

# Preparation and Evaluation of Celecoxib Nanoemulsion for Ocular Drug Delivery

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## Abstract

**Introduction:** Poor bioavailability of drugs from ocular dosage forms is mainly due to the tear production, nonproductive absorption, transient residence time, and impermeability of corneal epithelium. The aim of the present study was to design, characterize of a novel nanoemulsion (NE) system as an ocular delivery system for celecoxib (CXB) and to evaluate its physicochemical characteristics and rabbit corneal permeability to enhance the penetration of the drug. **Methods:** CXB NEs were prepared by testing its solubility in oils, surfactants, and cosurfactants. Utilizing pseudoternary phase diagram, the optimum ratios were chosen and full factorial design was used with 3 variables at 2 levels for preparing eight formulations. The prepared NEs were evaluated regarding their viscosity, pH, particle size, differential scanning calorimetry thermograms, stability, *in vitro* drug release, and corneal rabbit permeability. **Results and Discussion:** The results showed that the mean droplet size range of NE formulations was in the range of 6.96-26.65 nm and pH was 6.5-6.9, respectively. Viscosity range was 118-245 cps. Drug release profile showed that 82.6% of the drug released in the 24 h of the experiment. The maximum and minimum drug permeated percentage through rabbit cornea was observed in NE component (NEC)-5 (15.73%) and NEC-1 (6.1%), respectively. All NE formulations with different compositions and properties significantly increased partitioning, flux, and permeability coefficient from rabbit cornea. Dapp and Papp parameters in NEC-3 and NEC-5 formulation were 0.0233 cm<sup>2</sup> h<sup>-1</sup>, 0.13 cm/h, and 46.62, 7.23 times higher than those of control (CXB suspension, 1%), respectively. The flux (Jss) of CXB from NEC-5 was 0.65 mg cm<sup>-2</sup> h<sup>-1</sup>, 21.68 times higher than those of control. **Conclusion:** This present study showed that any change in content and composition of NEs could be changed physicochemical properties and permeability parameters during drug permeation from NE formulations. The phenomenon may be due to alteration of the cornea structural changes in the presence of NECs.

**Key words:** Celecoxib, nanoemulsion, ocular, permeability, release

## INTRODUCTION

The complex anatomical and biological structure of human eye make it almost impervious against foreign bodies, including drugs. Drug delivery to the eye is complicated due to several removal pathway of precorneal area, the effect that usually reduces drug efficacy. In spite of several scientific reports, effective ophthalmic drug delivery remains a challenge for pharmaceutical researchers.<sup>[1]</sup>

Nanoemulsions (NEs) were early described by Hoar and Schulman and are colloidal dispersion composed of oil phase and aqueous phase, which require surfactant and cosurfactant agents to stabilize the interfacial area.<sup>[2]</sup> They are optically isotropic and thermodynamically stable with a droplet diameter size usually

between 10 and 100 nm.<sup>[3]</sup> Their surface tensions are very low, and their droplet size is small which makes them highly absorbable and permeable.

Due to unique structure and properties of NEs, they may be considered as proper formulations for ocular delivery

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of many drugs. NEs are easily prepared and sterilized, relatively stable and capable of embracing both hydrophilic and lipophilic molecules.<sup>[4]</sup> The presence of surfactant and cosurfactant in oil-in-water (o/w) NEs enhances drug permeation and uptake through biomembrane.<sup>[5]</sup> NEs are thermodynamically stable and low viscose mixtures of oil and water that have been stabilized with a surfactant and usually in combination with a cosurfactant. NEs have shown several advantages for drug delivery such as; ease of preparation, perfect stability, increasing drug solubility, controlling drug delivery rate, improving the bioavailability of hydrophilic and lipophilic drug through different delivery routes.<sup>[5]</sup>

NEs were first observed by Schulman and Winsor in the 1950s.<sup>[5]</sup> Then, the term “NEs” has been used to describe multi-component systems comprising non-polar, aqueous, surfactant, and cosurfactant components. Conventional NEs can be classified o/w, water-in-oil, and bicontinuous phase NEs.<sup>[5]</sup> Some advantages offered by nanomulsions include improvement in poorly drug solubility, enhancement of bioavailability, protection of the unstable drugs against environmental conditions and a long shelf life.

