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Research Article

Emulsifying properties of novel biopolymer (Cola acuminata gum) extracted from pods of Cola acuminata: Macroscopic, Microscopic and Rheological Characterization

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Abstract

Gums are valuable excipients usually employed as emulsifiers in the preparation of pharmaceutical, food and cosmetic products. The emulsifying properties of *Cola acuminata* gum (CAG) derived from the pods of *Cola acuminata* were evaluated. Different batches of emulsions containing arachis oil (40% v/v) emulsified with distilled water were formulated respectively with tragacanth gum (TRG), acacia gum (ACG) and CAG at different concentrations (1.0-4.0% w/v). The different formulations were labelled as TRG1-TRG4, ACG1-ACG4 and CAG1-CAG4. Organoleptic properties real-time and accelerated stability studies of the emulsions were evaluated using centrifugation, thermal and freeze-thaw conditions. The rheology of the emulsions was measured using a Digital Synchroelectric viscometer (NDJ- 5S, China) with spindle 2 operated at various rotational speeds (6, 12, 30 and 60 rpm). In contrast, the microstructures of the emulsions were evaluated by photomicrography. CAG1, TRG1 and ACG1-ACG4 could not form stable emulsions. Real-time stability studies revealed that TRG2 and CAG2 cracked on days 3 and 7, respectively. TRG3 experienced creaming from the fourth week, while TRG4, CAG3 and CAG4 remained unchanged. Accelerated stability tests induced creaming in CAG3 and TRG4. The degree of creaming induced by various accelerated stability conditions was in the order of thermal > freeze-thaw > centrifugation. CAG4 showed no creaming throughout the experiment. All the emulsions exhibited pseudoplastic behaviour. There was no significant change in the viscosity of TRG3, TRG4, CAG3 and CAG4 after 8 weeks of storage. An increase in the concentration of the emulsifiers increased the emulsions' viscosity and stability. There was an increase in the emulsions' droplet size and a decrease in emulsions' droplet number during storage, indicating gradual coalescence. CAG showed superior emulsifying properties.

Keywords: Cola acuminata gum (biopolymer); emulsion; real-time stability studies; solvent extraction method; Rheology

Introduction

The quest to obtain pharmaceutical excipients from new materials has significantly increased in this new dispensation, especially in developing economies with abundant raw materials yet to be optimized. Exudates from trees and shrubs are potential commercial sources of functional biopolymer gums. These polysaccharides can be used as emulsifying agents in pharmaceutical, cosmetic and food preparations, many of which are formulated as emulsions [1,2].

Emulsions are biphasic liquid systems consisting of two immiscible liquids (commonly water and oil). The internal or dispersed phase is finely distributed as droplets in the external or continuous phase. Emulsions are thermodynamically unstable, thereby causing the coalescence of the dispersed droplets, leading to the separation of the two phases [3,4]. In order to prevent emulsion instability, emulsifiers are usually added. Emulsifiers can act by reducing the tension at the interface between the two immiscible liquids, form a wedge that is partitioned in between the two liquid phases or form a thin

layer of film that is adsorbed on the droplets' surface, thereby preventing the agglomeration of the dispersed phase. In most pharmaceutical emulsions, the stabilizing system comprises surfactants or, biopolymers or a combination of these [5,6]. Hydrophilic biopolymer emulsifiers can further enhance emulsion stability by acting as stabilizers to increase the viscosity of the external phase and retards the aggregation of droplets [2]. Tragacanth gum and Acacia gum have been used for decades as emulsifiers and for other purposes in the preparation of food and pharmaceutical products [7,8]. *Cola acuminata* gum (CAG) is a novel biopolymer obtained from the pods of *Cola acuminata,* a species in the genus Cola belonging to the family Sterculiaceae, native to tropical Africa. The pods are usually thrown away as waste products after removing the seeds or allowed to decay on farmland to form organic manure. Reports of the preliminary phytochemical and physicochemical investigations conducted by Bamigbola et al. [9] showed that CAG powder is an amorphous hydrophilic polysaccharide gum containing a mixture of monosaccharides such as ribose, xylose and glucose and possesses good compressibility and flow properties. CAG was sparingly soluble in water and produced viscous mucilage that exhibited pH-dependent swelling with pseudoplastic flow.

The biopolymer gum has been used previously in the formulation of suspensions and polymeric films. It has been documented that the suspending properties of CAG were better than those of acacia and tragacanth [10]. Biopolymer films prepared with CAG possessed good physical, mechanical and mucoadhesive properties with the potential for use as coating, encapsulating, sustained release formulations and mucoadhesive drug delivery systems. Oral and topical toxicological evaluations of the gum in rats indicated its safety for possible oral and topical formulations. Oral and topical toxicological evaluations carried out were acute toxicity (LD_{50}) tests at the maximum dose of 5000 mg/kg body weight and topical skin irritation tests, respectively [9,11]. To date, there has not been any reported work on the use of CAG as an emulsifying agent. Consequently, the objective of this work is to compare the emulsifying properties of CAG with both Tragacanth gum and Acacia gum.

