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MOXIFLOXACIN LOADED SOLID LIPID NANOPARTICLES (SLNs): PREPARATION AND CHARACTERIZATION

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ABSTRACT

As drug delivery systems Nanoparticulate widely investigated because of many advantages such as smaller size, controlled drug release potential, targeting ability, enhancement of therapeutic efficacy and reduction of toxicity. So, Solid Lipid Nanoparticles(SLNs) have recently received considerable attention as alternative drug delivery carrier. The aim of present work was to formulate Moxifloxacin loaded solid lipid nanoparticles (SLNS) using palmitic acid as a lipophilic material, Tween 80 and poloxamer as surfactants and methanol, butanol as cosolvents. The SLNs were prepared by o/w microemulsion technique which involves high speed homogenization. Moxifloxacin loaded SLNs seem to have dimensional properties useful for topical administration. The SLNs were characterized for particle size analysis, zeta potential, drug content, entrapment efficiency, Scanning Electron Microscopy (SEM) photographs, Differential scanning calorimetry (DSC) and IR studies. Results indicated mixed lipid-matrix produced nanosuspensions with low-crystallinity, smaller particle sizes, no drug-excipient incompatibility and higher drug entrapment. The optimized formulation was incorporated into various gels like carbopol and xanthan gum gel. Preliminary physical parameters of gels like viscosity, gel strength were evaluated. In vitro drug release studies were carried out for moxifloxacin SLN loaded gel preparations and % drug loading capacity was calculated. Ex vivo penetration studies were conducted for 12 hrs using Keshary Chien diffusion cell. The studies revealed a stable formulation without precipitation of drug and a sustained drug release from the gel matrix. SLN composed of moxifloxacin would prove to be a good topical drug delivery in treating bacterial infections.

Key words: Moxifloxacin, poloxamer, carbopol, xanthan gum, nanosuspensions, zeta potential.

INTRODUCTION

Solid lipid nanoparticles (SLNs) are introduced as a carrier system for poorly water soluble drug and cosmetic active drug. Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. They are synthesized from synthetic/natural polymers and suited to optimize drug delivery and reduce toxicity. They have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size [1,2]. They have some limitations due to their high cost and scarcity of safe polymers with regulatory approval. To overcome this limitation polymeric nanoparticle lipid is used as an alternative carrier. These nanoparticles are known as solid lipid nanoparticles (SLNs).

Solid lipid nanoparticles are also referred to as "zero-dimensional" nanomaterials [3]. SLNs are developed as an alternative system for polymeric nanoparticles, liposome and emulsion. SLNs have unique property like small size, large surface area, high drug loading and interaction of phase at the interphase. SLNs are attracting major attention in novel colloidal carrier for topical application. SLNs are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLNs are sub-micron colloidal carrier composed of physiological lipid, dispersed in water or in an aqueous surfactant solution.

Solid lipid nanoparticles (SLNs) composed of physiological lipid, dispersed in water or in an aqueous

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surfactant solution [4]. Distinct advantages of SLNs are their solid state of the particle matrix, the ability to protect chemically labile ingredients against chemical decomposition and the possibility to modulate drug release. They show some potential disadvantages like drug leakage during storage and insufficient drug load. To overcome the limitation of SLN, nanostructured lipid carriers (NLC) have been developed. Both carrier types are submicron size particles (50-1000 nm) and are based on solid lipids but they can be distinguishing by their inner structure [5]. SLNs consist of solid lipids while NLC are made of solid matrix entrapping variable liquid lipid noncompartmen. NLC is also called as an upgrade of the solid lipid nanoparticles even though SLNs is still intended to indicate the nanostructered lipid carriers, creating no clear differentiation.

MATERIALS AND METHODS

Moxifloxacin was a gift sample from Apex pharma, Hyderabad, Palmitic acid, Tween 80 were purchased from Panchi chemicals, Hyderabad and Methanol were purchased from Usha chemicals, Hyderabad, and Poloxamer was purchased from Ra Chem pharma. All other chemicals and solvents used were of analytical grade.

