Influence of Grewia polysaccharides on the stability of oil-in-water emulsions

Elijah I. Nep¹,³*, Chinwe U. Kemas², Adeola O. Adebisi³, Barbara R. Conway³, Alan M. Smith³

¹Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria; ²Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria; ³Department of Pharmacy, University of Huddersfield, Queensgate, Huddersfield, UK

INTRODUCTION

Emulsions are dispersed systems which are of great interest across several industries including the pharmaceutical, cosmetic, agro-chemical and food industry. They offer opportunity for encapsulation of bioactives and are thus suitable as delivery systems in functional foods and pharmaceuticals (McClements et al., 2007; Frank et al., 2011). Despite their versatility, the stability of emulsions is a major concern.

Plant-based hydrocolloids or polysaccharides have increasingly become the stabilizers of choice in the food industries due to the growing consumer demand for natural ingredients. Whereas some of these hydrocolloids or polysaccharides show a certain degree of surface activity (Dickinson, 2009), they are generally not considered to be surface active agents, due to their strong hydrophilic character. The long-term stability of oil-in-water emulsions is enhanced by the addition of “stabilizers” or “thickeners” (Walstra, 1993). These are non-surface active, usually plant hydrocolloids that increase viscosity of the water phase of an emulsion. The increase in the viscosity of the continuous phase of the oil-in-water emulsion, reduces droplet mobility so that creaming or sedimentation is suppressed and also, coalescence is decreased due to less droplet collisions (Sjoblom, 2006). Furthermore, stabilizers can also have an immediate effect on droplet breakup during the emulsification process due to their viscosity enhancing properties. The presence of galacturonic acid monomers possessing different functional groups (Thakur et al., 1997), their degree of esterification (DE) (Carti and Leser, 2001; Endreb et al., 2009; Schidt et al., 2015; Naji-Tabasi et al., 2016), and the molecular weight (Akhtar et al., 2002)

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The aqueous dispersion of the polysaccharide isolated from the inner bark of the stem of Grewia mollis exhibits a relatively high viscosity at low concentrations. Two extraction methods were adopted to isolate two different polysaccharide fractions from the plant – the native grewia polysaccharide gum (GPG) and the starch-free grewia polysaccharide (SFGP). The influence of these polysaccharides on the stability of oil – in – water emulsions was investigated at three concentrations – 0.5%, 1.0% and 1.5% w/v. The formulated emulsions were evaluated using parameters such as emulsion stability, storage stability, creaming index, heat stability, globule size and size distribution, and emulsion rheology. The results show that the GPG formulated emulsions were more stable than the SFGP emulsions at each parity level of concentration, exhibiting better emulsion/storage stability, lower creaming index and finer microstructure which were concentration dependent. The higher degree of esterification of SFGP did not result in corresponding improvement in emulsion stability over GPG-containing emulsions. GPG may be preferable to SFGP when stabilization of oil-in-water emulsions is indicated.

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has been reported to influence the overall performance of pectins as stabilizers and emulsifiers.

Grewia polysaccharide is obtained by extraction from *Grewia mollis*, a shrub native to sub-Saharan Africa belonging to the Malvaceae family. In some parts of Nigeria where it is cultivated, the inner bark from the stems of the shrub is pulverised and often used as a thickener in various food formulations. The polysaccharide isolated from the plant has been described as a pectin-like material (Nep et al., 2016) and studies have shown that the aqueous dispersions exhibit pseudoplastic flow with a relatively high viscosity at low concentrations (Okafor et al., 2001; Nep & Conway, 2011a; Nep et al., 2013). Previous studies have reported the potential of grewia polysaccharide extract as a pharmaceutical excipient in solid oral formulations: as a binder or sustained release matrix (Nep & Conway, 2011b), as bioadhesive (Nep & Okafor 2006; Nep & Conway, 2011c), and as a suspending agent (Nep & Conway, 2011d). Our literature search revealed limited study on the potential of this polysaccharide for stabilization of emulsions.

