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Physicochemical characterization and pharmacokinetic evaluation of rosuvastatin calcium incorporated solid lipid nanoparticles

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ABSTRACT

Rosuvastatin calcium (RCa) is a very efficient antihyperlipidemic agent, however, being a BCS class II drug, results in poor oral bioavailability. The present study focused on the enhancement of oral bioavailability of RCa with solid lipid nanoparticles (SLNs). Physicochemical properties of the particles were evaluated by particle size (PS), polidispersity index (PDI), zeta potential (ZP), DSC, FT-IR, XRD, ^{1}H NMR analyses. Entrapment efficiency (EE), drug loading capacity (DL), *in vitro* release and release kinetics were also analyzed. Safety and efficacy of the formulations were analyzed by cytotoxicity, permeability and pharmacokinetic studies. PS values were ranged between ~134 and 351 nm with homogenous size distribution (PDI ~ 0.130–0.33) and ZP data were valued within the range of ~ −17 mV to −41 mV. The SLN2 formulation showed the best cytotoxicity test results and had medium permeability (P_{app} 5.72×10^{-6} cm sec^{-1}) while pure RCa resulted in low permeability (P_{app} 3.08×10^{-7} cm sec^{-1}). According to the stability analyses (3 months) 5 ± 3 °C and 25 ± 2 °C were found suitable storage temperatures for SLNs. Pharmacokinetic studies confirmed significant improvement in C_{max} (1.4 fold) and AUC_{last} (8.5 fold) by SLNs in comparison with the pure drug indicating the enhanced biopharmaceutical performance of the RCa loaded SLNs.

1. Introduction

Acute coronary syndromes (ACS) are the major health problems and leading cause of death and disability worldwide. Low-density lipoprotein cholesterol (LDL-C) is one of the most studied risk factors for ACS, and it is now well established that there is a direct correlation between the levels of plasma LDL-C and the risk of coronary artery diseases. Reduction in plasma LDL-C level is a fundamental treatment for the prevention of ACS. Over the past 20 years, the results of many large-scale, randomized clinical trials have shown that use of statin therapy to lower LDL-C concentrations can significantly reduce the incidence of mortality, major coronary events, stroke and the need for revascularization in a broad spectrum of patients (Cheng, 2004; Fabbri and Maggioni, 2009; Sahebkar and Watts, 2013).

Rosuvastatin calcium (RCa); member of 'superstatin' group is a BCS class II drug having low bioavailability (20%) due to first-pass effect as well as binding to plasma proteins (88%) and its crystalline structure reduces its aqueous solubility (Martin et al., 2003; Suares and Parbhakar, 2016). Some pharmaceutical technologies such as solid dispersions, inclusion complexes, nanocrystal technology, liquisolid technology, microemulsion technology, liquid self-nanoemulsifying drug delivery system (SNEDDS), solid SNEDDS and solid lipid nanoparticles (SLNs) have been investigated in order to enhance solubility, dissolution rate and bioavailability of RCa (Demirel et al., 2014; Alshora et al., 2016; Beg et al., 2017; Dudhipala and Veerabrahma, 2017).

SLNs are new generation solid nanometer-scale delivery systems where the oil phase has been substituted by a solid lipid. They are mostly made of triglycerides, partial glycerides, fatty acids, steroids and waxes that are biocompatible, biodegradable, with low toxicity (Trucillo and Campardelli, 2019). SLNs exhibit unique characteristics such as small particle size, large surface area and the interaction of phases at the interfaces, and are appealing for their capability to enhance the delivery of pharmaceuticals, neutraceuticals and different

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materials (Reddy and Shariff, 2013). SLNs have been proposed for different administration routes, such as oral, topical, ophthalmic, inhalation and nasal administration as well as parenteral injection (Olbrich et al., 2002; Başaran et al., 2010; Sznitowska et al., 2017). Studies have shown that encapsulation of drugs in SLNs will not only ensure their controllable release, but also protect the incorporated drug from enzymatic degradations which results in the improvement of their absorption. SLNs enhances the bioavailability of the drug with longer retention and exposure durations at the targeted sites (Hanumanaik et al., 2013; Dara et al., 2019; Wang et al., 2019).

In this study RCa was incorporated into stearic acid (SA) and tripalmitin (TP) based SLNs for the enhancement of poor oral bioavailability. Since SLNs are one of the most preferred drug delivery systems when the aim is the enhancement of the oral bioavailability, sure there are studies about the incorporation of RCa to SLNs with both SA (Beniwal and Choudhary, 2017; Himavarshini and Sumaharshan, 2017; Singh et al., 2018) and TP (Suvarna et al., 2015). In our study nanoparticles were formulated by hot homogenization technique using high shear homogenization to avoid organic solvents and to formulate safer formulations. Detailed critical evaluations with cytotoxicity, permeability and pharmacokinetic studies were performed. Physicocemical properties were evaluated in detail and analyses results revealed the highest oral bioavailability of RCa approx. 8.5 fold with SA based SLN2 formulation was achieved (Suvarna et al., 2015; Gadad et al., 2016; Singh et al., 2018).

2. Materials and methods

2.1. Materials

Rosuvastatin Calcium and Ketoprofen were kindly gifted byAbdi İbrahim İlaç (İstanbul, Turkey); Stearic Acid, Phosphatidylcholine, Tween® 80, Acetonitrile, Formic Acid were purchased from Sigma-Aldrich (Steinheim, Germany); Tripalmitin was the product of Fluka (Cedex, France); Caco-2 Cell line (American Type Culture Collection (USA); Dulbecco's Modified Eagle's Medium, Fetal Bovine Serum, Penicillin/Streptomycin, Hank's Balanced Salt Solution, were the products of Biochrom AG (Berlin, Germany); HEPES and Trypan Blue were purchased from Sigma (USA); Dimethyl Sulphoxide (cell culture grade) and Thiazolyl Blue Tetrazolium Bromide were from AppliChem GmBH (Darmstadt, Germany); Ketamine and Xylazine were from EGE-VET (İzmir, Turkey); Heparin was the product of Mustafa Nevzat İlaç Sanayii A.Ş., (İstanbul, Turkey); Sprague Dawley Rats were provided by Eskişehir Osmangazi University Medical and Surgical Experimental Animals Implementation and Research Center (MSEAIRC). All other chemicals used were analytical grade.

2.2. Preparation of SLNs

SLNs were prepared by the hot homogenization technique with high shear homogenization using ultra-turrax (IKA T25, USA) (Mäder and Mehnert, 2001; Ekambaram and Abdul Hasan Sathali, 2011). Briefly; lipid was heated up to 10 °C above its melting point and RCa was added to the molten lipid with different concentrations. Surfactant solution was heated up to same temperature. Hot aqueous surfactant solution was added to the clear homogenous hot lipid phase to avoid the lipid loss, and homogenization was carried out by using ultra-turrax homogenizer for three minutes at 9500 rpm. Ultrasonication for 10 min at 20% amplitude (VCX 130 PB, USA) was applied during the cooling stage at 4 °C in an ice bath. Table 1 shows the compositions of the formulations. Non-ionic surfactant Tween® 80 (T80) was used in SLN1-SLN3 formulations. It was reported that the use of surfactants as combinations might prevent particle agglomeration more efficiently compared to one surfactant alone therefore the mixture of T80 and amphoteric surfactant phoshatidylcholine (PTC) was used for SLN4 (Mäder and Mehnert, 2001). Hot homogenization technique was selected to avoid the organic solvents to formulate safer formulations.

Table 1
Compositions of SLNs.

Code	RCa (mg)	SA (g)	TP (g)	T80 (g)	PTC* (g)	Bidistilled water (mL)
SLN1-P	–	1.960	–	1	–	50
SLN1	40	1.960	–	1	–	50
SLN2-P	–	1.980	–	1	–	50
SLN2	20	1.980	–	1	–	50
SLN3-P	–	–	1.980	1	–	50
SLN3	20	–	1.980	1	–	50
SLN4-P	–	–	1.995	0.5	0.5	50
SLN4	5	–	1.995	0.5	0.5	50

2.3. Physicochemical characterization analyses of SLNs

Malvern Zetasizer Nano-ZS (England) was used to measure particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of SLNs. Samples were prepared by diluting proper volume of SLNs dispersion in distilled water of 50 μS cm^{-1} with 0.9% NaCl constant conductivity and mixed by vortex (Müller and Heinemann, 1993). For each formulation three analyses were performed at 25 °C.

Prior to the structural analyses the formulations were lyophilized by Lyovac-GT 2 laboratory freeze-dryer (Leybold Heraeus GmbH, Germany). Melting transitions and heat capacity changes were analyzed using differential scanning calorimetry (DSC; Shimadzu DSC-60, Japan). Constant amount of lyophilized SLN samples were put in aluminum crucibles and purged with inert nitrogen gas at the flow rate of 50 mL min^{-1}. The thermograms were obtained at 30–350 °C with an increase rate of 10 °C min^{-1}. The crystalline sytucture of SLNs were also evaluated by X-ray diffraction (XRD; Rikagu, D/Max-3C, Japan) analyses. Samples were exposed to CuKα radiation (40 kV, 20 mA) and scanned from 2° to 40°, 2θ at a scanning rate 2° min^{-1}. For the determination of possible interactions between the drug and the lipids, Fouirer Transform Infrared Spektrofotometer (FT-IR; Perkin Elmer Spectrum 2000, UK) spectra were recorded at the wavelength range of 4000–500 cm^{-1} and also nuclear magnetic resonance (^{1}H NMR; Bruker, Ultra Shield CP MAS NMR 500 MHz, Germany) spectra of SLNs were also obtained to verify the modifications in the pure RCa and solid lipids during the formulation stages.

