Optimization Of Rheological Properties In The Formulation Of An Ointment Base From Natural Ingredients

R. K. Tsatsop, G. Djiobie, K. Regonne, V. Bama, A. Mbawala, M. Ngassoum

ABSTRACT: The purpose of this study was to optimize the formulation of ointment base by using statistical mixture design. Screening of hard thickeners (Palm Stearin, Beef Tallow and Beeswax) was done to choose the best hard thickener or combination of hard thickeners whose nature and proportions would give the best semi-solid and consistent ointment with desired rheological properties (Spreadability, viscosity, hardness). Contour plots of each response were depicted, based on the equation given by the statistical-fitted models. The optimum area for the ointment base properties, were located and the combination Beeswax:Palm stearin:Beef Tallow was chosen. Other optimization was done to check the contribution of different factors (Hard Thickener, Soft Thickener and Lubricant) on the rheological properties and their correlation. A strong inverse correlation was found to exist between the spreadability and the viscosity of the ointment base (91%). A multi-response optimization was done to obtain a combined optimum proportion of ingredients that gave us 5% HT, 84%ST and 11% L. It can be concluded that the ointment base obtained with optimal rheological and drug release properties can be used for cosmetic and pharmacological applications. The plant extracts can be incorporated in this matrix for herbal ointments.

Keywords: Formulation, hard thickeners, ointment base, rheological properties.

1. INTRODUCTION

A wide variety of topical dermatologic products, ranging from solids to liquids, is available for treatment of skin diseases. The majority of them comprise of semisolids: creams, gels, lotions and ointments. Ointments are generally prepared from fats, vegetable oils, lanolin, Vaseline, paraffin, beeswax, glycols, and alcohols. All ointments consist of a base, which mainly acts as a carrier or vehicle for the medicaments. The nature of the base also controls its performance; hence, selection of ointment base is a very important aspect of formulation.

- *R. K. TSATSOP: Department of applied chemistry, National advanced school of agro-industrial sciences, The University of Ngaoundere, Cameroon. rolitsatsop@yahoo.fr,*
- *G. T. DJIOBIE: Department of applied chemistry, National advanced school of agro-industrial sciences, The University of Ngaoundere, Cameroon*
- *K. REGONNE Department of applied chemistry, National advanced school of agro-industrial sciences, The University of Ngaoundere, Cameroon*
- *V. BAMA Department of applied chemistry, National advanced school of agro-industrial sciences, The University of Ngaoundere, Cameroon*
- *MBAWALA: Department of Food Science and Nutrition, National advanced school of agro-industrial sciences, The University of Ngaoundere, Cameroon*
- *M. B. NGASSOUM: Associate Professor, Department of applied chemistry, National advanced school of agro industrial sciences, University of Ngaoundere, Cameroon, Email: ngassoum@yahoo.fr*

Traditional ointment bases have been oleaginous in nature, which include hydrocarbons (petrolatum, beeswax, etc.), vegetable oils that do not allow inclusion of any water inversely to fatty alcohols (cholesterol, lanolin, wool alcohol, or stearyl alcohol, etc.). Due to their peculiar characteristics, ointments can adhere to the application site for sufficiently long periods before they are worn off. This property helps prolong drug delivery at the application site and allows achievement of the desired therapeutic effect (Emollient, Occlusive, Antiseptic, etc.). Ointments are advantageous in terms of ease of application, rapid formulation, and ability to topically deliver a wide variety of drug molecules. They exhibit complex flow behavior, including irreversible shear breakdown, thixotropy, viscoelasticity, etc., characterized by the methods of measurement [1]. However, the main problem associated with the formulation of ointments, is the establishment of products with optimal rheological properties (Viscosity, spreadability, Hardness) ameliorating their performance [2]. This problem has led to decreased levels of efficacy of these products on their targets. Consumer preference for such products depends on these properties and a few others like appearance, odour, extrudability (when applicable), initial sensations upon contact with the skin and residual greasiness after application [3]. Ultimate acceptability and clinical efficacy of such preparations require them to possess optimal mechanical properties (ease of removal from the container), rheological properties (viscosity that gives a semi-solid consistency, good spreadability on the substrate, minimum hardness) and desired drug release [4]. The optimum consistency of such a formulation helps ensure that a suitable dose is applied or delivered to the target site. The delivery of the correct dose of the drug thus depends highly on the rheological properties of the formulation. These properties intend depend on the nature and choice of ingredients used in the formulation matrix or base that serves as a carrier of the active principle responsible for the specific action of the product. It is necessary to balance the ingredients by using several different ingredients (excipient) to obtain the vehicle with desired properties (stability, ease of application and performance), since it is frequently impossible for a single ingredient to produce an ideal

vehicle (ointment base). A combination of several excipients frequently will give vehicles with enhanced aesthetics as well as increase drug potency and bioavailability [5,6]. This means the creation of a complex three-dimensional network structure that would retain the structure before the product is used, exhibit the proper flow when utilized, and recover the structure after the application. Mixture designs have been utilized to optimize mixture proportions in many product development areas in the food industry, pharmaceutical industry and engineering [7]. With these in mind mixture experiments along with non-linear modeling were used in the present study to investigate the interactions (synergism or antagonism) between the thickeners and lubricants in a blend of multiple components. Therefore, this work focused on the objective to formulate an ointment base with optimal rheological properties and drug release by the valorization of local resources as ingredients (Beeswax, Beef tallow, Palm stearin, shea butter and sesame oil).

