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Natural Polymer from *Sesamum radiatum* as an Emulsifier and Stabilizer in Fabrication of Macroemulsions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Sesamum gum was extracted from the leaves of *Sesamum radiatum* and evaluated as emulsifier or stabilizer in coarse emulsions.

Methods: The emulsion systems were assessed by monitoring of emulsion density, viscosity, globule size, and by evaluating the effects of temperature and salt over a four week period.

Results: It was observed that the known 4:2:1 ratio did not form emulsions when sesamum was used but other ratios such as 2:4:1, 2:4:0.5, 2:4:0.25 and 2:4:0.125 formed emulsions. The emulsions were however, unstable. When sesamum gum was used as a stabilizing agent (0.4%$^{w}/_{v}$ – 2.0%$^{w}/_{v}$), and acacia (7.5%$^{w}/_{v}$) as an emulsifying agent, stable emulsions were formed. At the end of four weeks, the emulsion systems exhibited lower globule sizes (1.68-1.95 µm) than the emulsion systems containing acacia alone (3.95 µm) as emulsifier. The emulsions creamed or cracked when exposed to temperatures of about 40°C over 4 weeks. They were however stable even in the presence of salt of 1.0%$^{w}/_{v}$ concentration.

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Conclusion: The presence of sesamum as a stabilizer influenced the globule size and size distribution, the viscosity and consequently the stability of the emulsions. The study suggests that sesamum gum possesses the desirable properties of emulsifier/stabilizer in emulsions.

Keywords: Dosage forms; drug delivery; emulsions; macroemulsions; natural polymers; naturapolyceutics; Sesamum radiatum; stability.

1. INTRODUCTION

Emulsions are widely employed colloidal formulations which are fabricated using two immiscible liquids to form a homogenous mixture. The immiscible liquids have to be agitated to form an emulsion. However, the mixture is unstable and will revert to the stable phases – immiscible liquids. Consequently, emulsions are stabilized with emulsifiers and stabilizers. They are used orally, topically, transdermally and parenterally. Although few macroemulsions are available in the market, emulsions can be utilized for administration of drugs with objectionable taste and lipophilic drugs, for diagnosis and nutritional administrations. In recent times, emulsions have been shown to exhibit sustained release of drugs and have been employed for encapsulation and delivery of actives, solubilization, lubrication, modification/alteration of rheological, organoleptic and optical properties [1,2]. In addition, emulsions have been used to reduce drug toxicity [3]; propel skin penetration of actives [4] and act as detoxifying systems [5]. Emulsions include the conventional /macroemulsions, microemulsions and nanoemulsions. The macroemulsions possess globule size diameters in the range of 100 nm – 100 µm; while the sizes of nano- and microemulsions are less than 100 nm. Despite the emergence of these advanced emulsions, macroemulsions still find usefulness in food, paint and pharmaceutical industries amongst others. The macroemulsions are advantageous in comparison to other advanced emulsions as they are easy to prepare and inexpensive.

Emulsifiers/ emulsifying agents and stabilizers/ stabilizing agents are basically the key ingredients in enhancing stability of emulsions. The phenomena precipitating instability of emulsions include gravitational separation (creaming), phase inversion, coalescence, flocculation, and Ostwald ripening [6,7]. Emulsifiers are known to act on the surface by adsorbing at the oil-water interface, reducing interfacial tension and producing a protective film around the dispersed phase [8], while stabilizers function by either adsorbing at the oil-water interface or by modifying the viscosity of aqueous phase [8]. In recent times, natural polymers or biopolymers such as proteins and polysaccharides are being used in place of synthetic surfactants or small molecule polymers to stabilize emulsions [9,10]. This shift stems from the need and quest to utilize less toxic and environmentally friendly materials. Several polysaccharides such as pectins [11]; xanthan gum, gum arabic [9] modified starches and celluloses [10], purified guar and fenugreek gums [12], corn fiber gum [13], carrageenans, hyaluronan, chitosan, alginates [2] have been used as emulsifiers/stabilizers in formulation of emulsions. These polysaccharides act as structuring or thickening or gelling agents in the aqueous medium thereby stabilizing emulsions through immobilization of the dispersed phase. In addition, it is suggested that they stabilize emulsions by steric stabilization mechanism [12]. Steric stabilization is produced by the multi-layers membranes (multimolecular layers) formed around the globules by oppositely charged biopolymers [2].

