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Research Article

FORMULATION AND OPTIMIZATION OF CELECOXIB NANOEMULGEL

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ABSTRACT

Objective: The main objective of this experiment was to prepare and optimized celecoxib nanoemulgel. This formulation can be used for acute rheumatoid arthritis patients.

Methods: Celecoxib is a poorly water soluble drug. We prepared celecoxib nanoemulgel to improve intrinsic solubility of celecoxib and enhance deeper permeation throughout the skin. After several screening, the combination of acetonitrile, triacetin, campul 908P was considered for oil phase; acconon MC8-2EP as surfactant, and capmul MCM C-10 as a co-surfactant accordingly. As per Box-Behnken surface design model, optimization was done for all the 13 formulations.

Results: Based on pseudo ternary plot, it was found that 4:1 S_{mix} ratio was optimum and possessed maximum drug solubility. Further, screening shown, 0.25-0.75% carbopol-940 can be a stable candidate for hydrogel preparation. Prepared nanoemulsions and hydrogels were admixed to prepare nanoemulgel. Based on overlay plot, EG14* formulation was consider as optimum one, and various evaluation parameters were performed along with other formulations. Using Franz diffusion cell, *in-vitro* diffusion studies was performed. Almost all the formulations produces good qualitative drug release profile. The EG14* shown 95.50% drug release after 12th hrs with standard Higuchi plot (R^2 value 0.9989). The optimum viscosity was found to be 521±0.81 mPas at 100 rpm. The appearance of the formulations was milky, yellowish white with expectable pH ranged from 5.8 to 6.7. The optimized formulation has good spreadability coefficient, good *ex-vivo* diffusion enhancement factor (3.03) as compare to marketed gel. Mostly, our formulations have less skin irritation and higher anti-inflammatory activity (92.56% of inhibition of paw edema for EG14*).

Conclusion: From the thermodynamic studies, it was confirmed that EG14* maintained excellent stability profile in various heating-cooling cycle, centrifugation, and freeze-thaw cycle condition. Hence, it can be conclude that, our formulation, can be consider for pilot scale up.

Keywords: Celecoxib, Rheumatoid arthritis, Capmul MCM C-10, Box-Behnken design, Pseudo ternary plot.

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INTRODUCTION

In autoimmune 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 tiredness. 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 synovium, 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 on 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 sedimentation rate, C-reactive protein, test for anemia, testing for antibodies such as rheumatoid factor and anticyclic citrullinated peptide, magnetic resonance imagining, ultrasound scan for distinctive characterization of RA [4]. Coming to the treatment point of view, there are many wide 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 cyclooxygenase-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. Campul 908-P (oil), Capmul MCM C-8 (co-surfactant), Acconon MC-8-2 EP (surfactant)-containing polyoxyethylenecapric glycerides were gifted by AbitecCorporation. USA. Menthol (penetration enhancer), dimethyl sulfoxide-extra pure (DMSO), triethanolamine was purchased from Siscoresearch laboratory Pvt. Ltd., Mumbai. Methyl paraben and propyl paraben were purchased from Lobachemical. Mumbai. Carbopol-940 (Gelling agent) 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, hydrophile-lipophile 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, cosurfactant, 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	Cosurfactant	% 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

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 cosurfactants showed better solubility for celecoxib and also retain 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 (S_{mix}) and oil phase were admixed using water titration method to form nanoemulsion. Acconon MC8-2EP and Capmul MCM C-10 (S_{mix}) weight ratios (1:1, 2:1, 3:1 and 4:1) were taken to construct pseudo-ternary diagram. Now, S_{mix} 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 nonoemulsion 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 (S_{mix}): Oil:water titration values, which represented in pseudo-ternary plots.

Preparation of nanoemulged

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 (S_{mix}) weight ratios provides maximum self-emulsifying property at ambient room temperature (0-25°C). With adequate S_{mix} 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

