amount of danazol in the supernatant was determined spectrophotometrically at λmax of 285nm.

With the aid of a calibration curve of known concentrations of danazol in the same solvent. The loading efficiency of nanoparticles was obtained from the theoretical and actual drug contents. The percent entrapment efficiency was calculated the following equation [15] :

Entrapment Efficiency%= Amount of drug actually present / Theoretical drug content expected × 100

The selected formula that being dried was weighed, which represented the actual weight of nanoparticles gained and the initial material polymer and drug) announced into the formula denoted the theoretical weight of nanoparticles. The percent yield was calculated using the following equation [16] :

%Yield = (weight of nanoparticles gained)/ (Theoretical weight of nanoparticles) x100

Flowability study of the lyophilized nanoparticles powder

The "angle of repose" has been determined for the lyophilized powder to note the flowing characters of the powder**.** Flow properties and the corresponding angle of repose are shown in the table (7). Fixed funnel method has been used in which the powder has been carefully filled into the glass funnel positioned over a lying flat surface, identified base diameter has been symbolized as **(D)**, up to the cone-shaped. Adjusted the height of the funnel at (4cm) from the topmost of powder heap with a view to diminishing the manipulate of the dropping powder on the cone edge. The tan of the angle of repose **(θ)** has been estimated after evaluating the height **(H)** of "the cone of the powder", consuming the equation [17]:

Tan Θ = H/ (0.5xD)

Nanoparticles surface morphology study by FESEM

The size and morphology of danazol nanoparticle were recognized using an FE-SEM (Zeiss Supra 55VP) at an accelerating voltage of 1 kV. For each sample was positioned on a carbon tape and was air-dried for 24 hrs [18]**.**

Powder x-ray diffraction (XRD)

X-rays diffraction patterns 'diffractograms" was used to verify whether the prepared materials are crystalline or amorphous. The study was established by p (XRD-6000, Shimadzu, Japan) at continuous scan range of 2θ = 5 - 50º, Cu-K radiation was generated at 30 mA current and 40 kV the operational voltage correspondingly [19].

Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared spectra of the raw danazol, physical blend, PVP K-15 and lyophilized powder of the chosen formula F12was achieved by grinding the powder with potassium bromide (KBr) and examined using IR Prestige-21 spectrometer (Shimadzu, Japan), obtained within the range from 4000 to 400 cm^{-1} [20]. The final FT-IR spectra's were established on a FT-IR spectroscopy was achieved and reviewed for evidence of any interactions between the drug and stabilizer.

Differential scanning calorimetry (DSC)

This analysis was manipulated to determine the compatibility or interaction between the drug and excipients and also manipulated to assess the crystalline state of the drug. Thermal analysis manipulating DSC was taken place individually on lyophilized nanoparticles, raw danazol, PVP-K15 and the physical mixture. Exactly weighed samples of 5mg were transferred into aluminum pans and sealed. Samples were tracked at a heating rate of 20° C $min^{-1}$and scanned in the temperature range of 50-300°C [21].

In vitro dissolution study of danazol

The in vitro dissolution study was done using USP dissolution test apparatus-II (paddle-assembly). The dissolution was performed on capsule filled with F12 in comparison with capsules have contained raw danazol (100mg) and a physical blend capsules of PVP-K15: danazol at ratio of (0.5:1) in 900 mL of 0.1N HCl (pH 1.2) and phosphate buffer solution (pH 6.8) as dissolution media (containing 2% w/v Brij-35) [22] which preheated and maintained at 37 ± 0.5° C and paddle were rotated at a speed of 75 rpm/min.

Samples of 5mL were withdrawn periodically at regular intervals of 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes. Each sample was replaced with fresh dissolution medium to preserve a constant volume afterward for each sampling. Samples were filtered through a 0.2µm membrane filter and suitably diluted on the need to be assayed spectrophotometrically at 286 nm wavelength [23]. This experiment was repeated in triplicate.

Statistical analysis

The outcomes of the experiments have been conducted at an average of triplicates ± standard deviation. The dissolutions have been analyzed for similarity using f1 and f2 analysis.

Results

Evaluations of prepared danazol nanoparticle

Analysis of particle size, polydispersity index, and surface area of prepared danazol nanoparticles

The outcomes of particle size, polydispersity index and specific surface area of the prepared danazol nanoparticles were shown in the table (2). The particle sizes of freshly prepared formulas were determined by "ABT_9000 particle size analyzer. Polydispersity index is a term to state the particle size distribution of nanoparticles. PDI is an index of width or spread or variation inside the particle size distribution and offers an indication of the long-term stability of nanosuspension. The monodisperse sample has a low PDI value, but higher value designates a wider particle dimension distribution of the system. The typical range of PDI standards is >0.7 for very polydisperse**,** 0.08-0.7 for mid-domain polydispersity, 0.05-0.08 for nearly monodisperse and < 0.05 for typical monodisperse (**[24]**. The specific surface area (SSA) of the particles is "the summation of the areas of the exposed surfaces of the particles per unit mass". It is an inversely· proportional with a particle size **[25]**.

As shown in the table (2), all the prepared danazol nanoparticles formulas showed particle size variation from 33 nm to 595 nm. PDI variation from 0.004 to 0.328, that representing an acceptable uniformity level and adequate homogeneity for most of them. As well; formulas showed a specific surface area variation from lowest SSA equal 3.60 m^2/g for F16 has an average particle size 595 nm in comparison with highest SSA of 67.10 m^2/g of the smallest particle size for F12 has an average particle size 33nm.

