Research Paper

Design and Evaluation of Microemulsion Gel System of Nadifloxacin

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Topical microemulsion systems for the antiacne agent, nadifloxacin were designed and developed to overcome the problems associated with the cutaneous delivery due to poor water solubility. The solubility of nadifloxacin in oils, surfactants and cosurfactants was evaluated to screen the components of the microemulsion. Various surfactants and cosurfactants were screened for their ability to emulsify the selected oily phase. The pseudoternary diagrams were constructed to identify the area of microemulsion existence. The influence of k_m (surfactant/cosurfactant) ratio on the microemulsion existence region was determined and optimum systems were designed. The systems were assessed for drug-loading efficiency and characterised for optical birefringence, pH and refractive index, robustness to dilution, globule size, drug content and thermodynamic stability. Optimised microemulsion systems were formulated into gel form and evaluated for viscosity, spreadability, drug content, *ex vivo* skin permeation and antibacterial activity. The maximum solubility of nadifloxacin in the microemulsion system was found to be 0.25%. The nadifloxacin microemulsions had a small and uniform globule size (67.3-121.23 nm). The stability results revealed that all formulations showed a stable globule size and the polydispersity index under stress conditions. Incorporation of nadifloxacin in microemulsion gel increased the *ex vivo* skin permeation and antibacterial activity when compared to marketed cream.

Key words: Acne vulgaris, antibacterial activity, *ex vivo* skin permeation, Nadifloxacin, topical delivery

Acne vulgaris is one of the most common diseases of the skin with a prevalence of 70-85% in adolescents and in cases of extreme disfiguration, sometimes have severe consequences for the personality development of young people, which is associated with a relatively high prevalence of depression and suicide. Adolescents suffering from acne show higher levels of anxiety and greater social inhibition and aggression compared to nonaffected individuals[1]. It is a multifactorial disease affecting the pilosebaceous follicles and arises from an enhanced sebum excretion, hypercornification of the sebaceous duct, ductal colonisation with *Propionibacteruim acne* and Staphylococcus aureus, which is associated with inflammation and host immunological reactions[2]. According to the aetiopathological factors in acne, the therapy consists of noncomedogenic, antiinflammatory and antimicrobial substances.

Nadifloxacin (NDFX), a fluorinated quinolone antimicrobial, is widely used for the treatment of multiple inflamed acne lesions as a topical agent. It has a potent bactericidal activity against *Propionibacteruim acnes* and other Gram-positive and Gram-negative bacteria[3]. It is poorly water soluble fluoroquinolone with log *P* value of 2.47. Commercially, NDFX is available as topical cream. Topical creams have demonstrated poor penetration of drug-loaded droplet into the deep layers of skin due to higher droplet size[4]. An approach, which will increase the drug solubility and promote cutaneous penetration, is highly desirable for optimising the therapeutic performance of NDFX. Microemulsion systems would be one such approach to achieve optimum NDFX delivery. Microemulsions are transperent systems and typically consist of oil, surfactant, cosurfactant and aqueous phase. They are also thermodynamically stable and has a droplet size <0.15 nm and does not have the tendency to coalesce[5,6]. Microemulsions have several advantages such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhanced penetration on topical delivery over conventional formulations[6,7]. High solubilisation capacity and small droplet size provides better adherence to biological-membrane transporting of the

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drug in a controlled manner. This is most useful for treatment of acne as it improves the skin attachment and consequently enhances the accumulation of antibacterial agent in the target area[8]. Increased drug loading and high penetration through biological membrane leads to increase in the bioavailability and less inter and intra individual variability in drug's pharmacokinetics. These advantages make the microemulsion an attractive drug delivery system. There are two basic types of microemulsion systems: oil-in-water (o/w) and water-in-oil (w/o), where the o/w microemulsions are important to improve the solubility of poorly water-soluble drugs[9].

Hence, the focus of our work was to investigate the potential of a microemulsion system for topical delivery of NDFX, so that improved and maximised therapeutic efficacy is achieved not only by enhancing its solubility but also the cutaneous penetration as compared to conventional topical preparation. The traditional way of preparation of microemulsion was to dissolve the drug in oils followed by microemulsification; this method can be applied for most of the drugs, such as triptolide[10], aceclofenac[11] and diclofenac diethylamine[12]. In this study, due to the higher solubility of NDFX in surfactants than that in oils, another approach was adopted to dissolve poorly water-soluble drugs into o/w microemulsion, which firstly involved dissolution of the drug in a hydrocarbon chain of surfactants followed by microemulsification[13].

MATERIALS AND METHODS

NDFX was a generous gift from Cipla Pharmaceuticals Ltd., Mumbai, India. Solutol HS-15, Poloxamer 188, Polaxomer 407 (BASF, Mumbai, India), Transcutol P, Capryol 90, Miglyol 812, Lauroglycol 90, Labrafil M 1944 CS, Labrafil M 2125 CS, Labrasol, Labrafac lipophile WL1349 (Gattefosse India Pvt. Ltd., Mumbai, India) Akolin MCM, Akomed E (Karlshamns AB., Sweden), Carbopol ETD 2020, Xanthan Gum (Signet Chemical Pvt. Limited, India), ethyl oleate, oleic acid, isopropyl myristate, Tea tree oil, PEG 400, n-butanol, n-propanol, ethanol, Tween 80 and Tween 20 were purchased from S. D. Fine Chemicals, Mumbai, India. *Staphylococcus aureus* ATCC No. 6538 was obtained as gift sample form MKR Drug Testing Laboratory, Mumbai, India. All the excipients and reagents were used as received. Double-distilled water was prepared freshly whenever required.