Preparation of Solid Lipid Nanoparticles (SLNs)

SLNs loaded with Moxifloxacin were prepared using microemulsion technique by dispersing warm o/w microemulsion in a cold aqueous medium under mechanical stirring [6,7]. Different formulations of drug loaded SLN were prepared by varying concentrations of palmitic acid as shown in the below Table 1. The lipid melt consists of poloxamer as a emulsifying agent, palmitic acid as a lipid phase and Tween 80 as a surfactant added to increase the stability of drug in lipid. Methanol and butanol as a cosolvent were added to the lipid phase. Drug was dissolved in the melted lipid phase. The microemulsion was obtained by constantly adding the lipid melt to the 100 ml of double distilled water (Dispersion medium). The hot preemulsions were homogenised at a pressure of 3000 rpm for 1 hr. Immediately the stirring was continued at room temperature for half-an-hour. The obtained milky microemulsion was rapidly cooled on an ice bath to obtain solid lipid nanoparticles (SLNs). The preparation outline was shown in fig 1.

Characterization of SLNs

Scanning Electron Microscopy (SEM) [8,9]

The morphology of the SLN was examined by SEM (Hitachi, Japan). The samples were stained with 2% (w/v) phosphotungstic acid for 30 s and placed on copper grids with films for viewing.

Particle size measurement

The average diameter and Polydispersity Index (PI) of SLN were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3 (Malvern, UK) at a fixed angle of 90°C and at 25°C. The aqueous SLN dispersions were diluted with distilled water before analysis. Each value is the average of 3 measurements.

Zeta potential measurement

The particle charge was quantified as Zeta Potential (ZP) using a Zetasizer 4 at 25°C.

Infrared spectroscopy (FTIR)

IR spectra of freeze dried SLN were obtained with a Win-IR, Bio-Rad FTS spectrophotometer, using the potassium bromide (KBr) disk technique (about 5 mg sample for 100 mg dry KBr).

Drug entrapment efficiency

The entrapment efficiency (EE), which corresponds to the percentage of Moxifloxacin encapsulated within and adsorbed on to the nanoparticles, was determined by measuring the concentration of free Moxifloxacin in the dispersion medium[8].

$$\% EE = \frac{[M_{initial\ drug} - M_{Free\ drug}]}{M_{initial\ drug}} \times 100$$

Where, $M_{initial\ drug}$ is the mass of initial drug used for the assay

$M_{Free\ drug}$ is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion

Drug loading efficiency

Drug loading efficiency (%DL) was calculated from the Moxifloxacin in the SLN sediments and the total amount of Moxifloxacin and the excipients added in the preparation of SLN according to the following[8]

$$\% DL = \left(\frac{\text{Amount of drug in SLN precipitations (mg)}}{\text{Amount of drug added (mg)} + \text{Amount of excipients added (mg)}} \right) X100$$

Formulation of Moxifloxacin SLN based gel [12]

Gels were prepared using 2 polymers: Carbopol 934P, sodium alginate. For the preparation of gel, glycerol (10%) and nanoparticulate dispersion (20%) were added. Required quantity of gelling agent was dispersed in the aqueous phase under continuous stirring.

Characterization of SLN loaded gels

Determination of pH

The pH of the 10% (w/w) gel was determined using digital pH meter calibrated using suitable buffer solutions [9].

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Spreadability

The spreadability of the gel was determined using the following technique: 0.5g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed [10]. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.

Measurement of Gel Strength

A sample of 50 gms of gel was placed in a 100ml graduated cylinder and gelled in a thermostat at 37^{O}C. The apparatus for measuring gel strength (weighing 10g) was allowed to penetrate in MSLN gels. The gels strength, which means the viscosity of the gels, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [10,11].

Rheological studies

Brookefield Viscometer was used for rheological studies. The sample was placed in a beaker and was allowed to equilibrate for 5min before measuring the dial reading using a T-C spindle at 0.5, 1, 2.5, and 5 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted.

