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In vitro Evaluation of Acetylsalicylic Acid Suppositories using Cow Fat Admixture as Bases

OLUSOLA I AREMU[1,3]*, JOHNPAUL I AJUZIE[3], MUSILIU O ADEDOKUN[2,3], CHRISTIANA Y ISIMI[3], GARBA A ABUH[3], MARTINS O EMEJE[3]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Nigeria
[3]Department of Pharmaceutical Technology and Raw Materials Development, National Institute Pharmaceutical Research and Development, Abuja, Nigeria

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ABSTRACT

Suppository formulation is a solid formulation intended to be inserted into the rectum where they melt or disperse and exhibit local or systemic effect. It is a valuable alternative to other dosage delivery systems as it tends to eliminate the latter's attendant side effects. A study has been made on the formulation and evaluation of Acetylsalicylic acid (ASA) suppositories using Cow fat (GF), its admixtures and Cocoa butter as reference comparatively. Rectal suppositories containing ASA (300 mg) in pre-calibrated mould were prepared by fusion method using CF, Cow fat and Palm Kernel oil (CP) at 3:1, Cow fat and Liquid Paraffin (CL) at 3:1 and Cocoa Butter (CB) as bases. Thereafter the suppositories were characterized using parameters such as appearance, crushing strength, weight variation, melting point, liquefaction time, content uniformity and *in-vitro* release in accordance with standard procedures. Liquefaction or disintegration time in minutes followed this order: ACB (4.40 ±0.84)<ACL(8.19±1.72) <ACP(11.28±1.84) < ACF(14.17±2.12) while that of cumulative drug release in percentage is ACL> ACP >ACF>ACB ($p<0.05$). Results obtained indicated that the bases used generally could be ranked in the order of CL > CP > CF > CB ($p<0.05$) in terms of favourable physicochemical properties investigated. The foregoing indicates that CL, CP or CF has promising potential and could be a substitute suppository base in the formulation of ASA suppositories.

INTRODUCTION

Fats are lipids obtained from different sources including animals such as pig, goat and cattle. They usually exist as solids of complex mixtures at room temperature, consisting of high proportion of saturated fatty acids [1]. Fats from different animals have different textures and melting point [2] and in some climes, are consumed as part of diet especially in paediatrics in their semi solid form [3]. They are sometimes incorporated as enhancers of food taste, texture, flavour and general acceptability. They also reduce gastric emptying as well as intestinal motility thereby affecting satiety. They provide also provide essential fatty acids (EFA) and promote the lipid-soluble vitamins absorption [4, 5].

Author for Correspondence:
Email: solabank@yahoo.com

Mulder [6] reported the nutritional and dietary aspects of fat obtained from cow milk (ghee); it is believed to aid digestion, improves eye sight and bone development in children [6]. In Indian traditional medicine, it has been used in treatment of eye disorders, anti-cholesterol and wound healing activity [6, 7]. Alferez et al [8] investigated the utilization of cow milk fat in malabsorption syndrome and came to the conclusion that, cow milk fat should be included in the diet, in cases of malabsorption syndrome.

Animal fats are largely used in the production of margarine, pastries and food flavours [9]. Technically, animal fats have been employed as adjuvants in food, paint, paper, cosmetic as well as pharmaceutical industries. Recently, they have been found application in renewable biofunctional building blocks for the manufacture of plastics or biopolymers. The latter have eventually yielded hydrolytically

stable as well as flexible materials with good water repellent property [10].

