EFFECT OF TERPENES AS Penetration Enhancers ON THE Release AND Penetration Kinetics OF MELOXICAM GELS FORMULATIONS

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ABSTRACT

Introduction: The transdermal route of administration has been extensively accepted as one of the potential routes for the local and systemic delivery of drugs. The greatest obstacle in drug absorption is the highly organized stratum corneum (SC), which hinders drug transport. The probable solution leads to inclusion of penetration enhancers for reversely disorganizing the barrier characteristic of stratum corneum. Objective: The main objective of the research work was to study the influence of peppermint oil, lemongrass oil, clove oil and turpentine oil as penetration enhancers on the percutaneous absorption of Meloxicam (ME) from a Carbopol 934 based gel formulation. Materials and Methods: ME gel sample was divided into 5 batches i.e., F1, F2, F3, F4, F5. Except F1, all other batches were incorporated with penetration enhancers (5% w/w) namely peppermint oil, clove oil, lemongrass oil and turpentine oil. The formulations were further evaluated for in-vitro drug release studies using a standard cellophane membrane at 37±0.5°C in phosphate buffer pH 7.4 and a comparative anti-inflammatory activity was conducted using rat paw edema method. Result and Discussion: In-vitro permeation studies using a standard cellophane membrane showed that the rank order of enhancement ratio (ERflux) for Meloxicam as peppermint oil (1.414) > clove oil (1.353) > lemongrass oil (1.326) > turpentine oil (1.272) proving peppermint oil as the most competent penetration enhancer for Meloxicam. Further In-vivo anti-inflammatory activity were carried out using the standard rat paw edema method. The in vivo studies revealed that gel containing peppermint, clove, lemongrass and turpentine exhibited 2.53, 2.0, 1.9 and 1.38 times higher anti-inflammatory effect as compared to meloxicam (standard). Conclusion: It can be concluded from the study that all the 4 terpenes significantly increases the permeation of meloxicam gels and can be used as effective penetration enhancers.

Keywords: Carbopol 934, Gel formulations, Meloxicam, Percutaneous, Penetration, Terpenes.
irritancy at low concentrations (1-5%). The purpose of this study was to use Meloxicam in different formulations by adding different oils (peppermint oil, lemongrass oil, clove oil and turpentine oil) obtained from plants; the source of terpenes as penetration enhancers. The second aim of the study was to evaluate the properties of Meloxicam formulations with oils like in vitro drug release studies using a standard cellophane membrane at 37±0.5°C in phosphate buffer pH 7.4 and comparative anti-inflammatory effect of these formulations using rat paw edema method. The purpose of this study was to use Meloxicam in different formulations by adding different oils (peppermint oil, lemongrass oil, clove oil and turpentine oil) obtained from plants; the source of terpenes as penetration enhancers. The second aim of the study was to evaluate the properties of Meloxicam formulations with oils like in vitro drug release studies using a standard cellophane membrane at 37±0.5°C in phosphate buffer pH 7.4 and comparative anti-inflammatory effect of these formulations using rat paw edema method.

MATERIAL AND METHODS

Meloxicam powder was obtained as a gift sample from Unimark Remedies Pvt. Ltd. Vapi, Gujarat. Carbopol 934 was purchased from Central Drug House Pvt. Ltd. New Delhi. Peppermint oil, lemongrass oil, clove oil and turpentine oil were purchased from Central Drug House Pvt. Ltd. Vapi, Gujarat. Carbopol 934 was purchased from RFCL Ltd. New Delhi. All the chemicals and reagents were of analytical grade obtained from Ranbaxy Fine Chemicals Ltd. New Delhi.

Formulation of Meloxicam Gels (1% w/w)

The gel samples were prepared by dispersing 1% Carbopol 934 in a mixture of water and propylene glycol (PG) (80:20 w/w). Meloxicam (1% w/w) was added to the mixture and kept under magnetic stirring for 6 hrs. The dispersion was then neutralized to pH 7.4 by adding 0.1N sodium hydroxide (NaOH) and their viscosity was improved by adding triethanolamine 0.01%. The prepared ME gel sample was divided into 5 batches and labeled as F1, F2, F3, F4, and F5. Except F1 gel formulation, penetration enhancers (peppermint oil, clove oil, lemongrass oil, turpentine oil) were incorporated in the concentration of 5%w/w respectively (Table 1). The mixture was shaken by using magnetic stirrer, until the uniform distribution takes place. The gels were then examined for permeation studies.