***In vitro* release studies were performed using modified Franz diffusion cell**

In vitro release studies can be performed in a modified Franz diffusion cell over a period of time 12 hours. [13, 14, 15] The sink condition was maintained by using 40% v/v PEG-400 in PBS in the receptor compartment and the temperature was maintained at 37± 1 ◦C with the help of a circulating water bath. Samples (1 ml) were withdrawn at appropriate time intervals. At specific time intervals, aliquots of samples containing the released drug are taken from the acceptor compartment and determined at 295nm using UV-Visisble spectroscopy.

RESULTS AND DISCUSSION

Particle size determination

Particle size measurement with Malvern mastersizer was performed after dilution of the samples, surprisingly it was found that the systems preserved their colloidal particle size between 160-210 nm. It was found that the particle size was less (160 nm) when the lipid content is as low as possible (SLN F1) as a dispersed phase. The observed particle size of all of the investigated system was in the colloidal ranges as shown in table 6.

Table 1. Formulation of optimized Moxifloxacin loaded solid lipid nanoparticles (SLNs)

INGREDIENTS	SLN F1	SLN F2	SLN F3	SLN F4
Moxifloxacin (g)	0.5	0.5	0.5	0.5
Poloxamer (g)	4	4	4	4
Palmitic acid (g)	1.5	2.5	3.5	4.5
Methanol+Butanol (ml)	4	4	4	4
Distilled water	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml

Fig.1. Schematic presentation of Moxifloxacin Solid lipid nanoparticles (SLNs)

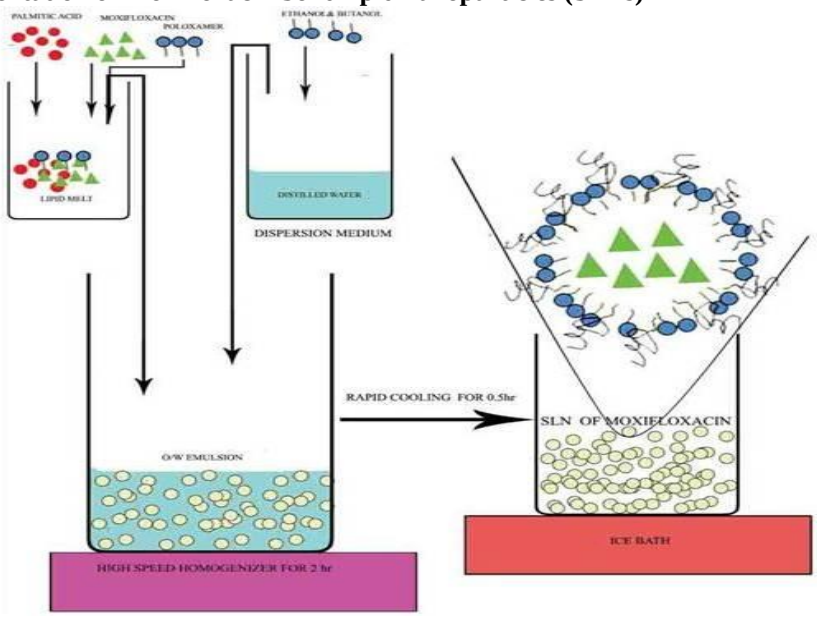

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Table 2. Formulation of various SLN loaded Sodium alginate gels

INGREDIENTS (%)	MSA gel 1	MSA gel 2	MSA gel 3	MSA gel 4
Moxifloxacin SLN	20	20	20	20
Sodium alginate	0.25	0.5	0.75	1.0
Glycerol	10	10	10	10

Table 3. Formulation of various SLN loaded Carbapol gels

INGREDIENTS (%)	MCP gel 1	MCP gel 2	MCP gel 3	MCP gel 4
Moxifloxacin SLN	20	20	20	20
Carbapol 934P	0.25	0.5	0.75	1.0
Glycerol	10	10	10	10

Fig. 2. Scanning electron photomicrograph of SLN

Fig. 3. FTIR of pure drug (Moxifloxacin)