2.4. Determination of encapsulation efficiency and drug loading capacity

For the determination of RCa in the samples a validated HPLC (Shimadzu, LC 20-AT, Japan) method was used (Kumar et al., 2006).

Analyses of RCa was conducted using C18 column (250 × 4.6 mm, 5 μm), maintained at 25 °C. Mobile phase was a mixture of formic acid (0.05 M) and acetonitrile (55:45; v/v). Analyses were performed at a flow rate of 1.0 mL min^{-1} of with an injection volume of 20 μL for *in vitro* and permeability test (100 μL for *in vivo* studies) with detection wavelenght at 240 nm (Kumar et al., 2006). For the reliabiliy of the data, validation studies of the HPLC method were performed. Analytical parameters such as linearity, precision, accuracy, specificity, and sensitivity were analyzed and statistically evaluated using the International Harmonization Committee (ICH), analytical process validation guidelines (ICH Q2B, 1996; ICH Q2A (R2), 2005).

For the determination of encapsulation efficacy (EE) and drug loading capacity (DL) Eqs. (1) and (2) were used respectively (Kheradmandnia et al., 2010).

$$EE\% = \frac{Drug\ \ in\ \ precipitate}{Total\ \ added\ \ drug} \times 100 \quad (1)$$

$$DL\% = \frac{Drug\ \ in\ \ precipitate}{Drug\ \ in\ \ precipitate + Added\ \ excipients} \times 100 \quad (2)$$

For the determination of EE and DL the accuratetly weighted SLNs

were dispersed in 2 mL of ethanol. Obtained solution was filtered using 0.2 μm nylon filter, and the amount of RCa in the filtrate was analyzed by HPLC.

2.5. In vitro release studies

A modified dialysis bag diffusion method was used to carry out the *in vitro* release of RCa from SLNs formulations (Adilya et al., 2014). A definite dispersion volume of the formulations that equivalent to same amount RCa respectively were transferred to a dialysis bag. The dialysis bag was put in a beaker contained 50 mL phosphate buffer of pH 6.8 at 37 ± 0.5 °C with a stirring speed of 100 rpm (Balakumar et al., 2013; Singh et al., 2018; Hirpara et al., 2018; Al-Shdefat et al., 2020). At predetermined time intervals (0.5, 1, 2, 3, 4, 6, 8, 12 and 24) 0.5 mL samples were taken and the same amount of fresh medium fluid was added to maintain sink conditions. The samples were analyzed by HPLC. The analyses were repeated in triplicate.

In vitro release analyses results were evaluated by DDSolver software program for the determination of the similarity factor (f_2) of the release profiles (Zhang et al., 2010).

2.6. Cytotoxicity and in vitro permeation studies

Thiazolyl blue tetrazolium bromide (MTT) analyses were carried out on Caco-2 cell line (with a passage number range of 25–30) for the determination of the cytotoxicity of the formulations prepared. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 50 units/mL penicillin and 50 μg/mL streptomycin. Hank's Balanced Salt Solution (HBSS) pH 7.4 containing 10 mM HEPES was used for transient studies. Cells were seeded in 96-well plates at 5×10^3cells/well, left to grow. After 24 h cells were incubated with 100 μL of serum-free medium containing serial dilutions of the tested SLN formulations (SLN2-P, SLN2, SLN4-P, SLN4). Cells were incubated with the SLN formulations for 24 h, then culture medium was removed and MTT solution was added to the wells and plates were incubated for 4 h. MTT dye in wells were removed and 200 μL of dimethyl sulfoxide (DMSO) was added to the wells to solubilize formazan crystals. Optical densities of wells were measured at 570 nm with a microplate reader (VERSAmax Molecular Devices Corporation, Sunnyvale CA, USA) for the determination of cell viability (%) (Yong et al., 2016).

$$\text{Cell viability}(\%) = \frac{\text{number of viable cells/mL(sample)}}{\text{average number of viable cells/mL(control)}} \times 100 \quad (3)$$

Permeation studies were carried out on Caco-2 cell lines (Yavuz et al., 2010; Netsomboon et al., 2016) with selected formulations according to the MTT analyses results. In permeation studies, the replicated Caco-2 cells were counted with trypan blue and the inserts (ThinCert ™, 12 wells, 1.0 μm por diameter) were added (6×10^4 cells/insert). Cells were incubated with 5% CO_2 at 37 °C. Cells were allowed to grow without any treatment for three days with changing the medium of the flask with fresh medium everyday. At the end of 21 days, cell monolayer integrity was tested by measuring transepithelial electrical resistance (TEER) with Millicell®-ERS (Merck, USA). When the resistance reached the range of 400–600 Ωcm^2, cell monolayer was used for transport studies (Yavuz et al., 2010; Netsomboon et al., 2016).

Culture medium was replaced from each well by 0.5 mL and 1 mL pH 7.4 HBSS containing 10 mM HEPES in the apical and basolateral side of the well and the cell monolayers were subsequently equilibrated for 30 min at 37 °C and these medium were thrown away. Formulations were prepared in HBSS containing 0.5 μM DMSO which contain 50 μM of RCa (0.5 mL) and were added to the apical side of the monolayer. The basolateral portion was supplemented with 1 mL of pH 7.4 HBSS containing 10 mM HEPES. Plates were placed in a horizontal shaker and incubated at 37 °C for 2 h at 60 rpm. Two hours later, 1 mL samples were taken from the basolateral compartment and the samples were stored at −20 °C until being analyzed by the HPLC.

Calculation of the apparent permeability coefficient (P_{app}) for the pure RCa and the studied formulations was carried out using the following Eq. (4).

$$P_{app}(\text{cm sec}^{-1}) = V_R \times dC_R/dt \times A \times C_{Do} \quad (4)$$

where P_{app} is an apparent permeability coefficient, V_R is a volume of receiver chamber, A is the surface area of the permeability barrier (cm^2), C_{Do} is the initial drug concentration in the donor chamber, and dC_R/d_t is the change in concentration of the compound in the receiver compartment over time (Blaser, 2007).

2.7. Stability studies

Considering the characterization, MTT and permeability test analyses results SLN2 was selected for stability studies. The formulation was put in a hermetically closed glass containers and were stored at three different conditions (5 ± 3 °C, 25 ± 2 °C and 40 ± 2 °C) (ICH Q1A-R2, 2003). At predetermined periods (day of production, 30th, 60th, and 90th day) the PS, PDI, ZP and remained RCa % were evaluated. All analyses were repeated in triplicate.

2.8. Pharmacokinetic studies

As well as the stability studies SLN2 formulation was selected also for the pharmacokinetic studies. Sprague Dawley rats (250–300 g) which were obtained from the Eskişehir Osmangazi University, Medical and Surgical Experimental Animals Implementation and Research Center (MSEAIRC) were used in this study. The animals were housed in a temperature controlled room (22 ± 2 °C; with a light/dark photoperiod of 12:12) with free access to food and water. To avoid the possible interactions with food only water was given to the animals for 12 h prior to the application. Animal care and research protocols were planned according to the principles of Guide for the Care and Use of Laboratory Animals (NIH Publication No: 85-23, revised in 1985) and study protocol was approved by Eskişehir Osmangazi University Local Ethical Committee (No: 561/2016).

In this study the rats were divided into two groups of five animals, where the first group was given 2.2 mg kg^{-1} standard RCa aqueous suspension and the second group was given SLN2 formulation (without lyopilization) with the equal RCa dose orally using 16-gauge 75 mm round tip oral-feeding needle (Fine Science Tools, USA). Animals were anesthetized using ketamine (90 mg/kg; as anesthetic agent) and xylazine (10 mg/kg; as muscle relaxant). Blood samples (0.25 mL) were withdrawn from the rat's tail at 0.5, 1, 2, 4, 6, 9, 24, 48, and 72 h after treatment into eppendorf tubes containing heparin (as anticoagulant agent) (Kumar et al., 2006).

A modified liquid-liquid extraction method was used for sample preparation (Kumar et al., 2006). Briefly, blood samples were centrifuged at 5000 rpm for 10 min at 25 ± 1 °C for the separation of plasma. Acetonitrile (ACN; 2 mL) was added to the separated plasma and vortexed for 2.5 min followed by sonication (5 min) and centrifugation (4500 rpm for 10 min) respectively. Supernatant was separated and samples were reconstituted using 200 μL mobile phase for analysis by HPLC with ketoprofen as an the internal standard.

2.9. Statistical analyses

All experiments were performed in triplicate and analyses results were expressed as mean ± standard error. The two-way analysis of variance (ANOVA) was performed using GraphPad Prism 7 software $P < 0.05$ was considered as statistically significant.

Table 2
Particle size (PS), polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE), and drug loading capasity (DL) of the SLN formulations (Mean ± SE; n = 3).

Code	PS (nm)	PDI	ZP (mV)	EE (%)	DL (%)
SLN1	351.13 ± 1.39	0.33 ± 0.03	−17.03 ± 0.53	71.50 ± 0.07	0.71 ± 0.01
SLN2	134.37 ± 0.91	0.13 ± 0.01	−18.77 ± 0.20	80.20 ± 0.06	1.57 ± 0.01
SLN3	240.20 ± 22.90	0.20 ± 0.00	−23.00 ± 1.50	103.10 ± 0.01	1.03 ± 2.00
SLN4	267.70 ± 18.70	0.30 ± 0.00	−40.80 ± 1.20	100.14 ± 0.65	0.25 ± 0.01

3. Results and discussion

3.1. Physicochemical characterization of SLNs

SLNs mostly consist of solid lipid(s), surfactant(s) and water. The term lipid is used here in a broader sense and includes triglycerides (e.g. TP), partial glycerides (e.g. Imwitor), fatty acids (e.g. SA), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate) (Mäder and Mehnert, 2001). Substances used to prepare these nanoparticles are generally recognized as safe compounds (GRAS ingredients); this aspect makes SLNs preferantial carriers for human use (Righeschi et al., 2016).

