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RESEARCH ARTICLE

APPLICATION OF MODEL INDEPENDENT APPROACH ON *IN VITRO* RELEASE OF EXTEMPORANEOUSLY PREPARED SEMISOLID FORMULATIONS CONTAINING METRONIDAZOLE WITH MARKETED SILVER SULFADIAZINE 1% CREAM, USP: A COMPARATIVE INVESTIGATION

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In an attempt for better treatment of bacterial infections, various semisolid formulations containing 5% *w/w* of metronidazole were prepared and evaluated for *in vitro* drug release and *in vitro* skin permeability using dialysis membrane and rat abdominal skin respectively using model independent approach. The f_1 lower than 15 and f_2 higher than 50 indicated similarities in the *in vitro* diffusion and permeation profiles of the extemporaneously prepared selected semisolid formulations and marketed silver sulfadiazine 1% cream, USP. Amongst all the semisolid formulations prepared, carbopol gel base was found to be most suitable dermatological base for metronidazole and the results obtained for *in vitro* diffusion and *in vitro* skin permeation studies are comparable with that of marketed silver sulphadiazine 1% cream, USP.

Key words: Semisolid formulation, Ointment, Metronidazole, Silver sulfadiazine.

INTRODUCTION

Topical antibiotics can play an important role in prevention and treatment of many primary cutaneous bacterial infections commonly seen in dermatological practice like localized superficial infections due to surgery, injury and abrasion. Topical antimicrobials help in preventing entry of microorganism into wound, which leads to fast healing of wounds (Benner *et al* 1999; Sable and Murakawa, 2003).

Metronidazole is a nitroimidazole antibiotic (**Figure 1**) classified in the WHO Essential Medicines List as antiamoebic, antigiardiasis, and antibacterial (WHO, 2009). Approved indications include treatment of trichomoniasis, vaginitis, and urethritis caused by *Gardnerella vaginalis*, giardiasis, amoebiasis, and infections caused by anaerobic bacteria (ANVISA, 2009),

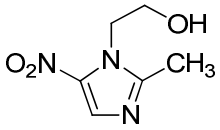

Fig. 1. Structure of metronidazole

which comprise intraabdominal infections, skin and skin structure infections, gynecologic infections, bacterial septicemia, bone and joint infections, central nervous system infections, lower respiratory tract infections, and endocarditis. The objective of the present study was to prepare various topical drug delivery systems such as gels and ointments and to evaluate and compare *in vitro* diffusion and permeation profile of prepared formulation with marketed silver sulfadiazine cream 1% USP.

MATERIALS AND METHODS

Metronidazole was received from Pfiscar India Ltd., Murthal, India and carbopol from Noveon, Mumbai, India. All other chemicals used were of analytical grade. UV spectrophotometer (Jasco V-530, Jasco Inc) was used in the study.

Preparation of semisolid dosage forms of metronidazole

Various semisolid formulations of metronidazole (MT) were prepared according to the composition given in **Table 1** with different dermatological bases using standard procedures. In each of the formulations, MT was incorporated at 5% *w/w* concentration respectively in the base with trituration using geometric dilution procedure to obtain homogeneous mass (Dua *et al* 2010).

In vitro diffusion studies

In vitro diffusion studies for all formulations were carried out using Keshary-Chein (KC) type diffusion cell (Rajni and Verma, 1995; Aqil *et al* 2003). The diffusion cell apparatus was fabricated locally as open-ended cylindrical tube with 3.7994 cm^2 area and 100 mm height having a diffusion area of 3.8 cm^2. 1% *v/v* acetic acid was used as receptor media. The dialysis membrane (25 cm^2) was soaked in water for a while, and then for 2 h in isotonic phosphate buffer (IPB) solution, pH 7.4 (100 ml) prior to be mounted on the diffusion cell. A weighed quantity of formulation equivalent to 25 mg of drug was taken on to the dialysis membrane and was immersed slightly in 20 ml of receptor medium which was continuously stirred. Entire system was maintained at 37±1°C. An aliquot of 2 ml was withdrawn at specific time intervals up to 6 h, suitably diluted and the MT content was estimated spectrophotometrically at 277.6 nm.

