



Research paper

Montelukast-loaded nanostructured lipid carriers: Part I Oral bioavailability improvement



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ABSTRACT

The purpose of the study was to formulate montelukast-loaded nanostructured lipid carrier (MNLC) to improve its systemic bioavailability, avoid hepatic metabolism and reduce hepatic cellular toxicity due to metabolites. MNLC was prepared using melt-emulsification-homogenization method. Preformulation study was carried out to evaluate drug-excipient compatibility. MNLCs were prepared using spatially different solid and liquid lipid triglycerides. CAE (DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester), a cationic, biodegradable, biocompatible surfactant was used to stabilize the system. MNLCs were characterized by FTIR, XRPD and DSC to evaluate physicochemical properties. MNLCs having a particle size of 181.4 ± 6.5 nm with encapsulation efficiency of $96.13 \pm 0.98\%$ were prepared. FTIR findings demonstrated no interaction between the drug and excipients of the formulation which could lead to asymmetric vibrations. DSC and XRPD study confirmed stable amorphous form of the montelukast in lipid matrix. *In vitro* release study revealed sustained release over a period of 24 h. *In vivo* single dose oral pharmacokinetic study demonstrated 143-fold improvement in bioavailability as compared to montelukast-aqueous solution. Thus, the result of this study implies that developed MNLC formulation be suitable to sustain the drug release with improvement in the bioavailability.

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1. Introduction

Asthma is a bronchial hypersensitivity disorder characterized by chronic, long lasting reversible airway obstruction. Asthma is produced by a combination of mucosal edema, constriction of the bronchial musculature, and excessive secretion of viscid mucus, causing mucous plugs. Primary problem associated with asthma is airway inflammation due to release of inflammatory mediators such as histamine, tryptase, leukotrienes and prostaglandins from bronchial mast cells, alveolar macrophages, T lymphocytes and

epithelial cells. These inflammatory mediators are triggered by exposure to allergens, irritants, cold air or exercise. Early-phase asthmatic response is characterized by acute bronchoconstriction whereas late-phase asthmatic response caused due to the direct activation of eosinophils and neutrophils. This causes injury that ultimately results in epithelial damage, airway edema, mucus hyper-secretion and hyper-responsiveness (or smooth muscle twitchiness) of bronchial smooth muscle. Predominantly hyperactivity is largely caused in response to activation of eosinophils, which are attracted into the bronchioles by leukotrienes (and other chemo-attractants). Eosinophils themselves also produce leukotrienes. Therefore, leukotrienes are critical both in triggering acute asthma attacks and in causing long term hypersensitivity of the airways in chronic asthma. Varying airflow obstruction leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough [1].

Montelukast (sodium) is a potent, orally active, has high affinity and selectively binds to the cysteinyl leukotriene 1 (CysLT1) receptor as compared to the prostanoid, cholinergic, or β -adrenergic airway receptor. Montelukast also inhibits physiologic actions of LTD4 at the CysLT1 receptor without any agonist activity. Montelukast (sodium) is a [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]

Abbreviations: NLC, nanostructured lipid carrier; MNLC, montelukast-NLC; CAE, DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester; CysLT1, cysteinyl leukotriene 1; EIB, exercise induced bronchoconstriction; ILS, intestinal lymphatic system; SLN, solid lipid nanoparticles; LCT, long chain triglycerides; MCT, medium chain triglycerides; FEV, forced expiratory volume; OATP, organic anionic transporting polypeptide; %EE, percent encapsulation efficiency; PBS, phosphate buffer saline; NTC, national toxicological centre; IS, internal standard; SL, solid lipid; LL, liquid lipid; GMS, Glyceryl monostearate; PDI, polydispersity index; LPN, lipid-polymer hybrid nanoparticles; MDI, metered dose inhaler.

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thio]methyl] cyclopropaneacetic acid, monosodium salt [2]. Montelukast is used for the prevention and treatment of asthma, chronic asthma attacks, to prevent exercise induced bronchoconstriction (EIB) and is also used to treat short-term (seasonal) and long-term (perennial) allergy symptoms such as sneezing, runny nose, itching or wheezing. It decreases the symptoms and the number of acute asthma attacks [3–7].

Currently, montelukast is marketed as conventional tablets, chewable tablets and granules for oral administration in the dose of 10 mg for adults and 4–5 mg dose for children of age 2–15 years. The conventional oral therapy of montelukast formulation is associated with hepatic first pass metabolism resulting short biological half-life (2.5–5.5 h) and thus reducing the bioavailability to 64%. Montelukast induces acute hepatocellular injury. Harugeri et al. confirmed abdominal distension and dyspnea due to montelukast in patient suffering from chronic persistent asthma treated with budesonide 200 µg plus formoterol 6 µg (dry powder inhaler, b.i.d.), albuterol 100 µg (MDI, two puffs) with montelukast 10 mg (tablet, o.d.). Upon clinical examination, the patient was diagnosed with jaundice and hepatomegaly [8]. These problems associated with conventional oral therapy make it a candidate for sustained release dosage form as well as for effective intestinal lymphatic system (ILS) targeting strategy. Improvement in the bioavailability and reduction in hepatotoxicity can be achieved by targeting ILS which bypasses the hepatic uptake and improves systemic toxicity profile [9]. Though the reported onset of action for orally administered montelukast is slower than the intravenously (IV) administered montelukast, difference in the AUC_{0–24} and maximum percent change in the forced expiratory volume (FEV) for IV and orally administered montelukast were insignificant [10]. IV route being invasive is less preferred by pediatric patients.

Extensive literature is available on formulation development of montelukast with more focus on the immediate release dosage forms such as fast dissolving tablet, mouth dissolving tablet, chewable tablet, in situ mucoadhesive nasal gel and film coated tablet to protect the drug from light [11–15]. However, very few reports are available on controlled release of formulations of montelukast. Pulsatile or timed release techniques with lag time are commonly used to control the release of the drug [16–19].

Lipid formulations such as lipid solutions, emulsions, liposomes, lipid micro- and nano-particles improve oral bioavailability [20–22]. Nanostructured lipid carriers (NLCs) are the second generation solid lipid nanoparticles (SLN). NLCs are preferred over other lipid formulations listed above because of higher drug loading capacity, less drug leakage during storage, improved oral bioavailability, physical stability and modulation of drug release profile. NLCs with more imperfections in the crystal structure as compared to SLN are prepared using a blend of solid lipid and spatially different liquid lipids. These imperfections result into improved drug loading and reduced drug expulsion during storage [23–25].

The presence of long chain triglycerides (LCT) in the formulations improves drug uptake via ILS by transcellular pathway, a major lipid uptake mechanism. Upon oral administration, after dissolution of LCT based formulation, nanoparticles are assimilated into nonpolar core of enterocytes generated chylomicron [9,26–28]. Zheng et al. demonstrated that a high percentage of LCT in the formulation resulted in larger particles whereas liquid lipids due to the ability to easily disperse in the aqueous phase results into smaller particles [29]. The presence of liquid lipid of different fatty acid composition than solid lipid increases imperfections in the lipid matrix crystals and thereby increases ability to entrap and retain drug molecules. Also, Tsai et al. have reported less hepatic uptake of particles <200 nm. Thus, it reduces the hepatic metabolism by cytochrome p450 3A4 and 2C9 enzymes, which in turn increases the bioavailability and also reduces hepatic cellular injury due to metabolites of drug [30].