In this study the effects of the type of components (lipid matrix and surfactant) and concentration of active agent on the properties of SLNs were evaluated. The characteristic properties of the prepared formulations were summarized in Table 2.

In SLN composition, many different surfactants can be used (with respect to charge and molecular weight) (Sznitowska et al., 2017; Mäder and Mehnert, 2001). The non-ionic surfactant; T80 was used in SLN1-SLN3 formulations. It was reported that the use of surfactants as combinations might prevent particle agglomeration more efficiently compared to one surfactant alone therefore the SLN4 was formulated with the combination of T80 and PTC together (Mäder and Mehnert, 2001).

Considering the analyses results, the PS mean values of SA based SLNs (SA-SLNs; SLN1, SLN2) and TP based SLNs (TP-SLNs; SLN3, SLN4) were ranged between 134.37 ± 0.91 and 351.13 ± 1.39 with a narrow PS distribution (PDI < 0.5) indicating the homogeneous dispersion which were correlated with the former statements (Table 2) (Senthil Kumar et al., 2015; Oehlke et al., 2017).

Surface charge is an important factor, influencing the stability of colloidal dispersion (Dudhipala and Veerabrahma, 2017). In our study SLN formulations were negatively charged due to the anionic characters of the lipids used (Righeschi et al., 2016). ZP values varied from −17.03 ± 0.53 to −40.80 ± 1.2 mV. Since the nanoparticles with ZP values greater than +25 mV or less than −25 mV typically recognized as stable due to strong electrostatic repulsions between particles and aggregation can be occured in the dispersions with a low ZP values due to Van Der Waal inter-particle attraction (Başaran et al., 2011; Senthil Kumar et al., 2015). Therefore our ZP analyses results indicated a relatively good dispersion stability for both of the SLNs (Table 2).

EE of SA-SLNs (SLN1, SLN2) and TP-SLNs (SLN3, SLN4) were ranged between ~72% and ~103%. SLN formulations prepared with TP as lipid matrices resulted in higher drug incorporation capacity than SA (Table 2). Such differences might be attributed to the structure of the lipid. TP has a long triglyceride chain and produced less ordered lipid crystals than SA which is composed solely of C18 carbon chains therefore there is much space for the incorporation of the drug within the polymeric matrix than in SA (Westesen et al., 1997; Hou et al., 2003).

Care needs to be taken considering the risk of drug expulsion when formulating SLNs with highly ordered crystalline lipids. Amorphous structure gives possibilities to achieve higher incorporation rates (Westesen et al., 1997; Hou et al., 2003). Therefore determination of the degree of lipid crystallinity and the modification changes of the lipids are very critical analyses for SLNs. DSC is widely used to investigate the structural changes of the lipids (Mäder and Mehnert, 2001). In our study DSC analyses were also conducted. The thermograms of the pure substances were used as references for the evaluation of the structural changes during the formulation stages. RCa showed a endothermic peak for water loss between the 75 and 80 °C, followed by multiple endothermic melting peaks within the temperature range of 180–290 °C showing semicrystalline structure Fig. 1 (Beg et al., 2016). SA and TP showed melting points of 72.20 °C and 65.29 °C respectively and for the formulations, sharp peaks of the lipids were revealed in the thermograms of the formulations whereas the peaks of RCa have disappeared because it was fully dissolved in the lipid matrix (Fig. 1).

Majority of active pharmaceutical ingredients (APIs) can exist in different solid-state forms like polymorphs, solvates, and the amorphous state. These solid forms can differ widely in their physicochemical properties, and thus can influence the quality, safety and efficacy of the drug product (Shete et al., 2010). In addition to DSC, semi-crystallinity of the drug was also determined using XRD analyses. XRD spectra of pure drug and pure lipids, SLNs were shown in Fig. 2. XRD pattern of RCa has indicated the semi-crystalline nature of the drug. Sharp crystalline peaks at 2Θ scattered angles at 6.8, 21.6, 24.2 and at 19.2, 23.0 and 24.0 were revealed in the XRD diffractograms of SA and TP respectively (Fig. 2). Decrease in the intensities of the peaks were observed for SLN1-SLN3 showing that the degree of crystallinity was being reduced (Fig. 2). For SLN4 formulation, crystalline state has been completely transformed to amforphous state (Fig. 2) which results in improved encapsulation efficiency (Table 2) (Li et al., 2016).

Interaction possibilities between SLN formulation components and active agent were investigated by FT-IR analyses. Characteristic peaks of RCa were detected at 2915, 1550, 1379, 1150 and 964 cm^{-1} (Fig. 3) (Sathali and Nisha, 2013). Characteristic peaks of RCa were not appeared in the spectra of SLNs showing that molecular dispersion of RCa in lipid structures (Fig. 3) (Zhang et al., 1999; Agarwal et al., 2015).

NMR is a powerful tool to investigate dynamic phenomena and the characteristics of nanocompartments in colloidal lipid dispersions. NMR's active nuclei of interest is ^{1}H. Due to the different chemical shifts, it is possible to attribute the NMR signals to particular molecules or their segments. Simple ^{1}H-spectroscopy permits an easy and rapid detection of supercooled melts therefore ^{1}H NMR spectroscopy analyses were performed in order to reveal the ionic interaction between RCa and solid lipids (Müller et al., 2000). The characteristic peaks were observed at 0.9 ppm (due to CH_3 group) and 1.3 ppm (due to CH_2 group) for both SA and TP (Fig. 4) (Jenning et al., 2000). The incorporated RCa signal were not revealed in the ^{1}H NMR specta of the formulations however only slight broadening of the characteristic lipid signals between 0.7–0.9, 1.0–1.5 ppm and 2.0–2.5 ppm for RCA loaded SLNs suggesting that RCa was incorportaed into lipid matrix without any chemical interaction with lipid structure (Fig. 4) (Polchi et al., 2016).

3.2. In vitro release studies

RCa is a weak acid in nature; therefore it is in the ionic form in basic media resulting in higher solubility values in buffers with high pH values when compared with low pH (1.2) buffers (Sarfraz et al., 2017). Therefore in our study *in vitro* release studies were performed using

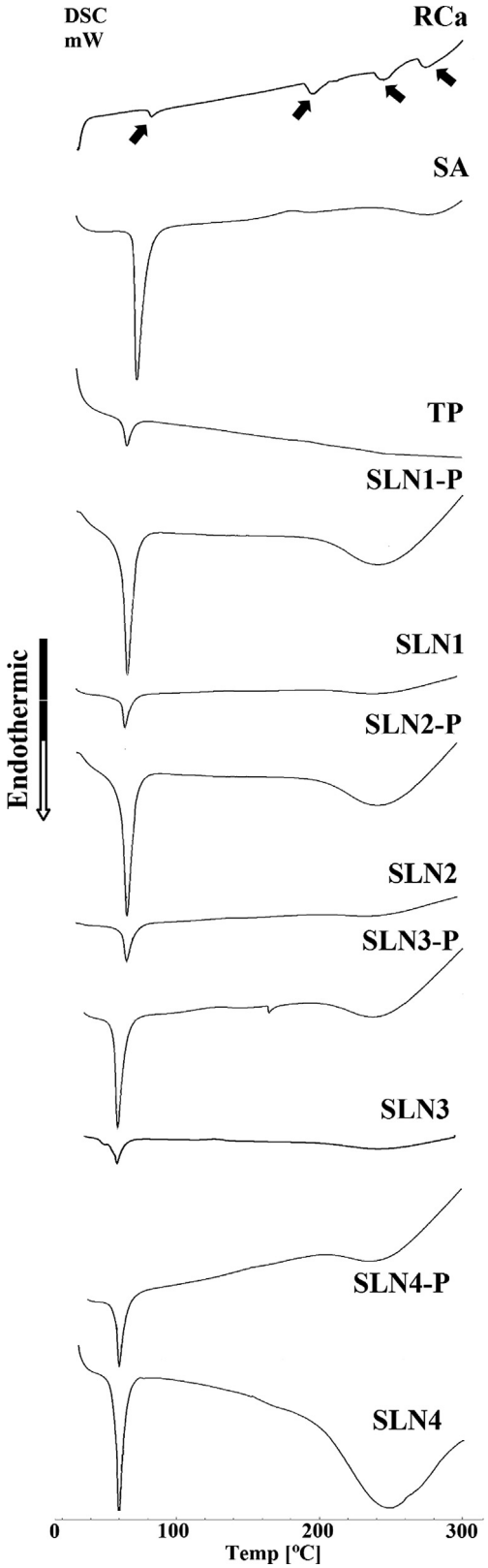

Fig. 1. DSC thermograms of RCa, SA, TP, SLN1-P, SLN1, SLN2-P, SLN2, SLN3-P, SLN3, SLN4-P and SLN4.

dialysis bag method in phosphate buffer (pH 6.8) (Adilya et al., 2014; Yenilmez et al., 2017).

