IJMPR 2021, 5(6), 114-120

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

ENHANCED ANTIMICROBIAL ACTIVITY OF CIPROFLOXACIN AGAINST *S. AUREUS* BY USING POLYSACCHARIDE IN MICROEMULSION SYSTEM

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Received on: 04/11/2021
Revised on: 25/11/2021
Accepted on: 15/12/2021

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ABSTRACT

Objective of the work is to formulate and evaluate microemulsion containing Ciprofloxacin within oleic acid and Chitosan for improvement of antimicrobial activity of Ciprofloxacin. Optimized formulation characterized for different parameter. Optimization was done on the basis of droplets size and stability of the formulation. Formulation prepared by chitosan polysaccharide showed improved antimicrobial activity as compared to marketed formulation of the same drug concentration. *In vitro* drug release was also in sustained manner because 76.15 % drug release in 24 hours means chitosan also sustained release of drug from formulation whereas formulation withought chitosan showed release of 84.93 %. Droplets size is also around 257.5 ± 50µm or 894 ± 20 nm by Malvern analyser and SEM showed droplets size around 1.1 µm. Thus it justifies term microemulsion. Drug entrapment in optimized formulation was around 63 percent, during formulation preparation different process variables also optimized such as shearing speed sonication time to reduce droplets in smaller size. We observed 4 min sonication and above 1000 rpm shearing speed is sufficient for formulation of microemulsion. Stability study showed optimized formulation was stable at 5, 25 and 40º C temperature, minor changes only observed but within acceptable limit. Thus microemulsion of CIFx containing chitosan in external phase can be use as a suitable delivery system for improvement of antimicrobial activity.

KEYWORDS: *Ciprofloxacin, Antimicrobial activity, Particle size, Zone of inhibition.*

INTRODUCTION

Fluoroquinolones are the class of broad spectrum antimicrobials. Clinically, fluoroquinolone class have been used to treat a variety of infections due to their broad spectrum activity against Gram-positive and Gram-negative bacteria. This class of drugs are very popular and safe so we tried to improve their antimicrobial activity by selecting suitable excipients and dosage form.[1],[2] Delivery of drugs for treatment of severe infections through conventional drug delivery systems, such as tablet, capsules is a considerable challenge to the treatment of severe diseases. Drug loss from the body by various factors like first pass metabolism are main obstacles.[3] Microemulsions used as drug carrier offers several favourable Pharmaceutical and biopharmaceutical properties such as their excellent thermodynamic stability, phase transition to liquid-crystal state, very low surface tension, and small droplet size, which may result in improved drug absorption, extended duration of action, high drug absorption, and permeation of loaded drugs.[4][5]

Microemulsion is a novel vesicular system for the delivery of drugs in to the topical, ocular, oral and parenteral route. Some advantages of microemulsion formulations are-The use of emulsion improved penetration of active constituent in the body, Microemulsion gives more flexibility for the delivery of the drug, Microemulsion gives controlled release of drugs, greater stability of active Pharmaceutical ingredients, microemulsion has better biocompatibility.[6] As compare to conventional dosage form submicron emulsion gives better absorption, long duration of drug for a particular amount of drug reduces the dosage frequency, reduce side effects and can be given by various routes like topical, oral and intravenous route to deliver the drug product, Rapid and efficient penetration of the drug moiety, Provides protection from hydrolysis and oxidation as drug in oil phase in o/w microemulsion is not exposed to attack by water and air, dur to liquid dosage form increases patient compliance.[7]

Chitosan, is a versatile hydrophilic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible. The activity dependence on polymeric molecular weight and degree of acetylation.