Materials and Methods

Materials

Cola acuminata pods (bought from Elele market in River State), Acetone (Avondale Laboratory Supplies and Service Limited, Bunbury Oxon, England.), Distilled water (Chafok scientific Co. Nigeria enterprises), Arachis oil (Silverline Chemicals India), Sodium metabisulphite, Tragacanth powder, Nigrosine dye (BDH, Chemical Ltd, England), Neubauer counting chamber, Homogenizer (Ormerod Engineer Limited, Rochdale England).

Methods

Extraction of Cola acuminata gum

Cola acuminata pods containing *Cola acuminata* nuts/seeds were purchased from the local market in Elele, Nigeria, from cola nut merchants who buy directly from cola nut farmers. The pods were cut open, and the nuts/seeds were removed and identified as *Cola acuminata* nuts in the Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele Campus, Rivers State and a voucher specimen (MU1346) is deposited in the Department Herbarium. CAG was extracted from the *Cola acuminata* pods in the Pharmaceutics and Industrial Pharmacy Laboratory, Faculty of Pharmacy, Madonna University, Elele Campus, Rivers State.

The method of Bamigbola et al. [9] was adopted to extract CAG from *Cola acuminata* pods using the solvent extraction method. Cola pods were freshly harvested, thoroughly washed with distilled water, and then cut into small pieces. A 2 kg quantity of the cola pods already sliced was weighed and immersed in 5 litres of distilled water containing 0.1% w/v sodium metabisulphite inside a plastic container, which was covered for 24 h. After that, the viscous mucilage produced was filtered from the pods using muslin cloth. CAG was precipitated from the viscous mucilage with the aid of acetone. The quantity of acetone used was three times the volume of the gum mucilage. The precipitated gum was further rinsed with more acetone to extract the remaining water until it was dry. The gum was later

dried at 60 °C for 2 h in a hot air oven (Gallen Komp, England) powderized, screened through a 150 μm sieve and preserved in an airtight amber-coloured bottle at room temperature.

Formulation of emulsions

Different batches of emulsions containing arachis oil (40% v/v) emulsified with distilled water were prepared with TRG, ACG and CAG, respectively, at the concentrations of 1.0%, 2.0%, 3.0%, and 4.0% w/v. A dry gum method of preparation of emulsion was used; the required amount of gum was weighed, dispersed into the specific amount of arachis oil and mixed in a mortar with a pestle. The already measured quantity of distilled water was later added and triturated vigorously. The emulsion produced was further homogenized by passing it through a homogenizer (Ormerod Engineer Limited, Rochdale, England). Distilled water was added to the emulsion in a glass measuring cylinder to make up the volume to 100 ml. The containers were tightly sealed, stored at 25 °C on a dark, vibration-free surface and protected away from sunlight. The formula used to prepare different batches of emulsions using different emulsifying agents is shown in Table 1 below.

Table 1. Formula used for the formulation of different batches of emulsions.

Ingredients	CAG1	CAG2	CAG3	CAG4	TRG1	TRG2	TRG3	TRG4	ACG1	ACG1	ACG1	ACG1
Arachis oil	40	40	40	40	40	40	40	40	40	40	40	40
Water	60	60	60	60	60	60	60	60	60	60	60	60
CAG (g)	1	2	3	4	-	-	-	-	-	-	-	-
TRG (g)	-	-	-	-	1	2	3	4	-	-	-	-
ACG (g)	-	-	-	-	-	-	-	-	1	2	3	4

Quantity of Arachis oil and water in ml.

Evaluation of emulsions

Macroscopic evaluation

Real time stability studies

Real-time stability studies were conducted on the emulsions. A 10 ml sample of each emulsion freshly prepared was added into a test tube, which was covered and kept at 25 °C. Immediately after preparation, the emulsions' stability was assessed on days 1, 3, 7, and subsequently on a weekly basis for the 8 weeks of storage. The samples were observed for possible physical changes such as colour, odour, creaming, cracking or phase separation to ascertain their stability. The samples that cracked into two distinct phases were exempted from further investigations. In contrast, the stable samples were monitored for creaming by observing the separation of the emulsion into an opaque layer and a transparent serum layer. The emulsion stability index (ESI) was depicted as the ratio of the final height of the emulsion to the initial height of the emulsion in test tubes, as shown below.

$$ES = \frac{Hf}{Hi}\ X\ 100 \qquad \text{….. (1)}$$

Where Hi was the emulsion's initial height and Hf was the creamed emulsion's final height [2].

