ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 3, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION AND PHYSICOCHEMICAL EVALUATION OF NANOSTRUCTURED LIPID CARRIER FOR CODELIVERY OF CLOTRIMAZOLE AND CIPROFLOXACIN

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Received: 03 March 2016, Revised and Accepted: 19 March 2016

ABSTRACT

Objective: The purpose of this research was to formulate nanostructured lipid carrier system (NLCs) in such a way that they can be applied for bacterial as well as fungal infectious diseases.

Methods: To achieve the prime objective, varying concentrations of clotrimazole (CLZ) and ciprofloxacin (CIPRO) were selected for formulations. Stearic acid (solid lipid polymer), oleic acid (liquid lipid polymer), and polyvinyl alcohol (surfactant) were utilized for formulating NLCs through solvent diffusion technique. NLCs were characterized for their surface morphology, Fourier transform infrared spectroscopy (FTIR) drug-polymer interaction, particle size distribution, zeta potential, loading capacity, drug entrapment efficacy (EE), and *in vitro* drug release profile.

Results: NLCs were fabricated with size range varying from 276 nm to 564 nm, possessing smooth spherical morphology. No drug-polymer interaction was observed through FTIR analysis. The highest drug EE for CLZ and CIPRO was found to be 78.6% and 65.8%, respectively. Formulated NLCs depict the biphasic release profile with initial burst release of 40% within 2 hrs followed by controlled release.

Conclusion: Better homing of drug molecules and controlled drug release through formulated NLCs makes them suitable carrier system for various anti-microbial and anti-fungal applications.

Keywords: Nanostructured lipid carries, Clotrimazole, Ciprofloxacin, Solvent diffusion method, *In vitro* release.

INTRODUCTION

Biocompatible lipids have attracted the attention of researchers in recent past as a suitable carrier system for delivery of different proteins, biomolecules, drugs, peptides, etc., to different target sites [1,2]. Lipid nanoparticle formulations with solid matrix, *viz*., solid lipid nanoparticles (SLNs) and nanostructured lipid nanoparticles (NLCs) are most popular and widely used. SLNs are formulated using solid lipids which maintain their solid state at room temperature as well as at body temperature and are considered as biodegradable and biocompatible as per generally recognized as safe status. SLNs possess some remarkable advantages in context to traditional carrier systems for controlled drug delivery such as microparticles, liposomes, emulsions, and polymeric nanoparticles. The associated beneficial aspects of SLNs are their high oral bioavailability, good tolerability [3], negligible toxicity [4], large-scale production through high-pressure homogenization, tissue targeting [5], topical drug delivery, and sustained drug release [3,6]. Besides, there are few limitations associated with SLNs such as drug expulsion on prolonged storage and limited drug loading (DL) capacity [3,5,7]. NLCs are new generation colloidal controlled drug carrier systems which possess excellent potential to overcome the limitations of its older generation SLNs. NLCs are based on spatially incompatible mixture of solid lipid and liquid lipid stabilized by suitable surfactant. These properties of NLCs can be applied to ease the administration of antimicrobial and antifungal drugs, also to overcome the associated limitations of traditional therapeutics [7].

Hospital acquired infections (bacteria or fungal infection) also termed as nosocomial infections are of great concern in healthcare sector, and these are increasing at an alarming rate leading to morality of thousands of patients worldwide [8-10]. There is an urgent need for the development new therapeutic delivery system to overcome this challenge and such problem can be easily tackled by the strategic co-delivery of two drugs which enable us to deal with these infectious diseases at the same time. Development of novel drug delivery system, encapsulated with two different classes of drugs within one carrier (NLCs) can have effective application in targeting fungal and bacterial diseases concomitantly [8].

Ciprofloxacin (CIPRO) is among the most widely available fluoroquinolone antibiotic which possesses potent antibacterial activity against broad range of Gram-negative and Gram-positive pathogens [11,12]. It is effective against a variety of bacterial infection including bone infection, sexually transmitted infections, gastrointestinal infections, skin infections, urinary tract infections (UTIs), etc. [13,14]. However, the efficacy of marketed drug is limited due to its poor aqueous solubility and lower bioavailability [14]. Likewise clotrimazole (CLZ) also possesses poor aqueous solubility which is a major disadvantage for its clinical applications CLZ is widely used broad-spectrum antifungal drug belonging to class of "Azoles" [15]. It is manly used to treat various types of mucus and skin diseases such as tineacrusis, tineapedis, vaginal yeast infections, oral thrush or oral candidiasis, ringworm, mycotic infections of the genitor-urinary tract, cancer, and sickle cell anemia [16]. Associated solubility problem with CIPRO and CLZ again is challenge to co-delivery of drugs through nanoparticles. For the same, sustained release NLCs formulations have been reported as a solution to this problem [15].

