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DEVELOPMENT AND IN VITRO CHARACTERIZATION OF NANOEMULSION EMBEDDED THERMOSENSITIVE *IN-SITU* OCULAR GEL OF DICLOFENAC SODIUM FOR SUSTAINED DELIVERY

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Keywords:

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ABSTRACT: The objective of this work was improvement in the ocular bioavailability of diclofenac sodium by enhancing drug permeation across cornea and promoting drug retention time on ocular surface. Using pseudo ternary phase diagrams transparent regions were identified and nanoemulsions were prepared by self-emulsification method, which were further optimized by emulsification study and drug content. Three best NE formulations were incorporated in 20% poloxamer 407 (thermosensitive polymer solution) to formulate nanoemulsion embedded thermosensitive *in-situ* ocular gel and further evaluated on the basis of gelation temperature and drug entrapment. Optimized nanoemulsion formulation was evaluated by TEM, particle size analysis and zeta potential. The optimized NE gel formulation changed from sol-gel phase at physiological temperature. Comparison of dissolution profile of developed formulation was carried out with the marketed formulation. The formulated NE *in-situ* gel showed drug release for a longer duration of time as compared to the marketed eye drops (0.1% w/v Voltaren Ophtha). The *in-vitro* release data was fitted to various kinetic models. It was found that the *in-vitro* drug release of diclofenac sodium nanoemulsion thermosensitive gel was best explained by zero order. *In-vitro* transcorneal permeation study was carried out on isolated goat cornea and comparison was done with marketed formulation, which showed that developed formulation exhibited higher permeation across goat cornea in 4 hours (44.65%) compared with that of the marketed formulation (31.25%). Hence this novel formulation was found to be a good replacement for conventional eye drops due to higher permeation and prolonged precorneal residence time.

INTRODUCTION: Ocular diseases are usually treated with topical administration of drug solutions (eye drops). These conventional dosage forms account nearly 90% of currently available marketed formulations.

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A major problem in conventional ophthalmic drug delivery is low drug bioavailability (less than 5% drug reaches intraocular tissue) due to ocular anatomical and physiological constraints, which include the poor permeability of cornea, nasolacrimal drainage effect, rapid turnover, dilution of drugs by tears, the short retention time in the precorneal area.[1]

Attempts to improve ocular bioavailability have been focussed on overcoming precorneal constraints through improving corneal penetration and prolonging the precorneal retention.[2]

Nanoemulsion is fine oil/water or water/oil dispersion stabilized by an interfacial film of surfactant molecules having droplet covering the size range 20 - 200 nm. Nanoemulsions have high surface area and low surface tension. With respect to their large surface area, small droplets size, low surface tension of the whole system and the low interfacial tension of o/w droplets, these emulsified systems have potential to enhance the permeability of drug molecules thereby increasing bioavailability of the drug. Presence of surfactants and co-surfactants increases membrane permeability, thereby increasing drug uptake. In other words, nanoemulsions act as penetration enhancers by disrupting tight junctional complexes to facilitate corneal drug delivery.[3]

In-situ gel-forming systems are viscous liquids that shift to a gel phase upon exposure to physiological conditions. The principal advantage of this formulation is the possibility of delivering accurate and reproducible quantities in contrast to already gelled formulations, and promoting precorneal retention.[4, 5]

Diclofenac sodium, a nonsteroidal anti-inflammatory drug which is a derivative of phenyl acetic acid, is administered topically as a 1 mg/ml aqueous solution in the eye for the management of pain in corneal epithelial defects in surgery or accidental trauma and symptomatic relief of seasonal allergic conjunctivitis and used to prevent intra-operative miosis during cataract surgery and post-operative chronic inflammations.

Diclofenac sodium is a weakly acidic drug having low water solubility. It has also limited therapeutic availability across cornea because of highly lipophilic corneal epithelium. To overcome the less bioavailability, commonly eye drops with higher concentrations are formulated or controlled release formulations have been formulated for several therapeutic agents. Diclofenac sodium is sparingly soluble in water hence high concentrated formulations are not suitable. Frequent dosing is needed to attain desired therapeutic concentration of the available eye drops of diclofenac (0.1% w/v) which may cause patient incompliance. [6]

In present work diclofenac sodium is formulated as thermosensitive *in-situ* gel forming nanoemulsion to overcome the problem of poor ocular bioavailability caused by low penetration rate through the cornea and rapid loss of drug from ocular surface due to above mentioned reasons. By virtue of their size and surface active properties, these NE_s are expected to be capable of delivering sufficient drug quantities into the cornea and aqueous humour. The development of *in-situ* NE gels would allow tailoring the rate of the drug release in such a manner that frequent instillation of the drops is avoided and the ocular drug bioavailability is improved as a result of prolonged drug retention time in *cul-de-sac* and high permeation of drug across cornea.

To exploit the benefits of these two dosage forms, nanoemulsion thermosensitive *in-situ* gelling system as a new vehicle for ophthalmic drug delivery is proposed. The essential idea is to prepare a nanoemulsion of the drug and then dispersing the drug loaded nanoemulsion in a polymer solution that gels upon triggering by temperature.

MATERIALS AND METHODS:

MATERIALS: The chemicals used were of laboratory grade and used as they were procured. The distilled water was used in all experiments.

TABLE 1: LIST OF MATERIALS

S. no.	Excipient/API	Source
1	Diclofenac sodium (API)	Alembic (Hyderabad)
2	Oleic acid	Molychem Pvt. Ltd
3	Tween 20	Molychem Pvt. Ltd
4	Ethanol	Changshu Yangyuan Chemical (China)
5	Poloxamer 407	BASF, Germany

METHODS:

Solubility studies: To find out appropriate oils, surfactants and co-surfactants as the components of nanoemulsion, the solubility of diclofenac Sodium in various oils (Ethyl oleate, Oleic acid, IPM, Myritol, MCT oil), surfactants (Kolliphor RH40, Kolliphor ELP, Tween 80, Tween 60, Tween 20) and co-surfactants (Glycerine, Propylene glycol, Ethanol, PEG 400, PEG 200, Propanol) was determined by using shake flask method .

The solubility of diclofenac sodium in various solvents was determined by dissolving excess amount of API in 3 ml of each of the selected

solvents in 5 ml capacity stoppered vials separately. Each glass vial was then mixed for 10 min using a vortex mixer. The mixture vials were then kept at 37 ± 1.0 ºC in a shaker bath for 72 h to get equilibrium.

The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45µm membrane to remove the remaining diclofenac sodium. After the appropriate dilution with methanol the concentration of diclofenac sodium in the filtrate was determined at 278 nm by UV Spectrophotometer and solubility of diclofenac sodium in different oils, surfactants, and co-surfactants was calculated with the help of standard calibration curve of drug in methanol. [7 - 9]

Optimization of Components for Nanoemulsion Formulation: [10] On the basis of solubility studies oil, surfactant and co-surfactant were selected respectively for nanoemulsion formulation. Selected components were further screened for their emulsifying ability to form a nanoemulsion.

Screening of Oil and Surfactants: Different surfactants were screened for emulsification ability. For this study 150 mg of surfactant was added to 150 mg of oily phase and then this mixture was heated at 60-70 ºC on a water bath for homogenization of the components. From each mixture prepared, 100 mg was withdrawn and diluted up to 100ml in a volumetric flask, and then it was inverted 50 - 60 times. The emulsions were allowed to stand for 24 hours and then % transmittance was determined by scanning in the range from 800 - 200 nm (wavelength 650 nm) by using UV spectrophotometer. They were also observed for turbidity or phase separation visually.

Screening of Co-surfactants: For screening of co-surfactants the best combinations of oil and surfactant as before were selected depending on the stability and % transmittance. For which the oil: surfactant: co-surfactant were taken as 600mg: 400mg: 200mg *i.e.* in the ratio of 6:4:2.The mixture was homogenised.