Based on the observations from the real-time stability studies, only four batches of emulsions (CAG3, CAG4, TRG3 and TRG4) remained stable throughout the 8 weeks of the experiments and therefore subjected to further investigations. The remaining eight unstable batches were exempted.

Determination of the emulsification degree

The method of Wen et al. (2019) was adapted to determine the emulsification degree in the emulsion samples kept for 8 weeks by calculating the mixing degree coefficient as shown below:

$$Cm = \frac{\acute{\omega}o}{\omega o} \qquad \text{….. (2)}$$

Where Cm is the mixing degree coefficient, ωo is the oil volume fraction of the initial emulsion, and ώo is the oil volume fraction of the final emulsion obtained at various storage time intervals [12].

Accelerated stability studies

Centrifugation or Mechanical stress stability test

The method of Estanqueiro et al. [13] was adapted. A 10 ml sample of each formulation was subjected to centrifugation at 3000 rpm for 30 min. The samples were examined for any change, and the (ESI) in terms of creaming was calculated as shown below:

$$ES = \frac{fev}{Iev} X\ 100 \qquad \text{….. (3)}$$

Where f_{ev} was the emulsion's final volume, and I_{ev} was the emulsion's initial volume [2].

Thermal stability test

The effects of heat on the stability of the emulsions were assessed using the method of Ebrahimi et al. [2]. A 10 ml sample of each emulsion formulation was heated in a water bath regulated at 80 °C for 30 min and then subjected to centrifugation at 3,500 rpm for 10 min. The ESI was then calculated using the formula in Equation 3 above.

Freeze-Thaw stability test

The method of Estanqueiro et al. [13] was employed to assess the effects of freeze–thaw cycles on the stability of the emulsions. Each of the emulsion samples (10 ml) was stored at 4 °C for 24 hr in a fridge. The samples were later transferred to an oven and stored at 40 °C for another 24 hr. The cycle was repeated three times. The ESI was also calculated using the formula in Equation 3 above.

Rheological study

The emulsion's rheological properties were evaluated. The viscosity (centipoise) of the four batches of emulsions (CAG3, CAG4, TRG3 and TRG4) was determined immediately after preparation at 25 °C using a Digital Synchroelectric viscometer (NDJ- 5S, China). Spindle 2 of the viscometer was operated at various rotational speeds (6, 12, 30 and 60 rpm), and the effect of shear rate on the viscosity of the samples was recorded. All the results were made in triplicates and recorded as the mean values. The result of the shear rate was plotted against shear stress to obtain a rheogram for the four samples. The rheological study was repeated on the four samples of the emulsions after 8 weeks of storage.

Microscopic evaluation

The microscopic examination of the four stable batches of emulsions was conducted immediately after the preparation and repeated on a weekly basis to monitor the effects of time of storage on the microstructure of the emulsions.

Emulsion microstructures

The microstructure of each emulsion was visualized under an optical microscope (BMS E1 Series China) equipped with a digital camera. A 0.05 ml of TRG3 emulsion was thoroughly mixed with 1ml of 50 % aqueous glycerol to give a dilution ratio of 1:20 dilution. Two drops of 10 % Nigrosine dye were added to the mixture to produce a contrasting blue-black aqueous background, which makes the oil droplets appear conspicuously as bright cycles. A drop of the diluted TRG3 emulsion sample was mounted on a microscope slide, covered with a cover slip and viewed under the microscope.

The microstructures of the emulsion were photographed at x10 objective magnification immediately after the preparation of the emulsions and weekly for 8 weeks, and the images were recorded accurately. The same procedure was chosen for TRG4, CAG3 and CAG4 batches for the 8 weeks [14].

Determination of droplet count and size

In order to determine the droplet count and size of the emulsions, the method of Abdulsamad et al. [14] was adopted. The counting of the emulsion's droplets was done using an upgraded Neubauer counting chamber. A drop of the diluted TRG3 emulsion (1:20 dilution) was mounted on the counting chamber, covered with a cover slip and viewed under an optical microscope (BMS E1 Series China) equipped with a digital camera. The images of the emulsions were observed at x10 objective

magnification. The entire depth of the droplets in the chamber was searched, after which 20 small squares of the chamber were randomly selected, and droplets observed in them were counted and recorded accurately. The droplet count was done for the freshly prepared emulsions weekly for 8 weeks. The same procedure was chosen for the TRG4, CAG3 and CAG4 batches.

The droplet number (N) obtained from an mm^3 of the emulsion was computed using the equation below:

$$N = C/256 \times F \times 100/P \times 10^4/2.5 \quad \text{..... (4)}$$

Where C is droplet count, F is the emulsion's dilution factor, and P is the percent composition of oil in the emulsion.

