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Research Article

FORMULATION AND EVALUATION OF NANOEMULSION GEL OF TENOXICAM FOR TOPICAL APPLICATION

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ABSTRACT

The aim of the present study to develop a nanoemulsion gel formulation of poorly water soluble drug tenoxicam for the transdermal delivery. Nanoemulsions have the particle size range in nano range. Particle size in nanoemulsion formulation determines the stability of formulation so it's very important to achieve the optimum size of particles to formulate the drug. To develop the nanoemulsion here ethyl oleate, tween-80 and propylene glycol were selected among various types of oils, surfactants and co-surfactants for preparing nanoemulsion. Various oil-in-water nanoemulsions were prepared by the spontaneous emulsification method. The nanoemulsion area was identified by constructing ternary phase diagrams. The prepared nanoemulsions were subjected to different thermodynamic stability tests to screen out the stable formulations among various prepared formulation having different smix ratios with oil phase. The Tenoxicam containing nanoemulsion gel was prepared and characterized for particle size, zeta potential, pH, In-vitro drug release and viscosity. Tenoxicam nanoemulsion gel treated dyliasis membrane employed to investigate the skin permeation mechanism of tenoxicam from nanoemulsion gel. The obtained results from the study suggested that nanoemulsions are potential vehicles for improved transdermal delivery of Tenoxicam.

Key words: *Nanoemulsion, Transdermal, Tenoxicam, In-vitro, Zeta potential, Dyliasis membrane*

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation [1]. Tenoxicam, an NSAID, has potential to treat inflammatory disorders like rheumatoid arthritis and osteoarthritis but not recommended orally due to development of gastric ulcers and other sever systemic disorders [2, 3]. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time.

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Therefore, an improved tenoxicam nanoemulsion formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body, such as bones, ligaments, joints, tendons, and muscles. There has been increased interest during recent years in the use of topical vehicle systems that could modify drug permeation through the skin. Many of the dermal vehicles contain chemical enhancers and solvents to achieve these goals [4]. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm [5]. Many studies have shown that nanoemulsion formulations possess improved transdermal

and dermal delivery properties in vitro [6-7] as well as in vivo [8-9]. Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions [10-11] and gels [12-13]. This article describes the potential of nanoemulsion systems in transdermal delivery of tenoxicam using pharmaceutically acceptable ingredients without using additional permeation enhancers, because excipients of nanoemulsions themselves act as permeation enhancers.

MATERIALS AND METHODS

Materials

Tenoxicam was gift sample from IPCA Laboratories Limited mumbai. Ethyl oleate, Tween 80, propylene glycol, tween-60 and Oleic acid were purchased from Merck Ltd (Mumbai, India). Cremophor RH 40 was purchased from BASF (cadilla, Gujrat). Carbopol 970 was kindly purchased from Glenmark, india. Methanol was purchased from Merck Pvt. Ltd. (Mumbai, India). All other chemicals were of analytical grade. Freshly distilled water was used throughout the work.

SOLUBILITY OF TENOXICAM

The solubility of tenoxicam in various oils (soyabean oil, ethyl oleate and isopropyl palmitate), surfactants (tween-20 and tween-80, tween-60) and co-surfactants (propylene glycol and cremophor RH 40) was determined by dissolving an excess amount of tenoxicam in 2 ml of each of the selected oils, surfactants, and cosurfactants in 5-ml capacity stoppered vials separately. A combination of oils was also used for determination of solubility. An excess amount of tenoxicam was added to each 5-mL-capacity stoppered vial and mixed using a vortex mixer. The mixture vials were then kept at 37 ± 1.0 C in an isothermal shaker for 72 hours to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45-µm membrane filter. The concentration of tenoxicam was determined in each oils, surfactants, co-surfactants in presence of methanol using spectrophotometer at their respective λ max.

DRUG – EXCIPIENT COMPATIBILITY STUDIES

Fourier Transform-IR Studies

An FTIR-8400s spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory was used to obtain the IR spectra of drug in the isotropic mixtures of excipients, pure drug, tween 80, propylene glycol, carbopol 970, physical mixtures of the drug with the individual excipients (1:1 ratio) were carried out using diffuse reflectance spectroscopy (DRS) - FTIR with KBr disc. To characterize possible interactions between the drug and excipients, infrared spectra was recorded.

