IN-VITRO RELEASE OF METRONIDAZOLE FROM SUPPOSITORIES FORMULATED WITH BLENDS OF COW FAT AND PALM KERNEL OIL

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ABSTRACT

The suitability of cow fat (CF) and palm kernel oil (PKO) blends as suppository bases was investigated using metronidazole as the model drug. Three blends of PKO and CF in the ratios of 1:2, 1:3 having melting points within acceptable range for suppository formulation were chosen. The suppositories including those of cocoa butter used as the Standard, were prepared by the fusion method and thereafter evaluated using the following parameters; appearance, weight uniformity, liquefaction time, absolute drug content and dissolution rate. The results of the evaluation indicated that the suppositories had uniform appearance and low variation in weight. Also their liquefaction times and absolute frug contents were acceptable ranges. The release studies carried out in phosphate buffer solution (pH 7.2) showed that the 1:2 (PKO:CF) base blend suppositories had the fastest release rate but may only be suitable for use in the temperate regions due to its melting point, while the 1:3 and 2:3 base blend suppositories had the slowest release but can be comfortably used in the tropics, Blends of CF and PKO have bright prospects as good suppository bases.

Keywords: *Cow Fat; Palm Kernel Oil, Metronidazole, Suppositories.*

INTRODUCTION

Several suppository bases have recently been developed to replace the age-long theobroma oil because of its inherent disadvantages [1]. A good suppository base should be chemically and physically stable during storage as a bulk product and after preparation into a suppository. It should have no incompatibility with drug moieties and should permit optimal release of the drug it contains [2]. In suppositories, the active ingredients are dissolved or dispersed in a suitable base that should either melt at body temperature or dissolve or disperse in the mucous secretions of the rectum. Suppositories may also contain excipients such as absorbents, absorption enchancers, diluents, lubricants, preservatives and surfactants [3]. Ideal suppository bases should be non-irritant, non-toxic and non-sensitizing, and should be compatible with a wide range of drugs, and should be readily moulded or formed into stable rigid shapes which should remain molten for a sufficient period of time to allow pouring into moulds [3].

Fatty suppository bases consist of natural and synthetic fatty bases such as theobroma oil and hydrogenated palm kernel oil. The first useful synthetic fatty suppository bases came from the hydrogenation and subsequent heat treatment of vegetable oils such as PKO and arachis oil [4]. Hydrogenation saturates, unsaturated glycerides and heat treatment splits some of the triglycerides into fatty acids and partial esters. Natural fats are known to contain a high proportion of unsaturated fatty acids. Water miscible suppository bases of varying melting points and solubility characteristics can also be prepared by blending polyethylene glycols of 1000, 4000 or 6000 molecular weights [4]. CF is a fat extracted from the adipose tissue of Bovine spp. (cattle) and has a waxy consistency and a whitish-cream colour. It is solid at room temperature and exhibits a characteristic offensive colour which necessitates deodorization. PKO is a bland lauric oil which melts rapidly and can be refined to very low colour level. The objective of this study was to establish the possibility of formulating stable suppositories with blends of CF and PKO.

MATERIALS AND METHODS

Material

Metronidazole (M&B), theobroma oil and potassium dihydrogen phosphate (Merck), and disodium hydrogen phosphate (Mallincrodt Chem.) were used as procured from the local suppliers. PKO was sourced locally, while CF was obtained from batch processed in our

laboratory. All other reagents and solvents were of analytical grade and were used as such. Distilled water was obtained from an all glass still.

Methods

Extraction of cow fat (CF)

CF was extracted from the adipose tissue of the peritoneum of cattle (Bovine spp.) by dry Rendering process [5]. The adipose tissue separated from the extraneous material, was subjected to dry heat at 115°C for 45 mins in an oven. The melted fat was then separated from the proteinous matter by filtering through a muslin cloth. The separated fat was then cooled and stored in a refrigerator at 10°C to avoid oxidation of the fat [5].

