

## ***In Vitro* Drug Release Studies of Metronidazole Topical Formulations Through Cellulose Membrane**

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**Three different topical formulations namely gel, cream and ointment, each containing 1% w/w metronidazole, were prepared and *in vitro* permeation studies carried out. The permeation of metronidazole from each of the topical formulation was determined using dialyzing cellulose membrane in a dissolution tester. Glycerin, a permeation enhancer, was incorporated in varying concentrations and the amount of permeated metronidazole analysed using high performance liquid chromatography. The drug release was found to be greatest in the gel formulation followed by cream and ointment preparations in that order. Incorporation of glycerine enhanced drug release profile.**

**Key words:** Metronidazole formulation, *in vitro* studies, permeation, glycerine

### **INTRODUCTION**

The release of a drug from a topical product formulation plays an important role in percutaneous absorption. Transdermal drug delivery is a very important route by which several drugs and medicaments can be administered. In the formulation of dermatological products, there are three basic classes of components to be considered: aqueous, active drug powder and oil. The permutation and combination of members of these classes, along with auxiliary thickening agents and surfactant emulsifiers, led to a complex number of topical medications.

Metronidazole, 1-( $\beta$ -hydroxyethyl)-2-methyl-5-nitroimidazole, is a nitroimidazole antimicrobial drug. Nitroimidazoles are used to combat anaerobic bacterial and protozoal infections such as those of the skin, vagina, gastrointestinal tract, joints and respiratory

tract [1]. Topical application of metronidazole is effective in the treatment of dermatological conditions and has also been used effectively to treat fungating tumours, and other chronic wounds [2,3]. Metronidazole is poorly absorbed after topical application with only trace serum concentrations reported after topical use [4,5]. It is essential to increase the percutaneous permeability of the drug so as to promote and maintain effective drug levels intended for treatment.

An attempt has been made in the present work to develop suitable topical formulations of metronidazole by conventional method that could be easily used. The objective of this study was to investigate the release characteristics of metronidazole from prepared topical formulations and compare these release characteristics with a similar innovator product in the market.

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## MATERIALS AND METHODS

### Materials

Metronidazole USP was donated by Cosmos Pharmaceutical Ltd., Nairobi, Kenya. Hydroxyethylcellulose (Natrosol<sup>®</sup> 250 HEC, grade HHX) was purchased from Hercules-Aqualon Division, Wilmigton-DE, USA. Cellulose membrane was from Sartorius Ag,

Germany. The other chemicals and reagents used were of analytical grade.

### Preparation of gel, cream and ointment

The following topical preparations were formulated: 1% w/w metronidazole gel A and B, 1% w/w metronidazole cream, and 1% w/w metronidazole ointment (Table 1).

**Table 1: Formulae of topical formulations containing metronidazole**

Ingredients (% w/w)	Formulations			
	Gel A	Gel B	Cream	Ointment
Hydroxyethylcellulose	5%	5%	-	-
Metronidazole	1%	1%	1%	1%
Ethanol	5%	5%	-	-
Benzyl alcohol	1%	1%	-	-
Glycerine	1%	2%	-	-
Emulsifying ointment	-	-	30%	-
Chlorocresol	-	-	1%	-
Emulsifying wax	-	-	-	30%
White soft paraffin	-	-	-	49%
Liquid paraffin	-	-	-	20%
Distilled water	q.s	q.s	q.s	q.s

### pH of the topical formulations

A digital glass electrode pH meter (pH 211, Hanna Instruments, Italy) was used to determine the pH of the topical preparation. The pH values were determined by inserting electrode of the pH meter into the topical formulations and allowing it to equilibrate for 1 min. Three reading were taken randomly from different zones of the topical formulation which was packed in round glass jar.

### Method of drug analysis

Metronidazole was analysed by validated high performance liquid chromatography (HPLC) method on a Merck Hitachi L7 series system using a UV detector set at 254 nm and a HPLC column (RP 4.6 mm × 25 mm with 5 μm L7 packing). The mobile phase consisted of 0.01 M monobasic potassium phosphate (pH 4) and absolute methanol (35:65% v/v) delivered at a flow rate of 1 ml/min. A formulation containing an equivalent of 3 mg

of the drug was weighed accurately and placed into a dry 50 ml volumetric flask and dissolved in 25 ml mobile phase. The contents were shaken using a mechanical stirrer for 20 min and diluted to volume using the mobile phase. The contents were further stirred for 1 h and filtered through 0.45  $\mu\text{m}$  nylon filter. The filtrate was analyzed by injecting 20  $\mu\text{l}$  into the HPLC system. Metronidazole content was determined from a standard curve prepared using different concentrations (1-60  $\mu\text{g/ml}$ ) of metronidazole reference substance.