Use of biodegradable, biocompatible surfactants prevents inflammation and fibrosis associated toxicity because of accumulation of surfactant in the lung on repeated use of NLC. CAE (DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester) is an amino acid based cationic surfactant derived from L-arginine, DL-pyrrolidone carboxylate and coconut fatty acid residue. Despite the fact that CAE is cationic, it is reported to be very safe, biodegradable surfactant with antimicrobial property [31].

Priyanka and Sathali have reported the preparation of montelukast sodium SLN by hot homogenization-ultrasonication method using ethanol as an organic solvent [32] but the use of organic solvent may interact more with a drug limiting solubility of lipid in the solvent [33]. In the present study, we demonstrated the green approach to formulate NLC with the mixture of LCT-MCT with improved encapsulation for oral administration of montelukast using biocompatible, biodegradable cationic surfactant. We also evaluated the *in vitro* release and *in vivo* pharmacokinetic parameters of montelukast in the form of MNLC.

2. Materials and methods

2.1. Materials

Montelukast (sodium) was obtained as a generous gift from Emcure Pharmaceuticals Ltd. (Pune, India). Telmisartan was received as a gift sample from Watson Pharmaceuticals Pvt. Ltd., India. Precirol ATO-5 and Capryol-90 were received as kind gift samples from Gattefosse (France). CAE (DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester) was obtained as a gift from Ajinomoto Co., Inc. (Tokyo, Japan). Milli Q water (Nanopure Diamond by Barnstead, Dubuque, IA, USA) was used in all the experiments. HPLC grade methanol was purchased from Merck (Mumbai, India). All other chemicals and reagents were of analytical grade.

2.2. Lipid Screening

Different solid and liquid lipids were screened for solubility of montelukast. Briefly, 5 mg increments of montelukast was added to melted solid lipid or liquid lipid (0.5 g) and mixed thoroughly using a vortex mixer to yield a clear solution. The addition of montelukast in melted solid lipid was continued until the saturation of it and the amount of montelukast dissolved was reported directly. Solubility in liquid lipid was estimated after separating the undissolved montelukast by centrifugation (Allegra™ 64R Centrifuge, Beckman-Coulter India Pvt. Ltd., Andheri (E), Mumbai, Maharashtra) at 25000 rpm, 25 °C for 20 min. The amount of dissolved drug was analyzed by HPLC after dissolving drug containing oil in an appropriate solvent followed by dilution with the mobile phase.

2.3. Preparation of montelukast (sodium) loaded nanostructured lipid carriers (MNLC)

Melt-emulsification-ultrasonication method was used to prepare MNLC. Briefly, the weighed quantities of Precirol ATO-5 (solid lipid, melting point 56 °C), Capryol-90 (liquid lipid) were mixed in the ratio of 7:3, melted together at 5–10 °C above their melting point. Montelukast sodium (0.2%) was added to the molten lipid mixture, vortexed to dissolve to form a uniform and clear lipid mix. The aqueous phase was prepared by dissolving surfactants (CAE; 1%) in Milli Q water and heated to the same temperature as that of the lipid phase (Table 1). The hot lipid phase was added into the aqueous phase under ultrasonication (Vibra-Cell, Sonics & Material, Inc., USA) at an amplitude of 75% for 3 min (30:05 on:off

Table 1
Composition of montelukast loaded nanostructured lipid carrier (MNLC).

Formulation ingredients	Quantity (% w/v)
Montelukast sodium	0.2
Precirol ATO5	1.4
Capryol-90	0.6
CAE	1.0
Milli Q water	100

cycle). The formed MNLC was cooled to room temperature and evaluated further.

2.4. HPLC analysis of montelukast

The amount of montelukast in the various samples was estimated using HPLC method reported earlier with slight modification [34]. The HPLC system consisted of a Thermo Scientific ODS Hypersil C-18 column (250 mm × 4.6 mm, 5 μ). A mixture of methanol: 20 mM ammonium acetate buffer with 0.2% of formic acid in the ratio of 88:12 (v/v) was used as mobile phase at the flow rate of 1 mL/min. The detector consisted of UV/VIS (Jasco UV 2075) model operated at a wavelength of 313 nm. Telmisartan was used as an internal standard (IS; 6 μg/mL in methanol) for the estimation of montelukast in plasma. The correlation coefficient was found to be 0.996 for the concentration range of 2–10 μg/mL.

2.5. Particle size and zeta potential analysis

Average particle size, polydispersity of the size distribution, and the zeta-potential were measured using a Malvern Zetasizer NANO ZS90 (Malvern, Worcs, UK). The cell temperature was 25 °C; the scattering angle was 90°. For better measurement and suitable signal intensity the samples were diluted at least 100-times with Milli Q water prior to the measurement and both measurements were taken in triplicate.

2.6. Drug content and entrapment efficiency (%EE)

The drug content of prepared MNLCs was determined by dissolving 1 mL of MNLC in 10 mL solvent mixture of ethanol:dichloromethane (1:3) and the solutions were analyzed for drug content by directly injecting into the HPLC system after appropriate dilutions with mobile phase. To determine the % entrapment efficiency (% EE) MNLC was centrifuged 20 °C for 30 min at 25,000 rpm. The amount of montelukast sodium entrapped in the pellet was determined by dissolving pellets in ethanol: dichloromethane (1:3) mixture and estimated by HPLC method followed by appropriate dilutions with mobile phase.

2.7. Transmission electron microscopy (TEM)

The morphology of MNLC was determined by TEM (TEM, JEM-2010, JEOL, Tokyo, Japan). Imaging was performed on TEM at a voltage of 80 kV having magnification of 60000X. A drop of NLC was placed on Formvar®-coated copper grids (Ted Pella, Redding, CA) and the grid was air-dried and images were captured using TEM.

2.8. Fourier transform infra-red spectroscopy (FTIR)

FTIR spectra of pure montelukast, Placebo-lipid matrix (Precirol ATO-5:Capryol-90; 7:3 ratio), lipids matrix with montelukast and MNLC were recorded using Jasco FTIR-4100 (Jasco, Japan). About

2–3 mg of the sample was mixed with dry potassium bromide and the prepared sample was scanned through the wave number range of 4000–400 cm^{−1}.

2.9. Differential scanning calorimetry (DSC)

Thermal characteristics of pure montelukast, Placebo-lipid matrix (Precirol ATO-5:Capryol-90; 7:3 ratio), lipids matrix with montelukast and MNLC were studied using DSC. DSC measurements were taken on a Mettler Toledo Star 821^e instrument with an intracooler (METTLER-Toledo, GmbH, Switzerland). The samples were accurately weighed (5–10 mg), hermetically sealed in aluminum pans and heated at a constant rate of 10 °C/min over a temperature range of 25–200 °C. Sealed empty aluminum pan was used as a reference. An inert atmosphere was maintained by purging with nitrogen gas at a flow rate of 50 mL/min. Indium/zinc standards were used to calibrate the DSC temperature and enthalpy scale.