Mucilage (the extract is actually mucilage but since such extracts are generally known as gum, it will be referred to as gum herein for ease of identification) from Sesamum (*Sesamum indicum and Sesamum radiatum*) leaves have been studied for suspending properties [14], rheological properties [15] and sustained release properties [16]. In comparison with acacia and mainly tragacanth, sesamum gum (gum from Sesamum sp) exhibited higher sedimentation volume, better ease of redispersibility and no formation of aggregates. It exhibited promising visco-elastic properties which would have influenced its controlled release ability. It was based on these potentials and the drive to replace synthetic materials in such applications as emulsifiers/stabilizers that this study was undertaken. Synthetic emulsifiers are from non-renewable resources and have higher environmental impact than their natural counterparts. Consequently, Naturapolyceutics, an emerging platform that utilizing natural polymers for the design and development of drug delivery systems [17] becomes imperative for the present and future. Natural emulsifiers are non-toxic, biodegradable, biocompatible and renewable. Hence, sesamum gum, an underutilized natural polymer was investigated in this study as possible emulsifier/ stabilizer for fabrication of emulsions.

2. MATERIALS AND METHODS

2.1 Materials

Sesamum gum (extracted from *Sesamum radiatum* leaves bought from the local market), ethanol 96%$^v/_v$, acacia gum, sodium chloride (BDH Chemicals, Poole, England), arachis oil (locally processed oil from peanuts), distilled water.

2.2 Extraction of Sesamum Gum

Sesamum gum was extracted by macerating 2.23 kg of the leaves in 8.4 liters of boiled water for 6 hours. Thereafter, the mucilage was filtered using a muslin cloth and the filtrate was precipitated and washed with 96%$^v/_v$ ethanol. Subsequently, the gum was air-dried for two days. The dried gum was milled using mortar and pestle and thereafter it was weighed in order to calculate the yield.

2.3 Rheological Characterization of Sesamum Gum

The viscosity, structural strength and viscoelastic behavior of sesamum gum had been undertaken amongst other parameters and published [14,15]. Data obtained indicated that sesamum gum is a viscous weak gel exhibiting non-Newtonian pattern of flow.

2.4 Formulation of Emulsion

Conventional emulsions were prepared in various ratios for oil, water and gum (the quantities are shown in Table 1) using either acacia gum or sesamum gum as an emulsifying agent. The primary emulsion was prepared and then water was added to make up to 50 mL. Wet and dry methods of preparing emulsion were utilized to determine the method that would produce stable emulsions.

2.5 Formulation of Emulsion using Sesamum Gum as Stabilizing Agent

Sesamum gum was utilized as a stabilizing agent in emulsions prepared with acacia gum as an emulsifier. Dry method (4:2:1) was employed; however, sesamum was blended with acacia gum in the concentrations shown in Table 2 before incorporation into each mix.

2.6 Stability Study

2.6.1 Globule size analysis

A water soluble dye such as amaranth dye was dissolved in water and the solution was utilized in the preparation of the primary emulsion to enhance the visibility of the globules under a binocular microscope. Thereafter, a drop of the emulsion was placed on a glass slide to which a drop of dilute glycerol was added and the slide

Table 1. Compositions of the emulsions prepared using acacia and sesamum gum as emulsifiers

S/No	Oil phase (mL)	Aqueous phase (mL)	Acacia gum (g)	Sesamum gum (g)
1	15	7.5	0.938	-
2	15	7.5	1.875	-
3	15	7.5	3.750	-
4	15	7.5	7.500	-
5	15	7.5	-	7.500
6	15	7.5	-	3.750
7	15	7.5	-	1.875
8	15	7.5	-	0.938
9	7.5	15	-	3.750
10	7.5	15	-	1.875
11	7.5	15	-	0.938
12	7.5	15	-	0.467
13	7.5	7.5	-	0.938
14	7.5	7.5	-	0.467

Table 2. Emulsion compositions using acacia as emulsifier and sesamum as stabilizer

Code	Qty of oil (mL)	Qty of water (mL)	Qty of acacia (g)	Qty of sesamum (g)
A	15	7.5	3.75	0.2
B	15	7.5	3.75	0.4
C	15	7.5	3.75	0.6
D	15	7.5	3.75	0.8
E	15	7.5	3.75	1.0
F	15	7.5	3.75	-
G*	15	7.5	-	0.938
H*	15	7.5	-	0.467

**These are stated here to clarify the codes as indicated in the chart for viscosity in discussion section*

was mounted on the microscope; focused and viewed at a magnification x10. A graticule was utilized to determine the diameters of the globules after it was calibrated with a stage micrometer. Globule size determination was undertaken weekly for 4 weeks to assess the stability of emulsions which can be affected by change in particle size.

