DELIVERY OF METRONIDAZOLE FROM PURIFIED NIGERIAN SHEABUTTER IN COMPARISON TO STANDARD AND MODIFIED OINTMENT BASES

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ABSTRACT

The drug delivery propensities of 1 %w/w metronidazole (MTZ) ointment formulations in purified Nigerian sheabutter base have been compared to those of similar formulations produced with standard or modified bases, with a view to determining bases capable of ensuring rapid release of MTZ from ointment formulations intended for topical treatment of rosaceae. Ten bases, having four different combined characteristics, were studied. MTZ ointment samples were prepared in each base by levigation technique. Drug release rates were determined by an *in vitro* dialysis method using the Erweka dissolution apparatus at pH 5, and 37°C in 180-min tests; and the dissolution samples quantified by UV analysis. The release pattern of MTZ from the bases was: hydrophilic hydrous bases > hydrophilic anhydrous bases > hydrophobic (hydrous or anhydrous) bases. The highest drug release rate and extent occurred from cetomacrogol cream (5.39 mg $min^{-½}$; 61.5%), while the lowest occurred from simple ointment (SO) base (0.30 mg $min^{-½}$; 4.2%). The drug release capacity of SO was significantly improved by incorporation of a surfactant (cetrimide). MTZ in sheabutter ointment exhibited intermediate drug release values (0.73 mg $min^{-½}$, 9.30%), which were neither enhanced by hydration nor by the surfactant.

Key Words: Metronidazole, Sheabutter, Ointment Bases, *In Vitro* Drug Release

INTRODUCTION

Metronidazole (MTZ) is classified in the WHO Essential Medicines List as antiamoebic, antigiardiasis, and antibacterial (WHO, 2009). It is a nitroimidazole compound (Figure 1) commonly used in the form of gel, injections, tablets, and suppositories (British Pharmacopoeia (BP), 2009), having a water-solubility of 10 mg/mL at 20°C and 10.5 mg/mL at 25°C (The Merck Index, 2006). Its topical use in ointments (containing 1% of MTZ) is not widespread but has been considered valuable for the treatment or management of acne and papulopustular rosacea, an inflammatory skin disease (Bannatyne, 1999; Dahl et al., 1998). Sheabutter is the natural fat obtained from seeds of the tree, *Butyrospermum parkii* (G. Don.) Kotschy, or *Vitellaria paradoxa* Gaertner F., family Sapotaceae, which grows naturally in West African Savannah and thrives in Southwest Nigeria (Irvine, 1963). Shea seeds collection and traditional sheabutter production are carried out by people in communities where the trees are located (Hall, 1996) and commercial sale and distribution of sheabutter contributes amply to the economy of such agrarian communities. Sheabutter is used in Nigeria primarily as cooking oil and illuminant, and is also exported to Europe and American countries where it is used in the manufacture of soaps, cosmetics, lubricants, paints, and as cocoa butter substitute for production of confectionaries (Addaquay, 2004).

Sheabutter has been variously studied for use in the formulation of pharmaceutical products: as a component of a suppository base in paracetamol formulation (Taylor *et al.*, 1993), as ointment base for salicylic acid, benzoic acid (Konning and Mital, 1978) and chlortetracycline hydrochloride (Thioune *et al.*, 2003). Purified Nigerian sheabutter is innocuous on human skin (Oyedele, 2002) and its water-sorption and rheological properties have been adjudged very suitable for ointment preparation (Odusote and Ifudu, 1987). Therefore, the objective of this study was to evaluate *in vitro* release of MTZ from purified Nigerian sheabutter in comparison to standard (official) and modified ointment bases.

MATERIALS AND METHODS

The shea butter used in the study was obtained locally and purified using previously reported technique (Oyedele, 2007). The standard ointment bases used (BP, 1988; Pharmaceutical Codex (PC), 1979) were compounded with ingredient grades (Evans Medical Ltd., Liverpool) and methods as specified by the compendia. MTZ

(May and Baker Plc., Nigeria) of particle size 90 m was used.

