Effects of formulation variables on the Candesartan cilexitil nanoparticles properties using polyvinyl pyrroledone

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ABSTRACT

Candesartan celexitil (CC), a non-peptide angiotensin II type 1 (AT1) receptor antagonist, is used in the treatment of hypertension. It has low solubility with low bioavailability thus nanoparticles approach is one of the recently technique used to enhance the solubility of drugs. The aim of this study is to improve the solubility of CC by preparing nanoparticles. Seven formulas of nanoparticles were prepared by antisolvent precipitation method utilizing polyvinylpyrrolidone (PVP) as polymer. Three formulas were prepared at different drugs: polymer ratio and another three formulas were at different solvent: anti solvent ratios. The prepared nanoparticles were characterized regarding the particle size by nano laser particle size analyzer, saturated solubility, and thermal analysis by differential scanning calorimetry (DSC). The prepared nanoparticles reveal that all formulas produce nanoparticle in range of (50 – 706) nm. Formula (F2) which utilizes (PVP) as polymer at polymer: drug ratio of (1:1) and solvent: anti solvent ratio of (1:10) was considered as the best formula which shows good evaluation parameters in addition to increment in solubility (10.54) times than that of pure drug. The thermal analysis of nanoparticle of the selected formula (F2) shows reducing of intensity of endothermic peak of the drug indicating reduced crystallinity of candesartan celexitil. Finally, it could be concluded that the selected formula is promising for preparation of candesartan cilexetil nanoparticles with improved solubility.

تأثيرات تكوين متغيرات على صفات الكانديسارتان سليكسيتيل الناتو جزيئية باستخدام البولي فنيل بايروليدون

الخلاصة

كانديسارتان سيليكسيتيل هو عقار غير مثبط غير ببتيدي لمستقبلات الانجيوتنسينوجين الثاني من النوع الأول يستخدم في علاج ارتفاع ضغط الدم العقار له قابلية ذوبان منخفضة في الماء مع انخفاض التوافر البايولجي وبالتالي تقنية النانونوتكنولوجي هي واحدة من التقنيات في الآونة الأخيرة التي تستخدم لزيادة ذوبان الأدوية . والهدف من هذه الدراسة هو تحسين ذوبان الكانديسارتان سليكسيتيل من خلال إعداده على شكل جسيمات نانويه . وقد أعدت سبعة صيغ من الجسيمات النانوية بطريقة الترسيب بمضاد المذيب واستخدام بولي فنيل بايروليدون على صيغ مختلفة من نسب الدواء إلى البوليمر ونسبة المذيب إلى المضاد للمذيب . وقد تم تقييم الجسيمات النانوية المحضرة بشان حجم الجسيمات باستخدام جهاز النانو الليزري المحلل

للجسيمات , ودرجة الذوبان, والتحليل الحراري باستخدام. النتائج أظهرت أن كل الصيغ أنتجت جسيمات متناهية الصغر في
قيم من (709- 50) نانو متر.واعتبرت الصيغة (2F) فيها نسبة البوليمر إلى الدواء هو (1:1) ونسبة المذيب إلى مضاد المذيب
هي (1:10) باعتبارها أفضل صيغة مع مواصفات جيدة بالإضافة إلى زيادة في الذوبان (10.5) مرات مقارنة بالدواء
النقي.ويظهر التحليل الحراري للجسيمات المتناهية الصغر من الصيغة المختارة (2F) إلى قلة في شدة ذروة إشارة الدواء في
التحليل مشيرا إلى انخفاض التبلور في الدواء . وأخيرا يمكن القول إن الصيغة المختارة واعدة لإعداد الصيغة النانوية
الكانديسارتان سيليكسيتيل مع تحسن واضح بالذوبان .