2.10. X-ray powder diffraction analysis (XRPD)

The XRPD patterns of pure montelukast, Placebo-NLC and MNLC were recorded on an X-ray diffractometer (PW 1729, Philips, Netherlands). The samples were irradiated with monochromatized CuK radiation (1.542 Å) and analyzed between 5 and 50° 2θ. The voltage and current used were 30 kV and 30 mA, respectively. The range and the chart speed were 1 × 10⁴ CPS and 10 mm/° 2θ, respectively.

2.11. In vitro release of montelukast from MNLC

In vitro drug release study was carried out as described by Luo et al. [35]. Cellophane membrane (Dialysis) bag was treated, thoroughly washed with boiling water twice for 15 min each time to remove impurities and was soaked overnight in the release medium. Briefly, the MNLC formulation equivalent to 10 mg montelukast was placed in the cellulose membrane bags (molecular weight cut off 12–14 kDa) which were previously soaked overnight in the dissolution medium. Both the ends of bag were tied to prevent any leakage. Dialysis bag was carefully placed in the beaker containing 100 mL phosphate buffer saline (PBS, pH 7.4) as the dissolution medium which was placed on the magnetic stirrer, rotated at 50 rpm for 48 h at 37 ± 0.1 °C. The drug release from MNLC was compared with the aqueous solution of 10 mg montelukast in distilled water placed in the dialysis bag as a control. The amount of montelukast released into the medium was determined with the help of HPLC analysis. The experiments were carried out in triplicate.

2.12. In vivo oral pharmacokinetic study

2.12.1. Animals

The guidelines of the Committee for the Purpose of Control and Supervision on Experimental on Animals (CPCSEA) were used to prepare an experimental protocol and were approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune. Concisely, male Wistar rats were procured from the National Toxicological Centre (NTC), Pune one week prior to experiments. During this one week period animals were allowed to acclimatize to the experimental conditions of temperature and humidity. Animals were housed together in plastic cages under standard conditions of temperature (24 ± 1 °C), relative humidity (55 ± 10%) and 12 h light/dark cycles throughout the experiment. Rats were allowed to standard pelletized chow (Pranav Agro Industries, Sangli, Maharashtra, India) and free access to filtered water but abstained for 12 h prior to experimentation. During this

acclimatization period, the health status of the animals was monitored daily.

2.12.2. Single dose oral administration

Montelukast aqueous solution and MNLC were given orally as a single dose using intra-gastric gavage technique. In this technique solution or suspensions are administered directly into the stomach by inserting small-diameter oral feeding needle into the esophagus [36]. Male Wistar rats were divided randomly into three groups with six animals in each group. Groups I, II and III were administered with plain distilled water, pure montelukast aqueous solution and MNLC (dose of 0.5 mg/kg of body weight) [37], respectively. 0.3 mL of blood sample was withdrawn from the retro-orbital plexus at predetermined time points of 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h. Collected blood samples were mixed thoroughly with di-sodium EDTA in order to prevent blood clotting. To separate the plasma tubes were centrifuged at 7000 rpm for 20 min at 4 °C and separated plasma was transferred into pre-labeled tubes and stored in a refrigerator until further analysis.

2.12.3. Plasma sample analysis

To prepare standard stock solutions of montelukast and telmisartan as IS, 5 mg of pure drug was dissolved in 5 mL of methanol (1000 ppm) separately. Working standard solutions of montelukast and IS were prepared by diluting the stock solutions to desired concentrations with methanol.

Plasma standard samples were prepared by spiking blank rat plasma with montelukast and IS standard solutions. These samples were processed by liquid–liquid extraction method. Briefly, to 100 μ L of plasma standard or sample, 300 μ L of IS in methanol (6 ppm) was added. Methanol used to prepare IS solution also serves as an extraction solvent. This mixture was vortexed for 10 min, followed by centrifugation at 7000 rpm for 15 min at 4 °C. The supernatant was collected and a volume of 20 μ L was injected into the HPLC system.

2.12.4. Data analysis

Pharmacokinetic parameters were calculated for each individual set of data using the pharmacokinetic software, WinNonlin version 4.0 (Pharsight, Mountain View, CA, USA) using non-compartmental method. The maximum concentration (C_{max}) was determined by observing individual animal concentration-versus-time curves. The area under the plasma concentration curve from the time of administration ($AUC_{0 \rightarrow \infty}$) was calculated using the trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was calculated as $AUMC_{0 \rightarrow \infty} / AUC_{0 \rightarrow \infty}$. The clearance (Cl) was calculated from the dose (D) divided by the $AUC_{0 \rightarrow \infty}$.

2.13. Stability studies

Effects of temperature and relative humidity on the particle size and the % EE of MNLC formulation were conducted as per ICH guidelines for stability study (25 °C $\pm$ 60% RH and 40 °C $\pm$ 75% RH) for 1, 2 and 3 months.

3. Result and discussion

3.1. Preformulation study for montelukast solubility and lipid screening

For the preparation of NLCs, solid and liquid lipids were selected based on the solubilization potential of the lipids for montelukast. Long chain triglycerides (LCT) having a carbon chain length of 16–18 atoms were selected for the preparation of drug encapsulated lipidic carriers as they are reported to enhance bioavailability by

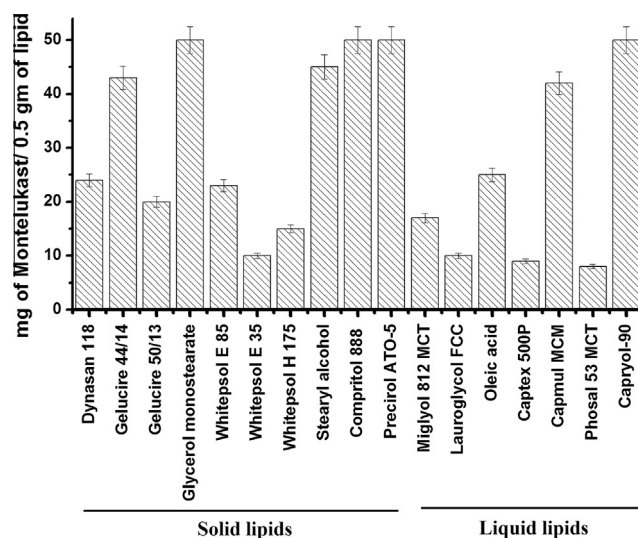


Fig. 1. Lipid screening for solubility of montelukast in various solid and liquid lipids. Values expressed as mean $\pm$ SD, $n = 3$.

the intestinal lymphatic system (ILS). As depicted in Fig. 1, montelukast showed maximum solubility in Precirol ATO5 (glyceryl palmostearate), GMS (Glyceryl monostearate) and Compritol 888. Muller et al. have reported that addition of oil or liquid lipid to solid lipid decreases crystallinity and thereby leakage of entrapped drug [38]. Therefore, solubility study in liquid lipid was also conducted to screen liquid lipids. It was found that montelukast had maximum solubility in capryol-90 (liquid lipid) (Fig. 1). Therefore, admixing capryol 90 with solid lipids such as Precirol ATO5, GMS or Compritol 888 can increase drug loading with higher entrapment efficiency as compared to SLN.