Ointment preparations

One percent (w/w) MTZ ointment samples were prepared by levigating the requisite quantity of MTZ with each of 10 ointment bases having four different characteristics, for testing namely: (1) *Hydrophobic anhydrous bases* Simple Ointment BP; and Sheabutter ointment base, consisting of 75% sheabutter and 25% arachis oil (Konning and Mital, 1978); (2) *Hydrophobic hydrous bases* Hydrous wool fat BP; and Hydrous sheabutter base, consisting of sheabutter hydrated 30%w/w with water; (3) *Hydrophilic hydrous (emulsion) bases* Cetomacrogol cream non-ionic (Formula A) BP; Aqueous cream anionic BP; and Aqueous cream cationic BP; and (4) *Hydrophilic anhydrous bases* Macrogol ointment BP; and each of Sheabutter ointment base and Simple Ointment BP base made hydrophilic by incorporation with 0.9% cetrimide (a surfactant) (PC, 1979).

Determination of drug release rates from ointments and analysis of samples

The method used for drug release determination from the medicated ointments was a modification of the *in vitro* dialysis method described by D'Souza and DeLuca (2005). The release compartment consisted of 4-g medicated ointment sample enclosed in 8-cm long cellulose 3787-D10 dialyzer tube (SIGMA) previously hydrated in water for 24 h at room temperature. The compartment was made air-tight at both ends by an inelastic silk cord, which also suspended it vertical on the rotary-shaft of the Erweka apparatus used (Heusenstamm Kr. Offenbach, West Germany) (BP, 1988), and set to revolve at 50 rpm. The dissolution medium was 900-ml of pH 5.0 acetate buffer solution maintained at 37 ± 0.5 °C (BP, 1988). pH 5 was used to simulate the normal human skin surface pH (Seidenari and Giusti, 1995). Samples (5 ml each) were taken at specified time intervals for up to 180 min and assayed for MTZ. The volume of the dissolution medium was kept constant by replacing the withdrawn volume of the sample with equal volume of fresh dissolution medium maintained at the same temperature. A minimum of three replicate release rate determinations were made for each ointment preparation. MTZ in samples were analyzed using an ultraviolet spectrophotometric method at 277 nm (Oladimeji *et al.*, 2006). A calibration curve was generated from a concentration range of the drug (0.1 to 2.0 g/ml) prepared in pH 5.0 acetate buffer solution and UV absorbance measured at 277 nm, from which the amount of MTZ in solution at each sampling time was determined.

Data analysis

The extent of drug release was assessed from the total amount of drug present in the dissolution medium at the end of the 180 min drug release experiment. The type of drug release kinetics applicable for the ointment bases was determined by evaluation of three models, viz: zero-order kinetic model (Q vs t), diffusion-controlled model (Q vs square-root of t) and first-order model (log(Qo Q) vs t), where Q is the amount of drug released at time 't' and Qo is the initial amount of the drug. The model that consistently produced the highest correlation among the ointment preparations was used for the assessment of drug release rates, and a slope obtained from linear regression analysis of the plot was determined as the drug release rate constant. The results expressed as mean ± SD were generated from replicate determinations for each ointment preparation. The data were subjected to *t* tests for significance. Analysis of variance and F-test were also conducted on the ointment groups' data.

RESULTS

The highest consistent correlation of linear plots obtained in the determination of MTZ release kinetics occurred when the drug amounts released were plotted against the square root of time. The correlation coefficient ranged from 0.96 to 0.99 for all the bases. On the other hand, the linear plots of MTZ amounts released versus time and those of logarithm of MTZ amounts remaining versus time for the ointment formulations had a correlation of not less than 0.95 for only two (HWF and HSB) and three bases (ACA, HWF and HSB), respectively. The kinetics for MTZ release from the bases was thereby established as the diffusion-controlled model.