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

Various phases	Name of the compounds used	Solubility (mg/ml)±SD	Remarks
Oils	Triacetin	37.67±1.87	Modestly soluble
	Isopropyl myristate	10.98±0.12	Partially soluble
	Olive oil	08.24±0.58	Poorly soluble
	Arachis oil	07.23±0.19	Poorly soluble
	Captex	16.89±0.98	Sparingly soluble
	Cottonseed oil	14.09±0.11	Sparingly soluble
	Maize oil	17.38±1.23	Sparingly soluble
	Captex	09.22±2.12	Poorly soluble
	Wheatgerm oil	06.34±1.34	Poorly soluble
	Capryol 90	08.34±0.14	Poorly soluble
	Soybean oil	28.34±1.23	Modestly soluble
	IPM	02.12±0.13	Very poorly soluble
	Clove oil	17.14±0.56	Sparingly soluble
	Campul 908P	43.18±2.11	Partial highly soluble
	Triacetin+Campul 908P	54.89±0.18	Highly soluble
	Acetonitrile+Triacetin+Campul 908P	65.21±2.12	Optimum soluble composition
Surfactants	Polyethylene glycol-40 stearate	21.34±0.15	Mostly soluble
	Sorbitan mono-Oleate (Span 80)	12.35±0.14	Sparingly soluble
	Polyoxy Ethylene SorbitanMonooleate (Tween 80)	39.24±0.13	Partial highly 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	Partial high soluble
Co-surfactants	Ethylene glycol	05.09±1.34	Less soluble
	Propylene glycol	07.87±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

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, propyl paraben, 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].

Table 3: Surfactant and cosurfactant ratio (1:1)

Water	S_{mix}	Oil
0	25	75
49.36	39.49	11.11
49.45	39.039	11.50
43.47	36.18	20.35
40.38	24.3	35.32
36.45	23.36	40.19
31.21	20.23	48.56
28.13	15.64	56.23
24.63	15.13	60.24

Table 4: Surfactant and cosurfactant ratio (2:1)

Water	S_{mix}	Oil
0	25	75
52.15	35.61	12.24
53.25	31.97	14.78
46.50	24.29	28.58
38.36	26.08	35.56
34.45	23.36	42.19
28.21	20.23	51.56
30.13	13.64	56.23
20.63	15.13	64.24

Table 5: Surfactant and cosurfactant ratio (3:1)

Water	S_{mix}	Oil
0	25	75
50.12	37	12.88
50.00	35.2	14.80
45.00	32.5	22.50
43.89	18.22	37.89
38.50	22.6	38.90
33.89	17.11	49.00
30.00	15	55.00
25.90	12.10	62.00

Table 6: Surfactant and cosurfactant ratio (4:1)

Water	S_{mix}	Oil
0.4	19.5	80.1
46	35	19
55.00	18.90	26.10
50.47	14.89	34.65
50.04	12.47	37.48
44.44	14.89	40.67
40.54	8.90	50.55
33.02	10.98	56.00
23.33	9.67	67.00

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 S90, 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 R^2 value, Predicted R^2 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 R^2 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:

$$Y = \beta_0 + X_1\beta_1 + X_2\beta_2 + X_2\beta_3 + X_1X_2\beta_4 + X_1 X_3\beta_5 + X_2X_3\beta_6 + X_1^2\beta_8 + X_3^2\beta_9 \quad (1)$$

β_0 represent the arithmetic outcomes average of all the outcomes of experimentation-13 batches. β_1 to β9 represents the coefficient of observed experimental values of Y_1 to Y_2. On the other hand, X_1, X_2, and X_3 are the coded level of factors. X_1 to X_n (n = any number) represent quadric 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 nanoemulged 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

(pH7.4). 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 side. Furthermore, 100 g weight was imposed over second glass top. Due to overweight, 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 was been carried out. Prepared nanoemulged (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-buffered 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/cm^2/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:

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

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 (0.8%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 −40-450°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 + Campul 908P was selected for oil phase [solubility: 65.21±2.12]. 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 MCM 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 + Campul 908P, exhibits maximum emulsification ability with Acconon MC8-2EP [97.38%]. The gradual addition of cosurfactant as Capmul MCM C-10 improves dispensability and shown maximum transmission of 96.23% followed by Captex-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_{mix} and water represent the nanoemulsion region and optimized concentration of mixture [17]. The various yellow

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	
	X_1	X_2	X_3	X_1	X_2	X_3	Y_1 (viscosity)	Y_2 (% drug diffusion)
EG1	−1.000	1.000	0.000	20.00	60.00	0.50	785	87.36
EG2	1.000	0.000	−1.000	60.00	45.00	0.25	689	88.32
EG3	−1.000	−1.000	0.000	20.00	30.00	0.50	876	81.108
EG4	0.000	−1.000	−1.000	40.00	30.00	0.25	593	83.01
EG5	1.000	0.000	1.000	20.00	45.00	0.75	1176	85.34
EG6	0.000	0.000	0.000	40.00	45.00	0.50	987	86.82
EG7	1.000	1.000	0.000	60.00	60.00	0.50	1098	92.703
EG8	0.000	−1.000	1.000	40.00	30.00	0.75	1351	85
EG9	1.000	0.000	−1.000	20.00	45.00	0.25	588	83.12
EG10	0.000	1.000	−1.000	40.00	60.00	0.25	557	88.86
EG11	1.000	0.000	1.000	60.00	45.00	0.75	1589	90.501
EG12	0.000	1.000	1.000	40.00	60.00	0.75	1284	91.1
EG13	1.000	−1.000	0.000	60.00	30.00	0.50	1077	86.28