3.2. Preparation of MNLC

In order to increase the % EE of hydrophilic montelukast, MNLC was prepared by melt-emulsification-ultrasonication method using Precirol ATO5 (solid lipid) and Capryol-90 (liquid lipid). CAE, an amino acid based cationic, safe and biodegradable surfactant was used for the stabilization of the formulation [31]. Precirol ATO5 is long chain triglyceride (LCT) with 18 'C' whereas Capryol-90 is medium chain triglycerides (MCT) with 12 'C' [39,40]. MNLCs were prepared with different solid lipid:liquid lipid ratios (SL:LL ratio) at different sonication amplitudes and time. It was observed that only SL:LL of 7:3 at sonication amplitude of 75% for 3 min resulted in MNLC with particle size <200 nm with % EE more than 90% and which were stable for 3 months. Batches prepared with high amplitude (90%) for 4 min resulted in aggregate formation due to increase in cavitation energy causing agglomeration of fine particle [41].

3.3. Nanoparticle characterization

3.3.1. Particle size and zeta potential (ζ)

Particle size, PDI and zeta potential are the physical properties of the colloidal dispersion determining stability of the formulation. As shown in Table 2 particle sizes of Placebo-NLC and MNLC were found to be 154.1 ± 7.8 and 181.4 ± 6.5 nm, respectively. Increase in the particle size for MNLC as compared to the Blank-NLC could be due to loading of hydrophilic drug in lipidic carrier. Whereas loading of the lipophilic drug in lipidic vesicle like liposome decreases the vesicular size because of the association of lipophilic drug with lipids of the formulation [42]. For both the formulations

Table 2
Physicochemical characterization of Placebo-NLC and MNLC (Mean $\pm$ SD; $n = 6$).

Parameters	Placebo-NLC	MNLC
Particle size (nm)	154.1 $\pm$ 7.8	181.4 $\pm$ 6.5
Zeta potential (mV)	36.3 $\pm$ 2.1	33.8 $\pm$ 1.8
Polydispersity index (PDI)	0.155	0.139
Drug content (mg/mL)	–	2.02 $\pm$ 0.05
% Encapsulation efficiency (%EE)	–	96.13 $\pm$ 0.98

PDI was less than 0.2 which indicated narrow size distribution of the nanoparticles.

Further, to predict the physical stability of this colloidal dispersion zeta potential (ζ) was determined. ζ of the montelukast dissolved in Milli Q water was -43.8 ± 0.7 mV. Zeta potential of the placebo batch (36.3 ± 2.1 mV) was slightly greater than the zeta potential of MNLC (33.8 ± 1.8 mV) (Table 2). This slight decrease in zeta potential for MNLC can be attributed to the presence of some free drug in the dispersion which is anionic in nature. Use of cationic surfactant (CAE) resulted into NLCs with positive zeta potential. Mechanism of cellular uptake of nanoparticles is the electrostatic attraction between the oppositely charged particles. Large negatively charged domain is present on the cell surface where cationic nanoparticles can be attracted leading to increased cellular uptake of either nanoparticles or released drug [43,44].

3.3.2. Encapsulation efficiency (% EE)

Due to the hydrophobic nature of the lipids, it is challenging to encapsulate and retain the hydrophilic drugs in the lipid matrix. However, to overcome this problem, selection of the formulation excipients was found to be crucial. Kumbhar and Pokharkar have proposed the engineered lipid-polymer hybrid nanoparticles (LPN) to improve the loading of hydrophilic drug, zidovudine [45]. In the present study, to achieve highest encapsulation and minimum drug leakage lipids were selected with the maximum solubility of the hydrophilic drug. Montelukast sodium, a hydrophilic drug showed maximum solubility in Precirol ATO5 and Capryol-90 and therefore, these lipids were selected for the study.

The drug content and entrapment efficiency (% EE) of the MNLC formulation were $0.202 \pm 0.001\%$ (i.e. 2.02 ± 0.15 mg/mL) and $96.13 \pm 0.98\%$ of montelukast, respectively. It was found that the addition of MCT lipid liquid (Capryol-90) to solid lipid (Precirol ATO5) leads to increase in encapsulation efficiency. The improved % EE can be due to reduction in particle crystallinity imparting improved stability and sustaining the release of encapsulated drug.

3.3.3. TEM

As depicted in Fig. 2 smooth spherical shaped lipid nanoparticles were observed. The size observed with TEM was in good

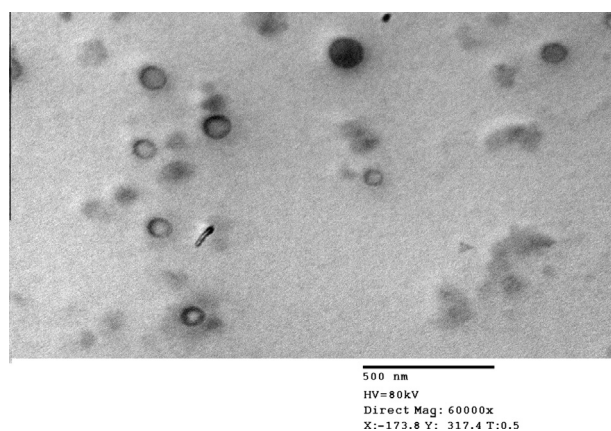


Fig. 2. TEM image of MNLC.

agreement with the particle size obtained by dynamic light scattering method.

3.3.4. FTIR

To study the interaction between the drug and excipients of the NLC formulation (lipids and surfactant), FTIR study was conducted and is depicted in Fig. 3. As demonstrated in Fig. 3a, montelukast exhibited broad peak at 3400 cm^{-1} which is characteristic of the tertiary hydroxyl group and a strong peak near 1700 cm^{-1} characteristic of a salt form of carboxylic acid. The peaks due to number of aromatic C–H groups are also observed between 2900 cm^{-1} and 3000 cm^{-1} whereas the C=O peak appeared at 1613 cm^{-1} . Fig. 3b revealed the characteristic peaks for the excipients used in the formulation. Fig. 3c and d shows all the characteristic peaks of the pure montelukast, which suggested the presence of montelukast in its pure form. These observations confirmed no major chemical bond formation between the montelukast and the components of NLC formulation that could result in asymmetric vibrations detectable by FTIR.

