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RESEARCH ARTICLE

PRELIMINARY MACROMOLECULAR ANALYSIS OF *ACACIA* GUM EXUDATES FROM *ACACIASENEGAL*AND *ACACIA SEYAL*

[*] I.B. Gashua[1,2], E.P. Tawari[1,3], M.N. Fakuta[4] and H. Bello[1,5].

1. School of Applied Sciences, University of Wolverhampton, Wolverhampton, United Kingdom.
2. Department of Science Laboratory Technology, Federal Polytechnic Damaturu, Yobe State, Nigeria.
3. Department of Chemical Pathology, Niger Delta University, Amassoma, Bayelsa State, Nigeria.
4. Rubber Research Institute of Nigeria, Gum Arabic Sub-Station, Gashua, Yobe State, Nigeria.
5. College of Science and Technology, Adamawa State Polytechnic, Yola, Nigeria.

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Corresponding Author

I.B. Gashua.

Abstract

Acacia senegal and *Acacia seyal* are important agro forestry cash crops indigenous to several countries of sub-Saharan Africa. The gum exudates produced by these species is termed gum Arabic which is an approved food additive (E414), primarily used as an emulsifier. In the current study, the molecular structure and biophysical properties of gum samples harvested from mature trees of *A.senegal* and *A. seyal* have been investigated. Preliminary analyses of the gums using transmission electron microscopy showed the presence of varied macromolecules, ranging in size from ~8 - ~60 nm. Furthermore, it was observed that molecular interaction among the molecules occurred in solution after incubation for five days at room temperature (25^0C).

Introduction:-

Acacia gum exudate (Gum Arabic) is a complex, polysaccharide-based plant gum, obtained from trees of selected *Acacia* species (i.e. *Acacia senegal*(L.) Wild. and*Acacia seyal* Del.), which are indigenous to the Sahelian region of the African continent, for which Sudanis the world's leading producer followed by Nigeria and Chad (UNCTAD, 2009). It is a highly heterogeneous substances which contain variation in monomer composition in the linking and branching of its monomer units. The gum also vary in the molecular mass distribution of the component macromolecules, both in terms of polysaccharides and glycoproteins/proteoglycans. The consequence of this heterogeneity is reflected in the variety of molecular species of the gum, which changes according to both the mode of separation and the method of detection used.

The precise molecular composition of the gums harvested from *A. senegal* and *A. seyal* differ, but the molecular structure recorded for the most abundant molecular constituent of both gums (i.e. arabinogalactan), is the same and consists of a core of 1,3-linked galactose units with branches linked through the 6 position consisting of galactose and arabinose terminated by rhamnose and glucuronic acid (Siddig, et al., 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, et al., 2005; Mahendran, et al., 2008).

The macromolecules present in gum Arabic have a tendency to self-aggregate as has been noted in the literature (Renard et al., 2014; Gashua et al, 2015). This phenomenon was described by Li et al., (2009) using rheological and dynamic light scattering to have significant influence on the rheological behaviour of the gum in solution.

The gum produced by *A.senegal* and *A. seyal* species is widely used in the food industry as an emulsifier, for flavour oils present in a wide variety of beverages where it prevents flocculation and coalescence, and inhibits destabilization caused by creaming. In food that contains fat and/or oil, gum Arabic is also used as an emulsifier to maintain uniform distribution of the fat through the product (Wyasu and Okereke, 2012). It is also extensively utilised in confectionery products, in which it is used to control texture and inhibit sugar crystallisation (Williams and Phillips, 2009).

The main objective of this work was to obtain a preliminary understanding of the molecular structure of two selected Acacia gum exudates harvested from *Acaciasenegal*and *Acacia seyal* using chromatographical technique and transmission electron microscopy (TEM) and too observe molecular aggregation, using TEM and scanning transmission electron microscopy (STEM).

Material and Methods:-

Plant Materials:-

*A. senegal*and *A. seyal* gum exudate samples were obtained from the Phillips Hydrocolloids Research Centre, Glyndwr University, United Kingdom. The gum samples were obtained in the form of raw gum nodules and had not been processed in any way prior to the current investigation.

