Original Research Article

Screening Study forFormulation Variables in Preparation and Characterization of Candesartan Cilexetil Loaded Nanostructured Lipid Carriers

ABSTRACT

The current study inspects the screening of the formulation components further, evaluates the quality issues 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.Firstly, the solubility of CC in different solid and liquid lipids is the major parameter for the selection of the best one. Precirol® ATO 5, Compritol ® 888 ATO andGlyceryl 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. However, Precirol® ATO 5 was found to hasgood physical compatibility with Transcutol® HP,Compritol ATO 888 was found to hashighphysical miscibility with Labrasol® ALF and last GMS was appeared in good affinity and compatibly with Capryol™ 90. Hence, the following binary lipid mixtures (Precirol® ATO 5 - Labrasol® ALF), (Compritol® 888 ATO - Transcutol® HP) and (GMS - Capryol™ 90)were selected for the preparation of NLCs. The liquid–solid lipid mixture in the ratio up to 30:70 was observed to have sufficient melting point (55-59 ◦C). LutrolF-68, Lutrol F-127, Cremophore EL and Cremophore® RH. In addition to, the combination of(Lutrol® F68:Cremophore® EL)and (Lutrol® F127: Cremophore® RH) were selected as the main surfactants for the preparation of NLCs formulations because of its good emulsification efficacy and homogeneity for the solid-liquid lipid mix. The prepared formulationswereinvestigated for the different quality issues. 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 to27.3 ± 3.7 mV). 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.

Keywords: Candesartan Cilexetil,Encapsulation efficiency, In-vitro release, Solid lipid, Nanostructured lipid carriers.

1. 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[1,2]. Candesartan Cilexetil is radiallyhydrolyzed to active form Candesartan during absorption from gastro intestinal tract[3,4].

Candesartan Cilexetil owngreatdrawbacks which influence on its oral efficacy and therapeutic applicationsuch as very low aqueous solubility and first-pass metabolism. Consequently, it has very low oral bioavailability not exceed 15%[2,4–8].

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 generations first, solid lipid nanoparticles (SLN) and second, nanostructured lipid carrier (NLCs) canimprove the lymphatic transport of the lipophilic drugs as CC and hence, increase its oral bioavailability [9–11]. LNs systems were recorded as an advanced drug carrier system than polymeric nanoparticle[12,13].

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 [14].

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[11,15–17]

Generally, nanostructured 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 aproper 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 themwere 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.

2. Materials and methods

The active Candesartan Cilexetil (CC) wasobtained as a gift fromMEMPHIS, El-Amirya – Cairo –EGYPT.

Compritol® 888 ATO (glyceryldibehenate), Precirol® ATO 5 (Glyceroldistearate type I), Maisine® CC (glycerylmonolinoleate), Labrafac™ PG (propylene glycol dicaprylate/ dicaprate), Labrafac™ CC (caprylic/ caprictriglycerides), Labrafac™ Lipophile WL 1349 (medium-chaintriglycerides), 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 triglycerideswith PEG esters of fattyacids), Gelucire® 39/01 pellets (Glycerol esters of saturated C12-C18 fattyacid ester), Transcutol® HP (Highlypurifieddiethylene glycol monoethylether), Labrafil® M 1944 CS (oleoyl macrogol-6 glycerides), Labrafil® M 2125 CS (linoleoyl macrogol-6-glycerides, corn oil PEG-6-ester), Peceol™(glycerylmonooleate), and Labrafil® M 2130 CS (lauroyl macrogol-6-glycerides) werekindlyprovided as a gift samplesfromGattefosse (France).

Glycerylmonostearate (GMS), Stearicacid, Oleicacid, Soybeanoil, Pluronic® F68 (polyoxyethylene-polyoxypropylene (150: 29) block copolymer), Pluronic® F127, Tween® 80 (polyoxyethylene (20) sorbitanmonooleate), Tween® 40 (Polyoxyethylenesorbitanmonopalmitate), Carboxymethylcellulose (CMC).

Phospholipon® 90 G (soy phosphatidylcholine) wasgiven as a gift fromLipoid, Ludwigshafen (Germany).

