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Research Article

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Development and Characterization of Ofloxacin & Prednisolone Loaded Nanostructured Lipid Carriers (Nlc) for Topical Route

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Abstract

Aim: *The present study was designed to develop and characterize nanostructured lipid carriers (NLC) of Ofloxacin and Prednisolone for topical use in case of infections associated with inflammation.*

Materials and Methods: *Ofloxacin was obtained as gift sample from Mankind Pharma Ltd, VillKyarta, P.O. Misserwal, Poonta Sahib, Sir Mour. H.P. Whereas Prednisolone was purchased from Yarrow chem., Mumbai. It was evaluated for its pre-formulation studies (organoleptic properties, melting point, solubility, compatibility, max. wavelength of absorption). NLCs were prepared through melt-emulsification followed by ultra-sonication technique. Further optimized batch of NLCs was incorporated into Gel. Formulated NLCs were evaluated in terms of morphological characteristics, particle size (Poly-dispersity Index), drug content, In-vitro drug release (using egg membrane), drug release kinetics (Ritger-Peppas diffusion method). Finally, gel containing NLCs was studied by physical characteristics, pH, viscosity, spreadability, drug content, In-vitro drug release and its kinetics.*

Results and Discussion: *In pre-formulation study, drugs were found having the similar properties as described in Indian Pharmacopoeia (IP) and United States Pharmacopoeia (USP). SEM photomicrograph revealed that NLCs were spherical with more or less smooth surface; particle size 512.3-1703 nm and PDI- 0.399-0.742 (ofloxacin) and particle size 539.3-1736.7 nm and PDI- 0.335 - 0.711 (prednisolone);drug content was found in range of 56.7 - 75.6% for ofloxacin and 65.9 – 81.8% for prednisolone. NLC1 demonstrated maximum release rate with 83.37±1.70% and NLC8 73.96±0.53%.NLC6 was best fitted in Korsmeyer - peppas model as the regression coefficients were 0.960, 0.964, 0.977, 0.950, 0.980 & 0.987 respectively and prednisolone NLC 9 (0.953) and they were close to 1.*

Conclusion: *In conclusion, the prepared NLCs had prolonged release effects with good potential for topical delivery of NLC based gel formulation of ofloxacin& prednisolone.*

Keywords: Ofloxacin, Prednisolone, Nanostructured Lipid Carriers (NLCs) and Poly - dispersity index (PDI).

Introduction

We are amidst a nano-era. Nanotechnology is one of the most innovative key technologies of the twenty-first century which wide opens new perspectives for developing innovative nanosized drug delivery for various drug carries as nanoparticles. The concept of nanoparticles and drug targeting was inspired by a visit of Paul Erlich to Karl Maria von Weber's opera "Der Freishütz" and presently is evolving into many exciting and clinically successful products [1, 2]. In recent years, studies have revealed that there is a wide number of new drug delivery system for the existing drugs and new technologies employed in drug discovery lead to find many new powerful substances as well [3]. Nanoparticles are colloidal particles with sizes ranging from 10 to 1000 nm in diameter [2]. Nanostructured lipid carriers are second generation of lipid nanoparticles which are produced by the blend of solid lipid and liquid lipid (oils). They contain solid lipids together with liquid lipids. These carriers have the usual particle diameter ranging 10–1000nm. To obtain the blend of particle matrix, solid lipids are mixed with liquid lipids, preferably in a ratio of 70:30 [4, 5]. The Nanostructured lipid carriers contain solid lipids together with liquid lipid, the produced NLC have less organized crystalline structure due to presence of different fatty acid chains in lipids and therefore provide better loading capacity for drug accommodation [6].

In previous studies, different methods have been described for development of lipid nanoparticles, especially NLCs. These methods are hot/cold homogenization, micro emulsion technique, solvent emulsification–evaporation, emulsification solvent diffusion, solvent injection (or solvent displacement), phase inversion technique, multiple emulsion method, and membrane contractor technique [7-10]. Various techniques like particle size analysis, zeta-potential,

transmission electron microscopy, differential scanning colorimetry (DSC), X-Ray scattering, polarized light microscopy, laser diffraction (LD), field-flow fractionation (FFF) were performed to investigate the structure, mobility and molecular environment of the compounds [3].

The present study was designed to develop and characterize nanostructured lipid carriers (NLC) of Ofloxacin and Prednisolone for topical use in case of infections associated with inflammation.

Materials and Methods

Materials

Ofloxacin was obtained as gift sample from Mankind Pharma Ltd, VillKyarta, P.O. Misserwal, Poonta Sahib, Sir Mour. H.P. Whereas Prednisolone was purchased from Yarrow chem., Mumbai. The sources of fatty acids, oils & surfactants were given in table no. 3.1. Other chemical solvents used were of analytical grade and their sources are given below:

Table 1: List of Materials

S.No.	List of Materials Used	Company Details
1.	Stearic acid	Merck Specialities Private Limited Shiv Naga Estate, A. Dr. Annie Besant Road, Worli, Mumbai-400018
2.	Neem oil	Local market
3.	Cholesterol, Methanol & Chloroform	Merck Specialities Private Limited Shiv Naga Estate, A. Dr. Annie Besant Road, Worli, Mumbai-400018
4.	0.1N HCl, Sodium hydroxide pellets, Potassium di hydrogen phosphate &Triethanolamine	Merck Specialities Private Limited Shiv Naga Estate, A. Dr. Annie Besant Road, Worli, Mumbai-400018
5.	Carbopol 934	SDFCL (S.D. Fine- chem315-317, T.V. Industrial Estate, 248, Worli Road Mumbai -30.