Out of the total 1200mg of the mixture 100 mg was withdrawn and added drop wise in a 100 ml volumetric flask and diluted with distilled water, then it was inverted 50 - 60 times and kept overnight. After which the % transmittance was determined by scanning in the range from 800-200 nm (wavelength 650 nm) using UV-visible spectro-photometer.

Construction of Pseudo-ternary Phase Diagram: The pseudo-ternary diagrams were constructed to determine the region of nanoemulsification, which will have the highest probability of forming a transparent nanoemulsion and to optimize the percentage of oil, surfactant and co-surfactant for the nanoemulsion formulation. The relationship between the phase behaviour of a mixture and its composition can be determined with the aid of phase diagram. Pseudo ternary phase diagrams are normally constructed with the oil phase, surfactant and co-surfactant, and the aqueous phase, which reveals the regions of liquid crystal, microemulsion and coarse emulsion. [11] For simplicity, the present study has ignored the effect of aqueous phase (dilution with excess water), and used only the oil, surfactant, and co-surfactant components to identify the nanoemulsification region. [12]

Ternary diagrams of surfactant, co-surfactant and oil were plotted (using Tri-plot vl-4software); each of them, representing an apex of the triangle and 100% of that particular component. The surfactant and co-surfactant was varied in mass ratios 1:1, 1:2, 2:1. These S_{mix} ratios were chosen once for increasing surfactant ratio to co-surfactant and then by increasing co-surfactant ratio to surfactant ratio for the detailed study of the phase diagrams. The different concentration ratios of oil and S_{mix} were taken as 0.5:9.5, 1:9, 2:8, 3:7, and 4:6. A series of ternary mixtures with varying compositions of the components were formed. The required amount of the three components were weighed accurately and vortexed for 3 min. The mixture was then homogenised on a water bath at 60-70 ºC for 20 min and then sonicated on a bath sonicator. Above mixtures were evaluated for nanoemulsion formation by adding the individual ternary mixture into 50 ml distilled water with simultaneous stirring for two minutes on a magnetic stirrer. The samples were kept for 24 hours to study any phase separation and turbidity. Transparent samples were considered desirable. These samples are then marked as points in the phase diagram. According to the mass ratio distribution the following distribution was formed of the formulation out of

which 0.1% w/w was diclofenac sodium rest formulation. The calculations as per the mass ratio of the various combinations for construction of pseudo ternary diagram are shown in **Tables 2** to **10**.

Combination-1: oleic acid + Tween 20 + Ethanol

TABLE 2: COMPOSITION OF COMBINATION-1 (S_{mix}Ratio 1:1)

O:S_{mix}	Formulation code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	SA1	4.995	47.4525	47.4525
1:9	SA2	9.99	44.955	44.955
2:8	SA3	19.98	39.96	39.96
3:7	SA4	29.97	34.965	34.965
4:6	SA5	39.96	29.97	29.97

TABLE 3: COMPOSITION OF COMBINATION-1 (S_{mix}Ratio 1:2)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	SB1	4.995	31.635	63.27
1:9	SB2	9.99	29.97	59.94
2:8	SB3	19.98	26.64	53.28
3:7	SB4	29.97	23.31	46.62
4:6	SB5	39.96	19.98	39.96

TABLE 4: COMPOSITION OF COMBINATION-1 (S_{mix}Ratio 2:1)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	SC1	4.995	63.27	31.635
1:9	SC2	9.99	59.94	29.97
2:8	SC3	19.98	53.28	26.64
3:7	SC4	29.97	46.62	23.31
4:6	SC5	39.96	39.96	19.98

Combination-2: Oleic acid + Kolliphor RH40 + PEG 400

TABLE 5: COMPOSITION OF COMBINATION-2 (S_{mix} Ratio 1:1)

O:S_{mix}	Formulation code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	MA1	4.995	47.4525	47.4525
1:9	MA2	9.99	44.955	44.955
2:8	MA3	19.98	39.96	39.96
3:7	MA4	29.97	34.965	34.965
4:6	MA5	39.96	29.97	29.97

TABLE 6: COMPOSITION OF COMBINATION-2 (S_{mix} Ratio 1:2)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	MB1	4.995	31.635	63.27
1:9	MB2	9.99	29.97	59.94
2:8	MB3	19.98	26.64	53.28
3:7	MB4	29.97	23.31	46.62
4:6	MB5	39.96	19.98	39.96

TABLE 7: COMPOSITION OF COMBINATION-2 (S_{mix} Ratio 2:1)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	MC1	4.995	63.27	31.635
1:9	MC2	9.99	59.94	29.97
2:8	MC3	19.98	53.28	26.64
3:7	MC4	29.97	46.62	23.31
4:6	MC5	39.96	39.96	19.98

Combination-3: Oleic acid+ Kolliphor RH40+ PEG 200

TABLE 8: COMPOSITION OF COMBINATION-3 (S_{mix} Ratio 1:1)

O:S_{mix}	Formulation code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	NA1	4.995	47.4525	47.4525
1:9	NA2	9.99	44.955	44.955
2:8	NA3	19.98	39.96	39.96
3:7	NA4	29.97	34.965	34.965
4:6	NA5	39.96	29.97	29.97

TABLE 9: COMPOSITION OF COMBINATION-3 (S_{mix} Ratio 1:2)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	NB1	4.995	31.635	63.27
1:9	NB2	9.99	29.97	59.94
2:8	NB3	19.98	26.64	53.28
3:7	NB4	29.97	23.31	46.62
4:6	NB5	39.96	19.98	39.96

TABLE 10: COMPOSITION OF COMBINATION-3 (S_{mix} Ratio 2:1)

O:S_{mix}	Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9.5	NC1	4.995	63.27	31.635
1:9	NC2	9.99	59.94	29.97
2:8	NC3	19.98	53.28	26.64
3:7	NC4	29.97	46.62	23.31
4:6	NC5	39.96	39.96	19.98

Preparation of Drug Loaded Formulae: From each pseudo-ternary phase diagram constructed, existing nanoemulsion region was identified and the concentration of oil, surfactant and co-surfactant at which clear and transparent nanoemulsion obtained was determined. Nine clear systems were selected (represented in **Table 11**) and prepared using oleic acid as oily phase, tween 20 and kolliphor RH 40as surfactant and PEG 200, PEG 400 and ethanol as co-surfactant. Accurately weighed diclofenac sodium (0.1% w/w) was placed in glass vial and oil, surfactant and co-surfactant were added. After adding all the components, the

mixture was vortexed for 3 min and then homogenized at 60 ºC for 20 min. After homogenization, the mixture was sonicated and in bath sonicator occasionally vortexed until diclofenac sodium was perfectly dissolved. [13] The mixture was stored at room temperature for further use for optimization (water would be added drop wise to preparation before incorporating it into gel solution)

TABLE 11: COMPOSITION OF SELECTED FORMULATIONS

Oil: S_{mix}	Formulation code	Oil (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)
0.5:9:5	SA1	4.94	47.53	47.53
0.5:9:5	SB1	4.94	31.78	63.28
0.5:9:5	SC1	4.94	63.28	31.78
0.5:9:5	MA1	4.94	47.53	47.53
0.5:9:5	MB1	4.94	31.78	63.28
0.5:9:5	MC1	4.94	63.28	31.78
0.5:9:5	NA1	4.94	47.53	47.53
0.5:9:5	NB1	4.94	31.78	63.28
0.5:9.5	NC1	4.94	63.28	31.78

All formulations contain 0.1% w/w (1 mg) of diclofenac sodium

Optimization of Drug Loaded Systems: A series of nanoemulsion formulations (SA1, SB1, SC1, MA1, MB1, MC1, NA1, NB1 and NC1) were selected on the basis of pseudo-ternary phase diagram study and prepared. The developed formulations were optimized to select best formulations which could then be transformed into the *in-situ* NE gels. Optimization was based on following parameters:

% Drug Content: Drug content was the crucial parameter for optimization of formulation as it indicates the amount of drug entrapped in the nano size globules of nanoemulsion. This study was carried out by diluting each ternary mixture with methanol and centrifuged for 2 hours at 1000-1500 rpm which were then analysed using UV spectrophotometer.

