ENHANCED ANTI-ANALGESIC MORPHINE BASED GEL: FORMULATION, CHARACTERIZATION, IN-VITRO AND IN-VIVO EVALUATION
Gunjan, Vijender Singh

Department of Pharmacy, Sharda University, Greater Noida, Uttar Pradesh

ABSTRACT: Non-steroidal anti-inflammatory drugs (NSAID) are used for relieving pain, and inflammation. Meloxicam is known for its efficacy to relieve pain, inflammation, and joints stiffness but still lacks in transdermal delivery of drug. In this paper, an attempt has been taken to develop meloxicacamemulgel formulation to enhance the delivery of drug within dermis. The active drug was mixed with various excipients and studies were undertaken to develop the formulation with enhanced topical application. It was observed that from all the excipients that were examined, linolenic acid, Azone®, propylene glycol and starch was found to be suitable for gel formulation. The formulated gel were then subjected to characterization studies, wherein drug content, globule size, stability test, in-vitro-release analysis, spreadability test, extrudability test, morphology test, dermis irritation studies, and stability studies were conducted. After analysis of all the test it was observed that, the meloxicacamemulgel formulation was proved to be potent analgesic for treatment of joint related issues.

KEYWORDS: Emulgel, Linoleic Acid, Melixocam, Topical Application.

INTRODUCTION
Meloxicam drug is used in diagnosis of arthritis related disorders with an analgesics and anti-inflammatory benefit. Meloxicam is a selective regulator of COX-2 type[1]. Meloxicam is moderately porous 7.12 mg / l soluble ingredient with 99.5 percent protein binding, which has gastrointestinal side consequences such as gastrical vomiting, ulceration with stomach perforation, both small and large intestines when provided orally[2].Topical distribution may be described as medication application over skin curing dermal problems like general disease with purpose of confining product to skin or dermal surface. The most commonly used semi-solids are wax, creams, salts etc.[3] According to britishpharmacophobeia gel formulation is defined as semi-solid solution composed of solubility of broad organic compounds or tiny inorganic elements that are closed and inter-dispersed by air. Gel formulation includes greater volume of aqueous or hydroalcoholic material[4][5]. Given several benefits of gels, availability of hydrophobic medications is greatly limited. In such sense emulsion-centered method is utilized to obtain special features of gels for hydrophobic clinical action[6]. Combination of gel and emulsions is known as emulgel[1]. In aqueous phase existence of gelling agents transforms the contemporary emulsion to emulgel. Emulsions are biphatic structures, wherein one immiscible substance is released onto another as mixture becomes fragile and balanced with an introduction of an emulsifying substance[1], [5]. The Emulgels are hydrogel comprising uniformly scattered oil micro-droplets. The gel forms a cross-related network wherein small drug particles are collected[7], [8]. Microemulsion used herein are transparent, consistent and stable solution that is further stabilized by adding surfactant along with co-surfactants. Many analgesic formulations are available in the market that are used to treat joint pains but mostly suffer from enhanced treatment and poor dispersity within skin. Thus there is a need to develope a formulation that is effective in treating joint pains.

REVIEW OF LITERATURE
Alex Bekker and collegues presented a review about pharmacological properties, comprising pharmacokinetics, negative impacts and resistance, of meloxicam are summarized. Analysis was done about a range of clinical studies recently conducted to access the effectiveness and protection of meloxicam in the pain management[9].
QinYu and co-workers produces a composition of nanocrystal for boosting transdermal melixocam (MLX) production. Nanoprecipitation technology center on acidic-basic neutralization was effectively prepared for MLX nanocrystals. MLX nanocrystals along particle sizes 175 nm were procured using the poloxamer 409 and Tween as mixed as stabilizers. Both differential scanning calorimetry and X-ray powder diffractometry verified the crystalline structure for MLX nanocrystals[10]. Conventionally many formulations are available in the market for treatment of joint pains but still lack in permeability within the skin and enhanced targeted delivery of the drug. Moreover many efforts have been made to improve the transdermal delivery of the drug but still some of them has lead to decreased effectivity. Thus, to overcome such disadvantages, there is a need to develop an effective formulation with increased penetrability, efficacy, bioavailability and economical safe and affordable.

**RESEARCH QUESTION**
How to enhance efficacy of meloxicam in pain management?

**METHODOLOGY**

**Design:**
The microemulsion formulation was prepared by aqueous titration with linioleic acid used as oil phase, Azone® and Polyethylene glycol as surfactant and co-surfactant respectively. The concentrations were decided centered on pseudoternary phase diagram. Further optimized emulsion was embedded within gel matrix i.e. carbopol ETD 2020 NF and corn starch was added as a binder

**Sample:**
Morphine was procured from a drug supplier industry, linoleic acid was procured from Makewell pharmaceutical Co-Ltd, Azone® and Propylene glycol was procured from drug supplier unit used as surfactant and co-surfactant respectively, corn starch was also procured from drug supplier and used as binder. Analytical grade chemicals were used.

