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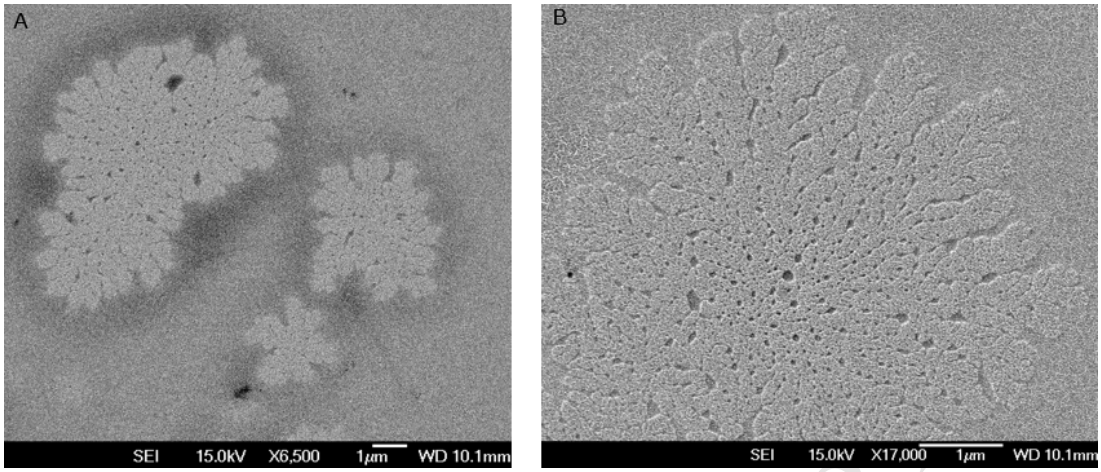

Molecular characteristics, association and interfacial properties of Gum Arabic harvested from both *Acacia senegal* and *Acacia seyal*.

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Abstract

The molecular composition of *Acacia senegal* and *Acacia seyal* gum exudate samples were studied using transmission electron microscopy. The molecules observed in both samples were found to have diameters of either ~ 20µm, ~60 µm or ~ 10µm. These most likely represent the arabinogalactan (AG), arabinogalactan-protein (AGP) and glycoprotein (GP) molecules present in *Acacia* gum exudates. Micrographs obtained for gum solutions that had been left to stand for up to 5 days, indicated that molecular aggregation had occurred, this was particularly evident for the *Acacia senegal* sample. This aggregation process was attributed to intermolecular electrostatic interactions. The adsorbed layer thickness of the gums adsorbed onto polystyrene latex particles was determined using dynamic light scattering. For the *Acacia senegal* gum sample, it was found that the adsorbed layer thickness increased over time and after 14 days had a value of 61nm. These findings are indicative of multilayer adsorption, through intermolecular electrostatic interaction. For the *Acacia seyal* gum sample the adsorbed layer thickness was only ~3nm and did not increase over time. Transmission electron microscopy revealed the presence of a distinct, thick adsorbed layer for the *Acacia senegal* gum and the presence of a much thinner, more diffuse layer for the *Acacia seyal* gum sample. Emulsification studies showed that the *Acacia senegal* gum was more effective at stabilising limonene oil-in-water emulsions than the *Acacia seyal* sample and that this was because markedly more *Acacia senegal* gum adsorbed at the oil-water interface compared to the *Acacia seyal* gum exudate.

Key words

Gum Arabic, molecular association, adsorbed layer thickness, transmission electron microscopy.

1. Introduction

Gum Arabic is a plant gum exudate obtained from the stems and branches of specific *Acacia* species, namely *Acacia senegal* and to a lesser extent *Acacia seyal* which grow across the Sahelian belt of Africa (Williams & Phillips, 2009; Williams, 2012; Williams, Phillips, Stephen, & Churms, 2006). Both of these gums have been shown to be primarily composed of complex polysaccharides, with a highly branched structure consisting of galactose, arabinose, rhamnose and glucuronic acid with the proportions of these varying between the two species (Al-Assaf, Phillips & Williams, 2005). These gums also contain a small amount of protein, namely ~2.5% (w/w) in the case of *Acacia senegal* and ~1% (w/w) in the case of *Acacia seyal*, which is present as an integral part of the structure of the gum. Recent studies by Lopez-Torrez, Nigen, Williams, Doco and Sanchez, (2015) have indicated that gum harvested from *Acacia senegal* has a higher degree of branching than gum harvested from *Acacia seyal*, with more branched galactopyranose residues, shorter arabinosyl side branches and more terminal rhamnopyranose residues.

