ANTI-ANALGESIC FORMULATION AND CHARACTERIZATION
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ABSTRACT: Analgesics refers to group of medication preferably utilized for relieving pain and inflammation. Diclofenac sodium is a non-steroidal anti-inflammatory drug that is widely involved in pain reduction. The present paper aims to develop a topical nanoemulsion in combination with gel i.e. nanoemulgel formulation with potent anti-analgesic activity, wherein eucalyptus oil was mixed with diclofenac sodium along with tween-20 and butyl glycerol to develop an emulsion. The emulsion was then converted to gel by using carbomer as gelling agent. Finally, the nanoemulsion gel was subjected to characterization studies which include pH, viscosity, excrudability, spreadability and in vitro analysis. Skin irritation test was also performed for the formulation. The pH was found to be 5.49 ± 0.15 and viscosity was 2.20 ± 0.06 which is appropriate for the formulation. Also spreadability, excrudability co-efficients were 14.05 and 17.43 gm/cm² respectively. There was no irritation or edema on application of the formulation on the skin.

KEYWORDS: Anti-analgesic, Diclofenac sodium, Eucalyptus, Nanoemulsion gel

INTRODUCTION
Non-steroidal anti-inflammatory drug (NSAIDs) are widely utilized drugs for reducing torment and inflammation[1]. They are specifically addressed as COX (cyclooxygenase) inhibitors that synthesize prostaglandins for mediating processes of torment and inflammation[1][2]. Diclofenac sodium is most acceptable drug that is used worldwide for treating pain and inflammation. Oral administration of drug may cause some harmful ailments like gastrointestinal problems, vomiting and alike[2]. Thus, to overcome such problems, there is a need for topical application that may be administered over pain affected areas and thus aid in pain reduction. Due to such reasons, topical administrations are considered to be suitable for drug delivery.

Topical application refers to application of drug on the skin with enhanced efficacy to deliver drug within skin through sebaceous glands for managing torment and inflammation. The benefit of topical application is to inhibit first-pass liver oxidation, which increases biological efficiency[3]. Nonetheless, there are certain difficulties involved with product ingestion and permeability when using this pathway. The skin cell membrane is the principal blocker in topical delivery of diclofenac sodium penetration within skin[1][4]. Diclofenac is excellent selection of topical non-steroidal anti-inflammatory drug centred for treating 1.8% 9.8% acute and chronic ailment respectively according to a report in Cochrane meta-analysis[5][6][7]. Many forms of diclofenac are available in the market such as tablet form, suspension form, solution form alike, but there are reports that they still lack in permeability of the active drug within the skin.

Many techniques were widely adopted to enhance drug permeation within the skin which may include but not limited to iontophoresis, microneedle technology, electroporation and alike but such techniques were observed to be not affordable[1][3]. Certain synthetic enhancers were used for enhancing the permeability of drugs but resulted in dermis irritancy or edema[1][3]. Moreover, many permeation enhancers were found to be toxic[1]. Therefore, there is a need to develop a formulation that surges the drug delivery without causing any skin irritation and is affordable also.

A study has shown that nanoemulsion plays important role in transdermal drug delivery. Nanoemulsions are thermodynamically robust isotropic system, wherein two immiscible liquids are combined for developing single phase by utilizing emulsifying agents which may comprise surfactant and co-surfactant[8]. Higher the concentration of emulsifying agent higher will be permeability of drug within the skin. When nanoemulsions are combined with gel leads to formation of nanoemulgels[9][1]. They are regarded as potent carriers for providing transdermal drug permeation due to their great potency of loading hydrophobic drugs. For instance, permeation of eprinomectin (EPR) presents
a challenge for permeation within skin[10]. This may be attributable due to hydrophobicity of said EPR. Thus, to enhance the transdermal delivery of drug within skin the present paper is represented that focuses towards enhanced drug delivery within the skin. In the present paper diclofenac sodium is mixed with different oil acting as natural permeation enhancer i.e. eucalyptus oil, lavender oil and Capsaicin oil are used. Further analysis was done to determine the compatibility of diclofenac sodium with above mentioned oils in order to determine the highest permeability. Firstly, a nanoemulsion is prepared by combining diclofenac sodium, essential oil, surfactant, co-surfactant, and emulsifying agent and humectant. After the formation of nanoemulsion, it was combined with a gelling agent i.e. carbomer to produce a nanoemulgel.

