DEVELOPMENT OF LIPID BASED FORMULATION OF A POORLY WATER SOLUBLE DRUG

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ABSTRACT:
The present work was aimed to formulate a self-emulsifying drug delivery system of lornoxicam and evaluating its in-vitro potential. The solubility of lornoxicam was determined in different oils, surfactants and co-surfactants. Pseudo ternary phase diagrams were used to evaluate the micro-emulsification existence area, and the release rate of the drug was investigated using an in-vitro dissolution test. Formulations were prepared in by simple mixing at 40°C. The formulations were evaluated for parameters like macroscopic evaluation, visual assessment, self-emulsification, transmittance test, droplet size, zeta potential and particle size distribution. From the solubility study, comparatively better solubility was seen in Triacetin (oil), Tween 80 (surfactant) and Capryol micro express (co-surfactant). Droplet size ranged between 18 and 24 nm. Formulations were clear and almost near to 100% transmittance after dilution with water. More than 90% of the active was released in 20 min. as compared to pure drug.

Keywords: Lornoxicam, Ternary phase diagram, Self-microemulsifying drug delivery system, Droplet size, Zeta potential, SEDDS.

INTRODUCTION
In recent years, much attention has turned to lipid-based formulations with the aim of improving the oral bioavailability of poorly water soluble drugs. Lipid-based formulations encompass a diverse group of formulations, very different in physical appearance, ranging from a simple tri-glyceride vehicle to more sophisticated formulations. Lipid based drug delivery systems offer a wide variety of options. They can be made as solutions, emulsions, suspensions, microemulsions, solid lipid nanoparticles, liposomes, self-emulsifying drug delivery systems (SEDDS), or dry emulsions. Moreover, it is possible to form blends that are composed of several excipients: they can be pure triglyceride (TG) oils or blends of different TG, diglyceride (DG) and monoglyceride (MG) oils, or blends of different TG, DG and MG. In addition different types of surfactants (lipophilic and hydrophilic) can be added, as well as hydrophilic co-solvents. The type of lipid component of the delivery system has a great influence on its capability to enhance absorption. Non-digestible lipids, including mineral oils, sucrose polyesters and others, are not absorbed from the gut lumen. They remain in the gastrointestinal lumen, tend to retain the lipophilic drug within the oil, and thus, may limit the absorption of the drug. Digestive lipids, including triglycerides, diglycerides, phospholipids, fatty acids, cholesterol and other synthetic derivatives, are suitable oils for drug delivery systems of lipophilic compounds.
Challenges in development of lipid based formulations

One of the major hurdles in developing a lipid based formulation involves the selection of a suitable excipient. An ideal excipient should:

- Be safe, inert and available at a purity level suitable for human use.
- Not degrade during manufacturing or storage.
- Be capable of solubilizing the drug dose in a volume not exceeding that of an oral capsule.
- Preferably possess surface active properties to enable self-emulsification or complete dissolution of the drug dose.
- Reliably and reproducibly enhance the oral bioavailability of the drug relative to a conventional formulation.
- Be physically and chemically stable and compatible with a wide range of drugs and other excipients.
- Be non-hygroscopic and inert to the capsule shell or other packaging components.

Lornoxicam, the newest member of the oxicam class, is a nonsteroidal anti-inflammatory drug (NSAID). It interferes in the synthesis of prostaglandins from arachidonic acid by the inhibition of the cyclooxygenase isozymes. The existence of an acidic group in NSAIDs is important for pharmacodynamic reasons. Because the acidity of oxicams is derived from enolic group, not from carboxylate moiety like other NSAIDs, it does not increase the frequency and severity of unwanted effects. Lornoxicam is absorbed rapidly and almost completely from the gastrointestinal tract. The absolute bioavailability of lornoxicam is 90% to 100% and almost 99.7% strongly binds to serum albumin. Unlike other oxicams, it has relatively short half-life (3–5 hr). Lornoxicam undergoes extensive hepatic metabolism in humans, and as many other NSAIDs, the cytochrome P450 2C9 appears to play a major role in the metabolism of Lornoxicam. Moreover, because lornoxicam has potent anti-inflammatory and analgesic activities, low dose therapy is possible and this results in less risk of the side effects of classical NSAIDs.

The main objective of the investigation is to formulate, optimize and stabilize SMEDDS containing lornoxicam with suitable surfactants and co-surfactants. Lornoxicam, which is poorly soluble in gastric fluid through conventional dosage forms (tablet), SMEDDS are prepared to increase their solubility in gastric fluid and improve bioavailability by increasing gastrointestinal absorption through passive diffusion.