The data indicate that the effectiveness of Chitosan varies and is dependent on species of target microorganisms. Chitosan generally showed stronger effects for gram-positive bacteria (e.g. Listeria monocytogenes, Bacillus megaterium, B. cereus, Staphylococcus aureus, Lactobacillus plantarum, L. brevis, L. bulgaris, etc.) than for gram-negative bacteria (e.g. E. coli, Pseudomonas fluorescens, Salmonella typhymurium, Vibrio parahaemolyticus, etc).[8]

Oleic acid, the most widely distributed of all the fatty acids, apparently occurring to some extent in all oils and fats**.** This acid is used in the preparations of microemulsion formulations as excipient due to its capacity to react with alkalis forming soaps with emergent properties.[9]

MATERIALS AND METHODS

Oleic acid as oil phase, Tween 80 as surfactant, ethanol as co-surfactant, Chitosan polysaccharide, All the materials were procured from the certified suppliers and of analytical grade only. Ciprofloxacin was kindly provided by SIFC, SIRT-P as a gift sample.

Preparation of emulsion

Different microemulsion containing Ciprofloxacin were prepared following standard procedure with minor modifications. Microemulsion was prepared using oleic acid as oil phase, Tween 80 as surfactant, ethanol as co-surfactant, and 0.5N NaOH solution as aqueous phase (for adjusting pH to 6.8–7.1). 0.5 % CH was added to the water phase of microemulsions. For drug-loaded microemulsion, Ciprofloxacin was slowly incorporated into the system under magnetic stirring at the concentration 0.3% (w/w). The drug has been added to the oil phase and mixed vigorously. The aqueous solution of Tween 80 and ethanol has been added to oily phase, and remaining water content has been added dropwise and mixed to form a transparent microemulsion.[10]

Process variables

Formulation of submicron emulsion can be affected by number of factors (process variables) which are directly affecting the properties of the microemulsion. The preparation of microemulsion involves various process variables, but out of them, following were selected:- Effect of homogenizer speed, sonication time, influence of temperature. Formulations were subjected to different homogenization speed 1500, 7000, 1600 rpm and different sonication time i.e. 4 min 6 min and 9 min. for effect of temperature formulations were subjected to different temperature 50℃, 55℃ and 60℃ and observed for any change in globules size with respect to temperature.

Optimization of process variables

The effects of variables were observed on the final particle size, drug loading during the preparation of a particular system, the other variables were kept constant. The observation is shown in table 8 and 9 in result discussion section after using different variables.

Characterization of submicron emulsion

Size and size distribution

The average particle size of the submicron emulsion dispersion was determined using a Zetasizer (Melvern Instruments, UK). The sample of dispersion was diluted to ensure that the light scattering intensity was within the instrument's sensitivity range.

Zeta potential

Zeta potential was determined by Melvern Zetasizer.

pH

The pH meter was used for the determination of the pH value of the emulsion at room temperature (25ºC ± 2).

Viscosity-Viscosity of formulation was determined by digital Brookfield viscometer

SEM: Sample for scanning electron microscopy was performed in SEM model Ultra Plus, software, ZESIS (Germany).

6.5.4 Drug entrapment

The submicron emulsion were centrifuged at 18,000×g and 4°C for 30 min in order to separate the incorporated drug from the un-incorporated drug. The supernatant was analyzed by UV for the un-incorporated drug concentration to determine the incorporation percentage from total amount of drug. [11] The entrapment efficiency of submicron emulsion was calculated as per equation given below:

Entrapment efficiency = Total drug – free drug/total drug×100

$$EE\,(\%) = (A2 - A1/A2) \times 100$$

In-vitro drug release (dialysis method)

In vitro release studies were carried out using bulk equilibrium reverse dialysis bag technique at 37°C. Dialysis membrane previously soaked for 24 hours in the dissolution medium. For the release experiment, 1ml of ME containing CIFx and was pipetted into a dialysis bag. The dialysis bag was kept in 100 ml of stirred sink solution (PBS pH 7.4) for 24 hours, temperature was maintained at 37°C & magnetically stirred at 50 rpm The samples were collected at different time durations i.e. 0, 15, 30, min, 1 hr, 2hr, 4hr, 6hr, 12hr and 24hr. the release medium was exchanged with equal volume of fresh PBS solution to maintain sink condition. At selected time intervals, aliquots were withdrawn from the release medium & replaced with same amount of the phosphate buffer. The sample was analyzed in triplicate using UV spectrophotometer at 276nm.[12]