1. INTRODUCTION

Candesartan celexitil (CC), a non-peptide angiotensin II type 1 (AT1) receptor
antagonist, is used in the treatment of hypertension. Candesartan cilexitil is a racemic
mixture containing one chiral center at the cyclohexycarbonyloxy ethyl ester group. It is
practically insoluble in water and sparingly soluble in methanol (1). Candesartan celexitil
is a prodrug that is hydrolyzed to candesartan during absorption from the gastrointestinal
tract (1) Candesartan cilexetil has log P value of 6.1 and the low aqueous solubility of CC
which may be the reason for very low bioavailability i.e. about 15%. (2). CC has poor
solubility in water and high permeability resulting in variable bioavailability and hence it
is belonging to class II of the biopharmaceutical classification system (3-5). Many new
chemical entities of very low solubility, oral bioavailability can be enhanced their
solubility by micronization but micronized product has the tendency of agglomeration,
which leads to decreased effective surface area for dissolution, thus recently the approach
was nanonization (6-8). There are different methods to prepare the nanoparticle such as
emulsification-solvent evaporation, high pressure emulsification and solvent evaporation,
salting out technique, solvent displacement nano precipitation, and supercritical fluid(9).
The aim of this study is to increase the solubility of poorly water-soluble Candesartan
Cilexetil by preparing nanoparticle by anti solvent nanoprecipitation.

2. MATERIALS AND METHODS

2.1 Materials

Candesartan Cilexetil was purchased from provizerpharma, India, polyvinyl pyrroledone
from HiMedia Laboratories, India. Tween20 was purchased from chemfine, chemicals-
Mumbai, India.

2.2 Methods

2.2.1 Determination of Candesartan Cilexetil Solubility

An equilibrium solubility determination for CC solubility was carried out using the shake
flask method (10-12) for different test media (water, HCL buffer pH 1.2 with 0.35%
polysorbate 20 and phosphate buffer solution pH 6.8 with 0.35% polysorbate 20. An
excess amount of the CC was added to 10 ml of medium in a test tube, and stirred in a
water bath with shaker at 37±2°C for 48 hours. Filtered samples were analyzed
spectrophotometrically for drug content at λmax of 255 nm.

2.2.2 Preparation of Candesartan Cilexetil Nanoparticles

CC nanoparticles were prepared by using solvent/antisolvent precipitation technique.
A certain amount of pure drug of CC was completely dissolved in 90% ethanol solvent.
The obtained drug solution was then injected at speed of 1ml/min using syringe infusion
pump into the water containing the stabilizer (PVP) of different percentages (0.5, 1 and

2%) with continuous stirring. Precipitation of solid drug particle occurred immediately upon mixing. The precipitated nanoparticles were sonicated at 37 °C for 30 min. and then lyophilized to obtain the nanoparticles powder (13). Different formulation variables affecting the properties of prepared nanoparticles were studied by utilizing the prepared seven formulas of composition shown in table

Table 1: Composition of CC nanoparticles formulas

Formula No.	Polymer	Solvent: anti solvent ratio Ethanol: Water	Polymer: drug ratio
F1	PVP	1:10	0.5:1
F2	PVP	1:10	1:1
F3	PVP	1:10	2:1
F4	PVP	1:10	1:0.5
F5	PVP	1:10	1:2
F6	PVP	1:05	1:1
F7	PVP	1:15	1:1

2.3.3 Characterization of the prepared nanoparticles

1- **Determination of CC content in nanoparticles**
To determine the drug content of the prepared nanoparticles, 200mg sample of each prepared formula was placed in a glass mortar and thoroughly triturated using methanol. After thoroughly rinsing all equipment, the total mixture was transferred to volumetric flask and the volume was completed to 100 ml with methanol. The resultant dispersion was sonicated for 15 min to ensure complete dissolution of CC. The mixture was filtered through a Whatman filter paper of 45µm, and the amount of the CC depending on absorbance of CC was determined spectrophotometrically (14).

2- Particle size analysis
Samples of all prepared nanoparticles were analyzed by using ABT-9000 nano laser particle size analyzer, and particle size distribution curves were obtained. The average particle size, polydispersity index (PDI), and the specific surface area (SSA) for each sample were recorded.

3- Determination of nanoparticles saturation solubility:
The solubility of the selected formula of CC nanoparticles was determined according to the same procedure which prescribed in 2.3.1

4- **Differential scanning calorimetry (DSC)**

Thermal analysis of the pure drug, polymer, and selected formula were determined by an automatic thermal analyzer system (Shimadzu DSC–60, Japan). Accurately weighed sample (5mg) were placed in none hermetically aluminum pans and heated at the rate of 20 °C/minute against an empty aluminum pan as a reference covering a temperature range of 50 °C to 300 °C

3. RESULTS AND DISCUSSION

3.1 Evaluation of particle size of the prepared nanoparticles:

The particle size of all the prepared nanoparticles of CC are listed in table 2 indicating that the lowest value was of formula (F2) equals (50) nm while the highest value of formula(F4) equals (706) nm. Although it seems that the ratio of drug to the polymer in(F2) and(F4) is same but the nanoparticle size in (F2) is less than in(F4). This is related to the concentration of polymer which in (F2) is more than F4.

