INTRAUTOIMMUNE DISEASE CLASS, RHEUMATOID ARTHRITIS (RA) IS ONE OF THE DEVASTATING DISEASES WHERE PATIENTS SUFFER ACUTE JOINT SWELLINGS, SERIOUS PAIN, AND STIFFNESS [1,2]. DURING FURTHER ADVANCEMENT OF THIS DISEASE, THE PATIENT MAY WITNESS WEIGHT LOSS, MODERATE FEVER, ANEMIA, AND EXTREME TREDNESS. RA EFFECTS PAIRED JOINTS AND OFTEN ELBOWS, ANKLES, SHOULDERS ETC. IN ACUTE CONDITION, RA CAUSES BONE DEFORMATIONS IN AFFECTED AREAS, SOMETIMES IT LEADS TO PERMANENT JOINT DAMAGE. SERIES OF COMPLICATIONS CAN BE SEEN DURING DEVELOPMENT OF THIS DISEASE, SUCH AS INFLAMMATION OF SYNOVIAUM, PROLIFERATING ACROSS THE JOINT SURFACE, THE INFLAMED JOINT BECOME RED AND WARMER AS OF MAXIMUM ACCUMULATIONS OF BLOOD CELLS ON AFFECTED AREAS, JOINT CAPSULE REMAINS STRETCH AND AFTER INFLAMMATION, WHICH MAKES IT UNFIT TO HOLD JOINT IN ITS PROPER POSITION [3]. RA DIAGNOSIS IS A CHALLENGING TASK BECAUSE NO SPECIFIC TEST WAS INVENTED TO SPECIFY RA WITHIN THE BODY; HOWEVER, DOCTORS OFTEN RELY ON X-RAY, ERYTHROCYTE SEGMENTATION RATE, C-REACTIVE PROTEIN, TEST FOR ANEMIA, TESTING FOR ANTIBODIES SUCH AS RHEUMATOID FACTOR AND ANTICYCLIC CITRULINATED PEPTIDE, MAGNETIC RESONANCE IMAGING, ULTRASOUND SCAN FOR DISTINCTIVE CHARACTERIZATION OF RA [4]. COMING TO THE TREATMENT POINT OF VIEW, THERE ARE MANY WIDES RANGES OF MEDICINES ARE AVAILABLE FOR RA, BUT DOCTORS PREFER PAINKILLERS, NON-STEROIDAL, ANTI-INFLAMMATORY DRUGS, DISEASE MODIFYING ANTI-RHEUMATOID DRUGS, STEROIDS ETC. [5]. IN THIS EXPERIMENT, WE USED CELECOXIB, A POTENTIAL CYCOOXYGENASE-2 (COX-2) INHIBITOR WHICH HAS VERY LESS ADVERSE EFFECT AS COMPARE TO ROFECOXIB, VALDECOXIB ETC. [6]. CELECOXIB MECHANISM OF ACTION IS VERY SIMPLE, IT SELECTIVELY INHIBITS COX-2, DUE TO WHICH COX-2-INDUCED INFLAMMATION AND PROSTANOIDS (PROSTAGLANDIN E2) SYNTHESIS CLEAVED [7]. DUE TO WHICH INFLAMMATION, EDEMA AND PAIN END. HOWEVER, CELECOXIB HAS VERY POOR ORAL BIOAVAILABILITY AND AQUEOUS SOLUBILITY. IT IS HIGHLY SOLUBLE IN ACETONITRILE [8]. IN THIS EXPERIMENT, WE ATTEMPTED TO PREPARE CELECOXIB NANOEMULGEL. NANOEMULGEL WHICH IS OTHERWISE KNOWN AS NANOEMULSION-BASED HYDROGEL, BY WHICH WE HAVE TO MAKE SOME EFFORT TO IMPROVE ITS PERMEABILITY AND DIFFUSIBILITY. THE MAIN ADVANTAGES OF THE NANOEMULGEL FORMULATION ARE DUE TO THE PRESENCE OF DUAL NATURE; MEANS HYDROPHILIC AND HYDROPHOBIC BASES WHICH CAN DEEPLY PENETRATE WITHIN THE SKIN [9-11]. MOREOVER, IT ALSO IMPROVES NANOEMULSION STABILITY BY DECLINING THE SURFACE AND INTERFACIAL TENSION AND ALSO INCREASES THE VISCOSITY OF THE AQUEOUS PHASE FOR PROPER DRUG ADMINISTRATION. NANOEMULGEL HAS ADDITION ADVANTAGES SUCH AS IT IS MORE ADHERE TOWARD SKIN SURFACE AND LEADS TO HIGHER CONCENTRATION GRADIENTS TOWARD SKIN HENCE ASSURED BETTER PENETRATION [12-14]. THE NANOEMULGEL FORMULATION HAS AN OUTSTANDING THIXOTROPIC PROFILE AND HAS EXCELLENT SPREADABILITY AND PROMINENT THERMODYNAMIC STABILITY.

METHODS
CELECOXIB (drug) was gifted by Prudence pharmachem-Ankleshwar, Gujarat. Triacetin (lipid) was procured from Himedia laboratory Pvt. Ltd., Mumbai. Capmul MCM C-8 (co-surfactant), Acconon MC-8-2 EP (surfactant)-containing polyoxyethylene acapric glycerides were gifted by Abitec Corporation, USA. Menthol (penetration enhancer), dimethyl sulfoxide-extra pure (DMSO), triethanolamine was purchased from Siscoresearch laboratory Pvt. Ltd., Mumbai. Celecoxib was purchased from Corel Pharma Chem., Ahmedabad. Rest of the chemicals and reagents used during experimentation were of analytical grades. Throughout the experiment deionized water was used.
Criteria for selecting excipients
Since we prepared this emulgel for topical usage, non-irritation and less sensitivity toward skin were our utmost priority. To maintain drug solubility throughout the nanoemulsion, the drug must have higher solubility in the oil phase. Furthermore, hydrophilic-lipophilic balance (HLB) value of surfactant has to be more than 10 for preparing a stable nanoemulsion. For maintaining a stable nanoemulsion, surfactant and co-surfactant containing higher and lower HLB value were considered for admixing [15,16].

Solubility study of celecoxib
The solubility of celecoxib in various oil, surfactant, and cosurfactant was screened out. An aliquot amount of celecoxib was added into 4 ml of different surfactant, cosurfactant, oils, and with deionized water in 10 ml vials containing stopper. Using cyclometer, the contents were vortexed and the temperature was maintained up to 25±1°C. The vials containing samples were kept in the isothermal bath for consistent 48 hrs to maintain equilibrium. After 48 hrs, samples were centrifuged at 4000 RPM for 15 minutes, and the supernatant was filtered using Accu-jet® membrane filter of 0.2 µm pore size (Sigma-Aldrich). The concentration of celecoxib in varying supernatants was determined by ultraviolet (UV) spectrophotometer at 255 nm. Surfactant, co-surfactant, and oils which show better solubility were used for nanoemulsion preparation containing 2% celecoxib (Tables 1 and 2).

