STUDY ON TRANSMUCOSAL PERMEATION OF DICLOFENAC DIETHYLAMINE MICROEMULSION GEL

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ABSTRACT
Non steroidal drugs are often the first choice of treatment for patients with acute mayo orthopaedic injuries, post-operative pain, chronic rheumatoid arthritis and osteoarthritis. Oral administration of these drugs leads to gastric irritancy/toxicity. Topical delivery of these drugs can bypass the difficulties of first-pass metabolism, enable absolute elimination of gastro intestinal toxic effects, maintain the steady plasma levels of drug for a prolonged period and deliver the drug at predetermined rate without the hazards of specialist care as is required in the intravenous infusion. Hence the present work aimed to develop formulation for microemulgel of diclofenac(diethylamine), carbomerETDT2020 and crodomol as permeation enhancer. Drug release studies carried out by dialysis method with phosphate buffer using franz diffusion cell. Drug release profile and diffusion coefficients were compared with brand formulation. Statistical data shows that the diffusion coefficients of the drug from emulgel formulations rank according to the following order F6 > F7 > MKT (4.01744, 3.17233, 0.16870) . Anti inflammatory activity was studied by carrageenin induced rat paw edema method. Emulgel containing crodomol as a permeation enhancer exhibited higher anti inflammatory activity than conventional gel and marketed product.

KEY WORDS: Microemulsion gel; Diclofenac diethylamine; Anti inflammatory activity.

INTRODUCTION
Topical administration of therapeutic agents offer many advantages than oral and intravenous administrations[1]. Several techniques have been explored to increase the penetration of drugs, including the use of enhancers, such as surfactants/solvents [2-3], essential oils and terpens [4], lipids [5-7], various forms of lipid vesicles such as liposomes, niosomes [8-10] and ethosomes [11-12] and transfersomes [13]. In most lipid formula-lipid aggregates or applied together with a lipid suspension in a hydrogel or more frequently, in an oil-in-water emulsion [14]. Diclofenac, a phenyl acetic acid derivative, is a potent member of the non-steroidal anti-inflammatory drugs (NSAIDs), which, due to its gastrointestinal disturbances, is topically administrated in the form of a 1.16% gel [15].

NSAIDs increases the relative risk of upper gastrointestinal tract bleeding is elevated in the elderly and it may be even higher for certain NSAIDs. In an attempt to reduce the relatively high incidence of serious adverse effects associated with the systemic use of NSAIDs, a growing number of topical formulations of these drugs have become commercially available. These topical formulations, either on their own or as adjuncts to reduced dosage of systems has proven to be useful in the management of a variety of musculoskeletal rheumatic diseases. Although the topical NSAIDs have mainly been studied regarding their transdermal diffusion kinetics, these might also have the applications when applied to the stratum corneum. Because of the rate-controlling effect of stratum corneum, the attainment of much-desirable zero-order drug delivery was relatively easy through skin. Prolonged exposure to a steady concentration of a drug could make the body immune to its beneficial therapeutic effects by inducing tolerance, so attempts were made to develop systems that would release variable amount of drugs at different time intervals, and the technique of enhancer depletion was attempted.

Diclofenac diethylamine (DDE) is a cyclooxygenase-2 inhibitor that is used in the management of rheumatoid arthritis, osteoarthritis, pain, and dysmenorrhea. DDE is freely soluble in methanol, alcohol and sparingly soluble in water. Because of its limited solubility in water the drug is incorporated in to microemulsion vehicles with hydrophilic polymers to enhance the solubility of drug and to get faster anti-inflammatory activity without any side effects.

The purpose of the present work was to formulate and evaluate a microemulsion-based gel containing DDE to enhance its permeation through skin using and its anti-inflammatory activity crodomol as a permeation enhancer.
and isopropyl myristate and propylene glycol are solvents to solubilise the diclofenac without using isopropyl alcohol and compare their in-vitro performance with that of a brand formulation (Voveran Emulgel®).