**MATERIALS AND METHOD**

Lornoxicam was procured as gift sample from M/s Amoli Organics Pvt., Captex 200, Capmul PG-8, Capmul MCM, Capmul MCM C8, Labrasol and Capryol micro express were gift samples from Abitec Corporation, U.S.A. Acrysol K140, Acrysol K150, Acrysol K135 were gifted by Corel Chemical Ltd, Ahmedabad. Labrasol, Capryol 90, Labrafel 2125 CS, Labrafac, Labrafac PG were gift from Gattefosse. Triacetin, Polyethylene glycol 400, Tween 80 and Tween 20 grade were purchased from S.D. Fine Chemical Ltd, Mumbai.

**Solubility study of lornoxicam in various excipients**

The solubility of lornoxicam in various components (oils, surfactants, and co-surfactants) was determined as follows: 500 mg of each of the selected vehicles was added to each cap vial containing an excess of lornoxicam. After sealing, the mixture was heated at 40°C in a water bath to facilitate the solubilisation. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25°C for 48 hours. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 5 minutes, and excess insoluble lornoxicam was discarded by filtration using a membrane filter (0.45 μm). The concentration of lornoxicam was then quantified by HPLC.

**Pseudoternary Phase Diagram**

Pseudo ternary phase diagrams of oil, surfactant/ co-surfactant (S/CoS), and water were developed using the water titration method. The mixtures of oil and S/CoS at certain weight ratios were diluted with water in a drop wise manner. For each phase diagram at a specific ratio of S/CoS (i.e., 1:0.5, 1:0.75, 1:1, 1:1.5, 1:2, 1:2.5 wt/wt) a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 minutes. Then each mixture was titrated with water and visually observed for phase clarity and flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio. To determine the effect of drug addition on the microemulsion boundary, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component. Phase diagrams were then constructed using sigma plot software. Self microemulsifying region could be obtained upon dilution and gentle agitation containing Triacetin as oil, Tween 80 as a surfactant and Capryol Micro Express as a co-surfactant.

**Fourier Transform-Infrared spectroscopy**

FT-IR spectroscopy was performed using FT-IR model Shimadzu 8400S, Japan. Lornoxicam drug and formulation was analyzed. A small amount of the
sample was directly placed on the disk and sample was scanned for absorbance over the range from 4000 to 4000 wave numbers (cm\(^{-1}\)) at a resolution of 1 cm\(^{1}\)

**PREPARATION OF SMEDDS FORMULATIONS**

A series of SMEDDS formulations were prepared using Twee 80 and Capryol Micro Express as the S/CoS combination and Triacetin as the oil. From the phase diagram study it was evident that 1:1 ratio of the Surfactant:Co-surfactant show highest microemulsion area. From this three different formulations (F1, F2 and F3) were prepared with 10%, 15% and 20% Oil and with 1:1 ratio of surfactant and co-surfactant were added. The components were mixed by gentle stirring until lornoxicam was perfectly dissolved. The mixture was stored at room temperature until further use\(^9\).

**Freeze Thawing**

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at -4°C for 24 hours followed by thawing at 40°C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies\(^9\).

**Self Emulsification time**

Different compositions were categorized on speed of emulsification, and clarity of the resultant emulsion. This was done by visual assessment that was performed by drop wise addition of the preconcentrate (SMEDDS) into 100, 250 and 1000 ml of distilled water, 0.1N HCl and pH 6.8 phosphate buffer. This was done in a glass beaker at room temperature, and the contents were gently stirred with glass rod\(^9\).

**Emulsion droplet size measurement**

Size analysis of microemulsion was carried out by dynamic light scattering with Zetasizer HSA 3000 (Malvern Instruments Ltd., Malvern, UK). Samples were placed in square glass cuvettes and droplet size analysis was carried out of optimized microemulsion formulation. Optimized microemulsion was diluted with excess (100 times) water and then droplet size of the system was also determined\(^10\).

**Refractive Index**

Refractive index proved the transparency of formulation. The refractive index of the system is measured by refractometer by placing drop of solution on slide and it compare with water (1.333). If refractive index of system is similar to the refractive index of water (1.333) and formulation have percent transmittance > 99 percent, then formulation have transparent nature.

**Zeta potential measurement**

Zeta potential for microemulsion was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd., UK). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment\(^11\).

**In-vitro drug release profile**

SMEDDS of lornoxicam (equivalent to 4 mg of lornoxicam) was filled in hard gelatin capsules. In-vitro release profiles of SMEDDS of lornoxicam, and pure lornoxicam powder were studied using United States Pharmacopeia (USP) XXIII apparatus I at 37±0.5°C with a rotating speed of 100 rpm in buffer pH 6.8 as the dissolution media. During the study, 5 ml of aliquots were removed at predetermined time intervals (5, 10, 20, 30, 40, 50 and 60 min) from the dissolution medium and replaced with fresh media. The amount of lornoxicam released was determined by HPLC method after filtration through 0.45μ membrane filter\(^12,13\).