Table 1: Percentage transmittance with surfactant and co-surfactant

| Surfactant | % Transmittance | Co-surfactant | % Transmittance |
|------------|-----------------|--------------|-----------------|
| Acconon MC8-2EP | 97.38±0.05 | Capmul MCM C-10 | 96.23±0.29 |
| Tween 80 | 93.90±0.12 | Captex-100 | 91.78±0.05 |

Table 2: Solubility studies of various oils, surfactants, cosurfactants

| Various phases | Name of the compounds used | Solubility (mg/ml)±SD | Remarks |
|---------------|---------------------------|-----------------------|---------|
| Oils          | Triacetin                 | 37.6±1.87             | Modestly soluble |
|               | Isopropyl myristate       | 10.98±0.12            | Partially soluble |
|               | Olive oil                 | 0.82±0.58             | Poorly soluble |
|               | Arachis oil               | 0.72±0.19             | Poorly soluble |
|               | Captex                    | 16.89±0.96            | Sparingly soluble |
|               | Cottonseed oil            | 14.09±0.11            | Sparingly soluble |
|               | Maize oil                 | 17.38±1.23            | Sparingly soluble |
|               | Captex                    | 0.92±0.12             | Poorly soluble |
|               | Wheatgerm oil             | 0.63±0.34             | Poorly soluble |
|               | Capryol 90                | 0.83±0.14             | Poorly soluble |
|               | Soybean oil               | 28.34±1.23            | Mostly soluble |
|               | IPM                       | 0.02±0.13             | Very poorly soluble |
|               | Clove oil                 | 1.71±0.56             | Sparingly soluble |
|               | Campul 908P               | 4.31±0.11             | Partially soluble |
| Surfactants   | Triacetin +Campul 908P    | 54.89±0.18            | Highly soluble |
|               | Acetonitrile+Triacetin-Campul 908P | 65.21±1.22 | Optimum soluble composition |
|               | Polyethylene glycol-40 stearate | 21.34±0.15 | Mostly soluble |
|               | Sorbitan mono-Oleate (Span 80) | 12.35±0.14 | Sparingly soluble |
|               | Polyoxy Ethylene SorbitanMonooleate (Tweed 80) | 39.24±0.13 | Partially soluble |
|               | Cremophor RH-40           | 29.01±0.02            | Moderately soluble |
|               | Acconon MC8-2EP           | 51.24±1.23            | Optimum soluble compound |
|               | Tween 20                  | 37.11±0.24            | Partially soluble |
| Co-surfactants| Ethylene glycol           | 0.05±0.34             | Less soluble |
|               | Propylene glycol          | 0.78±0.45             | Less soluble |
|               | PEG 400                   | 9.90±0.09             | Less soluble |
|               | Capmul MCM C-10           | 16.09±1.20            | Moderately soluble |
|               | Captex 100                | 11.29±0.62            | Sparingly soluble |
|               | Capmul PG8                | 4.89±0.19             | Poorly soluble |
|               | Transcutol                | 6.89±1.96             | Less soluble |
|               | Capmul MCM L-8            | 5.89±2.01             | Less soluble |

Construction of pseudo-ternary plot
From the solubility studies, it was confirmed that celecoxib possessed maximum solubility in acetonitrile + triacetin + Campul 908P oil mixture; hence, this mixture was considered for further extension. Acconon MC8-2EP as a surfactant and Capmul MCM C-10, as a cosurfactant showed better solubility for celecoxib and also retained good stability with oil phase and an aqueous phase. To investigate nanoemulsion region, maintaining ambient temperature up to 25±0°C, pseudo-ternary diagram was plotted using ProSim software. The surfactant and cosurfactant mixture (S1 and S2) and oil phase were admixed using water titration method to form nanoemulsion. Acconon MC8-2EP and Capmul MCM C-10 (S1) weight ratios (1:1, 2:1, 3:1 and 4:1) were taken to construct pseudo-ternary diagram. Now, S1 and oil were mixed in different ratios such as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and nanoemulsion was formed using water as a titrant. Since celecoxib is a hydrophobic drug, hence, our target was to prepare water in oil nanoemulsion. From the ternary plot, all the possible regions of nanoemulsion formation were estimated [9,17]. Following tables represent the surfactants, co-surfactant (S2): Oil-water titration values, which represented in pseudo-ternary plots.

Preparation of nanoemulgel
Nanoemulgel was prepared by implementing simple three steps.

Step 1: Formation of nanoemulsion
From the ternary phase diagram, it was clear that 4:1 ratio of acconon MC8-2EP and capmul MCM-C-10 (S1) weight ratios provides maximum self-emulsifying property at ambient room temperature (0-25°C). With adequate S1 concentration, required quantities of acetonitrile, triacetin, campul 908P, acconon MC8-2EP, capmul MCM C-10, and 5% methanol was taken and admixed with 2% of celecoxib, maintaining the proper ratio of aqueous and oil phase. The drug was dissolved in aqueous phase along with surfactant and co-surfactant. The aqueous phase then blended with oil phase at 15000 rpm, using high-pressure homogenizer (Micron Lab APV, Denmark) until a milky emulsion was formed.
formed. The celecoxib concentration was maintained constant (2%) for all the formulations.

**Step 2: Formation of hydrogel**

Hydrogels were prepared using carbopol-940, DMSO, methylparaben, propylparaben, and deionized water [9].

**Step 3: Formation of nanoemulgel**

Prepared nanoemulsion was incorporating into hydrogel to form nanoemulgel formulation (Table 8).

**Characterization of nanoemulsion and nanoemulgel formulations**

**Turbidimetric evaluation**

The aliquot amount of nanoemulsion (0.8 ml) was incorporated into 0.1 molar hydrochloric acid, and volume was maintained up to 200 ml using distilled water with continuous stirring using magnetic stirrer at ambient temperature. The turbidity was measured using digital nephew turbidity meter at particular equilibrium [18].

**Nanoemulsion particle size analysis and zeta potential studies**

Globular size and zeta potential determination are an essential part to identify the nanoemulsion behavior. Initially, samples were diluted with water at list 200 times and measured its particles/globular size using photon correlation spectrometer (Zetasizernano 90, Malvern Ltd. United Kingdom). Zeta potential can be measured by estimating the responses of the charged particles drift in a constant velocity. Furthermore, Zetasizer generates a high-frequency AC field to oscillate the charged particles. Using Nanotrac controlled reference technique, particle size distribution was measured by comparing oscillations of reference colloidal particles. Using MPS single zeta potential can be measured.

**Transmission electron microscopy (TEM)**

TEM was performed to characterize and evaluate morphological understanding of prepared nanoemulsion. TEM was performed using a JEM-ARM 200F instrument (JEOL solutions for innovation corporation-USA) which operates at 200 kv.