The gum obtained from *Acacia senegal* has been shown to consist of three distinct fractions, which can be isolated by hydrophobic affinity chromatography (Randall, Phillips, & Williams, 1989; Osman, Williams, Menzies, Phillips, & Baldwin, 1993). These are referred to as the arabinogalactan (AG), arabinogalactan-protein (AGP) and glycoprotein (GP) fractions which differ mainly in their molar mass and protein content (Randall, Phillips, & Williams, 1989; Osman, Williams, Menzies, Phillips, &

Baldwin, 1993; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). The AG component constitutes ~90% (w/w) of the total gum and has been reported to have a molar mass and hydrodynamic radius, R_h, of ~2.5 x 10^5 g/mol, and ~15nm respectively and it contains very little protein (<1% w/w) (Randall, Phillips, & Williams, 1988; Osman, Williams, Menzies, Phillips, & Baldwin, 1993). Work by Sanchez using transmission electron microscopy and atomic force microscopy has shown that the AG fraction consists of oblate ellipsoids with a ~ 20 nm diameter and a ~1.5 nm thickness with an inner, interspersed chain network (Sanchez, Schmitt, Kolodziejczyk, Lapp, Gaillard, & Renard, 2008).

Arabinogalactan – protein(s) accounts for ~10% (w/w) of the total gum and has a molar mass and R_h of 1-2 x 10^6 g/mol and ~23nm respectively and contains ~10% protein (Randall, Phillips & Williams, 1988; Osman, Williams, Menzies, Phillips, & Baldwin, 1993; Qi, Fong, & Lamport, 1991; Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012). This fraction can be degraded by proteolytic enzyme (Mahendran Williams, Phillips, Al-Assaf, & Baldwin, 2008; Renard, Lepvrier, Garnier, Roblin, Nigen, & Sanchez, 2014) and hence it has been suggested that the molecules which constitute this fraction display a “wattle blossom–like” structure with carbohydrate blocks attached to a polypeptide chain/core protein, as is thought to be typical for AGPs in general (Fincher, Stone, & Clark, 1983; Randall, Phillips, & Williams, 1989; Showalter, 2001). Studies by Mahendran have identified carbohydrate blocks of ~4.5 x 10^4 Da linked by O-serine and O-hydroxyproline to a polypeptide chain of approximately 250 amino acids in length (~30 kDa core protein) present in the AGP fraction. (Mahendran Williams, Phillips, Al-Assaf, & Baldwin, 2008). More recently,

Renard (2012) reported that the AGP present in the gum when in solution adopted two types of conformation, with two different molecular weights. The low molecular weight population, with long-chain branching had a compact structure while the high molecular weight population with a short chain branching had a more elongated conformation (aggregates of the smaller molecules). Transmission electron microscopy indicated the presence of two populations of disk – like molecules with thicknesses below 3-5nm and approximately 85% of the particles observed displayed an apparent diameter of 20 – 80nm. Small angle neutron scattering gave a maximum dimension for AGP of 64nm. Single molecules with a spheroidal shape and aggregated molecules with an elongated shape were both reported to possess an outer structure combined with an inner porous network of interspersed chains or interacting blocks (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012).

The GP fraction constitutes only ~1% of the total gum, has a molar mass of 2.5×10^5 and contains up to 50% protein. This fraction is not degraded by proteolytic enzyme. Studies of the morphology of the molecular components of this fraction have indicated that when in solution it too contains a mixture of single and aggregated molecules. These molecules displayed a high propensity to self-associate in to either linear or circular ring structures. The structure of the single molecules was that of a thick shell wrapped around a central hole and gave rise to a ring-like morphology with ~8 – 11 nm diameter (Renard, Lepvrier, Garnier, Roblin, Nigen, & Sanchez, 2014).

However, markedly less attention has been paid to the molecular characteristics and composition of gum Arabic obtained from *Acacia seyal*. It is known to have a significantly higher average molar mass than that obtained from *Acacia senegal*, but interestingly it has a lower intrinsic viscosity indicating a much more tightly packed structure (Al-Assaf, Phillips, & Williams, 2005; Gashua, Williams, Yadav, & Baldwin, 2015). Gel Permeation Chromatography studies have shown that this gum is also polydisperse and that the proteinaceous components are distributed across the range of molar mass species present (Al-Assaf, Phillips, & Williams, 2005; Gashua, Williams, Yadav, & Baldwin, 2015).

