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Formulation and Characterization of Nano Lipid Carrier Dry Powder Inhaler Containing Ciprofloxacin Hydrochloride and N-Acetyl Cysteine

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Abstract

Nanolipid carriers (NLC) are developed as an alternative to solid lipid nanocarriers in order to increase the payload and to prevent drug expulsion. In this study, NLCs loaded with ciprofloxacin hydrochloride (CIP) and N-Acetyl cysteine (NAC) were prepared and evaluated for its delivery to the lung for treatment of the symptoms of cystic fibrosis and chronic obstructive pulmonary disorder.NLCs prepared by emulsification and sonication technique using cetyl palmitate (the solid lipid, 2%) and oleic acid (as the liquid lipid, 2%) and Tween80 (surfactant, 0.25%) showed smaller particle sizes (of199.1 ±1.859 nm) and relatively high encapsulation efficiencies (72.143±1.8 %.) and optimum zeta potential (-38.27 ± 0.384 mV).A novel DPI formulations loaded with the NLC containing CIP(CIP-DPI), NAC (NAC-DPI) and CIP/NAC combination (CIP-NAC-DPI) wereprepared by freeze drying method using Lactose (8%w/v) as a cryoprotectant. The DPI prepared showed good flow properties, prolonged drug release and improved stability.*In-vitro* drug release profile of CIP HCl in case of CIP-NLC showed 55 % release in 15 hours while it was 60% in case of CIP-NLC-DPI formulation. Similar is the case with NAC formulations. Following intratracheal administration in rat model, the percentage of CIP extracted from lungs was 70.2% in case on CIP-NAC-DPI against 49.8% for CIP-DPI and 42.1% for plain CIP. This amount is about 1.6 times increase in CIP in lungs by co-administration with NAC. However, no appreciable change in the residence time of CIP in lungs after intratracheal administration of CIP-DPI and combined DPI (CIP-NAC-DPI) was noted.

Keywords: Ciprofloxacin Hydrochloride, N-Acetyl Cysteine, Nano lipid Carriers, Dry powder inhaler, cystic fibrosis

Introduction

Cystic fibrosis is an inherited chronic lung disease in which patients have mucus-clogged lungs that leave them vulnerable to repeated, ever more serious respiratory infections.Bacterial infections lead to biofilm formation and host inflammatory responses and the ultimate resistance to antibacterial therapies results in increased morbidity and mortality [1, 2]. This condition is difficult to treat by oral administration due to bio-availability problems to lungs and aerosol formulation containing antibiotic alone would be ineffective due to thick mucus which acts as barrier for penetration. These conditions warrants for the use of antibiotic combination with mucolytics by pulmonary delivery.

Drug delivery to the lungs using dry powder inhalers (DPI) represents a potential delivery route for the treatment of several pulmonary disorders[3].This non-injective route has many advantages over other sites of administration because it has a rapid onset of action, fewer systemic side effects, high bioavailability, reducing frequent dosing, avoidance of the first-pass metabolism, local action for pulmonary diseases, and convenience to patients when administered[4].However, for successful development of pulmonary drug delivery systems is a challenge in terms of inhalability, stability and size control [5]. Physicochemical characteristics of the formulation could influence aerodynamic size of the particles and ultimately affect the intrinsic tendency of aerosol particles to deposit in lungs, due to their shape, size and density.In several studies, size of the particles for deposition in the lungs was found to be in the 1–5 μm range.[6]. For an ideal pulmonary drug delivery system, drug carriers with average size in nanometer range such as liposome [7] nanoparticles [8], exhibit some well-defined and delicate characteristics, which are suited for pulmonary delivery of drugs with increased bioavailability, control release properties.

Nano lipid carriers (NLCs) are new generation nano particulate carrier system, developed in order to overcome the limitations of first generation lipid nanoparticles i.e. solid lipid nanoparticles (SLN). In NLC the oil component of the o/w emulsion is replaced by a blend of a solid lipid and an oil leading to a solid particle matrix at body temperature[9]. The NLCs agglomerates produced were found to have excellent inhalation properties and improved physicochemical properties.Bioadhesive properties of NLC due to their small particle size as well as their lipophilic character lead to longer residence time in the lung.

Ciprofloxacin hydrochloride (CIP HCl), a member of the fluorinated quinolone family, has a wide coverage against both gram-positive and gram-negative organisms and has shown good potential as an inhaled medicine.[10]It is efficient for the treatment of clinical complications of cystic fibrosis. It has also been shown to demonstrate good *in vitro* bactericidal activities against a number of pathogens that cause respiratory infections.*[11].* Oral and intravenous forms of ciprofloxacin though are used clinically to treat respiratory tract infections,they exhibit relatively unfavorable pharmacokinetics profile in the lower respiratory tract, including a relatively short elimination half-life (1.0 to 1.6 h) and a low area under the concentration-time curve of 43 to 113 mg h/liter[12]. Indeed, as a liposomal formulation, ciprofloxacin is currently in phase 2 safety and efficacy studies[13].