Table (2): Particle Size data of prepared danazol nanoparticles

Formula No.	PolymerType	Particle size nm	Surface area m^2/g	Poly dispersity PDI
F1	HPMC-A4C	375	1.50	0.004
F2	HPMC-E50	334	6.20	0.009
F3	PVP-K15	188	10.80	0.010
F4	HPMC-A4C	149	14.71	0.262
F5	HPMC-A4C	188	11.12	0.075
F6	HPMC-A4C	334	6.36	0.009
F7	HPMC-A4C	530.5	4.27	0.019
F8	HPMC-E50	94.3	23.51	0.083
F9	HPMC-E50	118	19.06	0.136
F10	HPMC-E50	211	10.04	0.132
F11	HPMC-E50	298	7.26	0.007
F12	PVP-K15	33	67.10	0.203
F13	PVP-K15	59.5	37.38	0.030
F14	PVP-K15	84.05	24.53	0.302
F15	PVP-K15	105.35	21.98	0.008

Determination of drug content in nanoparticles powder, **percent yield and loading efficiency**

The results showed that 5mg of lyophilized nanoparticles powder of F12 contained 3.043±0.001mg of danazol. The percent yield of the recovered quantity for processed nanoparticles was determined after Freeze - drying process. In Figure (13), photographic images for lyophilized powder of (F12) danazol nanoparticle and danazol nanosuspension**.** The percent yield of danazol was 82 ± 1.632, may be characterized as satisfactory. The loading efficiency of the drug in the prepared nanoparticle was found to be 91.2% ± 0.463.

Figure (2). Photographic images (A)for lyophilized powder of (F12) danazol nanoparticle and (B) danazol nanosuspension.

Flowability of the prepared nanoparticles

The angle of repose was measured for raw danazol and the selected nanoparticles formula of F12 in order to observe the flow properties of the powder. The angle of repose (Ɵ) is distinctive of the internal cohesion of the particles [(26)]. The angle of repose for raw danazol was 38.6 ± 0.08º while for nanoparticles was 24.56 ±0.94º.

Nanoparticles surface morphology study by **FESEM**

The size and shape of the nanoparticles were characterized by a field emission scanning electron microscope (Zeiss Supra 55VP). The images of the FESEM at different magnification power for raw danazol as shown in figure (3), at which it can be seen that bulk micronized danazol consisted of crystalline plates with smooth surfaces and fractured edges with sizes ranged from 1 to 20µm in length [(27)]. The images for nanoparticles were obtained for the selected formulas (F12)

at different magnification power as shown in figure (4) and indicate submicron sized particles likely <<(100nm) in diameter.

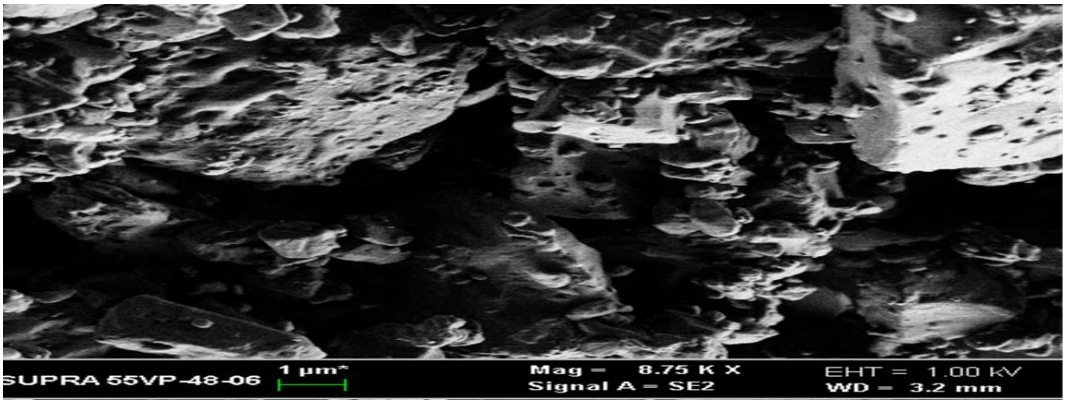

Figure (3): Field Emission Scanning Electron Microscope (FESEM) of raw danazol

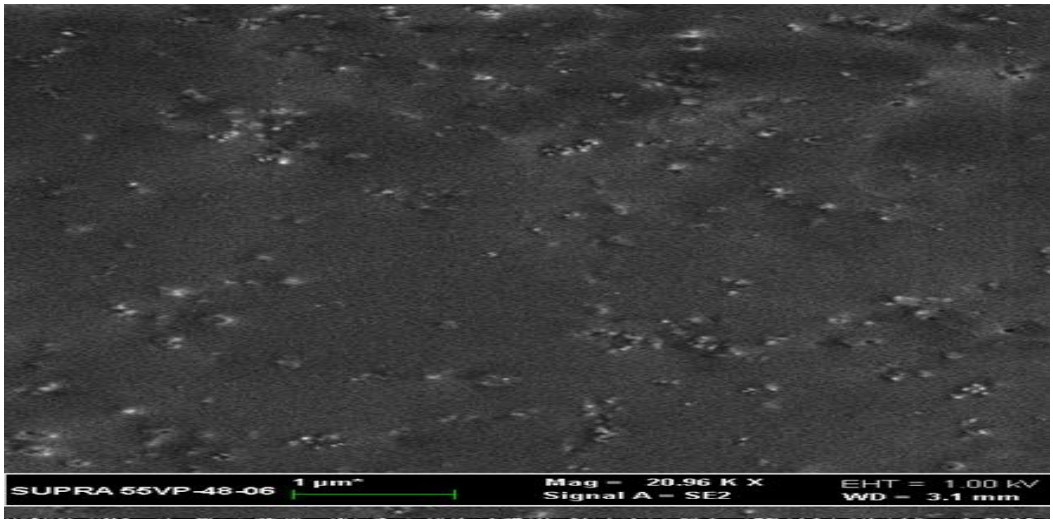

Figure (4): Field Emission Scanning Electron Microscope(FESEM) of nanoparticles

X –ray powder diffraction analysis

The recorded characteristic diffraction peaks of raw danazol were at 15.8, 17.2 and 19.0 (2θ) degrees**.** X –ray powder diffraction spectrum indicated that bulk micronized danazol was in the crystalline form [28] as shown in Figure (5). Figure (6) representing XRD diffractogram of lyophilized nanoparticle .On the other hand, the XRD patterns for the corresponding physical mixtures have been showen in Figure (7).

