Abstract
The present work was undertaken to develop and evaluate transdermal ointments of ibuprofen containing supersaturated drug and menthol as a penetration enhancer. Transdermal ointments were prepared using white petrolatum, beeswax, propylene glycol, PEG 400 and menthol using a fusion technique. Microscopy was used to determine the supersaturation of the drug in the vehicle. The formulated ointments were subjected to in vitro release studies and skin permeation studies. These studies were conducted in the diffusion cells developed in our laboratory, specifically for this purpose. Selected formulations were evaluated for their anti-inflammatory activity using the carrageenan-induced paw edema in rats. The formulation containing menthol demonstrated more transport across the skin. The final formulations selected for topical and systemic investigation had menthol in both the formulations. The results corroborated the fact that the drug was released into the systemic circulation from ibuprofen ointments after topical application with one containing penetration enhancer releasing more. The study clearly indicates that trans-dermal delivery of ibuprofen using a topical petrolatum base ointment is a viable option.

Key words: transdermal; ointment; ibuprofen; co-solvency; solubility; carrageenan

Introduction
Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) is used in several inflammatory conditions (1). Several semi-solid dosage forms for ibuprofen with variable drug content are used for topical inflammatory conditions such as backache and muscular pain (2). Gels and creams with 10% and 15% ibuprofen, respectively, are available. Although popular for topical inflammatory conditions, it is also useful in migraine, dental pain, pain associated with PMS, sore throat, cold/flu and fever, all of which needs systemic delivery of ibuprofen and is more effective than paracetamol in some of these conditions (3,4). For systemic delivery of drugs, dosage forms such as oral tablets, capsules, caplets, intravenous solution, oral suspension, oral solution and suppositories (5) are available. In pharmaceutical market, ibuprofen is also available in all these dosage forms and for systemic delivery of ibuprofen these dosage forms are popularly prescribed by physicians (6). However, on many of these occasions the drug has to reach the systemic circulation via the oral route where it could cause very significant side-effects like peptic ulcers. Thus, oral route is generally not a preferable route for ibuprofen. Other routes to reach systemic circulation could be conveniently attempted. In this regard, a transdermal route which has many advantages could be the choice for ibuprofen. We have conducted some preliminary literature search regarding the transport of ibuprofen across skin and entrance into the systemic circulation. Interestingly, several reports suggested that ibuprofen enters the systemic circulation from topical route at a very high rate and extent (7,8). However, transdermal formulations for ibuprofen are not yet available in the market. Further, literature search suggests
that this route and mode of delivery using semisols for systemic delivery is promising and is slowly gaining prominence (9). There are several advantages for systemic delivery of drugs with the ointment usage compared to a transdermal patch. Thus, this gave us enough leads to investigate further on this novel transdermal ointment approach for systemic delivery of ibuprofen.

Transdermal patches and its modifications such as electrically based enhancement techniques, photomechanical waves and microneedles are different topical approaches that could lead to drug levels in the systemic circulation (10). The very well known examples present in the market are nitroglycerin, fentanyl, lidocaine, estradiol patches, etc. On the other hand semisolids for transdermal delivery into systemic circulation can also be attempted for drugs with high penetration into the skin. There are some additional advantages to these semi-solids compared to transdermal patches and its modifications and these include ease of application, cosmetic appeal and reduced skin irritation (10). The aim of this investigation was to develop a petrolatum-based ibuprofen ointment that could lead to convenient systemic levels after topical administration. The selection of petrolatum base has several advantages for transdermal delivery of drugs and as well, currently high grade and high purity petrolatum with clear qualification and instructions, which was not previously available is sold in the market and for these reasons it was naturally, the selected ointment base in this study.

