Meloxicam (MLX) is a highly potent once daily non-steroidal anti-inflammatory drug (NSAID) of the enolic class. It is more selective in inhibiting cyclooxygenase COX-2 than COX-1 therefore; it possesses superior gastrointestinal safety profile and tolerability compared with regular NSAIDS (1). The drug is effective as analgesic for various pain conditions (2). After an oral dose, MLX is well absorbed from the gut, with an absolute bioavailability of 89%, with the plasma concentration peaking after 5–6 h, indicating the slow absorption of MLX after an oral administration (3). Consequently, intramuscular formulation of MLX is used to shorten the onset of action, since rapid pain relief is required in case of acute and painful exacerbations of rheumatoid arthritis (1). Moreover, due to the potential local tissue irritation and necrosis, intramuscular administration of MLX is not recommended for the chronic use (4). It is therefore reasonable to investigate the approaches that may facilitate oral absorption of MLX for treating acute pain. MLX is practically insoluble in water (8 µg/mL) at low pH value. Also it has a zwitterionic property with two pK_a values of 1.09 and 4.18 (3, 5). A zwitterionic drug possesses a large intramolecular multipole moment due to its multiplicity of oppositely charged groups. Consequently, most of these drugs show low solubility in polar and nonpolar media (3). MLX is classified as a Class II compound having a low solubility and high permeability according to the Biopharmaceutics Classification System (6). The pharmacokinetic profiles of these drugs can generally be changed by formulation techniques that increase their aqueous solubility (3), as the rate of dissolution remains one of the most challenging aspects in formulation development of poorly water-soluble drugs (7). To achieve adequate pharmacodynamic properties such as rapid onset of drug effect, improvement of dissolution is important factor (1, 7). Therefore, several attempts have been made to improve MLX solubility (8).

Self nanoemulsifying drug delivery system (SNEDDS) is a system which contains surfactants and usually, but not always, oils, co-solvents and a
drug (9). By optimizing various additives, a proper SNEDDS could be obtained. Upon dilution in aqueous media such as gastrointestinal fluids, these systems can form nanoemulsions (10). The ability of SNEDDS to enhance the oral bioavailability of poorly water-soluble drugs has been recognized. It is commonly believed that increased luminal solubilization and improved drug dissolution in gastrointestinal tract are the main mechanisms responsible for enhanced class II drug bioavailability. Increased drug solubilization is achieved by the interaction of lipid formulations and their digestion products with
endogenous bile salts and phospholipids to form a range of vesicular and micellar species which enhance drug solubilization in gastrointestinal tract (11).

SNEDDSs were classified by Lipid Formulation Classification System (LFCS). LFCS was initially introduced by Pouton in 2000 (12), then six years later he updated it by including an extra type of formulation (13). Lipid-based delivery systems range from simple oil solutions to complex mixtures of oils, surfactants, co-surfactants and co-solvents. The latter mixtures are typically self-dispersing systems often referred to as self-emulsifying drug delivery systems (SEDDS or SNEDDS). Many poor water soluble drugs are much more soluble in co-solvents than oils, and such drugs may also dissolve in polyoxyethylene-rich environment present in water soluble non-ionic surfactant materials (14). This naturally encourages formulators to add water soluble surfactants and co-solvents at the expense of lipids, ultimately resulting in complete exclusion of lipid excipients to produce Type IV formulations (14). Ultra-fine SNEDDS (UF-SNEDDS) were recently developed. These systems form clear, transparent emulsion, upon dilution with an aqueous media and have droplet size of less than 50 nm (15, 16).

UF-SNEDDS formulation is, in theory, comparatively simple. The key step is to find a suitable oil/surfactant mixture that can dissolve the drug within the required therapeutic concentration. SNEDDS mixture can be filled in either soft or hard gelatin capsules (17).

Rapid entry of MLX into the blood stream is especially beneficial in the treatment of acute pain. So, the aim of this research work was to investigate the feasibility of preparation of MLX UF-SNEDDS in order to achieve fast dissolution, which would presumably yield quick onset of peak plasma concentration.

