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Research Article

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF NADIFLOXACIN LOADED SOLID LIPID NANOPARTICLE BASED HYDROGEL

Prashant Kumar [1]*, Sunil Khatak [2]

[1]Raj Kumar Goel Institute of Technology (Pharmacy) 5th Km Stone Delhi Meerut Road, Ghaziabad, U.P. 201003 India

[2]Associate Professor, Raj Kumar Goel Institute of Technology (Pharmacy) Ghaziabad, U.P. India

*Corresponding Author Email: peelwanprashant@gmail.com

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ABSTRACT

The aim of this dissertation work was to develop and characterize an optimal formulation of solid lipid nanoparticles of nadifloxacin, which would then be incorporated into hydrogel. The SLN was developed with drug nadifloxacin, which is poorly water soluble. On the basis of solubility studies (i.e. partitioning effects), the lipid and components were chosen. In this study, the two variables amount of lipid and concentration of Poloxamer 188 were studied. The EE increased as the concentration of Poloxamer 188 increased. The particle size was observed to decrease as the concentration of Poloxamer 188 was increased. The EE increased in a similar way as the amount of lipid was increased. So the purpose was to formulate hydrogel with carbopol 940 with improved drug entrapment, sufficient viscosity, good extrudability, good homogeneity and improved drug release. Hydrogels are polymers that have swelling ability in water or aqueous solvent systems. Due to their increased water content, gels can provide a better feeling for skin than other conventional dosage forms. Hydrogels are insoluble in water. They are not easily removed from the application site.

Keywords: Inflammation, anti-bacterial, hydrogel, rheological activity, pH meter analyser, hot homogenization.

INTRODUCTION

Acne is the common chronic of the skin that affects a large proportion of the men and women worldwide. It is caused by increased sebum excretion and the sebaceous duct hyper-cornification. Acne is triggered by the inflammatory and host immunological activity of Staphylococcus aureus and Propionibacterium acnes. It is a multifactorial disease that affects the pilosebaceous follicles.[1, 2] Nadifloxacin is a fluoroquinolone having broad spectrum topical antibacterial activity used to treat several inflamed acne lesions.[3] The antibacterial action of nadifloxacin on methicillin-resistant Staphylococcus aureus, methicillin-susceptible S. aureus, P. acnes and S. epidermidis bacteria were evaluated and nadifloxacin was not reported resistant to test organism. The minimum inhibitory concentration (MIC90) values of nadifloxacin were 0.78 µg/ml for S. epidermidis and 0.20 µg/ml for P. acnes, while MIC50 values was 0.05 µg/ml and 0.20 µg/ml against S. epidermidis and P. acnes, respectively.[2,4,5] Nadifloxacin is commercially available on the market in traditional formulations such as cream to treat skin infections, where poor penetration through pilosebaceous follicle is the main obstacle. Therefore, a higher active drug concentration is not sustained for an extended period. Carrier based drug delivery by solid lipid nanoparticles based hydrogel is an advanced delivery technique for the treatment of acne through follicular targets.[6] The hydrogel of solid lipid nanoparticles composed of digestible and non-digestible lipid and water can deliver therapeutics to the site of action and achieve an effective concentration of drug than traditional formulations such as cream, gel and ointment. The higher amount of incorporation of drug and vesicle of reduced size allows higher drug penetration and produce sustained release effect at the action site.[7] This investigation aimed at developing and evaluating the nadifloxacin loaded solid lipid nanoparticles based hydrogel so that the drug is penetrated more deeply into the skin, sustained release of drug, increased degree of retention in the layers of the skin.

MATERIALS AND METHODS

Nadifloxacin (BP) was purchased from Sherincorps Solutions Inc., Indore, India. Glyceryl mono stearate (GMS), Carbopol 940, Poloxamer 188, Triethanolamine, Methyl-p-hydroxy benzoate, Methanol, and Propyl-p-hydroxy benzoate were obtained from Sigma-Aldrich Chemicals Pvt. Ltd. Bangalore, India. All the reagents and chemicals were of analytical grade. The experiments have been conducted using double distilled water. pH Meter (Systronics Water Analyser 371), Centrifuge (REMI R-8C Laboratory Centrifuge), High Speed Homogenizer (IKA® T18 Digital Ultra Turrax), Magnetic Stirrer (Jindal S.M. Scientific Instruments (P) Ltd.), Water Bath Shaker (HICON® ISO 9001:2000), Mechanical Shaker (Wrist Action Shaking Machine), Probe Sonicator (Orchid Scientifics Ultrasonic Homogenizer), Fourier Transform Infrared Spectrometer (JASCO FT/IR-4100typeA), UV Spectrophotometer (Shimadzu Corp. PharmaSpec UV 1700), Viscometer (Brookfield DV-E Viscometer), Zetasizer [Malvern (MAL1099378) Nano Series Zetasizer], Melting Point Apparatus (Decibel), Heating Plate (Laboratory Heating Plate ISO Certified), SEM, Photo Electron Microscope (Binocular Research Microscope RXL-5T).

Preformulation Studies

Pre-formulation studies are important for the determination of different physical and chemical properties of active pharmaceutical ingredients for their efficacy, safety and stability.

Physicochemical Properties

Nadifloxacin were characterized into based on physical chemical properties including colour, odour, physical shape as an amorphous or crystalline form and melting point. The water and phosphate buffer solubility was measured at various pH levels.