Here, we attempted to formulate NLCs for co-delivery of CLZ and CIPRO. The aim of this work is to formulate NLCs for simultaneous delivery of CLZ and CIPRO with improved solubility profile and to characterize the fabrication through; entrapment efficiency (EE), particle size estimation, DL efficiency, Fourier transform infrared spectroscopy (FTIR) analysis and morphological characteristics.

METHODS

Materials

CIPRO and CLZ were obtained as gift samples from *Optimum Pharmaceuticals* Private Limited; stearic acid, oleic acid and poly

vinyl alcohol (PVA) were purchased from Himedia (India). Unless and otherwise specified all chemical were procured from Merck chemicals (India).

Method for preparation of NLCs

The NLCs with or without drug were prepared by employing well established solvent diffusion method with little modification as per laboratory conditions [17]. Briefly, stearic acid (100 mg), oleic acid (45% w/v) and desired amount of drug were dissolved in a mixture of ethanol (5 ml) and acetone (5 ml) over water bath at 70°C. The resultant organic solution was immediately dispersed into 50 ml double distilled water having PVA (0.5% w/v) under moderate mechanical agitation (400 rpm) for 5 minutes at 70°C. Resulting mixture was then cooled at room temperature under continuous stirring until drug-loaded NLCs were obtained. Fabricated NLCs were washed thrice with double distilled water in order to remove unbound or loosely bound drug molecules from the formulation. Drug-free NLCs were prepared exactly in the same manner by only replacing the drug with the same amount of polymer. Compositions of various formulations are represented in Table 1.

Characterization of prepared NLCs

Shape and surface morphology

Surface morphology of NLCs was characterized by scanning electron microscopy (SEM). NLCs were mounted onto metal stubs using double-sided adhesive carbon conductive tap and placed inside vacuum chamber. Surface morphology of NLCs was studied by striking the beam of electron incident beam to the surface of NLCs from tungsten filament.

Drug polymer interaction

The infrared spectrums of drug and excipients were recorded to detect possible interactions of drug and excipients [18]. FTIR spectrum of CIPRO, CLZ, stearic acid, and drug polymeric NLCs was taken at ambient conditions. The FTIR spectra were recorded in the wavelength region ranging between 4000 and 400/cm. Spectrums were obtained by running Micro Lab software and interpretations were made for drug and drug loaded NLCs.

Particle size distribution, zeta potential and poly dispersity index (PDI) analysis

Beckman coulter zeta-sizer was used to determine particle size distribution, zeta potential and PDI of prepared NLCs. All samples were appropriately diluted with de-ionised water, and the pH was adjusted within the range of 6.8-7 followed by sonication for 5 minutes before size analysis.

Drug EE and DL

Drug EE and DL of prepared NLCs were analyzed by employing simple and well-established method by Hu *et al.* [17]. Briefly, from the prepared NLCs formulation, 1 ml of suspension was taken and dissolved in 1 ml methanol, this solution was further diluted 10 times and sonicated for 10 minutes. The resulted dispersion was centrifuged for 20 minutes at 10,000 rpm and the drug content in supernatant was measured by UV-spectrophotometer at 260 nm for CLZ and 261 nm for CIPRO. All the samples were analyzed in triplicate.

Percentage drug EE and percentage DL was calculated using equation given below:

$$EE(\%)=\frac{(\text{Total drug})-(\text{Free drug})}{(\text{Total drug})}\times 100 \quad (2)$$

$$DL(\%)=\frac{(\text{Initial drug})-(\text{Free drug})}{(\text{Mixed lipid})}\times 100 \quad (3)$$

***In vitro* release studies**

The drug release study of NLCs based formulation was performed according to method [17] with some modification. The dialysis tube (10 kDa molecular cut off, Himedia, Mumbai, India) was soaked in the release media (10 mM phosphate buffer at pH 7.4 with 2% Tween 80) overnight before the experiment. One end of the dialysis tube was tightly tied and 1 ml of prepared NLCs formulation was added to dialysis tube after which the other end of the tube was also tied and placed in glass vial. Preheated (37°C) 10 ml release media was then added to vial in which dialysis tube was kept and stirrered at 100 rpm maintained at 37°C. To determine the amount of drug released from the formulation at different time points up to 24 hrs, 2 ml of release media was withdrawn from bottle at predetermined time points and analyzed spectrophotometrically (U.V. Spectrophotometer - Systronics-Model-2202). Withdrawn release media was immediately replaced with 2 ml of fresh media to maintain constant volume and sink condition.