Suppository is a solid medicated formulation designed for insertion into the rectum where they melt, disperse and elicit local or systemic effect. Bases used for suppository formulation can be lipophilic or hydrophilic bases. Rectal drug delivery has a number of advantages over other routes of drug administration, such as reduced hepatic first pass, elimination and avoidance of gastric irritation associated with certain drugs such as acetylsalicylic acid. Acetylsalicylic acid is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic, anti-inflammatory and antiplatelet properties. It elicits these effects by inactivating cyclooxygenase enzymes (COX-1 and 2), an enzyme required for prostaglandin synthesis [11]. Low dose acetylsalicylic acid irreversibly blocks the formation of thromboxane A_2 in platelets, producing an inhibitory effect on platelet aggregation [12]. In 2016, El-Majri and El-Baseir were able to formulate ibuprofen, an NSAID, as suppository [13]. Oral NSAIDS including acetylsalicylic acid has been reported to cause upper and lower gastrointestinal disorder such as gastric ulcer due to direct irritation of gastric mucosa [14-16]. Formulation of acetylsalicylic suppository tends to reduce this deleterious effect on gastric mucosa [17].

This study was thus aimed at developing and evaluating acetylsalicylic acid suppository formulations using cow fat admixture as bases ultimately to eliminate the untoward effects of conventional oral delivery form of the drug.

MATERIALS AND METHODS

The following materials were used: Acetylsalicylic acid powder (Sigma), Aluminum foil (Novena foil, China), Liquid paraffin (BDH Chemicals ltd, Poole, England), Cocoa butter, Hydrochloric acid (EmproveExp Merck, Germany) Sodium dihydrogen orthophosphate, Sodium chloride, Palm kernel oil (derived from the kernel of *Elaeis guineensis*), Distilled water (prepared in the Pharmaceutical Technology laboratory of the National Institute for Pharmaceutical Research and Development (NIPRD), Nigeria).

Extraction of Cow Fat

Pure cow fat was extracted from the adipose tissue of cow as follows: The extraneous matter on the tissue was first manually removed before being blended using mortar and pestle. The blended sample was then melted on water bath and filtered with 250 mesh sieve. The extracted fat was then stored in refrigerator for subsequent use.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Cow Fat

The method of Okhale et al [18] was employed in analyzing the cow fat by GC-MS using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV), scan range of 45-400 amu, and scan rate of 3.99 scans/sec], and Shimadzu GCMS solution data system. The Gas chromatography column was Optima-5 ms fused silica capillary with 5 % phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for Gas chromatography oven temperature was 60-180 °C at a rate of 10 °C/min, then held at 180°C for 2 min, followed by 18-280 °C at a rate of 15 °C/min, then again held at 280 °C for 4 min. The injection port temperature was 250 °C while detector temperature was 280 °C. Helium was used as a carrier gas, at a flow rate 1.61 mL/min. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using autosampler and in the split mode with ratio of 10:90. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11).

Preparation of Acetylsalicylic Acid Suppositories using Different Bases

Various bases employed in the preparation of acetylsalicylic acid suppositories were (i) cow fat, (ii) cow fat/palm kernel oil (3:1) and (iii) cow fat/liquid paraffin (3:1). The suppositories were prepared in pre-calibrated metallic suppository mould with the above listed bases by employing fusion method. A quantity of each base was weighed as required into a beaker and placed in a water bath maintained at about 43 ˚C to melt. A required quantity of acetylsalicylic acid (targeted at 300mg per suppository) was weighed into a beaker, then a portion of the melted base was mixed together with the active drug and thereafter made up to the required weight and thoroughly stirred at about 38 ˚C using a magnetic stirrer for homogeneity. The mixture was poured into the mould until overflowed; then as the solidifying mixture was shrinking, more of the mixture was poured to top it up. After the solidification of the mould content it

was unscrewed and the suppositories were removed and wrapped in aluminium foil for further studies. The procedure was repeated using other bases listed above.