MATERIALS AND METHODS

Materials:

Diclofenac diethylamine (DDE) was obtained as a gift sample from Arti drugs, Crodomol procured from Croda chemicals, Triethanolamine (TEA), and carrageenan were purchased from S.D. Fine chemicals (Mumbai, India.). Propylene glycol, Carbomer ETDT2020, Cetomacrogol 1000, Liquid paraffin, Isopropyl alcohol (IPA), Isopropyl myristate (IPM), Cetosteryl alcohol, Methylparaben and dialysis membrane (MW cutoff 12,000) were purchased from Hi-Media Laboratories (Mumbai, India.). All the other chemicals and reagents used were of analytical grade samples.

Methods:

The emulsion gel that contains Diclofenac diethylamine within this formulation was prepared as follows for 50g (as drug content in 100g is 1.16) shown in Table 1&2. 0.58 gm of drug was dissolved in 5g isopropyl alcohol and 2.5 g of propylene glycol. Methylparaben and fragrance are mixed in suitable recipient. Carbomer 0.35gm was swelled in water and heated at 65ºC. 1.25g cromodol, 1g cetomacrogol 1000, 1g liquid paraffin were melted at 65ºC. This mixture was added to carbomer gel and stirred continuously to get homogenous phase and colourless emulsion gel and finally drug solution is added and the emulsion gel formulation was neutralized to the pH 7.2 with alkali.

Measurement of globule size:

Prepared microemulsions were analysed for their oil globule size by laser light scattering particle analyser (Master sizer 2000, Malvern, UK) in which the equipment was previously calibrated with polystyrene nanoparticles 100nm in size. The instrument was able to measure globule sizes ranging from 20nm to 2,000nm. Emulsion samples were diluted with 100mL of distilled water and charged into a wet sample holder. The instrument calculated globular sizes based on the scattering by laser light and by using a refractive index of IPM (1.435).

Spreadability:

Spreadability of conventional gel (CG) and microemulsion based gels (MEG) was measured in terms of diameter of gel circle produced when placed between two glass plates of defined weights. Weighed quantities (350mg) of emulsion-based gels or CG was taken on one glass plate and another glass plate was dropped from a distance of 5 cm. The diameter of the circle of spread gel was measured and mean was taken by repeating the experiment three times. The voveran Emulgel (Novartis Pharma) was considered as reference standard.

The pH of the gel formulations was determined by using a pH meter (7.0). The measurement was performed at 1, 30 and 60 days after preparation to detect any fluctuation with time.

In vitro permeation of DDE through the dialysis membrane:

In vitro permeation of DDE through dialysis membrane was studied using Franz diffusion cell. The dialysis membrane was cut in to small circular patches and fixed onto the donor compartment of the Franz diffusion cell. The ready-made donor compartment was checked immediately for possible leaks by taking distilled water before the in vitro drug release study. A weighed quantity of gel was spread on the donor compartment. The receiver compartment consists of phosphate buffer solution at pH (7.4) as the dissolution maintained at 37ºC by a circulating water bath (Grant, Model GR150, UK.). The dissolution media was constantly stirred at 100 rpm using magnetic stirrer (Jenwey, Model 1103, UK). Aliquots (5mL) were withdrawn at specific time intervals and replaced with the same volume pre warmed at 37ºC. Samples were analysed for DDE content by measuring the absorbance at 285nm using UV visible spectrometer.

Permeation data analysis:

Average values of three readings of in-vitro permeation data were calculated and the average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot was calculated as flux (J_\text{SS}) [Li et al., 2002] and the permeability coefficient was calculated using Equation 1:

$$Kp = J_{\text{SS}} / CV$$

$$Kp = J/C$$

Where,

J is flux, Kp is permeability coefficient, and CV is total amount of the drug.

