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Clarithromycin loaded lipid polymer hybrid nanoparticles: Fabrication, *in vitro* and *in vivo* evaluation

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Abstract: Clarithromycin; a Macrolide antibiotic is used to treat various bacterial infections. Unluckily, its hydrophobic nature adds to its variable and pitiable oral bioavailability. In the current study, clarithromycin loaded Lipid Polymer Hybrid Nanoparticles (LPHNs) were produced followed by in vitro and in vivo evaluation. Clarithromycin loaded LPHNs (CHNF-5) with particle size 125.31±2.5nm and PDI (0.312±0.01) showed high entrapment efficiency 96%, and drug loading capacity 0.596 % ± 0.014. Spherical shaped particles were confirmed by scanning electronic microscopy. Differential Scanning Calorimetry and Powder X-Ray Diffraction confirmed the crystalline properties of the LPHNs. The engineered nanoparticles exhibited high stability at different temperature. Dissolution studies demonstrated that LPHNs effectively sustained the drug release rate. *In vivo* pharmacokinetic studies of the produced LPHNs demonstrated significant increase in the maximum peak plasma concentration (1390 ng/mL, $P<0.001$), AUC (14146 ng h/mL, $P<0.001$), and eradication half-life (11.6 h, $P<0.01$)

Keywords: Lipid polymer hybrid nanoparticle, clarithromycin, stability, bioavailability, dissolution.

INTRODUCTION

In spite of the innovation in antibiotics, the therapeutic management of intracellular infections regularly flops thoroughly to eliminate the pathogens. Certainly, the venture is to devise the sources of carrying an antibiotic in such a shape that it is capable to easily penetrate the effective cells and then released into these cells (Uskokovic 2015). A variety of bacteria are the causes for the current predicament of infectious diseases that have an increased mortality rate globally. Morbidity/ death rate has been dwindled to the greater extent with the discovery of new antibiotics (Carrara *et al*., 2018). The discovered antibiotics being devised in the conventional dosage form have been reported with a number of issues including increased cytotoxicity, administration frequency, swift degradation, low water solubility, insufficient antibiotic concentration at target site and clearance in the bloodstream (Wang, Hu *et al*., 2017; Gao, Chen *et al*., 2018).

Clarithromycin (CLR) is comparatively novel macrolide antibiotic (Koseki *et al*., 2017) (fig. 1). Bacterial upper and lower respiratory infections and uncomplicated Gram-positive soft tissue infections are included in its major indications. Most recent indication includes combination therapy for pelvic inflammatory disease and triple therapy for Helicobacter pylori associated peptic ulcer disease. Since it is acid stable, clarithromycin is also used to treat the infections in the gastrointestinal tract. However, It belongs to Class-II of the Biopharmaceutical Classification System (BCS), which have high permeability and low solubility (Mishra, Gautam *et al*., 2016). Clarithromycin has poor oral bioavailability (50%) with plasma half-life of 4 to 7 hours (Awidat *et al*., 2016; Sahu *et al*., 2016). Poor water solubility of CLR (0.34 mg/L) has been reported as the major hindrance for its therapeutic potential and dosage form development.

Poor water solubility of numerous drugs is one of the major challenges in pharmaceutical drug development that contributes absorption and dissolution limitation. Dissolution rate and then the bioavailability are suggestively affected by the size and size distribution of

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pharmaceutical particles. The fine particles have high surface areas which lead to enhanced dissolution and subsequently to boosted bioavailability. Nanoparticles have been reported one of the best strategies to overcome the major issue of hydrophobic drug molecules (Yun *et al*., 2015).

There are a number of issues associated with most antibiotics, which include poor water solubility, concentration at the target site, intracellular delivery, pulmonary accumulation, release profile, dosing frequency and side effects. These issues have been reported to be effectively overcome by novel nanoparticle drug delivery approaches. The frequently employed nanocarriers consist of dendrimers (polymers), polymeric nanoparticles, metal (gold, silver), polymeric micelles, solid lipid nanoparticles, and liposomes. However, the use of liposomes in drug delivery system was reduced because of low encapsulation efficiency of poor aqueous-soluble drugs; ultimately the release of the entrapped drug happens instantly which results in the unsteadiness of the drug. Moreover, polymeric nanoparticles have hydrophobic drugs with elevated loading capacity in contrast to liposomes. Although, there are complications associated with this system, such as biocompatibility and modest circulation lifetime, polymeric nanoparticles and lipid-based nanocarriers are ideal delivery systems due to their solid condition of physiological lipid carriers and submicron sized particles (Yameen *et al*., 2016; Sánchez *et al*., 2016; Liu *et al*., 2017).

A new drug delivery system; lipid polymer hybrid nanoparticle has become an important and advanced alternative to solid lipid nanoparticles and liposomes (Fang *et al*., 2016; Omolo *et al*., 2019). Furthermore, the LPHNs is a hybrid nano-particulate drug delivery system that exhibits exceptional advantages of both systems while excluding many of their shortcomings.

It has become evident from the current published literature that there has not been reported any study for Clarithromycin loaded LPHNs yet. Consequently, it is important to devise CLR-loaded lipid polymer hybrid nanoparticles with advanced physicochemical and biological features against intracellular bacteria.