Methodology:-

Determination of Molecular mass parameters:-

The molecular mass properties of the samples were determined by multidetectional gel permeation chromatography (GPC). The system comprised of a Superose 6 column and a vacuum degasser (type 006150/4 Cambridge, U.K). The eluent used was 0.1 M sodium nitrate containing 0.005% sodium azide (which acts as a bactericide) filtered with a GSWP 0.22 µm Millipore membrane filter, under reduced pressure before use.

The samples were passed through a 0.45 µm pore size syringe filter prior to injection into the system, through a rheodyne injection valve (Rheodyne Inc., USA) fitted with a 200 µm volume loop and delivered at constant flow rate of 0.5 mL/min using an HPLC pump (Waters corporation 515). The eluent from the column was monitored by multi-angle laser light scattering (MALLS) in conjunction with an Optilab DSP refractive index (RI) detector (Wyatt Technology) and an Agilent 1100 UV spectrophotometer detector set at a wavelength of 280 nm. The refractive index increment (dn/dc) value used was 0.141 mL/g (Padala, Williams, and Phillips, 2009). Data was captured using ASTRA 4.90.80 software (Wyatt Technology) and was analysed using the first order polynomial (Zimm model) (Ratcliffe et al., 2005).

Transmission Electron Microscopy (TEM):-

Negative staining:-

Solutions of 1% (w/w) *A. senegal* and *A. seyal* gum samples were prepared in sterile deionised water.

Formvar/carbon-coated nickel (TEM) grids (200 mesh) were incubated on the surface of 30 µl drops of the sample solutions for 90 seconds. Excess liquid was carefully removed by touching the edge of the grids on to filter paper (wick).

The grids were then negatively stained by placement on a 30 µl drop of 2% (w/w) uranyl acetate for 90 seconds. Excess uranyl acetate was removed from the grids as described above. The negatively stained grids were then air dried, prior to being observed on a JEOL JEM-1200 EX Transmission Electron Microscope, at an accelerating voltage of 80 kV. The images were photographed using a GATAN retractable multi scan camera.

Scanning transmission electron microscopy (STEM):-

Scanning transmission electron microscopy (STEM) imaging was performed in a JEOL 7000F SEM using the transmitted electron detector. The work was performed at 20 kV. Samples were prepared as described previously on formvar/carbon-coated Nickel 200 mesh grids

Molecular interaction:-

Solutions of 1% (w/w) *A. senegal* and *A. seyal* gum samples were prepared with sterile distilled water for the aggregation experiments. The experiment was performed to study change in molecular size and shape of the gum Arabic molecules in aqueous solution over time. The sample solutions were incubated at room temperature (25 ^{0}C) in a stationary position, for a period of 5 days before the observations were recorded

A 30 µl of each sample solution was applied to a formvar/carbon-coated TEM nickel grid (200 mesh) for 90 seconds then air dried. The grids were negatively stained for 90 seconds with 2% (w/w) uranyl acetate. They were then air dried prior to observation as described for the negative staining procedure.

Results and Discussion:-

The GPC elution profiles for the *A.senegal* and *A. seyal* gum samples as monitored by light scattering (LS), refractive index (RI) and Ultra Violet (UV at 280 nm wave length) are presented in Figures 1 and 2. The distribution of sizes of the different molecular species are indicated by the LS, and it can be observed that there are more of the larger size molecules in the sample, as they are eluted from the column first. The RI give the concentration profile of the eluting species which showed that there is low concentration of the larger molecules eluting at ~8 mL arabinogalactan protein (AGP). Most of the molecules are found at ~9 – 10 mL arabinogalactan (AG). The UV gives the concentration and chemical nature of the molecules , especially the proteinaceous components glycoprotein fractions (GP).