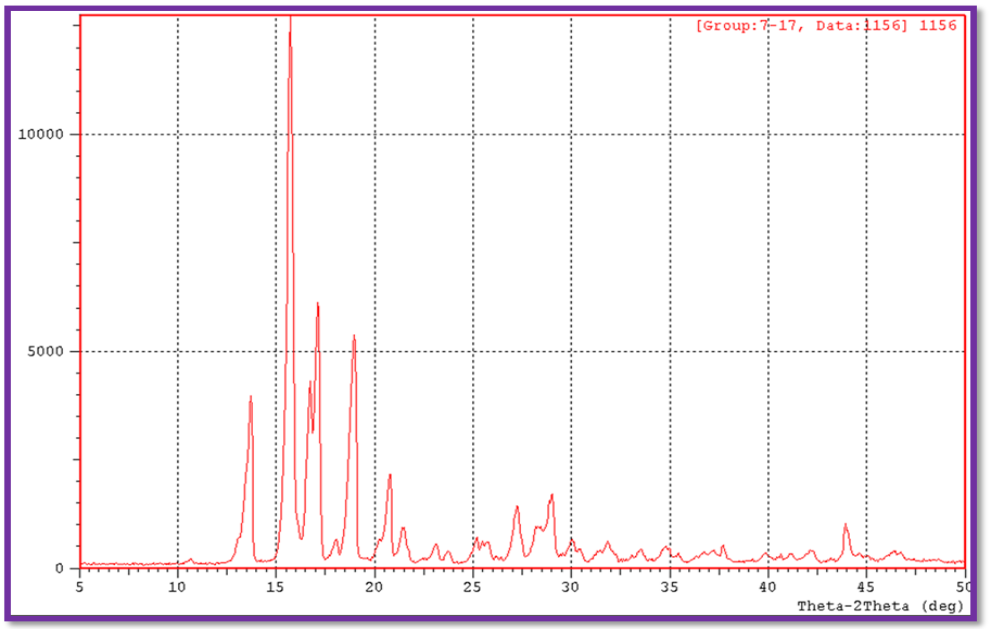

Figure (5): X-ray powder diffraction (XRPD)spectrum of raw danazol

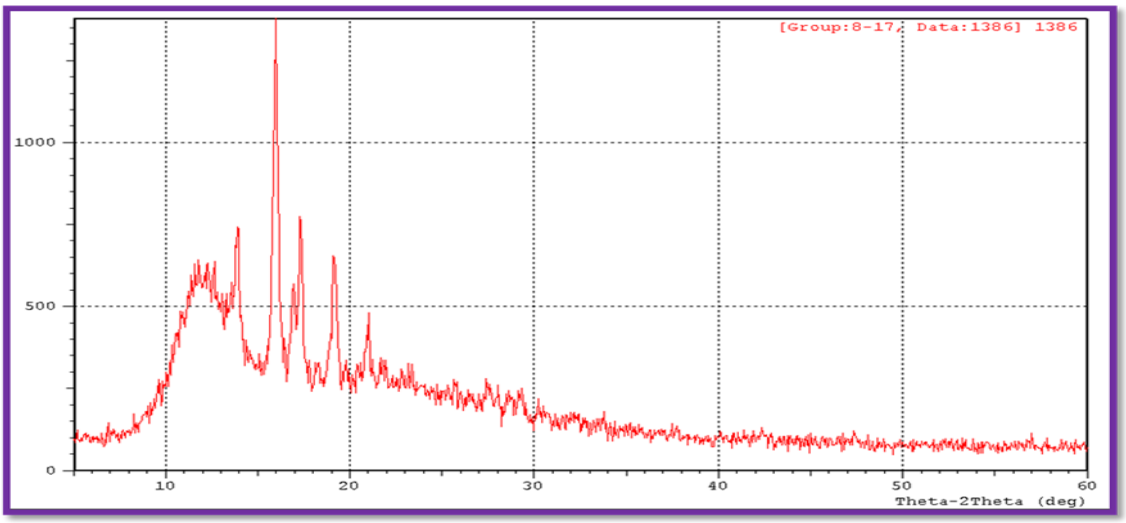

Figure (6): X-ray powder diffraction (XRPD) spectrum of nanoparticles

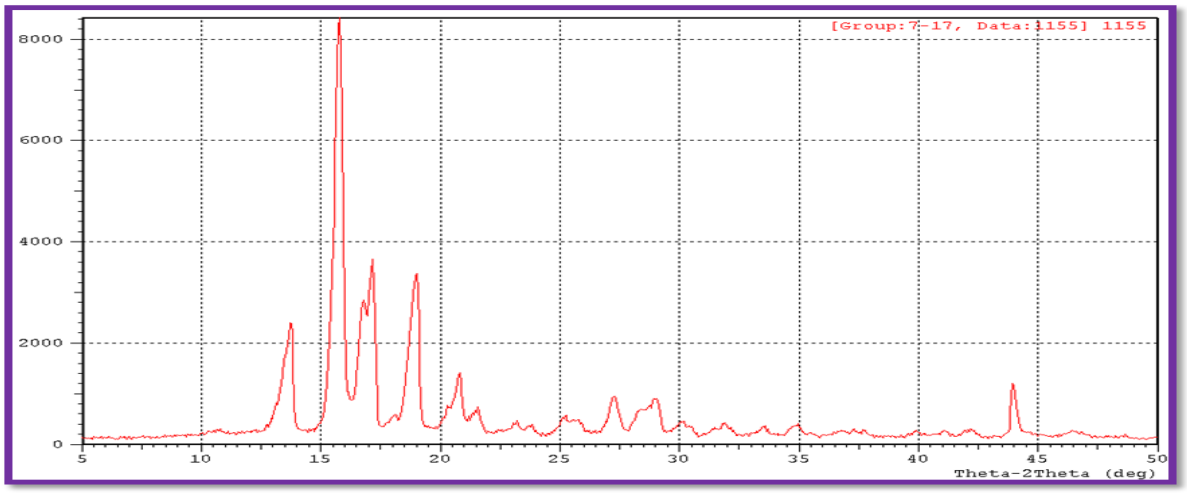

Figure (7): X-ray powder diffraction (XRPD) spectrum of physical mixture of PVP-K15 and raw danazol

Fourier Transform Infrared Spectroscopy (FT-IR)

The obtained FT-IR spectrum of raw danazol powder is shown in figure (8). The FT-IR spectra of raw danazol powder show a characteristic peak of 0-H stretching at the 17position at 3514.3 cm-1 [29], C≡CH stretch at 2099.0 cm^{-1}, C=N **stretch of isooxazole ring** at 1631.78 cm^{-1} [30]. The FTIR spectrum of the polymer was taken for the prepared danazol nanoparticles of selected formula (F12) and the physical mixture (PVP-K15: danazol) and PVP-K15 as represented in figures (9-12).