Cremophor® RH 40 (polyoxyl- 40- hydrogenated castor oil) and Cremophor® EL (polyethoxylated castor oil) weresuppliedfrom BASF (Germany).

Sodium hydroxide, potassium dihydrogen orthophosphate, Potassium Chloride and

Hydrochloricacid (HCL) weresupplied by El-Nasr Pharm. Chem. Company, Cairo (Egypt).

Membrane filter (0.45 µm) Millipore Iberica S.A.U. ; Madrid (Spain).

Methanol and Acetonitrile HPLC grade werepurchasedfrom Sigma Aldrich (USA).

All the above materials were in analytical grade and were used without further purification.

2.1. Selection of solidlipid

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. [18–20].

2.2. Selection of liquidlipid

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 37°C with continuous agitation at 100 rpm [14,21–24]. 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 254nm.

2.3. Physical compatibility of solid and liquidlipid

The miscibility of Selected Solidlipids and liquid lipids which possess the maximumaffinityforthedrug 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 43 ^{o}C which did not reveal any residue of oil droplets on the filter paper was selected for the development of CC – loaded NLCs [11,24–26]

2.4. Selection of a binary lipid phase ratios

The ratio of selected solid lipids and liquid lipids was determined based on the meltingpointofthe binary lipid mixture.Selectedsolidand 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 left to solidify at room temperature. The capillary method was used to determine the melting points of the congealed lipid mixtures. [27,28].

2.5. Selection ofsurfactant

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)wasdissolvedin3mLofmethylenechlorideandaddedto10mLof 5%surfactantsolutionsthen stirred by applyingmagneticstirrer.The organiclayerwas evaporatedat40°Candtheremainingsuspensionsweredilutedwith 10-fold distilled water. The transmittance percent of the resultant sampleswasdeterminedusingUV-Visspectrophotometerat510nm [22,24,29].

2.6. 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 [27,30–32]. 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.

2.7. Physicochemical characterization of CC-NLCs

2.7.1. Particle size and polydispersity index

The meandiameter and polydispersity index of particle of nanostructuredlipid carriers loadedwith CC wasdeterminedusing a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire (UK), equippedwith a 10 mW He-Ne laser employing the wavelength of 633 nm and a back-scattering angle of 90° at 25 °C. Before Photon correlationspectroscopic (PCS) analysis, CC-NLCs formulations shouldbedilutedwith a certain amount of double-distilled water (1:200) to getappropriatescatteringintensity. The analysis, of Particle size wasdeterminedusing Mie theorywith the refractive index and absorbance of lecithin at 1.490 and 0.100, respectively[33–36]

2.7.2. 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) [34,37].

2.7.3. Encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency and loadingcapacity of CC into NLC formulations weremeasured by the indirect method by measuring the concentration of the free CC. Initially, 2 ml of NLCs formulations werecentrifuged at 100,000 rpm for 1 h at 4 °C to evaluate the unentraped CC usingcooling ultracentrifuge (Beckman Instruments TLX-120 Optima Ultracentrifuge) [38–41]. The aqueous layer wasaspirated and filteredusing Millipore® membrane (0.2 μm) and dilutedwith an appropriateamount 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 loadingcapacity of CC intoNLCsweredeterminedthrough the followingequations

EE% = [(weight of initial drug - weight of free drug) / (weight of free drug)] X 100,

LC% = [(wt. of drug in nanoparticles) / (wt. of nanoparticles)] X 100.

2.7.4. *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 [42–44]. The donor compartment is cellulose membrane dialysis bags (MWCO‑12 000, 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.

3. RESULTS and DISCUSSION:

3.1. 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 [45]. 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 ofGelucire® 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. 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 [46–48]. Gelucire® 44/14 is Polyoxylglycerides mixture [49], Precirol® ATO 5composed of mixture of palmitostearate glyceride and Compritol® 888 ATO composition is mixture of behenate glyceride;[50] while Glyceryl Monostearate (GMS) is mixture of variable proportions of glyceryl monostearate and glyceryl monopalmitate[49].

