RESEARCH ARTICLE

SCREENING STUDY FOR FORMULATION VARIABLES IN PREPARATION AND CHARACTERIZATION OF CANDESARTAN CILEXETIL LOADED NANOSTRUCTURED LIPID CARRIERS

Walid Anwar[*] , **Hamdy M. Dawaba** , **Mohsen I. Afouna** , **Ahmed M. Samy**
Pharmaceutics Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.

Article Info:

Cite this article:
Anwar W, Dawaba HM, Afouna MI, Samy AM. Screening study for formulation variables in preparation and characterization of candesartan cilexetil loaded nanostructured lipid carriers. Universal Journal of Pharmaceutical Research 2019; 4(6):8-19.
https://doi.org/10.22270/ujpr.v4i6.330

*Address for Correspondence:
Walid Anwar, Pharmaceutics Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.Tel: 002 010 66429953,E-mail: *wanwar8182@gmail.com*

Abstract

Objective: The current study inspects the screening of the formulation components further, evaluates the physicochemical properties of the nanostructured lipid carriers (NLCs) for the antihypertensive drug as Candesartan Cilexetil (CC). The sequence screening of all excipients required for the preparation of NLCs should be performed.

Methods: The prepared formulations were investigated for the different quality issues. The screening studies were performed to select the appropriate one of solid lipid, liquid lipid and surfactant. Also, investigation of physical compatibilities of solid lipid with liquid lipid and the ratios of them were evaluated. Furthermore, the physical characterization and quality issues of developed formulations were described and determined. Firstly, the solubility of CC in different solid and liquid lipids is the major parameter for the selection of the best one.

Results: Precirol® ATO 5, Compritol ® 888 ATO and Glyceryl Monostearate (GMS) were showed the maximum solubility of the CC (1000 ± 4.12 mg, 1500 ± 4.15 mg and 1750 ± 3.16 mg), respectively. Hence, they were selected as the solid lipids for the development of NLCs. Liquid lipids Transcutol® HP (30 ± 2.21 mg/ml), Labrasol® ALF (25 ± 1.32 mg/ml) and Capryol™ 90 (18 ± 1.34 mg/ml) were observed to have good affinity for the drug on systematic screening of different liquid lipids. All designed formulations observed in nanometer size of particles ranged from (408.9 ± 11.5 to 114.6 ± 8.3 nm) with high encapsulation efficiency around 99%. Also, the obtained results revealed that the ZP of the various formulations was consistently negative surface charge in between (-13 ± 2.3 to 27.3 ± 3.7 mV).

Conclusion: Finally, formula number nine of CC (CC-NLC9) which composed of GMS (solid lipid), Capryol™ 90 (liquid lipid) and Lutrol® F127: Cremophore® RH (surfactants combination) was selected as the best formulation after the rank order for further investigations in the next work. The current work clarifies a sequence steps for selection of excipients for NLCs by employing simple experiments.

Keywords: Candesartan Cilexetil, encapsulation efficiency, *in-vitro* release, nanostructured lipid carriers, solid lipid.

INTRODUCTION

Candesartan Cilexetil (CC) is prodrug of candesartan, angiotensin II type 1 (AT1) receptor antagonist, widely used in the management of hypertension and heart failure[1]. Candesartan Cilexetil is radially hydrolyzed to active form Candesartan during absorption from gastro intestinal tract[2]. Candesartan Cilexetil own great drawbacks which influence on its oral efficacy and therapeutic application such as very low aqueous solubility and first pass metabolism. Consequently, it has very low oral bioavailability not exceed 15%[3].

To repair previously mentioned drawbacks and to enhance oral bioavailability, lipid–based drug delivery systems like nanostructured lipid carrier (NLCs) second type of lipid nanoparticles system can be employed. Lipid nanoparticles systems (LNs) which have to generation's first, solid lipid nanoparticles (SLN) and second, nanostructured lipid carrier (NLCs) can improve the lymphatic transport of the lipophilic drugs as CC and hence, increase its oral bioavailability[4]. LNs systems were recorded as an advanced drug carrier system than polymeric nanoparticles[5,6]. Advantages of nanostructured lipid carriers (NLCs) over the advantages of polymeric

nanoparticles because of the lipid component matrix and its properties, which is physiologically tolerated. Resulted in avoidance of acute and chronic toxicity. In addition to, as good biocompatibility, protection for the incorporated compound against degradation and controlled release of drugs[7]. Nanostructured lipid carriers (NLCs) composed of both solid and liquid lipids in certain proportion. Therefore, they offer various advantages over solid lipid nanoparticles (SLN) such as higher encapsulation efficiency, smaller size and low polymorphic changes[8]. Generally, nanostructure lipid carriers (NLCs) are nano-drug delivery carrier, which own the advantages of polymeric nanoparticles, emulsion, and liposomes. Furthermore, (NLCs) are essentially composed of a biocompatible lipid core with entrapped lipophilic drugs and surfactant at the outer shell. The major aim of this workwas to select a proper excipient for the development of NLCs using Candesartan Cilexetil (lipophilic anti-hypertensive agent) as a model drug. The screening studies were performed to select the appropriate one of solid lipid, liquid lipid and surfactant. Also, investigation of physical compatibilities of solid lipid with liquid lipid and the ratios of them were evaluated. Furthermore, the physical characterization and quality issues of developed formulations were described and determined. Therefore, this study can offer the sequence steps for the development of NLCs and evaluation of their quality characteristics.

MATERIALS AND METHODS

The active Candesartan Cilexetil (CC) was obtained as a gift from MEMPHIS, El-Amirya–Cairo –EGYPT. Compritol® 888 ATO (glyceryl dibehenate), Precirol® ATO 5 (Glyceroldistearate type I), Maisine® CC (glyceryl monolinoleate), Labrafac™ PG (propylene glycol dicaprylate/dicaprate), Labrafac™ CC (caprylic/ caprictri glycerides), Labrafac™ Lipophile WL 1349 (medium-chain triglycerides), Cpryol® 90 (propylene glycol monocaprylate type II), Lauroglycol® FCC (propylene glycol monolaurate type I), Labrasol® ALF (caprylocaproyl macrogol-8-glycerides), Gelucire® 44/14 (lauroyl macrogol-32 glycerides), Gelucire® 43/01 (mixtures of mono, di and triglycerides with PEG esters of fatty acids), Gelucire® 39/01 pellets (Glycerol esters of saturated C12-C18 fattyacid ester), Transcutol® HP (Highly purified diethylene glycol monoethylether), Labrafil® M 1944 CS (oleoyl macrogol-6 glycerides), Labrafil® M 2125 CS (linoleoyl macrogol-6-glycerides, corn oil PEG-6-ester), Peceol™ (glyceryl monooleate), and Labrafil® M 2130 CS (lauroyl macrogol-6-glycerides) were kindly provided as a gift samples from Gattefosse (France). Glyceryl monostearate (GMS), Stearic acid, Oleic acid, Soybean oil, Pluronic® F68 (polyoxy ethylene-polyoxypropylene (150: 29) block copoly - mer), Pluronic® F127, Tween® 80 (polyoxy ethylene (20) sorbitan monooleate), Tween®40 (Polyoxy ethylene sorbitan mono palmitate), Carboxy methyl cellulose (CMC). Membrane filter (0.45 μm) Millipore Iberica S.A.U.; Madrid (Spain). Methanol and

Acetonitrile HPLC grade were purchased from Sigma Aldrich (USA).
All the above materials were in analytical grade and were used without further purification.

Selection of solid lipid
The solid lipids screening was carried out by quantification of the saturation solubility of CC in different solid lipids which were determined by the test tube method. Precisely weighted amount of the CC (100 mg) putted in the test tube then the solid lipid was added in increments of (250 mg) to the test tube which could be heated to 4-5°C above the melting point of the solid lipid by saving in a controlled temperature water bath (Water path 4050, Romo, Cairo, Egypt). The quantity of solid lipids required to solubilize the drug in the molten state was recorded. The full dissolution state was completed by the formation of a clear, transparent solution[9].

Selection of liquid lipid
Screening of liquid lipids were achieved by determination of saturation solubility of CC in various oils which was performed by adding an excess amount of drug in small glass vials contain fixed volume (5 ml) of different liquid lipids. The vials were strictly closed and incubated in adjusted mechanical shaker (Oscillating thermostatically controlled shaker, Gallent Kamp, England) for 72 h at 37ºC with continuous agitation at 100 rpm[10]. Then the mixtures of liquid lipids and CC were centrifuged at high speed using (Biofuge Primo centrifuge maximum 17.000 rpm, England) centrifuge at 10,000 rpm for 15 min. The supernatant was separated and dissolved in an appropriate amount of methanol and the drug solubility was determined spectrophotometrically using UV-Vis spectrophotometer (Ultraviolet spectrophotometer, Shimadzu 1800, Japan) at λ_{max} 254 nm.