### ***In vitro* diffusion studies**

The cellulose membrane was cut into suitable size and soaked in distilled water overnight to hydrate and soften it. Hydrated cellulose membrane was used to simulate the epithelium layer of the skin. The *in vitro* diffusion studies were carried out using the Erweka DT6 (Germany) dissolution apparatus. A weight of 1.8 g of drug loaded topical formulation was placed on marked area of 2  $\text{cm}^2$  on a microscopic glass slide. This was completely covered with a hydrated cellulose membrane and firmly secured at the sides. The baskets were filled with 900 ml of potassium phosphate buffer, pH 7.2, which was used as the dissolution medium and the temperature maintained at 37°C. The slides were/ placed in the baskets and the speed of stirrer set at 100 rpm. The samples were prepared in triplicate for all the topical formulations as well as the innovator gel.

Aliquots of 10 ml were withdrawn at predetermined intervals and similar quantities replaced with fresh dissolution fluid to maintain sink conditions. The drug content in each drawn sample was assayed using HPLC method as mentioned above. All experimental units were evaluated in triplicate (n=3).

The mechanism of drug release from the topical formulations was studied from the drug release data using the modeling equations as follows:

For zero order kinetics [6]

$$Q = K_1t \quad (\text{Equation 1})$$

First order kinetics [7]

$$\text{Log } Q_0 = K_2t/2.303 \quad (\text{Equation 2})$$

Higuchi's square root of time diffusion equation [8]

$$Q = K_3t^{1/2} \quad (\text{Equation 3})$$

Where:

Q is the percentage drug released and  $Q_0$  is the percentage of drug unreleased at time t;  $K_1$ ,  $K_2$  and  $K_3$  are the release rate constants for zero order kinetics, first order kinetics and Higuchi's square root of time diffusion equation, respectively.

## **RESULTS AND DISCUSSION**

Three different topical formulations were prepared and compared with the innovator gel that had 1% w/w of metronidazole. The physical characteristics of the three different topical formulations are as follows: The gels were smooth, shiny, clear and transparent, with a yellow colouration due to the drug and easily spread on the skin to form a thin water soluble layer. The ointment was thick with a speckled yellow colouration and easily spread to form an oily film on the skin. The cream formulation was less viscous than the ointment and easily spread on the skin to form a water washable oily layer.

The surface pH of the topical formulations is as shown in Table 2. The surface pH values for the topical formulations were found to be close to neutral and were between 6.9 and 7.1. The pH range of the topical formulations thus indicated that they were non-irritant to the skin.

Upon analysis, all the prepared topical formulations exhibited uniform drug content comparable to the innovator gel. The drug content in the prepared topical formulations ranged from 92% to 97% (Table 2). The United States Pharmacopeia requires the

content of metronidazole gel to be 90.0% to 110% of the label claim [9].