Deodorization and bleaching of CF

Deodorization and bleaching were done together. This was done by heating the crude CF at 90°C for 30 mins with a combination of activated charcoal and bleaching earth (bentonite) in the ratio of 10:1 per 20g of CF, accompanied with continuous agitation. At the end of the heating, the hot mixture was filtered with a Whatman No. 3 filter paper and the filtrate allowed to solidify at 10°C [6,7].

Determination of the melting points of the bases blends

Bases containing varying portions were prepared by fusion to select bases of optimal consistency, melting and solidification points. The melting points of the base blends were determined in a melting point apparatus (model MFB-600-010F,) Gallenkamp, England). The values reported were averaged from five determinations. Based on the above criteria, the following ratios were selected and used: 1:2, 1:3, and 2:3 of PKO:CF, corresponding to batches A, B and C respectively.

Preparation of Suppositories

Using the displacement value of metronidazole for theobroma oil and consequently for other fatty bases [3], the correct quantities of the base in each batch was calculated. The suppositories were prepared to contain 200 mg of metronidazole per suppository. Enough quantities to yield fifty suppositories were calculated. The correct quantity of the drug was added to the molten base with continuous stirring until it was cool but pourable. The preparation was poured into a 1 g mould already lubricated with glycerine and cooled at 0°C for 30 mins. The suppositories were removed from the moulds and stored in a refrigerator for further experiments. The same procedure was carried out for all the batches including the standard (thoebroma oil-based suppositories). Prior to further experiments the suppository was equilibrated at room temperature (28°C) for 3 hrs.

Evaluation of suppositories

Appearance: The suppositories were selected randomly from each batch and cut longitudinally. The external and internal surfaces were examined with the naked eye and also with a hand lens. The suppositories were examined for the presence or absence of air bubbles brittle fracture, uniformity of mixing and for the presence or absence of contraction holes.

Uniformity of weight: Twenty suppositories were selected at random and weighed together using a torsion balance (model DWM 2-145184, New Jersey). They were also weighed individually and the mean and coefficient of variation for each batch determined

Liquefaction time determination: This was determined by the modification of a method described by Setnikar and Fantelli [8]. Each suppository was placed in a dialysis membrane which was previously soaked in water overnight and tied at both ends. The set-up was suspended in 250ml of phosphate buffer (pH 7.2) thermoregulated at 37°C. The time taken for the suppository to melt completely at that temperature was noted. The values reported were averages of five determinations for each batch.

Absolute drug content: A suppository whose weight equals the average weight of twenty suppositories was allowed to melt at 37°C in 70ml of phosphate buffer contained in a 100 ml volumetric flask. The flask was shaken very well, filtered and made up to volume (100 ml) through the filter and the content analyzed at 277 nm in a spectrophotometer (SP6-450 UV-VIS. Pye Unicam). This procedure was repeated for all

the batches including the standard. The absolute drug content was determined by reference to the standard Beer's law plot for metronidazole. The values reported are averages of five determinations for each batch.

Release studies: The method of Dal Zotto et al. [9] was adopted for this study. Each suppository from the tested batches was put in a piece of dialysis tube (Visking Tubing, London UK) 10 cm long, 2.5 cm diameter closed at one end, which had previously been soaked in water overnight at room temperature. After the addition of 5 ml of phosphate buffer (pH 7.2) the open side was tied with a thread to prevent leakage. The set-up was then suspended in a dissolution tester (DTD, Erweka Germany) containing 500 ml phosphate buffer maintained at a temperature of 37 +/-1 °C and agitation rate of 100 rpm, as the dissolution medium. At predetermined time intervals. 5 ml of the medium was filtered. The filtrate was analyzed in the spectrophotometer above at 277 nm.