2.6.2 Determination of density

Known volumes of the emulsions (50 ml) were weighed and used to determine the densities.

2.6.3 Evaluation of relative viscosity

The relative viscosity was determined using a U-tube – Ostwald viscometer. Thereafter the relative viscosity with respect to water was determined using the equation:

$$\eta_E = \eta_w \frac{t_E \rho_E}{t_w \rho_w} \quad (1)$$

Where η = viscosity (Pa.s); η_E is viscosity of emulsion and η_w is viscosity of water; ρ = Density (kgm^{-3}); t = Time (s)

The relative viscosity was determined weekly for 4 weeks.

2.6.4 Effect of temperature

Temperature influences the stability of dosage forms on storage. Hence the effect of temperature was assessed by subjecting the emulsions with stabilizers to elevated temperature of 40℃ for 8 hours in a drying oven, a lower temperature of 5℃ for 24 hours and ambient temperature for 4 weeks. The emulsions were observed for creaming or cracking.

2.6.5 Effect of salt concentration

After the primary emulsion was formed, a solution of sodium chloride (0.5 g) dissolved in distilled water needed to make up the dosage form to 50 mL was added to the primary emulsion. The emulsions were stored and observed for any changes such as creaming or cracking.

3. RESULTS AND DISCUSSION

The gum extracted was fibrous, brown, odorless and tasteless and the percentage yield was 1.89%. However, there is an indication the yield could be improved as the leaves were found to still be slimy after extraction. This perhaps implies that increase in volume of water utilized for maceration may enhance the yield.

3.1 Formation of Macroemulsions

Various ratios of oil:water:gum were explored in formulating macroemulsions (Tables 3 and 4). It was observed that the known 4:2:1 ratio did not form emulsions when sesamum gum was used but on the other hand was the only ratio that formed emulsion when acacia was used. The applications of other ratios were undertaken for sesamum when the 4:2:1 ratio did not form the primary emulsion. Other ratios such as 2:4:1, 2:4:0.5, 2:4:0.25 and 2:4:0.125 formed emulsions which suggest that sesamum gum can be used for oil-in-water emulsions. Emulsions with sesamum gum as emulsifier formed when water was twice or same quantity with oil and not when oil was more. Hence, sesamum gum can be used in formation of oil-in-water emulsions and not water-in-oil emulsions. This also suggests that sesamum gum may be a hydrophilic emulsifier having more hydrophilic ends than hydrophobic ends. Comparing the ratios of oil: water: Gum utilized, the ratio 2:4:0.25 produced the creamiest primary emulsion (on visual observation). One of the desirable properties of an emulsifier is for it to be effective at low concentrations. Less quantity (0.938 g) of sesamum gum can be utilized to form an emulsion in contrast to acacia (3.750 g).

However, acacia can be used for water-in-oil emulsions. Which liquid becomes the dispersed phase depends on the emulsifying agent utilized and the relative quantities of the liquids [18]. It is deduced that the quantity of emulsifier required to form emulsion is a factor of molecular weight. Molecular weight influences the visco-elastic properties of a material. Materials with higher molecular weights exhibit more viscosity than those with lower molecular weight. From these it is inferred that sesamum gum may have a higher molecular weight than acacia which may explain why low quantities of sesamum gum form emulsions. Although sesamum successfully formed emulsions, the emulsions can only be employed for immediate dispensing and not for storage as they are unstable by the third day. The instability of emulsions produced with sesamum gum may be attributed to microbial growth which imply that the emulsions will require preservatives. The possible breakdown of sesamum gum led to increase in interface tension and loss of protective film around the dispersed phase producing creaming of the emulsion and subsequent separation of the phases. Creaming is one of the instabilities of emulsion produced by the density difference of the two liquids in emulsion and gravitational force [8]. However, this study was undertaken deliberately without preservatives to assess the gum's performance unaided. In addition, the ability of a preservative to maintain stability on storage will depend on the microbial load. Hence, it may be imperative to sterilize the gum or reduce microbial load before use. In addition, although not ascertained, hydrolysis may not be ruled out as another factor to the breakdown of the emulsions as such lead to loss of viscosity and breakdown of emulsions.

