Ciprofloxacin surf-plexes as emulsion to improve antimicrobial efficacy

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Introduction

To deliver a therapeutic molecule through colloidal carrier in order to achieve optimum therapeutic efficacy remains a challenge for drug delivery scientists. Ciprofloxacin (CPF), a powerful broad-spectrum antibiotic, is useful in the treatment of several types of infections. However, development of delivery vehicle for CPF is difficult because of poor aqueous solubility and low retention at physiological milieu (pH=7.4) [Blondeau 2004]. This study emphasized on an alternative administration vehicle involving hydrophobic ion-pair complexes of CPF with sodium lauryl sulphate (SLS) and sodium deoxycholate (SDC), which were incorporated in the core of submicron-sized oil-in-water (o/w) emulsion. The proposed study demonstrates the suitability of these surf-plexes for improving payload efficiency and potentiality of developed system for prolonged retention with improved antimicrobial efficacy by imparting cationic charge on the surface, which can be used as potential drug delivery vehicle for topical and parenteral applications.

Material and methods

CPF was generously provided by Alkem Laboratories (Mumbai, India) as a gift sample. Soya-oil, lecithin, poloxamer 188 (Pluronic F68), chitosan, sodium lauryl sulphate (SLS) sodium deoxycholate (SDC), protamine sulfate (PRM), stearylamine (SA) and 3-(4, 5-Dimethythiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (MO, USA). For cell culture, Dulbecco's modified eagle medium (DMEM) with Glutamate, fetal bovine serum (FBS) and antibiotic solution (penicillin/streptomycin, 0.1% v/v) and Trypan blue solution were purchased from Sigma (St. Louis, USA). Well plates, for cytotoxicity studies were from greiner bio-one (Germany). All materials were used without further purification.

Preparation, optimization and characterization of ionic complexes of CPF

The ionic complexes of CPF with SLS and SDC were prepared by dissolving appropriate quantities (equimolar concentration) of surfactant in de-ionised water and gradually adding it CPF solution. The formation of ionic complex was optimized by preparing at different pH and taking different molar ratio of both the components. The developed ionic complex was characterized by FTIR, Mass spectra, H^1 NMR and for partition coefficient.

Preparation of lipid based submicron emulsion

Different submicron lipid emulsions containing CPF (SE), ionic complex of CPF with SLS (SE-SLS) and SDC (SE-SDC) were prepared by following standard procedure with minor modifications [Zurowaska et al. 1999]. The lipid phase was prepared by heating soya oil at 70°C containing lecithin. The CPF-ionic complexes (equivalent to 0.3% CPF) were added to the lipid mixture as mentioned above after dissolving in chloroform. This lipid phase was added gradually to aqueous phase containing pluronic F-68 at 70°C and magnetically stirred. The primary emulsion was prepared by stirring for 20 min using high shear mixer (Ultra Turrax, Janke & Kunkel, Staufen, Germany) at the speed of 22000 rpm, and subsequent emulsification was accomplished by sonication using ultrasonic probe at 20% amplitude for 5 min. The cationicity to the surface of

submicron lipid emulsion (SE-SDC) was imparted using stearylamine (SE-SA-SDC), protamine (SE-P-SDC) and chitosan (SE-CH-SDC).

Physicochemical characterization

The globule size and size distribution as well as zeta potential were measured by Zetasizer nanoZS (Malvern, UK). Transmission electron microscopy (TEM) was performed using negative staining with sodium phosphotungstate solution (0.2% w/v). Suspension were dispersed in the staining solution for 30 min at RT, placed on a copper grid covered with nitrocellulose, dried under vacuum for at-least 24 hr and observed under TEM (JEM-1200 EXII, instrument (JEOL, Tokyo, Japan). For entrapment efficiency emulsions with drugs were centrifuged at 48,000 ×*g* and 4°C for 30 min in a Beckman Optima MAX® ultracentrifuge (Beckman Coulter, Fullerton, CA, USA) in order to separate the incorporated drug from free drug. The supernatants were analyzed by RP-HPLC for the free drug (A1) concentration to determine the encapsulation percentage from total amount of drug taken (A2). Entrapment efficiency was calculated using the equation. [E.E (%) = (A2-A1 /A2) x 100].