Where, Independent variables, X_1: Acetonitrile+triacetin+Campul 908P (oil), X_2: Acconon MC8-2EP and Capmul MCM C-10 (S_{mix}), X_3: Carbopol 940 (thickening agent). Dependent variables, Y_1: Viscosity at 100 rpm, Y_2: % of drug diffusion at 12th hrs

color shade areas within the pseudoternary 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_{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, apseudo ternary diagram of S_{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 phase] 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.

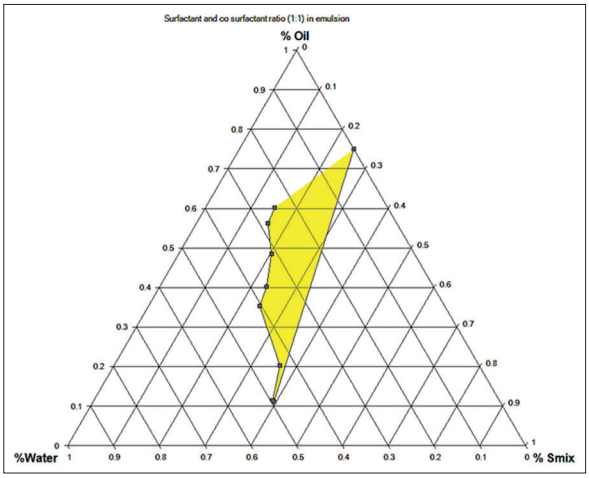

Fig. 1: Pseudo-ternary diagram of 1:1 S_{mix} ratio

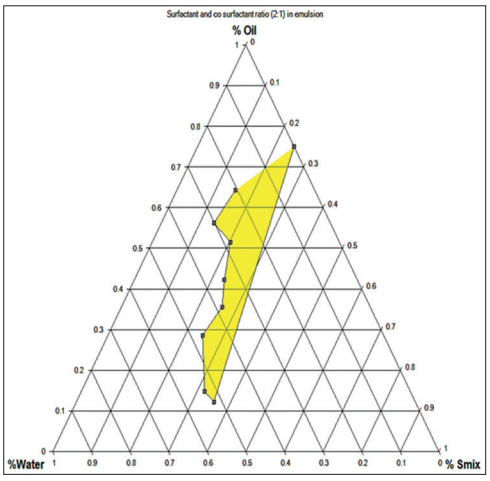

Fig. 2: Pseudo-ternary diagram of 2:1 S_{mix} ratio

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

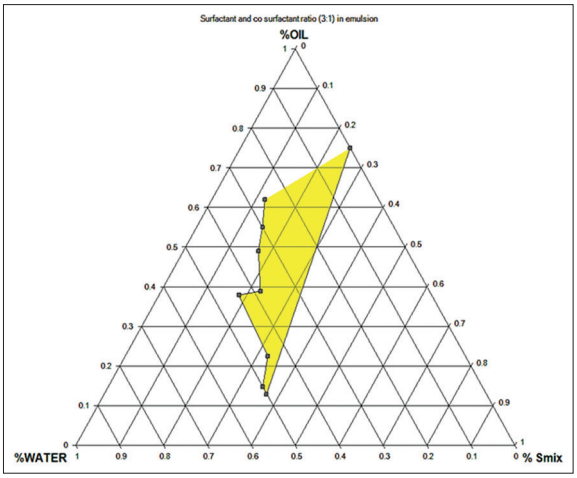

Fig. 3: Pseudo-ternary diagram of 3:1 S_{mix} ratio

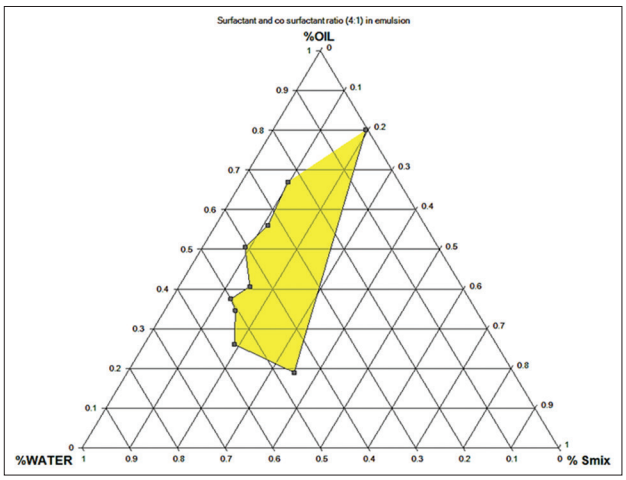

Fig. 4: Pseudo-ternary diagram of 4:1 S_{mix} ratio

quadric model has higher adjusted R^2 value (0.9996), less PRESS value, and expectable p-value (0.0029) [Table 12 and 14] and less PRESS value [Table 13]. Hence, quadric model was considered as optimum model. The polynomial equation as per the coded factor was incepted bellow.

$$\text{Viscosity at 100 rpm } (Y1) = +987.00 + 128.50X_1 - 21.63X_2 + 371.63X_3 + 28.00X_1X_2 + 78.00X_1X_3 - 7.75X_2X_3 + 18.13X_1^2 - 46.12X_2^2 + 5.37X_3^2 \quad (2)$$