REVIEW OF LITERATURE
Kantharao and colleagues developed an oral formulation i.e. Orodispersible Tablets of diclofenac sodium for managing pain and inflammation. The researchers involved two disintegrators preferably croscarmellose sodium (CCS) and sodium starch glycolate (SSG) at varying concentrations by applying direct compression technique. Further the solid dosage forms were evaluated for their efficacy with regards to oral delivery of drug in a rapid manner. The solid dosage forms were subjected to post compression test wherein it was found that the drugs were suitable for utilizing it on regular basis[11]. Guhmann and co-workers developed diclofenac taste-masked orodispersible tablets (ODTs) possessing rapid drug release features. Various taste-masking techniques and formulation conceptions were screened in vitro for suitable drug selection[12]. However, prolonged consumption of diclofenac directly may lead to certain adversities such as gastrointestinal ulcer, bleeding, kidney failure, poor efficacy and alike. Therefore there is a need to find an alternate way of delivering the drug at the targeted site. Although may improvements have been made for enhancing the drug delivery but still some of them lacks in terms of poor effectivity. Therefore there is a need to develop a formulation that easily penetrated within the skin and aids in enhanced drug delivery thereby curing the pain and inflammation in the people.

RESEARCH QUESTION
How to enhance drug delivery of diclofenac sodium for managing pain and inflammation?
How to reduce skin irritation?

METHODOLOGY
Design
The methods involved are: mixing oil and surfactant, co-surfactant in required amount and moved in vortexer to obtain a mixture. Different concentration of diclofenac was poured in the mixture followed by addition of distilled water to obtain a solution. Further the solution was subjected to characterization studies like size distribution, morphology and stability.

Sample
Diclofenac sodium procured from pharmaceutical industries, eucalyptus oil, Capsaicin oil and lavender oil were procured from vijay exporter, carbomer as gelling agent, tween 20 as surfactant, butylene glycol as co-surfactant.

Analyzing solubility of diclofenac sodium in different excipients
The first step in development of formulation was to check the solubility of diclofenac in various excipients such as oil, surfactant and co-surfactant. The steps comprise of: adding extra quantity of diclofenac sodium in 10 ml of vials followed by addition of 3 ml of each oil i.e. eucalyptus oil, Capsaicin oil and lavender oil in each vial. The vials were then subjected to vortexing to obtain a mixture. The mixture was then centrifuged at 1500 rpm for 5 min. to obtain a supernantant which was then filtered via filter paper. Finally drug content was assessed via UV spectrophotometer at 260 nm by diluting with ethanol[1].
Further surfactant was added to the vial. The surfactant used herein is tween 20 for determining drug solubility by analyzing through emulsification efficiency. Water titration was performed to determine emulsification potent, wherein 2µl of oil phase was added in a drop wise manner in 30% surfactant via vortex mixer until clear and transparent phase was obtained. The solution of oil mixed surfactant was then stored for 48 h under examination to analyze formation of turbidity within said sample. Co-surfactant i.e. butylene glycol was added with surfactant for lowering interfacial tension and surging interface fluidity for easing nanoemulsion preparation. Co-surfactant also aids in procuring nanoemulsion composition along with min. surfactant amount. This provides diclofenac sodium nanoemulsion.

Phase behavior study
Phase diagram was developed for studying different phases of utilized ingredients i.e. oil phase, water phase and surfactant phase. Phase diagram, was prepared by undertaking aqueous titration and analyzing stages behavior after simultaneous addition of water in drop wise manner within oil-surfactant-co-surfactant mixture. further, the sample was subjected to vortexing for 3-5 min physical appearance was observed to check presence of turbidity, liquid crystal phase and alike following which ternary plotting was done for identifying area of different phases[1].