Physical compatibility of solid and liquid lipid
The miscibility of Selected Solid lipids and liquid lipids which possess the maximum affinity for the drug could be achieved. Constant ratio 1:1 of solid lipids and liquid lipids were mixed and melted in different glass tubes. The molten binary lipid mixture was permitted to solidify at room temperature. After that, the glass tubes were determined visually for the absence of divided layers in congealed lipid mass. Furthermore, the miscibility between solid lipid and liquid lipid was inspected by smearing a cooled sample of congealed lipid mixture onto a filter paper, followed by visual observation to clear the presence of any residue of oil on the filter paper. A binary mixture distinguished a melting point over 43ºC which did not reveal any residue of oil droplets on the filter paper was selected for the development of CC–loaded NLCs[4].

Selection of a binary lipid phase ratios
The ratio of selected solid lipids and liquid lipids was determined based on the melting point of the binary lipid mixture. Selected solid and liquid lipids were blended in the ratio varying from 90:10 to 10:90, then the binary lipid mixtures were exhibited to be melted and stirred at 200 rpm for 1 h at 5°C above the melting point of solid lipid using hot plate magnetic stirrer (Magnetic stirrer, Wise-stir, Model MSH-20D, Hot

plate stirrer, Korea) then kept aside to solidify at room temperature. The capillary method was used to determine the melting points of the congealed lipid mixtures[11].

Selection of surfactant

The surfactant used for fabrication of NLCs should be screened selected depending on its ability to emulsify solid-liquid binary lipid mixture, binary lipid mixture (100 mg) was dissolved in 3 ml of methylene chloride and added to 10 mL of 5% surfactant solutions then stirred by applying magnetic stirrer. The organic layer was evaporated at 40°C and the remaining suspensions were diluted with 10-fold distilled water. The transmittance percent of the resultant samples was determined using UV-Vis spectrophotometer at 510 nm[12].

Fabrication of nanostructured lipid carriers (NLCs)

CC nanostructured lipid carriers (CC-NLC) were prepared by hot homogenization–ultrasonication technique but with some few modifications. Briefly, a weighted amount of selected solid-liquid binary lipids mixture (5% w/v) was melted at 5°C above the melting point of solid lipid. A known concentration of CC (5% w/v of lipids) was dissolved in the prepared oil phase (5% w/v mixture of solid and liquid lipid). The aqueous phase containing selected surfactant (2.5% w/v) was heated to the same temperature was added drop by drop to the lipid phase under magnetic stirring at 1500 rpm for 5 min. After that, homogenization of the resultant pre-emulsion was performed at high speed of mixing about 20,000 rpm using an Ultra-Turrax T25 homogenizer (WiseMix™ HG15A, Daihan Scientific, Seoul, Korea) for 10 min[11]. The resultant o/w nanoemulsions were subjected to probe sonication (ultrasonic processor, GE130, probe CV18, USA) at 60 % amplitude for 10 min. The obtained NLC dispersion was left beside to reach room temperature.

Physicochemical characterization of CC-NLCs

Particle size and polydispersity index

The mean diameter and polydispersity index of particle of nanostructured lipid carriers loaded with CC was determined using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire (UK), equipped with a 10 mW He-Ne laser employing the wavelength of 633 nm and a back-scattering angle of 90° at 25°C. Before Photon correlation spectroscopic (PCS) analysis, CC-NLCs formulations should be diluted with a certain amount of double-distilled water (1:200) to get appropriates cattering intensity. The analysis, of Particle size was determined using Mie theory with the refractive index and absorbance of lecithin at 1.490 and 0.100, respectively[13].

Zeta potential analysis

The zeta potential of NLC formulations was measured via electrophoretic mobility measurements using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire (UK). The zeta potential was calculated by applying the Helmholtz–Smoluchowski equation (n=3)[14].

Encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency and loading capacity of CC into NLC formulations were measured by the indirect method by measuring the concentration of the free CC. Initially, 2 ml of NLCs formulations were centrifuged at 100,000 rpm for 1 h at 4°C to evaluate the un entrapped CC using cooling ultracentrifuge (Beckman Instruments TLX-120 Optima Ultracentrifuge). The aqueous layer was aspirated and filtered using Millipore® membrane (0.2 μm) and diluted with an appropriate amount of methanol and measured by UV-Vis spectrophotometer (Shimadzu, the model UV-1800 PC, Kyoto, Japan) at 254 nm to measure the free amount of CC. Consequently, encapsulation efficiency and loading capacity of CC into NLCs were determined through the following equations[15,19].

$$EE\% = \frac{Wi - Wf}{Wi} x100$$

Where, Wi= Weight of initial drug, Wf= Weight of free drug

$$LC\% = \frac{Wd}{Wn} x100$$

Where, Wd= Wt. of drug in nanoparticles
Wn=Wt. of nanoparticles

In-vitro drug release study

The *in vitro* release of CC from CC suspension and CC-NLCs was performed by a dialysis bag diffusion technique. The receptor compartments consist of the following release media: 500 ml Hydrochloric acid solution (0.1 N) of pH 1.2 and phosphate buffer solution (PBS) of pH 6.8 and again, in the same previous media but with addition Polysorbate 20 (0.35-0.7% w/w) to confirm more achieve sink conditions of dissolution media[16]. The donor compartment is cellulose membrane dialysis bags (MWCO-12000, Sigma, USA) were soaked in dissolution media overnight prior experiment. One milliliter of freshly prepared CC-NLC and CC suspension (equivalent to 2.5 mg of CC) were diluted with 5 ml of dissolution media and which tightly closed from two sides by a thermo-resistant thread. The bags were immersed in the Dissolution apparatus, (six-spindle dissolution tester, Pharmatest, type PTWII, Germany) automatically adjusted at 37±2°C and 100 rpm. Two-milliliter sample was aspirated at a predetermined time interval (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h) and the same volume of media was added to maintain sink condition. The release of free CC from NLC was compared to that from suspension. The aspirated samples were measured using UV-Vis spectrophotometer at 254 nm.

RESULTS AND DISCUSSION

Selection of Solid Lipid

The efficient solubility of the drug in the solid lipid reflects the capacity of NLC formulations to accommodate high amount of specific drug[17]. Initially, Candesartan Cilexetil (CC) solubility in various solid lipids should be performed to select the appropriate ones, which allowed accommodation of high amount of the drug leading to maximizing an essential qualification of a carrier system as the loading capacity and encapsulation efficiency of the prepared NLC formulations.

Figure 1 represents the solubility of CC in different solid lipids. The experiments with solid lipids demonstrated that the affinity of CC to solid lipid was in order of Gelucire® 44/14>Precirol® ATO 5 >Compritol®888 ATO>Glyceryl Mono Stearate (GMS) >Stearic acid>Labrafil® M 2130 CS>Gelucire® 39/01>Gelucire® 43/01 where, Gelucire® 44/14, Precirol®ATO 5, Compritol® 888 ATO and Glyceryl Mono Stearate (GMS), showed higher CC solubilizing ability, with solid lipid values per 100 mg of CC (w/w) of (750±3.11 mg, 1000±4.12 mg, 1500±4.15 mg and 1750±3.16 mg), respectively.
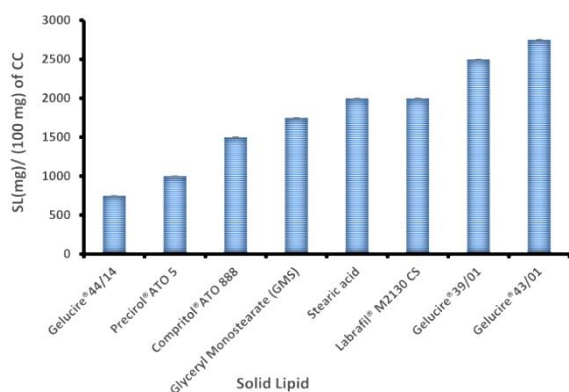

Figure 1: Solubility study of CC in different solid lipids.