The impact of variations in extraction methods on the functional properties of the polysaccharide extract have been explored (Ogaji, 2011; Nep et al., 2016) providing the potential to diversify the applications for extracts produced using different methods. Nep et al. (2016) also determined the degree of esterification and intrinsic viscosity to be higher (49.2% and 4.40 dL/g) in SFGP than GPG (38.4% and 3.78 dL/g), respectively. They also showed by the absence of O-methyl signals in 13C NMR spectra, that the uronic acid residues of SFGP were not methyl esterified, instead they were O-acetylated.

The aim of the present study was to determine the ability of GPG and SFGP to stabilize oil-in-water emulsions, thus filling the gap in the literature on the potential of grewia polysaccharides as emulsion stabilizers.

**MATERIALS AND METHODS**

The materials used for this study as procured from the manufacturers include: sodium metabisulphite, alpha-amylase (Termamyl 120 L), HCl, 95% ethanol, 95% propan-2-ol and sunflower oil were purchased from Sigma - Aldrich (Poole, Dorset, UK). Grewia polysaccharides - GPG and SFGP were extracted in our laboratory from the bark of the inner stem of *Grewia mollis*.

**Extraction of native polysaccharide from *G. mollis* stem**

The extraction of native grewia polysaccharide from the stem bark of *G. mollis* was carried out according to the method reported by Nep et al., (2016). Briefly, the dried and shredded inner bark of the material was macerated in 0.1% sodium metabisulphite for 24 hours. The swollen gum was separated from the residue by filtration and the filtrate was precipitated from solution using absolute ethanol. Further purification was achieved by re-dispersion in water and final precipitation in absolute ethanol to give gum fraction code named GPG which was then oven dried at 50 °C for 24 hours and stored at 20 °C.

**Extraction of starch-free polysaccharide from native *G. mollis* polysaccharide**

The extraction of starch-free grewia polysaccharide was performed as reported by Nep et al., (2016). Briefly, 1%w/v of Termamyl 120 L was added to 3 L of a 1%w/v dispersion of GPG and stirred at 70 °C for 4 hours. The Termamyl was pretreated by heating at 30 °C for 30 min to deactivate pectinases and arabinoylanases. At 1 hour time points, an aliquot of the dispersion was removed and tested for the presence of starch using 0.1% KI. Starch digestion was complete after 3 hours after which the sample did not test positive for starch. Subsequently, protein from the sample was precipitated by adjusting the pH to 4.5 with 2 M HCl and centrifuging at 4400 rpm for 20 min. Thereupon, the supernatant was dialysed against deionized water for 72 hours using cellulose membrane with MW cut-off at 12500 Da. The dialysed material was precipitated using 2 volumes of 95% ethanol followed by solvent exchange using 1 volume of 95% propan-2-ol. The precipitate was oven dried overnight at 40 °C. This sample was code named SFGP, and stored at 20 °C.

**Preparation of polysaccharide-stabilized emulsions**

The emulsions containing 0.5%, 1.0% and 1.5% (w/w) of GPG and SFGP were formulated using a previously reported method (Naji-Tabasi & Razavi, 2016). Gum solutions were prepared by dissolving the appropriate amount of polysaccharide gums or derivatives in water at 50 °C and keeping overnight on a stirrer to ensure hydration. Sunflower oil (30%, w/w) was added dropwise to the polysaccharide solution while stirring at 2000 rpm. The emulsion was homogenized (Ultra Turrax T-18, Heidolph, Germany) at 20000 rpm for 10 min in alternate cycles of homogenization (2 min) and rest (2 min).

**Determination of emulsion stability**

*Centrifugation assay*

The emulsions were centrifuged (Hettich, Germany) at 2000 g for 10 min immediately after production.
and the emulsifying stability was calculated using equation 1 as reported by Sciarini et al., (2009):

\[ \text{Emulsion stability} \% = \frac{e_v}{i_v} \times 100 \]  
Equation 1

where, \( e_v \) is the emulsion volume and \( t_v \) is the total volume.