Table 2. Particle size range of the prepared nanoparticles

Formulas No.	Particle size range nm	Particle size average nm
F1	62.9 - 79.2	71.05
F2	50 - 56.1	53.05
F3	70.6 - 79.2	74.9
F4	629 – 706	667.5
F5	500 – 561	530.5
F6	111 – 140	125.5
F7	250 -315	282.5

3.2 Formulation variables affecting the properties of prepared nanoparticles

The results shown in figure (1, 2, and 3) of nanoparticle of the seven formulas using (PVP) indicate that changing polymer concentration has an impact on CC nanoparticles mean size. Increasing polymer concentration lead to increase in mean particle size but observed only higher than drug: polymer equal ratio. This can be explained that increasing polymer concentration caused more coating of drug particles until a certain concentration where all drug particles are coated with polymer, then increasing polymer concentration would lead to increase the thickness of the polymer coat around each particle or may lead to aggregation of many particles and increase in the mean particle size. It has been shown that the solvent: antisolvent ratio 1:10 was the best ratio among the other which gave the lowest mean particle size.

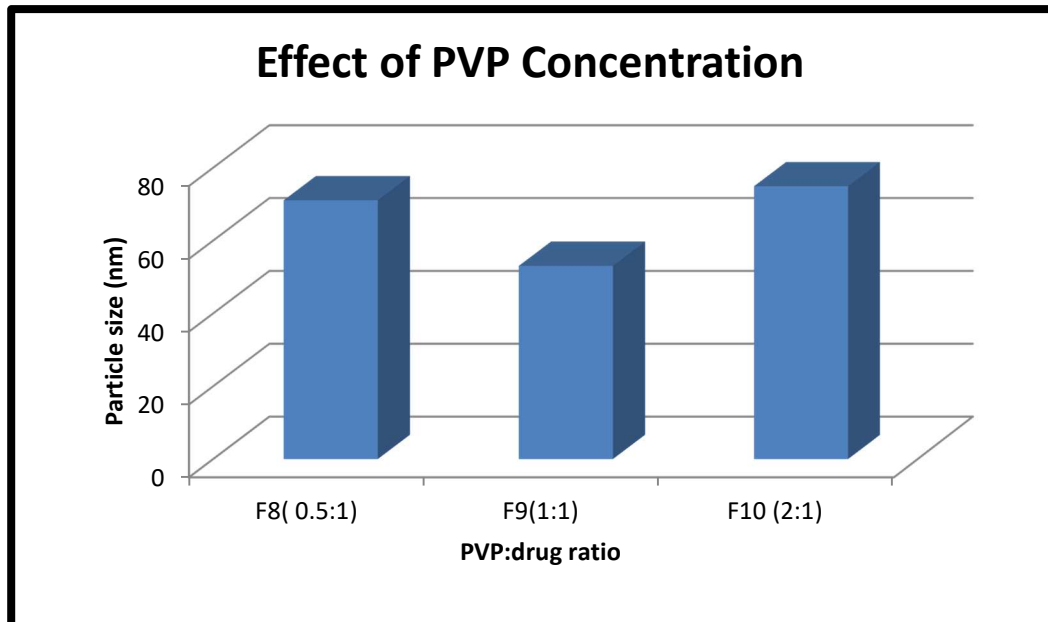**Figure 1. Effect of PVP concentration on nanoparticle size**

