Effects of formulation variables on the Candesartan cilexitil nanoparticles properties using poloxamer 188

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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 poloxamer 188 as polymer. Three of them at different drugs: polymer ratio and another three 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 DSC. The prepared nanoparticle reveals that all formulas produce nanoparticle in range of 70.6 - 1119 nm. Formula (F2) which utilizes PXM as polymer at polymer: drug ratio of (1:1) and solvent: antisolvent 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 cilexetil. 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 الكانديسارتن سلكستيل النانو جزئية باستخدام بلوكزامر 188

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الكلمات المفتاحية: كانديسارتان سلكستيل, نانو بارتكل, الذوبان, بولوكزامر 188 و التحلل

الخلاصة

كانديسارتان سيليكستيل (CC) هو عقار مثبط غير ببتيدي لمستقبلات الانجيوتنسين الثاني من النوع الاول. يستخدم في علاج ارتفاع ضغط الدم . العقار له قابلية ذوبان منخفضة في الماء مع انخفاض التوافر البيولوجي وبالتالي تقنية النانوتكنلوجي هي واحد من التقنيات في الآونة الأخيرة التي تستخدم لزيادة ذوبان الادوية. والهدف من هذه الدراسة هو تحسين ذوبان CC من خلال إعداده على شكل جسيمات نانويه. وقد أعدت سبع صيغ من الجسيمات النانوية بطريقة الترسيب بمضاد المذيب واستخدام بولوكسامير 188 على هيئة صيغ مختلفة كل من نسب (الدواء الى البوليمر) ونسبة المذيب الى المضاد للمذيب. وقد تم تقييم الجسيمات النانوية المحضرة بشأن حجم الجسيمات باستخدام جهاز نانو اليزري المحلل الجسيمات ، ودرجة الذوبان ، و التحليل الحراري باستخدام DSC . النتائج اظهرت ان كل الصيغ انتجت جسيمات متناهية الصغر في قيم من 70,6-1119 نانومتر. واعتبرت صيغة (F2) فيها نسبة البوليمر الى الدواء هو (1: 1) ونسبة مذيب الى المضاد للمذيب (1:10) باعتبارها أفضل صيغة مع مواصفات جيدة بالإضافة إلى زيادة في الذوبان 10.54 مرات مقارنة بالدواء النقي . ويظهر التحليل الحراري للجسيمات متناهية الصغر من الصيغة المختارة (F2) الى قلة في شدة ذروة اشارة الدواء في التحليل مشيرا إلى انخفاض التبلور في الدواء . وأخيرا يمكن القول أن الصيغة المختارة واعدة لإعداد الصيغة النانوية للكانديسارتان سيليكستيل 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 cilexetilis 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 cilexetil 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 antisolvent nanoprecipitation.

2. MATERIALS AND METHODS

2.1 Materials

Candesartan Cilexetil was purchased from provizerpharma, India, Poloxamer 188 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 Candesartan Cilexetil solubility was carried out using the shake flask method (10-12) for different test media (water, 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 drug 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 Candesartan Cilexetil 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 (Poloxamer 188) of different concentrations (0.5, 1 and 2%) with continuous stirring. Precipitation of solid drug particles occurred immediately upon mixing. The precipitated nanoparticles is sonicated at 37 °C for 30 min. and then lyophilized to obtain the nanoparticales powder (13). Different formulation variables affecting the properties of prepared nanoparticles were studied by utilizing the prepared seven formulas of composition shown in table 1.

Table 1: Composition of CC nanoparticles formulas

Formula No.	Polymer	Solvent : antisolvent ratio	Polymer : drug ratio
F1	PXM	1:10	0.5:1
F2	PXM	1:10	1:1
F3	PXM	1:10	2:1
F4	PXM	1:10	1:0.5
F5	PXM	1:10	1:2
F6	PXM	1:5	1:1
F7	PXM	1:15	1:1

2.3.3 Characterization of the prepared nanoparticles

- *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 Candesartan Cilexetil.The mixture was filtered through an ordinary filter paper, and the absorbance of Candesartan Cilexetil was determined spectrophotometrically. The amount of drug inside the nanoparticles was determined (14).

- Particle Size analysis

Samples of all prepared nanoparticales 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.