Solubility studies:

The solubility of NDFX in various oils, modified oils, 5% (w/w) surfactant solutions and cosurfactants was determined by using the shake-flask method, under ambient conditions of temperature and humidity. Briefly, an excess amount of NDFX was added to each vial containing 1 g of the selected vehicle, i.e., oil, surfactant solution (5% w/w) or cosurfactant. After sealing, the mixture was vortexed using a cyclomixer (Remi, Mumbai, India) for 10 min in order to facilitate proper mixing of NDFX with the vehicles, further shaken for 24 h in a reciprocating water bath shaker (Remi, Mumbai, India) maintained at room temperature. Water bath shaker was operated at approximately 100 shakes per min for 24 h. The supernatant was then withdrawn after centrifugation (Minispin, India) at 5000 rpm for 10 min and diluted appropriately with methanol. The concentration of drug was quantified spectrophotometrically at 290 nm using UV-160A double beam spectrophotometer (Shimadzu, Japan) with respective blanks.

Screening of surfactants and cosurfactant for emulsifying ability:

The turbidimetric method was used to assess the relative efficacy of the surfactant and cosurfactants to improve the microemulsification ability and also to select the best from the large pool available for topical delivery. Screening of surfactants for emulsifying ability was carried out at 45-60° for homogenizing the components. Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45-60° for homogenizing the components. For screening of cosurfactant, 0.2 g of surfactant was mixed with 0.1 g of cosurfactant. Capryol 90, 0.3 g, was added to this mixture and the mixture was homogenised with the aid of gentle heat (45-60°). The isotropic mixture, 50 mg, was accurately weighed and diluted with double-distilled water to 50 ml to yield a fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give a uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 638.2 nm using double-distilled water as blank.

Construction of pseudoternary phase diagrams:

For construction of pseudoternary phase diagrams two methods can be employed, such as water titration[12]

or oil titration[14]. In the present investigation, water titration method was employed to construct a phase diagram. The weight ratio of surfactant to cosurfactant (k_m) varied as 1:1, 2:1, 3:1. In water titration, mixtures of oil with surfactants and co-surfactants were prepared in the ratios (% w/w) of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 into different vials. A small amount of water, i.e., 10 µl increment was added into the vials. Following each addition, the mixtures in vials were vortexed for 2-3 min and were allowed to equilibrate at 25° for 30 min. After equilibration, the mixtures were examined visually for phase separation and transparency. In addition, the mixtures were observed through crossed polarisers (fabricated in house by using polarising lenses, Nikkon, Japan) for determining the optical isotropy of the systems. The point at which the mixture became turbid or showed signs of phase separation was considered as the end point of the titration. The area of microemulsion existence was determined and denoted as ME.

Preparation and optimisation of NDFX microemulsion system:

NDFX-loaded microemulsion (NDFX-ME) was prepared by dissolving NDFX in Capryol 90 with vortexing followed by ultrasonication. The required quantities of surfactant and cosurfactant were added and the mixture was cyclomixed to yield a homogenous solution. To this solution, water was added to yield a microemulsion and optimisation of NDFX-ME was carried out by assessing the effect of k_m values on microemulsion region, drug loading in microemulsion system and effect of drug loading on globule size of the microemulsion.

Characterisation of the NDFX-ME:

Robustness of NDFX-ME to dilution was studied by diluting it 100 times with water. The diluted microemulsions were stored for 6 h at room temperature to access dilution stability and observed for any signs of phase separation or drug precipitation.

The NDFX-ME was checked both visually and using crossed polarisers and viewed for optical isotropy to confirm absence of other phases. Optical birefringer was fabricated in-house using polarising lenses, Nikkon, Japan. The sample was placed between the two cross-polariser lenses and viewed.

The mean globule size and polydispersity index (PI) of NDFX-ME was determined by photon correlation spectroscopy that analyses the fluctuations in light scattering due to Brownian motion of the particles, using Beckman coulter N5 submicron particle size analyser (Coulter Corporation, USA). Light scattering was monitored at 25° and at 90° angle. The pH of the selected microemulsion was measured in triplicate using the digital pH meter (Universal enterprises, India) at room temperature.

The selected formulations were subjected to different thermodynamic stability tests to assess their physical stability. The formulations were subjected to centrifugation at 3500 rpm for evaluating the phase separation. Three freeze–thaw cycles between −21° and +25° with formulation preserved at each temperature for not less than 24 h were performed and assessed for physical instabilities such as phase separation and precipitation. The formulations that survived thermodynamic stability were selected for formulation of the microemulsion gel.

Formulation and optimisation of NDFX-microemulsion gel system:

NDFX-microemulsion gels (NDFX-MEG) were prepared using xanthan gum and Carbapol ETD 2020. Briefly, xanthan gum was directly added to the prepared microemulsion slowly with continuous stirring with the help of the overhead stirrer. In case of Carbopol ETD 2020, the dispersion was neutralised using 50% w/w triethanolamine to obtain a gel.

Characterisation of the NDFX-MEG:

The mean globule size and PI of NDFX-MEG was determined by using Beckman coulter N5 submicron particle-size analyser. NDFX-MEG was sufficiently diluted with double-distilled water in a volumetric flask and gently mixed for globule size analysis. The pH of the selected microemulsion gels were measured using the digital pH meter at room temperature. Spreadability of NDFX-MEG was determined as per procedure described by Bachhav, *et al*.[15]. The drug content in NDFX-MEG was determined using a UV spectrophotometric method at 290 nm. Rheological characterisations of samples were performed at IIT, Bombay using controlled stress rheometer (Antonn-Paar Physica MCR-51, Germany). The measurements were performed using cone and plate geometry with 40 mm diameter (cone angle 3.988°). Viscosity parameters were collected at 25° with 1 min equilibration time at every rpm. Experiments were performed in triplicates.