Melting Point

A capillary fusion process used to determine the melting point of the drug sample. In a capillary which has closed from one end, a small amount of drug has been inserted. This capillary was put in a melting point control device (Decibel, Digital auto melting point apparatus). The thermometer was used to detect the temperature at which the medicine begins to melt and compared the value in the literature references.

Partition-coefficient

The rate and the absorption degree of hydrophilic and lipophilic drug is crucial to predict. A drug's partition coefficient is important for the calculation of its efficiency for lipophilic characteristic. The lipophilic or hydrophilic nature of drug is thus characterized. P-value of drugs above 1 are considered lipophilic while those with nadifloxacin partition coefficient in n-octanol/water have been identified using separating funnel. 100mg drug was weighed precisely to 50ml per n-octanol and aqueous phase in a separating funnel. The bottle was put on a handle shaker for 6 hour before the balance was touched. Separation of the phase done by the separating funnel, and for the volume of the drug divided in it after sufficient dilution the aqueous phase has been analysed (Table 1). Absorption done by the Shimadzu UV-visible Spectrophotometer has been reported. Calculation of the partition coefficient done by the formula below:

$$P_{o/w} = (C_{oil} / C_{water}) \text{ equilibrium}$$

Where, C_{oil} = drug conc. in the oily phase (octanol), C_{water} = drug conc. in aqueous phase (water)

FTIR

IR spectrum of the sample drug was developed by the application of FTIR (Jasco). High grade KBr in 100mg was mixed with 1mg of the sample drug by trituration in a mortar and allow the mixture to condensed into a pellet at 10 ton/cm^2 with a pellet maker. The pellet obtained was scanned in between 4000-400 cm^1. The developed IR spectrum was compared with the officially reported monograph of Nadifloxacin.

Analysis of UV Spectra

Nadifloxacin standard solution (10 µg/ml) in the methanol and pH 6.8 phosphate buffer were prepared separately. In the spectrometric range between 200-400 nm, solutions were scanned. The λ_{max} were calculated from the spectra obtained and compared to λ_{max} of the official monograph of the respective solvents (methanol and phosphate buffer pH 6.8).

Analytical Methodology

Stock and Standard Solution Preparation

Preparation of Stock Solution

10mg of drug Nadifloxacin was dissolved in 10 ml of methanol to get stock solution of 1000 µg/ml in concentration.

Preparation of Standard Solution

5 ml of the stock solution that is 1000 µg/ml in concentration was further diluted with 50 ml of methanol to develop the standard solution of 100 µg/ml in concentration.

Determination of λ_{max}

Nadifloxacin has been set up separately and spectroscopically in a regular arrangement (100 µg/ml) about 200 to 400 nm in methanol. In each dissolvable drug spectrum was taken. In contrast to the one provided in the official monographs, the acquired λ_{max} of Nadifloxacin in methanol.

Preparation of Calibration Curve

Calibration Curve of Nadifloxacin was developed in Methanol as follows

Different concentrations or dilutions of the drug sample Nadifloxacin, varied from 2-20 µg/ml were prepared for the calibration curve. The absorbance by UV spectrophotometer against the reference blank of methanol was measured at 270 nm. Computer data have been analyzed and statistical parameters identified.

Formulation Procedure of SLNs

Methodology for Preparation of SLNs by Hot homogenization followed by Ultrasonication

Hot homogenization accompanied by ultrasonicator was the technique used to develop SLNs. A positive lipid measurement over its liquefying point was dissolved at around 10 °C and predetermined drug measurement was applied to obtain a simple soft arrangement of drug lipid (oil stage). Dissolving the surfactant in 100 ml double refined water, and warming it at the same temperature as the oil phase, has established a fluid level. The hot fluid phase was applied to the oil phase and homogenized at a temperature of 15000 rpm, which took 15 minutes with the Digital Ultra Turrax homogenizer. The resulting hot lipid was additionally sonicated by a test-sonicator for 5 minutes at 60 percent capacity. The NAD-SLN distribution obtained was cooled to room temperature.

Selection of Components for SLN

Following experiments were conducted to select the components for SLNs preparation:

Lipids Selection

The lipid selection for SLN formulation is a significant factor, and a partition coefficient, percent encapsulation and loading ability were considered dependent on lipid solubility. Different solid lipids have melting spots that have been screened for this study more than the room temperature such as Compritol 888 ATO, stearic acid, tristearate and GMS. (Table 2) Dissolving the drug into minimum quantity of ethanol, 5 mL of hot, 0.1 N of HCL solution, and 6 hours at hot bath water shaker (HICON Waterbath shaker) were used in each lipid melt. Dissolving and dispersing the drug was done in the drug. Following cooling, the water step was isolated by a syringe filter of 0.22 µm and the drug content was analyzed by UV spectroscopy. The partition coefficient of the drug / aqueous solution was determined by:

Partition coefficient = (Initial amount of drug – amount of drug in aqueous phase) / (Amount of drug in aqueous phase)

Surfactant Selection

Four non-ionic forms with an identical mechanism of stabilization were screened in the present study (Poloxamer 188, Propylene glycol, HPMC, Poloxamer 407). The selection by developing SLNs with lipid-surfactant ratio, that is to say 1:1 % w/w. Analysis for the size of particle and zeta potential all the SLNs preparations utilizing the above mentioned surfactants were presented in Table 3