TERNARY PHASE DIAGRAM

On the basis of the solubility studies and drug excipient compatibility studies, ethyl oleate was selected as the oil phase. Tween-80 and propylene glycol were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and co-surfactant (Smix) were mixed at different mass ratios (1:1, 2:1, 3:1 and 4:1) and prepared four phase diagrams. For each phase diagram, oil and Smix at a specific ratio was mixed thoroughly at different mass ratios from 1:9 to 9:1 in different glass vials [14]. The mixtures of oil, surfactant and co-surfactant at certain weight ratios were titrated by adding water drop wise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and at the same time examined for the transparency. Samples with low viscosity, single phase and transparent nature were considered as stable formulation. The data obtain after titration was used for the construction of ternary phase diagram.

SELECTION OF NANOEMULSION FORMULATION

From the constructed phase diagram, different formulations were selected from the nanoemulsion region so that the drug could be incorporated in the oil phase. 20 mg of tenoxicam was kept constant in all selected

formulations, were dissolved in a beaker containing oil, surfactant and co-surfactant.

PREPARATION OF NANOEMULSION FORMULATION LOADED WITH DRUG

The nanoemulsion formulations were prepared by spontaneous emulsification method as follow. Appropriate quantities of oil ethyl oleate, surfactant tween-80 and co-surfactant propylene glycol were weighed and mixed well. The drug was accurately weighted to 20 mg and added to the previous mixture and stirred with a magnetic bar on magnetic stirrer, at room temperature until the drug completely dissolved. The weighed amount of water then added drop wise with continuous mixing. Table no.1 showing the composition of selected nanoemulsion formulations [20].

Table 1: Formulation design

COMPOSITION (gm)					
FORMULATION CODE	***TENOXICAM (mg)***	***ETHYL OLEATE***	***TWEEN 80 & PROPYLENE GLYCOL***	***WATER***	***RATIOS OF TWEEN 80 & PROPYLENE GLYCOL***
F1	20	0.18	2.42	0.4	4:1
F2	20	0.20	2.40	0.4	4:1
F3	20	0.22	2.38	0.4	4:1
F4	20	0.18	2.42	0.4	1:1
F5	20	0.20	2.40	0.4	1:1
F6	20	0.22	2.38	0.4	1:1
F7	20	0.18	2.42	0.4	1:4
F8	20	0.20	2.40	0.4	1:4
F9	20	0.22	2.38	0.4	1:4

CHARACTERIZATION OF NANOEMULSION FORMULATION

Stability

The formulations (1% w/w) were tested for physical (dispersion) stability by using centrifugation, heating-cooling cycle. Only those formulations which survived dispersion stability tests were selected for further study. Formulations which become turbid during heating-cooling cycle which indicates that these formulations were unstable and screened out for further study.

Heating Cooling cycle

Six cycles between refrigerator temperature 4 ºC and 45 ºC with storage at each temperature of not less than 48 hrs was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

Centrifugation

In order to estimate metastable systems, the optimized nanoemulsion formulation was diluted with purified distilled water. Passed formulations were centrifuged at 3500 rpm for 30 minutes and observed for any change in homogenecity of nanoemulsions.

Droplet Size Measurements

Droplet size analysis was done by Dynamic Light Scattering (DLS). Dynamic light scattering (sometimes referred to as photon correlation spectroscopy or quasielastic light scattering) is a technique for measuring the size of particles typically in the submicron region. 2 gms of each formulation were diluted with 500 ml of phosphate buffer pH 7.4. The volumetric flasks were inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulations, particle size was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the

brownian motion of the droplets as function of time using a Zetasizer Nano series (Malvern Instrument, DTS.Ver.4.10 serial no.MAL 500999). Light scattering was monitored at 25 ºC [5].

Zeta Potential Determination

Zeta potential for nanoemulsion was determined using zetasizer hsa 3000 (Malvern instrument ltd., UK). 2 gms of each formulations were diluted with Milli Q water up to 100 ml. Zeta potential were measured by using Malvern Zetasizer. Equipped with a 4.0 mW He-Ne red laser (633nm) [20]

FORMULATION OF TENOXICAM NANOEMULSION GEL

Nanoemulsion base gel was prepared by dispersing the 1g of the Carbopol-970 in a sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24 hrs for complete swelling of carbopol-970. The carbopol dispersion was mixed with selected formulations containing 20 mg of tenoxicam in 2.6 gms of excipients. The mixture was stirred well to get homogenous solution so that concentration of carbopol 970 will become 0.5% w/w. 0.2 ml of triethanolamine was added with continuous stirring to get homogenous gel. Table no.2 showing the composition of drug and excipients.