Table 1. Composition of topical formulations of metronidazole

Ingredients	Quantity (in mg)								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
MT	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sodium CMC		5.0							
Cetostearyl alcohol					15.0			15.0	
Yellow Beeswax					2.5	5.0		1.0	2.0
Carbopol 940	2.0								
Triethanolamine	q.s.								
Tween 80					5.5				
Borax						0.2			
Methyl paraben	0.15	0.2	0.3		0.2	0.2		0.2	
Propyl paraben	0.05	0.1	0.2		0.1	0.1	10.5	0.1	
Sodium metabisulphite								0.1	
SLS								2.0	
Hard Paraffin						7.0			
HPMC			2.0						
PEG 4000				50.0					
PEG 300				45.0					
DMSO							10.5		
Isopropyl myristate							8.0		
Mineral oil						45.0	30.5		
White petrolatum						10.0	30.7		83.0
Propylene glycol								10.0	
Span 60					7.5				
Wool fat									10.0
Bees wax							4.8		
Glycerine		24.0	10.0						
Water q.s.	100	100	100		100	100		66.6	

CMC: carboxymethyl cellulose; DMSO: dimethyl sulfoxide; MT: metronidazole; PEG: polyethylene glycol; HPMC: hydroxylpropylmethyl cellulose; SLS: sodium lauryl sulfate; F_1- Carbopol gel base; F_2- Sodium CMC gel; F_3- HPMC gel; F_4- Macrogol gel; F_5- Water miscible base; F_6- Cold cream; F_7- Simple ointment base; F_8- Beller's ointment base; F_9- Oleagenous base.

After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium. Average of three determinations was used to calculate the cumulative percent drug release at each time interval (Ezzedeen *et al* 1990; Sanna *et al* 2009).

In vitro skin permeability studies

In vitro skin permeation studies were carried out for the best three formulations which exhibited the higher drug release through dialysis membrane using Keshary-Chein (KC) diffusion cell (Rajni and Verma, 1995; Aqil *et al* 2003) in a similar way as described for i*n vitro* diffusion studies using rat abdominal skin. The rat skin was obtained from the abdominal portion of albino rat after sacrificing the animal. The hair and fat were removed after treating the skin with 0.32 mol/l ammonia solution for 30 min (Nagia *et al* 2006). The skin was tied to the KC diffusion cell (donor cell) such that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell (Singh *et al* 2009). All experiments were carried out in triplicate.

Comparison of *in vitro* diffusion and *in vitro* permeation profiles

The *in vitro diffusion* and *in vitro* skin permeation profiles of the best among the three selected formulations in each case was compared for similarity with marketed silver sulfadiazine 1% cream, USP. A model independent approach was used employing a difference factor (f_1) and similarity factor (f_2) as given in equations 1 and 2, respectively (Moore and Flanner, 1996; Costa and Sousa Lobo, 2001).

$$f_1 = \frac{\sum[R_t - T_t]}{\sum R_t} \times 100 \qquad \text{Eq. 1}$$

$$f_2 = 50 \cdot \mathrm{Log}\left[\frac{1}{\sqrt{1 + \frac{1}{n}\sum (R_t - T_t)^2}} \times 100\right] \qquad \text{Eq. 2}$$

where, R_t and T_t are % dissolved for reference and test formulation at each time point and n is the number of time points in dissolution profile. The time intervals used to study the f_1 and f_2 were up to 420 min.

The f_1 value increase proportionally due to the dissimilarity between the two release profiles. If f_1 value lies between 0-15 and f_2 value of two drug release profiles is between 50 and 100, then these two drug profiles are considered similar. Value under 50 indicates difference between the release profiles. The value of f_2 = 50 reflects 10% difference; when value is > 50, the difference between R and T is less than 10% (FDA, 1997).

RESULTS AND DISCUSSION

In the present investigation, *in vitro* diffusion and permeation were found to be better for Carbopol gel formulations (MT_1) in comparison to the formulations containing various other dermatological bases. The effect of dermatological bases on drug release profiles has been well documented (Parikh *et al* 1986; Perez-Marcos *et al* 1991). The following cumulative amount of drug diffusion for MT_1 at 6 h was observed to be 69.12±1.82% and the cumulative amount of drug permeation at the same time interval was 14.12±1.75%. The comparative release of MT from various formulations is shown in **Figure 2**.

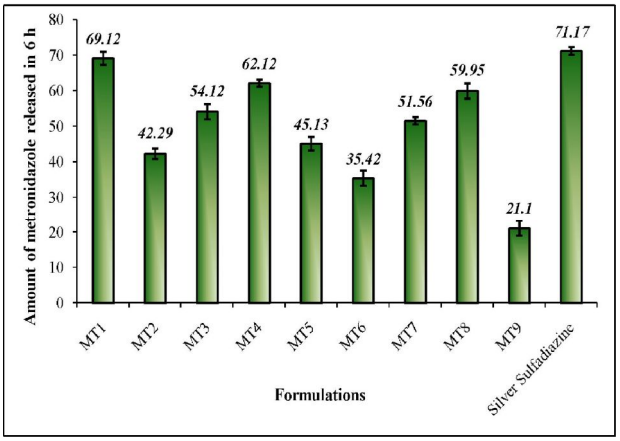

Fig. 2. Comparative *in vitro diffusion* of different metronidazole semisolid formulations with marketed silver sulfadiazine 1% cream, USP

The enhanced drug diffusion and drug permeation from the carbopol gel base may be attributed to the presence of pores in the gel which allow relatively free diffusion of the drug to the vehicle and lack of over-solubilization of the lipophilic drug in the aqueous vehicle and hence readily available for release (Dhavse and Amin, 1997; Pandey *et al* 1999). In creams and various oleaginous bases, owing to their biphasic nature, partitioning of the drug occurs in aqueous and oil phases which results in the slower release of drug. In case of gels, the drug diffusion occurs through the aqueous phase and hence they offer a greater drug diffusion and release.