Table 1: The various formulations of Meloxicam (1% w/w) gels

<table>
<thead>
<tr>
<th>Ingredients (%)w/w</th>
<th>F1 ME</th>
<th>F2 ME+P</th>
<th>F3 ME+C</th>
<th>F4 ME+L</th>
<th>F5 ME+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carbopol 934</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Peppermint oil</td>
<td>_</td>
<td>5</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Clove oil</td>
<td>_</td>
<td>_</td>
<td>5</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Lemongrass oil</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>5</td>
<td>_</td>
</tr>
<tr>
<td>Turpentine oil</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>5</td>
</tr>
<tr>
<td>Distilled water(qs)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

F1 – Meloxicam Gel without Penetration Enhancer
F2– Meloxicam Gel with Peppermint Oil as Penetration Enhancer
F3– Meloxicam Gel with Clove Oil as Penetration Enhancer
F4– Meloxicam Gel with Lemon Grass Oil as Penetration Enhancer
F5– Meloxicam Gel with Turpentine Oil as Penetration Enhancer

Evaluation

pH

The gel formulation was also evaluated for various physicochemical properties. The pH measurement of the gels were done by using digital type pH meter (Systronics Ltd, Ahmedabad, India) by dropping the glass electrode completely into the gels system. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Drug content

The various formulations were estimated by SHIMADZU-1700 UV spectrophotometer at λmax 269 nm in alcohol.

Spreadability

The Spreadability is very much important as show the behavior of gel comes out from the tube. Spreadability of the gel formulations was expressed in seconds i.e. time taken by two glass slides to slip off from 1g gel placed in between under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. Spreadability is calculated by the formula:

\[ S = \frac{M \times L}{T} \]

Where, S is spreadability, M - is weight tied to upper glass slide, L - length of glass slides and T - is time taken to separate the slides at an angle of 45°. Spreadability of the gel formulation decreases with the increases in the concentration of the polymer.

Extrudability

The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.

Consistency

The consistency measurement was carried out by dropping a cone attached to holding rod on the centre of glass cup filled with gel from a fixed distance of 10 cm. The distance travelled by the cone was noted down after 10sec by measuring the penetration from the surface of the gel to the tip of the cone inside the gel. Homogeneity

Homogeneity of various gel formulations was tested by visual observations.

In vitro drug release

From each formulation 1 gram sample was accurately weighed and placed on a semi permeable cellophane membrane (previously immersed in phosphate buffer, pH 7.4 for 20 hrs), the loaded membrane was stretched over the lower open end of a glass tube of
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2.9 cm diameter and made water tight by rubber band. The tube was placed in a 100ml beaker containing 50 ml of diffusion fluid i.e phosphate buffer (pH 7.4). Sodium lauryl sulphate 1% w/w was added to the medium to ensure sink condition. The system was maintained for 2 hr at 37°C in a thermostatic water bath shaker at 100 rpm. 1 ml sample of each formulation was withdrawn from the phosphate buffer at time intervals of 30, 60, 90 and 120 min and the volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analyzed with dilution for Meloxicam content using UV spectrophotometer at λmax 269 nm. The results were expressed as mean % cumulative drug release ±SD (n=3).

Calculation of permeation parameters

The cumulative amount of drug penetrated was plotted against time and steady state flux Jss (µg/cm²/h) was determined as the slope of linear regression line. Flux (Jss) can also be calculated by using Eq. (1)

\[ J_{ss} = \frac{dQ}{A dt} \]

Where, dQ is the change in the quantity of drug passing through the skin expressed in µg, A is effective diffusion area (cm²) dt is time (h)

Enhancement ratios of flux (ERflux) were calculated using Eq. (2)

\[ ER_{Flux} = \frac{Drug flux with percutaneous enhancer}{Drug flux without percutaneous enhancer} \]

Skin irritation test

Guinea pigs (400-500 g) of either sex were used for testing of skin irritation. The animals were maintained on standard animal feed and had free access to water and were kept under standard conditions. Hair was shaved from back of guinea pigs and area of 4cm² was marked on both the sides, one side served as control while the other side was test. Gel was applied (500 mg / guinea pig) twice a day for 4 days and the site was observed for any sign of edema and erythema.

In-vivo evaluation

Anti-Inflammatory Activity

In-vivo studies of ME gels were conducted on Albino rats of either sex, weighing (200-300gm) were procured from institute animal house. They were housed in propylene cages, kept under controlled conditions for one week before experiments and fed on a standard pellet diet. Animal were fasted overnight with free access to water prior to experiments. Animals were divided into 7 groups having 6 animals each (Table 2).

First group served as control and second served as standard while other 5 are served as test groups.

The Anti inflammatory activity was performed by the carrageenan (CAG) induced rat paw edema method and inflammation was measured by using plethysmometer. In each of the 5 groups of six animals, CAG solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1ml was injected subcutaneously into the sub plantar region of the right hind paw of each rat.

Readings were taken 1 hour after the administration of test formulations. One group was kept as control, receiving only 0.5% normal saline. The right hind paw volume (in mm) was measured by means of a plethysmometer. The percentage anti-inflammatory activity was calculated according to the following formula:

\[ \text{Percentage Edema Inhibition} = \left[ 1 - \left( \frac{V_t}{V_c} \right) \right] \times 100 \]

Where, \( V_t \) represents the mean increase in paw volume in rats tested with test compounds and \( V_c \) represents the mean increase in paw volume in control groups of rats.