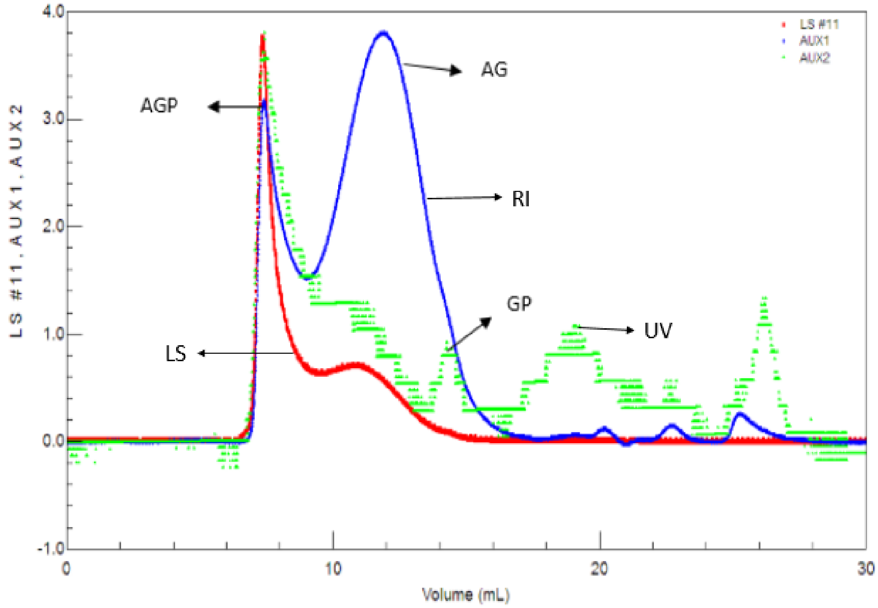

Figure 1. GPC elution profile of gum Arabic (*A. senegal*) monitored using LS, RI and UV detector at 280 nm. (where, AGP= Arabinogalactan Protein, AG= Arabinogalactan, and GP= Glycoprotein). This is applicable to Figure 2 for A. seyal.

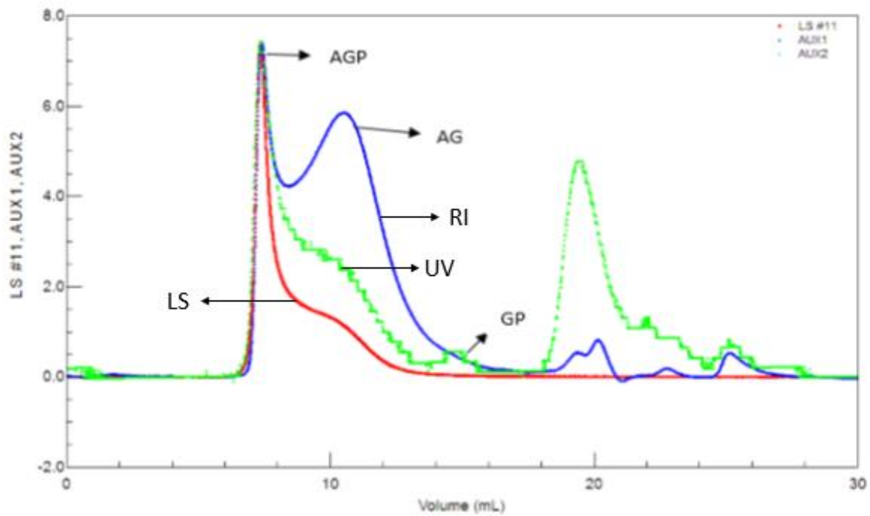

Figure 2. GPC elution profile of gum Arabic (*A. seyal*) monitored using LS, RI and UV detector at 280nm.

The Figures 3 and 4, reports the RI/R.M.S (Root mean square radius of giration-$R_{g)}$ profiles for the gum samples as a function of elution volume determined using the Astra software designed and accompanied with the specific GPC model. It is not possible to determine the R_g across the whole molecular mass profile since the samples contain molecules with R_g less than ~10 nm which is below the limits of this technique. The RI and R_g elution profiles for this samples showed that the R_g for the AGP component at the peak maximum of 8.5 mL is ~25 nm. It is not possible to determine the R_g at the peak maximum for the AG fraction since the values are too small, i.e. <10 nm which is beyond the limits of the technique used as mentioned earlier.