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) [48,51] In addition,

monoglycerides possess emulsification properties [52] which can also improve the drug solubility, such explain the potentiality of Precirol ATO 5® 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 [23,53–55]..

3.2. 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 [5,51,56]. Also, higher drug solubility in the oil phase brings down the necessities of surfactants in this way limiting their toxic impacts [56].

The solubility of CC in various liquid lipids were showed in figure (2).

It was evident that CC revealed highest solubility in peppermint oil (48 ± 2.14mg/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™Lipophil 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) [57] 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) [58].

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™LipophilandLabrafac™ CC) due to their known surfactant properties [39,59].

The high solubilizing effect of Transcutol® HP for CC is consistent with Cirri et al., 2018 [29]. 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, [60]. 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.

3.3. 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 [61,62]. 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.

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. Doktorovov et al 2010 [59,63]. 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.

3.4. 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 [24]. 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°C) (data not shown). Furthermore, the increment of liquid lipid concentration, the melting point of the blends were beneath the ideal level. In addition, 70:30 the most appropriate and common ratio and widely applied by most previous related studies [37,64–67]. Consequently, 70:30 was chosen as the proper preparing ratio for the solid-liquid lipid mixture in all NLC formulations.

3.5. 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. [22]. 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 [39,68].

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 % transmittance), 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 smalle particles. [23,69].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 (HLB = 15) >Cremophore® EL (HLB = 12-14) >Phospholibon® (HLB = 8) [23,70,71]. 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 [72].

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 were another factor such as the chemical structure of surfactants had a great impaction on the nanoemulsification 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 2012, 2008 and Kassem et al 2017 [73–75] 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.

3.6. 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 [39,76]. 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 [22,77–79].

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 [23,70,71]. Also, branched alkyl chain structure, as well as the length of hydrophobic chains of surfactants, had a countless effect on the nanoemulsion formation [73–75] as Cremophore® EL and Cremophore® RH.

hence, based on the introduced emulsification study of surfactants, Lutrol® F68 andLutrol® 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.

3.7. 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 2012 and Elbahwy et al 2017 [72,80] 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) [81]. 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.

3.8. Physicochemical characterization of CC-NLC formulations

3.8.1. 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, the 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 [82,83].

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 [22,77–79,83].

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 [84,85].

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 [86]. 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.

3.8.2. Zeta potential (ζ) 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 [87,88]. From the factors which mainly influence zeta potential of lipid-based nanoparticles structure of solid and liquid lipid and the medium composition [65,88]. 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 [6,36,38,81,89]. 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 ofglyceryl tripalmitostearate (25% - 35%), glyceryl dipalmitostearate (40% - 60%) and glyceryl monopalmitostearate (8% - 22%) [49,90] and Compritol® 888 ATO composed of glyceryl tribehenate (28% - 32%), glyceryl dibehenate (52% - 54%) and glyceryl monobehenate (12% - 18%) [49,88], 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%) [49]. 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,* 2008and López-García and Ganem-Rondero, 2015[88,91] 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 [59,92].

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^{TM} 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, 2007 [93], these revealed the higher ZP values of GMS nanoparticles than other nanoparticles.

3.8.3. 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 [94], physical and chemical nature of the lipid matrix and crystalline state of lipid matrix and also surfactant was found to affect encapsulation efficiency [38,46,95].

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 matrix [6,96]. 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 accommodated [14,38,83,96].

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 [23,45]. 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 [23,47,54].

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 [38].

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.

3.8.4 *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 [43,97]. It was found that all formulations exhibit a lack of drug release within 24 h except CC suspension showed almost complete drug release (100%) within 8 h. As CC had solubilities equal to 11 μg/mL in 0.1 M HCl and 1 μg/mL in PBS (pH 6.8)[8,98], 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 [8,99]. These observations are in line with the study reported by Zhang et al 2012 [8]. Previous studies ascertained that NLCs must be absorbed into the blood or lymphatic system after duodenal administration to rates [100]. 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 conformation through employing more investigations in next 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 (ζ), encapsulation efficiency and loading capacity of CC-NLCs wherein the formula F9 was chosen as the best formula for further investigations.

