

## Effect of Vehicles on *In vitro* Release of Metronidazole Through Different Membranes

### Taşıyıcıların Farklı Membranlardan *İn vitro* Metronidazol Salımına Etkisi

Mine Özyazıcı<sup>\*</sup>, Esra Baloğlu, Sinem Y. Karavana, Özgen Özer and Tamer Güneri

Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100-Bornova, Izmir, Turkey.

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#### Abstract

The invitro release tests is a measure of inprocess control and also as a finished product specification for creams, ointments and gels. The objective of this study was to evaluate the in vitro release and ex-vivo percutaneous absorption of Metronidazole (MTD) from different water soluble and oleaginous vehicles. All of the experiments were conducted using three different barriers (synthetic membrane, excised rat and rabbit skins). Within the barriers tested rat skin was found to be the best alternative to the others. Moreover water soluble vehicles could be suggested as good candidates for the topical delivery of MTD, giving higher drug release.

**Keywords:** Metronidazole, Topical delivery, *In-vitro* release, *Ex-vivo* release, Diffusion barrier.

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#### Introduction

Stratum corneum is the principal barrier for cutaneous penetration allowing slow absorption for the majority of drugs. In any case, the permeability of the stratum corneum is increased by using appropriate vehicle. It is generally assumed that the nature of the vehicle, selected strongly influences the rate and extent of drug release. Release may be improved by selecting the appropriate vehicle. The effect of

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<sup>\*</sup>Corresponding author e-mail: mine.ozyazici@ege.edu.tr

the vehicle on the release and the therapeutic activity of drugs have been investigated by various authors (Güngör and Berğişadi, 2004; El Gendy *et al.*, 2002; Özsoy *et al.*, 2004). The best vehicle for topical use has been described as the one which contributes a reversible decrease in the stratum corneum resistance and allows the diffusion of molecules into the vehicle itself (Ferreira *et al.*, 1995a).

Metronidazole (MTD) is a 5-nitroimidazole derivative used in the treatment and prophylaxis of various anaerobic infections (Nau *et al.*, 1998; Lopez-Nigro, *et al.*, 2003). Patients with rosaceae are treated with either MTD ointments or tablets (Campos-Aldrete and Villafuerte-Robles, 1997). Hence the efficacy of topical MTD has been reported and studied by some investigators (Ferreira *et al.* 1995a; Ferreira *et al.* 1995b).

The aim of the present study was to compare and investigate the influence of vehicles on release of MTD from different topical formulations *in vitro*. *Ex-vivo* permeation studies through a synthetic membrane, excised rat and rabbit skins were also evaluated.

## Materials and Methods

### Materials

MTD was provided by Ibrahim Ethem Ulugay Company, Turkey. All of the chemicals were of analytical grade.

Cellophane membrane (thickness, 30 $\mu$ m, extra corporeal EX-29, USA) was rinsed with distilled water and soaked into the receptor liquid (0.05 M phosphate buffer; pH 7.4) for 1 hour before starting the experiment (Santoyo *et al.* 1996).

New Zealand white rabbits (NZ 2.5-3kg) and Swiss Albino rats (SA 300-350g) were obtained from the Ege University, Veterinary Laboratories. Investigations on animals were carried out with the approval of the Ege University, Ethical Committee for Animal Research (Document No: 2001-04). For *ex-vivo* studies the abdominal hair of the animals were shaved and the skin samples were cut in full thickness, removed and washed with water. Fat and connective tissues were carefully removed and the skin was left in contact with receptor liquid (0.05 M phosphate buffer; pH 7.4) for 1 hour (Bozkır and Yüksel, 1995). Thickness of the NZ rabbit and SA rat skins were measured found between the range of 1.62 $\pm$ 0.03 mm and 0.59 $\pm$ 0.04 mm respectively.

### Preparation of formulations

The composition and codes of the formulations were shown in Table 1. The oleaginous (white ointment-F1, cold cream-F3) and water-soluble bases (stearate ointment-F2, polyethylene glycol ointment-F4) were prepared according to the USP monographs.

All formulations were prepared by simple melting method. Aqueous and oil phases were separately heated at 70 $\pm$ 5 $^{\circ}$ C, mixed together with continuous stirring. After

cooling ( $40-50 \pm 5^\circ\text{C}$ ) was added MTD and a homogeneous and uniform mixture was obtained.