In the mucolytic arena, dornase alfa[14], denufosol tetrasodium [15], lancovutide[16], hypertonic saline[17], heparin [18] and N-acetyl-cysteine[19, 20] have all found therapeutic efficacy. Inhaled N-acetyl-cysteine (NAC) is believed to cause an increase in the osmotic pressure of the fluid lining the mucosal surfaces. The hyper tonicity produced induces transcellular water migration, a reduction in mucous viscosity and increased clearance via the cilia transport mechanism. This increase in mucus removal is beneficial for patients suffering from cystic fibrosis and chronic bronchitis.[21]It alsobreaks disulfide bonds in mucus and liquefies that makes it useful in thinning the abnormally thick mucus in cystic and pulmonary fibrosis patients. NAC is known to enhance CF sputum penetration (Suk JS et al, 2011) and was reported to transfer promising synthetic nanoparticle genes effectively to lungs of CF patients [22]. In addition, NAC inhibited growth of both gram-negative and gram-positive bacteria. [23].Some studies have also shown that mucolytic agents may inhibit antibiotic activity when used in combination[24]. However, Heaf et al. (1983),[25] described how mesna (Mistabron®), a mucolytic agent, despite inhibiting pseudomonal growth, did not reduce the bactericidal activity of azlocillin (acylampicillin antibiotic). Furthermore, in a study by Roberts and Cole (1981), 1% NAC was shown to potentiate the anti-pseudomonal activity of carbenicillin in vitro.NAC was also reported to increase the therapeutic efficacy of CIP when used in combination for the treatment and eradication of biofilms formed on ureteral stent surfaces[26] and may be a useful therapeutic option in partial biliary obstruction.[27].

The administration of antibiotic and mucolytic in dry powder aerosol form is one of the proposed strategies that can be adopted to reduce doses and, consequently, to reduce the side effects.[28] Because inhaled powders work locally in the lung as a common site of action, lower doses are needed to achieve the same therapeutic effect as the oral doses.[29].There has been huge success in processing dry powder inhalers using a range of drug substances, including nifedipine, tacrolimus, and budesonide.[30] To the best of our knowledge, this is the only study demonstrating the use of CIP-NLC and NAC-NLC as DPI. Therefore, the aim of the present investigation is to prepare and evaluate stable DPI loaded with CIP-NLC and NAC-NLC for high pulmonary deposition, thereby enhanced accumulation in deeper parts of bronchi.

Material and Method

Material

Ciprofloxacin hydrochloride (CIP HCl) was obtained from Innova Captab, Baddi and N-Acetyl cysteine(NAC) by Crescent Therapeutic limited, Baddi (Himachal Pradesh , India) Cetyl palmitate and Oleic acid, Lactose, orthophosphoric acid, Trehalose, sucrose, mannitol, boric acid, Disodium hydrogen phosphate, potassium dihydrogen phosphate by Central Drug House, New Delhi, Tween 80 act as emulsifier was provided by Hi-Media Lab Pvt Ltd, Mumbai (India), 4- Chloro- 7 nitro-2, 1, 3-benzoxadiazole from Sigma Aldrich, (U.S.A.), dialysis tubing, Sephadex-G-50 from Sigma Chemical co.(U.S.A.), Ethanol, Methanol, Hydrochloric acid by Renkem Chemicals, New Delhi (India)

Methods

Nano lipid carrier loaded with CIP HCl and NAC were prepared by emulsification and ultrasonication method reported previously[31]. Briefly, Drug was grinded along with surfactant and was added with the molten mixture of solid and liquid lipid at 40º C. Surfactant solution in water heated to 80º C was added to the drug-lipid mixture and homogenized for 6 minutes at 400 rpm, maintaining the temperature at 80º C to obtain fine emulsion. The emulsion was sonicated for 3 minutes at 60 amplitude using probe sonicator. The resultant mixture was cooled up to 2 to 3º C in refrigerator to form NLCs.

The NLC dispersion obtained was added with lactose (8% w/v)as a cryoprotectant, mixed and was filled in 30 ml vials partially capped and lyophilized. Lyophlization was performedby freezing the mixture at -40ºC followed by lyophlization for 24 hours in a laboratory freeze-drier (VIRTIS) keeping vacuum at 50 to 60mTorr and condenser temperature -60ºC. The lyophilized product obtained in the form of dry mass was capped full in the chamber and preserved in freeze until its further use.

Optimization of process parameter

The properties of NLCs like particle size, shape, density, drug entrapment efficiency depends on formulation and operational

parameters like concentration of emulsifier, concentration of solid lipid, solid lipid: liquid lipid, concentration of drug, homogenization time, sonication time etc. Individual batches were designed, according to single factor variation, in order to screen optimal formulation.Particle size and Polydispersity index (PDI) were considered as response parameters for optimization.