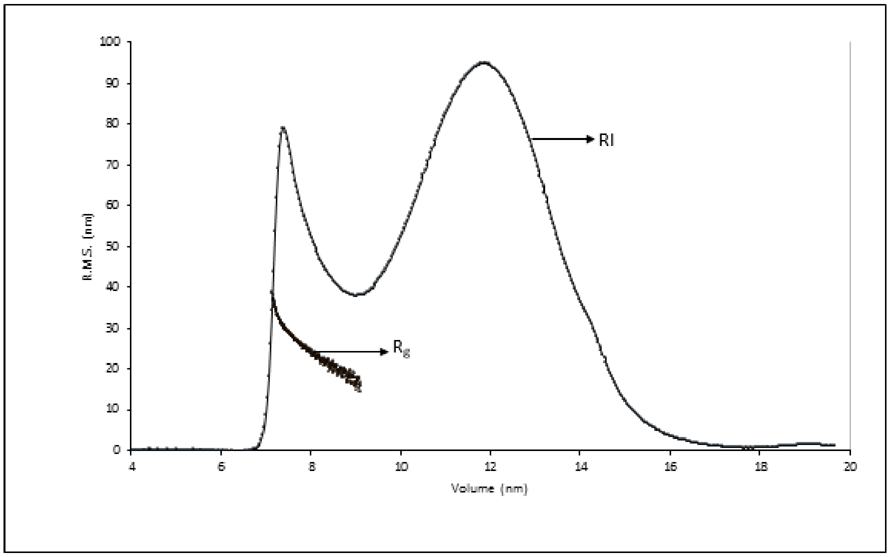

Figure 3. RI / R.M.S (Root mean square radius of gyration (R_g) elution profile of gum Arabic (*A. senegal*) gum sample. (R.M.S. = Root mean square radius of giration or R_g).

From the RI/R.M.S ($R_{g)}$ profile of *A. seyal* gum sample presented in Figure 2, it is interesting note that, the R_g profile shows a distortion. This observation occurs at elution volumes corresponding to the AG fraction indicating a different molecular structure. Schittenhelm and Kulicke, (2000) reported that a plot of log Root Mean Square (R.M.S) radius of gyration (R_g) versus log molecular weight (Mw) has been known to reveal useful information about the conformation of polymer solution. This will further be considered in the follow up research to this paper.

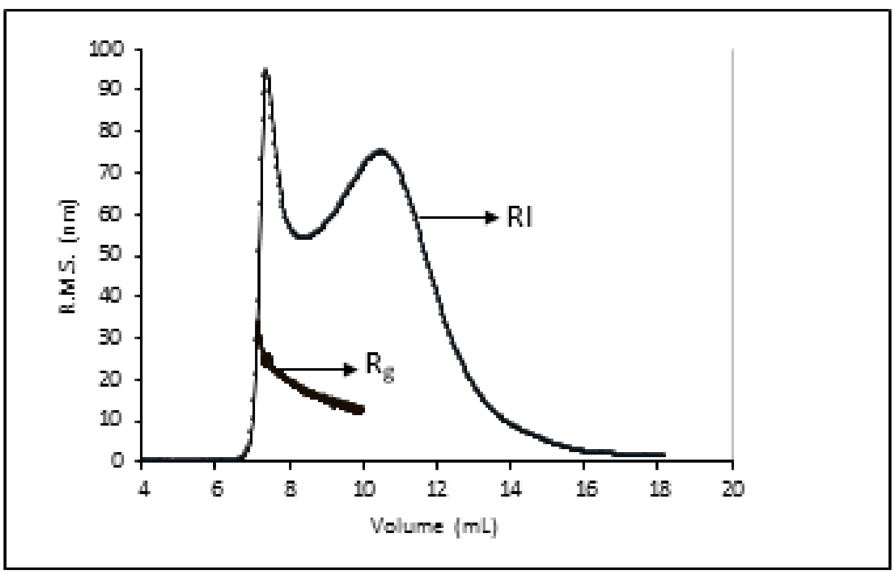

Figure 4. RI / R.M.S (Root mean square radius of gyration (R_g) elution profile of gum Arabic (*A. seyal*) sample

The weight average (Mw) and number average (Mn) molecular mass values obtained by GPC are given in Table 1. The values for the *A. senegal* gum sample of $6.52x10^5$ and $3.64x10^5$ for Mw and Mn respectively, are significantly lower in magnitude than the values of $1.02x10^6$ and $6.11x10^5$ recorded for the gum sample obtained from A. seyal. The average molecular weight of the gum collected from *A. seyal* has been previously reported to be higher than that of *A. senegal* and that *A. seyal* is more highly branched and more compact in structure than *A. senegal* (Hassan, et al., 2005). These results are consistent with the literature, which also showed, that such values can vary depending upon the precise molecular structure/composition of the gum, which is affected by an excess of biotic and abiotic factors (Renard, et al., 2006).