The droplet size diameter (d) was calculated from equation 5 below:

$$d = 10\sqrt[3]{6/\pi N} \quad \text{..... (5)}$$

Data obtained were used to plot the graph of droplet numbers against time to indicate the change in droplet distribution with time. Graph of the droplet size against time was also plotted to evaluate the emulsion stability.

Statistical analysis

ANOVA was carried out for data statistical analysis, using Duncan's post hoc comparison with $p < 0.05$ as a significance level.

Results and Discussion

Macroscopic Evaluation

Real time stability studies

Stability studies give helpful information about the stability of a formulated product. Real-time stability studies involve storage of the product under the recommended storage conditions and testing of the samples at intervals for possible changes in the physicochemical properties of the products for the time of the proposed shelf life [15].

From the results of the physical examinations, emulsions prepared with CAG exhibited brown colouration, while that of TRG and ACG exhibited light brown and cream colouration, respectively. There was no noticeable change in the colour of the emulsion formulations that remained stable throughout the 8 weeks of the study. CAG1, TRG1 and all four batches prepared with ACG cracked with noticeable phase separation into two layers from day 1. The respective concentrations of these emulsifying agents were not enough to form a stable emulsion. Furthermore, TRG2 and CAG2 cracked on days 3 and 7, respectively. The inability of acacia gum to form stable emulsion even at 4% is an indication of its weak emulsifying property. It has been reported that because of the weak emulsifying property of ACG, a high ratio (approximately 1:1) of gum to oil was needed to prepare stable emulsions [16]. The weak emulsifying property of acacia gum was corroborated by another report where 5% to 15% of ACG were used to obtain stable sunflower oil-based emulsions when compared with Amuniacum gum (*Dorema ammoniacum*) [2].

Using the method of Ebrahimi et al. [2], the ESI was calculated as the ratio of the final height of the emulsion to the initial height of the emulsion in test tubes, as shown in equation 1 earlier on. Table 2 shows the emulsification stability index (ESI) of emulsions formulated with different concentrations of CAG and TRG kept under normal storage conditions over 8 weeks.

Figure 1 shows the extent of creaming visually observed in the emulsion formulations during the actual time stability study for 8 weeks. Many emulsions usually experience creaming on standing. Creaming occurs when the density of the dispersed phase is less than that of the continuous phase, causing the droplets to float to the surface of the emulsion due to gravitational force. The phenomenon can be measured visually or by optical imaging [17].

Creaming usually leads to the separation of emulsion into the upper cream layer concentrated with the droplets and the lower watery layer depleted of droplets [18]. In the colloidal sense, creaming is not

considered physical instability because it is reversible. However, from the pharmaceutical point of view, creaming renders emulsions inelegant and is thus not desirable and can lead to coalescence followed by phase separation [19,20].

Table 2. Emulsification Stability Index (%) of emulsions formulated with different concentrations of CAG and TRG kept under normal storage conditions over 8 weeks.

Samples	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 88
CAG4	100	100	100	100	100	100	100	100
TRG4	100	100	100	100	100	100	100	100
CAG3	100	100	100	100	100	100	100	100
TRG3	100	100	100	97	93	87	78	67

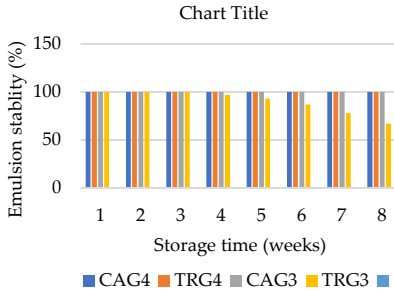

Figure 1. Real time stability of emulsions formulated with different concentrations CAG and TRG over 8 weeks.

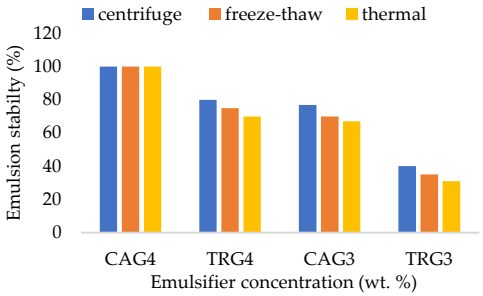

Figure 2. Effect of centrifugation, thermal and freeze-thaw conditions on the stability of emulsions formulated with different concentrations of CAG and TRG.

Table 2 shows that over the entire storage period, all of the ESI in all three batches (TRG4, CAG3, and CAG4) remained at 100%. TRG3, on the other hand, had a perfect ESI of 100% until the fourth week, when it dropped to 97% and then to 67% by the end of week 8. The creaming rate increases when the ESI decreases and vice versa. Figure 1 provides supporting evidence, showing that creaming in TRG3 was first spotted at week 4 and steadily grew until week 8. The creaming observed was represented as the percent stability of the emulsion. The creamed emulsion was easily re-dispersed after being gently agitated. The other three batches (TRG4, CAG3 and CAG4) remained unchanged throughout the 8 weeks. Therefore, it could be said that at concentrations between 2-3%, CAG exhibited superior emulsifying properties compared with TRG; however, at 4%, both gums formed stable emulsions throughout the 8 weeks.