Characterization of NLCs

Measurement of size and zeta potentials of NLC

The mean particle size diameter, PDI and zeta potential were measured in solution directly after synthesis using Beckman coulter Delsa Nano C Particle analyzer model DLS 4 C (Table 1&2). CIP-NLC and NAC-NLC (2 ml) were added to the quartz cell of the photon correlation spectroscope. Measurements were taken at 90° opposite the incident light source and mean droplet size was calculated from intensity.

Determination of Percentage Entrapment Efficiency

Entrapment efficiency was determined by minicolumn centrifugation method using sephadex® G 50 solution (10% w/v). NLCs were slowly charged on prepared column and centrifuged at 400 rpm for 3 mins and then the same procedure was repeated by adding 100 µl of water. The free drug gets bound to the gel when NLC passed through the gel and were collected from first and second stage of centrifugation. The eluted NLC were heated at 80◦C to rupture the NLC and centrifugation was done for 3 mins at 3000 rpm and liberated drug was estimated spectrophotometrically.

Entrapment efficiency (%) = [Entrapped drug/Total drug]*100

Solid state characterization of DPI

Solid state characterization of DPI formulations like Angle of repose, Both Bulk density (BD) and tapped density (TD) determined by standard Pharmacopoeia procedures and were tabulatedin Table 3.The Compressibility Index of the powder blend was determined by Carr's compressibility index. The formula for Carr's Index is as shown below;

Carr's Index (%) = [(TD-BD) x 100] / TD

Particle morphology

Particle morphology of the DPI was performed by Scanning Electron Microscopy (SEM) for determining the surface morphology, size and shape of formulation and to observe the aggregation property of NLC with carrier particles.

In Vitro Drug Release

*In vitro*release was evaluated by using a dialysis bag diffusion technique under sink condition. The drug release from CIP-NLCs and NAC-NLCs was performed in Phosphate buffer saline (PBS) (pH 7.4) using dialysis diffusion bag. Both thesuspensions were placed individually in dialysis bags (MWCO 12000 Da, Sigma Aldrich) and the dialysis bags were subsequently placed in flasks containing 200 ml dissolution medium (PBS pH 7.4) and stirred at 150 rpm in a 37◦C in the incubator shaker. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to the flask to maintain a constant volume. Drug concentration in the dissolution medium was determined using UV/Vis Spectrophotometry (Shimadzu 1700) at 278 nm for CIP HCl and at 410 nm for NAC. All experiments were carried out in triplicates. The release profile were fitted to kinetic equations (zero order, first order, Higuchi release and Korsmeyer-peppas release, Hixson Crowell) to understand the release mechanisms.

In vivo studies

The developed lyophilized powders were studied i*n vivo*for lung distribution on adult albino rats (wistar origin) of either sex weighing 150 ± 20 g. the animals were housed in animal house of I.S.F. College of pharmacy with free access to pelletized chow and water. The temperature was maintained at approximately 26℃ to 28℃. Animal experiments were approved by CPCSEA. *In vivo* studies of the selected formulations were performed after intra-tracheal administration with the help of a suitable delivery device (cannula).

Intratracheal Administration

Intratracheal instillation was performed as reported byShek*et al* (1990) and Joshi and Misra (2003)[32, 33]. Briefly, three wistar rats (180-220 g) were used in each group for each time interval. Rats were housed in individual plastic cages at a constant temperature. Animals were fasted overnight prior to each experiment. Rats were selected randomly and anaesthetized by intraperitoneal administration of ketamine (80 mg/kg). The trachea was exposed by blunt dissection of the sternohyoideus muscle and a small midline incision was made over the trachea between the fifth and sixth tracheal rings using a 20 gauge needle. The trachea was cannulated with PE200 tubing (5-7 cm) with the tip positioned approximately at the tracheal bifurcation. The PE50 (10-15 cm) tubing connected to a glass Hamilton syringe (Waters, Bangalore, India) was inserted into the cannula and advanced to the bifurcation of the trachea. Solutions containing 100 µg plain CIP HCl[34] and NAC[35] or NLC encapsulated CIP HCl and NAC prepared by rehydration (30 min) of powder with 250 µL triple-distilled water were instilled slowly over a 1 min period, followed by 50 µL normal saline. Animals that were to be killed at 1, 2, 4 and 8h after administration had the cannula secured with sutures and the access cannula excised to leave a 1 cm protrusion. Animals were allowed to recover and, after recovery, animals were housed in individual plastic cages with free access only to water.

Organ homogenate studies

Animals exposed to various formulations were sacrificed after 1, 2, 4, and 8 h and the lungtissue was removed, washed, dried using a tissue paper, weighed separately, and homogenized in phosphate buffer. The homogenized tissues (20%) were deproteinized with 100µl of acetonitrile and kept in dark for 30 min

and filtered. To the filtrate, 100µl of extraction fluid methanol was added with vigorous shaking and then centrifuged at 5000 rpm for 30 minutes. The supernatant was separated and drug content was measured using UV- VIS Spectrophotometer. The amount of drug was calculated as percent drug recovery at different time intervals.