MATERIALS AND METHODS

Materials
MLX was a gift from Riyadh Pharma (Saudi Arabia). Peanut oil was purchased from Uni-Chem Chemical Reagents (England). Olive oil was obtained from Avonchem, Cheshire (England). Sesame oil was purchased from Fluka (Switzerland). Ethyl oleate, polyethylene glycol 400 (PEG 400), castor and linseed oil were purchased from Winlab (England). Oleic acid was purchased from Riedel-de Haén (Germany). Cremophor RH40 was kindly supplied by BASF, Ludwigshafen (Germany). Capmul grades were kindly supplied by ABITEC (USA). Tween grades were purchased from Sigma (USA). High-performance liquid chromatography (HPLC) grade methanol was purchased from BDH Chemicals (England). All other chemicals were of reagent grade and all solvents were HPLC grade.

Methods

HPLC method of analysis
MLX was analyzed using Autosampler, model no. 717 plus, binary HPLC pump, model no. 1525, Dual λ Absorbance, model no. 2487, Nova-Pak C18 3.9 x 150 mm Column (Waters, USA). The flow rate was adjusted at 1 mL/min and the mobile phase consisted of methanol and phosphate buffer (0.1 w/v potassium dihydrogen phosphate adjusted to pH 6 by potassium hydroxide) in ratio of 40 : 60. UV detector was adjusted at 356 nm. Figure 1 shows typical chromatograph of MLX at different concentrations.

Determination of MLX solubility
Solubility of drug in SNEDDS components is very important to obtain nanoemulsion. Therefore, appropriate oils, surfactants and cosurfactants were selected to determine MLX solubility. MLX solubility in different oils, mixed glycerides and surfactants was determined by addition of an excess amount of MLX to each compound. The mixtures were shaken at 37°C for 72 h, then were centrifuged in Eppendorf tubes for 30 min at 9000 rpm using centrifuge, model no. MIKRO 120 (Hettich-Zentrifugen, Germany). MLX concentrations in each supernatant were determined by the previously mentioned HPLC method. MLX solubility results are shown in Figure 2.

Preparation of MLX SNEDDS
According to the solubility study, Cremophor RH40 was chosen as the main hydrophilic surfactant. MLX SNEDDSs were prepared according to the design shown in Table 1 by mixing the exact compositions of each compound. The mixtures were slightly heated in water bath to liquefy the components. The melted components were shaken until the entire MLX amount was dissolved.

Evaluation of MLX nanoemulsions

Visual observations
To assess the self-emulsification properties, specific amount of MLX formulation was dispensed in 25 mL of water in a glass flask at ambient temperature, and the contents were gently stirred manu-
ally. The tendency to spontaneously form a transparent emulsion was judged as good and it was judged bad when there was poor or no emulsion formation. The measurements are shown in Table 2.

**Turbidity measurements of MLX SNEDDS**

After diluting specific amount of MLX SMEDDS with 25 mL water in a stopped tube and gently mixed, the resultant nanoemulsions were evaluated for their turbidity. Turbidity given in nephelometric turbidity units (NTU) was measured using turbidity meter, model no. 415 (MARTINI Instruments, Romania). Turbidity measurements were performed with 10 mL of the nanoemulsion dispensed in clear screw-capped vials. The results are shown in Table 2.

**Particle size measurements of MLX SNEDDS**

A specific amount of MLX SNEDDS was dispensed in adequate amount of water in a stopper flask and gently mixed. The resultant nanoemulsions were evaluated for its droplet size. The droplet size distribution of the resultant nanoemulsions was determined by laser diffraction analysis using Zetasizer (Nano ZS, England), which has a particle size measurement range of 0.3 nm – 10.0 µm. The droplets size of MLX SNEDDS was determined in a small volume module. Samples were directly placed into the module and the data were collected for 10 min. Particle size was calculated from the volume size distribution. All studies were repeated in triplicates, with good agreement being found between measurements. The results are shown in Table 2.