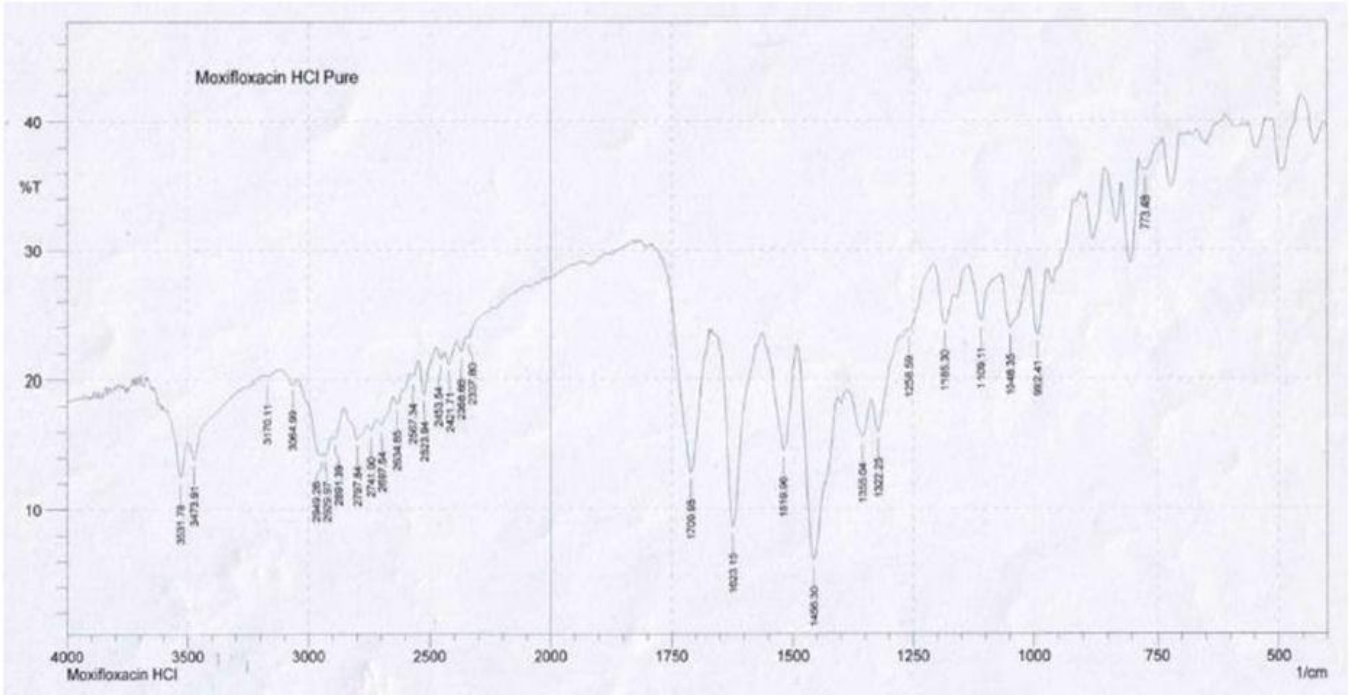

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Fig. 4. FTIR of Drug+excipients