Emulsification: This was the criterion important to investigate the spontaneity of the components to form the emulsion. The ternary mixture forming emulsions were analysed by adding drop wise the prepared mixture in a 100 ml volumetric flask filled with distilled water. In certain cases it turned translucent to turbid, signifies the possibility of unstable emulsion formed.

Visual Assessment: The samples giving good emulsification were further analysed for turbidity initially and after 24 h. It would indicate phase separation of the emulsion.

Preparation of Nanoemulsion-loaded Sol-gel Systems: The best nanoemulsion formulations were selected for embedding into poloxamer based *in-situ* gel base. 20 % Poloxamer 407 containing gels were prepared as they exhibit sol-to-gel phase transition at physiological temperature and hence, contributes to sustained and prolonged drug delivery. [14] Gels were prepared by employing cold method. [15] The weighed quantity of poloxamer 407 was dispersed in chilled distilled water with constant stirring under freezing conditions and refrigerated overnight (24 hours) for complete dissolution of the polymer. Firstly, water was added to previously optimized drug loaded nanoemulsion formulae and vortexed to form clear transparent nanoemulsion. These prepared nanoemulsion formulations were added drop wise to the poloxamer solution individually with gentle stirring by using magnetic stirrer. The pH of formulations was adjusted to 7.4 with few drops of sodium hydroxide solution (1 M NaOH) by using PH meter (Labindia Ltd., Mumbai, India) [16]. The nanoemulsion embedded in-*situ* gels were thus formed and stored for further evaluation.

Optimization of *in-situ* NE Gels:

Appearance: The appearance and clarity of the formulations after and before gel formation was observed by visual examination of the formulations under light, against white and black backgrounds alternatively. [17]

% Drug Content: The developed formulations were evaluated for drug content by UV spectro-photometry (Shimadzu, Japan, UV spectro-photometer). The drug content of NE *in-situ* gels was determined, firstly by diluting the samples with STF (pH 7.4) and then the samples were centrifuged for 2 hours at 1000-1500 rpm and analysed using UV spectrophotometer to calculate the percentage of drug content.

Measurement of Gelation Temperature (GT): Gelation temperature of *in-situ* NE gels was measured by taking 10 ml of sample solution and a magnetic bead in a transparent beaker, which was then kept on a low temperature water bath. A thermometer was inserted into the solution and heat is provided with continuous agitation. The

temperature, at which the magnetic bead stopped motion, was noted as gelation temperature. [18]

Evaluation of Optimized Nanoemulsion Formulation: After optimisation of nanoemulsion embedded gels, nanoemulsion formulation that was incorporated into final optimized gel formulation was further evaluated for transmission electron microscopy and particle size analysis.

TEM: This was done to analyse the morphology and structure of the formed nanoemulsion droplets which was studied using transmission electron microscopy. Malvern version 6.30 and it is capable of point to point resolution. In order to carry out study, a drop of the nanoemulsion was deposited on the holey film grid and studied after drying.

Particle Size Analysis: This study is mainly performed by using photon correlation spectroscopy; the technique analyses the fluctuations in light scattering due to Brownian motion of the particles. The instrument used was a Zetasizer 1000 HAS, Malvern Instruments Ltd. [19]

Zeta Potential: Zeta potential is a technique which is used to determine the surface charge properties, more over the long term physical stability of nanoemulsions. The instrument used was a Zetasizer, (Malvern Instruments Ltd.). The measurement was carried out with a diluted nanoemulsion preparation and its value was determined from electrophoretic mobility of the oil droplets. [20]

Evaluation of Nanoemulsion Embedded *In-Situ* Gel: Viscosity: The viscosity of the formulation was studied firstly at room temperature (solution form) then at physiological temperature *i.e*. 37 ºC (gel form) by using Brookfield viscometer.

***In-vitro* Drug Release Study:** *In vitro* drug release study was carried out by using Franz diffusion cell (shown in **Fig. 1**). The membrane was dipped in the medium for 24hrs before use. STF was used as receptor solution. Initially, the receiver chamber was filled with STF (pH 7.4).

The previously dipped cellophane membrane was mounted between the donor and receiver compartment of the Franz diffusion cell; on which the weighed quantity of formulated gel was spread completely to cover most of the area. The receiver fluid was stirred using a small magnetic bead, and the temperature was maintained to 37±2 ºC with the help of hot plate. At predetermined time intervals 1 ml sample solution was withdrawn and replaced with fresh STF to maintain sink condition. The collected samples were subjected to quantification of diclofenac sodium using UV-VIS spectrophotometer at 276 nm. Triplicate experiments were carried out for each release study. [21 – 23] *In-vitro* release profile of developed formulation was compared with release profile of marketed diclofenac sodium eye drops (Voltaren Ophtha) under similar conditions.

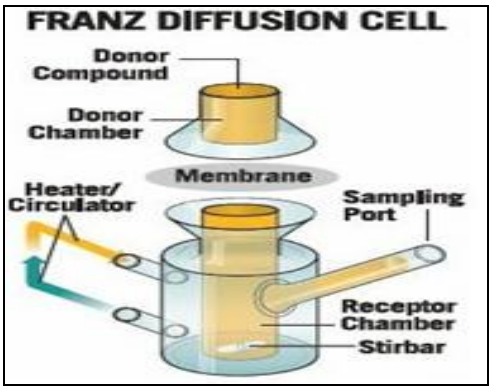


FIG.1 : FRANZ DIFFUSION CELL

Drug Release Kinetics Studies: [24, 25] To analyze the mechanism of drug release from diclofenac sodium nanoemulsion based gel the *in vitro* dissolution data were fitted to zero order, first order, Higuchi release model, and Korsemeyer-Peppa's model and the model with higher correlation coefficient (R^2) was considered to be the best model.

***In-vitro* Transcorneal Permeation Study:** [26, 27] Isolated goat corneas were used to determine transcorneal permeability of diclofenac sodium from the thermosensitive *in-situ* NE gel. Fresh whole eyeballs of goats were collected from a local butcher's shop immediately after the goat was slaughtered and brought to the laboratory in cold saline solution within 1 h of slaughtering.

Corneas were then excised carefully from the eyeballs along with 2 to 4 mm of surrounding scleral tissue and kept in freshly prepared artificial tear solution, pH 7.4. The study was carried out on a Franz diffusion cell. The upper chamber served as a donor compartment in which weighed quantity of

formulated *in-situ* NE gel/marketed formulation (Voltaren Ophtha) under study was placed. The excised goat cornea was sandwiched between donor and receptor compartments of the Franz diffusion cell so that its epithelial surface faced the donor compartment.

Freshly prepared STF (pH 7.4) was filled in receiver compartment and stirred using a small magnetic bead. The whole system was maintained at 37 ± 0.5 °C. Periodically, samples were collected for upto 4 hours and subjected to quantification of diclofenac sodium using UV-VIS spectro-photometer at 276nm.

FT-IR Analysis of Optimized Formulation: Fourier Transform infrared studies were carried out for pure drug alone and final optimized formulation by using ATR-FTIR (Bruker spectrophotometer), and spectra were recorded in the wavelength region of 4000 - 450 cm^{-1} to study the compatibility and to determine if there are any drug excipients interactions. [28]

RESULTS AND DISCUSSION:

Solubility Studies: Solubility of diclofenac sodium in different oils, surfactants and co-surfactants was determined. From **Table 12** it was concluded that oleic acid has maximum solubility and thus was selected for further study. From **Table 13** it was observed that kolliphor RH40 and tween 20 have more solubility for diclofenac sodium and hence selected for further screening. From the data obtained in **Table 14**, three co-surfactants selected for further studies were PEG 200, PEG 400 and ethanol.