A. Pre formulation studies

Drug identification test

The drug identification test are based on following parameters as given below:

Organoleptic Properties of the Drug

As per Indian Pharmacpoeia, Drugs (Ofloxacin& Prednisolone) were physically characterized on the basis of colour, odour and taste. All these parameters were recorded and compared with standard.

Melting Point

Melting point of the drugs (ofloxacin& prednisolone) was determined by capillary tube method. Small amount of powdered drug was filled separately inside the thin capillary tubes and sealed. The capillary tube was placed into the melting point apparatus one by one. Thermometer was also placed in the apparatus. The nobe of apparatus was rotated in order to increase temperature and the temperature at which drug melted was recorded.

Solubility

Solubility is defined as the spontaneous interaction of two or more substances to form a homogenous dispersion. The solubility of ofloxacin and prednisolone was studied in various aqueous and non - aqueous solvents. Accurately weighed 10 mg of drugs was taken in 10 ml of each solvent separately at room temperature and shaken for 24 hours. After that the solubility was observed visually for clear fluid.

Compatibility Studies of Drugs

FT-IR studies helps to confirm the identity of pure drug and detect the interaction of drug with Excipients. The compatibility between pure drug and mixture of drug with lipids was recorded on Perkin Elmer Spectrum Version 10.03.06 using a KBr disk. Accurately weighed 1mg of the drug and drug mixtures with lipids were mixed with 100mg of potassium bromide by trituration in a mortar and the mixture was compressed into a pellet at 10 ton/cm^2 in a pellet maker. The sample was scanned at 400cm^{-1}-4000cm^{-1} the results were compared with standard [11, 12].

Analytical methods for drug analysis by UV- Spectrophotometer

Determination of maximum wavelength (λmax) of ofloxacin in methanol

The standard stock solution of ofloxacin was prepared by dissolving accurately weighed 10 mg drug in 10 ml of methanol to get a concentration of 1000 µg/ml. from this solution, 1 ml of solution was pipette out and transferred into 100 ml volumetric flask. The volume was made up to the mark with methanol to give the standard solution of concentration 100 µg/ml. the resulting solution was scanned spectrophotometrically between 200 nm to 400 nm. The maximum wavelength was noted.

Determination of maximum wavelength (λmax) of prednisolone in methanol

The standard stock solution of prednisolone was prepared by dissolving accurately weighed 10 mg drug in 10 ml of methanol to get a concentration of 1000 µg/ml. From this solution, 2.5 ml of solution was pipette out and transferred into 25 ml volumetric flask. The volume was made up to the mark with methanol to give the standard solution of concentration 100 µg/ml. the resulting solution was scanned spectrophotometrically between 200 nm to 400 nm. The maximum wavelength was noted.

Standard Curve of Ofloxacin

The working standard solution of ofloxacin was prepared by further diluting the standard stock solution. The aliquots of 1ml, 2ml up to 10ml of stock solution of ofloxacin were taken into series of 10ml volumetric flask and volume was made up to the mark with methanol to give the final concentration of 1µg/ml to 10µg/ml. The solutions were filtered through the whattmann filter paper and filtrate analyzed at λ_{max} 299.5 nm by using UV-Visible spectrophotometer.

Standard Curve of Prednisolone

The working standard solution of prednisolone was prepared by further diluting the standard stock solution. The aliquots of 0.2ml, 0.4ml up to 2.0 ml of stock solution were taken into series of 10ml volumetric flask and volume was made up to the mark with methanol to give the final concentration of 2 µg/ml to 20 µg/ml. The solutions were filtered through the whattmann filter paper and filtrate analyzed at λ_{max} 238 nm by using UV-Visible spectrophotometer [13].

Simultaneous drug analysis of ofloxacin and prednisolone by UV Spectroscopy

Preparation of standard stock solution of ofloxacin and prednisolone

Accurately weighed 10 mg ofloxacin and 10 mg prednisolone was transferred into clean, dry 100 ml volumetric flask separately and dissolved with sufficient volume of methanol to get the concentration of 1000 µg/ml. From this solution, 1 ml of solution was pipette out and the volume was made up to 100 ml with methanol to obtain standard solution having concentration 10µg/ml of ofloxacin and 10µg/ml of prednisolone [14].

Preparation of Mixed Standard Working Solution of Ofloxacin& Prednisolone

Accurately measured standard solutions of Ofloxacin (1, 2, 3, 4, 5, 6, 7, 8, & 9 ml) and prednisolone (9, 8, 7, 6, 5, 4, 3, 2, & 1 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of the solutions were measured at 299.5 nm (λ-max of Ofloxacin) and at 238 nm (λ-max of prednisolone) against methanol as blank. The calibration curves were prepared by plotting absorbance versus concentration and the regression equations were calculated.