**Table 2: Surface pH and percent content of metronidazole**

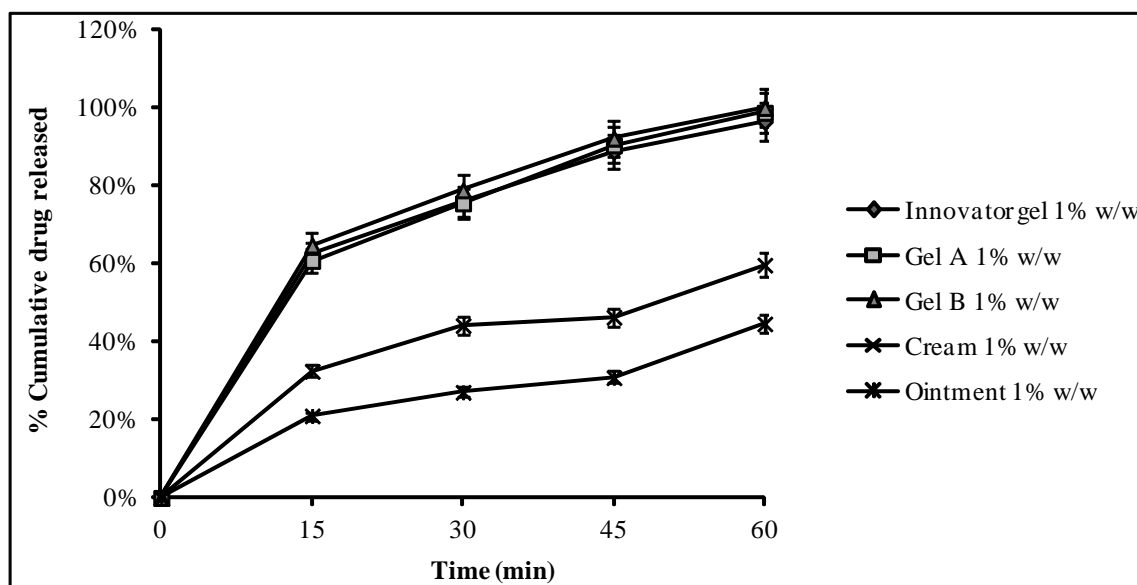
Formulation	Surface pH Mean $\pm$ SD	Drug content mean $\pm$ SD (%)
Gel A	7.10 $\pm$ 0.05	97.4 $\pm$ 0.5
Gel B	7.10 $\pm$ 0.05	97.3 $\pm$ 0.6
Cream	6.92 $\pm$ 0.15	96.7 $\pm$ 0.6
Ointment	7.10 $\pm$ 0.45	92.0 $\pm$ 0.5
Innovator gel	7.20 $\pm$ 0.05	97.4 $\pm$ 0.1

SD = standard deviation.

The *in vitro* release of metronidazole across the cellulose membrane from cream and ointment was only 59.59% and 44.55%, respectively, while gel A and gel B released

98.60% and 99.85% of metronidazole at the end of 1 h. The innovator gel released 96.29% of the drug at the end of 1 h. The poor release of metronidazole from the cream and ointment bases used in the formulation that did not allow the drug to diffuse from the base into the dissolution media. This could also be as a result of the cream and the ointment bases having more affinity towards the metronidazole drug molecule.

In general, it is the nature of the vehicle used that influences the rate and extent of drug release [10,11]. The release of the drug from the dosage form is also influenced by the physicochemical properties of both the vehicle and the drug. Gel B released more drug at the end of 1 h as compared to gel A and this is evidently due to the increase in glycerine content from 1% w/w to 2% w/w.



**Figure 1. *In vitro* release profiles of metronidazole through cellulose membrane from the different formulations.**

The regression data obtained from the kinetic indicated that the data fitted better to Higuchi's square root of time equation which

gave higher linear regression coefficient,  $r^2 \geq 0.98$ , for all the formulations (Table 3). This meant that the drug release from the matrix of

the polymer used for the topical formulations was due to diffusion. However, the cream and ointment did not follow the first order

kinetics but had higher linear regression coefficient values with Higuchi's square root equation (Table 3).

**Table 3: Kinetic data from *in vitro* release studies for metronidazole topical formulations**

Formulation	Zero order		First order		Higuchi's square root	
	Release rate constant	r <sup>2</sup>	Release rate constant	r <sup>2</sup>	Release rate constant	r <sup>2</sup>
Innovator	0.019	0.9017	0.049	0.9024	0.133	0.9878
Gel A	0.019	0.9168	0.054	0.9174	0.134	0.9927
Gel B	0.020	0.9021	0.049	0.9028	0.138	0.9880
Cream	0.011	0.9325	0.062	0.9372	0.076	0.9911
Ointment	0.008	0.9603	0.079	0.9604	0.052	0.9821

r = coefficient of linear regression.

Skin permeation enhancers are added to topical formulations to increase the skin permeability by reversibly altering the physicochemical nature of the stratum corneum to reduce its diffusion resistance. Penetration enhancers increase skin permeability by interacting with intercellular lipids and/or denaturing proteins [12]. Glycerine probably acted as a permeation enhancer by increasing the drug solubility by co-solvency effect [13]. Moreover, glycerine or glycerols are used in topical formulations for their emollient effects. Emollients are agents that keep the moisture intact on the skin and keep the skin soft. This moisture on the skins keeps the skin hydrated for a longer period of time by occlusion and thereby loosens the intercellular lipid layer and enhances permeation of drug molecules [14,15].

## CONCLUSION

From this study, it is discernible that metronidazole gels can be successfully prepared using polymers like hydroxyethylcellulose with simple permeation enhancers such as glycerine at 2% w/w concentration. The gels also possessed

acceptable aesthetic properties for patient use than the cream and ointment ( $p < 0.05$ ).

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