**In vitro dissolution of MLX SMEDDS**

*In vitro* MLX release was conducted by using one gram of each MLX UF-SNEDDS filled in hard gelatin capsules size 00 and also two commercial MLX products namely Mobic® and Mobitil® tablets (containing 7.5 mg MLX). The *in vitro* dissolution profiles were determined using USP2 rotating paddle apparatus (ERWEKA, DT-700, Germany) at

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Table 1. Composition of 1 g MLX UF-SNEDDS each containing 7.5 mg of MLX dissolved in 80% (w/w) of Cremophor RH 40.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Type</th>
<th>Capmul MCM C8</th>
<th>Tween 60</th>
<th>PEG 400</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F1</em></td>
<td>Type IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>Type IV</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>Type IV</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>F4</td>
<td>Type IIIB</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F5</td>
<td>Type IV</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>F6</td>
<td>Type IIIB</td>
<td>90</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>F7</td>
<td>Type IV</td>
<td>0</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>F8</td>
<td>Type IIIB</td>
<td>85</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>

*F1: containing 100% (w/w) of Cremophor RH 40

Table 2. Physical characterization of MLX UF-SNEDDS.

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Visual observation</th>
<th>Turbidity (NTU)</th>
<th>Particle size (nm)</th>
<th>% Release at 5 min</th>
<th>% Release at 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Good</td>
<td>4.8 ± 0.5</td>
<td>25.6 ± 1.3</td>
<td>10.6 ± 5.5</td>
<td>59.1 ± 7.1</td>
</tr>
<tr>
<td>F2</td>
<td>Good</td>
<td>3.7 ± 0.3</td>
<td>12.8 ± 1.4</td>
<td>22.2 ± 8.4</td>
<td>66.1 ± 8.6</td>
</tr>
<tr>
<td>F3</td>
<td>Good</td>
<td>4.2 ± 0.3</td>
<td>16.5 ± 1.6</td>
<td>30.1 ± 6.8</td>
<td>64.8 ± 2.3</td>
</tr>
<tr>
<td>F4</td>
<td>Good</td>
<td>3.6 ± 0.3</td>
<td>13.9 ± 1.3</td>
<td>40.8 ± 7.2</td>
<td>75.7 ± 1.8</td>
</tr>
<tr>
<td>F5</td>
<td>Good</td>
<td>3.3 ± 0.2</td>
<td>16.2 ± 1.3</td>
<td>12.5 ± 0.8</td>
<td>64.6 ± 4.9</td>
</tr>
<tr>
<td>F6</td>
<td>Good</td>
<td>2.3 ± 0.2</td>
<td>14.3 ± 1.3</td>
<td>48.3 ± 9.0</td>
<td>73.7 ± 2.4</td>
</tr>
<tr>
<td>F7</td>
<td>Good</td>
<td>2.5 ± 0.2</td>
<td>16.8 ± 1.5</td>
<td>20.7 ± 5.8</td>
<td>69.1 ± 6.1</td>
</tr>
<tr>
<td>F8</td>
<td>Good</td>
<td>1.9 ± 0.2</td>
<td>14.7 ± 1.3</td>
<td>55.2 ± 7.6</td>
<td>82.7 ± 0.5</td>
</tr>
</tbody>
</table>
Rotating speed of 50 rpm in 900 mL of 0.1 M HCL maintained at 37 ± 0.5°C. Samples were periodically withdrawn after 5, 10, 15, and 20 min using 1 µm cannula filters for MLX UF-SNEDDS and after 5, 10, 15, 20, 25, 30, 35 and 60 min for F8 capsules and Mobic® and Mobitil® tablets. MLX concentrations were assayed using HPLC method. The dissolution experiments were carried out in triplicates. The results are shown in Figures 3 and 4.