Table 3. Emulsification degree for emulsions formulated with different concentrations of CAG and TRG kept under normal storage conditions over 8 weeks.

Samples	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
CAG4	1	1	1	1	1	1	1	1
TRG4	1	1	1	1	1	1	1	1
CAG3	1	1	1	1	1	1	1	1
TRG3	1	1	1	0.93	0.83	0.68	0.45	0.18

Emulsification degree

The Emulsification degree is usually evaluated to investigate if there is complete or incomplete emulsification in a system, and it can be determined by calculating the mixing degree coefficient. Factors such as oil or water volume fraction, temperature, share rate and quantity of emulsifier can affect the emulsification degree. A value of C_m close to 1.0 indicates an efficient mixing degree or emulsification degree. The lower the value of C_m, the lesser the emulsification degree [12]. The various emulsification degrees obtained for the emulsion samples during the 8 weeks of storage are shown in Table 3.

The value of emulsification degree for CAG3. CAG4 and TRG4 remained at 1.0 for the 8 weeks, indicating excellent emulsification degree. The value for TRG3 started reducing from week 4, indicating a gradual decrease in the degree of emulsification. Since all the factors that influence the emulsification degree are the same for all four samples except the number of emulsifiers added to the emulsion, it can be assumed that an increase in the amount of emulsifier increased the mixing degree or emulsification degree. Emulsions with a low degree of emulsification are not completely emulsified, have poor stability and are prone to creaming, aggregation and eventual cracking. That may be the reason why creaming was observed in TRG3 [12].

Accelerated stability studies

Effects of Centrifugation, Thermal and Freeze-thaw conditions

There are issues with the stability of every medicinal product over time. When compared to a poor formulation, an appropriate formulation will take a significant amount of time before it becomes unstable. Instability in the formulated products is often undetectable until after a significant amount of storage time has passed under the standard conditions of real-time stability tests [15, 21]. Therefore, the purpose of accelerated stability testing is to speed up the process of product deterioration by subjecting the product under test to "high-stress" storage settings. These conditions include factors like temperature, humidity, centrifugation, vibration, and light intensity, among others, all of which have the potential to hasten product deterioration. Because of this, high-stress environments decrease the amount of time needed for testing. This is essential since, during long-term testing, reliable evaluation of the order of the reaction is impossible due to insufficient degradation [22]. As a result, this necessitates the implementation of this measure.

Table 4 shows the Emulsification Stability Index (%) of emulsions formulated with different concentrations of CAG and TRG when subjected to accelerated storage conditions such as centrifugation, thermal and freeze-thaw. CAG4 maintained 100% ESI for all three accelerated storage conditions. TRG4 and CAG3 were least affected, with similar ESI values of 80% and 77% ESI for centrifugation; 75% and 70% ESI for freeze-thaw and 70% and 67 % ESI for thermal, respectively. TRG3 was mostly affected with ESI values of 40% ESI for centrifugation, 35% ESI for freeze-thaw and 31% ESI for thermal.

Table 4. Emulsification Stability Index (%) of emulsions formulated with different concentrations of CAG and TRG subjected to centrifugation, thermal and freeze-thaw accelerated conditions.

Samples	Centrifugation	Freeze-thaw	Thermal
CAG4	100	100	100
TRG4	80	75	70
CAG3	77	70	67
TRG3	40	35	31

From Figure 2, it could be observed that centrifugation, thermal and freeze-thaw conditions induced creaming in TRG3, TRG4 and CAG3; however, no creaming was observed in CAG4, which indicated its superior stability compared to other samples. The extent of creaming observed in TRG4 and CAG3 were similar ($P > 0.05$), indicating equivalence in their emulsion stability properties. However, the extent of creaming observed in TRG4 and CAG3 was significantly ($P<0.05$) less than that of TRG3, which exhibited more pronounced creaming when subjected to accelerated stability studies.

The rate of collision of droplets can be increased by different high-stress conditions such as centrifugation, freeze-thaw and heat. These lead to the destruction of the protective films around the droplets, which can eventually lead to creaming, flocculation and coalescence [23]. According to Figure 2, the emulsions were more stable under centrifugation, followed by freeze-thaw and were more affected by thermal conditions. The reason for this observation is that most natural gums and synthetic polymers are degraded when subjected to thermal stress due to depolymerization and split of the polymer chains or elimination of low molecular weight fragments [1]. The thermodynamic effects of

high temperature employed for both freeze-thaw and thermal conditions can lead to the destruction of the protective films of emulsifying agents formed around the emulsion droplets, thereby affecting the stability. An increase in temperature leads to a decrease in stability, hence the more pronounced creaming effect induced by thermal stress. The impact of increased temperature can also reduce the water-retention capacity and thickening properties [24].