From the quadric equation 2, it can be postulated that Viscosity has higher average arithmetic outcome average +987(βo) (Table 11) carbopol-940 (X_3) has massive influence on increasing viscosity because coefficient of X_3 (371.63) was much higher than X_1 (128.50). On the other hand, S_{mix} concentration (X_2) has antagonistic effect on viscosity because X_2 coefficient was in minus (−21.63). The oil and carbopol-940 mixture (X_1X_3) 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 was depends on increasing concentration of oil phase. At

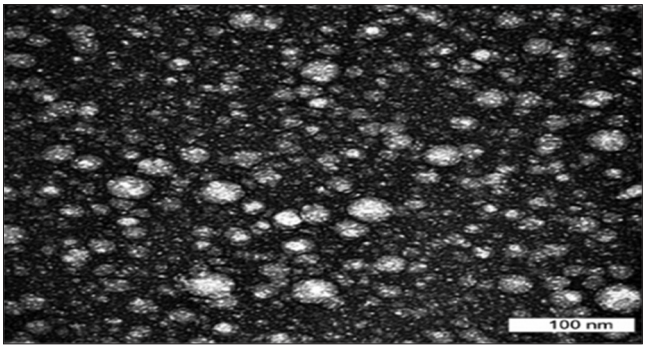

Fig. 5: Transmission electron microscopy analysis of prepared nanoemulsion

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 (Y1) with oil mixture (X1), Smix (X2) and carbopl-940 (X3) indicating good correlation between the variables (Table 9).

Effect of formulation variables on % drug diffusion on 12th 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 (0.9984), expectable p-value (<0.0001) [Table12 and 14] and less PRESS value (0.36) [Table 13]. Hence, a linear model was considered as an optimum model. The polynomial equation as per the coded factor was incepted bellow.

$$\%\text{ of Drug diffusion at } 12^{th}\text{ hr (Y2)} = +86.89+2.61X_1+3.08X_2+1.08X_3 \quad (3)$$

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 12th 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_2) has higher influence and concentration of Carbopl-940 (X_3) has less influence on drug diffusion at the 12th hr because of higher coefficient of X_2 (3.08) and lower coefficient of X_3 (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_2 on % drug release at the 12th 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 12th hrs (Y2) with oil mixture (X1), Smix (X2) and carbopl-940 (X3) 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 791.08 mPas and for percentage drug diffusion