The enhancement of drug penetration due to emulsion-based formulations was noted as enhancement factor (EF), which was calculated using equation 2.

$$EF = Kp(\text{MEGs}) / Kp(\text{CG})$$
In-vitro anti-inflammatory activity:
In-vitro anti-inflammatory activity studies of microemulsion gel and CG formulations were carried out using carrageenan-induced paw edema in albino rats. Albino rats of either sex weighing 160-220gm were fasted over night but provided with water. They were divided in to three groups: Control, Standard (treated with CG), and Test (treated with MEG) each of five animals. MEG and CG equivalent to 1.16mg of DDE/kg were applied topically on the right hind paw of rats of test and standard groups, respectively. For control rats, the gel base with out drug was applied. After 15min, 1% carrageenan was injected(100ml) in the right hind paw of rats to induce inflammation. The volume of paw of rats of control(Vc) and treated(Vt) groups at different intervals of time was measured by using plethysmograph.
The % inhibition of edema volume was calculated using Equation 3 with standard error.

\[
\text{% inhibition} = \frac{(V_c - V_t)}{V_c} \times 100
\]

Results and discussion:

Physical Examination:

In F1 formulation the drug was not dissolved due to insufficient quantity of IPA, carbomer enhances the viscosity of the gel and alteration in pH of the drug. The drug in F2 & F3 formulation was not dissolved in the mixture of IPA and PG and the consistency of gel also not desirable due to low quantity of carbomer. Formulation F4 the drug dissolved in the mixture of IPA and PG along with crodomol which increased the solubility carbomer gave good consistency to the gel. The addition of sodium hydroxide adjusted the pH to 7.2. F5, F6 the drug dissolved in the mixture of IPM because it has both solubilising and emulsifying property but when observed under microscope crystals were observed with F5, it might be due absence of active solvent. F7 containing drug dissolved in PG and pH was adjusted with 30% NAOH it gave smooth transparent gel. F8 formulation drug dissolved in PG and melted cetomacrogol along with crodomol. The drug was clear when observed under microscope. The prepared emulgel formulations were white viscous creamy preparations with a smooth and homogenous appearance, easily spreadable with acceptable bioadhesion and fair mechanical properties. The pH values of all the prepared formulations ranged from 7.0 to 7.2, which are considered acceptable to avoid the risk of irritation upon application to the skin. From the physical appearance and other parameters F6, F7, F8 formulations were used for invitro release studies.

Microbiological assay:
The use of control plates showed that the plain emulgel bases were microbiologically inert towards the test pathogens, total viable count, total yeast and mould count strain.

In-vitro release studies:

The in vitro release profiles of DDE from F6, MKT & F7 formulations are represented in Fig 1. It was observed that the release of the drug from its all emulgel formulations was higher than its release from its commercial product. The release of the drug from its emulgel formulations can be ranked in the following ascending order: F6 > F7 > MKT, where the amounts of the drug released after 3 hours were 99%, 84%, 69%, respectively. Thus the greatest release was observed with Formulation F6.

This finding may be due to the presence of crodomol (medium chain triglycerides) and the emulsifying agent in its high level which leads to an increase in the lipophilicity-hydrophilicity of the emulgel and diffusion of the drug from the emulgel. The presence of liquid paraffin led to retardation of diclofenac diethylamine release from its emulgel formulation. The lower drug release in MKT product is the solubility of the drug is done by incorporation of isopropyl alcohol along with propylene glycol which increased interfacial tension between the oil phase of the drug and increase the solubilisation of the drug, as time being where the alcohol evaporated and the drug release is lower as shown in Fig 1. The drug was precipitated and irritates the skin upon contact with the skin for a long period.

The steady state mean flux and diffusion coefficient of F6, F7 & MKT is shown in Table 2. F6 is having more steady state mean flux and diffusion coefficient than compared to F7 and MKT product. This finding indicated that the enhancing effect of the emulsifying agent on the drug release was more pronounced than the lowering effect of the liquid paraffin on the drug release. The drug release data was analysed according to zero and first-order kinetics as well as diffusion controlled mechanism using linear regression analysis.