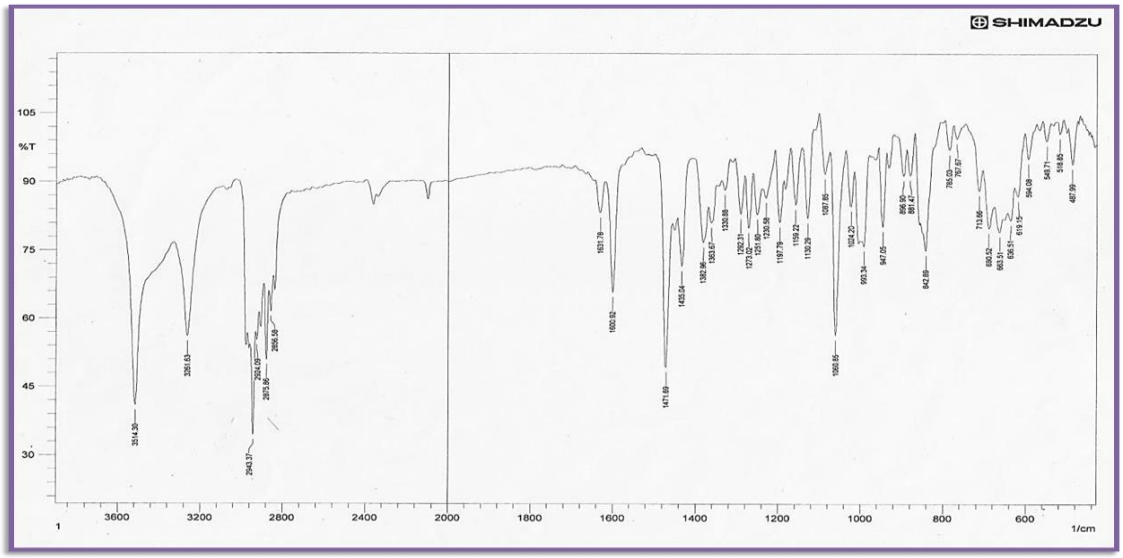

Figure (8): Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of raw danazol

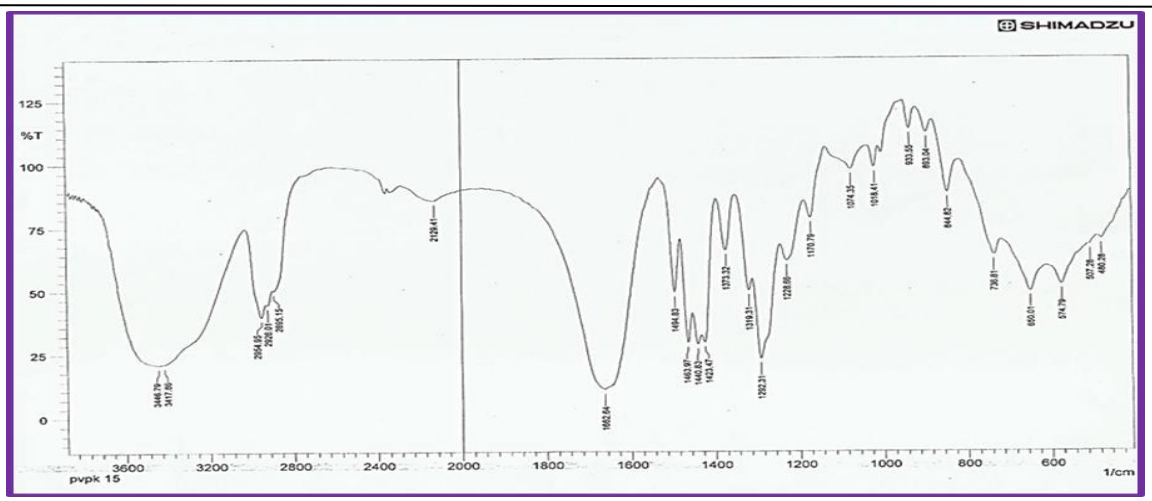

Figure (9): Fourier Transform Infrared Spectroscopy (FT-IR)spectrum of PVP-K15

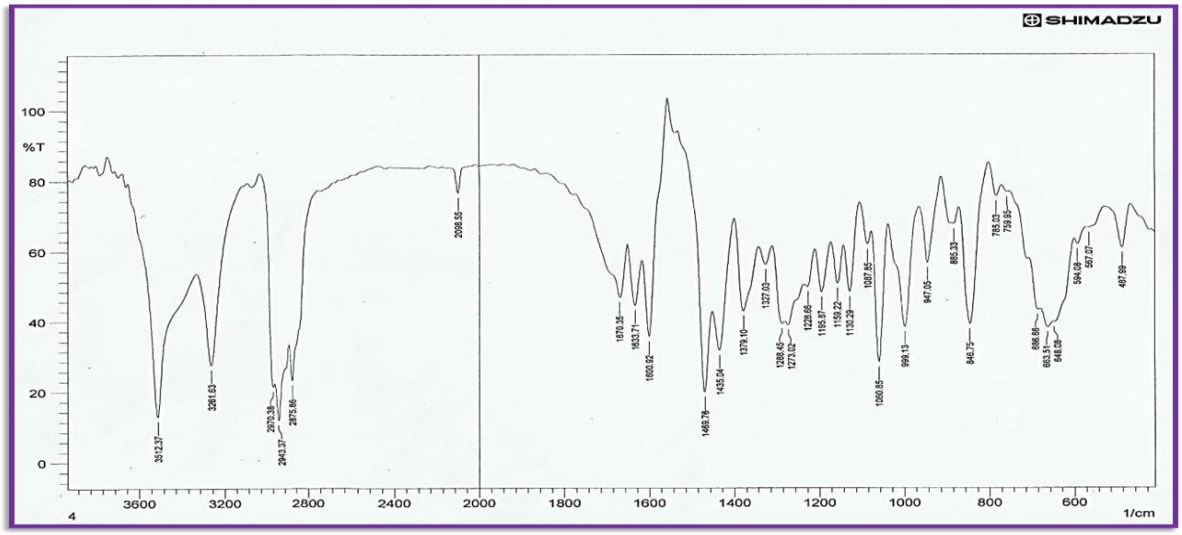

Figure (10): Fourier Transform Infrared Spectroscopy(FT-IR) spectrum of physical mixture of PVP-K15:raw danazol

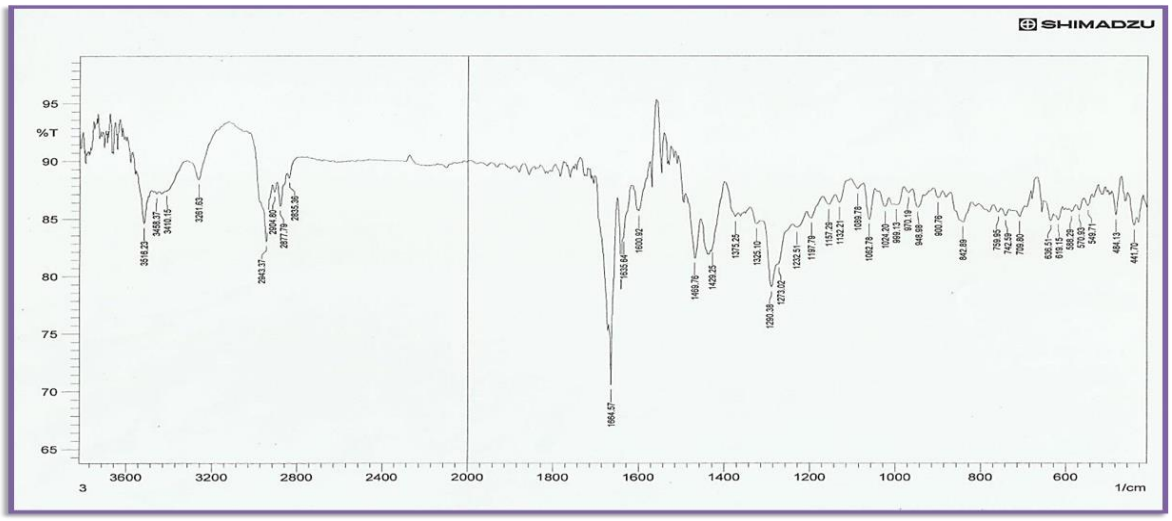

Figure (11): Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of nanoparticles **F12**

Differential scanning calorimetry (DSC)

The raw danazol showed clear and sharp characteristic endothermic peak around its melting point (229.48° C), such peak indicates that danazol in the crystalline and pure state as this result close to the that reported at 228.6° C [31]**.** The diffractogram of raw danazol was shown in figure (12). While PVP-K15 showed broad glass transition (Tg) peak at 120.73° C as in figure (13). In physical mixture as in figure (14), the intensity of danazol peak was reduced and slightly shifted the peak of PVP-K15 at 94.62° C.