These results related to the imperfect structure of matrix of Gelucire® 44/14, Precirol® ATO 5, Compritol® 888 ATO and Glyceryl Monostearate (GMS) molecules, which are formed due to its chemical nature (mono-, di-, and triglyceride contents) and its composition that containing different length of chain of fatty acid that offer loosely porous structural features that make the drug easier to modify and more soluble[18]. Gelucire® 44/14 is Polyoxylglycerides mixture[19], Precirol® ATO 5composed of mixture of palmitostearate glyceride and Compritol® 888 ATO composition is mixture of behenate glyceride;[20] while Glyceryl Monostearate (GMS) is mixture of variable proportions of glyceryl monostearate and glyceryl monopalmitate[19].
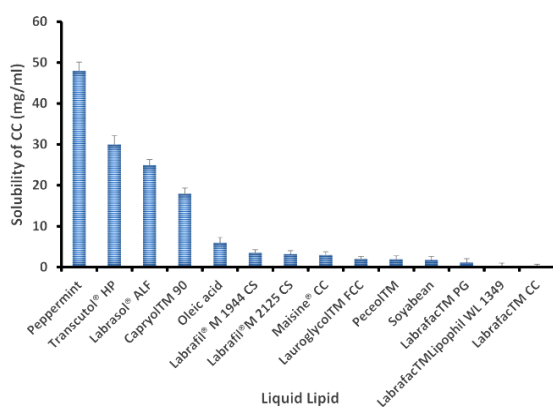

Figure 2: Solubility study of CC in different liquid lipids.

The variety of Precirol ATO 5®fatty acid (C 16 and C18) content with subsequent loosely porous structure and its higher relative monoglycerides content in

between different solid lipids used (more lipid monoglyceride, more lipid polarity)[21]. In addition, monoglycerides possess emulsification properties[22] which can also improve the drug solubility, such explain the potentiality of Precirol ATO5® to solubilize Candesartan Cilexetil than Compritol® 888 ATO than GMS. Stearic acid, Labrafil® M 2130 CS, Gelucire® 39/01, Gelucire® 43/01 proved to be lower CC solubilizing ability, with solid lipid values per 100 mg of CC (w/w) of (2000±3.14 mg, 2000±5.12 mg, 2500±3.15 mg and 2750±4.13 mg), respectively, than the above mentioned ones. So, the following three solid lipids, Precirol®ATO 5, Compritol® 888 ATO and GMS selected to be used as lipid core for the preparations of CC-NLCs in this study after discarding of Gelucire®44/14 because the addition of Gelucire®44/14 to liquid lipid reduces the melting point of NLCs formulations which was not appropriate to be administered orally[23, 26].

Table 1: Miscibility study of binary lipid mixtures with different surfactants.

SL: LL in ratio	SAA10 ml of 5% soln.	%Transmittance
Precirol® ATO 5: Labrasol® ALF	Lutrol® F68	98.106±5.3
	Lutrol® F127	97.972±8.1
	Cremophore®EL	94.756±3.3
	Cremophore®RH	81.847±9.1
	Tween® 40	69.650±1.8
	Tween® 80	65.850±2.3
	Phospholibon®	19.890±7.3
Compritol® ATO 888: Transcutol® HP	Lutrol® F68	97.324±7.2
	Lutrol® F127	95.079±1.4
	Cremophore®EL	89.438±1.3
	Cremophore®RH	82.680±6.3
	Tween® 40	67.435±3.3
	Tween® 80	67.256±7.2
	Phospholibon®	10.350±4.3
GMS: Capryol™ 90	Lutrol® F68	98.685±5.2
	Lutrol® F127	95.004±8.1
	Cremophore®EL	86.475±5.3
	Cremophore®RH	78.185±6.7
	Tween® 40	75.443±6.3
	Tween® 80	69.481±9.2
	Phospholibon®	30.989±8.3

Selection of Liquid Lipid
Proper dissolvability of CC in Liquid Lipid is basic for the successful formulation of nanostructured lipid carrier as well as encapsulation efficiency was directly influenced by solubility of the drug in liquid lipid. Screening of liquid lipids was evaluated depending on the solubility of CC in different liquid lipids. Also, higher drug solubility in the oil phase brings down the necessities of surfactants in this way limiting their toxic impacts. The solubility of CC in various liquid lipids was showed in Figure 2. It was evident that CC revealed highest solubility in peppermint oil (48±2.14 mg/ml), Transcutol® HP (30±2.21 mg/ml), Labrasol® ALF (25±1.32 mg/ml) and Capryol™ 90 (18±1.34 mg/ml) and the least solubility was observed in Labrafac™ PG (1.1±0.97 mg/ml), Labrafac TMLipophil WL 1349 (0.166±0.81 mg/ml) and Labrafac™ CC (0.087±0.65 mg/ml). The relatively

high solubility of (CC) in peppermint oil (48±2.14 mg/ml) may be attributed to the composition mixture of peppermint oil with various alcohols, ketones and terpenes (menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, b-pinene, a-pinene and pulegone)[27] that might be aided in solubilization of CC through interaction with one or more of the functional groups of CC (such as –NH and –C=O). Furthermore, surface active properties of components of peppermint oil (HLB=12.3)[28].
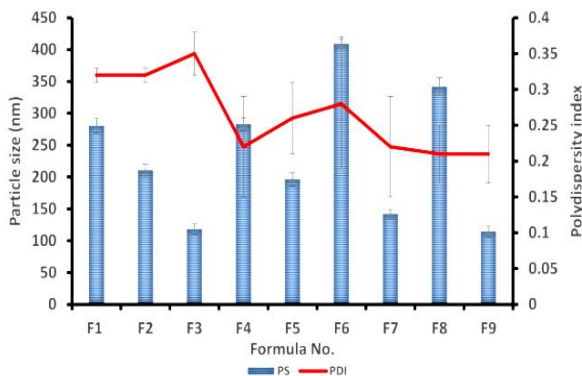

Figure 3: Mean particles size and polydispersity index of CC-NLCs formulations.

The solubilization ability of Transcutol® HP (30±2.21 mg/ml), Labrasol® ALF (25±1.32 mg/ml) and Capryol™ 90 (18±1.34 mg/ml) for CC was attributed to their intrinsic self-emulsifying property and their chemical structure (PEG-medium chain triglycerides) because of the affinity of a broad range of hydrophilic and lipophilic drug molecules to be encapsulated into lipid carriers, increased with PEG-glycerides than that glycerides free from PEG moieties such as (Labrafac™ PG, Labrafac™Lipophil and Labrafac™ CC) due to their known surfactant properties[29].
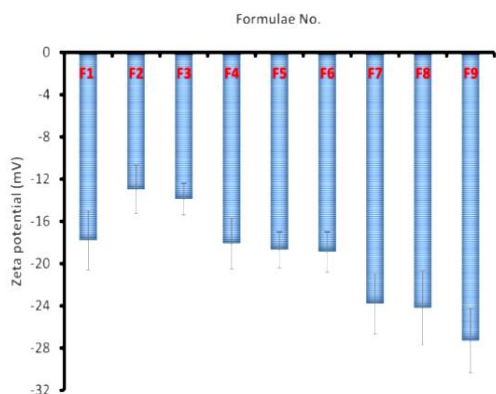

Figure 4: Zeta potential of CC-NLCs formulations.

The high solubilizing effect of Transcutol® HP for CC is consistent with Cirri *et al*.,[12]. Furthermore, presence of Caprylic acid (C8) in oil composition had great impaction on drug solubility, where the oils of the more Caprylic acid content were found to be the higher solubilizing one for drug such as (Caprylic acid content in Labrasol® ALF and Capryol 90) are 80 and 90%, respectively,[30]. This phenomenon may be attributed to the Caprylic acid polarity making it more

efficient solubilizing one for the poorly water-soluble drug. Thus, Transcutol® HP, Labrasol® ALF and Capryol™ 90 were selected as a liquid lipid for further investigation because of the high solubilizing extent of CC after discarding of peppermint oil due to the low of its flashpoint (66.1°C) than the temperature that needed during the formulation preparation process.

Table 2: Miscibility study of binary lipid mixtures with combinations of surfactants.