Stability studies:
The formulation trial F6 was packed and stored at 40°C/75%RH, 30°C/65%RH. After one month the samples were analysed for appearance, pH, assay and in-vitro dissolution, anti-inflammatory activity up to 3 months. The results show the samples kept at higher temperature and humidity conditions in 3 months study were stable. The formulation F6 was stable at various stress as well as ambient temperature and its in-vitro release profile is comparatively similar with marketed sample.
Statistical analysis:

The cumulative % of DDE released from gel F6 and marketed sample in dissolution medium at 3 hrs after storage were compared and the statistically significance was tested, the student t-test showed that the two-tailed P value is 0.0003, t = 5.3 considered extremely significant.

Anti inflammatory activity studies:

The percent inhibition of carrageenan-induced edema formation by F6 and marketed gel shown in Fig 2. The mean volume of rat paw edema for the control group was 0.025, 0.056, 0.2, 0.332 ml at 30min, 1, 2, 3hr, respectively. Where as conventional form were comparatively less 0.016±0.032, 0.149, 0.19 and the test formulation shows 0.02, 0.032, 0.11, 0.054ml respectively indicating that the formulation has more anti-inflammatory activity than control and conventional formulations. The F6 inhibited 73% of the carrageenan-induced edema formation, while the marketed formulation exhibited 42% reduction in swelling at the end of the study. F6 containing 0.58% diclofenac diethylamine showed percent inhibition of edema after topical application of 0.58% diclofenac diethylamine gel at various times intervals between 0-3 hrs prior to the carrageen injection, initially upto 120 min at (2 hrs) the standard group showed greater percent edema inhibition (68±0.0074 at 2 hrs) and then effect reduced significantly with time at 180 min (3hrs). The test group percent inhibition was slow almost all test points initially, however with time upto 3hrs the percent inhibition gradually increased to 73±0.0016.

On the basis of these results, it can be conclude that greater anti-inflammatory activity was achieved with the F6 than marketed product, the increase in crodomol content solubility of drug with propylene glycol and melted cetomacrogol 1000 gives maximum percent edema inhibition. The increase in edema volume is lower in F6 when compared to marketed product. This finding is due to evaporation of alcohol in MKT and precipitated the drug hence showed percent inhibition in MKT is lower than F6.

Statistical analysis:

The percent inhibition of carrageenan-induced edema formation by F6 and marketed gel at 3 hrs were compared and the statistically significance was tested, P<0.01 was considered significant. The t value of test versus control is 6.1 which is compared with t-table; P<0.01, the test results are more significant.

Conclusion:

Invitro drug release and anti-inflammatory studies shown that superior drug release rates, diffusion coefficients and percentage edema inhibition from F6 compared to brand formulation. In absence of IPA in F6 formulation peeling effect is also not observed, hence it was considered as optimised formulation.

Acknowledgements:

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References:

Table 1 Composition Of Formulations

<table>
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<tr>
<th>Formulation code</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
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<th>F5</th>
<th>F6</th>
<th>F7</th>
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</table>

Water upto 50g in all formulations

Table 2 Steady state mean flux and diffusion coefficients of F6,F7 & MKT

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cumulative amount at 3 hrs (Q_{3h}, mg/cm²)</th>
<th>Mean Flux (±SEM) (mg/min)</th>
<th>Diffusion coefficient (cm²/sec)</th>
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<tbody>
<tr>
<td>F6</td>
<td>0.011397(±0.000136)</td>
<td>9.516 (±0.20897)</td>
<td>4.01744</td>
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<tr>
<td>MKT</td>
<td>0.010306(±1.97321)</td>
<td>3.66 (±2.9194)</td>
<td>0.16870</td>
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<tr>
<td>F7</td>
<td>0.010317(±0.000308)</td>
<td>4.4366(±2.226)</td>
<td>3.17233</td>
</tr>
</tbody>
</table>

Figure 1 In vitro drug release profiles of F6, F7, Marked product (MKT)
Figure : 2 Mean % edema inhibition v/s Time

LEGENDS
Table :1 Composition Of Formulations
Table:2 Steady state mean flux and diffusion coefficients of F6,F7 & MKT
Figure:1 In vitro drug release profiles of F6,F7,Marketed product(MKT)
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