Influence Of Some Dissolution Enhancing Agents On The Pharmacokinetic Profile Of Meloxicam Delivered From Hydrophilic Ointments

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ABSTRACT Meloxicam, a very poorly soluble in water selective COX2 non steroidal antiinflammatory drug, was included in Carbopol 990 hydrogels using dissolution enhancing agents. Carbopol 990 is a high molecular weight polymer, easily soluble in water even at low concentrations, with increased viscosity, transparency and filmogen gel forming properties. To increase the solubility of Meloxicam, in this study we used: Tween 80, Triethanolamine and polyethylene glycol 400 (PEG 400). The kinetic profile of Meloxicam in vitro release from pharmaceutical formulations was studied using Franz diffusion cell. Methylcellulose artificial membrane was used as diffusion membrane.

KEY WORDS Meloxicam, ointments, Franz diffusion cell, enhancers

Introduction

Semisolid formulations are considered to be optimal for the disposal of active substances through the skin, cornea, rectal, nasal, vaginal and urethral tissue. The ointments are semisolid formulations administered on the skin or mucous membranes for protective or therapeutic purposes. The ingredients of the ointment consist in the active substances dispersed in the ointment base, with or without the help of auxiliary agents. Depending on the degree of dispersion of the drug, the ointments include: solution type ointments, emulsion type ointments, suspension type ointments or polyphasic ointments[1].

An optimized formulation offers the advantages of lower toxicity, lower cost (with a useful minimum effective concentrations) and a maximum opportunity for clinical efficacy. The first pharmacokinetic stage is the release of the active substance from the applied topical formulation. The release rate depends on the thermodynamic activity of the drug. A low rate of the release generally corresponds to a low bioavailability after topical administration. An optimum concentration of active substance with high affinity towards the ointment base is insufficient to ensure an increased bioavailability due to the deposit effect exerted by the vehicle.

Topical formulations containing non steroidal anti-inflammatory drugs (NSAIDs) are widespread in the world. Diclofenac, indomethacin, piroxicam, phenylbutazone, indomethacin, ibuprofen, ketoprofen are among the most commonly used NSAIDs. In our country, no product used for topical application containing Meloxicam is available on the market. In this paper, we tried to establish and optimize some topical formulations with Meloxicam, a representative COX-2 selective oxicam[2-5].

Like other NSAIDs, Meloxicam’s incorporation in the formulation has been problematic due to its low solubility in the ointment. Studies have shown that the solubility of NSAIDs may be increased by alcohol, propylene glycol, dimethylsulfoxide. The concentration of active substance does not exceed 10% for NSAIDs formulated and marketed for topical application[6,7].

Surface-active substances (such as Tween 80), used in topical preparations, may have an effect on penetration by reducing the interfacial tension in the hair follicle, and by protein conformation changes in the stratum corneum. Almost all substances penetrate more easily through the hydrated stratum corneum than through the dry tissue. So, any pharmacologically inactive substance that does not affect the stratum corneum and increases its hydration, is regarded as skin penetration enhancer[8].

In vitro transdermal release and penetration studies are the easiest and most inexpensive way to characterize the absorption and skin penetration profiles. These studies are conducted during the formulation of topical forms in order to select the optimum absorption and release profile of the active substance. These tests provide useful information related to drug disposal and storage...
through the different layers of the skin: the stratum corneum, epidermis or dermis. Transdermal diffusion cells simulate the diffusion through animal skin. They consist of two compartments separated by a diffusion membrane. The donor compartment allows the application of tested formulation, while the receiver compartment is filled with liquid simulating the blood.

This system protocol uses a static or recirculating receiver liquid system. Static diffusion cell system maintain the receiver liquid without recirculation. Recirculating diffusion cell system provides a constantly refreshed receptor liquid. However, it’s volume is constant during the experiment. In this study we used the Franz diffusion cell with a static system.

Worldwide, NSAIDs are used in situations requiring the treatment of inflammation and pain [9-11]. Also, in studies developed in the same period of time, was noted that the topical applied NSAIDs have low gastrointestinal or kidney side effects, while these effects occur more frequently when administered orally [12-15].

Meloxicam

Meloxicam is a non steroidal anti-inflammatory oxicam drug (NSAID) with analgesic, antipyretic, anti-inflammatory and uricosuric properties. In terms of physical properties, Meloxicam is a light yellow powder with a crystalline structure, practically insoluble in water, highly soluble in acid and alkaline solutions. It is very slightly soluble in methanol. Meloxicam has an apparent partition coefficient (log P) app = 0.1 in n-octanol/ pH 7.4 buffer. pKa values are 1.1 and 4.2 for Meloxicam [16].