Table 2: Division and description of animals in groups for anti-inflammatory activity

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DESCRIPTION</th>
<th>TEST/CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>WITHOUT CAG SOLUTION I</td>
<td>CONTROL</td>
</tr>
<tr>
<td>II</td>
<td>CAG SOLUTION</td>
<td>INDUCED MODEL</td>
</tr>
<tr>
<td>III</td>
<td>CAG+F1</td>
<td>TEST</td>
</tr>
<tr>
<td>IV</td>
<td>CAG+F2</td>
<td>TEST</td>
</tr>
<tr>
<td>V</td>
<td>CAG+F3</td>
<td>TEST</td>
</tr>
<tr>
<td>VI</td>
<td>CAG+F4</td>
<td>TEST</td>
</tr>
<tr>
<td>VII</td>
<td>CAG+F5</td>
<td>TEST</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. The anti-inflammatory activity of test formulation with enhancer was compared with formulation containing the standard drug. A protocol for in-vivo was approved by Institutional Animal Ethical Committee (IAEC), I.T.S College of Pharmacy, Muradnagar with approval No. ITS/04/IAEC/2013.

RESULT AND DISCUSSION

The results for pH, drug content, spreadability, extrudability, consistency and homogeneity are displayed in Table 3. The results were in the official limits. The results are expressed as mean ± SD (n=3).
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Table 3: Table shows the evaluation of Meloxicam gels *

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>6.94±0.002</td>
<td>7.42±0.006</td>
<td>6.96±0.02</td>
<td>7.12±0.003</td>
<td>7.25±0.005</td>
</tr>
<tr>
<td>2</td>
<td>Drug Content (in %)</td>
<td>99.1±0.03</td>
<td>100.21±0.43</td>
<td>100.12±0.33</td>
<td>99.63±0.01</td>
<td>99.98±0.02</td>
</tr>
<tr>
<td>3</td>
<td>Spreadability (gm/cm/sec)</td>
<td>7.66±0.0049</td>
<td>7.98±0.0054</td>
<td>8.05±0.0033</td>
<td>7.94±0.0049</td>
<td>8.11±0.0021</td>
</tr>
<tr>
<td>4</td>
<td>Extrudability (mg)</td>
<td>200±0.34</td>
<td>190±0.44</td>
<td>185±0.23</td>
<td>170±0.78</td>
<td>195±0.56</td>
</tr>
<tr>
<td>5</td>
<td>Consistency (60secs)</td>
<td>1.1±0.0011</td>
<td>1.3±0.0021</td>
<td>1.2±0.0023</td>
<td>1.1±0.0032</td>
<td>1.4±0.0014</td>
</tr>
<tr>
<td>6</td>
<td>Homogeniety</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

* Results are expressed as mean ±SD (n=3)

In vitro drug release

The *in vitro* drug release from the gels displayed in Figure 1, revealed that the % cumulative drug release obtained from the gels containing ME and peppermint oil, clove oil, lemongrass oil, turpentine oil as permeation enhancer were 40.48±1.42, 64.59±1.41, 59.43±1.39, 55.41±1.42 and 51.32±1.43 respectively. The results were expressed in % (mean ± SD, n=3) and the % cumulative drug release follow the order peppermint oil > clove oil > lemongrass oil > turpentine oil > ME. It was observed that formulations containing permeation enhancer was better than ME (F1) only.

![In vitro drug release of meloxicam gels with and without penetration enhancers](image)

Table 4: Table shows the comparative flux of the Meloxicam formulations

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Formulation code</th>
<th>Flux(Jss) $\mu$g/cm$^2$/hr after 2 hrs</th>
<th>ER flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>273.24</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>386.455</td>
<td>1.414</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>369.705</td>
<td>1.353</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>362.547</td>
<td>1.326</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>347.608</td>
<td>1.272</td>
</tr>
</tbody>
</table>

Skin irritation test

The guinea pigs displayed no sign of edema and erythema.
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**In vivo studies**

The various batches of topical gel were also screened for their topical anti-inflammatory potential. At the same subcutaneous dose of carrageenan, ME shows 30.79± 0.37 % inhibition of rat paw edema where as the tested compound showed percentage inhibition ranging from 72.72 ±0.16 % to 39.53 ±0.16 % after 24hrs (Figure 2). The *in vivo* studies shown in Fig 3 revealed that gel containing peppermint, clove, lemongrass and turpentine exhibited 2.53, 2.0, 1.9 and 1.38 times higher anti-inflammatory effect as compared to ME (standard).

![Figure 2: Comparative data of % inhibition of different test sample (Mean±SEM, n=6) after 24hrs](image)

**CONCLUSION**

It can be concluded from the study that all the 4 terpenes significantly increases the permeation of meloxicam gels and can be used as effective penetration enhancers.

**REFERENCES**