In-vitro drug release study

In-vitro release studies were carried out using bulk equilibrium reverse dialysis bag (cutoff Mol. wt. 12000 Dalton, Sigma, USA) technique at 37°C, as previously described. At given time intervals, a dialysis bags was withdrawn from the stirred release solution and the content of the dialysis bag was assayed for CPF content by RP-HPLC as described previously.

In-vitro antimicrobial efficacy

The antimicrobial activity of selected formulations was determined in comparison to free drug by determining the MIC values against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* strains. MIC tests were performed by broth micro dilution method according to the NCCLS standards. Serial two-fold dilutions from 20.00 to 0.02 µg/ml of CPF, SE, SE-SLS, SE-SDC, SE-SA-SDC, SE-CH-SDC and SE-P-SDC was carried out. For the experiment, bacterial strains were inoculated onto agar plates, incubated for 18 hr at 35°C, and then diluted in PBS to make its optical density equal to McFarland No. 0.5. Ten µl of bacterial culture was diluted 1:10 (approximate concentration: 10^7 cells/ml) and added to the microtitre wells containing the drug solution and incubated at 35°C. After 2 hr, 100 Mueller Hinton II Broth (MHB) (USA) were added to each well. The final concentration of microorganisms was 5×10^5 cfu/ml. The plates were then incubated for 18h at 35°C. MIC was defined as the lowest concentration of ciprofloxacin or its ionic complex loaded formulation at which no visible growth of bacteria was observed after 18h. Positive controls (growth) consisted of bacteria in broth and bacteria with empty formulation in broth. Negative controls (sterility) consisted of un-inoculated broth and each of the drug/formulation dilutions in broth.

Results and Discussion

Ciprofloxacin is commercially available as CPF-HCl salt form and it shows pH governing solubility profile. It is an amphoteric quinolone antibiotic having an acidic pKa at 6.0 and a basic pKa at 8.8 [Sun et al. 2002] and its solubility or retention in oil or lipidic phase is very low. However it is moderately soluble in water at neutral pH. At acidic pH it exists in protonated form, which can form ion pair complex with negatively charged counter ions. An improvement is needed in payload/encapsulation efficiency of CPF and ultimately to shoot-up antimicrobial efficacy is the

demand of present scenario**.** It has been postulated that formation of ion pairs complex with CPF (CPF-surfplexes) would increase the lipophilicity of CPF and submicron emulsion would constitute as an optimal carrier for encapsulation of the same. SDC and SLS were selected as ion pairing agent because they are negatively charged in a wide range of pH. The amount of CPF recovered in supernatant was less at lower pH (pH 3.5) which gradually increases as pH of the buffer approached to neutral (7.4) or basic pH (9.2) as depicted in Fig. 1. This may be attributed to the fact that as the pH of the medium decreases, the proportion of protonated CPF increases, and thus available for complexation with negatively charged surfactant and as pH increased, the zwitter-ionic and anionic form of CPF becomes more dominant which do not favor complex formation. . Therefore; further complexation was carried out at acidic pH. The CPF solubility decreases up to molar ratio of 3 and 6 in case of SLS and SDC and regain beyond that point (Fig. 2). Therefore (1:1 molar ratio) of ciprofloxacin-surfactant were chosen for complex formation. These studies, further confirm that ionic complexation occurs mainly due to electrostatic interaction between protonated amino group of ciprofloxacin and sulfate and carboxyl group of SLS/SDC respectively.

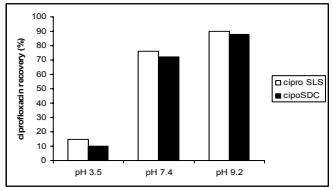

Fig. 1. Effect of pH on complexation.

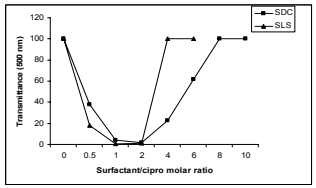

Fig.2. Effect of molar ratio on complexation.