SL:LL in ratio	SAA Combination (1:1) 10 ml of 5% soln.	% Transmittance
Precirol® ATO 5: Labrasol® ALF	Lutrol® F68: Tween® 40	53.8±6.7
	Lutrol® F127: Tween® 40	50.2±9.5
	Lutrol® F68: Cremophore® EL	18.0±7.3
	Lutrol® F127: Cremophore® RH	89.8±8.5
Compritol® ATO 888: Transcutol® HP	Lutrol® F68: Tween® 40	71.0±5.2
	Lutrol® F127: Tween® 40	59.0±4.3
	Lutrol® F68: Cremophore® EL	54.1±7.3
	Lutrol® F127: Cremophore® RH	26.7±8.9
GMS: Capryol™ 90	Lutrol® F68: Tween® 40	28.3±4.4
	Lutrol® F127: Tween® 40	23.2±8.7
	Lutrol® F68: Cremophore® EL	98.2±7.3
	Lutrol® F127: Cremophore® RH	96.7±6.3

Physical Compatibilities between Solid lipid and Liquid lipid
An essential for the improvement of a stable NLC development and permits taking into account that the fluid lipid is completely entrapped inside solid lipid matrix thus, physical compatibility between solid lipids and liquid lipids must be achieved.
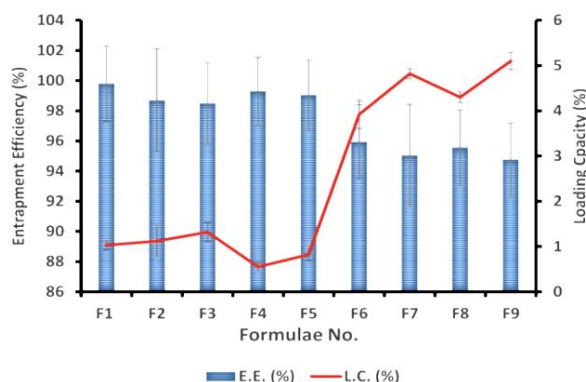

Figure 5: Encapsulation efficiency and loading capacity of CC-NLCs formulations.

All three selected solid lipids (Precirol® ATO 5, Compritol® 888 ATO and GMS) were further evaluated for the physical compatibility with three

selected liquid lipids (Transcutol® HP, Labrasol® ALF and Capryol™ 90) by applying visual and filter paper examination. The obtained results indicate that (Precirol® ATO 5- Transcutol® HP) and (Precirol® ATO 5-Capryol™ 90) mixtures showed phase separation and residue of liquid oil droplets on filter paper indicating formation of inhomogeneous mixtures (data not shown). The reduction in the melting temperature of the combined lipid mixtures was the reason for such observation.
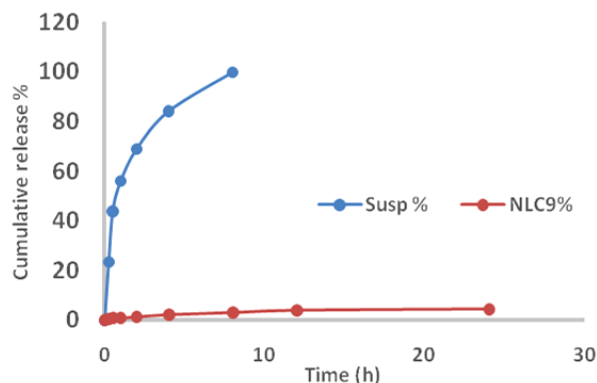

Figure 6: *In vitro* release profiles of CC from CC-NLC9 and CC-Susp in phosphate buffer (pH 6.8).

On the other hand, no presence of more than one layer was observed in the solidified mass and no residue of liquid lipid droplets on the filter paper of (Precirol® ATO 5-Labrasol® ALF) mixture indicate that formation of homogenous mixture. These results are consistent with S. Doktorovova *et al*.,[31]. However, (Compritol® 888 ATO-Labrasol® ALF) and (Compritol® 888 ATO-Capryol™ 90) mixtures also showed phase separation and presence of liquid oil droplets on filter paper indicating formation of inhomogeneous mixtures. Such an observation could be attributed to the same previous reason mentioned above. On the other side, there was no separation was showed in the congealed mass and no residue of liquid oil droplets on filter paper of (Compritol® 888 ATO-Transcutol® HP) mixture indicate that formation of homogenous mixture. While, GMS as solid lipid showed good miscibility and homogeneity with all three selected liquid lipids (Transcutol® HP, Labrasol® ALF and Capryol™ 90). Therefore, based on the screening study of the solid and liquid lipids for CC and physical compatibility between two types of lipids, (Precirol® ATO 5-Labrasol® ALF), (Compritol® 888 ATO - Transcutol® HP) and (GMS-Capryol™ 90) mixtures were selected as solid and liquid lipids, respectively for further investigation. Since Precirol® ATO 5 is one of three selected solid lipids has a high affinity for the CC was found to has also good compatibility for Labrasol® ALF liquid lipid. While Compritol® 888 ATO sloid lipid has a high affinity for CC was found to has good compatibility for Transcutol® HP liquid lipid and GMS solid lipid has a high affinity for CC was found to has good compatibility for Capryol™ 90 liquid lipid while the remained lipids were excluded from further designing of the formulation.

Determination of the SL: LL ratios using melting point technique

Solid-liquid lipids proportion was chosen with the goal to have enough drug loading capacity with a legitimate liquefying point to keep up the solid-semisolid uniformity of the particles at room temperature. As (Transcutol® HP, Labrasol® ALF and Capryol™ 90) were seen to have good drug solubilization limit, a higher proportion of (Transcutol® HP, Labrasol® ALF and Capryol™ 90) as liquid lipids could be helpful for the higher drug encapsulation[32]. In any case, at the same time, the consistency of the (solid-liquid) lipids blend can't be undermined. It was seen that the (solid-liquid) lipids blend in the proportion up to 70:30 were having an adequate melting point (55–59^{0}C) (data not shown). Furthermore, the increment of liquid lipid concentration, the melting point of the blends was beneath the ideal level. In addition, 70:30 the most appropriate and common ratio and widely applied by most previous related studies[33]. Consequently, 70:30 was chosen as the proper preparing ratio for the solid-liquid lipid mixture in all NLC formulations.

Screening study of surfactants: assessment of dispersion properties

The most important character for surfactant selection is the ability and capacity of surfactant to emulsify the produced emulsion with keeping its stability. A higher transmission rate is consistent with smaller particles and therefore greater emulsification[34]. Furthermore, the surfactant plays a necessary role in stabilization of NLC by decreasing the interfacial tension between the aqueous phase and the lipid phase of nanoemulsion and thus inhibits coalescence and agglomeration of particles. As depicted in the Table 1, results revealed that Lutrol® F68 showed maximal emulsification capacity for binary lipids of three selected lipid mixtures (Precirol® ATO 5-Labrasol® ALF), (Compritol® 888 ATO-Transcutol® HP) and (GMS-Capryol™ 90) where (98.106±5.3, 97.324±7.2 and 98.685±5.2% transmittance), respectively. Followed by Lutrol® F127 (97.972±8.1, 95.079±1.4 and 95.004±8.1 % transmittance). Then, Cremophore® EL (94.756 ±3.3, 89.438±1.3 and 86.475±5.3 % transmittance) and last Cremophore® RH (81.847±9.1, 82.680±6.3 and 78.185±6.7% transmittance). On the other hand, binary selected lipid mixtures mentioned above exhibited poor emulsification and formed turbid nanoemulsion with Phospholibon® (19.890±7.3, 10.350±4.3 and 30.989± 8.3 % transmitta -nce), respectively. The transmittance percentage which clearly distinguished and reflect the ability of surfactants to emulsify and stabilize binary selected lipid mixtures. Where a high percentage of transmittance indicates enough emulsified and stabilized emulsion. This fact is attributed to the hydrophilic-lipophilic balance (HLB) value of surfactant used, which results in high HLB values accompanied by higher transmission lead to smallparticles[26]. HLB values of surfactants used in screening studies are in order of lutrol® f68 (HLB=29) >lutrol® f127 (HLB=23)>Cremophore® RH (HLB= 14-16)Tween®40(HLB=15.4)>Tween®80 (HL B=15) >Cremophore® EL (HLB=12-14)> Phospho libon® (HLB=8)[35]. Phospholibon® was a poor sufficient to

emulsify the selected binary lipid mixture because it has a low value of HLB which was not adequate for o/w emulsion formation[36]. Despite there were no major variation of HLB values of the most surfactant used, Cremophore® EL (HLB= 12-14) had high emulsification effect than Cremophore® RH (HLB =14-16), Tween® 40 (HLB= 15.4) and Tween® 80 (HLB=15). Apart from HLB value, there was another factor such as the chemical structure of surfactants had a great impaction on the nano-emulsification process. Tween® 80 is derived from polyoxylatedsorbitan and oleic acid. Cremophor® EL is polyethoxylated castor oil, which is a mixture of polyethylene glycol ethers and polyethylene glycol esters of glycerol and ricinoleic acid. Cremophor® EL possesses a branched structure of alkyl chain, whereas Tween® 80 possesses a linear shape structure. The obtained results were in confirmation with Borhade *et al.*, and Kassem *et al.*,[39] stated that surfactants whose branched alkyl structure had good emulsification properties on nanoemulsion formation. Therefore, based on the emulsification ability of surfactants for selected binary lipid mixtures, Lutrol®F68, Lutrol®F127, Cremophore®EL, Cremophore®RH, Tween® 40 and Tween®80 were selected for further investigation as surfactant combination study.

