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Original Research Article

Preparation and evaluation of carvedilol-loaded solid lipid nanoparticles for targeted drug delivery

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Abstract

Purpose: *To develop suitable solid lipid nanoparticles (SLN) containing carvedilol (CL) for controlled delivery to site of action.*
Methods: *Solid lipid nanoparticles (SLNs) containing carvedilol (CL) were prepared by hot homogenization and ultrasonication methods. The SLNs were characterized in terms of entrapment efficiency, particle size, zeta potential, polydispersity index, cytotoxicity, solid state characterization and drug release. The stability of the formulations was investigated by monitoring their properties for a period of 3 months.*
Results: *The mean size of the nanoparticles was in the range of 130.70 ± 1.80 to 154.40 ± 2.40 nm. Solid state analysis showed that carvedilol was uniformly dispersed in the lipid nanoparticles. Drug entrapment efficiency ranged from 96.03 ± 0.13 to 93.46 ± 0.21 % while in vitro cumulative drug release from the nanoparticles in simulated intestinal fluid (SIF) and phosphate buffer containing 30% PEG (pH 6.8) was 96.57 ± 0.40 and 75.13 ± 0.15 %, respectively, at the end of 24 h. In vitro release of carvedilol from SLNs followed fist order kinetics and Higuchi diffusion model.*
Conclusion: *The SLNs developed in this study represent a promising safe system for the sustained and controlled delivery of carvedilol.*

Keywords: *Carvedilol, Solid lipid nanoparticles, Antihypertensive, Sustained release*

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INTRODUCTION

Cardiovascular diseases cause a significant number of deaths around the world. Considering the number of patients worldwide who experience cardiovascular problems, concerted efforts have been made to develop drugs to prevent and/ or treat cardiovascular diseases. Consequently, there are now several drugs that are available in the market, including carvedilol (CL) [1] which is used for the management of cardiovascular diseases.

CL, (±)-1-(carbazol-4-yloxy)-3-((2-(o-methoxy-phenoxy)ethyl)amino)-2-propanol, is a non-selective β-blocking agent with vasodilating action due primarily to its blockage of α_1 – receptors. This makes it usefull for the treatment of hypertension and angina pectoris [2,3]. However, preliminary data suggest that it is a strong β-adrenergic antagonist but a weak vasodilator [4].

Although CL is well absorbed in the gastrointestinal tract, it is extensively metabolized in the liver leading to decreased bioavailability of about 25 % [5]. The oral bioavailability remains low because of significant

first-pass hepatic metabolism by cytochrome P450 and also because of its short plasma half-life [6]. Long-term therapy of cardiovascular diseases by orally administered CL may result in poor patient compliance because of low bioavailability.

Lipid particles are of great importance to drug researchers and developers because of their ability to avoid hepatic first pass metabolism and because they enhance intestinal permeability [6,7].

Various controlled delivery systems have been developed from lipids and introduced with the aim of improving solubility, stability and bioavailability of poorly absorbed drugs [8]. Solid lipid nanoparticles (SLNs) have received increased research interest in recent years due to their superior characteristics and advantages over biopolymer-based colloidal nanoparticles [9]. Solid lipid nanoparticles are evaluated as alternative drug delivery system in order to bypass the first pass metabolism [10]. As a way of improving the bioavailability of orally administered CL, drug-loaded solid lipid nanoparticles were developed using trimyristin as lipid and polyethylene glycol sorbitan monooleate as surfactant. It has also been shown to be non-toxic for human use and officially recognized as a pharmaceutical excipient [5]. In this study, carvedilol-loaded SLNs were prepared using hot homogenization and ultra-sonication method, and studied.

EXPERIMENTAL

Materials

Carvedilol was a kind gift from Santa-Farma (İstanbul, Turkey). Dynasan 114 (glyceryl trimyristate) was purchased from Condea (Witten, Germany), and Tween 80 (polyoxyethylene-80 sorbitan monooleate) from Merck (Hohenbrunn, Germany). Purified water (MilliQ, Millipore,USA) was used whenever water was required throughout the study. All other chemicals were of analytical grade.

Preparation of carvedilol loaded solid lipid nanoparticles

SLNs were prepared by hot homogenization followed by ultrasonication method based on the formula given in Table 1. Lipid (5 %) was melted at about 75 ºC (10 ºC above melting point of the lipid) and the drug (carvedilol, 5 % relative to lipid) was added to obtain a clear melted solution. An aqueous phase was prepared by dissolving surfactant (Tween 80, 2 - 5 %) in 10 ml double-distilled water and heated to same temperature of the oil phase. The hot aqueous phase was added to the oil phase and homogenization was carried out at a pestle speed of 8000 rpm at a temperature 75 ºC, using Ultra Turrax® T 25 basic homogenizer (IKA, Staufen, Germany) for 5 min. Hot oil in water emulsion was obtained and the mixture was sonicated for 5 min at 60 % power using a sonicator (Sonics, USA). Carvedilol loaded SLNs were obtained by allowing hot nanoemulsion to cool to room temperature. Thermal stability of carvedilol was not evaluated because published reports attest to the thermal stability of carvedilol [11].

Table 1: Composition of carvedilol-loaded solid lipid nanoparticles

Ingredient (% w/w)	Carvedilol (%)	Dynasan 114 (%)	Tween 80 (%)	Distilled water (%)
F1	0.05	0.5	0.2	9.25
F2	0.05	0.5	0.3	9.15
F3	0.05	0.5	0.4	9.05
F4	0.05	0.5	0.5	8.95
Placebo	-	0.5	0.4	9.10

Evaluation of particle size and zeta potential

Mean diameter of the bulk population and the particle distribution via the polydispersity index (PI) and zeta potential of SLNs were analyzed by a Zetasizer Nano ZS (Malvern Instruments, UK). Distilled water with a conductivity value of 50 µS/cm was adjusted using sodium chloride (0.1 N) at pH 7.4 and used in zeta potential analyses. Electrostatic mobility was converted to zeta potential using Helmholtz-Smoluchowski equation. For the analyses, constant volume (20 µL) of formulations were dispersed in 1 mL of distilled water and particle size (PI) and zeta potential were recorded (n=3).