Clarithromycin loaded LPHNs were produced by using a modified combinative approach of magnetic stirring followed by probe sonication. Stearic acid was utilized as the solid lipid and Eudragit Rs 100 was used as a polymer. Sodium lauryl sulphate was employed as surfactant. Ethyl cellulose was used as helper polymer and oleic acid as helper lipid to augment significant properties like drug loading capacity, drug encapsulation, and anti-bacterial action.

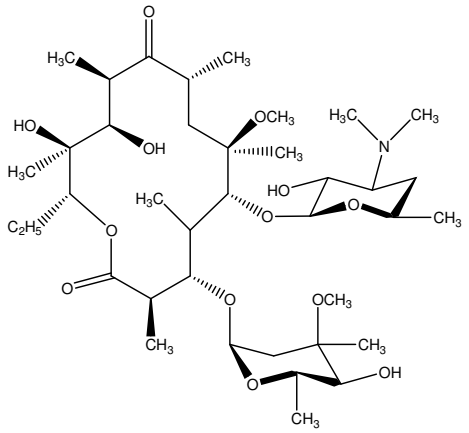

Fig. 1: Chemical Structure of Clarithromycin

MATERIALS AND METHODS

Materials

Clarithromycin (B.No.128-170113-1) were received as gift from Ferozsons Laboratories Limited, Nowshera. While Stearic Acid (B.No.D-7664-4400), Eudragit Rs 100 (B.No.D-6958-4406), Ethyl cellulose (B.No.D-4154-4405), sodium Lauryl Sulphate (B.No.D-7592-1405) and oleic acid (B.No.D-5635-4400) were purchased from Musa-G Chemicals, Peshawar, Pakistan. Other exploited materials and Solvents were found of analytical status.

Methods

Engineering of LPHNs

Unloaded as well as Clarithromycin loaded LPHNs were produced via combinative approach; magnetic stirring and sonication (probe) (Kalhapure *et al*., 2016). Stearic acid was melted by heating to 80ºC. Solution of Eudragit Rs 100 and sodium Lauryl Sulphate (surfactant) in 80% ethanol was prepared and heated to the same temperature (80°C) followed by mixing with the melted lipid phase. To remove the organic solvent, the resultant mixture was stirred followed by volume adjustment with water. Furthermore, the mixture was subjected to high intensity sonication (30 % amplitude) followed by cooling to produce the desired LPHNs dispersion. The process and experimental conditions are shown in Table 1. Once the process and experimental conditions were optimized, then the most favorable formulation was selected to be loaded with clarithromycin (20mg) without adding the helping polymers and lipid. For fabrication of drug loaded LPHNs; 20 mg of clarithromycin was included in the organic solution of surfactant and polymer, and it followed the same process. For co encapsulation, both the ethyl cellulose and oleic acid were mixed in the organic solution of CLR, surfactant and polymers with the subsequent addition to the melted lipid phase (table 2). The procedure that followed thereafter was the same as above.

Table 1: Optimization of experimental and process conditions for preparation of blank nanoparticles

Formulation Code	Stearic Acid (gm)	Eudragit (gm)	Sonication (Hz)	SLS	Sonication time (Min)	Stirring time (Min)
BF-1	0.5	1.0	30%	0.2	2	20
BF-2	0.5	1.0	30%	0.3	2	20
BF-3	0.5	1.0	30%	0.5	5	20
BF-4	0.5	1.0	30%	0.6	8	20
BF-5	0.5	1.0	30%	0.8	8	40
BF-6	0.5	1.0	30%	1.0	8	60

Table 2: Optimization of helper polymer and lipid for hybrid nanoparticles

Formulation Code	Oleic Acid (ml)	Ethyl Cellulose (gm)
CHNF-1	0	0
CHNF-2	0.1	0
CHNF-3	0.2	0
CHNF-4	0.2	0.3
CHNF-5	0.2	0.5

Lyophilization

Freeze dryer (Heto Power Dry LL1500-Thermo Electron Corporation, USA) was used for the lyophilization of CLR- LPHNs. Before drying, 10% Glucose solution was put in as cryoprotectant. In addition, CLR-LPHNs remained at -20 ºC overnight and were then transferred to the freeze dryer for lyphilization at -75 ºC and the duration was 48 hrs at an increasing pace of 5 ºC/h.

Characterization

Dynamic light scattering

Macrotac instrument was employed to carry out particle size measurements. An analysis was made for zeta potential, PDI, and z-average particle size. To gain particular scattering intensity; deionized water was used to dilute the entire LPHNs formulations that was then measured at 25 ºC and at a 90° scattering angle.

Entrapment efficiency and drug loading capacity

The following formula and reported method was used for drug loading capacity and drug entrapment efficiency of the created CLR-LPHNs (Ghanshyam, Patel *et al*., 2011; Sadiq and Abdul Rassol 2014).

$$EE\% = \frac{\text{(Whole quantity of drug added – Unloaded Drug)}}{\text{(Total sum of drug added)}} \times 100$$

$$DLC\% = \frac{\text{(Whole quantity of drug in LPHNs)}}{\text{(Sum of drug added + sum of Excipients)}} \times 100$$

For EE% and DLC% a total of five different nano-formulations were prepared having specified quantity (constant quantity) of Clarithromycin (20 mg), stearic acid (0.5 gm), Eudragit (1.0 g), Sodium Lauryl Sulphate (1gm). Some of the processing parameters like stirring time (60 min) sonication time (8min) and sonication frequency 30% were also kept constant for all the nano-formulations.

