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Abstract

The chemical and physicochemical characteristics of gum exudate samples harvested from mature trees of *Acacia senegal* at two new specific ecolocations in Nigeria, have been investigated together with gum samples harvested from *Acacia senegal* and *Acacia seyal* originating from Sudan. The monosaccharide sugar compositions for the *Acacia senegal* gum samples were found to be similar, but the protein contents for the Nigerian samples were significantly higher than recorded for the Sudanese sample. Gel Permeation Chromatography coupled to light scattering, refractive index and U.V. detectors, has shown the presence of arabinogalactan, arabinogalactan-protein and glycoprotein fractions within the *Acacia senegal* gums and has also shown the presence of an additional small proportion of very low molar mass proteinaceous material all the samples which has previously been ignored. The plot of radius of gyration, Rg, as a function of elution volume showed a discontinuity for one of the Nigerian samples and for the *Acacia seyal* gum sample at elution volumes corresponding to the AGP component suggesting a different molecular structure. Plots of Mw − v − Rg confirmed that the molecules had a compact structure. The hydrodynamic size of the molecules was followed using dynamic light scattering as a function of time and it was found that molecular association occurred in solution. The extent of association increased as the protein content in the sample increased and was inhibited in the presence of electrolyte, it was concluded that association was due to electrostatic interaction between the protein moieties and glucuronic acid groups on individual macromolecules.

Key words:

Gum Arabic, molecular association, Acacia gum.

1. Introduction

Essentially, gum Arabic is a complex, polysaccharide-based plant gum exudate, obtained from trees of selected *Acacia* species (i.e. *Acacia senegal and Acacia seyal*), which are indigenous to the Sahelian region of Africa, and for which Sudan is the world's leading producer. This gum is widely used in the food industry as an emulsifier, for flavour oils present in soft beverages, it is also extensively used in confectionery products in which it is used to control texture and inhibit sugar crystallisation (Williams, Phillips, Stephen, & Churms, 2006; Williams and Phillips, 2009).

The molecular composition of the gums harvested from *Acacia senegal* (*A. senegal*) and *Acacia seyal* (*A. seyal*) differ, but the molecular structure recorded for the most abundant molecular constituent of both gums (i.e. arabinogalactan), consists of a core of β 1,3 linked galactose units with branches linked through the 6 position consisting of galactose and arabinose terminated by rhamnose and glucuronic acid (Street and Anderson, 1983; Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Al-Assaf, Phillips & William, 2005). Both gums also contain a small percentage of proteinaceous material, with an almost identical amino acid composition, some of which is covalently attached to arabinogalactan (Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Mahendran, Williams, Phillips, Al-Assaf & Baldwin, 2008).

Characterisation of gum Arabic using gel permeation chromatography (GPC), coupled to light scattering, refractive index and UV detectors has shown that the gum exudates obtained from both *A. senegal* and *A. seyal* consist of three main fractions, referred to as the arabinogalactan (AG), arabinogalactan - protein (AGP) and glycoprotein (GP) components, which differ mainly in their molecular size and protein content (Randall, Phillips & Williams, 1989; Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Osman, Menzies, Williams, Phillips & Baldwin, 1993; Renard, Lavenant-Gourgeon, Ralet & Sanchez, 2006; Mahendran, Williams, Phillips, Al-Assaf & Baldwin, 2008). The AG, AGP and GP fractions account for ~90%, ~10% and ~1% of

the total gum respectively. They have molar masses of ~ 250Kg/mol, 1-2,000kg/mol, and 200Kg/mol and contain <1%, ~10% and 25-50% proteinaceous material. The amino acid content/composition of the three components differs significantly, with that recorded for the GP fraction showing least similar to the other fractions. The major amino acids found in the AG fraction in descending order are Hyp, Ser, Glu, Pro and Gly , the major amino acids in the AGP fraction are Hyp, Ser, Thr, Pro, Leu and His, whereas the most abundant amino acids in the GP component are Asp, Phe, Ser, Glu and Gly (Randall, Phillips, & Williams, 1989). These differences suggest that the glycoprotein(s) present in the GP fraction are less likely to include hydroxyproline-rich glycoproteins (HRGPs) than those present in the other two fractions. This is of particular interest, as this is the least intensively studied fraction of gum Arabic, and one or more of these proteins may play an important role in some of the food applications for the gum, such as in oil-in-water emulsions used in the beverage industry.

Considerable progress has been made over the past thirty years regarding our understanding of the molecular structure and composition of gum Arabic using fractions isolated by hydrophobic affinity chromatography (Randall, Phillips & Williams, 1989; Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Renard, Lavenant-Gourgeon, Ralet & Sanchez, 2006). It is important to note that the fractions obtained using this separation technique have been shown to have a broad molecular mass distribution, but each fraction contains a predominance of either the AG, AGP or GP components (Osman, Menzies, Williams, Phillips & Baldwin, 1993; Renard, Lavenant-Gourgeon, Ralet & Sanchez, 2006).

With regards the structure of the macromolecules present in these three fractions, recent work using transmission electron microscopy, atomic force microscopy and neutron scattering has indicated that the AG fraction has a disk-like molecular structure with a diameter of ~20nm and a thickness below 2nm (Sanchez et al., (2008)). As to the AGP fraction, Mahendran, Williams, Phillips, Al-Assaf & Baldwin, (2008), undertook a series of alkali and enzyme hydrolysis experiments and concluded that the AGP component has a wattle blossom type structure with highly

branched carbohydrate blocks of molar mass ~ 40Kg/mol attached to a polypeptide chain.

Furthermore, deglycosylation of whole gum using hydrofluoric acid by Mahendran and co-workers, (2008) and subsequent analysis by SDS polyacrylamide gel electrophoresis, indicated the presence of two core proteins, consisting of ~ 250 and 45 amino acids respectively. It was assumed that the larger polypeptide is associated with the AGP component and that the smaller one may be present in the AG or GP fractions (Mahendran, Williams, Phillips, Al-Assaf & Baldwin, 2008). More recently, the AGP fraction has been reported to consist of two populations of molecules with a maximum diameter of 64nm and a thickness below 5nm, one with long chain branching and a globular shape and the other a more elongated shape (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012).

Whereas, the GP fraction was recently shown using GPC/MALLS, small angle X-ray scattering, synchrotron radiation circular dichroism and transmission electron microscopy to consist of a mixture of spheroidal ring-like glycoprotein modules with a diameter of ~9nm, which were reported to be due to the formation of hydroxyproline-arabinogalactan subunits (Renard et al., 2014).

The determination of the precise molecular structure and composition of gum Arabic is further complicated by the fact that the macromolecules present in the gum tend to self-associate when in solution, as demonstrated by Li et al using dynamic light scattering (Li et al., 2009). These authors hypothesised, that this self-association is brought about through hydrogen bonding, and is responsible for the changes in rheological properties of gum Arabic solutions over time, as also reported by Sanchez et al. (Sanchez, Renard, Schmitt, & Lefebvre, 2002). Molecular association can also occur when the gum is subjected to heat as, for example, during maturation and spray drying as reported by Aoki et al., (2007). They followed the maturation process using GPC and demonstrated that the intensity of the peaks for the AG and GP fractions decreased and AGP increased over time and this was attributed to

association of the proteinaceous moieties present. This is likely to occur through hydrophobic bonding.

The aim of the current study, was to investigate the physiochemical characteristics and associative behaviour of two samples of gum Arabic harvested from trees of *Acacia senegal* at two different geographical locations in Nigeria, which have not been studied previously and to compare their properties with those of previously studied samples of gum Arabic, harvested from trees of *Acacia senegal* and *Acacia seyal* originating from Sudan.

2. Materials and methods

2.1 Gum samples

Acacia senegal gum exudates were obtained from two provenances/eco-locations in the North Eastern (Sahelian) region of Nigeria namely; Gashua (NG1) at latitude $12^0 53' 10'' N$, longitude $10^0 52' 38'' E$ and altitude 370m which has a sandy, Sahelian soil type condition, and at Gujba (NG2) at latitude $11^0 24' 39'' N$, longitude $11^0 59' 38'' E$ and altitude 456m which has a clay-loam type soil. Both locations are known locally for gum Arabic production, which is mostly harvested from indigenous trees. However, in recent years, *Acacia* plantations have been established in this region, by staff of the Rubber Research Institute of Nigeria (RRIN) substation, located in Gashua, Yobe state (which was established for the specific purpose of enhancing gum Arabic research, by the Federal Government of Nigeria). Both samples of the Nigerian gum Arabic were authenticated by the RRIN substation in Gashua. Authenticated Sudanese samples of gum Arabic, harvested from Sudanese trees of *A. senegal* and *Acacia seyal* were obtained from the Phillips Hydrocolloids Research Centre, Glyndwr University, United Kingdom. All four gum samples were in the form of nodules and had not been processed.

2.2 Moisture content

1g of each gum Arabic sample was placed in a crucible and dried at $105^0 C$ overnight using a Sanyo Convection Oven, Model MOV 212F, Japan. The samples were then

transferred to a desiccator and cooled at room temperature to obtain the moisture content. This analysis was performed in triplicate and the average value recorded.