Stability tests on the formulations F1-F4 were carried out at  $25 \pm 2^\circ\text{C}$  and  $40 \pm 2^\circ\text{C}$  macroscopically.

### *Solubility*

The excess amount of MTD was added to 10 ml of pH 7.4 phosphate buffer and placed in an ultrasonic water bath for 24 hours. The samples withdrawn were filtered through a  $0.4\mu\text{m}$  filter and assayed spectrophotometrically at 320 nm ( $n=6$ ).

**Table 1.** The codes of the formulations containing 1% MTD.

Formulation	F1	F2	F3	F4
Beeswax	50 g		120 g	
White vaseline	950 g			
Stearic acid		200 g		
Cetyl alcohol		10 g		
Spermaceti wax		10 g	125 g	
Glycerin		50 g		
Potassium hydroxide		10.5 g		
Vaseline oil			560 g	
Sodium borate			5 g	
Polyethylene glycol 400				60 g
Polyethylene glycol 4000				40 g
Distilled water		719.5 g	190 g	

### *Partition coefficient*

The partition coefficient ( $P_o$ ) of MTZ (Kim *et al.*, 2001; Mrestani *et al.*, 2004; Gao *et al.*, 2005) was determined in aqueous phosphate buffer (pH 7.4) pre-saturated with n-octanol. The drug was dissolved in this buffer at concentrations of  $10^{-2}$  M. The aqueous phase:n-octanol ratio was adjusted to 1:1. The mixture was agitated for 6 h, at 600 rpm. After separating the phases by centrifugation, samples, before and after partition, were quantified by using UV-vis spectrophotometer, at 320 nm ( $n=6$ ). The partition coefficient in water was also determined according to the method mentioned above.

### *In vitro release and permeation*

The dialysis cell method was used to determine the amount of the drug diffused from different ointment formulations (Roseman, 1981). The tubes were covered with either a cellophane membrane or excised abdominal rat and rabbit skins. The tubes were immersed into a 100 ml beaker containing 50 ml the receptor phase (0.05M phosphate buffer; pH 7.4). The receptor phase was continuously stirred with a small magnetic bar at a speed of 100 rpm during the experiments to ensure homogeneity and maintained at  $37 \pm 0.5^\circ\text{C}$  (Santoyo *et serial al.*, 1996). After the application of the formulations (F1-F4) on membranes, serial sampling was

performed serial sampling at specified time for 8 hours. The samples were then assayed spectrophotometrically (Shimadzu UV-1208, Japan) at 320 nm (Ferreira *et al.*, 1995b). The determination was carried out in triplicate and the calibration curve was used for the determination of the amount of MTD dissolved.

Permeability coefficients of the formulations were calculated according to Fick's Law (Higuchi, 1962);

$$Q = P \cdot A \cdot C_0 \cdot t$$

Where P is the permeability coefficient (cm/s), Q is the amount of the drug released (mg); A is the area of the diffusion membrane (cm<sup>2</sup>); C<sub>0</sub> is the initial concentration of the drug in the formulations (mg/cm<sup>3</sup>); and t is the time (Shah, 1993; Çelebi *et al.*, 1993).

#### *Statistical Analysis*

Tests for significant differences between means were made by analysis of variance (ANOVA). Reference to significant difference denotes that the test was carried out at the level  $p < 0.05$ .