B. Methods of Preparation of formulations

Preparation of Ofloxacin& Prednisolone loaded NLCs

The nanostructured lipid carriers of ofloxacin and prednisolone were prepared by Melt-emulsification followed by ultra-sonication technique. In this technique the lipid phase and aqueous phases were prepared separately. The oil phase consisted of oils (solid lipid and liquid lipid), and drugs while the aqueous phase consisted of double-distilled water and hydrophilic emulsifiers. The lipid phase containing solid lipid (stearic acid) and liquid lipid (neem oil) were blended and melted at 60°C above the melting points of lipids to form a uniform and clear oil phase. To this drug (100 mg w/w) was added. Meanwhile, the aqueous phase containing emulsifying agent (10% tween 80w/w) dispersed in distilled water (Q.S). The oil phase was added drop wise to the aqueous phase at the same temperature by the aid of agitation at 600rpm for 10min. The coarse emulsion was further treated by probe-type sonicator (at 100% amplitude and 6 cycles) for 20 min. Subsequently the dispersion was cooled in ice water bath to room temperature and stored at 4°C [15].

Table No. 1: Formulation table of Ofloxacin

Ingredients	Formulation batches (in mg)					
	NLC 1	NLC 2	NLC 3	NLC 4	NLC 5	NLC 6
Ofloxacin	100	100	100	100	100	100
Stearic acid	500	600	700	800	900	400
Neem oil	500	400	300	200	100	100
Cholesterol	0.15	0.15	0.15	0.15	0.15	0.15
Span 20	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
Tweens 80	3600	3600	3600	3600	3600	3600
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Table No. 2: Formulation table of Prednisolone

Ingredients	Formulation batches (in mg)		
	NLC 7	NLC 8	NLC 9
Prednisolone	100	100	100
Stearic acid	500	700	800
Neem oil	500	300	200
Cholesterol	0.15	0.15	0.15
Span 20	1.5%	1.5%	1.5%
Tweens 80	6%	6%	6%
Distilled water	100 ml	100 ml	100 ml

Preparation of optimized NLC containing gel

The optimized batch of NLCs was incorporated into the gel. Carbopol (1%w/v) was used as a gelling agent. It was added to the NLC dispersion with overhead stirring at 800 rpm (Remi, Mumbai, India). Stirring was continued until carbopol dispersed. Methyl paraben was added as preservative. The carbopol dispersion was neutralized using 0.05% (w/w) triethanolamine and pH of gel was adjusted to 7.4.

Table No. 3: Composition of gel containing NLCs of ofloxacin& prednisolone

S. No.	Ingredients	Quantity
1.	NLC dispersion of ofloxacin	Equivalent to 50 mg
2.	NLC dispersion of prednisolone	Equivalent to 50 mg
3.	Carbopol 934 P	1%
4.	Methyl paraben	0.05%
5.	Triethanolamine	Q.S.
6.	Distilled water	Q.S. to make 50gm

C.Evaluation parameters

Morphological studies

Surface morphology of nanostructured lipid carriers of ofloxacin& prednisolone was observed by scanning electron microscope (SEM JSM-6490LV) using gold sputter technique. The dried samples of NLCs were mounted on the aluminium stub and coated the sample with gold sputter coater. The image was scanned at an acceleration voltage of 15 kV with a chamber pressure of 0.8 mmHg [12].

Particle size analysis

The diameter (Z-ave) and particle-size distribution (evalu¬ated by the polydispersity index [PI]) are parameters that have a direct impact on the physical stability of NLC dispersion. Photon correlation spectroscopy, also known as dynamic light scattering (DLS), and laser diffractometry (LD) are the most widely used techniques for measuring the size and dis¬tribution of particles in colloidal dispersions. Particle size of NLC was determined by using zeta sizer (Nano plus 3). For particle size analysis, NLC dispersions were suspended in double distilled water, in order to avoid the multi dispersion of the light caused by a high concentration of particles. All experiments were performed under controlled temperature condition at 25±1°C [16].

Poly dispersity index: The polydispersity index (PDI) is a measure of the width of the particle size distribution. Poly dispersity index value was used to characterize the mono dispersed and poly dispersed nature of nano particles. Higher the Polydispersity index values indicate the high level of non-uniformity [12]. It was determined by using Nano plus zeta sizer.

Drug content

A fixed amount of NLC dispersion (200 mg) was taken in volumetric flask and suspended in methanol with continuous shaking for 30 min to ensure the release of entrapped drug in the media. This solution was transferred into a centrifuge tube and centrifuged at 12000 rpm

for 15min at 20˚C. The supernatant was filtered through a 0.45 um membrane filter and diluted with sufficient volume of methanol. The amount of the drug (ofloxacin& prednisolone) in the supernatant was determined spectrophotometrically (UV-VIS Spectrophotometer, Shimadzu Japan 1601) at 299.5nm and at 238nm respectively using regression equation derived from the standard curve. Each value was measured in triplicates, and the results are presented as mean ± standard deviation [17].