2.3 Sugar composition

The sugar compositions of the four gum samples were determined by HPAEC-PAD, using methanolysis combined with TFA hydrolysis using the methodology described previously (Yadav, Johnston, Hotchkiss, & Hicks, 2007) with some minor modifications. In brief, the samples were first dissolved in deionized water (1 mg/ml). An aliquot of 100 nmoles myo-inositol (internal standard) was then added to the gum solution and the mixture dried in a Teflon-lined screw cap glass vial, by blowing with filtered nitrogen, followed by drying in a vacuum oven at 50°C overnight. The samples were then methanolyzed with 1.5 M methanolic HCl, in the presence of 20% (v/v) methyl acetate for 16 h, cooled to room temperature and dried by blowing with filtered nitrogen after the addition of five drops of t-butanol. The methanolyzed samples were subsequently hydrolyzed with 0.5 ml 2M TFA at 121°C for 1 h, evaporated by blowing with filtered nitrogen at 50°C and the residue was then washed by sequential addition and evaporation of three aliquots (0.5 ml) of methanol. Into five separate glass vials, were placed 100, 200, 300, 500 and 1000 nmoles of a mixture of standard sugars containing fructose, arabinose, rhamnose, galactose, glucose, xylose, glucuronic acid and galacturonic acid. Afterwhich, 100 nmoles of myo inositol (internal standard) was added to each vial, evaporated and dried as above. These standard samples were also methanolyzed and hydrolyzed as described above, and used for quantification.

The resultant hydrolysates were then analyzed for neutral and acidic sugars by HPAEC-PAD, using a Dionex DX-500 system that included a CarboPac PA20 column and guard column, a GP 50 gradient pump, an ED40 electrochemical detector utilizing the quadruple potential waveform (gold working electrode and pH reference electrode), an AS3500 auto sampler with a thermal compartment (30°C column-heater), and a PC10 pneumatic controller post column addition system. The mobile phase consisted of isocratic 12 mM NaOH eluant for 10 min followed by 100 mM NaOH and 6 mM CH_3COONa for 3 min, 100 mM NaOH and 12 mM CH_3COONa for 17

minutes at a flow rate of 0.5 mL/min. at ambient temperature. The column was washed with 1 M CH$_3$COONa for 0.10 min and with 100 mM NaOH for 10 minutes, followed by 30-min equilibration with 12 mM NaOH at a flow rate of 0.5 mL/min at ambient temperature was required to yield highly reproducible retention times for the monosaccharides. The total run time was ca. 70 minutes. In order to minimize baseline distortion due to changes in the pH of the eluant during monosaccharide detection (by PAD) 730 mM NaOH was added to the post column effluent via a mixing tee.

2.4 Glucuronic Acid Content

The glucuronic acid content of the samples was determined using a modified version of the method for quantitative determination of uronic acid (Blumenkrantz & Asboe-Hansen, 1973). Glucuronic acid, meta-hydroxydiphenyl, sodium tetraborate, concentrated sulphuric acid and sodium hydroxide were obtained from Fisher Scientific. A 0.15% solution of meta-hydroxydiphenyl in 0.5% sodium hydroxide and a 0.0125M solution of sodium tetraborate in concentrated sulphuric acid were prepared. A calibration curve was initially determined using glucuronic acid. 0.4 mL solutions containing 0.5 to 20 μL glucuronic acid were pipetted into screw cap glass tubes and 2.4 mL of sulphuric acid/sodium tetraborate reagent was added and screw caps fitted. The tubes were then heated in a water bath at 100^{0}C for a period of exactly 5 minutes. The samples were then cooled immediately in a water ice bath for 5 mins and 40 μL of meta-hydroxydiphenyl reagent was then added. The tubes were shaken and the absorbance at 520nm was measured within 5 minutes using a Lambda 25 spectrophotometer.

A blank run was then performed without the addition of meta-hydroxydiphenyl reagent, which was replaced with 40 μL of 0.5% sodium hydroxide. This blank run was performed since the other sugars present in gum Arabic produce a pinkish chromogen with sulphuric acid/tetraborate at 100^{0}C. The absorbance for the blank sample was subtracted from the original absorbance and the glucuronic acid content was thus determined from the calibration curve.

2.5 Protein content

Protein content analysis was carried out using the AOAC approved Kjeldahl method (AOAC, 1999). A nitrogen conversion factor of 6.60 was used (Nx6.60) as suggested by (Anderson, 1986).

2.6 Amino acid composition

Amino acid analysis was performed by Alta Bioscience, University of Birmingham, U.K. The gum Arabic samples were first hydrolysed in a special vacuum hydrolysis tube, in which accurately weighed samples were dissolved with 6 mol dm^{-3} HCl of known volume ($1cm^3$). A custom designed and built, automatic amino acid analyser was then used to measure the amino acids in the samples, which was based on modular HPLC components controlled by a Nascom III computer. Each sample was injected onto the top of the column containing a strong cation exchange resin, maintained at temperature of 55^0C. The amino acids were separated and eluted off the cation exchange column, in order of their net charge value, using a series of sodium citrate buffers, producing a stepwise gradient with increasing pH and ionic strength. The flow rate of the eluent (citrate buffers) was maintained at $0.22cm^3$/min using an LKB pump. A Waters WISP sampler was used to sample the eluting amino acids, which were mixed with a colouring reagent (ninhydrin) that was eluted at a rate of $0.3cm^3$/min using an ACS 400 pump. The colorimetric reaction was carried out at 120^0C for 3 mins. Then, the absorbance was monitored at 440 and 550 nm using a Waters UV detector. A Linseis recorder was used for recording the chromatogram.

Hydroxyproline, a principal amino acid present in gum Arabic, has a low response factor and was contained within the Aspartic acid peak. The two peaks were resolved by lowering the temperature of the cation exchange column to 40^0C. Detection was carried out at 440 nm and data expressed as the ratio of each amino acid, these were normalised with the initial experimental data by comparing the hydroxyproline/leucine ratios.

2.7 Molecular mass distribution

The molar mass distribution for each gum sample was obtained by Gel Permeation Chromatography (GPC). The system consisted of a Suprema 3000A 10µm column, HPLC Pump, (Waters P-500) connected to a rheodyne injector (series 7125) with a 200µm loop. 0.1M $NaNO_3$ was used as eluent and was passed through a vacuum degasser (type 006150/4 Cambridge, U.K.) prior to the rheodyne. A DAWN DSP laser light scattering photometer (Wyatt Technology), Optilab DSP interferometric refractometer and Agilent UV-spectrophotometer, set at wavelength of 280 nm, were used as the detectors. The refractive index increment (dn/dc) value used was 0.141 ml/g. All samples were filtered through 0.45µm nylon filters prior to injection. Data were captured using the Astra software (Wyatt Technology) and analysed using the Zimm method.

2.8 Hydrodynamic size

The hydrodynamic size of the gum Arabic molecules were measured by dynamic light scattering using the Zetasizer Nano series ZS, Malvern Instrument. **'**Raw' gum Arabic nodules were ground to powder using a pestle and mortar, then dissolved in a 0.1M solution of sodium nitrate in order to prepare a series of concentrations of gum, ranging from 0.1 to 0.5% (w/v). The samples were first filtered using a 0.45 µm pore size filter, and were then injected into a disposable cuvette DTS0012 which was immediately inserted into the measuring chamber of the instrument. All measurements were performed at 25^0C using a refractive index of 1.476 and an absorbance of 0.010 as input parameters. The results presented, represent the mean of 10 sub runs.

2.9 Molecular association

The molecular association of gum Arabic, was investigated by measuring the hydrodynamic size as a function of time, in both water and in 0.1M sodium nitrate using the Zetasizer Nano series ZS, (Malvern Instruments). 10% (w/v) gum Arabic solutions were prepared in deionized water and in 0.1M sodium nitrate. The hydrodynamic size was then determined as a function of time, using the procedure as described above.

3. Results and discussion

3.1 Sugar, protein and amino acid content

The monosaccharide sugar composition and protein content of the gum samples are given in Table 1 and their corresponding amino acid compositions are presented in Table 2. It is noted that the *A. seyal* gum has significantly lower rhamnose and glucuronic acid content, but a higher arabinose content than the gums obtained from *A. senegal,* as has been reported previously (Anderson, Douglas, Morrison, & Weiping, 1990; Williams and Phillips, 2009). Since the core of the AG macromolecules present in gum Arabic (which constitute ~ 90% of the gum by weight) is believed to consist of a backbone of $\beta, 1,3$ -linked galactose residues, the increased arabinose content recorded for *A. seyal* gum sample suggests that it possesses more and/or longer branches, which may account for its more compact structure. (Al-Assaf, Phillips, & Williams, 2005; Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Hassan, Al-Assaf, Phillips, & Williams, (2005).