Screening study of surfactants combination: Assessment of dispersion properties

From previous literature, it was clearly distinguished that the kind and quantity of surfactant influence the size of the nanoparticles and their storage stability. The quantity of surfactant should be enough to cover the surface of the hydrophobic nanoparticles[29,38]. The combination of two or more surface-active agents exhibits to form blended surfactant films at the surface of the particle size. The formed blended surfactant films were produced in sufficient amount to cover the surface of particles successfully and produce nanoparticles with small size as well as keeping storage stability by production of requisite viscosity[34]. In present art, it was observed in Table 2, 1:1 ratio of Lutrol® f127 and Cremophore® RH showed good emulsification ability and promote nanoemulsion stability of first binary lipids mixture (Precirol® ATO 5 - Labrasol® ALF), combination of selected surfactants in ratio as the same previous exhibited poor emulsification properties of second binary lipids mixture (Compritol® 888 ATO- Transcutol® HP) while combination of selected emulsifying agent at the mentioned above ratio (Lutrol® f68 and Cremophore® EL) and (Lutrol® f127 and Cremophore® RH) showed the higher emulsification capability as well as stability of emulsion of third binary lipids mixture (GMS-Capryol™ 90). The obtained results may be attributed to the same reasons mentioned above under explanation of screening study of surfactants wherein the hydrophilic-lipophilic balance (HLB) value of the surfactant play a great role in this fact[26]. Also, branched alkyl chain structure, as well as the length of hydrophobic chains of surfactants, had a countless effect on the nanoemulsion formation[37,39],0 as Cremophore® EL and Cremophore® RH. Hence, based on the introduced emulsification study of

surfactants, Lutrol® F68 and Lutrol® F127 were selected as surfactants for every binary lipid mixture for the preparation of NLC. Addition to (Lutrol® f127: Cremophore® RH) at equal ratio for (Precirol® ATO 5 -Labrasol® ALF) binary lipid mixture and (Lutrol® f68: Cremophore® EL) and (Lutrol® f127: Cremophore® RH) at equal ratio for (GMS - Capryol™ 90) binary lipids mixture as surfactant combination for preparation of NLC.

Fabrication of CC-NLCs

Based on screening and solubility studies, the NLC formulations were designed, formulated and improved using lipid phase composed of Precirol® ATO 5, Compritol® 888 ATO and GMS as solid lipid and Labrasol® ALF, Transcutol® HP and Capryol™ 90 as liquid lipid which were chosen based on CC solubility in the lipid phase. Lutrol® F68, Lutrol® F127, Cremophore® EL and Cremophore® RH as surfactants which constituted the aqueous phase.

The concentration of the lipid phase to surfactant was constant at 5% (w/w) and 2.5% (w/w), respectively and the concentration of CC was fixed to 5% (W/W) of the lipid phase. The lipid phase should not be exceedingly beyond 5% w/w. The observations are in line with studies reported by Das *et al.*, and Elbahwy *et al.*,[36,39] who discovered that an increased concentration of lipid leads to an enormous increase of particle size. As formulations are designed to be orally used, surfactants have been established at a pleasant 2.5% concentration (w/w)[42]. The composition of the formulation is given in the Table 3. Preparation of CC-NLCs were performed using homogenization followed by probe sonication technique. The influence of the lipids and surfactants variation on the particle size and the PDI was studied. Also, other physical characterization should be achieved for every formulation to select the best one for further investigations.

Physicochemical characterization of CC-NLC formulations

Particle size, polydispersity index (PDI)

Determination of physical properties as particle size and PDI are essential for predicting the stability of NLCs formulations. Particle sizing is a significant method for confirming nanosized particle manufacturing. Also, the smallest particle size, more absorbable and uptake through the gastrointestinal tract then, efficiently phagocytosed by the reticuloendothelial system. Therefore, the accuracy in particle size evaluation was necessary. Usually, the recommended particle size requisite for transportation through the intestine should not be more than 300 nm[43]. As represented in Table 4 and Figure 3 the observations revealed that all the designed formulations were showed in the nanometer range (<408 nm). It can be concluded that particle size of Precirol® ATO 5 nanoparticles (F1 to F3), Compritol® 888 ATO nanoparticles (F4 and F5) and GMS nanoparticles (F6 to F9) ranged from (280.6±11.8 to 118.6±8.1 nm), (283±9.9 and 196.5±10.2 nm) and (408.9±11.5 to 114.6±8.3 nm), respectively. The obtained results were clearly distinguished that formulations that contain more than one surfactant give the small particle size than that contain one surfactant as in F3 and F9

(118.6±8.1 and 114.6±8.3 nm) this behavior was attributed to the same reason discussed above under the screening study of surfactants combination. Also, these results were in accordance with the following reported studies[34]. On the other hand, the largest particle size was exhibited in GMS formulations which contain surfactant Lutrol® F68 alone or in combination with other surfactants as in F6 and F8 (408.9±11.5 and 392.1±13.8 nm). This observation may be attributed to the tendency of GMS nanoparticles to form a gel after 24 h storage at room temperature due to polymorphic transitions in GMS after cooling at room temperature. Furthermore, the interaction between GMS and Lutrol® F68. The polymorphic transitions in the lipids

after cooling to the room temperature and the interaction between surfactant and lipid are known to cause gel formation and subsequently influence the PS in NLC and SLN dispersions[44]. The polydispersity index as an indicator of the size distribution width of the particle. The PI value that reflects dispersion quality typically varies between 0 and 1. Most researchers recognize PI values ≤0.3 as optimum values; however, values ≤0.5 are also acceptable[45]. Table 4 and Figure 3 give an overview of the results of polydispersity index measurements. The prepared NLC dispersions had a PI value ≤0.35±0.01 due to the preparation method used indicating a homogenous and narrow size distribution of nanoparticles of NLCs.

Table 3: Suggested formulae of CC-NLCs.

F. No.	SL (70%) (5%) of Total Formula(g)			LL (30%)			SAA (2.5%) of Total Formula (g)				Drug (5%) of Lipids	Water (92.5%) (g)
	P	C	GMS	L	T	Cp	L F68	L F127	L F127 + Cr RH (1:1)	L F68 + Cr EL (1:1)	CC	(mg)
F1	3.5			1.5			2.5				250	92.5
F2	3.5			1.5				2.5			250	92.5
F3	3.5			1.5					2.5		250	92.5
F4		3.5			1.5		2.5				250	92.5
F5		3.5			1.5		2.5				250	92.5
F6			3.5		1.5		2.5				250	92.5
F7			3.5		1.5		2.5				250	92.5
F8			3.5		1.5					2.5	250	92.5
F9			3.5		1.5				2.5		250	92.5

SL = Solid lipid, LL = Liquid lipid, SAA = Surface active agent, P = Precirol®ATO 5, C = Compritol®ATO 888, GMS = Glyceryl Monostearate, L = Labrasol® ALF, T = Transcutol® HP, Cp = Capryol™ 90, L F68 = Lutrol® F68, L F127 = Lutrol® F127, Cr RH = Cremophore®RH, Cr EL = Cremophore®EL, CC=Candesartan Cilexetil.