Result and Discussion

Optimization of process parameters

Results of the optimization experiments with respect to particle size, PDI, Zeta potential and entrapment efficiency of CIP-NLCs by varying parameters like homogenization time, sonication time, and amount of solid lipid, liquid lipid and drug are tabulated inTable 1.Mean particle size as well as (PDI) werefound to be reduced with increasing homogenization (400 rpm) time up to 6 mins,after which, particle size increased due to increase in kinetic energy of particle which lead to agglomeration. Similarly, the size of the NLCs decreased as the time of sonication increased from 1 to 3min (Amplitude 60) and beyond this sonication failed to reduce the size. The phenomenon of excess energy input leading to an increase in droplet size of Nano Lipid Carrier by inducing re-aggregation. The effects of Tween 80 concentration (0.1 % to 0.25% w/v) was studied keeping lipid concentration constant(1% w/v). With increase in Tween-80 concentration up to 0.25 % (w/v), mean particle size dramatically reduced butno appreciable decrease was found with further increase in Tween-80 concentration. On increasing the amount of solid lipid, size was found to be decreased. However, the mean particle size of lipid nanoparticles usually depends on various factors,e.g. type and concentration of lipids and surfactants, and on the viscosity of lipid phase.

Concentration of liquid lipid showed much influence on size andentrapment efficiency (EE)of NLCs. Liquid lipid due to less crystalline structure exhibit imperfection of lipid matrix and so offersmore space for incorporation of drug. On increasing the liquid lipid concentration, entrapment efficiency was first increased followed by marginal decrease. Increase in sizes was also shown by increasing the amount of drug payload andthe highest drug amount(20mg) showed acceptable size. Further increase in drug amountresulted in poor drug incorporation and larger particle size. The above optimized experimental parameters were also applied for the preparation of NAC NLC and the response parameter values of the batch prepared are given in Table 2.

Particle morphology, size, zeta potential

The size of the CIP loaded NLCs was found to be 199±5 nm and PDI was 0.196±0.94. In order to confirm the size of NLC and to observe surface morphological characteristics, TEM and SEM were carried out respectively. TEM (Figure 1) and SEM (Figure 2) photographs showed uniform size; mono-dispersed round shaped NLCs. Slight rough surface could be observed in majority of NLCs in SEM photographs. Zeta potential of NLVs estimated by electrophoretic mobility technique using zetameter was found to be -38.27mV indicating the stability of nanoparticle during storage.

Entrapment efficiency

Nanolipid carriers are capable to provide more room for accommodation of drug. Entrapment efficiency of CIP-NLCs was found to be 72.143% and that of NAC-NLCs was 68.14%. Entrapment was found to be increased with the addition of liquid lipid. In addition to the influence of liquid lipid, presence of surfactant (Tween 80) in the formula could have improved the loading capacity. The presence of limited amount free drug (Not more than 20%) however, has advantage that it will contribute for initial raise in Cmax followed by steady state drug concentration due to sustained release of entrapped drug.

Solid state Characteristics

Solid state characteristics like angle of repose, bulk and tapped density, compressibility are essential parameters that determine the delivery efficiency of DPI formulations. These characteristics determined for DPI formulations are given in Table 3. Angle of repose, an index of flow characteristics of the DPIs prepared were well below 30ºensuring good flow. Bulk and tapped density values were very low in comparison to plain drug crystals showing the possibilities of good aerodynamic flow behavior. True density of CIP powder is 2.1g/cm^3 and that of NAC powder is 1.48g/cm^3.

In vitro drug release

In-vitro drug release profile of CIP HCl from various CIP-NLCs is given in Figure 3 along with their comparison with respective DPI formulation (CIP-NLC-DPI). Percentage of CIP HCl release from the NLCs after 8h and 15 h was 30% and about 55% respectively.The initial drug release (8h) is because the of burst effect that do not correspond to the real mechanism of the drug release from NLCs. It is due to immediate dissolution of drug particles adsorbedon the surface of NLCs. About 60% of drug was released from NLCs within the first 24 h and the 70% drug was released upto 48 hrs. Drug release followed Higuchi kinetics(Table 4) indicating the release predominantly by diffusion. Increased diffusional distance due to the formation of drug depletion zone at particle surface and presence on the surrounding lipid shell barrier might have slowed down the release rate resulting sustained drug release profile.

The release profile of NACfrom NAC-NLCs and NAC-NLC-DPI is depicted in Figure 3. It showed a biphasic release profile with 25 % release in first 4 hrs followed by sustained release ofabout 55% and 65% in 24 h and 48 h. Drug release followed Higuchi kinetics.