Considering the instability of emulsions formed using sesamum gum, we decided to assess its behavior as an emulsion stabilizer. When sesamum gum was used as a stabilizing agent and acacia as an emulsifying agent employing the compositions as shown Table 2, emulsions formed. These emulsions were then characterized for stability by determining particle size, density, viscosity and effects of temperature and salt.

3.2 Stability Studies

Stability studies are paramount in drug delivery systems; even more vital in macroemulsions as they are known to be thermodynamically unstable [19]. The shelf-life and the physicochemical properties of emulsion-based delivery systems are determined by the selection of stabilizers utilized in the fabrication [7]. The outcome of the assessed physicochemical properties such as globule size, viscosity, density, effects of salt and temperature are discussed.

Table 3. Compositions of macroemulsions using sesamum gum as an emulsifying agent

S/No	Qty of oil (mL)	Qty of water (mL)	Quantity of gum (g)	Remarks
1	15	7.5	7.500	-
2	15	7.5	3.750	-
3	15	7.5	1.875	-
4	15	7.5	0.938	-
5	7.5	15	3.750	+
6	7.5	15	1.875	+
7	7.5	15	0.938	+
8	7.5	15	0.467	+
9	7.5	7.5	0.938	+
10	7.5	7.5	0.467	+

+ implies that emulsion formed; - implies that emulsion did not form

Table 4. Formulation of macroemulsions using various concentrations of acacia gum

S/No	Qty of oil (mL)	Qty of water (mL)	Qty of acacia gum (g)	Remarks
1	15	7.5	7.500	-
2	15	7.5	3.750	+
3	15	7.5	1.875	-
4	15	7.5	0.938	-

+ implies that emulsion formed; - implies that emulsion did not form

3.2.1 Globule size analysis

Vital in maintaining stability of emulsions are the average globule size and distribution [20]. Broad globule size distributions were observed in emulsions formed with acacia gum without stabilizer and those of 0.2 g stabilizer. As the concentration of the stabilizer increased, smaller globule sizes were obtained and the size distributions narrowed. As the concentration of stabilizer increased, the globules sizes were maintained within 0.5-5 µm eliminating globule sizes greater than 5 µm. The data corroborates with McClements and co-workers' report which stated that increasing the concentration of emulsifier produced small droplets size [7]. The result obtained in this study also corroborates the result obtained when xanthan gum was used [21]. Xanthan gum decreased the average globule size and narrowed the globule size distribution. Polysaccharides form protective films/layers around the globules thereby increasing the steric or electrostatic repulsion between the globules [22]. Globule size distribution of an emulsion depicts the portions of globules in different classes of size and can be represented by plots. Figs. 1a-g represent the globule size distributions of samples A – F and then a comparative plot for the samples at week IV of globule size assessment. Table 5 shows the average globule sizes over four weeks for samples A-F explicating the impact of stabilizer on the globule size. As the concentration of stabilizer increased, the average globule sizes were relatively maintained implying that the emulsions may be stable. While samples B-E may withstand further storage, Samples A and F may continue to experience increase in particle size. As globules size increases, coalescence rate will increase and then the emulsions will become unstable with time [23].

3.2.2 Density of the emulsion

The densities of the emulsions are functions of the molecular weights of the materials utilized. Increase in concentration of materials will invariably increase the density of emulsions. This is shown in Fig. 2. Higher concentrations of emulsifier produced denser emulsions. It is being postulated that increasing the concentration of the emulsifier/stabilizer will thicken the protective films around the droplets which will ensure that the density difference between the two phases (dispersed and continuous phases) are small. In addition, the biopolymers may have formed multimolecular layers around the dispersed globules [2] ensuring small difference in density. When the density difference is small, the emulsion is stable as the two phases will not separate.