EVALUATION OF NANOEMULSION-BASED GEL

Determination of pH

The pH of 10%w/v suspension of nanoemulsion gel in distilled water was determined using µ pH meter 361.

Drug Content Studies

Naoemulsion based gel, 2 gms from each formulations were taken in 100 ml volumetric flask having 10ml methanol and stirred by vortex mixer for 5 minutes. The solutions were filtered using whatmann filter paper. The absorbance of the solution was estimated spectrophotometrically (UV 1800, Shimadzu, Japan) at 364.60 nm using standard curve.

Viscosity Measurement

The viscosity of the formulations (0.5gm) was determined as such without dilution using a Brookfield viscometer Digital model LV TD-230V, serial – 214 cone plate rheometer (Brookfield Engineering Laboratories, USA) using spindle ≠CPE 50-100 at 25° C.

Droplet Size Analysis

Droplet size analysis was carried out by Dynamic Light Scattering (DLS) for topical nanoemulsion gel. For the analysis of particle size in formulation 2 gms of each formulation were diluted with 500 ml of phosphate buffer pH 7.4. The volumetric flasks were inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulations, particle size was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a Zetasizer Nano series (Malvern Instrument, DTS. Ver.4.10, serial No. MAL 500999). Light scattering was monitored at 25 ºC.

In-Vitro Drug Diffusion Studies

Franz diffusion cell is used to obtain the drug release profile of the nanoemulsion formulation in the case of formulations for transdermal application. The membrane was mounted between the donor and receptor compartments. Cylindrical glass tube open at both ends with an exposed surface area of 3.14 cm^2 was used as diffusion cell. A dialysis membrane was allowed to hydrate in distilled water for 24 hrs. Dialysis membrane was fixed to one end of the cylinder with rubber band.

1 gm of gel was spread over the cellophane membrane. Precautions were taken to ensure uniform thickness of gel over the membrane and to remove all air bubbles between the gel and the membrane.

With the help of another cylinder tube dialysis membrane should be in contact with the receptor media. The cell was immersed in a beaker containing 25ml of 5% v/v methanolic phosphate buffer pH 7.4 (receptor media). The system was maintained at 37±2°C. Precaution was taken to keep the buffer below the rubber

band. The buffer was stirred with a magnetic stirrer during the entire 8 hrs release study. Samples of 2ml were withdrawn at different time intervals for a period of 8 hrs from the receptor compartment and replaced with an equal volume of buffer at 37±2°C. The samples after diluting suitably were analyzed spectrophotometrically, at a wavelength of 368.20 nm for tenoxicam content [22].

Table 2: The composition of drug and excipients

EXCIPIENTS	*MASS (gm)*
Tenoxicam	20 mg
Ethyl Oleate	2.6
Tween 80	2.6
Carbopol 970	1
Propylene Glycol	2.6
Distilled Water	q.s.
Trietanolamine	0.2 ml

RESULT AND DISCUSSION

Screening of components by solubility studies

The excipients selected needed to be pharmaceutically acceptable, nonirritating and non sensitizing to the skin and to fall into the GRAS (generally regarded as safe) category. Among the selected oils that were screened, maximum solubility of tenoxicam was found in ethyl oleate as compared to the other oils as shown in table 3. Non-ionic surfactants are less toxic than ionic surfactants. The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form. The right blend of low and high hydrophilic lipophilic balance (HLB) surfactants leads to the formation of a stable nanoemulsion formulation [17]. Tween 80 selected as a surfactant having the HLB value 15. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of a single surfactant, usually necessitating addition of a co-surfactant. The presence of co-surfactant decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form a nanoemulsion. Propylene glycol is here used as co-surfactant as the solubility of drug in propylene glycol is maximum 8.80 mg/ml as depicted in table no.3.