**Storage stability evaluation**

The emulsions were placed in glass containers and stored at room temperature for 4 weeks. The changes in the emulsion volume were measured on days 7, 14, 21, 28 and 35. The emulsion stability was calculated according to equation 1.

**Heat stability**

Stability of the emulsions against high temperature was determined by heating the sample emulsions in a water bath at 80 °C for 30 min followed by centrifugation at 2700 g for 10 min. Emulsion stability was calculated using equation 2 as reported by Sciarini et al., (2009):

\[ \text{Emulsion stability} \% = \frac{f_{ev}}{i_{ev}} \times 100 \]  
Equation 2

where, \( f_{ev} \) is the final emulsion volume and \( i_{ev} \) is initial emulsion volume.

**Visual phase separation**

Visual phase separation of the emulsions was evaluated by transferring the emulsions into 30 mL test tubes. The test tubes were sealed with plastic tapes and stored at 20 °C, for 21 days. Any evidence of physical phase separation was monitored during this period.

**Light microscopy**

The microstructure of the emulsions was studied using an optical microscope (Keyence VHX-2000E, Tokyo, Japan) connected to software installed on the video output unit of the microscope. Briefly, a drop of the undiluted emulsion was deposited on a microscope slide and covered with a coverslip. The images were scanned and acquired at x1000 magnification.

**Droplet size measurement**

The droplet size distribution and mean diameter of the prepared emulsions were measured immediately after preparation and after storage at room temperature for 7, 14 and 28 days. This was measured using a Mastersizer 2000 (Malvern Panalytical, Worcestershire, UK) laser diffraction particle size analyser equipped with a small volume sample dispersion unit Hydro 2000SM (Malvern Panalytical, Worcestershire, UK). The measurements were taken under the conditions that the refractive indices of sunflower oil and dispersion medium (deionized water) were set to 1.469 and 1.333 respectively. The droplet size was described using the surface-weighted mean diameter (d3, 2).

**Creaming index**

The creaming index of the emulsions was determined by observing the separation of cream layer. Sample emulsions (6 mL) were transferred into screw-capped test tubes (10 mm internal diameter, 100 mm height) and stored at 25 °C for 7 d. The total height of the emulsion (\( H_e \)) and the heights of the cream layer (\( H_c \)) were measured and the extent of creaming, expressed as creaming index (CI), was calculated using equation 3:

\[ CI = \frac{H_c}{H_e} \times 100 \]  
Equation 3

**Determination of foaming capacity and stability**

The foam capacity and stability was determined at 0.5% (w/w). Albumin (0.5%, w/v) was added to the solution (50 mL) after complete hydration of gum. The samples were mixed at 20000 rpm for 2 min with a homogenizer (Ultra Turrax T-18, Heidolph, Germany). Foam volumes were recorded immediately after production and foam capacity and stability were calculated using equations 4 and 5, respectively:

\[ \text{Foam capacity} \% = \frac{f_{fv}}{i_{fv}} \times 100 \]  
Equation 4

where, \( i_{fv} \) is initial foam volume and \( t_{fv} \) is total suspension volume.

\[ \text{Foam stability} \% = \frac{f_{fv}}{i_{fv}} \times 100 \]  
Equation 5

where, \( f_{fv} \) is foam volume after 30 min and \( i_{fv} \) is initial foam volume. All samples were prepared for 4 times.

**Rheological properties**

Steady shear viscosity and small deformation oscillatory measurements (frequency sweep, and heating and cooling scans) of the emulsions were performed on a Bohlin Gemini HR Nano rheometer (Malvern Panalytical, UK) fitted with a 55 mm, 2° cone and plate geometry with a gap of 70 mm. Steady shear viscosity measurements were performed at 25 °C across a shear rate of 0.01-1000 s⁻¹. Small deformation oscillatory measurements of storage modulus (\( G' \)) and loss modulus (\( G'' \)) were taken across a frequency range of 0.1-100 rad s⁻¹ at 20 °C and a constant strain of 1% (using the same geometry parameters used for the viscosity measurements) which was within the linear viscoelastic region of the emulsions determined from a strain sweep.