**Statistical analysis**

IBM® SPSS® statistics (version 19.0.0) software was used to analyze the data. One-way ANOVA plus post hoc least significant difference (LSD) were applied to compare more than two groups or plus post hoc Dunnett test to compare groups with a control. A value of $p < 0.05$ was denoted significant throughout the analysis of data.

**RESULTS AND DISCUSSION**

**Solubility of MLX**

Drug solubility in the formulation and the ease of dispersion remain important design criteria especially in preparing SNEDDS (11). MLX was found to be very slightly soluble in oleic acid, ethyl oleate,
castor oil, peanut oil, and linseed oil and practically insoluble in olive oil and sesame oil (Fig. 2). MLX solubility in fatty acid, fatty acid ester and natural oils was ranged from 0.072 to 0.528 mg/g. The highest solubility was in oleic acid which is a fatty acid which belongs to the general group of polar oils (14).

Capmul products are mono-, di- and triglyceride emulsifiers prepared through the glycerolysis of selected fats and oils. They can be prepared by esterification of glycerin with specific fatty acids. They are lipophilic, insoluble in water (14). They are used to produce stable emulsions and to modify viscosity. MLX solubility in Capmul grades had shown a slight increase comparing to natural oils and fatty acids.

MLX solubility in non-ionic surfactants like Tween 40, 60, 80 and 85 were ranged between 5.06 to 2.77 mg/g. The lowest solubility was obviously with Tween 85. This decrease in solubility may be due to decreasing hydrophilic-lipophilic balance (HLB) value of this surfactant (5). In another words, increasing HLB increases MLX solubility.

The highest MLX solubility was found to be in Cremophor RH 40 (9.78 mg/g). Cremophor RH 40 is a polyoxyl 40 hydrogenated castor oil. Polyoxyethylene castor oil derivatives are nonionic solubilizers and emulsifying agents used in oral, topical, and parenteral pharmaceutical formulations. MLX solubility in Cremophor RH 40 may be due to the changing happen in lipophilic moiety of the hydrogenated castor oil upon condensation with polyethylene. Also, Cremophor RH 40 has pH value of 6–7 which enhance MLX solubility. Many poor water soluble drugs are much more soluble in co-solvents than oils, and such compounds also dissolve in the polyoxyethylene-rich environment present in watersoluble non-ionic surfactants. This naturally encourages formulators to add water-soluble surfactants and co-solvents at the expense of lipids, which results in complete exclusion of lipid excipients to produce type IV lipid based formulations (14). It is worthy to mention that the regulatory status of Cremophor RH 40 is included in the FDA Inactive Ingredients Database (IV and ophthalmic solutions), parenteral medicines licensed in the UK and the Canadian List of Acceptable Non-medicinal Ingredients (18).

Also, Cremophor RH 40 has been used in about 35 FDA-approved drugs as seen from the FDA inactive ingredient database (19).

PEG 400 has the ability to form complexes with large number of poor water drugs. That is why it is widely used as solvent and solubilizing agent mostly in liquid and semisolid formulations (20). Regarding MLX solubility in PEG 400, it was found to be 5.1 mg/g. However, for Type III and IV lipid based formulations which contain large quantities of water miscible surfactants and co-solvents, the likelihood of drug precipitation upon dispersion in water increases and care should be taken to minimize the quantity of formulation components such as PEG 400 that have limited ‘on going’ solubilization capacity after diluting the formulation with water or in gastrointestinal fluids (11).

Visual observations

For the development of self-emulsified formulations, a right blend of low and high HLB of the components is necessary for formation of a stable nanoemulsion (12). This is possible as surfactant strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermomechanically spontaneous dispersion. Furthermore, co-surfactant increases interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (21).

Droplet size and turbidity of dispersed MLX UF-SNEDDS

The droplet size of nanoemulsion is one of the most important parameters, because it governs the effective drug release (22). It has been confirmed that smaller droplet size resulted in large surface area providing high drug release for absorption (23). SNEDDS containing high proportions of water soluble surfactants provide small particle sizes on dispersion (11). MLX UF-SNEDDS Type IIIB and IV exhibit very small droplet size after dispersion in water (less than 25.6 nm). As a result, these systems are considered as UF-SNEDDS due the tiniest of the droplet size (15). Also these systems gave clear solutions with turbidity up to 4.8 NTU. The nanometer range particle size could enhance drug absorption and bioavailability.