Zeta potential measurement
The main parameter which influences the storage stability of colloidal nanocarrier is zeta potential, which measures the nanoparticle's surface charge and provides the repulsion degree between the nanoparticles preventing its agglomeration[46]. From the factors which mainly influence zeta potential of lipid-based nanoparticles structure of solid and liquid lipid and the medium composition[47]. Also, it depends on higher steric stabilization and lowers an electrostatic stabilization of nonionic surfactants which perfectly forming a coat around the particles of NLCs. Result in surface coverage of NLC decreases the electrophoretic mobility of nanoparticles and thus lower the zeta potential values[3,15]. This phenomenon explains the higher stability of NLC formulations despite having a lower zeta potential value. Zeta potential values ofall designed formulations are shown in Table 4 and represented in Figure 4. The results revealed that the ZP of the various formulations was a consistently negative surface charge. ZP values of Precirol® ATO 5 nanoparticles (F1 to F3), Compritol® 888 ATO nanoparticles (F4 and F5) and GMS nanoparticles (F6 to F9) in between (-13 ±2.3 to -17.8±2.8 mV), (-18.1±2.4 and -18.7±1.7 mV) and (-18.9 ±1.9 to 27.3±3.7 mV), respectively. Due to the non-ionic behavior of used surfactants for stabilization of nanoparticles so; these molecules had not any role in

the obtained zeta potential charges. Furthermore, the solid lipids were used in developed NLCs composed of mixture of acylglycerols: Precirol® ATO 5 composed of glyceryl tripalmitostearate (25-35%), glyceryl dipalmitostearate (40-60%) and glyceryl monopalmito-stearate (8-22%)[48] and Compritol® 888 ATO composed of glyceryl tribehenate (28%-32%), glyceryl dibehenate (52-54%) and glyceryl monobehenate (12-18%)[49], both of them being glycerol esters of long chain-length fatty acids (C18, C16) and (C22) respectively. So, that they provide neither charge nor polarity that participates to zeta potential. whereas, GMS composed of triacylglycerols (5–15%), diacylglycerols (30–45%) and monoacylglycerols (40–55%)[19]. In such a case due to the high content of partial emulsifying glycerides (mono and diglycerides) of GMS and the presence of non-esterified hydroxyl groups of glycerol, this molecule showed some of the polarity that participates to zeta potential. On the other hand, the liquid lipids were used in developed NLCs composed of diacylglycerol of medium-chain-length fatty acids. Liquid lipids provide the majority impaction and contribute to zeta potential due to its polarity which results from a free hydroxyl group of the glycerol that not subjected to the esterification process and the chain length of the fatty acids. These observations are in line with studies reported by Teeranachaideekul *et al*., and López-García and Ganem-Rondero[49] which stated that it might be due to

presence of liquid lipid at the surface of NLC. Being the melting point of liquid lipid lower than that of the solid lipid, during the fabrication process of NLC, the solid lipid recrystallizing again first, with encapsulating an apart of the liquid lipid inside the solid lipid matrix. Subsequently, the remained amount of liquid lipid was covered the outer layer of formed nanoparticles[31,50].

The obtained results can be concluded that GMS nanoparticles (F6 to F9) possessed high ZP values than that of Precirol®ATO 5 nanoparticles (F1 to F3) and Compritol® 888 ATO nanoparticles (F4 and F5) this fact can be explained by the following reasons: certain polarity and emulsifying properties of GMS resulted from none esterified hydroxyl group of glycerol and the length of chain of the fatty acids. Another reason was attributed to the negative charged carboxylic groups of MCT (capryol™90) which composed mainly monoesters and a small fraction of diesters of caprylic/capric triglyceride. A similar explanation has been reported by Teeranachaideekul *et al.*,[51], these revealed the higher ZP values of GMS nanoparticles than other nanoparticles.

Table 4: Particle size, polydispersity indices and zeta potential of CC-NLCs formulations.

Formula No.	PS (nm)	PDI	ZP (mV)
F1	280.6±11.80	0.32±0.01	-17.8±2.80
F2	210.7±9.10	0.32±0.01	-13.0±2.30
F3	118.6±8.10	0.35±0.03	-13.9±1.50
F4	283.0±9.90	0.22±0.07	-18.1±2.40
F5	196.5±10.20	0.26±0.05	-18.7±1.70
F6	408.9±11.50	0.28±0.09	-18.9±1.90
F7	141.8±7.10	0.22±0.07	-23.8±2.90
F8	342.1±13.80	0.21±0.04	-24.2±3.50
F9	114.6±8.30	0.21±0.04	-27.3±3.07

Entrapment efficiency, drug content and drug loading of CC-NLCs

The quantity of drug encapsulated in the nanoparticles and the drug content in the lipid matrix is a further significant consideration for the optimization of NLC. The quantity of drug encapsulated in the lipid matrix depends on many factors as: the type of lipids used, physicochemical properties of the drug, miscibility and solubility of drug in the molten lipid[52], physical and chemical nature of the lipid matrix and crystalline state of lipid matrix and also surfactant was found to affect encapsulation efficiency[15]. Encapsulation efficiency and loading capacity of all NLC formulations are showed in the Table 5 and demonstrated in Figure 5. The entrapment efficiency and drug loading were determined and found to be in between 94.76±2.44 % to 99.80±2.50% and 0.55±0.11% to 5.10±0.19%, respectively. These high entrapment efficiencies and drug loading of CC in NLCs could be attributed to the high lipophilic nature of CC (log p~6.2) which enhance the solubility of CC in various lipids and subsequently easily incorporated into the lipid matrix3[,53]. Moreover, the using of a mixture of perfect ordered with less ordered lipids, which caused several crystal defects in lipid matrix and provided much imperfections leading to void spaces in which more drug molecules could be

accommodated7[,15,43,53]. It was observed that E.E and LC of CC in Precirol® ATO 5 nanoparticles (F1 to F3) were varied from 98.5±2.70% to 99.8±2.50 and 1.03±0.10 to 1.32±0.21, respectively, in Compritol® 888 ATO nanoparticles (F4 and F5) were varied from 99.04±2.35 to 99.30±2.25% and 0.82±0.13 to 0.55±0.11, respectively and in GMS nanoparticles (F6 to F9) also were ranged from 94.76±2.44% to 95.94±3.45% and 3.92±0.31% and 5.10±0.19%, respectively. From the results, it was clearly distinguished that Precirol® ATO 5 nanoparticles (F1 to F3) and Compritol® 888 ATO nanoparticles (F4 and F5) showed higher entrapment efficiency around 99% than that of GMS nanoparticles (F6 to F9) around 95%. Such a fact was attributed to the chemical composition of each one. Where, the imperfect and less ordered matrix structure of Precirol® ATO 5 and Compritol® 888 ATO molecules, which are formed from a combination of mono-, di- and triglyceride that expected to exhibit lower crystallinity and more structure porosity which allows higher solubility and easier accommodation of more drug molecules[17,26].

Also, Precirol® ATO 5 is a di-glyceride with two different chain length fatty acids palmitic and stearic acid (C16 and C18); therefore, it is expected to have less ordered lipid network compared to GMS, and thus lead to the more drug molecules could be entrapped[18,26]. Further, subsequent to cooling, Precirol®ATO 5 and Compritol®888 ATO recrystallize in a progression of polymorphs. In like manner, with respect to the conditions utilized during the preparation, CC could be homogenously dispersed[15].

Table 5: Encapsulation efficiency and loading capacity of CC-NLCs formulations.

Formula No.	E.E. (%)	L.C. (%)
F1	99.80±2.50	1.03±0.10
F2	98.70±3.40	1.12±0.35
F3	98.50±2.70	1.32±0.21
F4	99.30±2.25	0.55±0.11
F5	99.04±2.35	0.82±0.13
F6	95.94±3.45	3.92±0.31
F7	95.04±3.40	4.82±0.11
F8	95.56±2.50	4.30±0.12
F9	94.76±2.44	5.10±0.19

Regarding the type of surfactant, it was clearly observed that NLCs formulation prepared using Lutrol® F68 higher E.E. than that prepared using other surfactants. This behavior repeated with every nanoparticle prepared using Lutrol® F68 alone (F1, F4 and F6) or in combination with Cremophore® EL (F8). This fact might be attributed to the high value of the hydrophilic-lipophilic balance of Lutrol® F68 (HLB ~ 29) compared to other surfactants.

***In-vitro* release study**

In-vitro release study was achieved for all formulations in addition to pure CC suspension. The release condition monitored in 0.1 M HCl (pH 1.2) and PBS (pH 6.8) and at the same conditions with adding tween 20 (0.35-0.7%w/w) to achieve "sink" conditions during a dissolution test for all formulations [54,55]. It was found

that all formulations exhibit a lack of drug release within 24 h. The *in-vitro* release of the best formula (CC-NLC9) was reached to less than 5% as showed in (Figure 6) but, CC suspension showed almost complete drug release (100%) within 8 h. As CC had solubility equal to 11 µg/ml in 0.1 M HCl and 1 µg/ml in PBS (pH 6.8). The very difficult release of CC results from its poor aqueous solubility and high lipophilicity (log *p*~6.2). Thus, CC possesses a high affinity to the lipids consequently the drug becomes more entrapped and retained inside the core of the lipid matrix preventing it from the release. Furthermore, the high efficient solubility and compatibility of CC with the lipid components as previously discussed before under screening studies[56,57]. These observations are in line with the study reported by Zhang *et al*.,[56]. Previous studies ascertained that NLCs must be absorbed into the blood or lymphatic system after duodenal administration to rates.