The mean extents and rates of release of MTZ from the different ointment bases are shown in Table 1. The release rate was significantly higher with hydrophilic hydrous (emulsion) bases than from hydrophilic anhydrous bases ($p < 0.05$), which in turn was higher than from hydrophobic bases ($p < 0.05$). Base by base comparison revealed that MTZ was released at significantly different rates in all ($p < 0.05$) except HWF vs. HSB, and SBO vs. SBO+S, where the compared

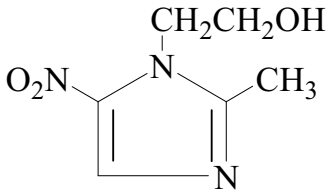

Figure 1. Chemical Structure of metronidazole; molecular weight: 171.16

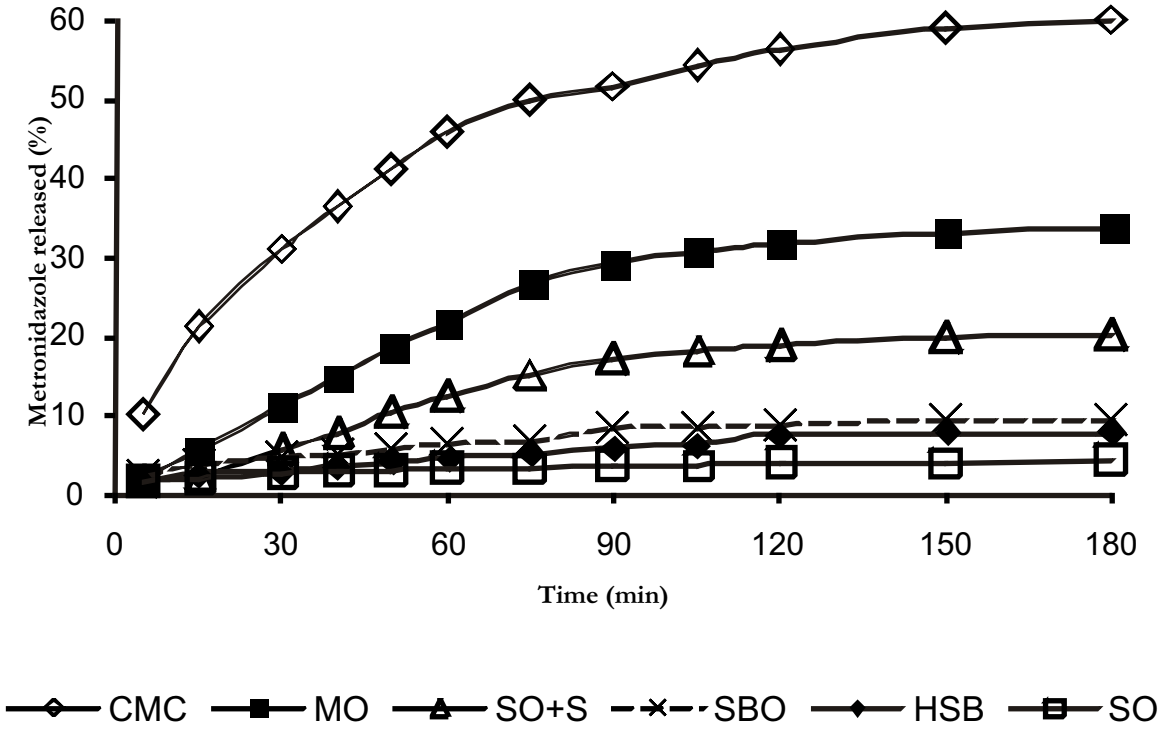

Figure 2. Release profiles of metronidazole from ointment bases, each containing 1%w/w of drug.