MLX UF-SNEDDS dissolution profiles

It is clear from Figure 3 that MLX UF-SNEDDS prepared with Cremophor RH 40 (F1) showed the lowest MLX release rate among all formulations. It was also observed that incorporation of Tween 60 (F2) and PEG 400 (F3) separately, significantly improved MLX release rate in these formulations. Also the presence of Tween 60 and PEG in the same MLX UF-SNEDDS (F7) at a ratio of 85 : 15 has slight significant effect on MLX release rate while a ratio of 90 : 10 has no significant effect (F5),
suggesting that increasing PEG 400 percentage over that of Tween 60 could enhance MLX release rate. However, F3 slightly enhanced drug release, which could be due to a decrease in the solubilization capacity of this formulation in the dissolution medium. As was mentioned, incorporation of large quantities of water miscible surfactants and co-solvents like PEG 400 increases the chance of drug precipitation upon dispersion. Also from Figure 3 it could be observed that there is a delay in MLX release at 5 min. This may be due to fact that surfactants often take a considerable time to dissolve, due to the formation of viscous liquid crystalline (or gel crystalline) phases at the surfactant-water interface (14). There was no significant difference found between F2, F3 and F7.

MLX release was highly significant from F4, F6 and F8 comparing to that of F1. The enhancement in drug release from these formulations could be due to the presence of Capmul MCM C8. It was reported that Capmul MCM C8 (HLB < 6) is likely to increase the interfacial fluidity of Cremophor grad (HLB > 12) boundaries in micelles due to the entrapment of low HLB surfactant into high HLB one (22). This entrapment is due to lipophilic properties of Capmul MCM C8. As a result, the solubilization capacity of the formulation increases. There was no significant difference found between F4, F6 and F8.

Comparative dissolution study

Mobic® is a brand of MLX tablets which contain 7.5 mg of MLX with inactive ingredients including colloidal silicon dioxide, crospovidone, lactose monohydrate, magnesium stearate, microcrystalline cellulose, povidone and sodium citrate dehydrate. Mobitil® is a novel formulation of MLX tablet also containing 7.5 mg of MLX with 37.5 mg β-cyclodextrin (β-CD) in an inclusion complex. Upon administration, an association-dissociation equilibrium is rapidly established with free MLX molecules readily available for absorption (in contrast to clumping which usually occurs with conventional tablet and capsule forms). Single MLX molecules are instantaneously absorbed and the equilibrium is rapidly shifted to the right hand side (MLX free side).

After 5 min, % dissolution of MLX from Mobic®, Mobitil® and MLX UF-SNEDDS (F8) were 3.5, 11.8 and 74.7%, respectively.

Mobitil® dissolution profile was increased by 7% dissolution over 60 min in acidic pH medium compared to Mobic®, may be due to the effect of UF-SNEDDS formulation. The benefits of micellar solutions produced by MLX UF-SNEDDS as drug delivery system arise mainly from the solubilization power of surfactants and thus the elimination of dissolution as a rate-limiting step in the absorption process (24).

CONCLUSION

MLX was successfully formulated as a UF-SNEDDS that showed significant improved in vitro percentage of MLX released when compared to a commercially available MLX tablets (Mobic® and Mobitil®). The results of this study revealed the better action of Capmul MCM C8 dispersion over Tween 60 for formulating MLX UF-SNEDDS.

The dissolution of MLX seems to depend on formulation and excipients. Using UF-SNEDDS could increase the dissolution rate in the stomach, and thereby, could potentially increase the absorption rate and bioavailability.

Acknowledgment

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project No. RGP-VPP-287.

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Received: 6. 11. 2013