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QUANTIFICATION OF FATTY ACIDS AND EMULSION FORMULATION STUDIES OF OIL EXTRACTED FROM CASHEW (*ANACARDIUM OCCIDENTALE* LINN) NUTS

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ABSTRACT

Excipients developed from locally available materials are increasingly becoming significant. This is especially so because they serve as alternatives/replacements to the already over exploited traditional sources of excipients. The aim of this research was to extract, characterize and evaluate the self-emulsifying property of the oil from cashew nut seed (CNO) using soxhlet, cold press and aqueous techniques. The physicochemical analysis of CNO was as described by Association of Official Analytical Chemists (AOAC, 2016) and include determination of moisture content, specific gravity, refractive index, saponification value, acid value, iodine value and peroxide value, while the quantitative analysis of the oleic acid content of CNO was determined using the High-Performance Liquid Chromatography (HPLC). Emulsions containing CNO were prepared in the presence and absence of an emulsifying agent (Tween 80) at 5 % v/v concentration. The organoleptic properties, such as the pH, viscosity, creaming index, globule sizes and emulsion type of the prepared emulsions were determined using standard protocol. Stability of the prepared emulsions stored at 25 ° C and 8 ° C were also evaluated at predetermined time interval. These results showed that the soxhlet method gave the highest oil content (39 %) than the cold press (32 %) and aqueous (15%) methods of extraction. Quantitative analysis of the oil using HPLC showed that CNO contained 78.18 % of oleic acid. Stability studies showed that the emulsions containing Tween 80 were homogenous and stable at 25 ° C and 8 ° C after 24 h, day 30 and 6 months and their physicochemical properties were not significantly altered. On the other hand, emulsions prepared without emulsifying agent were found to be stable after 24 h but pH, viscosities, creaming indexes and globule sizes were observed to increase significantly by day 30. These increased indices were also found not to differ significantly by 6 months. In conclusion, this study has been able to show that even though CNO possess self-emulsifying property, it cannot be used to prepare stable emulsions on its own without the inclusion of an emulsifying agent most especially for CNO emulsion that will not be use immediately (within 24 hrs).

Key Words: Emulsion, Linoleic acid, Oleic acid, Stearic acid, Tween 80

INTRODUCTION

An emulsion can be defined as a dispersion of two immiscible or partially miscible liquids in which one is uniformly distributed in the form of droplets throughout the other liquid (Park *et al.,* 2020). These two immiscible liquids are mainly oil and water i.e. polar and non-polar solvents. Emulsions may be oil-in-water (o/w), in which oil droplets are dispersed in water or water-in-oil (w/o), in which water is dispersed in the

oil (Gillian, 2021). Emulsions can also be multiple in nature in which the re-emulsification of w/o or o/w emulsions occurs in either water or oil to give o/w/o or w/o/w (Barkat *et al.*, 2017).

When two liquids that do not mix (such as oil and water) are brought together, they form two separate layers with minimum area of contact due to low surface free energy (G). When energy is imputed into the system in the form of mixing or agitation, the liquids will form various droplet sizes with resultant increase in surface free energy of the system. Thus, emulsions are thermo-dynamically unstable. This increase in surface free energy ΔG and the resultant increase in surface area ΔA is represented in equation (1) (Gillian, 2021) in which the surface tension is given by γ.

$$\Delta G = \gamma \Delta A \quad (1)$$

When droplets come in contact with each other, they will coalesce (merge together) in an attempt to reduce the interfacial area (ΔA) and the free energy (ΔG). Thus in emulsification, two competing processes are occurring together, one process requires input of energy and the formation of droplets while the other process involves the coalescence of droplets and the reduction of surface area and free energy.

For partially miscible oil and water phases, the droplets will eventually grow by Ostwald ripening; an irreversible mechanism that allows the growth of large droplets at the expense of smaller droplets (Halim *et al.,* 2021).

Even though emulsion can be formulated for most of the routes of drug administration, the topical, oral and parenteral routes are mainly utilised. Oil-in-water emulsions are mainly employed in the preparation of intravenous and oral dosage forms while the w/o emulsions are utilised in the formulation of subcutaneous, intramuscular and dermatological dosage forms. When compared to tablets and suspensions, absorption from emulsions are usually faster and complete (Mahato and Narang, 2018). This is due to solubilisation of the drug in the oil phase, as such, eliminating the dissolution step which occurs before absorption. But due to the unpredictable nature of drug delivery with emulsions in the gastro-intestinal environment, self-emulsifying drug delivery systems (SEDDS) are now available to reduce the instability. These systems are made up of drug, oil, surfactant and co-surfactant and can form emulsions by little agitation once in the environment of the gastrointestinal tract (Mahato and Narang, 2018).

The largest forms of emulsions are those intended for dermatological purposes such as- lotions, creams and liniments. Both the water-in-oil and oil-in-water emulsions for dermatological use enhance drug penetration by either occlusion, evaporation or addition of penetration enhancing agents. Even though emulsions have the advantage of increasing bio-availability and reducing unwanted effects, they are not fully utilized due to the challenge of instability which may lead to inadequate dosing and even toxicity (Saeed and Shola, 2015).

Emulsions can be used to deliver drugs that are poorly soluble in water but readily soluble in oils (Dash *et al*., 2014). Emulsions can be used to mask the unpleasant taste and odour of drugs, when the drug is dissolved in the internal phase of an o/w emulsion. The external phase can then be formulated to contain the appropriate sweetening or flavouring agents. Drugs that are more stable in an oily phase compared to an aqueous medium can show improved stability in an emulsion dosage form. Intravenous emulsions of contrast

media have been developed to assist in diagnosis. Emulsion can also be used to prolong the release of drugs (especially semisolid emulsions) thereby providing sustained release action. The oily phase can serve as a reservoir of the drug, which slowly partitions into the aqueous phase for absorption (Dash *et al*., 2014).

Emulsions are thermodynamically unstable and therefore should be formulated by stabilizing the emulsion from separation of the two phases by the addition of a third agent called surfactant. Pharmaceutical emulsions may be difficult to manufacture. Storage conditions may affect stability. Emulsions are also bulky, difficult to transport, and prone to container breakages. They are liable to microbial contamination which can lead to cracking. This will make uniform and accurate dosing difficult to be achieved (Anukam *et al*., 2015).