3.2.3 Evaluation of relative viscosity

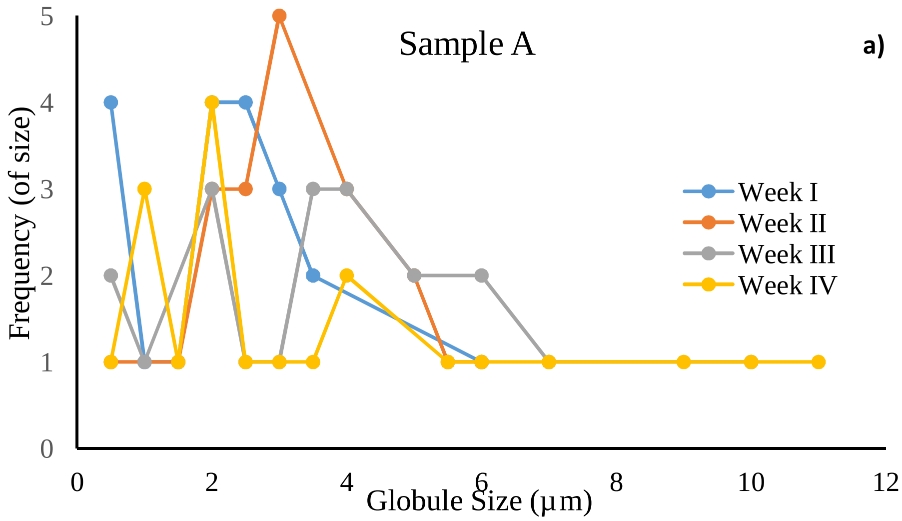
Viscosity is a factor that influences ease of use, spreadability and dose uniformity. In addition, increase in viscosity is one of the factors influencing the stability of emulsions. As the concentration of the stabilizer, sesamum gum increased, the viscosity increased (Fig. 3). This may further explain the relative progressive stability of the globule size as the concentration of sesamum gum increased to 1.0 g (Fig. 1g and Table 5). Increase in viscosity reduces the movement of globules which consequently minimizes creaming leading to long term stability of emulsions [11]. In addition, the reduction in globule movement minimizes globule collisions which prevents coalescence. Consequently, sesamum gum possesses another desirable property of an emulsifier/stabilizer which is to increase the viscosity of an emulsion. However, emulsions fabricated with sesamum gum as emulsifier in the absence of acacia gum, samples G and H lost viscosity in days possibly due to microbial degradation as stated earlier. It is worthy to note there was no drastic loss of viscosity when sesamum gum was blended with acacia gum or used as a stabilizer. This requires further investigation as if we suggest that the loss in viscosity may be due to microbial impact, same should also occur when used as stabilizer. Even if it is possibly catalytic hydrolysis as known to be experienced by some natural polymers, it should also occur when used as stabilizer. Unless some form of interactions may have occurred between acacia and sesamum gum assuring the stability of sesamum gum.

3.2.4 Effect of temperature

Emulsions were found to be stable at ambient temperature (room temperature: 25-28℃). As the emulsions were exposed to the two temperatures (5 and 40℃), creaming and cracking were observed. Creaming is an upward or downward movement of the dispersed phase leading to subsequent separation of the phases. The direction of the movement of the dispersed phase depends on the type of emulsion – O/W or W/O. Cracking is also a form of separation of the two liquid phases. However, while creaming is reversible, cracking is irreversible. These forms of instabilities occurred when the emulsions were cooled or heated. When an emulsion is cooled, the oil (arachis oil) crystallizes thereby encouraging coalescence which may eventually lead to the breakdown of the emulsion. The emulsions were also unstable at 40℃ possibly due to increase in fluidity and subsequent mobility/movement leading to globule collision and thereafter coalescence. This of course will be a concern in tropical regions where temperatures are elevated. However, the instabilities expressed in this study is not peculiar to the materials utilized as conventional emulsions are known to be unstable when exposed to environmental stresses such as heating, cooling, pH extremes and high mineral concentrations [7].

3.2.5 Effect of salt

Neither creaming nor cracking was observed when salt was added to the emulsions. This suggests that the films or layers provided by the emulsifier/stabilizer around the globules prevented the aggregation and subsequent phase separation/creaming that would have been precipitated by the presence of salt. In addition it is suggested that the improved stability may be due to the steric and electrostatic repulsion between the globules which has been enhanced by the presence of a polysaccharide [22], such as sesamum gum as employed in this study. Furthermore, the effect of mineral salt on emulsion is known to be dependent on presence or absence of interactions with emulsifier/ stabilizer and the concentration of the mineral salt.



