

Research & Reviews: Journal of Pharmaceutics and Nanotechnology

Development and Optimization of Site Targeted Topical Delivery Of Norfloxacin Emulgel

Vijay Swami, Kuchekar BS, Swati Jagdale*

MAEER's Maharashtra Institute of Pharmacy, MIT Campus, S. No. 124, Kothrud, Pune 411038, Savitribai Phule Pune University, India

Research Article

Received date: 25/06/2015

Accepted date: 24/09/2015

Published date: 01/10/2015

*For Correspondence

Swati C Jagdale, Professor and Head, Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, Pune, (MS), 411038, India, Tel: +91-9881478118

E-mail: jadgaleswati@rediffmail.com

Keywords: Norfloxacin, Topical, Hydroxypropyl methylcellulose, Emulgel, Antibacterial.

ABSTRACT

Norfloxacin is a synthetic fluoroquinolone having wide range of antibacterial activity. It is used in treatment of systemic as well as local infections. Norfloxacin has short half-life, poor oral bioavailability and very slight solubility in water. The aim of the present work was to develop and optimize site targeted emulgel delivery of Norfloxacin for topical antibacterial activity with enhance bioavailability. The major objective was to formulate an emulgel of hydrophobic drug (Norfloxacin) and to study effect of concentration of gelling agent and emulsifiers on the system. The oil and aqueous phases were prepared separately and mixed to form emulsion. Emulsion was then mixed with gel in 1:1 proportion to form emulgel. 32 full factorial designs were used to optimize the concentration of gelling agent and emulsifier. Emulgels were evaluated for physical appearance, pH, spreadability, viscosity, drug content, in-vitro release study through cellophane and egg membrane, ex-vivo permeation study, antibacterial activity and skin irritation test. Optimized batch F4 (2% HPMC K4M and 4.2: 3.2% of Tween 80: Span 80) showed 98.81% of drug release with average flux 2.52 $\mu\text{g}/\text{cm}^2/\text{min}$ and followed Korsmeyer-Peppas kinetics. Amount of drug permeated from optimized batch F4 was $71.66 \pm 2.52\%$ with average flux 2.19 $\mu\text{g}/\text{cm}^2/\text{min}$. Emulgel of Norfloxacin thus can act as a potential topical delivery system.

INTRODUCTION

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical route. Skin is one of the most readily accessible organ of human body and is one of the main route for topical drug delivery system^[1]. Potential advantages of topical drug delivery is delivering the drug directly at the site of action and delivering the drug for extended period of time. Topical delivery system increases the contact time and mean resident time of drug at the applied site leading to an increase in local drug concentration^[2]. Widely used topical formulation includes ointment, cream and lotion which had numerous disadvantages. They are usually very sticky causing uneasiness to the patient when applied topically. They also have less spreading coefficient and need to apply with rubbing. They also face problem with stability of the formulation. Due to all these factors, use of transparent gels has been increased. Hydrophobic drugs are not able to be delivered through gels. To overcome this problem, emulsion based approach was used^[3]. When the emulsions and gels are used in combined form, the dosage forms are referred as emulgel. Presence of gelling agent in water phase converts emulsion into emulgel^[4]. Emulgel have several advantages as being thixotropic, easily spreadable, water soluble, transparent, greaseless, non-staining, having pleasing appearance etc^[5].

Several antibacterial and antifungal agents are available in different topical formulations. Norfloxacin is a fluoroquinolone having broad spectrum antibacterial activity. It is active on both actively dividing as well as dormant bacteria by inhibiting bacterial DNA gyrase enzyme^[6]. Norfloxacin has short biological half-life (3-4 hrs) and dosage of drug is usually 400 mg of tablet twice a day.

Bioavailability of drug is 30% post oral administration. Literature survey indicated that work on palatable dry syrup, gastro retentive tablets and extended release matrix tablets has been carried for Norfloxacin [7-8]. Presently no topical Norfloxacin preparation is available in the market. Norfloxacin is used mainly in the treatment of urinary tract infections. It is also used for the treatment of gonorrhoea. It has shown to have superior antibacterial activity against both gram positive and gram negative bacteria. Norfloxacin possesses poor water solubility and hydrophobicity. The aim of the present work was to enhance the bioavailability, to prevent first pass metabolism of drug and to deliver the drug at the site of action. The objective of work was to optimize an emulgel formulation of Norfloxacin. The work investigates the influence of concentration of gelling agent (HPMC K4M) and emulsifiers (Tween 80: Span 80) on *in-vitro* release profile of drug.

MATERIALS AND METHODS

Materials

Norfloxacin was received as a gift sample from JCPL Pharma Pvt. Ltd. Jalgaon. HPMC K4M was received as a gift sample from Colorcon Asia Pvt. Ltd. Mumbai. Oleic acid, Span 80, Tween 80, Propylene glycol, Carbopol 940, Gellan gum, Gelatin, Triethanolamine, Methyl and Propyl paraben were purchased from Analab Chemicals Pvt. Ltd. Mumbai. All other chemicals and reagents used were of analytical grade.

Characterization of Norfloxacin

Physical characterization: Pure drug was observed visually for its appearance and color.

Melting point: The melting point of drug was determined using capillary method.

IR spectroscopic study: Purity of drug and drug excipient interaction was determined using FTIR Spectrophotometer (Varian 640 IR).

UV spectroscopic study: UV Spectroscopic analysis of drug was carried out using UV spectrophotometer (UB Varian carry 100 scan). Standard solution of Norfloxacin were prepared as 100 µg/ml. Different concentrations 2-12 µg/ml of drug in phosphate buffer solution pH 7.4 were prepared and scan in UV spectrophotometer at λ_{\max} 271.