The surface tension theory is associated with the adsorption of surface–active agents (surfactants) at each globule interface; this will reduce the o/w interface and hence stabilize the emulsion. However, the characteristic of the interfacial film is important in ensuring the stability of the emulsion e.g. acacia stabilizes the emulsion by forming a strong viscous film around the globule. Oriental wedge theory is associated with the formation of a mono molecular layer of emulsifying agent around each droplet of the internal phase of the emulsion. The internal film theory proposes the formation of a film of emulsifying agent which prevents the contact of the dispersed phase (Anukam *et al*., 2015).

When formulating an emulsion, the route of administration and the intended use of the emulsion will determine the choice of surfactant, oil and the type of emulsion that will be produced (w/o or o/w). In addition, the cost of production, chemical incompatibility with other excipients in the formulation and toxicity of all the excipients will have to be considered (Wahlgren *et al*., 2015).

MATERIALS AND METHODS

Materials

The following materials were used during the investigation: Cashew Nut Oil, Tween 80 ®, Oleic acid, Linoleic acid, Stearic acid, Palmitic acid, Methyl paraben, Propyl paraben. All other materials used were of analytical grade.

Methods

Collection, authentication and processing of cashew nuts

Cashew nuts were collected from a farm land in Udi Local Government area of Enugu state, Nigeria. It was authenticated in the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria, Kaduna state, Nigeria and assigned a verification number ABU 00184 for future reference. The nuts were slit open with the help of a simple knife cutter, the kernels were removed from the shell and then roasted in an oven at 70 ° C for 30 min in order to dry the kernel and remove the testa. The nuts were then blended using an automated blender to obtain cashew nut powder (CNP). This powdered material was placed in a desiccator until further use.

Extraction of Cashew Nut Oil

Soxhlet extraction

The process described by Yahaya *et al.,* (2020) was adopted. A sample of the powdered nut (100 g) was placed into the extracting chamber of the soxhlet extractor, then the extracting solvent (200 mL of n-hexane) was added into the chamber and the process allowed to run for 7 h at 25 º C. After the process was completed, the oil was

recovered by means of a rotary evaporator and the yield determined using equation 2.

$$\frac{W0-W1}{W0} \times 100\% \text{---------- (2)}$$

Where: W_0 is weight of the powder sample

W_1 is weight of the residue after extraction

Cold press extraction

A known weight (100 g) of the blended nuts was introduced into a hydraulic press machine. The oil was obtained through the pressure exerted on the powdered nuts by means of the press. The yield of the oil was determined using equation (2) above (Saeed and Shola, 2015).

Aqueous extraction

A known weight (100 g) of the blended nuts was introduced into beaker and 400 mL of distilled water added to it. This was heated for 2 h at 50 º C in a water bath with intermittent stirring to allow complete leaching of the oil into the water. The oil inter-phase with water was transferred into a glass container and the heating and stirring process was repeated until all the oil was extracted from the residue (Yahaya *et al.,* 2020). The oil was then separated from the water using a separating funnel.

Extraction of fatty acids from cashew nut oil extracted by soxhlet method

The cashew nut oil (5 g) was added to 50 mL of 0.5 M NaOH at 100 º C for 1 h to achieve hydrolysis of the oil in a reflux chamber. The mixture was cooled and 50 mL of petroleum ether added twice to remove any unsaponifiable fraction. The remaining fractions were acidified with 1 M HCl until a pH of 2.9 was achieved. After this, the free fatty acid was extracted twice with 50 mL petroleum ether. The petroleum ether was then removed at 40 º C in a water bath. The acids were finally recovered with 4 mL of methanol and filtered with 0.22 µm Millipore filter. This was stored at room temperature for further HPLC analysis (Guarrasi *et al.,* 2019).

Preparation of standard solution

The standard stock solution of oleic acid of 40 mg/L was prepared in methanol and the working solutions of varying concentrations (40, 80 and 120 mg/L) were also prepared (Guarrasi *et al.,* 2019).

High performance liquid chromatography (HPLC) of fatty acids in cashew nuts oil

Agilent HPLC (USA) 1260 infinity consisting of quaternary pump and a UV detector equipped with sampler TCC under computer control was used. The sample (10 µL) was analysed in a column (250 × 4.6 mm i.d., particle size 5 µm). The mobile phase consisted of acetonitrile/water (85: 15 v/ v) and was degassed to remove air bubbles. Total run time for the HPLC analysis was 2 min at a flow rate of 1 mL/min while UV detection was carried out at 364 nm. The solution was acidified with 0.2 % acetic acid to stabilize the fatty acids. The composition of the fatty acids was determined after calculation of each content of the fatty acid from the cashew nut oil based on the calibration graph prepared using standard fatty acids (Guarrasi *et al.,* 2019).

Preparation of cashew nuts oil emulsions with and without Tween 80 as surfactant

Three (3) batches of emulsions containing 20, 25 and 30 % v/v of CNO (cashew nut oil extracted by soxhlet method) were prepared according to the composition in Table 1. Formulation (F1) containing 20 % v/v of CNO was prepared by measuring 20 mL of CNO into a 500 mL beaker, Tween 80 (5 mL) was also put into the beaker and the contents were mixed gently by shaking. Distilled water (15 mL) was transferred into the beaker and the contents shaken

vigorously in a unidirectional manner with a glass rod until a uniform mixture was obtained. The resulting mixture was further diluted with distilled water (10 mL) then methyl paraben (0.18 g) and propyl paraben (0.09 g) were added. The mixture was mixed using an automatic blender at 28,000 rotations per minute (rpm) for 10 min. The resulting emulsion was packaged into glass bottles and stored at 25 ° C for further analysis (Anukam *et al*., 2015). Similarly, the other formulations (F2 and F3) were prepared according to the formula in Table 1.

For formulations that do not contain Tween 80, the quantities of ingredients as stated in Table 1 were used. Batch F4 was prepared as follows; CNO (20 mL) was measured into a 500 mL beaker, methyl paraben (0.18 g) and propyl paraben (0.09 g) were added into the beaker and the volume was made up to 100 mL with distilled water, the mixture was mixed using an automatic blender as earlier described for 10 min. The resulting emulsion was packaged in a glass container and stored at 25 ° C for further analysis. Other formulations of emulsions (F5 and F6) were also prepared accordingly.

Table 1: Formula for the Preparation of CNO Emulsion with and Without Tween 80 as Surfactant

Ingredients (g)	F_1	F_2	F_3	F_4	F_5	F_6
CNO	20	25	30	20	25	30
Tween 80	5	5	5	-	-	-
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.09	0.09	0.09	0.09	0.09	0.09
Water to	100	100	100	100	100	100

Evaluation of the Emulsions

Organoleptic characterization

All the formulations were tested for colour, texture and phase separation by visual observation immediately after preparation. The feel of the preparations after application on the skin were also determined.