![Figure 1 - The chemical structure of Meloxicam](image)

In literature, the topical bioavailability of Meloxicam was 1.05% compared to oral administration. These low levels make this path the best in order to avoid gastrointestinal side effects and intestinal first-pass metabolism. Although systemic absorption after topical administration was much lower compared to oral administration, Tmax was significantly prolonged. This is due to the skin's barrier and deposit role, which control the release of the active substance from the skin. To achieve therapeutic effects, the literature indicates a local concentration of 100 ng/mL [17].

The mechanism of action of Meloxicam is based on inhibition of prostaglandin synthesis and of inflammatory leukocytes migration, inhibiting phagocytosis and lysosomal hydrolase release.

Compared with other NSAIDs, Meloxicam has a low activity on COX-1 and with an activity 18 times weaker than indomethacin and 29 times weaker than diclofenac. These studies were performed on guinea-pig peritoneal macrophage cultured cells by inducing the expression of COX1 and COX2 with lipopolisacharids (LPS) [18,19].

Meloxicam may be included in topical formulations. In our country there is no topical formulation based on Meloxicam.

Agents used in this study to increase the diffusion through the skin have several mechanisms of action [20-22],

- Triethanolamine increases the solubility of the active substance in the vehicle. The dissolution of sparingly soluble compounds is sometimes based on their transformation into more easily soluble salts, provided that the therapeutic effects remain the same. The increase of NSAIDs solubility using ethanolamine is based on this mechanism [23,24],

- Polyethylenglycols increase the partition coefficient,

- Tween 80 increases Meloxicam’s degree of dissolution of, and also acts by solubilizing the lipids in the stratum corneum of the skin.

This study was focused on observing the pharmacokinetic profile of Meloxicam by determining it’s concentration in the receptor solution after the application of the ointment on the artificial methylcellulose membrane of Franz diffusion cell.

One of the objectives of the study was to obtain a hydrophilic ointment that can incorporate easily the Meloxicam, and from which Meloxicam may be released with an optimal pharmacokinetic profile.

By adding agents to increase the solubility, we could incorporate Meloxicam in the hydrogel, resulting a hydrophilic solution type ointment from which the active substance is released more easily than from emulsion or suspension type ointments. We chose to obtain hydrophilic ointments because of the physical properties of the dissolution enhancer agents, and because of the much wider spreading of this formulations in the last period of time.
Matherial and method

The substances and solutions that we used were: Meloxicam (Unichem Laboratories Ltd.), Carbopol 990 (TM Medchim), Tween 80 (Merck), Triethanolamine (Fluka Chemie AG), Polyethylene glycol 400 (PEG400) (Merck), Glycerol (SC Chimreactiv LLC), Sodium Hydroxide (Merck), Phenylhydragryri Boratis (Medchim TM), Saline Solution 0.9% m / v (BBraun).

We used Hanson Research 58-001-455 vertical diffusion cells with 7ml capacity, diffusion hole of 15 mm (recommended FDA and SUPAC SS) and Helix stirrer. The absorbance was read at 362nm wavelength using a JASCO UV-VIS V530 Spectrophotometer and Spectra Manager software.

25 mm diameter Teknokroma Methylcellulose membranes were used characterized by a pore size of 0.45 m and 3.14cm2 releasing surface.

Preparation of hydrophilic ointments

Three formulations with a concentration of 0.3% Meloxicam were obtained: G1, G2 and G3.

Table 1 The Composition of Hydrogels

<table>
<thead>
<tr>
<th>Components</th>
<th>Experiment 1 Formula G1</th>
<th>Experiment 2 Formula G2</th>
<th>Experiment 3 Formula G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>0.3 g</td>
<td>0.3 g</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.1 g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>-</td>
<td>3 g</td>
<td>-</td>
</tr>
<tr>
<td>PEG400</td>
<td>-</td>
<td>-</td>
<td>30 g</td>
</tr>
<tr>
<td>Carbopol 990</td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Glycerol</td>
<td>12 g</td>
<td>12 g</td>
<td>12 g</td>
</tr>
<tr>
<td>10% Sodium Hydroxide Solution</td>
<td>3 g</td>
<td>6.5 g</td>
<td>3 g</td>
</tr>
<tr>
<td>0.2% Phenylhydragryri Boratis solution</td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Distilled Water q.s.ad.</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>

In a tared vial with a rod inserted in it, we weighted the glycerin, the 0.2% Phenylhydragyri Boratis solution and about 70 g distilled water. Then, we sprinkled the Carbopol with continuous stirring and let it soake for 30 minutes. After soaking, 10% NaOH solution diluted with 10 g of water was added to transform the mixture into gel. Meloxicam was weighted in a porcelain cup and mixed with the required amount of Tween 80 (for G1), Triethanolamine (for G2) and PEG 400 (for G3). The dispersed Meloxicam was then incorporated into carbopol gel and the formulas were completed with water as in the provided table. The pH of each gel was determined by potentiometry, pH falling within the limits imposed by the Xth Romanian Pharmacopoeia (4.5-8.5) [1,25].