Drug-Lipid Ratio Estimation

An accurately weighed NAD (250 mg) was done to optimize the drug-lipid ratio. This fixed quantity of drug has been has been dispersed into the lipid melt (GMS) with various ratios of drug-lipid (1:1 to 1:5). The surfactant concentration (Poloxamer 188) at 0.25% for all formulations was kept constant. (Table 4)

Lipid-Surfactant Ratio Estimation

The lipid-surfactant ratio determination was done by weighing precisely the screened lipid (GMS, 500 mg). The certain volume of surfactant solution (Poloxamer 188) was distributed in the lipid melt (GMS) with various lipid-surfactant ratios (1:0.5, 1:1, 1:1.5, 1:2). For all formulations the lipid concentration (GMS) was maintained constantly. (Table 5)

Preparation of Optimized SLNs

The final optimized formulation of NAD-SLNs was based on the utilization of estimated and selected surfactant and lipid. The table 6 includes the estimated quantity of the selected surfactant and lipid.

The SLNs have been developed by the utilization of hot homogenization method followed by probe sonication.

Characterization of Optimized SLNs

In order to estimate their different characteristics, the optimized SLNs were determined based on certain parameters:

Particle size and Zeta Potential

Zeta potential is an important method for characterizing the surface that is useful to assess the stability and surface charge on the nanoparticle system. A Zeta sizer was used to analyse the mean particle size and distribution of particle size of the bulk dispersion of prepared SLNs. Zeta sizer was MAN0318-4-0 Malvern, Zetasizer (Nanoseries) ZSP available at Maharshi Dayanand University, Rohtak, Haryana, India utilized for the analysis.

Determination of Percentage Entrapment Efficiency

Entrapment efficiency is the percentage of a drug which is entrapped in a nanoparticle or micelle. With the determination of the free drug amount in the dispersion medium, the percentage of entrapment efficiency of the developed NAD-SLNs was determined. For 20 minutes, the formulation of nano-lipid dispersion was centrifuged (utilizing REMI R-8C Laboratory Centrifuge). Appropriate dilution with DMSO resulted in a transparent supernatant after centrifugation analysed by an ultraviolet spectrophotometer at 270 nm. The % EE was based on the formula described below:

$$\% \text{ EE} = [(W_{\text{total drug}} - W_{\text{free drug}}) / W_{\text{initial drug}}] \times 100$$

$W_{\text{initial drug}}$ = initial drug weight, $W_{\text{free drug}}$ = free drug weight tested for aqueous dispersion supernatant after centrifugation

Loading Capacity

Loading capacity is determined by the estimation of the entrapped drug per unit weight of the nanoparticle, referring to the weight level as a consequence of the entrapped drug. The measurement of the absolute drug entrapped separated by the overall weight of the SLNs tends to be determined. With the use of the following formula the loading capacity can be calculated:

$$\% \text{ Drug loading} = [(W_{\text{total drug}} - W_{\text{free drug}}) / W_{\text{SLNs}}] \times 100$$

Where; $W_{\text{initial drug}}$ = initial drug weight, $W_{\text{free drug}}$ = free drug weight tested for aqueous dispersion supernatant after centrifugation, W_{SLNs} = SLNs total weight

Scanning Electron Microscopy

Scanning electron microscopy is a method utilized for the estimation of particles surface morphology to determine their size. SEM has been used to analyse the morphology of the particles for the optimized freeze-dried SLNs.

Preparation of SLNs based Hydrogel

SLN formulation were selected at desirable component ratios to prepare hydrogel, after SLNs particle size and morphology have been characterized. The Carbopol 940 used as a polymer to develop the SLNs-based hydrogel to enhance the viscosity of the SLNs for topical use. The hydrogel was mixed slowly with SLNs under constant stirring. The viscosity of the SLNs-based hydrogel system decreased as the SLNs were incorporated to the hydrogel. Therefore, hydrogels were formulated in different concentrations (1, 1.5, and 2% w/w) to obtain desirable viscosity of SLNs based hydrogel and the final concentration was selected based on transparency and viscosity. (Table 7)

Characterization of Hydrogel

Physical Appearance

The consistency, homogeneity, colour, phase separation and grittiness were observed for all the prepared hydrogel.

Extrudability

The gel formulation were filled in regular collapse aluminium tubes and clamped to the end. The tubes have been recorded with respect to their weight. The gel was extruded through applying pressure by the finger on the tube. The extrudability of the gel formulation is determined by measured the gel extruded from the tube.

Determination of pH

Digital pH meter was used to calculate the pH. The hydrogel loaded with SLN was correctly weighed and dispersed in 25ml of distilled water. Before using the pH meter, the buffer solution pH 4.0, 7.0, and 9.0 was calibrated. The formulation pH was measured in all three different buffers and an average value were determined.

Spreadability

It is the degree to which gel spreads easily on the skin. The paddle and drag process is done using a modified propagation device. The block is made of wood and a glass toboggan of 10×10 cm is placed above it. An excess of 2 gm hydrogel has been set on the fixed panel, on which another panel is mounted to add weights. The weight for the expulsion of air is 1 Kg. 80 gm has been applied to the saucepan, and the requisite slipping time is noted. The less time it takes to detach, the greater the diffusion.

$$S = M \times L / T$$

Where, M is attached mass to the top slide, L is the glass slide length, and T is time taken.

Viscosity
Utilizing the Brookfield Viscometer, the SLN-based hydrogel viscosity was calculated. 175 mg of SLN-based hydrogel was filled into a beaker of 250 ml to assess its viscosity and the Spindle number LV4 was used to measure its viscosity.