Development of diclofenac sodium nanoemulsion
The methods involved are: mixing 1.5 ml of eucalyptus oil followed by addition of 1ml tween 20 and butylene glycol each and moved in vortexer to obtain a mixture. Different concentration of diclofenac was poured in the mixture followed by addition of distilled water to obtain a nanoemulsion. Further the nanoemulsion was subjected to characterization studies like size distribution, morphology and stability.

Stability analysis
Diclofenac sodium nano-emulsion prepared was further subjected to examination for stability analysis wherein, triple heating-cooling phases at 47°C and 4°C for 2 days were conducted and morphological change was observed which preferably includes color change, state separation and drug content precipitation. Further, optimized formulation was centrifuged at 2500 rpm for 25min. to procure state separation/ drug content precipitation[1]. Lastly, freeze thaw period was conducted at least seven times at -10°C- 30°C for one day under deep freezer at ambient temperature for analyzing any difference in morphology comprising state separation, drug content precipitation and color change[1].

Particle size along with zeta potential inspection
The above-mentioned analysis was conducted within SurPASS and litesizer (UK). This involves, diluting sample for 150 times with D.M. water and mixing thoroughly by analyzing at 30°C with wavelength of 644nm at 95° scattering angle[1].

Particles morphology
Particles size and morphology was examined via digital microscope (Chennai, India) by performing dilutions using distilled water[1].

Development of diclofenac sodium nanoemulsion gel
Gel formulation was prepared by undertaking following steps: 0.5% w/w of carbomer was dissolved in distilled water and stirred for 30 min followed by storing for at least 24 hour to obtain a homogenized swelled product. Then 4.5% glycerine was poured in the swelled product followed by adding triethanolamine in a drop wise manner to obtain a gel matrix. The pre-formed nanoemulsion was subsequently poured within the gel matrix to obtain diclofenac gel formulation (table 1). Further, characterization studies were conducted for prepared gel formulation which preferably comprises the following discussed below.

**Table 1: Ingredient names and compositions used for preparation of diclofenac sodium nanoemulsion gel formulation**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Amount</th>
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pH
pH of the formulation was measured via pH meter (Labman, India), wherein 3 gm of nanoemulsion gel was dissolved in 30 ml distilled water[1].

Viscosity
Viscosity of gel formulation was determined by utilizing viscometer at 27 ± 1.5°C[1].

Spreadability
Spreadability of formulated diclofenac gel was measured by a procedure, wherein the gel formulation was spread over a glass plate which was already premarked with an area covering 2cm with the help of marker. Then the next glass plate was kept over first plate and a gentle pressure is applied over it for at least 5 min in order to press the plated so that the gel formulation may spread easily. At last, spreaded area was calculated[1].

Extrudability
Extrudability has been calculated using a collapsible aluminum frame[1]. The proportion of fluid extruded from aluminum film. Gram weights were noted that must extrude at least a 0.4-cm gel strip in 15 seconds. Extrudability is related similarly to volume of extruded material.

Drug content analysis
Drug content analysis was performed by dissolving 1.5 gm of diclofenac sodium loaded nanoemulsion gel in 50ml of 70% v/v ethanol and vortexed for 15min to obtain a mixture. The mixture was then centrifuged at 4000 rpm for 10 min to obtain a supernatant. The supernatant was then filtered and filtrated obtained was diluted via phosphate buffer mobile phase followed by analysis under HPLC (Shimadzu, Japan) at 256nm with 6min. retention time.

In-vitro drug release analysis
Altered franz diffusion cells were utilized for measuring in-vitro drug release. 0.5 gm of Gel formulation was spreaded homogeneously over dialysis membrane and 25 ml of phosphate buffers was added in it to obtain a dissolution media. The media obtain was then stirred for 20 min at ambient temperature. After that it was replaced with fresh media to obtain prepared samples. Spectrophotometer analysis was adopted to measure percentage drug release[1].