Determination of pH

The Mettler Toledo pH meter was standardized using a pH 7.0 phosphate buffer solution. The meter was dipped in 30 mL of the emulsion at room temperature and the pH was recorded. Triplicate measurements were recorded for each emulsion formulation (AOAC, 2016).

Determination of Viscosity

The viscosity of the emulsion was determined using Brookfield viscometer with spindle number S-06. The emulsion (30 mL) was transferred into a 50 mL beaker, the spindle was placed in the beaker and viscosity of the emulsion at room temperature was obtained at 100 rpm. Triplicate determinations were obtained for each formulation of emulsions (Idah *et al.,* 2014).

Creaming index

The formulated emulsions (10 mL) was transferred into universal bottles and tightly sealed with a cap and then stored at room temperature for 24 h. The height of total emulsion (HE) and the height of the droplet-depleted lower layer (HD) was measured using a meter rule. Creaming index was then calculated using equation 3 (Gillian, 2021).

$$\text{Creaming Index} = 100\left(^{HD}/_{HE}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Globule size determination

This test was conducted with light microscope by placing two (2) drops of the sample on a glass slide, a drop of glycerol was added unto it and the cover slip was placed over the first glass slide. The mounted emulsion was viewed using a calibrated eye piece at x1000 magnification, the diameter of 100 individual globules were measured and recorded (Cassidy, 2014).

Identification of Emulsion Type

Test using methylene blue

The formulated emulsion was viewed under a microscope to determine their types (o/w or w/o). A drop of the emulsion was placed on a slide and mixed with a drop of methylene blue (water soluble dye) with the aid of a spatula, the slide was covered with a cover slip and the colour of the droplets observed against the background under the microscope (Dash *et al.,* 2014).

Stability study

The formulated emulsions (100 mL) were stored in a refrigerator (8 ° C) and at 25 ° C (relative humidity of 75 %) for 30 days and 6 months. After this period, the emulsions were evaluated for organoleptic properties, pH, viscosity, globule size and creaming index (Gillian, 2021).

RESULTS

There should have been a narrative on the Table 2 here

Table 2: Cashew Nut Oil Yield Extracted by Soxhlet, Cold Press and Aqueous Methods

Method	Yield (%)
Soxhlet	39
Cold Press	32
Aqueous	15

Fatty Acid Composition of Cashew Nuts Oil

High performance liquid Chromatography (HPLC) was used as a means of studying the fatty acid composition of cashew nut oil. Peaks were obtained as shown in the chromatogram (Figure 1). The fatty acid peaks were identified and quantified from the calibration curve of the respective standard. Oleic acid, linoleic acid, palmitic acid and stearic acid were quantified to be 78.18, 14.04, 7.12 and 0.10 % respectively. Table 3 gives the quantity of the fatty acids extracted.

Table 3: Soxhlet Extracted Fatty Acid Composition of Cashew Nut Oil by HPLC Method

Fatty Acid	Composition (g/100g)
Oleic acid	78.18
Linoleic acid	14.04
Palmitic acid	7.12
Stearic acid	0.10

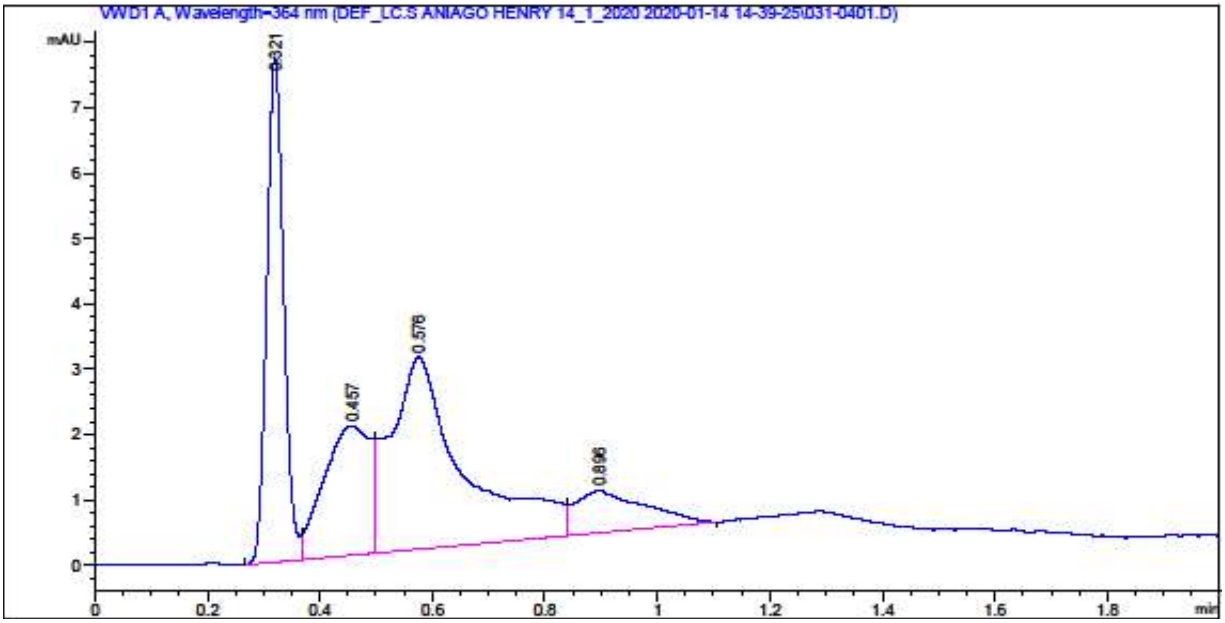

Figure 1: Chromatogram of Oleic Acid from Cashew Nut Oil Extracted by Soxhlet Method 39.18 mg/l (0.32 min)