In vivo studies

Concentration of drug and percentage drug recovery in lung tissue

Lung tissue distribution and percentage recovery in lungs of CIP HCl and NAC following intra tracheal administration of their free

Table1: Optimization of homogenization time, sonication time, amount of solid lipid, liquid lipid and drug

Step	Batch Code	H.t.(S.t)*	Surfactant	Solid lipid	Liquid lipid	Drug	Optimized Parameter (level)	Dependent variable(optimized value)			
								Size (nm)	PDI	ZP	E.E.
Step1	1-4	2-8	0.1	1	-	-	h.t (6mins) **Batch 3**	522-983 (522±18)	0.217-0.241 (0.217±0.05)	-	-
Step2	5-8	6 (1-4)	0.1	1	-	-	s.t (3mins) **Batch 7**	364-510 (364±17)	0.24-0.31 (0.24±0.43)	-	-
Step3	9-12	6(3)	0.1-0.25	1	-	-	Surfac-tant (0.25%) **Batch 12**	276-364 (267±39)	0.22-0.49 (0.220±0.53)	-	-
Step4	13-16	6(3)	0.25	10-40	-	-	SL (40mg) **Batch 16**	220-260 (220±12)	0.15-0.19 (0.15±0.01)	-	50-60 (55.24± 49.2)
Step5	17-20	6(3)	0.25	25-40	5-15	-	LL (5mg) **Batch 17**	200-210	0.110-0.213 (0.199±5)	-	60-70(70.14± 8.3
Step6	20-24	6(3)	0.25	35	5	10-40	Drug (20mg) **Batch 21**	199-289 (199±5)	0.19-0.29 (0.19±0.94)	38.27	65-72(72.143± 2.08)
Optimized		**6min(3 min)**	**0.25%**	**35 mg**	**5mg**	**20mg**	**Batch 21**	**199±5**	**0.196± 0.94**	**38.27**	**72.143 ± 2.08**

*Homogenization time (Sonication time)

Table 2: Optimized formulation of NAC NLC

Size(nm)	PDI	Zeta potential	Entrapment efficiency (%)
22.4nm±2.4	0.163±1.3	6.14±1.2	68.143±13

Table 3:Solid State properties of DPI

Sample	Angle of repose(◦)	Bulk density (gm/ml)	Tapped density(gm/ml)	Compressibility index(%)
CIP-NLC- DPI	21.4	0.312	0.386	23.718
NAC-NLC-DPI	24	0.395	0.448	17.83

Table 4: Kinetics modeling of release profile

	Linear correlation coefficient (R^2)			
	CIP HCl NLC	CIP HCl NLC DPI	NAC NLC	NAC NLC DPI
Zero order	0.881	0.8712	0.8943	0.8646
First order	0.881	0.8712	0.8943	0.8646
Higuchi model	**0.9703**	**0.9691**	**0.9905**	**0.9736**
Peppas-Korsemeyer model	0.8885	0.906	0.9351	0.9741
Hixson-Crowell	0.9204	0.9192	0.9425	0.9201

Table 5: Conc. obtained and % recovered of CIP HCl with respect to time in lung tissue when given through intratracheal administration from free and DPI form respectively.

Time	CIP HCl		CIP-NLC-DPI		CIP-NLC-DPI and NAC-NLC-DPI	
	Concentration(µg/ml)	% recovered	Concentration(µg/ml)	% recovered	Concentration (µg/ml)	% recovered
1	21.05±2.98	42.1±3.0	24.5±1.48	49.8±2.5	35.1±1.98	70.2±1.0
2	16.2±2.94	32.4±2.5	22.4±3.34	44.8±2.0	31.49±2.43	62.9±3.4
4	9.49±2.93	18.98±1.6	19.03±3.39	38.06±1.0	25.46±1.11	50.9±2.8
8	1.15±2.02	2.3±1.0	14.7±3.83	29.4±0.8	17.2±1.96	34.4±1.9

Table 6: Conc. obtained and % recovered of NAC with respect to time in lung tissue when given through intratracheal administration from free and DPI form respectively

Time	NAC		NAC-NLC-DPI		NAC-NLC-DPI and CIP-NLC-DPI	
	Concentration(µg/ml)	% recovered	Concentration(µg/ml)	% recovered	Concentration(µg/ml)	% recovered
1	33.6±2.09	47.14±3.0	46.8±1.48±2.98	65.6±2.5±	49.98±1.98	70±2.34
2	26.13±3.91	36.36±2.5	39.12±3.34±4.57	54.8±2.0±	42.84±2.03	60±2.97
4	13.5±3.98	18.98±1.6	33.7±3.39±2.43	47.2±1.0±	39.2±1.45	54.98±3.05
8	1.4±1.46	2.03±1.0	24.21±3.83±1.04	33.9±0.8±	25.8±1.94	36.2±1.84

Table 7: Residence time of CIP HCl and NAC in lungs

	CIP			NAC		
Residence time (hours)	CIP HCl Plain	CIP-NLC DPI	CIP-NAC-DPI	NAC-Plain	NAC-NLC-DPI	CIP-NAC-DPI
	1.6	9.3	6.8	1.5	7.7	7.9