Table 3: Solubility of drug with different oils, surfactants and co-surfactants

COMPONENT	*SOLUBILITY (mg/gm)*
Oleic acid	5.6±.02
Ethyl Oleate	10.3±.03
Soyabean oil	3.10±.14
Tween 80	9.80±.36
Tween 60	6.2±.21
Propylene glycol	8.80±.13
Cremophor RH 40	5.9±.25

FTIR Study

To characterize possible interactions between the drug and excipients, infrared spectra was recorded. IR-spectra of tenoxicam, individual excipients, and physical mixtures of the drug with individual excipients in ratio 1:1 showing no interaction between drug and selected excipients by retaining the λ_{max} of drug in spectrum. FTIR spectrum of tenoxicam with excipients showed characteristic amide peaks for tenoxicam at 3097.78, 3115.04, 3089.96, 2966.62 and 3107.43 cm^{-1} carbonyl stretching (N-H Stretching), vibrations at 1043.52, 1151.50, 1197.79, 1151.50 and 943.22 cm^{-1} ; C=O stretching at 1857.51, 1328.95, 1328.95, 1330.88 and 1915.38 cm^{-1}. The FTIR spectrum data showing in figure 1-3 confirms that all the excipients do not alter the performance characteristic of the drug, indicating their compatibility.

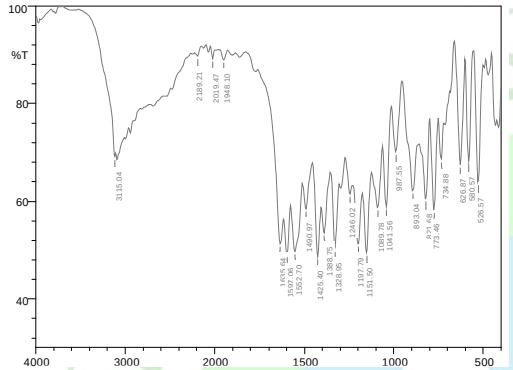

Fig.1. FTIR Spectrum of Tenoxicam +Propylene glycol

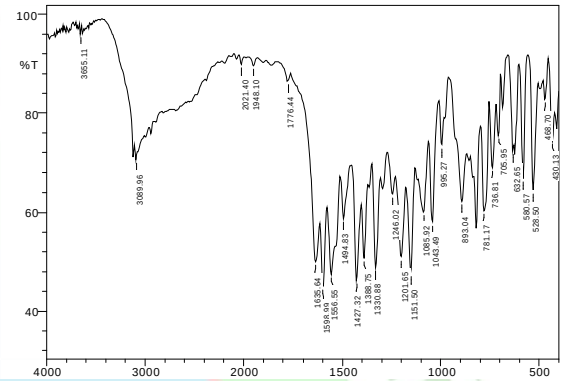

Fig.2. FTIR Spectrum of Tenoxicam +Tween 80

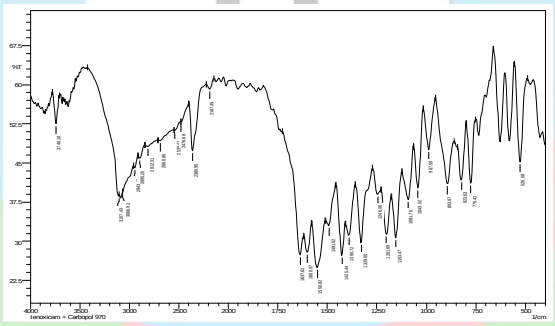

Fig.3.FTIR Spectrum of Tenoxicam+ Carbopol 970

Ternary phase diagram

The construction of pseudoternary phase diagrams is used to determine the concentration range of components in the existence range of nanoemulsion. The pseudoternary phase diagrams with various weight ratios of tween 80 to propylene glycol are depicted in Figure 4. Care was taken to ensure that observations were not made on metastable systems.although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous [17]. Pseudoternary phase diagrams were constructed separately for each Smix ratio (Figure 4), so that o/w nanoemulsion regions could be identified and nanoemulsion formulations could be optimized. Ternary phase system with tween80:propylene glycol (4:1) exhibited maximum area for nanoemulsion formation. It is well known that large amounts of surfactants cause skin irritation. It is therefore important to determine the surfactant concentration properly and use the optimum concentration of surfactant in the formulation. From pseudoternary phase diagrams, the formulations in which the amount of oil phase completely solubilized the drug and which could accommodate the optimum quantity of smix and distilled water were selected for the study [18, 19].

EVALUATION OF NANOEMULSION FORMULATION

Stability of nanoemulsion

Stability study testing conducted on 1% w/w nanoemulsion formulations from the ternary phase diagram done by centrifugation and heating cooling cycle and depicted in table 4. Changes occurred in formulation F7-F9 which have Smix ratio of 1:4. These formulations are rejected for further study as they are not thermodynamically stable. The only formulations which passed the stability test by tolerating physical changes applied onto them were selected for further study.