Gum Arabic is widely used in the Food Industry as an emulsifier to stabilise flavour oil-in-water emulsions for application in beverages (Williams & Phillips, 2009). It has been proposed that the protein present within the structure of the gum molecules facilitates their adsorption onto the surface of the oil droplets with the carbohydrate component protruding into the aqueous phase providing an electrosteric barrier preventing droplet flocculation and coalescence (Padala, Williams, & Phillips, 2009). Whilst the gum obtained from *Acacia senegal* is very effective at forming oil-in-water emulsions this is not the case for that harvested from *Acacia seyal* (Flindt, Al-Assaf, Phillips, & Williams, 2005).

Therefore, the objective of the current study was to gain a clearer understanding of the mechanisms underlying the differences in the emulsification properties exhibited by gums obtained from trees of *A. senegal* and *A. seyal*. For this investigation, we chose to use polystyrene latex particles as a model system to determine the adsorbed layer thickness, which was studied using a combination of

dynamic light scattering and transmission electron microscopy. In addition to which, the molecular composition and self-assembly of the molecules present in the two gum samples was investigated by transmission electron microscopy and scanning transmission electron microscopy.

2. Materials and methods

2.1. Materials

The samples of *Acacia senegal* and *Acacia seyal* gum exudates used in this study have been previously described and characterised (Gashua, Williams, Yadav & Baldwin, 2015) and a summary of their chemical and physicochemical characteristics are presented in Table 1.

Polystyrene latex particles of 0.1µm mean particle size were purchased from Sigma-Aldrich Chemie GmbH, Germany and were in the form of a 10% aqueous dispersion. The bovine serum albumin (BSA) was obtained from Sigma Aldrich, Gillingham, UK. Sodium nitrate (analytical grade) and D-Limonene reagents were obtained from Fisher Scientific, UK. The density of the D-limonene was 0.843g/cm^3.

2.2. Methods

2.2.1. Molecular size

2.2.1.1. Dynamic light scattering

The hydrodynamic size of the *Acacia senegal* and *Acacia seyal* gum samples and BSA were determined by dynamic light scattering (Zetasizer, Nano series, Malvern Instruments). Powdered samples were dissolved in standard deionized water to give solutions with a concentration of 0.05% w/w. These were filtered using a 0.45µm pore size filter in order to remove extraneous materials before immediately transferring 1.5 mL of each into a DTS0012 disposable cuvette and placing them into the measuring chamber of the instrument. All measurements were performed at room temperature (25^0C) and the values reported are the average of 10 subruns.

Transmission electron microscopy (negative staining for molecular size and aggregation experiments)

A 1% (w/w) solution of the *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/v) 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 (for aggregation experiments)

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.

2.2.2. Adsorbed layer characteristics

2.2.2.1. Dynamic light scattering

The thickness of the gum Arabic layer adsorbed onto the polystyrene latex particles was determined by dynamic light scattering using the Malvern Zeta Nano ZS (Malvern, UK). 9.5 mL of gum Arabic or BSA solutions at concentrations of 0.01 – 0.05% in water and in presence of 0.5M $NaNO_3$ were added to 0.5 mL of a 0.5% polystyrene latex dispersion. For the dynamic light scattering measurments, sample times and delay times were chosen automatically by the instrument sorftware and analysed by cumulant analysis. Consistent values for the diffusion coefficient were found from multiple ten sub-run repeats and the particle size calculated. The hydrodynamic radius of the particles with ($R_{h,polymer}$) and without (R_h) polymer

adsorbed was determined and the adsorbed layer thickness, δ, was calculated using equation (1)

$$\delta = R_{h,polymer} - R_h \quad (1)$$

2.2.3. Transmission electron microscopy (negative staining for measurement of absorbed layer thickness)

The nature of the adsorbed layer for the two gum Arabic samples on polystyrene latex particles was further investigated by transmission electron microscopy. 9.5 mL of the *A. senegal* and *A. seyal* solutions at a concentration of 1% in water were added to 0.5 mL of a 0.5% polystyrene latex dispersion.

Formvar coated nickel TEM grids were washed in 30µl of tris buffer saline (1xTBS) by placement on a drop of the TBS on the surface of a sheet of dental modelling wax, for 5 minutes. The grids were rinsed with sterile deionized water (SDW) by floatation on a 30ul drop of SDW for 5 minutes and were then incubated on the surface of 30µl drops of the sample solutions for 90 seconds. Subsequently the grids were rinsed with deionized water as described previously, and excess liquid was carefully removed by touching the edge of the grids on to filter paper.