Finally, a graph was plotted between % cumulative drug release CDR v/s time and release profile of all formulations was compared with each other.

RESULTS AND DISCUSSION

Surface morphology analysis

Surface analysis was performed to investigate the morphological characteristics for different formulations. SEM results revealed that formulated NLCs were of spherical shape with smooth surface morphology (Fig. 1).

Drug polymer interaction

FTIR spectroscopic analysis was carried out to study the polymer drug (CLZ and CIPRO) interactions (Fig. 2). FTIR spectrum of stearic acid depicts prominent band at 1700/cm (C=O stretching) and other peaks at 2916/cm and 2849/cm which corresponds to C-H bond consisting saturated carbon atoms (Fig. 2a). The pure CLZ has characteristic IR peaks at 1495/cm, 1216/cm (C-N stretch) and 769/cm (C-Cl stretch) correspondingly depicted in Fig. 2b. CIPRO showed the IR peaks at 3528/cm (O-H stretch), 2426/cm (R=COOH stretch), 1707/cm(COOH stretch), 1451/cm (N-H stretch) and 1272/cm (C-F stretch), respectively (Fig. 2c). Representative bands of polymer and drugs appeared with substantial compatibility in drug loaded NLCs (Fig. 2d) revealing drugs stability within the NLCs without any chemical interaction.

Particle size distribution, zeta potential and PDI analysis

Particle size distribution was studied, and the obtained mean particle size for formulations F1-F6 varied from 276 nm to 564 nm as shown

Table 1: Composition of NLCs formulation

Formulation code	Drug		Solid lipid stearic acid (mg)	Liquid lipid oleic acid (%w/v)	PVA (%w/v)
	CIPRO (mg)	CLZ (mg)			
F1	0	0	100	45	0.5
F2	25	0	100	45	0.5
F3	0	25	100	45	0.5
F4	25	25	100	45	0.5
F5	12.5	25	100	45	0.5
F6	25	12.5	100	45	0.5

CIPRO: Ciprofloxacin, CLZ: Clotrimazole, NLCs: Nanostructured lipid carrier system, PVA: Poly vinyl alcohol

Table 2: Particle size, zeta potential, PDI, percent EE and DL of the respective formulations

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	%EE		%DL	
				CIPRO	CLZ	CIPRO	CLZ
F1	276	–14.3	0.629	0	0	0	0
F2	335	–16.7	0.678	83.6	0	16.72	0
F3	347	–21.1	0.653	0	78.6	0	15.73
F4	476	–13.2	0.767	61	56.26	12.2	11.25
F5	517	–18.6	0.824	56.4	63.2	6.26	12.64
F6	564	–22.9	0.821	65.8	45.33	13.16	5.03

PDI: Poly dispersity index, EE: Entrapment efficiency, DL: Drug loading, CIPRO: Ciprofloxacin, CLZ: Clotrimazole

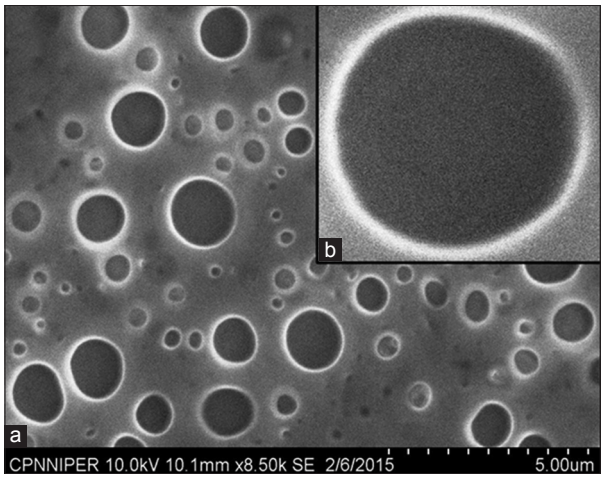

Fig. 1: Scanning electron micrographs of nanoparticles (a) nanostructured lipid carrier system (NLCs) in bulk and (b) individual NLCs