Table 1: Suppository formulations and their composition

Ingredients	ACF	ACL	ACP	ACB
Acetylsalicylic acid	6g	6g	6g	6g
Cow fat to	38.7g			
Cow fat + liquid paraffin (3:1) to		38.4g		
Cow fat + palm kernel oil (3:1) to			38.9g	
Cocoa butter to				39.8g

ACF= acetylsalicylic acid + cow fat; ACL= acetylsalicylic acid +cow fat /liquid paraffin (3:1); ACP= acetylsalicylic acid + cow fat/palm kernel oil (3:1); ACB= acetylsalicylic acid + cocoa butter

Evaluation of Suppositories

Appearance

From each batch, ten suppositories were randomly selected and observed as an intact unit and also after splitting longitudinally. The presence or otherwise of fissuring, pitting, exudation, sedimentation, migration of the active ingredients as well as the colour, odour and shape were evaluated.

Weight Uniformity

The suppositories were assessed for weight variation following official procedure [19]. Twenty suppositories were randomly selected from each batch of the prepared suppositories and weighed individually on an analytical balance (Mettler Toledo, Switzerland). The mean weights and standard deviations were then computed.

Hardness/Crushing Test

The crushing strength, a measure of mechanical strength or hardness of the suppository was determined using the hardness tester (Erweka, GmbH Germany). The load at which each suppository cracked was recorded as the hardness. This parameter was determined in triplicate for each batch of the suppositories.

Liquefaction Time

One suppository was placed in a beaker with a thermometer inserted and placed on a thermo-regulated heating mantle to ensure that the temperature of 39 ± 1°C was maintained. The period in time required for the suppository to totally melt was recorded as the liquefaction time. This evaluation was also carried out in triplicate.

Melting Point Determination

The method adopted by Adebayo and Akala [20] was employed to determine the melting point of the suppositories. A suppository was randomly selected from each batch and placed in a beaker with a thermometer inserted. The beaker was placed on water bath and regulated to a gradual temperature increase of 1°C per 2 min. The temperature at which the suppository sample began to melt was recorded as melting point. The results obtained were mean values of five determinations.

Content Uniformity

Drug content uniformity assay was individually carried out on six suppositories randomly selected from each batch. One suppository was placed in a beaker containing 60 ml of 0.1N HCl solution that was pre-heated to, and maintained at 39 ± 1°C. After the complete melting and dispersion of the suppository, the resultant mixture was made up to 100 ml with the 0.1N HCl and thoroughly stirred at 100 rpm for 5 minutes using magnetic stirrer and filtered through a cotton plug. The absorbance of 5ml of filtered portions was measured in UV-Vis spectrophotometer (Jenway 6505, UK) at 230 nm. The concentration of the acetylsalicylic acid content was computed using a standard Beer-Lambert curve.

Release Studies

Dissolution test was carried out in 100 ml of 0.1N HCl maintained at temperature of 38 ± 1°C. A suppository from each batch was placed in the solution and the magnetic stirrer was set at 50 rpm. A 5ml portion of the release medium was withdrawn at 5 minutes' interval and was filtered through a cotton plug. The volume of the release medium was kept constant by replacing the amount withdrawn with 0.1N HCl. Absorbance of 5 ml of filtered portions was spectrophotometrically determined at 230 nm (UV-Vis spectrophotometer, Jenway 6505, UK).

RESULTS AND DISCUSSION

GC/MS has long been used for the selective qualitative and quantitative analysis of non-polar compounds particularly fatty acids with long chain alkyl groups [21]. The detection of structural molecular ions generated from the MS source provides more sensitive and selective assay of

varied arrays of fatty acids present in lipid samples.

Results from NIST library (Fig. 1) show that, the cow fat samples are rich in long chain fatty acids and fatty acid methyl esters with different alkyl/allyl side chains attached. It chiefly contained oleic acid, heptanoic acid (19.69%) and decanoic acid which is also known as capric acid a medium chained fatty acid. Many studies suggest the anti-inflammatory property of Oleic acid which is a monounsaturated fatty acid. Capric acid is known to be non-irritant to the skin. This effect is highly beneficial to cow fat as a base in suppository formulations. In vitro release of suppository formulated with blend of cow fat (obtained from the adipose tissue) and palm kernel oil, documented in literature, showed fast release of the active ingredient used. The chemical and physical stability, non-reactive and widely compatible properties of these fatty acids confer on cow fat the properties of a good suppository base. The presence of heptanoic acid suggests the slight odour perceived from cow fat.