RESULTS AND DISCUSSION

Typical analytical values for the rendered CF are 52% saturated fatty acids; 44% monounsaturated fatty acids; 32-54% Iodine value; 190-202 mg KOH per g fat saponification value, 16 max mEq peroxide oxygen per kg peroxide value; 2.5 max KOH per g acid value and 1.2 max % by weight unsaponifiable matter. Rendered animal fats contain insignificant levels of natural antioxidants (tocopherols) compared to vegetable oils and are more prone to oxidation. That necessitated the storage pf CF in a refrigerator ro reduce oxidation [5]. The melting point of CF was 45°C which is too high to be used as a suppository base since it is expected to melt before absorption takes place because of its insolubility in aqueous fluid. Three admixtures of PKO and CF were found to possess melting points within the range suitable for suppository formulation. They include 1;2 (340 +/- 0.01°C) 1:3 (37.2 +/- 0.02 °C) and 2:3 (35.2 +/- 0.0°C) The suppositories were torpedo-shaped, smooth in texture with absence of entrapped air bubbles, holes or brittle fracture. The internal and external surfaces of the suppositories were uniform in appearance when examined with the naked eye and a hand lens. This indicated satisfactory subdivision and dispersing of suspended material. Thus all the suppositories passed the test according to BP specifications [10]. From the result of the weight uniformity test, the variation in weight of the suppositories was very low. Their mean weights and coefficients of variation were: 1.29g +/- 1.28%; 1.25 g +/- 0.44%; 1.27 g +/- 1.29%; and 1.22g +/- 1.37% for batches A,B,C and standard respectively. The variation in weight of the suppositories may have resulted from inappropriate mixing or sedimentation of the dispersed drug during pouring. All the batched nevertheless, passed the test according to BP specifications [10].

The result of the liquefaction time test carried out on the suppositories are 11.5 +/- 2.1 mins; 14.2 +/- 3.4 mins; 24.0 +/- 1.4 mins and 11.7 + /-1.9 mins for batches A,B,C, and standard respectively. The monographs [10,11] did not specify any range for the liquefaction time of metronidazole suppositories since the liquefaction time data naturally depends to some extent, upon the size and shape of the rectal suppositories used [8]. From the result, the batches of the suppositories performed creditably well since their liquefaction times were relatively short except for batch C. Liquefaction time takes considerable time although it can be shortened by the addition of drugs, propylene glycol or water [8], and for drugs with a local action, the softening or liquefaction is not always essential, but it is essential for those including a drug for a general action like metronidazole, release of which depends in first instance on the liquefaction of the suppositories. The knowledge of liquefaction time is essential because a suppository which takes too long to liquefy may be expelled before liquefaction together with the drug it includes. Besides, it may exert a mechanical irritant action on the rectal ampulla even if the base and the drug per se are not irritant. A suppository with a high liquefaction time may still release to contents because of pressure exerted on the formulation by rectal contraction or posture. Thus maximun limit of 10 mins suggested for suppositories that melt before absorption [8] may not be realistic, as pressure exerted on a softened suppository ultimately leads to its ingression in the rectal cavity. The result of the absolute drug content analysis

conformed to BP specifications [10].

Fig. 1 shows the release profile of metronidazole from the suppositories.