**Instruments:**
Vials, centrifuge, spectrophotometer, magnetic stirrer, particles size analyzer, zeta-potentiometer, pH meter.

Solubility within oils, surfactant and co-surfactant

Adding large amount of drug in 20 ml vials comprising 20ml oil which may include but not limited to Castor oil, Aura cassia, thyme thymol, linoleic acid, surfactants which may comprise but not restrict to Azone®, tween 120, span, tween 80, co-surfactant which may comprises propylene glycol, cremophorEL, tween 20 respectively followed by shaking on mechanical shaker for 48 hours to obtain a solution. The solution was centrifuged for 15 min at 3000 rpm and then supernatant formed was filtered followed by noting UV absorbance at 340 nm by appropriately diluting with ethanol[1], [5].

Pseudoternary phase diagram build-up

Chemix school and 4.12 software was used for developing pseudoternary phase illustration. Aqueous Titration methodology was utilized for studying and determining the component concentration range. Three phases diagram was made with 0.5:1.5, 2.5:5, 3.5: 1.5 ratio of azone to propylene glycol respectively. Oil, surfactant and co-surfactant mixture, binder was prepared at varying weight ratios and diluted with water in a drop-wise manner to obtain a mixture solution. The solution was further vortexed by a vortex equipment after being equilibrated and the mixture was finally assessed by naked eyes and were distinguished between micro emulsion, crude emulsion or a gel[1], [5].

Emulsion preparation
Emulsion preparation was done by dissolving meloxicam in the mixture of oil, surfactant and co-surfactant at differing concentrations (Table 1) followed by adding required amount of water to the mixture in a drop wise manner to obtain a solution. The solution was stirred continuously at 37 °C, to obtain a clear phase.

**Table 1: Meloxicam formulation chart**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulation code</th>
<th>S:Cos ratio ($S_{mix}$)</th>
<th>Oil:S ratio</th>
<th>Drug amount incorporated (mg)</th>
<th>Theoretical drug content (mg)</th>
<th>Total volume of mixture (ml)</th>
<th>Amount of water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>0.5:1:5</td>
<td>1:7</td>
<td>150</td>
<td>200.25</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>0.5:1:5</td>
<td>2:6</td>
<td>150</td>
<td>195.34</td>
<td>20</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>A3</td>
<td>0.5:1:5</td>
<td>3:5</td>
<td>150</td>
<td>180.56</td>
<td>20</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>B1</td>
<td>1.5:1:5</td>
<td>1:7</td>
<td>150</td>
<td>199.32</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>B2</td>
<td>1.5:1:5</td>
<td>2:6</td>
<td>150</td>
<td>201.34</td>
<td>20</td>
<td>2.9</td>
</tr>
<tr>
<td>6</td>
<td>B3</td>
<td>1.5:1:5</td>
<td>3:5</td>
<td>150</td>
<td>239.43</td>
<td>20</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>C1</td>
<td>2.5:1:5</td>
<td>1:7</td>
<td>150</td>
<td>167.43</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>C2</td>
<td>2.5:1:5</td>
<td>2:6</td>
<td>150</td>
<td>156.45</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>C3</td>
<td>2.5:1:5</td>
<td>3:5</td>
<td>150</td>
<td>161.50</td>
<td>20</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The meloxicam formulation was then subjected to characterization studies to determine the efficacy of the formulation. Characterization studies involve:
- Determination of drug content
- Emulsion (1.5 ml) was drawn in graduated cylinder followed by volume making. After that dilutions were made with ethanol and absorbance was measured at 340 nm. The concentration of drug within emulsion was measured against a blank.
- Globule size measurement
- Globule size was determined by particle size analyser (Sigma Aldrich), wherein 2 ml of emulsion was diluted to 300 ml with ethanol and readings were procured.
- Zeta potential
- Zeta potential of all prepared formulations were procured via utilizing Zeta potentiometer (Microtrac) [1], [5].

**Emulgel formulation**

1% w/w of Carbopol ETD 2020 NF and 1.5% w/w of corn starch were mixed in distilled water followed by adding 0.6% of pre-formulated emulsion over gel base. Triethanolamine was added to adjust pH glyceryl triacetate, sorbate was incorporated as preservative [1], [5]. Table 2 describes emulgel formulation at different concentration.