***Ex vivo* skin permeation studies:**

The topical microemulsion gels were evaluated for *ex vivo* skin permeation profile as per the procedure described by Dalmora *et al*.[16]. The *ex vivo* skin permeation study was done on developed NDFX-MEG as well as on commercial formulations to compare the permeation rate of NDFX from respective formulation. The *ex vivo* permeation studies were performed with Franz diffusion cell at 37° under magnetic stirring using abdominal skin of male wistar rat. The skin samples were hydrated in phosphate buffer pH 6.8 for 1 h before use.

Permeation data analysis:

The cumulative amount of drug permeated through the skin (μg/cm^2) was plotted as a function of time (t) for each formulation[7,17,18]. Drug flux (permeation rate) at steady state (J_{ss}) was calculated by dividing the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (K_p) was calculated by dividing J_{ss} by the initial concentration of drug in the donor cell ($C0$): Kp=Jss/Co. Enhancement ratio (Er) was calculated by dividing the Jss of the respective formulation by the Jss of the control formulation: Er=Jss of formulation/ Jss of control. The permeation data were analysed statistically by paired Student's t-test (P<0.05) at 95% signifi cance level.

Evaluation of antibacterial activity:

The microbiological efficacy of NDFX in the optimised NDFX-MEG, placebo-MEG, NDFX standard (0.20% w/v NDFX in DMSO) and Nadoxin® cream was evaluated against *Staphylococcus aureus* ATCC No. 6538 by using cup plate method. Mueller-Hinton agar plates were prepared by pouring 10-15 ml of the medium into each sterile Petridish and were allowed to set at room temperature. The bacterial cell suspension was standardised to the optical density of 0.1 at 620 nm using a spectrophotometer. Thus final concentration of micro-organisms in the inoculum was adjusted to 10^8 cfu/ml and was inoculated over the surface of agar medium using a sterile cotton swab. The cups were scooped in each plate using a sterile borer. Sample equivalent to 0.2% w/w NDFX was quantitatively transferred to these plates. Positive control and negative control plates were also maintained to validate the experiment. The petri plates were then incubated in the anaerobic jar at 37° for 48 h. The zones showing complete inhibition were measured and diameters of the same were recorded to the nearest mm with the help of a measuring scale[19,20]. After incubation, the mean zone of inhibition was recorded for all the test samples (n=3). The results were analysed statistically by one-way ANOVA followed by Bonferroni's multiple comparison test (P<0.05) at 95% significance level.

RESULTS AND DISCUSSION

Being a poorly water-soluble drug, solubility of NDFX in the oily phase of microemulsion is very critical because it may affect the stability as well as the percutaneous delivery performance of the formulation. In order to screen appropriate solvents for the preparation of microemulsions, the solubility of NDFX in various oils, nonionic surfactants and cosurfactant was measured and the results are shown in figs. 1-3, respectively. The solubility of NDFX in Capryol 90 was 2065.26±0.04 μg/ml, which was the highest amongst the oils investigated. Amongst the various surfactants and cosurfactants screened, NDFX was found to exhibit good solubility in surfactants such as Tween 80 (12.73 μg/ml) and Labrafil M 2125 CS (36.18 μg/ml), whereas Transcutol P (7123.58±0.54 μg/ml) exhibited superior solubility amongst the cosurfactants screened in the preliminary studies. The selection of surfactant or cosurfactant in the further study was governed by their emulsification efficiency of oil in which NDFX had maximum solubility rather than their ability to solubilise NDFX.

Emulsification studies were performed to evaluate the ability of various surfactants to emulsify the selected oily phase. For the oil–surfactant mixture which forms microemulsion, it is essential to determine the condition under which they could disperse efficiently to form microemulsions with the selected oil. The percentage transmittance values of various dispersions are given in Tables 1 and 2. Emulsification studies with various surfactants showed that SolutolHS-15 had very good ability to emulsify Capryol 90 followed by Tween 80 and Tween 20, whereas, Labrasol appeared to be a poor emulsifier for Capryol 90. These observations are in line with the investigations reported by Malcolmson *et al*.[21] and Warisnoicharoen *et al*.[22] who concluded that microemulsification is also influenced by the structure and chain length of the surfactant. In case of Polaxomer 407, good transmittance (99.7%) was observed, but was not selected as it showed less solubility for NDFX as compared to Solutol HS-15 and Tween 80. whereas, Labrafil M 2125 CS which exhibited good solubility

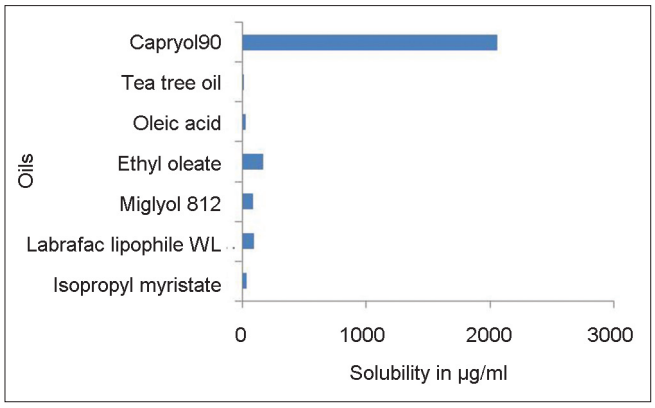

Fig. 1: Solubility of Nadifloxacin in various oily phases. Data are expressed as mean±SD (*n*=3)

