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Influence of storage conditions on the physical and release properties of piroxicam suppositories formulated with lipophilic bases

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Abstract

This study investigated the effect of storage conditions on physical and release properties of lipophilic-based piroxicam suppositories with a view to determining the most desirable conditions for their storage. Piroxicam 20 mg suppositories were prepared by fusion method using cocoa butter, Witepsol H15® and Witepsol W35® to which 2 %w/w Tween 20® was added. Physical and dissolution properties of the suppositories were determined by established methods after preparation and at 4-month intervals for 12-month storage on the shelf (27.5 ± 0.9 °C to 32.5 ±0.8 °C) and in the refrigerator (4 ± 1 °C). The suppositories stored in the refrigerator hardened at the fourth month, with fat "blooming" observed on cocoa butter-based suppositories at 8th month. Melting points of cocoa butter-based suppositories remained below 37.5 °C on storage, while those formulated with Witepsol H15® and Witepsol W35® increased to 42.1 °C and 44.0 °C, respectively. Cocoa butter-based suppositories stored on the shelf showed decrease in melting range and mechanical strength compared with those stored in the refrigerator. There was increase in mechanical strength and disintegration time of the witepsol-based suppositories on aging. Release of piroxicam from witepsol bases decreased significantly ($P < 0.05$) on storage either in the refrigerator or on the shelf, while those formulated with cocoa butter base increased. In conclusion, piroxicam suppositories formulated with cocoa butter would require storage in the refrigerator, while long-term storage of those formulated with Witepsol H15® and Witepsol W35® in the refrigerator might not be desirable.

Keywords: Piroxicam suppositories; Lipophilic bases; Storage conditions; Physical properties; Release properties

INTRODUCTION

Suppository bases, acting as vehicles for drugs are important factors in the formulation, physical and release properties of suppositories [1-3]. While suppositories are relatively stable in the temperate regions, their stability, especially for the fat-based suppositories, is of great concern in tropical climates, which has warranted the recommendation that they should be stored in a refrigerator or cold condition [4]. However, the physicochemical properties of formulated suppositories have been reported to change during storage [5-7]. Prolonged storage of suppositories has been found to result in changes in physical appearance, hardness, softening and melting points of the suppositories [6,8,9], depending on the nature of the base [10-12]. Hardening phenomenon due to storage may also result in little or no melting of the suppositories, thus causing local irritation in the rectum or bowel

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obstruction [13]. Changes in the physical properties of suppositories have been reported to affect the extent of drug release and release mechanism from the formulations [5]. Such adverse effect of storage may be highly pronounced in formulations where the active drugs constitute a minute portion of the formula relative to the suppository base. Among such drugs of interest is piroxicam, a poor water soluble drug with a formulation dose of 20 mg [14,15].

Piroxicam is classified as a potent nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic affects [15]. It has been used in acute and chronic musculoskeletal and joint disorders, acute gout and pain associated with inflammation [15,16]. The formulation of piroxicam as suppository for rectal administration offers advantages over oral administration that presents gastrointestinal side effects [17,18], and first-pass effects in the liver [19]. Previous studies have shown that the poor water solubility of the drug influenced the release of the drug from fat-based suppositories [17,20,21]. However, such studies were not extended to the effect of storage period and conditions on the physical properties and release profiles of the drug from the suppositories.

Therefore, the current study aims at evaluating the effect of storage on the physical and release properties of fat-based piroxicam suppositories stored in the two most common storage conditions in the tropics (open shelf and refrigerator), with the objective of determining the more desirable storage condition for the suppositories.

EXPERIMENTAL

Materials. The following materials were used: piroxicam powder (donated by Drugfield Pharmaceuticals Ltd, Sango Otta, Nigeria), Cocoa butter (Starmark Cocoa Processing Company Ltd, Ondo, Nigeria), Witepsol W35® and Witepsol H15® (AXO Industry International, Chaussee de Louvain 171, Belgium), Sodium Hydroxide (BDH Laboratory, Poole BH15, England), Potassium dihydrogen orthophosphate (Surechemproducts Ltd, England), Polysorbate 20 (Tween 20®) (Sigma Chemical Co., St. Louis, USA).

Preparation of piroxicam suppositories. The bases used in this study were cocoa butter, Witepsol H15® and Witepsol W35®. The suppositories were prepared by fusion method [11] using a 1g metal mould with six cavities. Suppositories, each containing 20 mg piroxicam powder and 2 %w/w Tween 20® were prepared. The quantity of base required in each formula was determined by the drug's displacement value [22]. The base was melted on a water bath at temperature not higher than 38 °C in order to avoid over-heating and degradation of the base. The quantity of Tween 20® required in each formula was added to the molten base, with subsequent addition of required quantity of the sifted piroxicam powder, followed by thorough mixing to form homogeneous mass. The mixture was poured into the lubricated stainless steel mould, allowed to cool, and the excess congealed mass trimmed off. The suppositories were then removed from the mould, wrapped with aluminum foil, packed in a wide-mouthed opaque plastic container, labelled appropriately and stored at 24 ± 1 °C for not more than 7 days before analysis. Suppositories without piroxicam (placebo) were also prepared using the same method. The compositions of the formulated suppositories and their codes are as in Table 1.