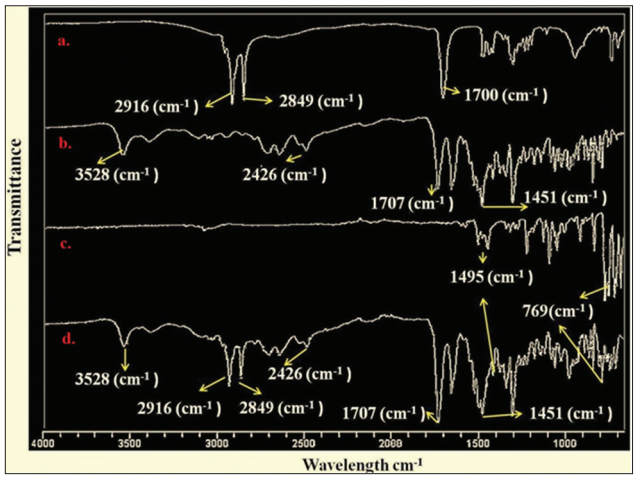

Fig. 2: Fourier transform infrared spectroscopy spectroscopic graphs for: (a) Stearic acid, (b) ciprofloxacin (CIPRO), (c) clotrimazole (CLZ) and (d) physical mixture of stearic acid, CIPRO and CLZ

in Fig. 3. NLCs with formulation-1 (F1) showed lowest particle size (276 nm), whereas NLCs with formulation-2 (F4) showed highest particle size (564 nm). Moreover, the poly-dispersity index for all formulations was found to be in the range of 0.629-0.821 as shown in Table 2, which suggests the homogenous size distribution of all formulated NLCs. To evaluate stability of colloidal dispersion zeta potential is a key factor and in general, dispersion of particles is said to be stable when the absolute value for zeta potential is above + or - 10 mV due to electrical repulsion in between particles of dispersion [19]. Obtained zeta potential was in between –13.2 mV to –22.9 mV as represented in Table 2, which depicts the good stability profile for all the formulations.

Drug EE and DL capacity

EE and DL capacity of drug loaded NLCs were investigated for all formulations in triplicate and the corresponding values have been listed in Table 2. Percentage DL capacity of the NLCs ranges from 5.03% to 16.72% and the percentage of EE values varies from 45.33% to 83.6 %. It has been reported that the assimilation of liquid lipids to solid lipids facilitates imperfections in crystal lattice of the lipid mixture, leaving sufficient space to house more drug molecules which improve DL and encapsulation efficiency of NLCs [20].

Drug *In vitro* release

In vitro release curves of all drug-loaded nanoparticles are shown in Fig. 4. From the figure, we observed that the fabricated NLCs showed biphasic drug release pattern, i.e. initial burst release of drug followed by sustained release at constant rate for 24 h. For CIPRO (25 mg) loaded NLCs formulation F2, F4 and F6, approximately 40 % of drug was release within first 2 hrs and most of the remaining drug was released within the 24 hrs. However for formulation F5 where the concentration of CIPRO was 12.5 mg, displayed initial drug release of approximately 35% within 2 hrs and the remaining drug was released within successive 24 hrs. Likewise for CLZ (25 mg) loaded NLCs formulation F2, F4 and F5 similar drug release pattern was obtained (40% of initial released followed by controlled release of drug) and for formulation F6 initial drug release was found to be of 35% and the remaining drug was released within 24 hrs. Loosely bound drug particles to the surface of NLCs may be the possible reason for the burst release pattern of drug as observed in the release studies.

CONCLUSION

Drug loaded nanostructured lipid carriers were formulated by solvent diffusion method, possessing improved incorporation of drug molecules and controlled drug release profile. Fabricated NLCs show the spherical morphology with particle size distribution ranging from 276 nm to 564 nm with no polymer-drug interaction, which suggest that physical mixture of drugs and polymer are chemically compatible and can be utilized to formulate NLCs. Formulated NLCs were stable as depicted by zeta potential and poly-dispersity index and showed the highest drug EE and DL. Biphasic drug release pattern was exhibited by drug-loaded NLCs with initial burst release followed by sustained release up to 24 hrs. All these physicochemical properties of the NLCs make them a suitable carrier system to deal with various microbial and fungal diseases. For better insight into the anti-fungal and anti-microbial activities of these NLCs biological evaluation are suggested.