The stability of CAG4 and TRG4 increased significantly ($p < 0.05$) compared with CAG3 and TRG3, respectively, when subjected to centrifugation, freeze-thaw and thermal conditions. This is probably due to an increase in the amount of the emulsifier leading to an increase in the viscosity of the continuous liquid phase, thereby restricting the velocity of droplet movement and reducing the rate at which fluid is drained between two droplets during a collision to prevent coalescence. This observation was similar to the reports of research works conducted on Persia gum (*Amygdalus scoparia* Spach) [24] and that of Ebrahimi et al. [2] conducted on Amuniacum gum (*Dorema ammoniacum*). All four emulsion formulations can still be considered to be stable because even under the various high-stress conditions of accelerated stability tests, none of the samples experienced cracking, which is irreversible. 3% - 4% of CAG and TRG can produce stable arachis oil-based o/w emulsions even when subjected to accelerated stability studies.

Rheology

Rheology is one of the vital parameters used to assess the stability of emulsion during storage because the coalescence of oil droplets is usually inhibited by high viscosity [25]. The impact of the shear rates on the apparent viscosities of the emulsions when freshly prepared and after 8 weeks of storage are shown in Figures 3 and 4, respectively. The concentration of emulsifiers positively affects the emulsion's apparent viscosity. An increase in the concentration of TRG and CAG increased the apparent viscosity of the emulsions, with CAG having values higher than that of TRG for all the range of shear rates employed. The apparent viscosity of the CAG and TRG emulsions decreased when the shear rate increased, indicating that the emulsions displayed shear-thinning (pseudoplastic) behaviour. The observed results agreed with that of many workers who had reported earlier that many natural biopolymers exhibited pseudoplastic behaviour [24, 26-28].

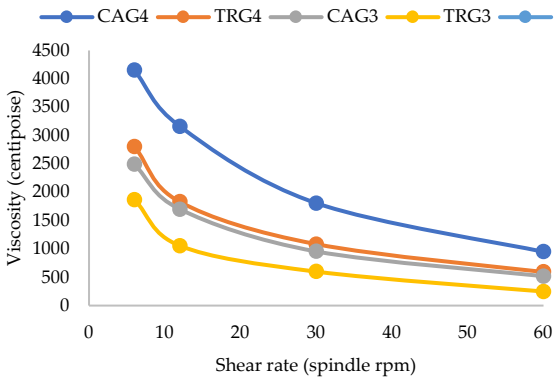

Figure 3. Effects of shear rate on the viscosity of emulsions freshly prepared with different concentrations of CAG and TRG.

The pseudoplastic behaviour of the emulsions causes the breakage of the linkages between droplets when the Newtonian profile is reached at higher shear rates. The observation from the studies indicated that the apparent viscosity of the formulated emulsions is dependent on the concentration of CAG and TRG, which was in agreement with what some authors have previously reported [24,26]. No significant difference ($p > 0.05$) was observed between the viscosity of the freshly prepared emulsions and after the 8 weeks of storage, indicating their relative stability throughout the storage period. No significant difference ($p > 0.05$) was also observed between the viscosity of CAG3 and TRG4. The degree of viscosity of the emulsions is in the order of CAG4 > CAG3/TRG4 > TRG3. However, a significant difference ($p < 0.05$) was observed between CAG4 and other batches, and there was also a significant difference ($p < 0.05$) between CAG3/TRG4 and TRG3.

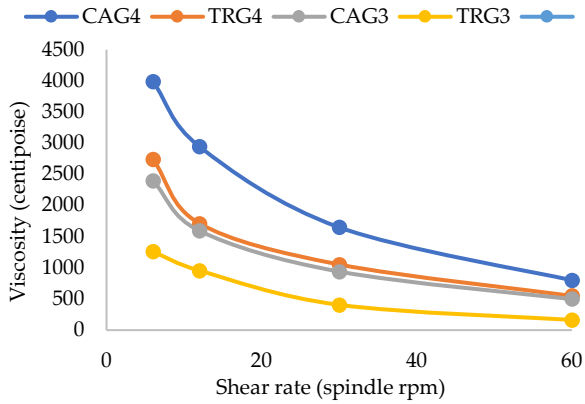

Figure 4. Effects of shear rate on the viscosity of emulsions prepared with different concentrations of CAG and TRG after 8 weeks storage.