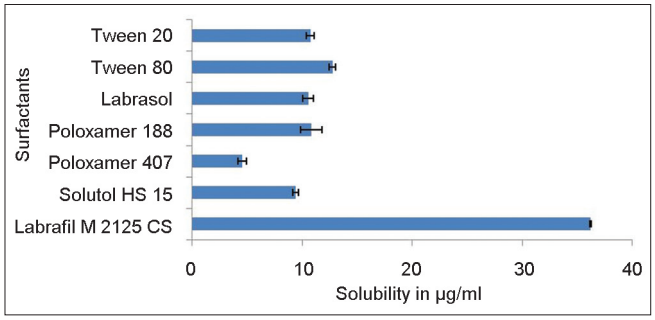

Fig. 2: Solubility of Nadifloxacin in 5% w/w surfactant solution. Data are expressed as mean±SD (*n*=3)

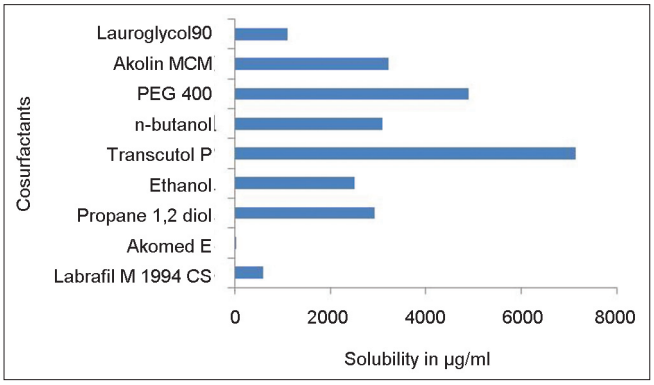

Fig. 3: Solubility of Nadifloxacin in cosurfactants. Data are expressed as mean±SD (*n*=3)

for NDFX, was not able to show good % transmittance indicating poor microemulsifying ability for the selected oily phase and hence rejected.

The short-chain alcohols and Transcutol P were widely used as cosurfactants[23,24]. In this experiment, Lauroglycol, PEG-400, n-butanol, Akomed E, Labrafil M 1944 CS, propane-1,2-diol, Akolin MCM, ethanol, isopropyl alcohol and Transcutol P as cosurfactants were investigated with Capryol 90 as the oil phase, Tween 80/Solutol HS 15 as surfactant at

TABLE 1: EMULSIFICATION EFFICIENCY OF VARIOUS NONIONIC SURFACTANTS

Surfactant	% Transmittance
Tween 20	89.82
Tween 80	94.01
Labrasol	64.02
Labrafil 2125	12.51
Polaxomer 188	82.92
Polaxomer 407	99.71
Solutol HS 15	100.20

Data expressed as mean (*n*=3)

TABLE 2: EMULSIFICATION STUDIES ON SURFACTANT/ COSURFACTANT COMBINATIONS

Cosurfactant	% Transmittance	
	Tween 80	Solutol SH 15
Lauroglycol	58.30	75.50
n-Butanol	89.6	98.81
PEG 400	85.08	98.96
Akomed E,	90.50	97.38
Labrafil 1944 CS	92.96	98.47
Akolin MCM	68.70	98.49
Ethanol	84.55	98.15
Propane-1,2-diol	81.59	98.85
Transcutol P	88.76	98.38

Data expressed as mean (*n*=3)

the fixed k_m of 1:1. All the hydrophilic cosurfactants were equivalent in improving the microemulsification ability of Solutol HS 15 and Tween 80. In case of lipophilic cosurfactants based on emulsification ability, Akomed E and Labrafil M 1944 CS were found to be the best followed by Akolin MCM whereas Lauroglycol 90 was less effective as a cosurfactant. Thus, there was good correlation between the structure and chain length of cosurfactant and transmittance value. This correlation was applicable to all lipophilic cosurfactants except Akomed E and Labrafil M 1944 CS. Labrafil M 1944 CS (oleic and linoleic acid backbone) was superior than Lauroglycol 90 (lauric acid backbone) due to its hydrophilic and surfactant-like properties. These investigations are in line with the investigation reported by Date *et al.*[2]. Though all cosurfactants showed good spontaneity of microemulsions, due to high solubility of NDFX, Transcutol P was selected as cosurfactant.

The pseudoternary phase diagrams were constructed in order to obtain the concentration range of components for the existence range of microemulsions. The phase diagrams of Capryol 90–Solutol SH 15–Transcutol P–water and Capryol 90–Tween 80–Transcutol P–water are shown in figs. 4 and 5. The transparent or translucent o/w microemulsion area was presented as

a shaded region in the phase diagrams. It is evident that Capryol 90–Tween 80–Transcutol P–water system had larger microemulsification region as compared to Capryol 90–Solutol SH 15–Transcutol P–water system (figs. 4 and 5). The optimum surfactant/cosurfactant ratio of microemulsion system was found at k_m 1:1 for Capryol 90–Tween 80–Transcutol P–water system. The emulsified area was the lowest at k_m 3:1 as the concentration of cosurfactant was less. System at k_m 1:1 formed a larger single-phase region than the systems at other k_m. It was reported that at the optimum k_m value, the cosurfactant gets entrapped into the cavities between the surfactant molecules, and the formed microemulsion will have the maximum solubilisation capacity[25]. In the current investigation, due to larger microemulsion region Capryol 90–Tween 80–Transcutol P–water system at k_m value 1:1 was selected for further studies.