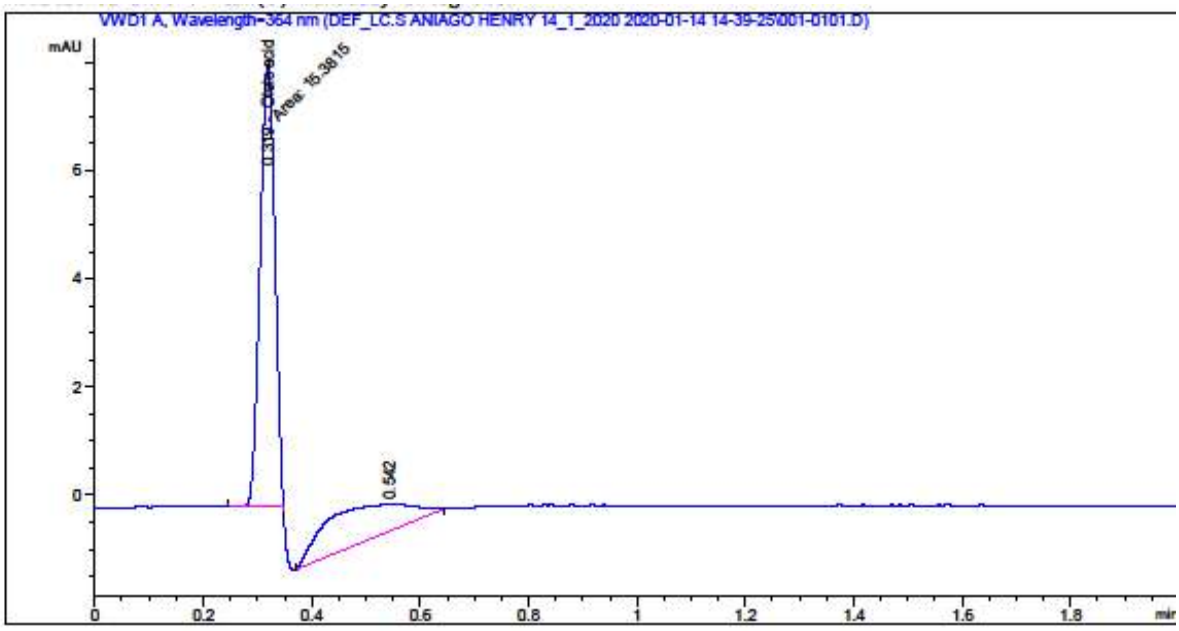

Figure 2: Chromatogram of Standard Oleic Acid

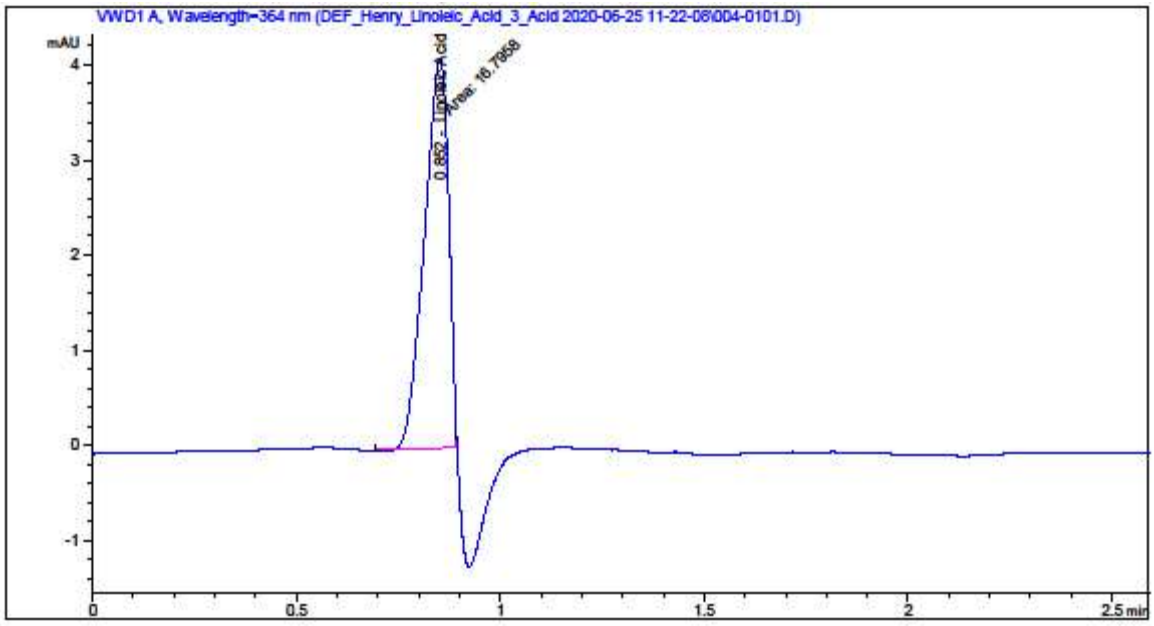

Figure 3: Chromatogram of Standard Linoleic Acid

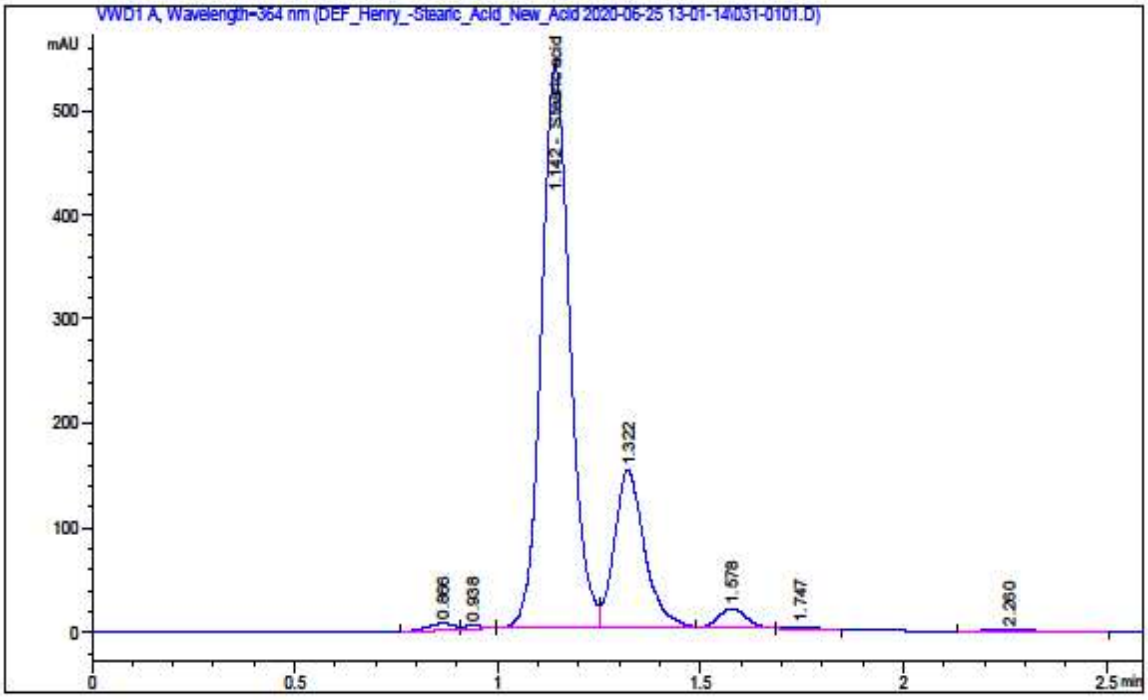

Figure 4: Chromatogram of Standard Stearic Acid

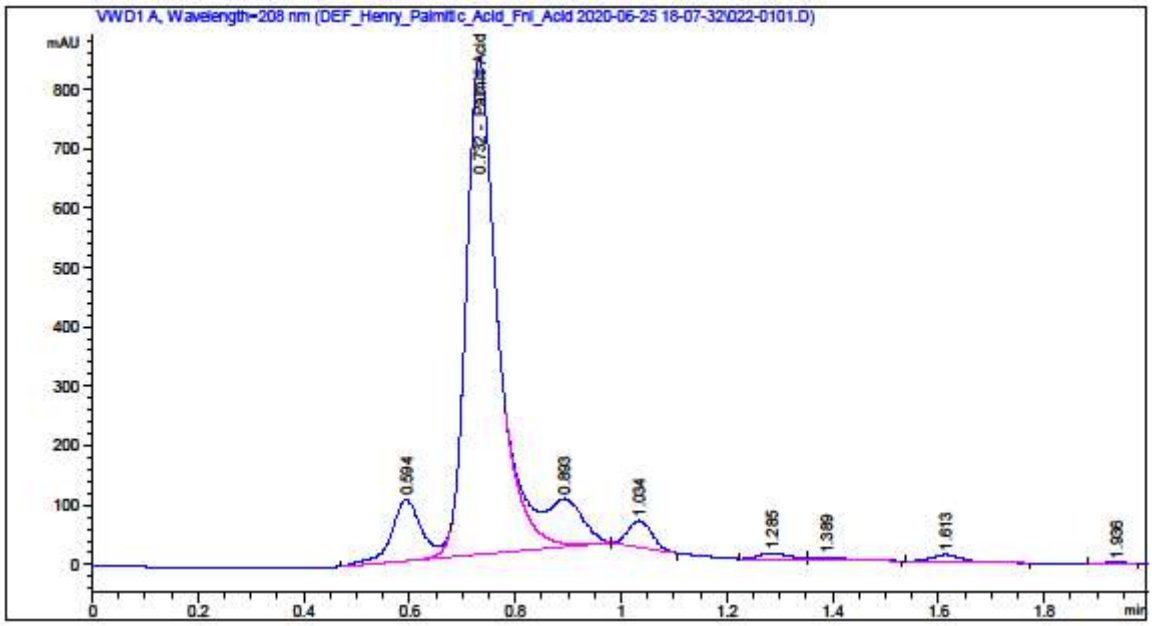

Figure 5: Chromatogram of Standard Palmitic Acid

PLATE I: Visual Appearance of Emulsions at 24 h after Formulation

Plate II: Visual Appearance of Emulsions Stored at 25 ^{o}C for 30 Days

Plate III: Visual Appearance of Emulsions Stored at 8 ^{o}C on Day 30

Table 4: Organoleptic Properties of CNO Formulation at 24 h, 30 Days and 6 Months

Formulations	25 ^{o}C			8 ^{o}C	
	24 h	Day 30	6 M	Day 30	6 M
F1	C, W, P	C, W, Mp	C, W, Mp	C, W, P	C, W, P
F2	C, W, P	C, W, Mp	C, W, Mp	C, W, P	C, W, P
F3	C, W, P	C, W, Mp	C, W, Mp	C, W, P	C, W, P
F4	C, W, P	M, Wb, Mp	M, Wb, Mp	M, Wy, Mp	M, Wy, Mp
F5	C, W, P	M, Wb, Mp	M, Wb, Mp	M, Wy, Mp	M, Wy, Mp
F6	C, W, P	M, Wb, Mp	M, Wb, Mp	M, Wy, Mp	M, Wy, Mp

KEY:C= Cloudy; W= Whitish; P= Pleasant;M= Milky;Mp= Mildy pleasant; Wb= Whitish brown; Wy= Whitish yellow;