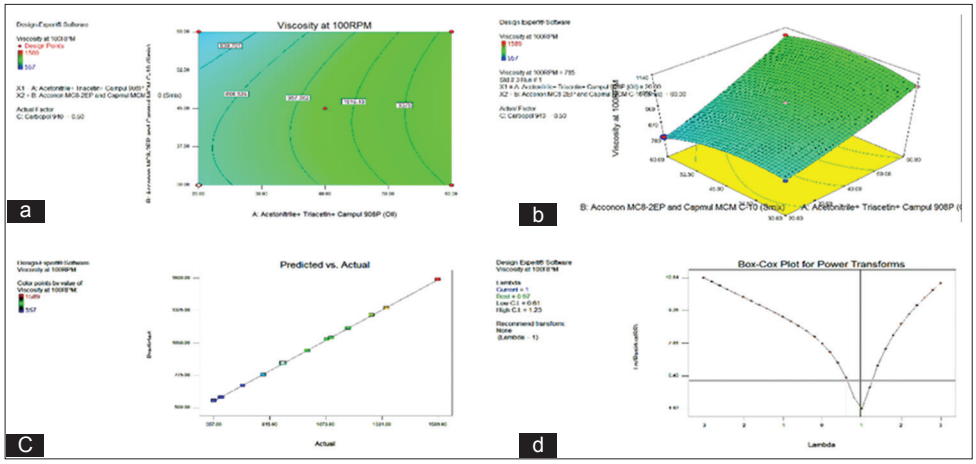

Fig. 6: (a-d) Counterplot, 3D modeling, predicted versus actual value and box-cox plot of viscosity at 100 rpm as per Box-Behnken surface design output

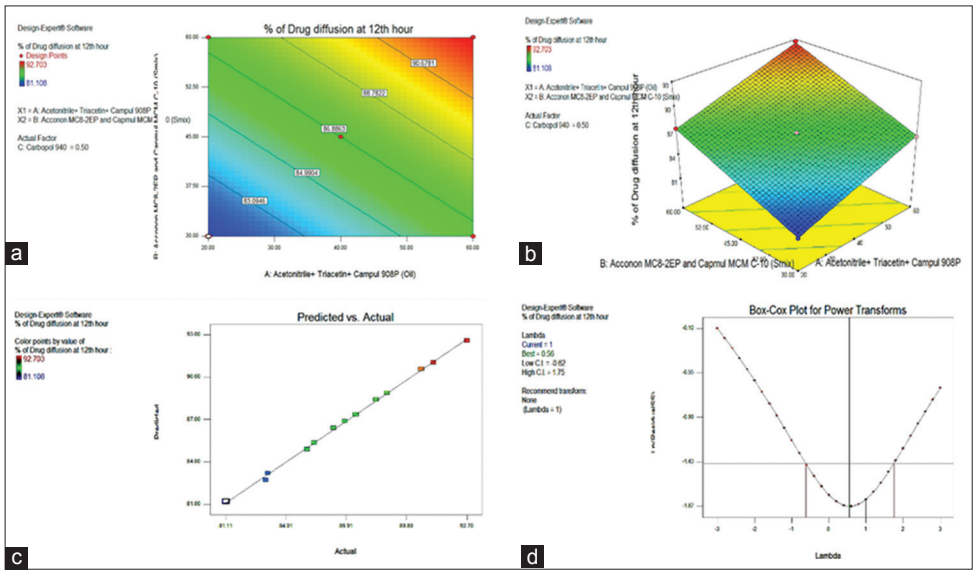

Fig. 7: (a-d) Counterplot, 3D modeling, predicted versus actual value and box-cox plot of % drug diffusion at the 12th hr as per Box-Behnken surface design output