Storage of the formulated suppositories. Suppositories from each formulation were divided into two portions, with a portion stored in the refrigerator (4 ± 1 °C), while the second portion was stored on the shelf at room temperature over a period of 12 months that spanned September to August. The room temperature within the period varied from 27.5 ± 0.9 °C to 32.5 ± 0.8 °C, while the

relative humidity measured with Dry and Wet Bulb hygrometer (OMSONS, India) varied from 54 ± 5 % to 79 ± 5 %.

Evaluation of physicochemical properties of the suppositories. The physiochemical properties of the suppositories were evaluated every 4 months for 12 months. Initial evaluation of physicochemical properties of the suppositories was carried out within 7 days of their preparation. The data obtained within 7 days of preparation served as base line (0 month) for comparison of those obtained on storage.

Visual assessment of the suppositories. Visual assessment of the physical appearance of the suppositories was carried out using the characteristics itemized in Table 2.

Uniformity of weight test. Twenty suppositories were randomly selected from each batch of the formulations and weighed individually using a Mettler analytical balance (AB54 Toledo, Switzerland). The mean weight and percentage relative standard deviations (RSD) were determined immediately after preparation and on storage. The deviations of the individual weight from the theoretical weight of the suppositories were also calculated.

Determination of content uniformity. The method described by Setnikar and Fontani [23] was used. A suppository taken randomly from each batch was weighed, sliced and placed in a beaker containing 100 ml of phosphate buffer solution (pH 7.2). The suppository was melted by heating the beaker gradually on a water bath. The beaker was shaken gently while the melting proceeded. When the suppository had been completely dispersed, the mixture was chilled and the oil layer was removed by filtration through a cotton plug. The aqueous portion was further filtered through Sinter glass number 3 (DURAN Group GmbH, Germany), having a porosity of 30 µm. The aqueous filtrate (1 ml) was diluted to 100 ml using phosphate buffer solution. The absorbance was measured by UV spectrophotometer (mini-1240 model, Germany) at 350 nm. The concentration of the piroxicam solution was calculated from a standard Beer-Lambert curve in the concentration range of 2.5×10^{-4} to 2.5×10^{-3} %w/v, and the drug content of each suppository determined. The result was an average of four determinations per batch of suppositories.

Determination of softening and melting points. The softening and melting points of piroxicam suppositories were determined using the modified method of Adebayo and Akala [24]. Each suppository sample was placed in a clean test tube with a thermometer inserted. The tube was clamped vertically, immersed at 8 cm depth in a water bath. Temperature of the water bath was gradually increased (1 °C/ 2 min). The temperature at which the suppository sample began to melt was recorded as the softening point, while the temperature of its complete liquefaction was defined as the melting point. The difference between the two temperature values gave the melting range of the suppository. The results obtained were average of four determinations.

Determination of disintegration time. The disintegration time of the suppositories was evaluated with the Manesty tablet disintegration apparatus using the BP [25] method for uncoated tablets. The apparatus consists of six cylindrical glass tubes, each filled with 160 ml of distilled water, immersed in a water bath maintained at constant temperature (37 ± 1 °C) and held by a basket rack attached to a vertical metal rod. The rod was fixed to a mechanical device capable of raising and lowering the device through a distance of 60 mm at a frequency of 32 cycles per minute. For disintegration time testing, one suppository was placed in each glass tube and a metal disc weighing 50 g was added to each tube [24]. The time required for complete deformation of each suppository sample was determined.

Determination of mechanical strength. The hardness of suppository samples was determined using the Monsanto Hardness Tester [26]. Ten suppositories randomly selected from each batch were used. The weight required for the suppository to collapse was taken as a measure of its hardness.

Evaluation of release profile of piroxicam from the suppositories. The United States Pharmacopeia [4] basket method was employed for the dissolution rate studies using digital tablet dissolution test apparatus (Model VDA-8D, PharmChem Machineries, Mumbai, India). Phosphate buffer solution (900 ml) at pH 7.2 was used as the dissolution medium. A suppository was randomly selected from each batch, its weight determined and placed inside the dissolution basket which was then lowered into a flask containing the dissolution medium maintained at constant temperature (37.0 ± 0.5 °C). The basket was rotated at the constant speed of 100 rpm. At determined time intervals, 5 ml samples were withdrawn over a period of 180 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, diluted appropriately with the buffer solution and the absorbance determined by UV spectrophotometer (mini-1240 model, Germany) at 350 nm. The amount of drug released was calculated from a standard Beer-Lambert calibration curve. The mean of four determinations was used in calculating drug release from each batch of suppositories. The drug release parameters: percentage of drug released at 180 min ($\%D_{180min}$), 60 min ($\%D_{60min}$), the time (min) for 50 % ($T_{50\%}$) and 75 % ($T_{75\%}$) of the drug to be released were calculated.