3.3.5. DSC

Pure montelukast and Precirol ATO5 showed endothermic peaks at 60.06°C (Fig. 4a) and 66.67°C (Fig. 4b), respectively corresponding to their melting points. To study the thermal behavior of solid lipid after addition of liquid lipid, a bulk mixture of solid lipid and liquid lipid was prepared in the same ratio as that used in the NLC formulation (Precirol ATO5:Capryol-90; 7:3 ratio). This placebo bulk mixture of lipids was subjected to DSC study. Fig. 4c revealed approximately 12° depression in melting of Precirol ATO5 after addition of Capryol-90 in 7:3 ratio as compared to pure Precirol ATO5. This depression in melting point and broadening of peak can be attributed to the dissolution of Capryol-90 in Precirol ATO5 with the formation of less distinct crystalline structure. Further, a melting behavior of ternary bulk mixture of lipids with montelukast was evaluated and is depicted in Fig. 4d. Fig. 4d did

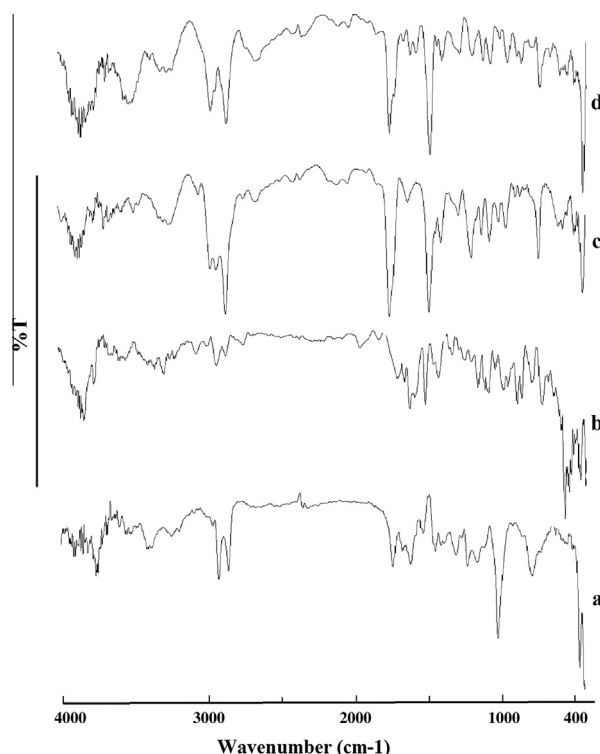


Fig. 3. FTIR spectra of (a) Montelukast sodium, (b) placebo-lipid matrix, (c) lipid matrix with montelukast and (d) lyophilized MNLC.

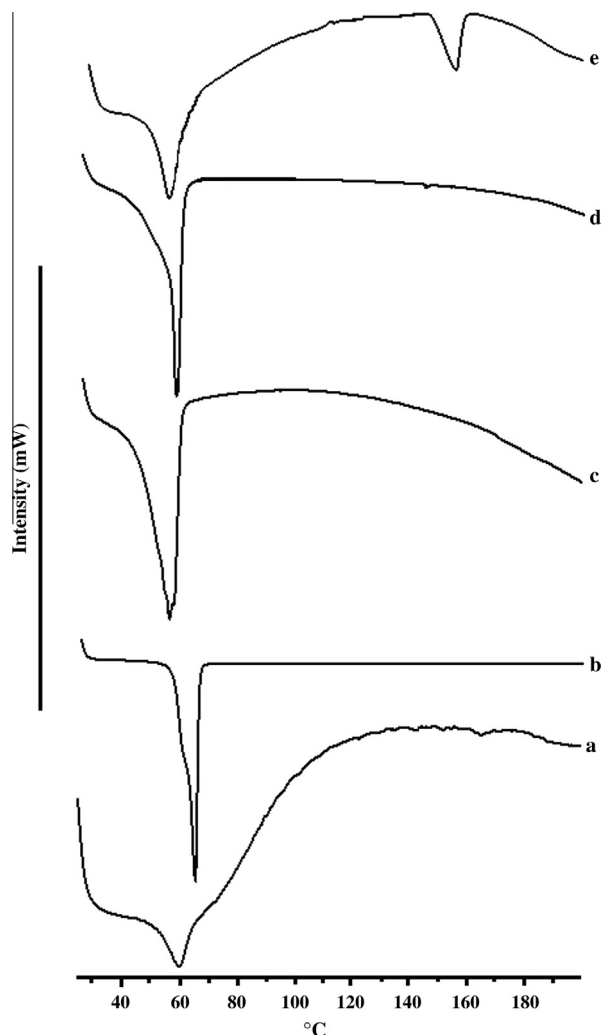


Fig. 4. DSC thermograms of (a) montelukast sodium, (b) precirol ATO5, (c) placebo-lipid matrix, (d) lipid matrix with montelukast and (e) lyophilized MNLC.

not reveal endotherm corresponding to the melting of montelukast suggesting complete solubilization of drug in the lipid matrix. Thermogram for melting behavior of lyophilized MNLC (Fig. 4e) revealed further depression in the melting of lipid matrix which could be attributed to nanocrystalline size of lipids in the NLC [46]. Thermogram of lyophilized MNLC also showed an endothermic peak at 154.51 °C for melting of mannitol used as cryoprotectant in the formulation with no endothermic peak for the drug. This indicated that even after lyophilization drug was in solubilized form in the lipid matrix.

3.3.6. XRPD

XRPD scans for montelukast, Placebo-NLC and lyophilized MNLC are depicted in Fig. 5. X-ray diffractogram for pure montelukast revealed diffused peaks characteristic of its stable amorphous nature (Fig. 5a). Fig. 5b and c shows sharp, separate peaks for Placebo-MNLC and MNLC, respectively which confirmed the crystalline nature of lipids in lyophilized formulations. Also, X-ray diffractogram for Placebo-MNLC and MNLC was exactly overlapping confirmed no amorphous to crystalline conversion of montelukast during processing. Thus, this study confirmed the presence of montelukast as molecular dispersion in the lipid matrix of the formulation.

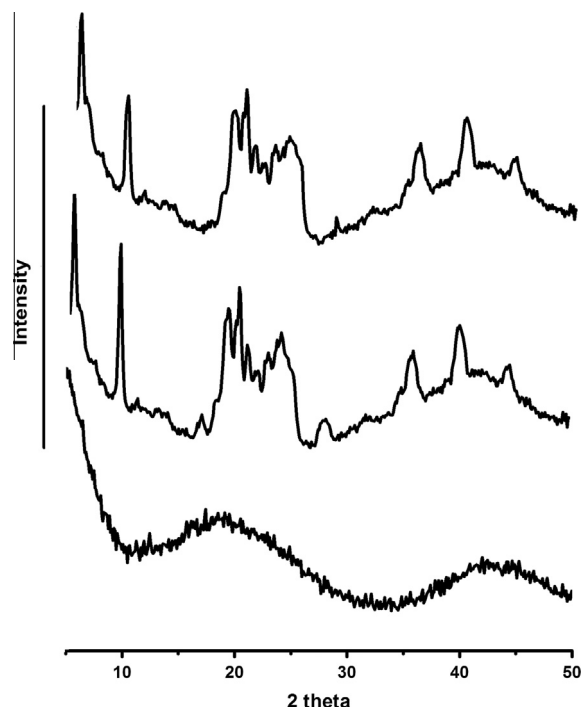


Fig. 5. X-ray diffractograms of (a) montelukast sodium, (b) placebo-NLC and (c) lyophilized MNLC.

3.3.7. In vitro release study

The release behavior of montelukast from prepared MNLC formulation was studied using the bulk-equilibrium reverse dialysis method and was compared with montelukast-aqueous solution.