Antimicrobial Activity (cup plate method)

Sterile marketed Ciprofloxacin eye drops was used as a standard preparation. The standard solution and the developed formulations (test solution) between the ranges of 10 and 100 µg per ml were poured into

separate cups or well bored into sterile agar solution previously seeded with organism *Staphylococcus aureus.* After allowing diffusion of solutions for 2 hrs, the plates were incubated for 24 hrs. A scale was used to measure zone of inhibition. The zone of inhibition measured around each cup was compared with that of the standard.[13]

Physical stability of microemulsion formulation[14]

Selected formulation were stored at 5°C and 25°C and 40°C for 3 month. viscosity, pH Particle size, and phase separation, were observed. The creaming and the phase separation were assessed visually at given time intervals. The centrifuge tests at 12,000 rpm at 25°C for 30 min were carried out to assess the physical stability of microemulsions.

RESULT AND DISCUSSION

Identification of drug

FTIR spectra of Ciprofloxacin was performed on prestige spectrophotometer in KBr disc to perform drug indemnification and it's confirmed that drug is Ciprofloxacin as shown in fig 1.

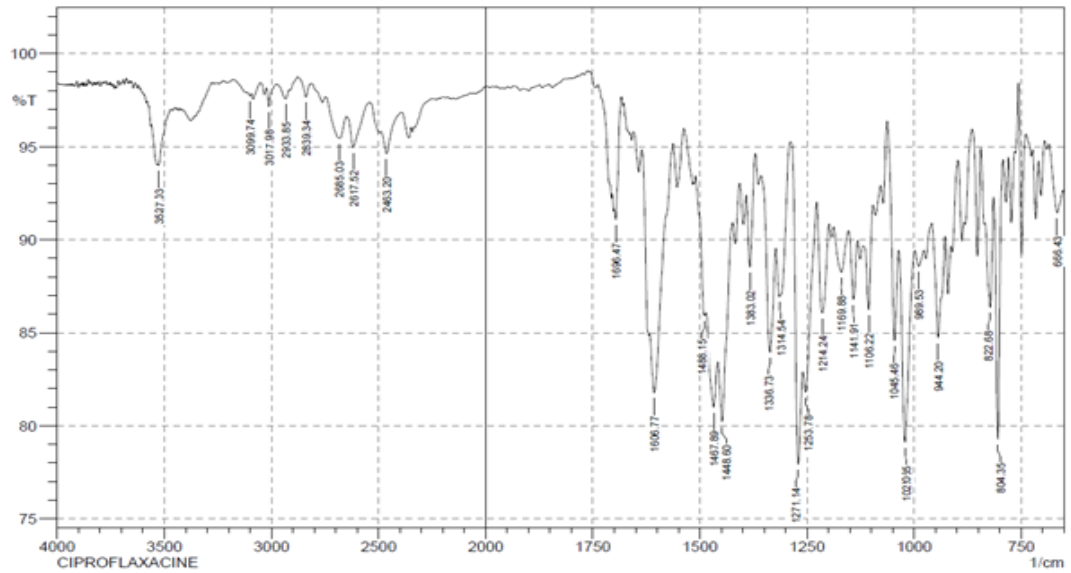

Fig. 1: FTIR spectra of Ciprofloxacin.

Table 1: Interpretation of FTIR spectra of Ciprofloxacin.

Wave Number (cm^{-1})	Characteristic absorption
3527	Hydroxyl group(O-H) st
3017	Aromatic cyclin(O=CH&Ar-H) st
1696	Carboxylic (C=O)st
1448	Carbonyl group(C-O)
1020	Fluorine group

Formulation optimized on the basis of stability and particle size. Different formulation prepared by changing the percentage of surfactant, co-surfactant and oil percentage. We selected 80 as surfactant, ethanol as co-surfactant, combination of both required to produce stable formulation. The effects of variables were observed on the final particle size, drug loading during the preparation of a particular system, the other variables were kept constant. The observations are shown in table 2.