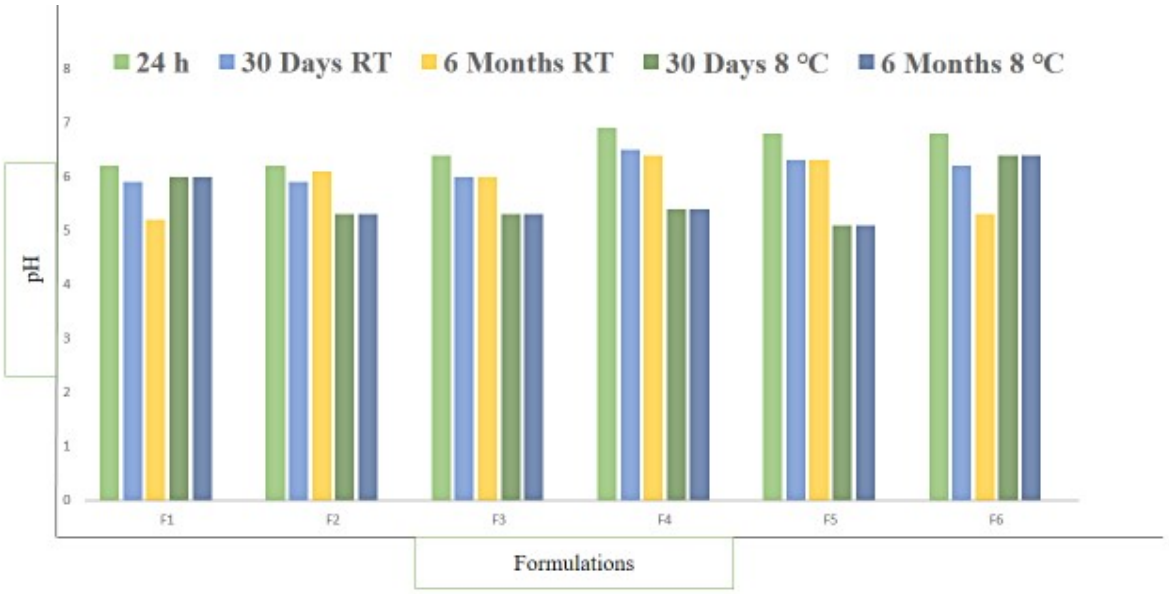

Figure 6: Result of pH of the Formulated Emulsion at 24 h, 30 days and 6 Month

Key: M=Month, RT= Room Temperature

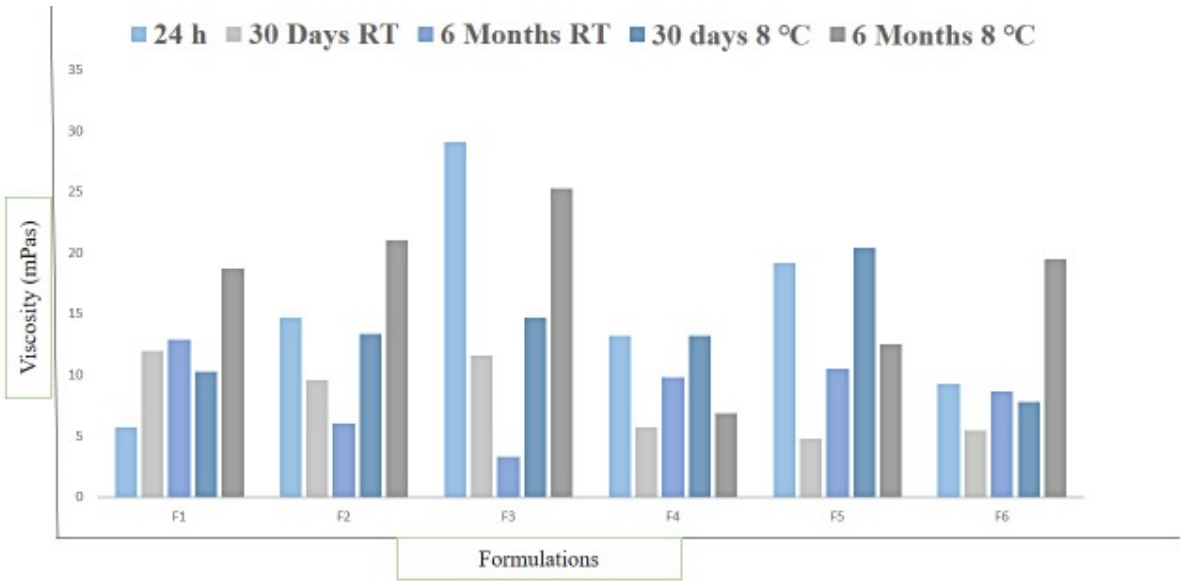

Figure 7: Viscosities of Formulated Emulsions at 24 h, 30 days and 6 Months

Key: M=Month, RT= Room Temperature

Table 5: Creaming Index (%) of Formulated Emulsion at 24 h, 30 Days and 6 Months

%Creaming/	25 ° C			8 ° C	
Formulations	24 h	Day 30	6 Months	Day 30	6 Months
F1	69	67	67	68	68
F2	67	67	67	67	67
F3	52	51	51	52	52
F4	83	PS	PS	PS	PS
F5	77	PS	PS	PS	PS
F6	64	PS	PS	PS	PS

KEY: PS (Phase Separation)