An acceptable dosage formulation is expected to contain uniform amount of drug content within standard limits for consistent therapeutic activities. The determination of drug content of various formulated suppositories outcome is presented in Fig. 2.

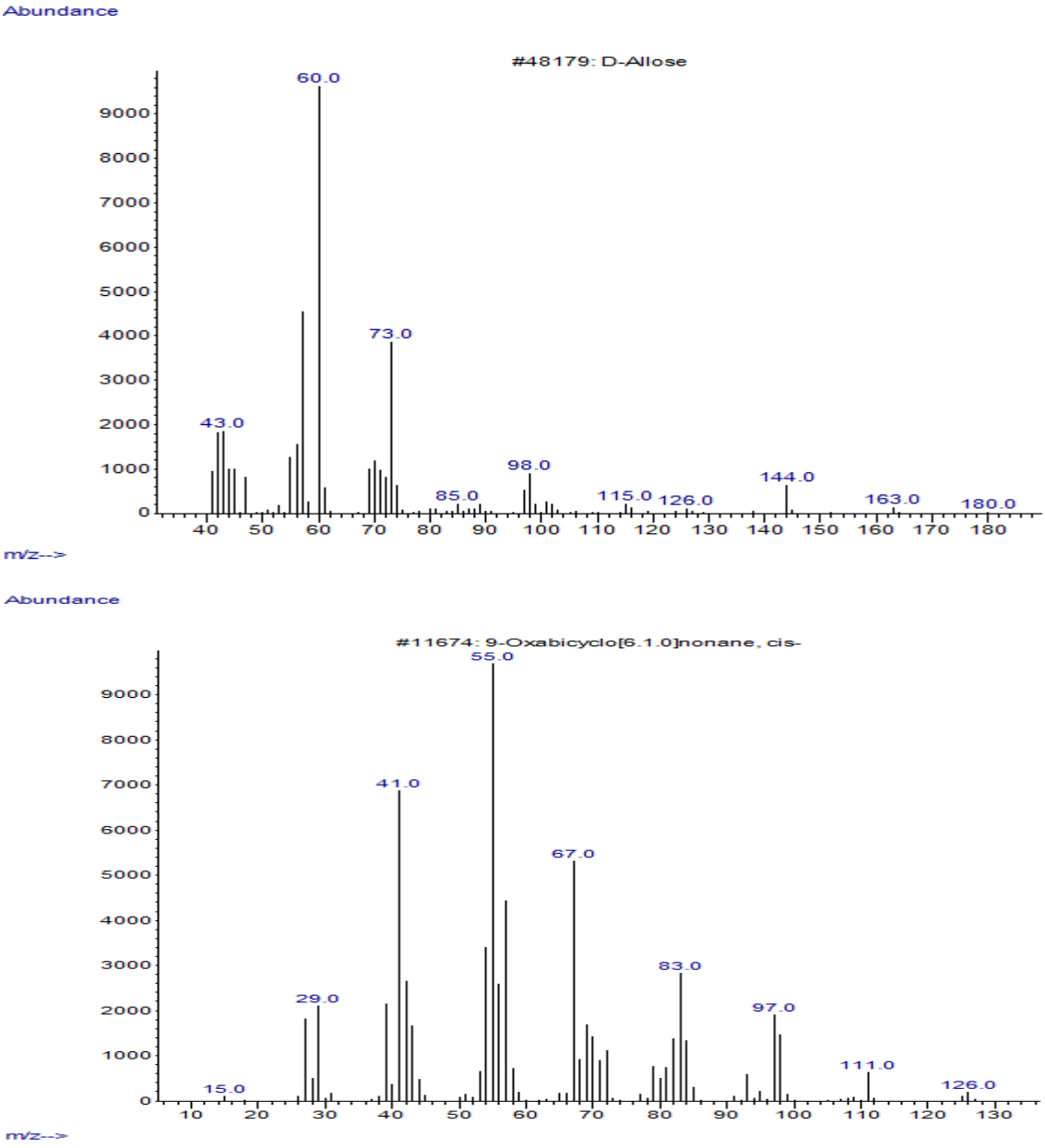

Figure 1: Gas chromatography spectra of cow fat showing the saturated fatty acids (i) hexadecanoic acid and (ii) octadecanoic acid