2. MATERIALS AND METHODS

2.1. Materials

The hard thickeners included, Palm Stearin (obtained from SOPROICAM (Soybeans Processing Industry of Cameroon)), Beeswax and Beef tallow. The soft thickener was Shea butter purchased from the local Dang market together with Beeswax and Beef tallow. The lubricant, sesame oil was extracted by Soxhlet method. All other chemicals and reagents used were of analytical grade and procured from VWR.

2.2. Selection of variables (independent, dependent) for screening and optimization plan

The different variables used in this study are shown in Table 1 bellow.

Table 1: *Selection of Variables*

Independent (Ingredients)	Dependent (Responses)
Hard thickeners (Beeswax, Palm stearin and Beef tallow),	Spreadability
Soft thickener (Shea butter),	Hardness
Lubricant (Sesame oil)	Viscosity
	Diffusion (Drug release)

2.3. Mixture Design of experiments for screening of hard thickeners

This screening was done in the way to choose the right hard thickener or mixture of hard thickeners that gives the desired consistency for ointment base and to optimize.

Table 2: *Screening Matrix: Domain without constraint*

Component	Abbreviation	Lower limit	Upper limit
Beeswax	BW	0	1
Palm stearin	PS	0	1
Beef tallow	BT	0	1

The screening plan proposed by the STATGRAPHICS Centurion program XVI.I is shown in Table 3 below. It was developed according to the augmented simplex-centroid mixture design with 14 points (Fig. 1). In this figure, the different combinations of the three hard thickeners are numbering from 1 to 13 corresponding to different run in Table 3.

Table 3: *Screening matrix for hard thickeners: Number of experiments*

				Responses (Dependent variables)			
Run	BW	PS	BT	Spreadability	Hardness	Viscosity	Diffusion
1	1.0	0.0	0.0				
2	0.0	1.0	0.0				
3	0.0	0.0	1.0				
4	0.333	0.666	0.0				
5	0.0	0.333	0.666				
6	0.333	0.333	0.333				
7	0.0	0.666	0.333				
8	0.166	0.166	0.666				
9	0.666	0.166	0.166				
10	0.666	0.166	0.166				
11	0.666	0.0	0.333				
12	0.333	0.0	0.666				
13	0.666	0.333	0.0				
14	0.333	0.333	0.333				

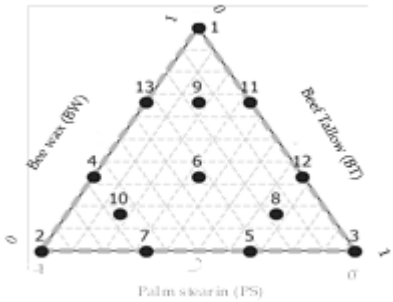

Fig. 1. *The Augmented Simplex Centroid Design for screening of hard thickeners*

2.4. Design experiments for optimization of formulation ointment base

Giving the three families of ingredients for ointment base formulation, which are Hard thickener, Soft thickener and Lubricant, the following domain with constraint (Table 4) was used. It was defined according to previous experiments.

Table 4: *Implicit Domain for optimization plan*

Ingredient	Abbreviation	Lower limit (%)	Upper limit (%)
Selected Hard thickener	HT	4	6
Shea butter	ST	84	88
Sesame oil	L	8	12

Base on the above implicit plan proposed by the STATGRAPHICS Centurion program, the following design of experiments were obtained for optimization (Table 5).

Table 5: *Mixture design for optimisation of ointment base formulation*

		Factors		Responses			
Run	HT	ST	L	Spreadability	Hardness	Viscosity	Diffusion
1	4	88	8				
2	6	86	8				
3	6	84	10				
4	4	84	12				
5	4.5	86.75	8.75				
6	5.5	85.75	8.75				
7	5.5	84.75	9.75				
8	4.5	84.75	10.75				
9	5	87	8				
10	4	86	10				
11	6	85	9				
12	5	84	11				
13	5	85.5	9.5				
14	4	88	8				
15	6	86	8				

2.5. Preparation of ointment base process

Ointment bases were prepared by fusion method as described by Alalor *et al.*[8]. The constituents of the base were placed together in a melting reactor and allowed to melt at 70ºC. After melting, the ingredients were stirred gently at 70ºC for 5 minutes and then cooled in open air [8].

2.6. Spreadability evaluation of ointment base

Spreadability of formulation was determined by an apparatus which was set up in the laboratory as described by Panda [9]. The ointment was placed between two glass slides and a weight of 1000g was placed on the slide for 5 minutes to compress the sample to a uniform thickness. A weight M of 463g was tied to the upper slide. Now the time in seconds required to move the slides across a distance of 10cm was taken as the measure of Spreadability [9]. The following formula Eq. (1) was used for the calculation of Spreadability.

$$S = \frac{M \times L}{T} \quad (1)$$

Where S is Spreadability, M is the weight tied to upper slide in g (463), L is the length of slide in 10 cm and T the time taken to separate two slides. Spreadability was calculated and expressed in g.cm/s

2.7. Hardness evaluation of ointment base

Hardness of formulations was determined by using the LFRA TEXTURE ANALYSER. It is based on the speed of displacement of probe into sample (ointment) at a given distance. The probe was moving down at a speed of 1mm/s till a 1 g surface trigger was attained. At this point the probe was in full contact with the sample surface. Then the probe continued to penetrate to a depth of 5 mm at a speed of 1 mm/s. At this point the probe returned to its starting position. The peak load (maximum force) was registered, and is considered a measure of firmness of the product – the bigger the force the thicker/harder is the sample. Values of peak force were expressed N.

