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Effects of some Interacting Variables on the Physical and Release Properties of Metronidazole Suppositories Formulated with Modified Natural Fatty Bases

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Abstract : The objective of this study was to assess quantitatively the independent and interaction effects of type of suppository base, concentration of drug and incorporation of Tween 20® as formulation variables influencing the physical and release properties of metronidazole suppositories with a view to enhancing these properties of the suppositories. The variables were investigated at "low" and "high" levels using a 2^3 factorial experimental design. Cocoa butter and shea butter modified with 20 %w/w beeswax were employed as bases in formulating 200 mg and 800 mg metronidazole suppositories with or without 4 %w/w Tween 20® using the fusion method. Physical and dissolution properties of the suppositories were determined by standard methods. The independent and interaction coefficients of the variables on physical and release properties of the metronidazole suppositories were calculated. The inclusion of Tween 20® in the formulations was the most influential independent variable that affected the rank-investigated release parameters of the metronidazole from the suppositories, while the highest dual interaction effect was that between the drug and Tween 20®. The effect of the base as an independent variable was less pronounced on the physical and release of the metronidazole suppositories. The effect of drug-base ratio was manifested by higher release of drug from 800 mg metronidazole suppositories than from 200 mg formulations. The interaction coefficients showed a departure from zero value, implying existence of interaction between the variables, thus suggesting the need for careful combination of the variables in order to obtain suppositories of desired physical and release properties.

Keywords: Metronidazole suppositories, formulation variables, interacting effects, physical and release properties.

Introduction

Metronidazole, an amoebicidal, bactericidal and trichomonicidal drug[1,2,3], is noted for gastrointestinal disturbances and nausea when taken orally[4]. Suppository formulations of metronidazole for rectal and vaginal administration are becoming more attractive due to their ability to overcome the side effects associated with oral administration of metronidazole. The suppository formulations of the drug have also been identified, in some cases, as alternative to intravenous infusion but which requires more technical expertise in its administration[3,5,6,7], a situation that may not be available in most rural clinics in the developing world. The suppository formulations are useful in paediatric, geriatric and unconscious patients, and when oral intake of the drug is restricted such as before surgery or in patient having disease of the upper gastrointestinal tract[8]. In order for the metronidazole suppository to present comparative systemic effect to that of the oral or parenteral dosage

forms, there is need to optimize the formulation variables for effective release of the drug from the suppository[9,10]. Among the influential formulation variables are the suppository bases which, in most cases, constitute the major component of the formula and additives that function as the drug release enhancer[11,12,13,14].

There are array of bases and drug release enhancers for formulation of suppositories, which affect the physical and release properties of the suppositories to different extents[4,11,14,15] and, by extension bioavailability of the drug[13]. Among the suppository bases are the natural fats, which melt at body temperature to release the drug content[16]. The use of such natural fatty bases in the tropics has limitations in terms of handling and stability on storage due to their low softening points[16,17]. In a previous study, natural fatty bases derived from *Theobroma cacao*, family Sterculiaceae (cocoa butter) and *Vitalleria paradoxa*, family Sapotaceae (shea butter) were modified with 20 %w/w beeswax to formulate metronidazole suppositories suitable for storage and handling in the tropics[16]. However, the modification of the natural fats decreased the drug release propensity of the suppositories which necessitated incorporation of Tween 20® at an optimal concentration of 4 %w/w as a drug release enhancer. Among the discernable independent variables that affected the physical and release properties of metronidazole suppositories formulated with the modified natural fatty bases were the base type, amount of drug incorporated and the surface active agent employed[16], which thus warranted further studies on ways to optimise the variables in achieving enhanced physical and release properties of the suppositories.

This study, therefore, aimed at investigating quantitatively the effects of formulation variables (base type, drug concentration and incorporation of surface active agent) on the physical and release properties of metronidazole suppositories with a view to optimising the rectal formulation of the drug using modified natural fatty bases. Quantitative assessment of the variables using 2^3 factorial experimental designs[18,19] helps in determining individual or interacting effect of the variables on physical and release properties of the formulations.

Materials and Methods

Materials

Metronidazole powder (donated by Fidson Healthcare Ltd., Sango-Otta, Nigeria); cocoa butter (theobroma oil) (Starmark Cocoa Processing Company Ltd., Ondo, Nigeria); sheabutter (procured at market in Shaki, Nigeria); beeswax (Fluka, Switzerland); sodium hydroxide (BDH Laboratory, Poole BH15, England), potassium dihydrogenorthophosphate (Surechem Products Ltd, England), polysorbate 20 (Tween 20®) (Sigma Chemical Co, St. Louis, USA).

Methods

Modification of Suppository Bases

The shea butter was purified as earlier described prior to use in this study[16,20]. White beeswax at 20 %w/w was employed in the modification of physical properties of the cocoa butter and shea butter bases using the method described by Adegoke *et al*[16]. The beeswax was melted in a platinum dish on a water bath regulated at about 5^{o}C above the melting point of beeswax[21]. The cocoa butter and shea butter, respectively, were chopped into tiny bits and added to the molten beeswax while congealing and stirred until cool. The modified bases were stored for 48 h after which their softening and melting points were determined[16].