There are no significant differences observed between the sugar compositions of the *A. senegal* gums from Sudan and Nigeria. It is evident, however, that the protein content of the Nigerian gums is significantly higher than observed for the sample originating from Sudan. In addition, Since the gum is known to be secreted within a zone between the inner bark and the cambial zone as part of the plants wound/stress response, Joseleau & Ullman, (1990), the observed difference in protein content could be due to genotypic differences between the Nigerian and Sudanese trees from which the gum was harvested, the age of the trees, prevailing climatic conditions, the soil in which the trees are planted, attack by herbivores, plant pathogens or a combination of factors (Idris, Williams & Phillips, 1998; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005; Al-Assaf, Phillips, & Williams, 2005; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). Furthermore, the Nigerian samples were harvested from a small number of trees, of known genotype, grown in two different eco-locations

In relation to the application of the gum in the Food Industry, it has been shown previously that the ability of *Acacia* gum exudates to stabilise oil-in-water emulsions is due to the proteinaceous components present within the gum. Hence, the Nigerian samples may have enhanced emulsification properties (Padala, Williams, & Phillips, 2009). The *A. senegal* gum samples were shown to contain approximately twice the amount of protein as compared to the gum obtained from *A. seyal,* which in agreement with previous findings (Idris & Haddad, 2012). The gum obtained from *A. seyal* is not able to effectively stabilise oil-in-water emulsions. The principal amino acids present in all the gum samples are hydroxyproline, serine, aspartine, threonine and proline which are present in a ratio which approximates to 4: 2: 1: 1: 1, which is in agreement with literature values (Anderson, Douglas, Morrison, & Weiping, 1990). The only significant difference observed between the three Acacia senegal samples is that a significantly higher value for phenylanaline (0.130g/100g) was observed for the NG2 sample compared to the NG1 (0.016g/100g) and the Sudanese sample (0.0745g/100g).

3.2 Molecular mass

The weight average, Mw, and number average and Mn molecular mass values obtained by GPC are given in Table 3. The values for the Sudanese and Nigerian *A. senegal* gum samples, are both of the same order of magnitude and are significantly lower than the values obtained for the gum sample obtained from *A. seyal*. These results are consistent with other data published in the literature, which also shows, as mentioned previously, that such values can vary depending upon the precise molecular structure/composition of the gum, which is affected by a plethora of biotic and abiotic factors (Idris, Williams & Phillips, 1998; Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Al-Assaf, Phillips, & Williams, 2005; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006).

The GPC elution profiles obtained using light scattering (LS), refractive index (RI) and UV detection at 280nm are presented in Figure 1. For the three *A. senegal* gum samples (Figure 1a, b and c) the RI profile shows a large peak, with a peak maximum at ~9.4 mL, together with a small shoulder on the high molecular mass side. The main

peak is attributed to the arabinogalactan (AG) fraction and the shoulder is attributed to the arabinogalactan-protein (AGP) fraction as reported previously (Randall, Phillips & Williams, 1989). The small peak at ~12.5 mL is the salt peak, (V_t). The UV elution profiles are significantly different to the RI profiles due to the fact that UV is sensitive not only to the concentration of the eluting material, but also to its chemical nature. We expect that the species detected by UV at 280nm are mainly proteinaceous in nature, but cannot rule out the additional presence of aromatic compounds such as polyphenols. The AGP peak has a higher intensity than the main AG peak, since it has previously been shown to contain more protein (Randall, Phillips & Williams, 1989). The very small shoulder observed at elution volumes ~ 10.0 -11.0 mL is attributed to the glycoprotein (GP) fraction previously identified (Randall, Phillips & Williams, 1989). It is of interest to note that, there is also a peak corresponding to V_t which may be due to single amino acid or phenolic molecules present in the samples. Interestingly, there are also a number of peaks observed at elution volumes greater than V_t, indicating that these components must interact with the column matrix. The LS profiles clearly demonstrate the presence of two molecular mass species, eluting at 8.0 mL and 9.0 mL, corresponding to the AGP and AG fractions respectively. It is noted that there is no significant difference observed between the molecular mass characteristics of the Sudanese and Nigerian samples.

The RI profile for the *A. seyal* gum sample (Figure 1d) shows a peak maximum at ~9.0 mL, but there was little evidence of a shoulder present corresponding to the AGP fraction as observed for the *A. senegal* samples. This is consistent with previous studies (Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Hassan, Al-Assaf, Phillips, & Williams, (2005), which have indicated the presence of a much smaller AGP content for the gum obtained from *A. seyal* and a lower protein content than for gum Arabic harvested from *A. senegal* (Table 1). The RI profile for this sample also shows a peak at ~11.2mL in addition to the salt peak at V_t and the UV profile indicates that both of the peaks contain UV absorbing material. It is also apparent that some UV absorbing species elute after V_t as for the *A. senegal* gum samples.

The RI and Mw elution profiles for the *Acacia* gum samples are presented in Figure 2 and show that for the *A. senegal* fractions (2a, b and c), the Mw for the AGP fraction eluting at the peak maximum (8.0 mL) for each of the samples is ~2M g/mol, while that for the AG fraction eluting at the peak maximum (9.4 mL) is ~250Kg/mol. For the *A. seyal* sample (Figure 2d) there is no observed peak for the AGP fraction, but the Mw for the AG fraction at the peak maximum (9.0 mL) is ~600Kg/mol. These results are consistent with previously published data (Siddig, Osman, Al-Assaf, Phillips & Williams, 2005; Idris, Williams & Phillips, 1998; Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012).

The RI and Rg elution profiles for the *Acacia* gum samples are presented in Figure 3 and show for the *A. senegal* Sudan and NG1 samples (Figures 3a and b) that the Rg for the AGP component at the peak maximum (8.0mL) is ~25nm. This is consistent with the observations of Renard et al (2012) as discussed above. It is not possible to determine the Rg at the peak maximum for the AG fraction since the values are too small, i.e. <10nm which is beyond the limits of the technique used. The Rg profile for the *A. senegal* NG2 sample is given in Figure 3c and shows an unexpected distortion in the line at an elution volume of ~8.0mL. Interestingly, the Rg profile for the *A. seyal* sample also shows a distortion in the line. We are unable to explain this observation, other than to say that the distortion occurs at elution volumes corresponding to the AGP fraction in the case of the NG2 sample indicating a different molecular structure. As discussed above, Renard et al (2012) have shown that the AGP component can exist in two different structural forms.

Rg is related to the molecular mass by the following equation.

$$Rg = K M^{\nu} \quad \text{..Equation 1.}$$

Where, K is a constant, M is the molar mass, and the exponent, ν, has a value that is dependent on the shape of the molecules. ν, can be determined from the slope of the line of the plot of log Rg – ν- log M and has typical values of 0.60, 0.33 and 1.0 for

random coils, homogeneous spheres and rod-like molecules respectively (Burchard (1999)). The log Rg - v- log M plots for the gum samples are presented in Figure 4. Figures 4a and 4b for the *A. senegal* Sudan and NG1 samples are linear and give values for ν of 0.43 and 0.48 respectively. These values are similar to data reported previously (Williams and Langdon 1995) and are consistent with a highly branched compact molecular structure as reported by Sanchez et al 2008 and Renard et al 2012. The plot for the *A. senegal* gum sample (NG2) gave a significantly higher value for ν of 0.67 indicating some structural differences (Figure 4c). The RI elution profiles (Figures 2a-c) indicate that this sample has a slightly higher AGP component that the other two *A. senegal* samples. The plot for *A. seyal* gum was not linear and a meaningful value for ν could not be obtained.

3.3 Hydrodynamic radius

The z-average hydrodynamic radius, R_h, of the gum samples was determined in the presence of 0.1M $NaNO_3$ as a function of their concentration and the results are presented in Figure 5. R_h was obtained by extrapolation to zero concentration and the results are presented in Table 3. The ratio of Rg / R_h also has characteristic values depending on the shape of the molecules with values of 0.778 for a homogeneous sphere and 1.78 for a random coil (Burchard, 1999). The values obtained for the *Acacia* gum samples of 1.0-1.4 are similar to those reported previously and are consistent with a highly branched compact structure (Idris, Williams, & Phillips, 1998).

3.4 Molecular association

The molecular association of the *Acacia* gum samples was followed by monitoring the hydrodynamic size as a function of time using dynamic light scattering. The natural log of the ratio of the initial hydrodynamic diameter to the diameter at time, t, (ln d/d_0) is given as a function of time in Figures 6a and 6b. Figure 6a presents the results obtained in deionised water and shows that ln d/d_0 increases over the 6h period for the three *A. senegal* gum samples in the order NG1>NG2>Sudan indicating that molecular association occurs. It is noted that there is an almost negligible increase for the *A. seyal* sample. The results presented in Figure 6b in the presence of

0.1M NaNO$_3$, show that ln d/d$_0$ remains constant over time for all of the samples indicating that molecular association does not occur. Li et al., (2009) also determined the hydrodynamic size of *A. senegal* gum samples by dynamic light scattering in both water and 6M urea. Samples were dissolved overnight and they found that the hydrodynamic size increased with increasing gum concentration and became particularly significant above gum concentrations of ~3%. The increase in hydrodynamic size was more pronounced in water compared to 6M urea and was reduced in both solvents after filtration. The increase in size was attributed to molecular association and since the size was lower in the presence of 6M urea compared to water it concluded that association was due to hydrogen bonding. In our study, it is interesting to note that the increase in hydrodynamic size for the samples follows the same trend as the sample protein content i.e. NG1>NG2>Sudan>Seyal. It is our belief that the molecular association results from electrostatic interaction between glucuronic acid and protein residues on different molecules, thus forming electrostatic complexes. The fact that the hydrodynamic size does not change in the presence of 0.1M NaNO$_3$, supports this observation since the electrolyte would screen electrostatic interactions. This concept also supports our previous hypothesis that gum Arabic forms multilayers at the oil-water interface due to protein - glucuronic acid electrostatic interaction (Padala, Williams, & Phillips 2009; Williams, 2012).