Analytical method validation

a. Accuracy: Accuracy was determined for three different concentration levels i.e. 80%, 100%, and 120% of Norfloxacin in phosphate buffer solution pH 7.4

b. Precision: Precision was determined in terms of intraday and interday precision.

c. Linearity: Linearity of Norfloxacin was studied for various concentrations of drug (2-12 µg/ml) in phosphate buffer solution pH 7.4

d. Limit of detection (LOD) and limit of quantification (LOQ): Limit of detection and limit of quantification was calculated using equation 1 and 2 given in ICH guidelines.

$$\text{LOD} = 3.3 \text{ SD/Slope} \dots \text{Eq. 1}$$

$$\text{LOQ} = 10 \text{ SD/Slope} \dots \text{Eq. 2}$$

SD=Standard Deviation

e. Differential scanning calorimetry (DSC) study: DSC of drug and polymer was studied by using differential scanning calorimetry (DSC 60, Shimadzu Corporation, Japan). The samples were analyzed over a range of 50 - 300°C at a heating rate of 100°C per minute under nitrogen atmosphere.

Compatibility study of drug and excipient

Norfloxacin and excipient (HPMC K4M, oleic acid, Tween 80 and Span 80) were mixed in 1:1 ratio and were kept in the stability chamber (Thermo Lab) for 1 month at $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$ according to ICH guidelines. At the end of 1 month the samples were removed and evaluated by FTIR and UV spectrophotometer.

Solubility study

An excess amount of Norfloxacin was added to selected oils and was stirred magnetically. After stirring for 2 hrs, it was filtered and diluted. The concentration of Norfloxacin was then determined at 271nm by UV spectrophotometry.

Preparation of emulgel [9]

Preliminary trial batches: The composition of Norfloxacin emulgel formulation is as shown in **Table 1**. Oil, surfactant, co-surfactant were mixed in quantity as shown in **Table 1**. 1% Norfloxacin was dissolved in this oil phase. Both the oil and aqueous phases were separately heated to 50-60°C. Then oil phase was added to the aqueous phase with continuous stirring upto 1 hour

to get an emulsion. The gel was then mixed with the emulsion in 1:1 ratio to get the emulgel. The pH of resultant emulgel was adjusted to 6 to 7 using triethanolamine.

Table 1. Preliminary trial batches.

Sr. No.	Ingredient (%w/w)	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
1	Norfloracin	1	1	1	1	1	1	1	1	1	1	1
2	Oleic acid	20	20	20	20	20	20	20	20	20	20	20
3	Propylene glycol	5	5	5	5	5	5	5	5	5	5	5
4	Tween 80	4.2	3.2	2.2	4.2	3.2	2.2	4.2	3.2	2.2	4.2	3.2
5	Span 80	3.2	2.2	1.2	3.2	2.2	1.2	3.2	2.2	1.2	3.2	2.2
6	HPMC K4M	2	2	2	-	-	-	-	-	-	-	-
7	Carbopol 940	-	-	-	1	1	1	-	-	-	-	-
8	Gellan gum	-	-	-	-	-	-	2	2	2	-	-
9	Gelatin	-	-	-	-	-	-	-	-	-	3	5
10	Water	64.56	65.56	66.56	64.56	65.56	66.56	64.56	65.56	66.56	64.56	65.56
11	Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
12	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
13	Triethanolamine	q.s. Adjust pH 6 to 7										

Design of experiment (DOE)

On the basis of evaluation of trial batches concentration of the polymer were decided. 32 level factorial design was applied to study the effect of independent variables i.e. concentration of gelling agent (X1) and concentration of emulsifier (X2). Dependent variables were % cumulative drug release at 1 hour (Y1) and viscosity of emulgel. Software program used was Design Expert version 9.0.3.1. The independent variables are listed in **Table 2** while all the batches were prepared according to the experimental design as indicated in **Table 3**. The batches were formulated by same procedure as what is followed in formulation of preliminary batches. ^[10]

Table 2. Coded level for the actual level of variable (F1-F9).

Independent variables	Coded levels		
	+1	0	-1
Concentration of gelling agent	3%	2%	1%
Concentration of emulsifiers ratio	4.2-3.2%	3.2-2.2%	1.2-2.2%

Dependent variables. 1. Cumulative drug release 2. Viscosity

Table 3. Formulation of factorial batches.

Sr. No. (%w/w)	Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Norfloracin	1	1	1	1	1	1	1	1	1
2	Oleic acid	20	20	20	20	20	20	20	20	20
3	Propylene glycol	5	5	5	5	5	5	5	5	5
4	Tween 80	4.2	3.2	2.2	4.2	3.2	2.2	4.2	3.2	2.2
5	Span 80	3.2	2.2	1.2	3.2	2.2	1.2	3.2	2.2	1.2
6	HPMC K4M	3	3	3	2	2	2	1	1	1
7	Water	64.56	65.56	66.56	64.56	65.56	66.56	64.56	65.56	66.56
8	Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
9	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
10	Triethanolamine	q.s. Adjust pH 6 to 7								

EVALUATION OF EMULGEL

Appearance and pH

Appearance of gel was evaluated on the bases of visual inspection. pH of the formulation was checked by directly dipping the electrode in the emulgel.

Viscosity

The viscosity of batches was determined using Brookfield's viscometer (spindle No.7).

Spreadability

One of the main criteria for an emulgel is to possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreadability. Spreadability was measured on the basis of "Slip" and "Drag" characteristics of gel. A ground glass slide was fixed

on block. An excess of gel (about 1 gm) of different formulations were placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 20 gm, lesser the time taken for separation of two slides better the spreadability^[11]. Spreadability was calculated using the following formula:

$$S=M \times L/T.....Eq. 3$$

Where, S=spreadability,

M=the weight in the pan (tied to the upper slide),

L=the length moved by the glass slide

T=the time taken by upper slide to travel 10cm. distance.

In-vitro diffusion study: Cellophane membrane

In-vitro drug release of Norfloxacin from emulgel were studied through cellophane membrane (cellophane membrane 12 mol. wt. approx. 12,000 dalton, pore size 2.4 nm) using Franz diffusion cell. The cellophane membrane was previously soaked in boiling water for 1 hour. Then it is soaked for half hour in alcohol. Then the cellophane membrane was soaked overnight in the phosphate buffer 7.4 at refrigeration temperature. The treated cellophane membrane was sandwiched between donor and receptor compartment of Franz diffusion cell. Formulation equivalent to 1 mg of Norfloxacin was evenly spread on the cellophane membrane. A magnetic bar was continuously stirred in diffusion medium at 50 rpm to avoid diffusion layer effect. 1 ml sample was withdrawn at appropriate time. These samples were analyzed by UV spectrophotometer at 271 nm. Batches with good results were further evaluated for permeation study through egg membrane and rat skin^[11].