The results obtained for *in vitro* diffusion and *in vitro* skin permeation studies with MT are comparable with that of silver sulphadiazine 1% cream, USP (SS: 71.17±2.10%; 15.95±0.68% respectively for *in vitro* diffusion and skin permeation) available in market.

The maximum extent of *in vitro* permeation of drug through skin after 6h was observed to be in the range 14.12±1.75 to 10.83±0.15. The extent of permeation of the drug is not sufficient to exert a systemic action but it is sufficient enough to exert a local action at the site of application. This inadequate permeation through the skin is possibly due to a strong affinity of hydrophobic drug to the lipophilic stratum corneum or the barrier effect of the latter despite using permeation enhancers.

However, the diffusion of MT from the three selected formulations was in the same order as that from dialysis membrane. The comparative *in vitro* diffusion and *in vitro* skin permeation of selected metronidazole semisolid formulations with marketed silver sulfadiazine 1% cream, at 6 h is shown in **Figure 2**. The values of f_1 and f_2 for the MT_1 as compared to that of the marketed silver sulfadiazine 1% cream, USP, were given in **Table 2** and **Figure 3-5**.

Table 2. Analysis of f_1 (difference factor) and f_2 (similarity factor) value of MT_1 with marketed silver sulfadiazine 1% cream, USP

Formulation	Drug release study	f_1	f_2
MT_1	*In vitro* diffusion	9.81	63.84
	In vitro skin permeation	7.23	90.12

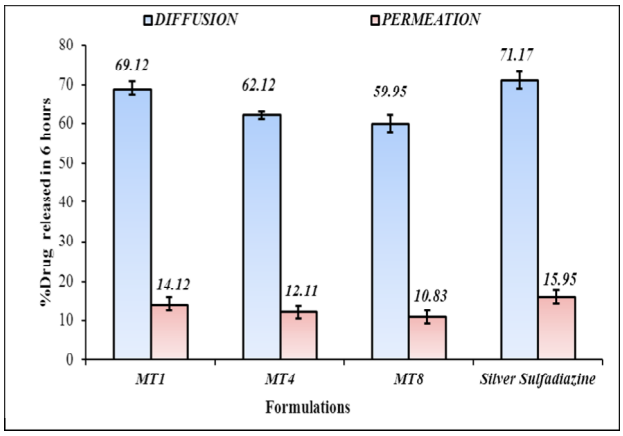

Fig. 3. Comparative *in vitro* diffusion and *in vitro* skin permeation of selected metronidazole semisolid formulations with marketed silver sulfadiazine 1% cream, at 6 h

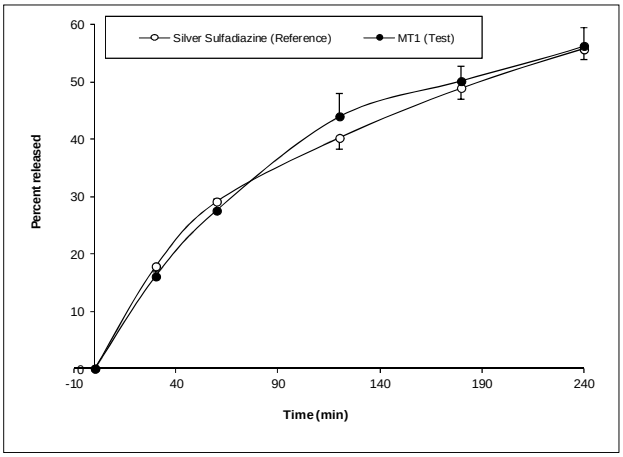

Fig. 4. Analysis of *in vitro* diffusion profile for f_1 (difference factor) and f_2 (similarity factor) of MT_1 with marketed silver sulfadiazine 1% cream, USP