For optimization, in terms of EE% and DLC% varied concentration of ethyl cellulose (Co-polymer) and oleic acid (Co-lipid) were employed.

Drug-excipients interaction

To probe drug-excipient interaction, Fourier Transform Infrared Spectroscopy (IR Prestige 21 Shimadzu, Japan) was employed (Tiţa, Fuliaş *et al*., 2011). Over a frequency rate of 4000-450 cm^{-1}, spectrum of processed (FCLR) and unprocessed CLR were scanned. For matching of formulation constituent, the pattern and peaks shaped by the processed CLR (FCLR) were compared with unprocessed CLR.

Morphological study

Surface morphology of the unprocessed CLR and engineered LPHNs was identified using Scanning Electron Microscopy (SEM) JSM 5910 (JEOL, Japan), (Sahu *et al*., 2013) Acceleration voltage of 15 kV was used to record SEM micrographs at various magnification levels.

Powder x-ray diffraction (P-XRD)

To validate fresh solid state composition, analysis of Powder X-ray Diffraction was implemented. The unprocessed CLR, produced LPHNs and major excipients including helping polymers and lipids were characterized using X-Ray Diffractometer JDX-3532 (JEOL, Japan). Cu Kαa radiation in scanning range of $2\phi = 5^{\circ}–50^{\circ}$ was used with tube current 30 mA, operated voltage of 40 kV, step size 0.05°, step time 1.0 sec, scattering slit 1.0 degree, divergence slit 1 degree, and receiving slit 0.2 mm for measurement.

Thermal analysis

Differential Scanning Calorimetry (DSC) is a thermo analytical technique applied to investigate melting and recrystallization manners of diverse samples. Precisely weighted unprocessed CLR, Oleic acid, ethyl cellulose, stearic acid and engineered CLR (CLRF-) were investigated by Differential scanning calorimeter (DSC)

(Perkin Elmer, Diamond Series DSC Equipment-USA). In crimped aluminum pans, the analysis was made at heating speed of 10 ◌C/min ranging 40–400◌.

Stability study

Physical stability has been reported a key issue with nanosuspensions (Wang, Zheng *et al*., 2013). For 90 days, a stability study was carried out at different temperatures. PDI and particle size values of samples hoarded at various temperatures were observed intermittently. The newly formulated sample was split into two parts. Two plain sealed glass vials were used for each part and were stored at variant temperatures (5±2°C and 25±3ºC) for 90 days. Samples were collected on the 1st, 15th, 30th, 60th, and 90th storage day and subjected to PDI and particle size measurements. Two tailed *t*-test was implemented for the data analysis. Probability <0.05 was regarded as important.

***In-vitro* drug release study and kinetics models**

Dialysis bag method was employed to perform an in Vitro drug release study (Bhardwaj and Burgess 2010). Deionized water was used to dampen the dialysis bags for 12 hours before use. CLR-LPHNs dispersion (1ml) from each sample was put into the dialysis bag and kept in 250ml phosphate buffer solution (pH 7.4) at a speed of 50 rpm. Samples were collected and equivalent volume of phosphate buffer solution was replaced after specific time (1-12 hr). UV spectrophotometer (λmax 210 nm) was utilized to explore samples against blank phosphate buffer solution (pH 7.4) (Moffat, Osselton *et al*., 2004) . Data acquired from *in vitro* drug release study was fixed into various kinetic models to sort out both drug release rate and later mechanism.

Pharmacokinetic evaluation

For the pharmacokinetic evaluation of clarithromycin nanoparticles and its optimized formulation, Sprague-Dawley rats weighing 150-200 g were used. These animals were provided with water but no food for 24 hours before the experiment. Four random groups of animals (six in each group) were made. For animal studies, the procedure was officially approved by the departmental ethical and research committee of the University of Malakand and applicable Bye-Laws 2008 (Scientific Procedure Issue-1). Rats were orally administered pure clarithromycin suspension, clarithromycin nanoparticles and its formulation, and marketed drug at an amount equal to 5 mg/kg. Blood samples were collected from the retro-orbital plexus at 0.25, 0.50, 0.75, 1, 2, 4, 8, 12, and 24 h. Animals were injected with an equal quantity of normal saline to recompense blood loss. To detach plasma, the samples of blood were centrifuged at 5000 rpm at 4°C for 10 minutes. A high performance liquid chromatography technique was applied to process and examine the accumulated samples of plasma as (reported previously by Gupta *et al*., 2016). WinNonLin (v 4.0; Pharsight Software, Mountain View, CA, USA) was employed to verify pharmacokinetics parameters of maximal plasma concentration (C_{max}), the area under the concentration-time curve (AUC), half-life ($t_{1/2}$), and time to reach maximal plasma concentration (T_{max}).