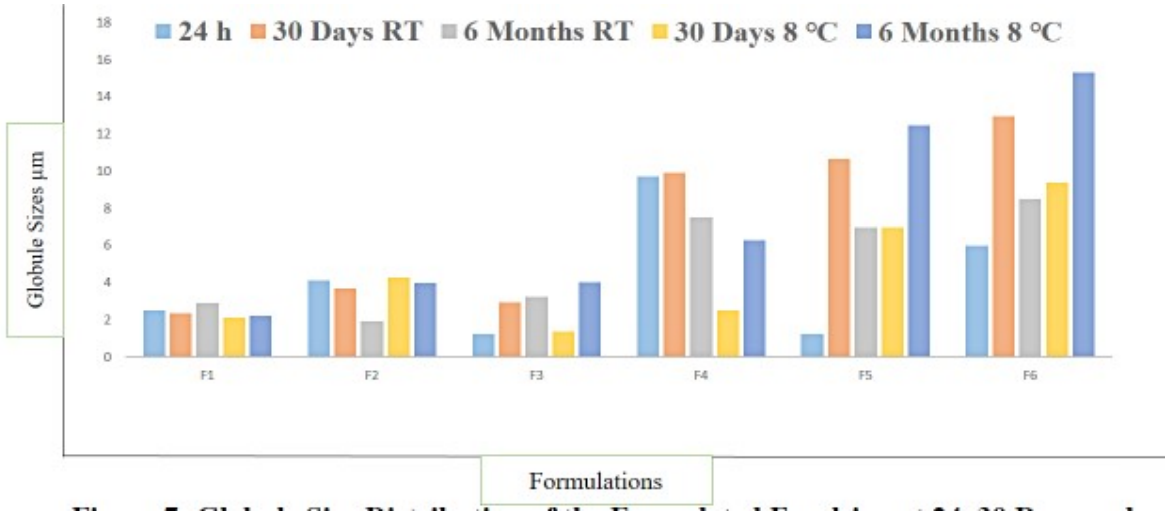

Figure 8: Globule Sizes of Formulated Emulsions at 24 h, 30 days and 6 Months

Key: M=Month, RT= Room Temperature

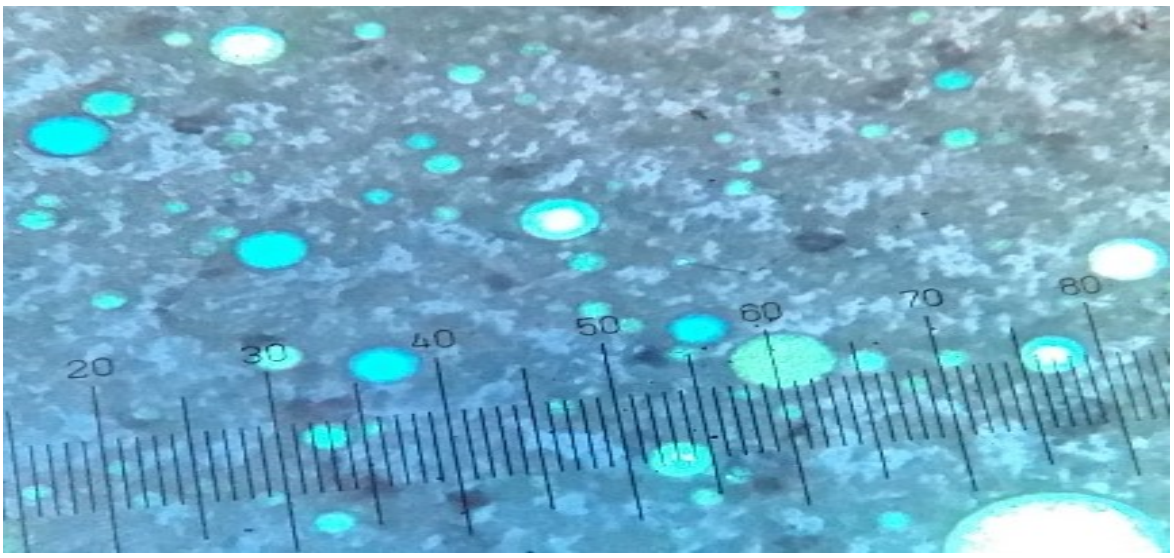

Figure 9: Microscopic Images at x1000 Magnification of CNO Prepared from Soxhlet Extracted oil after 24 h

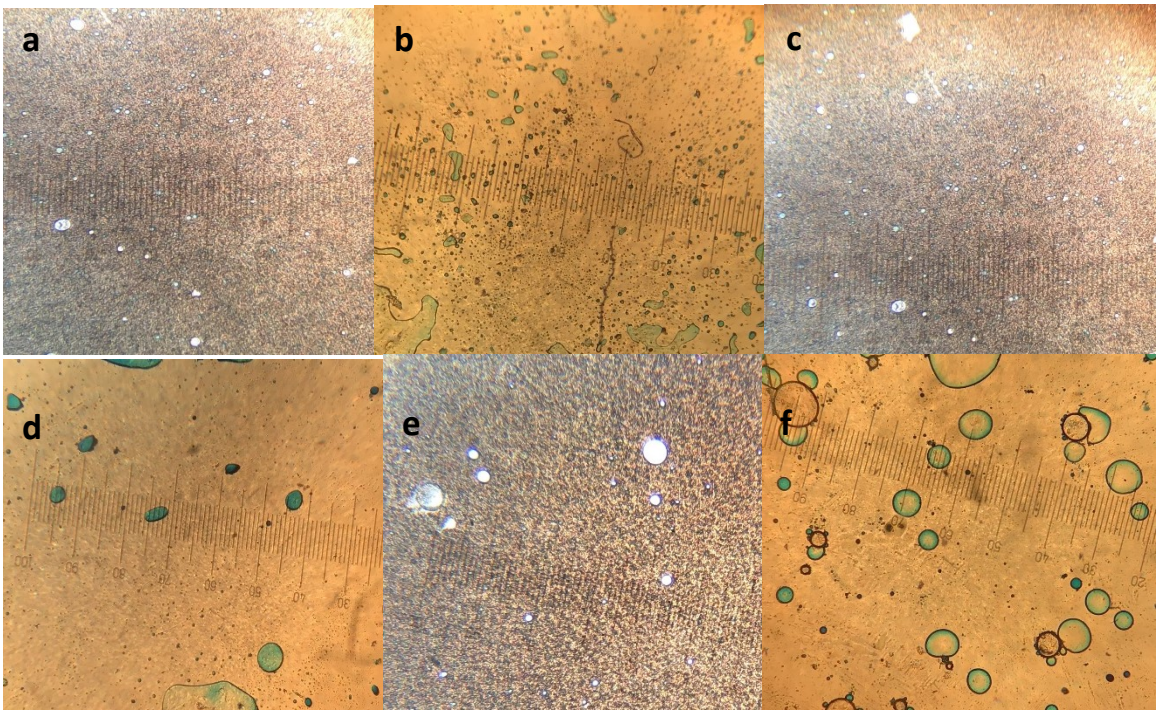

Figure 10: Microscopic images at x100 magnification of CNO emulsion prepared after 6 Months stored at 8 °C. (Figure a, c, e = F_1, F_2, F_3 while b, d, f= F_4, F_5, F_6)

DISCUSSION

From the results obtained, extraction using the soxhlet process gave higher yield 39 % w/w than the cold press 32 % w/w and the aqueous methods 15 % w/w (Table 2). The extraction yield of the Soxhlet process can be ascribed to enhanced solvent action on the oil due to the soxhlet process which involves heating the solvent to reflux. These results differ from 48.81, 33.81 and 29.41 % w/w obtained for soxhlet, cold press and aqueous methods respectively in study on extraction of cashew nut oil by Yahaya *et al.*, (2012). These differences in oil yield from these two studies could be attributed to factors such as age of the cashew tree used, available information of cashew cultivation to the farmer, the sources of cashew planting material, cost of farm labour etc. (Agbongiarhuoyi *et al.,* 2015). This present result shows that employing the soxhlet method of extraction can be considered economical for commercial production of cashew nuts oil (CNO) in Nigeria.

The fatty acid composition of CNO extracted by soxhlet method revealed the presence of oleic acid (78.18 %), linoleic acid (14.04 %), palmitic acid (7.12%) and stearic acid (0.1 %) (Table 3). This is similar to the report by Nandi (2019) in the study on 'nutritional constituents of cashew nut' with oleic acid being 73.3 %, linoleic acid 7.67% and palmitic acid being 0.89 %. High oleic acid constituent (78.18 %) as observed in this study, suggests that CNO could possesses self-emulsifying properties (Gillian *et al.*, 2013).

All the emulsions formulated using CNO extracted by Soxhlet method were white in color, smooth non-gritty in appearance with mildly pleasant odour 24 h after preparation (Table 4). There was however, a colour changes from white to whitish-brown on prolong storage (30 days to 6 Months) at 25

º C for both categories of emulsions (with Tween 80 and without Tween 80).

At prolong storage (30 days to 6 Months) of the 6 batches of emulsions at 8 º C, F_1, F_2 and F_3 retained their organoleptic properties while (F_4, F_5, F_6) were found to be milky, whitish-yellow and mildly pleasant. In addition, the intensity of the colouration was found to increase with increase in the concentration of the oil from 20 to 30 %. This shows that the stability of an emulsion is influenced by its storage condition, concentration and the presence or absence of a Tween 80. Pharmaceutically, the emulsions prepared without Tween 80 will lose acceptability by users which will result in non-compliance of the formulation.