2.8. Viscosity evaluation of ointment base

The rheological behaviour of different formulations was done by measuring the viscosity. This viscosity expressed in centipoise (cP) was determined by CAP-2000 Brookfield viscometer by modified method of Akanksha *et al.*[10]. Test sample was taken in a clean and dry 250 ml beaker, and the viscosity of the test sample was determined by standard operating procedure of Viscometer using spindle N° 5. This spindle was used for finding the viscosity of the sample at speeds of rotated at 25, 50 and 80 rpm. The reading, near to 100% torque was noted. Samples were measured at 25 ± 1°C.

2.9. Drug release evaluation of ointment base

The drug release of different formulations was studied by dialysis method using cellophane membrane under sink conditions. Of each formulation containing 1% of gallic acid, 100 mg was placed on a circular area of cellophane membrane previously soaked in phosphate buffer pH 7.4 for 30 min. The loaded membrane was then firmly stretched using a rubber band over one of the open ends (circular) of a glass tube with a surface area of 0.7855 cm^2. The tube was then immersed in 100 mL beaker containing 25 mL of phosphate buffer pH 7.4 serving as a receptor medium. The receptor compartment was kept at 32 °C to reflect the usual skin temperature in a thermostatically controlled water bath. At time intervals of 30, 60, 90, 120, 150, 180, 210 and 240 min, aliquots of 1 mL were withdrawn from the receptor media and immediately replaced by equal volume of fresh phosphate buffer kept at the same temperature [9]. To this 1ml of aliquot, was added 1ml of Na_2CO_3 and 0.5ml of Folin Ciocalteau reagent, the mixture was heated in a water bath for 15 min which after cooling was read spectrophotometrically at 760nm for drug content against a suitable blank [11] and the cumulative amount of drug released was calculated. Diffusion was obtained as the slope of the graph of concentration of drug released as a function of time expressed in µg/cm^2.min.

2.10. Statistical analysis

Four experiments were carried out at each experimental design point and the mean values were stated as observed responses. Experimental runs were randomised, to minimise the effects of unexpected variability in the observed responses. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Duncan's test. Statistical analyses ($p < 0.05$) were performed using Statgraphics centurion software (Version XVI.I). The optimal extraction conditions for ointment base formulation were estimated through regression analysis and contour plots (obtained using Minitab 16.0 software) of the independent variables and each dependent variable.

3. RESULTS AND DISCUSSION

3.1. Screening of hard thickeners

The results for the analysis of different formulation bases aimed at choosing the best hard thickener are shown on the table below (Table 6). The rheological properties considered during analysis were Spreadability, Hardness, Viscosity and Diffusion. The experimental results obtained are between 1.93 and 15.44g.cm/s, 2.3 and 53.5N, 4320 and 15880cP and 0.018 and 0.308 µg/cm^2.min, for Spreadability, Hardness, Viscosity, and Diffusion respectively. The maximum value for spreadability 15.44g.cm/s is for experiments 2 and 7. The minimum value for hardness 2.3N is for experiment 12, the minimum value for viscosity 4320cP is for experiment 2 and the maximum value for diffusion is for experiment 8. Consequently, it was very indispensable to realize an optimization in order to obtain desired properties of ointment base.

Table 6: *Mixture design for Screening of hard thickeners*

				Observed Responses				Adjusted Responses			
Run	BW	PS	BT	Spread.	Hard.	Viscos.	Diff.	Spread.	Hard.	Viscos.	Diffus.
1	1.0	0.0	0.0	2.31	41.62	14960.0	0.01	2.20	42.44	14989.9	0.02
2	0.0	1.0	0.0	15.44	5.66	4320.0	1.13	15.45	6.17	4386.45	1.13
3	0.0	0.0	1.0	7.72	4.18	1820.0	0.09	7.56	4.39	1707.42	0.09
4	0.333	0.666	0.0	2.10	25.86	10320.0	0.02	1.04	27.57	9770.93	0.04
5	0.0	0.333	0.667	7.72	5.25	10800.0	0.16	8.49	5.77	11971.9	0.15
6	0.333	0.333	0.333	2.43	21.32	12800.0	0.23	1.91	23.12	12407.5	0.25
7	0.0	0.667	0.333	15.44	6.32	15880.0	0.30	14.25	6.20	14903.6	0.31
8	0.167	0.167	0.667	3.86	21.73	12880.0	0.31	3.46	12.74	11208.9	0.31
9	0.333	0.333	0.333	2.43	28.76	12080.0	0.26	1.91	23.12	12407.5	0.25
10	0.167	0.667	0.167	3.56	21.11	10200.0	0.27	5.79	16.98	11393.2	0.23
11	0.667	0.0	0.333	1.93	25.40	12840.0	0.06	1.83	22.48	12192.8	0.06
12	0.333	0.0	0.667	2.57	2.30	11160.0	0.28	2.98	6.74	12222.1	0.27
13	0.667	0.333	0.0	3.56	53.50	14800.0	0.07	3.95	51.49	14689.9	0.06
14	0.667	0.167	0.167	2.57	41.36	12240.0	0.16	2.86	42.19	12848.0	0.16

The Table 7 gives Significance of different effects of models (Cubic Models) and the indications for the validation of models for the different responses respectively. Lack of fit was also given in Table 7 in order to check the quality of the fitted models. The given response would give a relative good fit to the mathematic model if the lack of fit is greater or equals to 0.05.