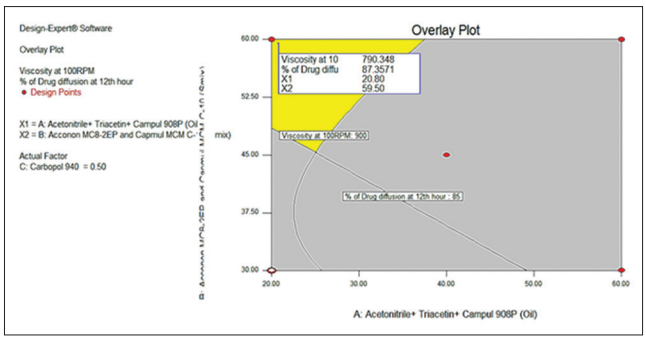

Fig. 8: Overlay plot and screening out optimum formula from the graph

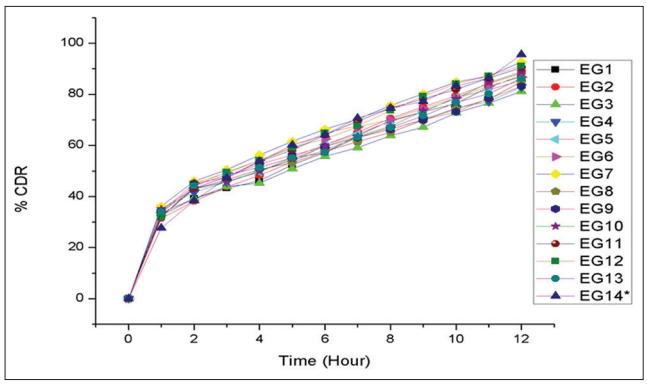

Fig. 9: % Cumulative drug release profile of all the nanoemulgel formulations

it was 88.12%. 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

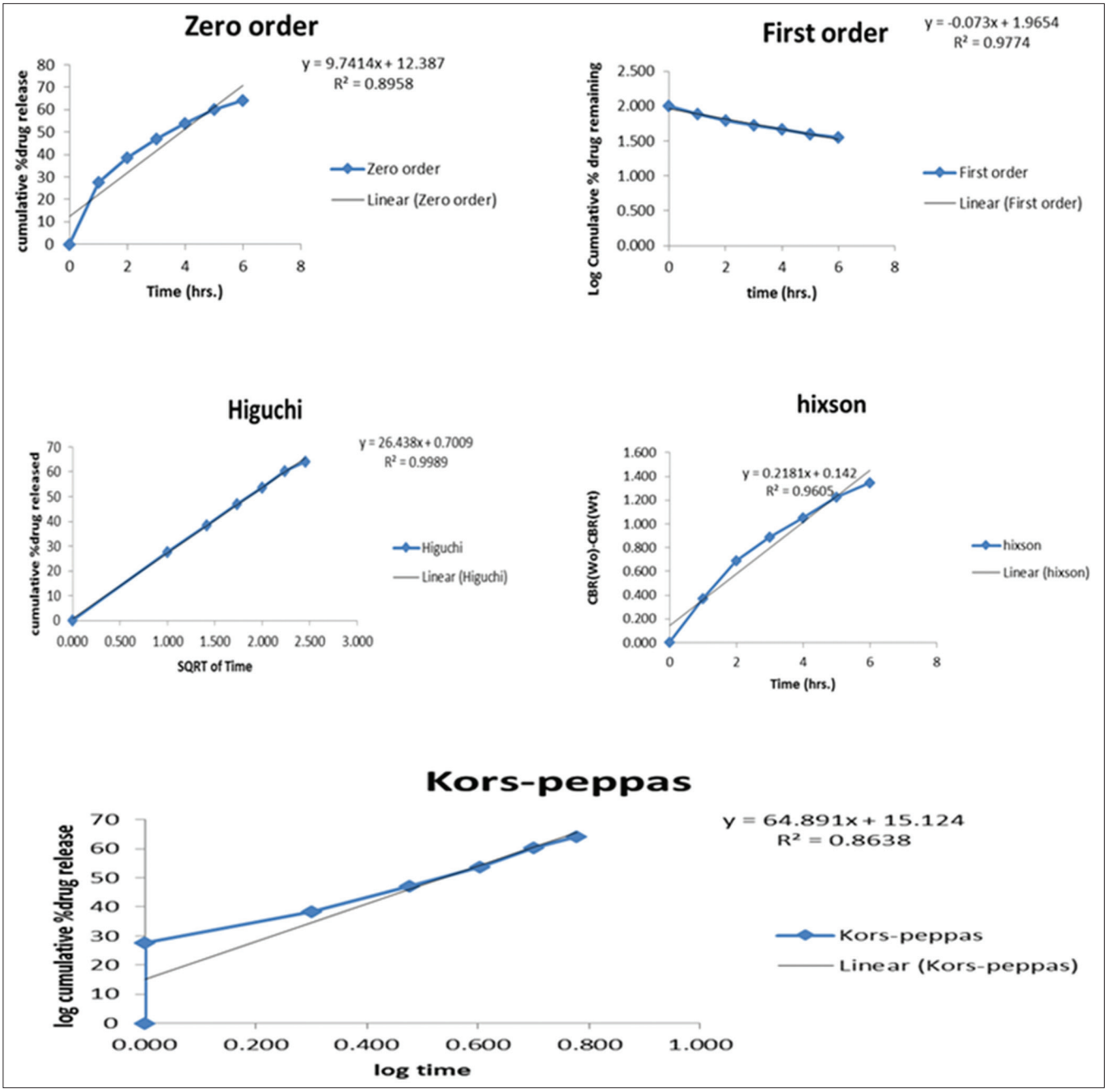

Fig. 10: EG14* of checkpoint batch showing excellent kinetic profile and maximum R^2 value in Higuchi kinetic model