Experimental Materials and Methods

Ibuprofen was obtained from Boots India Ltd., Mumbai. White bees wax, hard paraffin were purchased from Loba Chemic, Mumbai. White soft paraffin was purchased from Burgoyn Urbidges & Co., Mumbai. Polyethylene glycol 400, propylene glycol, sodium carboxymethyl cellulose (CMC) and menthol were obtained from S.D. Fine Chemicals, Bombay. Methanol was obtained from Ranbaxy Chemicals, Delhi. Magnetic stirrers were obtained from Remi Equipments Pvt. Limited. A Double Beam UV-Vis Spectrophotometer (SL 164) used to analyze the samples was obtained from Elico, Mumbai. A diffusion cell used to study drug release from the ointments was designed in our laboratory. Carrageenan sodium salt was obtained from SD fine Chemicals Ltd., Mumbai. Microscope was obtained from Ajay Optics. Centrifuge, vortex mixer and magnetic stirrer were obtained from Remi Industries Ltd. Menthol was obtained from Final Chemicals. Diethyl ether was obtained from Finar Chemicals. Plethysmograph used to determine the extent of inflammation in a rat was locally made.

Development of the ointments

To develop the appropriate Ibuprofen ointment, petrolatum based ointment excipients which include white beeswax, and hard paraffin were used (Table 1). In its preparation PEG 400 and propylene glycol were used as co-solvents. This could lead to higher solubility of the drug in the base. Ointments containing increased concentration from 3% to 7% of PEG 400 and propylene glycol were prepared and the solubility of the drug was determined using a microscopic method previously published (11). The final ointment formulation is anticipated to have high drug levels in the soluble form. A high drug level especially in the soluble form can lead to therapeutic drug levels in the systemic circulation after ointment administration than compared to the existence of the same drug in the insoluble form. As a reason, a 12.5% drug containing formulation was selected. This concentration is well below 10% of its solubility in the selected formulation with a minimum amounts of the cosolvent used in this study (its solubility in the minimum cosolvent containing formulation is
Although gels and creams with such a high content are available, there could be significant precipitation of the ibuprofen in these formulations of higher aqueous nature because of its poor water solubility. This may not result in systemic therapeutic levels. Thus, these are used only for local applications. On the other hand, petrolatum base can incorporate more drug in soluble form and thereby can lead to higher systemic levels. Paraffin/PEG/Propylene glycol ointment was prepared by melting white bees wax, hard paraffin, to which the drug dissolved in PEG 400 or propylene glycol with or without menthol added while stirring. The entire mixture was stirred while cooling to form Ibuprofen ointment.

**Drug release into dissolution medium**

*In vitro* release studies are important for a number of reasons including product optimization and *in vitro*-*in vivo* correlations. Drug release measurements were carried out in a diffusion cell designed in our laboratory (Figure 1) in optimized dissolution media using all the four formulations prepared in this study. A dialysis membrane (gelatin paper soaked in water at 50ºC for 10 min) was placed between the donor and the receiver. The donor always contained 500 mg of the ointment. Since the ointment was prepared using a fusion technique and contained the drug in the soluble form and was used for the release studies just after it was manufactured, it was assumed that the content of the drug in all the ointments applied on the donor side is the same. As a reason, we did not estimate the drug amount in the ointments prior to its usage in the release studies and thus the drug content, content uniformity, spreadability and viscosity were not determined. The dissolution media was optimized by investigating the drug release from a 5% ibuprofen suspension prepared using CMC as the suspending agent into different compositions of methanol:water (30 : 70) (Media 1), methanol:water (15 : 85) (Media 2) and pure water (Media 3). Pure water offered a better sink compared to PBS that occasional showed interference in the UV assays in the presence of methanol. Addition of methanol in the media can result in a better sink condition. Release drug contents were measured using UV-double beam spectrophotometer at wave length 221nm. The media which supported sink conditions the best was taken as an optimized media. The dissolution studies were conducted as previously described (11) and the diffusion coefficient calculations are based on Higuchi equation (12) which is shown below:

\[ Q = 2C_0(Dt/\delta)^{1/2} \]  
\[ \text{eqn 1} \]

Where ‘\(C_0\)’ is the initial drug concentration in the donor,  
‘\(Q\)’ is the cumulative amount of drug,  
‘\(t\)’ is the release time and  
‘\(D\)’ is the diffusion coefficient.