Table 4. Characterization for nanoemulsion formulations F1-F6

FORMULATION CODE	*STABILITY STUDY*		*DROPLET SIZE MEASUREMENT [nm]*	*ZETA POTENTIAL(mV)*
	HEATING-COOLING CYCLE	*CENTRIFUGATION*		
F1	No change	No Phase separation	9.42±.23	-13.6±.5
F2	No change	No Phase separation	7.15±.39	-11.9±.5
F3	No change	No Phase separation	10.44±.32	-15.9±.5
F4	No change	No Phase separation	10.00±.23	-17.7±.5
F5	No change	No Phase separation	8.26±.12	-27.3±.5
F6	No change	No Phase separation	12.02±.10	-31.6±.5

Droplet size measurement

The mean particle size was observed as 9.54 nm after 48 hrs post-dilution in dilution media phosphate buffer pH 7.4. The particle size for the formulation F1- F6 is obtained in range between 7.5-12.2 nm depicted in table 4.

Zeta Potential Determination

Many physiological studies have proved that apical potential of absorptive cells, as well as that of all other cells in the body, is negatively charged with respect to the epidermal cells. A nanoemulsion which results in the positively charged dispersed oil droplets upon dilutions with an aqueous phase, leads to adhesion to the epidermal cell. All the formulations acquire zeta potential, which varied between -31.8and -11 mV, suggesting increased adhesion of the droplets to the cell surface because of electrostatic attraction shown in table 4.

EVALUATION OF NANOEMULSION GEL

pH Measurements

pH of 10% w/v of nanoemulsion gel ranged between 5.12 – 5.36 (table 5). The result indicates that pH of all the nanoemulsion gel (F1-F6) is in close approximation to the skin pH (4-5.6), inferring the compatibility of formulation with skin.

Drug Content

The drug content of formulation F1-F6 was found in the range of 98.95-99.91% showing in table 5 indicating that uniform distribution of tenoxicam in formulation.

Viscosity determination

The viscosity of the formulations (F1-F6) was determined as such without dilution and tabulated in table 5.

Droplet Size Analysis

Droplet size analysis was carried out by Dynamic Light Scattering (DLS) for topical nanoemulsion gel (F1-F6). Change in mean particle size was observed. The mean particle size obtained as 32.25 nm after gelling of nanoemulsion formulations. The particle size range obtained for gel formulations F1-F6 are found between 33.5-58.29 nm which is shown in table 5.

Table 5: Evaluations for nanoemulsion gel formulations F1-F6

FORMULATION CODE	*DROPLET SIZE (nm)*	*pH*	*% DRUG CONTENT*	*CONSISTANCY INDEX (m PAS)*
F1	33.55±.5	5.12 ± 0.01	99.37 ± 0.12	114.43
F2	21.53±.5	5.14 ± 0.02	99.91 ± 0.01	90.52
F3	27.29±.5	5.29 ± 0.01	99.71 ± 0.03	122.80
F4	42.09±.5	5.32 ± 0.01	98.48 ± 0.03	101.72
F5	37.33±.5	5.32 ± 0.01	98.41 ± 0.01	88.56
F6	58.29±.5	5.36 ± 0.005	98.40 ± 0.01	163.76