Different batches of ASA suppositories with the exception of those formulated with cocoa butter were found to be solid at room temperature. This is important because of convenient usage by the patient which will invariably enhance compliance. The suppositories were observed for physical integrity and were found after dissection not to contain air bubbles, not brittle and were not fragile. This shows that the suppositories will be able to withstand rigours of transportation and other mechanical stress.

Various characteristics of the formulated suppositories as evaluated are presented in Table 2. All the four batches of ASA suppositories satisfy BP requirement for weight uniformity (Table 2). Not more than two individual suppositories deviated from the average weight by more than 5% and no suppository differed from the average weight by more than 10% [19]. The relative standard deviations (RSD) of the mean weight of the suppositories were less than 3.5%. This is relevant in order to ensure consistency in different doses of the drug.

Table 2: Physicochemical parameters of the suppositories

Physicochemical parameters	ACF	CF	ACL	CL	ACP	CP	ACB	CB
Shape	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo
Colour	Light yellow	yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
Mean weight (g)	2.05±0.04	-	1.99±0.03	-	2.05±0.04	-	2.01±0.01	-
Hardness strength (N)	7.5±0.52	9.15±2.27	1.31±0.57	2.29±0.57	4.74±1.02	1.31±0.57	-	-
Melting point (°C)	37.9±0.92	38.6±0.26	37.9±0.69	38.5±0.63	37.8±0.29	36.4±0.40	34.0±0.61	32.1±0.70
Liquefaction time (min)	14.17±2.12	18.32±2.01	8.19±1.72	9.30±2.16	11.28±1.84	11.52±0.63	4.40±0.84	3.20±0.63
Displacement value	1.46	-	1.35	-	1.48	-	1.12	-

[a]ACF= acetylsalicylic acid + cow fat; [b]CL= cow fat; [c]ACL= acetylsalicylic acid +cow fat /liquid paraffin (3:1); [d]CL= cow fat /liquid paraffin (3:1); [e]ACP= acetylsalicylic acid + cow fat/palm kernel oil (3:1); [f]CP= cow fat/palm kernel oil (3:1); [g]ACB= acetylsalicylic acid + cocoa butter; [h]CB= cocoa butter.

The crushing strength mirrors the ability of the suppositories to withstand the exposure to shipment, packaging and handling. The crushing strength of the placebo suppositories and those containing medicament exhibit significant difference ($p<0.05$) from batch to batch, they are: CF (9.15N) > CL (2.29N) > CP (1.31N) and ACF (7.5N) > ACP (4.74N) > ACL (1.31N) respectively. There is no official value stated in the monograph for the strength of the suppositories for comparison except those stated by other workers. It has been stated that the acceptable crushing strength force is 17.7-19.6N [22]. Although values gotten in the present work are at variance with the reported one, it is of the opinion that forces of ACF (7.5N) and ACP (4.7N) are strong enough to resist breaking down in structural integrities of the suppositories. The crushing strength of ACB could not be determined because of its physical instability at prevailing room temperature. It is a common knowledge that cocoa butter exhibits different polymorphic forms that could transform interchangeably from stable to unstable and vice versa [23]. This is one of its drawbacks that has necessitated search for alternatives as suppository bases.