Conclusions

This study has shown that two *Acacia senegal* gum samples originating from two eco-locations in Nigeria, possess similar physicochemical characteristics to a *Acacia senegal* gum sample from Sudan, and that all three are distinctly different to a gum sample of Sudanese origin, harvested from *Acacia seyal*. Interestingly the Nigerian samples were found to contain significantly more protein than the Sudanese samples. This may have a positive impact on the ability for these gums to stabilise oil-in-water emulsions, whether this is due to genotypic differences between the trees harvested or is merely due to biotic/abiotic stressors or a combination of the two is unknown. GPC/MALLS experiments have shown, as expected, that the samples contain AGP, AG and GP fractions and from the knowledge of their Mw, Rg and Rh it

is evident that the molecules have a highly branched compact molecular structure. It has been shown that the molecules will readily associate in solution and it is believed that the association is as a result of electrostatic interactions between proteinaceous components and glucuronic acid residues present within the molecules. This is borne out by the fact that the association occurs to a greater extent as the protein content increases and is inhibited in the presence of 0.5M NaNO3, which has clear implications for its use as an emulsifier in the beverage industry.

References

Anderson, D. M. W. (1986). Nitrogen conversion factors for the proteinaceous content of

gums permitted as food additive. *Food Additives & Contaminants*, *3(3),* 231–234.

Aoki, H., Al-Assaf, S., Katayama, T. & Phillips, G.O. (2007) Characterisation and properties of

Acacia senegal (L.) Willd.var.*senegal* with enhanced properties (Acacia (*sen*)

SUPERGUM): Mechanism of the maturation process. *Food Hydrocolloids,* 21, 329-337.

Association of Analytical Communities (AOAC) (1999). Official methods of analysis method

988.05. Gaithersburg, Maryland: AOAC International.

Blumenkrantz, N. & Asboe-Hansen, G. (1973). New method for quantitative determination

of uronic acid. *Analytical Biochemistry, 54(2),* 484–489.

Burchard, W. (1999). Solution properties of branched macromolecules. Advances in Polymer

Science, 143, 113–194.

Idris, O. H. M., & Haddad, G. M. (2012). Gum Arabic's (Gum Acacia's) journey from tree to

end user. Arabic' In *Gum Arabic* eds. J.F Kennedy, G.O Phillips and P.A Williams,

(pp. 3–19) RSC Publishing, Cambridge, UK.

Idris, O. H. M., Williams, P. A. & Phillips, G. O. (1998). Characterisation of gum from *Acacia*

senegal trees of different age and location using gel permeation chromatography. *Food*

Hydrocolloids, 12(4), 379–388.

Joseleau, J., & Ullmann, G. (1990). Biochemical evidence for the site of formation of gum

Arabic in *Acacia senegal. Phytochemistry, 29(11),* 3401–3405.

LI, x., Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K. & Zhang, H. (2009). Rheological study

of gum Arabic solutions: Interpretation based on molecular self-association*. Food*

Hydrocolloid, 23(8), 2394–2402.

Mahendran, T., Williams, P. A., Phillips, G. O., Al-Assaf, S. & Baldwin, T. C. (2008). New

Insights into the Structural Characteristics of the Arabinogalactan-Protein (AGP) Fraction

of Gum Arabic. *Journal of Agricultural and Food Chemistry, 56(19),* 9269–9276.

Osman, M. E., Menzies, A. R., Williams, P. A., Phillips, G. O. & Baldwin, T. C. (1993). The

molecular characterisation of the polysaccharide gum from *Acacia senegal*.

Carbohydrate Research, 246(1), 303–318.

Padala, S. R., Williams, P. A. & Phillips, G. O. (2009). Adsorption of gum Arabic, egg white

protein, and their mixtures at the oil-water interface in Limonene oil-in-water

emulsions*. Journal of Agriculture and food chemistry, 57(11),* 4964–4973.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1989). Fractionation and characterization of

gum from *Acacia* senegal*. Food Hydrocolloids, 3(1),* 65–76.

Renard, D., Lavenant-Gourgeon, Ralet, M. C., & Sanchez, C. (2006). Acacia senegal gum:

Continuum of molecular species differing by their protein to sugar ratio, molecular

weight and charges. *Biomacromolecules, 7(9),* 2637–2649.

Renard, D., Garnier, C., Lapp, A., Schmitt, C. & Sanchez, C. (2012). Structure of

arabinogalactan-protein from *Acacia* gum: From porous ellipsoids to supramolecular

architectures. *Carbohydrate Polymers, 90(1),* 322–332.

Renard, D., Lepvrier, E., Garnier, P., Roblin, P., Nigen, M. & Sanchez, C. (2014. Structure of

glycoproteins from *Acacia* gum: An assembly of ring-like glycoproteins modules.

Carbohydrate Polymers, 99(8), 736–747.

Sanchez, C., Renard, D. Robert, P., Schmitt, C., & Lefebvre, J. (2002). Structure and

rheological properties of *Acacia* gum dispersions. *Food Hydrocolloids, 16(1),* 257–267

Sanchez, C., Schmitt, C., Kolodziejczyk, E., Lapp, A., Gaillard, C., & Renard, D. (2008). The

Acacia gum Arabic fraction of a thin oblate ellipsoid: A new model based on small-angle

neutron scattering and A Initio calculation. *Biophysical Journal, 94(2),* 629–639.

Siddig, N. E., Osman, M. E., Al-Assaf, S., Phillips, G.O., & Williams, P.A. (2005). Studies on

Acacia exudate gums, part IV. Distribution of molecular components in *Acacia seyal* in

relation to *Acacia senegal*. *Food Hydrocolloids, 19(4),* 679–686.

Street, C. A. & Anderson, D. M. (1983). Refinement of structures previously proposed for

gum Arabic and other *Acacia* gum exudates. *Talanta, 30(11),* 887–893.

Williams, P. A. (2012) 'Structural characteristics and functional properties of gum Arabic' In

J.F Kennedy, G.O Phillips & P.A Williams (Eds.), *Gum Arabic* (pp. 179–187) Cambridge:

RSC Publishing, UK.

Williams, P.A., & Phillips, G.O. (2009). Gum Arabic. In G.O. Phillips and P.A. Williams (Eds.)

Handbook of Hydrocolloids (pp.252–273. Cambridge: Woodhead publishers Ltd Press.

Williams, P. A., Phillips, G. O., Stephen, A. M., & Churms, S. C. (2006). Gums and Mucilages.

In Stephen, A. M., Phillips, G. O., & Williams, P. A., (Eds.), *Food polysaccharides and their*

applications (p455). Boca Raton: CRC Press Taylor and Francis Group, FL.

Williams, P. A. & Langdon, M. J. (1995). Characterisation of gum Arabic by gel permeation

chromatography. *Chromatography and Analysis, Oct/Nov. 39,* 5–7.

 Yadav, M. P., Johnston, D. B., Hotchkiss, A. T., & Hicks, K. B. (2007). Corn fibre: A potential

 gum Arabic replacer for beverage flavour emulsification. *Food Hydrocolloids, 21(7),*

1022–1030.

List of Tables

Table 1. Sugar composition and protein content of *Acacia* gum exudates (% w/w) on dry weight basis

	A. senegal (Sudan)	*A. senegal* (NG1)	*A. senegal* (NG2)	*A. seyal* (Sudan)
Rhamnose	9.5	9.2	7.7	2.0
Arabinose	25.7	24.9	23.6	32.5
Galactose	38.9	45.3	38.8	44.2
Glucuronic acid	21.5	15.5	20.2	13.0
Total sugar (%)	95.6	94.9	90.1	91.7
Protein[a]	2.09	2.93	2.66	0.99
Protein [b]	1.69	2.12	2.13	0.69

[a]=From Kjeldhal method using NCF 6.60 as suggested by Anderson, (1986)

[b]=From amino acid analysis

Table 2. Amino acid composition of *Acacia* gum exudates (g/100g) on a dry weight basis

	A. senegal (Sudan)	*A. senegal* (NG1)	*A. senegal* (NG2)	*A. seyal* (Sudan)
Hydroxyproline	0.453	0.521	0.476	0.201
Aspartic acid	0.104	0.144	0.167	0.0409
Threonine	0.139	0.170	0.168	0.0463
Serine	0.217	0.278	0.267	0.100
Glutamic acid	0.0726	0.109	0.136	0.0262
Proline	0.114	0.140	0.140	0.0531
Glycine	0.0500	0.0627	0.0660	0.0162
Alanine	0.0295	0.0402	0.0435	0.0162
Valine	0.0578	0.0810	0.0923	0.0307
Methionine	0.00260	0.00276	0.00176	0.00113
Isoleucine	0.0237	0.0302	0.0272	0.0108
Leucine	0.139	0.174	0.174	0.0534
Tyrosine	0.0188	0.0234	0.0171	0.00982
Phenylalanine	0.0745	0.016	0.130	0.0208
Histidine	0.124	0.143	0.135	0.0443
Lysine	0.0509	0.0702	0.0688	0.0117
Arginine	0.0208	0.0268	0.0245	0.00933

Table 3. Molar mass and hydrodynamic size of *Acacia* gum exudates obtained by GPC/MALLS.