The profiles of *in vitro* release studies of pure RCa and the SLNs formulations as a function of time were illustrated in Fig. 5. In order to

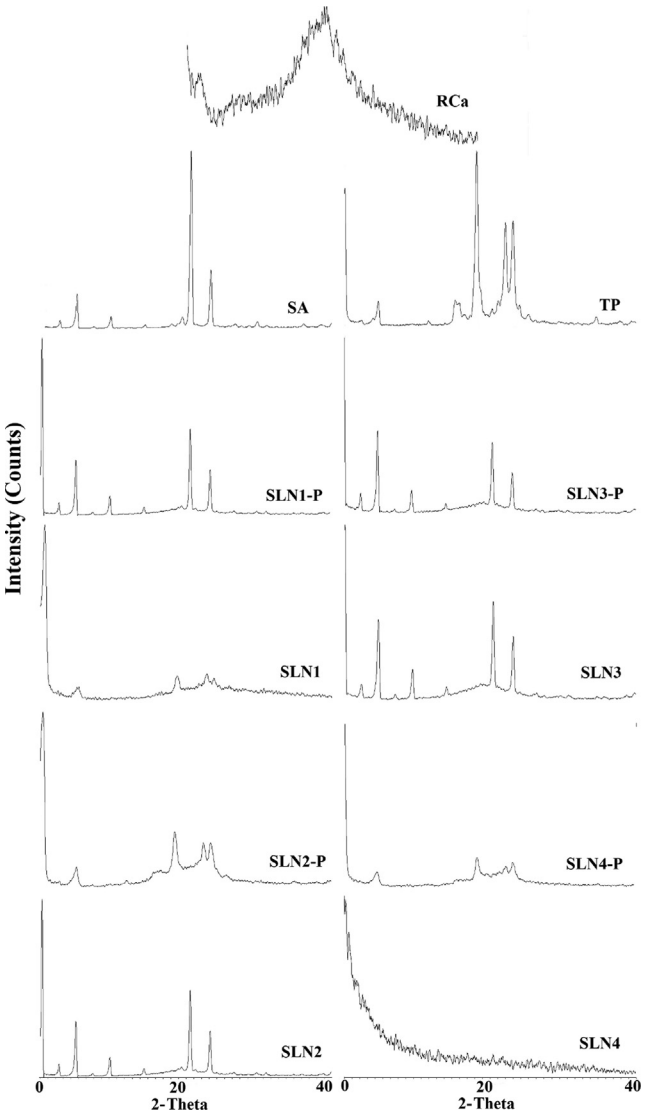

Fig. 2. XRD spectra of RCa, SA, TP, SLN1-P, SLN1, SLN2-P, SLN2, SLN3-P, SLN3, SLN4-P and SLN4.

evaluate controlled/sustained release potential of the SLNs, the *in vitro* releases were monitored for 24 h (Fig. 5).

The release rate of pure RCa was 75.81% within 2 h and reached to 95.46% just after 6 h. For SLN formulations, sustained drug release profiles were revealed in 24 h with initial burst releases within 6 h. The release of a drug from the SLN can be influenced by structural differences of the lipid matrix, surfactant concentration and production parameters (Müller et al., 2000). SLN1 and SLN2 displayed similar biphasic drug release patterns with initial burst release within 6 h (78.88% and 74.36% cumulative release rates for SLN1 and SLN2 respectively), followed by relatively prolonged releases up to 24 h (97.62% and 89.34% for SLN1 and SLN2 respectively) resembling that, surface located RCa generates the burst release while drug-enriched core gives posibility to prolong the release up to 24 h for both SLN1 and SLN2 (Wissing et al., 2004; Kumar et al., 2012; Sathali and Nisha, 2013; Kumar et al., 2020). Similarity factors (f_2) of release profiles were evaluated by DDSolver Program and analyses results revealed that significant differences between the formulations and pure RCa release profile (f_2 factors of 30.36 and 30.38 for SLN1 and SLN2 respectively) were obtained (Zhang et al., 2010).

The cumulative releases were valued as 31.96%, 33.53% and 21.50% at 0.5 h and 95.46%, 92.16%, 92.40% just after 6 h for pure

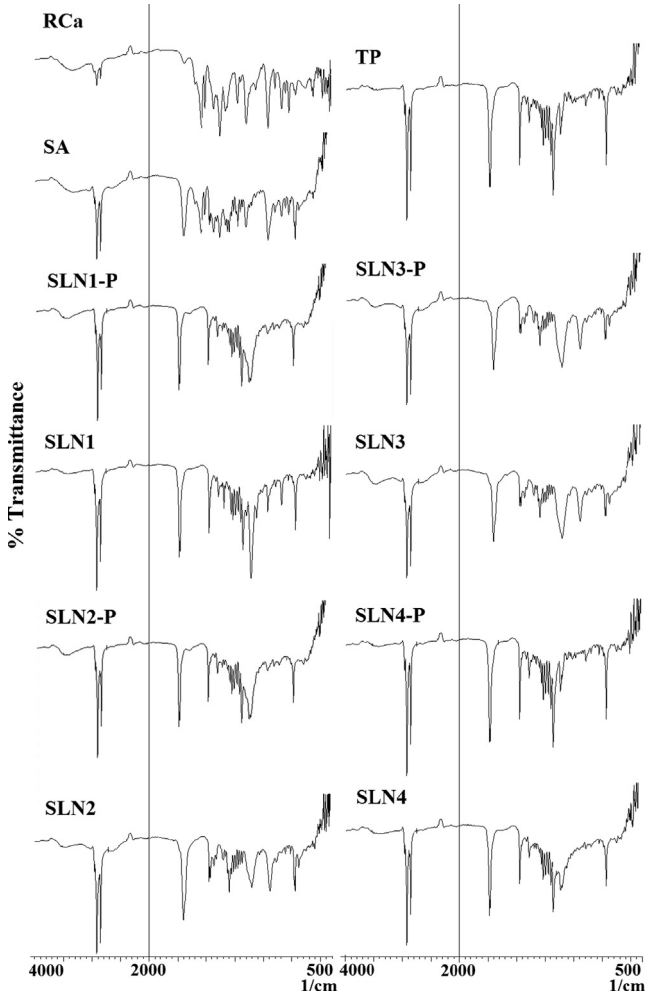

Fig. 3. FT-IR spectra of RCa, SA, TP, SLN1-P, SLN1, SLN2-P, SLN2, SLN3-P, SLN3, SLN4-P and SLN4.

RCa, SLN3 and SLN4 respectively (Fig. 5) (Chantaburanan et al., 2017). Drug release profiles of SLN3 and SLN4 were also compared with pure RCa release profiles considering f_2 factor. SLN3 formulation showed higher similarity with pure RCa profile while significant differences were revealed with SLN4 formulation (f_2 factors of 75.37 and 45.81 for SLN3 and SLN4 respectively).

Drug release profiles revealed that the RCa releases from TP-SLNs (SLN3 and SLN4) were not as extended as SA-SLNs (SLN1 and SLN2) most probably due to the differences in the amount of RCa located on the outer surface of the nanoparticles. Analyses results showed that, also the type of lipid has great influence on drug release characteristics of the particles (Fig. 5) (Zur Muhlen et al., 1998; Müller et al., 2000; Wissing et al., 2004; Sathali and Nisha, 2013; Chantaburanan et al., 2017).

When single solid lipid matrix was used as a carrier material, the incorporation of the drug was limited by the highly ordered lattice of solid lipid matrix (Li et al., 2016). Since TP forms more ordered lipid crystals than SA, the majority of the RCa located on the surface of the TP-SLNs resulting in higher burst effect rates with poor prolonged release of the incorporated drug (Westesen et al., 1997; Hou et al., 2003; Righeschi et al., 2016). From the drug release profiles, it is clear that the prepared SLNs are suitable for the sustained delivery of RCa while shortening t_{max} duration due to the rapid initial burst release (Din et al., 2015; Kumar et al., 2020).

On the basis of the best properties in terms of PS, PDI, ZP, EE, DL and release analyses results, SLN2 formulation was selected for further stability and pharmacokinetic studies.

3.3. Cytotoxicity and in vitro permeation studies

For the determination of the effect of lipid type on cytotoxicity of nanoparticles, SLN2-P, SLN2 and SLN4-P, SLN4 were selected as SA and TP based SLNs respectively. The IC_{50} cytotoxic values as a result of the MTT analyses were calculated to be 335.238 µM, 87.852 µM, 52.138 µM, and 52.137 µM for SLN2-P, SLN2, SLN4-P and SLN4 respectively (Fig. 6). The cytotoxicity studies showed cell viability were over 70%, 50% for SLN2-P, SLN2 and SLN4-P, SLN4 respectively for 50 µM concentration. According to the analyses results the SLN2 formulation with the best cytotoxicity test results was selected for permeability studies (Fig. 6).

Schöler et al. (2002) reported previously that the cytotoxicity of SLN is influenced by the lipid matrix and reduced cell viabilities of SLN consisting of stearic acid is attributed to free stearic acid which is released after intracellular enzymatic degradation of these SLN. In cell culture medium, at high SLN concentrations, protein-SLN interactions are more likely to occur and thus an increased SLN aggregation is possible (Schöler et al., 2002). According to Shah et al. (2016) cellular uptake of SLNs was energy-dependent, and the endocytosis of SLNs was mainly dependent on clathrin-mediated mechanisms. It is considered that cellular uptake of SLN will decrease due to aggregation at high SLN concentrations and thus intracellular enzymatic degradation of SLN will decrease and relevant cytotoxicity will be reduced (Oh and Park, 2014; Shah et al., 2016). Erratic dose-response curve of SLN-2 formulations in our cell viability results may be attributed to the lower cellular uptake of aggregated SLN (at high SLN concentration). Lower cellular uptake is thought to lead a decrease in enzymatic degradation of SLN. Consequently, the release of the free stearic acid which is the cytotoxic component of SLN will be decreased hence the erratic cell viability results.