Determination of drug release kinetics. The dissolution data were fitted into 4 release kinetic models [27-29] namely; Zero-order (Q vs t), First-order (log (Qo – Qt) vs. t), Higuchi square root model (Q vs. $t^{½}$) and Korsmeyer-Peppas' model (log Qt vs. nlog t), where Q is the amount of drug released at time t, Qo is the initial amount of the drug, Qt is the amount remaining at time t and *n* the release exponent from Korsmeyer-Peppas' model. In the application of the Korsmeyer-Peppas' model, the first 60 % drug release data were used [27,29]. Dissolution data were evaluated using Microsoft Excel spreadsheet and DDSolver software [30,31]. The best-fit dissolution model was identified by $R^2_{adjusted}$, Model Selection Criteria (MSC) and Akaike Information Criterion (AIC), where model with the highest $R^2_{adjusted}$ (≥ 0.990), MSC (≥ 3.00) values and lowest AIC value within the set of the models was considered the best fit [31].

Statistical analyses. Statistical differences among the results from the various physical tests and dissolution rate studies were assessed by employing Microsoft Excel and GraphPad Prism 5 software with minimum level of significance established at 5 %.

RESULTS

Physical appearance. All the suppositories had smooth surfaces without colour mottling. They were hard with dry surfaces. The prominent change in physical appearance of the suppositories was hardening which occurred from the 4th month among those stored in the refrigerator (Table 2). Colour change occurred among the cocoa butter-based suppositories on the shelf at the 8th month, while fat "blooming" occurred at the 8th month among those stored in the refrigerator. Mold growth was observed among the medicated suppositories formulated with witepsol bases (HD, WD) that were stored on the shelf.

Physicochemical properties. The physical mean weights of medicated suppositories were 1.004 ± 0.013 g, 1.028 ± 0.005 g and

1.035 ± 0.004 g for CD, HD and WD, respectively. All the suppositories fell within 95 and 105 % of the average weights, and there was no significant difference ($P > 0.05$) between the theoretical and physical weights of the formulated piroxicam suppositories. A comparison of the weights of the suppositories on preparation with those stored for 12 months on the shelf or in the refrigerator showed deviations not greater than 3.5 %.

The mean piroxicam contents of the suppositories were 102.4 ± 2.8 %, 96.6 ± 3.5% and 98.9 ± 3.7 % for CD, HD and WD, respectively. On analysis of the drug contents of the suppositories at 12^{th} month, there was no significant difference ($P > 0.05$) observed with those obtained on preparation.

The effect of storage condition and aging on physical properties of piroxicam suppositories prepared with cocoa butter, Witepsol H15® and Witepsol W35® bases are indicated in Table 3. The inclusion of piroxicam into the bases did not significantly ($P > 0.05$) affect the melting range of the resulting piroxicam suppositories on preparation (CP vs. CD; HP vs. HD; WP vs. WD) as indicated in Table 3. There was increase in the melting range of the suppositories on storage in either the refrigerator or on the shelf, with witepsol-based suppositories (HP, HD, WP, WD) having significantly higher values ($P < 0.05$) than those of cocoa butter based suppositories (CP, CD).

Storage of the medicated suppositories (CD, HD, WD) in the refrigerator led to increase in their mechanical strength, which was highly significant ($P < 0.05$) for those formulated with witepsol bases. The differences between mechanical strength on preparation and on storage for 12 months in the refrigerator were 0.4 kg, 3.2 kg and 2.8 kg for CD, HD and WD, respectively.

The initial disintegration times of all the suppositories were within 15 min on preparation (Table 3). The disintegration times of the cocoa butter-based suppositories (CP, CD) decreased on storage, while there was increase in disintegration time of those formulated with witepsol bases (HP, HD, WP, WD) for all the storage conditions. The observed differences in the disintegration time of witepsol-based suppositories stored on the shelf and those stored in the refrigerator were not significant ($P > 0.05$).

Release profiles of piroxicam suppositories. Release parameters derived from dissolution-time curves of piroxicam suppositories (CD, HD, WD) stored on the shelf or in the refrigerator at zero, 4^{th}, 8^{th} and 12^{th} month are indicated in Table 4. Typical dissolution-time curves for piroxicam suppositories on preparation (zero month) and after storage for 12 months on the shelf or in the refrigerator are depicted in Fig. I. As indicated in Table 1, the release of piroxicam from the suppositories was in the order of WD > HD > CD. None of the formulations released up to 90 % of piroxicam content within 180 min of the study. On storage, cocoa butter-based suppositories (CD) showed increase in the release of piroxicam, with those stored in the refrigerator being significantly higher ($P < 0.05$) than those stored on the shelf. The release of piroxicam from witepsol-based suppositories (HD, WD) decreased significantly ($P < 0.05$) on storage for 12 months either on the shelf or in the refrigerator. Figure II also depicted the effect of the suppository base, storage condition and aging on release of piroxicam from the formulations. None of the fat- based suppositories released up to 75 % of their drug content within 60 min.

