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Effect of Permeation Enhancers on the Release and Permeation Kinetics of Oxytetracycline Hydrochloride Organogel Formulations

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ABSTRACT

Oxytetracycline HCl is an antibacterial drug used in the treatment of acne vulgaris and dermatitis. Its oral bioavailability is intermediate i.e., 60% and it is rapidly excreted through renal route. This drug has many side effects such as epigastric pain, nausea, vomiting, diarrhea, hypersensitivity, and tooth discoloration when taken orally. To overcome the side effects and to increase the bioavailability of Oxytetracycline HCl, topical formulations were prepared. Organogels were prepared using sorbitan monostearate as gelling agent and isopropyl myristate as vehicle with different permeation enhancers. The prepared formulations were evaluated for drug content, viscosity, extrudability, homogeneity, skin irritation test, *in vitro* drug release, and antimicrobial activity. A formulation containing eucalyptus oil with isopropyl myristate, showed better *in vitro* permeation and higher antimicrobial activity as compared to other formulations with different penetration enhancers.

Key words: Eucalyptus oil, organogel, Oxytetracycline HCl, penetration enhancer

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INTRODUCTION

For a drug to be an effective topical agent, it should penetrate the skin as readily as possible. Drug delivery across the skin is a great challenge. The greatest obstacle to this is the stratum corneum, which is the outermost layer of skin and is considered as the primary rate limiting barrier to the permeation of the drugs across the skin. It consists of dead, flattened cells, filled with keratin embedded in a lipid matrix. Stratum corneum has been described as hydrophilic protein 'bricks' in a hydrophobic mortar. This barrier function of stratum corneum creates difficulties for the formulation of the scientists aiming to deliver drugs via the skin. The search for a solution to this problem led the investigators to employ several penetration enhancement techniques such as the use of penetration enhancers in the formulation.[1]

Oxytetracycline HCl is an antibacterial drug used in the treatment of skin disorders such as acne vulgaris and dermatitis. Its oral bioavailability is intermediate i.e., 60% and it is rapidly excreted through renal route. On oral administration it shows many side effects such as epigastric pain, nausea, vomiting, diarrhea, hypersensitivity, and tooth discoloration. To reduce the side effects and increase the bioavailabity, organogel formulations of Oxytetracycline HCl were prepared by using penetration enhancers.[2]

This study was aimed to evaluate the enhancing activity of different penetration enhancers i.e., propylene glycol,

polyethylene glycol, ethanol, isopropanol, n-octanol, and eucalyptus oil by applying them concomitantly with drug (Oxytetracycline HCl) to investigate the contribution of vehicle (isopropyl myristate) and accelerant to the observed enhancement.

MATERIALS AND METHODS

Materials

The following materials were used in the study: Oxytetracycline HCl (Siemen laboratories, Gurgaon, India); Sorbitan monostearate (CDH Pvt. Ltd., Mumbai); Isopropyl myristate (Qualikems Fine Chemical Pvt. Ltd., New Delhi); Eucalyptus oil and n-octanol (CDH Pvt. Ltd., New Delhi); Ethanol (Changshu Yanguan Chemical, China); Himedia Dialysis membrane-150 (Himedia laboratories Pvt. Ltd., Mumbai).

Preparation of organogels

Organogel formulation was prepared by a two-step method. First, organogel base was formed by melting the sobitan monostearate on a water bath at the temperature range of 60–70°C, adding isopropyl myristate to it with continuous stirring, and then allowing it to cool at room temperature; thus, forming an organogel base. Then drug (Oxytetracycline HCl) along with different penetration enhancers was added to this base to form the desired organogel. The ratios for the different formulation ingredients have been given in Table 1.[3]

EVALUATION

Viscosity

Brookfield viscometer DV II model attached with T-bar spindle (no. 94) was used for determination of viscosity. Organogel was filled in a beaker of suitable size and spindle was lowered perpendicularly taking care in such a way that spindle does not touch the bottom of beaker. The spindle was rotated at such a speed so as to generate the torque >30%. The viscosity of organogel was then obtained by multiplying the viscometer reading with the multiplication factor given in Brookfield viscometer catalogue.[4]

Drug content determination

A specified quantity (100 mg) of gel was shaken with 10 ml mixture of ethanol: PBS (pH 7.4) in 30:70 ratio. The resulting solution was filtered using Whatman filter paper. The filtrate was appropriately diluted and the drug content was determined by measuring the ultra violet (UV) absorbance of filtrate at 356 nm.[4]

Extrudability

Extrudability was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 sec from collapsible tube.[5]

Homogeneity

Homogeneity of various formulations was tested by visual observation and was ranked as follows:[4] +++ = Excellent, ++ = Very Good, + = Good, - = Poor.