Preparation of Metronidazole Suppositories

Suppository batches containing 200 mg and 800 mg metronidazole were prepared by fusion method in 2 g moulds[16]. The quantities of the modified cocoa butter and shea butter required in each formulation were determined by the drug's displacement value[9]. The metronidazole powder was sifted through mesh 60 (250 µm) before being incorporated into the melted modified bases. Batches of suppositories containing 4 %w/w Tween 20®, in addition, were also prepared. The Tween 20®was mixed with the melted bases prior to the addition of the sifted metronidazole. The suppositories were stored in a refrigerator (4±1^{o}C) and analysis carried out 24 h after formulation. The composition of each suppository formulation is indicated in Table 1.

Evaluation of PreparedMetronidazole Suppositories

Weight Variation

The weight variation test was carried out as described in the British Pharmacopoeia (BP)[22]. Twenty suppositories were randomly selected from each batch of the formulations, weighed individually using a Mettler analytical balance(Mettler Toledo PB 153 analytical balance, Switzerland), and the mean weights and standard deviations calculated[16].

Table 1: Codes and composition of formulated metronidazole suppositories

Formulation code	Composition
2CB	200 mg MTZ + cocoa butter
2CBT	200 mg MTZ + cocoa butter + 4 %w/w Tween 20®
8CB	800 mg MTZ + cocoa butter
8CBT	800 mg MTZ + cocoa butter + 4 %w/w Tween 20®
2SB	200 mg MTZ + shea butter
2SBT	200 mg MTZ + shea butter + 4 %w/w Tween 20®
8SB	800 mg MTZ + shea butter
8SBT	800 mg MTZ + shea butter + 4 %w/w Tween 20®

Content Uniformity

Six suppositories were randomly selected from each batch and assayed individually for drug content using the method described by Adegoke *et*al[16]. A suppository was weighed and placed in a beaker containing 100 ml of phosphate buffer solution (pH 7.2). The suppository was melted by gentle heating of the beaker on a water bath. The beaker was agitated while the melting proceeded. When the suppository had been completely dispersed, the mixture was adjusted to 100 ml with phosphate buffer, chilled and the congealed oil layer removed by filtration through a cotton plug. The aqueous portion was further filtered through sinter glass number 3 (DURAN Group GmbH, Germany) and 1 ml of the filtrate diluted to 100 ml using the phosphate buffer. The absorbance of the diluted solution was measured by UV spectrophotometer (mini-1240 model, Germany) at 326 nm. The concentration of metronidazole in the solution was calculated from a standard Beer-Lambert curve in the concentration range of 0.05 mg to 0.3 mg/ml, and the drug content of each suppository determined.

Softening and Melting Points

The softening and melting points of metronidazole suppositories were determined according to the method of Adebayo and Akala[23] as modified by Oladimeji and Bankole[24]. A suppository, randomly selected from each batch was placed in a clean test tube with a thermometer inserted. The tube was clamped vertically, immersed at 8cm depth in a water bath regulated to a gradual temperature increase of 1 °C/2 min. The temperatures at which the suppository sample began to melt and that of its complete liquefaction were defined as the softening point and melting point, respectively. The results obtained were average of four determinations. The softening and melting points of bland modified suppository bases (placebos) were also similarly determined.

Crushing Strength

The crushing strength, a measure of mechanical strength or hardness of the suppository, was determined[8]using the Monsanto Hardness Tester (Copley Erweka, Germany). Ten suppositories randomly selected from each batch were used for the determination. The weight under which each suppository collapsed was recorded in kilogram force and converted to Newton[16].

Disintegration Time

The disintegration time of six suppositories randomly selected from each batch formulation was determined with the Manesty tablet disintegration apparatus (Manesty Machines Ltd., Liverpool, England) using the method described in the British Pharmacopoeia[22]. The apparatus, which consisted of six cylindrical

glass tubes each filled with 160 ml of distilled water, was held by a basket rack immersed in a water bath maintained at 37±1 °C . One suppository was placed in each glass tube and a metal disk weighing 50 g was placed on the suppository[23]. The device was set to oscillate through a distance of 60 mm at a frequency of 32 cycles per min, the time required for complete deformation of each suppository was recorded and the average value for the six suppositories calculated[24].

In Vitro Release of Metronidazole from Suppositories

The United States Pharmacopeia (USP)[25]basket method was employed forthe dissolution studies, using digital tablet dissolution test apparatus (Model VDA-8D, PharmChem Machines, Mumbai, India). The procedures were as described by Adegoke *et*al[16]. Phosphate buffer solution (900 ml) at pH 7.2 was used as the dissolution medium. Each suppository was embedded in a bed of 12 glass beads (to prevent floating of the suppository) within the dissolution basket and lowered into a flask containing the dissolution medium maintained at constant temperature (37.0 ±0.5 °C). The basket was rotated at a constant speed of 100 rpm. At appropriate time intervals, 5ml samples were withdrawn over a period of 360 min. The volume of the dissolution medium was kept constant by replacing the volume of the sample withdrawn with an equal volume of fresh buffer solution maintained at the same temperature. The withdrawn samples were filtered, 2 ml of this was diluted with an equal volume of the buffer solution and the absorbance of the diluted sample was determined by UV spectrophotometer (mimi-1240 model, Germany) at 326 nm. The amount of drug released at each sampling time was calculated from a standard Beer-Lambert calibration curve. The mean of three determinations was used in calculating drug release from each batch of suppositories. The percentage of drug released at 60 min ($\%D_{60min}$), 360 min ($\%D_{360min}$), the time (min) for 25 % ($T_{25\%}$) and 50 % ($T_{50\%}$) of the drug to be released as well as the dissolution lag time (min) were computed as the release parameters.

