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Determination of the Most Effective Concentration of Deet and Permethrin in A Mosquito Repellent Soap and Assay of the Active Ingredients

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Abstract

The study was carried out to investigate the most effective concentration of DEET and Permethrin in a mosquito repellent soap and to develop a method for the assaying of the active ingredients. The effect of perfume in a repellent soap was also investigated. In the study nine soaps were prepared and the concentrations of DEET and Permethrin were varied with six soaps having both ingredients. Efficacy tests were carried out on the different soaps. The most effective and economic soap in repelling mosquitoes was the one which had 50 % DEET. Physical and chemical tests revealed that the soaps had an average total fatty matter of 71.6 %, average moisture content of 14.35 % and average free alkali of 0.04.

Keywords: active ingredients, spectroscopy, mosquito repellent, soap, validation

INTRODUCTION

N,*N*-Diethyl-meta-toluamide, (DEET) (Figure 1), is the most common active ingredient in insect repellents. It is intended to be applied to the skin or to clothing, and provides protection against mosquitoes, ticks, fleas, chiggers, and many other

biting insects [1]. The US army developed DEET in 1946 to protect soldiers in jungles from mosquito-borne illnesses and was developed by the U.S. Department of Agriculture and registered for use by the general public in 1957 and today about 30 percent of the U.S. population uses DEET each year [2-3]. DEET can be applied directly to the skin or clothing, but you should never be ingested. Oddly enough, scientists aren't even completely certain why DEET works. Perhaps it prevents the mosquito from recognizing you as prey. Or perhaps it coats you with a scent mosquitoes simply find revolting. Either way, it doesn't kill the insects, it just repels them [4-5].

Permethrin (Figure 2) is a broad spectrum insecticide, used to kill a variety of insects [6]. Permethrin is referred to as a synthetic pyrethroid insecticide because, while manmade, it resembles naturally-occurring chemicals with insecticidal properties, called pyrethroids [7]. Permethrin is used against a number of pests, on nut, fruit, vegetable, cotton, ornamental, mushroom, potato and cereal crops [8]. It is used in greenhouses, home gardens and for termite control [9]. It also controls animal ectoparasites, biting flies, and cockroaches. Permethrin is available in dusts, emulsifiable concentrates, smokes, ULV (ultra low volume), and wettable powder formulations [10]. Permethrin is used in tropical areas to prevent mosquito-borne disease such as dengue fever and malaria [11]. Permethrin kills ticks on contact with treated clothing. Permethrin-containing products (Permanone) are recommended for use only on clothing, shoes, bednets and camping gear—never on skin [4].

Permethrin acts as a stomach poison when it is ingested by insects or as a contact poison through direct contact with target pests [8]. It kills adults, eggs, and larvae, and has a slight repellent effect against insects. The insecticidal activity of this material lasts up to 12 weeks after application. It functions as a neurotoxin, affecting neuron membranes by prolonging sodium channel activation [12].

Literature survey reveals that there are a few analytical methods that are available for the determination of the most effective concentration of DEET and Permethrin in mosquito repellent soaps. Hence, we made an attempt to develop and validate a simple method that quantitatively determines the active ingredient in soap using UV Spectroscopy.

EXPERIMENTAL

Instrumentation

UV-vis spectra of the samples were obtained on an Agilant Cary 5000 Spectrometer.

Chemicals and reagents

Sodium hydroxide AR grade (NaOH), potassium hydroxide AR grade (KOH), liquid paraffin were purchased from Sigma Aldrich. Bentonite, palm kernel oil, ocean perfume, Permethrin and DEET were donated by Zimbabwe Pharmaceuticals. All other chemicals were obtained from the National University of Science and Technology Applied Chemistry Laboratory.

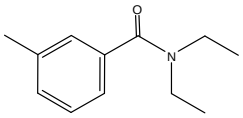

Figure 1: Chemical Structure of DEET

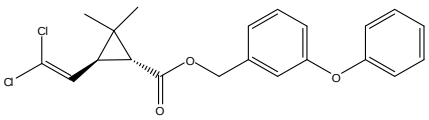

Figure 2: Chemical Structure of Permethrin

Preparation of a 100 g soap

49.74 g Palm kernel oil was heated in a beaker up to 45 ˚C and 0.136g BHT added. In a second beaker 20.10 g DEET and 0.54 g Permethrin were added. In a third beaker, 8.8 g NaOH was dissolved in 18.8 ml of water and the solution was cooled to 25 ˚C. After cooling KOH was added and mixed for 15 minutes using a hand mixer. 1.084 g bentonite was added while mixing. Liquid paraffin was added into a tray for lubrication and the prepared soap mixture was transferred into the tray and covered with a blanket and left covered for 24 hours after which it was cut into small bars.