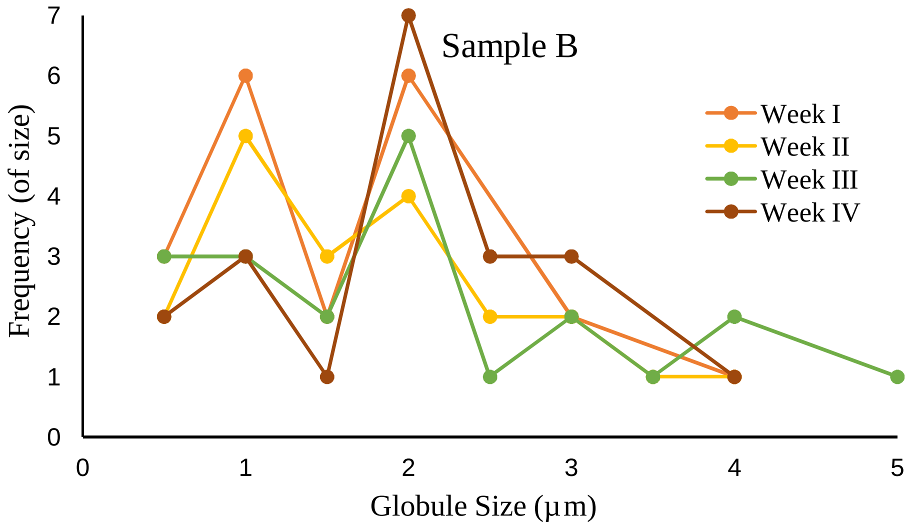


Table 5. Average globule size for the different macroemulsions

Period	Sample A (µm)	Sample B (µm)	Sample C (µm)	Sample D (µm)	Sample E (µm)	Sample F (µm)
Week I	1.89±1.73	1.63±1.30	1.93±0.94	1.58±0.94	1.40±0.94	1.93±1.78
Week II	3.20±1.90	1.75±1.22	1.60±0.94	1.73±1.34	1.53±1.21	3.23±2.94
Week III	3.40±2.81	1.98±1.47	1.73±1.22	1.75±1.29	1.70±1.23	3.80±3.60
Week IV	3.93±3.42	1.95±1.21	1.88±1.41	1.78±1.08	1.68±1.21	3.95±3.92

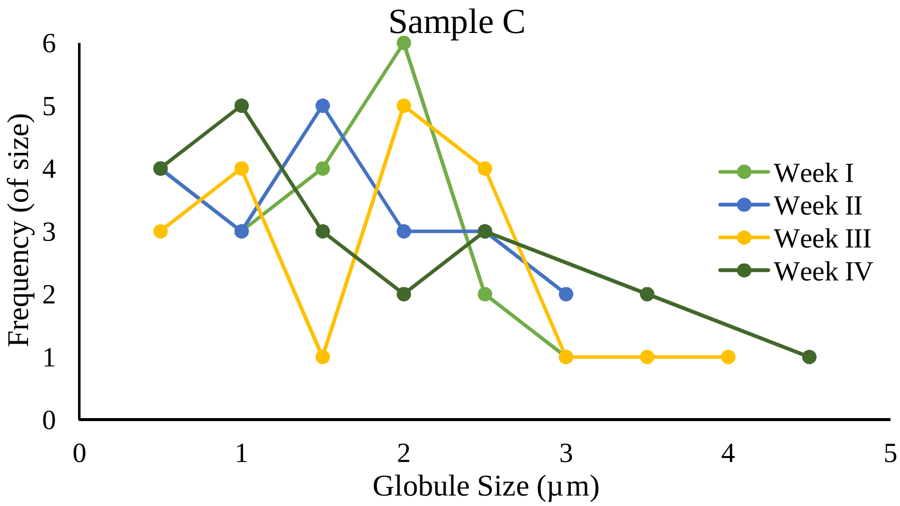
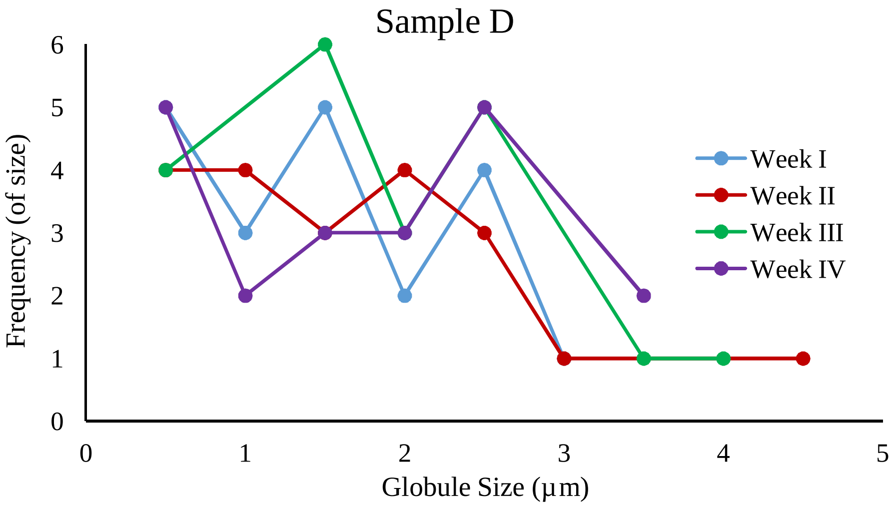
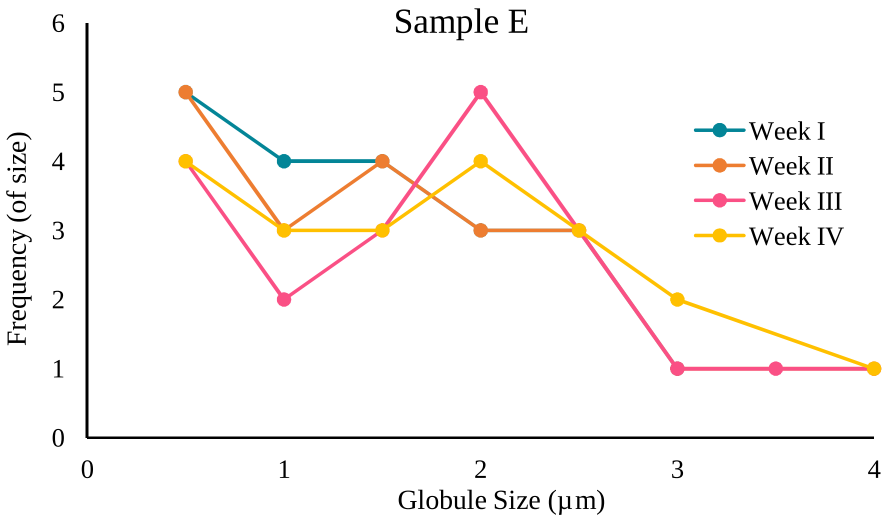