Scanning electron microscopy

The morphology of placebo and CL-loaded SLNs were determined with a scanning electron microscopy using Carl Zeiss Microscopy (SUPRA 50VP model, Oberkochen, Germany) instrument. Solid lipid nanoparticles were deposited on metallic stubs placed in liquid nitrogen and dried under vacuum. Then sputter was coated with gold in a cathodic evaporator [12].

Solid-state characterization

Differential scanning calorimetric curve of pure carvedilol, polymer and mixture of drug and polymer measurement were carried out by using

Shimadzu (DSC-60, Shimadzu, Japan) instrument equipped with a liquid nitrogen sub-ambient accessory. The samples (2-6mg) were accurately weighed in aluminium pans hermetically sealed and heated at a rate of 10 ºC per min in a 30 to 250 ºC temperature range, under nitrogen flow of 50 mL min^{-1}. Temperature-dependent structure and crystallinity changes in the lipids were analysed.

Characteristics of the solid lipid structures of SLNs as well as stability changes in CL after the preparation of the formulation was also analysed using powdered X-ray diffractometry (PXRD), product of Rigaku, Tokyo, Japan. The data were recorded over the 2θ range from 3° to 45° at a scan rate of 0.04°.

In order to examine the lipid matrix of the improved SLNs dispersion relative to the pure drug and pure solid lipid, ^{1}H- NMR techniques were used to examine the possible effect of CL on the crystallization process. High-resolution proton nuclear magnetic resonance (^{1}H-NMR) spectra of samples prepared, were obtained on an NMR instrument (Ultra Shield CP MAS, Germany) operating at 500 MHz and 20°C. NMR analysis (^{1}H-NMR) of the solution was performed after dissolving the materials in deutero chloroform ($CDCl_3$).

Interaction of drug with the lipid was determined by FT-IR Spectroscopy (Shimadzu IR-Prestige-21, Japan). The pellets were produced by mixing 1 mg of sample with 200 mg potassium bromide at high compression pressure. The scan range selected was 400 to 4000 cm^{-1} and the revolution was selected was 4 cm^{-1}. The pellets were examined and the spectra were compared between pure drug, lipid and the formulations [13].

Assessment of entrapment efficiency

The entrapment efficiency (EE) of carvedilol-loaded SLNs was determined by measuring the concentration of drug encapsulated within and adsorbed onto the nanoparticles. About 1 ml of CL-SLNs dispersion was diluted using 5 mL of phosphate buffer (pH 6.8) containing 30 % PEG 400 and centrifuged at 11,000 rpm (Eppendorf Centrifuge 5417R, Germany) for 15 min. The amount of free drug in the supernatant was estimated by using HPLC at wavelength of 242 nm, and EE was calculated using Eq 1.

$$EE\ (\%) = \{(WA - Wb)/WA\}100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where *W*A is the amount of initial drug used for the assay, and *W*b is the amount of free drug in the supernatant after centrifugation of the aqueous dispersion [14].

Stability studies

Formulations were stored in amber bottles at different temperatures and relative humidity. The effects of duration of storage and storage condition on entrapment efficiency were calculated. Stability studies revealed the physical stability of these lipid particles after 3 months of storage at different temperatures. The SLNs were evaluated for particle size, zeta potential value, PDI and EE on 30th, 60th and 90th days.

***In vitro* drug release studies**

In vitro drug release of CL from optimized SLN formulations were studied through dialysis bag (cellulose membrane) which was sealed with clamps and stirred at 50 rpm using magnetic stirrer. The temperature was maintained at 37 ± 0.5°C under sink conditions. The dispersion was transferred into dialysis membrane which was previously soaked in double distilled water for 12 hours, tied properly at both the ends and kept inside the glass. The *ın vitro* release studies were conducted both in simulated intestinal fluid (SIF) and phosphate buffer (pH 6.8) containing 30 % PEG 400.

The dialysis medium (50 mL) was stirred at 100 rpm over a magnetic stirrer maintained at 37 ± 1 °C. At pre-determined time intervals, 1-mL samples were withdrawn from the dissolution media respectively and analyzed for the drug content using HPLC (Shimadzu Corporation, Kyoto, Japan) at 242 nm and fresh 1 mL, of each medium was added to the appropriate system. By determining the amount of CL released at various time intervals, the % release versus time graphs were plotted for the formulations.

The drug content of each formulation was determined by HPLC (Shimadzu Corporation, Kyoto, Japan). The mobile phase was a mixture of 0.03M potassium dihydrogen phosphate (pH 3.0) buffer: acetonitrile: methanol (60:50:10, v:v:v:), prepared daily and de-gassed by sonication and filtered through 0.45 µm membrane filter before the experiment. The flow rate was set at 1.0 mL/min resulting in a run time of 10 min per sample. The injection volume was 20 µL. Detection was performed at 242 nm and samples were analyzed at room temperature [15]. In order to study the mechanism of drug release from the SLNs, the release data were fitted to different equations with DDSolver programme [16].

Evaluation of cytotoxicity

Colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for the quantitative determination of cytotoxicity of the formulations on 3T3 mouse embryo fibroblast cell lines. The cells (2×10^4) were seeded in a 96-well plate (Greiner, Sigma-Aldrich, Germany), with RPMI 1640 (Sigma Aldrich, Germany) medium. The RPMI 1640 medium was supplemented with 10 % FBS and 1 % antibiotics (penicillin/streptomycin). The cells were incubated at 37 °C for 24h in a humidified atmosphere containing 5 % CO_2.

At the end of the 24-hour incubation, the formulations, with the cell culture medium were added to the cells at the rates determined. The cells were left for 24 and 48 hours of incubation. At the end of the 24 and 48 h incubation periods, the formulations were withdrawn from the wells and 20 µL of MTT dye (Greiner, Sigma-Aldrich, diluted with PBS to 5 mg/mL concentration) solution was added. The plates were incubated for 4 h under the same conditions earlier mentioned. Thereafter, 200 µL spectrophotometric DMSO was added to each well to dissolve the resultant formazan crystals. After 30 min of incubation, absorbance of the content of each plate was measured at 570 nm using a multi-mode microplate reader (Cytation 5, BioTek Instruments, Germany) [17].

Statistical analysis

Each experiment was carried out three independent times and the data are presented as mean ± SEM (standard error of mean). Statistical analysis of the data were carried out using SPSS software, version 11.5. Differences were considered statistically significant when the *P*-value was less than 0.05.