TABLE 12: SOLUBILITY PROFILE OF DILOFENAC SODIUM IN OILS

S. no.	Oils	Solubility (mg/ml)	Solubility (as per IP)
1	Oleic acid	20.13	Sparingly soluble
2	Ethyl oleate	1.47	Slightly soluble
3	Myritol	0.29	Very slightly soluble
4	MCT oil	0.23	Very slightly soluble
5	IPM	0.20	Very slightly soluble

TABLE 13: SOLUBILITY PROFILE OF DILOFENAC SODIUM IN SURFACTANTS

S. no	Surfactants	Solubility (mg/ml)	Solubility (as per IP)
1	Kolliphor RH40	10.74	Sparingly soluble
2	Tween 20	9.21	Slightly soluble
3	Tween 60	7.33	Slightly soluble
4	Tween 80	5.07	Slightly soluble
5	Kolliphor ELP	1.55	Slightly soluble

TABLE 14: SOLUBILITY PROFILE OF DILOFENAC SODIUM IN CO-SURFACTANTS

S. no.	Co-surfactants	Solubility (mg/ml)	Solubility (as per IP)
1	PEG 200	40.07	Soluble
2	Ethanol	37.21	Soluble
3	PEG 400	13.35	Sparingly soluble
4	Propanolol	9.97	Sparingly soluble
5	Propylene glycol	7.71	Slightly soluble
6	Glycerin	1.01	Very slightly soluble

Optimization of Components for Nanoemulsion Formulation: Oil, surfactants and co-surfactants having maximum solubility for API were further screened.

This was done by determining the parameter of % transmittance which was used to differentiate the clear and transparent appearing solutions.

TABLE 15: SCREENING RESULTS OF OIL+ SURFACTANTS

Formulation Code	Oil	Surfactants	% Transmittance
A1	Oleic acid	Tween 80	30.1%
A2	Oleic acid	Tween 20	65.1 %
A3	Oleic acid	Tween 60	11.8%
A4	Oleic acid	Kolliphor RH40	39.8%

Table 15 Results inferred that the oily phase oleic acid exhibited high emulsification efficiency with tween 20 ranking first (65.8%) and kolliphor RH40 ranking second (39.8%) compared to other combinations. Hence combinations A2 and A4 were selected for further screening on the basis of % transmittance.

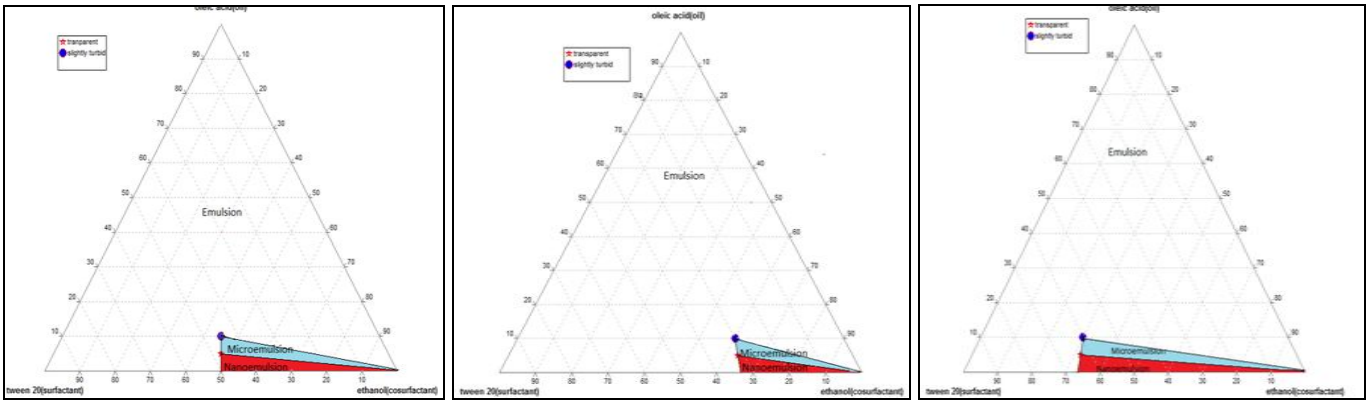
TABLE 16: SCREENING RESULTS OF CO-SURFACTANTS

Formulation code	Oil	Surfactants	Co-surfactants	% Transmittance
F1	Oleic acid	Kolliphor RH40	Ethanol	40.0%
F2	Oleic acid	Kolliphor RH40	PEG 200	54.8%
F3	Oleic acid	Kolliphor RH40	PEG 400	61.6%
F4	Oleic acid	Tween 20	Ethanol	65.8%
F5	Oleic acid	Tween 20	PEG 200	54.0%
F6	Oleic acid	Tween 20	PEG 400	50.6%

Out of all screening studies conducted the results show that F2, F3 and F4 have the best % transmittance and were further considered for construction of pseudo- ternary phase diagram for determining the nanoemulsion formation region.

Construction of Pseudo-Ternary Phase Diagram: While conducting the screening studies it was observed that 3 combinations involving two different surfactants and three co-surfactants which have same oil combination have desirable % transmittance. Thus, it became necessary to finalize the final combination by construction of pseudo-ternary phase diagram.

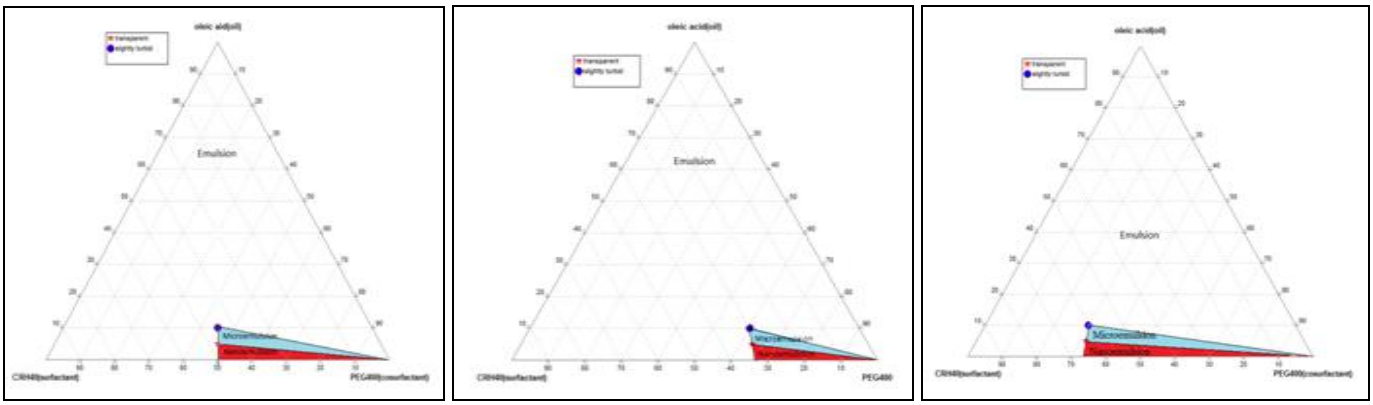
The pseudo-ternary diagram helps to determine the percentage of components for formation of clear and transparent NE. The mass ratio of surfactant to co-surfactant were varied as 1:1, 1:2, and 2:1 and the ratio of oil: Smixwere varied as 0.5:9.5, 0.5:9, 2:8, 3:7, 4:6.