Table 2: Process variables for ME-CIFx.

S. NO.	Formulation code	Speed (rpm)	Particle stability	Sonication time (min)	Particle size (nm)	Percentage entrapment
1.	ME-CIFx	15000	Stable	4	540.6	60.3%
2.	ME-CIFx	7000	Unstable	2	620.4	54.5%
3.	ME-CIFx	1600	Unstable	1	690.8	53.2%

Table 3 Process variables for ME-CIFx- CHn.

S. NO.	Formulation code	Speed (rpm)	Particle stability	Sonication time (min)	Particle size (nm)	Percentage entrapment
1.	ME-CIFx- CHn	15000	Stable	4	895.5	63.3%
2.	ME-CIFx- CHn	7000	Unstable	2	930.4	61.4%
3.	ME-CIFx- CHn	1600	Unstable	1	980	59.1%

Thus high shearing speed and sonication time 4 min was resulted with 890 nm droplets size and it is selected as optimized process for formulation in case of ME-CIFx-CP, and similar results were observed with ME-CIFx formuation

Physicochemical characterization

Particle size

Formulation optimized on the basis of particle size. Different formulation prepared by changing the percentage of surfactant, co-surfactant and oil percentage.

Table 4: Formulation optimization.

Sr. N.	Drug %	Oil Phase %	Surfactant/ Co-surfactant:(Tween 80: ethanol)	Chitosan (%)	Particle size
1	**0.3 %**	**10**	**2:1**	**0.5**	**900**
2	0.3	15	2:2	1	2020
3	0.3	20	2:3	1.5	2533
4	0.3	25%	1:1	2	3000
5	0.3	30%	1:2	2.5	3600

Table 5: Optimized formulation composition.

Formulation	Drug (%)	Oil Phase (%)	Surfactant/ Co-surfactant Ratio by weight (Tween: ethanol)	Chitosan (%)	Water
ME-CIFx	0.3	10	2:1 (40:20 ml)	-	30 ml
ME-CIFx-CHn	0.3	10	2:1 (40:20 ml)	0.5	30 ml

Formulation F1 was optimized formulation coded as ME-CIFx-CP. and ME-CIFx is formulation withput chitosan.

Paricle size analyse by Malvern for formulation with chiotan and without chitosan particle size shown in fig 2

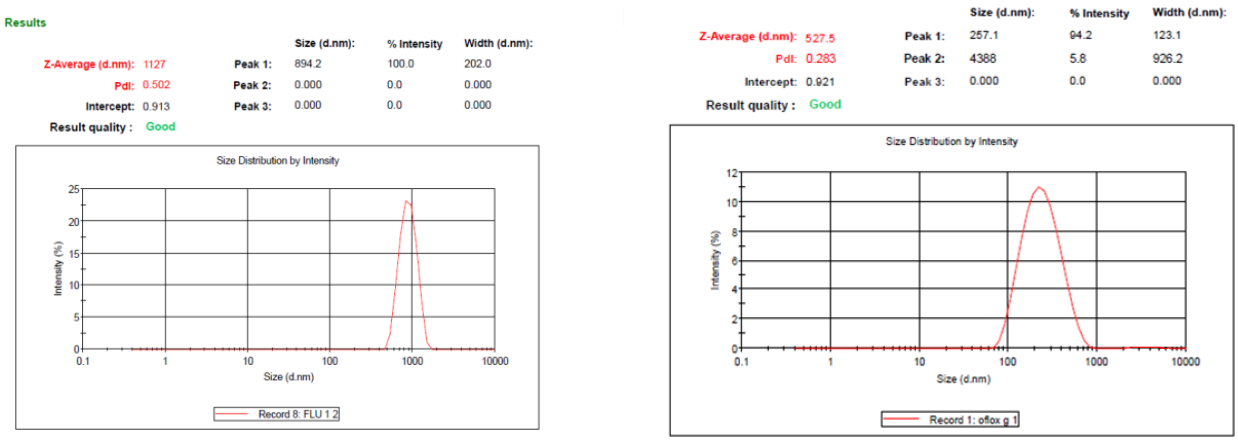

Fig 2 Particle size of optimized formulation by Malvern instrument.