Table 7: *Significance of different effects and Indications for Validation of models*

	Spreadability		Hardness		Viscosity		Diffusion	
Terme	Coefficient	Probability	Coefficient	Probability	Coefficient	Probability	Coefficient	Probability
Beeswax (BW)	2.201		42.436		14959.9		0.0168	
Palm stearin (PS)	15.445		6.171		4386.450		1.129	
Beef Tallow (BT)	7.563		4.388		1707.420		0.0909	
BW*PS	-28.482	0.0134	68.517	0.091	11440.10	0.165	-0.346	0.0001
BW*BT	-11.143	0.177	-39.620	0.271	17364.7	0.061	0.512	0.0215
PS*BT	-0.614	0.932	9.904	0.765	46.758.6	0.002	-1.696	0.0003
BW*PS*BT	-54.656	0.233	165.742	0.404	-81.441.5	0.101	6.348	0.0014
BW*PS* (BW-PS)	49.405	0.0192	79.555	0.250	9345.140	0.507	2.673	0.0006
BW*BT* (BW-BT)	4.280	0.759	20.640	0.746	-30.053.4	0.079	-1.280	0.0088
PS*BT* (PS-BT)	21.051	0.151	-21.348	0.737	13.761.3	0.348	-1.266	0.0091
R^2(%)	96.75		94.16		95.38		99.60	
R^2adjusted (%)	89.46		81.04		85.03		98.71	
Lack of fit	0.08		0.523		0.21		0.10	

3.1.1 Modelization of Spreadability of ointment base

Base on the analysis of results obtained using Statgraphics software, the fitting model for spreadability was special cubic (Eq. 2);

$$Spreadability = 2.2 \times BW + 15.4 \times PS + 7.5 \times BT - 28.4 \times BW \times PS - 11.1 \times BW \times BT - 0.6 \times PS \times BT - 54.6 \times BW \times PS \times BT + 49.4 \times BW \times PS(BW - PS) + 4.2 \times BW \times BT(BW - BT) + 21.8 \times PS \times BT(PS - BT) \quad (2)$$

It is observed that the binary interactions BW*PS*(BW-PS) with coefficient 49.4 and PS*BT*(PS*-BT) with coefficient 21 have a positive effect on spreadability, which confirmed that the strongest synergism could be obtained by using more beeswax than palm stearin or more palm stearin than beef tallow in the formulation base. However, the strongest antagonism will be observe in the reverse case. On the contrary, the ternary combinations BW*PS*BT with coefficient 54.65 and the following binary interactions BW*BT with coefficient 11.14 and PS*BT with coefficient 0.61 exerted an antagonistic effect on spreadability. The coefficients of determination (R^2) of the special cubic model chosen was 96.75%, whereas p-values for lack of fits were 0.08 (>0.05) (Table 7). The predicted models can reasonably represent the observed values. Thus the response is sufficiently explain by the model.

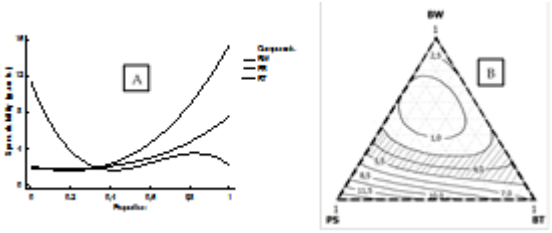

Fig. 2. *Effect of hard thickeners on Spreadability (A) and its Contour Plot (B).*

Ointment spreadability can be categorized into three groups: low, moderated, and high. After screening, it was found to be inversely proportional to the concentration of Beeswax (Fig. 2-A). As the amount of Beeswax increased, the ointment became thicker and, consequently spreadability decreased. The greater the saturated fatty acid fraction of the wax structure, the more viscous the ointments will be, and, conversely, the spreadability of the ointments will decrease with increasing content of unsaturated fatty acids [1]. Palm stearin and beef tallow initially did not show any significant effect on spreadability but as they increased, they behaved more like softeners by increasing spreadability. This can be due to the fatty composition of these fats. The palm stearin is made up of the same amount of saturated and unsaturated fatty acid [12], while the beef fat is made up of about 61% of unsaturated fatty acid (oleic, palmitic and linoleic) [13]. The final formulation we need is supposed to be semisolid, spread easily but not flow off the skin. Consequently, we need beeswax that will increase the viscosity and one of the others to modulate its effect. For this the moderate range of spreadability [3-5] g.cm/s has been consider (Fig. 2-B).

3.1.2 Modelization of hardness of ointment base

Base on the analysis of results obtained, the model for hardness gave equation below (Eq. 3);

$$Hardness = 42.4 \times BW + 6.1 \times PS + 4.3 \times BT + 68.5 \times BW \times PS - 39.6 \times BW \times BT + 9.9 \times PS \times BT + 165.7 \times BW \times PS \times BT + 79.8 \times BW \times PS \times (BW - PS) + 20.6 \times BW \times BT(BW - BT) - 21.3 \times PS \times BT(PS - BT) \quad (3)$$

The coefficients of determination (R^2) of the special cubic model chosen was 94.16%, whereas p-values for lack of fits

were 0.52 (>0.05) (Table 7). This indicates that the model is adequate for the data obtained at a confidence level of 95%. The Fig. 3-A showed the relationship between hardness and different hard thickeners.