**RESULTS AND DISCUSSIONS**

Phase behavior study
Phase activity of Diclofenac loaded emulgel (emulsion+gel) was conducted by constructing pseudoternary phase diagram with utilization of aqueous titration method. Within ternary phase model, high liquid crystals zones and a comparatively lower nanoemulsification field were found. The ternary structure step model comprises Liquid crystal region close to apex of surfactants and nanoemulsion towards edge of aqueous plants. Also, the comparison of butylene glycol with Tween 20 turned the crystals field into rapidly flowing nanoemulsion zone in phase transition. The hydrophobic region of surfactants is presumably attributed to improved intrusion of oil process. Surfactant-co-surfactant ratio (4:2) has major impact on diclofenac loaded emulgel system. It was observed that rise in cosurfactant concentrations in relation to surfactants resulted increase in surface region of nanoemulsifying and also enhanced spontaneity was observed in nanoemulsified product. This may be attributable due to significant surge in oil amount solubilization utilized in emulgel formation. Phase study also confirmed that tween 20 along with

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<tr>
<td>1.</td>
<td>Diclofenac Sodium</td>
<td>1.5gm</td>
</tr>
<tr>
<td>2.</td>
<td>Eucalyptus oil</td>
<td>7.55 ml</td>
</tr>
<tr>
<td>3.</td>
<td>Isopropyl alcohol</td>
<td>5.5 ml</td>
</tr>
<tr>
<td>4.</td>
<td>Tween 20</td>
<td>32.23 ml</td>
</tr>
<tr>
<td>5.</td>
<td>Butylene glycol</td>
<td>15.15 ml</td>
</tr>
<tr>
<td>6.</td>
<td>Glycerin</td>
<td>4.5 ml</td>
</tr>
<tr>
<td>7.</td>
<td>Distilled water</td>
<td>q.s. to 150 ml</td>
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butylene glycol was appropriate for preparing diclofenac loaded nanoemulsion in presence of oil phase i.e. eucalyptus oil along with isopropyl alcohol in ratio of 4:2. (Figure 1).

Figure 1: Pseudoternary phase diagram of diclofenac loaded emulgel formulation to identify effect of components on formulation for in-vitro performance. $S_{\text{mix}}$ denotes ratio of surfactant and co-surfactant (4:2).

Analyzing solubility of diclofenac sodium in different excipients

Diclofenac was dissolved in different concentration of oil phases and surfactant and co-surfactant as shown in table 1. The solubility of diclofenac in different oil, cosurfactant and surfactant is represented in figure 2. It was observed that, diclofenac was highly soluble in eucalyptus oil i.e. 7.55 mg/ml as compared to Capsaicin oil and Lavender oil with 5.55 mg/ml and 4.45 mg/ml respectively. Similarly, solubility of diclofenac in tween 20 and butylene alcohol is 32.23mg/ml and 15.15 mg/ml

Table 2: Ingredients used for diclofenac emulgel formulation

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<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Amount</th>
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<tbody>
<tr>
<td>1.</td>
<td>Diclofenac Sodium</td>
<td>1.5gm</td>
</tr>
<tr>
<td>2.</td>
<td>Capsaicin oil</td>
<td>10.0 ml</td>
</tr>
<tr>
<td>3.</td>
<td>Isopropyl alcohol</td>
<td>5.5 ml</td>
</tr>
<tr>
<td>4.</td>
<td>Tween 20</td>
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</tr>
<tr>
<td>8.</td>
<td>Distilled water</td>
<td>q.s. to 150 ml</td>
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Figure 2: Represents solubility of diclofenac in different oils. From the graph it can be inferred that diclofenac was highly soluble in eucalyptus oil.

Stability analysis
Thermodynamic stability refers to a system within its lowest energy phase or in chemical equilibrium with its surrounding environment. Through stability analysis, existence of metastable formulations are elucidated. Stability analysis of diclofenac sodium nanoemulsion lead to an observation that, no precipitation, stage separation and color change was observed in nanoemulsion formulation. Also coalescence formation and cracking was also not observed in nanoemulsion during stress stability analysis.

Particle size along with zeta potential inspection
Mean particle size of diclofenac sodium nanoemulsion was noted to lie in the range of 45.07 ± 65.08 with polydispersity index falling in the range of 0.140 ± 0.34. From the results obtained it was clear that the particles were of uniform size. Emulsion stability was also accessed from magnitude of surface charge. At large electrical repulsion force, formation of coalescence was observed. State separation was achieved by decrease in electrostatic repulsion.