Kinetic Analyses of the Release Data

In describing the release model, the in vitro release data were fitted into Zero-order kinetic model(Q vs t), First-order kinetic model ($\log(Q_o - Q_t)$ vs t), Higuchi model(Q vs$t^{½}$) and Korsmeyer-Peppas model ($\log Q_t$ vs nlogt)[26,27], where Q is the amount of drug released at time t, Q_o is the initial amount of the drug, Q_t is the amount remaining at the time t and "n" the release exponent from Korsmeyer-Peppas model. Dissolution data were evaluated using Microsoft Excel spreadsheet and DDSolver software[28,29]. Three statistical criteria namely: Adjusted Coefficient of Determination ($R^2_{adjusted}$), Model Selection Criteria (MSC) and Akaike Information Criterion AIC) were used in selecting the best-fit dissolution model[11,29].

Factorial Experimental Analysis

Quantitative analysis of the three formulation variables on the physical and release properties of the metronidazole suppositories was based on 2^3 factorial experimental designs[18,19,30]. The variables: nature of the suppository base (B), concentration of the drug (D) and concentration of the surfactant, Tween 20®(T), were employed at a "low" level (denoted by subscript, L) and a "high" level (denoted by subscript, H) resulting in 8 factorial levels. Using the above nomenclatures, the various combinations of variables used in the design were:

$B_LD_LT_L$, $B_LD_LT_H$, $B_LD_HT_L$, $B_LD_HT_H$, $B_HD_LT_L$, $B_HD_LT_H$, $B_HD_HT_L$, $B_HD_HT_H$
Where;
B_L= Nature of the suppository base (modified cocoa butter)
B_H= Nature of the suppository base (modified shea butter)
D_L= Concentration of drug (200 mg)
D_H= Concentration of drug (800 mg)
T_L = Concentration of surfactant (Tween 20) (0 %w/w)
T_H = Concentration of surfactant (Tween 20) (4 %w/w)

The combinations were grouped into appropriate sets to enable the assessment of each variable on the values of physical and release parameters of the suppositories. The effect of increasing a variable from "low" to its "high" level on any parameter was determined by summing all the results from samples containing "high" level of the variable and subtracting the sum of the results from samples containing "low" level of the variable as indicated in Table 2. The amount by which the result of the treatment departed from zero, irrespective of whether positive or negative was a quantitative measure of the effect of the variable on the parameter being investigated[30].

To determine whether there was any interaction between two variables, the results of the combination in which the two variables appeared together at either "high" or "low" levels were summed and the sum of other combinations subtracted from this to obtain the interaction coefficient[18] as indicated in Table 2. A result of zero indicated no interaction, but a significant departure from zero implied that the two variables were interacting with each other, with the extent of departure from zero being a measure of the magnitude of the interaction[18,19].

Table 2: Procedure for quantitative assessment of independent and interaction variables on the physical and release parameters of the suppositories

Variables Independent	**Summation procedure of results obtained from physical and release parameters of metronidazole suppositories**
B_L and B_H	¼[($B_HD_LT_L$+$B_HD_LT_H$+$B_HD_HT_L$+$B_HD_HT_H$) – ($B_LD_LT_L$+$B_LD_LT_H$+$B_LD_HT_L$+ $B_LD_HT_H$)]
D_L and D_H	¼[($B_HD_HT_L$ + $B_HD_HT_H$ + $B_LD_HT_L$+ $B_LD_HT_H$) – ($B_HD_LT_L$+$B_HD_LT_H$ + $B_LD_LT_L$+$B_LD_LT_H$)]
T_L and T_H	¼[($B_HD_LT_H$ + $B_HD_HT_H$ + $B_LD_LT_H$ + $B_LD_HT_H$) – ($B_HD_LT_L$+$B_HD_HT_L$ + $B_LD_LT_L$++$B_LD_HT_L$)]
Interaction	
B-D	¼[($B_HD_HT_L$ + $B_HD_HT_H$ + $B_LD_LT_L$+$B_LD_LT_H$) – ($B_HD_LT_L$+$B_HD_LT_H$ + $B_LD_HT_L$+ $B_LD_HT_H$)]
B-T	¼[($B_HD_LT_H$ + $B_HD_HT_H$ + $B_LD_LT_L$+$B_LD_HT_L$) – ($B_HD_LT_L$+$B_HD_HT_L$ + $B_LD_LT_H$ + $B_LD_HT_H$)]
D-T	¼[($B_HD_LT_L$+$B_HD_HT_H$ + $B_LD_LT_L$+$B_LD_HT_H$) – ($B_HD_LT_H$ + $B_LD_HT_L$+ $B_HD_HT_L$ + $B_LD_LT_H$)]

Results and Discussion

Physical Properties of Metronidazole Suppositories

All the suppositories satisfied the BP requirement for weight uniformity. The relative standard deviations (RSD) of the mean weights of the suppositories were less than 3.5 % (Table 3), while the calculated deviations of the mean weight of the metronidazole suppositories from the theoretical weight were within ± 1.6 %, which indicated perfect calibration of the moulds.