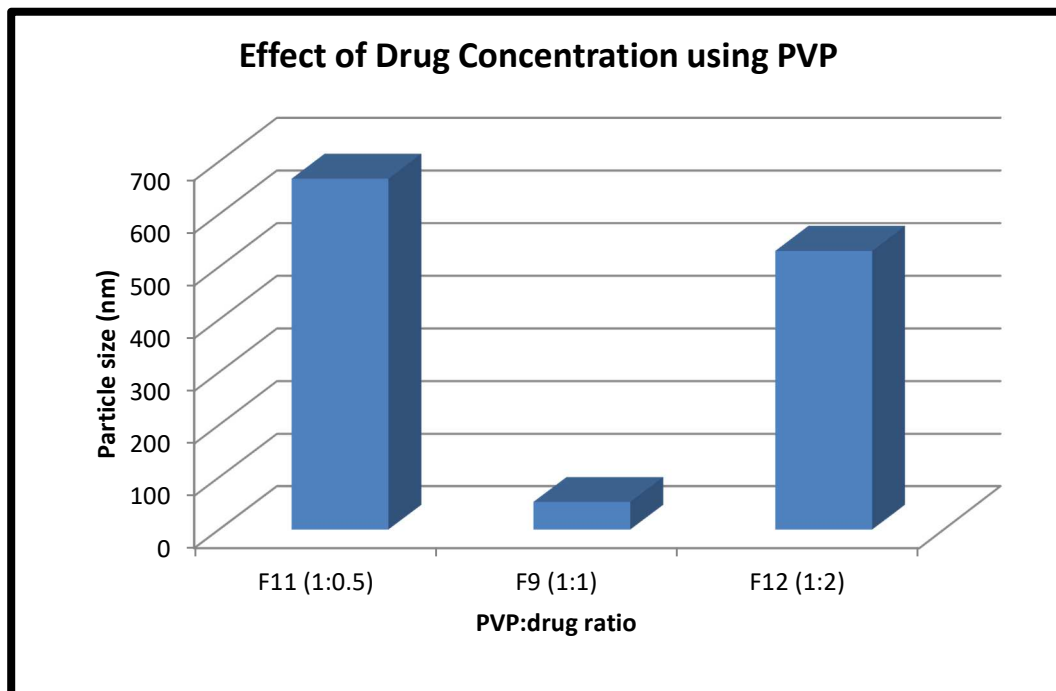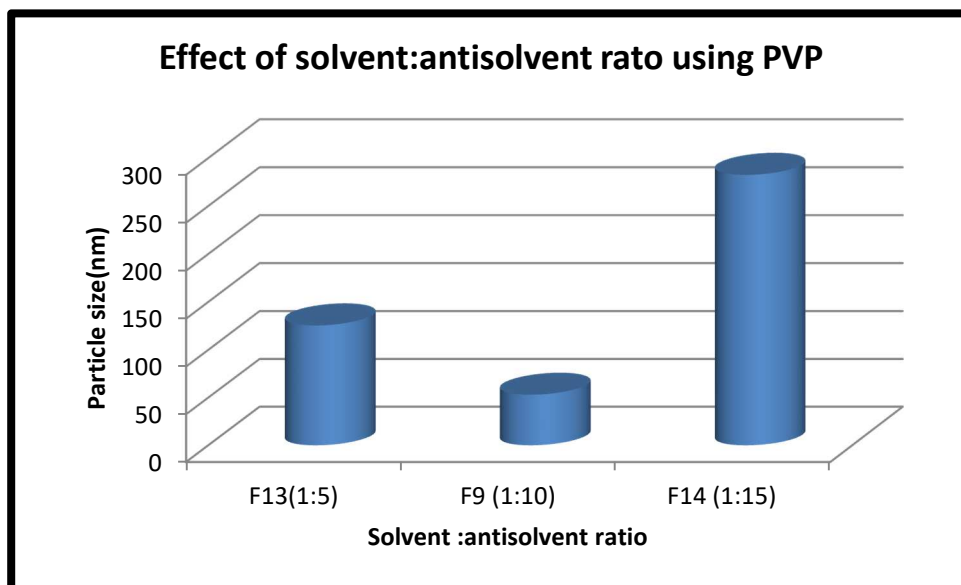**Figure 2. Effect of drug concentration using PVP on nanoparticles size**

Figure 3. Effect of solvent: anti solvent ratio using PVP on nanoparticle size

3.3 Saturated solubility study of the prepared nanoparticles

Solubility of CC nanoparticles of the selected formula in different solvents was determined as shown in table 3. CC nanoparticles saturation solubility increased of the selected formula (F2) with higher increment in buffer pH 6.8 in comparison to buffer pH 1.2 and this attributed to pH dependent property of drug which confirmed by Shilpa Bhilegaonkar *et al* (15). The saturation solubilities of CC nanoparticles of the selected formulas in water were increased 10.54 folds relative to pure drug for selected formula (F2). The increase in saturation solubility is mainly due to nanonization effect. Such results also reported by Hecq *et al*, where they prepared nifedipine nanocrystals and found that nanonization lead to increase saturation solubility in water from 19.5± 0.1 µg/ml to 25.9±1.4 µg/ml (16).