STATISTICAL ANALYSIS

The important data was generated and reported by taking mean of the triplicate of the samples with standard error mean and ±standard deviation values. Additionally, ANOVA (one way analysis of variance) and t-tests were employed to statistically analyze the obtained data. P values <0.05 were considered the significant range for evaluation of different data. WinNonLin (v 4.0; Pharsight Software, Mountain View, CA, USA) was used for evaluation of imperative PK (pharmacokinetics) parameters.

RESULTS

Fabrication of lipid polymer hybrid nanoparticles

The optimized process conditions for unloaded LPHNs including stearic acid (0.5%) ,eudragit (1%), SLS (1%), magnetic stirring time (60minutes) and probe sonication (30% hz) and sonication time (08minutes) are shown in table 3. The particle size of the blank nanoparticles were found to be decreased with increasing concentration of the surfactant and polymers (table 3). It was observed that by increasing the sonication and stirring times, the particle size was reduced. (Khan, Matas *et al*., 2013). The optimized unloaded formulation BCLR- (BF6) showed particle size 115.25± 4.0nm. Subsequently, after drug (CLR) loading, size of the particle was increased to

Table 3: Process and experimental conditions for preparation of blanks nanoparticles

Formulation Code	Stearic Acid (gm)	Eudragit (gm)	Sonication (Hz)	SLS	Sonication time (Min)	Stirring time (Min)	Particle Size (nm)±SD
BF-1	0.5	1.0	30%	0.2	2	20	605.41±5.0
BF-2	0.5	1.0	30%	0.3	2	20	445.34±4.5
BF-3	0.5	1.0	30%	0.5	5	20	310.76±5.0
BF-4	0.5	1.0	30%	0.6	8	20	140.82±4.0
BF-5	0.5	1.0	30%	0.8	8	40	129.53±3.0
BF-6	0.5	1.0	30%	1.0	8	60	115.25±2.5

Table 4: Impact of helper lipid and polymer on entrapment efficiency and DLC

Formulation Code	Oleic Acid (ml)	Ethyl Cellulose (gm)	EE (%)	DLC (%)
CHNF-1	0	0	60 ± 2.7	0.397± 0.017
CHNF-2	0.1	0	66 ± 2.9	0.423± 0.015
CHNF-3	0.2	0	74 ± 2.9	0.459±0.017
CHNF-4	0.2	0.3	83 ± 2.5	0.471±0.016
CHNF-5	0.2	0.5	96 ± 3.45	0.596± 0.014

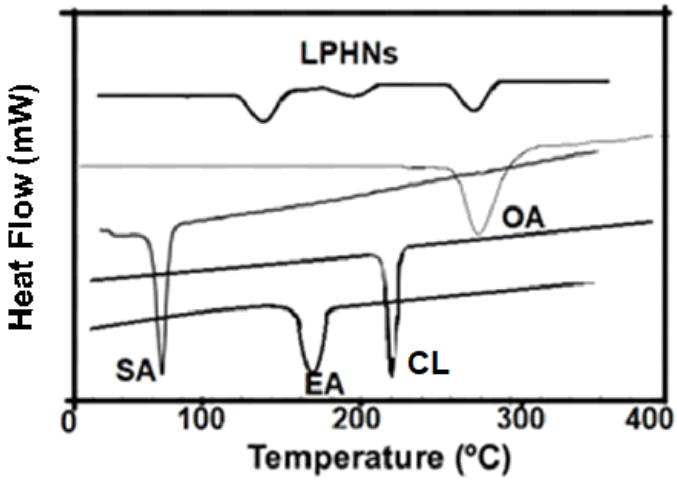

Fig 2: DSC thermograms, Clarithromycin (CL), Oleic Acid (OA), Ethyl cellulose (EC), Stearic Acid (SA) and Lipid polymer hybrid nanoparticles (LPHNs).

125.31±3.5.0 nm (table 4). The zeta potential of optimized LPHNs (CHNF5) formulation was (-37) mV. The EE and DLC of loaded clarithromycin without helper lipid and helper polymer were noticed 60 % ±2.7 and 0.397 % respectively. However, EE and DLC for the optimized clarithromycin loaded formulation with the helping polymers and lipids were found 96% and 0.596 % respectively (table 4).

Characterisation of optimized LPHNs

Differential scanning calorimetry (DSC)

DSC study was performed for unprocessed clarithromycin, processed clarithromycin, stearic acid, oleic acid and ethyl cellulose. The unprocessed CLR showed a sharp melting point peak around 220°C (fig. 2). On the other hand, the major excipients including SA, Ethyl cellulose and OA showed the melting point peaks around 80°C, 180°C and 280°C respectively. However, the DSC thermogram of the produced CLR loaded LPHNs clearly showed melting point peaks for the helping lipids and polymers with little broader peak for CLR.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of the produced LPHNs was compared with the unprocessed CLR sample. Fig. 3 shows C-N bending at 1171 cm-1, C=O stretching at 1727 cm-1, CH2 stretching at 2827 cm-1, OCH3 stretching at 2880 cm-1 and C-CH3 Stretching at 2943 cm-1(Mohammadi, Nokhodchi *et al*., 2011). For both the unprocessed and processed CLR samples, the peaks were around the same wave length which confirmed the intact chemical integrity of CLR in LPHNs.