Pulsatile or timed release approach is reported in the literature to develop the montelukast oral tablets using press coating technique [16–19]. Krishnaveni et al. developed press coated tablet of montelukast using natural polysaccharides. These press coated tablet contained inner immediate release core tablet which was then press coated with the natural polysaccharides to obtain timed controlled release. A lag time of approximately 3 h was achieved with less than 11% release and more than 90% drug was released within 9 h [16]. Also, Ranjan et al. have developed pulsatile release capsule of montelukast which was osmotically controlled by a drill in cap coated with cellulose acetate toward plug side. Lag time of 4.5 h with 96.29% *in vitro* release was obtained at the end of 12 h [18]. Peroni et al. have reported significant protective effect of montelukast at 12 h whereas no protective effect was obtained at 24 h post dosing of conventional montelukast formulations in exercise-induced asthma patients [47]. Hence, with the aim to obtain protective effect even after 12 h montelukast-NLC was developed.

As graphically represented (Fig. 6), pure drug and MNLC showed approximately 95 and 20% release, respectively at the initial 1 h whereas to release 95% drug from MNLC it took 24 h.

Montelukast being a water soluble molecule showed the complete release at the end of 1 h from its aqueous solution. The release study confirmed the sustained release of drug from NLC and this significant reduction in the drug release rate of a hydrophilic molecule can be ascribed to drug association with lipid matrix.

The drug release data obtained for MNLC were fitted into different kinetic models to understand the mechanism of drug release. Since the release of drug from montelukast aqueous solution was rapid, these data were not fitted into release models. However, release data of montelukast from MNLC were found to follow

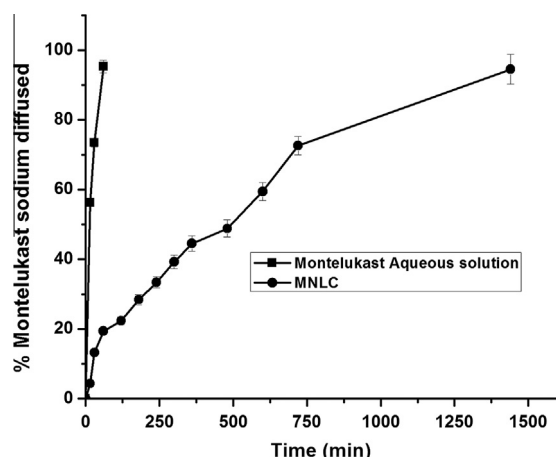


Fig. 6. In vitro release profile of Montelukast-aqueous solution (—■—) and MNLC (—●—). Values expressed as mean $\pm$ SD, $n = 3$.

Higuchi kinetics with the best fit (r^2) value 0.986 indicating drug releases by diffusion mechanism [48].

3.3.8. Single dose oral pharmacokinetic study

Montelukast-aqueous solution and MNLC formulation were orally administered as a single dose to Wistar rats. The plasma concentration–time profile for a period of 24 h is depicted in Fig. 7 and pharmacokinetic parameters are reported in Table 3. After oral administration, aqueous solution of montelukast showed maximum concentration (C_{max}) of 86.40 ± 11.30 ng/mL at the end of 3 h (T_{max}), while MNLC showed C_{max} of 769.15 ± 27.23 ng/mL at the end of 4 h. Significantly higher C_{max} values ($p < 0.0001$) of MNLC against montelukast-aqueous solution and C_{max} for commercially available 4 mg montelukast oral granules (175 ng/mL) [49] indicated improvement in bioavailability when encapsulated in NLC. A slight increase in T_{max} for MNLC compared to montelukast aqueous solution can be attributed to the slow release and therefore slow absorption of the drug from MNLC [23] whereas dissolved drug from the aqueous montelukast solution was immediately absorbed. This resulted in faster absorption and faster elimination of the montelukast from the body.

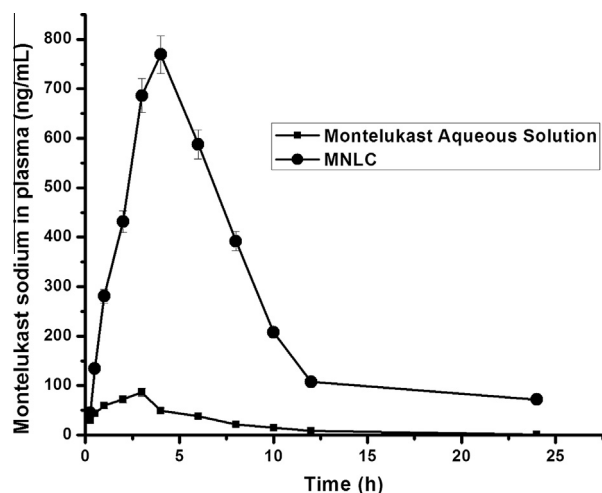


Fig. 7. Mean plasma concentration of montelukast-time profile after oral administration of Montelukast-aqueous solution (—■—) and MNLC (—●—). Values expressed as mean $\pm$ SD, $n = 6$.

Table 3

Single dose oral pharmacokinetic parameters for montelukast-aqueous solution and MNLC (Mean $\pm$ SD; $n = 6$).

Pharmacokinetic parameters	Montelukast-aqueous solution	MNLC
C_{max} (ng/mL)	86.40 ± 11.30	$769.15 \pm 27.23^{***}$
T_{max} (h)	3	4 [*]
AUC_{0-t} (h * ng/mL)	7.14 ± 1.32	$429.90 \pm 76.24^{***}$
$AUC_{0-\infty}$ (h * ng/mL)	14.42 ± 2.53	$2067.35 \pm 102.35^{***}$
$AUMC_{0-\infty}$ (h ² * ng/mL)	131.84 ± 41.64	$57070.92 \pm 1024.3^{***}$
K_{el} (h ⁻¹)	0.164 ± 0.02	$0.0437 \pm 0.005^{***}$
$t_{1/2}$ (h)	4.24 ± 0.98	$15.84 \pm 2.58^{**}$
MRT (h)	9.14 ± 0.62	$27.61 \pm 2.73^{***}$
Vd	160.09 ± 8.11	$8.03 \pm 0.8^{***}$
Cl	17.51 ± 2.54	$0.291 \pm 0.073^{**}$
Fr	–	143.34

^{*} $p < 0.01$, respectively as compared to Montelukast-aqueous solution.

^{**} $p < 0.001$, respectively as compared to Montelukast-aqueous solution.

^{***} $p < 0.0001$, respectively as compared to Montelukast-aqueous solution.

It was noted that plasma concentration of montelukast at all the time points was significantly higher for MNLC than pure montelukast. Also at the end of 24 h MNLC, showed plasma concentration of 71.65 ± 10.07 ng/mL as compared to plasma concentration of pure drug which was negligible. After oral administration, approximately 60-fold increase in the AUC_{0-24} was obtained for MNLC against pure drug. This increase in AUC_{0-24} demonstrated the significant enhancement in systemic absorption of montelukast from MNLC. Decrease in K_{el} and therefore increase in MRT (9.14 h vs 27.61 h) can be correlated with increased magnitude of AUC_{0-24} . 20-fold decrease in Vd implies reduction in extravascular tissue distribution and therefore nonspecific tissue toxicity. Obtained *in vivo* results were superior to the available literature which reports T_{max} of 7 h from the developed pulsatile, osmotically released capsule of montelukast.