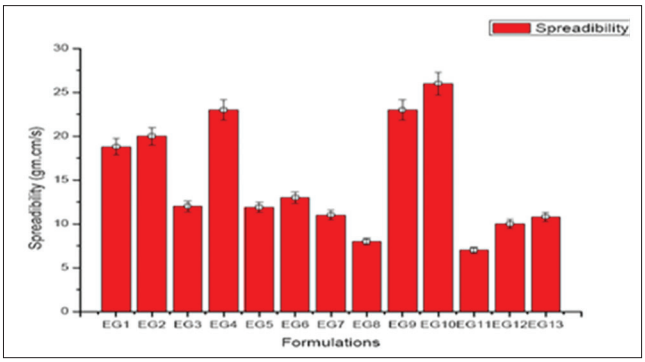

Fig. 11: Spread ability profile of all the formulation

decrease in viscosity [27]. It was because unarranged particles started arranging within the direction of flow in the longitudinal axis. However, increase concentrations of carbapol-940 enhance viscosity. Further, addition of S_{mix} 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
Celecoxibe	2	2	2	2	2	2	2	2	2	2	2	2	2
Acetonitrile+triacetin+Campul 908P [X_1]	20	60	20	40	20	40	60	40	20	40	60	40	60
Acconon MC8-2EP+Capmul MCM C-10 [X_2]	60	45	30	30	45	45	60	30	45	60	45	60	30
Menthol	5	5	5	5	5	5	5	5	5	5	5	5	5
DMSO	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben	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
Propyl paraben	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 [X_3]	0.50	0.25	0.50	0.25	0.75	0.50	0.50	0.75	0.25	0.25	0.75	0.75	0.50
Triethanolamine (To adjust the Ph up to 6-6.5)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs
Deionized water (%)	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100

Table 9: Regression analysis of viscosity (Y_1) with oil mixture (X_1), S_{mix} (X_2) and carbopl-940 (X_3)

Source	df	SS	MS	F	Significance F
Regression	3	1240680.25	413560.0833	99.74203066	3.17473E-07
Residual	9	37316.67308	4146.297009	-	-
Total	12	1277996.923	-	-	-

Table 10: Regression analysis of % drug diffusion at 12th hrs (Y_2) with oil mixture (X_1), S_{mix} (X_2) and carbopl-940 (X_3)

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 Y_1 and Y_2

Coefficient	Y_1 (viscosity)	Y_2 (% drug diffusion)
b_0	987.00	86.82
b_1	128.50	2.61
b_2	−21.63	3.08
b_3	371.63	1.08
b_{12}	28.00	0.043
b_{13}	78.00	−9.750E-003
b_{23}	−7.75	0.062
b_{11}	18.13	−0.065
b_{22}	−46.12	0.11
b_{33}	5.37	0.065

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

Source	Y_1		Y_2	
	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

consistency. The pH of these formulations was ranged between 5.8 and 6.7. Which are considerably acceptable and less irritable for use in human skin, as human skin pH is 5.5.

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 EG10 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_{mix} 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/cm^2/h) as compare to CG formulation (135.67 µg/cm^2/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 standard deviation on skin irritating score (0.8164) as compare to a standard irritant (1.34). No sign of erythema was observed in the test as compare to a standard irritant. Hence, our product maintained less skin irritation.

Table 13: Model summary statistics of response to select the best model to fit data

Sources	Linear			2FI			Quadric		
Responses	**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	82170.45	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)-quadric 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	2887.39	<0.0001	2974.72	<0.0001
X_2	81.77	0.0029	4139.08	<0.0001
X_3	24149.53	<0.0001	508.48	<0.0001
X_1X_2	68.55	0.0037	-	-
X_1X_3	531.93	0.0002	-	-
X_2X_3	5.25	0.1058	-	-
X_1^2	16.41	0.0271	-	-
X_2^2	106.29	0.0019	-	-
X_3^3	1.44	0.3158	-	-

ANOVA: Analysis of variance

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

Formulation	Independent variable's	Composition in %W/W	Responses	Predicted value	Actual responses value	% Error
EG13	% Oil [X_1]	60	Viscosity % drug diffusion	1025	1077	4.82
	% S_{mix} [X_2]	30		84.67	86.28	1.86
	% Carbopol-940 [X_3]	0.50				
EG14*	% Oil [X_1]	20.80	Viscosity % drug diffusion	790.34	791.08	0.093
	% S_{mix} [X_2]	59.50		87.35	88.12	0.873
	% Carbopol-940 [X_3]	0.50				
EG6	% Oil [X_1]	40	Viscosity % drug diffusion	984	987	0.303
	% S_{mix} [X_2]	45		85.67	86.82	1.32
	% Carbopol-940 [X_3]	0.50				