In-vitro diffusion study: Egg membrane

The same procedure was used for *in-vitro* drug release study as followed in cellophane membrane. Instead of cellophane membrane egg membrane was obtained and used to study *in-vitro* drug release. Raw egg was taken and a small hole was made at the bottom to remove all its contents. Then the egg shell was dipped into 0.1N HCl for 3 hrs. The egg shell was dissolved and the membrane was collected. Egg membrane was then washed with distilled water and used for study.

Ex-vivo permeation study

Male rat free from any visible sign of disease were selected. The abdominal hair was removed from the skin and the cleared area was washed thoroughly with distilled water. Abdominal skin of full thickness was excised from the rat. Adhering subcutaneous fat was carefully removed. This was mounted on the donor compartment with the epidermis facing the donor compartment. The receptor compartment was filled with phosphate buffer solution pH 7.4. Receptor phase was maintained at $37 \pm 0.50C$. 100 mg emulgel was placed over it and spread evenly and the permeation study was carried out in the similar manner as that with cellophane membrane.

Release kinetics, mechanism of drug release and flux

The diffusion profile of all the batches was fitted to PCP DISSO.v2.08 software to confirm the kinetics of drug release and flux^[12-13].

Drug content determination

Norfloxacin content in emulgel was measured by dissolving known quantity of emulgel in phosphate buffer solution pH 7.4 and stirred for 4 hrs. Absorbance was measured after suitable dilution at 271 nm in UV/VIS spectrophotometer. The drug content was determined in triplicate. Then drug concentration of the diluted sample solution was calculated as follows.

$$X=Y-C/M.....Eq. 4$$

Where, X=concentration in $\mu\text{g/ml}$.

Y=absorbance of solution at 271 nm.

C=intercept of standard curve.

M=slope of standard curve

Further, the % drug content was calculated from the concentration using the equation...

$$\% \text{ drug content} = \frac{\text{concentration of sample solution}}{\text{Equivalent concentration of drug taken}} * 100 \quad .Eq. 5$$

Photomicrography

Morphology of emulsion was studied under light microscope. F1 and F4 batch of the emulgel were viewed under light

microscope to study their shape. The emulgel was carefully evenly spread on glass slide and cover slip was placed over it. Then it mounted and viewed by light microscope under magnification of 40X.

Microbiological assay

The microbiological assay was carried out to determine the activity of prepared formulation with the strain. Batch F1 and F4 were selected for the antimicrobial test. Cup plate method with agar medium (I.P) was used. This technique is used for evaluation of bacteriostatic or fungistatic activity of a compound. Cups were made with sterile glass bore and labeled. 100 mg of emulgel was placed in cup A and B for batch F1 and F4 respectively in the plate. 1000 µg/ml Norfloxacin solution was filled at cup C in the plate. After incubation for 24 hrs at 37°C ± 0.5, the zone of inhibition was measured and % Inhibition was calculated as follows:

$$\% \text{ Inhibition} = L2/L1 \times 100 \dots \dots \dots \text{Eq. 6}$$

Where L1- Total length of the streaked culture

L2- Length of inhibition.

Skin irritation test

Various preparations, when applied dermally, might elicit skin irritation. Therefore, to access the skin sensitizing potential of the formulation, formulated emulgel was applied to dorsal skin of Wistar albino rats. Skin irritation study was conducted in accordance with the approval of the Animal Ethical Committee. The animals were housed in propylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before experimentation.

The animals were divided into five groups, each consisting of six animals.

Group A: treated with normal saline- Control Group

Group B: treated with optimized gel from HPMC K4M based gels without drug.

Group C: treated with F1 batch.

Group D: treated with F4 batch.

Group E: treated with 1% aq. Formaline solution used as standard irritant.

A 0.1 gm sample of the test emulgel was applied to the site to each rat which was approximately 1" × 1" square. The skin was shaved with electric razor. Animals were returned to their cages. After 24 hour exposure, the test site was wiped with distilled water to remove any remaining test emulgel residue. The skin was observed for any redness, edema or rashes.

Stability study

Short-term accelerated stability for emulgel (F4 batch) was carried out for 3 months at 300 ± 2°C/65% ± 5 RH. Emulgels were evaluated for appearance, pH and drug content and % cumulative drug release.

RESULTS AND DISCUSSION

Characterization of Norfloxacin

Physical Characterization indicated a pale yellow, crystalline powder which was practically odorless. The melting point of drug was found to be 224°C which was close to reported melting point 228°C.

Compatibility study

From **Figure 1**, it can be observed that one prominent characteristic peak was obtained between 3550 and 3500/cm, which was assigned to stretching vibration of OH group and intermolecular hydrogen bonding by single bridge. A band at 3500 to 3300/cm suggested the NH stretching vibration of the imino moiety of piperaziny group. The peak at 2750-2700/cm indicated the presence ethyl group. FTIR study indicated absence of any chemical interaction between drug and excipients. From **Figure 2**, in UV spectra there was no change in λ_{max} of drug in all combinations indicating that no chemical change took place in drug. Thus drug and excipients were found to be stable. Results of FTIR spectroscopy and UV spectroscopic study indicated drug excipient compatibility as shown in **Figures 1 and 2**.

UV Spectroscopic study

UV absorption of Norfloxacin in phosphate buffer solution pH 7.4 showed λ_{max} at 271 nm. The graph of absorbance versus concentration was found to be linear. The drug obeys Beer's- Lamberts law in the range of 2 to 12 µg/ml. The line equation for the calibration curve was found to be $y=0.1163 \times (\text{Eq. 7})$ and $R^2=0.9975$.

Analytical method validation

Accuracy: Percent recovery mean for 80%, 100% and 120% of Norfloxacin in phosphate buffer solution pH 7.4 was found to be 99.35%, 99.15% and 98.19% respectively.