Fig. 1a-c. Globule size distribution plot; a) Sample A; b) Sample B and; c) Sample C





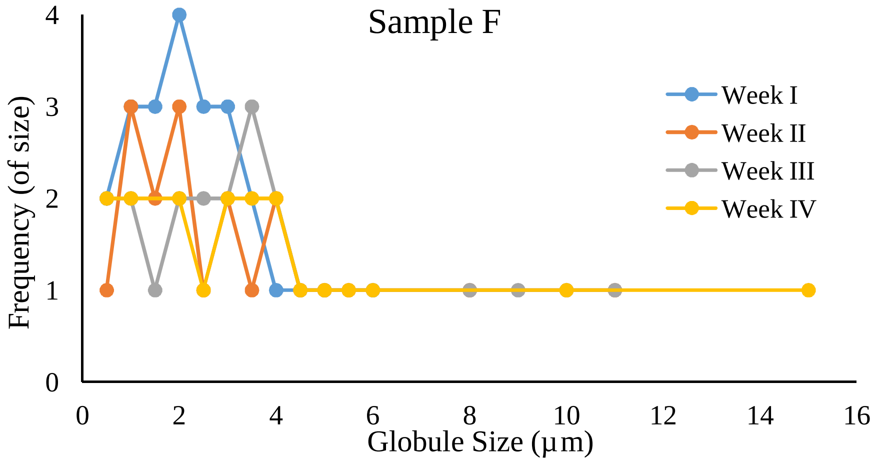


Fig. 1d-f. Globule size distribution plot; d) Sample D; e) Sample E and; f) Sample F

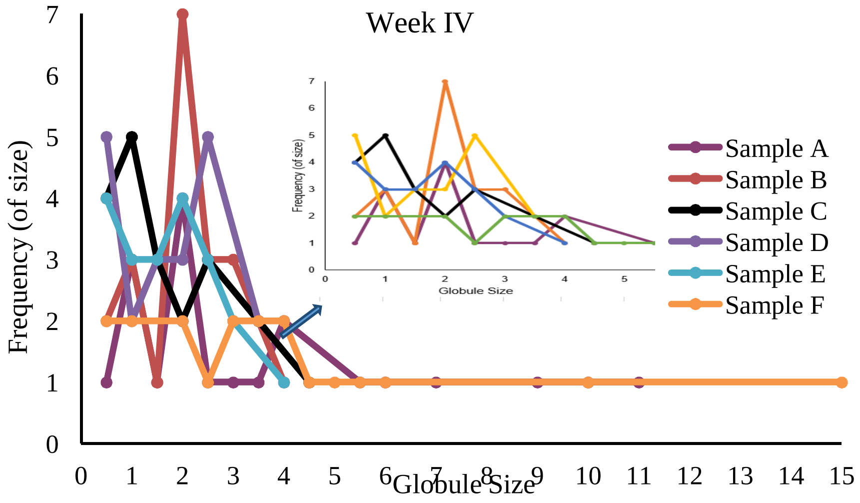


Fig. 1g. Globule size distribution plot for Sample A-F at the fourth week (Week IV)