Release kinetics of piroxicam suppositories. The best fit selection criteria from Higuchi, Zero-order and First-order release kinetics models based on dissolution data obtained at zero and 12^{th} month storage of the suppositories (Fig. I) are indicated in Table 5. The $R^2_{adjusted}$, MSC and AIC values indicated

that at zero month, formulation CD could fit into Higuchi (diffusion) model, while formulations HD and WD could be fitted into First-order model (Table 5). On storage for 12 months, the three formulations (CD, HD, WD) were perfectly fitted into First-order kinetics model based on the values of the selection criteria. None of the formulations fitted into Zero-order kinetics model immediately after preparation or on storage for 12 months (Table 5).

The derived release parameters and selection criteria from the dissolution profiles in Fig. I, using the Korsmeyer-Peppas model are indicated in Table 5. The release exponents, *n* ranged between 0.64 and 0.78, indicating non-Fickian diffusion release mechanism. While the release constants (K_{kp}) significantly changed ($P < 0.05$) on storage, there was no significant difference ($P > 0.05$) in the release exponents on storage (Table 5). The release constants (K_{kp}) of piroxicam suppositories formulated with cocoa butter (CD) increased on storage, while those formulated with Witepsol H15® (HD) and Witepsol W35® (WD) decreased.

Table 1: Codes and composition of formulated piroxicam suppositories

Code	Formulation
CP	Cocoa butter with 2 %w/w Tween 20® (Placebo)
CD	Cocoa butter with 2 %w/w Tween 20® and 20 mg piroxicam
HP	Witepsol H15® with 2 %w/w Tween 20® (Placebo)
HD	Witepsol H15® with 2 %w/w Tween 20® and 20 mg piroxicam
WP	Witepsol W35® with 2 %w/w Tween 20® (Placebo)
WD	Witepsol W35® with 2 %w/w Tween 20® and 20 mg piroxicam

Table 2: Physical appearances of suppository formulations on storage

Physical property	Storage period (month)	Formulation code (see Table 1)/ Storage condition											
		CP		CD		HP		HD		WP		WD	
		SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS
Cracking	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-	-	-	-
Fat blooming	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	+	-	+	-	-	-	-	-	-	-	-
	12	-	+	-	+	-	-	-	-	-	-	-	-
Colour change	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
	8	+	-	+	-	-	-	-	-	-	-	-	-
	12	+	-	+	-	-	-	-	-	-	-	-	-
Mold growth	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	+	-	-	-	+	-
	12	-	-	-	-	-	-	+	-	-	-	+	-
Softening	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-	-	-	-
Hardening	0	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	+	-	+	-	+	-	+	-	+
	8	-	-	-	+	-	+	-	+	-	+	-	+
	12	-	+	-	+	-	+	-	+	-	+	-	+

SS: Shelf storage; RS: Refrigerator storage; - No change in physical appearance; + change in physical appearance;

Table 3: Physical properties of suppository formulations on storage

F/C	S/C	Storage period / Physical properties											
		0 month*			4th month			8th month			12th month		
		MR (°C)	MS (kg)	DT (min)	MR (°C)	MS (kg)	DT (min)	MR (°C)	MS (kg)	DT (min)	MR (°C)	MS (kg)	DT (min)
CP	SS	32.6-35.1	3.8 ± 0.2	15.0 ± 1.0	31.5-34.0	3.7 ± 0.2	12.6 ± 0.8	31.3-34.2	3.6 ± 0.2	10.2 ± 0.8	30.8-34.5	3.5 ± 0.3	9.2± 1.2
	RS				32.6-35.5	3.9 ± 0.2	11.5 ± 0.3	32.2-35.7	3.9 ± 0.2	9.1 ± 0.6	32.4-36.3	4.2 ± 0.2	8.3 ± 0.8
CD	SS	33.3-35.7	3.6 ± 0.3	14.7 ± 0.6	33.1-36.6	3.6 ± 0.3	12.4 ± 1.0	32.6-36.5	3.4 ± 0.1	9.8 ± 0.8	31.7-37.0	3.4 ± 0.3	8.7 ± 0.7
	RS				34.7-36.4	3.8± 0.9	11.4± 0.6	34.9-36.8	4.0± 0.2	7.9± 0.7	35.2-37.4	4.2± 0.1	7.2± 0.7
HP	SS	33.3-36.9	3.2 ± 0.3	14.7 ± 0.9	34.3-38.4	3.9 ± 0.5	17.9 ± 0.7	34.4-41.9	4.2 ± 0.3	18.0 ± 0.8	34.5-41.8	4.3 ± 0.2	21.2 ± 1.2
	RS				34.0-38.9	4.5 ± 0.4	16.8 ± 0.4	35.0-39.9	5.6 ± 0.2	17.5 ± 0.9	35.2-40.9	5.7 ± 0.3	21.5 ± 0.8
HD	SS	34.0-37.3	3.3 ± 0.2	14.0 ± 0.1	34.0-38.3	3.8 ± 0.2	17.3 ± 0.5	34.1-41.4	4.0 ± 0.5	17.9 ± 0.6	34.5-42.1	4.0 ± 0.1	21.4 ± 1.0
	RS				34.5-38.0	5.1 ± 0.2	18.1 ± 0.6	35.6-41.4	6.2 ± 0.3	18.0 ± 0.7	35.9-42.1	6.5 ± 0.3	21.1 ± 0.5
WP	SS	34.3-38.0	3.0 ± 0.3	14.3 ± 0.2	34.4-38.9	3.8 ± 0.4	19.5 ± 0.6	34.5-43.8	3.8 ± 0.2	19.4 ± 0.8	34.6-44.0	4.0 ± 0.3	22.7 ± 0.9
	RS				35.1-39.1	4.2 ± 0.2	17.7 ± 0.2	35.4-41.1	4.8 ± 0.3	18.7 ± 0.9	35.6-41.9	5.3 ± 0.1	23.2 ± 0.7
WD	SS	33.4-37.6	3.6 ± 0.2	13.9 ± 0.6	34.8-39.2	4.3 ± 0.1	18.2 ± 0.8	35.0-41.9	4.5 ± 0.2	18.4 ± 0.4	35.1-43.5	4.8. ± 0.1	21.5 ± 0.7
	RS				34.7-38.6	5.0 ± 0.4	16.2 ± 0.6	34.5-40.5	5.9 ± 0.5	17.2 ± 0.8	35.5-41.9	6.4 ± 0.2	21.4 ± 1.0