## **Results and Discussion**

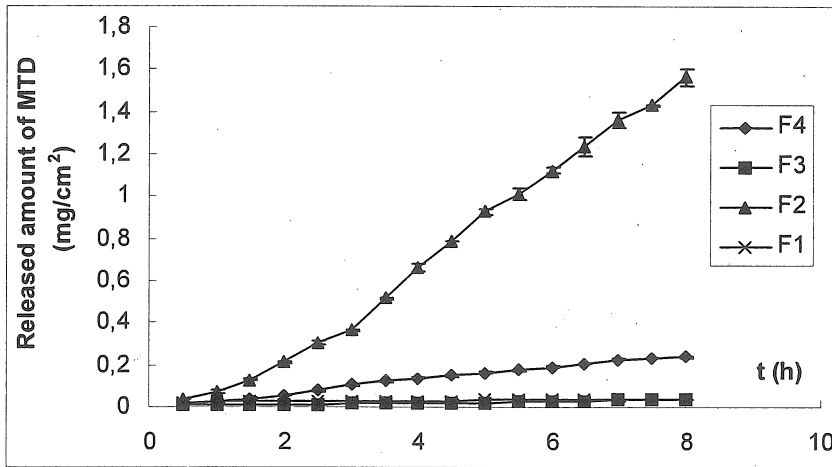
The release of the from the dosage forms depends directly on the physicochemical properties of the vehicle and the drug employed. Solubility of the drug is one of the most important physical properties that affect the release in both base and its surrounding medium (Gürol *et al.*, 1996). The solubility of MTD in phosphate buffer (pH 7.4) was found as  $11.1 \pm 4.8$ , indicating that the solubility of the drug in this medium did not constitute a limiting factor in the absorption process. Lipophilicity is a very useful physicochemical parameter reflecting the transfer properties of a compound. The aqueous phase:n-octanol partition coefficient (P<sub>o</sub>) is commonly used in the pharmaceutical industry to reflect the lipophilicity of a drug (Comer and Tam, 2001). The partition coefficient of MTD was calculated as  $0.560 \pm 0.2$  and  $0.689 \pm 0.1$  in water and phosphate buffer (pH 7.4), respectively. According to the data, MTD was found to have more affinity to phosphate buffer than water.

Macroscopical observations on formulations F1-F4 to check for sign of phase separation or microbial contamination showed no physical changes were observed during the period of 3 months. The physical aspects and homogeneity of the samples were not altered.

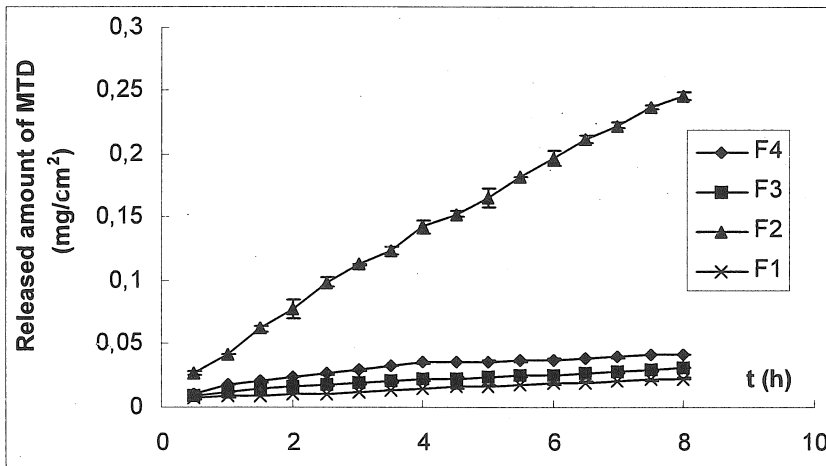
Different release profiles of MTD were observed due to the vehicles examined. According to the release results, the highest drug release obtained with F2 formulation calculated as  $1.563 \pm 0.040$ ,  $1.006 \pm 0.022$  and  $0.245 \pm 0.002$  mg/cm<sup>2</sup> for cellophane membrane, excised rabbit and rat skins, respectively. Released amount of MTD was dependent on the solubilizing effect of the vehicle.

The effect of vehicles on the released amount of MTD was found different. However, no significant difference was observed between the oily based vehicles.

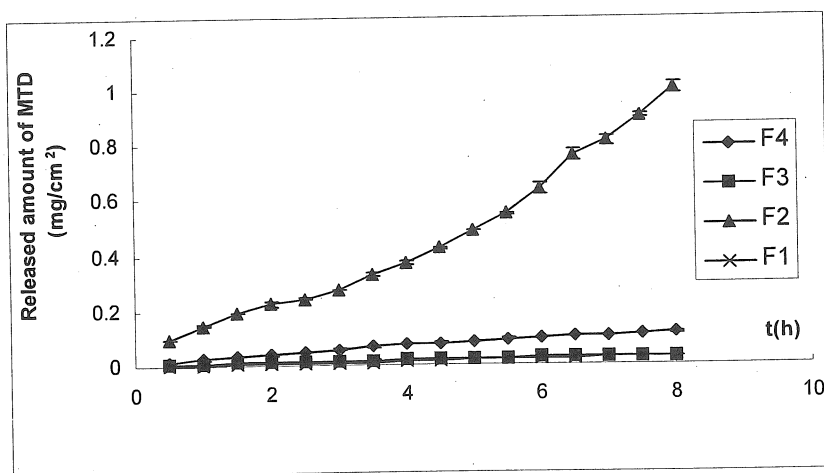
The release profiles of MTD from different formulations through different membranes were shown in Figures 1-3 and Table 2.