Emulsion microstructures

The degree of creaming is directly proportional to the square of the diameter of the droplet; therefore, when droplet size decreases, the creaming rate decreases. Generally, the smaller the size of the droplet, the higher the emulsion's stability. The size of the emulsion's droplet is indicative of the emulsifier's efficiency. A good emulsifier produces tiny droplets to produce a stable emulsion [13,24,29].

Figure 5 shows the microstructures of emulsions formulated with different concentrations of CAG and TRG. Based on the microscopic observations, at both concentrations of 3% and 4%, CAG produced a relatively more significant number of smaller droplets than TRG. The greater number of droplets produced by CAG indicated its superior emulsifying properties over TRG. The formation and maintenance of emulsion droplets in their subdivided state depends on various factors such as emulsifier's capacity, emulsifier's concentration and the process of shearing. In this case, the concentrations of the emulsifiers and the shearing process are similar for the emulsions prepared with CAG and TRG. Therefore, the more significant number of smaller droplets observed in CAG-stabilized emulsions are possibly connected to the superior emulsifying capacity of CAG over TRG. The various factors required for stability of emulsion as observed in this study are in agreement with the observation of different workers [13,24,30].

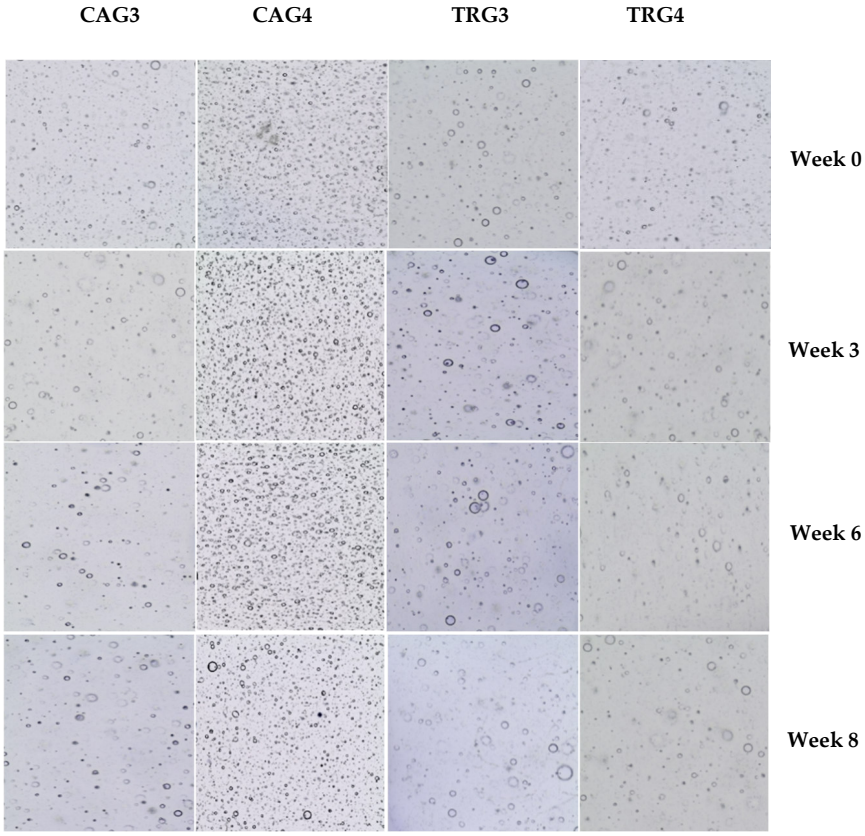

Figure 5. Microstructures of emulsions formulated with different concentrations of CAG and TRG over 8 weeks storage time.

An increase in the concentration of both gums from 3% to 4% showed an increase in the droplets' number and a decrease in the size of the droplets. The concurrent increase in droplets' number and decrease in the size of droplets indicated that an increase in the emulsifiers' concentration tends to improve the emulsifying properties by increasing the viscosity of the system, which restricts droplet movement and reduces the removal of fluids between droplets when a collision occurs, which in turn may retard the rate of coalescence of dispersed droplets and enhance the emulsion stability. The rheology and viscosity of oil in water emulsion are greatly affected by the size and distribution of droplets [24,30]. The degree of the emulsions' droplet size is in the order of CAG4 < CAG3/TRG4 < TRG3. No significant difference ($p > 0.05$) was observed between the droplet size of CAG3 and TRG4. However, the droplet size of the CAG4 emulsion was significantly less ($p < 0.05$) than the droplet size of other batches. In comparison, the droplet size of CAG3/TRG4 emulsions was also significantly less ($p < 0.05$) than the droplet size of TRG3.