**Mathematical modeling**

Using design expert® 7.0 software, it was possible to find which model is best suits for correlation between independent and dependent variables. The software itself selects a suitable model on the bases of individual parameters generated from regression analysis, such as adjusted R² value, Predicted R² value and predicted residual sum of square (PRESS) and p value. At 5% level of significance, analysis of variance was implemented. Here, more than one model was found to be significant; hence, best-fit model was screened out by analyzing adjusted R² value, which has to be higher in denomination but <1, and PRESS value which has to be lower in value. The general quadric equation for three independent variables is as follows:

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_1\beta_2X_1X_2 + \beta_1\beta_3X_1X_3 + \beta_2\beta_3X_2X_3 + \beta_1\beta_2\beta_3X_1X_2X_3 + \beta_0
\]

(1)

\(\beta_0\) represent the arithmetic outcomes average of all the outcomes of experimentation-13 batches. \(\beta_1\) to \(\beta_9\) represents the coefficient of observed experimental values of \(Y\) to \(Y\). On the other hand, \(X_1, X_2, \ldots\) and \(X_n\) are the coded level of factors. \(X_1, X_2, \ldots\) and \(X_n\) represent quadratic terms and interaction, respectively. The coefficient of one factor signifies the effect of particular factor and interaction of two-factor represents the quadric nature and effect between those two factors, respectively. In front of factors if the negative sign arose, the nit indicates that it has an antagonistic effect on design; on the other hand, the positive sign represents the synergistic effect on design model (Table 7).

**Appearance and pH determination**

The prepared nanoemulgel were evaluated based on physical examination first. The product homogeneity, consistency, color, and pH were determined. The freshly prepared products were stable and maintained light milky yellowish appearances. The pH was determined using digital pH meter (Mettler instrument, Germany) by taking 1% of prepared nanoemulgel in double distilled water [19].

**Viscosity**

The viscosity of the different nanoemulgel was determined using Brookfield Digital Viscometer (LVDV III Ultra, Brookfield Engineering Laboratories, and the USA) at 25°C. The t-92 number spindle was taken, and viscosity was recorded at different rotational speeds of 10, 20, 50, and 100 rpm [20].

**Drug content**

1 ml of nanoemulgel was diluted to 20 ml of methanol and sonicated. The volume was maintained up to 100 ml using phosphate buffer...
(pH 7.4). The UV-VIS spectrophotometer (SHIMADZU-1880) was used to measure celecoxib content after several dilutions of nanoemulgel at 255 nm.

Spread ability studies
As per Jain et al., spreadability of prepared nanoemulgel was measured using two different glass slides (7.5×2.5 cm). The first slide was bound with wooden frame. On top of the first glass slide, 1 g of nanoemulgel was allocated, and second glass slide was placed over first glass slide. Furthermore, 100 g weight was imposed over second glass top. Due to over-weight, entrapped air between the sandwiched nanoemulgel was removed. Using thread and progressive weights, the second glass slide was pulled up to pre-set distance of 7.0 cm. The time (second) and weight (g) required to mobilized second slide up to 7 cm was measured. The spreadability can be calculated using following formula:

\[ S = \frac{M \times L}{T} \]

where M is the weight (g) tied to the second slide. L is the length of the glass slide. T is the time taken to separate two glass sides [21].

In-vitro diffusion studies
Using Franz diffusion cell, dialysis studies were been carried out. Prepared nanoemulgel (1 g) was eventually applied onto the surface of the dialysis membrane. The dialysis membrane was clasp between donor and receptor compartments. The receptor compartment was filled with pH 7.4 phosphate buffer solution. The receptor compartment solution was stirred constantly using 2.5 cm long magnetic beads maintaining the temperature at 25°C. The aliquots amount of samples were (1 ml) withdrawn conservatively from 1 to 12 hrs interval, and simultaneously, 1 ml of fresh 7.4 pH phosphate buffer solution was poured into donor compartment. The various samples of each time interval were analyzed using UV-VIS spectrophotometer (SHIMADZU-1880) at 255 nm at appropriate dilutions.

Ex-vivo diffusion studies
Using averted rat skin of a pre-sacrificed animal, ex-vivo diffusion studies were carried out. Same in-vitro diffusion procedure was been followed for this experiment. Ex-vivo parameters were calculated by calculating cumulative correlation of drug diffused per unit time. The average cumulative amount of the drug permitted through the unit surface of the skin was plotted against time in an hour. From the plot, the slope of the linear portion was calculated [22]. It was considered as flux Jss (µg/cm²/hr). The drug permission coefficient was calculated by this following formula:

\[ Kp = \frac{Jss}{Cv} \]

Hear, Cv and Jss stands for the total amount of the drug and permeability coefficient of the drug, respectively [23]. Due to the formation of nanoemulgel formulation, the enhancement of the drug permeation would be higher than the marketed product [Note: Due to unavailability of marketed product, we prepared celecoxib gel as per Karade et al. article] [24]. The enhancement factor was calculated as per the following equation:

The enhancement factor \([EF] = \frac{Kp \text{ of nanoemulgel formulation}}{Kp \text{ of marketed gel}}\)

Acute skin irritation studies
Skin irritation studies were carried out at Deshpande laboratory, Bhopal-India, according to the approval of Animal Ethical Committee [1410/c/11/CPCSEA]. As per modified Draize et al. (1944) method, the selected three rabbits for experiments were acclimatized according to the laboratory condition prior 1 week of the experiments. Humidity and temperature of the experimental room were maintained up to 45% RH and 25°C, respectively. The dorsal side of the skin of the selected three rabbits was trimmed (5 cm), and the first rabbit was considered for negative control, where no treatment was given. The second rabbit was considered for the test, where 4 g of nanoemulgel was introduced into its trimmed dorsal skin, and the third animal was treated with formalin (0.6%v/v) which is consider as a standard irritant. This process was continued for 6 days. On the 7th day, the dorsal skin of all the three rabbits was cleaned with distilled water. The treated skin was examined by visual observation for erythema and edema [25].

Anti-inflammatory activity studies
As per Animal Ethical Committee [1410/c/11/CPCSEA] approval, anti-inflammatory activity was performed for prepared nanoemulgel. Totally, three groups (3×3) of Wistar rats containing average 200 g weight of either sex were selected. The animals were caged in polypropylene boxes, and adequate diets were given before starting the experiment. The animals were maintained as per standard laboratory conditions, at 25±1°C and 50-55% RH. Controlled group (first group) of animals was maintained untreated. Remaining two groups of rats were induced with 1% w/v carrageenan solution by subcutaneous route to produce paw edema. After injection, 1 day was waited to observe edema effect on animals paw. Next day, the second group of animals was treated with optimized 20-25 mg of nanoemulgel formulation followed by the third group of animals with 30 mg marketed product (Karade et al.). The paw volume was measured using plethysmometer for consecutive 1-12 hrs. The percentage inhibition of edema was determined against the controlled group [26,27].

Thermodynamic stability studies
Thermodynamic stability studies are an integral part to screen out metastable formulations. Since we prepared nanoemulgel, which has to be free from phase separation, cracking and creeming, and all other associated stability issues. To understand that in details we investigate our all products in three different conductions. At first, formulation was centrifuged at 4000 rpm for 30 minutes. Those formulations which did not shown any phase separation considered for extreme heating and cooling studies, where temperature maintained up to −40 to +450°C. Best formulation which passed the previous experiment, was considered for freeze-thaw cycle test, where formulations were charged for 48 hrs at −210°C to +250°C. The formulation which intact good stability was considered as best formulation.