After the microemulsion regions in the phase diagrams were identified, the microemulsion formulations were selected at different component ratios as described in Table 3. The microemulsions containing excess amount of drug were prepared and optimised for drug-loading efficiency and effect of amount of surfactant:cosurfactant and oil phase on globule size and PI of drug-loaded microemulsions were investigated. Formulations in which the oil content was 10% and surfactant–cosurfactant k_m (1:1) were 30, 35, 40 and 60%, respectively, were investigated for drug loading.

The drug-loading efficiency was found to be increased, as the content of surfactant:cosurfactant mixture was varied from 30 to 60% for microemulsion system. The results demonstrated that the increased drug load ing of microemulsion was obtained due to the higher concentration of surfactant and cosurfactant in the microemulsion system. Hence, this ensured that concentration of surfactant–cosurfactant had a major effect on the

TABLE 3: COMPOSTION OF NDFX MICROEMULSION FORMULATION

Formulation	Capryol 90:Tween 80-Transcutol (1:1):water (%w/w)	Drug loading (%)	Globule size (nm)	PI
F1	10:30:60	0.1±2.23	108.45±0.096	0.890±0.124
F2	10:35:55	0.15±2.68	96.43±0.01	1.113±0.057
F3	10:40:50	0.2±1.35	77.23±0.08	1.102±0.02
F4	10:60:30	0.25±1.37	65±1.35	1.132±0.006
F5	15:35:50	0.18±2.41	119.45±1.35	1.073±0.001
F6	20:35:45	0.2±3.27	121.64±1.35	1.07±0.049

Data were expressed as mean±SD (n=3). PI-Polydispersity index, NDFX-Nadifloxacin

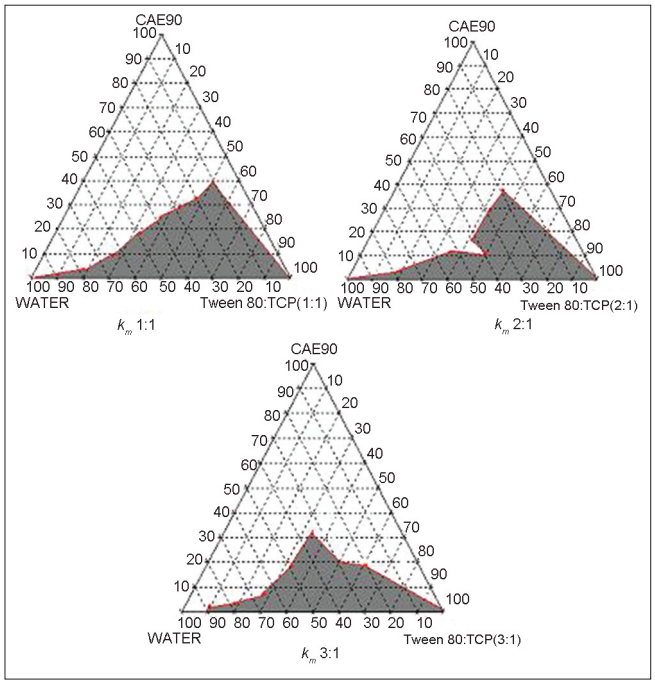

Fig. 4: Pseudoternary phase diagrams of microemulsions. Pseudoternary phase diagrams of microemulsions composed of oil (Capryol 90), surfactant (Tween 80), cosurfactant (Transcutol P) and water at various k_m values

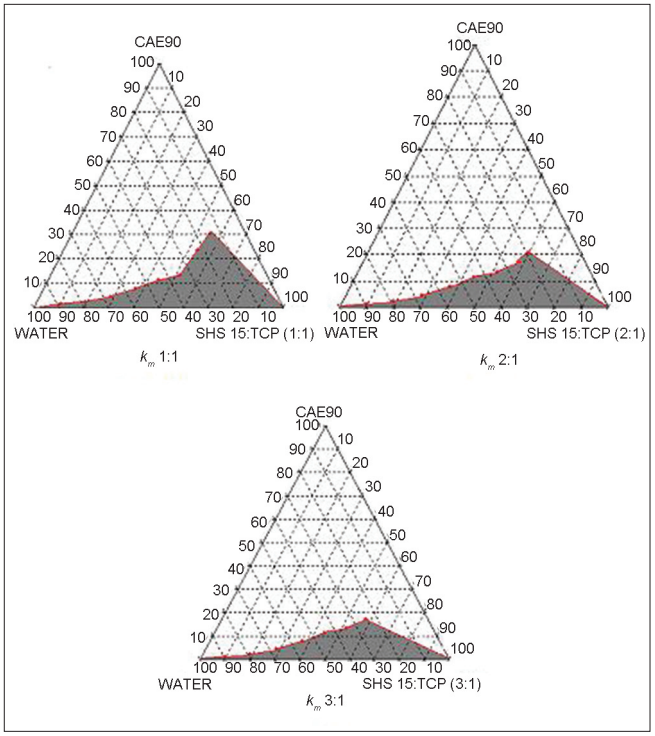

Fig. 5: Pseudoternary phase diagrams of microemulsions. Pseudoternary phase diagrams of microemulsions composed of oil (Capryol 90), surfactant (Solutol HS 15), cosurfactant (Transcutol P) and water at various k_m values

drug loading. This could be due to the highest solubilisation capacity of surfactant and cosurfactant for NDFX as investigated in the preliminary studies.