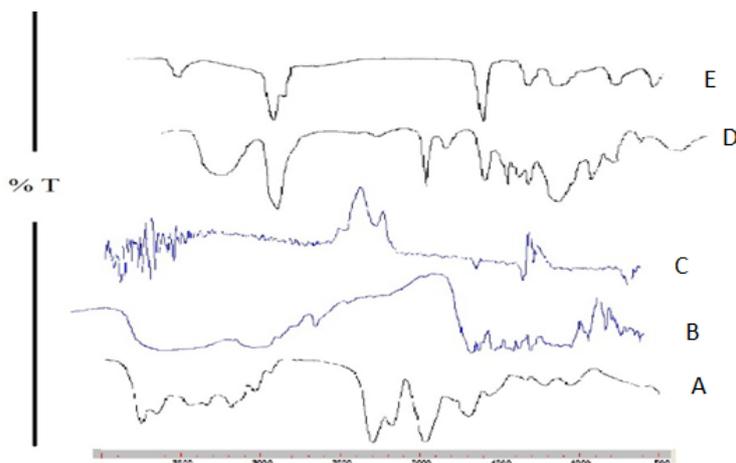


Figure 1. IR spectra of A) Norfloxacin B) Norfloxacin+HPMC K4M C) Norfloxacin+Span 80 D) Norfloxacin+Tween 80 E) Norfloxacin+oleic acid.

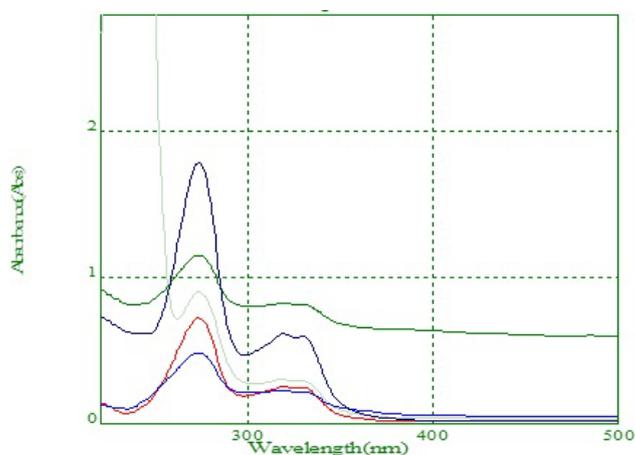


Figure 2. UV compatibility study of A) Norfloxacin+phosphate buffer pH 7.4 B) Norfloxacin+Span 80 C) Norfloxacin+Oleic acid D) Norfloxacin+Tween 80 E) Norfloxacin+PG.

Precision: The method was found to be precise in terms of intraday and interday precision.

Linearity: The method was found to be linear in concentration range of 2 to 20 µg/ml of Norfloxacin in phosphate buffer solution pH 7.4

Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ values for Norfloxacin were found to be 0.150 µg/ml and 0.456 µg/ml respectively.

Differential scanning calorimetry (DSC) study: DSC spectrum of pure drug as shown in **Figure 3** indicated melting point of Norfloxacin sharp at 222.52°C. HPMC K4M showed melting point at 79.54°C. Emulgel showed flat point at 112°C which is due to predominance of Tween 80 which has flash point at 114°C.

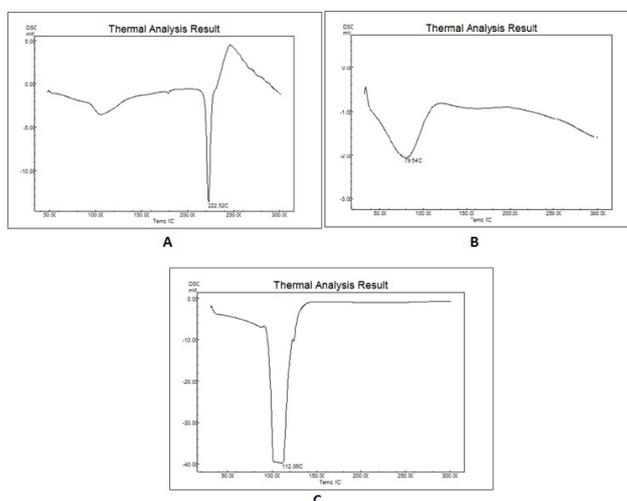


Figure 3. DSC Thermogram of pure drug A. Norfloxacin, B. HPMC K4M and C. Emulgel.

Solubility

Highest solubility of Norfloxacin was found in oleic acid amongst oils, Tween 80 and Span 80 amongst surfactants and propylene glycol amongst co-surfactants. Hence these components were finalized for preparation of emulgel system.

Preliminary trial batch

All the preliminary batches were evaluated for the viscosity, spreadability, % drug release, pH, % drug content. Batch A4, A5 and A6 with carbopol as gelling agent showed less cumulative drug release (70-82%) as compared to emulgel with HPMC K4M. Emulgels were found to remain intact on cellophane membrane in *in-vitro* drug release study. Batch A7 and A8 with gellan gum showed 85%-95% of cumulative drug release but those emulgels were not stable. Oil phase was found to be separating from gel phase after 72 hrs storage at room temperature. Emulgels with gelatin showed % cumulative drug release in the range of 50-70% at the end of 240 mins. Viscosity of gelatin emulgel was found to be very high than emulgels with HPMC K4M, carbopol and gellan gum. Emulgels with HPMC K4M liquefies over membrane during *in-vitro* and *ex-vivo* diffusion study. Emulgels with HPMC K4M showed % cumulative drug release in 85-98% at the end of 240 mins in *in-vitro* and *ex-vivo* study. From these results, 2% HPMC K4M was selected as the gelling agent and Tween 80:Span 80 as emulsifier (3.2:2.2). These concentrations were used for the formulation of the factorial batches.

Evaluation of emulsions and emulgels

Appearance: All emulsions formulation batches were found to be homogenous milky while emulgels were found to be yellowish white viscous creamy preparation.