Scanning electron microscopy (SEM)

Fig. 4 shows scanning electron micrographs of the unprocessed CLR and CLR loaded LPHNs. The CLR raw particles were found with plates, prism and rectangle shapes. All the particles were homogenously distributed and there was not observed agglomeration and aggregates of the particles. The approximate particle size of the unprocessed CLR particles was found to be below 200 micron. On the other hand, the produced CLR loaded LPHNs were found with spherical shapes and homogenous distribution.

X-ray diffraction

It has become evident from fig. 5 that the produced Clarithromycin loaded lipid polymer hybrid showed peaks of the crystalline helper lipids and polymers with disappearance of some of the major peaks of clarithromycin. In addition, some of peaks corresponding to the CLR have appeared with short peaks intensities

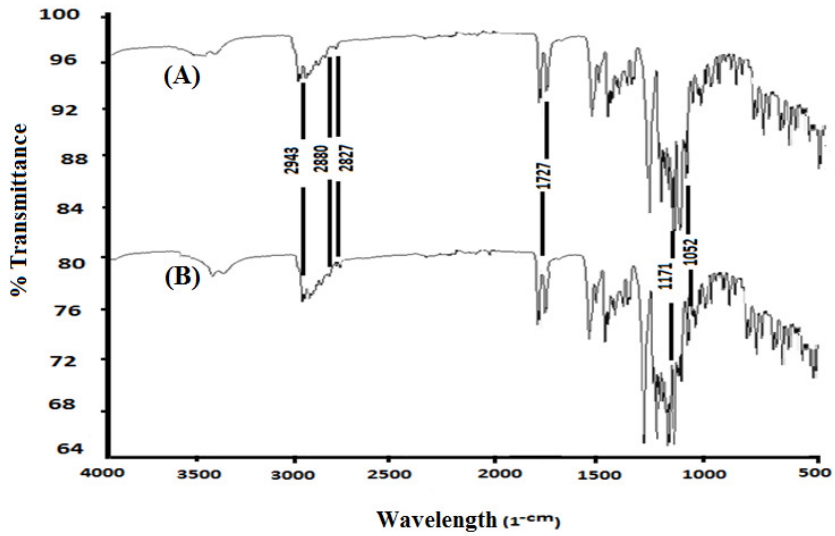

Fig. 3: FT-IR spectra of unprocessed CLR (A) and processed CLR (B).

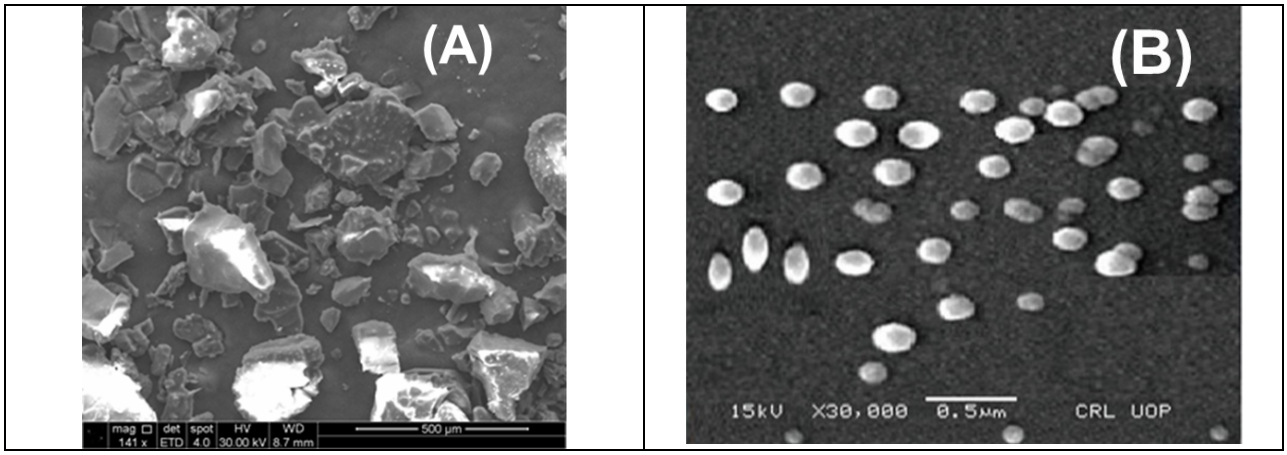

Fig. 4: SEM micrographs of raw CLR (A) and CLR loaded LPHNs (B)