The permeation studies carried out on pure RCa and SLN2. The results of permeation studies after two hours indicated that the permeation of RCa from SLN2 formulations through tissue membrane were better than the pure RCa. The P_{app} values for SLN2 and plain drug were 5.72×10^{-6} and 3.08×10^{-7} cm sec^{-1} respectively. Digestive lipids comprised of dietary lipids such as fatty acids, glycosides, phospholipids, cholesterol esters as well as various synthetic derivatives enhance the transport of the drug through the intestine. Surfactants that are the other formulation components of SLNs mitigate the intestinal efflux by inhibition of the P-glycoprotein efflux pump therefore lipid nanoparticles have a major role in the enhancement of the drug transport from the intestine (Neupane et al., 2013).

In general, substances with P_{app} values less than 1×10^{-6} cm sec^{-1} are classified as low permeability substances while substances with P_{app} values between 1×10^{-6} and 1×10^{-5} cm sec^{-1} and P_{app} of $> 1 \times 10^{-5}$ cm sec^{-1} are regarded as medium and highly permeable substances respectively. In general, permeability profiles of model drugs are the key determining factors for the assessment of the drug permeability and according to the analyses results, pure RCa valued within the low permeability range, while SLN2 resulted in the medium permeability range (Yee, 1997). Analyses results indicated that SLN formulation has enhanced the permeability of the incorporated drug which probably result in enhanced clinical bioavailability.

3.4. Stability studies

The selected formulation; SLN2, was stored at 5 ± 3 °C, 25 ± 2 °C and 40 ± 2 °C in hermetically closed glass containers (to avoid the the effect of relative humidity of the storage conditions) for stability studies during the storage period of 3 months. The stability of the particles were evaluated with PS, PDI, ZP and remained RCa (%) analyses and the analyses results were presented in Table 3.

Freshly prepared SLN2 formulation had 134.4 ± 0.91 nm mean PS

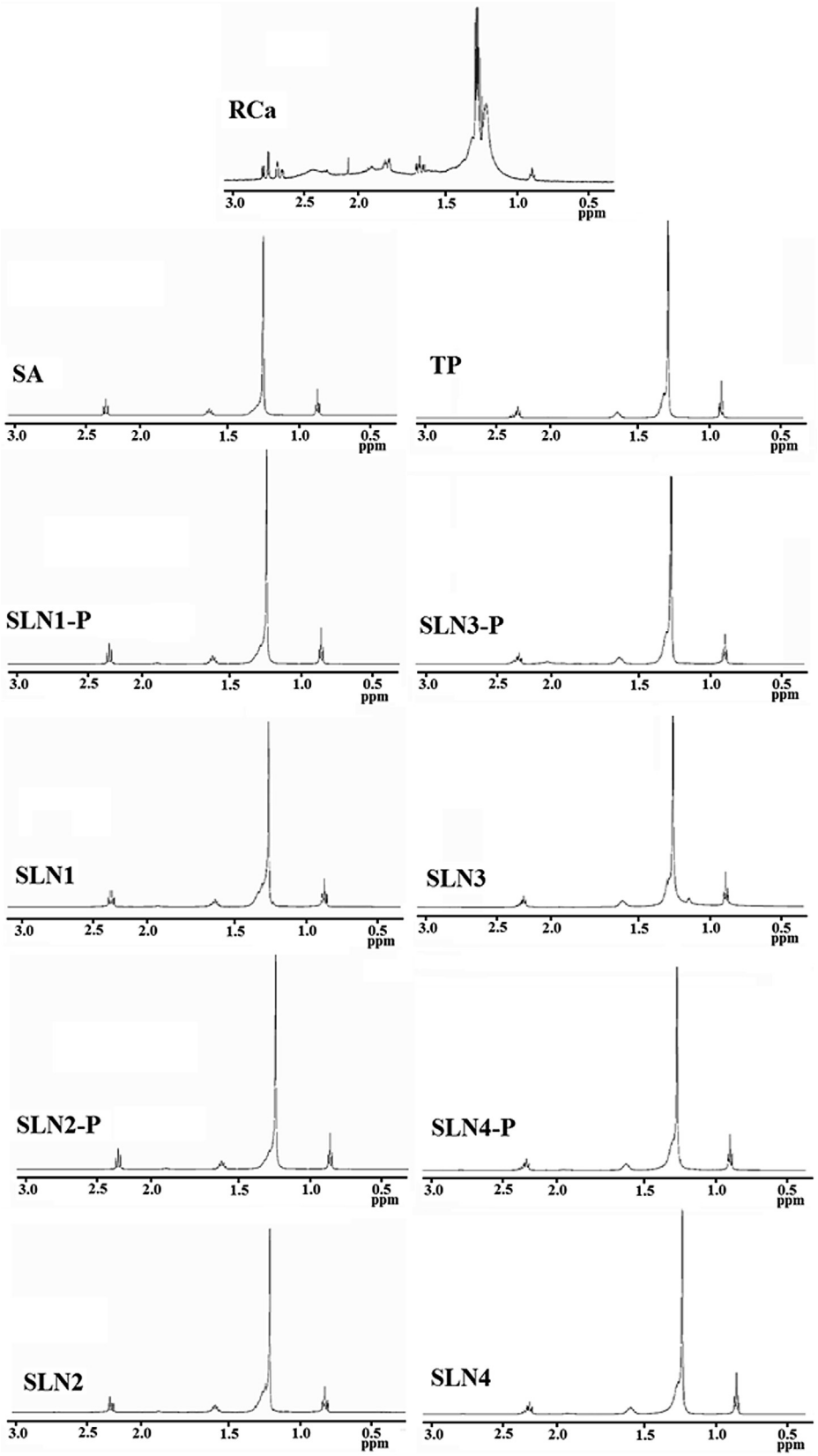

Fig. 4. ^{1}H NMR spectra of RCa, SA, TP, SLN1-P, SLN1, SLN2-P, SLN2, SLN3-P, SLN3, SLN4-P and SLN4.

(PDI = 0.13 ± 0.01) and at the end of 3 months mean PS of SLN2 showed significant differences and the detected PS were 286.3 ± 5.55 nm ($p \leq 0.0001$); (PDI = 0.43 ± 0.01; $p \leq 0.0001$), 348.0 ± 6.35 nm ($p \leq 0.0001$); (PDI = 0.46 ± 0.01; $p \leq 0.0001$), 621.0 ± 16.6 nm ($p \leq 0.0001$); (PDI = 0.77 ± 0.02; $p \leq 0.0001$) for the formulations kept at 5 ± 3 °C, 25 ± 2 °C and 40 ± 2 °C respectively (Table 3). Despite the variations in PS, particles remained in nanometer range during the storage period of 3 months.

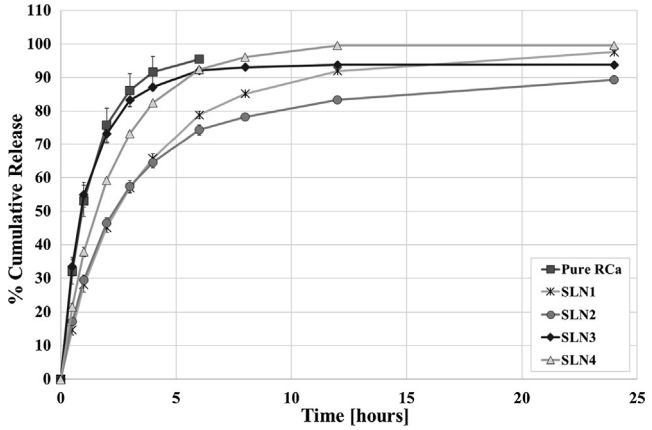

Fig. 5. In vitro release analyses results of RCa from SLN formulations (mean ± SE; n = 3).

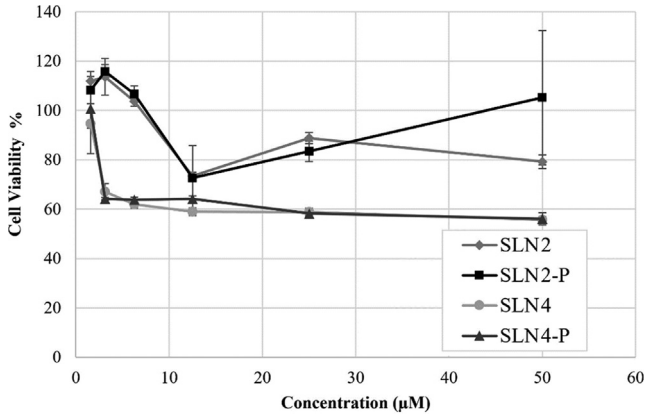

Fig. 6. Cell viability % of SLN2, SLN2-P, SLN4, and SLN4-P formulations (mean ± SE; n = 3).

Specific surface characteristics, morphology, and the smaller particle sizes are the key factors for enhanced oral bioavailability of the incorporated drug. It has been reported that increase in surface area due to the reduced particle size leads to enhanced absorption in the GIT of active agents (Hirunpanich et al., 2008; Poovi and Damodharan, 2018).

ZP analyses revealed that SLNs were negatively charged due to the characteristic properties of SA and TP. Freshly prepared SLN2 formulation had −18.77 ± 0.20 mV ZP value, while ZPs were recorded as −36.43 ± 0.69 mV (0.01 > p > 0.0001), −36.10 ± 0.98 mV (0.01 > p > 0.0001), −35.77 ± 0.78 mV (0.001 > p > 0.0001) for the formulations kept at 5 ± 3 °C, 25 ± 2 °C, and 40 ± 2 °C respectively (Table 3). Colloidal dispersions are considered as physically stable when their ZP values are greater than ± 30 mV (Souto et al., 2004). Analyses results revealed that despite significant changes in ZP values (0.01 > p > 0.0001), formulations transformed to more stable dispersions during the storage period of 3 months with ZP values up to ~ −37 mV (Table 3) (Souto et al., 2004).