	A. senegal (Sudan)	*A. senegal* (NG1)	*A. senegal* (NG2)	*A. seyal* (Sudan)
Mw	6.4×10^5	4.85×10^5	7.43×10^5	1.14×10^6
Mn	3.52×10^5	2.77×10^5	3.94×10^5	6.84×10^5
Mw/Mn	1.9	1.8	1.9	1.7
Rg	18.5	13.0	17.9	24.0
Rh	15.2	13.0	15.4	17.3
Rg/Rh	1.2	1.0	1.2	1.4
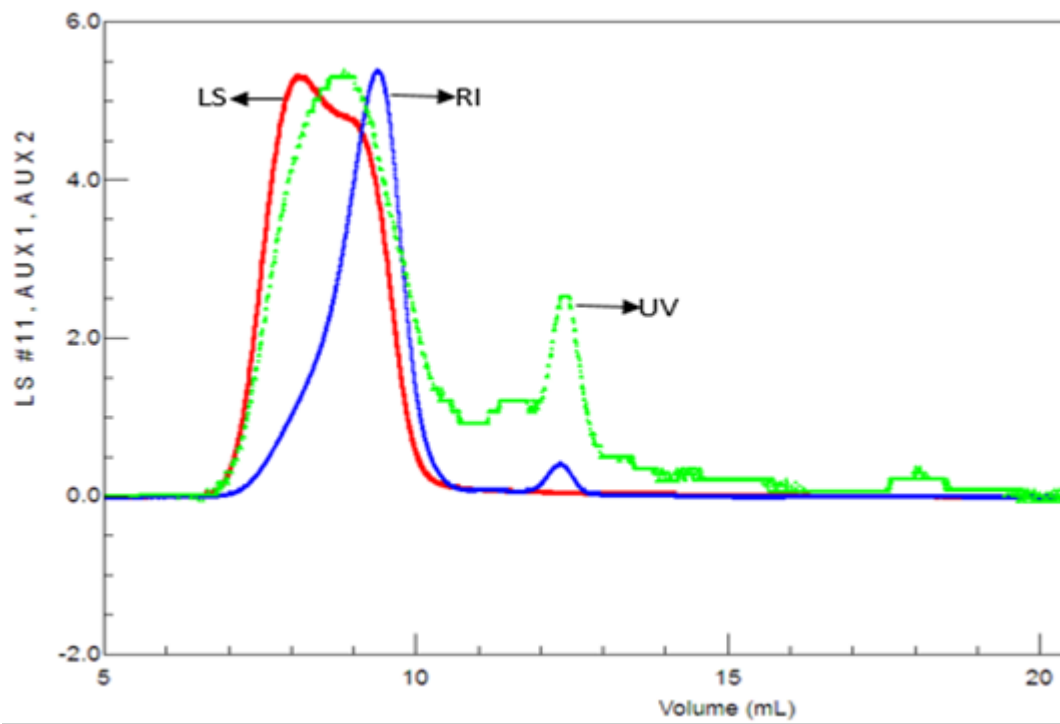
Fig. 1a. GPC elution profiles of *Acacia senegal* (Sudan) gum sample using LS/RI/UV detectors
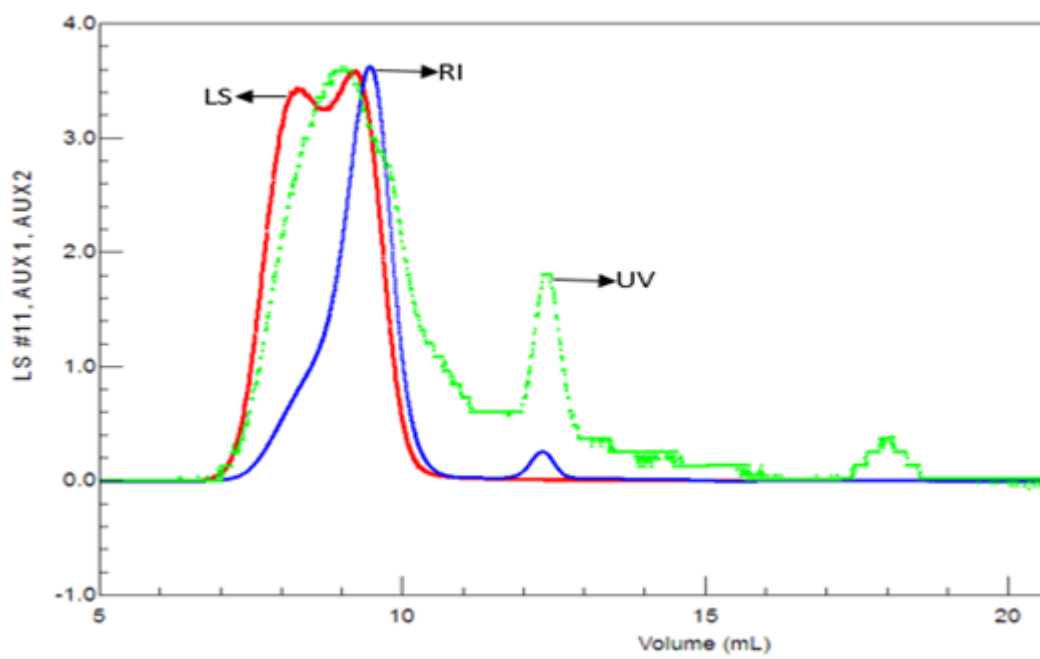
Fig.1b. GPC elution profiles of *Acacia senegal* (NG1) gum sample using LS/RI/UV detectors
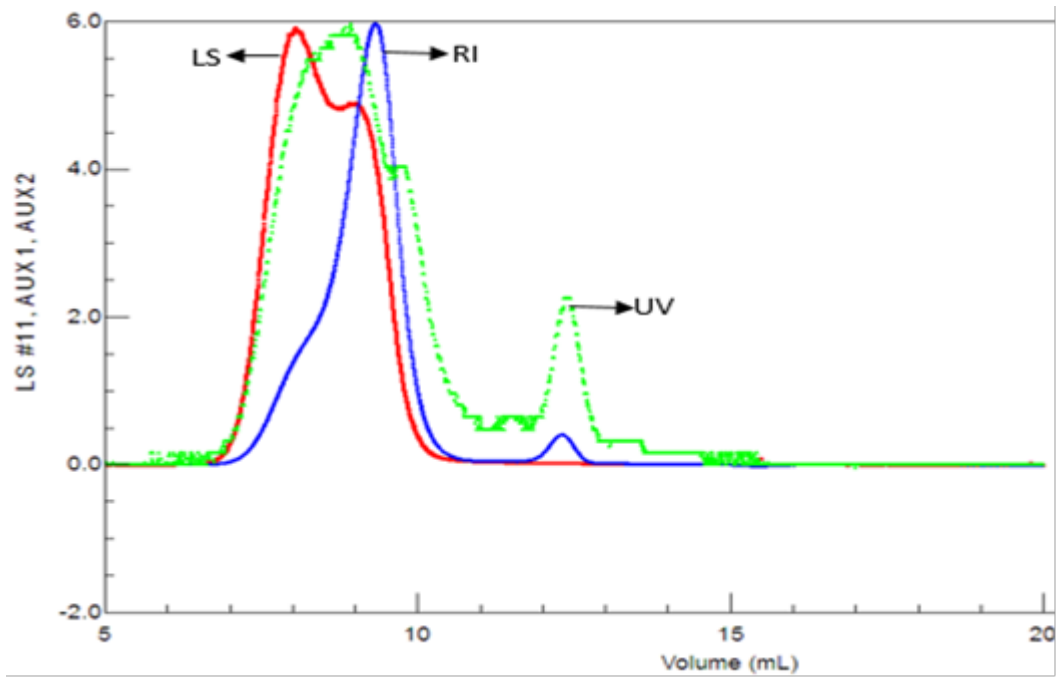
Fig.1c. GPC elution profiles of *Acacia senegal* (NG2) gum sample using LS/RI/UV detectors
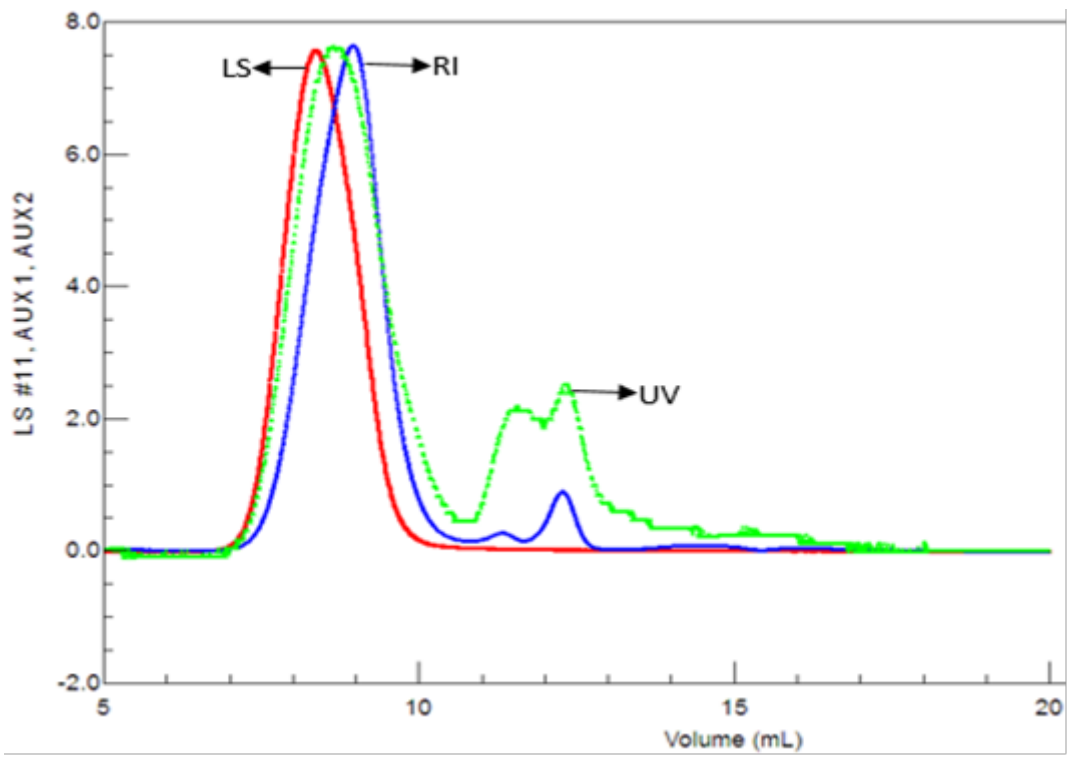
Fig.1d. GPC elution profiles of *Acacia seyal* (Sudan) gum sample using LS/RI/UV