Increase in concentration of the drug in the formulation led to increase in the physical weights of the suppositories, while inclusion of 4 %w/w Tween 20® improved the weight uniformity of the suppositories as evidenced from decrease in the RSD (Table 3). As indicated in Table 3, inclusion of Tween 20® in the formulation lowered the softening points of the suppositories, which might have resulted in maintaining the molten mass in a relatively mobile state while being poured into the mould cavities, thus enabling uniform filling and low deviation in the weight uniformity of the suppositories.

The mean drug content for the suppositories met the BP requirement for content uniformity. The mean drug content of the 8 products fell between 95.2 and 98.7 % of the expected metronidazole content. However, the variability (SD) in the assayed drug content of the suppositories was dependent on the drug loading (2CB vs. 8CB; 2Sb vs. 8SB) and incorporation of Tween 20® (2CB vs. 2CBT; 2SB vs. 2 SBT) (Table 3). Surfactants like Tween 20® have been reported to improve drug dispersion into hard or soft excipients[14]. Tween 20® decreased the softening points of the bases (Table 3), making the bases more pliable for the incorporation of the metronidazole. Tween 20® is also capable of reducing contact angle between the suppository bases and the metronidazole, making the drug readily miscible with the bases.

The modified cocoa butter and shea butter bases had softening points of 35.5 °C and 33.5 °C, respectively, with a common melting point of 37.0 °C (Table 3). The need to modify the bases with beeswax was due to their having softening/melting points close to the average room temperature in the tropics[16,17]. However, incorporation of metronidazole further increased the softening and melting points of the modified bases (Table 3). While a softening point of not more than 36 °C has been suggested for fast release of drugs from fatty bases[17], increase in melting point above 37 °C as observed for those formulations without Tween 20® (2CB, 8CB, 2SB, 8SB) (Table 3) may retard the release of metronidazole. The effect of drug loading on the softening and melting points was counteracted by incorporation of 4 %w/w Tween 20® to the formulations, e.g.,

2CB vs. 2CBT (Table 3). There was a decrease in both the softening and melting points of the suppositories which has been attributed to alteration in the rheological properties of the suppositories[16]. Such decrease in softening points of the metronidazole suppositories formulated with the modified fatty bases caused by the addition of Tween 20® may create storage problem in the tropics as well as difficulty in handling during administration.

The crushing strengths of the modified cocoa butter and shea butter suppositories that did not contain metronidazole and Tween 20® were determined as 20.85 ± 0.92 N and 13.00 ± 1.36 N, respectively (Table 3). However, the inclusion of metronidazole and Tween 20® altered the crushing strengths of the suppositories significantly ($P < 0.05$) (Table 3). The addition of metronidazole to the modified bases seemed to potentiate the effect of the beeswax used in stiffening the fatty bases. This was observed to be drug concentration dependent, e.g., 2CB vs. 8CB (Table 3). Incorporation of 4 %w/w Tween 20® into the formulations significantly reduced ($P< 0.05$) the crushing strength of the suppositories. It may, therefore, be necessary to consider the effect of the drug and the surfactant on the suppository bases' rheology before adjusting their consistency with beeswax.

The disintegration times of all the suppositories were less than 13.5 min (Table 3). A short disintegration time of suppositories is expected to favour prompt release of the active constituents. The disintegration times were comparable for both bases. A concentration dependent increase in the disintegration times of the suppositories was observed when metronidazole was incorporated into the modified bases, e.g., 2CB vs. 8CB (Table 3). This may be due to a stiffening effect of the drug on the suppository bases. Inclusion of Tween 20® in the formulation, however, decreased the disintegration times of the metronidazole suppositories significantly ($P < 0.05$). This has been explained to be due to deplasticizing effect of the Tween 20® on the matrix of the suppositories resulting in low consistency and reduction in the disintegration time[16].