The concentrations of these two active ingredients were varied as shown in Table 1.

Table 1: Concentrations of permethrin and DEET

Sample	% Permethrin conc	% DEET conc
1	0.5	0
2	0.5	20
3	0.5	30
4	0.5	50
5	0	20
6	1.0	20
7	1.5	20
8 (perfumed)	0.5	20
9 (control)	0	0

EFFICACY

Protocol for repellent testing

Mosquitoes

Laboratory reared *Anopheles arabiensis* unfed female mosquitoes were used.

Application of repellents

An area measuring 25cm X 25cm was cut on the glove so that the skin was exposed. The repellents were applied lightly, evenly and completely on the exposed skin area by gently rubbing them over the skin surface.

Repellence

Each mosquito repellent was applied on the mentioned area and left to dry for 5 minutes (30 seconds was taken as a standard measure for each repellent). Thus, the repellents were applied for 30 seconds before commencing repellent tests. A total of 200 starved female laboratory bred *Anopheles arabiensis* mosquitoes were used per experiment. The mosquitoes were placed in mosquito cages made from 5-liter buckets that were opened on top and covered with mosquito netting and provided with a sleeve for introducing mosquitoes. Each palm was placed into a mosquito cage containing mosquitoes for 1.5 minutes and the number of mosquitoes probing to bite was recorded [13].

The calculation was carried out as follows [14]:

$$\frac{Bc - Bt}{Bc} \times 100$$ Where: Bc - mean number of bites on control; Bt - mean number of bites on treated

Repellence was monitored every 30 minutes until protection was lost.

Tests done on the soap

Determination of moisture content

Moisture dishes were washed and placed in oven to dry for 20 minutes before cooling and weighing. A small sample of soap *ca* 1.0 g was grated and placed in the oven for 1 hour 30minutes at 120 $^{\circ}$C and allowed to cool before weighing again.

Determination of total fatty matter

150 ml of distilled water was added to 5.0 g of grated soap and warmed on a hot plate to dissolve.The solution was poured into a separating funnel and shaken. A few drops of methyl orange indicator solution were added and then from a burette, 1N sulphuric acid was added until there was an excess of 5 ml. The contents of the flask were cooled to room temperature and about 100 ml of 40/60 petroleum ether were added. The shaking was repeated until the aqueous layer had become clear and then it was allowed to stand. The aqueous layer was drawn off into a second separating funnel and again extracted with 40/60 petroleum ether. The extraction process was repeated and the 3-petroleum ether extracts were combined in the first separating funnel and washed by shaking with water until the washings were neutral to methyl orange. When most of the solvent had been distilled off 5ml of acetone were added, then the flask was warmed on a steam bath and the acetone was removed under a stream of dry air.

Determination of free alkali

5.0 g of soap sample was weighed into a conical flask and 100 ml of neutralized ethanol were added and the contents were placed on a hot plate to dissolve. 5 ml of 10% $BaCl_2$ were added followed by 1 ml of phenolpthalein indicator. The solution was titrated against 1N H_2SO_4 to a colourless endpoint.

Assay of active ingredients
UV Spectroscopy method
A 1% solution of both the analytes were scanned using the UV-VIS Spectrometer. The machine was calibrated before use. From the UV scan, it was found that the analytes absorb at a wavelength of 254 nm, therefore this was used as a wavelength of analysis.

Preparation of standard solution
DEET
2.0 g of DEET standard was accurately measured into a 50 ml volumetric flask and diluted to the mark with ethanol.

Permethrin
1.0 g of Permethrin standard was accurately measured into a 50 ml volumetric flask and diluted to the mark with ethanol. Ethanol was used as the blank solution for both the standards before measuring the absorbance of the sample solutions.

Sample preparation
Permethrin
A crushed 2.0 g soap was dissolved in 100 ml distilled water. Distilled water was used as the blank for measuring the absorbance of Permethrin.

Extraction of DEET from the soap sample
100 ml of distilled water was added to a 2.0 g of grated soap and warmed on a hot plate to dissolve. The solution was carefully poured into a separating funnel. The beaker was washed with 3 portions of hot water and the washings were poured into the separating funnel. A few drops of methyl orange indicator solution were added and then from a burette, 1N sulphuric acid was added until there was an excess of 5 ml. The contents of the flask were cooled to room temperature and 50 ml of 40/60 petroleum ether was added and shaken. The aqueous layer was drawn off into a second separating funnel and again extracted with 40/60 petroleum ether. The extraction process was repeated and the 3-petroleum ether extracts(from 100ml petroleum ether divided into 3 portions) were combined in the first separating funnel and washed by shaking with water until the washings were neutral to methyl orange. The absorbance of the extracted sample was measured using petroleum ether as a blank solution at a wavelength of 254 nm.