**Table 1. Compositions of Ointments Investigated for Drug Release and Transport across the Skin**

<table>
<thead>
<tr>
<th>Composition/Formulation (% W/W)</th>
<th>Form 1</th>
<th>Form 2</th>
<th>Form 3</th>
<th>Form 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>PEG 400</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Menthol</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Petrolatum and Beeswax (upto 100)</td>
<td>upto 100</td>
<td>upto 100</td>
<td>upto 100</td>
<td>upto 100</td>
</tr>
</tbody>
</table>
Transport across mice skin

The skin used for transport studies was removed from the mice and the section was prepared as described previously (13). The skin section thus prepared was clamped carefully to one end of the hollow glass tube (dialysis cell) so that the stratum corneum faced up on the receiver compartment. The dissolution medium selected by optimization technique previously mentioned was used as receiver compartment. The donor compartment was immersed into the receiver compartment so that the edge just touched the receiver compartment. For first 30 min skin washing was performed. Then the receiver fluid was replaced with fresh dissolution media. The known quantity (500 mg ointment) was spread uniformly and the experiment was continued as mentioned in the release studies section. Permeation profiles were constructed by plotting the cumulative amount of the drug permeated Vs time.

Antiinflammatory effects (Carrageenan rat paw method)

The anti-inflammatory activities of the formulations under investigation were studied using the carrageenan-induced edema model as previously mentioned (14). Male wistar rats (140-
175 g) were used. Formulations were prepared just before the administration. Drug formulations administered were: two selected ointments and one oral suspension formulations. Carrageenan 1% solution to be injected into a rat was prepared by adding 250 mg of carrageenan in 25 ml of Normal saline. The solution was injected into the hand paw of the rat to cause inflammation. For investigating the systemic effects, the ointments (500 mg) were applied to the shaved surface on the abdomen of the rat and for investigating the local effects the ointments were applied near the paw at the site of inflammation (a fairn electronic shaver with trimmer were used to shave the abdomen of the rat). It is assumed that the drug diffuses from the ointment, reaches the systemic circulation via transdermal route and thereby elicits the action. Inflammation was measured by the equipment called plethysmograph (Narsaiah Enterprises, Warangal, India). The percentage increased in the volume of paw was calculated using the formula:

\[
\text{% Increase in paw swelling} = \frac{V - Vi}{Vi} \times 100
\]

Where \( V \) = Volume of the paw 2 hr after the carrageenan injection

\( Vi \) = The initial paw volume

**Results**

Upon dissolving ibuprofen in propylene glycol and PEG 400 at a ratio of 7:3 in the total ointment composition and thereby dispersing into the petrolatum based ointment base, a 12.5% w/ w ibuprofen ointment containing drug in the solubilized form. A 3% menthol could be conveniently incorporated as a penetration enhancer into this. The final formulations have drug with 10% of excess in solubilized form. The compositions are tabulated (Table 1). To investigate the release of the drug from the prepared 12.5% ibuprofen ointment, tailoring (optimizing) of dissolution medium is essential such that sink conditions are maintained during the release. Three different media (Media 1, Media 2 and Media 3) were investigated for this purpose and finally Media 1 was found to be optimum and we used this dissolution medium to investigate drug transport in this study (Figure 2). Drug release studies were investigated to determine the rate and extent of drug release from the ointment. The drug release from all the four formulations was investigated. The release depended on the composition of the medium (Figure 3). From cumulative amount release data, Form 4 was more effective than Form 2, which was more effective than Form 1, and Form 1 was more effective than Form 3. When square root time vs cumulative amount drug release was plotted (Higuchi plot), it yielded a straight line for all the formulations (Figure 4). Thus, using Higuchi equation, we could calculate the diffusion coefficient of the drug from the vehicle. The calculated diffusion coefficients for Form 1, Form 2, Form 3 and Form 4, were 8.86 *10E7, 11.34 *10E7, 5.57 *10E7 and 14.33 *10E7, respectively. In drug transport across mice skin studies, it was found that ibuprofen transported across the skin from all the formulations. The transport was enhanced in the presence of menthol (Figure 5). Local and systemic effects of the drug after topical application in the form of the ointment were tested in a carrageenan-induced rat paw inflammatory model. Based on the drug release from the ointments and skin permeation, Formulation 2 and Formulation 4 were selected to investigate this. The percentage inhibition of inflammation in the rat paw method in case of Formulation 4 which contained a penetration enhancer and administered at a remote location was 65%, while with Formulation 2 that contained no penetration enhancer had 35% percent inhibition. However, in case of local effects the inflammation reduction with both the ointments was 100% suggesting that this mode of administration better suits for ibuprofen ointment. In either case, a placebo control both for administering at the inflammatory site (local application) as well as at the remote location (systemic application) was used.