RESULTS

Physicochemical characteristics of SLNs

The results of the particle size and other characteristics of the formulations analysed are presented in Table 2. The particle size of all the formulations ranged from 130 to 154 nm; PDI of formulations ranged from 0.147 to 0.247, while the zeta potential were from −14.7 to −27.7 mV. From the results obtained, formulations containing 4 % Tween 80 (F3) showed relatively lower particle sizes. The optimum formulation was selected as F3 because of the smaller particle size.

Table 2: Particle size, zeta potential and PDI of carvedilol-loaded SLNs

Formulation code	Particle size (nm)	Zeta potential (mv)	PDI
F1	136.7±2.4	-5.6±1.45	0.228±0.032
F2	135.6±1.7	-4.7±1.06	0.247±0.051
F3	130.7±1.8	-2.7±1.76	0.197±0.032
F4	154.4±2.4	-3.9±1.68	0.150±0.129
Placebo	138.4±1.5	-8.4±1.56	0.147±0.051

Figure 1 shows the images of surface morphology of placebo, pure CL, pure lipid and optimum CL-SLN formulations. The results showed that the particles were flakes with a narrow size distribution, and had smooth surfaces. Particle size results and SEM images pointed out that particles were different not only in size, but also in shape., Carvedilol pure powders had a non-uniform shape, while SLN formulations had a flaky shape [18,19].

The solid state of CL in formulation was measured by differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD).

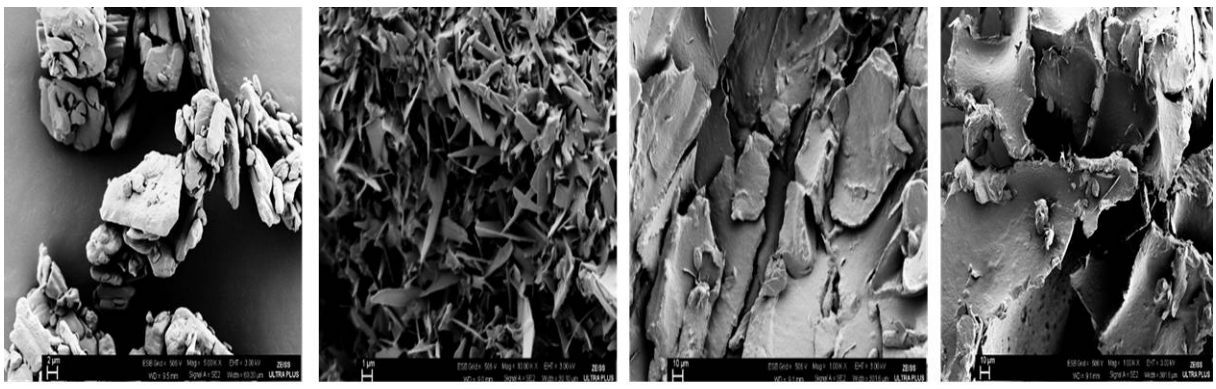

Figure 1: SEM images of CL (a), Dynasan-114 (b), Placebo (c), F3 (d)

As shown in the DSC patterns (Figure 2), pure carvedilol showed a pure and clear endothermic melting peak at 116.7°C, which is indicative of the crystalline.

Characteristics of CL

DSC studies were performed to confirm the absence of drug-excipient interactions. The DSC study revealed the absence of any chemical interaction between CL and lipids. CL endothermic peak was not observed in DSC thermogram of SLN formulations owing to the molecular inclusion of CL in the lipid matrix of Dynasan-114. This suggests that CL exists in amorphous state in SLNs. After incorporation into SLN formulations, the characteristic peak of CL disappeared completely.

In order to confirm the physical state of carvedilol, PXRD measurement was performed. Figure 3 shows the PXRD curves of pure carvedilol, Dynasan-114 powder, lyophilized CL-SLN.

In Figure 3, pure CL and lipid showed compact and characteristic diffraction peaks, respectively. However, no sign of the typical crystalline peaks of CL was observed for both placebo and SLN formulations. The results obtained from DSC and PXRD combined, show that carvedilol lyophillized formulations were in the amorphous state. Numerous diffraction peaks of carvedilol were observed at 2θ of 12.4°, 24.4° and 26.1°, and this reveals the crystalline nature of carvedilol [1,18,19].

The ^{1}H- NMR measurements of pure Carvedilol, Dynasan-114, Placebo and Cl-SLN were as seen in Figure 4 [19].

Analysis of the FTIR spectra as seen in Figure 5 revealed absorption bands at 3345 cm-1 corresponding to the stretching of the NH and OH peaks merged together. İn addition, CL samples showed bands at 2924 cm-1, related to C-H stretching, and at 1598 cm^{-1}, related to the bending vibrations of the NH group. A few characteristic peaks of CL were seen in both CL-SLN FT-IR patterns. Compared to pure lipids, and placebo formulation, FT-IR peaks of F3 CL-SLN were less intense.

Entrapment efficiency and stability

Entrapment efficiency is an important parameter for characterizing the SLN formulations. F3 formulation was analyzed for entrapment efficiency by HPLC, and the results are shown in Table 3. From the results obtained, the formulation showed good entrapment efficiency of 96.03 ± 0.13 %.

Stability studies were conducted for optimized formulation (F3) which showed better size, better PDI, better zeta potential, and better EE.

The kinetic models used were zero-order, first-order, Higuchi and Korsemeyer-Peppas models.