Analytical Method Validation
The developed method was validated with respect to Linearity and Range, assay precision, ruggedness and robustness.

Linearity and Range
The linearity of an analytical method is its ability to produce test results that are proportional to the concentration of the analyte in the sample solutions, within a specific range of (50-150%) of the working concentration. Linearity is usually

expressed as the variance around the slope of the regression line. The importance of linearity depends on how wide ranging the method is. The linear range of detectability that obeys Beer's law is dependant on the compound analysed and detector used. The recommended range should be not less than 20%. Under most circumstances, regression coefficient (r) is approximately 0.999. Intercept and slope should be indicated.

Assay Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied to multiple sampling of a homogeneous sample .The precision of an analytical method is usually expressed as the percentage RSD of the assay results. It should not be more than 2% for not less than 5 samples [15].

Ruggedness

The ruggedness of an analytical method is the degree of some reproducibility of test results obtained by analysing the samples under a variety of test conditions. It is also a measure of the test results under normal, operational conditions from laboratory to laboratory and from analyst to analyst.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. It provides an indication of the method's reliability during normal usage.

RESULTS AND DISCUSSION

Efficacy tests

The different soaps prepared had variations in the quantity of the active ingredients, DEET and Permethrin. Therefore the physical appearance and effectiveness differed. The effectiveness of the prepared soap was measured according to the 100% repellence period the soap had when applied on the skin that is the number of hours taken before mosquitoes start landing or biting the treated skin. (Table 2)

Table 2: Effectiveness of prepared soap

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
DEET concentration	0	20	20	20	0	50	30	20	20
Permethrin concentration	0	0.5	1	1.5	0.5	0.5	0.5	0	0.5
100 %repellence period /hr	0	4.5	4.5	4.5	0	7	5	4.5	5

As the percentage of DEET increased, the 100% repellence period increased. Permethrin did not offer any repellence and the soap which had Permethrin had a repellence period of 0 hours. This was the same result as that of the control which had

0 hours repellence period because it had no active ingredient. This showed that Permethrin only in a mosquito repellent soap does not offer any repellence against mosquitoes. The soaps which had a combination of DEET and Permethrin also indicated that the repellence period was dependent on DEET only. Increasing the concentration of Permethrin and keeping constant the concentration of DEET did not increase the repellence period. 4.5 hours was the repellence period for the soaps which had 20% DEET and a concentration range of 0.5% to 1.5% for Permethrin. Increasing the concentration of DEET and keeping constant the concentration of Permethrin affected the repellence period. The soap which had a higher DEET concentration had a repellence period of 7 hours, the one with 30% had 5 hours, the one with 20% had a repellence period of 4.5 hours. However at 60% DEET concentration, the repellence period was constant at 7 hours. This shows that increasing the concentration of DEET beyond 50% will give the same result as that of 50% concentration in a soap formulation.

Adding Permethrin to a mosquito repellent soap formulation does not affect the repellence period which the soap will give. It therefore can be removed from the formulation since its absence does not affect any physical appearance or property of the soap beyond the acceptable limits. The soap which had no Permethrin had a moisture content, total fatty matter and other tests in the acceptable range. The soap which had perfume did not have a significant difference from the soap which had the same composition of every other material but with no perfume. The perfumed soap had a repellence period of 5 hours and the one with no perfume but of the same active ingredient concentration had 4.5 hours. The slight difference therefore suggests that the perfume may have properties that help repel mosquitoes. Adding perfume to a mosquito repellent also helps the user to have a relaxing scent instead of the DEET or Permethrin smell which is not as pleasant.

Moisture content

The average moisture content of the prepared soaps was 14.3462 as shown in Table 3

Table 3: Moisture content

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Moisture content /%	17.1814	16.2979	14.1985	14.9890	16.8386	14.9965	17.1249	17.3409

The soaps with Permethrin only or higher concentration of Permethrin were soft and had a higher moisture content than all other soaps. The moisture contents for these soaps were from 17.1814 to 17.3409 %. Soaps with DEET only of higher percentages of DEET than Permethrin were the hardest and had the lowest moisture content which ranged from 14.1985 to 14.9965%. A combination of the two ingredients, DEET and Permethrin had an average moisture content of 16.8386%.

Total Fatty Matter

Total fatty matter is the total amount of fatty material mostly fatty acids that can be separated from a sample after splitting with a mineral acid. It is the indication of soap quality. The more the fatty matter the better the quality of soap. The average total fatty matter for the soap was 71.60% as shown in Table 4.