F/C: Formulation code (see Table 1); S/C: Storage condition; SS: Shelf storage; RS: Refrigerator storage;
MR: Melting range (°C); MS: Mechanical strength (kg); DT: Disintegration time (min)
0 month* provides baseline data for both the shelf and the refrigerator storage; **See Table 1 for formulation code

Table 4: Release parameters for piroxicam Suppository Formulations on storage

Storage period (month)	Drug release parameter	**Formulation code/Storage condition					
		CD		HD		WD	
		SS	RS	SS	RS	SS	RS
0*	$T_{50\%}$ (min)	>180		73.5 ± 4.8		56.7 ± 2.5	
	$T_{75\%}$ (min)	>180		145.4 ± 4.3		128.3 ± 1.2	
	$\%D_{180min}$	31.3 ± 1.1		85.7 ± 2.9		89.9 ± 2.7	
4	$T_{50\%}$ (min)	>180	147.4 ± 2.5	87.2 ± 4.6	93.6 ± 4.0	95.5 ± 3.2	84.8 ± 2.5
	$T_{75\%}$ (min)	>180	>180	>180	165.9 ± 3.7	160.0 ± 2.7	146.1 ± 3.0
	$\%D_{180min}$	41.9 ± 2.1	57.7 ± 1.1	72.4 ± 3.0	79.6 ± 1.7	80.3 ± 2.0	87.8 ± 2.3
8	$T_{50\%}$ (min)	176.5 ± 2.0	93.8 ± 4.3	152.1 ± 4.8	108.5 ± 1.0	108.1 ± 2.0	95.6 ± 3.6
	$T_{75\%}$ (min)	>180	>180	>180	>180	>180	174.1 ± 2.6
	$\%D_{180min}$	49.9 ± 1.8	71.3 ± 1.8	54.2 ± 2.0	73.2 ± 0.9	73.7 ± 1.1	76.4 ± 1.7
12	$T_{50\%}$ (min)	168.0 ± 3.6	96.3 ± 4.2	>180	132.3 ± 2.4	150.9 ± 3.0	127.3 ± 3.5
	$T_{75\%}$ (min)	>180	118.8 ± 2.3	>180	>180	>180	>180
	$\%D_{180min}$	53.4 ± 1.9	79.9 ± 3.7	49.2 ± 0.9	62.5 ± 1.4	56.4 ± 1.3	62.1 ± 2.6

SS: Shelf storage; RS: Refrigerator storage; **See Table 1 for formulation code
0* month provides baseline data for both the shelf and the refrigerator storage

Table 5: Comparative release rate constants and model fitting parameters for release kinetics of piroxicam suppositories immediately after formulation and on storage for 12 months

Release Kinetic Model	Release parameter	Storage period (month)/storage condition/Formulation code**								
		0 month*			12th month					
					Shelf			Refrigerator		
		CD	HD	WD	CD	HD	WD	CD	HD	WD
Higuchi	K_H (mg/min$^{1/2}$)	0.418	1.194	1.284	0.746	0.692	0.758	1.074	0.814	0.833
	$R^2_{adjusted}$	0.968	0.965	0.971	0.960	0.955	0.941	0.956	0.918	0.924
	MSC	3.3	3.2	3.4	3.1	3.0	2.7	3.0	2.4	2.4
	AIC	51.1	82.5	81.1	71.5	70.6	78.9	83.2	86.7	86.0
Zero-order	K_o (min^{-1})	0.024	0.109	0.117	0.069	0.063	0.070	0.099	0.076	0.076
	$R^2_{adjusted}$	0.876	0.885	0.833	0.893	0.871	0.930	0.896	0.962	0.949
	MSC	1.9	2.0	1.7	2.1	1.9	2.5	2.1	3.1	2.8
	AIC	70.6	99.7	105.7	85.3	85.4	81.2	95.3	75.8	80.5
First-order	K_1 (mg/min)	0.0004	0.002	0.0022	0.001	0.0008	0.001	0.0016	0.001	0.0012
	$R^2_{adjusted}$	0.922	0.994	0.993	0.972	0.966	0.989	0.985	0.998	0.998
	MSC	2.4	4.9	4.8	3.4	3.2	4.3	4.1	6.3	6.1
	AIC	63.5	59.0	61.7	66.6	66.9	55.7	67.8	32.4	34.5
Korsmeyer -Peppas	K_{KP} (mg/min^n)	0.221	0.541	0.563	0.358	0.366	0.288	0.537	0.227	0.249
	n	0.64	0.68	0.70	0.66	0.64	0.71	0.65	0.78	0.76
	$R^2_{adjusted}$	0.998	0.995	0.981	0.998	0.995	0.997	0.987	0.998	0.995
	MSC	5.8	5.0	3.6	5.8	5.1	5.5	4.1	6.0	5.1
	AIC	15.9	29.4	37.7	33.7	40.3	39.8	43.9	29.3	41.8
Best fit release kinetic model		Higuchi	First-order	First-order	First-order	First-order	First-order	First-order	First-order	First-order