In stability studies, the remaining amount of RCa % in the formulations were evaluated. HPLC analyses results revealed that RCa amounts were 98.10 ± 0.5%, 95.19 ± 1.6% and 65.35 ± 10.6% for the formulations kept at 5 ± 3 °C, 25 ± 2 °C, and 40 ± 2 °C respectively after 3 months (Table 3). There are significant differences in the remained RCa% values at 40 ± 2 °C indicating possible chemical degradation to RCa impurity C (Machairas et al., 2018). The remained RCa% values are not extensively changed for other temperatures with valued as 98.10 ± 0.5 and 95.19 ± 1.6 for the formulations kept at 5 ± 3 °C and 25 ± 2 °C respectively. Changes in RCa amounts were not exceeded limit of ± 10% therefore formulations were regarded as stable at 5 ± 3 °C and 25 ± 2 °C during the storage period of 3 months (Başaran et al., 2010; Lingayat et al., 2012).

Considering the physical and chemical changes, it can be concluded that 5 ± 3 °C and 25 ± 2 °C seem to be better storage temperatures than 40 ± 2 °C for SLN2 formulation considering short term (3 months) stability studies results.

3.5. Pharmacokinetic studies

Oral administration is the most preferred route for drug delivery because of its simplicity, convenience, and patient compliance, especially in the case of repeated dosing for chronic therapies (Xu et al., 2013). However, many of the drugs remained poorly absorbed when administered by oral route, owing to the physicochemical properties of the drug (e.g., pKa, solubility, stability, lipophilicity, polar-nonpolar surface area, presence of hydrogen bonding functionalities and crystal form) and factors related to the dosage forms (Agüeros et al., 2011). The bioavailability of oral drugs is strongly influenced by two important parameters, solubility and permeability rates of the APIs (Müller et al., 2006; Xu et al., 2013). In the past several years, SLNs have been extensively investigated and developed as a potential nanocarriers for oral drug delivery. Similar to liposomes, lipid nanoemulsions, and micelles, SLNs could improve oral absorption of many drugs because of their higher encapsulation efficiency (Demirel et al., 2001; Chai et al., 2016).

Pharmacokinetic studies were performed on male Sprague Dawley rats to evaluate the efficiency of the selected SLN2 formulation on the enhancement of the oral bioavailability of RCa. The actual dose range for RCa in adults is 5–80 mg orally once daily and the usual starting dose for treatment is 10–20 mg once a day (Rubba et al., 2009; Luvai et al., 2012; http1–http3). It was reported that there were no mortalities in rats given an oral dose of 1000 mg kg^{-1} or 2000 mg kg^{-1}, and other than depression of bodyweight at 2000 mg kg^{-1} (http3) therefore 2.2 mg kg^{-1} standard RCa suspension and SLN2 formulation (with

Table 3
Stability analyses results of SLN2 formulation after the storage period of 3 months (Mean ± SE; n = 3).

Code	Storage temperature	PS (nm)	PDI	ZP (mV)	Remained RCa (%)
SLN2-Day 0	–	134.4 ± 0.91	0.13 ± 0.01	−18.77 ± 0.20	100 ± 0.0
SLN2 1st month	5 °C ± 3 °C	155.3 ± 6.38	0.23 ± 0.06	−19.10 ± 0.51	–
	25 °C ± 2 °C	181.0 ± 1.53	0.26 ± 0.02	−28.10 ± 1.97	–
	40 °C ± 2 °C	527.7 ± 11.8	0.41 ± 0.04	−36.43 ± 0.69	–
SLN2 2nd month	5 °C ± 3 °C	255.0 ± 6.10	0.37 ± 0.02	−28.10 ± 1.97	–
	25 °C ± 2 °C	264.7 ± 8.65	0.44 ± 0.01	−30.77 ± 1.00	–
	40 °C ± 2 °C	561.0 ± 23.5	0.60 ± 0.05	−37.43 ± 1.36	–
SLN2 3rd month	5 °C ± 3 °C	286.3 ± 5.55	0.43 ± 0.01	−36.43 ± 0.69	98.10 ± 0.5
	25 °C ± 2 °C	348.0 ± 6.35	0.46 ± 0.01	−36.10 ± 0.98	95.19 ± 1.6
	40 °C ± 2 °C	621.0 ± 16.6	0.77 ± 0.02	−35.77 ± 0.78	65.35 ± 10.6

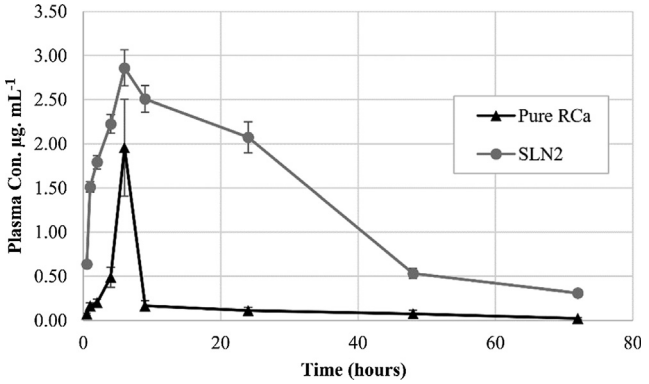

Fig. 7. The plasma concentration curves of pure RCa and SLN2 formulation (Mean ± SE, n = 5).

comparable amount of RCa) were given orally considering not exeeding the toxic level. The plasma concentration profiles of pure RCa and SLN2 after a single dose with oral administration were shown in Fig. 7.

The results of pharmacokinetic studies indicated remarkably superior drug absorption potential from SLN2 formulation with respect to the pure RCa (8.5 fold increase). SLN2 showed significant improvement ($p \leq 0.0001$) in the extent of drug absorption (AUC_{last} and AUC_{total}) compared to pure RCa. In our former study almost 8 fold oral bioavailability enhancement compared to the pure RCa was achieved with RCa incorporated cyclodextrin-polyanhydride nanoparticles (Al-Heibshy et al., 2019). Since the lipid based drug delivery systems enhance the lymphatic transport of the incorporated lipophilic drugs, the success of SLN2 can be also attributed to enhanced lymphatic transport of drug from SLN2 formulation and also it is possible to degrease the applied doses with SLN formulations while maintaining comparable drug plasma levels (Suresh et al., 2007; Rani et al., 2017).

The average AUC_{last} and AUC_{total} of pure RCa were 10.62 ± 2.12 and 12.00 ± 2.48 µg h mL^{-1} (mean ± SE) respectively, whereas for SLN2, AUC_{last} and AUC_{total} were valued as 90.64 ± 5.74 and 98.87 ± 5.68 µg h mL^{-1} (mean ± SE) respectively. Almost 1.41, 2.06 and 2.46-fold improvement in C_{max}, MRT and $t_{1/2}$ were observed for the SLN2 with respect to pure RCa, while t_{max} value (6 h) remained unchanged. It has been widely reported that due to the characteristic properties of the lipids and surfactants used for the formation of SLNs, the lipids and the surfactants act as good permeation enhancers for the drug and enhance the solubility of the drug in the GIT milieu while reducing the first-pass metabolism of the drug by transportation of the incorporated drug through a lymphatic route to the systemic circulation (Neupane et al., 2013; Sznitowska et al., 2017). In this study pure RCa concentration has reached the lowest plasma concentration point just after 9 h while SLN2 showed very high RCa concentration at same point showing that it is possible to extend repeated dose application period as well as maintaining enhanced oral bioavailability of RCa which was the main aim of the study.

4. Conclusions

In our study, RCa-loaded SLNs were successfully formulated by high shear homogenization followed by ultrasonication technique. With the avoidance of organic solvents and use of ingredients with GRAS status, safer formulations were formulated. The physicochemical properties of the RCa-loaded SLNs showed that the SLNs have PS in a nanometer range with relatively high ZP values. The sustained release of RCa could be achieved with SLNs with the ratio of 97.78% up to 24 h. The SA SLNs exhibited less cytotoxicity effect than TP SLNs, therefore among the formulations prepared, SA based SLN2 formulation was selected for stability and pharmacokinetic studies. The SLN2 has enhanced the oral bioavailability of RCa compared to the pure RCa (8.5 fold increase) and able to provide sustained release profile up to 72 h in rats. According to pharmacokinetic studies analyses results pure RCa concentration has reached the lowest plasma concentration after 9 h while SLN2 showed very high RCa concentration at same point showing that it is possible to extend repeated dose application period as well as maintaining enhanced oral bioavailability of RCa which was the main aim of the study. In stability analyses SLN2 formulation was found to be stable considering PS, PDI, ZP and remained RCa (%) at 5 ± 3 °C and 25 ± 2 °C over 3 months. As a conclusion SLN2 formulation has great potential on the enhancement of the oral bioavailability of RCa while maintaining the sustained release up to 3 days.

CRediT authorship contribution statement

Fawaz N.S. Al-Heibshy: Formal analysis, Investigation, Validation, Writing - original draft. **Ebru Başaran:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Rana Arslan:** Formal analysis. **Naile Öztürk:** Formal analysis. **Kevser Erol:** Formal analysis. **Müzeyyen Demirel:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2020.119106.

References

Adilya, N.P., Macedo, A.S., Doktorovova, S., Souto, E.B., Kim, S., Chang, P.-S., Ko, S., 2014. Development and evaluation of lipid nanocarriers for quercetin delivery: a comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE). Food Sci. Technol.-LEB. 59 (1), 115–121.

Agarwal, R., Malthar, H.P., Chaitanya, B., 2015. Development and pharmacodynamic evaluation of rosuvastatin-loaded nanostructured lipid carriers. J. Pharm. Pharm. Sci. 4 (7), 699–716.