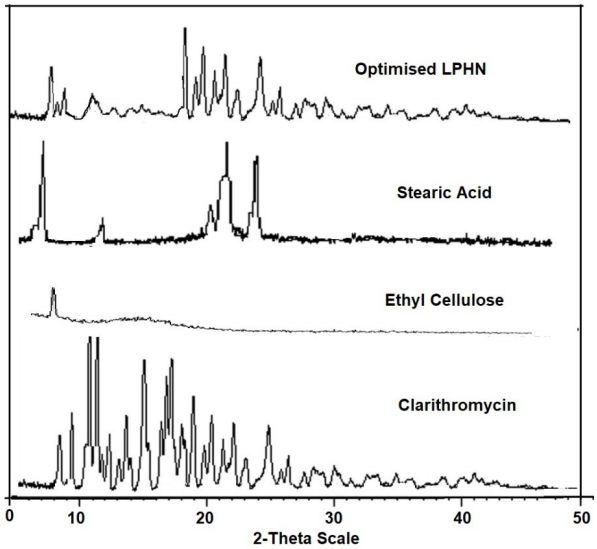

Fig. 5: PXRD patterns of unprocessed CLR, helper lipids and polymers CLR loaded LPHNs

Physical stability

The optimized CLR loaded LPHNs were found very stable for 90 days both at room (25°C) and refrigerated temperature (4°C). After 90 days, both particle size and PDI values were found very similar to the fresh prepared samples (figs. 6 and 7).

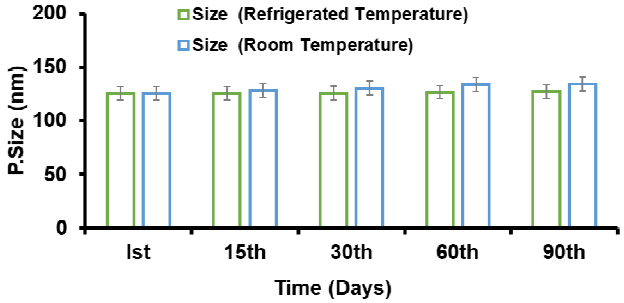

Fig. 6. Particle size distribution of CHNF-5 as a function of time.

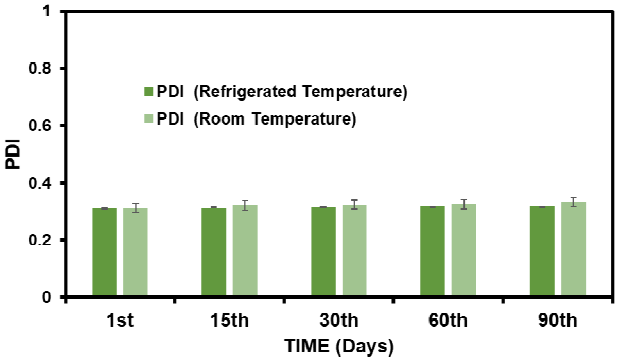

Fig. 7: PDI of CHNF-5 as a function of time

Drug release kinetics and dissolution study

The dissolution data represented through Figure 8 clearly revealed that Clarithromycin loaded LPHNs formulations demonstrated maximum (burst) release in the outset. Almost 13% to 28% of the total Clarithromycin was released during the first hour followed by sustained release. All the CLR loaded LPHNs demonstrated release of almost 99% of Clarithromycin in 12 hours (fig. 8). Whereas, the optimized LPHNs (CHNF5) nanoformulation showed more effective sustained release rate of the CLR and only 77.87% of Clarithromycin was released in 12 hours.

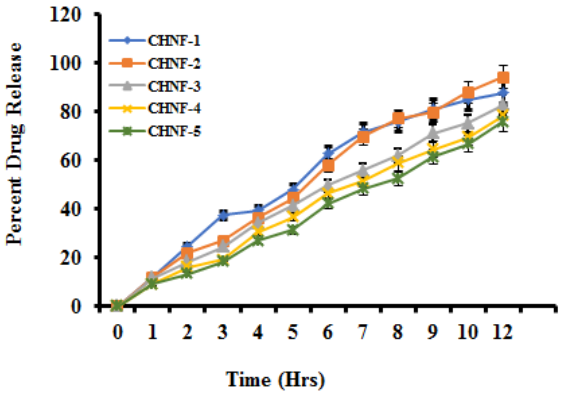

Fig. 8: Comparative drug release profile of different LPHNs formulations.

In-vitro drug release data was incorporated into mathematical kinetic models. It highlighted that it fixed appropriately into a zero-order kinetic model. For instance, drug release from LPHNs is not reliant on the quantity of drug still existing in LPHNs with R^2 values in the range of 0.950-0.998 for Clarithromycin (Costa and Lobo 2001) (table 5).

Korsmeyer-Peppas model provided more insight into the mechanism of drug release from the produced LPHNs formulations. In this model, the value of n (release exponent) was more than 0.5 (n>0.5) in the presented model. This corroborated non-Fickian diffusion kinetics (anomalous transport). For example, the drug release followed both diffusion of drug from LPHNs and erosion/dissolution of the lipid matrix as well (Barzegar-Jalali 2008; Sadiq and Abdul Rassol 2014).

Pharmacokinetics evaluation

Plasma concentration-time profile underlying the pharmacokinetic of clarithromycin suspension, clarithromycin optimized nanoparticles and its optimized nano-formulation along with marketed formulation after oral administration of an equal dose of 5 mg/kg body weight is shown in fig. 9. The various pharmacokinetic parameters including area under the concentration-time curve, biological half-life, time to reach maximal plasma concentration, and maximal plasma concentration are represented in table 6.