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; 0 month* provides baseline data for both the shelf and the refrigerator storage; **See Table 1 for formulation code

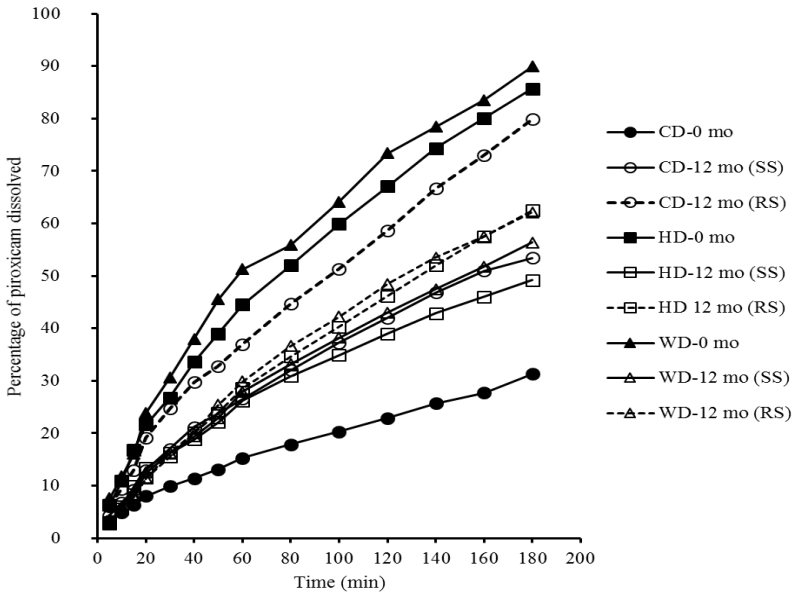

Figure I: Effect of storage conditions on release of piroxicam from fat-based suppositories SS: Shelf storage; RS: Refrigerator storage; 0 month: provides baseline data for the shelf and the refrigerator storage; CD, HD, WD: See Table 1 for formulation code

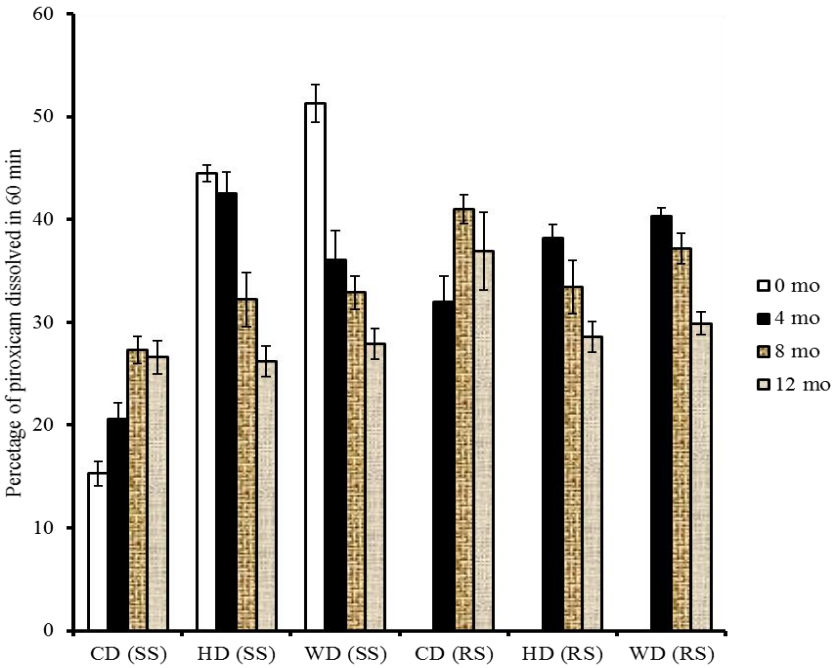

Figure II: Percentage of piroxicam released in 60 minutes from fat-based suppositories stored on the shelf and in the refrigerator over a period of 12 months
SS: Shelf storage; RS: Refrigerator storage; 0 month: provides baseline data for the shelf and the refrigerator storage; CD, HD, WD: See Table 1 for formulation code