RESULTS AND DISCUSSION
Screening of excipients and solubility studies
Based on higher solubility within the drug molecule and emulsification ability, the combination of acetonitrile + triacetin + Camul 908P was selected for oil phase [solubility: 65.21±0.12]. Acconon MC8-2EP was selected as surfactants because of its optimum solubility [51.24±1.23] and maximum emulsification ability, at the same time, our surfactant has HLB value more than 10. Due to the usage of single surfactant transient, negative interfacial tension and fluid interfacial film are rarely achieve. After much more screening, Capmul BCM C-10 was selected as cosurfactant because of its higher solubility potential with the drug [51.24±1.23]. Our surfactant was less ionic in nature; hence, less toxicity can be escalated. Furthermore, powerful biological expectancy and permeation enhancement could be possible.

From the obtained results, it can be concluded that the combination of acetonitrile + triacetin + Camul 908P exhibits maximum emulsification ability with Acconon MC8-2EP [97.38%]. The gradual addition of cosurfactant as Capmul BCM C-10 improves dispensability and shown maximum transmission of 96.23% followed by Captop-100 of 91.78% transmission (Tables 1 and 2).

Construction and outcome of pseudo-ternary diagram
Formation of various pseudo ternary phase diagram by utilizing the ratio of oil S_oil and water represent the nanoemulsion region and optimized concentration of mixture [17]. The various yellow
color shade areas within the pseudo ternary phase diagrams represent stable emulsion phase, where clear and transparent w/o nanoemulsion was formed (Figs. 1-4). Rest of the white area represents conventional and turbid non-optimized emulsion. The various output of Acconon MC8-2EP and Capmul MCM C-10 (S\textsubscript{mix}) weight ratios (1:1, 2:1, 3:1, 4:1) were tabulated and represented bellow in Tables 3-6, respectively.

It was observed that increased concentration of surfactant (4:1) produces maximum nanoemulsion region. Further increasing the concentration of surfactants could produce toxicity. Hence, a pseudo ternary diagram of S\textsubscript{mix} 4:1 was selected for drug loading nanoemulsion.

**TEM**

The TEM study was performed to correlate morphology, the structure of the particles along with obtained particle size. The prepared nanoemulsion was diluted with double distilled water in 1/100 times. One drop of the diluted emulsion was poured in holey film grid of TEM and dried. After drying point-to-point estimation of particles was done using TEM. The droplet size of nanoemulsions was found to be aligned with previously obtained particle size results (Fig. 5).

**Turbidimetric evaluation**

Turbidity of the prepared nanoemulsions were determined using Manti Lab 0-100 NTU Digital Turbidity Meter MT-134. The various formulations such as EG2, EG7, EG11, and EG13 possessed maximum turbidity because of higher percentage of oil phase. The globular size of the particles was also higher in those formulations. However, EG1, EG7, EG10, and EG12 formulations were constrained with less turbidity, because of higher percentage of surfactant, which considerably governs particle size and its distribution.

**Particle size analysis**

Using laser scattering microscopy particle size has been determined. The droplet size increase with increase in concentration of acetonitrile + triacetin + Campul 908P (oil) concentration. On the other hand, decreased concentration of oil in EG1, EG3, EG5, and EG9 produces lesser droplet size. Maximum droplet size was around in nanoscale. The particle size was alter with HLB value of the surfactant and co-surfactant concentration. EG13 formulation possessed maximum concentration of oil and minimum concentration of surfactant, hence, it produces maximum droplet size within all the formulations (456±1.67 nm). However, the droplet size of the particles ranged from 234 to 456 nm. Zeta potential was around −6.23±0.23 to −1.39±1.90, which is indicating that the nanoemulsion particles are non-aggregative and cationic in nature.

### Table 7: Observation and variable responses of Box-Behnken factorial design for various nanoemulgel formulations (mean±SD, n=3)

| Formulations | Independent variable (coded) | Independent variables (actual) | Dependent variables | Y\textsubscript{1} (viscosity) | Y\textsubscript{2} (% drug diffusion) |
|--------------|-------------------------------|--------------------------------|---------------------|-------------------------------|-------------------------------------|
| EG1          | X\textsubscript{1} = −1.000, X\textsubscript{2} = 1.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 20.00, X\textsubscript{2} = 60.00, X\textsubscript{3} = 0.50 | Y\textsubscript{1} | 785                          | 87.36                              |
| EG2          | X\textsubscript{1} = 1.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = −1.000 | X\textsubscript{1} = 60.00, X\textsubscript{2} = 45.00, X\textsubscript{3} = 0.25 | Y\textsubscript{1} | 689                          | 88.32                              |
| EG3          | X\textsubscript{1} = −1.000, X\textsubscript{2} = −1.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 20.00, X\textsubscript{2} = 30.00, X\textsubscript{3} = 0.50 | Y\textsubscript{1} | 876                          | 81.108                             |
| EG4          | X\textsubscript{1} = 0.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 40.00, X\textsubscript{2} = 30.00, X\textsubscript{3} = 0.25 | Y\textsubscript{1} | 593                          | 83.01                              |
| EG5          | X\textsubscript{1} = 1.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 1.000 | X\textsubscript{1} = 20.00, X\textsubscript{2} = 45.00, X\textsubscript{3} = 0.50 | Y\textsubscript{1} | 1176                         | 85.34                              |
| EG6          | X\textsubscript{1} = 0.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 40.00, X\textsubscript{2} = 45.00, X\textsubscript{3} = 0.75 | Y\textsubscript{1} | 987                           | 86.82                              |
| EG7          | X\textsubscript{1} = 1.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 60.00, X\textsubscript{2} = 60.00, X\textsubscript{3} = 0.50 | Y\textsubscript{1} | 1098                         | 92.703                             |
| EG8          | X\textsubscript{1} = 0.000, X\textsubscript{2} = −1.000, X\textsubscript{3} = 1.000 | X\textsubscript{1} = 40.00, X\textsubscript{2} = 30.00, X\textsubscript{3} = 0.75 | Y\textsubscript{1} | 1351                         | 85                                 |
| EG9          | X\textsubscript{1} = 1.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 1.000 | X\textsubscript{1} = 20.00, X\textsubscript{2} = 45.00, X\textsubscript{3} = 0.25 | Y\textsubscript{1} | 588                           | 83.12                              |
| EG10         | X\textsubscript{1} = 0.000, X\textsubscript{2} = 1.000, X\textsubscript{3} = −1.000 | X\textsubscript{1} = 40.00, X\textsubscript{2} = 60.00, X\textsubscript{3} = 0.25 | Y\textsubscript{1} | 557                           | 88.86                              |
| EG11         | X\textsubscript{1} = 1.000, X\textsubscript{2} = 0.000, X\textsubscript{3} = 1.000 | X\textsubscript{1} = 60.00, X\textsubscript{2} = 45.00, X\textsubscript{3} = 0.75 | Y\textsubscript{1} | 1589                         | 90.501                             |
| EG12         | X\textsubscript{1} = 0.000, X\textsubscript{2} = 1.000, X\textsubscript{3} = 1.000 | X\textsubscript{1} = 40.00, X\textsubscript{2} = 60.00, X\textsubscript{3} = 0.75 | Y\textsubscript{1} | 1284                         | 91.1                               |
| EG13         | X\textsubscript{1} = 1.000, X\textsubscript{2} = −1.000, X\textsubscript{3} = 0.000 | X\textsubscript{1} = 60.00, X\textsubscript{2} = 30.00, X\textsubscript{3} = 0.50 | Y\textsubscript{1} | 1077                         | 86.28                              |