detectors
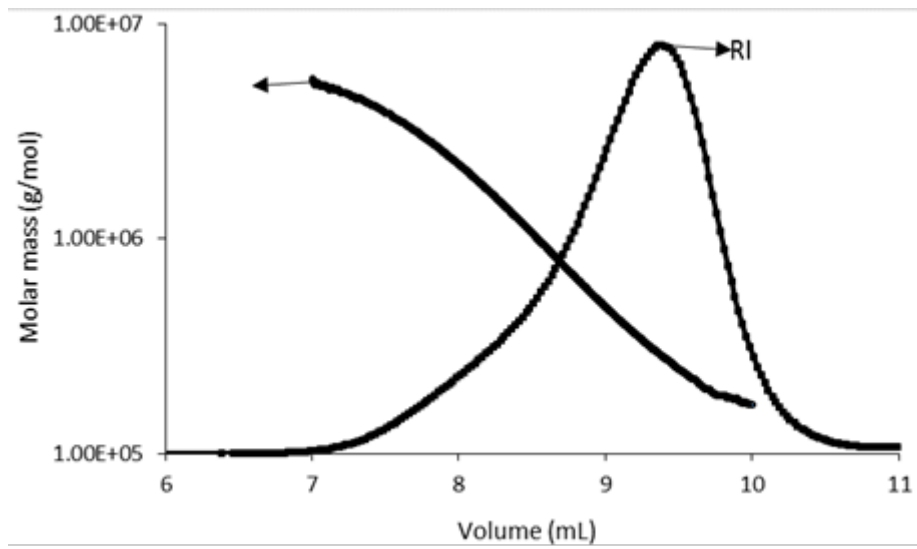
Fig 2a. RI/Mw profile for *Acacia senegal* (Sudan) gum sample
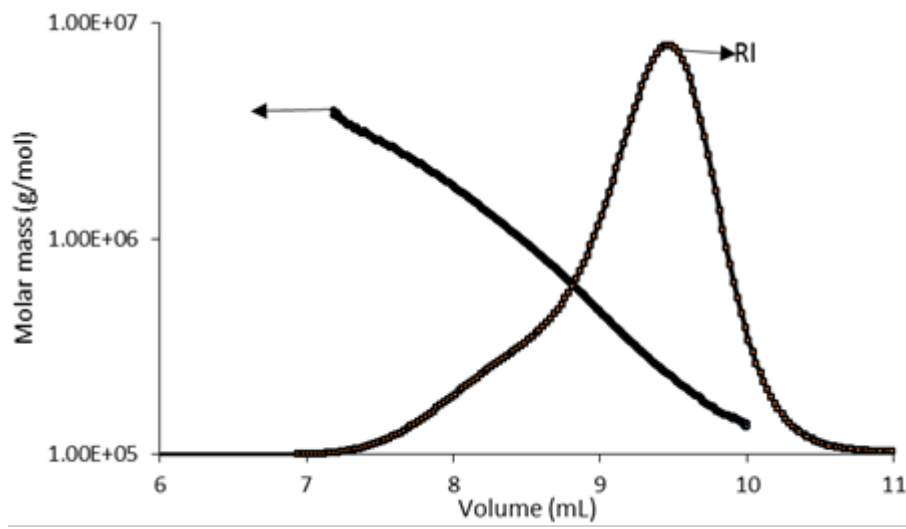
Fig 2b. RI/Mw profile for *Acacia senegal* (NG1) gum sample
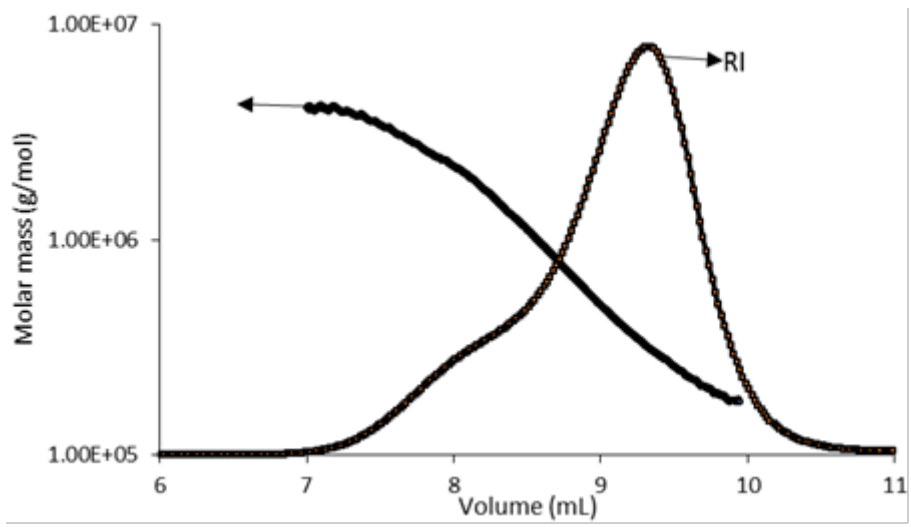
Fig 2c. RI/Mw profile for *Acacia senegal* (NG2) gum sample
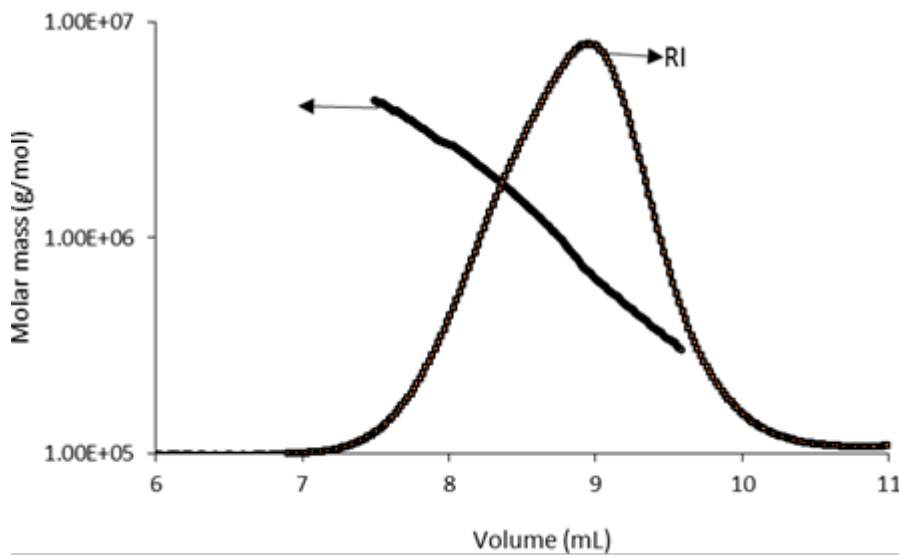
Fig 2d. RI/Mw profile for *Acacia seyal* (Sudan) gum sample
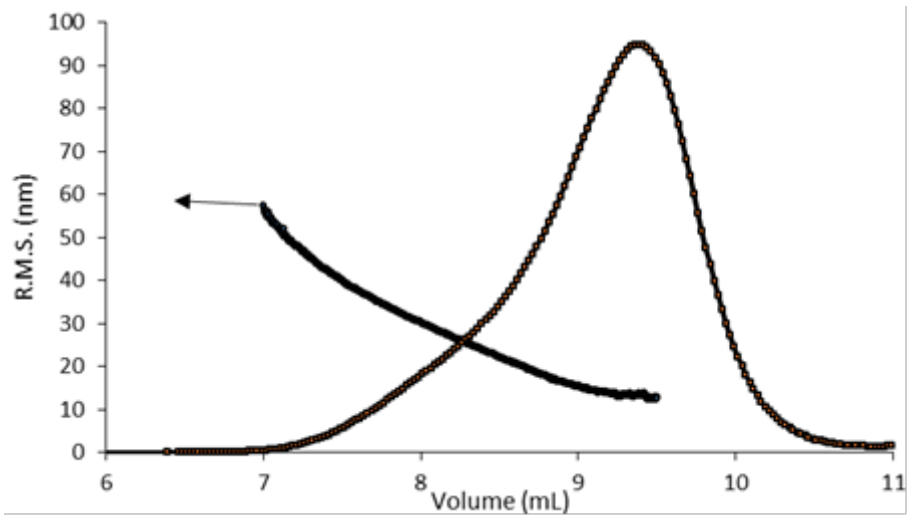
Fig. 3a. RI/Radius profile of *Acacia senegal* (Sudan) gum sample
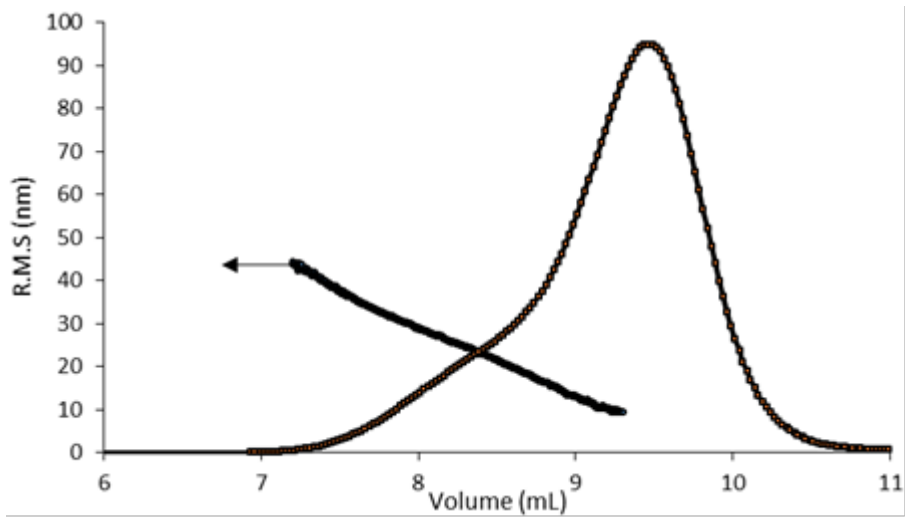
Fig. 3b. RI/Radius profile of *Acacia senegal* (NG1) gum sample
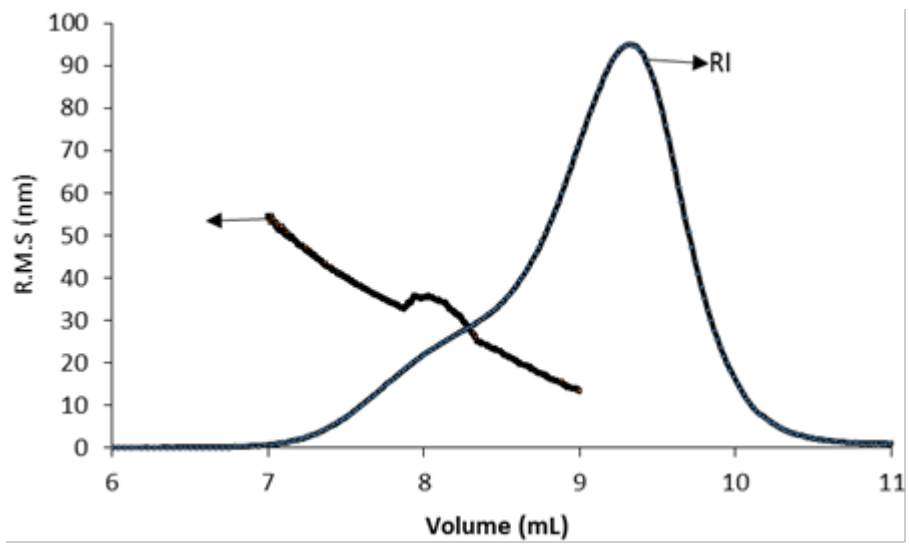
Fig. 3c. RI/Radius profile of *Acacia senegal* (NG2) gum sample
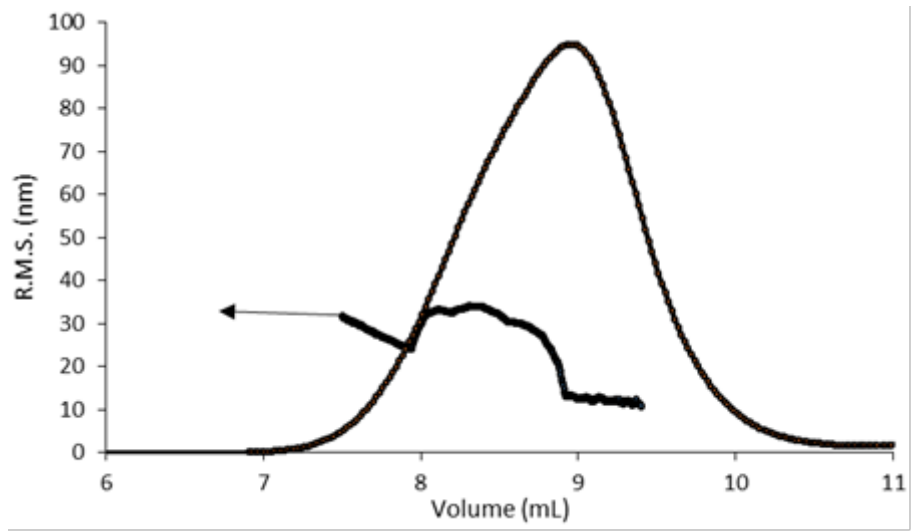
Fig 3d. RI/Radius profile of *Acacia seyal* (Sudan) gum sample
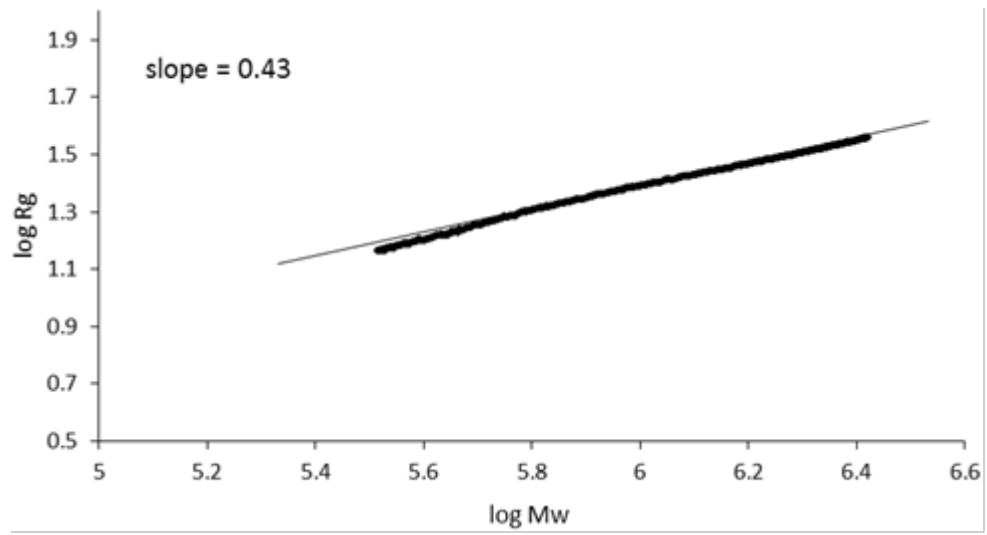
Fig. 4a. log Rg against log Mw of *Acacia senegal* (Sudan) gum sample
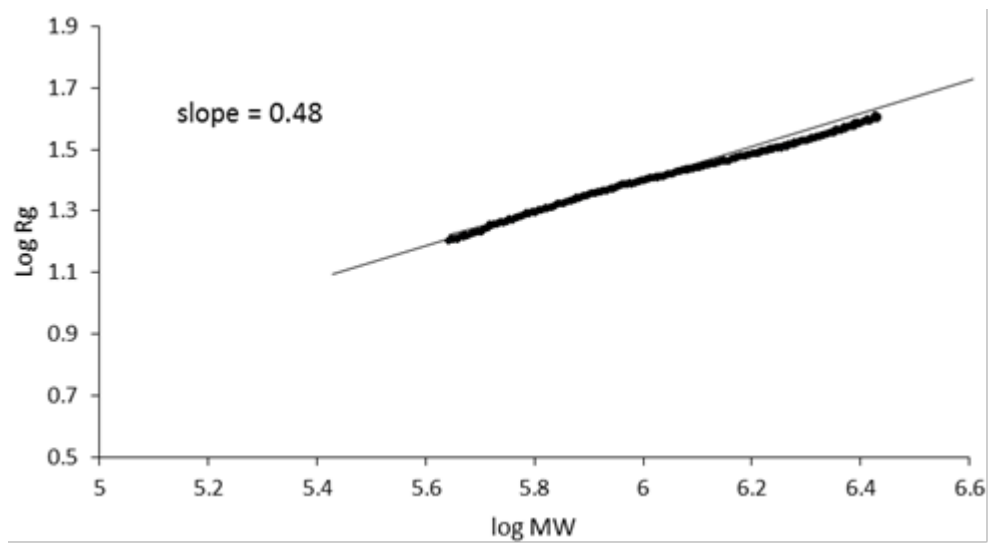
Fig. 4b. log Rg against log Mw of *Acacia senegal* (NG1) gum sample
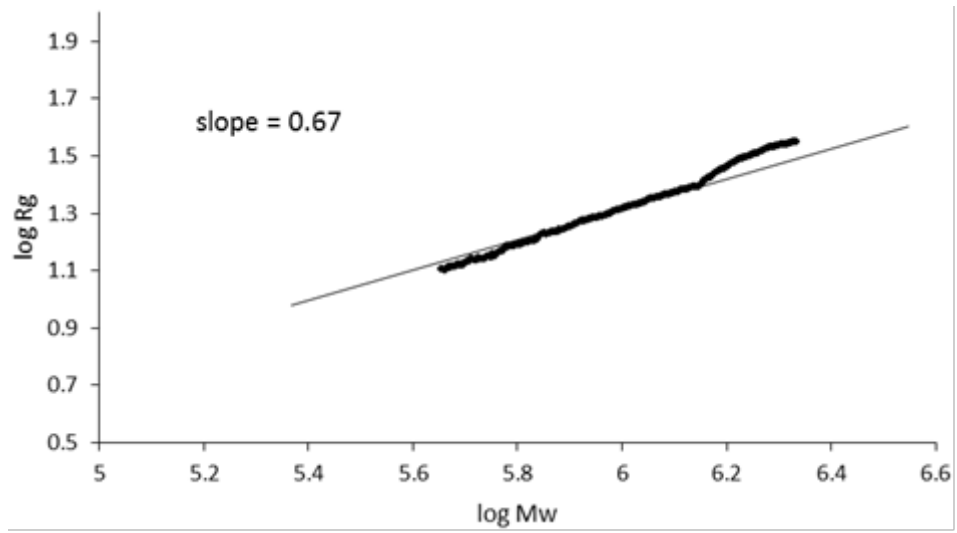
Fig. 4c. log Rg against log Mw of *Acacia senegal* (NG2) gum sample
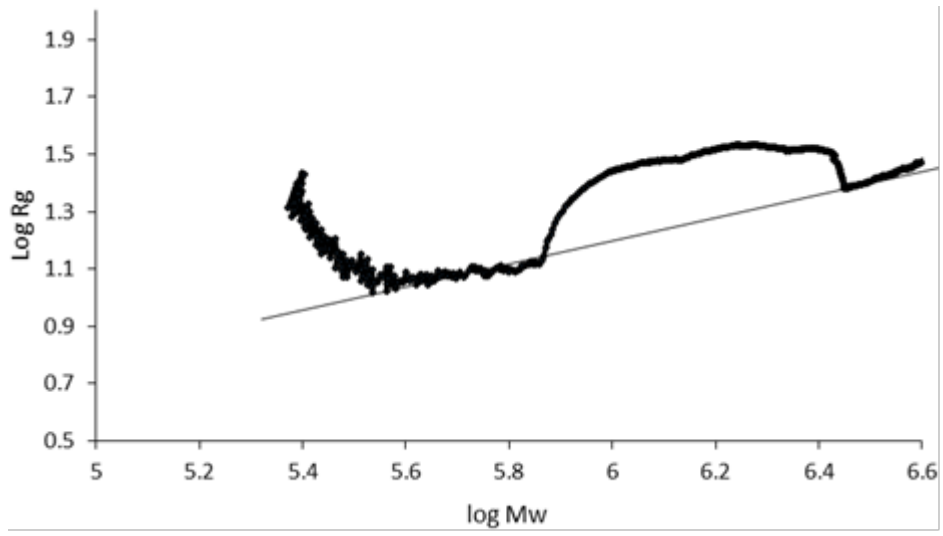
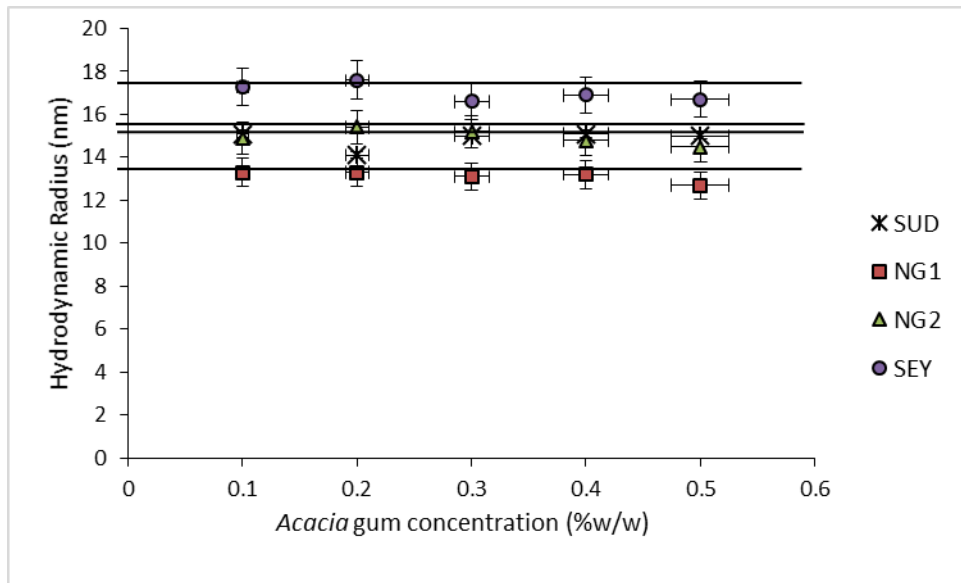Fig. 4d. log Rg against log Mw of Acacia seyal (Sudan) gum sample