Novel Transdermal Ointment
Placebos did not demonstrate any anti-inflammatory effects.

Discussion

Currently, delivery of drugs into systemic circulation via topical route by applying ointments is the state of art in this area of research (9). In this study, we aimed at investigating this issue taking ibuprofen as the drug of choice as it has several systemic applications as well it has been previously shown that it is taken at a very high level into systemic circulation after topical application, although this issue regarding its systemic delivery was not the focus (7, 8). To facilitate drug transport into the skin and thereby into the systemic circulation, methods like hydrating the skin, saturating the vehicle with the drug or adding chemical penetration enhancers (15) have been researched. All these three factors were incorporated in the formulation development of a transdermal ibuprofen ointment. A petrolatum base hydrates the skin very well. Thermodynamic activity of the drug in the formulation, which is one of the deciding factors for enhanced absorption into deeper layers of the skin and also into the systemic circulation, can be enhanced by increasing the solubility of the drug in the vehicle (16). On these lines supersaturation of the drug in the vehicles is the need of the hour. Additionally,
we have incorporated a penetration enhancer. We have opted to prepare a 12.5% ibuprofen ointment containing the active in a soluble form so that super-saturation of the drug occurs in the presence of co-solvents and also optimum viscosity or thixotropic properties are the characteristics of the formulae investigated. Propylene glycol and PEG was used as co-solvents to enhance the solubility of the drug in the ointment base. Methods of increasing the solubility of the drug in the petrolatum based ointment by cosolvency techniques have been previously described (17). As the concentration of propylene glycol was increased to 7% the solubility reached to more than 12.5%. The solubility was determined using a microscopic method. The final topical ointment base incorporated PEG 400: 3%, propylene glycol: 7%, drug: 12.5% and rest petrolatum base. Further, another set of ointment base for systemic delivery of the drug was prepared. This additionally incorporated 3% and 5% of menthol in it.

Subsequently, the ointments were characterized for in vitro drug release into the dissolution medium and drug transport across mice skin and diffusion coefficients from the release data and drug transport across the skin determined. The calculations and interpretation of drug release studies followed a modification of protocols published by Ozsoy et al., 2004 (11) and Zhang et al., (2002) (18). In the in vitro drug transport across the mice skin, the amount of drug transported through unit area of skin was more in case of formulation containing penetration enhancer, suggesting that for systemic delivery second formulation is better. The results with the two formulations in carrageenan-induced inflammation model corroborated the fact that the drug was released into the systemic circulation from ibuprofen ointments after topical application with the one containing penetration enhancer releasing more. Results also indicated that local use is better, however, systemic administration was also able to subside the inflammation. Thus, the study clearly indicated that transdermal delivery system for ibuprofen is a viable option. However, more studies are to be conducted to further develop an effective and clinically viable ointment for ibuprofen for systemic delivery.

References