In–vitro drug release

The in vitro drug release was done using a modified vertical diffusion cell using egg membrane to analyze the release profile of each drug from the polymer matrices. Phosphate buffer of pH7.4 was used as receptor medium. A fixed amount of NLC dispersion was placed in the donor compartment and the recipient compartment was filled with Phosphate buffer of pH 7.4. The temperature of the receptor compartment was maintained at 37± 0.5˚C under continuous stirring. At predetermined time intervals 1 ml sample was withdrawn from the sampling port and replaced by the same volume of freshly prepared phosphate buffer solution. The samples were than analyzed spectrophotometrically at 299.9 nm and at 238nm [18].

Drug release kinetics study

The release of drug loaded nanostructured lipid carriers statistics was studied using, zero-order (cumulative amount of drug release versus time), first-order (log cumulative percentage of drug remaining versus time), higuchi (cumulative percentage of release versus square root of time) and korsmeyer-Peppas (log cumulative percentage of drug released versus log time) equation models [19, 20, 21].

Table No. 4: Ritger-Peppas diffusion exponent and mechanism of diffusional release from various swellable controlled release systems

Release Exponent (n)			
Thin Film	Cylendrical Sample	Spherical Sample	Drug Release Mechanism
0.5	0.45	0.43	Fickian Diffusion
0.5<n<1.0	0.45<n<0.89	0.43<n<0.85	Anomalous(non-fickian) Diffusion
1	0.89	0.85	Non-Fickian case II
n>1.0	-	-	Non-Fickian Super case II

Table No. 5: Ritger-Peppas diffusion exponent and mechanism of diffusional release from various non-swellable controlled release systems

Release Exponent (n)			
Thin Film	Cylindrical Sample	Spherical Sample	Drug Release Mechanism
0.5	0.45	0.43	Fickian Diffusion
0.5<n<1.0	0.45<n<1.0	0.43<n<1.0	Anomalous(non-fickian) Diffusion
1	1.0	1.0	Non-Fickian case II
n>1.0	-	-	Non-Fickian Super case II

D.Evaluation parameters of gel containing NLCs

The basic physicochemical parameters were studied for the developed formulation as follows-

Physical examination of NLC containing gel

The physical appearance of developed formulations was visually observed for their color, homogeneity, consistency and phase separation [22].

pH of NLC containing gel

The pH of gel was measured by using digital pH meter. One gram of gel was dissolved in 50ml of distilled water and stored for two hours. The pH meter probe immersed into it and the observed pH value was recorded in triplicates [23].

Viscosity of NLC containing gel

A Brookfield digital viscometer (DV-E Viscometer, Brookfield Engineering laboratories) was used to measure the viscosity of ofloxacin and prednisolone loaded NLC gel. Sample holder of the viscometer was filled with gel and then the spindle was inserted into sample holder. The spindle was rotated at 100 rpm. Whole experiment was carried out at room temperature with a no. 6 spindle and viscosity measurement was done in triplicates.

Spreadability of NLC containing gel

0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted [18].

Drug content of gel

The concentration of ofloxacin and prednisolone in NLC gel was determined by simultaneous method as reported in Kumar et al., 2016. A weighed quantity of gel was diluted with 100ml methanol and the resulting solution was filtered through 0.45 um membrane filters. The samples were suitably diluted and analyzed spectrophotometrically. A simultaneous estimation for ofloxacin and prednisolone at 299.5 and 238 nm, respectively was employed using UV-visible spectrophotometer [22].

In–vitro drug release study of gel

In vitro release study of the formulated NLC gel was performed using self modified diffusion cell assembly through excised hairless abdominal goat skin (Obtained from slaughter house). Abdominal skin of the goat was excised; hair was removed carefully by using a hair depilatories. The dermal surface was carefully cleaned to remove subcutaneous tissue and fats without damaging the epidermal surface and prepared into specified size of 2 cm in diameter and soaked in phosphate buffer 7.4 pH for15 min. The receptor compartment of the diffusion cell was filled with 50 ml of methanol along with a, magnetic bead. The optimized gel (1gm) was taken in the donor compartment. Samples were withdrawn from the receptor compartment at predetermined interval up to 8 hours and then replaced with the same amount of methanol. Drug concentration was determined at the wavelength 299.5nm and 238nm (for ofloxacin & prednisolone respectively) by UV spectrophotometer. All the measurements were carried out in doublet.

Drug release kinetics of gel

The obtained data from in – vitro drug release studies was plotted as percent drug release vs. time (Zero order equation), percent drug release vs. square root of time (Higuchi's model equation), log percent of drug remaining vs. time (First order equation) and log percent of drug released vs. log time (Korsmeyer's equation) to evaluate the drug release mechanism. Standard values of release

mechanism with variations were noted.

Results and Discussion

Pre-formulation studies

Organoleptic Property of the Drug

The Organoleptic properties of Ofloxacin& Prednisolone were characterized on the basis of their colour, odour and taxture. The results of these parameters are given below:

1- Ofloxacin: Colour - Pale yellow or yellowish white Texture - Crystalline powder Odour - Odourless

The physical properties compiled to that described for standards [24].

2- Prednisolone: Colour - White Texture - Crystalline powder Odour - Odourless

The above properties were similar to that described for standard drug [25].

Melting Point

The observed melting point of ofloxacin was found to be in a range of 250 - 257°C and the observed melting point of prednisolone was found to be in a range of 230°C. These results are then compiled with IP standards and found to be same as reported in reference, which indicates the purity of both drugs.