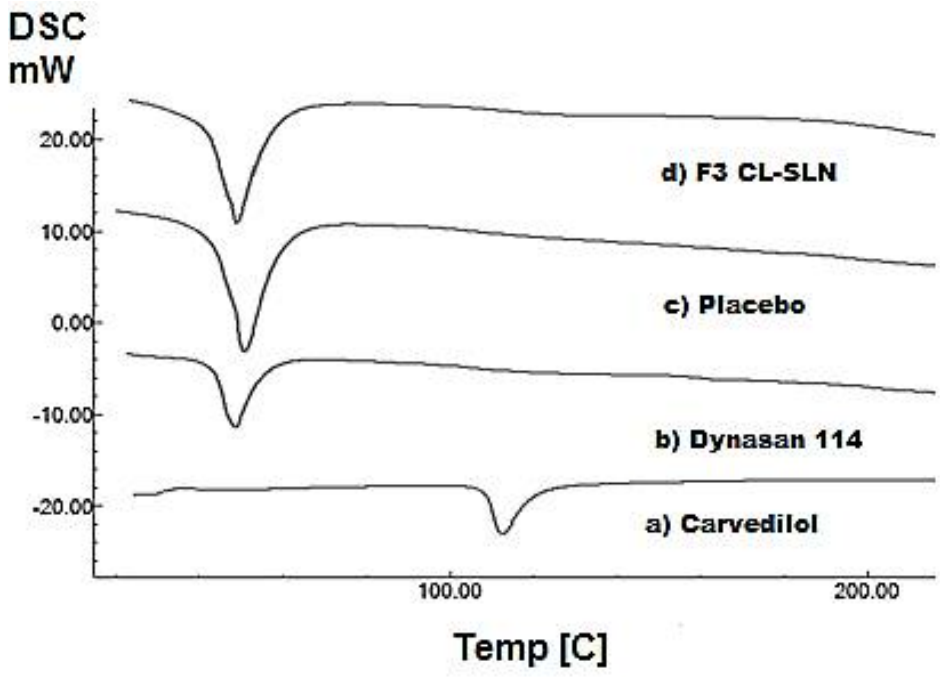

Figure 2: DSC profiles of; CL (a), Dynasan-114 (b), Placebo (c), F3 (d)

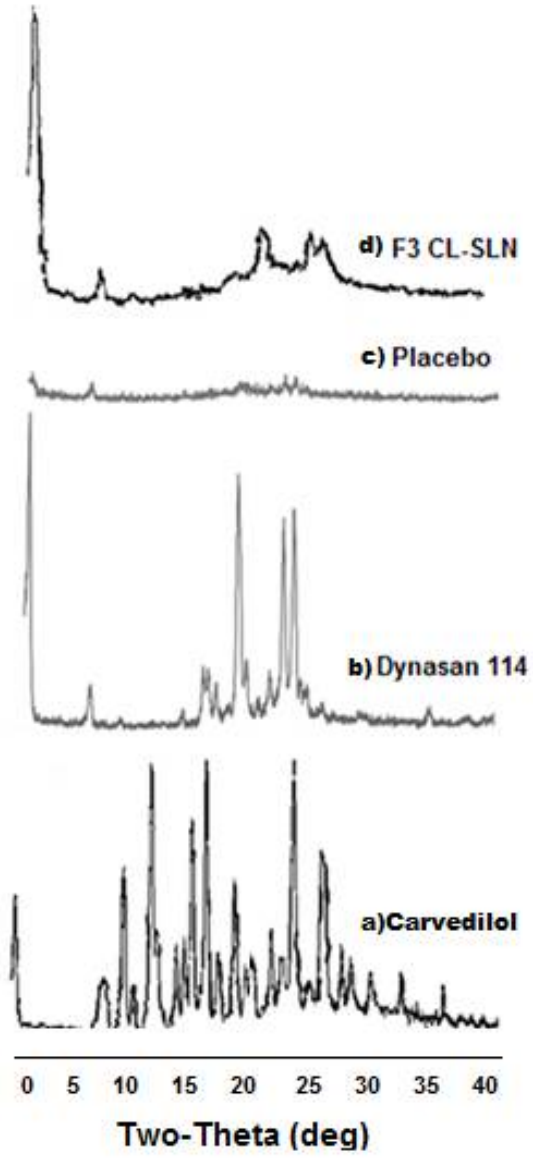

Figure 3: PXRD patterns of; CL (a), Dynasan-114 (b), placebo (c), F3 (d)

In vitro drug release

The results from studies on release of carvedilol from SLN optimum formulations (F3) both in simulated intestinal fluid (SIF) and in phosphate buffer + 30 % PEG 400 showed that there was controlled release of drug from the solid lipid nanoparticles. An amount of 75.13 ± 0.15 % of carvedilol was released in SIF in 24 h, while 96.57 ± 40 % of carvedilol was released in phosphate buffer + 30 % PEG 400 in 24 h. When compared, the cumulative release in pH 6.8 phosphate buffer was more than that of SIF.

In order to study the mechanism of drug release from solid lipid nanoparticles and release of pure drug, the release data were fitted to different equations with DDSolver programme and the results are presented in Table 4.

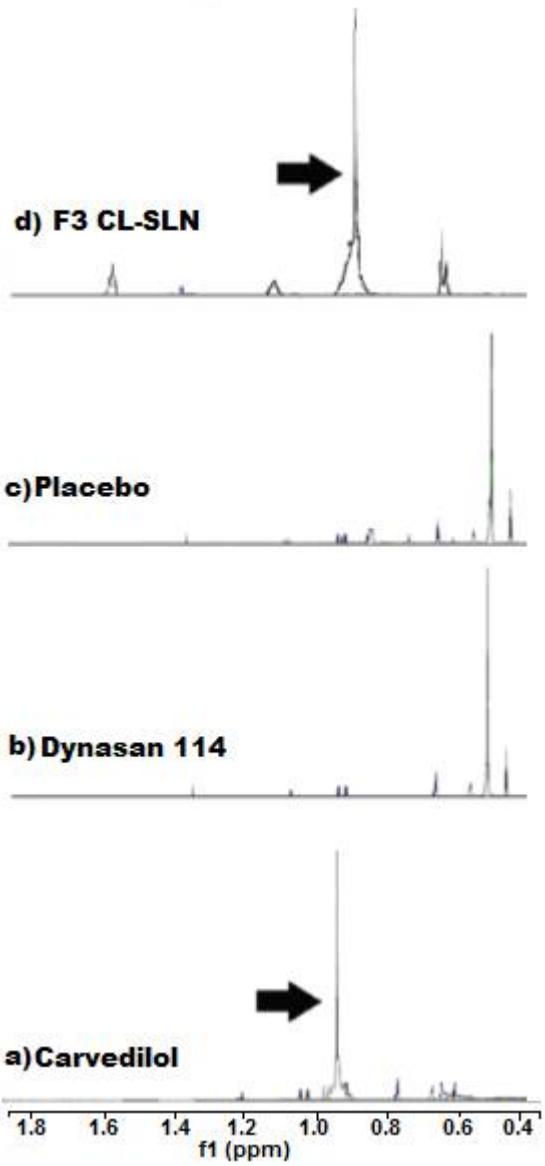

Figure 4. Proton nuclear magnetic resonance spectroscopy (^{1}H- NMR) spectra of CL (a), Dynasan-114 (b), placebo (c), F3 (d)

In vitro cytotoxicity

The results obtained from SLN and carvedilol application to fibroblast cells after 24 and 48 hours are presented in Figure 7.