Table 4: Total fatty matter

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Empty beaker/g	180.7802	180.7800	180.7805	180.7800	180.7801	180.7805	180.7802	180.7805
Beaker + matter	184.4604	184.8890	184.5055	184.2014	183.9961	184.8048	183.9377	184.1222
Sample weighed/ g	5.0011	5.0100	5.0000	5.0021	5.0016	5.0210	5.0000	5.010
Total fatty matter/%	73.56	82.02	74.50	68.40	64.30	80.15	63.15	66.70

The soap had a higher TFM than the specifications because it was not an ordinary soap for dirt removal, it has active ingredients which also contribute to the total fatty matter. In this case, the extracted material included DEET since it is soluble in petroleum ether. Therefore the fatty matter increased with increase in the quantity of DEET added in the soap formulation. The different soaps had different compositions of the active ingredient therefore the TFM increased as the active ingredient quantity increased. Higher TFM is good for the soap because soaps with high TFM are good on dry skin. The skin is re-hydrated making it smooth after application. Additionally the high oil content within the soap acts as a lubricant giving soap its soapy feel [16].

Free alkali as Na_2O

Free alkali is determined in soap to establish that all the caustic soda has reacted with the oil and that none is left in the soap to harm the skin. The experimental soaps had an average of 0.0357 free alkali as shown in Table 5.

Table 5: Free alkali

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Weight/g	10.0004	10.0001	10.0015	10.0022	10.0010	10.0009	10.0003	10.0000
Titre/cm^3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Free alkali	0.0357	0.0357	0.0357	0.0357	0.0357	0.0357	0.0357	0.0357

This was within the acceptable range. There is 0.01 % unreacted alkali in the prepared soap, but it is insufficient to cause harm on human skin. Free alkali should not be zero since it enhances bleaching and helps increase shelf life [17]. It should also not be too

much since its corrosive and considering that this soap will be applied on the skin, free alkali should not exceed the acceptable range. Free alkali for all the prepared soaps was almost the same since the added base was uniform throughout.

Assay of active ingredients

Instead of simultaneously analysing the two active ingredients separate methods of analysis were developed but both used the UV-VIS Spectrophotometer. This was because the soap was soluble in water and insoluble in most solvents like acetonitrile, petroleum ether and ethanol. Permethrin is soluble in water and DEET is insoluble in water and soluble in petroleum ether. Therefore dissolving the soap in water, Permethrin could be easily analysed using the Spectrophotometric method. DEET needed an extraction method after dissolving the soap in water and it was extracted using Petroleum ether.

Method precision

Method precision was aimed at finding out how close the experimental results are to each other. Three samples from a homogenous sample of the soap gave an RSD of less than 2 for both DEET and Permethrin in Table 6, Table 7 and Table 8.

Table 6:Assay results for DEET (20%) and Permethrin (0.5%)

Sample	**Active Ingredient**	**Absorbance**	**Absorbance**
1	DEET (20%)	**Sample**	**Standard**
		0.203	0.02
		0.201	0.02
		0.202	0.02
Mean		0.202	0.02
%RSD		0%	0%
	Permethrin (0.5%)	0.016	0.030
		0.016	0.030
		0.016	0.030
Mean		0.016	0.030
%RSD		0%	0%

Table 7: Assay for DEET (20%)

Sample	**Active Ingredient**	**Absorbance**	**Absorbance**
2	DEET (20%)	Sample	Standard
		0.200	0.02
		0.201	0.02
		0.200	0.02
Mean		0.200	0.02
%RSD		0.00025%	0%

Table 8: Assay for Permethrin (0.5%)

Sample	**Active Ingredient**	**Absorbance**	**Absorbance**
3	**Permethrin (0.5%)**	**Sample**	**Standard**
		0.017	0.030
		0.016	0.030
		0.017	0.030
Mean		0.017	0.030
%RSD		0.0029%	0%

The methods were very precise for the assaying of the two active ingredients in a repellent soap.

Method accuracy
This was done to find out how close the experimental results are to the true value. The method were very accurate as the standard deviation was almost zero for both the assay of DEET and Permethrin. Therefore the developed methods were accurate.

Ruggedness
Similar results were obtained when the analysis was performed on a different day. This proved that the developed method can be performed universally at different conditions and at different times.

CONCLUSION

Higher concentrations of DEET gave higher repellence against mosquitoes and Permethrin does not help repel mosquitoes when used in a soap formulation. Therefore a repellent soap with DEET only at a high concentration of 50 % is the most effective. Spectrophotometric methods were developed for assaying of the active ingredients, Permethrin and DEET. The methods were validated with respect to accuracy, precision and ruggedness.

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