Table 5: Kinetics models for different formulations

Formulation Code	Zero Order(R^2)	First Order(R^2)	Higuchi (R^2)	Kors Meyers Peppas (R^2)	Release exponent(n)
CHNF-1	0.9502	0.9833	0.9602	0.965	>0.05
CHNF-2	0.9788	0.9421	0.9377	0.9344	>0.05
CHNF-3	0.9887	0.9776	9485	0.9395	>0.05
CHNF-4	0.9907	0.9813	0.953	0.936	>0.05
CHNF-5	0.998	0.971	0.990	0.991	>0.05

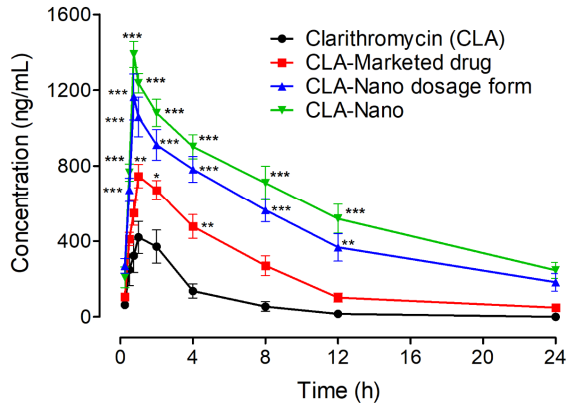

Fig. 9: Pharmacokinetic profile of clarithromycin (CLA), clarithromycin nanoparticles (CLA-Nano), clarithromycin nano-dosage form (CLA-Nano dosage form) and clarithromycin marketed drug (CLA-Marketed drug). Plot of plasma concentration (ng/mL) *vs* time (h). Data presented as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to pure clarithromycin suspension treated rats at respective time-period. Two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis.

The pharmacokinetic profile of clarithromycin at 5 mg/kg show a maximum concentration value of 419.7 ng/mL at 1.21 h, with area under the concentration-time curve from time zero to 24 h observed as 1734 ng h/mL. The elimination half-life was noted as 2.36 h and the volume of distribution was observed as 11.23 mL. The comparative marketed formulation of clarithromycin showed a statistically non-significant increase in the maximum plasma concentration as 745.7 ng/mL at 1.12 h. However, a significant change was observed in the elimination half-life, which was significantly increased to 6.6 h ($P<0.05$), with an AUC of 5356 ng h/mL. Significant changes in the pharmacokinetic profile of clarithromycin was produced only when administered as optimized nanoparticles. Thus, the functionalized clarithromycin nanoparticles showed a noteworthy improvement in the maximum peak plasma concentration (1390 ng/mL, $P<0.001$), AUC (14146 ng h/mL, $P<0.001$), and elimination half-life (11.6 h, $P<0.01$), with an important reduction in the time to arrive at maximum plasma concentration (0.79 h, $P<0.05$), as compared to the pure clarithromycin suspension. Formulation of clarithromycin nanoparticles also showed an enhancement in the bioavailability as revealed from the significant decrease in the time for peak plasma concentration (0.81 h, $P<0.05$) and a considerable amplification in the maximum plasma concentration achieved (1165 ng/mL, $P<0.001$), the extent of plasma exposure of clarithromycin as AUC (11184 ng h/mL, $P<0.001$), and the requisite time for the plasma clarithromycin concentration to dwindle by 50% (12.6 h, $P<0.001$), when compared to the respective pharmacokinetic parameters of pure clarithromycin drug suspension.

DISCUSSION

CLR loaded LPHNs with proper size, Zeta potential and PDI were effectively produced using controlled process and experimental conditions. Besides, vital properties of the LPHNs system such as, drug release and drug encapsulation were more improved by adding helper polymer ethyl cellulose and helper lipid Oleic acid. Combinative approach of magnetic stirring and probe sonication were found very useful for fabrication of stable CLR loaded LPHNs. The higher Concentration of the surfactant can potentially provide an improved firmness to the small lipid droplets that avert them from coalescence (Garg, Singh *et al*., 2015). Both of these parameters can lead to high energy input and efficient micromixing, which in turns can result into narrow size distribution of the particles and small particle size (Rahim, Sadiq *et al*., 2017). The resulted zeta potential for the optimized LPHNs system is an adequate and sufficient for electrostatic stability (Ullah, Khan *et al*., 2018). Additionally, zeta potential ± 30 and PDI<0.5 divulged that optimized LPHNs (CHNF5) would be unwavering during storage at optimum conditions (Lestari, Müller *et al*., 2015). Drug loading capacity and entrapment efficiency were considerably improved with the addition of helper polymer ethyl cellulose and helper lipid oleic acid (table 4). It has been illustrated that in lipid and polymer based nanoparticulate drug delivery systems, the combined energy of drugs with the lipids and polymers

Table 6: Summary of pharmacokinetics parameters for clarithromycin (CLA), its nanoparticles (CLA-Nano), its nano-dosage form (CLA-Nano dosage form) and clarithromycin marketed drug (CLA-Marketed drug)