S_{mix} ratio (1:1) **S_{mix} ratio (1:2)** **S_{mix} ratio (2:1)**

FIG. 2: TERNARY PHASE DIAGRAM FOR COMBINATION -1 (OLEIC ACID +TWEEN 20 +ETHANOL)

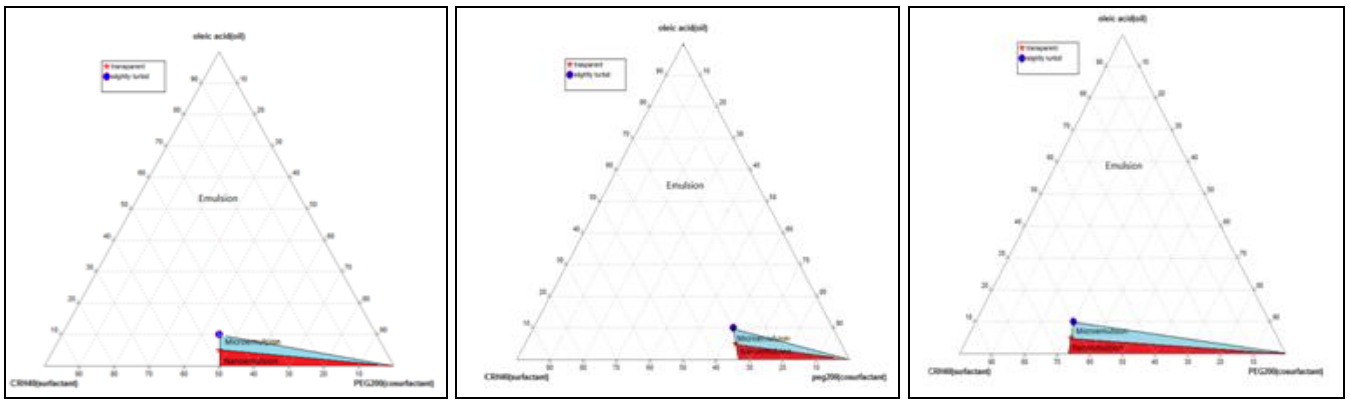
Three combinations SA1, SB1 and SC1 (shaded red) were finalize by construction of pseudo-ternary phase diagram.



S_{mix} ratio (1:1) **S_{mix} ratio (1:2)** **S_{mix} ratio (2:1)**

FIG. 3: TERNARY PHASE DIAGRAM FOR COMBINATION -2 (OLEIC ACID +CRH 40+PEG 400)

Three combinations MA1, MB1 and MC1 (shaded red) were finalize by construction of pseudo-ternary phase diagram.



S_{mix} ratio (1:1) **S_{mix} ratio (1:2)** **S_{mix} ratio (2:1)**

FIG. 4: TERNARY PHASE DIAGRAM FOR COMBINATION - 3 (Oleic acid + CRH 40 + PEG 200)

Three combinations NA1, NB1 and NC1 (shaded red) were finalize by construction of pseudo-ternary phase diagram

Preparation of Drug Loaded Formulae: Nine transparent formulations *i.e.* SA1, SB1, SC1, MA1, MB1, MC1, NA1, NB1, and NC1 were selected from the above pseudo-ternary phase diagrams, and then prepared with low energy method.

Optimization of Nanoemulsion: Optimization of nanoemulsions was done based on following parameters:

% Drug Content: Entrapment efficiency of the selected nine formulations (SA1, SB1, SC1, MA1, MB1, MC1, NA1, NB1 and NC1) was checked. The formulations SC1, SA1, NA1, SB1 and MB1 with drug content 99.06 %, 95.09 %, 91.19 %, 82.04 %, and 65.32 % respectively were selected for further study.

Emulsification and Visual Assessment: On diluting the formulations up to 100 ml, SB1 turned turbid. It indicates the instability of the emulsion. SA1, SC1 and NA1 were selected for incorporating in gel base.

Preparation of Nanoemulsion-Loaded Sol-Gel Systems: Formulations SA1, SC1 and NA1 were added in gel base (20% poloxamer 407) with constant stirring on a magnetic stirrer and designated as SA1-G, SC1-G and NA1-G.

Optimization of *in-situ* NE Gels: From **Table 17** all three formulations were found to have desirable gelation temperature, so optimization was done on the basis of percentage drug content. SC1-G was selected as the best formulation with maximum percentage drug content (89.70%).

TABLE 17: ANALYSIS OF NANO EMULSION EMBEDDED THERMOSENSITIVE GELS

Nanoemulsion formulation code	Gel formulation code	Appearance	Gelation temperature	% Drug content
SA1	SA1-G	Transparent	34 ºC	83.89%
SC1	SC1-G	Transparent	34 ºC	89.70%
NA1	NA1-G	Transparent	34 ºC	60.63%

Evaluation of Optimized Nanoemulsion Formulation: Nanoemulsion formulation that was embedded in SC1-G *i.e.* SC1 was further evaluated.

TEM: The nanoemulsion globules of optimized formulation were visualized by Transmission Electron Microscope (TEM). These results confirmed that the droplets were spherical in shape and in nano size range and thus formulated nanoemulsion was in range.

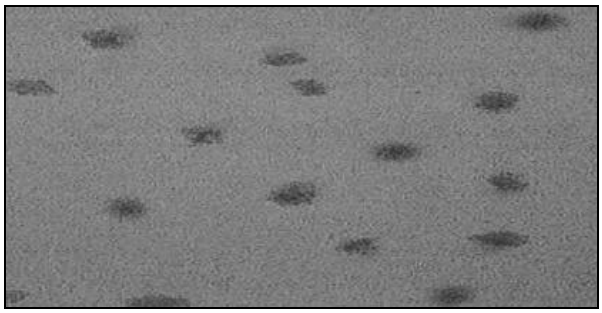


FIG. 5: TEM IMAGE OF NANOEMULSION FORMULATION

Particle Size Analysis: The globule size of the optimized emulsions was determined by photon correlation spectroscopy. The average particle size of the formulation was 119.5 d.nm. Thus results showed that the particle size of the formed nanoemulsion was in the desirable range (less than 200 nm) therefore a transparent nanoemulsion was formed successfully.

TABLE 18: PARTICLE SIZE DISTRIBUTION

		Size (d.nm)	% Intensity	St Dev (d.nm)
Z-Average (d.nm): 119.5	Peak 1:	7.816	59.0	1.611
Pdl: 0.362	Peak 2:	174.3	41.0	38.66
Intercept: 1.01	Peak 3:	0.000	0.0	0.000

Zeta Potential: Zeta potential values lower than -10 mV generally indicates a high degree of physical stability. The formulation possesses good physical stability since the value of zeta potential was -8.39 mV.

TABLE 19: ZETA POTENTIAL OF OPTIMIZED FORMULATION

		Mean (mV)	Area (%)	St Dev (mV)
Zeta potential (mV): -8.39	Peak 1:	-8.39	100.0	5.02
Zeta Deviation: 5.02	Peak 2:	0.00	0.0	0.00
Conductivity: 0.390	Peak 3:	0.00	0.0	0.00
Result quality: Good				

Evaluation of *In-situ* NE Gel:

Viscosity: The viscosity of the formulation was measured by using Brookfield viscometer at room temperature and physiological temperature at varying % Torque viscosity study showed that at room temperature, the formulation was in a liquid

state and exhibited low viscosity and at physiological temperature it got transformed into gel with high viscosity as required for an *in-situ* forming system, therefore the results were desirable.

TABLE 20: VISCOSITY DETERMINATION

% Torque	Viscosity at room temperature (25 °C)	Viscosity at physiological temperature (37 °C)
10	1247	3032
20	965	2492
50	732	1853
100	605	1100