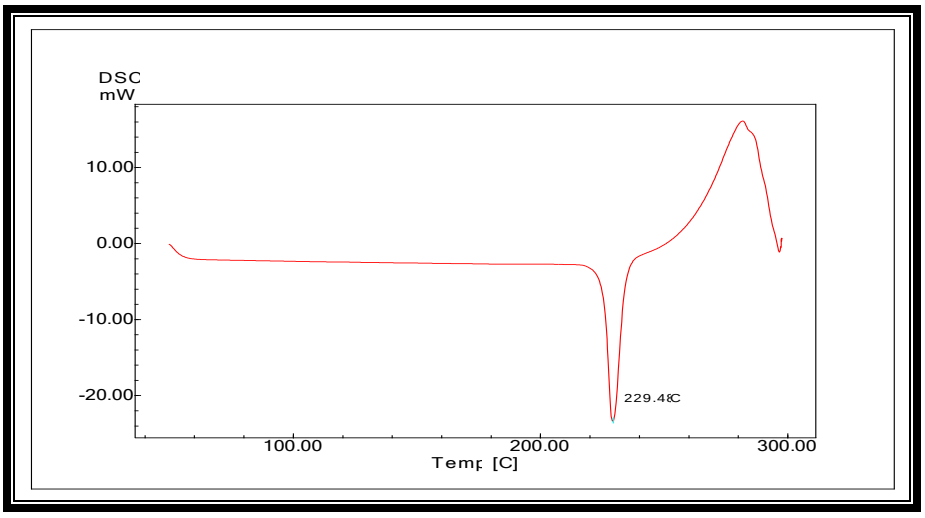

Figure (12): Differential scanning calorimetry (DSC) spectrum of raw danazol.

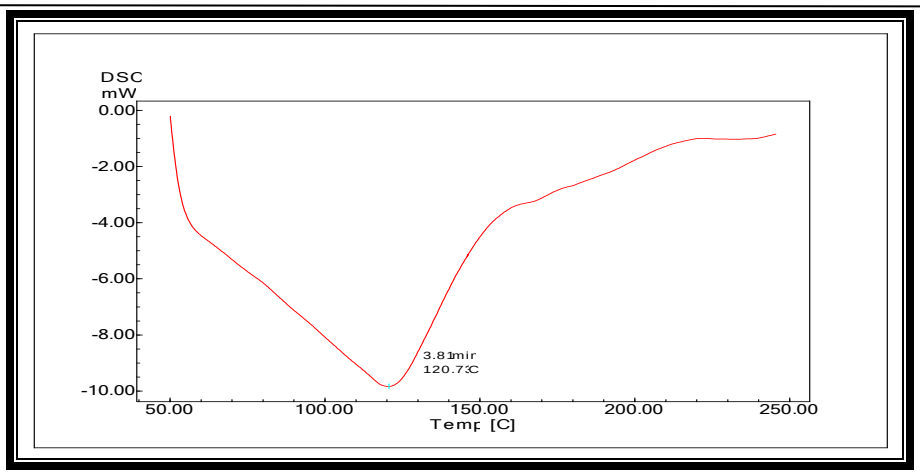

Figure (13): Differential scanning calorimetry (DSC) spectrum of PVP-K15

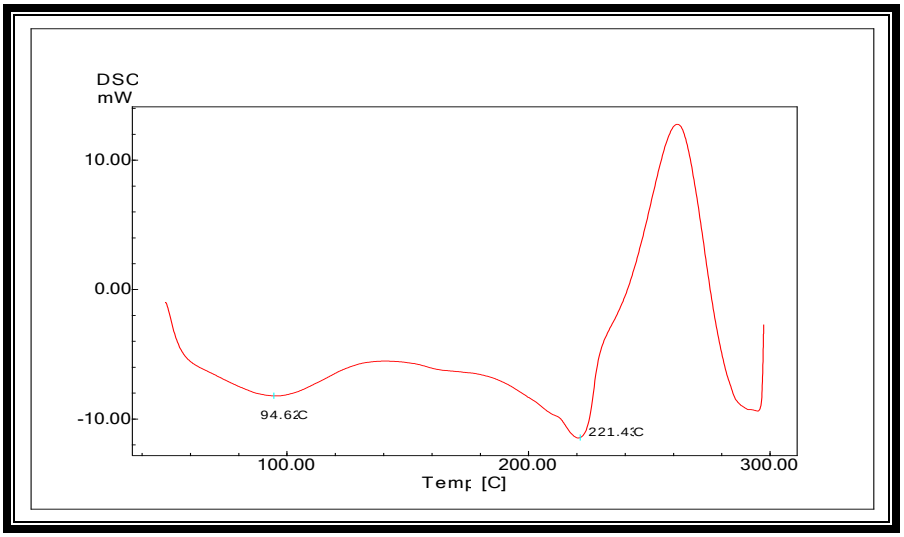

Figure (14): Differential scanning calorimetry (DSC) spectrum of physical mixture of PVP-K15:raw danazol