In vitro Studies

In order to estimate the release of drug (*in vitro)*, dialysis diffusion has been utilized. Nanoparticles were kept in the dialysis bag (Sigma Aldrich D9277) utilizing dialysis membrane. Double-distilled water was employed for the activation of dialysis membrane for 24 hours before use. Lyophilized NAD-SLNs of 200 mg were dispersed in 2 ml of phosphate buffer pH 6.8 and poured into the dialysis bag, whose ends were thread-bound. The dialysis bag of solid dispersion were placed in conical flasks having 50 ml phosphate buffer pH 6.8. The conical flasks were placed on the magnetic stirrer with hot plate at 37 ºC at 100 rpm. Samples of 2ml had to be taken from different time intervals (0.5 h, 1h, 2h, 4h, 6h, 8h, 12h, 18h and 24h) and the same volume of fresh medium were replaced. The samples have been allowed to pass through a nylon syringe filter of 0.22 µm and followed by UV analysis.

***In vitro* Release Kinetics**

Different kinetic models were utilized to express the release kinetics for the estimation of *in vitro* release results. The following are several models expressing the release kinetics of the drug:

Zero Order Kinetic Model
The kinetic model of zero order that provides a representation of percentage cumulative drug release vs. time in graphical form. It describes:

$$C = K_0 t$$

Where, C = drug conc. at time t, K_0 = rate constant of zero order (concentration / time), t = time

First Order Kinetic Model
The first order kinetic model that explains the release from the system with a concentration-dependent release rate. The log % cumulative drug remain vs. time is expressed in the graphical representation.

$$\text{Log } C = \text{Log } C_0 K_1 t / 2.303$$

Where; Log C_0 = drug initial conc. at time t, K_1 = first-order rate constant

Higuchi Model
Higuchi defined the drug release from as a square root of time related process depend on Fickian diffusion. The cumulative % of the drug release versus square root of time is graphical representation.

$$Q = Kt^{1/2}$$

Where; Q = drug release amount at time t, K = diffusion rate constant

Korsmeyer-Peppas Model
The log % cumulative drug release vs. log time is graphically represented by Korsmeyer-Peppas model.

$$Mt/M\infty = K\ t\ n$$

Where; $Mt/M\infty$ = fractional drug release, K = constant (drug properties for geometric and structural), n = release exponent indicative [determined from linear, regression of log ($Mt/M\infty$) vs. log t]

The value of n is less than 0.5 for Fickian diffusion; value of n ranging from 0.5 to 1 for non-Fickian (abnormal / zero order) release; and value of n is equal to 1 for zero order drug release.
The models discussed above were represented by graphs depicted with respective kinetic model equation, that is, Higuchi model, zero order, first order, and Korsmeyer-Peppas model.

RESULTS AND DISCUSSION

Preformulation Studies
In preformulation-studies the following parameters have been evaluated.

Physicochemical Characteristics
The drug sample of NAD was purchased from Sherincorps Solutions Inc., Indore, India and evaluated for different characteristics as follows:

Melting Point
Capillary fusion method was used for the melting point determination and was found to be within a range of 141-142 ºC, comparable to the value reported (Table 8). This illustrates the drug's confirmation.

Partition Coefficient
Calculation of the partition coefficient done by the formula below:

$$P_{o/w} = (C_{oil} / C_{water})\ \text{equilibrium}$$

The estimated average value of $P_{o/w}$ is shown in the Table 9.

Compared to the aqueous phase, NAD showed more affinity in the oily phase. The avg. partition coefficient of NAD was determined to be 1.095, indicating that the drug's nature is lipophilic since its P value is more than 1.

Fourier Transform Infrared Spectroscopy
FTIR was used to assess the IR spectra of Nadifloxacin. As listed in the Table 10, the chromatogram has been compared to its recorded peaks. There were slight variations from all the peaks of the drug sample. Figure 1 shows the IR spectrum of the drug sample.

Analysis by UV Spectrum
After scanning them between the range of 200-400nm, the UV-range of the Nadifloxacin std. solution (100 µg/ml) in methanol was independently filtered. The λ_{max} in dissolvable solvent was estimated from spectra. The λ_{max} of Nadifloxacin was found to be 270 nm, which was consistent with the reference appreciation[9] that confirmed the identifiable evidence of the drug. Figure 2 shows the representation of UV spectrum of Nadifloxacin in methanol.

Analytical Method
The results were obtained using the analysis method based on UV spectroscopy.

Standard and Stock Solution Preparation
The standard and stock solution of NAD in methanol solvent has been developed successfully for further study.

λ_{max} Determination
The λ_{max} of the NAD was determined from the UV analysis in methanol solvent. NAD showed maximum absorbance at 270 nm, corresponding to the reference value.[9] This determined λ_{max} of NAD has been utilized for all UV spectrometric testing.