Viscosity: Viscosity of the F1 to F9 batches is as shown in **Figure 4**. Viscosity of the formulated emulgel was found to be proportional to the concentration of the gelling agent i.e. HPMC K4M. Thus batch F1, F2, and F3 showed higher viscosities than the other batches. From the result it was found that viscosity of emulgel decreases with increase in shear stress indicating a shear thinning system.

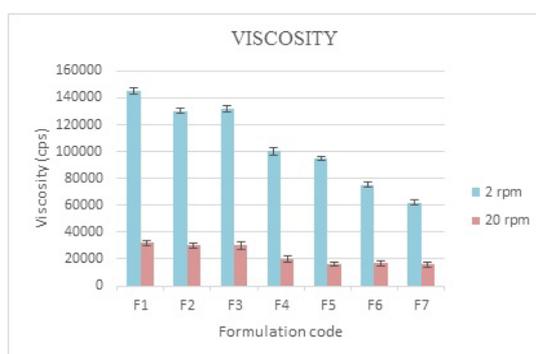


Figure 4. Viscosity of the optimized batches F1- F7.

Spreadability: Spreadability of emulgel is an important parameter. It was found that spreadability increases with decrease in viscosity as shown in **Figure 5**. Batch F7 showed highest spreadability.

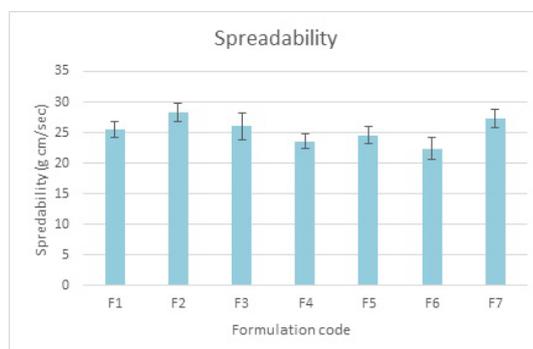


Figure 5. Spreadability of optimized batches F1-F7.

In-vitro drug diffusion study

***In-vitro* diffusion study: cellophane membrane:** The results of *in-vitro* drug release study are as shown in **Figure 6**. Formulation batch F4 showed release of drug faster compared to other formulation due to the lower concentration of HPMC K4M and higher concentration of emulsifiers. With increase in concentration of gelling agent, it leads to retard the drug release from formulation mainly due to high viscosity.

Batch F4 showed highest drug release of 98.81 ± 2.74 . Batch F3 containing showed $47.57 \pm 2.51\%$ drug release. This low

drug release was mainly due to higher concentration of gelling agent and lower concentration of emulsifiers (3% HPMC K4M and Tween 80:Span 80 2.2:1.2%). Higher concentration of gelling agent hinders the drug release. The batches according to the % drug release can be arranged as F4>F1>F7>F5>F2>F6>F3. F4 batch showed higher drug release rate may be due to higher concentration of emulsifiers and lower concentration of gelling agent than F1. From appearance, % drug release, viscosity and spreadability, batch F1 and F4 were selected for the further permeation study through egg membrane and rat skin.

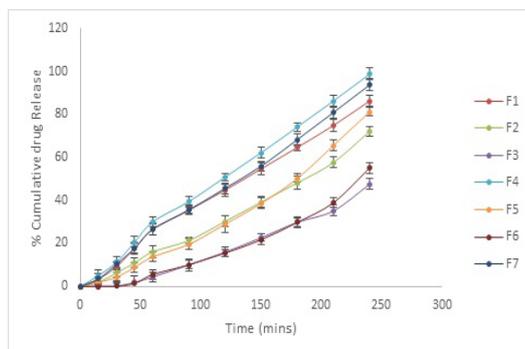


Figure 6. In-vitro % cumulative drug release F1-F7.

Kinetic study and mechanism of drug release

The correlation coefficient value (R2) of each formulation for zero order, first order, matrix, Hixon Crowell and value of release exponent for Korsmeyer Peppas model are as shown in **Table 4**. The release kinetics data indicated that the release of drug from emulgel followed Korsmeyer Peppas kinetics. Based on the Korsmeyer-Peppas equation, values of the 'n' exponent equal to or less than 0.5 were characteristic of Fickian or quasi-Fickian diffusion, whereas values in the range of 0.5 to 1 were an indication of an anomalous mechanism for drug release. On the other hand, a unity value that is n=1 for n would be expected for zero-order release. Here batch F1, F2, and F4 showed Fickian diffusion mechanism for drug release. When the drug diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian. Batch F3, F5, F6 and F7 showed release component values less than 1 but more than 0.5. When 0.5<n<1, the release is called "anomalous" and in such situation both swelling and diffusion play an important role. When the drug diffusion rate and the polymeric relaxation rate are of the same order of magnitude, anomalous diffusion is observed and the value of n falls between 0.5 and 1.0 [14].

Table 4. Kinetics and release mechanism of F1-F7.

Batch	R2 Value					Release component	
	Zero order	First order	Matrix Higuchi	Hixon Crowell	Korsmeyer Peppas	n	k
	F1	0.3056	0.3074	0.9261	0.3068	0.9799	0.3031
F2	0.7578	0.7583	0.9713	0.7581	0.9722	0.3780	0.0249
F3	0.9038	0.9040	0.9810	0.9039	0.9818	0.5036	0.0123
F4	0.2062	0.2092	0.9221	0.2082	0.9898	0.3879	0.0467
F5	0.9009	0.9012	0.9863	0.9011	0.9770	0.5736	0.0168
F6	0.9233	0.9234	0.9526	0.9234	0.9621	0.5266	0.0104
F7	0.5013	0.5025	0.9536	0.5021	0.9896	0.5268	0.0375

Design of Experiment

Response surface methodology as experimental design was used to determine the effect of independent variables on dependent variables. Results of contour plot and response surface plot are as shown in **Figures 7 and 8**.