Consequently, lack of *in-vitro* release of CC from NLCs suggesting that NLCs could be absorbed via the enterocytes after oral administration, the most sought after therapeutic effect which required confirmation through employing more investigations in this work.

The rank order was performed for all prepared NLCs formulations (F1 to F9) in order to choose the best formula based on the previously measured characterization as Particle size, polydispersity index (PDI), Zeta potential, encapsulation efficiency and loading capacity of CC-NLCs wherein the formula F9 was chosen as the best formula for further investigations.

CONCLUSIONS

Nanostructured lipid carriers (NLCs) are adaptable nanoparticles with multipurpose applications. However, quality and successful incorporation of CC into NLC to develop more efficient formulation based on proper selection of the components and optimization. The current work clarifies a sequence steps for selection of excipients for NLCs by employing simple experiments. Screening studies were performed for whole excipients to select appropriate ones to prepare CC loaded NLCs. Furthermore, the developed formulations were subjected to physic-chemical characterization. The resulted formulations appeared in nanoparticles size with high encapsulation efficiency.

ACKNOWLEDGEMENTS

AUTHOR'S CONTRIBUTION

Anwar W: writing original draft, methodology, investigation. **Dawaba HM:** formal analysis, data curation, conceptualization. **Afouna MI:** writing, review and editing. **Samy AM:** methodology, formal analysis.

All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST

There are no any conflicts of interest.

REFERENCES

1. Gurunath S, Nanjwade BK, Patil PA. Oral bioavailability and intestinal absorption of candesartan cilexetil: Role of naringin as P-glycoprotein inhibitor. Drug Dev Ind Pharm 2015; 41: 170–176. *https://doi.org/10.3109/03639045.2013.850716*

2. Gurunath S, NanjwadeBK, Patila PA. Enhanced solubility and intestinal absorption of candesartan cilexetil solid dispersions using everted rat intestinal sacs. Saudi Pharm J 2014; 22: 246–257. *https://doi.org/10.1016/j.jsps.2013.03.006*

3. Dudhipala N, Veerabrahma K. Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: Characterization, pharmacokinetic and pharmacodynamic evaluation. Drug Deliv 2016; 23: 395-404. *https://doi.org/10.3109/10717544.2014.914986*

4. Mathur P, Sharma S, Rawal S, Patel B, Patel MM. Fabrication, optimization, and *in vitro* evaluation of docetaxel-loaded nanostructured lipid carriers for improved anticancer activity. J. Liposome Res 2019;1–15. *https://doi.org/10.1080/08982104.2019.1614055*

5. Attama AA, Momoh MA, Builders PF. Lipid nanoparticulate drug delivery systems: a revolution in dosage form design and development in recent advances in novel drug carrier systems.2012;107–140. *https://doi.org/10.5772/50486*

6. Poonia N, Kharb R, Lather V, Pandita D. Nanostructured lipid carriers: versatile oral delivery vehicle. Futur Sci OA 2, FSO 2016; 135. *https://doi.org/10.4155/fsoa-2016-0030*

7. Mendes IT, *et al*. Development and characterization of nanostructured lipid carrier-based gels for the transdermal delivery of donepezil. Colloids Surf B Bioint 2019; 177, 274–281. *https://doi.org/10.1016/j.colsurfb.2019.02.007*

8. Rawal S, Patel MM. Threatening cancer with nanoparticle aided combination oncotherapy. J Control Release 2019; 301: 76-109. *https://doi.org/10.1016/j.jconrel.2019.03.015*

9. Joshi M, Patravale V. Formulation and evaluation of nanostructured lipid carrier (NLC)-based gel of valdecoxib. Drug Dev Ind Pharm 2006; 32, 911–918. *https://doi.org/10.1080/03639040600814676*

10. Gadhave DG, Tagalpallewar AA, Kokare CR. Agranulo cytosis protective olanzapine loaded nanostructured lipid carriers engineered for CNS delivery: optimization and hematological toxicity studies. AAPS Pharm Sci Tech 2019; 20, 1–15. *https://doi.org/10.1208/s12249-018-1213-y*

11. Cortesi R, *et al*. Nanostructured lipid carriers (NLC) for the delivery of natural molecules with antimicrobial activity: production, characterisation and *in vitro* studies. J Microencapsul 2017; 34, 63–72. *https://doi.org/10.1080/02652048.2017.1284276*

12. Cirri M, *et al*. Design, characterization and in vivo evaluation of nanostructured lipid carriers (NLC) as a new drug delivery system for hydrochlorothiazide oral administration in pediatric therapy. Drug Deliv 2018; 25, 1910–1921. *https://doi.org/10.1080/10717544.2018.1529209*

13. Gu Y, Tang X, Yang M, Yang D,Liu J. Transdermal drug delivery of triptolide-loaded nanostructured lipid carriers:

Preparation, pharmacokinetic, and evaluation for rheumatoid arthritis. Int J Pharm 2019; 554, (Elsevier B.V.). *https://doi.org/10.1016/j.ijpharm.2018.11.024*

14. Marques AC, Rocha AI, Leal P, Estanqueiro M, Lobo JM. Development and characterization of mucoadhesive buccal gels containing lipid nanoparticles of ibuprofen. Int J Pharm 2017; 533: 455–462. *https://doi.org/10.1016/j.ijpharm.2017.04.025*

15. Tatke A, *et al*. In situ gel of triamcinolone acetonide-loaded solid lipid nanoparticles for improved topical ocular delivery: Tear kinetics and ocular disposition studies. Nanomat 2019; 9:1–17.*https://doi.org/10.3390/nano9010033*

16. Gruberová L, Kratochvil B. Biorelevant dissolution of candesartan cilexetil. ADMET DMPK 2017; 5:39. *http://dx.doi.org/10.5599/admet.5.1.346*

17. Vivek K, Reddy H,Murthy RSR. Investigations of the effect of the lipid matrix on drug entrapment, *in vitro* release, and physical stability of olanzapine-loaded solid lipid nanoparticles. AAPS Pharm Sci Tech 2007; 8: 16–24. *https://doi.org/10.1208/pt0804083*

18. Seyed YA, Shahidi F, Mohebbi M, Varidi M, Golmohammadzadeh S. Preparation, characterization and evaluation of physicochemical properties of phycocyanin-loaded solid lipid nanoparticles and nanostructured lipid carriers. J Food Meas Charact 2018; 12: 378–385. *https://doi.org/10.3109/03639049809085644*

19. Rowe RC, PJS, MEQ. Handbook of Pharmaceutical Excipients, sixth Edition. The Pharmaceutical Press, London 2009.

20. Abd-Elbary A, Tadros MI,Alaa-Eldin AA. Sucrose stearate-enriched lipid matrix tablets of etodolac: modulation of drug release, diffusional modeling and structure elucidation studies. AAPS Pharm Sci Tech 2013; 14: 656–668. *https://doi.org/10.1208/s12249-013-9951-3*

21. Kaithwas V, Dora CP, Kushwah V,Jain S. Nanostructured lipid carriers of olmesartan medoxomil with enhanced oral bioavailability. Colloids Surfaces B Biointerfaces 2017; 154, 10–20. *https://doi.org/10.1016/j.colsurfb.2017.03.006*

22. Jensen LB, *et al*. Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles. Int J Pharm 2010; 390: 53–60. *https://doi.org/10.1016/j.ijpharm.2009.10.022*

23. Kasongo KW, Pardeike J, Müller RH,Walker RB. Selection and characterization of suitable lipid excipients for use in the manufacture of didanosine-loaded solid lipid nanoparticles and nanostructured lipid carriers. J Pharm Sci 2011; 100, 5185–5196. *https://doi.org/10.1002/jps.22711*

24. Bargoni A, *et al*. Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. Pharm Res 1998; 15: 745–750. *https://doi.org/10.1023/a:1011975120776*

25. Wong HL, Bendayan R, Rauth AM, Li Y, Wu XY. Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Adv Drug Deliv Rev 2007; 59: 491–504. *https://doi.org/10.1016/j.addr.2007.04.008*

26. Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AO. Natamycin solid lipid nanoparticles-sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. Int J Nanomed 2019; 14: 2515–253. *https://doi.org/10.2147/IJN.S190502*