**RESULTS AND DISCUSSION**

**Emulsion Stability**

Emulsion stability is a measure of the rate at which an emulsion creams, flocculates or coalesces. The
term is used generally to describe the ability of an emulsion to resist alterations in its properties over time (Huang et al., 2001; McClements, 2004). The dominant mechanisms of emulsion instability are gravity creaming, Ostwald ripening, flocculation and droplet coalescence (Dickinson, 2009). In the present study three methods were used to measure emulsion stability.

Centrifugation assay

The stability of emulsions by centrifugation assay is an important parameter used in accessing and predicting the emulsion stability to coalescence (Khan et al., 2010). It gives the physical characteristics of the system at a given pressure or centrifugal force and the percent stability is obtained from the level of phase separation of the emulsion system due to different densities of the individual components that make up the system. The lower the phase separation levels of the system the higher the stability and vice versa. The effect of polysaccharide concentration on the emulsion stability is presented in Fig. 1.

**Heat Stability**

GPG stabilized emulsions exhibited greater heat stability when compared with SFGP stabilized emulsions at the same levels of concentration (Fig. 2), and heat stability increases with increasing polysaccharide concentration. However, there was no corresponding increase in heat stability for SFGP-containing emulsions when polysaccharide concentration was raised from 1.0% to 1.5%. Stability to denaturation by heat can be attributed to the formation of a protective film by hydrocolloids around oil droplets that are not unfolded at higher temperatures to expose non-polar groups. McClements, (2004) reported that heating favors hydrophobic interaction in emulsion systems and thereby leading to droplet aggregation.

**Storage Stability**

Even though centrifugation is a fast method for investigation of emulsion stability, it sometimes does not reflect the true behavior of the emulsion during storage. Therefore, in the present study, emulsion stability was also screened during 4 weeks of storage at 20 °C. The results are presented in Fig 3. It was observed that although stability decreased with time, it increased with increasing polysaccharide concentration. The gradual decrease in storage stability has been attributed to the swelling of internal droplet size as a result of rupture of the oil layer or coalescence of the oil globules. (Akhtar et al., 2010)
Fig. 3. Effect of concentration of GPG and SFGP on storage stability of the oil-in-water emulsion over 4 weeks

Visual Phase Separation of the Emulsions
Fig. 4 illustrates the changes in the emulsion samples over 4 weeks. The emulsions containing lowest concentrations of polysaccharides (0.5%) exhibited creaming in the first week with obvious boundary lines separating the cream layer from the serum phase (Fig 4A). While the creaming became visible in the emulsions containing 1.0% of the polysaccharides in the second week (Fig 4B), it became visible only in the fourth week for emulsions containing 1.5% polysaccharide (Fig 4C).

In addition, the results show that SFGP-containing emulsions exhibited a faster onset of separation than the GPG-containing emulsions at all concentration levels indicating that the GPG-containing emulsions may be more stable than the SFGP-containing emulsions. The cream layer in the emulsion containing 0.5% polysaccharide was evidently more distinct. This has been attributed to free mobility of droplets owing to weak viscous forces in the aqueous phase, resulting in well-defined and distinct layer of separation (Koocheki et al., 2009). These observations are consistent with results from previous studies (Huang et al., 2001; Sun et al., 2007; Taherian et al., 2007) confirming the positive effect of polysaccharides on the inhibition of creaming in emulsions.

Light microscopy and droplet size
The microstructure of the emulsions immediately after preparation is presented in Fig. 5. The micrographs are in agreement with the results obtained for droplet size measurement (Fig. 6).