**Figure 1.** The release profiles of MTZ from different formulations through cellophane membrane. All samples were run in triplicate. Values are means  $\pm$  SD, in some cases the error bars are smaller than the symbols.



**Figure 2.** The release profiles of MTZ from different formulations through rat skin. All samples were run in triplicate. Values are means  $\pm$  SD, in some cases the error bars are smaller than the symbols.



**Figure 3.** The release profiles of MTZ from different formulations through rabbit skin. All samples were run in triplicate. Values are means  $\pm$  SD, in some cases the error bars are smaller than the symbols.

**Table 2.** Drug release of the formulations (n=3) ( $p < 0.05$ ).

Formulations	Diffusion barrier	Amount of drug released $\pm$ SD (mg/cm <sup>2</sup> )	J <sub>ss</sub> (mg/cm <sup>2</sup> /h)	r <sup>2</sup>
F1	Cellophane	0.038 $\pm$ 0.001	0.002	0.975
	Rat Skin	0.023 $\pm$ 0.0003	0.002	0.993
	Rabbit Skin	0.025 $\pm$ 0.0001	0.004	0.960
F2	Cellophane	1.563 $\pm$ 0.040	0.217	0.991
	Rat Skin	0.245 $\pm$ 0.002	0.029	0.994
	Rabbit Skin	1.006 $\pm$ 0.022	0.117	0.958
F3	Cellophane	0.036 $\pm$ 0.0004	0.004	0.983
	Rat Skin	0.029 $\pm$ 0.0002	0.003	0.978
	Rabbit Skin	0.032 $\pm$ 0.0003	0.003	0.991
F4	Cellophane	0.236 $\pm$ 0.005	0.032	0.987
	Rat Skin	0.042 $\pm$ 0.001	0.004	0.892
	Rabbit Skin	0.115 $\pm$ 0.003	0.014	0.978

According to the data release studies, the highest and the faster release of MTD was obtained from water-soluble bases. This could be attributed to favorable partitioning of MTD toward the aqueous phase. In these formulations, the free concentration of MTD was greater than those oleaginous vehicles. In contrast, for the oleaginous vehicles, the partitioning towards the internal aqueous phase would render the drug almost unavailable in the external oil phase (Ferreira *et al.*, 1995b).

In all cases, the highest MTD release was obtained from cellophane membrane. This observation could be explained by the polar structure of cellophane and

lipophilic barrier role of the skin (Laugel *et al.*, 1998). The synthetic membranes could be used to assess product performance in quality assurance, but the use of physiological conditions especially rat skin was essential for estimating the real drug release characteristics (Taş *et al.*, 2003). In the case of rabbit and especially rat membrane, the amount released from formulations were markedly lower than that obtain from cellophane membrane suggesting that these membranes were rate-controlling barriers.

In conclusion, it was possible to assume that these results could be related mainly to the solubility of the drug in the vehicle. It could be stated that F2 formulation was a good candidate for the topical delivery of MTD, giving significantly higher drug release than the other vehicles.

## Özet

Kremler, merhemler ve jeller için, in vitro salım testleri, bitmiş ürün özelliklerinin bir ölçüdür. Bu çalışmanın amacı, metronidazolün su ve yağ bazlı farklı taşıyıcı sistemlerden in vitro salım ve ex-vivo perkütan absorpsiyonunu değerlendirmektir. Bütün denemeler, üç farklı membran (sentetik membran, sıçan ve tavşan derileri) kullanılarak yapılmıştır. Denemelerde kullanılan membranlar arasında, sıçan derisi diğer membranlara göre en iyi alternatif olarak bulunmuştur. Ayrıca, su bazlı taşıyıcılar daha fazla ilaç salımı sağladığı için, metronidazolün topikal uygulaması için uygun bir taşıyıcı olarak önerilebilirler.

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