27. Lin W, *et al*. Chemical composition and biological properties of essential oils of two mint species. Trop J Pharm Res 2013; 12: 577–582. *https://doi.org/10.3390/molecules15042326*

28. Orafidiya LO, Oladimeji FA. Determination of the required HLB values of some essential oils. Int J Pharm 2002; 237: 241–249. *https://doi.org/10.1016/s0378-5173(02)00051-0*

29. Safwat S, Ishak RAH, Hathout RM, Mortada ND. Nanostructured lipid carriers loaded with simvastatin: effect of PEG/glycerides on characterization, stability, cellular uptake efficiency and *in vitro* cytotoxicity. Drug Dev Ind Pharm 2017; 43: 1112–1125. *https://doi.org/10.1080/03639045.2017.1293681*

30. Bandivadeka MM, Pancholi SS, Kaul-Ghanekar R, Choudhari A, Koppikar S. Self-microemulsifying smaller molecular volume oil (Capmul MCM) using non-ionic surfactants: A delivery system for poorly water-soluble drug. Drug Dev Ind Pharm 2012; 38: 883–892. *https://doi.org/10.3109/03639045.2011.631548*

31. Doktorovová S, Araújo J, Garcia ML, RakovskýE, SoutoEB. Formulating fluticasone propionate in novel PEG-containing nanostructured lipid carriers (PEG-NLC). Colloids Surfaces B Biointerfaces 2010; 75, 538–542. *https://doi.org/10.1016/j.colsurfb.2009.09.033*

32. Negi LM, Jaggi M, Talegaonkar S. Development of protocol for screening the formulation components and the assessment of common quality problems of nano-structured lipid carriers. Int J Pharm 2014; 461: 403–410. *https://doi.org/10.1016/j.ijpharm.2013.12.006*

33. Scioli MS, *et al*. Carbamazepine-loaded solid lipid nanoparticles and nanostructured lipid carriers: Physicochemical characterization and *in vitro/in vivo* evaluation. Colloids Surfaces B Biointerfaces 2008; 167:73–81. *https://doi.org/10.1016/j.colsurfb.2018.03.052*

34. Rizwanullah M, Amin S, Ahmad J. Improved pharmaco - kinetics and antihyperlipidemic efficacy of rosuvastatin-loaded nanostructured lipid carriers. J Drug Target 2017; 25, 58–74. *https://doi.org/10.1371/journal.pone.0061922*

35. Malkani A, Date AA, Hegde D. Celecoxib nanosuspension: Single-step fabrication using a modified nanoprecipitation method and *in vivo* evaluation. Drug DelivTransl Res 2014; 4:365–376. *https://doi.org/10.1007/s13346-014-0201-3*

36. Elbahwy IA, Ibrahim HM, Ismael HR, Kasem AA. Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles. J Drug Deliv Sci Technol 2017; 38: 78–89. *https://doi.org/10.1016/j.jddst.2017.02.001*

37. Kassem AM, Ibrahim HM, Samy AM. Development and optimisation of atorvastatin calcium loaded self-nanoemulsifying drug delivery system (SNEDDS) for enhancing oral bioavailability: *in vitro* and *in vivo* evaluation. J Microencapsul 2017; 34: 319–333. *https://doi.org/10.1080/02652048.2017.1328464*

38. Manjunath K, Reddy JS. Solid lipid nanoparticles as drug delivery system. Methods Find Exp Clin Pharmacol 2005; 27: 1–20. *https://doi.org/10.1358/mf.2005.27.2.876286*

39. Borhade V, Nair H, Hegde D. Design and evaluation of Self-Microemulsifying Drug Delivery System (SMEDDS) of Tacrolimus. AAPS PharmSciTech 2008; 9: 13–21. *https://doi.org/10.1208/s12249-007-9014-8*

40. Borhade V, Pathak S, Sharma S, Patravale V. Clotrimazole nanoemulsion for malaria chemotherapy. Part I: Preformulation studies, formulation design and physicochemical evaluation. Int J Pharm 2012; 431:138–148. *https://doi.org/10.1016/j.ijpharm.2011.12.040*

41. Das S, Ng WK, Tan RBH. Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): Development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? Eur J Pharm Sci 2012; 47:139–151.*https://doi.org/10.1016/j.ejps.2012.05.010*

42. Burra M, *et al*. Enhanced intestinal absorption and bioavailability of raloxifene hydrochloride via lyophilized solid lipid nanoparticles. Adv Powder Technol 2013; 24: 393–402.*https://doi.org/10.1016/j.apt.2012.09.002*

43. Wang Q, *et al*. Nanostructured lipid carriers as a delivery system of biochanin A. Drug Deliv 2013; 20:331–337. *https://doi.org/10.3109/10717544.2013.838716*

44. Date AA, Nagarsenker MS. Single-step and low-energy method to prepare solid lipid nanoparticles and nanostructured lipid carriers using biocompatible solvents. Eur J Pharm Res 2019; 1: 12–19. *https://doi.org/10.34154/2019-EJPR.01(01).pp-12-19/euraass*

45. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles in brain targeting. J Control Release 2008; 127: 97–109. *https://doi.org/10.1016/j.jconrel.2007.12.018*

46. Montenegro L, *et al*. From nanoemulsions to nanostructured lipid carriers: A relevant development in dermal delivery of drugs and cosmetics. J Drug Deliv Sci Technol 2016; 32: 100–112.*https://doi.org/10.1016/j.jddst.2015.10.003*

47. Noh GY, Suh JY, Park SN. Ceramide-based nanostructured lipid carriers for transdermal delivery of isoliquiritigenin: development, physicochemical characterization, and *in vitro* skin permeation studies. Korean J Chem Eng 2017; 34: 400–406. *https://doi.org/10.1007/s11814-016-0267-3*

48. Martins S, Tho I, Ferreira DC, Souto EB, Brandl M. Physicochemical properties of lipid nanoparticles: Effect of lipid and surfactant composition. Drug Dev Ind Pharm 2011; 37: 815–824.*https://doi.org/10.3109/03639045.2010.545414*

49. López-García R, Ganem-Rondero A. Solid lipid nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC): occlusive effect and penetration enhancement ability. J Cosmet Dermat Sci Appl 2015; 05: 62–72. *https://doi.org/10.1002/chin.200623260*

50. Jores K, Haberland A, Wartewig S, Mäder K, Mehnert W. Solid Lipid Nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and raman spectroscopy. Pharm Res 2005; 22: 1887–1897. *https://doi.org/10.1007/s11095-005-7148-5*

51. Teeranachaideekul V, Souto EB, Junyaprasert VB, Müller, RH. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q10-Development, physicochemical characteri-zation and *in vitro* release studies. Eur J Pharm. Biopharm 2007; 67: 141–148. *https://doi.org/10.1016/j.ejpb.2007.01.015*

52. Kaur IP, Bhandari R, Yakhmi. Lipids as biological materials for nanoparticulate delivery. Handbook of Nanomaterials Properties 2014. *https://doi.org/10.1007/978-3-642-31107-9* *https://doi.org/10.4103/0250-474X.57282*

53. Tran TH, *et al*. Preparation and characterization of fenofibrate-loaded nanostructured lipid carriers for oral bioavailability enhancement. AAPS Pharm Sci Tech 2014; 15: 1509–1515.*https://doi.org/10.1208/s12249-014-0175-y*

54. Hoppe K, Sznitowska M. The Effect of Polysorbate 20 on Solubility and Stability of candesartan cilexetil in dissolution media. AAPS Pharm Sci Tech 2014; 15: 1116–1125. *https://doi.org/10.1208/s12249-014-0109-8*

55. Hassan HA, Charoo NA, Ali AA, Alkhatem SS. Establishment of a bioequivalence- indicating dissolution specification for candesartan cilexetil tablets using a convolution model. Dissolution Technol 2015; 22: 36–43. *https://doi.org/10.14227/DT220115P36*

56. Zhang Z, Gao F, Bu H, Xiao J, Li Y. Solid lipid nanoparticles loading candesartan cilexetil enhance oral bioavailability: *In vitro* characteristics and absorption mechanism in rats. Nanomedicine Nanotechnology Biol Med 2012; 8: 740–747. *https://doi.org/10.1016/j.nano.2011.08.016*

57. Nekkanti V, Karatgi P, Prabhu R,Pillai R. Solid self-microemulsifying formulation for candesartan cilexetil. AAPS Pharm Sci Tech 2010; 11: 9–17. *https://doi.org/10.1208/s12249-009-9347-6*