GPG and SFGP – containing emulsions exhibited the finest microstructure at a concentration of 0.5% under light microscopy. At higher concentrations of
1.0% and 1.5% a coarser microstructure was observed for both gum samples, however the globule microstructure of SFGP-containing emulsions was coarser than that of GPG-containing emulsions. The mean droplet size increased with concentration of polysaccharide and age of the emulsion. This was more prominent for SFGP-containing emulsions than for GPG containing emulsions. Increasing the concentration of GPG from 1.0% to 1.5% exhibited little effect on mean droplet size. From the results presented, it is obvious that emulsions formulated using GPG as emulsifier are more stable compared with SFGP-containing emulsions at 1.0 and 1.5 % concentration. It must be noted that, while the results from light microscopy and mean droplet size shows that emulsions containing 0.5% polysaccharide exhibited the finest microstructure and lowest mean droplet size respectively, this did not however translate to greater stability of the emulsions as seen from the visual phase separation. This result is comparable to earlier studies by Garti et al., (1999) who reported that flocculation is more likely to occur in emulsions containing low polysaccharide concentrations. This is according to the mechanism of depletion flocculation which predicts that an increase in the concentration of non-adsorbing biopolymers at lower concentration of emulsifier (hydrocolloid gums) causes an increase in the inter-droplet attraction (Makri & Doxastakis, 2006).

Fig. 6. Effect of Polysaccharide Concentration and Age of Emulsion on Mean Droplet size

The mean droplet size (d3,2) presented in Fig. 6 supports the results from light microscopy (Fig. 5A-C). It can be seen that immediately after preparation (Day 0), emulsions containing 1.5% of SFGP has the largest droplet size. This was followed closely by emulsions containing 1.0% of SFGP with mean droplet size greater than emulsions containing 1.5% of GPG. Emulsions containing 0.5% GPG exhibited the lowest mean droplet size on Day 0. Any advantage conferred by virtue of having a small globule size in stabilizing the emulsions containing 0.5% of polysaccharide was compromised by the low viscosity of the emulsions at that concentration (Huang et al., 2001). This explains the fast phase separation observed with emulsions containing 0.5% of polysaccharides. Conversely, at higher concentrations of polysaccharide, the high viscosity of the emulsions provided a stabilizing effect against coalescence of the rather large droplets and as a result slowed down the rate of phase separation. The increase in emulsion viscosity reduces droplet mobility so that creaming or sedimentation is suppressed and also, coalescence is decreased due to fewer droplet collisions (McClements, 2005; Sjoblom, 2006; Dluzewska et al., 2006).

Creaming index

Under normal conditions, the creaming index has been reported to be related to the rate of globule aggregation in an emulsion (Ye & Singh, 2006). The stability of emulsions against creaming and phase separation is shown in Fig. 7.

Fig. 7. Effect of Polysaccharide Concentration and Storage on Creaming and Phase Separation

The curves clearly show that the emulsion stability can be enhanced by increasing the gum concentration. At low concentrations of polysaccharide (0.5%), distinctive and progressive creaming was observed between 7 and 28 days, with SFGP-containing emulsions displaying higher values of creaming index at all concentrations thus, indicating that GPG emulsions were more stable to creaming. Conversely, at higher concentrations (1.0% and 1.5%), no visual changes or creaming took place and the emulsions maintained integrity until after the 7th -14th day of storage. The least creaming was exhibited by GPG-containing emulsions (1.0 % and 1.5%). This concurs with previous studies by Dickinson (2009), who found that low concentrations of polysaccharide in an emulsion can have a destabilizing effect due to the mechanism of depletion flocculation induced by non-absorbing
polysaccharides. At higher polysaccharide concentration creaming is retarded since droplet aggregation is immobilized by the high viscosity of the polysaccharides. This has been reported elsewhere (Semenova, Dickinson, Burlakova & Zaikov, 2010).

Foaming capacity and Foam stability
The impact of 0.5% gum on foaming capacity and stability of albumin solution (0.5%) is presented in Fig. 8. The foaming capacity and stability of albumin at 0.5% concentration was 16.6 and 50.5% respectively. GPG altered the foaming capacity and foam stability of albumin by 10% and 45% respectively, while SFGP altered the foaming capacity and foam stability of albumin by 15% and 42% respectively. This represents a significant improvement on the foaming capacity and foam stability of albumin.