***In Vitro* Drug Release Profile of Metronidazole Suppositories**

The dissolution profiles of the metronidazole suppositories are indicated in Figure 1. The drug release parameters ($\%D_{360min}$ and $T_{50\%}$) indicated in Table 3 showed that none of the formulations released up to 80 % of the drug content at 360 min. The release parameters also indicated that only suppositories to which Tween 20® was incorporated were able to release up to 50 % of the drug content within 360 min. The dissolution lag times of metronidazole from the suppository formulations are indicated in Table 3. Metronidazole released from formulations without Tween 20® (2CB, 2SB and 8SB) exhibited longer lag time than those formulations to which Tween 20® was incorporated (2CBT, 8CBT, 2SBT and 8SBT). Dissolution lag time has been defined as the time in which less than 5 % of the drug has gone into dissolution[31]. In lipophilic suppository formulations, the lag phase is as a result of the need for the base to melt prior to drug release[31]. The observed lag times for the formulations were not the function of the melting points of the suppositories only but also that of their drug content and the added Tween 20® (Table 3). The release parameters presented in Table 3 showed that none of the formulations released 25 % of their drug content within 60 min. The effect of the base type on drug release from the suppositories was not as significant as those of the drug concentration and incorporation of Tween 20® into the formulations (Table 3). The *invitro* metronidazole release promoting effects of Tween 20® in modified cocoa butter and shea butter can be attributed to the surfactant's ability to decrease the surface and interfacial tensions of the molten bases and to facilitate the drug's penetration of the dissolution medium thereby aiding desorption of the embedded drug out of the suppository bases matrix[32]. The addition of Tween 20® to the metronidazole suppositories caused reduction in the softening and melting points, crushing strength and disintegration time of the suppositories (Table 3) which, to a large extent, possibly aided the release of the drug from the bases. Hydrophilic surfactants like Tween 20® are noted for their ability to reduce viscosity of molten fatty bases and facilitate the pathway of drug particles to the interface[14]. The surfactant is also capable of decreasing the interfacial tension between the suppository base and the dissolution medium[13,32]and/or may enhance the wettability of lipophilic drugs like metronidazole[32].

The drug release pattern common to the two suppository bases (modified cocoa butter and shea butter) was a faster release rate of the drug in 800 mg formulations (Table 3). In this present study, the 200 mg metronidazole incorporated into a 2 g base represents a minute portion of the formulation compared with the fatty base. Thus, the fatty base present in higher proportion may promote entrapment of the drug within its matrix (more so that the drug is lipophilic) and hinder the drug release as observed in this study. Since the melting of a fatty base and its deformation are prerequisite for drug release[12], it is therefore expected that the observed increase in melting point and disintegration time with increase in concentration of the drug in the

formulations would result in decrease in drug release. The melting points of 8CB and 8SB formulations were higher than 41 °C (Table 3). However, an increase in the release of the drug from these formulations was observed when compared with formulations 2CB and 2SB.This indicated that the process of drug release from the bases may not rely solely on melting of the bases, other process like wearing off of the drug from the surface of the suppositories might take place. The higher the concentration of the drug in the formulation, the greater the amount of drug particles located at the surface of the suppository that are prone to such drug release process.

Table 3: Physical properties and dissolution parameters of metronidazole suppositories for factorial experimental design

Physical and release parameters	Formulation code/Variables combination code							
	2CB	2CBT	8CB	8CBT	2SB	2SBT	8SB	8SBT
	$B_LD_LT_L$	$B_LD_LT_H$	$B_LD_HT_L$	$B_LD_HT_H$	$B_HD_LT_L$	$B_HD_LT_H$	$B_HD_HT_L$	$B_HD_HT_H$
Determined mean weight (g)‡	1.966 ± 0.061 (3.1)	1.962 ± 0.027 (1.4)	2.225 ± 0.073 (3.3)	2.298 ± 0.023 (1.0)	2.013 ± 0.065 (3.2)	2.025 ± 0.043 (2.1)	2.302 ± 0.066 (2.9)	2.332 ± 0.024 (1.0)
Assayed drug content (%)	95.2 ± 16.2	97.0 ± 10.5	98.1 ± 6.1	98.7 ± 4.2	94.6 ± 14.6	96.2 ± 7.5	97.5 ± 5.9	98.3 ±2.7
Softening point (°C)*	35.5 ± 0.5	33.4 ± 0.6	37.0 ± 0.9	34.7 ± 0.4	34.3 ± 0.6	32.8 ± 0.4	36.5 ± 0.5	34.9 ± 0.8
Melting point (°C)**	38.6 ± 0.7	36.5 ± 0.2	41.8 ± 0.6	38.5 ± 0.5	37.8 ± 0.4	36.2 ± 0.2	41.2 ± 0.7	38.6 ± 0.9
Crushing strength (N)†	27.6 ± 1.0	17.0 ± 0.2	30.4 ± 2.8	26.9 ± 2.5	15.4 ± 0.6	10.3 ± 0.4	23.6 ± 0.5	17.1 ± 0.5
Disintegration time (min)	10.7 ± 0.9	6.3 ± 0.7	13.5 ± 1.2	12.8 ± 1.7	10.3 ± 1.2	5.8 ± 0.8	12.5 ± 1.6	10.9 ± 1.2
% drug released in 360 min	31.8 ± 0.9	62.8 ± 0.7	39.6 ± 1.6	75.1 ± 1.9	29.0 ± 1.7	60.5 ± 3.6	36.6 ± 1.7	72.6 ± 2.5
Dissolution lag time (min)	13.5 ± 1.9	< 10.0	< 10.0	<< 10.0	17.0 ± 1.5	< 10.0	11.3 ± 1.7	< 10.0
$T_{50\%}$ (min)	>> 360	255.1 ± 2.9	> 360	143.5 ± 2.2	>> 360	316.1 ± 2.8	> 360	216.8 ± 2.5
Percentage released in 60 min ($\%D_{60min}$)	11.2 ± 0.9	20.1 ± 0.7	13.2 ± 1.0	24.5 ± 0.8	10.9 ± 0.4	17.9 ± 1.2	11.1 ± 0.6	20.1 ± 1.4
Time for 25 % release ($T_{25\%}$) (min)	227.5 ± 0.8	76.1 ± 2.9	132.5 ± 2.4	66.2 ± 0.3	257.4 ± 1.1	85.0 ± 1.3	201.3 ± 2.6	74.5 ± 2.2