The cornea is an important mechanical and chemical barrier, and its main function is to protect the intraocular tissues of the eye. The cornea is characterized by lipophilic and hydrophilic structures and represents an effective barrier to the absorption of both hydrophilic and lipophilic molecules.<sup>[6,7]</sup>

Due to the tightness of the corneal barrier and the rapid loss of the instilled drug solution from the precorneal area, bioavailability decreases. Poor bioavailability of drugs from ocular dosage forms is mainly due to the tear production, nonproductive absorption, transient residence time, and impermeability of corneal epithelium corneal bioavailability is predicted to range between 1% and 5% for lipophilic molecules and to be <0.5% for hydrophilic molecules.<sup>[8]</sup>

Celecoxib (CXB) is a selective cyclooxygenase-2 (COX-2) inhibitor used for the treatment of rheumatoid arthritis and osteoarthritis. CXB has analgesic, antipyretic, and anti-inflammatory activity as a result of selective inhibition of the enzyme COX-2 and does not inhibit platelet aggregation.<sup>[5]</sup> In contrast with other non-steroidal anti-inflammatory drugs, it has neither acute nor chronic gastrointestinal toxicity. CXB is also used for the treatment of colon cancer, ultraviolet (UV) light-induced skin cancer, breast cancer,<sup>[9]</sup> and ocular disorders such as age-related macular degeneration and diabetic retinopathy.<sup>[10]</sup>

The aim of the present study is to develop a newly NE for ocular delivery of CXB and to evaluate its physicochemical characteristics and rabbit corneal permeability to enhance the penetration of the drug.

## MATERIALS AND METHODS

### Materials

CXB powder was purchased from Hakim Company (IR Iran). Tween 80, span 20, and oleic acid were purchased from Merck (Germany). Diethylene glycol monoethyl ether (Transcutol P) was gifted from GATTEFOSSE Company (France). All of the chemicals and solvents were of the analytical grade. Fresh double distilled water was used in the experiments. Dialysis bag was purchased from the Tuba Azma Co. (Tehran, Iran).

### Animals

Male New Zealand white rabbits weighing 2-2.5 kg were used in the present study which was conducted with the approval of the Animal Ethical Committee, Ahvaz Jundishapur University of Medical Sciences (permit no. IR. AJUMF. REC.1395.131).

### Methods

#### CXB assay

The amount of celecoxib was measured by UV spectrophotometer apparatus at 292 nm in buffer phosphate solution (PBS) (pH = 7.4) medium.

#### Solubility of CXB

The solubility of CXB was determined in oils (oleic acid, Transcutol P and oleic acid + transcutol P [10:1]), surfactants (Tween 80, Span 20), and cosurfactant (propylene glycol) by dissolving an excess amount of CXB in 3 ml of oil, and other components using a stirrer at 37°C ± 0.5°C for 72 h. The equilibrated samples were then centrifuged at 5000 rpm for 30 min to remove the undissolved drug; then, the clear supernatant liquid was decanted. The solubility of CXB was measured by validated UV spectrophotometric method at 292 nm.

#### Construction of phase diagram

The pseudo-ternary phase diagrams were mapped by titration method of liquid mixtures of surfactant, cosurfactant, and oil with water at room temperature to obtain the concentration range of the components for the existing boundary of NEs without drug. Two phase diagrams were prepared with the 2:1 and 3:1 mass ratios of (Tween 80-Span 20/propylene glycol), respectively. For each phase diagram, oil phase (oleic acid-Transcutol P) and the surfactant mixture were then mixed at the mass ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, ght ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:38:2, and 9:1. These mixtures were diluted drop wise with double distilled water at 25°C ± 1°C, under moderate agitation. The samples were classified as NEs when they appeared as clear liquids.<sup>[11]</sup>

## Preparation of NEs

NE samples selected from the constructed phase diagrams were prepared based on the composition as shown in Table 1. The full factorial design was used concerning with 3 variables at 2 levels for preparing eight NE formulations. Major variables take part in the determination of NEs properties include surfactant/cosurfactant ratio (S/C), percentage of oil (% oil), and water percentage (%W). Eight different NE formulations with low and high levels of oil (5% and 50%), water (5%, 10%), and S/Co mixing ratio (2:1, 3:1) were selected for preparing NE formulations. CXB (1%)<sup>[10]</sup> was added to oil phase, and then S/Co mixture and an appropriate amount of double distilled water were added to the mixture drop wise and continued by stirring the mixtures at ambient temperature until a transparent mixture was obtained.<sup>[12]</sup>

## Droplet size examination

The droplet size of NEs was determined at room temperature by dynamic light scattering with a nanosizer (SCATTER SCOPE 1 QUIDIX, South Korea).