4. CONCLUSION

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 workclarifies 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 physicochemical characterization. The resulted formulations appeared in nanoparticle size with high encapsulation efficiency.

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Tables

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

SL: LL in ratio	SAA 10 ml of 5% soln.	Transmittance ± SD %
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

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

Table (3): Suggested formulae of CC-NLCs

F No.	SL (70%) (5%) of Total Formula (g)			LL (30%) (5%) of Total Formula (g)			SAA (2.5%) of Total Formula (g)				Drug (5%) of Lipids (mg)	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	
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.

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

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

Figures

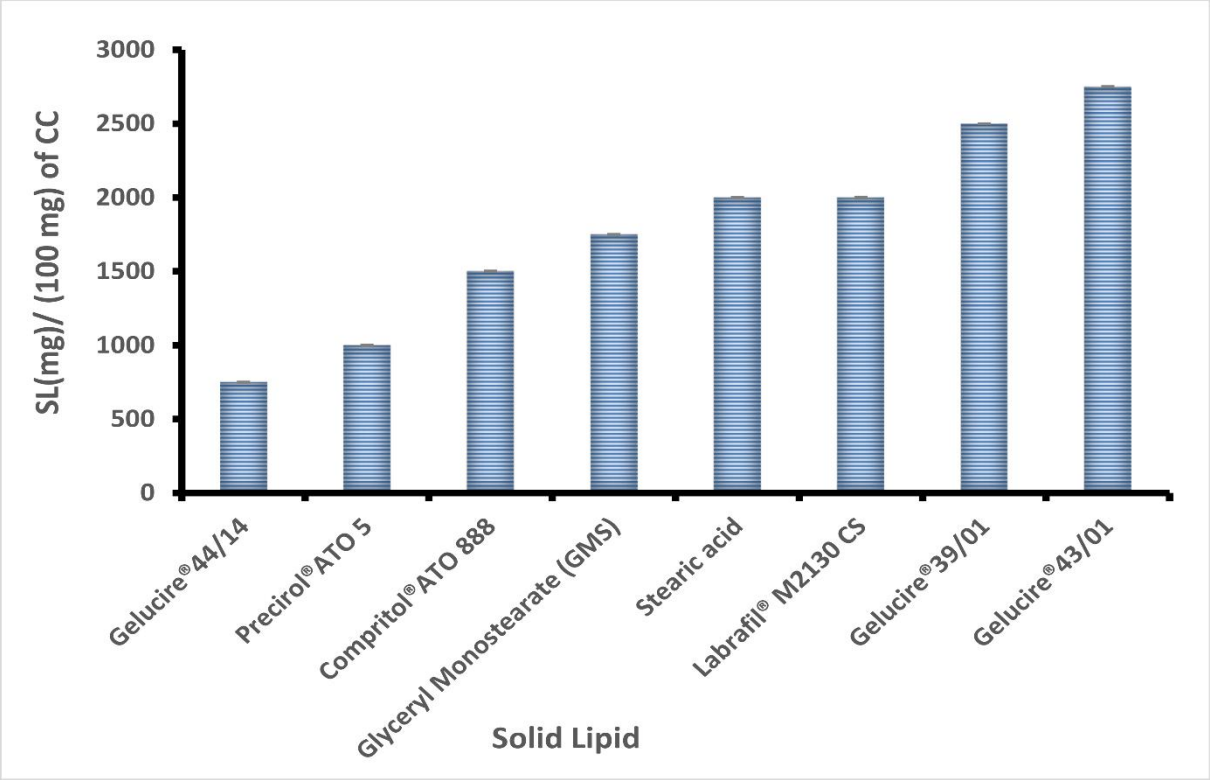

Figure (1): Solubility study of CC in different solid lipids

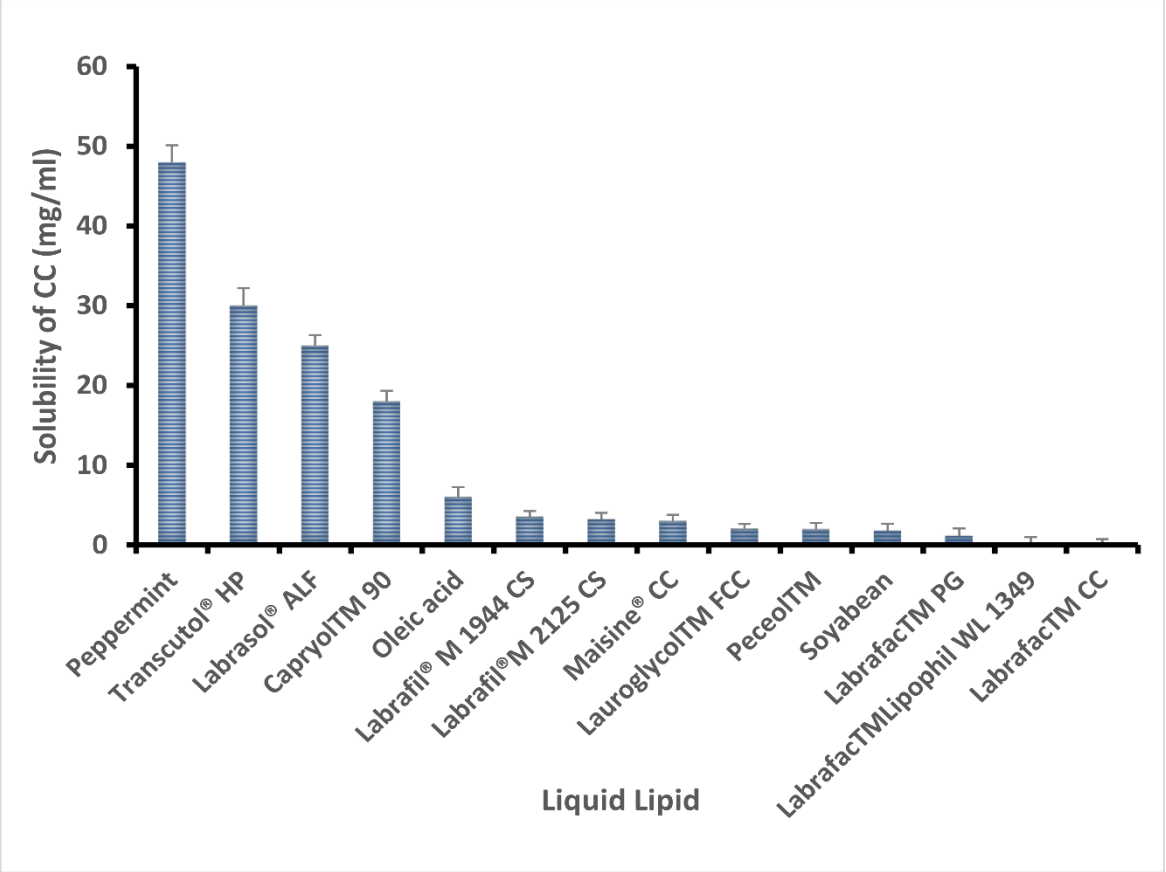