Skin Irritation Test

The organogel containing drug was applied on the skin. The test gel and cotton swab covering it were secured firmly on the applied surface with the help of adhesive tapes. Then observations were made for any sign of erythema and ranked as follows as per the state of the applied site[5]: +++ = Severe erythema, ++ = Moderate erythema, + = Slight erythema, - = No irritation.

In vitro permeation studies through dialysis membrane

The *in vitro* diffusion study of the organogels was performed using semipermeable dialysis membrane (dry,

Table 1: Composition of oxytetracycline HCl organogel containing different penetration enhancers with nonaqueous phase i.e., isopropyl myristate

Ingredient (10 g organogel)	P1	P2	P3	P4	P5	P6	Control
Drug	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Isopropyl myristate	5.9	5.9	5.9	5.9	5.9	5.9	6.2
Propylene glycol	0.3						—
Polyethylene glycol		0.3					—
Ethanol			0.3				—
Isopropanol				0.3			—
n-octanol					0.3		—
Eucalyptus oil						0.3	—
Sorbitan monostearate	3.5	3.5	3.5	3.5	3.5	3.5	3.5

unwashed, pre-cut and open ended; flat width: 35 mm; inflated diameter: 21 mm; length: 30 mm), carried by using Franz diffusion cell. The membrane was soaked in phosphate buffer, pH 7.4 (PB) for 6–8 hours, and clamped carefully between donor and receptor compartment as shown in Figure 1. The recipient chamber was filled with 22 ml of ethanol: IPBS (pH 7.4) 30:70 solution. Ethanol was added to maintain sink condition and temperature was maintained at 37 ± 0.5°C by circulating water in surrounding jacket. 1 g of gel was evenly spread on semipermeable dialysis membrane, which was clamped between donor and receptor compartment. Air bubbles, if any, were removed carefully and medium was stirred by external driven Teflon coated magnetic bar. At predetermined time intervals, 2 ml of solution was pipetted out from the receptor compartment and immediately replaced with 2 ml fresh phosphate buffer. The drug concentration of the receptor fluid was