Melting point determination is important in order to establish the solidity of the suppositories at room temperature and then the ability to melt at body temperature. Also from Table 2, all the formulations except those of cocoa butter fall within acceptable range. The melting points of ACF and ACL (37.9°C) were found to be lower than their respective placebo bases which were 38.6 and 38.46°C respectively while that of ACP was higher than its blank base. The temperature of heterogeneous system may differ from its original homogeneous state depending on the different temperatures of its constituents as a result of physical interaction. It has been reported (reference) that this change in temperature is as a result of inclusion of active ingredient and this may also alter the release of the drug from the vehicle (base). The melting point of cocoa butter with the drug was 34°C, this is quite low for storage in tropics. The liquefaction times for all the suppositories

showed significant ($p<0.05$) difference. They are of the trend: ACF (14.17±2.12>ACP (11.28±1.84)>ACL (8.19±1.72) >ACB (4.40±0.84). These values fall within acceptable limits. Liquefaction should not take longer than 30 minutes. The absorption from the drug released and the time to reach peak plasma concentration for therapeutic activity to begin is shortened. Invariably there is better patient compliance unlike when there is a prolonged liquefaction time. On the other hand, a suppository that takes a longer time to liquefy may exert irritant action on the rectal mucosa, increase in release time and delay onset of action.

The mean drug content for ACL (99.15%), ACP (97.11%) and ACF (96.8%) suppositories met the USP requirement for the content uniformity test. The mean drug content of these three batches fall within (96.7- 99.2%) as can be seen in Fig. 2. This is significant in order to ensure accurate dosing. Mean drug content for ACB was 94.2%, this is slightly outside the acceptable range. Variation in drug content of the suppositories could either be as a result of improper mixing of the active ingredient with the molten bases or intrinsic affinity between the base and the active ingredient [24].

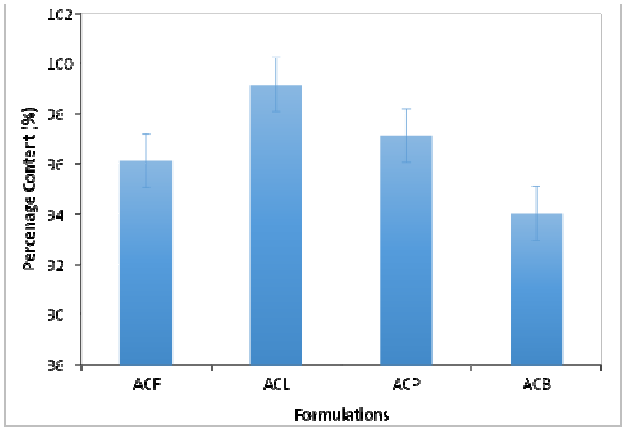

Figure 2: Percentage content of Acetylsalicylic acid suppositories

ACF= acetylsalicylic acid + cow fat; ACL= acetylsalicylic acid +cow fat /liquid paraffin (3:1); ACP= acetylsalicylic acid + cow fat/palm kernel oil (3:1); ACB= acetylsalicylic acid + cocoa butter

For biological activity to be effected, drug must diffuse out of the bases. *In vitro* assessment of rate and extent of release of the drug from various batches of formulated suppositories is presented in Fig. 3. Bioavailability of a drug has direct relationship with the drug's therapeutic effect. The amount of drug released at a given time interval contributes to the rate and extent of the bioavailability of the drug. As can be seen in Figure 3, there is no significant difference ($p<0.05$) in the rate and extent of release of the medicament from ACP, ACL and ACF; implying that, there is little or no undesirable affinity or interaction between the bases and the drug. Barring physiological interactions in the rectum, there is relative assurance that the drug in these suppositories will be biologically available to elicit its action. The case is different for ACB, as can be seen in Figure 3. The rate and extent of release was the least of all the bases used in this study, suggesting that, there might be some interactions between the drug and the base.