The grids were then negatively stained by placement on a 30ul drop of 2% (w/v) Uranyl acetate for 90 seconds. Excess Uranyl acetate was removed as described above. The negatively stained grids were air dried, prior to being observed on a

223 JEOL JEM-1200 EX Transmission Electron Microscope, at an accelerating voltage of
224 80Kv. The images were photographed using a GATAN retractable multi scan
225 camera.

226

227 *2.2.4. Emulsification properties*

228 20%w/w limonene oil-in-water emulsions were prepared according to the method
229 described by Padala, et al., (2009). 32g of 0.5% (w/w) gum Arabic (*A. senegal* and *A.*
230 *seyal*) in water was accurately weighed into a container followed by the addition of
231 8g of D-limonene. The system was sheared using an Ultra Turrax T25 mixer (IKA
232 Werke GmbH and co DE) equipped with a S25 N18 G rotor set at maximum 24,000
233 rpm for 4 minutes at a temperature of 25^0C.

234 The droplet size and mean specific surface area of the gum Arabic oil-in-water
235 emulsions was determined by laser diffraction using the Mastersizer 2000 (Malvern
236 Instruments). The dispersion unit of the instrument was first cleaned with distilled
237 water while simultaneously varying the agitation speed until the laser intensity
238 display was about 80%. The emulsion was then added to the water in the dispersion
239 unit dropwise using a pipette, until the obscuration was between 10-15%. All
240 measurements were carried out in triplicate and at room temperature and the
241 average value taken.

242 The prepared emulsions were left to equilibrate for 72 hours before centrifugation,
243 the supernatant was collected and the molecular mass distribution then determined
244 by Gel Permeation Chromatography using a Superose 6 column as described

previously (Padala, Williams, & Phillips, 2009). The difference in the RI peak areas before and after emulsification was obtained using the Astra software 4.2.0 and the amount of gum Arabic adsorbed onto the oil droplets was calculated.

3.0. Results and discussion

3.1. Molecular size and aggregation of molecules over time

The hydrodynamic radii of the biopolymer samples obtained by dynamic light scattering are presented in Table 2. The results for both the *Acacia* gums are in close agreement to the values reported previously (Gashua, Williams, Yadav, & Baldwin, 2015). The value obtained for BSA is also consistent with values reported in the literature (Robinson & Williams 2002).

Electron micrographs of the negatively stained *Acacia* gum samples (1% w/w) obtained by transmission electron microscopy are presented in Figure 1. It can be observed that both gum samples contain a majority of molecules with a diameter of ~20 nm which is the size reported for the AG component of *Acacia senegal* gum (Sanchez, Schmitt, Kolodziejczyk, Lapp, Gaillard, & Renard, 2008). Both of the samples were also shown to contain a proportion of molecules with a diameter of ~60 nm which corresponds to the diameter of the AGP molecules present in gum Arabic (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012). In addition, a population

of much smaller molecules with a diameter of ~ 10nm was also observed to be present which corresponds to the diameter of the GP component of the gum (Renard, Lepvrier, Garnier, Roblin, Nigen, & Sanchez, 2014).

We have previously shown by dynamic light scattering that the *Acacia* gum molecules have a tendency to aggregate in solution and we concluded that this was due to electrostatic interaction between protein and glucuronic acid residues present within the gum structure (Gashua, Williams, Yadav, & Baldwin, 2015). In the present study, the degree of aggregation of the molecules present in both the gum samples was monitored over a period of time, using transmission electron microscopy and scanning transmission electron microscopy. The results of which are presented in Figures 2 and 3; for samples left standing for 1 and 5 days respectively. Figure 2 shows the molecular structures present in the samples after 1 day and it is observed that aggregates are present in the *A. senegal* sample (Figure 2 A) with the majority of molecules with a diameter of ~40 nm as indicated with the red arrows and larger molecules with a diameter of ~66 nm shown with the blue arrows. However, there is no apparent aggregation in the *A. seyal* sample at this stage (Figure 2 B). Figure 3 shows the samples after leaving the solution to stand for 5 days. It was observed that distinct, snowflake-like aggregates were present in the *Acacia senegal* gum sample, which had a diameter of ~ 180 nm (Figure 3 A). The *Acacia seyal* gum was also found to contain aggregates, with a diameter of ~ 60 nm, but the molecules were not organised into a snowflake-like pattern, rather, they seemed to be ellipsoidal in shape (Figure 3 B). These data are

289 consistent with our previous findings, obtained from dynamic light scattering
290 studies, which showed that the *Acacia senegal* gum sample aggregated to a much
291 greater extent than the gum obtained from *Acacia seyal*. This is attributed to the
292 fact that the *Acacia senegal* sample contains more protein and a greater number of
293 glucuronic acid groups than the *Acacia seyal* sample.