*Softening points obtained for modified cocoa butter and shea butter without drug and Tween 20 were 35.5 ± 0.5 °C and 33.5 ± 0.0 °C, respectively.

** Melting point obtained for both modified cocoa butter and shea butter without drug and Tween 20 was 37.0 ± 0.0 °C,

† Crushing strength values for modified cocoa butter and shea butter without drug and Tween 20 were 20.85 ± 0.92 N and 13.7 ± 1.36 N, respectively.

‡Figures in parentheses are % relative standard deviations (RSD)

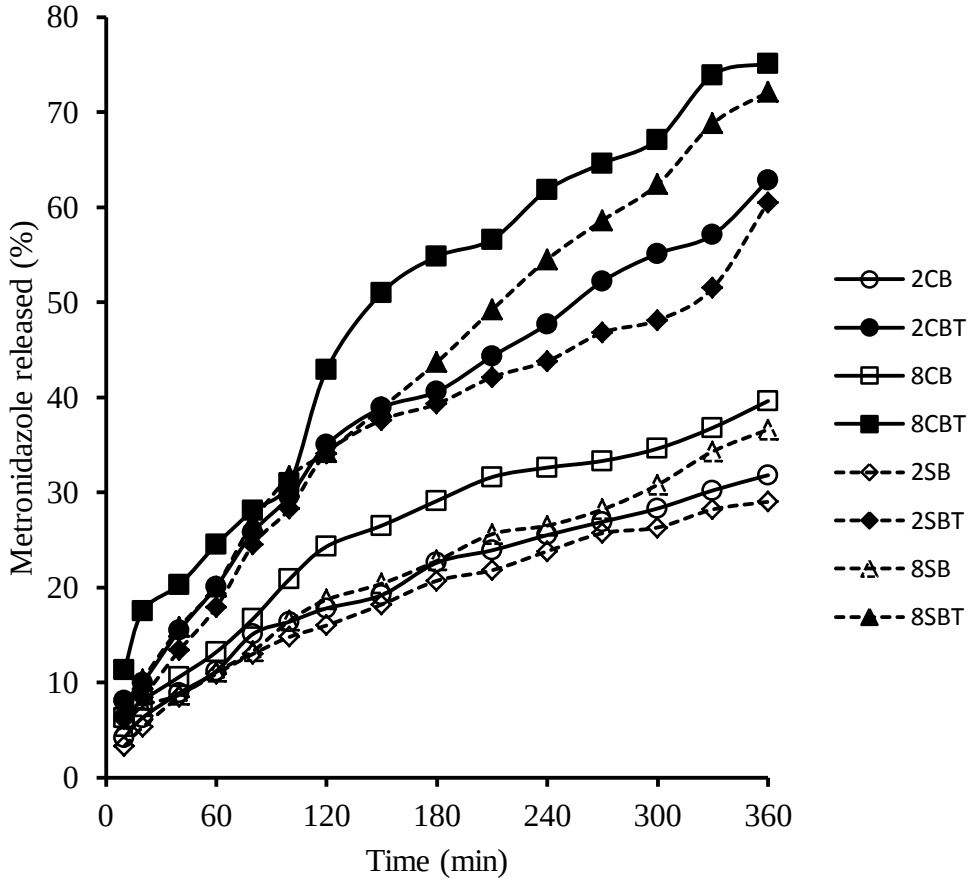

Figure 1: Dissolution profile of metronidazole suppositories formulated with modified cocoa butter and shea butter bases

Release Kinetics of Metronidazole from Suppositories

The release profiles depicted in Figure 1 were fitted into the Higuchi, Zero-order and First-order release models and the selection criteria derived from the models are indicated in Table 4. Based on the selection criteria, formulation 8SBT could be fitted into the First-order model, while the remaining seven formulations are perfectly fitted into the Higuchi square root model. Drug concentration and incorporation of 4 %w/w Tween 20® to the formulations significantly ($P < 0.05$) increased the release constants of the drug.

The release mechanism of metronidazole from the suppositories was also analysed with Korsmeyer-Peppas model as indicated in Table 4. The release exponents, *n*, for all the formulations range from 0.55 to 0.67 indicating anomalous (non-Fickian diffusion) drug release mechanism[26,33,34]. Anomalous transport (non-Fickian) refers to a combination of both diffusion-controlled and erosion-controlled drug release mechanisms[35,36]. The value of *n* was independent of the nature of the base but highly influenced by the inclusion of Tween 20® in the formulations.