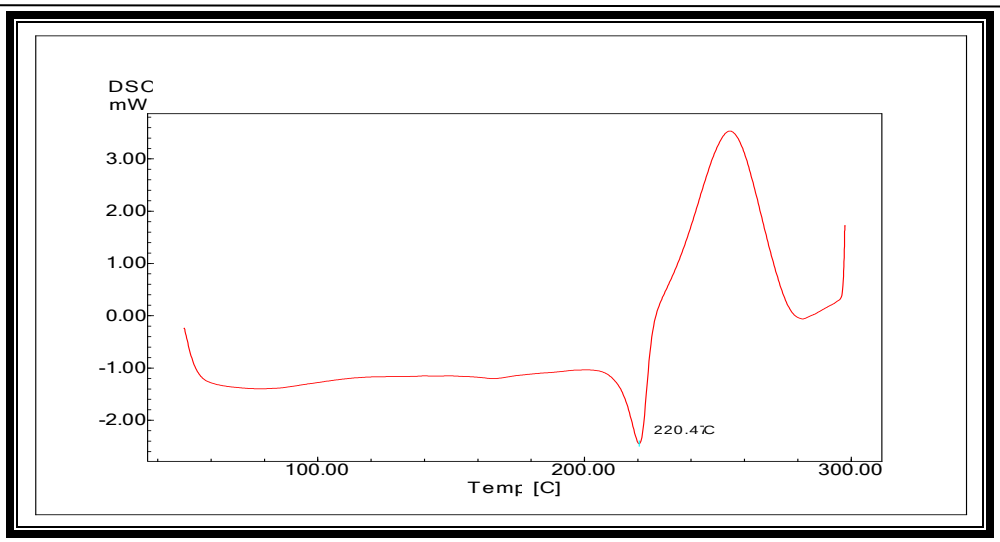

Figure (15): Differential scanning calorimetry (DSC) spectrum of danazol nanoparticles F12

***In-vitro* dissolution study of danazol nanoparticles from capsule dosage form**

The release profiles of danazol from capsules containing drug nanoparticles of F12, Physical mixture of PVP-K15: Danazol at ratio of 0.5:1 and the raw danazol were tested separately in each medium of 0.1N HCl of pH 1.2 with 2%w/v Brij-35 and phosphate buffer solution of pH 6.8 with 2% w/v Brij-35 as shown in figures (16) and (17); respectively.

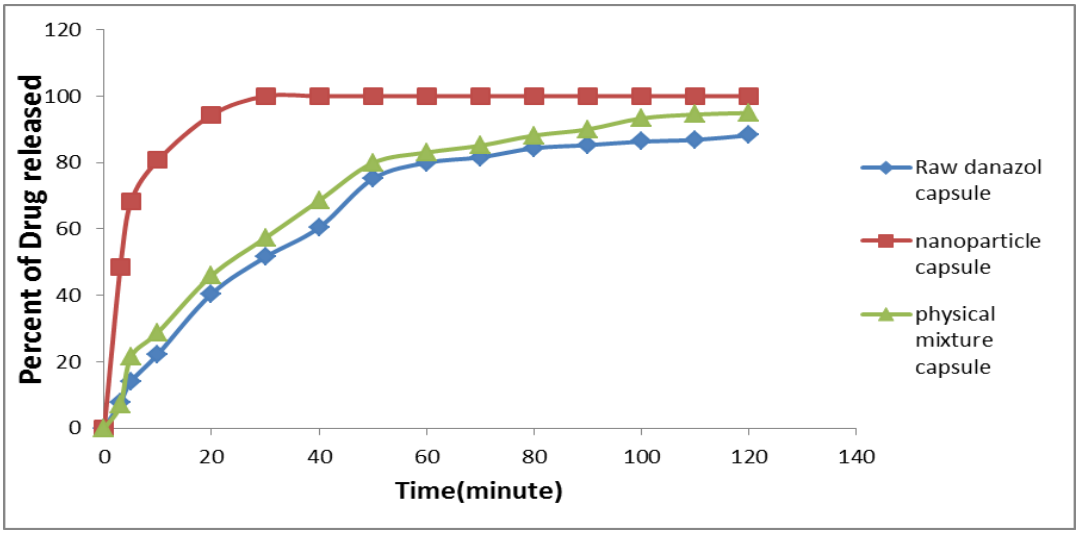

Figure (16): The *In-vitro* release of the raw danazol, F12, physical mixture of PVP-K15:raw danazol (0.5:1) in 0.1N HCl of pH 1.2 with 2% Brij-35 at 75 rpm and 37° C (n=3).

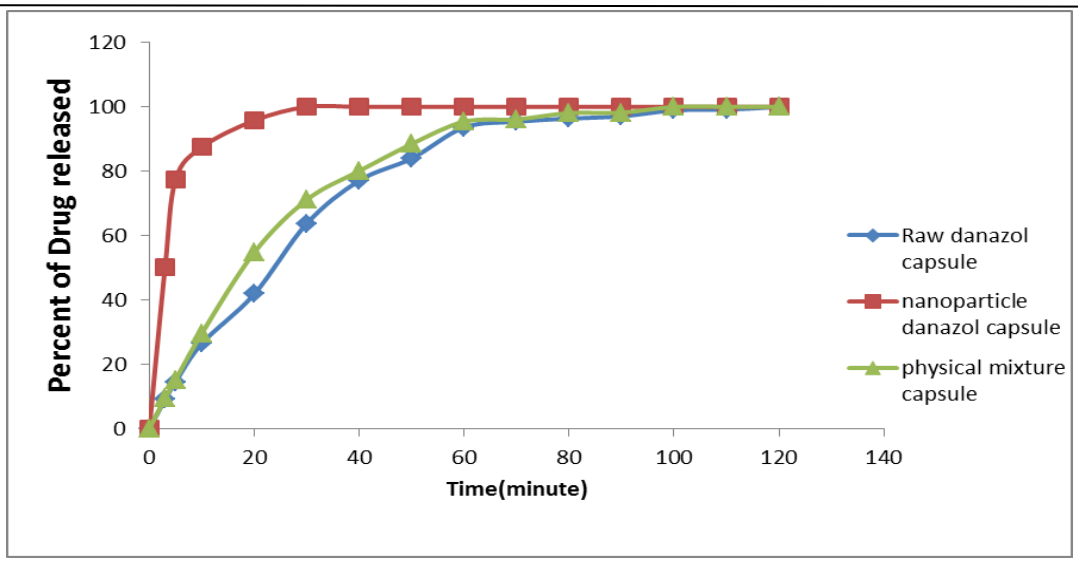

Figure (17): The *In-vitro* release of the raw danazol, F12, physical mixture of PVP-K15: raw danazol (0.5:1) in phosphate buffer of pH 6.8 with 2% Brij-35 at 75 rpm and 37° C (n=3).