Table 3. Solubility data of the selected formulas in different media

Solvent	Solubility of selected formulas (µg/ml)		
	Pure Drug	F2	
Water with 0.35% Tween 20	1.2	16.34	
HCL Buffer pH 1.2 with 0.35% Tween 20	5.7	19.32	

Phosphate Buffer pH 6.8 with 0.35% Tween 20	112.5	8423.62	

3.4 Differential scanning calorimetry (DSC)

DSC thermograms of Candesartan Cilexetil in figure (4) shows sharp endothermic peak at 172° corresponding to its melting point indicates pure crystalline state of drug (17). Although the thermograms of nanoparticles of the selected formula (F2) as shown in figure (6) reveal a reduce in the intensity of endothermic peak of drug in comparison to that of pure drug and with relation to the thermograms of pure polymer PXM?? PVP which shown in figure (5), but it is available relatively at same temperature and this may be due reducing in percent of crystallinity of drug amorphization during preparation which may participate in solubility enhancement results. These results is in agreement with research by Cheow *et al* which study the amorphization of drug nanoparticles (18).
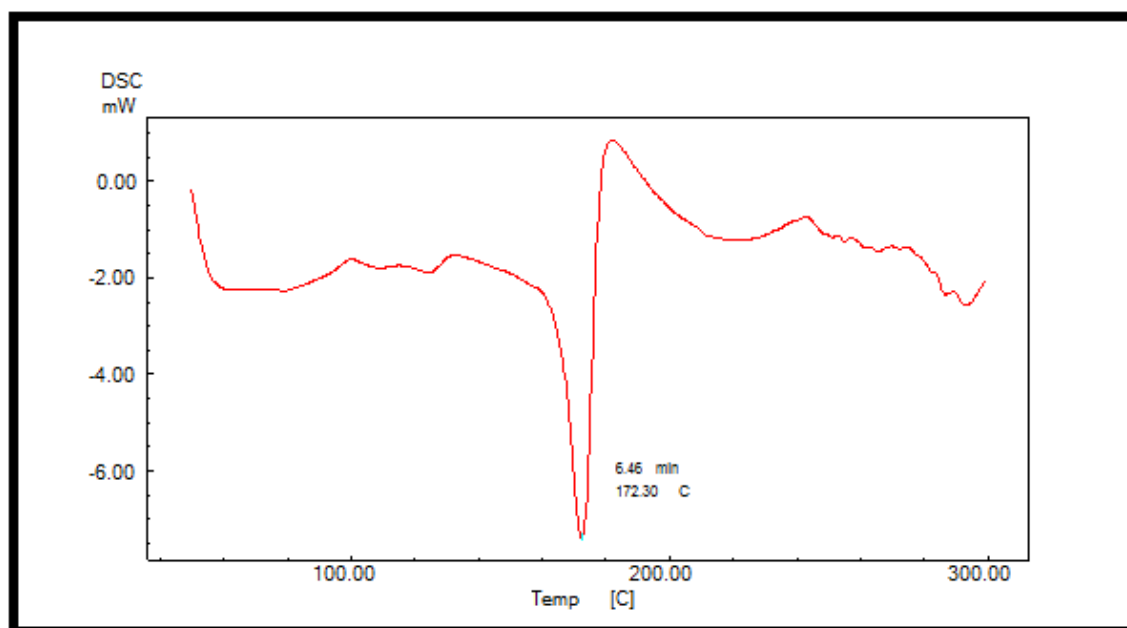
Figure 4. DSC thermo gram of pure candesartan cilexitil