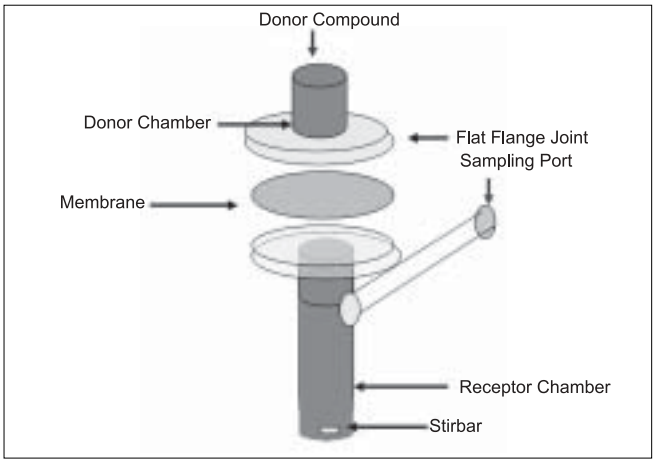

Figure 1: Franz diffusion cell

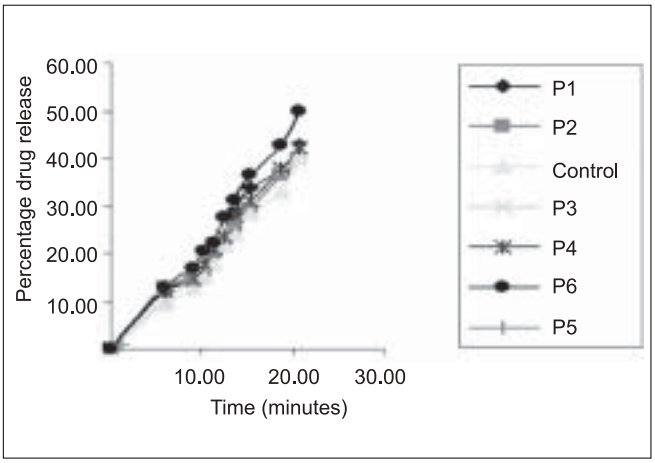

Figure 2: Comparative study of different penetration enhancers on percentage drug release of oxytetracycline HCl containing sorbitan monostearate, iso propyl myristate, and different penetration enhancers

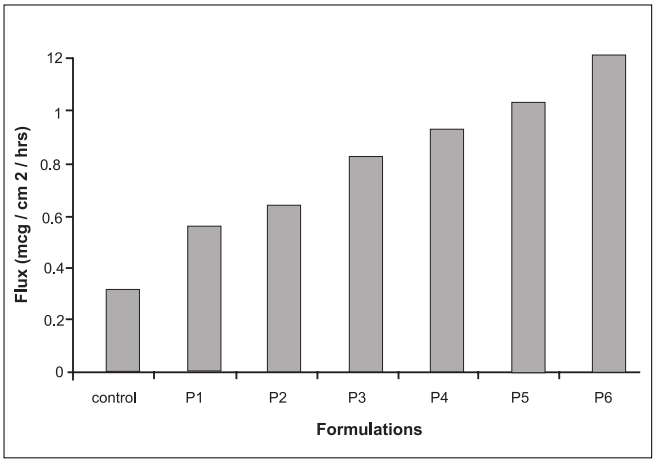

Figure 3: Flux for organogel formulation of sorbiton monosterate with isopropyl myristate along with different penetration enhancers

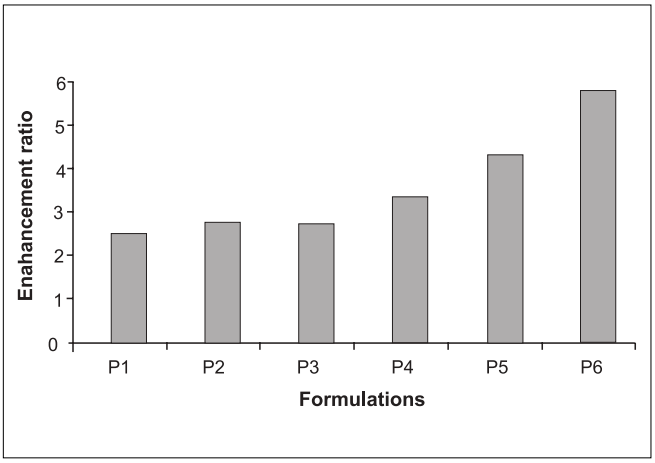

Figure 4: Enhancement ratio of organogel formulation containing sorbiton monosterate with isopropyl myristate along with different penetration enhancers

Table 2: Evaluation of organogel formulations with isopropyl myristate, sorbitan monostearate, and eucalyptus oil

Parameter	P1	P2	P3	P4	P5	P6	Control
Viscosity (Centi poise)	15,440	14,800	14,600	1,328	1,311	1,023	26,000
Extrudability	+	+	+	++	++	+++	+
Drug Content (%)	95.19 ± 0. 009	96.20 ± 0.01	97.21 ± 0. 04	97.61 ± 0. 009	98.31 ± 0.004	99.62 ± 0. 015	80.22 ± 0.013
Homogeneity	+++	+++	+++	+++	+++	+++	+++
Skin Irritation*	—	—	—	—	—	—	—

Key (homogeneity and extrudability) Key* (skin irritation), +++ Excellent, ++ Moderate erythema, ++ Very Good, +++ Severe erythema, + Good, + Slight erythema, — No Irritation

determined spectrophotometrically (Shimadzu, Tokyo, Japan) against appropriate blank solution.[6]

Antimicrobial activity

Antimicrobial activity was by ditch plate technique. This technique has been used for evaluation of bacteriostatic and fungistatic activity of compounds especially in semisolid preparations. Agar plates were prepared and sterilized as per standard procedure. A cup (2.5 X 0.5 cm^2) was made in the center of agar plates and formulations (0.5 g) were filled in the cup. The prepared culture loops were streaked across the agar at right angle from ditch to the edge of the plate. The organogel formulations containing different penetration enhancers were compared with control. The bacterial growth was observed and percentage inhibition was calculated as follows:[7]

Percentage Inhibition = L_2/L_1 X 100,

where L_1 = Total length of streaked culture, L_2 = length of inhibition.