Where, Independent variables, X\textsubscript{1}: Acetonitrile+triacetin+Campul 908P (oil), X\textsubscript{2}: Acconon MC8-2EP and Capmul MCM C-10 (S\textsubscript{mix}), X\textsubscript{3}: Carbopol 940 (thickening agent). Dependent variables, Y\textsubscript{1}: Viscosity at 100 rpm, Y\textsubscript{2}: % of drug diffusion at 12\textsuperscript{th} hrs

**Effect of formulation variables on viscosity of the prepared nanoemulgel**

As per Box-Behnken surface design output, quadric model output was projected. After the screening of analytical data, it was found that...
viscosity was found to be 791.08 mPas and for percentage drug diffusion the linear relationship was established between S_{mix} and % oil. The steeper ascent of the graph progressively ascending from the midpoint of S_{mix} and % oil, indicating agonizing effect of X_1, X_3 and X_2 on % drug diffusion at the 12^{th} hr. As per design, the drug optimum diffusion would be around 88%. The linear plot with expected and predicted value signifies the perfect correlation of the model. From the box-cox plot of power transfer graph, it was observed that the blue color line was found to be within the red color line, indicating the model is in the optimized zone and no significant changes require for response transformation. Regression analysis of % drug diffusion (Y_2) with oil mixture (X_1), Smix (X_2) and carbopol-940 (X_3) indicating good correlation between the variables (Table 9).

\[ \% \text{Drug diffusion at 12^{th} hr} = +86.89 +2.61X_1 +3.08X_2 +1.08X_3 \]

(3)

From the equation 3, it can be confirmed that % drug diffusion has lower average arithmetic outcomes as compare to viscosity and all the independent (Table 11) variables (X_1, X_2 and X_3) have agonistic effect with drug diffusion at the 12^{th} hr because of positive sign of all the coefficient associated with X_1, X_2 and X_3. Furthermore, it can be concluded that S_{mix} (X_1) has higher influence and concentration of Carbopol-940 (X_3) has less influence on drug diffusion at the 12^{th} hr because of higher coefficient of X_3 (3.08) and lower coefficient of X_1 (1.08).

From the 3D plot, it can be assumed that the linear relationship was established between S_{mix} and % oil. The steeper ascent of the graph progressively ascending from the midpoint of S_{mix} and % oil, indicating agonizing effect of X_1, X_3 and X_2 on % drug release at the 12^{th} hr. As per design, the drug optimum diffusion would be around 88%. The linear plot with expected and predicted value signifies the perfect correlation of the model. From the box-cox plot of power transfer graph, it was observed that the blue color line was found to be within the red color line, indicating the model is in the optimized zone and no significant changes require for response transformation (Fig. 7). Regression analysis of % drug diffusion at 12^{th} hrs (Y_2) with oil mixture (X_1), Smix (X_2) and carbopol-940 (X_3) indicating good correlations between the variables (Table 10).

**Optimization and screening from overlay plot**

The yellow color surface indicating optimized zone in which EG14* has a best-optimized viscosity at 790.34 mPas, % drug diffusion at 87.35%. These statistical responses are predicted by Design Expert 7.0 software. Considering these facts, our experimental or actual responses for viscosity was found to be 79.108 mPas and for percentage drug diffusion was showing around 780 mPas. Design points also indicating viscosity was depended on carbopol-940 concentration. The linear plot with expected and predicted value signifies the perfect correlation of the model. From the box-cox plot of power transfer graph, it was observed that the blue color line was found to be within the red color line, indicating the model is in the optimized zone and no significant changes require for response transformation. Regression analysis of viscosity (Y_1) with oil mixture (X_1), Smix (X_2) and carbopol-940 (X_3) indicating good correlation between the variables (Table 9).

**Effect of formulation variables on % drug diffusion on 12^{th} hrs**

As per Box-Behnken surface design output, linear model output was projected. After the screening of analytical data, it was found that linear model has higher adjusted R^2 value (0.9994), expected p-value (<0.0001) [Table12 and 14] and less PRESS value [Table 13]. Hence, a linear model was considered as an optimum model. The polynomial equation as per the coded factor was incepted bellow.

\[
\text{Viscosity at 1 rpm} \ (Y_1) = +987.00 +128.50X_1 -21.63X_2 +371.63X_2 +28.00X_1X_2 +78.00X_1X_3 -7.75X_2X_3 +18.13X_2^2 -46.12X_2^2 +5.37X_3^2
\]

(2)

From the quadratic equation 2, it can be postulated that Viscosity has higher average arithmetic outcome average +987.80 (Table 11) carbopol-940 (X_2) has massive influence on increasing viscosity because coefficient of X_2 (371.63) was much higher than X_1 (128.50). On the other hand, S_{mix} concentration (X_1) has antagonistic effect on viscosity because coefficient was in minus (-21.63). The oil and carbopol-940 mixture (X_1) have maximum susceptibility to produce maximum viscosity because it has maximum coefficient value (78.00) as compare to oil and surfactant mixture (X_1X_2), which is 28.00. The mixture of S_{mix} and carbopol-940 produces antagonistic results. Further analysis shows double concentration of oil can increase viscosity (as coefficient was 18.13) but double concentration of carbopol-940 may have less influence on viscosity, due to negative relation of carbopol-940 within the system (Fig 6).

Non-linearity of the 3D model was inclined toward oil phase, indicating viscosity depends on increasing concentration of oil phase. At an optimum concentration of carbopol-940 (0.50), the viscosity was showing around 780 mPas. Design points also indicating viscosity was depended on carbopol-940 concentration. The linear plot with expected and predicted value signifies the perfect correlation of the model. From the box-cox plot of power transfer graph, it was observed that the blue color line was found to be within the red color line, indicating the model is in the optimized zone and no significant changes require for response transformation. Regression analysis of viscosity (Y_1) with oil mixture (X_1), Smix (X_2) and carbopol-940 (X_3) indicating good correlation between the variables (Table 9).
The % error for viscosity and percentage drug diffusion was estimated to be as 0.093% and 0.873% respectively, which was far lesser than 9% of the actual limit. Hence, it can be concluded that EG14\* formulation turn out to be best-optimized formulation for nanoemulgel preparations. (Fig. 8 and Table 15).