294

295 Scanning transmission electron microscopy was also performed on an *Acacia*
296 *senegal* gum sample which had been left to stand for 5 days in order to further
297 elucidate the structure of the aggregates. The micrographs obtained are presented
298 in Figure 4 and the snowflake-like structure of the aggregated molecules is clearly
299 visible.

300

301 *3.2. Adsorbed layer characteristics*

302 *3.2.1. Adsorbed layer thickness*

303 Initial studies were undertaken to determine the adsorbed layer thickness of BSA
304 adsorbed onto polystyrene latex particles and the results are presented in Figure 5.
305 The adsorbed layer thickness values obtained in water were found to be ~3nm and
306 are similar to values reported by others (Robinson & Williams, 2002). This value is
307 consistent with the fact that the molecules have a z-average diameter of 6.6nm as
308 dertermined by dynamic light scattering. It is recognised that the adsorption of
309 globular proteins onto hydrophobic surfaces is facilitated through molecular

unfolding which exposes the hydrophobic groups within the protein core and enables them to adsorb onto the particle surface. The adsorbed layer thickness values obtained in the presence of 0.5M $NaNO_3$ are much higher than can be expected and indicate that bridging flocculation has occurred. It is evident that the protein adsorbed layer thickness is too small to facilitate stabilisation through steric repulsive forces.

The adsorbed layer thickness values of the *Acacia senegal* and *Acacia seyal* gums adsorbed onto the polystyrene latex particles in water and in the presence of 0.5M $NaNO_3$ are shown as a function of the concentration of gum added and after various equilibration times in Figures 6a-b and 7. Figure 6a shows the adsorbed layer thickness for *Acacia senegal* gum adsorbed in water. The first point to note is that the thickness increases as the amount of gum present increases and then tends to plateau. The increase is due to an increase in the amount of gum adsorbed at the surface as the gum concentration is increased. The value obtained for the adsorbed layer thickness in the presence of 0.05% gum (at plateau coverage) on Day 0 is 21nm which compares to a value of 29.8nm for the z-average hydrodynamic diameter of the gum molecules determined by dynamic light scattering. Since the AGP molecules present within gum Arabic are believed to have a disk or cylinder-like structure with a thickness of below 3-5nm (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012) this would indicate that the molecules do not lie flat on the surface, but must adsorb end-on or else adsorb as multilayers. It is particularly interesting to note that the adsorbed layer thickness also increases significantly with time. For example, for the polystyrene latex particles in the

presence of 0.05% gum solution the thickness increases from 21nm to 61nm over a 14 day period. This could be due to molecular rearrangements, molecular exchange or an increase in the amount of gum adsorbed. The additonal adsorption could be either directly onto the PS latex particle surface or perhaps most likely due to multilayer adsorption. As reported above and in our previous study, *Acacia senegal* gum alone self associates in solution through electrostatic interactions between protein and carboxylate moieties within its structure (Gashua Williams, Yadav, & Baldwin, 2015). We have also previously reported multilayer adsorption to occur for sugar beet pectin through a similar mechanism (Siew, Williams, Cui, & Wang, 2008).

The adsorbed layer thickness for *Acacia senegal* gum in the presence of 0.5M $NaNO_3$ is shown in Figure 6b as a function of the concentration of gum and also after various time periods. Again, we see an increase in the adsorbed layer thickness as the concentration of gum is increased which then reaches a plateau value. The values obtained on Day 0 are significantly greater than for samples prepared in the absence of electrolyte, for example, an adsorbed layer thickness of 44nm was obtained in the presence of 0.05% gum solutions in 0.5M $NaNO_3$ compared to 21nm in water. This value is significantly greater than the value we obtained for the z-average diameter by dynamic light scattering (29.8nm), but could be accounted for if preferential adsorption of higher molar mass molecules occurred. We have shown in the current study by TEM and have reported previously from studies using GPC/MALLS, that the diameter for the high molar mass species is typically 40-60nm. These findings are also consistent with the