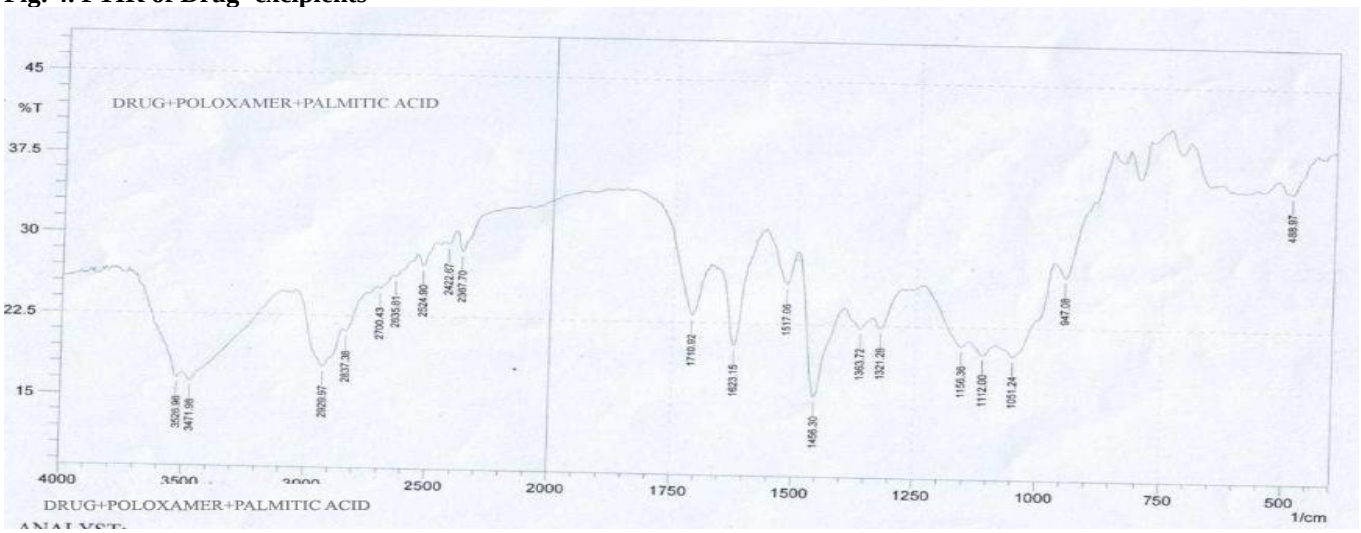

Table 4. Effect of varying concentrations of lipid (Palmitic acid) on the parameters of the Moxifloxacin SLNs

FORMULATION	% ENTRAPMENT EFFICIENCY	POLYDISPERSITY INDEX	DRUG LOADING CAPACITY (%)	SIZE (nm)	ZETA POTENTIAL (mv)
SLN F1	62.42±1.5	0.229±0.9	74.6	160±2.2	-19.48±2.4
SLN F2	77.85±0.6	0.226±1.0	79.5	182±3.4	-17.4±5.2
SLN F3	86.28±2.0	0.210±1.2	88	190±2.6	-30.2±5
SLN F4	92.05±1.6	0.212±1.4	96.2	210±1.8	-31.5±1.4

Table 5. Evaluation parameters of optimized SLN4 loaded gels

Formulation	Viscosity (mpas)	Gelling strength (sec)	Spreadability g/sec	Mucoadhesive force (dynes/cm2)
MSA gel 2	41.56	61	8.75	11.1
MSA gel 4	45.22	100	17.82	19.5
MCP gel 2	35.74	82	2.77	26.7
MCP gel 4	37.5	126	4.86	34.2

Table 6. *In vitro* skin permeation studies – Cumulative drug release of optimized SLN loaded gel formulations

Time (hrs)	MSA gel 2 (0.5% w/v)	MSA gel 4 (1 % w/v)	MCP gel 3 (0.75 %w/v)	MCP gel 4 (1% w/v)
1	10.54	14.2	15.52	20.54
2	19.77	22.4	24.26	29.74
3	31.89	34.3	36.62	36.78
4	48.01	44.1	48.24	45.48
5	56.56	49.2	51.7	54.12
6	65.17	52.8	58.2	59.91
7	77.48	61.5	66.4	70.12
8	90.69	69.4	78.42	75.69
9	96.78	75.06	82.78	82.77
10		81.32	98.14	87.31
11		98.21		90.54
12				98.49

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Table 7. Release kinetics of optimized formulation (MCP gel_4)

	ZERO ORDER PLOT	HIGUCHI PLOT	FIRST ORDER PLOT	KORSEMEYER - PEPPAS	INFERENCE
R^2	0.997	0.991	0.925	0.871	Zero order kinetics
m	7.062	7.062	6.987	6.864	