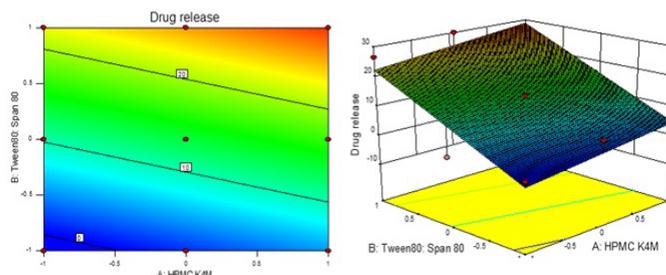


Figure 7. (A) Contour plot and (B) Response surface plot showing relationship in between % drug release at 60mins and polymer concentration.

Effect of formulation variables on drug release at 60 min

The regression equation obtained for the drug release at 60 min is as follows:

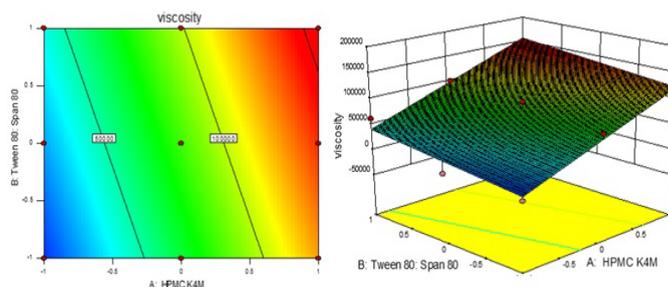


Figure 8. (A) Contour plot and (B) Response surface plot showing relationship in between viscosity of emulgel and polymer concentration.

$$\text{Drug release (at 60 min)} = +13.52000 + 3.23167A + 12.01333B \dots \text{Eq. 7}$$

Where, A: HPMC K4M conc. B: Tween 80:Span 80 conc

The model terms for the drug release at 60 min were found to be significant with high value of R² 0.8421 which indicated the adequate fitting to a linear model. Values of prob F was less than 0.05 which confirmed that the model terms were significant. Also, pred R-squared value was 0.6592 which is in reasonable agreement with the adj R-squared value of 0.8421. Concentration of both HPMC K4M and Tween80: Span 80 showed significant effect on drug release as shown in **Table 5**. As it can be seen from the equation, the concentration of emulsifier showed greater effect on the drug release than concentration of HPMC K4M. Overall both the variables have positive effect on the % drug release as shown in **Figure 7**.

Table 5. ANOVA study of optimized batches.

Sr. No.	Response Model	Sum of Squares	Df	Mean Square	F value	P value	R2	Adequate Precision
1	Drug Release (60 min.)	928.58	2	464.42	16.00	0.0039	0.8421	9.804
2	Viscosity	2.150E+010	2	1.075E+010	38.83	0.0004	0.9284	15.452

Effect of formulation variables on gel viscosity

The regression equation obtained for the gel viscosity is as follows:

$$\text{Gel Viscosity} = +82111.11111 + 57500.00000A + 16666.66667B \dots \text{Eq. 8}$$

Where, A: HPMC K4M concentration; B: Emulsifiers concentration

The model terms for the gel viscosity was found to be significant with high value of R² 0.9284 which indicates the adequate fitting to a linear model. Values of prob F was less than 0.05 indicated that the model terms were significant. The pred R-squared of 0.8273 is in reasonable agreement with the adj R-squared 0.9045; i.e. the difference is less than 0.2 as shown in **Table 5**. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq. 8, it was found that both the polymers individually showed positive effect on viscosity of emulgel. The viscosity increased with the increase in the concentration of individual polymer. HPMC K4M showed greater effect on viscosity as compared to Tween 80:Span 80 as shown in **Figure 8**.

Validation of statistical model

After statistical analysis by the design expert software, the experimental values for the % cumulative drug release at 60 mins and viscosity were found very close to the applied predicted values as indicated in **Table 6**, hence the model was successfully validated for batch F4.

Table 6. Comparison of predicted and actual values.

Polymers	Code levels	Actual levels	Response	% drug release at 1 hr	Viscosity
			Predicted value	25.53	98777
			Observed value	30	100000
HPMC K4M	0	2	Standard deviation	5.386	16626
Tween80. Span 80	1	4.2. 3.2	Standard error mean	2.839	8763.14

In-vitro diffusion study: egg membrane

As shown in **Figure 9** formulations F1 and F4 showed less drug release as compared to the drug release through cellophane membrane at a given point of time. This may be due to complexity of the egg membrane.

Ex-vivo drug permeation study

It was found from **Figure 10** that ex-vivo release of F1 and F4 were less than in-vitro release through cellophane membrane and egg membrane. This decrease in drug release was observed which may be due to the fat content and thickness of rat skin. F4 showed better release than F1, this may be due to the high viscosity of F1 [14-15].

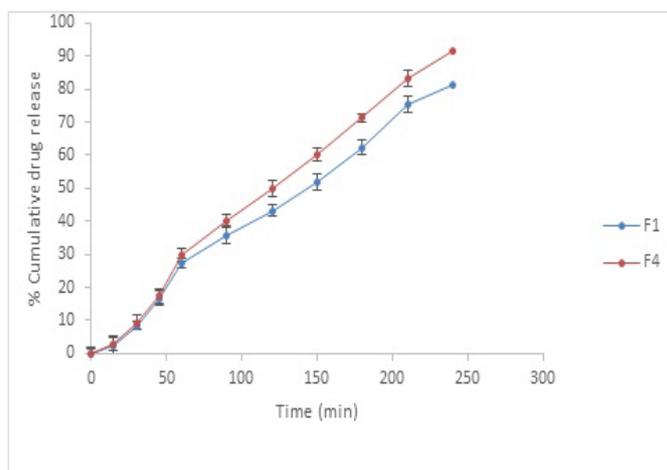


Figure 9. In-vitro % cumulative drug release for F1 and F4 batch.