Table 4: Release rate constants and model fitting parameters for release kinetics of different strengths of metronidazole from suppository formulations

Release kinetic model	Release parameter/ Selection criterion	Formulation code							
		2CB	2CBT	8CB	8CBT	2SB	2SBT	8SB	8SBT
Higuchi	K_H ($mg/min^{1/2}$)	3.274	6.216	16.496	31.064	3.026	5.764	14.032	27.656
	$R^2_{adjusted}$	0.992	0.982	0.981	0.971	0.991	0.967	0.972	0.957
	MSC	4.72	3.86	3.89	3.40	4.59	3.28	3.44	3.01
	AIC	34.05	67.54	53.14	80.83	34.31	74.44	56.88	85.81
Zero-order	K_o (min^{-1})	0.206	0.394	1.040	1.968	0.192	0.366	0.896	1.770
	$R^2_{adjusted}$	0.745	0.815	0.702	0.794	0.773	0.793	0.844	0.913
	MSC	1.23	1.55	1.08	1.45	1.35	1.44	1.73	2.31
	AIC	86. 31	102.21	95.37	110.18	82.84	102.04	82.55	96.37
First-order	K_1 (mg/min)	0.002	0.006	0.016	0.032	0.002	0.006	0.008	0.024
	$R^2_{adjusted}$	0.835	0.949	0.831	0.961	0.851	0.924	0.908	0.987
	MSC	1.67	2.84	1.64	3.10	1.77	2.45	2.25	4.22
	AIC	79.77	82.88	86.90	85.31	75.51	87.00	74.69	67.69
Korsmeyer-Peppas	K_{KP} (mg/min^n)	2.578	4.020	14.040	16.144	2.204	4.104	7.752	11.304
	n	0.55	0.58	0.53	0.63	0.56	0.56	0.61	0.67
	$R^2_{adjusted}$	0.996	0.994	0.983	0.954	0.998	0.977	0.990	0.997
	MSC	5.28	4.84	3.87	2.81	5.80	3.57	4.44	5.70
	AIC	25.56	46.59	53.46	49.86	16.05	62.26	41.80	28.50

k_o, k_1, k_H =zero-order, first-order and Higuchi release constants, respectively;k_{KP}, *n* = release constant and release exponent in Korsmeyer-Peppas model, respectively; $R^2_{adjusted}$ = adjusted coefficient of determination; MSC = Model Selection Criterion; AIC = Akaike Information Criterion;

In the Korsmeyer-Peppas model, a release exponent (*n*) of 0.5 corresponds to Higuchi square root model classified as Fickian diffusion[27]. The values of *n* for all the formulations were greater than 0.5 suggesting non-diffusion models for the release mechanism of the drug from the suppositories. A comparison of the selection criteria (R^2-adjusted, MSC and AIC) shows that the release mechanism of the suppositories was best fitted to Korsmeyer-Peppas model than the Higuchi model that was earlier postulated. This should be expected as the release of drug from natural fatty bases involves melting of the base, partitioning and diffusion of the drug through the molten base into the dissolution medium[18] rather than one type of release phenomenon postulated by Higuchi model.

The use of Korsmeyer-Peppas model in predicting the release mechanism of metronidazole from the suppositories became more expedient as in vitro dissolution profiles of the metronidazole suppositories indicated in Figure 1 were not linear. Thus, the Korsmeyer-Peppas model, as a "power law"[37], seemed to predict the fractional release of the drug as related to time in an exponential manner better than the Higuchi model.

Effects of Interacting Variables

Some of the values of the physical properties and dissolution parameters of the metronidazole suppositories presented in Table 3 were used in calculating the independent and interacting coefficients of the formulation variables (Table 5). In comparing the formulations, the ranking of the individual independent coefficient values for softening point was T > D >> B; on melting point was D > T >> B; on crushing strength was B > D > T; on disintegration time was D >> T > B; on $\%D_{60min}$ was T >>> D > B and on$T_{25\%}$ was T >>> D > B. Changing the base from modified cocoa butter (B_L) to modified shea butter (B_H) gave negative effects on softening point, melting point, crushing strength, disintegration time and $\%D_{60min}$ indicating a decrease in these parameters. However, a positive value was obtained for $T_{25\%}$, indicating an increase in the time required for 25 % of the drug to be released. With lower melting point, crushing strength and disintegration time obtained for suppositories prepared with modified shea butter as compared with modified cocoa butter, it is expected that the modified shea butter would give a lower time for $T_{25\%}$. The result obtained may therefore be due to differences in chemical composition of the cocoa butter and shea butter, which may affect the affinity of the drug to the bases and its diffusion from them.

The effects of drug concentration on $\%D_{60min}$ and $T_{25\%}$ were positive and negative, respectively, suggesting that an increase in concentration of metronidazole from 200 mg (D_L) to 800 mg (D_H) in the

suppositories increased the amount of drug released in 60 min and decreased the time required for 25 % of the drug to be released. This suggests that a faster release of the drug is obtained from higher dose than lower dose. It also denotes that the inherent poor aqueous solubility of the drug[21] did not inhibit its dissolution at higher drug loading (D_H). The positive effects of the drug loading on softening and melting points suggest reduction in the premature liquefaction problems that are normally encountered by fatty bases in the tropics.