## pH and viscosity measures

The pH values of NEs were determined directly in the samples using digital pH meter (Mettler Toledo seven easy, Switzerland) at room temperature.

The viscosity of samples was determined at 25°C using a Brookfield viscometer (DV-II+Pro Brookfield., USA) with spindle number 34.

## Differential scanning calorimetry (DSC)

DSC measurements were carried out by means of a MettlerToldo DSC<sup>1</sup> star<sup>®</sup> system equipped with the refrigerated cooling system. Approximately, 5-10 mg of each NE samples were weighted into hermetic aluminum pans and

quickly sealed to prevent water evaporation from NE samples. Simultaneously, an empty hermetically sealed pan was used as a reference. NE samples were exposed in a temperature ranging from +30°C to -50°C (scan rate: 5°C/min). Changes of enthalpy quantities ( $\Delta H$ ) were calculated from endothermic and exothermic peaks of DSC thermograms.<sup>[13]</sup>

## Release of NEs

Franz diffusion cells (contact area 3.4618 cm<sup>2</sup>) with a cellulose membrane were used to determine the drug release rate of CXB from different NEs. Before each experiment, the cellulose membrane was first hydrated in double-distilled water at 25°C for 24 h. Then, it was mounted between donor and receptor compartments. CXB samples (5 g NE) were accurately weighed and placed on the membrane. Each diffusion cell was filled with 25 ml of buffer PBS (pH =7.4). The receptor fluid was continuously stirred by externally driven magnetic bars at 200 rpm throughout the experiment. At definite time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h), 2 ml sample was removed from receptor compartments and then analyzed spectrophotometric determination and to maintain sink conditions, replaced immediately with an equal volume of fresh receptor medium. Samples were analyzed by UV spectrophotometer at 292 nm. The cumulative percentage of released drug was plotted versus time and their behavior was described by fitting on different kinetic models. The maximum  $r^2$  was considered as the most probable release mechanism.<sup>[13]</sup>

## Physical stability of NEs

The physical stability of each NE formulation was evaluated by centrifuge stress test and the temperature stability. NEs were kept in various temperature conditions (4°C, 25°C, 37°C, and 75%  $\pm$  5% RH) as per the ICH guidelines for 6 months and then evaluated by monitoring time- and temperature-dependent changes of the physicochemical characteristics, such as clarity, odor, color, phase separation, pH, viscosity, and particle size. Furthermore, NEs were centrifuged by High-Speed Brushless Centrifuge (MPV-350R, POLAND at 12000 rpm for 30 min at ambient temperature. After centrifugation, the physical instability of the formulations was visually determined by the degree of phase separation.<sup>[14]</sup>

## The *ex vivo* cornea permeation experiments

Male New Zealand albino rabbits were sacrificed, and their corneas together with sclera rings were separated. The underlying tissue was completely removed without causing any injury using the scissors or a scalpel. The rabbit corneas were kept in a DexSol solution (chondroitin-sulfate-based, commercial storage media for the preservation of corneal epithelium, Chiron ophthalmic, Irvine, California).<sup>[15,16]</sup> The *ex vivo* cornea permeation study was performed using

**Table 1:** Composition of selected nanoemulsion of celecoxib

Formulation	Factorial design	S:C	% Oil	% S+C	% Water
NEC-1	+++	3:1	50	40	10
NEC-2	++-	3:1	50	45	5
NEC-3	+--	3:1	5	85	10
NEC-4	---	3:1	5	90	5
NEC-5	---	2:1	5	85	10
NEC-6	---+	2:1	5	85	10
NEC-7	--+	2:1	50	45	5
NEC-8	-++	2:1	50	40	10