The results of this study revealed significant improvement in the bioavailability of montelukast when it was encapsulated in the lipid mixture of LCT and MCT as compared to montelukast solution. In the studied NLC formulation, major component was Precirol ATO-5, a LCT which is reported to improve bioavailability by intestinal lymphatic uptake in the form of micelle. Montelukast (sodium) is a substrate of transmembrane organic anionic transporting polypeptide (OATP), especially OATP2B1 which carries the solute across the plasma membrane. This bidirectional transport is driven by the concentration gradient of the solute across the membrane [50]. Hence, it surmised that slow release of montelukast from lipid nanoparticles could maintain the concentration gradients across the membrane resulting in enhanced uptake from outside to inside of the cell.

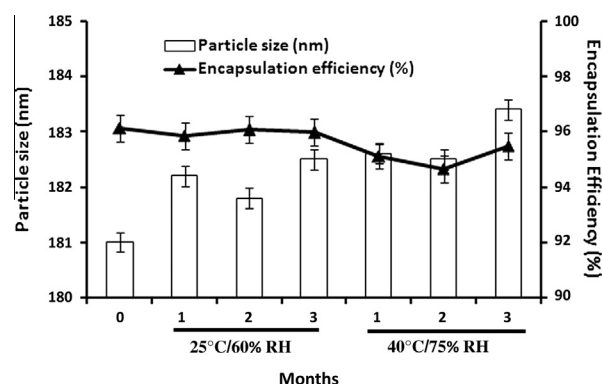


Fig. 8. Stability evaluation of MNLC as per ICH guidelines. Values expressed as mean $\pm$ SD, $n = 3$.

3.3.9. Stability study

The prepared MNLC formulation was subjected to different conditions of temperature and humidity as per the ICH guidelines to evaluate its stability. The stability study was performed to ascertain the changes in particle size and % EE as a function of temperature and humidity. As graphically represented in Fig. 8, no significant changes were observed in particle size and % EE ($p < 0.05$) throughout the study period of 3 months. Also the microscopic examination of MNLC at each time point did not show the drug crystals due to leakage of drug from the lipid matrix. Thus, the results confirmed the satisfactory stability of prepared lipidic nanoparticles.

4. Conclusion

Developing nanoparticles with particle size <200 nm improves lymphatic uptake, reduces hepatic uptake and therefore reduces hepatic cellular toxicity due to metabolites. This study successfully demonstrated the use of lipid matrix prepared by melt-emulsification-homogenization method using a combination of medium and long chain triglycerides to improve oral bioavailability. Pharmacokinetic parameters of MNLC were significantly different than pure drug. Fourfold decrease in elimination rate, prolonged $t_{1/2}$ and increased in MRT for MNLC demonstrated the superiority of MNLC over montelukast aqueous solution. MNLC demonstrated sustained release profile over 24 h with drug release by Higuchi diffusion mechanism. Thus, the findings of this study revealed suitability of NLC to improve performance of the drug *in vivo*.

Conflict of interest

Authors report no conflict of interest.