Table 1. Molar mass parameters of *Acacia* gum exudates (*A.senegal* and *A. seyal*) obtained by GPC-MALLS.

	Acacia senegal	*Acacia seyel*
Mw	$6.52x10^5$	$1.02x10^6$
Mn	$3.64x10^5$	$6.11x10^5$
R_g	16.5	21.0
Mw/Mn	1.8	1.7

Mw= Average molecular weight, Mn= Average number molecular mass, R_g= Root mean square radius of gyration (R.M.S), Mw/Mn= Polydispersity index.

The RI/R_g elution profiles for the *A. senegal* (figure 3) sample indicated that the R_g for the AGP component at the peak maximum (8.5 mL) is ~25 nm. This is consistent with the observations of Renard et al., (2012). The RI elution profiles also indicated that the samples contain a higher AGP component than the *A. seyal*.

The results for the transmission electron microscopy (TEM) showed that both the A. *senegal* and A. *seyal* gums comprised of an array of different sized molecules identified as putative AG, AGP, and GP molecules. The sizes of the molecules observed ranged from ~20 nm for AG, ~60 nm for AGP and ~8 - 12 nm GP Figures 5 and 6. In this study, molecules with ~12 nm diameter (Figure 5A) and ~8 nm diameter (Figure 5B) were observed in the midst of the more frequently observed ~20 nm (AG) and ~60 nm (AGP) particles. These values are similar to those reported by Renard et al., (2014) for the component of gum Arabic.

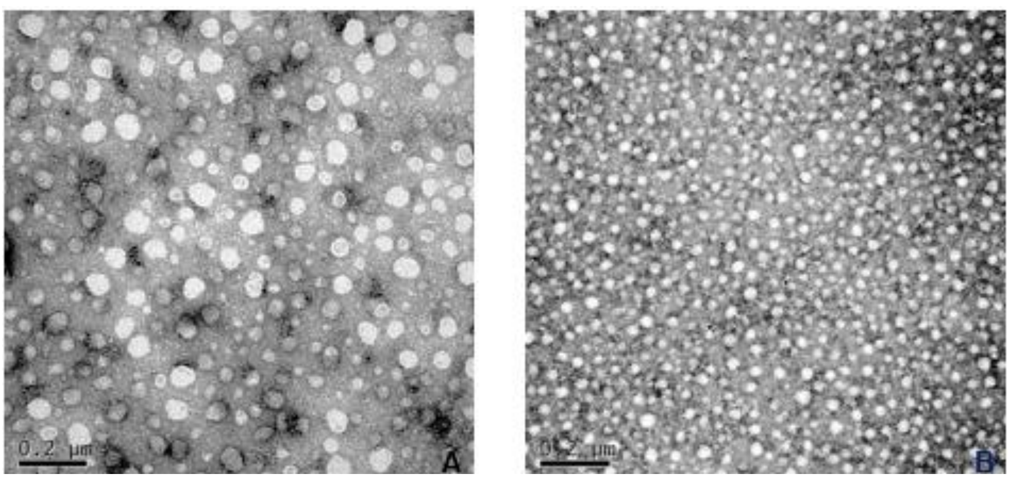

Figure 5.Transmission electron micrograph of *A. senegal* (A) and *A. seyal* (B) gum samples (1% w/w) negatively stained, showing the distribution of molecules present in the sample. It can be observesd that the A. senegal gum sample has distinctly larger molecules than the *A. seyal* gum sample. This could be attributed to the difference in the molecular properties and/or chemical nature of the two gums.

The images of *A. senegal* obtained using the Scanning Transmission Electron Microscope (STEM) are presented in Figure 6. The Images are taken at two different magnifications as shown in the figure, with A= X6, 500 and B= X16, 000. Larger aggregated molecules were observed. This is to further elucidate the structure of the molecules. The structure of aggregated molecules is clearly visible using this technique which confirms the structure observed using the negative staining of TEM.