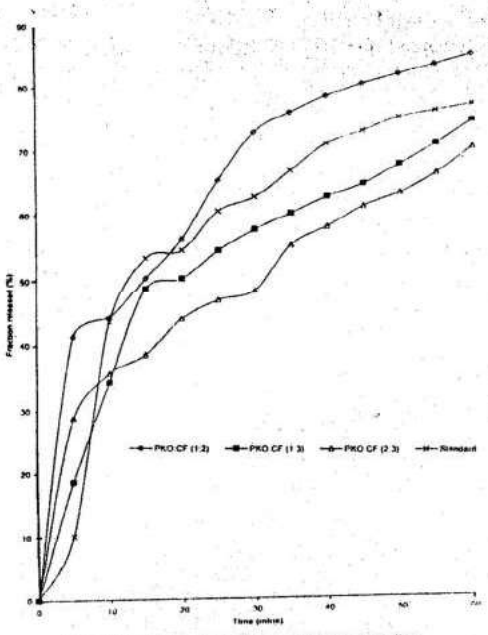

Fig. 1. Release profile of metronidazole from the suppository

There was a high initial release in all the suppositories. However, there were differences in their percentage initial release and percentage maximum release. The release of a drug which is for a general action depends on the liquefaction time of the suppositories [8], and water soluble additives help the fatty bases to emulsify and contribute to the liquefaction process. The release of metronidazole from the bases was generally high since it is a water soluble drug and would have a favourable partition coefficient to the dissolution medium. The suppositories formulated with 1:2 (PKO:CF) blend had the fastest release followed by the standard which was closely followed by the 1:3 blend of PKO:CF. The 2:3 (PKO:CF) base blend suppositories had the slowest release. The trend can be related to their liquefaction times, since the blend with lower liquefaction time had the fastest release rate while the blend with higher liquefaction time had the slowest rate. All the same, all the suppositories could be used when a formulation with fast action is needed.

To understand the release mechanisms of metronidazole from the suppositories, the release rate was described by using the following equations:

$$\frac{M_t}{M} = kt^n \qquad (1)$$

$$\text{Log}\ \frac{M_t}{M} = \log k + n\log t \qquad (2)$$

where M_t/M is the fraction of released drug at time t, k is a characteristic constant which incorporates the geometry of the suppositories, and n is an indicative of release mechanism.

As the k value become higher the drug is released faster. The n value of 1 corresponds to zero-order release kinetics. $0.5 < n < 1$ means a non-Fickian (anomalous) release model and $n = 0.5$ indicates Fickian diffusion [12]. From the plots of log M_t/M versus log t (Fig 2), the kinetic parameters n and k were calculated, and presented in Table 1.

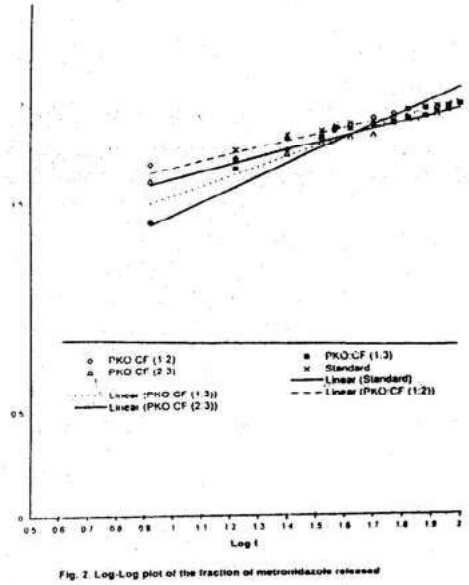

Fig. 2. Log-Log plot of the fraction of metronidazole released

From the table, most of the n values are close to 0.5 except the standard, suggesting that metronidazole might be released from the suppositories prepared with the base blends by Fickian diffusion.

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Table 1. Result of the kinetic parameters

Batch (PKO:CF)	Release exponent (n)	Kinetic constant (k)	Correlation coefficient ®
1:2	0.335	1.3391	0.9484
1:3	0.4894	1.0453	0.92245
2:3	0.3577	1.6228	0.9823
STANDARD	0.6477	0.7900	0.7690

Release from the standard suppositories was found to be anomalous (n>0.5). Also, the higher k values of the base blend suppositories indicated that the release of metronidazole was faster in them than in the standard suppositories.

CONCLUSION

Suppositories formulated with PKO.CF (1:2) admixture had the fastest release (k =1.3391) but can only be stored at room temperature in the temperate regions. Ideal suppositories for use in the tropics may be formulated with 1:3 blend of PKO:CF. In all, admixtures of PKO and CF can be used in preparing physically stable suppositories and could thus replace the age-long theobroma oil with many desirable characteristics.

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