DISCUSSION

The placebo suppositories (CP, HP and WP) gave baseline values for the comparison of physical properties of the piroxicam suppositories. There was no colour mottling on preparation, indicating that the piroxicam was uniformly dispersed within the bases. However, a colour change from yellow to off-white was observed among suppositories prepared with cocoa butter (CP and CD) stored on the shelf. This could be due to bleaching caused by oxidation of the initial colours of the bases. Hardening effect that was observed with those suppositories stored in the refrigerator could be due to crystallization of the base [9]. Another age-related change in the physical appearance was fat "blooming" observed with cocoa butter based suppositories stored in the refrigerator. Fat "blooming" in cocoa butter had been reported to be due to fat migration through its micro-fissures which crystallizes at the surface of the base [32]. While inclusion of 1-10 %w/w Tween 20® in the formulation has been suggested to inhibit such defect [32], the present study showed that addition of 2 %w/w Tween 20® to suppositories formulated with cocoa butter did not inhibit the formation of fat "blooming". The insignificant change ($P > 0.05$) in weights of the piroxicam suppositories on storage was an indication of physical stability of the suppositories without the attendant shrinking or moisture absorption despite the high hydroxyl value of Witepsol W35® (WP and WD).

The softening and melting points as denoted by melting ranges are crucial in the release of drug from suppositories formulated with fatty bases [33]. A softening point between 32 °C and 37 °C is considered desirable for suppositories formulated with fatty bases in order to maintain the suppository in its solid state at ambient temperature in the tropics as well as enable fast release of active ingredients from these bases when inserted into the body [34]. The results in Table 3 showed that all the suppositories had softening points below 37

°C on preparation. However, there was a decrease in the softening points of cocoa butter-based suppositories (CP and CD) stored on the shelf which may be due to change in the polymorphic form of the base on exposure to fluctuating high tropical temperatures over the storage period [35]. The reverse was obtained with witepsol-based suppositories (HP, HD, WP and WD) stored on the shelf as there was gradual increase in their softening points on aging. Generally, all the suppositories stored in the refrigerator showed increase in the softening points on aging, which was more pronounced with those suppositories formulated with witepsol bases than cocoa butter base.

While the melting points of suppositories formulated with cocoa butter (CP and CD) remained under 37.4 °C on aging, those formulated with Witepsol H15® (HP and HD) and Witepsol W35® (WP and WD) were as high as 42.1 °C and 44.0 °C, respectively. The same trend was observed for the placebos formulated with these bases (Table 3). This finding reflected the hardening phenomenon that has been report for semi-synthetic fatty bases on storage [12,13,35,36], as a result of differences in their fatty acid composition. Cocoa butter is composed of triglycerides with considerable amount of unsaturated oleic acid, while the witepsol bases (H15 and W35) have mainly mono- and di- glycerides [19]. The semi-synthetic fatty bases (witepsol) are readily subjected to hardening as a result of crystallization, especially those with high hydroxyl value (Witepsol W35®) than cocoa butter.

The inclusion of piroxicam in the cocoa butter (CD) and Witepsol H15® (HD) gave no significant change ($P > 0.05$) in the mechanical strength of the suppositories. This was in contrast to the reported repression of mechanical strength of some suppository bases by some phenolic drugs [37] and concentration-related increased in mechanical strength of metronidazole suppositories reported [38]. However, the low piroxicam content (2 %w/w) in each suppository may be responsible for the insignificant effect of the drug on the mechanical strength of the suppositories. Suppositories stored in the refrigerator showed continuous increase in their mechanical strength over the 12-month storage which was significantly ($P < 0.05$) higher than those stored on the shelf. This may be due to the chilling temperature the suppositories were subjected to which made them stiffer and more compact than those on the shelf. Such hardening and increase in mechanical strength of those suppositories formulated with witepsol bases were found to affect significantly ($P < 0.05$) drug release from the suppositories.

While all the formulations were found to satisfy the BP [25] requirement for disintegration (Table 3), the continuous increase in disintegration time of the witepsol-based suppositories (HD and WD) on aging unlike the decrease observed for cocoa butter-based suppositories might have contributed to decrease in the release rate of the drug from formulations HD and WD.

The release profile of piroxicam from the suppositories was in the order: Witepsol W35® (CD) > Witepsol H15® (HD) > cocoa butter (CD) (Table 4), which reflected the hydroxyl values of the bases and their monoglycerides contents. Piroxicam being a lipophilic drug has high affinity for the three fatty bases. However, the presence of hydroxyl groups in witepsol bases and with their monoglycerides content conferring emulsifying property, made them more hydrophilic and readily miscible with the dissolution medium than the cocoa butter, thus enhancing desorption of piroxicam from the witepsol bases faster than from cocoa butter. Also, the presence of high hydroxyl value in Witepsol W35® imparted a more hydro-dispersible character on the base than Witepsol H15 [2,8,39], hence reflecting the faster release rate of the drug in Witepsol

W35® than in Witepsol H15® (Table 4, Fig. II).