ACKNOWLEDGMENT

Authors would like to acknowledge Jaypee University of Information Technology, Waknaghat, Solan (H.P.) India, Himachal Pradesh University, Shimla (H.P.), India, and National Institute of Pharmaceutical Education and Research, Mohali (Punjab), India, for providing facilities to do this work.

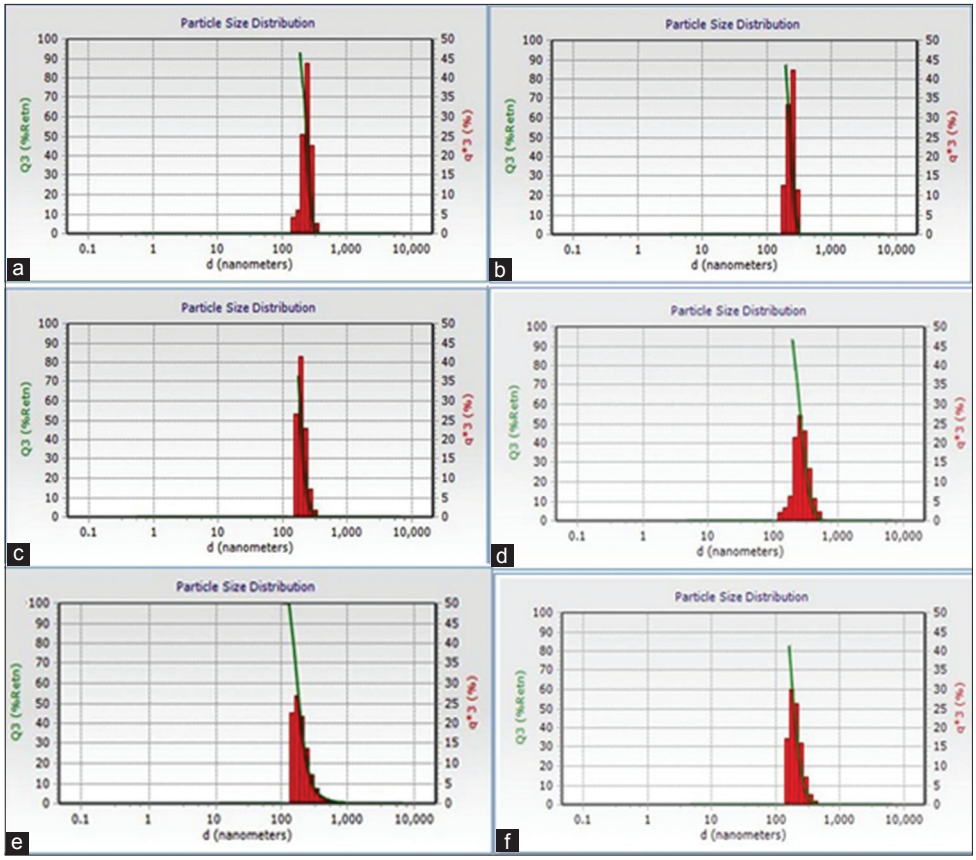

Fig. 3: Particle size distribution graph of; (a) formulation-1, (b) formulation-2, (c) formulation-3, (d) formulation-4, (e) formulation-5, and (f) formulation-6

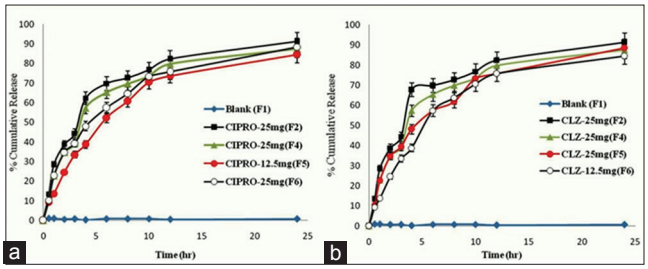

Fig. 4: *In vitro* dug release profiles for (a) ciprofloxacin - loaded nanostructured lipid carrier system (NLCs) and (b) clotrimazole - loaded NLCs.

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