Fig. 5. Zero order plot of various SLN loaded gel formulations

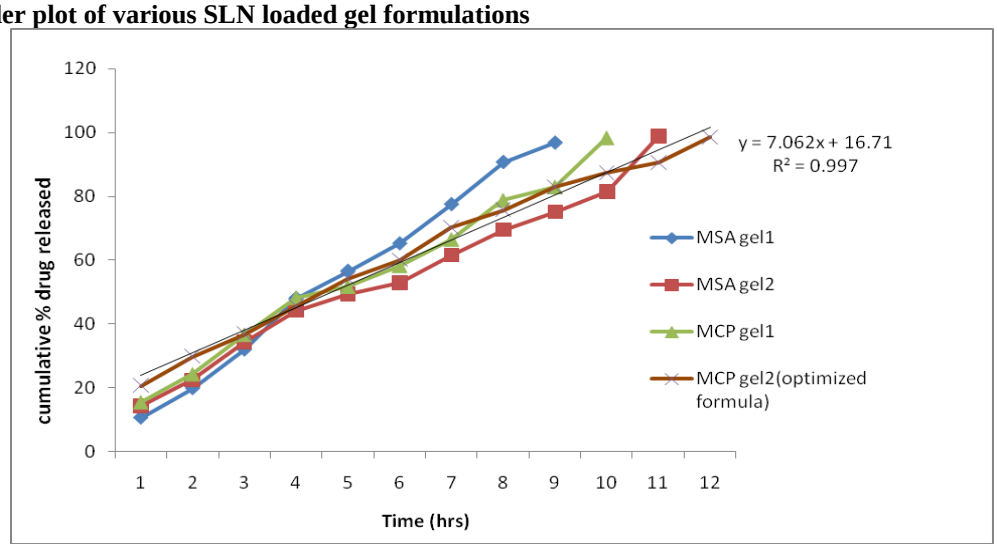
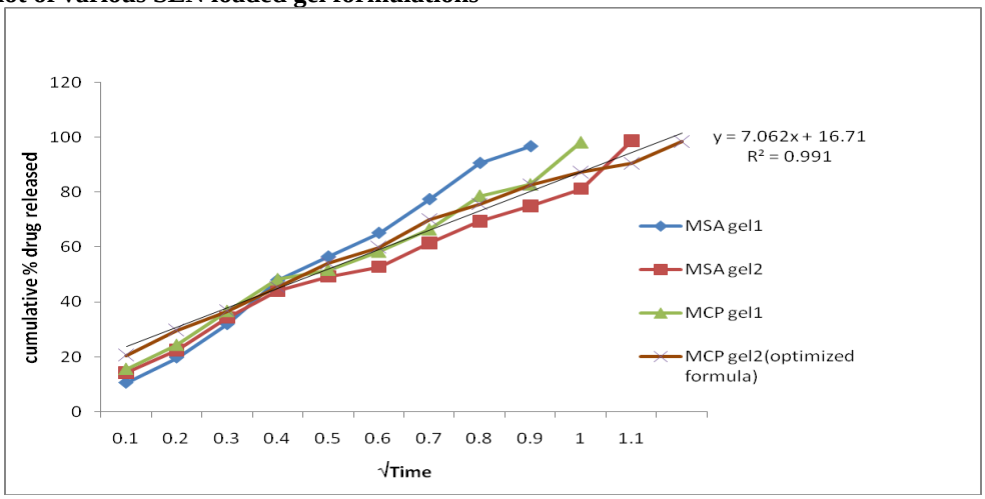


Fig.6. Higuchi plot of various SLN loaded gel formulations



FTIR analysis

Solid state analysis of the prepared SLN was also performed by IR spectroscopy. Figure 3 and 4 shows stacked IR spectra of pure drug, physical mixture of drug, lipid and surfactant. From the spectral study it was observed that there was no significant change in the peaks of pure drug and drug lipid mixture and therefore no interactions were found.