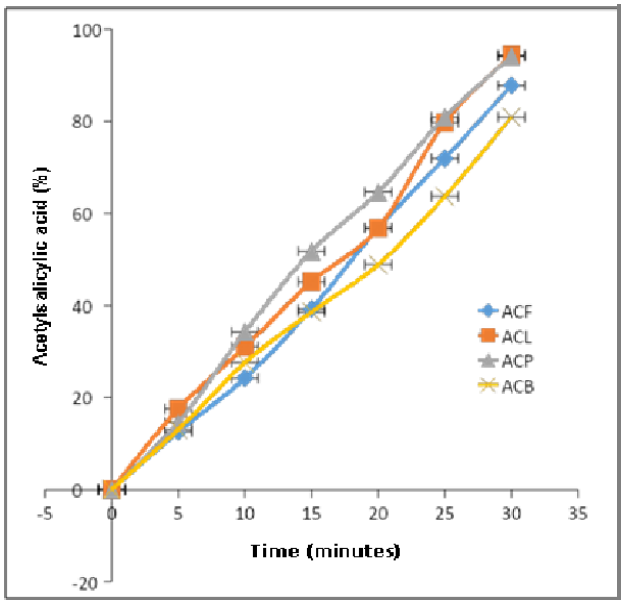

Figure 3: Release profile of Acetylsalicylic acid suppositories.

ACF= acetylsalicylic acid + cow fat; ACL= acetylsalicylic acid +cow fat /liquid paraffin (3:1); ACP= acetylsalicylic acid + cow fat/palm kernel oil (3:1); ACB= acetylsalicylic acid + cocoa butter.

Values for release rate constant and model fitting parameters for release kinetics of of all the suppository formulations are presented in Table 3. Drug release kinetic studies were analyzed using Zero-order, First-order and Higuchi release models. Correlation of Coefficient of Determination (R adjusted) was used in selecting the best-fit release method. (ACF: R^2=0.997, ACL: R^2=0.991, ACP: R^2=0.996 and ACB: R^2=0.993) were found to follow Zero-order model. ACP (R^2=0.996) also fitted perfectly on Higuchi

model. indicating diffusion controlled drug release.

Table 3: Release rate constant and model fitting parameters for release kinetics of different Acetylsalicylic acid suppository formulations

Release Kinetic Mode	Selection Parameters	[a]ACF	[c]ACL	[e]ACP	[g]ACB
Zero-Order	K0 (min)	3.068	3.098	3.145	2.616
	R^2	0.997	0.991	0.995	0.993
First-Order	K_1 (mg/min)	0.033	0.028	0.030	0.029
	R^2	0.951	0.966	0.892	0.939
Higuchi	K_H ($mg/min^{1/2}$)	23.559	23.718	24.484	20.090
	R^2	0.971	0.959	0.996	0.967
Korsmeyer-Peppas	K_{KP} (mg/min^n)	1.104	0.939	1.032	0.986
	R^2	0.997	0.992	0.993	0.995

[a]ACF= acetylsalicylic acid + cow fat, [c]ACL= acetylsalicylic acid +cow fat /liquid paraffin (3:1), [e]ACP= acetylsalicylic acid + cow fat/palm kernel oil (3:1), [g]ACB= acetylsalicylic acid + cocoa butter

The release mechanism of acetylsalicylic acid from the suppositories was analyzed with Korsmeyer-peppas model. The release exponents n, for all the formulations ranges from (0.93 to 1.104). ACP which fitted perfectly on Higuchi model, has release exponent of 1.032 which implies that the drug release from the system follow super case II transport since the release exponent is more than 0.89 [25]. These values of 'n' for all the formulations were greater than 0.5 suggesting non-Fickian diffusion mechanisms of drug release from the suppositories.

CONCLUSION

Acetylsalicylic acid suppositories using cow fat, cow fat/liquid paraffin (3:1), cow fat/palm kernel oil (3:1) were successfully formulated and characterized. All the three bases especially, cow fat/liquid paraffin (3:1) have the potential for usage in the formulation of suppositories that could withstand storage in the tropics.

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