**The viscosity of nanoemulgel**

Viscosity was determined using the previously mentioned procedure. Initially prepared hydrogel viscosity was depended on sharing the stress. However, it was observed that increase rpm could lead to
Fig. 10: EG14* of checkpoint batch showing excellent kinetic profile and maximum R² value in Higuchi kinetic model

Fig. 11: Spread ability profile of all the formulation

decrease in viscosity [27]. It was because unarranged particles started arranging within the direction of flow in the longitudinal axis. However, increase concentrations of carbopol-940 enhance viscosity. Further, addition of Sₘₛ and oil mixture alters the viscosity and share thinning profile of the prepared nanoemulgel. The various viscosity profile at different rpm was mention in Table 18.

Content uniformity
Almost all the formulation retained good drug content. However, EG11 possessed higher drug content as 98.18%, and E10 formulation scored lest in drug content as 92.01%.

Physical appearance and pH determination
The various formulated nanoemulgels was found to have milky yellowish white in appearance. Almost all the formulations have a good
Table 8: Formulation table

| Ingredients (% w/w)          | EG1   | EG2   | EG3   | EG4   | EG5   | EG6   | EG7   | EG8   | EG9   | EG10  | EG11  | EG12  | EG13  |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Celecoxib                    | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     |
| Acetomtriol triacetin·Capmul | 60    | 45    | 30    | 30    | 45    | 45    | 60    | 30    | 45    | 60    | 45    | 60    | 30    |
| 908P [X3]                    | 0.00   | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| Acconon MC8-2EP·Capmul       | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     |
| MCM C-10 [X4]               | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     | 5     |
| Menthol                      | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  |
| DMSO                         | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| Methylparaben                | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  |
| Propylparaben                | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| Carbopol-940 [X5]           | 0.50  | 0.25  | 0.25  | 0.25  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  |
| Triethanolamine              | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    | Qs    |
| (To adjust the Ph up to 6-6.5)| 100   | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to | Qs to |

Table 9: Regression analysis of viscosity (Y1) with oil mixture (X1), Smax (X2) and carbopol-940 (X3)

| Source     | df  | SS          | MS           | F            | Significance F |
|------------|-----|-------------|--------------|--------------|----------------|
| Regression | 3   | 1240680.25  | 41256.00833  | 99.7403066   | 3.1743E-07     |
| Residual   | 9   | 37316.67308 | 416.297009   | -             | -              |
| Total      | 12  | 1277996.923 | -            | -             | -              |

Table 10: Regression analysis of % drug diffusion at 12th hrs (Y2) with oil mixture (X1), Smax (X2) and carbopol-940 (X3)

| Source     | df   | SS          | MS           | F            | Significance F |
|------------|------|-------------|--------------|--------------|----------------|
| Regression | 3    | 139.5865    | 46.52884    | 2540.762     | 1.72E-13       |
| Residual   | 9    | 0.164817    | 0.018313    | -            | -              |
| Total      | 12   | 139.7513    | -           | -            | -              |

Table 11: Polynomial coefficient for Y1 and Y2

| Coefficient | Y1 (viscosity) | Y2 (% drug diffusion) |
|-------------|----------------|-----------------------|
| b0          | 987.00         | 86.82                 |
| b1          | 128.50         | 2.61                  |
| b2          | -21.63         | 3.08                  |
| b3          | 371.63         | 1.08                  |
| b4          | 28.00          | 0.043                 |
| b5          | 78.00          | -9.750E-003           |
| b6          | -7.75          | 0.062                 |
| b7          | 18.13          | -0.065                |
| b8          | -46.12         | 0.11                  |
| b9          | 5.37           | 0.065                 |

Table 12: Fit summary of highest order polynomial measured responses of the independent variables

| Source        | Y1     | Y2     |
|---------------|--------|--------|
| f value       | p value| f value| p value|
| Linear versus mean | 99.74 | <0.0001 | 2540.76 | <0.0001 |
| 2FI versus linear | 5.77  | 0.0335  | 0.33     | 0.8047  |
| Quadratic versus 2FI | 68.98 | 0.0029  | 0.96     | 0.5141  |

Spread ability studies

Spread ability studies were performed as per described procedure. The spread ability of the prepared nanoemulgel was depended on polymer consistency and viscosity of oil phase and polymer concentration. More viscous formulation would have less spread ability. It is expressed to understand the maximum surface area of the skin, in which the formulation spreads over. On the contrary, spread ability has a direct impact on drug distribution and penetration throughout the skin. From this column graph, it was estimated that EG1 formulation secured maximum spread ability coefficient (Fig. 11).

In-vitro diffusion studies or cumulative drug release

In-vitro diffusion studies using Franz diffusion cell and dialysis membrane helps to identify overall release patterns of the formulations. Almost all the formulation maintained good release profile, but EG1, EG7, EG10, and EG12 possessed optimum releases, as $S_{max}$ concentration was higher in those formulations. Moreover, most formulations turn out liquid after finishing of this experiment, indicating higher diffusion throughout dialysis membrane. After Box-Behnken factorial design of the experiment, EG14* formulation was developed out, within all those 13 formulations. The drug release patterns or diffusion from EG14* was very steady, after 12th hr it released 95.50% of drug within that formulation (Fig. 9 and Table 16). EG14* had also shown excellent kinetic profile. From the R2 value (0.9989) it was predicting that mechanism of drug diffusion is Higuchi (Fig 10 and Table 17).

Ex-vivo diffusion studies

For this study, we took EG14* as our optimized formulation and prepared celecoxib gel as per Karade et al. article as CG. It was observed that after the 12th hr of diffusion EG14* possessed 95.50% cumulative drug release, where else CG formulations delivered 56.90% cumulative drug release only. The optimized formulation exhibits maximum permeation coefficient (412.51 µg/cm²/h) as compare to CG formulation (135.67 µg/cm²/h) when drug concentration was consider as 20 mg. The permeation enhancement factor was compared between EG14* and CG formulation. The enhancement factor was found to be 3.03 (Fig 12).

Acquit skin irritation test

Prepared nanoemulgel skin irritation study was performed to estimate the safety index of the formulation. It was observed that prepared nanoemulgel was very tolerated by rabbit’s skin and shown less skin irritation.
**Table 13: Model summary statistics of response to select the best model to fit data**

| Sources | Linear | 2FI | Quadratic |
|---------|--------|-----|-----------|
|         | Adjusted $R^2$ | Predicted $R^2$ | PRESS | Adjusted $R^2$ | Predicted $R^2$ | PRESS | Adjusted $R^2$ | Predicted $R^2$ | PRESS |
| $Y_1$  | 0.9611  | 0.9357  | 8217.045 | 0.9850  | 0.9587  | 52802.27 | 0.9996  | -    | ±     |
| $Y_2$  | 0.9984  | 0.9974  | 0.36     | 0.9980  | 0.9945  | 0.77     | 0.9979  | -    | +     |