dimensions of the AGP reported by Renard and colleagues (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006) who suggested that the molecules have a cylinder-like structure with a diameter of ~60nm and a thickness below 3-5nm. Since the adsorbed layer thickness is 61nm in the presence of 0.5M $NaNO_3$ this indicates that the molecules may adsorb end-on at the surface. Snowden previously reported that the adsorption capacity for *Acacia senegal* gum adsorbed onto polystyrene latex particles was ~1mg m^{-2} in water and that this increased to ~5mg m^{-2} for adsorption from electrolyte (Snowden, Phillips, & Williams, 1987). The increase in the amount adsorbed is explained by the fact that the electrolyte screens lateral electrostatic interactions between the gum molecules adsorbed at the surface allowing more to be accommodated. In addition, as the gum absorbs there is an increase in the surface charge (Padala, Williams and Phillips, 2009) which prevents further molecules in solution from adsorbing due to charge repulsions. This behaviour is typical for polyelectrolytes generally. The fact that the adsorbed layer thickness does not increase over time, as is the case in water alone, can be explained by the fact that the electrolyte would also inhibit intermolecular protein-carboxylate interactions through charge screening and prevent multilayer adsorption from occurring. This also agrees with the fact that we observed that association of the *Acacia senegal* gum molecules alone in aqueous solution was inhibited in the presence of electrolyte solution (Gashua, Williams, Yadav, & Baldwin, 2015).

Figure 7 shows the adsorbed layer thickness for samples prepared with *Acacia seyal* gum in water and in the presence of 0.5M $NaNO_3$. The adsorbed layer thickness is

379 of the order of ~2-3nm in water and ~0.5nm in 0.5M $NaNO_3$ which is much less than
380 the average hydrodynamic diameter of the gum molecules of 34.2nm obtained
381 from dynamic light scattering measurments. If we assume that the AGP molecules
382 present in this gum have similar disk or cylinder-like molecular characterstics to
383 those observed in *Acacia senegal* gum, this suggests that the gum molecules in the
384 *A.seyal* sample lie flat on the surface of the polystyrene latex particles rather than
385 end-on. *Acacia seyal* has a lower protein content than *Acacia senegal* and the
386 protein is less accessible as evidenced by the fact that it is not completely
387 hydrolysed by proteolytic enzyme hence the molecules will have a lower affinity for
388 the hydrophobic surface (Flindt, Al-Assaf, Phillips, & Williams, 2005). Our results as
389 described above and in previous studies (Gashua, Williams, Yadav, & Baldwin, 2015)
390 also indicate that the molecules present in *Acacia seyal* gum do not self associate
391 in solution to the same extent as those present in gum obtained from *Acacia*
392 *senegal* .

393 Electron micrographs of the polystyrene latex particles in the presence and absence
394 of *Acacia senegal* and *Acacia seyal* gums are presented in Figure 8 (a – f). From
395 Figure 8 (a and b) it can be observed that the particles themselves in the absence of
396 either of the *Acacia* gum exudates seem to have been stained by the uranyl acetate,
397 which is probably due to the fact that the stain is cationic in nature. It is also
398 apparent that the polystyrene latex particles are polydisperse and display a variety
399 of sizes. Figures 8 c) and d) confirm that the *Acacia senegal* gum molecules have
400 formed a distinct, thick adsorbed layer surrounding the particles and Figures 8 e)

401 and f) demonstrate that the *Acacia seyal* molecules have formed a much thinner,
402 more diffuse layer around the particles.

403 The thickness of the adsorbed layer of *A. senegal* gum (Figure 8 c) and d)) is
404 approximately 15.5nm which is in reasonable agreement with the value of 21nm
405 recorded on day 1 for a 0.05 % w/w solution in water from the dynamic light
406 scattering measurements. The difference is most likely due to the fact that the
407 adsorbed layer observed by TEM using negative staining has been air-dried prior to
408 imaging and any residual water will be removed once subjected to the high vacuum
409 present within the entry chamber of the transmission electron microscope. In direct
410 contrast, there is no such distinct adsorbed layer on the particles incubated in the
411 presence of *A.seyal* gum, which is also consistent with the fact that the adsorbed
412 layer thickness values obtained by dynamic light scattering was very small, namely,
413 ~2-3nm.

414

415 *3.3. Emulsification properties*

416 The droplet size distributions of the emulsions prepared with the *Acacia senegal*
417 and *Acacia seyal* gum exudates are presented in Figure 9a and 9b respectively. It is
418 observed that the droplet size for the freshly prepared emulsions is smaller for
419 *Acacia senegal* than for *Acacia seyal*. In addition, while the droplet size for the
420 emulsion prepared using the *Acacia senegal* gum remains constant on storing for a
421 week, the droplet size for the emulsion prepared using *Acacia seyal* increases. This
422 is as expected, as it is well known that the gum obtained from *Acacia senegal* has

423 superior emulsification properties compared to that harvested from *Acacia seyal*
424 (Flindt, Al-Assaf, Phillips, & Williams, 2005). The fact that the *Acacia seyal* gum is
425 able to form emulsions at all, indicates that it must adsorb at the oil-water interface
426 to some degree. The amount of gum adsorbed onto the emulsion oil droplets was
427 calculated from the difference in the areas of the GPC RI elution profiles and was
428 found to be 3.53 mg/m^2 and 0.77 mg/m ^{2} for *Acacia senegal* and *Acacia seyal*
429 respectively. The adsorption data points are included on the adsorption isotherms
430 previously reported for the adsorption of *Acacia senegal* gum onto limonene
431 droplets (Padala, Williams, & Phillips, 2009) and shows very good agreement with
432 these earlier findings (Figure 10) .