Solubility

Solubility studies of ofloxacin in different solvents at room temperature revealed that it was freely soluble in water, glacial acetic acid, 0.1N HCl, phosphate buffer (pH 7.4), chloroform, slightly soluble in ethanol, dichloro methane, sparingly soluble in methanol and insoluble in acetone. Solubility studies of prednisolone in different solvents at room temperature revealed that it was very slightly soluble in water, phosphate buffer (pH 7.4) soluble in methanol, dioxane and sparingly soluble in 0.1N HCl.

Compatibility Studies of Drugs

FT-IR studies were done to confirm the identity of pure drug and detect the interaction of drug with excipients used in the preparation of nanostructured lipid carriers. Formulation NLC 1, NLC 2 and NLC 7 were analysed for FTIR scanning after scanning of ofloxacin, prednisolone, stearic acid, neem oil and cholesterol.

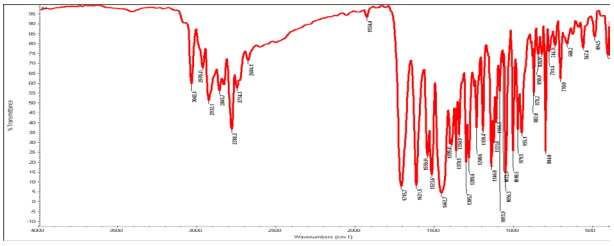

Figure No. 1: FT-IR Spectra of Ofloxacin

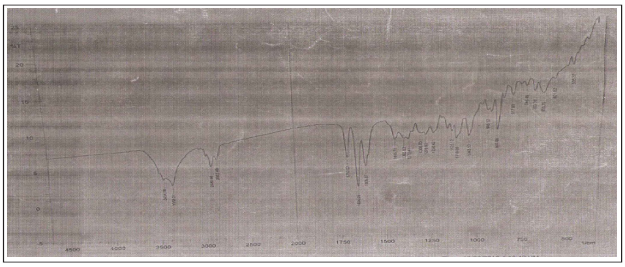

Figure No. 2: FT –IR spectra of Prednisolone

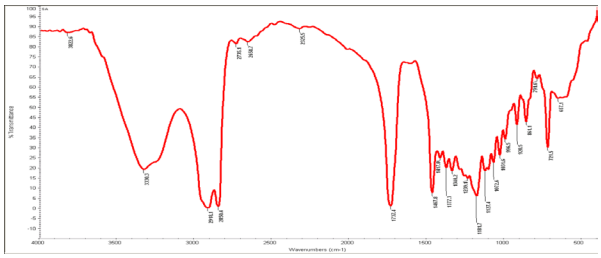

Figure No. 3: FT-IR spectra of Stearic acid

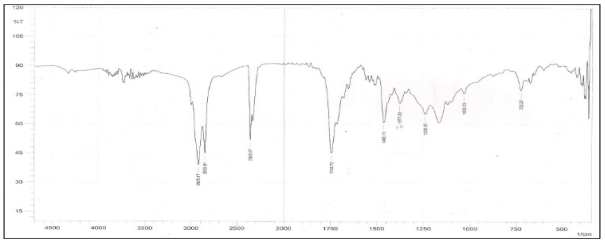

Figure No. 4: FT-IR Spectra of Neem oil

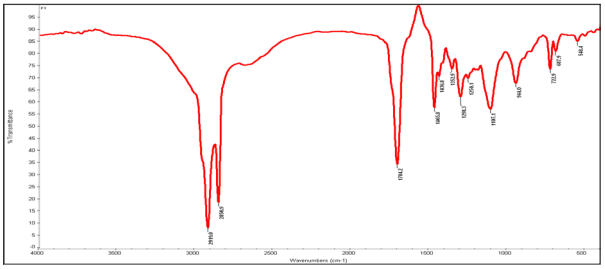

Figure No. 5: FT-IR Spectra of NLC 1

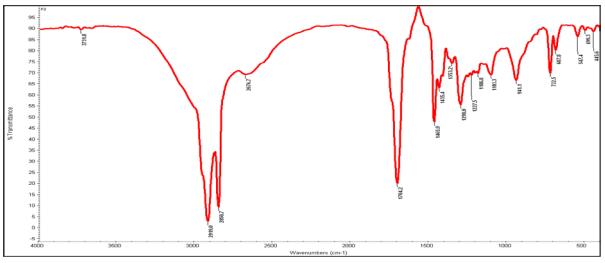

Figure No. 6: FT-IR Spectra of NLC 2

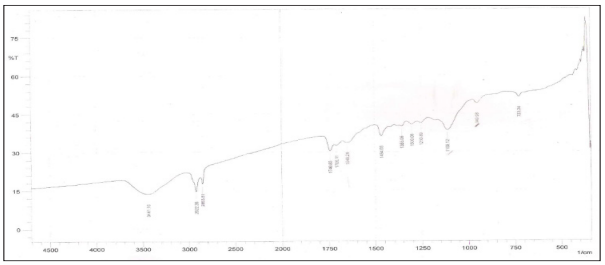

Figure No. 7: FT-IR Spectra of NLC 7

Determination of maximum wavelength (λmax) of ofloxacin and prednisolone in methanol

The standard stock solution of concentration 100 µg/ml of ofloxacin and prednisolone was prepared in methanol and the resulting solution was scanned in UV- spectrophotometer at 200 nm to 400 nm. The maximum wavelength was found 299.5 nm and 238 nm respectively.