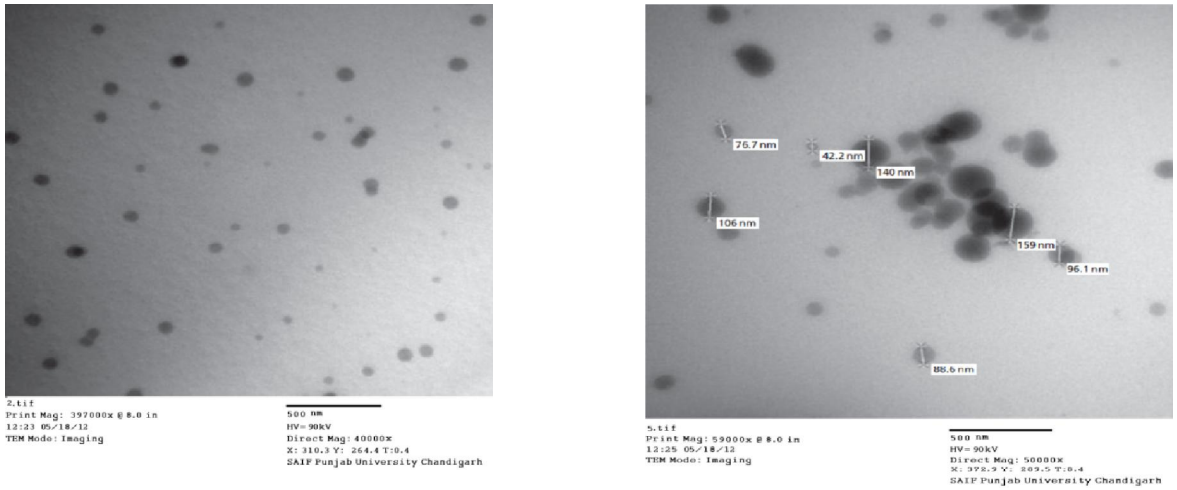

Fig1 TEM images of CIP-NLC and NAC-NLC

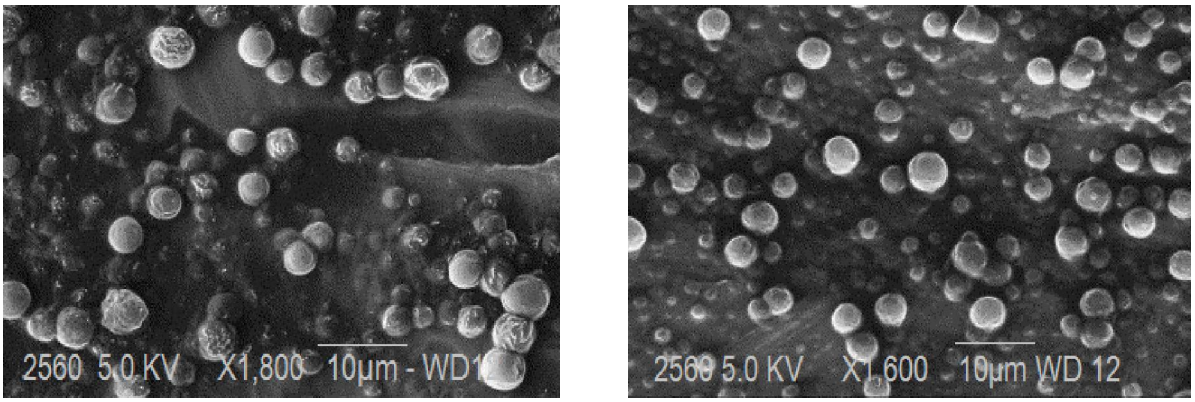

Fig 2: SEM images of CIP-DPI and NAC-DPI

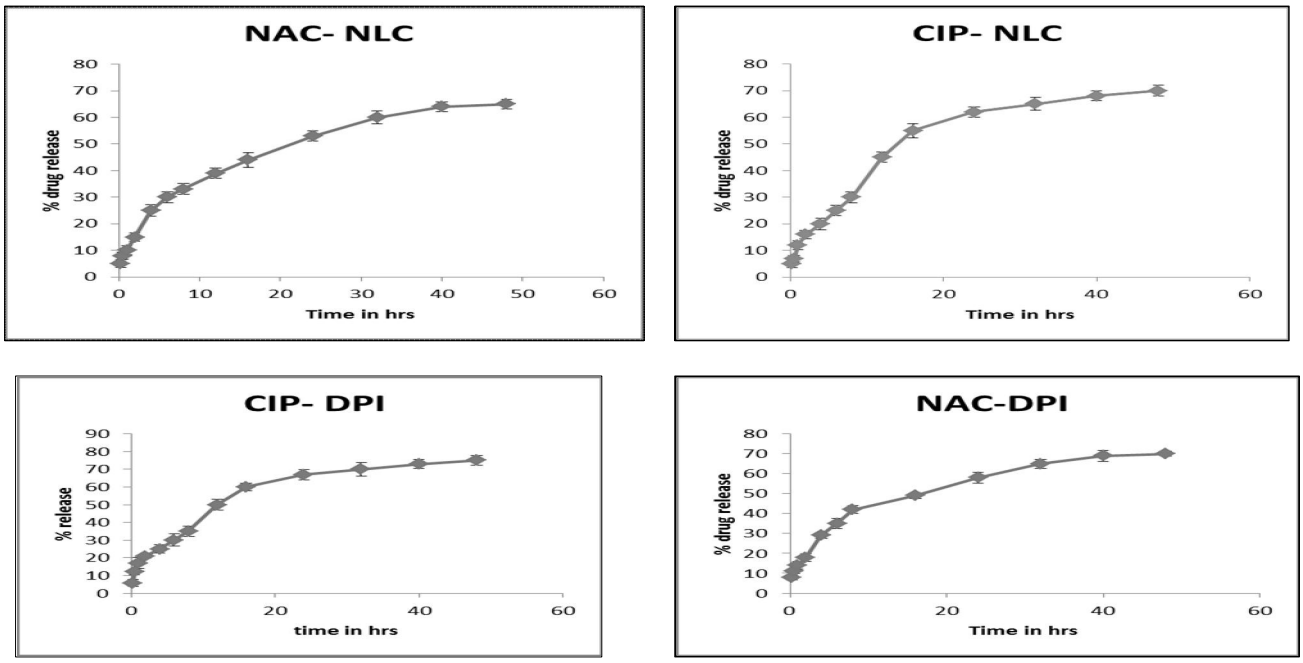

Fig: 3*In vitro* drug release profilefrom a) CIP HCl NLC b) NAC NLC c) CIP HCl DPI d) NAC NLC DPI

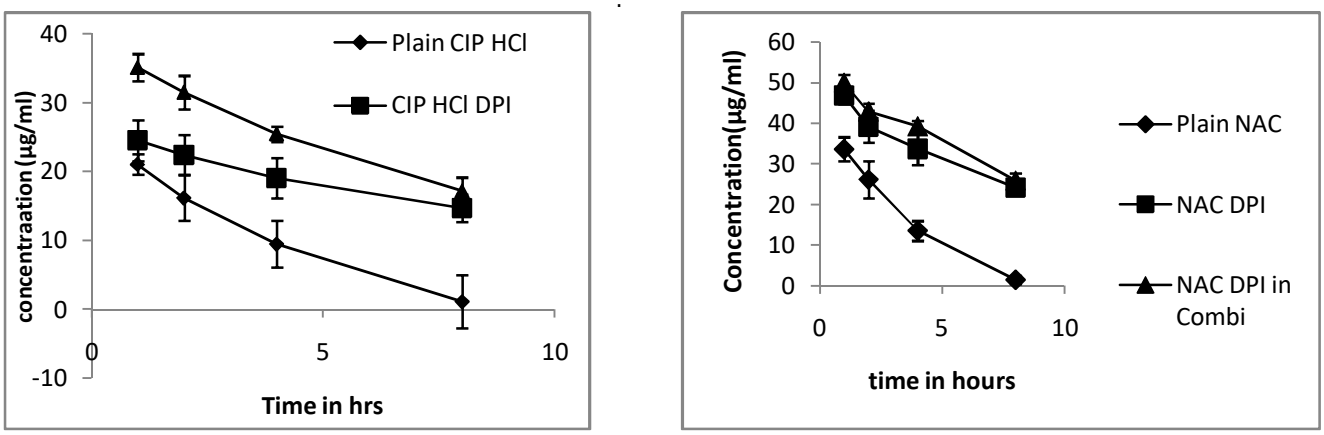