Based on the 24 h cytotoxicity test results (Figure 7(A), Dynasan showed cell viability over 50 % (59.50 ± 1.54 %) even at the highest doses. CL and CL-loaded solid lipid nanoparticles begun to show high cytotoxicity after 25 µg/mL, with IC_{50} values of 41.3 ± 2.30 and 38.5 ± 1.32 µg/mL, respectively).

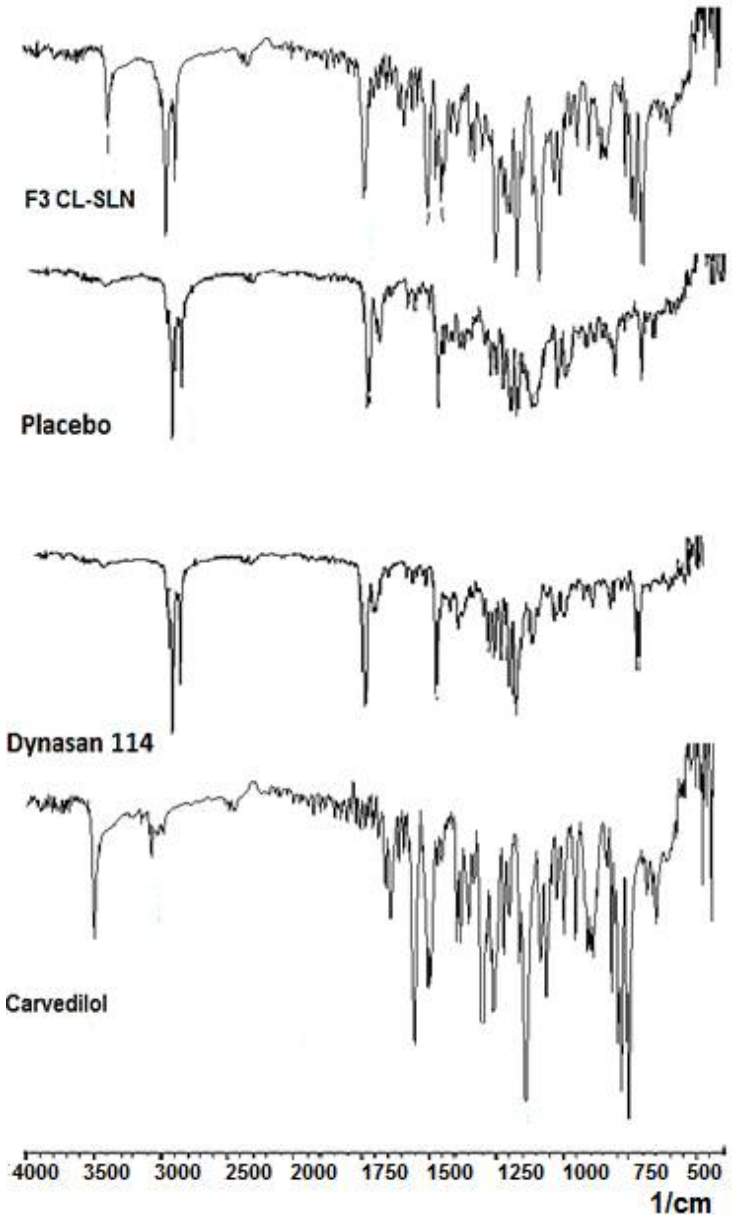

Figure 5: Infrared absorption spectra of CL (a), Dynasan-114 (b), placebo (c), and F3 (d)

Table 3: Stability results of optimized (F3) CL-SLN formulation (ZP: Zeta potential)

DAY	25°C/60 ± 5%RH				4°C			
	Size (nm)	***ZP (mV)***	***PDI***	***% EE***	***Size (nm)***	***ZP (mV)***	***PDI***	***% EE***
0	130.7 ± 1.8	-22.7 ± 1.8	0.197 ± 0.030	96.0 ± 0.1	130.7 ± 1.8	-22.7 ± 1.8	0.197 ± 0.030	96.0 ± 0.1
30	132.7 ± 1.4	-21.2±1.7	0.185 ± 0.060	94.1 ± 0.2	130.9 ± 1.6	-22.10 ± 0.96	0.195 ± 0.010	95.0 ± 0.2
60	176. 5 ± 0.8	-20.6 ± 1.0	0.189 ± 0.130	94.1 ± 0.1	132.6 ± 1.1	-21.8 ± 1.6	0.193 ± 0.110	94.9 ± 0.2
90	189.0 ± 1.8	-19.8 ± 1.2	0.176 ± 0.030	93.5 ± 0.2	138.7 ± 1.3	-20.9 ± 1.4	0.191 ± 0.230	94.8 ± 0.1