Discussion

The loading efficiency of the drug in the prepared nanoparticle was found to be 91.2% ± 0.463, thus indicating minimal loss of drug during efficient nanoparticles processing with slight batch to batch variability. It appeared that the high yield and loading efficiency of the drug indicate that the technique applied in the construction of nanoparticles was appropriate and reliable, moreover these results are in agreement with that obtained by El-Gendy's group on preparation of budesonide nanoparticles [(32)]. The angle of repose for raw danazol was 38.6 ± 0.08º which indicated fair flow property. While for nanoparticles was 24.56 ±0.94º, that revealed that the selected formula had excellent flowability. So, it confirms efficient filling into capsules during the manufacturing procedure.

XRD indicated that bulk micronized danazol was in the crystalline form [(28)] as shown in Figure (5). The X-ray powder diffraction (XRD) patterns proved that the crystalline habit of lyophilized (F12) powder was not totally converted into an amorphous form. Figure (6) representing XRD diffractogram of lyophilized nanoparticle that showed less intensity of the diffraction peak when compared to that of the raw drug. This crystallinity reduction would lead to a greater surface disorder, as a result, give higher saturated solubility than crystalline matters and therefore enhanced dissolution rates [(33)]. The reduced peak intensity confirmed that the particle size was marked smaller after the precipitation process[(34)] On the other hand, the XRD patterns for the corresponding physical mixtures have been produced similar physical

characteristics to that of bulk danazol that the peaks of danazol crystals could be still detectable in mixture as the danazol content has been relatively more or PVP has little influence on physical state of drug, Figure (7) representing XRD diffractogram of physical mixture. Danazol is hydrophobic and has a high propensity to crystallize. Even if produced as amorphous particles, it usually crystallizes during storage **(35)** .To overcome recrystallization, large quantities of crystallization-inhibiting polymers must be added **(36)** . The lower polymer to drug ratio of 0.5:1 was selected as gave lowermost particle size while PVP in small quantity may be not sufficient enough to convert danazol from crystalline into an amorphous state completely and part of it converted into an amorphous form. All **(FT-IR)** data from (figure 8) indicate the purity of the drug. The results exposed as represented in figures (9-12). the presence of all main peaks of drug which indicates that there is no significant interaction between drug and polymer; So, we ruled out any incompatibility during the preparation of nanoparticles

From DSC profiles, changes in crystallinity were small, A decrease was seen in the melting temperature of danazol nanoparticles (figure 15) in comparison to the raw material. Although the melting point decreased by approximately 9°C (from 229.48 to 220.47°C) for danazol, it was assumed to be mostly influenced by the incorporation of polymer within nanocrystals to form nanoparticles. Consequently, it was believed that the crystalline nature of danazol nanoparticle would not influence oral absorption significantly [37]. The only reduction in crystalline nature of drug was detected but the drug was partially transferred to amorphous as already established from X-ray diffraction. In both dissolution media, the rate of release of danazol from a capsule containing F12 was the fastest one.

Within 30 minutes, the released from the F12 capsule was 100% in comparison with a raw capsule that released 51.53% in 0.1N HCl and 63.75% in phosphate buffer solution; While the physical mixture showed 57.3 % and 71% at the same time. In addition, 100% of danazol was released from a raw and physical mixture containing capsules after 120 and 100 min in phosphate buffer solution, whilst 140 min is required in 0.1N HCl from both of them. Also, it is noticed that there is a slightly higher release of drug from physical blend than that from the raw. This indicates that the solubilizing efficiency of PVP-K15.

A comparison between the dissolution profiles of danazol from different samples was made using ***f*1** and ***f*2**. According to the food and drug administration's guidelines, f1 values lower than 15 (0–15) and *f*2 values greater than 50 (50–100) mean similarity of the release profiles **(38)**.

From this analysis, it was observed that the release profiles of danazol from nanoparticles when compared with the raw drug were not similar, in addition, they were similar from a raw and physical mixture containing capsules. So that, we can conclude that the danazol nanoparticles showed significant advantage and better *in vitro* release profile than that the raw drug or physical blend with a stabilizer which might be due to a reduction in drug crystallite size, increase the wettability and enlargement of surface area. This suggested that the danazol capsules incorporating drug nanoparticles ensures a high prospective for oral dosage and can boost the bioavailability of the drug in which the dissolution activity limits bioavailability.

Conclusions

Based on outcomes attained from the study one can conclude that, the achievability of the antisolvent-precipitation method is an easy, efficient method to prepare danazol nanoparticles successfully using different types of stabilizers at polymer: drug ratios of 0.5:1, 1:1, 2:1 and 3:1. High concentration of drug in solvent solution leads to the maximum reduction in particle size resulted. All the studied polymers (PVP and HPMC) are suitable to stabilize danazol nanoparticles and prevent particles growth. The selected formula of F12, containing PVP-K15 as stabilizer showed faster *in vitro* dissolution rate than raw drug and its physical blend with stabilizer.

Future Work

A clinical study is crucial to evaluate the in vivo bioavailability of danazol capsules incorporating drug nanoparticles that show in *vitro–in vivo* correlation and to settle the effectiveness.

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