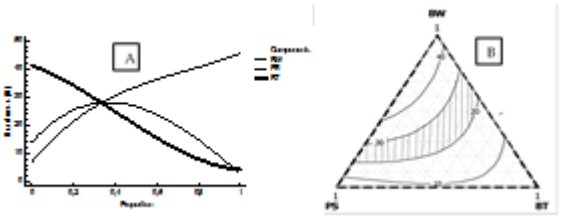

Fig. 3: *Effect of hard thickeners on Hardness (A) and its Contour Plot (B)*

From the Fig. 3-A, it is observed that hardness increases simultaneously with increase in Beeswax and the reverse trend with Beef tallow. Palm stearin started by increasing hardness, then decreased continuously. Base on the equation validated by the cubic model, we observe that interaction between the three hard thickeners BW*PS*BT with coefficient 165.7, greatly increased the hardness of the ointment base formulated, this indicates, the proportion of the three must be well controlled in the same formulation for optimal hardness. Therefore to obtain a semi-solid, we need one which will increase slightly the hardness and another which will decrease hardness, because when the product is too hard, spreadability will be difficult and thus the efficacy will be retarded. From the graph (Fig. 3-B), it will be best that a hardness be between [20-30] N.

3.1.3 Modelization of viscosity of ointment base

The equation of the adjusted model of viscosity is as follows:

$$\text{Viscosity} = 14989 \times \text{BW} + 4386 \times \text{PS} + 1707 \times \text{BT} + 11440 \times \text{BW} \times \text{PS} + 17364 \times \text{BW} \times \text{BT} + 46758 \times \text{PS} \times \text{BT} - 81441 \times \text{BW} \times \text{PS} \times \text{BT} + 9345 \times \text{BW} \times \text{PS}(\text{BW} - \text{PS}) - 30083 \times \text{BW} \times \text{BT}(\text{BW} - \text{BT}) + 13761 \times \text{PS} \times \text{BT}(\text{PS} - \text{BT}) \quad (4)$$

The model explains that 95.38% of viscosity was achieved. The p-values for lack of fits was 0.21 which suggest a good fit to the mathematical model (special cubic model) (Table 7). The Fig. 4 shows the relationship between viscosity and different hard thickeners.

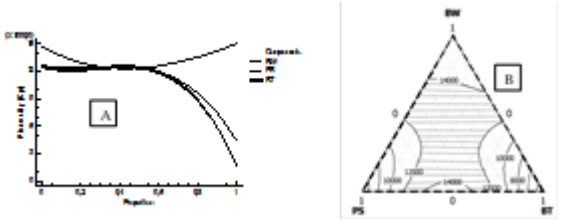

Fig. 4: *Effect of hard thickeners on viscosity (A) and its Contour Plot (B)*

Viscosity is a measure of the resistance to flow of a material. An ointment is semi-solid and must have a viscosity that maintains it at this state. Ointment viscosity can be also categorized into three groups: low, moderated, and high. We notice from the graph it kept increasing with increase in BW (Single action of BW has a coefficient of 14989.9) unlike the other two HT. There is need for a viscosity-increasing ingredient like beeswax but this alone will lead to very high undesirable viscosity of product and so, its effect must be complemented with another, which decreases viscosity. In fact, the major compounds in beeswax are saturated and unsaturated monoesters, diesters, saturated (30-32%) and unsaturated hydrocarbons, free acids and hydroxy polyesters [14]. The greater the saturated fatty acid fraction of the wax structure, the more viscous the ointments will be, and, conversely, the viscosity of the ointments will decrease with increasing content of unsaturated fatty acids [1,15]. This composition give them the more viscous property. We also observe from the equation (Eq. 4) or Table 6 that singly, BT and PS have an increasing effect on viscosity (1707.42 and 4386.45 respectively) which is negligible as compared to that of BW (14989.9). The low viscosity obtain with palm stearin can be explain by the composition of this hard thickener which has similar proportion in saturated and unsaturated fatty acids [12]. From these values, we also observe that BT has a lower value compared to PS and will therefore make a better complement for BW than PS to maintain the desired semi-solid state of the ointment. This can also explained by the fact that beef fat is made up of about 61% of unsaturated fatty acid (oleic, palmitic and linoleic) [13]. These unsaturated fatty acids allow them to easily flow. From this single effect study, the moderated viscosity [12000-14000] cP will be the best option (Fig. 4-B).

3.1.4 Modelization of Drug release of ointment base

The Eq. 5 of the adjusted model of the drug release (diffusion) is as follows.

$$\text{Diffusion} = 0.017 \times \text{BW} + 1.12 \times \text{PS} + 0.09 \times \text{BT} - 2.34 \times \text{BW} \times \text{PS} + 0.51 \times \text{BW} \times \text{BT} - 1.69 \times \text{PS} \times \text{BT} + 6.34 \times \text{BW} \times \text{PS} \times \text{BT} + 2.67 \times \text{BW} \times \text{PS}(\text{BW} - \text{PS}) - 1.28 \times \text{BW} \times \text{BT}(\text{BW} - \text{BT}) - 1.26 \times \text{PS} \times \text{BT}(\text{PS} - \text{BT}) \quad (5)$$

The coefficient of determination (R^2) of drug diffusion obtained was 95.38%. The p-values for lack of fits was 0.10 which suggest a good fit to the mathematical model (special cubic model) seen Table 7. The following graph shows (Fig. 5-A) the relationship between diffusion and different hard thickeners.