NEC: Nanoemulsion of celecoxib

Modified Franz diffusion cells fabricated in the house with an effective diffusion area of approximately 0.348 cm<sup>2</sup>. The excised rabbit corneas were placed between the donor and receptor compartments of the cell, so that sclera ring clamped between two chambers and cornea facing the receptor without any damage. The receptor phase was 7 mL of buffer PBS (pH = 7.4), and its temperature was regulated at 37°C ± 0.5°C. 0.5 g CXB NE samples (containing 1% drug) were accurately weighed and placed to the corneas. The receptor phase was continuously stirred using magnetic beads at 200 rpm throughout the experiment. The experiments were performed under non-occlusive condition to allow air permeation to corneal tissues. At definite interval times (0.5, 1, 2, 3, 4, 5 h), a 0.5 ml sample was withdrawn from the receptor chamber for spectrophotometric analysis and immediately replaced by an equivalent volume of fresh PBS to maintain sink condition. Samples were analyzed by UV visible spectrophotometer at 292 nm. A drug-free NE was used as a blank. The same test was performed for the 1% drug, and thus, the amount of permeated drug between NE and suspension were compared. The results were plotted as cumulative permeated drug percentage versus time.<sup>[17,18]</sup>

### Calculation of permeation parameters

Different corneal permeability parameters were measured using corneal permeation data including flux ( $J_{ss}$ ), permeability coefficient ( $P$ ), lag time ( $T_{lag}$ ), and diffusivity coefficient ( $D$ ). The cornea permeation rate at steady state ( $J_{ss}$ , mg/cm<sup>2</sup>h) was determined from the linear portion of the slope of the permeation curve. Since the thickness ( $h$ ) of cornea did not show the real pathway for drug permeation, so diffusivity coefficient is defined as appearance  $D$  ( $D_{app}$ ). Apparent permeability coefficient ( $P_{app}$ , cm/s) and apparent diffusivity coefficient ( $D_{app}$ , cm<sup>2</sup>/h), parameters were calculated from the equations ( $P_{app} = J_{ss}/C_0$ ) and ( $D_{app} = h^2/6 T_{lag}$ ), respectively. The lag time ( $t_{lag}$ , hr) was determined by extrapolating the steady-state line to the time axis.

### Statistical analysis

All the experiments were repeated three times and data were expressed as the mean value ± standard deviation. A one-way analysis of variance (ANOVA) was used to see any significant differences and  $P < 0.05$  was the level of statistical significance with 95% confidence intervals.

## RESULTS AND DISCUSSION

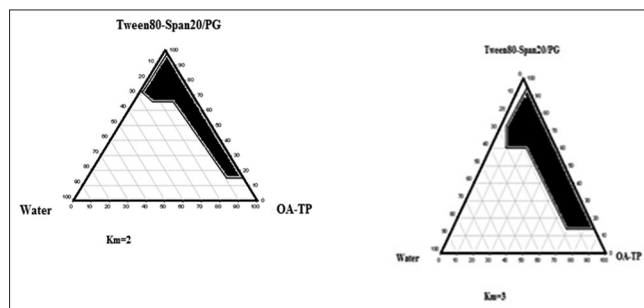
### Solubility of CXB

The solubility of CXB is shown in Table 2. To develop and design NE formulations the suitable oil was selected by determining the concentration of CXB that would dissolve. Based on the solubility experiments of CXB in oil, surfactant

**Table 2:** Solubility of CXB in various oils, surfactants, and cosurfactants (mean±SD,  $n=3$ )

Phase type	Excipient	Solubility (mg/ml)
Oil	Transcutol P	6.494±0.125
	Oleic acid	2.01±0.001
	Oleic acid+TP (10:1)	6.9±0.1
Surfactants	Tween 80	1.15±0.3
	Span 20	0.01±0.001
Cosurfactant	Propylene glycol	1.825±0.1

CXB: Celecoxib, SD: Standard deviation



**Figure 1:** The pseudo ternary phase diagrams of the oil-surfactant/cosurfactant mixture-water system at the 2:1 and 3:1 weight ratio or Tween 80/Span 20/PG at ambient temperature, dark area show nanoemulsions zone

and cosurfactant, we found that oleic acid-Transcutol P (10:1), Tween 80, Span 20, and PG could be the most appropriate combinations for the preparation of CXB NE.