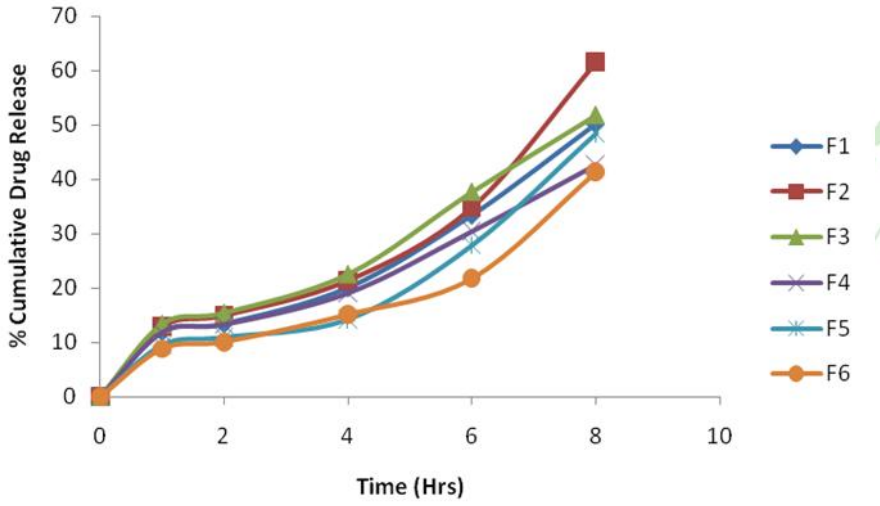

Fig.5. Comparative diffusion studies of all formulations

In vitro skin permeation studies

Dissolution studies by using semipermeable membrane were performed to compare the release of drug from six different nanoemulsion based gel formulations (F1−F6). The comperative drug release profiles are shown in the table 6 and shown in graphical form in fig.5. All nanoemulsion gel formulations having the same quantity (20 mg) of tenoxicam. The maximum release is found in F2 could be due to the smallest droplet size and

lowest viscosity compared to nanoemulsion gels F1-F6. Reduction in the droplet size leads to higher surface area and higher dissolution of tenoxicam in oily phase of nanoemulsion based gel formulations eventually permitted drug release at faster rate from nanoemulsion based gel formulations showing the significance of the nano sizing of the oils globules [15]. By the study of *in vitro* drug release we can conclude that formulations F2 which having Smix ratio of 4:1 show better drug release. In all the formulations, formulation F2 with drug, tween 80: propylene glycol (4:1), found to give a better result i.e.; drug 61.68 % release in 8 hours.

Table 6.Diffusion profiles of formulations F1- F6

S.NO	*TIME (Hrs)*	*% CUMULATIVE DRUG RELEASE*					
		F1	*F2*	*F3*	*F4*	*F5*	*F6*
1	0	0	0	0	0	0	0
2	1	11.925	12.901	13.395	11.860	9.504	8.854
3	2	13.400	14.973	15.406	13.264	10.91	10.104
4	4	20.053	21.421	22.550	19.062	14.21	15.136
5	6	33.401	34.928	37.687	30.446	27.85	21.812
6	8	50.173	61.683	51.811	42.697	48.41	41.397

In order to understand the complex mechanism of drug release from the nanoemulsion based gel formulations, the *in-vitro* tenoxicam release data were fitted to Korsmeyerpeppa's release model and interpretation of release exponent values (n) enlightens us in understanding the release mechanism from the formulations which is tabulated in table 7. The release exponent values thus obtained were from 0.9580 to 0.8096. Based on these values we can say that the formulation exhibited non-fickian transport. The formulations showed higher (r) values for zero order plots indicating that drug release from F2 followed zero order kinetics.

DISCUSSION

- In the present study, seven excipients were explored for solubility studies of tenoxicam. For each excipient, λ_{max} of the drug in methanol (i.e., 364.60nm) was found to be retained indicating that each of these excipients is well compatible with the drug at 37±2°C. The solubility data of tenoxicam revealed that the best combination of excipients in order to formulate nanoemulsion of tenoxicam was the combination of ethyl oleate, tween 80 with propylene glycol.
- Optimization of the tenoxicam nanoemulsion was performed using ternary phase diagram. On the basis of solubility studies, ethyl oleate was selected as the oil; tween 80 and propylene glycol were selected as surfactant and co-surfactant. Distilled water used as an aqueous phase. Nine formulations were selected from nanoemulsion region of ternary phase diagram (monophasic region) and were evaluated to get optimum formulations with desired droplet size and drug release characteristics. Viscosity studies of the optimized formulations indicate the pseudo plastic behavior.
- In the present work, studies on spectroscopic absorbance, drug content, droplet size, viscosity and drug release characteristics indicates that the tenoxicam nanoemulsion gel formulation F2 employing ethyl oleate as the oil and tween 80, propylene glycol as the surfactant and co-surfactant in ratio of 4:1 exhibited excellent results.
- The objective behind development of nanoemulsion was to have maximum drug release while maintaining the particle size at lowest level, using proper combination of excipients (oil/surfactant/co-surfactant). Further, the optimized formulation was

selected among the six formulations based upon higher % cumulative drug release of nanoemulsion. Nanoemulsion gel formulation F2 showing the higher % cumulative drug release.

- On the basis of observation, it can be predicted that optimum amounts of ethyl oleate, tween 80 and propylene glycol are able to produce nanoemulsion with desirable particle size and release characteristics.

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