Table 1: Concentration of components used for the measurement of Partition coefficient for Nadifloxacin

S. No.	Drug amount (NAD) taken in mg	Volume of water (ml)	Volume of Octanol (ml)
1.	100	50	50
2.	100	50	50
3.	100	50	50

Table 2: Numerous lipids utilized for the selection

Lipids for the selection process	Initial amount of drug
GMS	10 mg
Tristearin	
Stearic acid	
Compritol 888 ATO	

Table 3: Constituents utilized for selection of surfactants

S. No.	Surfactant	Lipid	Surfactant-Lipid Ratio (%w/w)
1.	Poloxamer 188	GMS	1:1
2.	Propylene glycol		
3.	HPMC		
4.	Poloxamer 407		

Table 4: Determination of the following Drug-Lipid ratio

Drug	Lipid	Surfactant	Drug-Lipid ratio
NAD	GMS	Poloxamer 188 (0.25% w/v)	1:1
			1:2
			1:3
			1:4

Table 5: Drug-Surfactant ratio determination

Lipid-Surfactant ratio	Amount of lipid utilized
1:0.5	500 mg
1:1	
1:1.5	
1:2	

Table 6: Estimated quantity of the selected surfactant and lipid

Drug	Drug: Lipid ratio	Lipid : Surfactant ratio
Nadifloxacin (1 gm)	NAD : GMS (1:2 % w/w)	GMS : Poloxamer (1:0.5 % w/w)

Table 7: Final concentration of components for the SLNs based hydrogel

Drug Sample	Polymer	pH Modifier	Preservatives	
SLN formulation	Carbopol 940 (1% w/w)	Triethanolamine	Methyl Paraben (0.1%)	Propyl Paraben (0.05%)

Table 8: Physicochemical Characteristics of Nadifloxacin

S. No.	Characteristics	Nadifloxacin	
1.	Physical form	Crystalline powder	
2.	Odour	Odourless	
3.	Colour	Off white to pale yellow	
4.	Melting point	Literature reported[8]	145-147 ºC
		By capillary method	141-142 ºC

Table 9: Partition coefficient of NAD

S. No.	Water : n-octanol	Drug conc. in water (µg/ml)	Drug conc. in n-octanol	Log P (Partition coefficient)	Avg. value of Log P
1.	1:1	4.732	0.432	1.039	1.095
2.	1:1	4.871	0.341	1.154	
3.	1:1	4.739	0.383	1.092	

Table 10: Nadifloxacin identification with FTIR

Types of stretching vibrations	Frequency (cm^{-1})	
	Reported	Observed
C–O	1180.44	1176.36
C=O	1604.77	1604.48
C–H	3610.74	3617.80

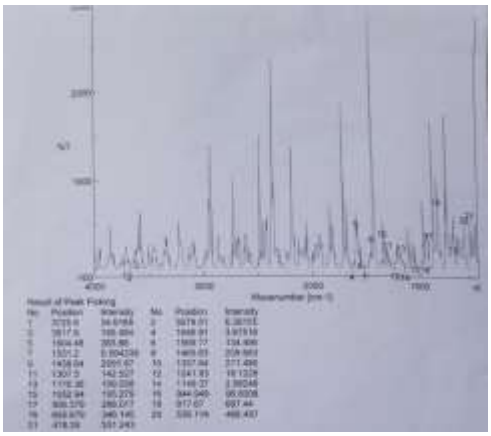

Figure 1: IR Spectrum of pure drug sample (Nadifloxacin)

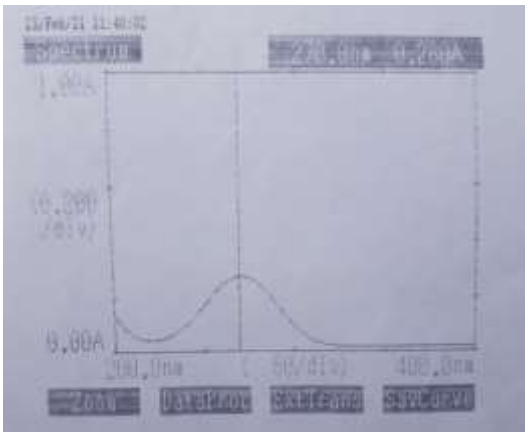

Figure 2: Spectral analysis of Nadifloxacin in methanol

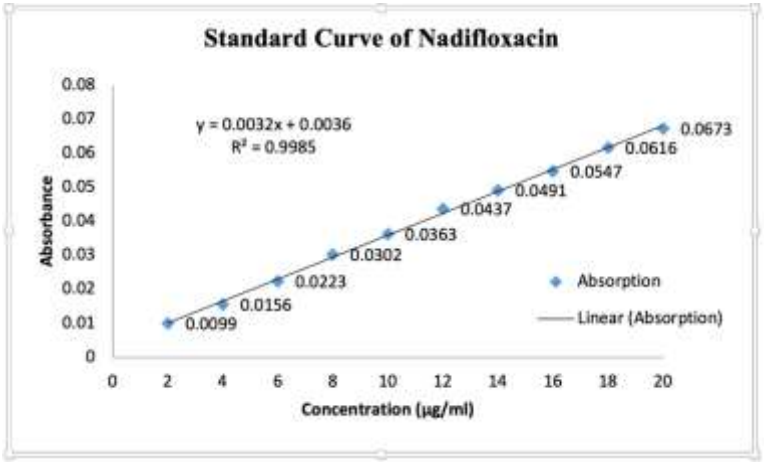

Figure 3: Calibration curve of NAD in methanol

Table 11: Statistical parameters derived from calibration curves

Statistical Parameters	In Methanol
R^2	0.9985
Slope	0.0032
Equation of line	y = 0.0032x + 0.0036