Standard Curve of Ofloxacin& prednisolone

Variable concentrations of ofloxacin& prednisolone (1µg/ml to 10µg/ml) were prepared from the stock solution (100µg/ml) in methanol and the absorbance of drug was noted at 299.5 nm against reference. The regression equation of the curve was found to be y = 0.080x + 0.016 (ofloxacin)y = 0.413x + 0.024 (prednisolone) and the regression coefficient of the drug was found to be close to 1, this indicates that the curve obey Beer's law as depicted follows:

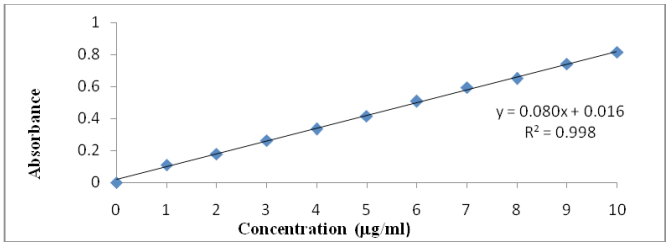

Figure No. 8: Standard curve of ofloxacin in methanol at λmax 299.5

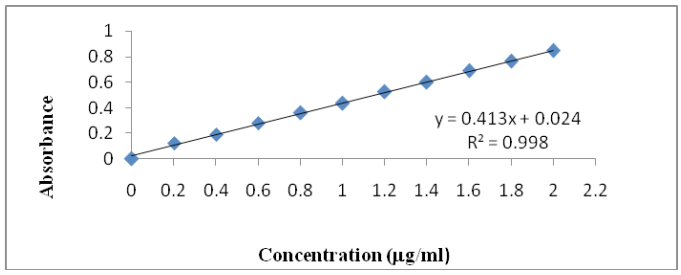

Figure 9: Standard curve of prednisolone at λ_{max} 238 nm

Simultaneous drug analysis by UV spectroscopy

The two wavelengths 299.5 nm (λ-max of OFL) and 238 nm (λ-max of PRD) were used at which the calibration curves were prepared for ofloxacin (2-20µg/ml) and prednisolone (0.1-1µg/ml). The regression equation of curve was found to be y = 0.005x + 0.004 for ofloxacin and y = 0.003x + 0.004 for prednisolone with R2 value 0.998 &0.996 respectively.

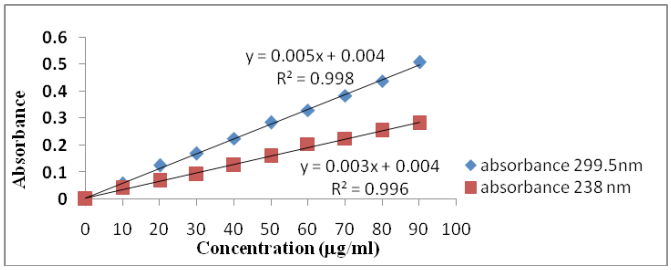

Figure 10: Standard curve of ofloxacin and prednisolone at λ_{max} 299.5 nm &λ_{max} 238 nm.

B.Evaluation parameters of NLCs of ofloxacin& prednisolone

Morphological studies

Surface morphology of nanostructured lipid carriers of ofloxacin& prednisolone was studied using scanning electron microscope (SEM JSM-6490LV). The SEM photomicrograph revealed that the NLCs of selected formulations were spherical with more or less smooth surface.

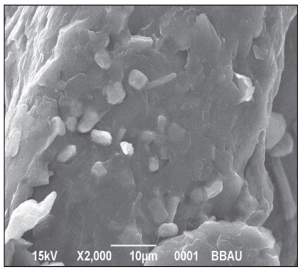

Figure 11: SEM image of optimized ofloxacin NLC

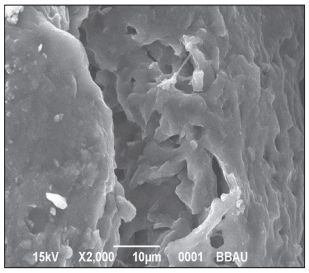

Figure 12: SEM image of optimized prednisolone NLC

Particle Size analysis

It showed that NLCs loaded with ofloxacin had the particle size in range from 512.3nm to 1703nm and the value of polydispersity index was found to be in a range from 0.399 to 0.742. The NLCs loaded with prednisolone had showed the particle size was between 539.3nm to 1736.7nm and poly dispersity index found between 0.335 - 0.711.