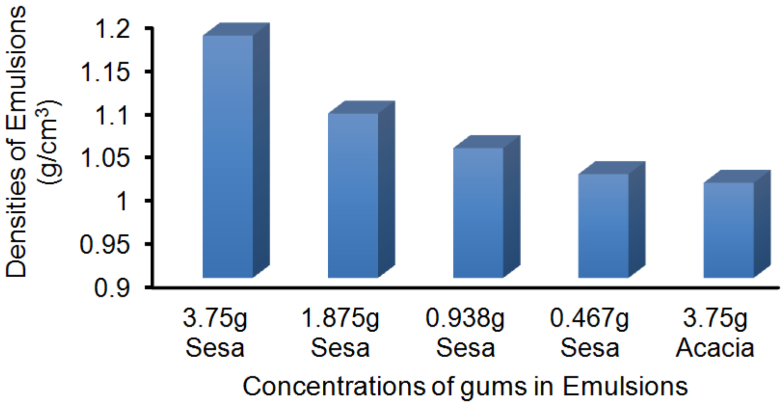


Fig. 2. Comparative densities of emulsions prepared with different concentrations of emulsifiers

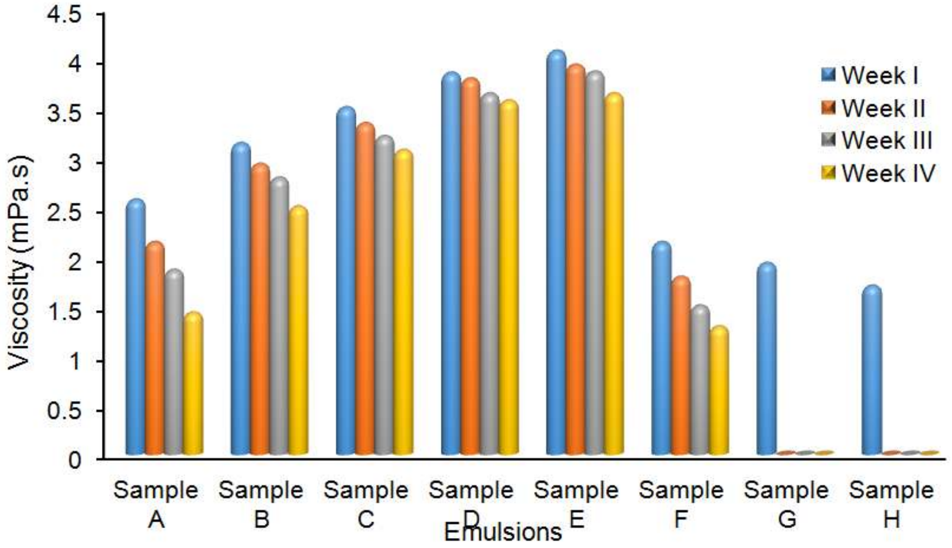


Fig. 3. Viscosity chart of emulsions - samples A-H over four weeks

4. CONCLUSIONS

The presence of sesamum as a stabilizer influenced the globule size and size distribution, the viscosity and consequently the stability to the emulsions. The study suggests that sesamum gum possesses the desirable properties of emulsifier/stabilizer: acts on the surface to reduce interfacial tension, adsorbs around the dispersed globules to prevent instabilities such as creaming and coalescence, effective at low concentration and increases viscosity of emulsions. Sesamum gum exhibited emulsifying ability for O/W emulsions. The breakdown of emulsion when sesamum was used as an emulsifier suggests microbial impact. Consequently, further studies will be undertaken to prepare sterile emulsions with sesamum gum as an emulsifier and also in the presence of preservatives, the stability of the emulsions will be assessed. In addition, further characterization of sesamum gum as an emulsifier/stabilizer will be undertaken.

CONSENT

This is not applicable.

ETHICAL APPROVAL

This is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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