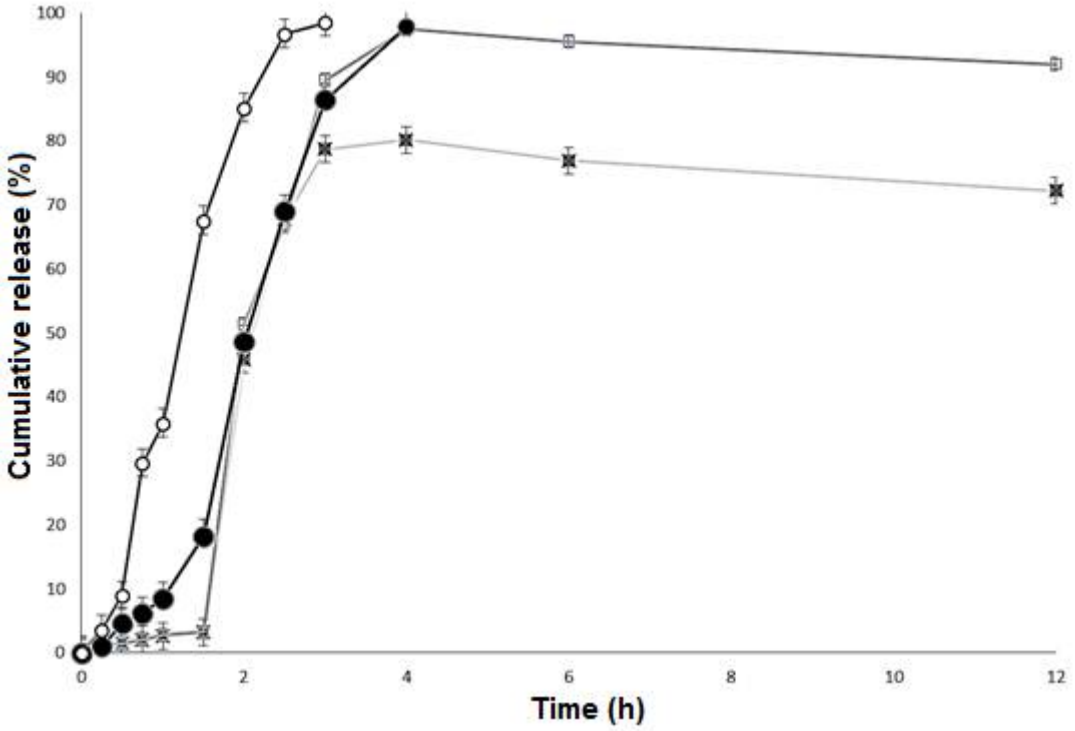

Figure 6: *In vitro* drug release profiles: CL-SLN (F3) in SIF (▪); CL-SLN (F3) in SIF + pH 6.8 + 30 % PEG (▫); pure CL in SIF (●); pure CL in SIF + pH 6.8 + 30 % PEG (◦)

Table 4: Release kinetics of CL- SLN (F3) and pure CL (*AIC: Akaike information criterion)

Formulation	First order			Higuchi		
	k_1	R	AIC	k_H	r	AIC
Pure CL (pH 6.8 + 30 % PEG)	1.172	0.697	76.495	51.844	0.830	71.306
Pure CL (SIF)	0.664	0.520	89.432	36.337	0.680	85.374
CL-SLN (SIF)	0.137	0.583	116.744	22.352	0.547	117.797
CL-SLN (pH 6.8 + 30 % PEG)	0.294	0.820	110.759	27.218	0.622	120.399

From the 48 h cytotoxicity test results [(Figure 7(B)], it was shown that the formulations showed cytotoxicity. The IC_{50} values for CL and C-loaded SLN formulations were 25.68 ± 3.33 µg/mL and 15.48 ± 1.85 µg/mL, respectively, but the IC_{50} for Dynasan was 88.21± 6.80 µg/mL.

DISCUSSION

Carvedilol-encapsulated SLNs were successfully developed using hot homogenization and ultrasonication methods according to the formulae given in Table 1 [20]. The mean particle size of formulations ranged from 130.7 ± 1.8 to 154.4 ± 2.4 nm with low polydispersity indices indicating narrow particle size distribution (Table2). It was also clear that increasing the concentration of the surfactant from 1 to 4 % (w/v) resulted in a significant decrease in the particle size [21].The mean diameter of the particles make them suitable for gastrointestinal absorption by M-cells on Peyer's patches [22]. The PDI used as a measure of a unimodal size distribution was within the acceptable limits for all the formulations. Usually, a small value of PDI indicates a homogenous population, while a larger PDI means a high heterogeneity in particle size [21]. Pure carvedilol had an irregular shape, while the lipid Dynasan shape was cluster-like, and the CL-SLN (F3) exhibited a flaky shape [18].

Pure CL shows a single sharp endothermic melting peak at 116.5 °C, indicating the crystalline characteristics of carvedilol.

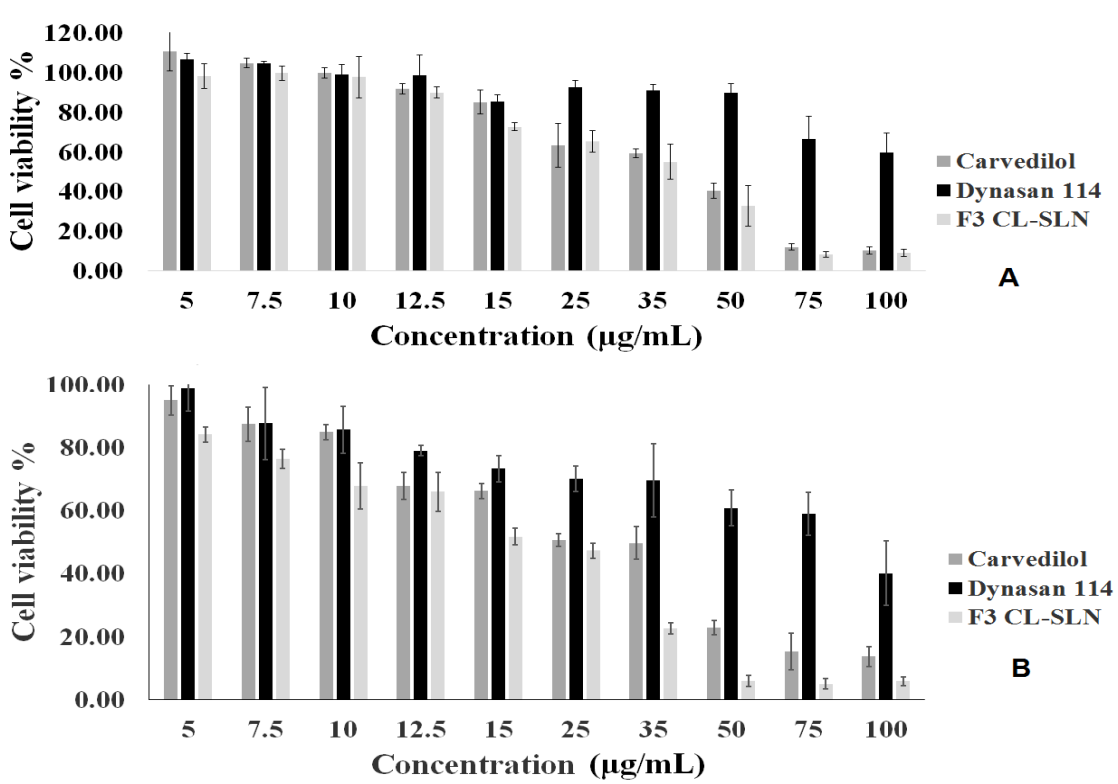

Figure 7: Cytotoxicity profiles of carvedilol loaded solid lipid nanoparticles incubated for A) 24 h and B) 48 h, via MTT assay

This peak was not evident in the thermograms of the SLN formulations indicating that CL was not in the crystalline state, but rather present in amorphous state with the drug completely entrapped within the solid lipid [6].