- Determination of nanoparticles saturation solubility:

The solubility of the selected formula of Candesartan Cilexetil nanoparticles was determined according to the same procedure which prescribed in 2.3.1

- 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 aluminium pans and heated at the rate of 20 ˚C/minute against an empty aluminium 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 candesartan cilexetil are listed in table 2 indicating that the lowest value was of formula F2 equals 70.6 nm while the highest

value of formula F4 equals 1119 nm. Although it seems that the ratio of drug to the polymer in F3 and F4 is same but the nanoparticle size in F3 is less than in F4. This is related to the concentration of polymer which in F3 is more than F4.

Table 2. Particle size range of the prepared nanoparticles

Formula No.	Particle size range	Particle size average
F1	99.7 -125	112.35
F2	70.6 – 79.2	74.9
F3	792 - 997	894.5
F4	997 - 1119	1058
F5	561 - 706	633.5
F6	627 - 997	812
F7	561 - 889	725

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 PXM 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.

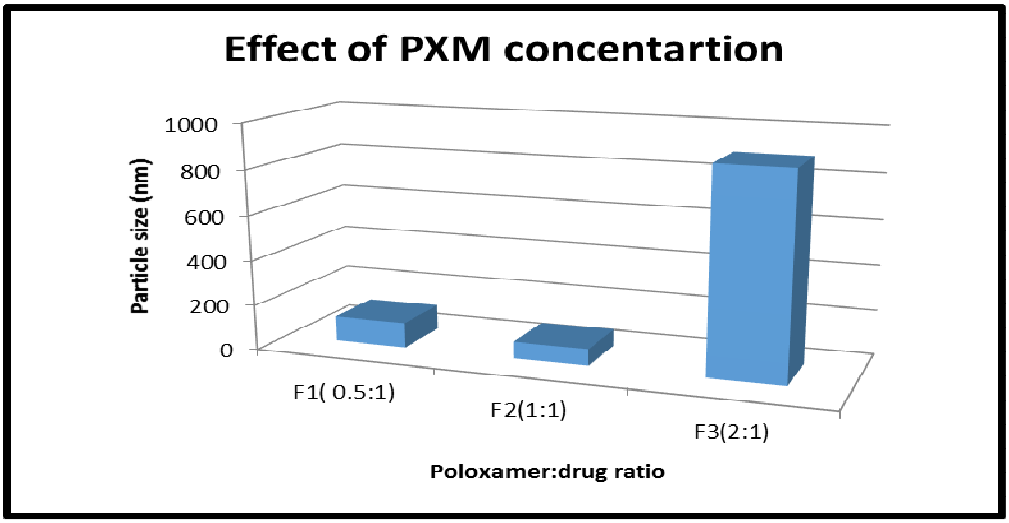