The components that have been used in the preparation of hydrogels have distinct roles:

1. **Carbopols** are carboxvinil polymers, soluble in solutions of alkali hydroxides and in water. They tend to agglomerate in water and therefore, they must be sprinkled in water with continuous stirring. They must be left to soake for up to 24 hours. The jellification occurs simultaneously while adding the NaOH solution. The Triethanolamine can be used for neutralization. When using Triethanolamine in the composition, the quantity of NaOH solution must be decreased. Carbopol gel is stable at a pH between 5.5 to 10. An excess of NaOH leads to a decrease in consistency, which it is not influenced by temperature.

2. **Polioxiilen derivatives of sorbitan fatty acid esters** (esters of spans with PEGs) are known as Tweens or Polysorbates. The Xth Romanian Pharmacopoeia formalizes Tween 80 as Sorbimacrogoli oleas 300 or polysorbate 80. Tweens can be used as dissolving and humectant agents. They have the disadvantages of bad taste and facilitate absorption of toxic substances in the human body, which limits its use in concentrations up to 2-3%. It is allowed to be used in concentrations that does not exceed a proportion greater than 25 mg / kg body weight when administered internally. This surfactant is a substance that reduces the solid liquid interfacial tension, with a HLB 7-9. The concentration varies with the specific surface of dispersed substances, typically 0.1% of the amount of the dispersion.
3. Triethanolamine, along with mono and diethanolamine are able to form salts with some of the poorly soluble in water substances. By converting them into salts, the solubility of those compounds is greatly increased. It also circulates the idea of increasing the skin permeability due to formation of the complexes with ethanolamines.

4. Macrogols or Carbowax or polyethilenglycols (PEGs) are condensation polymers of ethylene oxide with water. The aggregation state depends on molecular weight: macrogol 400 is a colorless liquid, macrogol 4000 is a white solid. The ratio of the two in the macrogol ointment may vary depending on desired consistency. They have advantage of increasing the degree of solubilization of poorly soluble in water substances.

5. Glycerin prevents drying the skin after application of hydrophilic ointments.

6. 0.2%Phenylhydragyri Boratis solution is an agent that provides a long-term stability (preservative agent).

**Diffusion tests protocol**

Diffusion tests were performed using a diffusion cell with a capacity of 7 ml, receiver solution 0.9% saline solution and 0.1% Tween 80. All experiments were performed in triplicate. Artificial membrane was pre-moistened with the receiver solution for 24 hours before the beginning of the experiment. Diffusion cell was filled with receptor solution and thermostated at 32°C for 1h before the start of the experiment. During the experiment, the cell was continuously thermostated maintaining the temperature at 32°C.

Time 0 is considered the moment when the formulation was applied on the membrane with the immediate assembling of the diffusion cell. 0.3g gel with a concentration of 0.3% Meloxicam as active ingredient was applied for each experiment.

0.5ml samples were taken at 5min, 10min, 15min, 30min, 1h, 2h, 3h, 6h, 24h. Each taken sample was diluted with 2.5ml receptor solution. The volume of solution extracted was then replaced with fresh receptor solution, so the amount of the cell receptor solution remained unchanged. Care was taken to avoid bubbles formation, that could decrease the surface of diffusion. The amount of gel that remained on the membrane at the end of the experiment was tanken and diluted with receiver solution and was spectrophotometrically read using JASCO V530 Spectrophotometer Spectra Manager program, along with the samples taken at the specified time interval.

**Qualitative and quantitative determinations**

Samples were read using Jasco V530 spectrophotometer and Spectra Manager programe at a wavelength specified in the literature (362nm). Triplicates average, standard deviation and confidence levels was determined using TTEST. Calculations and graphs were performed using Microsoft Excel.

**Results**

The standard solutions were obtained by dissolving 0.01g Meloxcam in 10g saline solution alkalized with 0.1g 20%NaOH. The maximum absorption peak was verified by spectrum measurement between 190nm and 1100nm. The spectrum is represented in the following figure.

![Figure 3 - The spectrum of Meloxicam](image)

The calibration curve was plotted function of the dilution absorbence at the wavelength 362nm (slope = 0.182418223).