Table 12: NAD partition in the screened lipids

S. No.	Lipid	Melting Point (ºC)	Amount of drug partitioned into different lipids (mg ± SD)
1.	Stearic acid	67-70	8.01±0.24
2.	GMS	55-60	8.71±0.62
3.	Compritol 888 ATO	70-75	7.52±1.32
4.	Tristearin	70-73	6.93±1.19

Table 13 Surfactants selection for the SLNs preparation

S. No.	Surfactant	Mean particle size (nm)	Particle size distribution (nm)	PDI
1.	Poloxamer 407	368.0	112.3	0.438
2.	Poloxamer 188	315.8	120.7	0.312
3.	HPMC	2369	928.3	0.643
4.	Propylene Glycol	2073	4877.3	0.529

Table 14: Drug-Lipid ratio estimation

F. Code	Drug-Lipid ratio (% w/w)	Poloxamer 188 (% w/v)	Mean Particle Size (nm)	PDI	%EE
DL1	1:1	0.5	365.3	0.436	65.3±2.46
DL2	1:2	0.5	317.5	0.452	73.2±3.27
DL3	1:3	0.5	443.6	0.407	72.9±4.64
DL4	1:4	0.5	437.1	0.512	74.8±3.14

Table 15: Lipid-Surfactant ratio estimation

Batch No.	Poloxamer 188 (% w/w)	Zeta Potential	Average Particle Size	PDI
LS1	1:0.25	-25.6	224.4	0.332
LS2	1:0.5	-28.9	211.7	0.483
LS3	1:0.75	-27.1	398.4	0.519
LS4	1:1.0	-22.3	422.8	0.599

Table 16: Screened NAD-SLNs preparation components

Drug	Drug : Lipid ratio	Lipid : Surfactant ratio
Nadifloxacin (1gm)	Drug and GMS ratio (1:2 % w/w)	GMS and Poloxamer 188 ratio (1:0.5 % w/w)

Table 17: Parameters of characterization

% Entrapment Efficiency	Zeta potential	Particle size	PDI
76.32%	-21.2	126.61±7.413 nm	0.048

Figure 4: Particle size of the NAD-SLNs

Figure 5: Zeta potential of the NAD-SLNs

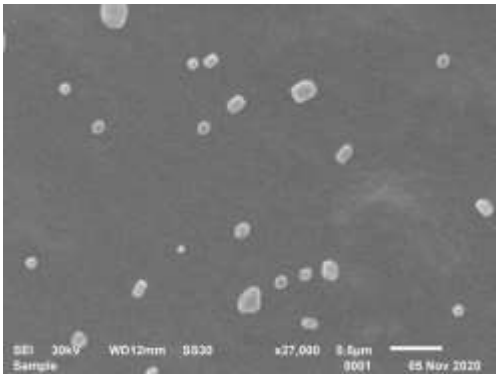

Figure 6: The final formulation as seen through SEM

Table 18: Components for the NAD-SLNs based hydrogel

Drug Sample	Polymer	pH Modifier	Preservatives	
SLN formulation	Carbopol 940 (1% w/w)	Triethanolamine	Methyl Paraben (0.1%)	Propyl Paraben (0.05%)

Figure 7: Hydrogel of the SLNs formulation

Table 19: Physicochemical Characteristics of hydrogel preparation

Colour	Homogeneity	pH	Grittiness	Phase separation
Milky white	Homogenous	6.67	No	No

Table 20: Hydrogel formulation spreadability

Weight (g) (M)	Length (cm) (L)	Time (sec) (T)	Spreading coefficient (gcm/min) (S=M*L/T)
50	12	25	24.00±1.5

Table 21: Viscosity of hydrogel preparations

Spindle number	Revolution per minute (RPM)	Torque (%)	Viscosity (cps)
S-6	50	22.8	9360±84

Table 22: Hydrogel formulation extrudability

Weight of sample in collapsible aluminium tube (g)	Pressure applied	Weight extruded from the tube (g)
10	Finger pressure	0.24±0.02

Table 23: *In vitro* release profile of NAD in PBS pH 6.8

Time (h)	% CDR of NAD in PBS pH 6.8			Average ± SD % CDR in PBS pH 6.8
	A	B	C	
0.5	1.88	2.18	2.28	2.11±0.17
1	4.57	5.73	7.69	6.00±1.29
2	11.97	129.06	12.24	12.09±0.11
4	18.43	18.77	18.82	18.67±0.17
6	32.69	32.62	32.29	32.53±0.17
8	37.24	35.32	36.65	36.40±0.80
12	47.82	48.33	48.97	48.37±0.47
18	65.22	64.73	65.56	65.17±0.34
24	75.86	75.72	76.86	76.15±0.51

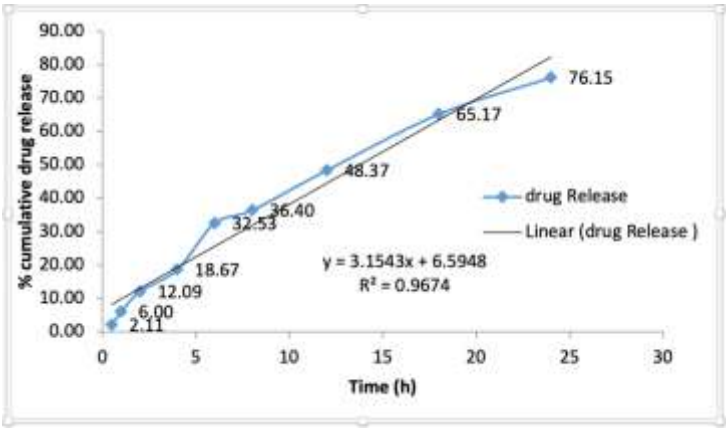

Figure 8: % cumulative drug release vs time.