### Phase studies

The pseudo ternary phase diagrams of oleic acid - Transcutol P (10:1)/Tween 80 - Span 20/propylene glycol/water are presented in Figure 1. It seems that phase behavior depended on surfactant and cosurfactant mass ratios. The mass ratio of surfactant/cosurfactant is an important and critical parameter affecting phase behaviors of NE. The extent of NE zone increasing with increasing relative amount of surfactant was reported in the previous research.<sup>[19]</sup> The phase diagrams revealed that NE region extended with large amount in the weight ratio of surfactant/cosurfactant ( $km = 2-3$ ). Phase diagrams indicated more width NE region with a rise in S/C ratio.

### Characterization of the CXB NEs

Eight different NEs were selected from the pseudo-ternary phase diagram with 2:1, and 3:1 mass ratio of Tween 80 - Span 20/PG. The composition of selected NEs is shown in Table 2. The pH, mean particle size, polydispersity index, and viscosity of CXB NEs are shown in Table 3.

The NE samples in this study showed the average viscosity range (118-245 cps), pH value (6.5 to 6.9), and particle



**Table 3:** pH, viscosity, mean particle size, polydispersity index of selected celecoxib nanoemulsions (mean±SD,  $n=3$ )

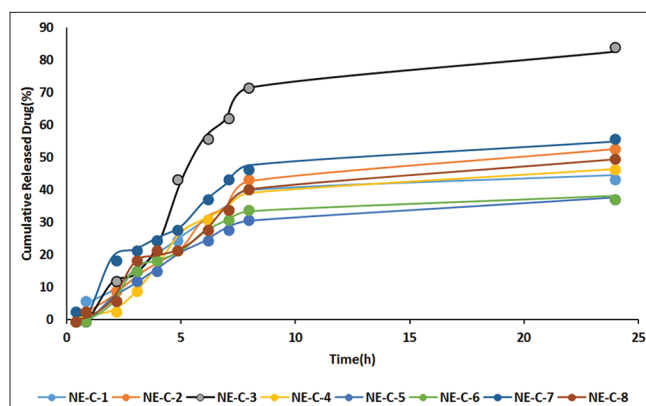
Formulation	pH	Viscosity (cps)	Mean droplet size (nm)	Polydispersity index
NEC-1	6.5±0.1	147±2.5	9.41±0.2	0.433±0.002
NEC-2	6.6±0.2	153±1.5	14.33±1	0.447±0.003
NEC-3	6.5±0.1	224±2	7.53±0.7	0.453±0.002
NEC-4	6.5±0.3	245±1.6	26.65±2.1	0.483±0.004
NEC-5	6.8±0.2	137±1.3	7.59±0.9	0.459±0.002
NEC-6	6.9±0.1	196±1.5	7.5±0.8	0.461±0.003
NEC-7	6.8±0.1	128±1.1	6.96±0.5	0.445±0.001
NEC-8	6.9±0.2	118±0.9	15.53±1	0.465±0.006

SD: Standard deviation, NEC: Nanoemulsion of celecoxib

size (6.96-26.65 nm) the correlation between particle size with independent variables is not statistically significant ( $P > 0.05$ ). ANOVA showed that correlation between pH and independent variable S/C is significant ( $P < 0.05$ ). It seems that the pH is increased with less percentage of S/C phase in some of nanoemulsions (NEs).

ANOVA represented that correlation between viscosity with independent variables (% oil) is significant ( $P < 0.05$ ). It seems that the viscosity is increased with less percentage of oil phase CXB NEs.

The results of NE formulations indicated the average viscosity range (118-245), pH value (6.5-6.9), and particle size (6.96-26.65 nm). ANOVA showed that correlation between mean particle sizes with independent variables is not significant ( $P > 0.05$ ). Particle size is one of the most important properties in nano-sized drug delivery systems. The decrease in particle size is connected with a great increase in surface area that would lead to enhanced bioavailability.<sup>[20]</sup> In the current study, the droplet size of all NE formulations was below 30 nm. The droplet sizes of the NEs prepared are far below the particle size of 10  $\mu\text{m}$  that could cause irritation.<sup>[20]</sup> The polydispersity value described the uniformity of the droplet size. All polydispersity values were smaller than 0.5. Therefore, these obtained results indicate that the droplet size narrow distribution in NE samples. ANOVA showed that correlation between pH and independent variable S/C is statistically significant ( $P < 0.05$ ). It seems that the pH is increased with less percentage of S/C phase in some of NEs. The finding is in consistent with the previous reports.<sup>[21]</sup> The pH value of all NE formulations was around 6.5. In the present study, viscosity ANOVA represented that correlation between viscosity with independent variables (% oil) is significant ( $P < 0.05$ ). It seems that the viscosity is increased with less percentage of oil phase CXB NEs. The findings are in agreement with the previous reports by other researchers.<sup>[22]</sup> Increased viscosity might help to improve the preocular retention time and thus the amount of the drug permeated through corneal. All of the NEs systems prepared in our study were more viscous in comparison to aqueous suspension.