Table 6: Particle size and Poly - dispersity index of Ofloxacin formulations (NLC1 NLC6)

Formulation Code	Particle size (nm)	Polydispersity index (PDI)
NLC 1	512.3	0.399
NLC 2	543.4	0.742
NLC 3	1703	0.680
NLC 4	873.6	0.430
NLC 5	1697.8	0.523
NLC 6	1128.1	0.536

Table 7: Particle size and Poly - dispersity index of Prednisolone formulations (NLC7- NLC9)

Formulation Code	Particle size (nm)	Polydispersity index (PDI)
NLC 7	539.3	0.335
NLC 8	1736.7	0.711
NLC 9	727.5	0.400

Drug content

The content of ofloxacin and prednisolone was determined in all the selected NLC preparations (NLC1 to NLC9). The content was found in range of 56.7 - 75.6% for ofloxacin and 65.9 – 81.8% for prednisolone, which indicates good entrapment efficiency of ofloxacin and prednisolone in the NLCs.

Table 8: Drug content of Ofloxacin (Mean± SD, n=3)

S. No.	Formulation code	Drug content (%)
1.	NLC 1	63.61±0.79
2.	NLC 2	63.45±1.20
3.	NLC 3	71.03±1.01
4.	NLC 4	75.61±0.38
5.	NLC 5	56.71±0.69
6.	NLC 6	63.78±1.78

Table 9: Drug content of Prednisolone (Mean± SD, n=3)

S.No.	Formulation code	Drug content (%)
1.	NLC 7	69.93±0.64
2.	NLC 8	65.98±1.69
3.	NLC 9	81.89±0.90

In–vitro drug release

In- vitro release study of ofloxacin and prednisolone loaded nanostructured lipid carriers were carried out. Table 10 and 11 exhibits the in vitro release.

Table 10: *In - vitro* release profile of ofloxacin loaded NLC (Mean ± S.D., n=3)

Time (inhrs)	NLC1 % release	NLC2 % release	NLC 3 % release	NLC 4 % release	NLC 5 % release	NLC 6 % release
0	0	0	0	0	0	0
0.5	5.065±0.50	2.704 ±0.94	3.612 ±0.43	7.084±0.45	2.192 ±0.75	1.193±0.98
1	11.224±0.53	5.555 ± 1.02	7.719 ±0.41	12.609±1.24	5.307±0.01	2.910±1.27
2	24.406±1.30	10.594 ±0.71	14.894±0.31	23.531±0.58	11.552 ±1.52	6.883±0.23
3	33.760±1.66	15.766±1.12	20.569±0.99	33.627±1.34	16.236±0.16	13.793±0.85
4	44.877 ±3.52	25.411±0.49	35.015±1.35	41.464±0.13	26.132±5.44	17.479±0.04
6	54.904±1.62	37.465±2.29	47.683±1.29	51.935±0.54	31.904±1.44	29.479±0.43
8	68.637±0.73	53.047±3.86	57.894±0.98	62.508±1.05	47.431 ±4.00	41.241±1.59
10	75.008±0.58	63.389±3.9	69.545±0.83	73.077±0.69	59.747±1.49	49.340±2.61
24	83.371±1.70	70.118±4.5	81.214±3.17	80.688±0.33	79.396±1.53	66.773±1.97

Table 11: *In vitro* release profile of prednisolone loaded NLC (Mean ± S.D., n=3)

Time (in hrs)	NLC 7 % release	NLC8 % release	NLC 9 % release
0	0	0	0
0.5	7.855±0.68	7.473 ±1.01	3.930 ± 0.61
1	13.043 ±0.0.58	15.756 ±1.72	7.596 ±1.2
2	19.185 ±0.36	26.173 ±1.87	11.865 ±0.66
3	30.052 ±0.80	39.490 ±0.89	22.574 ±1.32
4	42.664 ±0.27	48.981 ±1.74	30.345 ±1.10
6	51.105 ±1.59	54.380 ±1.62	38.290 ±1.63
8	59.869 ±0.50	62.294 ±0.23	46.425 ±0.72
10	65.238 ±0.90	68.230 ±0.74	58.645 ± 3.01
24	71.673 ±1.57	73.961±0.53	71.364± 0.50

In-vitro drug release kinetics

The regression coefficients (R2) value for all formulations was calculated and the best fit model was selected on the basis of values of regression coefficients. Among the model tested, the drug release profiles of ofloxacin formulations NLC1, NLC2, NLC3, NLC4, NLC5& NLC6 were best fitted in Korsmeyer - peppas model as the regression coefficients were 0.960, 0.964, 0.977, 0.950, 0.980 & 0.987 respectively and they were close to 1. On the other hand, the drug release profile of prednisolone NLC formulations (NLC7 & NLC8) were closed to higuchi model based on the regression coefficients 0.899, 0.883 respectively whereas, NLC 9 was closed to Korsmeyer model as R2 value shown 0.953.