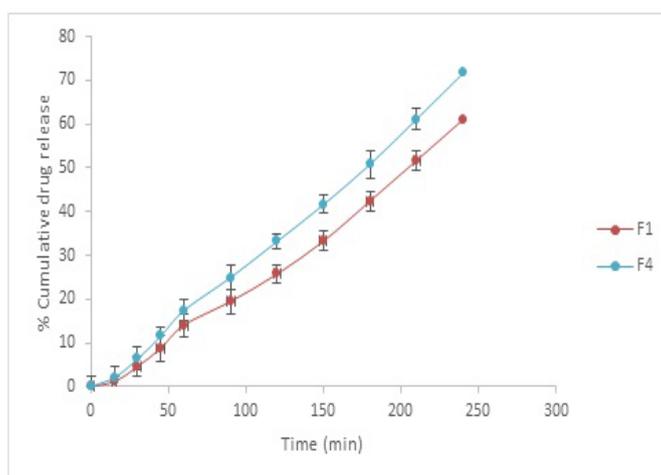


Figure 10. Ex-vivo % cumulative drug release through rat skin for F1 and F4 batch.

Kinetic study, mechanism of drug release and rat skin and flux

From **Table 7** it can be seen that release of drug follows Korsmeyer-Peppas model. Release component n values for Korsmeyer-Peppas model were found to be between 0.1-0.5, which indicated that the mechanism of release of drug through egg membrane and rat skin was Fickian.

Table 7. Kinetics and release mechanism of F1 and F4.

Sr. No.	Formulation R2	Zero order R2	First order R2	Matrix R2	Hixon- Crowell R2	Korsmeyer-Peppas		
						R2	n	k
I.	In-vitro permeation through egg membrane							
a.	F1	0.5735	0.5804	0.9592	0.5801	0.9813	0.3444	0.0315
b.	F4	0.48867	0.4878	0.9542	0.4874	0.9926	0.3359	0.0352
II.	Ex-vivo permeation through rat skin							
a.	F1	0.6676	0.6682	0.9775	0.6680	0.9923	0.3686	0.0239
b.	F4	0.6334	0.6342	0.9749	0.6339	0.9970	0.3572	0.0277

The flux of drug through different membranes was calculated with an area of 3.12cm². Average flux across cellophane membrane for F1 and F4 was 2.38 and 2.63 µg/cm²/min, whereas across egg membrane it was 2.15 and 2.52 µg/cm²/min. Average flux across rat skin membrane was 1.95 and 2.19 µg/cm²/min. The results of drug permeation through the cellophane membrane, egg membrane and rat skin confirmed released and permeation of drug from emulgel and hence could possibly permeate through the human skin.

Photomicrography

The suitably diluted emulsions of optimized batches (F1 and F4) were observed under light microscope at 40X. From the photomicrograph as seen in **Figures 11 and 12**, spherical globules of emulsion were observed. The size of emulsion globule in emulgel was found in range of 0.2-0.5 µ. Though this study does not give any exact estimate of size of these globules however it gives a general idea about formation of emulsion and success of the method used for the formulation of the emulgel.

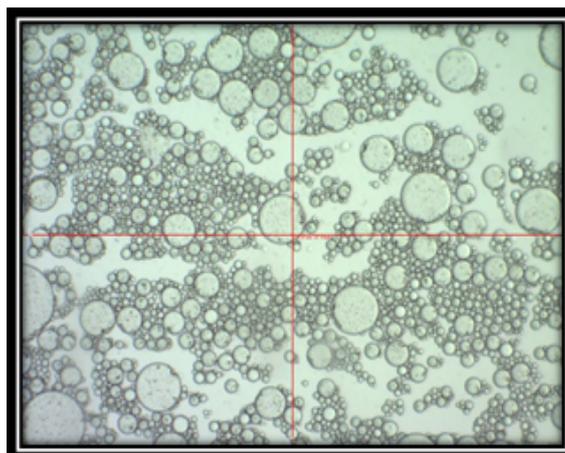


Figure 11. Photomicrograph of emulsion.

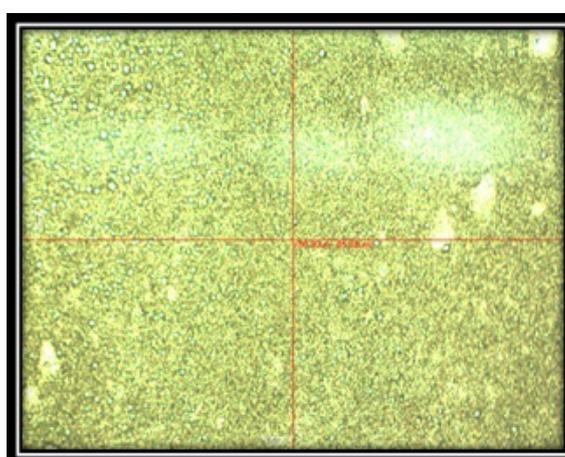


Figure 12. Photomicrograph of emulgel.

Microbiological assay

The use of control plates allowed that the plain emulgel bases were microbiologically inert toward the staphylococcus aureus strain. The antimicrobial activity of Norfloxacin in its emulgel formulations is as shown in **Figure 13**. Percentage inhibition was taken as a measure of the drug antibacterial activity. The activity was observed with F1 and F4 where the percentage inhibition found to be $34.3 \pm 1.52\%$ and $28.6 \pm 1.5\%$. The % inhibition by the pure Norfloxacin solution was found to be $45.36 \pm 1.74\%$. This indicated effectiveness of emulgel formulation.

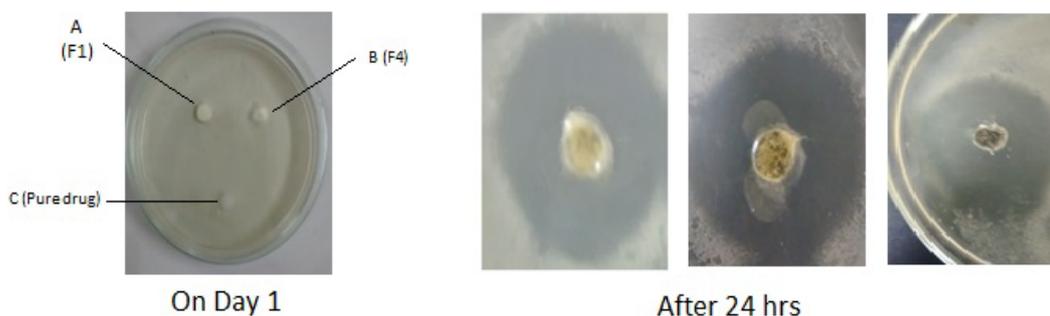


Figure 13. Antimicrobial study of formulation F1, F4 and pure drug.