433

434 **4. Conclusions**

435 Transmission electron microscopy experiments have confirmed that the gum Arabic
436 harvested from both *Acacia senegal* and *Acacia seyal* contain spheriodal shaped
437 molecules, which vary in size. The majority of these molecules have a diameter of
438 ~20nm, which is consistent with values previously reported for the AG component
439 of gum Arabic obtained from *A. senegal*. Whilst a smaller proportion have
440 diameters of ~ 60nm and ~10nm which most likely correspond to the AGP and GP
441 components known to be present. It was also observed that the molecules tended
442 to aggregate in solution over time and this was found to occur to a greater extent
443 for the gum from *Acacia senegal* compared to *Acacia seyal*. This aggregation
444 process has been attributed to electrostatic interaction between proteinaceous and

445 uronic acid moieties present within the structure of the gums. *Acacia senegal* gum
446 contains higher levels of both protein and glucuronic acid than *Acacia seyal* gum
447 which is consistent with the fact that it aggregates to a greater extent.

448 The thickness of the adsorbed layer of *Acacia senegal* gum molecules on the surface
449 of polystyrene latex particles was found to increase over time and this has been
450 attributed to the formation of multilayers which occur as a consequence of
451 electrostatic interaction. This conclusion is supported by the fact that the thickness
452 did not change over time for experiments undertaken in the presence of electrolyte
453 which would act to screen electrostatic interactions between the molecules.
454 Transmission electron microscopy also showed that gum obtained from *Acacia*
455 *senegal* formed a distinct, thick layer around the polystyrene latex particles. In
456 contrast, both dynamic light scattering and transmission electron miscroscopy
457 showed that the gum from *Acacia seyal* formed a very thin, diffuse layer on the
458 surface of the polystyrene latex particles and that there was no evidence of
459 multilayer adsorption.

460 In emulsification studies, the gum from *Acacia senegal* was found to produce
461 emulsions with a smaller droplet size than for gum from *Acacia seyal.* Furthermore,
462 the droplet size for emulsions prepared with gum from *Acacia senegal* remained
463 constant over time while the droplet size increased for emulsions prepared using
464 gum from *Acacia seyal* indicating that droplet flocculation and/or coalescence had
465 occurred. This behaviour is believed to be due to the fact that the amount of
466 *Acacia senegal* gum adsorbed onto the oil droplets was found to be ~five times that

for the gum from *Acacia seyal* which is consistent with the adsorbed layer thickness studies carried out using polystyene latex particles.

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	Rha	Ara	Gal	GluA	Protein[a]	Protein[b]	Mw x 10^5(g/mol)	Mn x 10^5(g/mol)	R_g(nm)	R_h(nm)
A. senegal	9.2	24.9	45.3	15.5	2.93	2.12	4.85	2.77	13.0	13.0
A. seyal	2.1	32.5	44.2	13.0	0.99	0.69	11.4	6.84	24.0	17.3

Table 1. Sugar, protein composition, Molar mass and hydrodynamic size of *Acacia* gum exudates.

All results experessed as % w/w on a dry weight basis. Rha= Rhamnose, Ara=Arabinose, GluA=Glucuronic Acid, Mw=Molecular weight, R_g=Radius of gyration, R_h=Hydrodynamic radius, [a]=by Kjeldhal analysis using a conversion factor of 6.60 as suggested by Anderson, (1986), [b]=From amino acid analysis (Source; Gashua, et al., 2015).

	A. senegal	*A. seyal*	BSA
z-average R_h (nm)	14.9	17.1	3.3

565

566 Table 2. Hydrodynamic radii of the biopolymers

FIGURE CAPTIONS

Figure 1. Transmission electron micrographs of *Acacia senegal* (A) and *Acacia seyal* (B) gums, negatively stained at day 0. (Scale bar = 0.2µm). Single molecules of varied sizes are observed in both gum samples as indicated by the arrowed letters (a), (b) and (c). The letter (a) indicates molecules of ~ 60nm (putative AGP), and the letter (b) indicates molecules of ~ 20nm (putative AG), while the smaller molecules, are indicated by the letter (c) which represents molecules ~ 10nm (putative GP).