Table 12: *In – vitro* release kinetics of ofloxacin loaded NLCs

Formulation code	Zero order		First order		Higuchi		Korsmeyer– peppas model	
	R^2	t_{50} (hr)	R^2	t_{50} (hr)	R^2	t_{50} (hr)	R^2	t_{50} (hr)
NLC 1	0.702	8.669	0.399	11.999	0.910	6.271	0.960	3.752
NLC 2	0.802	13.065	0.521	14.402	0.924	11.111	0.964	8.090
NLC 3	0.789	10.498	0.498	13.216	0.925	8.170	0.977	4.396

NLC 4	0.724	9.253	0.388	12.273	0.925	6.800	0.950	4.523
NLC 5	0.880	12.159	0.545	14.138	0.948	10.290	0.980	5.134
NLC 6	0.874	14.886	0.567	15.535	0.939	13.873	0.987	9.588

Table 13: *In – vitro* release kinetics of prednisolone loaded NLCs

Formulation code	Zero order		First order		Higuchi		Korsmeyer– peppas model	
	R^2	t_{50} (hr)	R^2	t_{50} (hr)	R^2	t_{50} (hr)	R^2	t_{50} (hr)
NLC 7	0.679	10.591	0.381	12.917	0.899	2.825	0.884	7.411
NLC 8	0.633	9.410	0.340	12.254	0.883	2.608	0.878	6.683
NLC 9	0.815	12.683	0.486	14.192	0.949	3.256	0.953	7.715

Evaluation parameters of gel containing NLCs of ofloxacin& prednisolone

Physical examination of NLC containing gel

NLC gel was examined against black and white background for transparency, smoothness and homogeneity in appearance. On the basis of physical appearance, ofloxacin and prednisolone loaded NLC gel was found to be transparent viscous with smooth and homogenous in appearance.

Determination of pH, spreadability& viscosity of NLC containing gel

The pH of optimized NLC gel was found to be between 7.3±0.03, which is within the acceptable limits and may be rendered its solubility for topical application to skin without irritation. The spreadability of gel was found to be 6 cm and viscosity of gel was found to be 1500 cps.

Drug content of NLC containing gel

The amount of ofloxacin as well as prednisolone was determined at 299.5 and 238nm respectively using UV-visible spectrophotometer (1601, Shimadzu, Japan). Drug content of ofloxacin and prednisolone of optimized NLC gel was found to be 56.66 ± 0.27 & 48.38 ± 0.24% respectively.

In vitro drug release study of NLC containing gel

In vitro drug diffusion study was performed using self-modified diffusion cell assembly. 1gm of the sample was withdrawn at fixed time intervals and the same volume of fresh medium (methanol) was added accordingly. Samples were analyzed using UV spectroscopy method at 299.5 nm and 238 nm wavelengths respectively. All the operations were carried out in doublets.The particle size analysis of ofloxacin and prednisolone loaded NLC revealed a variation in the sizes of the different formulations. The results indicate that particle size was influenced by most of the formulation variables such as concentration of lipids, surfactant concentration, stirring speed, and temperature. Leaving all other parameters constant, in this study variable was concentration of solid lipid and liquid lipid. The results indicate that the amount of stearic acid and neem oil had great impact on the NLC dispersions. It has been observed that the NLC prepared from mixture of stearic acid and neem oil (1:1) showed fairly lowest particle size (NLC 1 & NLC 7 formulations having particle size 512.3nm & 539.3nm respectively) and NLCs prepared from mixture of stearic acid and neem oil ratio 9:1 & 7:3 showed increase in particle size up to 1697.8 nm and 1736.7nm in NLC5 & NLC8 respectively.

The results were in accordance to that reported by some researchers in terms of lipid aggregates generated with high lipid concentration. Hence increase in concentration of solid lipid increased particle size of NLCs. Ratio 1:1 was appropriated for preparation of NLC.The choice of surfactant and their concentration also has great impact on the quality of NLC dispersion. After investigating the influence of surfactant concentration on the particle size of NLC dispersions, 6% Tweens 80 solution was found to be sufficient to reduce the interfacialtension between the aqueous and lipid phase which leads to the formation of emulsion droplets of smaller size. Additionally, higher surfactant concentration was enough to cover the tiny lipid droplets which stabilized and prevented the coalescence of the nano-emulsion droplets [26].

Influence of surfactant concentration is not well defined on the drug content. Higher percentage entrapment efficiency of prednisolone NLCs can be attributed to the lipophilic nature of drug prednisolone, as it should have higher affinity for lipid matrix. No relationship with solid lipid: liquid lipid ratio could be established with NLCs of prednisolone. NLC 9 has solid lipid: liquid lipid ratio 8:2 showed maximum drug content of 81.19%.

In the mechanism of NLC loaded gel, zero order model is more pronounced than first order plot, Higuchi &Korsmeyer model based on R2 values. A lag time was observed (0-5 hrs) in Higuchi model & (0-1hr) in Korsmeyer's model. This could be because in gel formulation the drug has to cross two diffusion barriers one is the gel and the other is NLC matrix before being absorbed from skin.

Conclusion

Formulation NLC1 (ofloxacin) & Formulation NLC7 (prednisolone) showed low particle size, low PDI, high drug entrapment and better release. So, Formulation NLC1 (ofloxacin) & Formulation NLC7 (prednisolone) were considered as best optimized formulations and were further incorporated into gel base using carbopol 934 P as gelling agent.

Ofloxacin and prednisolone NLCs loaded topical gel formulation was successfully prepared and the methodology used for the preparation of formulation was simple and economic. Present concentration of the gelling agent (Carbopol 934) had significantly affected the residence time and drug release properties of the formulation.

This study provides supplementary evidences that the prepared NLCs had prolonged release effects with good potential for topical delivery and NLC based gel formulation of ofloxacin& prednisolone in combination shows potential as new delivery system for topical application.

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