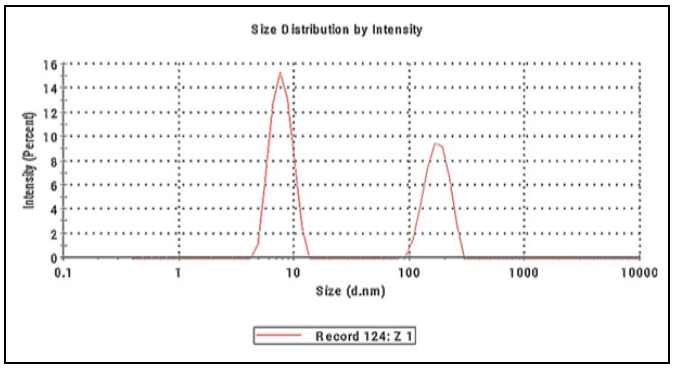


FIG. 6: GRAPHICAL REPRESENTATION OF PARTICLE SIZE DISTRIBUTION

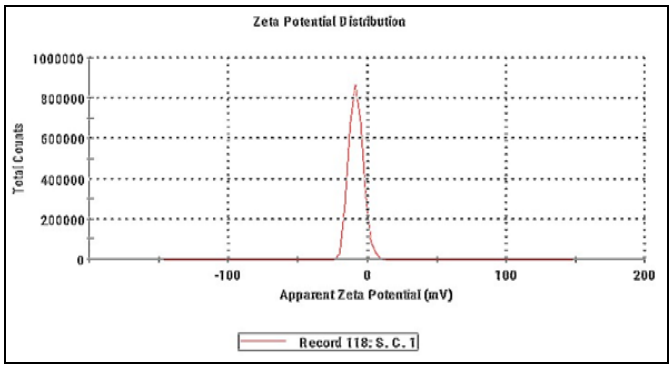


FIG.7: GRAPHICAL REPRESENTATION OF ZETA POTENTIAL

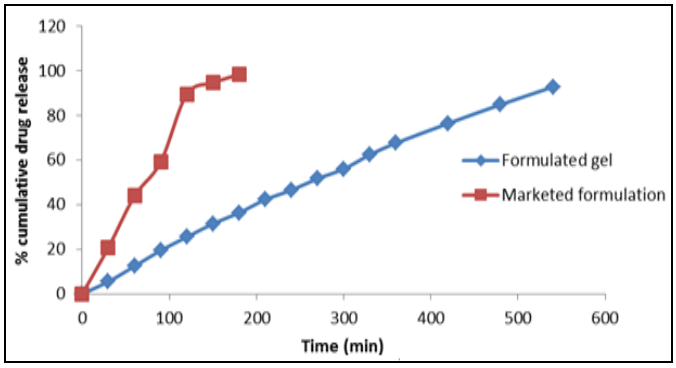


FIG. 8: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE STUDY OF FORMULATED GEL AND MARKETED FORMULATION (VOLTAREN OPHTHA)

***In-vitro* release study:** *In-vitro* release study was carried out on Franz diffusion cell; the results of drug release profile (**Fig. 8**) depict extended drug release period. The drug release profile of formulated and marketed the formulation (0.1% w/v Voltaren Ophtha) was compared which shows that the formulated NE in-situ gel showed drug release for a longer duration of time as compared to the marketed eye drops, thus sustained drug delivery was achieved.

Drug release kinetics studies: The release data of optimized formulation was subjected to drug release kinetics study. It was observed that zero order release kinetics was best suited based on R^2 value

TABLE 21: DRUG RELEASE DATA OF DICLOFENAC SODIUM NANAOEMULSION EMBEDDED THERMOSENSITIVE *IN-SITU* OCULAR GEL

Time	Log time	S.R. of time	% cumulative release	Log % cumulative release	% cumulative remaining	Log % cumulative remaining
0	0	0	0	0	100	2
30	1.4771	5.4772	5.4824	0.7390	94.5176	1.9755
60	1.7782	7.7460	12.3835	1.0928	87.6165	1.9426
90	1.9542	9.4868	19.4494	1.2889	80.5506	1.9061
120	2.0792	10.9545	25.4565	1.4058	74.5435	1.8724
150	2.1761	12.2474	31.4165	1.4972	68.5835	1.8362
180	2.2553	13.4164	36.3412	1.5604	63.6588	1.8039
210	2.3222	14.4914	42.2071	1.6254	57.7929	1.7619
240	2.3802	15.4919	46.4965	1.6674	53.5035	1.7284

270	2.4314	16.4317	51.6094	1.7127	48.3906	1.6848
300	2.4771	17.3205	55.9694	1.7480	44.0306	1.6438
330	2.5185	18.1659	62.3059	1.7945	37.6941	1.5763
360	2.5563	18.9737	67.5129	1.8294	32.4871	1.5117
420	2.6232	20.4939	76.3906	1.8830	23.6094	1.3731
480	2.6812	21.9089	84.8212	1.9285	15.1788	1.1812
540	2.7324	23.2379	92.7106	1.9671	7.2894	0.8627

Zero Order Model: Graph was plotted between % Cumulative release *vs.* Time

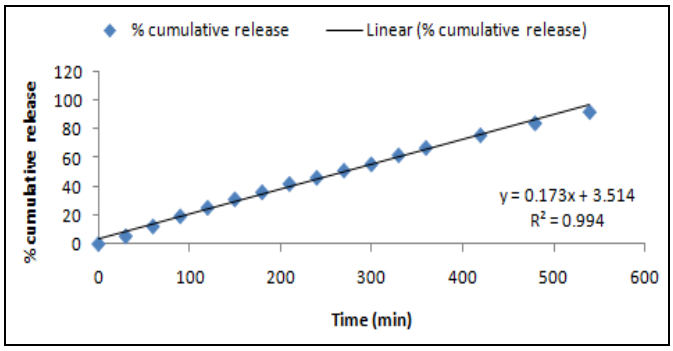


FIG. 9: ZERO ORDER PLOTS FOR DRUG RELEASE OF DICLOFENAC SODIUM NANAOEMULSION BASED *IN-SITU* GEL

First Order Model**:** Graph was plotted between Log % Cumulative remaining *vs.* Time

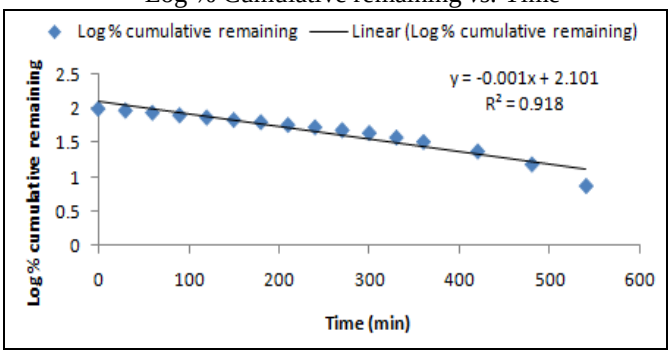


FIG. 10: FIRST ORDER PLOTS FOR DRUG RELEASE OF DICLOFENAC SODIUM NANAOEMULSION BASED *IN-SITU* GEL

Higuchi Model: Graph was plotted between % Cumulative release *vs.* Square root time

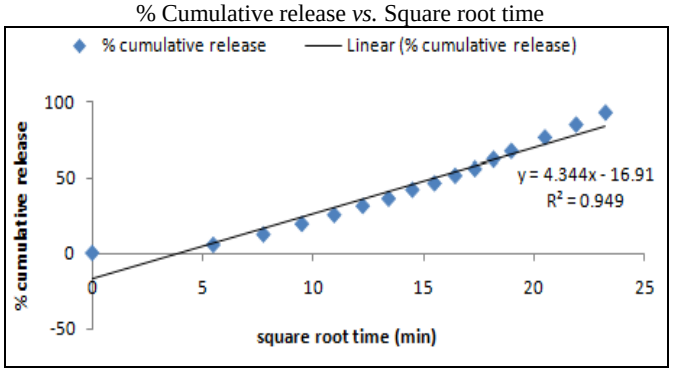


FIG. 11: HIGUCHI PLOT FOR DICLOFENAC SODIUM NANO EMULSION *IN-SITU* GEL

Korsmeyer - Peppas Model: Graph was plotted between Log % Cumulative release *vs.* Log time

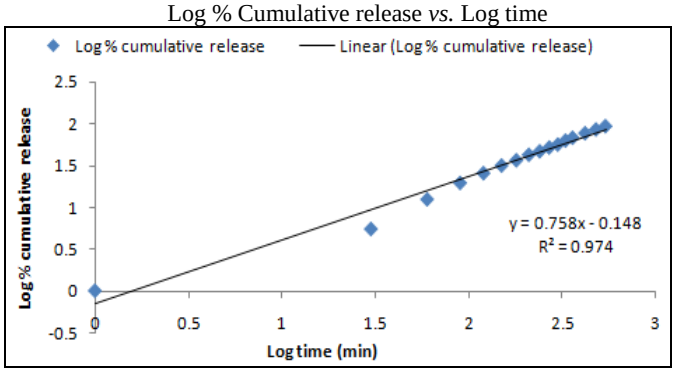


FIG. 12: KORSMEYER-PEPPAS PLOT FOR DICLOFENAC SODIUM NANOEMULSION *IN-SITU* GEL

Data obtained in **Table 21** were fitted into equation for the zero order, first order and higuchi and korsmeyer-peppas release models. The interpretation of data was based on the value of the resulting regression coefficients; as depicted in **Table 22**.