In order to determine the effect of the amount of oily phase on drug-loading efficiency, the content of oily phase varied from 10 to 20%, whereas the content of surfactant and cosurfactant mixture was fixed at 35% as in formula 2, 5, 6 in microemulsion system. The increase in drug loading with increase in oil content could be attributed to enhanced solubility of NDFX in oil. The amount of oily phase affected the drug-loading efficiency.

The amount of oily phase also affected the globule size. The average globule sizes were found to increase significantly with more oil, which can be attributed to the expansion of oil drop of microemulsion by increased amount of oil. This finding was consistent with a previous report that the average droplet sizes of tripolide microemulsion containing 1.5 and 60% oil were 12.5 and 9.8 nm, respectively[10]. Small droplet size was preferred in terms of skin permeation, so the oil content was selected as 10%.

The globule size was varied from 120 to 57 nm as the content of surfactant–cosurfactant mixture was varied from 30 to 60% microemulsion system. The result showed that the smaller globule size of microemulsion was obtained due to the higher concentration of surfactant and cosurfactant in microemulsion system. The decrease in the globule size can be attributed to the solubilisation of internal phase within a larger number of surfactant micelles, which are consequently swollen to a lesser extent[26,27]. When the content of oil was at 10% and surfactant cosurfactant mixture 60%; the formulations exhibited the smallest globule sizes as in case of formula 4. Based on the high drug-loading efficiency formula 3, 4 and 6 were further characterised and investigated for thermal stability.

Microemulsions were found to be robust to all dilutions and did not show any separation even after 24 h of storage. Optical isotropicity of the microemulsion was confirmed by visualisation through crossed polarisers. Birefringence is a light-scattering phenomenon, which is a characteristic of liquid crystalline systems. Birefringence results from unequal refractive indices of light in which the light passing through the matter is divided into two components having different velocities. Therefore, when liquid crystalline phase is observed between crossed polarisers, an intense bands of colours, i.e., birefringence is observed whereas, microemulsions appear completely black. Formulations appeared completely dark when observed between polarising plates in crossed position because of inability of light to pass. These observations, therefore, confirmed the optical isotropy of the resulting microemulsions[8,28]. The globule size for all NDFX-ME formulations revealed from light-scattering experiments were in the range 65.83-121.21 nm, which is generally considered to be the globule size of microemulsion (≈10-150 nm). From Table 4, NDFX-ME had slightly increased globule size of the internal droplet as compared to those without drug indicating that drug would immerse in the surfactant film around oily droplet of o/w microemulsion. PI is the measure of globule homogeneity and it varies from 0 to 1. PI value to zero is the indication of higher the homology between the globules. PI for all formulations was around one. The selected NDFX-ME formulations (Table 3) were subjected to various thermodynamic stability tests, which included centrifugation and freeze–thaw cycle tests. No phase separation, creaming or drug precipitation was observed while performing these tests and all the formulations survived these stress tests. The results showed that all the formulations had a good physical stability. Very low interfacial tension between oil and water and small droplet size made these systems thermodynamically stable. The increased concentration of oil and surfactant in microemulsions may retain the drug in droplets of microemulsion formulation that will decrease its permeation in the skin[29,30,31]. Hence, based on these studies, the microemulsion formulation containing low oil and surfactant: cosurfactant content, i.e., F3 was formulated into gel.

From the results shown in Table 5, it was noted that there was no significant change in globule size when microemulsion was formulated in gel form using xanthan gum and Carbopol ETD 2020. When the microemulsion was gelled by incorporating a gelling agent, globule aggregation was not observed which was indicated by no change in PI. The

TABLE 4: CHARACTERIZATION OF NDFX MICROEMULSION

Formulations	Globule size (nm)		PI	pH
	Placebo	Drug loaded		
F3	63.3±1.12	77.23±0.08	1.123±0.23	6.30±0.21
F4	58.05±0.36	65±1.35	1.018±0.02	6.08±0.10
F6	102.5±1.10	121.21±0.46	0.854±0.10	6.4±0.17

Data were expressed as mean±SD (*n*=3). PI-Polydispersity index, NDFX-Nadifloxacin

TABLE 5: CHARACTERIZATION OF NDFX MICROEMULSION GEL

Formulations	Globule size (nm)	PI	pH	Drug content (%w/w)	Viscosity at 20 rpm	Spreadability diameter (cm)
Xanthan gum gel	73.10±0.5	1.193	6.32±0.02	97.53±1.26	34000	7.5±0.15
Carbopol ETD 2020 gel	72.59±0.39	1.254	7.26±0.05	98.53±1.53	53000	8.8±0.15

Data were expressed as mean±SD (*n*=3). PI-Polydispersity index, NDFX-Nadifloxacin