The melting of a fatty base is a pre-requisite for drug release. The lower the melting range, the faster the drug release. It was therefore expected that the release rate of piroxicam should be faster in cocoa butter with lower melting point than witepsol bases with higher melting ranges (Table 3). However, an examination of melting range (softening and melting points) of the fatty bases (Table 3) relative to the piroxicam released (Table 4) revealed that the melting points of the fat-based suppositories played no significant role in the release of the drug at zero month. This finding was in contrast to that of Ilomuanya *et al.* [2] on the release of paracetamol from fatty bases. It, however, agreed with some previous studies [10,17] in which cocoa butter with lower melting range (32-35 °C) gave a lower release of medicament contents compared with Fattibase®, Witepsol H15®, Witepsol W35® and Suppocire AM® which have relatively higher melting ranges. The obtained trend has been attributed to the presence of monoglycerides in the Witepsol H15® and Witepsol W35® bases which act as emulsifying agents, thus facilitating the dispersion of the drug to the surrounding medium [17]. Thus, the high hydroxyl values and monoglycerides content of the witepsol bases appeared to have greater impact on the release rate of piroxicam from the fatty bases than their melting ranges. However, this observation was limited to suppositories analysed at zero month as prolonged storage, resulting to hardening of suppositories with increased melting range, gave a different release pattern.

The observed increase in piroxicam released from cocoa butter-based suppositories on aging has been previously reported for amoxicillin formulated in cocoa butter [12]. Witepsol bases, on the other hand, showed continuous decrease in the amount of piroxicam released during the storage period (Table 4, Fig. II). On storage, semi-synthetic fatty bases (Witepsol H15® and Witepsol W35®) have been shown to be subjected to hardening with a corresponding increase in melting points [40]. The increase in hardness was more pronounced with suppositories stored in the refrigerator than on the shelf. This has been previously explained to be as a result of increase in crystallinity and transesterification occurring in the Witepsol H15® and Witepsol W35® bases due to their mono- and di- glycerides contents [9,35], compared with polymorphic phase transitions that is synonymous with cocoa butter. The consequence of hardening as a result of storage was also the increase in the softening and melting points of the witepsol-based suppositories. The melting points of the witepsol based-suppositories were above 37 °C from the 4th month of storage (Table 3). Since the temperature of the dissolution medium was regulated to 37.0 ± 0.5 °C, any fatty suppository with melting point above 37 °C will show poor drug release. Such suppository may remain in the dissolution medium like a "plug" in which, instead of melting, the release of the drug occurs by wearing off from the surface of the intact suppository. This may be responsible for the decrease in the release of piroxicam from the witepsol bases as they aged.

The mechanism of piroxicam release from the formulations immediately after preparation and on storage for 12 months was best described with Korsmeyer-Peppas model ($R^2_{adjusted} \geq 0.981$; MSC ≥ 3.6; AIC ≤ 43.9) with release exponents, *n* ranging between 0.64 – 0.78, indicating non-Fickian diffusion (anomalous) transport drug release mechanism (Table 5). Such release mechanism has been associated with more than one type of release phenomenon facilitating drug release from formulations [41]. In case of fat-based suppositories, this involves melting of the base, partitioning and

diffusion of the drug through the molten base to the dissolution medium [22,42]. The $R^2_{adjusted}$, MSC, and AIC values obtained for Higuchi, Zero and First order kinetics models for the release of piroxicam immediately after preparation indicated Higuchi diffusion model for CD and First-order release kinetics model for HD and WD (Table 5). The time-release profiles of piroxicam from the suppositories as indicated in Fig. II were parabolic, indicating that the drug release mechanism from the suppositories did not follow Zero-order kinetics model [43,44]. The release mechanism changed to First-order kinetics model for all the formulations on storage for 12 months either on the shelf or in the refrigerator (Table 5). Under the First-order release model, there was a decrease in the drug release constants (K_1) on aging for suppositories prepared with Witepsol H15® (HD) and Witepsol W35® (CD), while those prepared with cocoa butter (CD) showed increase in the release rates. First-order release model is drug concentration dependent which may be related to the poor solubility of piroxicam in the dissolution medium and the change in geometry of the suppositories as they melt.

Conclusion. This study has shown that long term storage of piroxicam suppositories could bring changes in the physical and release properties of the suppositories depending on the nature of the base. The storage of fat-based suppositories in the refrigerator is desirable for their stability, however this report showed that hardening and increase in melting range of witepsol-based suppositories on storage are major issues to be considered as they affect the release property of the suppositories. Also, fat "blooming" as a physical appearance defect in cocoa butter-based suppositories was accentuated by storage in the refrigerator. The release of piroxicam from the suppositories on preparation was significantly ($P < 0.05$) dependent on the chemistry (glycerides composition and hydroxyl value) of the bases, in contrast, the softening/melting points and mechanical strength of the witepsol bases became the determinant factors in the release of piroxicam from suppositories formulated with these bases on aging. It is, therefore, suggested that long-term storage of suppositories formulated with semi-synthetic fatty bases (Witepsol H15® and Witepsol W35®) in the refrigerator as required for all fat-based suppositories may not be desirable due to their hardening and increase in melting range that resulted in marked reduction in the amount of piroxicam released from the formulations.

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