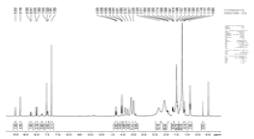

Fig. 3. ^{1}H NMR Spectra of CIPRO-SLS

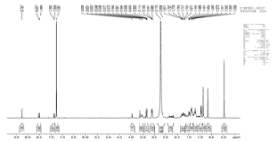

Fig. 4. ^{1}H NMR Spectra of CIPRO-SDC

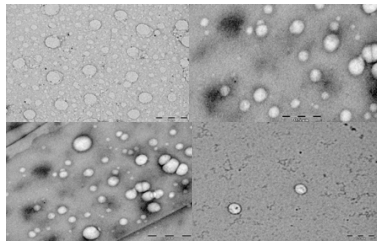

Fig 5. TEM microphotograph of different CPF-Surfplexes

Formulation	Zeta potential (mv)		Average globule size (nm)	Polydispersity	Viscosity (Cp)	pH
	Blank	With drug				
SE-SDC	-35.8±2	-25.3±1	278±12	0.189±0.06	2.6±0.3	7.2±0.4
SE-SLS	-35.1±3	-25.2±2	225±15	0.234±0.05	2.3±0.5	7.1±0.5
SE-CH-SDC	+34.8±4	+28.2±2	324±16	0.197±0.08	3.1±0.7	5.8±0.8
SE-PE-SDC	+14.8±0.5	+12.0±1	298±09	0.161±0.04	3.3±0.5	7.3±0.4
SE-SA-SDC	+28.4±4	+23.3±3	256±10	0.201±0.03	2.8±0.4	7.1±0.2

Table 1: Physicochemical characterization of different CPF-Surfplexes formulations.

The formation of ciprofloxacin-SLS (cipro-SLS) and ciprofloxacin-SDC (cipro-SDC) ionic complex were confirmed by ^{1}H NMR spectroscopy (Fig. 3 and 4). The entrapment efficiency of CPF-ionic

complex improves significantly as depicted in Table 1. The loading efficiency was found to be nearly 27±3.38% in emulsions when CPF was used without complexation. Contrary to this, the payload efficiency was dramatically improved to almost four times (≥ 80%) in all formulations when CPF was loaded in the form of ionic complex. The average globule size of all the formulations was well controlled and found to be in the range of 225-325 nm with low polydispersity index as shown in Table 1. TEM microphotographs, further confirm that globules size was found to be in the range of ≤ 500nm (Figure 5)**.** The zeta potential of the formulations was determined in order to assess the contribution of cationic inducer on charge distribution and shown in Table 1. Drug release profile from different CPF surf-plexes shown in Fig. 6 and was obtained in sustained manner As evinced from Fig.7 that SE-SLS, SE-SDC, SE-SA-SDC, SE-CH-SDC, SE-P-SDC released 66±2%, 64±3%, 51±2%, 41±0.5%, 57±2 % of the drug respectively within 24h. The antimicrobial efficacy of CPF & its ionic complexes has been represented in Table 2. The antimicrobial activity of submicron emulsion was found to be negligible while among cationic emulsion, chitosan and protamine based submicron emulsion shows partial activity (data not shown) when tested without CPF. However the antimicrobial efficacy of CPF-ionic complex loaded submicron emulsion was significantly increased (Table 2). It may be due to destructive effect of polycationic substance like chitosan and protamine on outer cell membrane. The MIC value was observed in the order of SE-SA-SDC > SE-CH-SDC > SE-P-SDC.

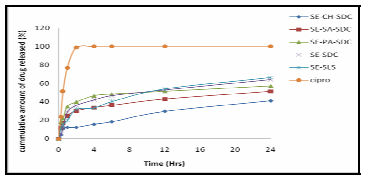

Fig. 6. In-vitro release profile of different CPF-surf-plexes in PBS (pH=7.4)

Formulation	MIC (nominal) (µg/ml)			(%) Drug encapsulation	Cumulative (%) drug released after 24h
	E.coli	*S.aureus*	*P.aeruginosa*		
Free drug	0.195	0.195	0.391	---	---
SE-Cipro	0.195	0.195	0.391	27±3	100±1
SE-SLS	0.0487	0.0487	0.0975	86±2	66±2
SE-SDC	0.0975	0.0975	0.195	82±2	64±3
SE-SA-SDC	0.0975	0.0975	0.195	90±1	51±2
SE-CH-SDC	0.0487	0.0487	0.0975	93±2	41±0.5
SE-P-SDC	0.0243	0.0243	0.0487	84±2	57±2

Table 2. Antimicrobial efficacy of different CPF-surf-plexes.

Conclusions

The problem of low payload efficiency is frequently encountered with variety of drugs including ciprofloxacin due to intrinsic properties. The present study indicates that surfplexes could be a best alternative to improve the payload efficiency and not to mention antimicrobial efficacy. Thus surf-plexes based drug delivery has proven its potential in the field of drug delivery which can be administered through different routes for various applications.

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