Drug content: The drug content for all the formulations was found in the range of 80-92%.

pH: The pH of all the formulations was found to be within the range of 6 to 7. The pH of the skin is in the range of 5.5 to 7. So it is concluded that all the formulation are compatible to the skin pH and good for topical delivery of the drug.

Stability study: Emulgels were found to be pale yellow viscous creamy preparation with the smooth homogenous appearance which is similar as that on first day. Appearance of emulgel was yellowish white viscous creamy in appearance with 6.28 ± 0.58 pH and $89.48 \pm 0.61\%$ of the cumulative drug release. After 3 month of stability study appearance it was found to be same with 6.92 ± 0.39 pH and $87.26 \pm 0.25\%$ of cumulative drug release.

Skin irritation test (Patch test)

As seen from **Figure 14** and **Table 8**, group E animals to which formaline was applied as a standard irritant showed redness and scar. Group A, B C, D have not shown any edema or erythema at the site of application. Group E shows well defined erythema and edema at the application site of formaline. It was observed that emulgels were very well tolerated by rats with no signs of erythema or edema after 24 hrs. Hence both the formulations i.e. F1 and F4 are non-sensitizing and safe for use ^[16].



Figure 14. Skin Irritation test.

Table 8. Results of skin irritation test.

Skin Responses	Score	Observed score				
		A	B	C	D	E
Erythema and Eschar Formation						
No Erythema	0	0	0	0	0	0
Very slight erythema (barely perceptible)	1	-	-	-	-	-
Well-defined erythema	2	-	-	-	-	2
Moderate to severe erythema	3	-	-	-	-	-
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4	-	-	-	-	-
Edema formation						
No edema	0	0	0	0	0	-
Very slight edema (barely perceptible)	1	-	-	-	-	-
Slight edema (edges of area well-defined by definite raising)	2	-	-	-	-	-
Moderate edema (raised approximately 1.0 mm)	3	-	-	-	-	3
Severe edema (raised more than 1.0 mm and extending beyond exposure area)	4	-	-	-	-	-
Total possible score for irritation	8	0	0	0	0	5

CONCLUSION

The purpose for formulating emulgel of Norfloxacin was to enhance the bioavailability of drug by avoiding first pass metabolism and to obtain site specific topical antibacterial activity. Optimization was carried out using factorial design. The release of Norfloxacin was good fit to the Korsmeyer Peppas model following the Fickian flow. In optimization of the formulation, from the polynomial equation and contour plots generated, both independent factors i.e. conc. of HPMC K4M and Tween 80: Span 80 showed significant effect on dependent variables i.e. % drug release and viscosity. In conclusion, a stable, effective and elegant Norfloxacin emulgel formulation, exhibiting good *in-vitro* drug release and viscosity, was formulated using HPMC K4M as a gelling agent and tween 80:span 80 as emulsifying agent.

ACKNOWLEDGMENT

The authors are grateful to JCPL Pvt. Ltd. Jalgaon, for providing gift sample of drug, Colorcon Asia Pvt. Ltd. Mumbai for providing HPMC K4M. Authors are thankful to Management of MAEER's Maharashtra Institute of Pharmacy, Pune to provide all the facilities to carry out the research work.

REFERENCES

1. Ajazuddin KN. Recent expansions in an emergent novel drug delivery technology: Emulgel. J Control Rel 2013; 171 : 122-132

2. McGrath JA, et al. Anatomy and organization of human skin, Oxford Blackwell publisher, 1993; UK, pp. 3.1-3.15.
3. Rieger M, et al. The theory and practice of industrial pharmacy (3rd edn.) PA Lea and Febiger, Philadelphia, 1986; 502-33.
4. Khullar R, et al. Emulgels: A surrogate approach for topically used hydrophobic drugs. *Int J Pharm Bio Sci* 2011; 1:117-28.
5. Joel LZ and Gregory PK. In: Liberman HA, Rieger MM, Banker GS: *Pharmaceutical dosage forms: disperse systems*, Marcel Dekker, 1989; New York, 2: 502.
6. Sateesha SB, et al. Formulation and stability study of palatable Norfloxacin dry syrup: comparison among different preparation methods. *Asian J Pharm Sci* 2010; 6: 57-66
7. Thahera PD, et al. Formulation and evaluation of Norfloxacin gastro retentive drug delivery systems using natural polymers. *Int Curr Pharm J* 2012; 1: 155-164
8. Oliveira PR, et al. Formulation development and stability studies of norfloxacin extended-release matrix tablets. *BioMed Research Int* 2013; 9 pages
9. Dignesh MK. Formulation design & development of piroxicam emulgel. *Int J Pharm Tech Res* 2012; 4(3): 1332-1344.
10. Jagdale SC, et al. Study on the effect of HPMC on compression coated floating pulsatile delivery of bisoprolol. *Curr Drug Ther* 2014; 9: 93-103.
11. Jagdale SC, et al. Development and evaluation of pluronic lecithin organogel topical delivery of tapentadol. *American J Pharm Sci Nanotech* 2015; 2: 1-21
12. Dash S, et al. Kinetic modeling of drug release from controlled drug delivery systems. *Acta Poloniae Pharm Drug Res* 2010; 67: 217-23
13. Jagdale SC, et al. Development and optimization of troxipide floating delivery for treatment of peptic ulcer. *Curr Drug Ther* 2014; 9: 83-92.
14. Costa P and Lobo M. Modeling and comparison of dissolution profile. *Eur J Pharm Sci* 2001; 13: 123-33.
15. Malay KD. Formulation and ex vivo evaluation of rofecoxib gel for topical application. *Acta Poloniae Pharm Drug Res* 2007; 63: 461-467.
16. Jain S, et al. Skin irritation study of intradermal patch of chitosan containing trazodone-HCl on rat skin. *Int J Res Pharm Biomed Sci* 2011; 2: 1082-1084.