**Table 2: Meloxicam emulgel formulation**

<table>
<thead>
<tr>
<th>Ingredient(s)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Sample 7</th>
<th>Sample 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam emulsion</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Carbopol</td>
<td>1.5%</td>
<td>1.5%</td>
<td>2.0%</td>
<td>2.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceryl triacetate</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>
Characterization of gel formulation

Physical characterization

The physical characterization involves: color, homogeneity, consist nature.

Drug Amount

Drug content was determined by weighing 1.5 gm emulgel followed by dissolving in 100 ml ethanol enhanced sonicated for 1 hour to ally the obtain a solution. Further the solution was passed via filter paper to obtain a filtrate. Finally the absorbance was measured via spectrophotometer at 340 nm against emulgel formulation. Drug content was determined by a linear regression wherein slope and intercept was used at standard calibration. Measurements were conducted out in triplets[1].

pH

pH was analyzed by utilizing a pH meter. 1.5 gm emulgel dissolved within 150 ml distilled water and kept for 1.5 hrs[1].

Viscosity

Brookfield Rheometer utilized for measuring viscosity of the prepared formulation, wherein formulation was moved at 3 rpm and subsequently readings were noted to determine viscosity of the sample. Measurements were conducted in triplets[1].

Spreadability

Spreadability is the measure of spread of formulation when applied on skin. to measure this, two slides were procured of 7 cm each. Out of this, one slide was fixed and 1gm formulation was poured over it. After that, next slide was kept over the top of first slide for 4-5 min. then the second slide was removed after 4-5 min. the time for removing the slides was noted. Minimum the time required to separate two slides gives better spreadibility of the formulation[1].

Extrudability

The extruding method is centered over weight determination needed to extrude 0.3 cm emulgel ribbon in 9 sec from collapse of lacquered aluminium film. The study was done in three separate models, estimating the mean values. The extrudability was then measured utilizing calculation below[1], [5].

\[
\text{Extrudability} = \frac{\text{weight applied to extrude emulgel from tube (gm)}}{\text{Area (cm}^2\text{)}}
\]

In vitro release

In vitro tests of formulation’s drug release profile were performed utilizing dialysis film in updated Franz Diffusion cells. Throughout dialysis surface, formulation (1.5 gm) has been spread equally. When Dissolution Media applied to Receptor sector, 30 ml of phosphate buffer pH 6.8 has been included. This whole arrangement was held on magnetic agitator and solution on sensor side was constantly stirring utilizing a magnetic perk, cell temperature were kept at ambient temperature. Further sample was taken off at appropriate intervals and substituted with same volume of fresh dissolving material. Samples were taken at appropriate intervals. At 360 nm, samples were tested and total percentage of drug release determined. Analysis was done in three variants. The figure displays total medication releases relative to time[5].

Stability studies

The prepared formulations was carried out to determine short-term stability test, wherein temperature and relative humidity levels were observed in the formulation for 3 months: At 1 m
period samples were collected and rheological features, product composition and percent CDR were measured[1].

RESULT AND DISCUSSIONS

Solubility within oils, surfactant and co-surfactant

Figure 1 and 2 represents solubility of active drug (meloxicam) in different oils, co-surfactant and co-surfactant. It was observed that meloxicam was highly soluble in linoleic acid i.e. 6.234 mg/ml and in Azone® and propylene glycol, Melixocam’s solubility was 30.112 mg/ml and 25.68 mg/ml respectively in comparison to other surfactant, co-surfactant and oils used. Thus linoleic acid, Azone® and propylene glycol were considered convenient for the gel formulation.

![Solubility Analysis](image1.png)

**Figure 1:** Representation of solubility of Meloxicam in different oils. It was observed that meloxicam was highly soluble in linoleic acid.

![Drug samples](image2.png)

**Figure 2:** Ingredients used in Melixocam emulgel formulations. The concentration (µg/ml) of the ingredients used in the preparation of the formulation is represented graphically.

Pseudoternary phase diagram build-up

Figure 3 represents pseudoternary phase diagram of microemulsion. It was observed that, for microemulsion area was highest for concentration of 3.5: 1.5 of surfactant: co-surfactant which may be attributable due to surfactant Azone® because increase in concentration of azone leads to increase in microemulsion area. Increased Azone® concentration also leads to increased water
requirement that ultimately leads to increased drug solubility. Therefore surfactant: co-surfactant ratio i.e. 3.5:1.5 was considered important.

![Surfactant: Co-surfactant](3.5:1.5)

**Figure 3: Pseudoternary phase diagram of surfactant:co-surfactant (3.5:1.5)**

Drug content
Drug content of gel formulation varied from 70% to 95%. From table 1, it was observed that, formulation B3 showed maximum drug content of approx. 95.21%. This may be due to greater drug solubility.