Figure 1. Effect of poloxamer concentration on nanoparticle size

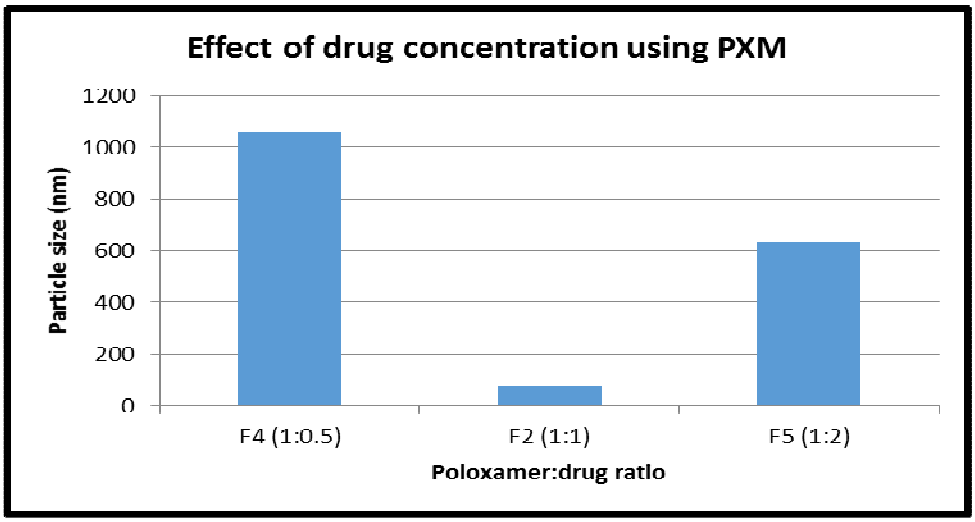

Figure 2. Effect of drug concentration using PXM on nanoparticles size

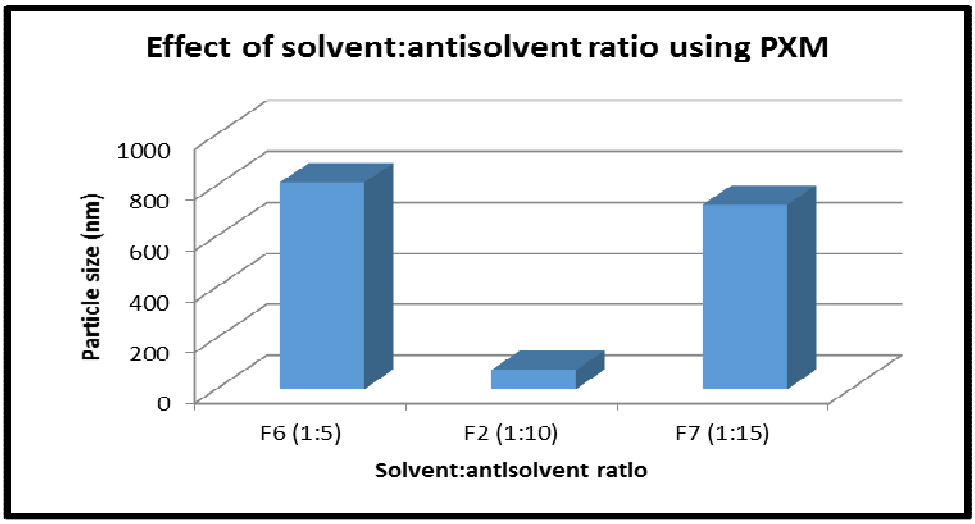

Figure 3. Effect of solvent: antisolvent ratio using PXM on nanoparticle size

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 Candesartan Cilexetil nanoparticles of the selected formulas in water were increased 10.54folds 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 nanocrystales and found that nanonization lead to increase saturation solubility in water from 19.5± 0.1 µg / ml to 25.9±1.4µg/ml (16).

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

Solvent	Saturated solubility (µg/ml)	
	Pure drug	Selected formula F2
Water	1.2	12.65
Buffer pH 1.2 with 0.35% Tween 20	5.7	15.41
Buffer pH 6.8 with 0.35% Tween 20	112.5	6583.47

3.4 Differntial scanning calorimetry(DSC)

DSC thermogram 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 which shown in figure (5), but it is available relatively at same temperature and this may be due reducing in percent of crystallintity 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).