Figure 4: Concentration of CIP HCl and NAC obtained with time from free and DPI form in lung tissue following intratracheal administration in rat model.

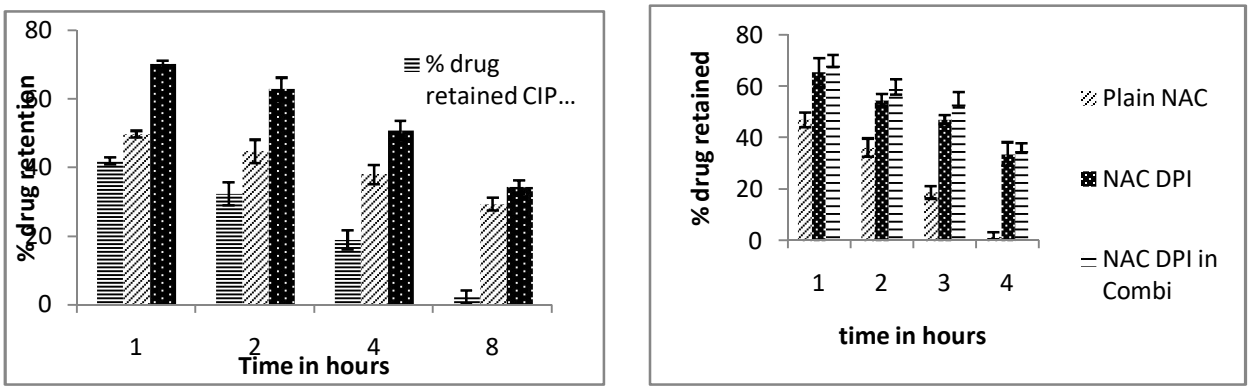

Figure 5: Percentage CIP HCl and NAC recovered with time from free and DPI form in lung tissue following intratracheal administration in rat