Globule size measurement
Table 4 represents globule size measurement of formulation. Decrease in globule size was observed with surge in surfactant amount. G1 represented lowest globule size of 15.34 nm and highest globule size was represented for H1 i.e. 130 nm that was further selected as required microemulsion formulation.

Zeta potential
Zeta potential was observed within the range of -30.6 to -56.67. It was observed that, formulations showed average to better stability.

Emulgel characterization

**Physical characterization**

**Color, homogeneity and consistency:** The drug formulations were found to be light yellow in color, were homogeneous and consistent. Drug percentage was found to lie in the range of 85.34%-96.34%. (table 3). The highest drug content was found in formulation F4 i.e. 96.46±3.45. pH of gel formulations prepared were observed to lie in the range of 6.34-7.2 that were ideal for skin and do not cause any irritation. No lumps were observed in formulations.

**Table 3: Characterization studies of emulgel formulations.** The table represents that, highest drug content (%), globule size and zeta potential was observed in formulation F4.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Appearance</th>
<th>Drug content (%)</th>
<th>Globule size (nm)</th>
<th>Zeta potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Light yellow</td>
<td>96.46±3.45</td>
<td>130</td>
<td>-56.67</td>
</tr>
<tr>
<td>F2</td>
<td>Light yellow</td>
<td>95.45±2.43</td>
<td>112.34</td>
<td>-45.34</td>
</tr>
<tr>
<td>F3</td>
<td>Light yellow</td>
<td>95.67±3.23</td>
<td>123.2</td>
<td>-53.21</td>
</tr>
<tr>
<td>F4</td>
<td>Light yellow</td>
<td>96.45±2.34</td>
<td>23.43</td>
<td>-37.32</td>
</tr>
<tr>
<td>F5</td>
<td>Light yellow</td>
<td>94.34±45</td>
<td>15.34</td>
<td>-30.6</td>
</tr>
</tbody>
</table>
Viscosity
Viscosity of all the formulations were analysed, wherein it was observed that lowest viscosity was in formulation F₁ and highest viscosity was observed in F₄ i.e. 385671 cPs. Therefore, formulation F₁ comprising linoleic acid, azone surfactant, propylene glycol as co-surfactant showed highest viscosity. This may be attributable to polymer concentration used in formulation preparation.

Spreadability
Figure 4 shows the spreadability of all the formulations. The spreadability was found to be highest in F₁ with spreading co-efficient of 13.45. The spreading co-efficient of other formulation lies in the range of 13.21-9.34 gm.cm/sec respectively. This may be due to viscosity and polymers concentration of formulation. i.e. great viscous formulation showed highest spreadability.

Extrudability
Figure 5 shows the extrudability of all the gel formulations prepared. It was observed that, highest extrudability was found in formulation 1 i.e. F₁ which is 16.43 gm/cm². Higher the viscosity, higher is the extrudability of the formulation.
Figure 5: Graphical representation of extrudability of formulations. Extrudability is measure of weight required to extrude amount 0.5 cm of emulgel within 10 sec. from crumbling aluminium foil.

Stability test
The drugs were found to be stable under stability chamber after 3 months also. There were no damage to the formulation.

In vitro release
Percentage drug release was observed for all the developed formulations. It was observed that, the highest drug release was in F1, i.e. 93.34%. After 8 hrs pure drug release was approx. 59.34%. This was due to higher polymer content within the formulation. Higher the polymer content, highest is the viscosity that ultimately leads to higher in vitro release.

The anti-analgesic formulation developed herein was found to showed spreadability coefficient of 13.45, which clearly indicates that the emulgel spreads evenly over the skin, extrudability coefficient of 16.43, which indicates that the drug can easily be extruded from the aluminium foil by applying very slight weight and also the drug was found to be stable after three months also without showing any change in appearance, color and also no damage was seen on the formulation. The percentage drug release was also found to be 93.34% which is sufficient enough for transdermal delivery to cure the joint pains. The developed formulation thus proves to be effective than the other known formulations in terms of enhanced permeability.

CONCLUSION
From the saturation solubility linoleic acid, Azone®, propylene glycol was selected to prepare emulsion. Aqueous titration method was used for preparation of emulsion. Various tests were conducted wherein globule size was found to be 130 nm which was considered suitable for microemulsion formulation. Average stability was observed in the formulation from the value of zeta potential and stability chamber studies. The morphology was also analysed wherein it was observed that, formulation was light yellow, homogeneous and was consistent. Thus, meloxicam gel was found to be effective in treatment of arthritis and pain management.

REFERENCES