**Figure 2:** *In vitro* release profile of nanoemulsions formulation of celecoxib

Figure 2 shows the release profiles of CXB NEs. Drug release profile showed that 82.6% of the drug released in the 24 h of an experiment for NEC-3. There was Higuchi kinetic for NEC-3. Drug released percentage and kinetic of release in selected NE formulations are displayed in Table 4.

ANOVA represented that correlation between drugs released in the 2 hours ( $R_{2h}$ ) with independent variables (% oil) is significant ( $P < 0.05$ ) in CXB formulations, so that, the  $R_{24h}$  is increased with more percentage. Furthermore, The correlation between drug  $R_{24h}$  with independent variables (% water) is significant ( $P < 0.05$ ), so that, the  $R_{24h}$  is increased with less percentage of water phase. In the current study, CXB NEs droplet size was obtained very small. It is known that small particle size contributes the quick release.

Figure 3 shows DSC cooling thermograms of CXB NEs. Cooling NEs transition temperature and enthalpy are provided in Table 5. DSC study was used for water behavior in NEs and distinction between bulk (free) and bound (interfacial) water.<sup>[23]</sup>

In cooling curves of the NE samples, bulk water (free water) and bound water are obtained in 0°C and -20 to -20.4°C, respectively. According to ANOVA results, a significant

**Table 4:** Percentage release and kinetic release of selected nanoemulsions (mean  $\pm$  SD,  $n=3$ )

Formulation	% Release (24 h)	Kinetic of release	R <sup>2</sup>	% Release (2 h)
NEC-1	44.5 $\pm$ 0.53	Higuchi	0.9153	8.98 $\pm$ 0.29
NEC-2	52.59 $\pm$ 1.72	Higuchi	0.9455	7.13 $\pm$ 1.09
NEC-3	82.60 $\pm$ 2.03	Higuchi	0.9193	11.49 $\pm$ 0.12
NEC-4	46.61 $\pm$ 2.62	Higuchi	0.9260	2.84 $\pm$ 0.94
NEC-5	37.62 $\pm$ 3.37	Higuchi	0.8800	6.73 $\pm$ 0.03
NEC-6	38.13 $\pm$ 3.92	Higuchi	0.8063	5.08 $\pm$ 1.49
NEC-7	54.89 $\pm$ 1.49	Higuchi	0.9232	19.12 $\pm$ 0.66
NEC-8	49.41 $\pm$ 1.33	Higuchi	0.9280	6.13 $\pm$ 1.39

NEC: Nanoemulsion of celecoxib

**Table 5:** Transition temperature and enthalpy of selected nanoemulsions (mean $\pm$ SD,  $n=3$ )

Formulation	TM <sub>2</sub> (°C)	$\Delta H_2$ (mJ/mg)
NEC-1	-20 $\pm$ 0.05	32.74 $\pm$ 0.15
NEC-2	-20 $\pm$ 0.06	36.08 $\pm$ 0.04
NEC-3	-20.4 $\pm$ 0.1	0.243 $\pm$ 0.7
NEC-4	-20.2 $\pm$ 0.02	0.345 $\pm$ 0.01
NEC-5	-20 $\pm$ 0.4	0.749 $\pm$ 0.01
NEC-6	-20.4 $\pm$ 0.03	0.148 $\pm$ 0.005
NEC-7	-20 $\pm$ 0.04	7.93 $\pm$ 0.01
NEC-8	-20 $\pm$ 0.03	15.66 $\pm$ 0.14