N.B.: + Case(s), in above of 1.0000: PRESS statistic not defined

**Table 14: ANOVA table for measured responses**

| Model terms | Viscosity ($Y_1$)-quadratic model | % Drug diffusion ($Y_2$)-linear model |
|-------------|------------------------------------|-------------------------------------|
| f value     | p value                            | f value                             | p value |
| Model       | 3103.48 <0.0001                     | 2540.76 <0.0001                     |
| $X_1$       | 2287.39 <0.0001                     | 2974.72 <0.0001                     |
| $X_2$       | 81.77 0.0029                        | 4139.08 <0.0001                     |
| $X_3$       | 24149.53 <0.0001                    | 5084.48 <0.0001                     |
| $X_4$       | 68.55 0.0037                        | -                                   | -       |
| $X_5$       | 531.93 0.0002                       | -                                   | -       |
| $X_6$       | 5.25 0.1058                         | -                                   | -       |
| $X_7$       | 16.41 0.0271                        | -                                   | -       |
| $X_8$       | 106.29 0.0019                       | -                                   | -       |
| $X_9$       | 1.44 0.3158                         | -                                   | -       |

**Table 15: Composition and results from checkpoint batches containing predicted and experimental values**

| Formulation | Independent variable/s | Composition in %W/W | Viscosity % drug diffusion | % Carbopol-940 (%X) | % Drug diffusion |
|-------------|------------------------|---------------------|---------------------------|---------------------|-----------------|
| EG13        | % Oil [X1] L           | 60                  | 1025                      | 984.67              | 86.28           |
| EG14*       | % S_m  [X1] L          | 30                  | 2790.34                   | 87.35               | 88.12           |
| EG6         | % S_m  [X1] L          | 45                  | 984.67                    | 85.67               | 86.82           |

**Table 16: % In-vitro cumulative drug release of nanoemulgel EG1 to EG14**

| Time in hour | EG1 | EG2 | EG3 | EG4 | EG5 | EG6 | EG7 | EG8 | EG9 | EG10 | EG11 | EG12 | EG13 | EG14* |
|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| 0            | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    |
| 1            | 32.67 | 31.47 | 33.67 | 35.12 | 33.87 | 34.12 | 36.12 | 32.12 | 34.11 | 35.18 | 32.18 | 31.87 | 33.98 | 27.68 |
| 2            | 39.23 | 38.19 | 39.18 | 41.65 | 43.17 | 45.18 | 46.01 | 43.11 | 45.19 | 42.98 | 43.19 | 44.11 | 44.19 | 38.39 |
| 3            | 43.23 | 43.76 | 43.87 | 45.71 | 45.87 | 49.32 | 50.51 | 46.53 | 47.38 | 45.62 | 47.72 | 49.56 | 48.52 | 47.12 |
| 4            | 46.11 | 48.25 | 45.17 | 50.11 | 49.58 | 54.18 | 56.28 | 50.61 | 52.67 | 49.82 | 53.78 | 54.17 | 51.34 | 53.78 |
| 5            | 52.47 | 53.67 | 50.91 | 54.17 | 55.43 | 59.28 | 61.57 | 53.18 | 56.18 | 54.71 | 58.13 | 59.01 | 55.21 | 60.20 |
| 6            | 57.28 | 60.21 | 55.71 | 58.17 | 60.34 | 62.48 | 66.29 | 57.71 | 59.52 | 59.72 | 64.11 | 64.88 | 57.21 | 64.11 |
| 7            | 63.78 | 65.19 | 59.14 | 63.17 | 64.44 | 67.21 | 70.27 | 61.41 | 63.09 | 63.62 | 69.42 | 67.66 | 63.18 | 70.62 |
| 8            | 69.72 | 70.67 | 63.81 | 66.28 | 69.41 | 70.17 | 75.61 | 65.12 | 66.32 | 67.51 | 74.61 | 73.71 | 76.03 | 74.61 |
| 9            | 73.15 | 75.16 | 67.17 | 70.12 | 73.09 | 74.48 | 80.17 | 69.81 | 69.98 | 73.57 | 78.24 | 79.32 | 71.62 | 77.24 |
| 10           | 76.41 | 79.45 | 72.71 | 74.62 | 78.72 | 78.61 | 84.85 | 74.28 | 73.19 | 78.34 | 81.98 | 84.16 | 76.83 | 83.23 |
| 11           | 82.78 | 84.19 | 76.51 | 77.15 | 81.78 | 83.19 | 87.11 | 78.18 | 78.42 | 84.58 | 86.41 | 87.23 | 80.18 | 86.41 |
| 12           | 87.36 | 88.32 | 81.108 | 83.01 | 85.34 | 86.82 | 92.703 | 85   | 83.12 | 88.86 | 90.51 | 91.1 | 86.28 | 95.50 |

**In-vivo anti-inflammatory effect**

As per Winter et al., paw edema test, optimized formula EG14* and marketed gel of ceclohex (as per Karade et al. article-CG) was evaluated for carrageenan-induced edema and anti-inflammatory activity. It was found that after EG14* formulation induction, the paw edema volume was significantly reduced and it was estimated using plethysmometer. After 12 hrs of nanoemulgel administration in induced edema area, it was found that higher of 92.56% edema volume was gone compare to marketed 79.67%. Its shows better penetration of drug throughout the skin and due to less viscosity, the canonization of drug and effective spread ability (Fig. 13).

**Thermodynamic stability studies**

As per previously described method, thermodynamic stability testing was done and it was cross-verified that no characteristic creaming, cracking and phase separation was observed. Various stress testing experiment such as heating-cooling, centrifugation, the freeze-thaw cycle was performed. Almost all the formulation passes the stability stress testing.
Table 17: Kinetic studies of drug release profile of formulation batches

| Formulation code | R² value | Zero order | First order | Higuchi | Hixon-Crowell | Korsmeyer-peppas | Best fit model |
|------------------|----------|------------|-------------|---------|---------------|-----------------|---------------|
| EG1              |          | 0.7938     | 0.9323      | 0.9627  | 0.9122        | 0.7283          | Higuchi       |
| EG2              |          | 0.8397     | 0.9586      | 0.9812  | 0.9406        | 0.7759          | Higuchi       |
| EG3              |          | 0.7584     | 0.9113      | 0.9460  | 0.8907        | 0.690           | Higuchi       |
| EG4              |          | 0.7626     | 0.9163      | 0.9524  | 0.8943        | 0.7052          | Higuchi       |
| EG5              |          | 0.7858     | 0.9234      | 0.9614  | 0.9018        | 0.7302          | Higuchi       |
| EG6              |          | 0.7936     | 0.9228      | 0.9695  | 0.8997        | 0.7553          | Higuchi       |
| EG7              |          | 0.8077     | 0.9458      | 0.9739  | 0.9229        | 0.7585          | Higuchi       |
| EG8              |          | 0.7662     | 0.8899      | 0.9569  | 0.8679        | 0.7323          | Higuchi       |
| EG9              |          | 0.7608     | 0.8946      | 0.9538  | 0.8720        | 0.7225          | Higuchi       |
| EG10             |          | 0.7678     | 0.9192      | 0.9526  | 0.8974        | 0.7071          | Higuchi       |
| EG11             |          | 0.8346     | 0.9502      | 0.9839  | 0.9291        | 0.7911          | Higuchi       |
| EG12             |          | 0.8329     | 0.9455      | 0.9858  | 0.9235        | 0.7969          | Higuchi       |
| EG13             |          | 0.7386     | 0.8711      | 0.9444  | 0.8495        | 0.7071          | Higuchi       |
| EG14*            |          | 0.8958     | 0.9774      | 0.9989  | 0.9605        | 0.8638          | Higuchi       |