Sample	Pharmacokinetic parameter			
	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng-h/mL)
Clarithromycin	2.369 ± 0.396	1.21 ± 0.621	419.7 ± 85.29	1734 ± 479.2
CLA-Nano	11.66 ± 1.964**	0.79 ± 0.985*	1390 ± 68.21***	14146 ± 1397***
CLA-Nano dosage form	12.68 ± 1.118***	0.81 ± 0.741*	1165 ± 121.4***	11184 ± 1289***
CLA-Marketed drug	6.651 ± 1.455*	1.12 ± 0.895	745.7 ± 61.78	5356 ± 792.7

Values are expressed as mean ± SD. One-way repeated measures ANOVA followed by Dunnett's *post hoc* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to pure clarithromycin drug suspension, $n = 6$.

play a key role in successful encapsulation of the drugs(Liu, Pan *et al*., 2010). In this case, it may be ascribed to the elevated strapping energy of stearic acid with CLR, OA, eudragit and SLS, which results into drug loading capacity and utmost entrapment efficiency.

Once a link between drug and the helping polymer/lipid is increased it would lead to high entrapment efficiency compared to the formulations without helping polymers and surfactants (Kalhapure *et al*., 2016). In addition, we can also rationalize the interaction of the copolymer and lipid together with the drug molecules. The helping copolymers and lipids can potentially form a favorable complex which in turn potentiates the interaction with clarithromycin. Furthermore, the stability studies for 90 days indicated that the engineered CLR loaded LPHNs were very stable at different conditions (figs. 6 and 7). Both particle size and PDI measurements of the stored samples were monitored at different time of intervals and were found to be very stable. This exhibited that both the process and experimental conditions were well controlled to produce stable hybrid nanoparticles. There was observed very less and insignificant growth in the size of the particles for the samples evaluated at bit higher temperature compared to the samples stored at refrigerated samples. This little enhancement in the particle size may be attributed to boost in solubility of the drug at high temperature which in turn can lead to growth of the particles (Shah, Ullah *et al*., 2016). The DSC studies confirmed effective entrapment of CLR within the lipid polymer hybrid system. There was observed a small increase in the width of the endothermic peak for the CLR which shows significant decrease in the particle size and transition to amorphous form. The dominant peaks of the helping polymers and lipids in the produced LPHNs explicitly show an effective entrapment of the drug molecules within the hybrid delivery system. The FTIR study confirmed compatibility of the CLR with the chosen hybrid system. The produced LPHNs did not produce a new peak. We did not observe any chemical interaction between CLR and polymers/surfactants. Scanning Electron Microscopy was exercised to scrutinize the surface morphology and shape of the unprocessed and engineered CLR LPHNs There was not observed any aggregates of the particles. This shows that the process and experimental parameters were adequately controlled to engineer the hybrid nanoparticles with homogenous distribution. Powder X-ray diffraction was executed to determine crystallinity of the optimized formulations and compared with the unprocessed CLR and helping polymers and lipids. Some of the XRD peaks for CLR in the LPHNs system appeared with small intensities, which happens due to reduction in the particle size (Khan, Matas *et al*., 2013). The resulted peaks of the helper polymers and lipids in the engineered LPHNs show the homogeneous distribution of the Clarithromycin within the lipid polymer hybrid delivery system and transition to amorphous form as well. *In vitro* release of drugs from LPHNs can be changed by selecting relevant lipid form, surfactant, polymer concentrations and fabrication variables as well (Seedat, Kalhapure *et al*., 2016). For the optimized LPHNs, there was observed strong sustained release of the drug ($p<0.05$) compared to the nanoparticles without helping polymers and lipid. This indicated that helper polymers and lipid with the optimized concentrations can effectively provide strong sustained release rate of the drugs from the polymer hybrid drug delivery systems. Tighter interactions between the drug molecules and polymer shall lead to a stable drug–polymer complex and may result in a more sustained drug release profile compared to the looser interaction/binding (Ahmed, Govender *et al*., 2018; Hameed, Khan *et al*., 2020). Different kinetics models were employed to investigate the drug release mechanism from the hybrid system.This illustrated that the release mechanism of drug from LPHNs has been changed to anomalous transport (non-Fickian diffusion kinetics) from diffusion-controlled. In non-Fickian diffusion kinetics, both erosion/dissolution and diffusion is controlling drugs release from LPHNs.

The produced CLR loaded LPHNs demonstrated strong pharmacokinetics (PK) attributes compared to the raw drug and marketed formulations. The comparative PK evaluation supported the in vitro dissolution studies and the polymer hybrid nanoparticles were found very effective because of the enhanced plasma concentration of the drug and the improved half-life

CONCLUSION

The stable CLR loaded LPHNs were successfully produced using simple stirring and probe sonication method. The key process and experimental conditions including concentrations of polymers and lipids, stirring rate, sonication and stirring time were optimized for stable LPHNs. The impact of helping polymers and lipids were found significant on EE and DLC of the CLR. The sustained release rate of the CLR from LPHNs were strongly increased by addition of the helping polymer; Ethyl cellulose and helper lipid; oleic acid into the developed formulations. *In vivo* pharmacokinetic studies of the engineered LPHNs resulted into significant increase in the drug plasma concentration and elimination half-life compared to the raw and marketed formulations.

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