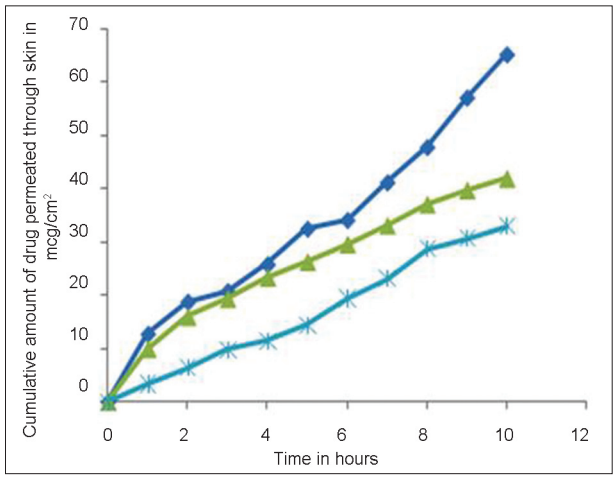

Fig. 6: Cumulative amount of drug permeated in µg/cm² versus time in hours.
▲ Carbopol ETD 2020 ME gel, ♦ Xanthan gum ME gel, Ж Nadoxin® cream

pH values of all formulations were found to be compatible for topical formulations. The pH range for skin is 6-8. The spreadability diameter of all microemulsion-based gels was in the range 7-8.6 cm, whereas the diameter for marketed formulation was 6.32 cm, indicating that the spreadability of microemulsion-based gels was better than that of marketed formulations. This could be because of the loose gel matrix nature of microemulsion-based gel due to presence of oil globules rather than the conventional cream formulation. Although good spreadability was observed for all the formulations, the formulation gelled with xanthan gum showed better spreadability and had a diameter that ranged 8.2-8.7 cm as compared to those gelled with Carbopol ETD 2020 with diameter ranged 7.1-7.6 cm.

The drug content in the NDFX-MEG is as shown in the Table 5, and was well within limits. There was no degradation of drug in the microemulsion when formulated as microemulsion-based gels. Rheological data obtained from microemulsion gel showed non-Newtonian, thixotropic behaviour. The thixotropic system is very advantageous and is more convenient than an ideally viscous system from the technological as well as the topical application viewpoints of the formulation[32]. Viscosity of microemulsion formulation gelled with Carbopol ETD 2020 was higher, i.e., in the range 199,000-220,000 cps as compared to those formulation which were gelled with xanthan gum had viscosity in the range 97,000-99,900 cps. Viscosity results at 20 rpm are shown in Table 5. The lower viscosity of xanthan gum NDFX-MEG could be attributed to ease of spreading. This was confirmed in a spreadability test where in xanthan gum NDFX-MEG showed larger diameter compared to Carbopol ETD 2020 NDFX-MEG. *Ex vivo* permeation studies were carried out using abdominal skin of male Wistar rat to assess the release of selected NDFX-MEG and compared with commercial formulation. Phosphate buffer (pH 6.8) containing 20% ethanol was selected as a diffusion medium on the receptor side. Ethanol was added in the release media to maintain the sink condition. The result of percutaneous penetration of marketed cream formulation (Nadoxin®) containing 1% NDFX and o/w microemulsion-based gel containing 0.2% w/w NDFX were shown in fig. 6. In the *ex vivo* skin permeation studies, it was demonstrated that microemulsion-based gels improved the permeation as compared to the marketed formulation at the end of 10 h.

Conventional creams have a mean droplet size ranging 10-100 µm. Such formulations have demonstrated poor penetration of drug-loaded oil droplets into deep skin layers. Gupta and Garg[4] have reported that microparticles with diameters ranging from 3 to 10 µm selectively penetrate follicular ducts, whereas particles >10 µm remain on the skin surface, and those <3 µm are distributed randomly into hair follicles and stratum corneum. As shown in fig. 6, microemulsion gel improved the skin permeation of NDFX over the commercial creams. This could be due to the presence of surfactant and cosurfactant in microemulsion which may affect the stratum corneum structure and reduce the diffusional barrier by acting as a permeation enhancer[30]. The microemulsion is expected to penetrate the stratum corneum and exist intact in the whole horny layer. Once it enters into the stratum corneum, the microemulsion may simultaneously alter both the lipid and the polar pathways[6,31]. The lipophilic domain of the microemulsion can interact with the stratum corneum in many ways. NDFX dissolved in the lipid domain of a microemulsion can directly partition into

the lipids of the stratum corneum or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilising its bilayer structure. In effect, these interactions would lead to an increase in the permeability of the lipid pathway to NDFX. On the other hand, the hydrophilic domain of the microemulsion can hydrate the stratum corneum to a great extent[29]. There is a general experience that hydration of the skin plays an important role in the percutaneous uptake of poorly soluble drug. When the aqueous fluid of the microemulsion enters the polar pathways, it increases the interlamellar volume of stratum corneum lipid bilayers, resulting in disruption of the interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers; a lipophilic drug like NDFX can then permeate more easily through the lipid pathway of the stratum corneum. The greater drug penetration enhancing activity of microemulsions may be attributed to the combined effects of both the lipophilic and hydrophilic domains of microemulsions[33].

The permeability parameters of different formulations are given in Table 6. The results showed that the optimised microemulsion formulation when gelled with xanthan gum, the flux (J_{ss}) and permeability coefficient (*K*p) was greatly increased as compared to the marketed formulation, i.e., xanthan gum NDFX-MEG showed highest flux of 2.761±0.051 µg/cm^2/h. At the end of 10 h, the cumulative drug release by xanthan gum NDFX-MEG was found to be 65±0.45 µg/cm^2 and 2.7-fold increase in flux as compared to marketed formulation with highest enhancement ratio (*E*r) 2.832. Also steady-state flux and permeability coefficient and enhancement ratio were significantly increased in Carbopol ETD 2020 NDFX-MEG as compared with marketed formulation. Results of statistical analysis of permeation studies also showed that NDFX-MEG were statistically significant as compared to marketed cream.