The positive and negative effects of Tween 20® on $\%D_{60min}$ and $T_{25\%}$, respectively, indicate an increase in the percentage of the drug dissolved in 60 min and a decrease in the time required for 25 % of the drug to be in solution. The use of surface active agents like Tween 20® in improving the dissolution of a poorly soluble drug like metronidazole has been documented[13,38]. The values obtained for the independent coefficients (Table 5) show that Tween 20® had the highest individual effect on the release properties ($\%D_{60min}$ and $T_{25\%}$) of the suppositories. The effects of Tween 20® on the disintegration time and crushing strength were negative (Table 5). Tween 20® was reported earlier to change the consistency of the modified bases, leading to softer suppositories. This is apparently responsible for the decrease in disintegration time and crushing strength of the suppositories.

The interactive coefficients indicate the effects of the variables in combination. The ranking of the effects on softening point was B-D > B-T >> D-T; on melting point was D-T > B-T > B-D; on crushing strength was D-T >> B-T > B-D; on disintegration time was D-T >> B-D > B-T; on $\%D60_{min}$ was D-T > B-T > B-D; and on $T_{25\%}$ was D-T > B-T > B-D. All the interacting variables (B-D, B-T and D-T) had the highest effect on $T_{25\%}$. While the interaction between the drug and Tween 20® (D-T) led to increase in $\%D_{60min}$, a decrease in $T_{25\%}$ was obtained (Table 5). This corresponds to an increase in the release profile of the drug from the suppositories. The interaction coefficients of D-T on disintegration time and crushing strength of the suppositories were positive, whereas, the individual effects of variable T on disintegration and crushing strength were negative. This suggests that increase in the drug concentration (D_H) was able to override the decrease in disintegration time and crushing strength as a result of inclusion of 4 %w/w Tween 20® (T_H) in the formulations.

The levels of interaction between the three variables (B-D-T) on the physical and release properties of the metronidazole suppositories are indicated in Table 5. The interaction coefficients obtained for melting point was zero (no interaction), while that for disintegration time was significantly low. Such results were expected to show direct correlation with the interaction coefficient obtained for $T_{25\%}$ since the release of drug from fatt-based suppositories depends on their melting point and deformation time[15]. However, the high interaction coefficient obtained for $T_{25\%}$ (Table 5) indicated a complex interaction between the three variables, in which the release of the drug from the suppositories was to a large extent independent of the melting point and deformation time of the suppositories.

Table 5: Calculated independent and interaction coefficient values as measure of quantitative effects of the nature of the base (B), drug concentration (D), Tween 20® (T) and their interacting effects on physical and release properties of metronidazole suppositories.

Variables	Coefficient values					
	Softening point	**Melting point**	**Crushing strength**	**Disintegration time**	**Percentage released in 60 min ($\%D_{60min}$)**	**Time for 25 % release ($T_{25\%}$)**
Independent variable						
B	-0.53	-0.40	-8.88	-0.95	-2.25	28.98
D	1.78	2.75	6.93	4.15	2.20	-42.88
T	-1.88	-2.40	-6.43	-2.80	9.05	-129.23
Interaction variable						
B-D	0.38	0.15	0.58	-0.50	-1.00	9.58
B-T	0.33	0.30	0.63	-0.20	-1.05	-20.38
D-T	-0.08	-0.55	1.43	1.65	1.10	-32.68
B-D-T*	-0.60	0.00	-10.50	0.20	8.90	-153.00

*Coefficient values obtained from $B_HD_HT_H$ - $B_LD_LT_L$

Conclusion

The three formulation variables, i.e., nature of the suppository base (B), concentration of the drug (D) and incorporation of Tween 20® (T) as independent and interacting variables significantly affected the physical and release properties of the metronidazole suppositories. The inclusion of Tween 20® (T) in the formulations was the most influential independent variable that affected the rank-investigated release parameters ($\%D_{60min}$ and $T_{50\%}$) of the metronidazole from the suppositories, while the highest dual interaction effect on the physical and release properties of the metronidazole suppositories was that between the drug and Tween 20® (D-T). The effect of the base (B) as an independent variable was less pronounced on the physicochemical properties of the metronidazole suppositories. While seven of the eight suppository formulations fitted into the Higuchi model based on the selection criteria, the release exponents obtained from the Korsmeyer-Peppas model for all the formulations ranged between 0.55 and 0.67, indicating anomalous (non-Fickian) drug release mechanism. Faster drug release was obtained from the 800 mg metronidazole suppository formulations than from the 200 mg metronidazole suppository formulations, suggesting that the drug-base ratio has significant effect on the drug release from the suppositories. This finding thus places limitation on extrapolation of the dissolution data obtained from suppositories with lower drug content to that with higher drug loading. Also, all the dual interaction coefficients showed a departure from zero value, implying interaction between the variables, thus suggesting the need for careful combination of the variables in order to obtain suppositories of desired physical and release properties.

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