**Stability Studies**

In order to evaluate the stability of the optimized SMEDDS the formulation was subjected to stability studies at 40°C ±2 °C /75%±5% RH. Samples were charged in stability chambers (Thermolab, Mumbai, India) with humidity and temperature control for a period of three months. At predetermined interval samples were evaluated for visual inspection, Emulsification time, PDI, Zeta potential, Droplet size, Drug content and in-vitro drug release\(^9\).

**RESULTS AND DISCUSSION**

**Solubility study of lornoxicam with various Excipients**

Solubility is major concern when formulating self emulsifying drug delivery system. Selection of right component is important prerequisite for formulation of stable SMEDDS. The drug should have good solubility in components of microemulsion so as the precipitation of drug during shelf life of formulation and after dilution in GI lumen can be avoided. Therefore, the solubility of lornoxicam was determined in various oils, surfactants and cosurfactant mixtures. Among the various components studied Triacetin was selected as oil, Tween 80 as surfactant and Capryol micro Express as co-surfactant for the further studies.

**Pseudo ternary Phase Diagrams**

Self microemulsifying systems form fine oil/water emulsions with only gentle agitation upon their introduction into aqueous media. Surfactant and
co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation. Therefore, the selection of oil and surfactants, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion.

A series of SMEDDS formulations were prepared (Fig 1) using Tween 80 and Capryol Micro Express as the S/CoS combination and Triacetin as the oil. From the phase diagram study it was evident that 1:1 ratio of the S:CoS show highest microemulsion area. From this study, three different formulations (F1, F2 and F3) were prepared with 10%, 15% and 20% oil and a 1:1 ratio of S:CoS in them. Amongst all the formulations, F3 shows maximum water uptake and highest microemulsion zone compared to the other formulations.

The FTIR spectrum of lornoxicam showed (Fig 2) a characteristic peak at 3066 cm$^{-1}$ and 2926 cm$^{-1}$ corresponding to $-\text{NH}$ stretching vibration. Intense absorption peak was found at 1645 cm$^{-1}$ due to the stretching vibration of the C=O group in the primary amide. Other peaks were observed at 1427 cm$^{-1}$ and 1452 cm$^{-1}$ and were assigned to bending vibrations of the N–H group in the secondary amide. The stretching vibrations of the O=S=O group appeared at 1311 cm$^{-1}$ and 1327 cm$^{-1}$. Other prominent peaks appeared at 831.34 cm$^{-1}$ corresponding to $-\text{CH}$ aromatic ring bending and hetero-aromatics and at 788.20 cm$^{-1}$ due to the C–Cl bending vibration. All these prominent peaks of lornoxicam were present in formulation spectrum as seen in Figure 3. It clearly indicates that the drug has retained its identity without losing its characteristics.
Freeze Thawing
The thermal cycling study created a thermodynamically unstable microemulsion, which had larger droplet size distribution upon dilution. Visual observation indicated that there was no phase separation in all three formulations and the physical appearance of all formulations was also similar. The overall stability of the formulation under normal conditions was found to be acceptable.

Self Emulsification
Emulsification time was assessed visually. Result was shown in Table 1. Emulsification time is most important parameter for SMEDDS and microemulsion formulation. A microemulsion that is formed within a 1 min. is said to be of Grade I. In case of all the batches F1, F2 and F3 Grade I type self emulsion was obtained.

Emulsion droplet size measurement
The stable formulations were subjected to size analysis by Zetasizer. It was concluded by size analysis study that initially, as the amount of surfactant increases, globule size decreases due to an increase in adsorption of surfactants around the oil water interphase of a droplets and a decrease in interfacial tension as shown in Table 1. After reaching a particular amount of surfactant, further increase in surfactant amount results in increased globule size. It could have occurred because excess adsorption of surfactant on the interphase resulted in retardation of efficiency of emulsification and more energy was required to produce an emulsion. In all the formulations droplet size ranges between 14-16 nm and PDI was also near to zero.

Zeta potential measurement
Zeta potential can be defined as the difference in potential between surface of the tightly bound layer (shear plane) and the electroneutral region of an emulsion. It has got practical application in the stability of emulsion since Zeta -potential governs the degree of repulsion between adjacent, similarly charged, dispersed droplets. If the Zeta -potential is reduced below a certain value (which depends on a particular system being used), the attractive forces exceed the repulsive forces, and the particles come together leading to flocculation. The zeta potential of the formulations was found between −13.45 to −18.17 mV as given in Table 1. In general, the zeta potential value of ±30 mV is desirable for the stability of a microemulsion. All formulations comply with the requirement of the zeta potential for stability.