EG14* batch produces more accurate results as compare to overlay plot

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	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	67.03	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	90.501	91.1	86.28	95.50

***In-vivo* anti-inflammatory effect**

As par Winter *et al.* paw edema test, optimized formula EG14* and marketed gel of celecoxib (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	R^2 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	0.9838	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	4898±0.55	2778±0.55	1176±0.29
EG6	4378±1.75	3813±0.28	1983±0.39	987±0.19
EG7	4920±0.89	4281±0.77	2590±0.11	1098±0.37
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	1077±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 $\mu g/cm^2/hr$	Kp in $cm/hr \times 10^{-3}$	Cumulative percentage drug diffused at 12^{th} 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
		1	2	3	4	5	6	7		
Negative control	No treatment	0	0	0	0	0	0	0	0	0
Test	4 g prepared nanoemulgel	3	3	2	2	2	1	1	2	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		
Formulations	**Heating-cooling cycle**	**Centrifugation**	**Freeze-thaw cycle**
EG1	×	√	√
EG2	√	√	√
EG3	√	√	√
EG4	×	√	√
EG5	×	×	√
EG6	√	√	√
EG7	×	√	√
EG8	√	√	√
EG9	√	√	√
EG10	√	√	√
EG11	√	√	√
EG12	√	√	√
EG13	√	√	√
EG14*	√	√	√

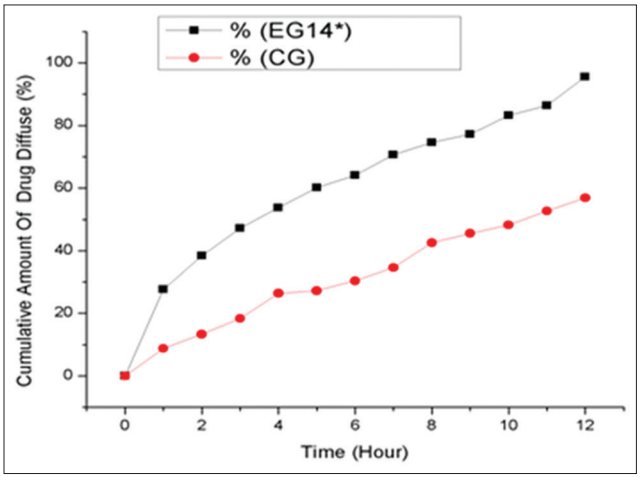

Fig. 12: Percentage cumulative amount of drug diffusion profile of EG14* and CG at 12th hr

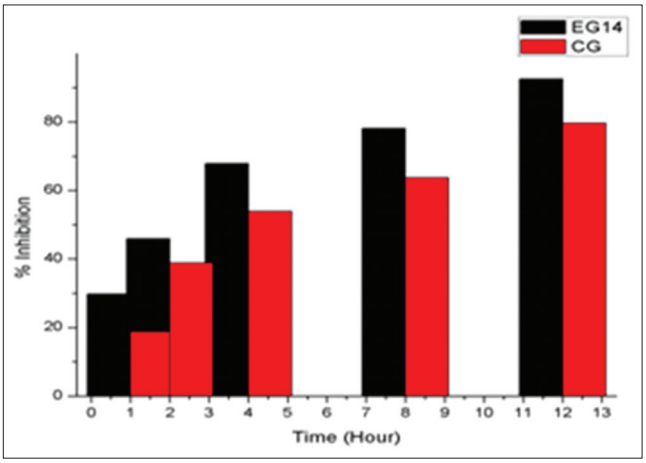

Fig. 13: Comparison of effect of anti-inflammatory activity of EG14* and CG gel

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 optimizenanoemulgel formulations. Almost all the 13 formulations

possessed good appearance and pH, good viscosity, drug diffusibility, spread ability, and the cumulative percentage of drug release. The overlay plot we had given best out of best formulation named as EG14* and found it has good percentage cumulative drug release profile, diffusion profile, almost 92.56% of inflammatory inhibition, and possessed handsome stability profile. Hence, it can be concluded that prepared celecoxib nanoemulgel was a good candidate for topical drug delivery system.

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