In order to find out the physical state of CL in the formulations, the XRD analyses were performed. The diffraction pattern of CL showed that it is highly crystalline in nature as indicated by its infinite typical peaks with the major characteristic diffraction pattern visible at a diffraction angle. The XRD interpretation of CL-SLN (F3) formulation showed more amorphous characteristics relative to the pure CL. There were some sharp peaks also seen, which may be due to the presence of solid lipid in the formulation. The lost peaks of CL in F3 formulation may be due to incorporation of CL between parts of crystal lattice of the lipid, leading to changes in the crystallinity of CL [17,19,20].

In order to characterize the integration of CL with lipid, ^{1}H- NMR measurements were also carried out. A pattern of spin diffusion between protons of the solid lipid and protons of the CL has been observed [23]. This is in harmony with the finding in this study, and clearly indicates that CL was incorporated into solid lipid. Simple ^{1}H-NMR spectroscopy permits an easy and rapid detection of supercooled melts due to the low line widths of the lipid protons. It also allows for the characterization of liquid nanocompartments in recently developed lipid particles, which are made from blends of solid and liquid lipids [19]. ^{1}H- NMR investigations were used to obtain information about the mobility and structure of both the nanoparticle system and the incorporated drug. The spectrum of the drug-free SLN dispersion confirmed the assumption of immobilized molecules of the dispersed lipid phase. The spectrum was dominated by the signals of the emulsifier, Tween, and the huge water signal. These results excluded formation of supercooled melts of the colloidal dispersed lipid after the production process. Comparing the drug-loaded SLN formulations with the drug-free SLN, there is an essential difference in the spectrum peaks and sharpness which could be observed due to the integration of Carvedilol [1,20,22].

The typical Fourier transform-infrared (FTIR) spectrum pattern of the CL was in the range of 400 to 4,000 cm^{-1}. The IR spectrum of pure carvedilol showed a peak at 3,344.88 cm^{-1} (N-H, str), which corresponds to N-H stretching. The hetero-aromatic structure, when present is revealed by the occurrence of the C-H stretching vibrations which is the characteristic region of active agent. These characteristic bonds are evident in the CL-SLN (F3) formulations. The bands are sharp but of weak-to-minimum

intensity. Infrared spectroscopy primarily provides information about the interactions between different atoms and is very useful for displaying information about hydrogen bonds [1]. Hydrogen bonding results showing that active agent was successfully loaded to particles.

In vitro release studies showed that the release of CL from SLN was over 20 hours. The slow release of CL is an indication that the active agent was at the core and surrounded by the lipid. Due to the increased diffusional distance the drug would normally have sustained release [6,22]. According to highest k and r^2 values and according to lowest AIC values from DDsolver programme, *in vitro* release of CL from formulations followed first order and Higuchi models showing the controlled release property of formulation.

Among the SLN formulations, F3 was chosen as optimum formulation due to its lowest particle size and best zeta potential value. The stability studies revealed the physical stability of SLN after 3 months storage and there was no significant change observed in the data. Zeta potential is a key Factor used for the evaluation of the stability of colloidal dispersions [5,20]. As observed from the results F3 formulation was negatively charged, indicating a relatively good stability and quality. An important parameter with respect to SLNs as drug carriers is their capacity for drug encapsulation. As mentioned earlier CL showed a high entrapment capacity of 96.03 ± 0.13 % in formulation F3, which after 3 months storage, decreased by only 2.68 % to 93.46 ± 0.21 %.

Cytotoxicity analysis of nanoparticles is vital to ensure that it does not present any risk to the patient or elicit an acute toxicity response. The toxicity of Dynasan nanoparticles of carvedilol was studied using 3T3 mouse embryo fibroblast cell lines for 24 and 48 h.

In the literature, there is no evidence that significant stotoxicity was associated with SLNs preparations using Dynasan as a lipid at rates ranging from 0.01 % to 10 %. Generally, over 60 % cell viability was shown [24,25].

The results obtained in this study also showed a reduction of up to 60 % only at the end of the 48-hour incubation period in formulation prepared with Dynasan [25,26]. It was found by the inventors that carvedilol caused cytotoxicity on different human tumor cell lines depending on the dosage and cell-line used. In a study carried out by Burman *et al* [27] carvedilol shows significant *in vitro* cytotoxicity. The ED_{50} for carvedilol was found to be 7 µg / mL on PTC (colon) cells whereas 33 µg / mL was found in MiaPaCa.2 (pancreas) cells.

Cheng *et al* [28] showed that carvedilol to be non-cytotoxic at concentrations equal to or less than 10 µM. Yang *et al* demonstrated that higher dosages (≥ 20 µM) were cytotoxic because at such high doses it induced T cell apoptosis, with attendant decrease in cell viability [29].

In this study, carvedilol showed high cytotoxicity after 25 µg/mL throughout the 24 h and 48h incubation period. When all the results were evaluated the formulations showed dose and time-dependent toxicities. However, the results obtained in this study have similarities and differences with the studies of other workers [27-29] found in literature. The lipid ratio, type and amount of the surfactants, the method of preparation, the incubation times used in MTT and the selected cell type are possible reasons for the differences.