Agüeros, M., Espuelas, S., Esparza, I., Calleja, P., Peñuelas, I., Ponchel, G., Irache, J., 2011. Cyclodextrin-poly(anhydride) nanoparticles as new vehicles for oral drug delivery. Expert Opin. Drug Deliv. 8 (6), 721–734.

Al-Heibshy, F.N.S., Başaran, E., Arslan, R., Öztürk, N., Vural, I., Demirel, M., 2019. Preparation, characterization and pharmacokinetic evaluation of rosuvastatin calcium incorporated cyclodextrin-polyanhydride nanoparticles. Drug. Dev. Ind. Pharm. 45 (10), 1635–1645.

Al-Shdefat, R., Anwer, K., Fayed, M.H., Alsulays, B.B., Tawfeek, H.M., Abdel-Rahman, R.F., Soliman, G.A., 2020. Preparation and evaluation of spray dried rosuvastatin calcium-PVP microparticles for the improvement of serum lipid profile. J. Drug. Deliv. Sci. Tec. 55 (101342), 1–9.

Alshora, D.H., Haq, N., Alanazi, F.K., Ibrahim, M.A., Shakeel, F., 2016. Solubility of rosuvastatin calcium in different neat solvents at different temperatures. J. Chem. Thermodyn. 94, 230–233.

Balakumar, K., Raghavan, C.V., Tamilselvan, N., Hariprasad, R., Abdu, S., 2013. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. Colloid. Surf. B 112,

337–343.
Başaran, E., Demirel, M., Sırmagül, B., Yazan, Y., 2010. Cyclosporine-A incorporated cationic solid lipid nanoparticles for ocular delivery. J. Microencapsul. 27 (1), 37–47.
Başaran, E., Demirel, M., Sirmagül, B., Yazan, Y., 2011. Polymeric cyclosporine-A nanoparticles for ocular application. J. Biomed. Nanotechnol. 7 (5), 714–723.
Beg, S., Jain, S., Kushwah, V., Bhatti, G.K., Sandhu, P.S., Katare, O., Singh, B., 2017. Novel surface-engineered solid lipid nanoparticles of rosuvastatin calcium for low-density lipoprotein-receptor targeting, a quality by design-driven perspective. Nanomedicine 12 (4), 333–356.
Beg, S., Raza, K., Kumar, R., Chadha, R., Katare, O.P., Singh, B., 2016. Improved intestinal lymphatic drug targeting via phospholipid complex-loaded nanolipospheres of rosuvastatin calcium. RSC Adv. 6 (10), 8173–8187.
Beniwal, A., Choudhary, H., 2017. Rosuvastatin calcium-loaded Solid Lipid Nanoparticles (SLN) using design of experiment approach for oral delivery Int. J. Chem. Lifesci. 6 (5), 2029–2038.
Blaser, D.W., 2007. Determination of drug absorption parameters in Caco-2 cell monolayers with a mathematical model encompassing passive diffusion. J. Int. Adsorpt. Soc. 25–34.
Chai, G.-H., Xu, Y., Chen, S.-Q., Cheng, B., Hu, F.-Q., You, J., Du, Y.-Z., Yuan, H., 2016. Transport mechanisms of solid lipid nanoparticles across Caco-2 cell monolayers and their related cytotoxicology. ACS Appl. Mater. Interfaces 8 (9), 5929–5940.
Chantaburanan, T., Teeranachaideekul, V., Chantasart, D., Jintapattanakit, A., Junyaprasert, V.B., 2017. Effect of binary solid lipid matrix of wax and triglyceride on lipid crystallinity, drug-lipid interaction and drug release of ibuprofen-loaded solid lipid nanoparticles (SLN) for dermal delivery. J. Colloid Interface Sci. 504, 247–256.
Cheng, J.W.M., 2004. Rosuvastatin in the management of hyperlipidemia. Clin. Ther. 26 (9), 1368–1387.
Dara, T., Vatanara, A., Meybodi, M.N., Vakilinezhad, M.A., Malvajerd, S.S., Vakhshiteh, F., Shamsian, A., Sharifzadeh, M., Kaghazian, H., Mosaddegh, M.H., 2019. Erythropoietin-loaded solid lipid nanoparticles: Preparation, optimization, and *in vivo* evaluation. Colloids. Surf. B. Biointerfaces 178, 307–316.
Demirel, M., Yazan, Y., Müller, R.H., Kılıç, F., Bozan, B., 2001. Formulation and *in vitro – in vivo* evaluation of piribedil solid lipid micro- and nanoparticles. J. Microencapsul. 18 (3), 359–371.
Demirel, M., Büyükköroğlu, G., Sırmagül, B., Kalava, B.S., Öztürk, N., Yazan, Y., 2014. Enhanced bioavailability of cinnarizine using solid dispersion: *in vitro* and *in vivo* evaluation. Curr. Drug. Theraphy 9, 294–301.
Din, F., Mustapha, O., Kim, D.W., Rashid, R., Park, J.H., Choi, J.Y., Ku, S.K., Yong, C.S., Kim, J.O., Choi, H.-G., 2015. Novel dual-reverse thermosensitive solid lipid nanoparticle-loaded hydrogel for rectal administration of flurbiprofen with improved bioavailability and reduced initial burst effect. Eur. J. Pharm. Biopharm. 94, 64–72.
Dudhipala, N., Veerabrahma, K., 2017. Improved anti-hyperlipidemic activity of Rosuvastatin Calcium via lipid nanoparticles, pharmacokinetic and pharmacodynamic evaluation. Eur. J. Pharm. Biopharm. 110, 47–57.
Ekambaram, P., Abdul Hasan Sathali, A., 2011. Formulation and evaluation of solid lipid nanoparticles of ramipril. J. Young Pharm. 3 (3), 216–220.
Fabbri, G., Maggioni, A.P., 2009. Cardiovascular risk reduction: what dorecent trials with rosuvastatin tell us? Adv. Ther. 26 (5), 469–487.
Gadad, A.P., Tigadi, S.G., Dandagi, P.M., Mastiholimath, V.S., Bolmal, U.B., 2016. Rosuvastatin loaded nanostructured lipid carrier: for enhancement of oral bioavailability. Indian J. Pharm. Ed. Res. 50 (4), 605–611.
Hanumanaik, M., Patel, S.K., Sree, K.R., 2013. Solid lipid nanoparticles; a review. Int. J. Pharm. Sci. Res. 4 (3), 928–940.
Himavarshini, J., Sumaharshan, J.R., 2017. Formulation development and in vitro charaterization of rosuvastatin calcium solid lipid nanoparticles. Int. J. Pharm. Therap. 8 (3), 123–129.
Hirpara, M.R., Manikkath, J., Sivakumar, K., Managuli, R.S., Gourishetti, K., Krishnadas, N., Shenoy, R.R., Jayaprakash, B., Rao, C.M., Mutalik, S., 2018. Long circulating PEGylated-chitosan nanoparticles of rosuvastatincalcium: development and in vitro and in vivo evaluations. Int. J. Biol. Macromol. 107, 2190–2200.
Hirunpanich, V., Sugiyama, E., Sato, H., 2008. Ethyl docosahexaenoate decreased Neoral® absorption due to particle size enlargement. Int. J. Pharm. 361, 251–252.
Hou, D., Xie, C., Huang, K., Zhu, C., 2003. The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials 24 (10), 1781–1785.
http1: https://www.rxlist.com/crestor-drug.htm#dosage..
http2: https://www.medicines.org.uk/emc/search?q=rosuvastatin.
http3: https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+7317.
ICH Q1A-R2, 2003. International Conference On Harmonisation of Technical Requirements For Registration of Pharmaceuticals For Human Use, ICH Harmonised Tripartite Guideline: Stability Testing Of New Drug Substances And Products.
ICH Q2A-R2, 2005, International Conference On Harmonisation of Technical Requirements For Registration of Pharmaceuticals For Human Use, ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text And Methodology.
ICH Q2B, 1996. International Conference On Harmonisation of Technical Requirements For Registration of Pharmaceuticals For Human Use, ICH Harmonised Tripartite Guideline: Guidance for industry, validation of analytical procedures, methodology.
Jenning, V., Mäder, K., Gohla, S.H., 2000. Solid lipid nanoparticles (SLN™) based on binary mixtures of liquid and solid lipids: a ^{1}H-NMR study. Int. J. Pharm. 205, 15–21.
Kheradmandnia, S., Vasheghani-Farahani, E., Nosrati, M., Atyabi, F., 2010. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. Nanomed.-Nanotechnol. 6 (6), 753–759.
Kumar, P.P., Gayatri, P., Sunil, R., Rao, Y.M., 2012. Atorvastatin loaded solidlipid nanoparticles, formulation, optimization and *in - vitro* characterization. IOSR J. Pharm. 2 (5), 23–32.
Kumar, R., Singh, A., Sharma, K., Dhasmana, D., Garg, N., Siril, P.F., 2020. Preparation, characterization and in vitro cytotoxicity of Fenofibrate and Nabumetone loaded solid lipid nanoparticles. Mat. Sci. Eng. C-Mater. 106 (110184), 1–13.
Kumar, T.R., Shitut, N.R., Kumar, P.K., Vinu, M.C.A., Pavan Kumar, V.V., Mullangi, R., Srinivas, N.R., 2006. Determination of rosuvastatin in rat plasma by HPLC, validation and its application to pharmacokinetic studies. Biomed. Chromatogr. 20 (9), 881–887.
Li, M., Zahi, M.R., Yuan, Q., Tian, F., Liang, H., 2016. Preparation and stability of astaxanthin solid lipid nanoparticles based on stearic acid. Eur. J. Lipid Sci. Technol. 118 (4), 592–602.
Lingayat, V.J., Zarekar, N.S., Shendge, R.S., 2012. Solid lipid nanoparticles, a review. Nanosci. Nanotechnol. Res. 2 (1), 80–102.
Luvai, A., Mbagaya, W., Hall, A.S., Barth, J.H., 2012. Rosuvastatin: a review of the pharmacology and clinical effectiveness in cardiovascular disease. Clin. Med. Insights Cardiol. 6, 17–33.
Martin, P.D., Warwick, M.J., Dane, A.L., Brindley, C., Short, T., 2003. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. Clin. Ther. 25 (10), 2553–2563.
Mäder, K., Mehnert, W., 2001. Solid lipid nanoparticles, production, characterization and applications. Adv. Drug Deliv. Rev. 47 (2–3), 165–196.
Müller, R.H., Heinemann, S., 1993. Fat emulsions for parenteral nutrition II: Characterisation and physical long-term stability of lipofundin. MCT/LCT. Clin. Nutr. 12, 298–309.
Müller, R.H., Mäder, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.
Müller, R.H., Runge, S., Ravelli, V., Mehnert, W., Thünemann, A.F., Souto, E.B., 2006. Oral bioavailability of cyclosporine, solid lipid nanoparticles (SLNs) versus drug nanocrystals. Int. J. Pharm. 317 (1), 82–89.
Netsomboon, K., Feßler, A., Erletz, L., Prüfert, F., Ruetz, M., Kieninger, C., Kräutler, B., Bernkop-Schnürch, A., 2016. Vitamin B12 and derivatives—In vitro permeation studies across Caco-2 cell monolayers and freshly excised rat intestinal mucosa. Int. J. Pharm. 497, 129–135.
Neupane, Y.R., Sabir, M.D., Ahmad, N., Ali, M., Kohli, K., 2013. Lipid drug conjugate nanoparticle as a novel lipid nanocarrier for the oral delivery of decitabine, *ex vivo* gut permeation studies. Nanotechnology 24 (41), 1–11.
Oehlke, K., Behsnilian, D., Mayer-miebach, E., Weidler, P.G., Greiner, R., 2017. Edible solid lipid nanoparticles (SLN) as carrier system for antioxidants of different lipophilicity. PLoS ONE 12 (2), 1–19.
Olbrich, C., Kayser, O., Müller, R.H., 2002. Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN) - effect of surfactants, storage time and crystallinity. Int. J. Pharm. 237 (1–2), 119–128.
Oh, N., Park, J.H., 2014. Endocytosis and exocytosis of nanoparticles in mam-malian cells. Int. J. Nanomed. 9 (1), 51–63.
Polchi, A., Magini, A., Mazuryk, J., Tancini, B., Gapiński, J., Patkowski, A., Giovagnoli, S., Emiliani, C., 2016. Rapamycin loaded solid lipid nanoparticles as a new tool to deliver mTOR inhibitors: formulation and in vitro characterization. Nanomaterials 6 (87), 1–20.
Poovi, G., Damodharan, N., 2018. Lipid nanoparticles: A challenging approach for oral delivery of BCS Class-II drugs. J. Pharm. Sci-US. 4, 191–205.
Rani, R., Dahiya, S., Dhingra, D., Dilbaghi, N., Kim, K.-H., Kumar, S., 2017. Evaluation of anti-diabetic activity of glycyrrhizin-loaded nanoparticles in nicotinamide-streptozotocin-induced diabetic rats. Eur. J. Pharm. Sci. 106, 220–230.
Reddy, N.R., Shariff, A., 2013. Solid lipid nanoparticles, an advanced drug delivery system. Int. J. Pharm. Sci. Res. 4 (1), 161–171.
Righeschi, C., Bergonzi, M.C., Isacchi, B., Bazzicalupi, C., Gratteri, P., Bilia, A.R., 2016. Enhanced curcumin permeability by SLN formulation, The PAMPA approach. LWT - Food Sci. Technol. 66, 475–483.
Rubba, P., Marotta, G., Gentile, M., 2009. Efficacy and safety of rosuvastatin in the management of dyslipidemia. Vascular Health Risk Manage. 5, 343–352.
Sahebkar, A., Watts, G.F., 2013. New LDL-cholesterol lowering therapies: pharmacology, clinical trials, and relevance to acute coronary syndromes. Clin. Ther. 35 (8), 1082–1098.
Sarfraz, R.M., Ahmad, M., Mahmood, A., Minhas, M.U., Yaqoob, A., 2017. Development and evaluation of rosuvastatin calcium based microparticles for solubility enhancement, an in vitro study. Adv. Polym. Technol. 1–9.
Sathali, A.A.H., Nisha, N., 2013. Development of solid lipid nanoparticles of rosuvastatin calcium. J. Pharm. Res. 1 (5), 536–548.
Schöler, N., Hahn, H., Müller, R.H., Liesenfeld, O., 2002. Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of macrophages. Int. J. Pharm. 231 (2), 167–176.
Senthil Kumar, P., Arivuchelvan, A., Jagadeeswaran, A., Punniamurthy, N., Selvaraj, P., Richard Jagatheesan, P.N., Mekala, P., 2015. Formulation of enrofloxacin SLNs and its pharmacokinetics in emu (Dromaius novaehollandiae) birds. Appl. Nanosci. 5 (6), 661–671.
Singh, H., Gupta, R.D., Gautam, G., 2018. Formulation development, characterization, and in vitro-in vivo study of antihyperlipidemic drug rosuvastatin calcium - solid lipid nanoparticles. Asian J. Pharm. Clin. Res. 11 (7), 436–443.
Shah, R.M., Rajasekaran, D., Ludford-Menting, M., Eldridge, D.S., Palombo, E.A., Harding, I.H., 2016. Transport of stearic acid-based solid lipid nanoparticles (SLNs) into human epithelial cells. Colloids. Surf. B. Biointerfaces. 140, 204–212.
Shete, G., Puri, V., Kumar, L., Bansal, A.K., 2010. Solid state characterization of commercial crystalline and amorphous atorvastatin calcium samples. AAPS PharmSciTech. 11 (2), 598–609.
Souto, E.B., Wissing, S.A., Barbosa, C.M., Müller, R.H., 2004. Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations. Eur. J. Pharm. Biopharm. 58, 83–90.
Suares, D., Parbhakar, B., 2016. Oral delivery of rosuvastatin lipid nanocarriers,