A- With Chiosan (ME-CIFx-CHn) B- Microemulsion withought Chitosan (ME-CIFx)

Table 6: Characterization of optimized formulation.

Formlation	Average particle size (nm)	pH	Viscosity (cP)
ME-CIFx	257.5 ± 50	7.2 ± 0.4	2
ME-CIFx- CHn	894 ± 20	7.1 ± 0.2	4

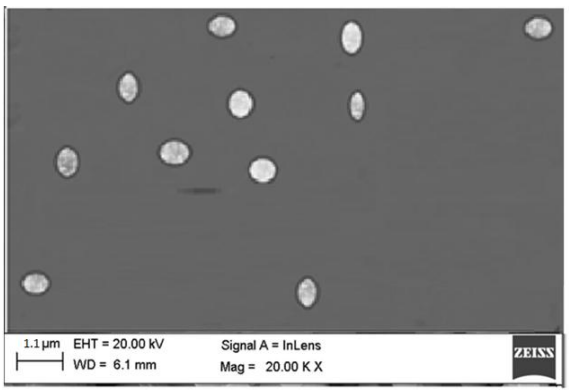

Fig. 3: SEM image of optimized formulation shows droplets size 1.1 µm.

Drug entrapment

The entrapment efficiency was found to be more than ~62% in optimized formulations. We found that CHn coated droplets shows more entrapment as compared to formulation with with ought CIFx because it coat the droplets and showed more entrapment.

***In vitro* drug release**

It has been revealed from the graph that the drug release by ME-CIFx-CHn formulation was in sustained manner as compared to drug release from microemulsion without Chitosan formulation only 76.15 % drug release in 24 hours Fig- 4.

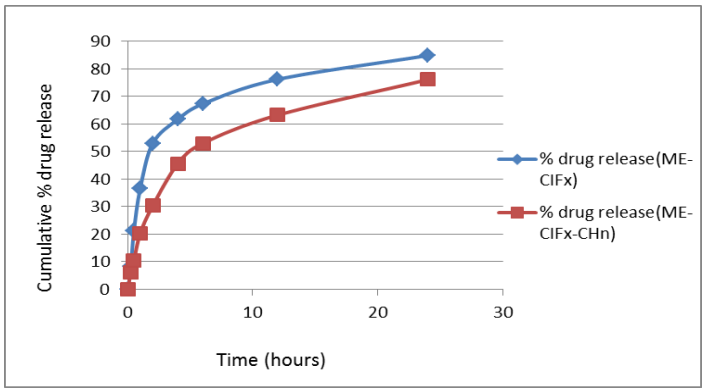

Fig. 4: *In vitro* Drug release from microemulsion containing CHn, and withought CHn.

Antimicrobial Activity by cup plate method

Table showing zone of inhibition of optimized formulation and compared with marketed preparation of same drug (0.3 % Ciprofloxacin).

Table 7: Zone of inhibition of test and standard formulation.

Conc (µg/ml)	ZOI in mm (standard)	ZOI in mm of ME-CIFx	ZOI in mm of ME-CIFx- CHn
10	-	-	1.1
50	1.2	1.4	2.2
100	1.5	1.7	3.3

ZOI- Zone of inhibition
Standard formulation- CIFx marketed eye drop solution
ME-CIFx- Microemulsion containing ofloxacin
ME-CFx-CP- Microemulsion containing ofloxacin with chitosan

The formulation ME-CIFx-CHn showed more zone of inhibition as compared to without chitosan formulation ME-CIFx and simple marketed Ciprofloxacin eye drop solution formulation.

More antimicrobial activity observed by ME-CIFx- CHn by cup plate method in formulation because chitosan polysaccharide able to kill this microorganism ant this could be due to electrostatic interaction between formulation and bacterial cell wall components like lipotheicocic and teichuronic acids in *s. aureus.* Thus such binding of chitosan with bacterial components, disturb its integrity and improved antimicrobial activity of ME-CIFx- CHn.