References

- [1] K.M. Gross, C.D. Ponte, New strategies in the medical management of asthma, *Am. Fam. Phys.* 58 (1998) 89–100.
- [2] <https://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=SearchDrugDetails> (accessed 12.03.14).
- [3] R. Ramakrishnan, E. Migoya, B. Knorr, A population pharmacokinetic model for montelukast disposition in adults and children, *Pharm. Res.* 22 (2005) 532–540.
- [4] E. Migoya, G.L. Kearns, A. Hartford, J. Zhao, J.V. Adelsberg, C.A. Tozzi, B. Knorr, P. Deutsch, Pharmacokinetics of montelukast in asthmatic patients 6 and 24 months old, *J. Clin. Pharmacol.* 44 (2004) 487–494.
- [5] W. Storms, Update on montelukast and its role in the treatment of asthma, allergic rhinitis and exercise-induced bronchoconstriction, *Expert. Opin. Pharmacother* 8 (2007) 2173–2187.
- [6] F.M. de Benedictis, S. Vaccher, D. de Benedictis, Montelukast sodium for exercise-induced asthma, *Drugs Today (Barc)* 44 (2008) 845–855.
- [7] A. Nayak, R.B. Langdon, Montelukast in the treatment of allergic rhinitis: an evidence-based review, *Drugs* 67 (2007) 887–901.
- [8] A. Harugeri, G. Parthasarathi, J. Sharma, G.A. D'Souza, M. Ramesh, Montelukast induced acute hepatocellular liver injury, *J. Postgrad. Med.* 55 (2009) 141–142.
- [9] H. Shete, V. Patravale, Long chain lipid based tamoxifen NLC. Part I: Preformulation studies, formulation development and physicochemical characterization, *Int. J. Pharm.* 454 (2013) 573–583.
- [10] R.J. Dockhorn, R.A. Baumgartner, J.A. Leff, M. Noonan, K. Vandormael, W. Stricker, D.E. Weinland, T.F. Reiss, Comparison of the effects of intravenous and oral montelukast on airway function: a double blind, placebo controlled, three period, crossover study in asthmatic patients, *Thorax* 55 (2000) 260–265.
- [11] K.G.B. Kumar, E. Mahesh, Formulation and evaluation of montelukast sodium fast dissolving tablets, *Asian J. Biomed. Pharm. Sci.* 2 (2012) 75–82.
- [12] A. Patil, T. Aman, N. Bhargava, P. Abhilash, M. Turaga, S. Kulkarni, Formulation and evaluation of mouth dissolving tablets of montelukast sodium, *Res. J. Pharm. Bio. Chem. Sci.* 2 (2011) 268–274.
- [13] Priyanka, P.B. Kumar, Formulation development and evaluation of montelukast sodium chewable tablets, *J. Chem. Pharm. Sci.* 6 (2013) 35–40.
- [14] Nerella, P. Dontha, A. Uppuluru, S.K. Konda, Formulation and evaluation of in situ mucoadhesive nasal gel of montelukast sodium, *Der Pharm. Sinica* 5 (2014) 1–8.
- [15] A.N. Zaid, S. Natour, A. Qaddomi, A.A. Ghoush, Formulation and *in vitro* and *in vivo* evaluation of film-coated montelukast sodium tablets using Opadry® yellow 20A82938 on an industrial scale, *Drug Des. Devel. Ther.* 7 (2013) 83–91.
- [16] G. Krishnaveni, M. Muthukumar, B. Krishnamoorthy, Development and evaluation of pulsatile drug delivery system containing montelukast sodium by press coated tablet using natural polysaccharides, *Int. J. Adv. Pharm. Gen. Res.* 1 (2013) 41–51.
- [17] B.U. Janugade, S.S. Patil, S.V. Patil, P.D. Lade, Formulation and evaluation of press-coated montelukast sodium tablets for pulsatile drug delivery system, *Int. J. ChemTech Res.* 1 (2009) 690.
- [18] O.P. Ranjan, U.Y. Nayak, M.S. Reddy, S.J. Dengale, P.B. Musmade, N. Udupa, Osmotically controlled pulsatile release capsule of montelukast sodium for chronotherapy: Statistical optimization, *in vitro* and *in vivo* evaluation, *Drug Deliv.* (2013), <http://dx.doi.org/10.3109/10717544.2013.853209>.
- [19] P. Bailpattar, P. Karwa, K. Patel, M.M. Sahidullah, P.M. Irshad, Formulation and evaluation of time controlled drug delivery system of montelukast sodium, *Int. J. Pharm. Inno.* 2 (2012) 1–12.
- [20] A. Patil-Gadhe, V. Pokharkar, Single step spray drying method to develop proliposomes for inhalation: a systematic study based on quality by design approach, *Pulm. Pharmacol. Ther.* 27 (2014) 197–207.
- [21] R. Pandey, G.K. Khuller, Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis, *Tuberculosis (Edinb)* 85 (2005) 227–234.
- [22] S. Weber, A. Zimmer, J. Pardeike, Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) for pulmonary application: a review of the state of the art, *Eur. J. Pharm. Biopharm.* 86 (2014) 7–22.
- [23] C.Y. Zhuang, N. Li, M. Wang, X.N. Zhang, W.S. Pan, J.J. Peng, Y.S. Pan, X. Tang, Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability, *Int. J. Pharm.* 394 (2010) 179–185.
- [24] C.M. O'Driscoll, B.T. Griffin, Biopharmaceutical challenges associated with drugs with low aqueous solubility—the potential impact of lipid-based formulations, *Adv. Drug Deliv. Rev.* 60 (2008) 617–624.
- [25] R.H. Müller, M. Radtke, S.A. Wissing, Solid nanoparticle (SLN) and nanostructured lipid carrier (NLC) in cosmetic and dermatological preparations, *Adv. Drug Deliv. Rev.* 54 (2002) 131–155.
- [26] S.M. Caliph, W.N. Charman, C.J. Porter, Effect of short-, medium-, and longchain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymphannulated and non-cannulated rats, *J. Pharm. Sci.* 89 (2000) 1073–1084.
- [27] C.J. Porter, W.N. Charman, Intestinal lymphatic drug transport: an update, *Adv. Drug Deliv. Rev.* 50 (2001) 61–80.
- [28] C.J. Porter, N.L. Trevaskis, W.N. Charman, Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs, *Nat. Rev. Drug Discov.* 6 (2007) 231–248.
- [29] M. Zheng, M. Falkeborg, Y. Zheng, T. Yang, X. Xu, Formulation and characterization of nanostructured lipid carriers containing a mixed lipids core, *Colloids Surf. A* 430 (2013) 76–84.
- [30] M.J. Tsai, P.C. Wu, Y.B. Huang, J.S. Chang, C.L. Lin, Y.H. Tsai, J.Y. Fang, Baicalein loaded in tocol nanostructured lipid carriers (tocol NLCs) for enhanced stability and brain targeting, *Int. J. Pharm.* 423 (2012) 461–470.
- [31] P.U. Singare, J.D. Mhatre, Cationic surfactants from arginine: synthesis and physicochemical properties, *Am. J. Chem.* 2 (2012) 186–190.
- [32] K. Priyanka, A.A. Sathali, Preparation and evaluation of montelukast sodium loaded solid lipid nanoparticles, *J. Young Pharm.* 4 (2012) 129–137.
- [33] R. Nair, K.S.A. Kumar, K.V. Priya, M. Sevukarajan, Recent advances in solid lipid nanoparticle based drug delivery, *J. Biomed. Sci.* Res. 3 (2011) 368–384.
- [34] K.N. Raju, T.G. Swamy, A.L. Rao, Development and validation of RP-HPLC method for the determination of montelukast sodium in bulk and in pharmaceutical formulation, *Int. J. Pharm. Chem. Biol. Sci.* 1 (2011) 12–16.
- [35] Y.F. Luo, D.W. Chen, L.X. Ren, X.L. Zhao, J. Qin, Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability, *J. Control. Rel.* 114 (2006) 53–59.
- [36] Z. Atcha, C. Rourke, A.H.P. Neo, C.W.H. Goh, J.S.K. Lim, C.C. Aw, E.R. Browne, D.J. Pemberton, Alternative method of oral dosing for rats, *J. Am. Assoc. Lab. Anim. Sci.* 49 (2010) 335–343.
- [37] R. Zhao, W.Z. Shi, Y.M. Zhang, S.H. Fang, E.Q. Wei, Montelukast, a cysteinyl leukotriene receptor-1 antagonist, attenuates chronic brain injury after focal cerebral ischaemia in mice and rats, *J. Pharm. Pharmacol.* 63 (2011) 550–557.
- [38] R.H. Müller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, *Adv. Drug Deliv. Rev.* 54 (2002) S131–S155.
- [39] R.M. Khalil, A.A. El-Bary, M.A. Kassem, M.M. Ghorab, M.B. Ahmed, Solid lipid nanoparticles for topical delivery of meloxicam: Development and *in vitro* characterization, in: 1st Annual International Interdisciplinary Conference, AIIIC 2013, 24–26 April, Azores, Portugal – Proceedings, pp. 779–798.
- [40] A. Azeem, N. Rizwan, F.J. Ahmad, Z. Iqbal, R.K. Khar, M. Aqil, S. Talegaonkar, Nanoemulsion components screening and selection: a technical note, *AAPS PharmSciTech* 10 (2009) 69–76.
- [41] G.A. Morris, J. Castile, A. Smith, G.G. Adams, S.E. Harding, The effect of prolonged storage at different temperatures on the particle size distribution of tripoly-phosphate (TPP) – chitosan nanoparticles, *Carbohydr. Polym.* 84 (2011) 1430–1434.
- [42] M.N. Padamwar, V.B. Pokharkar, Development of vitamin loaded topical liposomal formulation using factorial design approach: drug deposition and stability, *Int. J. Pharm.* 320 (2006) 37–44.
- [43] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems – a review Part 1, *Trop. J. Pharm. Res.* 12 (2013) 255–264.

- [44] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems – a review Part 2, *Trop. J. Pharm. Res.* 12 (2013) 265–273.
- [45] D.D. Kumbhar, V.B. Pokharkar, Physicochemical investigations on an engineered lipid–polymerhybrid nanoparticle containing a model hydrophilic active, zidovudine, *Colloids Surf. A* 436 (2013) 714–725.
- [46] L. Montenegro, M.G. Sarpietro, S. Ottimo, G. Puglisi, F. Castelli, Differential scanning calorimetry studies on sunscreen loaded solid lipid nanoparticles prepared by the phase inversion temperature method, *Int. J. Pharm.* 415 (2011) 301–306.
- [47] D.G. Peroni, G.L. Piacentini, M. Ressa, A. Bodini, A. Loiacono, R. Aralla, A.L. Boner, Time efficacy of a single dose of montelukast on exercise-induced asthma in children, *Pediatr. Allergy Immunol.* 13 (2002) 434–437.
- [48] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, *Eur. J. Pharm. Sci.* 13 (2001) 123–133.
- [49] http://www.merck.ca/assets/en/pdf/products/SINGULAIR-PM_E.pdf.
- [50] E.K. Ayman, M. Varma, Oral absorption, intestinal metabolism and human oral bioavailability, in: J. Paxton (Ed.), *Topics on Drug Metabolism*, InTech Europe, 2012, pp. 1–34.