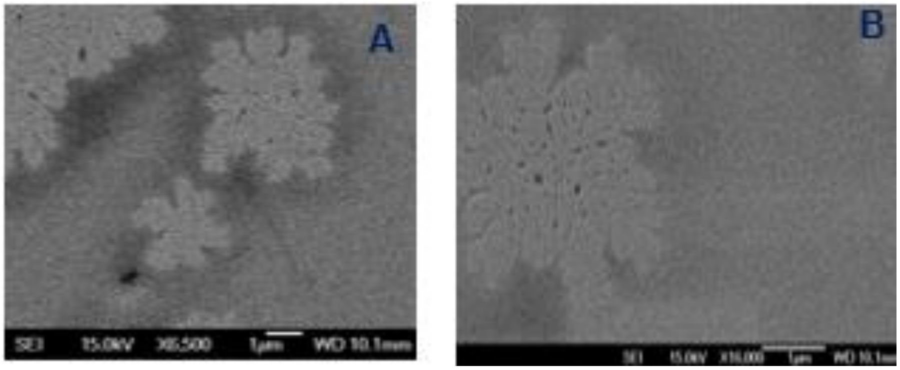

Figure 6. Scanning transmission electron micrographs of *A.senegal* sample after incubation for 5 days at room temperature. Images taken at two magnifications are shown A, X6, 500 and B, X16, 000.

Figure 6 is the STEM image of *A. senegal* after 5 days. The image for *A. seyal* was not reported due to technical hitch encountered during the course of the analysis. (The scale bar = 0.2 µm in both micrographs).
The level of interaction of molecules in aqueous solutions of both *A.senegal*and *A. seyal* gum samples were monitored after five days so as to assess their level of interaction with time using TEM, and the results are presented in figures 7 (A) and (B). In this study, molecules with ~12 nm diameter and ~8 nm diameter were observed in the midst of the more frequently observed ~20 nm (AG) and ~60 nm (AGP) particles.These values are similar to those reported by Renard et al., (2014)for the glycoprotein component(s) of the gum Arabic.

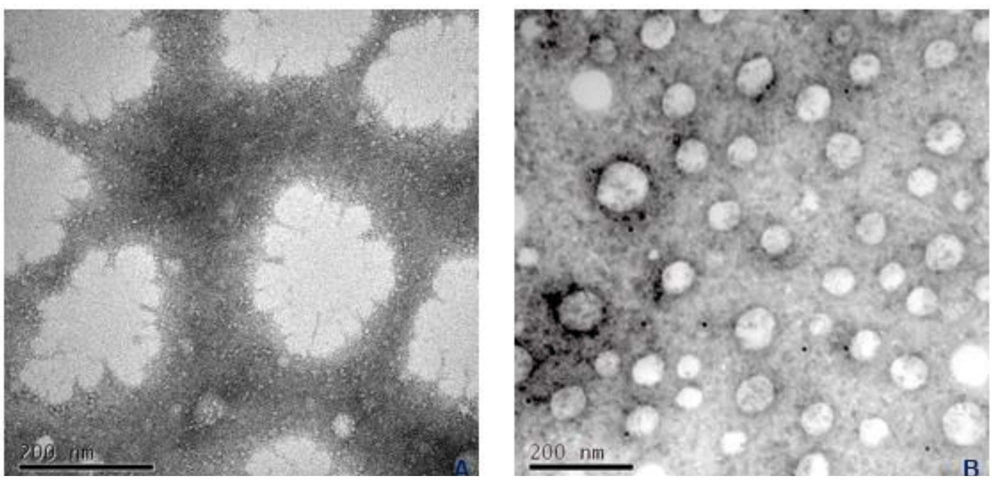

Figure 7. Transmission electron micrographs of *A.senegal* (A) and *A. seyal* (B) gums negatively stained and observed after 5 days. Distinct disc-like aggregates were observed in the *A.senegal* gum in varied sizes with an average diameter of ~184 nm. The *A. seyal* gum also contains aggregates, with average diameter range of ~37 nm but the aggregated are not disk-like manner, they seem to be more ellipsoidal in shape. (The scale bar = 200nm in both micrographs).

The rate of aggregationdue to interaction of gum Arabic macromolecules could be attributed to the nature of interaction of the molecules in the different gums due to the amount and accessibility of the protein components of the different gums. Similar observation was made by Williams, (2012), in which multilayers were reported to be formed as a result of aggregation of molecules due to interaction in solution. In the case of *A. seyal* gums, it shows less aggregation in solution. This is because, its protein is less accessible and therefore have a lower affinity for hydrophobic surfaces as reported previosly by Flindt et al., (2005).

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