Table 24: Data for *in vitro* drug release kinetics

Time (h)	Log time	SQRT	% CDR in PBS pH 6.8	Log % CDR in PBS pH 6.8	Log % drug remain in PBS pH 6.8
0.5	-0.301	0.707	2.11	0.324	1.990
1	0	1	6.00	0.777	1.973
2	0.301	1.414	12.09	1.082	1.944
4	0.602	2	18.67	1.271	1.910
6	0.778	2.449	32.53	1.512	1.829
8	0.903	2.828	36.40	1.561	1.803
12	1.079	3.464	48.37	1.684	1.712
18	1.255	4.242	65.17	1.814	1.541
24	1.380	4.898	76.15	1.881	1.377

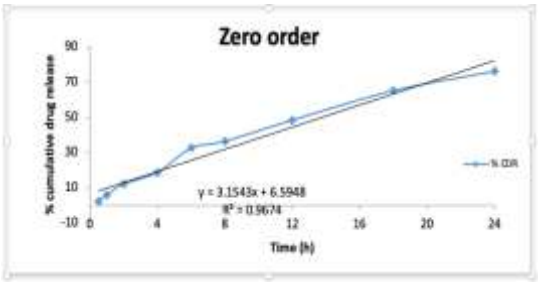

Figure 9: Zero order release model in PBS pH 6.8

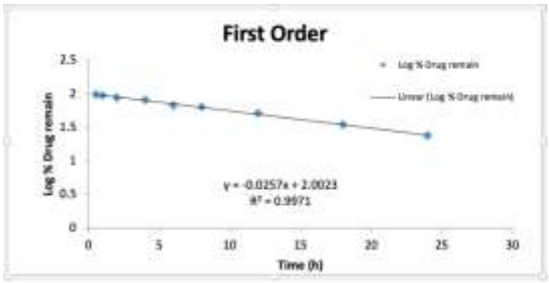

Figure 10: First order release model in PBS pH 6.8

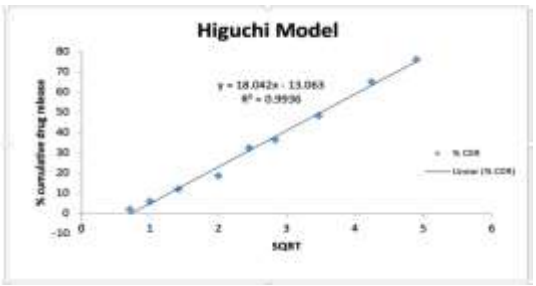

Figure 11: Higuchi release model in PBS pH 6.8

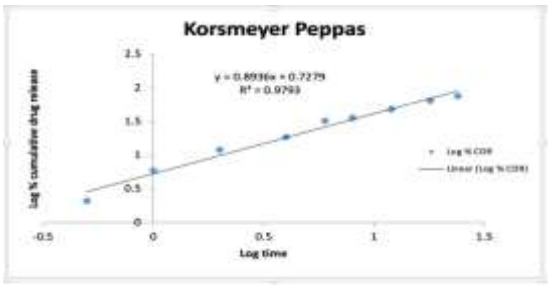

Figure 12: Korsmeyer Peppas model in PBS pH 6.8

Table 25: Correlation coefficient values calculated by different mathematical models

Release model		Regression Coefficient in PBS pH 6.8
Zero order	R^2	0.967
First order	R^2	0.997
Higuchi model	R^2	0.993
Korsmeyer Pepas model	R^2	0.979
	n	0.893

Calibration Curve Preparation
The calibration curve plotted in methanol drawn to the following results:

The absorbance by UV spectrometer against reference blank of methanol was measured at 270 nm in various aliquots from 2-20 µg/ml developed from the standard solution of the drug (NAD). Calibration curve of NAD in methanol was plotted as absorbance vs concentration (Figure 3). The statistical parameters involving line equation, regression coefficient (R2), and slope were calculated from the curve after data were processed. The plot demonstrate a linear relationship between the given ranges of concentration. Table 11 provides statistical parameters derived from calibration curves.

Components Selection for SLNs Preparation

Selection of Lipids
It is stated and expected to lead to high drug encapsulation due to high partition coefficient/lipid solubility. The test results lead to higher partition of NAD in glyceryl monostearate is higher. (Table 12)

With GMS, nadifloxacin has highest partition coefficient, thus for experimental research GMS was therefore chosen as the lipid carrier.

Selection of Surfactants
The selection of surfactants like Poloxamer 188, Propylene glycol, HPMC, Poloxamer 407 were utilized for the development of SLNs with a fixed ratio of lipid-surfactant i.e. 1:1. The SLNs developed with Poloxamer 188 has smallest mean particle size that is 315.8 nm with monomodel size distribution. However, some micron average particle size of 368.0 nm, 2369 nm, and 2073 nm was provided by other surfactants like Poloxamer 407, HPMC and Propylene glycol, respectively as table 13.

In formulations utilizing Poloxamer 188, the PDI and mean particle size are comparatively low i.e. 0.312 and 315.8 nm, respectively. Poloxamer 188 has therefore been selected as surfactant to prevent particle growth/agglomeration and provides enough charge for formulation stabilization.