The pH as a physicochemical parameter plays an important role in molecular, metabolic and cell regulating processes (Gillian, 2021). The pH requirements for topical preparations is recommended to be between 4 and 6 (Gillian, 2021); changes in the pH of formulations affect the barrier functions of the epidermis and cause skin damages or more untoward effects on the skin (AOAC, 2016). In this present study, the pH of all the formulated emulsions were found to be between 5.9 and 6.8 (Figure 6) across the storage conditions and time. There was no significant difference in the pH values between the different batches of emulsions 24 h after formulation ($p>0.05$). There was also no significant difference in pH between emulsion with Tween 80 (F_1) and those without Tween 80. There was significant statistical difference when compared between emulsions stored at 24 h and those stored at 8 º C for 6 Months. However, emulsion containing Tween 80 (F_1, F_2, F_3) were found to have slightly lower pH (5.8) than those prepared without Tween 80 (F_4 F_5 F_6) (6.5). In addition, the formulations were observed to have higher pH when stored at 25 º C for 30 days and 6 Months as compared to emulsion stored at 8 º C for the same duration (Figure 6). Also, the pH was found to be same when stored at 8 º C for 30 days and 6 Months. In addition, increase in the concentration of the oil from 20 to 30 %, did not result to appreciable increase or decrease in the pH of the formulations. This implies that storing the emulsion at high temperature increases the rate of instability as the formulations were found to become more acidic (Dash *et al.,* 2014).

Viscosity is a measure of a fluid resistance to flow (Barkat *et al*., 2017). Generally, viscosities of the formulations with Tween 80 present a regular pattern with viscosities decreasing with increasing storage duration at 25 º C and increasing with increasing duration of storage at 8 º C. The viscosities of the formulated emulsions 24 h after preparation were between 5.70 and 29.10 mPas at a shear rate of 100 rpm and was found to increase with increasing concentration of the oil phase (Figure 7). Also, the viscosity of the emulsions was observed to decrease with increasing concentration of the oil (for formulations without Tween 80) but that of batch F_6 was lower which could be a result of the oil phase exceeding the required quantity of the internal phase. However, viscosities of emulsions prepared with the Tween 80 were observed to be higher than those prepared without the Tween 80. This shows that Tween 80 increased viscosity of emulsion. The viscosity of the emulsions prepared without Tween 80 stored at 25 º C for 30 days was observed to be 4.8 mPa (formulation F_5). On the other hand, emulsions stored at 8 º C for 30 days had higher viscosity; 8.4 mPa (formulation F_2) (Figure 7). But formulations stored at 25 º C for 6 Months showed a decrease in viscosity with increasing concentration. This implies that the storage temperature affects the stability of the emulsions by reducing the

viscosity. The result shows that there is no significant difference between the batch of emulsions containing Tween 80 (F_1) and those without Tween 80 (F_4) ($p>0.05$) on storage for 30 days and 6 Months. Also, there is no significant difference between emulsions formulated at 20 % (F_1, F_4) and those formulated at 30 % (F_3, F_6). These uneven viscosities in formulations prepared without Tween 80 implies that the emulsion will not flow as required during dispensing leading to irregular dosing.

Creaming occurs when there is a density difference between the two phases of an emulsion. It is the precursor of coalescence which will eventually lead to phase separation (Maphosa and Victoria, 2018). This means that for an emulsion to be stable, the rate of creaming should be slow. Increasing the concentration of oil in all the emulsions was found to decrease the creaming rate (Table 5). In addition, emulsions prepared with the Tween 80 (F_1, F_2, F_3) were found to have lower creaming rates (between 52 and 69 %) than those prepared without the Tween 80 F_4, F_5 and F_6 which were between 64 and 83 %. This can be attributed to the reduction in interfacial tension brought about by the presence of the Tween 80. The creaming rate of the emulsions prepared with the Tween 80 was found to decrease upon storage at 25 º C and at 8 º C and can be attributed to the effect of the Tween 80. However, those prepared without the Tween 80 were found to completely separate out (phase separation) upon storage at 25 º C and at 8 º C which is as a result of the absence of Tween 80. This shows that the emulsions are unstable without Tween 80; it also infers that these emulsions prepared without Tween 80 will lose their aesthetic nature and acceptance by users (Barkat *et al*., 2017).

The globule sizes is used to differentiate an emulsion from nano emulsion or micro emulsion (Maphosa and Victoria, 2018). An emulsion will be stable if the globule sizes are uniformly distributed throughout the continuous phase. Aggregation of globules, will lead to flocculation and finally phase separation. The average sizes of all the emulsion globules ranges between 1.25 µm and 12.37 µm (Figure 8). There is no significant difference in the globule sizes of the emulsion 24 h after formulation ($p>0.05$) and on storage for 30 days and 6 Months at 25 º C and 8 º C. But formulations F_4 and F_5 shows significant difference ($p< 0.05$) in globule sizes on storage for 30 days and 6 Months in 25 º C. When the emulsions were stored for 30 days at 25 º C and 8 º C, the globule sizes were found to increase with increase in concentration. Emulsions containing Tween 80 were observed to have smaller globule sizes than those without Tween 80. Lager globule sizes were observed for emulsions stored at 25 º C (12.37 µm) than those stored at 8 º C (9.27 µm) for 30 days and 6 months as shown in Figure 8. Thus, emulsions with larger globules will eventually lead to phase separation. The difference in globule sizes of emulsions containing Tween 80 and those without Tween 80 was not significant ($p>0.05$) after storage for 6 Months. Also, globule sizes of the formulation were found to increase with increase in the concentration of the oil phase from 20 to 30 %. As a result, emulsions containing Tween 80 are stable and are able to retain uniformity of active pharmaceutical ingredients as against those formulated without Tween 80.

The dye test reveal whether an emulsion is water-in-oil or oil-in-water and can also reveal the change that occurs in emulsion phases i.e., when an emulsion changes from oil-in-water (o/w) to water-in-oil (w/o) as a result of change in surfactant affinity or temperature difference. Figure 9 shows that the emulsions were formulated as oil-in-

water (o/w). However, on storage for 30 days and 180 days at 25 ° C and 8 ° C, the formulations with Tween 80 (F_1 F_2 F_3) were seen to remain as o/w while formulations without Tween 80 (F_4 F_5 F_6) changed to w/o (Figure 10). This means phase inversion has taken place in the formulations without Tween 80 as such will not be considered fit for pharmaceutical use.

CONCLUSION

The soxhlet extraction method is the most efficient method of extracting CNO producing the highest yield. The oil can be exploited in the formulation of stable emulsions at a concentration of 20 % and in the presence of a surfactant (Tween 80).

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