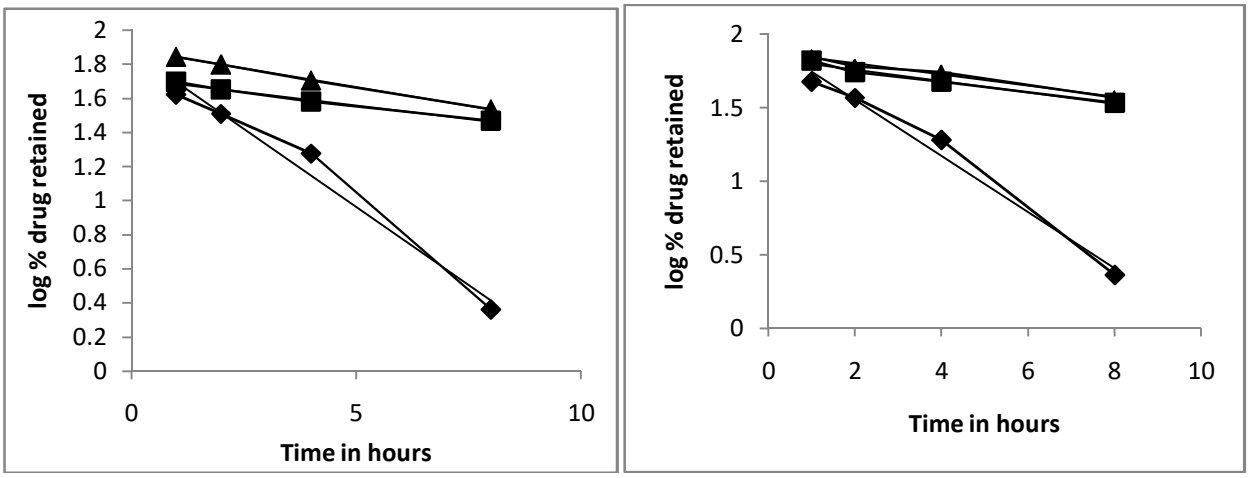

Figure 6: Percentage of CIP (Left) and NAC (Right) recoveredin lungs with time following intratracheal administration of Plain (♦), Single drug DPI ( ) and DPI containing NLCs of both the drugs ( ).

and DPI form are given in Table 5 & 6 respectively. Drug retention and half-lifein lung tissue were calculated with the help of software kinetic.Lung uptake of the drug from the DPI was higher as compared to plain drug suspension. The % CIP HCl recovered at 1 h and 8h after intratracheal administration of CIP-DPI was found to be 49.8 % and 29.4 % respectively. The values are 1.2 times at 1h and 12.8 times at 8 h when compared to free NLC.

Residence time of CIP HCl and NAC in lung tissue was more in CIP-DPI and NAC-DPI than in the free form as shown in table7. Because in case of lyophilized NLC, clearance of the drug from the organ was less due to slow release of the drug from the NLC. CIP HCl and NAC longevity in lung tissue was a direct consequence of the retention of the Lipid nanoparticles at the site and the retention of the drugs in lipid nanoparticles.

The potential for pulmonary delivery of CIP HCl and NAC may have clinical significance in the light of recent data demonstrating that CIP-NLC-DPI and NAC-NLC-DPI were highly effective, following intratracheal administration, in rat lungs.

When the CIP HCl was given in combination with NAC DPI the percentage dose recovered in the lungs was found to be higher. The observation suggests that the mucolytic removes the physicochemical barrier i.e. mucus. Hence the concentration of antibiotic was higher in the lungs in case of combinational therapy. In case CIP HCl in combination with NAC DPI, the % dose recovered at 1h and 8 h after intratracheal administration was found to be 70.2% and 34.4% respectively. The values are 1.6 and 17 times at 8h when compared to free CIP HCl plain.

Conclusion

In the present investigation, an attempt was made to enhance the bioavailability of CIP HCl in lungs by administering as its NLC and as the NLC loaded DPI in combination with NAC for efficient treatment of symptoms of Cystic fibrosis. The NLCs were prepared by emulsification and ultrasonication technique using Cetyl palmitate as solid lipid, oleic acid as liquid lipid and Tween80 as surfactant. The in vitro release studies confirmed that in case of both the CIP-NLCs and NAC-NLCs the drug was released in a sustained manner. These types of release profiles of CIP-NLCs and NAC-NLCs resemble the drug enriched core model. In such a model, the drug enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusional path and hindering effects by the surrounding solid lipid shell, the drug has a sustained release profile. The DPI formulation wasstable at refrigerated condition.Based on above results it was concluded that, nano lipid carriers are suitable carriers for incorporating CIP HCl and NAC. The effect of NLCs encapsulation on CIP HCl and NAC levels in lungs following administration was determined in rat model. NLC in DPI preparations offer several potential advantages such as higher patient compliance, and reduction in systemic side effects.

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