**Table 2 Values of the absorptions of the original Meloxicam solution dilutions**

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (mg/100g)</th>
<th>Absorption at 362 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.0975</td>
<td>0.0233</td>
</tr>
<tr>
<td>3</td>
<td>0.195</td>
<td>0.0359</td>
</tr>
<tr>
<td>4</td>
<td>0.39</td>
<td>0.0707</td>
</tr>
<tr>
<td>5</td>
<td>0.78</td>
<td>0.1429</td>
</tr>
<tr>
<td>6</td>
<td>1.56</td>
<td>0.2822</td>
</tr>
</tbody>
</table>

3.15 0.5777

8 6.25 1.2505

9 12.5 2.0629

10 25 2.2279

11 50 2.2807

12 100 2.3397
The evolution of Meloxicam diffusion rates function of time and also the percentages of diffused Meloxicam are presented in the following graphs, for the three formulations that we used.

Discussions
In the first experiment, after applying Formula G1, starting with the time of application until 10 minutes after application, the diffusion rate was in
a continuous increase. Beginning with \( t = 15 \) minutes, a decrease of the diffusion rate was observed. It can be noticed that Meloxicam is delivered in very small amounts from the formulation up to 24 hours after the beginning of the experiment. The other two gels have approximately the same trend, while in exchange, Formula G2 has higher speed values in delivering Meloxicam.

Cumulative results can be seen in the figure posted below.

The evolution of diffusion rate of Meloxicam through membrane, function of time – Cumulative results

The highest value for delivery rate of Meloxicam at 10 minutes for Experiment 2 - G2 Formula can be justified by the conversion of poorly soluble in water Meloxicam with Triethanolamine in a much more soluble salt, easily soluble in the Carbopol hydrogel dispersion medium. By increasing the solubility of Meloxicam, the degree of its diffusion through the methylcellulose hydrophilic membrane is increased also. Delivery rate is highest at 10 minutes after application, which shows an almost immediate effect after topical application of the non-steroidal anti-inflammatory drug.

Although Tween 80 and PEG 400 had the same role in increasing the solubility of Meloxicam, adding them to the formulations G1 and G3 did not have the same effect as using Triethanolamine. One explanation would be the too high affinity of Meloxicam for PEG 400, which makes it less available for the diffusion membrane.

The release of Meloxicam form the formulation is difficult because of its high-affinity. PEG400, on the one hand ensure a good dissolution of Meloxicam, which otherwise is very poorly soluble in hydrophilic environments, and on the other hand provides a deposition effect due to the increased affinity for the dissolution agent. Observing the evolution of Meloxicam delivery from Formula G3, it can be seen that the speed of delivery of Meloxicam is almost the same during the first 6 hours of experiment.

The percentage of Meloxicam delivered reaches the highest value for Formula G2, and the lowest value is found for Formula G1.

Tests have been shown to be statistically significant using Microsoft Excel TTEST test, where \( p \) values <0.05.

<table>
<thead>
<tr>
<th>( p ) value</th>
<th>Diffusion speed of Meloxicam through membrane, function of time - significance test</th>
<th>Procentage of the diffused Meloxicam function of time – significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p ) Exp1 and Exp2</td>
<td>0.000638</td>
<td>0.002442</td>
</tr>
<tr>
<td>( p ) Exp1 and Exp3</td>
<td>0.019762</td>
<td>0.005353</td>
</tr>
<tr>
<td>( p ) Exp2 and Exp3</td>
<td>0.001904</td>
<td>0.001172</td>
</tr>
</tbody>
</table>

Tails specify the number of distribution tails. If tails = 1, TTEST uses the one-tailed distribution. If tails = 2, TTEST uses the two-tailed distribution.Type is the kind of TTEST performed.

Conclusions

1. In all the experiments pursued, there was an increase in the rate of diffusion starting with the time of application until 10 minutes after the application, when the diffusion rate decreased. It can be observed that Meloxicam is delivered in very small amounts from the formulation up to 24 hours after the beginning of the experiment. Formula G2 has higher speed values in delivering Meloxicam, while the other two gels have approximately the same trend.

2. The concentrations determined are similar to those obtained in the literature, ensuring a desired local anti-inflammatory therapeutic effect when topical administered.

3. The possible systemic effects after topical application would be greatly reduced considering...
the concentrations obtained by sampling the receiving solution. So, a local anti-inflammatory effect can be obtained after topical administration of Meloxicam, while avoiding side effects encountered in the case of oral administration of NSAIDs.

4. The delivery rate of the active substance from the formulation provides an analgesic and anti-inflammatory effect with rapid onset and effect. For approximately 6 hours after the beginning of the experiment, the concentrations of the active substance are maintained at the same levels, while Meloxicam can be detected at 24 hours after the single dose membrane application.

5. This study brings the latest information on Meloxicam topically applied pharmacokinetics.

6. Any of the formulas may be an option for a topical Meloxicam formula and can be used in the treatment of rheumatic disease or any condition where the pain is localized to muscles and tendons.

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