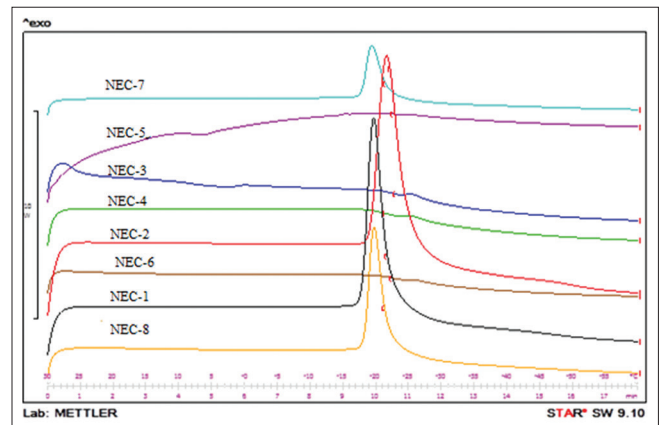
NEC: Nanoemulsion of celecoxib

correlation ( $P < 0.05$ ) was found between the bound melting transition temperature (TM<sub>2</sub>) and independent variables, so that any decrease in oil amount and significantly increased the temperatures. In addition, the independent variables in affected enthalpy of exothermic peak of bound water ( $P < 0.05$ ), for example, the enthalpy was increased due to increase in oil, and S/C ratio. Our findings are agreement with the previous reports by Podlogar *et al.*<sup>[24]</sup> Similar behavior was found by Podlogar *et al.*, So that, free water and bound water are obtained in -8 to 0°C and -17 to -26°C, respectively.

The permeability parameters of selective NEs are indicated in Table 6. The amount of CXB that had permeated through the rabbit cornea membrane per the area of ocular cells (mg/cm<sup>2</sup>) was plotted as a function of time (hours).

In permeability studies, the correlation between Papp with independent variables was not significant ( $P > 0.05$ ). Hence, the Jss of CXB from NEC-5 was 0.65  $\pm$  0.001 mg cm<sup>-2</sup> h<sup>-1</sup>, 21.68 times higher than those of control (CXB suspension, 1%). The correlation between Jss with independent variables (% oil) is significant ( $P < 0.05$ ). So that, any decrease in oil phase percentage significantly increased the Jss parameters.

The correlation between Tlag with independent variables (% water) is significant so that, any decrease in water phase percentage a significantly increased the Tlag parameters. The

**Figure 3:** Differential scanning calorimetry cooling thermograms of celecoxib nanoemulsions

correlation between apparent diffusivity coefficients ( $D_{app}$ ) with independent variables was not significant ( $P > 0.05$ ).  $D_{app}$  and  $P_{app}$  parameters in NEC-3 and NEC-5 formulation were 0.0233 cm<sup>2</sup> h<sup>-1</sup>, 0.13 cm/h, and 46.62, 7.23 times higher than those of control (CXB suspension, 1%), respectively. The correlation between ER<sub>flux</sub> with independent variables (% oil and s/c ratio) is significant ( $P < 0.05$ ). So that, any decrease in oil phase percentage and S/C ratio significantly increased the ER<sub>flux</sub> parameter. The correlation between ERp and ERD with independent variables was not significant ( $P > 0.05$ ).

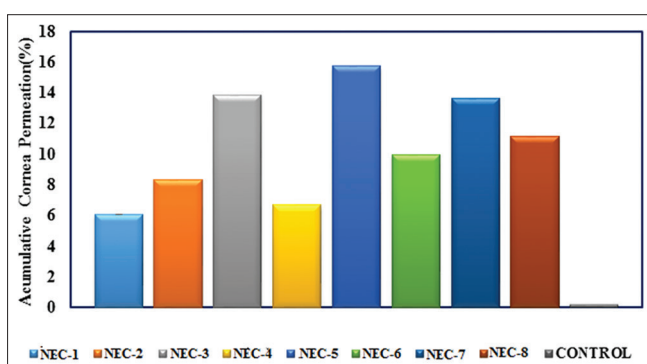
Figure 4 shows the accumulated permeated percentage in the 5 hours (%P<sub>5h</sub>) of CXB through rabbit cornea from different NEs. The correlation between drug permeated percentage in the 2 hours (%P<sub>2h</sub>) and %P<sub>5h</sub> with independent variable (% oil) is statistically significant ( $P < 0.05$ ), therefore, the %P<sub>2h</sub> and %P<sub>5h</sub> are increased with any decrease in oil percentage phase. The minimum and maximum of %P<sub>5h</sub> are obtained in NEC-1 (6.1%) and NEC-5 (15.73%), respectively.