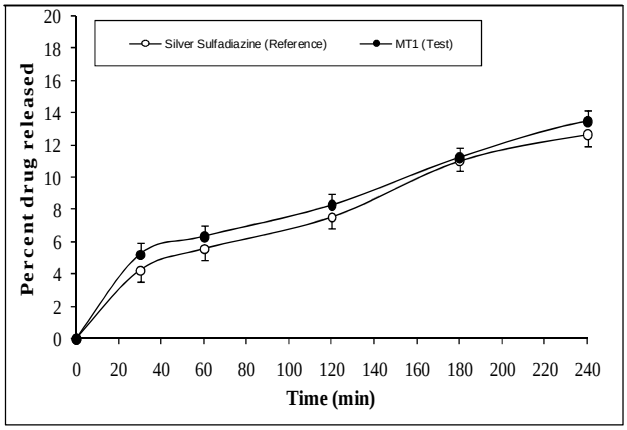

Fig. 5. Analysis of *in vitro* skin permeation profile for f_1 (difference factor) and f_2 (similarity factor) of MT_1 with marketed silver sulfadiazine 1% cream, USP

The f_1 lower than 15 and f_2 higher than 50 indicated similarities in the *in vitro* diffusion and permeation profiles. Thus, the data indicated that the release mechanism of the drug from all the formulation followed the same pattern.

CONCLUSION

The *in vitro* release characteristics of the prepared topical formulations of metronidazole were quite encouraging and in agreement with marketed Silver Sulfadiazine 1% Cream, USP. Amongst all semisolid formulations prepared, carbopol gel base was found to be most suitable dermatological base for metronidazole in comparison to various other dermatological bases. It also possessed aesthetic appeal, which other bases lacked, which is an important aspect from patient compliance and consumer point of view.

REFERENCES

Aqil M, Sultana Y, Ali A. Matrix type transdermal drug delivery systems of metoprolol tartrate: *in vitro* charaterization. *Acta Pharm.* 2003;53(2):119-25.

Benner MW, Bencon PM, James WD. Topical Antibiotics in Dermatology, 5th ed, McGraw Hill: New York, 1999; 25-7.

Brazilian Health Surveillance Agency (ANVISA). 2009. Flagyl label. Accessed December 15, 2009.

Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* 2001;13(2):123-33. [DOI: 10.1016/S0928-0987(01)00095-1]

Dhavse VV, Amin PD. Formulation and evaluation of topical bases of ketoprofen. *East Pharm.* 1997;480:133-6.

Dua K, Pabreja K, Ramana MV. Aceclofenac topical dosage forms: *In vitro* and *in vivo* characterization. *Acta Pharm.* 2010;60(4):467-78. [DOI: 10.2478/v1007-010-0036-5]

Ezzedeen FW, Shihab FA, Husain EJ. Percutanous diffusion of cefalexin, sulfamethoxazole and diphenhydramine from ointments. *Pharmazie* 1990;45(7):512-4.

FDA, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms (BP1). Centre for drug evaluation and research, Food and drug administration. 1997; Rockville, MD, P9.

Moore JW, Flanner HH. Mathematical comparison of dissolution profiles. *Pharm. Technol.* 1996;20(6):64-74.

Nagia AE, Hanan ME, Gehan FB. Formulation and evaluation of meloxicam gels for topical administration. *Saudi Pharm. J.* 2006;14(3-4):155-62.

Pandey S, Basheer M, Roy S, Udupa N. Development and evaluation of transdermal formulations containing metronidazole and norfloxacin for the treatment of burn wound. *Indian J. Exp. Biol.* 1999;37(5):450-4.

Parikh NH, Babar A, Plakogiannis FM. Medicament release from ointment bases: II. Testosterone: *in vitro* release and effects of additives on its release. *Drug Dev. Ind. Pharm.* 1986;12(14):2493-2509. [DOI: 10.3109/03639048609063195]

Perez-Marcos B, Iglesias R, Gomez-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A. Mechanical and drug-release properties of atenolol-carbomer hydrophilic matrix tablets. *J. Control. Rel.* 1991;17(3):267-76. [DOI: 10.1016/0168-3659(91)90145-4]

Rajni V, Verma PRP. Diffusion study of ibuprofen from ointment bases. *Ind. J. Pharm. Sci.* 1995;57(1):1-6.

Sable D, Murakawa GJ. Quinolones in Dermatology. *Clin. Dermatol.* 2003;21(1):56-63. [DOI: 10.1016/S0738-081X(02)00322-X]

Sanna V, Peana AT, Moretti MD. Effect of vehicle on diclofenac sodium permeation from new topical formulations: *in vitro* and *in vivo* studies. *Curr. Drug Deliv.* 2009;6(1):93-100.

Singh S, Gajra B, Rawat M, Muthu MS. Enhanced transdermal delivery of ketoprofen from bioadhesive gels. *Pak. J. Pharm. Sci.* 2009;22(2):193-8.

World Health Organization (WHO). 2009. WHO Model Lists of Essential Medicines, 16th ed, Accessed November 20, 2009.

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