Table 1. Mean extents and rates of release of metronidazole from different ointment bases containing 1 %w/w of drug

Ointment base	Symbol	Release rate constant (mg min$^{-½}$)[a]	Extent of release (%)[a]
Hydrophilic-hydrous (emulsion) bases			
Cetomacrogol cream non-ionic (Formula A) BP	CMC	5.39 (0.02)	61.50 (4.33)
Aqueous cream anionic BP	ACA	4.42 (0.01)	54.28 (9.56)
Aqueous cream cationic PC	ACC	3.65 (0.01)	31.10 (1.68)
Hydrophilic-anhydrous bases			
Macrogol ointment BP	MO	3.80 (0.01)	33.60 (2.97)
Simple Ointment BP base + surfactant	SO+S	2.23 (0.01)	19.22 (1.30)
Sheabutter ointment base + surfactant	SBO+S	0.73 (0.03)	9.33 (0.63)
Hydrophobic-anhydrous bases			
Sheabutter ointment base	SBO	0.73 (0.02)	9.30 (0.56)
Simple Ointment BP	SO	0.30 (0.01)	4.20 (0.23)
Hydrophobic-hydrous bases			
Hydrous wool fat BP	HWF	0.62 (0.04)	7.70 (0.17)
Hydrous sheabutter base	HSB	0.62 (0.05)	7.05 (0.53)

Standard deviation values in parenthesis

mean values were not significantly different ($p > 0.05$).

The drug release rates showed correlation with the extents of release. Figure 2 depicts the release profiles of MTZ from selected representative bases. A maximum of 61.5, 33.6 and 9.30 % of the drug was released from CMC, MO, and SBO bases, respectively, within the 3-h drug release testing period. All the hydrophobic bases, regardless of hydration status, delivered less than 10% of their MTZ content in 3 h, in rates ranging from 0.30 to 0.73 mg $min^{-½}$ (Table 1). The presence of surfactant (cetrimide) in SO base improved the drug release rate of the base 7-fold (from 0.30 to 2.23 mg ($min^{-½}$)), as well as the extent of release almost 5-fold (from 4.2 to 19.2 %). However, the surfactant produced no enhancing effect in SBO base (Table 1).

DISCUSSION

Adequate characterization of drug release rate from ointments requires the determination of its appropriate release kinetics model. The kinetics may vary from zero-order through first-order to diffusion-controlled. Data generated in this study affirm that the kinetics of MTZ release from different ointment bases hydrophilic, hydrophobic, hydrous or anhydrous bases is mainly the diffusion-controlled model. This implies that MTZ is liberated from the drug in solution at equilibrium with the solid drug dispersed in the base. Similar diffusion-controlled release mechanism has been reported for salicylic acid formulated in various dispersion ointment bases (Erös *et al*., 2000).

The study of the influence of different ointment base properties on MTZ release is important toward evaluating the drug's prospective bioavailability and efficacy from the different base types. This study has thus revealed that hydrophilic hydrous bases will deliver MTZ faster and more efficiently than other types of bases (Table 1). This finding is consistent with other reports which show that hydrophilic character of an ointment base promotes the release of a water-miscible drug (Odusote and Ifudu, 1987; Erös *et al*., 2000).

The possibility of increasing the hydrophilicity and thereby enhance MTZ release from hydrophobic ointment bases (SBO and SO) was evaluated in this study, by incorporation of cetrimide at the same effective concentration (0.9 %) as in ACC base. This strategy was partially successful, in that the MTZ release rate and extent in SO were significantly enhanced, but not in SBO (Figure 2, Table 1). The non-improvement of MTZ release in SBO by cetrimide is, however, not unusual, as other studies indicate that surfactant incorporation may increase or decrease drug release (Sieg and Robinson, 1979; Jain et al., 2003).

In conclusion, purified Nigerian sheabutter has been found to perform better than Simple Ointment (BP) in terms of ability to deliver MTZ from ointment formulations but was surpassed by more hydrophilic standard bases. Hydration of sheabutter (HSB) or incorporation of a surfactant (SBO+S) did not appreciably improve the drug release capacity of the base.

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