Estimation of Drug-Lipid Ratio
Drug lipid ratios for optimum drug encapsulation with the lowest average particle size were calculated to maximize the lipid material. For an estimate of particle size and the percentage entrapment efficiency of SLNs, the different drug-lipid ratios (1:1 to 1:4) were analysed (Table 14).

The lipid concentration increased by 1:1 to 1:4 while the surfactant constant was held, there was a noticeable increase in particle size, but no substantial increase in entrapment efficiency at 1:2 drug ratios. This was due to lipid matrix saturation and increased load. With an improvement in the drug-lipid ratio, particle size greatly increased. For further research, the drug lipid ratio (1:2) was thus selected.

Estimation of Lipid-Surfactant Ratio
The surfactant concentrations were ranging from 0.25-1.0 % w/w for determination of the surfactant concentration based on zeta potential, PDI and particle size were screened for the surfactant, Poloxamer 188 (Table 15).

The surfactant concentration effect has been analysed and the lipid surfactant ratio 1:1 has to be stabilized. The LS2 is of highest zeta potential, that is to say, -28.9mV. The size of the particle is in the range of the nano size (211.7 nm), with effective PDI i.e. 0.483. The LS-1 formulation also showed a lowest PDI, i.e. 0.332, range of nano-size (224.4 nm). Compared to LS1 and LS2, the formulation LS3 and LS4 were of higher particle size. The formulation LS2 was chosen as an optimized formulation for all the formulation parameters due to its lowest mean particle size and highest zeta potential.

Preparation of Optimized NAD-SLNs
The selected components, involving NAD (drug), GMS (lipid) and Poloxamer 188 (surfactant), have been used in the predetermined amounts by means of hot homogenization method followed by ultra-sonication for the successful preparation of NAD-SLNs. (Table 16)

NAD-SLNs Characterization
Described in Table 17, Figure 4 and 5

Scanning Electron Microscopy
Scanning Electron Microscopy is a method for determining particle size by investigating the surface morphology of the particles. SEM was used to examine the morphology of the optimized freeze dried SLNs. (Figure 6)

Preparation of NAD-SLNs based Hydrogel
For hydrogel formulation, SLNs preparation were selected at determined component ratios (Table 16). SLNs particle size and morphology have been characterized (Figure 7, Table 18).

Characterization of NAD-SLNs based Hydrogel

Physical Appearance
There was a homogenous formulation that was non-gritty and which does not have a phase separation, summarized in Table 19.

Spreadability
Spreadability is one of hydrogel's essential property. Increased viscosity leads to poor spreadability. The spreadability of hydrogel has been found to be great and summarized in Table 20. The ideal spreadability coefficient for hydrogel was found. In the range of 24-18.75gcm/min.

pH Determination
The pH of formulation was 6.67 which summarized in Table 19.

Viscosity
The hydrogel viscosity has been recorded in Table 21. Formulation viscosity increases with increasing polymer concentration.

Extrudability
Extrudability of hydrogel formulation was determined and summarized in Table 22. The extrudability was 0.24g.

***In Vitro* Studies**
For *in-vitro* release of NAD from SLNs the dialysis diffusion technique was utilized. The 24 h have been utilized for determining NAD release pattern (Figure 8) from lipid particles. As shown in Table 23, the percent cumulative release of NAD (% CDR) in 24 h was 76.15% in PBS pH 6.8.

***In Vitro* Release Kinetics**
To prove the drug's design and performance, its release kinetics were examined. The mechanism and drug release pattern of optimized NAD-SLNs were evaluated using a variety of kinetic models. Fitting the *in vitro* release data (Table 24) into the appropriate equations, such as a zero order, first order, Higuchi, and Korsmeyer Peppas model, yielded the graphs representing the

kinetic models. Table 25 shows the correlation coefficient values for all models. (Figure 9-12)

Based on the values of correlation coefficients determined by various mathematical models, it was discovered that the first order release model had the highest correlation coefficient R^2 is 0.997. The highest R^2 value for the first order release model, on the other hand, suggests that the release mechanism was primarily diffusion regulated.

DISCUSSION

The aim of this dissertation work was to develop and characterize an optimal formulation of solid lipid nanoparticles of nadifloxacin, which would then be incorporated into hydrogel. The SLN was developed with drug nadifloxacin, which is poorly water soluble. On the basis of solubility studies (i.e. partitioning effects), the lipid and components were chosen. In this study, the two variables amount of lipid and concentration of Poloxamer 188 were studied. The EE increased as the concentration of Poloxamer 188 increased. The particle size was observed to decrease as the concentration of Poloxamer 188 was increased. The EE increased in a similar way as the amount of lipid was increased.

Particle size was increased as the amount of lipid was increased. ZP was increased as the Poloxamer 188 concentration was increased, but not dramatically. Since Poloxamer 188 coat the particle surface, the charge on the particle surface is shielded. As a result, it can be suggested that SLN of nadifloxacin incorporated into hydrogel can be utilized as a possible dosage form to achieve our objective.

CONCLUSION

The purpose was to formulate hydrogel with Carbopol 940 with improved drug entrapment, sufficient viscosity, good extrudability, good homogeneity and improved drug release. Hydrogels are polymers that have swelling ability in water or aqueous solvent systems. Due to their increased water content, gels can provide a better feeling for skin than other conventional dosage forms. Hydrogels are insoluble in water. They are not easily removed from the application site.

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