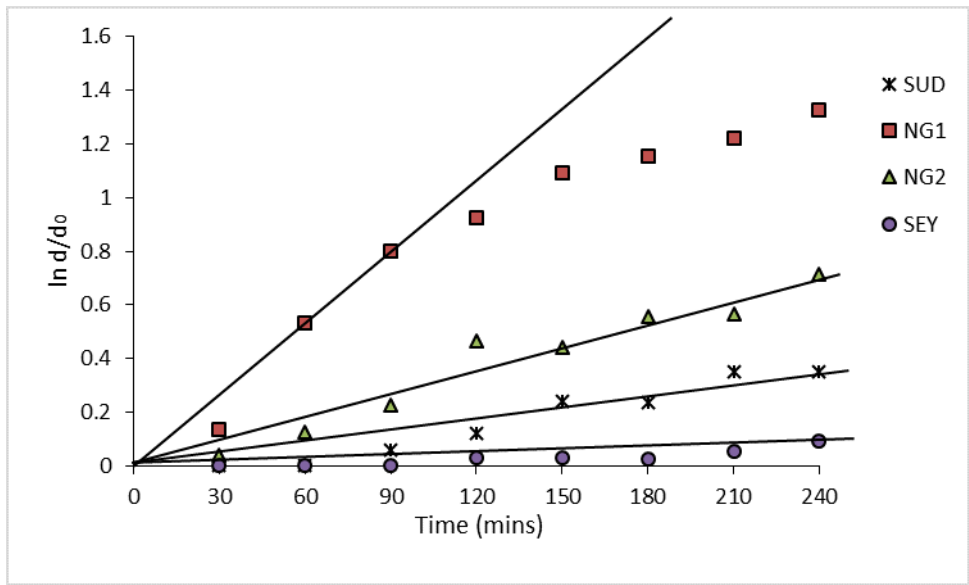Fig. 5. Hydrodynamic radius of Acacia gums as a function of concentration in 0.1M NaNO$_3$