Statistical analysis

The flux of the *in vitro* release studies was calculated using PCP Disso software. Flux at the end of 480 minute time period was used to compare the different formulations:

$$\text{Enhancement ratio (ER)} = \frac{\text{Flux of test formulation}}{\text{Fluctuation of control formulation}}$$

RESULTS AND DISCUSSION

Viscosity is an important parameter for characterizing the gels as it affects the extrudability and release of drug. Formulation P_6 showed the lowest viscosity and P_1 showed the highest viscosity. The results for viscosity, drug content, extrudability, homogeneity, and skin irritation of all the prepared organogel formulations are shown in Table 2. The drug content of the gel preparations were found to be the highest in P_6 formulation i.e., 99.62 ± 0.015. The drug content determination also showed that the drug was uniformly distributed throughout the gel. The viscosity of the gels was found in the range of 15,440 to 1,023 cps for different formulations. All the formulations were homogenous in texture and showed no skin irritation upon application for eight hours. Among all the formulations, P_6 formulation showed excellent extrudability .

The *in vitro* release profile of oxytetracycline HCl from its various organogel formulations are shown in Figure 2. It can be observed from the figure that the drug release from formulations containing penetration enhancers were higher than its release from control. The drug release from organogels containing penetration enhancers can be ranked in the increasing order as per their respective penetration enhancers as propylene glycol < polyethylene glycol < ethanol < isopropanol < n-octanol < eucalyptus oil. The nonionic surfactant (propylene glycol) showed significant enhancement compared to control formulation. Glycol is a sorption promoter and has high thermodynamic activity, which produces a driving force and thus carries the drug along with it by carrier mechanism; glycols when applied to skin also tend to partition into the skin and thereby promote the movement of drug. Alcohol shows greater release compared to glycol. Ethanol penetration enhancing effect can be attributed to two factors: (a) increase in thermodynamic activity due to evaporation of ethanol known as "push effect"; (b) "pull effect" in which permeation of drug molecules is increased due to reduction in barrier property. Propanol and octanol showed higher release rates than ethanol and this is probably because they contain longer carbon chain, which in turn increases their lipophilicity and hence partition coefficient, which in turn enhances their push and pull effect. The highest percentage of drug release and flux is shown by formulation containing eucalyptus oil as penetration enhancer. Eucalyptus oil contains 1,8-cineole as its main constituent and a series of 17-monoterpene and terpenoids that have proven penetration enhancement effect for hydrophilic drugs.[8]

The order of percentage drug release was: eucalyptus oil > n-octanol > isopropanol > ethanol > polyethylene glycol > propylene glycol. The result indicates that eucalyptus oil has the strongest penetration enhancement activity among the six penetration enhancers studied. When formulated in to organogel using isopropyl myristate with eucalyptus oil, the flux was found to be 1.18 and enhancement ratio was 5.57, which clearly indicates the synergistic effect [Figures 3 and 4]. There is also potential synergistic effect observed between octanol and isopropyl myristate, the flux was approximately 0.90, the enhancement ratio was 4.11. Thus, it can well be concluded that the amount of drug released and flux show potential synergism when formulated with isopropyl myristate, but in case of eucalyptus oil have penetration enhancement ability of the oil predominant.

The antimicrobial activity was ranked in the same order as that of *in vitro* drug release. The greatest activity was observed in formulation containing eucalyptus oil with oxytetracycline HCL where percentage inhibition reached up to 95.4% (*Staphylococcus aureus*) and less activity was shown by formulation containing propylene glycol with

oxytetracycline HCL where percentage inhibition was 50%. Hence, organogel formulation containing eucalyptus oil as penetration enhancer are more effective compared to other organogel formulations.

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