The results show that all NEs increased drug flux through skin more than they did diffusion. The results show that all NEs increased drug flux through Rabbit cornea more than they did diffusion. All NEs formulations with different compositions and properties significantly increased partitioning, flux and permeability coefficient from Rabbit cornea. The staining

**Table 6:** *Ex vivo* permeability parameters of celecoxib NE formulations through rabbit cornea ( $n=3$ , mean $\pm$ SD)

Formulation No.	$J_{ss}$ , mg/cm <sup>2</sup> h	$T_{Lag}$ , h	$D_{app}$ , cm <sup>2</sup> /h	$P_{app}$ (cm/h)	$ER_{flux}$	$ER_D$	$ER_P$
Control	0.03 $\pm$ 0.001	3.4 $\pm$ 0.1	0.0005 $\pm$ 0.0001	0.018 $\pm$ 0.02	-	-	-
NEC-1	0.253 $\pm$ 0.034	0.5 $\pm$ 0.01	0.0034 $\pm$ 0.00008	0.05 $\pm$ 0.006	8.41 $\pm$ 1.14	6.72 $\pm$ 0.153	2.8 $\pm$ 0.39
NEC-2	0.139 $\pm$ 0.004	1.13 $\pm$ 0.080	0.0015 $\pm$ 0.0001	0.027 $\pm$ 0.007	4.64 $\pm$ 1.21	2.95 $\pm$ 0.21	1.54 $\pm$ 0.04
NEC-3	0.332 $\pm$ 0.008	0.14 $\pm$ 0.001	0.0233 $\pm$ 0.003	0.066 $\pm$ 0.001	11.05 $\pm$ 0.001	46.62 $\pm$ 0.11	3.68 $\pm$ 0.09
NEC-4	0.334 $\pm$ 0.003	2.64 $\pm$ 0.05	0.006 $\pm$ 0.00001	0.066 $\pm$ 0.006	11.12 $\pm$ 0.11	1.261 $\pm$ 0.02	3.7 $\pm$ 0.04
NEC-5	0.65 $\pm$ 0.001	1.04 $\pm$ 0.01	0.0016 $\pm$ 0.00002	0.130 $\pm$ 0.0001	21.68 $\pm$ 0.016	3.199 $\pm$ 0.033	7.23 $\pm$ 0.005
NEC-6	0.337 $\pm$ 0.122	1.29 $\pm$ 0.81	0.0022 $\pm$ 0.0001	0.072 $\pm$ 0.003	11.24 $\pm$ 4.09	4.42 $\pm$ 1.37	4.02 $\pm$ 1.31
NEC-7	0.329 $\pm$ 0.012	1.03 $\pm$ 0.006	0.0016 $\pm$ 0.00001	0.066 $\pm$ 0.002	10.96 $\pm$ 0.41	3.22 $\pm$ 0.020	3.65 $\pm$ 0.14
NEC-8	0.315 $\pm$ 0.08	1.008 $\pm$ 0.001	0.0016 $\pm$ 0.0000002	0.063 $\pm$ 0.01	10.49 $\pm$ 2.78	3.30 $\pm$ 0.0005	3.49 $\pm$ 0.92

NE: Nanoemulsion, SD: Standard deviation, NEC: Nanoemulsion of celecoxib



**Figure 4:** Celecoxib permeation percent from various nanoemulsions formulations after 5 hours (mean $\pm$ standard deviation,  $n=3$ )

test of CXB NEs is established o/w NE structures. In previous studies, It was demonstrated that the o/w NEs may be advantageous because the presence of a surfactant and cosurfactant compositions increases barrier permeability.<sup>[5]</sup> In this research, CXB NE formulations could be act as permeation enhancers and to improve corneal drug delivery. Our findings are agreement with the previous reports by Naveh *et al.*<sup>[25]</sup> They show increase the corneal absorption of pilocarpine by an especial o/w NE system. The rabbit cornea model was used for drug delivery studies due to its similarity to human corneas.<sup>[26]</sup>

## CONCLUSION

In NEs, the total amount of surfactant and cosurfactant (s+c), water and oil have deterministic effects on physicochemical characteristics, *in vitro* release and permeability of CXB through rabbit cornea.

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