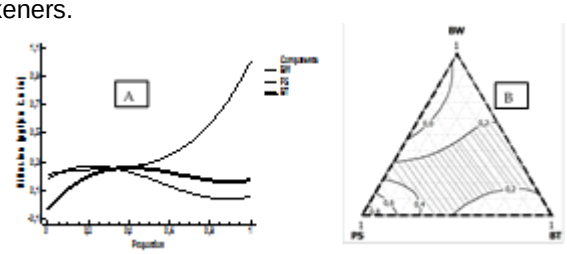

Fig. 5: *Effect of hard thickeners on Drug release (diffusion) (A) and its Contour Plot (B)*

From the above results (Fig. 5-A), increasing the concentration of BW and BT did not show any significant effect on diffusion. The aim was to maximise diffusion to a certain level that ensures that the ointment remains on its target. We also see from the graph that as PS increased, diffusion equally followed suit (this is confirmed by its positively high coefficient 1.12). This is not good because, the active component will not last on the site where it is supposed to perform its function thereby slowing down the

healing process. From this single effect study, the drug release [0.2-0.3] µg/cm^2.min will be the best option (Fig. 5-B) for combination of hard thickeners in our ointment. To obtain optimized area of all properties, the selected areas of all responses were superimposed. Fig. 6 shows the optimized area which had all properties in the selected criteria. The point X is the selected point for scale up formulation in the second part of study. At this point, the proportion of BW:PS:BT was 25:50:25.

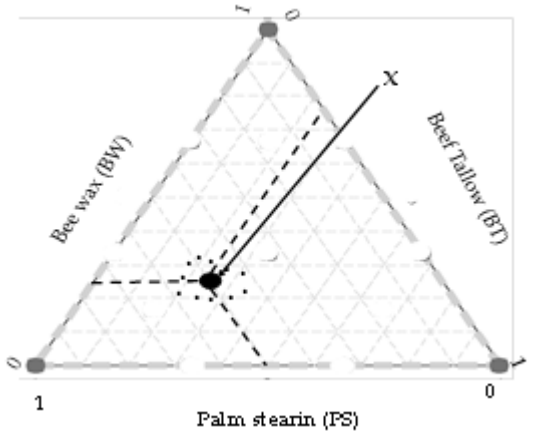

Fig. 6: *The optimised area of ointment base formulation and the selected point X*

Table 8: *Mixture design for optimization of formulation of ointment base*

Run	HT	ST	L	Observed responses Spread.	Hard.	Viscos.	Diff.	Adjusted responses Spread.	Hard.	Viscos.	Diff.
1	4	88	8	1.22	30.40	15240.0	0.44	1.26	30.66	15390.3	0.51
2	6	86	8	1.88	40.59	13080.0	0.11	1.90	39.33	12725.4	0.12
3	6	84	10	1.72	32.54	14160.0	0.11	1.83	31.64	14023.4	0.15
4	4	84	12	2.21	23.15	13040.0	0.04	2.24	22.87	13023.7	0.05
5	4.5	86.75	8.75	1.60	28.15	14920.0	0.10	1.71	26.63	14344.6	0.10
6	5.5	85.75	8.75	1.71	27.64	13040.0	0.23	1.69	27.60	12732.9	0.27
7	5.5	84.5	9.75	1.60	26.36	13040.0	0.49	1.78	24.09	12325.8	0.47
8	4.5	84.75	10.75	1.72	21.57	12960.0	0.48	1.76	20.77	12514.1	0.50
9	5	87	8	1.28	27.18	14280.0	0.17	1.14	28.56	14622.6	0.14
10	4	86	10	1.93	19.74	10120.0	0.46	1.84	20.66	10371.0	0.45
11	6	85	9	1.72	31.93	13680.0	0.25	1.40	34.82	14501.0	0.20
12	5	84	11	3.31	20.30	10560.0	0.39	3.15	21.88	10939.6	0.37
13	5	85.5	9.5	1.60	22.24	12160.0	0.34	1.66	22.76	12969.6	0.38
14	4	88	8	1.28	31.01	15520.0	0.57	1.26	30.66	15390.3	0.51
15	6	86	8	1.78	39.47	12600.0	0.10	1.90	39.33	12725.4	0.12

3.2. Optimization of formulation of ointment base

The results for the analysis of different formulation bases aimed at choosing the best proportion of ingredients (Hard thickener, Soft thickener and lubricant) are shown on the Table 8. The responses observed in the course of these analysis are: Spreadability, Hardness, Viscosity and Diffusion. The experimental results obtained are between 1.22 and 3.31g.cm/s, 19.74 and 40.59N, 10120 and 15520cP and 0.1 and 0.57 µg/cm^2.min, for Spreadability, Hardness, Viscosity, and Diffusion respectively. Concerning the viscosity, the range obtained 10120 to 15520cP is slightly comparable to result to the values obtained by Akanksha *et al.* [10] ranging from 14410 to 15213cP. During their formulation, the Emulsifying wax, white soft paraffin and liquid paraffin were used as hard thickener, soft thickener and lubricant respectively. Also the slight difference in value can be due to their ratio 30:50:20 used for formulation. From this, our formulation ointment base from natural ingredient could be used as matrix for antiseptic ointment. Because the viscosity governs many properties of the ointment formulation such as, spreadability, pourability of the product from the container etc. [16] The tables 9 gives the indications for the validation and Significance of different effects of models for the different responses. All responses as shown above were fitted to the cubic model.