Refractive index
Refractive index of all the three formulations were found near to 1.33 (Table 1) which showed transparency of formulations were good. So we can say that all the formulations have transparent in nature.

Table 1 Evaluation data of SMEDDS formulations

<table>
<thead>
<tr>
<th>Batches</th>
<th>Emulsification time (sec)</th>
<th>Droplet size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta potential (mV)</th>
<th>Refractive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (10%oil)</td>
<td>80</td>
<td>14.82</td>
<td>0.187</td>
<td>-13.45</td>
<td>1.3615±0.0002</td>
</tr>
<tr>
<td>F2 (15%oil)</td>
<td>80</td>
<td>14.73</td>
<td>0.236</td>
<td>-12.24</td>
<td>1.3585±0.0003</td>
</tr>
<tr>
<td>F3 (20%oil)</td>
<td>25</td>
<td>15.26</td>
<td>0.176</td>
<td>-18.17</td>
<td>1.335 ± 0.0002</td>
</tr>
</tbody>
</table>

In-vitro drug release profile
Drug release from the SMEDDS formulations (batches F1, F2 and F3) was found to be significantly higher as compared with that of plain lornoxicam. It could be suggested that the SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain lornoxicam. Thus, this greater availability of dissolved lornoxicam from the SMEDDS formulation could lead to higher absorption and higher oral bioavailability. The in-vitro drug release is shown in Fig. 3. Amongst the obtained data, formulation F3 shows around 94% drug released at the end of 20 minutes, which is much is significant in comparison to other two formulations and the pure drug. Thus based on the various studies carried out formulation F3 was selected for further study.

Fig 3: In-vitro drug release of formulations

Stability Studies
No change in the physical parameter was observed during the stability studies. Interestingly, no significant decline in the lornoxicam content was observed at the end of three months at accelerated condition (40°C/75% RH) indicating that lornoxicam remained chemically stable in SMEDDS formulation. It was also seen that no significant change in parameters such as emulsification time, Polydispersity index, zeta potential,
drug content, droplet size, drug content, refractive index and % drug release was observed for the lornoxicam SMEDDS formulation (Table 2).

The similarity factor was calculated for comparison of dissolution profile before and after stability studies of F3. (n=6))

Table 2: Stability Studies data of Formulation batch F3

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Visual inspection</th>
<th>Emulsification time (sec)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>Droplet size (nm)</th>
<th>Refractive index</th>
<th>% Drug Content (%)</th>
<th>Similarity factor (f2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>Clear</td>
<td>25</td>
<td>0.176 ± 0.019</td>
<td>18.17 ± 0.09</td>
<td>15.26 ± 0.12</td>
<td>1.335 ± 0.0002</td>
<td>99.98 ± 0.01</td>
<td>91.25</td>
</tr>
<tr>
<td>3 Months</td>
<td>Clear</td>
<td>28</td>
<td>0.183 ± 0.022</td>
<td>18.19 ± 0.05</td>
<td>15.29 ± 0.15</td>
<td>1.337 ± 0.0006</td>
<td>99.95 ± 0.03</td>
<td>88.44</td>
</tr>
<tr>
<td>6 Months</td>
<td>Clear</td>
<td>30</td>
<td>0.186 ± 0.017</td>
<td>18.19 ± 0.12</td>
<td>15.33 ± 0.09</td>
<td>1.339 ± 0.0011</td>
<td>99.94 ± 0.02</td>
<td>88.44</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD (n=6)

Fig 4: Comparison of dissolution profile before and after stability studies of F3. (n=6)

CONCLUSION
The present study dealt with the formulation of SMEDDS of a poorly soluble candidate, lornoxicam using Triacetin as oil, Tween 80 as surfactant and Capryol micro Express as co-surfactant. The studies revealed that the drug can be successfully formulated into an oil-surfactant mixture that improves the dissolution of lornoxicam in a simple and economic manner. Rapid improvement in the in-vitro dissolution rate proves the possible high advantage of such formulations in oral drug therapy, with faster onset of action and better therapeutic efficacy. The physicochemical characterization of the formulation indicated that the enhancing effect on dissolution was mainly attributed to the solubilization of lornoxicam in oil and the utilization of the surfactants to reduce the tension between the oily and aqueous phases. The formulation can be further taken for in-vivo studies.

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REFERENCE