Drug entrapment efficiency (%EE) and loading efficiency (%DL)

Percentage entrapment efficiency of SLN F3 and SLN F4 were found to be satisfactorily high that is, 86.28±2.0 and 92.05±1.6 respectively. The high drug incorporation may be attributed to the fact that rapid quenching of drug occurred in lipid phase due to presence of poloxamer and Tween 80 as surfactant phase and the drug incorporation followed core-shell model with drug-enriched core. It was observed that increase in the lipid content which reduced the partition of Moxifloxacin in outer phase (when in o/w microemulsion state) lead to the increase in the entrapment efficiency, that is, entrapment is proportional to the increase in the lipid content as evidenced by lipid.

Drug loading efficiency of SLN F3 and SLN F4 was found to be satisfactory, that is, 88% and 96.2%,

respectively. There was an increase in the entrapment and loading efficiencies in case of SLN F4 as higher percentage of lipid mixture was used in its preparation.

SLN loaded gels

Two polymers namely Carbapol and Sodium alginate were used to entrap the prepared SLNs in different concentrations ranging from 0.25-1.0%. The optimized formulation of SLNs was selected based on there Zeta potential, entrapment efficiency and incorporated in gel. From the results it was concluded that increase in gel concentration leads to optimized formulation with desired characteristics as in the case of mucoadhesive force and gelling strength. MCP F1-F4 showed better results than MSA F1-F4. The optimised concentration of carbapol was found to be 1%w/w as it has high viscosity (37.5mpas), gelling strength (126 sec) and mucoadhesive force (34.2 dynes/cm^2) which are shown in Table 5.

***In Vitro* Release Rate Studies**

The optimized moxifloxacin SLN was chosen as SLN F4 which shows a least Zeta potential and good Entrapment efficiency. It was then incorporated in different gels and in vitro release studies were carried out.

A different release kinetic was observed for the SLN-gel formulations (as shown in table), Fick's law of diffusion seems not to be applicable in each case. An initial rapid drug release was noted in the SLN dispersions, whereas a lag time (15 min) was observed with SLN-gel formulations which could result from the time taken by the drug to diffuse across the gel. The direct exposure of SLN dispersion to diffusion media and quick release of drug may account for rapid initial release in SLN dispersions. SLN-gel formulations showed controlled drug release over 12 hr and an increase in release rate was observed after 12 hrs.

The values of diffusional release exponent (η) from the straight lines which showed that the release of drug from formulations followed a non-Fickian pattern. From the percent cumulative drug released versus time plot, the slope values were determined as release rate constants. The percent cumulative drug released was maximum for the MCP gel_4 (98.49%) formulation with release rate constants of 7.062 %/cm^2/hr. MSA gel_2 gel formulation shows a poor sustained release of drug i.e., before 12hrs the complete release taken place . Hence, moxifloxacin SLNs incorporated in carbopol 934P (concentration 1%w/v) showed better results when compared to sodium alginate based SLN loaded gels.

MCP gel_4 slowly releases the drug as compared with SLN-F4 dispersion, accounted for by the time the drug takes to diffuse through gel. The slower release of drug from MCP gel_4 maintained the drug concentration for longer period of time. Burst releases as well as sustained release both are of interest for dermal application. Burst release can be useful to improve the penetration of drug. Sustained release supplied the drug over a prolonged period of time.

CONCLUSION

O/w microemulsion dispersed in cold aqueous medium followed by mechanical mixing method is suitable to produce SLN in nanometric size range. Hydrophilic drugs like Moxifloxacin can also be successfully incorporated in the solid lipids (palmitic acid and poloxamer) matrix. The drug Moxifloxacin could very well be entrapped in the solid SLNs and their characteristics could be monitored by making changes in various formulation and process variables. We concluded that Moxifloxacin could be incorporated successfully in SLNs with the desired characteristics of size, shape, and entrapment with reasonable stability. Ex-vivo skin penetration studies show that drug SLN loaded gel has a sustained release action. It could also be an ultimate route of administration in case of bacterial infections. Further *in vivo* studies can prove to have enriched drug release and abridge side effects.

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