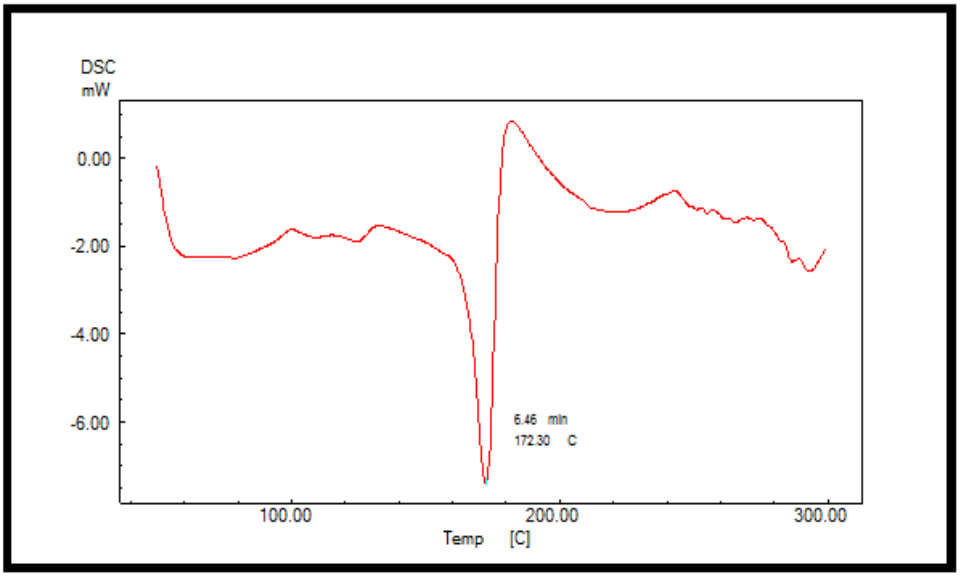

Figure 4. DSC thermogram of pure candesartan cilextil

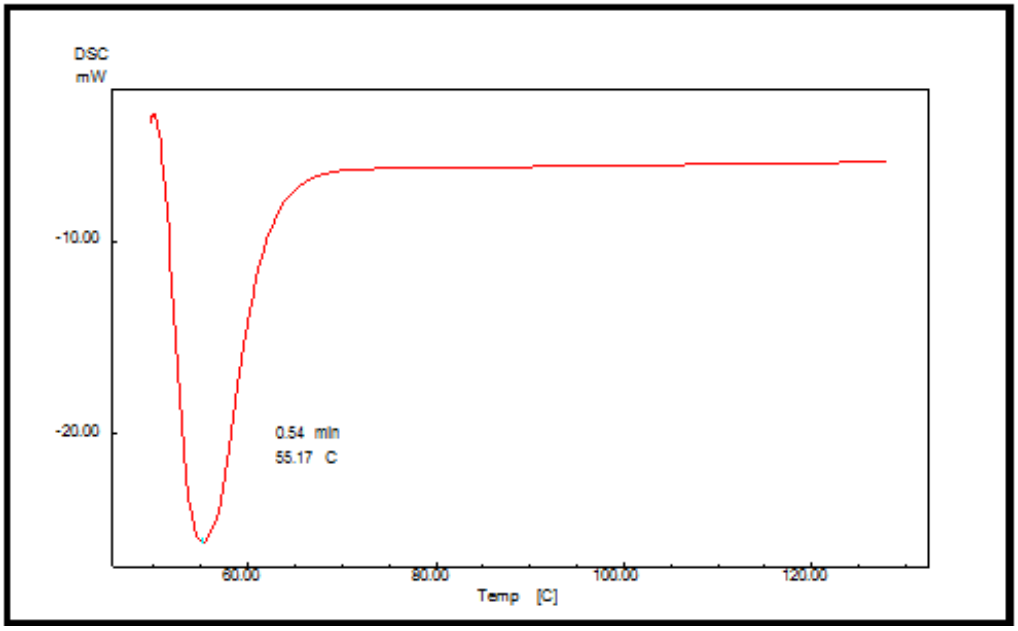

Figure 5. DSC thermogram of pure Poloxamer

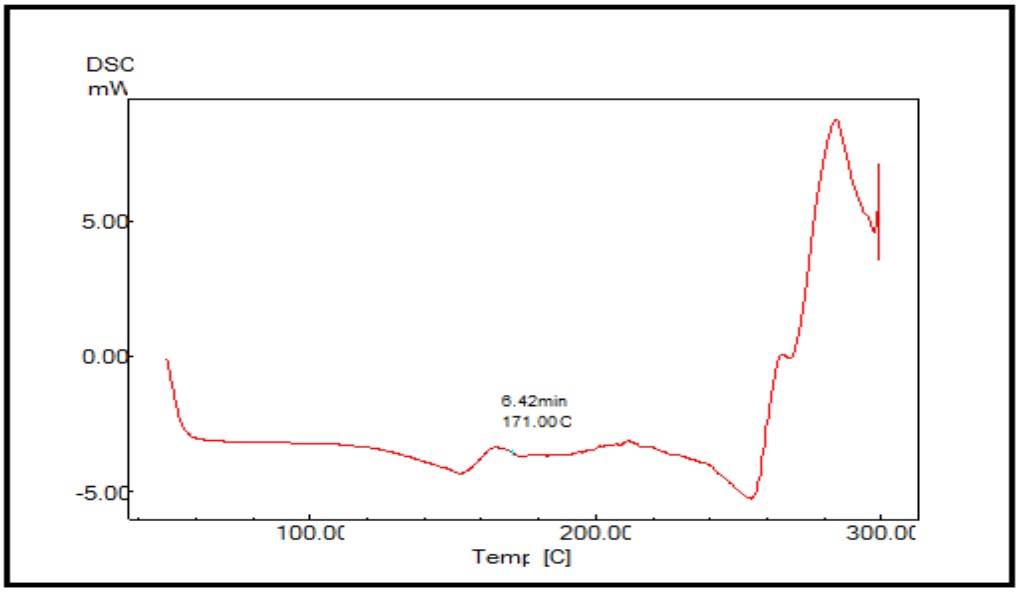

Figure 6. DSC thermogram of F2 (poloxamer) nanoparticles

4. CONCLUSIONS

Among the prepared formulas of nanoparticles, the formula prepared using drug: polymer and solvent: antisolvent 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 shows increment in saturated solubility about 10.54folds that of pure drug, thus it can be consider promising formula for enhancement of solubility of candesartan cilexitil.

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