investigation of *in vitro* and *in vivo* profile. Int. J. Pharm. Sci. Res. 7 (12), 4856–4864.
Suresh, G., Manjunath, K., Venkateswarlu, V., Satyanarayana, V., 2007. Preparation, characterization, and in vitro and in vivo evaluation of lovastatin solid lipid nanoparticles. AAPS Pharm. Sci. Tech. 8 (1), 2779–2785.
Suvarna, G., Narender, D., Kishan, V., 2015. Preparation, characterization and in vivo evaluation of rosuvastatin calcium loaded solid lipid nanoparticles. Int. J. Pharm. Sci. Nanotech. 8 (1), 2279–2785.
Sznitowska, M., Wolska, E., Baranska, H., Cal, K., Pietkiewicz, J., 2017. The effect of a lipid composition and a surfactant on the characteristics of the solid lipid microspheres and nanospheres (SLM and SLN). Eur. J. Pharm. Biopharm. 110, 24–30.
Trucillo, P., Campardelli, R., 2019. Production of solid lipid nanoparticles with a supercritical fluid assisted process. J. Supercrit. Fluids. 143, 16–23.
Wang, L., Wang, C.-Y., Zhang, Y., Fu, H.-J., Gao, Y., Zhang, K.-R., 2019. Preparation and characterization of solid lipid nanoparticles loaded with salmon calcitonin phospholipid complex. J. Drug. Deliv. Sci. Technol. 52, 838–845.
Westesen, K., Bunjes, H., Koch, M.H.J., 1997. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J. Control. Release. 48, 223–236.
Wissing, S.A., Kayser, O., Muller, R.H., 2004. Solid lipid nanoparticles for parenteral drug delivery. Adv. Drug Deliv. Rev. 56, 1257–1272.
Xu, W., Ling, P., Zhang, T., 2013. Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. J. Drug Deliv. 2013, 1–15.
Yavuz, B., Bilensoy, E., Vural, İ., Şumnu, M., 2010. Alternative oral exemestane formulation: improved dissolution and permeation. Int. J. Pharm. 398 (1–2), 137–145.
Yee, S., 1997. *In vitro* prmeability across caco-2 cells (colonic) can redict *in vivo* (small intestinal) absorption in man - fact or myth. Pharm. Res. 14 (6), 763–766.
Yenilmez, E., Yurtdaş Kırımlıoğlu, G., Şenel, B., Başaran, E., 2017. Preparation, characterization and *in vitro* evaluation of dirithromycin loaded Eudragit® RS 100 nanoparticles for topical application. Lat. Am. J. Pharm. 36 (11), 2203–2212.
Yong, Y., Saleem, A., Guerrero-Analco, J.A., Haddad, P.S., Cuerrier, A., Arnason, J.T., Harris, C.S., Johns, T., 2016. Larix laricina bark, a traditional medicine used by the Cree of Eeyou Istchee, antioxidant constituents and *in vitro* permeability across Caco-2 cell monolayers. J. Ethnopharmacol. 194 (3), 651–657.
Zhang, Q., Yie, G., Li, Y., Yang, Q., 1999. Studies on the cyclosporin a loaded stearic acid nanoparticles. Int. J. Pharm. 34 (4), 311–312.
Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., Xie, S., 2010. DDSolver, an add-in program for modeling and comparison of drug dissolution profiles. AAPS J. 12 (3), 263–271.
Zur Muhlen, A., Schwarz, C., Mehnert, W., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery drug release and release mechanism. Eur. J. Pharm. Biopharm. 45, 149–155.