The xanthan gum formulations showed good permeability as compared to Carbopol ETD 2020 formulations, so optimised microemulsion formulation gelled with xanthan gum was selected for antibacterial studies. Mueller-Hinton agar was selected as the media from the available literature, supporting the growth of the aerobic microorganisms. The inoculum was grown on nutrient agar broth and turbidity (indicating growth of the aerobic microorganisms) was observed. When the microorganisms were inoculated in petri plates containing Mueller-Hinton agar, followed by incubation, a matted growth was obtained. This confirmed the growth of the organisms, suitability of the media and maintenance of successful conditions for the growth. After addition of microemulsion-based gels, the zone of inhibition was visible and prominent at the end of 48 h and reported in Table 7. A distinct zone of inhibition was observed in the petri plate containing standard solution of the drug and the drug-loaded microemulsion-based gels as well as one containing marketed cream. Petri plates with placebo microemulsion gel formulation as well as DMSO blank did not show any zone of inhibition. Results of disc plate method are as shown in figs. 7 and 8 indicates an antibacterial effect of NDFX-MEG compared with marketed cream and NDFX in DMSO. The One-way ANOVA followed by Bonferroni's multiple comparison test showed that the NDFX-MEG had statistically significant antimicrobial activity as compared to marketed cream and NDFX in DMSO (P<0.05). The enhanced *in vitro* antibacterial activity of NDFX-MEG may be attributed to enhanced penetration of oil globules containing NDFX through bacterial cell wall to inhibit the enzyme DNA gyrase, thus inhibiting bacterial multiplication[15].

Thus, foregoing results indicate that under optimised conditions, NDFX can be successfully incorporated in microemulsion system using GRAS-listed and topically

TABLE 6: PERMEABILITY PARAMETERS OF DIFFERENT FORMULATIONS

Formulations	Flux (J_{ss}±SD) (µg/cm^2/h)	Permeability coefficient (*K*p) in cm/h×10^{-3}	Enhancement ratio (*E*r)
Marketed	0.975±0.057	0.975±0.057	2.832
Xanthan gum gel	2.761±0.051	2.761±0.051	
Carbopol ETD 2020 gel	1.362±0.057	1.362±0.057	0.581

Data were expressed as mean±SD (n=3), statistically significant at P<0.05 by Student's *t*-test

TABLE 7: DIAMETER OF ZONE OF INHIBITIONS RECORDED IN THE ANTIMICROBIAL STUDY

Sample	Zone of inhibition (mm) (n=3)
NDFX-ME xanthan gum gel	50.5±0.1
Marketed formulation	29.4±0.11
Placebo ME xanthan gum gel	No zone observed
Standard drug solution in DMSO	42.3±0.15

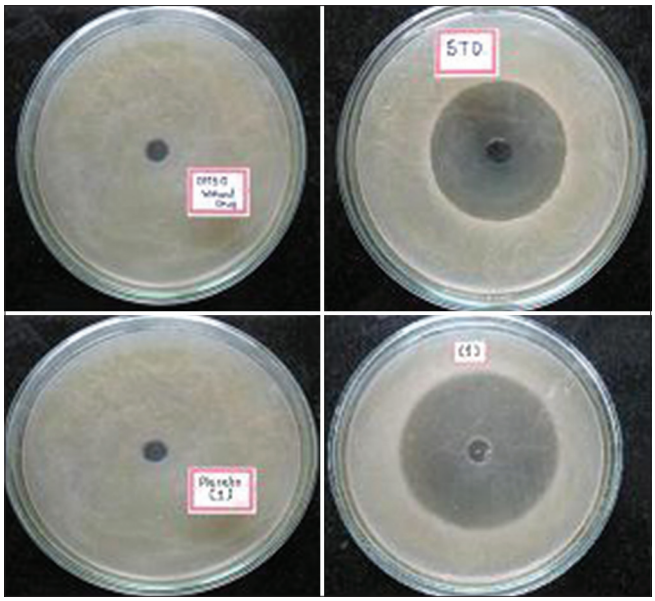

Fig. 7: Zone of inhibition antibacterial activity.
Diameter of zone of inhibition antibacterial activity of (a) DMSO, (b) Nadifloxacin (NDFX) in DMSO, (c) placebo microemulsion gel, (d) NDFX-microemulsion gel (F1)

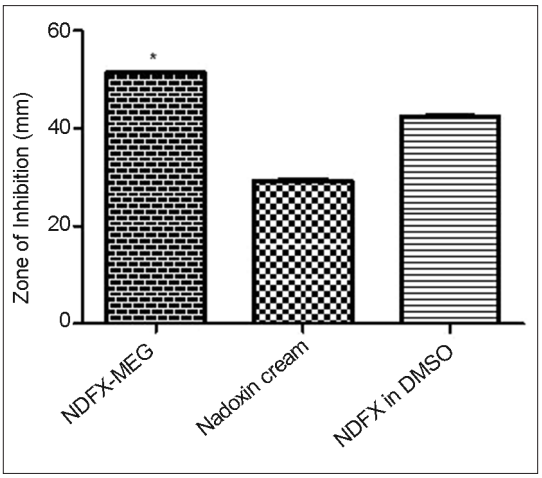

Fig. 8: Effect of antibacterial activity of different formulations of Nadifloxacin.
***Indicates significance at P<0.05. Mean±SD, n=3**

acceptable surfactants, cosurfactants and oily phase. The results provide a basis for the successful design of NDFX microemulsion which resulted in improved penetration of drug and antimicrobial activity in comparison with commercial formulation of NDFX.

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