Figure (2): Solubility study of CC in different liquid lipids

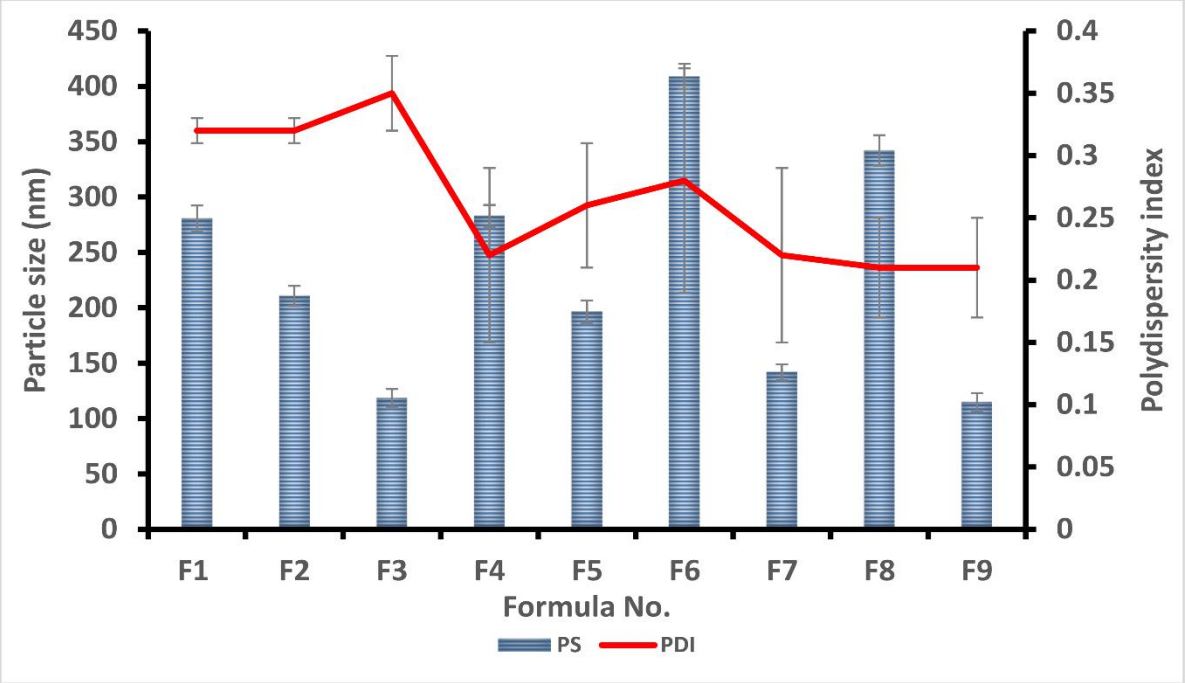

Figure (3): Mean particles size and polydispersity index of CC-NLCs formulations

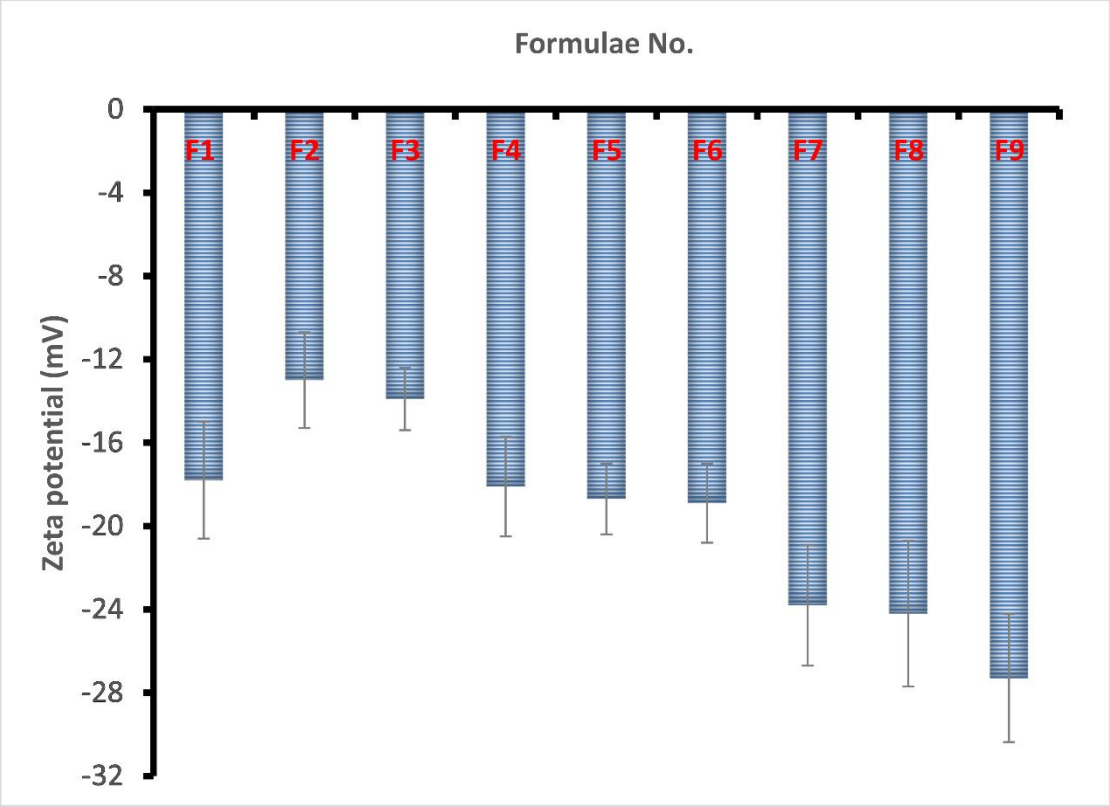

Figure (4): Zeta potential of CC-NLCs formulations

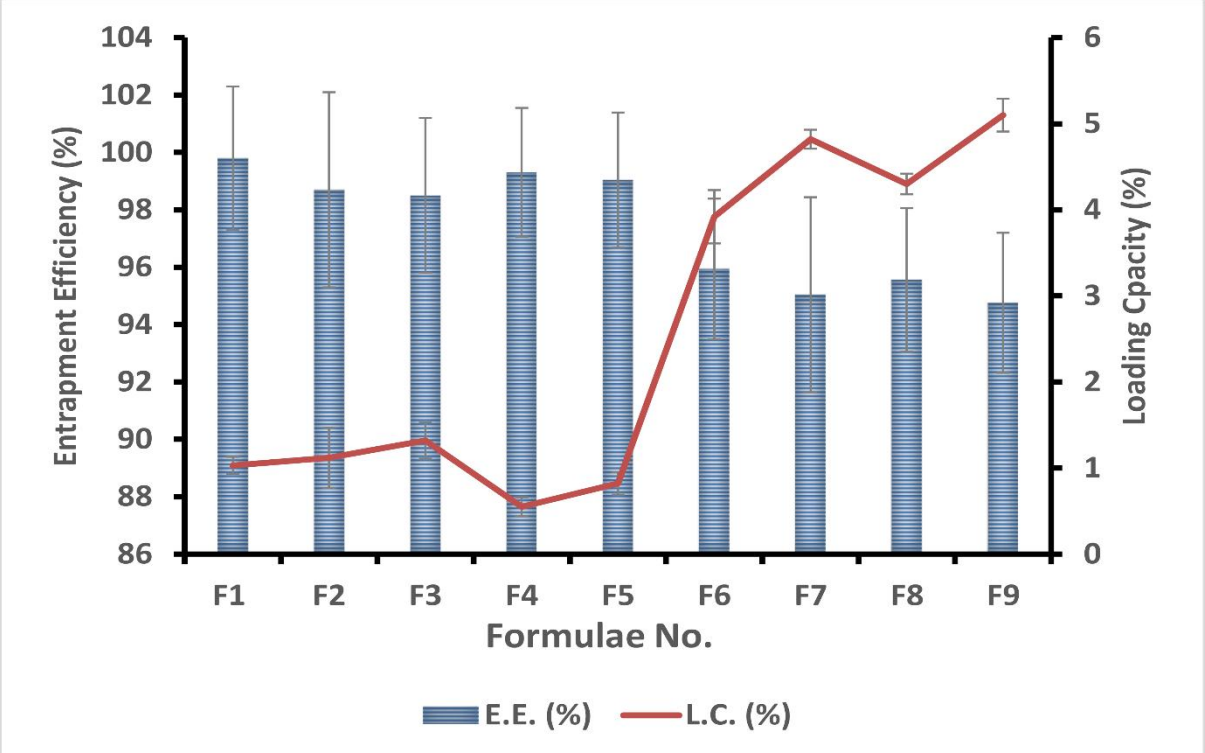

Figure (5): Encapsulation efficiency and loading capacity of CC-NLCs formulations