CONCLUSION

The findings of the present study show that carvedilol loaded solid lipid nanoparticles were prepared successfully by hot homogenization and ultrasonication process. The molecular state of carvedilol changed from the crystalline state to the amorphous state following incorporation into solid lipid nanoparticles. The developed formulation is stable and safe, and represents a promising system for the sustained and controlled delivery of carvedilol to target cells, tissues and organs.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Alves JM, Prado LD, Rocha HV. Evaluation and correlation of the physicochemical properties of carvedilol. Pharm Dev Technol. 2016 Nov; 21(7): 856-866.*
2. *Hokama N, Hobara N, Kameya H, Ohshiro S, Sakanashi M. Rapid and simple micro-determination of carvedilol in rat plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 1999; 732(1): 233-238.*
3. *Cátia VD, Cláudia MD, Magdalena LA, Aleksandra W, Mariusz RW, Paulo JO. Carvedilol and antioxidant proteins in a type I diabetes animal model. Eur J Clin Invest. 2017; 47 (1): 19–29.*
4. *Li J, Wang L, Wang S, Chen M, Gu E, Hu G, Ge R. Simultaneous quantification of carvedilol and its metabolites in rat plasma by ultra-performance liquid chromatography tandem mass spectrometry and pharmacokinetic application. J Chromatogr B Analyt Technol Biomed Life Sci. 2015; 1 (974): 138-146.*
5. *Aboud HM, El Komy MH, Ali AA, El Menshawe SF, Abd Elbary A. Mohammed HE, Adel AA, Shahira FM, Ahmed AE. Development, optimization, and evaluation of carvedilol-loaded solid lipid nanoparticles for ıntranasal drug delivery. AAPS PharmSciTech. 2016; 17 (6): 1353-1365.*
6. *Mishra A, Imam SS, Aqil M, Ahad A, Sultana Y, Ameeduzzafar, Ali A. Carvedilol nano lipid carriers: formulation, characterization and in-vivo evaluation. Drug Deliv. 2016; 23(4): 1486-1494.*
7. *O'Driscoll CM, Griffin BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility- the potential impact of lipid-based formulations. Adv Drug Deliv Rev. 2008; 60: 617–624.*
8. *Luo Y, Teng Z, Li Y, Wang Q. Solid lipid nanoparticles for oral drug delivery: chitosan coating improves stability, controlled delivery, mucoadhesion and cellular uptake. Carbohydr Polym. 2015; 122: 221-229.*
9. *Üner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. International Journal of Nanomedicine. 2007; 2(3): 289-300.*
10. *Venishetty VK, Chede R, Komuravelli R, Adepu L, Sistla R, Diwan PV. Design and evaluation of polymer coated carvedilol loaded solid lipid nanoparticles to improve the oral bioavailability: A novel strategy to avoid intraduodenal administration. Colloids Surf B Biointerfaces. 2012; 95: 1-9.*
11. *Shah MK, Madan P. Elucidation of intestinal absorption mechanism of carvedilol-loaded solid lipid nanoparticles using Caco-2 cell line as an in-vitro model. Pharm Dev Technol. 2014; 29: 1-9.*
12. *Jenning V, Thunemann AF, Gohla SH. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm. 2000; 199(2): 167-177.*
13. *Ranga PM, Jeevitha N. Semi-solid dispersion of carvedilol solid lipid nanoparticles for topical delivery, EJPMR, 2016; 3(3): 231-238.*
14. *Pallavi V, Singh K. Development and evaluation of colloidal modified nanolipid carrier: Application to topical delivery of tacrolimus. Eur J Pharm Biopharm.2011; 79(1): 82–94.*
15. *Patel LJ, Suhagia BN, Shah PB, Shah RR. RP-HPLC and HPTLC methods for the estimation of carvedilol in bulk drug and pharmaceutical formulations. Indian J Pharm Sci. 2006; 68 (6): 790-793.*
16. *Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. The AAPS Journal. 2010; 12(3): 263-271.*
17. *Sharma A, Jain CP, Tanwar YS. Preparation and characterization of solid dispersions of carvedilol with poloxamer 188. J. Chil. Chem. Soc. 2013; 58(1): 1553-1557.*
18. *Liu D, Pan H, He F, Wang X, Li J, Yang X, Pan W. Effect of particle size on oral absorption of carvedilol nanosuspensions: in vitro and in vivo evaluation. International Journal of Nanomedicine. 2015; 10: 6425–6434.*
19. *Morel S, Terreno E, Ugazio E, Aime S, Gasco MR. NMR relaxometric investigations of solid lipid nanoparticles (SLN) containing gadolinium(III) complexes, Eur J Pharm Biopharm. 1998; 45(2): 157-163.*
20. *Mehnert W, Mäder K. Solid lipid nanoparticles Production, characterization and applications. Adv Drug Deliv Rev. 200; 47(2-3): 165-196.*
21. *Zhang L, Han L, Sun X, Gao D, Qin J, Wang J. The use of PEGylated liposomes to prolong the circulation lifetime of salvianolic acid B. Fitoterapia. 2012; 83(4): 678-689.*
22. *Prasanthi NL, Gondhi S, Manikiran SS, Kumar SN, Rao NR. Solid lipid nanoparticles of carvedilol by hot homogenization: Formulation and evaluation. 2011; 2(2): 1-16.*
23. *Wissing SA, Müller RH, Manthei L, Mayer C. Structural characterization of Q10-loaded solid lipid nanoparticles by NMR spectroscopy. Pharm Res. 2004; 21(3): 400-405.*
24. *Olbrich C, Schöler N, Tabatt K, Kayser O, Müller RH. Cytotoxicity studies of Dynasan 114 solid lipid nanoparticles (SLN) on RAW 264.7 macrophages-*

impact of phagocytosis on viability and cytokine production. J Pharm Pharmacol. 2004; 56(7): 883-891.

25. *Schöler N, Hahn H, Müller RH, Liesenfeld O. Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of Macrophages. Int J Pharm. 2002; 231(2): 167-176.*
26. *Rigon RB, Fachinetti N, Severino P, Santana MHA, Chorilli M. Skin Delivery and in Vitro Biological Evaluation of Trans-Resveratrol-Loaded Solid Lipid Nanoparticles for Skin Disorder Therapies. Molecules 2016; 21: 116.*
27. *Burman AC, Mukherjee R, Jaggi M, Singh AT. (2003). U.S. Patent No. 6,632,832. Washington, DC: U.S. Patent and Trademark Office.*
28. *Cheng SM, Yang SP, Ho LJ, Tsao TP, Chang DM, Lai JH. Carvedilol Modulates In-Vitro Granulocyte-Macrophage Colony-Stimulating Factor-Induced Interleukin-10 Production in U937 Cells and Human Monocytes, Immunol Invest. 2003; 32(1-2): 43-58.*
29. *Yang SP, Ho LJ, Lin YL, Cheng SM, Tsao TP, Chang DM, Hsu YL, Shih CY, Juan TY, Lai JH. Carvedilol, a new antioxidative b-blocker, blocks in vitro human peripheral blood T cell activation by downregulating NF-kB activity, Cardiovasc Res. 2003; 59(3): 776-787.*