Droplet count and droplet size

An emulsion is a thermodynamically unstable disperse system prepared when subjected to shear stress to reduce the droplet size and increase the droplet surface area. Due to the thermodynamic instability of dispersed systems, the ageing of emulsion usually results in the coalescence of the droplets in order to reduce the excess free energy caused by increased droplet surface area due to shear stress [14]. Creaming, Ostwald ripening, flocculation, and coalescence of the oil droplets are different types of instability that can lead to phase separation in emulsion [3]. Progressive increase in droplet size and decrease in droplet number usually precede cracking and phase separation [4,31]. However, emulsifying agents tend to adsorb at the droplet interface to prevent or slow down the rate of coalescence [2]. Microscopic evaluations of CAG3, CAG4, TRG3 and TRG4 stored for 8 weeks at 25 °C showed that the droplet size increased gradually and progressively with time in Figure 6 and a corresponding decrease in the droplets' number with time in Figure 7.

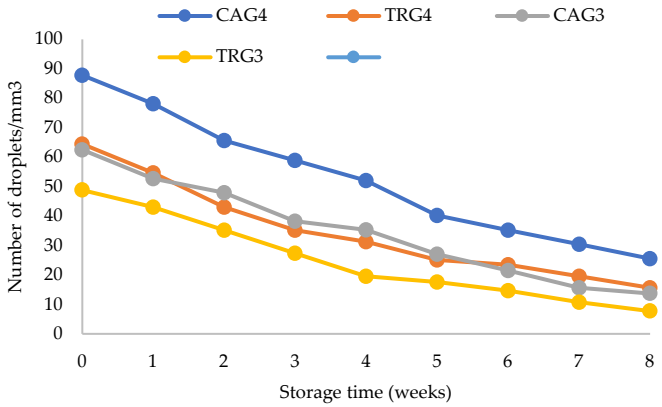

Figure 6. Size of droplets of emulsions formulated with different concentrations of CAG and TRG determined on weekly basis for eight weeks.

This is indicative of the gradual process of coalescence of the droplets. A similar observation of the coalescence of droplets was reported by Abdulsamad et al. [14] in the emulsions prepared using cashew gum as the emulsifying agent. An increase in droplet size is in the order of TRG3 > TRG4/CAG3 > CAG4, while a decrease in droplet number is in the reverse order of CAG4 < TRG4/CAG3 < TRG3. There is a significant similarity in the rate of coalescence of CAG3 and TRG4. The similarity in the rate of coalescence of CAG3 and TRG4 is confirmed by their superimposed graphs, as shown in Figures 6 and 7. The size of the droplets of the dispersed phase in many pharmaceutical emulsions is usually between 0.1-10 µm [31]. The range of the size of the droplets for the four emulsions throughout the 8 weeks of storage are as follows: CAG4 (2.49-4.41 µm); CAG3 (3.13-5.46 µm); TRG4 (3.07-5.19 µm) and TRG3 (3.59-6.10 µm).

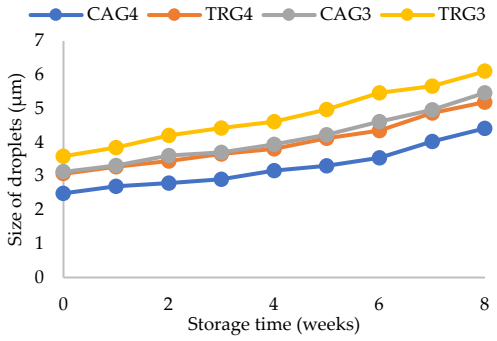

Figure 7. Number of droplets of emulsions formulated with different concentrations of CAG and TRG determined on weekly basis for eight weeks.

Despite the gradual onset of coalescence observed in the emulsions, the size of the droplet of the emulsions at the end of the 8th week is still within the acceptable range for pharmaceutical emulsions. The maintenance of the droplets size of all the emulsion within the acceptable range for pharmaceutical emulsions at the end of the 8th week. This indicates that the four emulsions remained stable throughout the storage time.

Conclusion

In this experiment, the results of the comparative macroscopic, microscopic, and rheological assessments carried out on CAG, TRG, and ACG showed that CAG possessed greater emulsifying characteristics in the development of arachis oil emulsion within the concentration range of 3% and 4%. This was determined by the fact that CAG produced a smoother, less viscous end product during the rheological testing. In comparison to TRG and ACG-stabilized emulsions, CAG-stabilized emulsions displayed superior stability under both normal and accelerated (high-stress) storage conditions, as well as a significantly greater number of droplets that were significantly smaller. In the range of 2% to 4%, it was discovered that CAG exhibits higher emulsifying characteristics compared to tragacanth. It has been found that 3% CAG has emulsifying capabilities that are equivalent to those of 4% TRG. When it comes to the production of pharmaceutical emulsions, CAG that has been derived from the waste product of Cola acuminata pods has the potential to be an extremely useful raw ingredient.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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