Stability studies

Initially, creaming and cracking was observed in both the emulsions but system gets redispersed after little shaking. No significant changes of particle size, viscosity, pH, phase separation were observed during 3 months. Data shown in table 15, 16 and 17. The centrifuge tests showed that all microemulsions had good physical stability.

Table 8 Influence of temperature on viscosity and pH at 5°C.

Time (Days)	Viscosity(cp) ± (s.d.)		pH ± (s.d.)	
	ME-CIFx	ME-CIFx-CHn	ME-CIFx	ME-CIFx-CHn
0	8.5± 0.1	8.5±0.2	6.5± 0.2	6.5± 0.4
15	8.7±0.3	8.6±0.5	6.5±0.1	6.5± 0.3
30	8.7±0.4	8.6±0.3	6.5± 0.5	6.6±0.3
45	8.9±0.2	8.8±0.4	6.6± 0.3	6.6±0.5
60	9.0±0.1	9.1±0.1	6.7±0.6	6.7± 0.2
90	9.2±0.4	9.6±0.2	7.1± 0.3	6.8± 0.3

Table 9: Influence of viscosity and pH at 25°C.

Time (Days)	Viscosity(cp) ± (s.d.)		pH ± (s.d.)	
	ME-CIFx	ME-CIFx-CHn	ME-CIFx	ME-CIFx-CHn
0	8.5± 0.2	8.4± 0.4	6.4± 0.5	6.7± 0.1
15	8.5± 0.4	8.4± 0.2	6.4± 0.4	6.5± 0.5
30	8.3± 0.5	8.3± 0.6	6.4± 0. 6	6.6± 0.3
45	8.2± 0.1	8.3± 0.1	6.5± 0.7	6.6± 0.2
60	8.1± 0.6	8.0± 0.6	6.5± 0.3	6.6± 0.2
90	7.9± 0.6	7.9± 0.4	6.7± 0.4	6.7± 0.7

Table 10: Influence of viscosity and pH at 40°C.

Time (Days)	Viscosity(cp) ± (s.d.)		pH ± (s.d.)	
	ME-CIFx	ME-CIFx-CHn	ME-CIFx	ME-CIFx-CHn
0	8.6± 0.2	8.6± 0.4	6.4± 0.5	6.5± 0.2
15	8.1± 0.2	8.4± 0.2	6.6± 0.3	6.5± 0.6
30	7.8± 0.4	8.1± 0.5	6.8± 0. 5	6.6± 0.3
45	7.5± 0.2	7.9± 0.1	6.9± 0.7	6.7± 0.2
60	7.1± 0.7	7.6± 0.6	6.9± 0.4	6.9± 0.2
90	6.4± 0.5	7.1± 0.3	7.1± 0.4	6.9± 0.8

CONCLUSION

Formulation prepared by chitosan polysaccharide showed improved antimicrobial activity as compared to marketed formulation of the same drug concentration. *In vitro* drug release was also in sustained manner because 76 % drug release in 24 hours means chitosan also sustained release of drug from formulation whereas formulation withought chitosan showed release of 84.95 %. SEM showed droplets size around 1.1 µm, thus it justifies term microemulsion. Drug entrapment in optimized formulation was around 60 % however this entrapment is less due to ciprofloxacin hydrochloride partitioning is less in oil phase, but antimicrobial activity of formulation was improved. During formulation preparation different process variables also optimized such as shearing speed sonication time to reduce droplets in smaller size. We observed 4 min is sufficient for formulation of microemulsion and homogenization above 1000 rpm is good for stable formulation. Stability study showed optimized formulation was stable at 5, 25 and 40º C temperature minor changes only observed but within acceptable limit. Thus microemulsion of CIFx containing chitosan in external phase can be used as suitable drug delivery system for antimicrobial activity.

ACKNOWLEDGEMENT

Rajeshwari Harode is thankful to SIRT-P and Durga pandey for their consistent support and kind cooperation for my work.

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