Table 18: Viscosity of nanoemulgel formulation at different rpm

| Formulation code | Determination of viscosity in mPas at different RPM, maintaining temperature at 25°C |
|------------------|-------------------------------------------------------------------------------------|
|                  | 10 rpm | 20 rpm | 50 rpm | 100 rpm |
| EG1              | 3567±0.78 | 3086±0.18 | 1678±0.22 | 785±0.67 |
| EG2              | 3171±1.98 | 2607±0.32 | 1349±0.11 | 689±0.37 |
| EG3              | 3678±0.89 | 3291±0.28 | 1790±0.39 | 876±0.11 |
| EG4              | 2987±1.78 | 2589±0.28 | 1290±0.71 | 593±0.38 |
| EG5              | 5209±0.86 | 4890±0.55 | 2778±0.55 | 117±0.29 |
| EG6              | 4378±1.75 | 3813±0.28 | 1983±0.39 | 987±0.19 |
| EG7              | 4920±0.69 | 4281±0.77 | 2890±0.52 | 1351±0.62 |
| EG8              | 5518±1.28 | 5089±0.29 | 2890±0.52 | 1351±0.62 |
| EG9              | 2890±0.88 | 2471±0.37 | 1289±0.72 | 588±0.45 |
| EG10             | 2789±0.11 | 2390±0.73 | 1027±0.52 | 557±0.31 |
| EG11             | 5678±0.48 | 5078±0.38 | 2467±0.19 | 1589±0.54 |
| EG12             | 5467±0.28 | 5036±0.73 | 2411±0.73 | 1284±0.58 |
| EG13             | 5024±0.61 | 4690±0.49 | 2247±0.39 | 1075±0.11 |
| EG14*            | 2741±0.11 | 2490±0.13 | 1011±0.30 | 521±0.81 |

Table 19: Comparison of optimized parameters of EG14* and CG formulation in ex-vivo studies

| Formulation | Jss in µg/cm²/hr | Kp in cm/hr×10⁻³ | Cumulative percentage drug diffused at 12th hr |
|-------------|-----------------|------------------|-----------------------------------------------|
| EG14*       | 412.51          | 20.62            | 95.50%                                        |
| CG          | 135.67          | 6.78             | 56.90%                                        |

Table 20: Acquit skin irritation study outcomes on rabbits

| Groups        | Treatment           | Score after days | Mean score | Standard deviation |
|---------------|---------------------|------------------|------------|--------------------|
| Negative control | No treatment       | 0 0 0 0 0 0 0 0 0 | 0          | 0                  |
| Test          | 4 g prepared nanoemulgel | 3 3 2 2 2 1 1 2 | 2.04       | 0.8164             |
| Standard irritant | 0.8%/v/v formalin    | 8 7 7 6 6 5 4    | 6.14       | 1.34               |

Table 21: Anti-inflammatory effects of NEG 14* and CG in carrageenan induced rat paw edema

| Group | Formulation | N  | Time (hour) | Mean % oedema±SD | % Inhibition |
|-------|-------------|----|-------------|------------------|-------------|
| I     | Controlled  | 3  | 1           | 2.56±0.12        | -           |
|       |             |    | 2           | 3.78±1.81        | -           |
|       |             |    | 4           | 2.78±0.17        | -           |
|       |             |    | 8           | 1.83±0.01        | -           |
|       |             |    | 12          | 0.94±1.23        | -           |
| II    | Carrageenan induce edema | 3 | 1           | 6.78±0.02        | -           |
|       |             |    | 2           | 7.11±1.26        | -           |
|       |             |    | 4           | 8.17±0.26        | -           |
|       |             |    | 8           | 5.81±0.28        | -           |
|       |             |    | 12          | 4.88±0.88        | -           |

(Contd...)
(Table 21: Continued)

| Group | Formulation          | N | Time (hour) | Mean % oedema±SD | % Inhibition |
|-------|----------------------|---|-------------|------------------|-------------|
| III   | EG14* (optimized formula) | 3 | 1           | 4.89±1.34        | 29.78       |
|       |                      |   | 2           | 3.78±0.18        | 45.89       |
|       |                      |   | 4           | 2.66±0.02        | 67.90       |
|       |                      |   | 8           | 2.18±0.03        | 78.19       |
|       |                      |   | 12          | 1.99±0.05        | 92.56       |
| IV    | CG (marketed)        | 3 | 1           | 3.98±0.02        | 18.72       |
|       |                      |   | 2           | 2.98±0.04        | 38.88       |
|       |                      |   | 4           | 1.78±0.05        | 53.89       |
|       |                      |   | 8           | 0.89±0.17        | 63.71       |
|       |                      |   | 12          | 0.45±1.67        | 79.67       |

Table 22: Output from thermodynamic stability of various formulations

| Surfactant and co-surfactant ratio (4:1) | Thermodynamic stability study |
|----------------------------------------|-------------------------------|
|                                        | Heating-cooling cycle | Centrifugation | Freeze-thaw cycle |
| Formulations                           | √                           | √              | √                 |
| EG1                                    | ×                           | √              | √                 |
| EG2                                    | ✓                           | ✓              | ✓                 |
| EG3                                    | ✓                           | √              | ✓                 |
| EG4                                    | ×                           | ✓              | ✓                 |
| EG5                                    | ×                           | ✓              | ✓                 |
| EG6                                    | ✓                           | ✓              | ✓                 |
| EG7                                    | ×                           | ✓              | ✓                 |
| EG8                                    | ✓                           | ✓              | ✓                 |
| EG9                                    | ✓                           | ✓              | ✓                 |
| EG10                                   | ✓                           | ✓              | ✓                 |
| EG11                                   | ✓                           | ✓              | ✓                 |
| EG12                                   | ✓                           | ✓              | ✓                 |
| EG13                                   | ✓                           | ✓              | ✓                 |
| EG14*                                  | ✓                           | ✓              | ✓                 |

It can be a conclusive evidence that efficacy of surfactant, cosurfactant, and oil was unaffected after exposing it to hostile conditions.

CONCLUSION

In topical drug delivery system, nanoemulgel formulations could be considered as a very recent approach, in which both the hydrophobic and hydrophilic drug can be formulated and quantified desire effects. Our study highly emphasized on proper optimization, design, development, delivery approach of a poorly water soluble drug called celecoxib. Based on higher solubility and HLB value, we categorically screened out proper oil, surfactant, and co-surfactant to prepare nanoemulsion first. Later, we have selected proper polymer for nanoemulgel formulation. From the pseudo-ternary phase diagram, it has been reviled that 4:1 ratio of Smix could produce good solubility, stability, and penetrability. Moreover, turbidimetric studies, particle size determination studies, and TEM concluded that prepared nanoemulsion retained within the nano range. Furthermore, Box-Behnken factorial design was used to optimizennanoemulgel formulations. Almost all the 13 formulations

Fig. 12: Percentage cumulative amount of drug diffusion profile of EG14* and CG at 12th hr

Fig. 13: Comparison of effect of anti-inflammatory activity of EG14* and CG gel
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