Fig. 6a. ln d/d$_0$ of 10% solutions of Acacia gums in water as a function of time
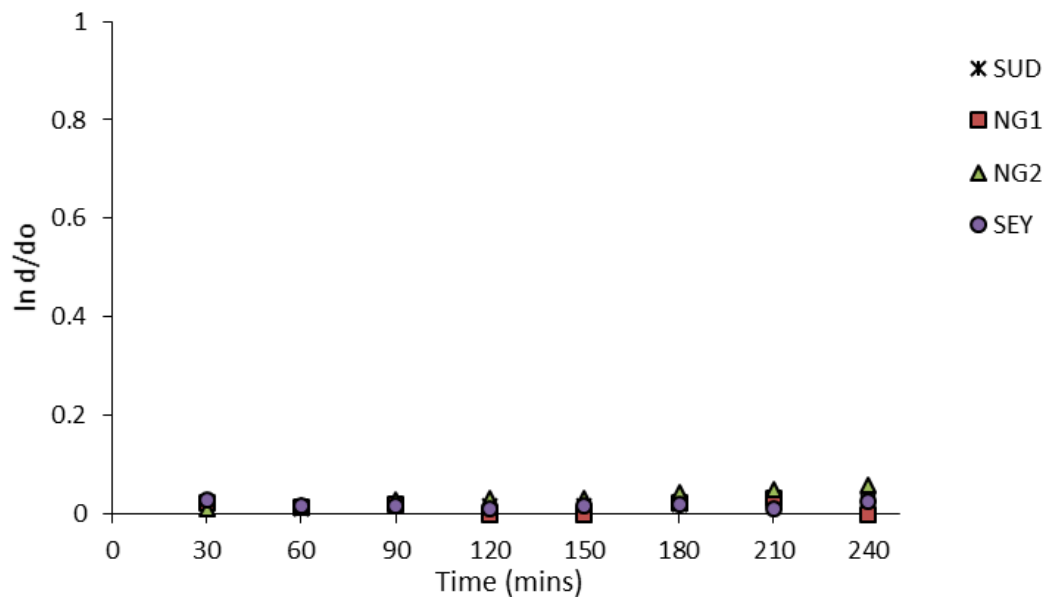
Fig. 6b. ln d/d_0 as a function of time for 10% solutions of *Acacia* gums in 0.1M $NaNO_3$