Table 9: *Significance of different factors and the indicators of validation of different responses*

Parameter	Spreadability Coefficient	Probability	Hardness Coefficient	Probability	Viscosity Coefficient	Probability	Diffusion Coefficient	Probability
Hard thickener (HT)	-12.095		271.377		110.755		-1.94062	
Soft thickener (ST)	1.26		30.5679		153.623		0.514	
Lubricant (L)	2.22		23.03		130.697		0.042	
HT*ST	29.16	0.113	-44.591	0.027	-20.1006.	0.0095	5.331	0.5723
HT*L	27.12	0.135	-46.437	0.024	-19.1905.	0.0115	6.575	0.4922
ST*L	0.42	0.639	-25.10	0.036	-15.642.2	0.0032	0.595	0.2.601
HT*ST*L	-44.18	0.095	455.43	0.039	301.617.	0.0068	-5.619	0.6.655
HT*ST (HT-ST)	23.89	0.064	-22.678	0.063	-13.9548.	0.0076	5.553	0.3.534
HT*L (HT-L)	5.87	0.557	-24.574	0.045	-99.1233	0.0279	1.667	0.7.559
ST*L (ST-L)	5.18	0.015	25.09	0.196	-21.4452	0.7800	-3.126	0.0.627
R^2 (%)	94.99		97.28		94.66		87.06	
R^2 adjusted (%)	85.97		92.40		85.00		63.76	
Lack of fit	0.35		0.67		0.60		0.053	

3.2.1 Effect of formulation ingredients on Spreadability

The equation (Eq. 6) shows the special cubic model obtained after analysis of spreadability.

$$\text{Spreadability} = -12 \times \text{HT} + 1.26 \times \text{ST} + 2.2 \times \text{L} + 29.1 \times \text{HT} \times \text{ST} + 27.1 \times \text{HT} \times \text{L} + 0.42 \times \text{ST} \times \text{L} - 44.18 \times$$

$$HT \times ST \times L + 23.89 \times HT \times ST(HT - ST) + 5.87 \times HT \times L(HT - L) + 8.18 \times ST \times L(ST - L) \quad (6)$$

The coefficient of determination (R^2) obtained was 94.99%. The p-values for lack of fits was 0.35 which suggest a good fit to the mathematical model (Table 9). Concerning the contribution of different factors on spreadability, it is observed that the interactions HT*ST (18.89%) and HT*L (17.56%) have a positive effect on spreadability while HT (7.84%) and interaction between the three mixture components HT*ST*L (28.16%) show the contrary (Fig. 7).

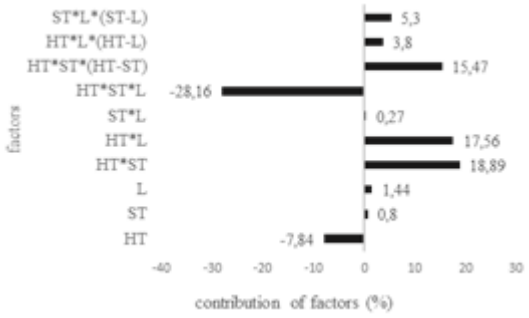

Fig. 7: *Contribution of factors on Spreadability*

This means that the binary effects hard thickener and soft thickener, hard thickener and lubricant exert a synergistic effect on the spreadability. The synergistic effect is due to the high content in unsaturated fatty acid of lubricant (sesame oil) which tend to moderate the negative effect of hard thickener on spreadability [15, 17]. However, the strongest antagonism could be obtained by the mixture of the three groups of ingredients (hard thickener, soft thickener and lubricant).

3.2.2 Effect of formulation ingredients on Hardness

The equation bellow (Eq. 7) shows the special cubic model obtained after analysis of Hardness. The coefficient of determination (R^2) obtained, 97.28% of hardness was achieved. The p-values for lack of fits was 0.67 (Table 9) which suggest a good fit to the mathematical model.

$$Hardness = 271.5 \times HT + 30.5 \times ST + 23.3 \times L - 445.9 \times HT \times ST - 464.4 \times HT \times L - 25.1 \times ST \times L + 488.4 \times HT \times ST \times L - 226.8 \times HT \times ST(HT - ST) - 248.7 \times HT \times L(HT - L) + 25.1 \times ST \times L(ST - L) \quad (7)$$

Hardness was equally dependent on different factors for optimization aimed at minimizing hardness. This was the case with some interactions such as HT*ST (19.82%) and HT*L (20.65%). The ternary interaction of HT*ST*L (21.72%) and HT alone (12.07%) will give antagonistic effects (Fig. 8). Giving that the hard thickener is made up of Beeswax and beef tallow, the antagonistic effect is due to their chemical composition. In fact, Waxes are a large and complex group of lipophilic compounds that generally consist of mixtures of esters of fatty acids with long-chain monohydric alcohols [1]. Lubricant here which is sesame oil is made up of unsaturated fatty acid (90%) this will tend to reduce the hardness of the ointment base.

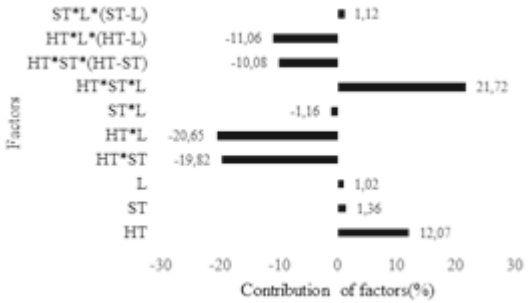

Fig. 8: *Contribution of factors on hardness*

3.2.3 Effect of formulation ingredients on Viscosity

The equation (Eq. 8) shows the special cubic model obtained after analysis of viscosity. The coefficient of determination (R^2) obtained was 94.26% for viscosity. The p-values for lack of fits was 0.60 which suggest a good fit to the mathematical model (Table 9).

$$Viscosity = 110755 \times HT + 15362.3 \times ST + 13069 \times L - 201006 \times HT \times ST - 191905 \times HT \times L - 15642.2 \times ST \times L + 301617 \times HT \times ST \times L - 139348 \times HT \times ST(HT - ST) - 99123 \times HT \times L(HT - L) - 2144 \times ST \times L(ST - L) \quad (8)$$

The interaction HT*ST*L (30.46%) will lead to a very high viscosity. This is the reason why right proportions of each component must be respected in order to avoid the production of a solid instead of a semi-solid formulation. The following graph is a representation of the different contributions of different factors to viscosity (Fig. 9).