TABLE 22: KINETICS OF DRUG RELEASE OF OPTIMIZED FORMULATION

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Peppas (R^2)
Diclofenac sodium nanoemulsion based *in-situ* gel(SC1-G)	0.9941	0.9186	0.9498	0.9748

The zero order rates describes the system where the drug release independent of its concentration shows the cumulative amount of drug release *vs.* time for zero order kinetics. The first order rate describes the release from systems where the release of drugs from a matrix as a square root of a time- dependent process based on Fickian diffusion.

It was found that the *in-vitro* drug release of diclofenac sodium nanoemulsion thermosensitive gel was best explained by zero order equation as the plot showed the highest linearity which was followed by the Korsmeyer-Peppas model. Therefore the release pattern seems to fit the zero order model. The value of R^2 found to be highest for the zero order model.

***In-vitro* Transcorneal Permeation Study:** Transcorneal permeation profile of the optimized formulation was compared with the marketed diclofenac sodium eye drop (0.1% w/v Voltaren

Ophtha) using isolated goat's cornea on Franz diffusion cell using STF (pH 7.4). The samples were withdrawn at regular intervals and analyzed for drug content. The percent drug permeated was plotted against time to get comparative permeation profile. **Fig. 13** shows various stages involved in *in -vitro* transcorneal permeation study.

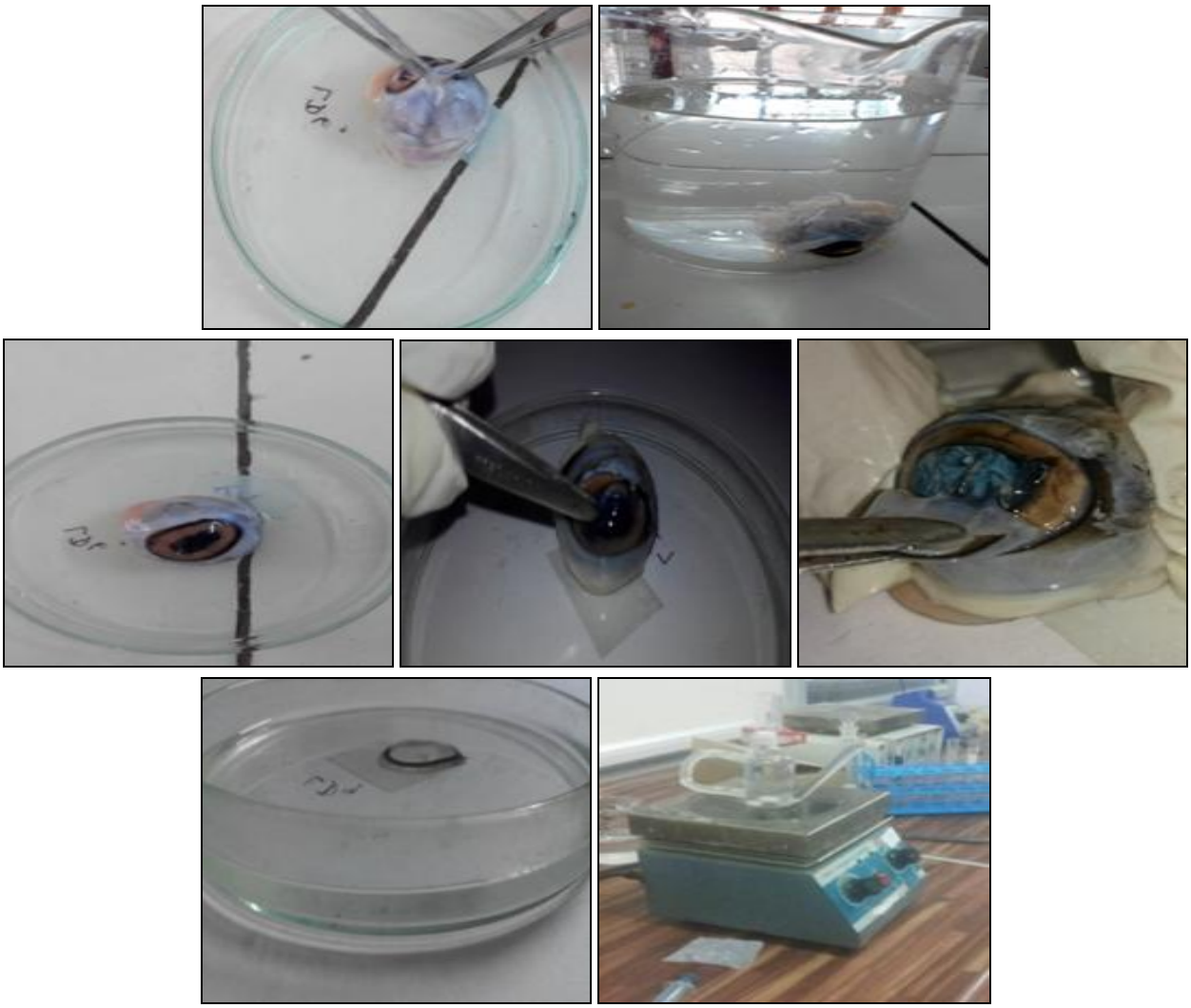


FIG. 13: VARIOUS STAGES IN *IN-VITRO* TRANSCORNEAL PERMEATION STUDY

From **Fig. 14** it was observed that developed formulation showed higher permeation across goat cornea in 4 hours (44.65%) compared with that of the marketed formulation (31.25%).

FT-IR of Optimized Formulation: Structural compatibility between drug and excipients were studied with the help of FT-IR spectra of drug and FT-IR spectra of mixture of drug and excipients. The main peaks in the spectrum of the drug diclofenac sodium both free and in formulation did not show any substantial difference. The FT-IR spectral analysis of the pure diclofenac sodium showed characteristic peaks at wave numbers 742, 1284, 3355, 1789, 1502 cm^{-1}.

The IR spectrum of optimized formulation has shown peak at 731.51 cm^{-1}, which shows presence of C-Cl stretch (760-540 cm^{-1}). Peak at 3395.48 cm^{-1} signifies presence of N-H stretching of secondary amine (3500-3300 cm^{-1}). Peak at 1722.94 cm^{-1} indicates the presence of C=O functional group. Peak at 1267.14 shows the presence of C-N stretching (1360-1180 cm^{-1}).

Peak at 1450.71 indicates presence of C=C stretch (1600-1450 cm^{-1}). All peaks were observed in the finger print region of FT-IR spectra. This proves the fact that there was no chemical interaction between drug and excipients.