Rheological properties
The rheological behaviour of emulsions is a significant property used in considering the stability of emulsions during storage since high viscosity conditions inhibits the coalescence of oil droplets. The instantaneous viscosity as a function of the shear rate is presented in Fig. 9A. The emulsions exhibited pseudoplastic flow behaviour at all concentrations of polysaccharide but occurred at a greater extent with increasing concentration. The instantaneous viscosity decreases with increase in shear rate that overcomes Brownian motion and aligns the emulsion droplets in the direction of flow with lower viscosity (McClements, 1999). Such a characteristic is desirable in topically used pharmaceutical and cosmetic products.

Fig. 9B presents the effect of storage for 28 days on the viscosity of the emulsions. It was observed that viscosity of the formulations reduces after storage for 28 days. This decrease was greater for the GPG formulations than for the SFGP formulations. Natural polysaccharides exhibit a reduction in viscosity of their dispersions upon storage. This is attributable to microbial degradation of the polysaccharide. The extent of microbial contamination may be accountable for the extent of loss of viscosity with storage time. Furthermore decrease in viscosity upon aging could be attributed to the coarsening of the emulsion which increases droplet size and hence decreases viscosity (Pal, 1996). This is in agreement with the data on droplet size presented in section 3.4 which shows increase in droplet size with age of emulsion.

The tan delta at a frequency of 2.0 rad s$^{-1}$ from the mechanical spectra of the emulsion formulations are presented in Fig. 9C. The results show that the emulsions exhibited more elastic behaviour with increasing concentration of polysaccharide. At a polysaccharide concentration of 0.5% and 1.5% w/v, GPG-containing emulsions were more elastic than SFGP-containing emulsions.

Nep et al. (2016) reported that SFGP has a higher intrinsic viscosity (4.40 dL/g) than GPG (3.78 dL/g) in deionized water. It has been shown that a reduction in molecular weight results in the improvement of the emulsifying capacity of pectins (Akhtar et al., 2002). Increasing the DE has also been reported to significantly improve the emulsifying behavior of citrus pectins (Schidt et al., 2015). Following this, one would expect that SFGP, with a higher DE (49.2%) than GPG (38.4%), would exhibit better emulsifying capacity. However, it has been shown (Nep et al., 2016) that the uronic acid residues
of SFGP unlike the citrus pectins were O-acetylated and not methyl esterified. Morris et al., (2000), earlier reported that, while an increase in DE may enhance coiling leading to increase in mobility and flexibility of the molecule and consequent faster adsorption at the oil interface, it also makes hydrophobic groups inaccessible, thereby compromising surface activity. Furthermore, Schimidt et al., (2015) working on citrus pectins, reported that a high degree of acetylation increases surface tension, and this would imply that O-acetylation of SFGP maybe a disadvantage.

While Funami et al. (2009) showed that the proteinaceous moiety in sugar beet pectin is most likely the source for its good emulsifying capacity, the present study revealed that the higher protein content (5.2%) of SFGP over GPG (2.3%) did not result in the improvement in the stabilizing effect of SFGP in oil-in-water emulsions. This may be because the increase protein content of SFGP is attributed to inefficient precipitation of α-amylase after starch hydrolysis (Nep et al., 2016) and not the protein fraction associated with the polysaccharide.

CONCLUSIONS

Oil-in-water emulsions were stabilized with GPG and SFGP at concentrations - 0.5%, 1.0% and 1.5%. The results showed that emulsion stability, creaming index, optical microscopy and droplet size were concentration dependent. At similar concentrations, GPG-containing formulations demonstrated better emulsifying capacity. Furthermore, GPG-containing emulsions exhibited a finer microstructure than SFGP-containing emulsions at the same level of concentration. The ability of GPG and SFGP to improve emulsion stability appears to be predominantly due to the increased viscosity of the aqueous continuous phase of the emulsions. The higher viscosity of GPG containing emulsions in addition to their finer microstructure accounts for their better stabilizing effect in the emulsion formulations. When the stabilization of oil-in-water emulsions is implicated, GPG may be preferable over SFGP.

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