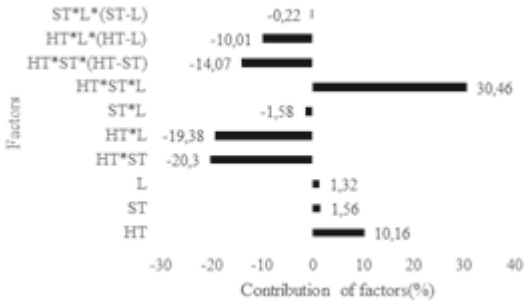

Fig. 9: *Contribution of factors on viscosity*

Hard thickener contributes to increase viscosity of the ointment base. This is due to the lipophilic compounds that generally consist of mixtures of esters of fatty acids with long-chain monohydric alcohols which constitute the wax constituting the hard thickener. In fact, waxes are recognized as a potential excipient or enhancing the aesthetic and maximizing the therapeutic benefits of topical formulations by increasing the viscosity [1]. However, there exists an antagonist effect between the hard thickener and lubricant. The interaction between the two variables tends to reduce viscosity of the ointment base. This can be due to the fact that the lubricant here which is sesame oil is made up of high content (90%) unsaturated fatty acid [18]. It was observed that when the amount of saturated fatty acids in the vegetable oil increases, the absolute viscosity increases also [15, 17].

3.2.4 Effect of formulation ingredients on drug release

The equation of the model is,

$$\text{Diffusion} = -2.94 \times \text{HT} + 0.51 \times \text{ST} + 0.04 \times \text{L} + 5.33 \times \text{HT} \times \text{ST} + 6.57 \times \text{HT} \times \text{L} + 0.59 \times \text{ST} \times \text{L} - 5.61 \times \text{HT} \times \text{ST} \times \text{L} + 5.55 \times \text{HT} \times \text{ST}(\text{HT} - \text{ST}) + 1.66 \times \text{HT} \times \text{L}(\text{HT} - \text{L}) - 3.12 \times \text{ST} \times \text{L}(\text{ST} - \text{L}) \quad (9)$$

The hard thickener contribute to reduce the drug release by the ointment base (Table 10). This is due to the lipophilic compounds that generally consist of mixtures of esters of fatty acids with long-chain monohydric alcohols which constitute the wax constituting the hard thickener. In fact, waxes are recognized as a potential excipient or enhancing the aesthetic and maximizing the therapeutic benefits of topical formulations by increasing the viscosity and prolonging the drug retention on the skin surface [1]. However, the interactions HT*ST*(HT-ST) with coefficient 17.43%, HT*L (20.64%) and HT*ST (16.73%) have a positive effect on diffusion (synergism) of the drug in the formulation (Table 9). This means that the soft thickener tend to reduce the effect of hard thickener therefore ameliorate its properties.

3.2.5 Optimization of formulation of ointment base

In order to have a stable and optimal formulation, it is necessary to understand the proportion and nature of each component. In addition, each formulation must be specifically designed according to the desired purpose of its use, site of application, and patient acceptability. From the above observations, the HT must not be high in the formulation in order to facilitate spreadability, the lubricant must not equally be too much to avoid formula from flowing off its target. Thus a multi-response analysis was done to obtain the combined optimum that was used to formulate the final product with HT:ST:L to be 5:84:11. Correlation studies of different responses is given in the following table (Table 10).

Table 10: *Parameters of reverse and linear correlation of responses*

Response		Correlation coefficient (r)	Goal
Spreadability	Hardness	0.88	Moderately strong
Spreadability	Viscosity	0.91	Strong
Diffusion	Hardness	0.77	Moderately strong
Diffusion	Viscosity	0.71	Moderately strong

From the above table, a strong inverse correlation was observed between Spreadability and Viscosity. This can be compared to the work of Hegdahl and Gjerdet [19] and Vennat *et al*. [20], who showed the existence of a strong negative correlation between the spreadability and viscosity of elastomeric materials. In addition, Pattanayak *et al*. [21] had shown by comparative study that when the viscosity of formulation increases, the spreadability decreases and vice versa.

4. CONCLUSION

This work aimed to formulate an ointment base with optimal rheological properties by the valorisation of local resources as ingredients (Beeswax, Beef tallow, Palm stearin, shea butter and sesame oil). Using the mixture design experiment, it was found that the mixtures of Beeswax, Beef tallow, Palm stearin not only improve spreadability of ointment base but also modify its viscosity, hardness and drug release. The optimum area for the ointment base properties, were located, using the statistical fitted models and the contour plot of responses. A strong inverse correlation was found to exist between the spreadability and the viscosity of the ointment (91%). A multi-response optimization was done to obtain a combined optimum proportion of ingredients. This ointment base with desired rheological and drug release properties can be used for further work as matrix for incorporation of extracts for cosmetic and pharmaceutical usages.

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