Zeta potential analysis of diclofenac sodium nano-emulsion values were found to lie in the range of -35.05 ± 1.34 mV indicating that emulsion possess negative charge, which may be due to existence of anionic group in fatty acid and glycols presence within oils, surfactants or co-surfactants. Particle size of diclofenac sodium nano-emulsion gel were observed to be spherical and less than 150 nm under microscope.

Drug content analysis
Amount of drug content of diclofenac sodium within formulation was analyzed with UV spectrophotometer at 255 nm. In the streamlined model, the amount of Diclofenac sodium material was 99.96 ± 0.53%.

Characterization of diclofenac sodium loaded nanoemulsion gel
pH
pH analysis was carried out to check compatibility of formulation with skin. pH of nanoemulsion gel formulation observed herein includes, 5.49 ± 0.1 5 which is a compatible pH for skin.

Viscosity
Viscosity of nano-emulsion formulation was done to determine the quality of the drug formulation wherein, viscosity was observed to be 2.20 ± 0.06 Pa.s at sheer stress of 45 Pa and also sheer rate of 50 s⁻¹.

Spreadability


Spreadability of all the formulations was conducted wherein it was found that diclofenac in combination with eucalyptus oil possessed highest spreadability co-efficient of about 14.05, while for other formulations spreadability co-efficient lies in the range of 9.45-13.25 gm.cm/sec respectively (Figure 3). The increased spreadability may be due to high viscosity of the diclofenac emulgel formulation. When the pressure was applied over glass plates it was observed that the formulation spreaded evenly crossing the 2 cm area marked on the glass plate.

Figure 3: Graphical representation of spreadability of diclofenac formulation in combination with essential oils. Spreadability refers to measuring the spread of formulation over the skin. The present graph, combination of diclofenac and eucalyptus howed highest spreadability (spreadability co-efficient=14.05).

Extrudability
On conducting extrudability of all the formulations it was observed that highest extrudability was found to be of diclofenac sodium containing eucalyptus oil i.e. 17.43 gm/cm². Increased extrudability may be due to high viscosity.

In vitro release
In-vitro analysis was done via Franz-diffusion cell utilizing PVDF membrane with pore size of 0.23 μm on diclofenac sodium nanoemulsion gel, wherein a comparison was made between a known formulation and newly synthesized formulation. The release profile was checked after a period of 8 hrs and results were presented by a graph plotted between cumulative drug release percentage and time. It was observed that, drug release profile of known formulation was 928.67±20.35 and newly prepared nanoemulsion formulation was 1847.94±38.35. This may be attributable to low concentration of carbomer utilized as gelling agent. Lower the concentration of gelling agent greater is the drug release.

The efficacy of transdermal drug delivery was thus enhanced by combining diclofenac with eucalyptus oil as it act as penetration enhancer for the skin. Presence of 1,8 cineole, in eucalyptus which is particularly a monospecific terpene aids in is able for enhancing penetration of both hydrophilic and lipophilic drugs[13]. This was proved from the spreadability, extrudability, test wherein spreadability co-efficient was found to be 14.05 and extrudability co-efficient was found to be 17.43. Also in-vitro drug release was also found to be 928.67±20.35 i.e. From this it can be inferred that combination of eucalyptus with diclofenac was found to be effective for transdermal drug delivery thereby reducing pain and inflammation in concerned person. In comparison to oral administration of pain killers, transdermal delivery is more advantageous as it does not cause
systemic side effects because it is applied only on the affected portion of the skin. Moreover, their mode of action is very fast. The topical formulation is also advantageous because they can be combined with any formulation and their mode of enhancement can be increased.

CONCLUSION

The present study deals with development of carbomer centered nanoemulgel wherein diclofenac was mixed with eucalyptus along other excipients which preferably includes tween 20, butylene glycol, glycerine to formulate a nanoemulgel. The characterization studies conducted such as Ph, viscosity, spreadability, extrudability and drug content was found to be satisfactory. Also there were no negative effect on skin after conducting dermal analysis. Also in vitro release, excrudability, spreadability was also seen to be enhanced. Therefore, diclofenac nanoemulgel was found to be potential to treat pain and inflammation.

REFERENCES