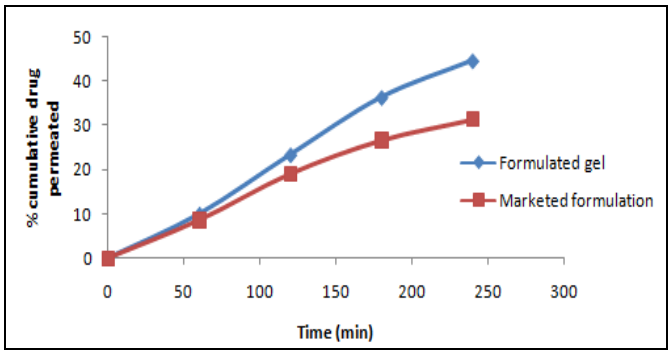


FIG. 14: *IN-VITRO* TRANSCORNEAL PERMEATON PROFILE OF MARKETED EYE DROPS AND FORMULATED GEL

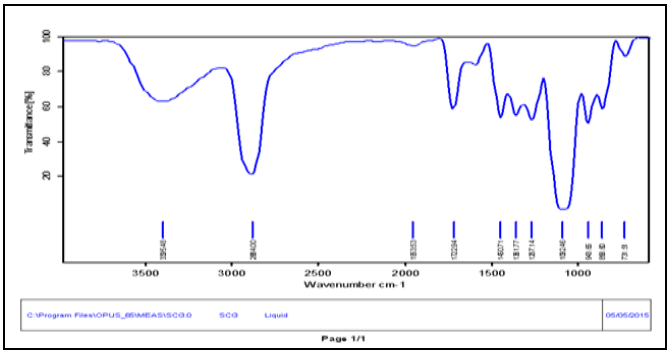


FIG. 15: FT-IR OF OPTIMIZED FORMULATION

CONCLUSION: An attempt was made to develop a novel nanoemulsion embedded thermosensitive *in-situ* gel for ophthalmic delivery of diclofenac sodium to enhance the permeation and residence time of the formulation, by overcoming the limitations associated with protective ocular barriers. The formulation exhibited prolonged drug delivery and greater permeation confirmed by *in-vitro* drug release and permeation studies. Developed formulation showed an optimum particle size and shape revealed by TEM and particle size analysis, which could easily permeate through the cornea and, thus permeation could be enhanced. Surface active properties of the nanoemulsion system and presence of penetration enhancers could have been contributed to increase the permeation of API.

Developed temperature sensitive *in-situ* NE gels showed optimum viscosity at room temperature and physiological temperature that revealed the phase transition of drug solution to gel after its administration to the eye and increased precorneal residence time of API which could lead to improve ocular bioavailability of the drug. The formulation also promises to reduce the frequency of drug administration, thus improving patient compliance.

Hence this novel formulation was found to be a good replacement for conventional eye drops due to higher permeation, prolonged precorneal residence time and sustained drug release along with greater patient compliance. Thus, it could be inferred that the objectives are achieved; further animal studies are required for the possible ocular irritation potential and the drug pharmacokinetics in the aqueous humour.

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REFERENCES:

1. Davies NM: Biopharmaceutical considerations in topical ocular drug delivery. Clin. Exp. Pharamacol, Physiol. 2000; 27(7): 558-562.
2. Kaur IP and Smitha R: Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. Drug Dev. Ind. Pharm. 2002; 28(4): 353-369.
3. Azeem A, Ahmad FJ, Khar RK and Talegaonkar S: Nanocarrier for the transdermal delivery of an anti-parkinsonian drug. AAPS Pharm Sci Tech. 2009; 10: 1093-103.
4. Kute PR, Gondkar SB and Saudagar RB: Ophthalmic in-situ gel: an overview. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(4): 549-568.
5. Kim EY, Gao ZG, Park JS, Li H and Han K: rhEGF/HP-beta CD complex inpoloxamer gel for ophthalmic delivery. Int. J. Pharm. 2002; 233: 159-67.
6. Ara T, Sharma S and Bhat SA: Preparation and evaluation of novel ocular inserts of diclofenac sodium amino acid conjugate for controlled drug delivery. International Journal of Scientific and Research Publications. 2015; 5(6):1-6.
7. Patil P, Joshi P and Paradkar A: Effect of formulation variables on preparation andevaluation of gelled self-emulsifying drug delivery system (SEDDS) of ketoprofen. AAPS Pharm Sci Tech. 2004; 5(3): 42.
8. Kanga KB, Lee SJ, Chona KS, Jeong YS, Yuk HS and Khanga G: Development ofSelf microemulsifying drug delivery systems for oral bioavailability enhancement of simvastatin in Beagle Dogs. Int. J. Pharm. 2004; 274-275.
9. Land L M, Li P and Bummer PM: The influence of water content of triglyceride oils on the solubility of steroids. Pharm. Res. 2010; (2): 254-260.
10. Goyal U, Arora R and Aggarwal G: Formulation design and evaluation of a self microemulsifying drug delivery system of lovastatin. Acta. Pharma.2012; 62: 357-370.
11. Constantinides PP: Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharm. Res. 1995; 12: 1561-1572.
12. Kommuru TR, Gurley B, Khan MA and Reddy IK: Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability

assessment. Int. J. pharm. 2001; 212: 233-246.
13. Ammar HO, Salama HA, Ghorab M and Mahmoud AA: Nanoemulsion as a potential ophthalmic delivery system for dorzolamide hydrochloride. AAPS Pharma Sci Tech. 2009; 10: 808-819.
14. Dorraj G, Hamid R and Moghimi HR: Preparation and characterization of thermosensitive in situ forming poloxamer hydrogel for controlled release of Nile red-loaded solid lipid nanoparticles. IJPS. 2013; 9(4): 39-50.
15. Schmolka IR: Artificial skin. Preparation and properties of Pluronic F127 gels for the treatment of burns. J. Biomed. Mater. Res. 1972; 6: 571-582.
16. Gupta H, Jain S. Mathur R, Mishra AK, Mishra P and Velpandian T: Sustained ocular drug delivery from a temperature and pH triggered *in-situ* gel system. Drug Deliv.2007; 14: 507-15.
17. Srividya B, Rita M, Cardoza and Amin PD: Sustained Ophthalmic delivery of ofloxacin from pH triggered in-situ gelling system. Journal of Controlled Release.2001; 73: 205-211.
18. Tong-Ying Jiang, Chang-Shan Sun, XueShen, Tian-Yi Wang, Si-Ling Wang *et al*., Development of a poloxamer analogs/bioadhesive polymers-based *in-situ* gelling ophthalmic delivery system for tiopronin. Journal of Applied Polymer Science.2009; 114: 775-783.
19. Craig DQM, Barker SA, Banning D, *et al*., An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm. 1995; 114(1): 103-110.
20. Kumar P and Gaur A: Feasibility Biowaiver, Extension of immediate release oral acyclovir 800 mg tablet formulations: A BCS class III drug. Int. J. Pharm and Pharm Sci. 2011; 3(4): 56-74.
21. Kulkarni SV, Sandeep HN, Shankar MS, Kumar RP, Rao SB, Ramesh B and Kumar AP: Effect of a single drop of latanoprost ophthalmic gel on intraocular pressure in the treatment of glaucoma. International Journal of Pharmaceutical Sciences.2010; 2(1): 429-435.
22. Sindhu A, Sharon F, Bharath S, Basavaraj BV, Deveswaran R and Madhavan V: Sustained ophthalmic delivery of ofloxacin from an ion activated in-situ gelling system. Pak J Pharm Sci. 2009; 22(2): 175-9.
23. Katarina E, Johan C, Roger, P. Rheological evaluation of Poloxamer as an in situ gel for ophthalmic use. Euro.J. Pharm. Sci. 1998; 6: 105-112.
24. Singhavi G and Singh M: Review: *in-vitro* drug release characterization models. International Journals of Pharmaceautics.2011; 2(1): 77-84.
25. Dash S, Murthy PN, Nath L and Chowdhary P: Kinetic modelling on drug release from controlled drug delivery systems. Acta Poloniae Pharmacy Drug Research.2010; 67(3): 217-223.
26. Gupta H, Jain S, Mathur R, Mishra P and Mishra AK: Sustained Ocular Drug Delivery from a Temperature and pH Triggered Novel *in-situ* Gel System, Drug Delivery.2007; 14: 507-515.
27. Gupta H, Mohammed Aqil, Khar RK, Ali A and Bhatnagar A: Sparfioxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine.2010; 6: 324-333.
28. Zavonar A, Berginc K, Kristl A AND Gasperlin M. Microencapsulation of self microemulsifying system: Improving solubility and permeability of furosemide. International Journal of Pharmaceutics 2010; 388: 151-158.

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