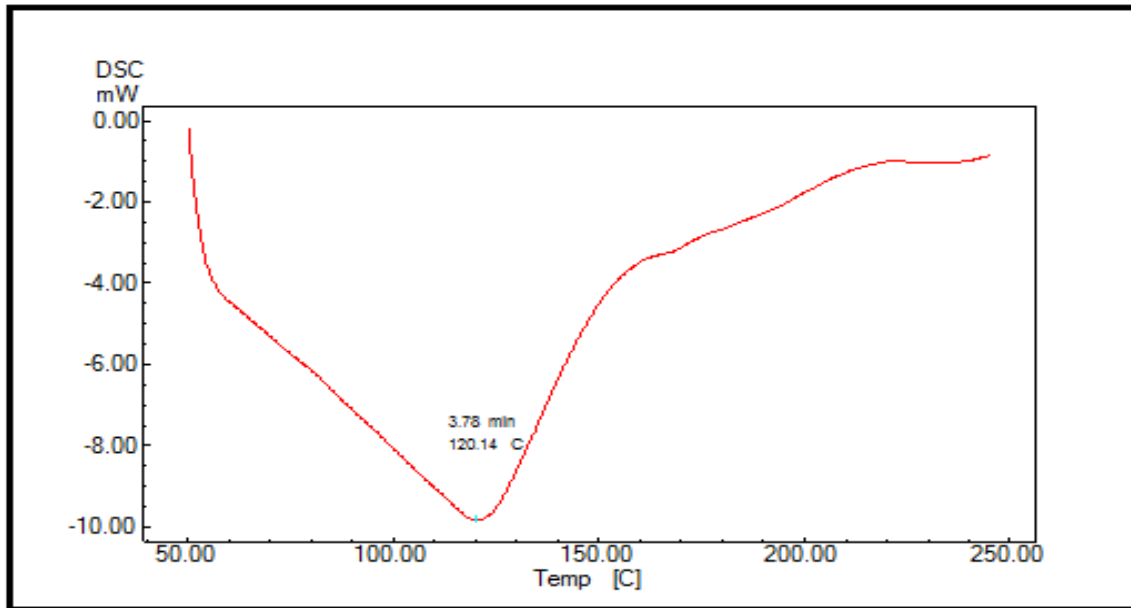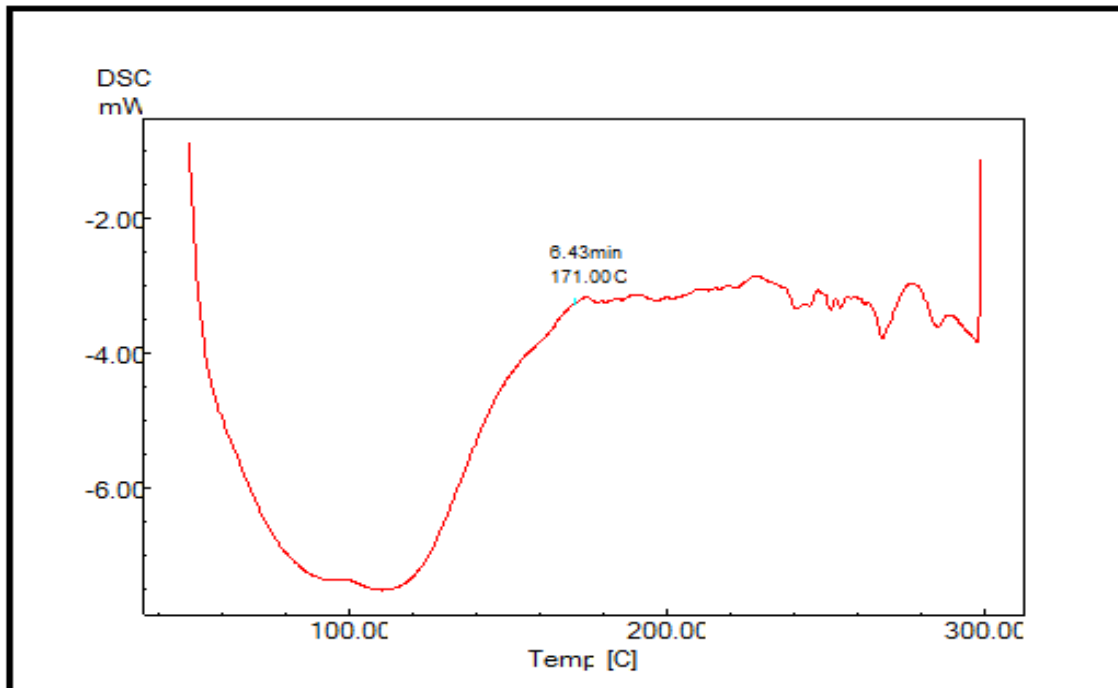**Figure 5. DSC thermograms of pure PVP**

Figure 6. DSC thermogram of F2 (PVP) nanoparticles

4. CONCLUSION:

Among the prepared formulas of nanoparticles, the formula prepared using drug: polymer and solvent: anti solvent ratios of 1:1 and 1:10 respectively was selected as the optimum formula (F2) that produces the smallest nanoparticle size. In addition, the optimum formula nanoparticles show increment in saturated solubility about 10.54 folds that of pure drug, thus it can be consider promising formula for enhancement of solubility of candesartan cilexitil.

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