Figure 2 Transmission electron micrographs of *A. senegal* (A) and *Acacia seyal* (B) gums negatively stained and observed after 1 day. Aggregation is observed to have started to occur in the *A. senegal* sample (A) with a number of ~ 40nm aggregates observed, (as indicated with the arrowed letter (a)) and a larger aggregate of ~66nm (indicated with the arrowed letter (b)). However, there is no apparent aggregation present in the *A. seyal* gum sample at this stage. The scale bar = 0.2 µm in both A and B.

Figure 3. Transmission electron micrographs of *Acacia senegal* (A) and *Acacia seyal* (B) gums negatively stained and observed after 5 days. Distinct snowflake-like aggregates were observed in the *A. senegal* gum in varied sizes with an average diameter of ~ 184nm (a), with another aggregate of ~ 420nm (b). The *A. seyal* gum also contains aggregates, with an average diameter range of ~ 37nm (a) and ~ 80nm (b), with a larger aggregate of ~ 320nm (c), but the aggregated molecules are not arranged in a snowflake-like manner, rather they seem more ellipsoidal in shape. The scale bar = 0.2 µm in both A and B.

Figure 4. Scanning transmission electron micrographs of *Acacia senegal* sample after incubation for 5 days at room temperature. Images taken at two magnifications are shown (A) X6, 500 and (B) X17, 000).

Figure 5. Adsorbed layer thickness of BSA as a function of concentration in water and in 0.5M $NaNO_3$.

Figure 6a. Adsorbed layer thickness of *Acacia senegal* gum sample as a function of concentration in water over 14 days period.

Figure 6b. Adsorbed layer thickness of *Acacia senegal* gum sample as a function of concentration in 0.5M $NaNO_3$ over 7 days period.

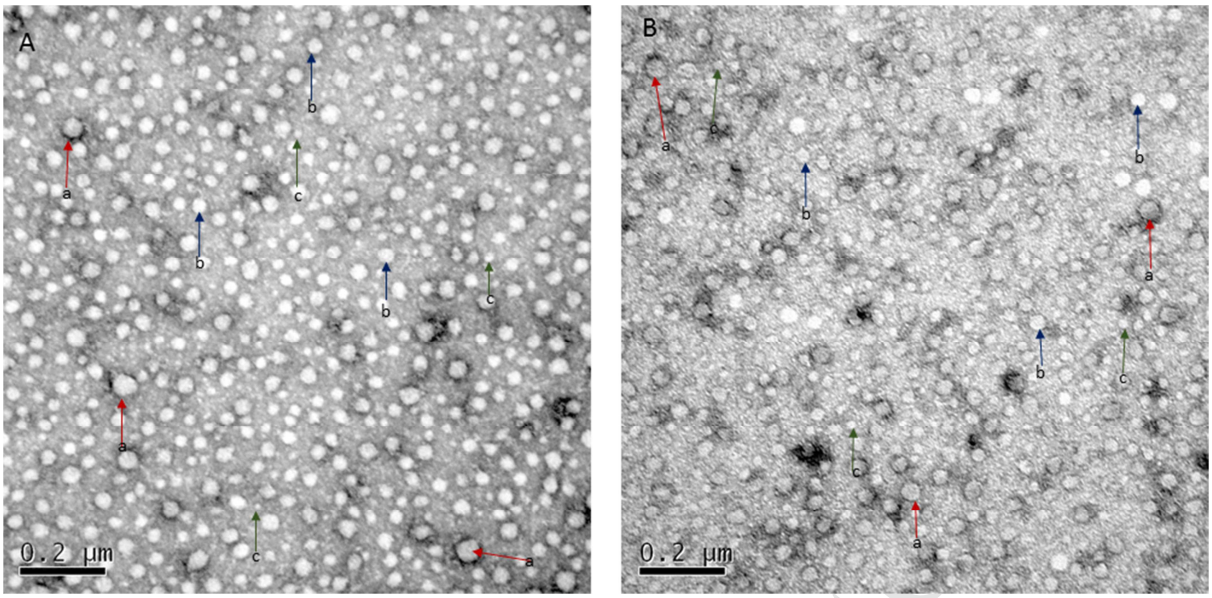
Figure 7. Adsorbed layer thickness of *Acacia seyal* gum sample as a function of concentration in water and in 0.5M NaNo3 over 7 days period. (Where d=day, W=water and S=salt, i.e 0.5M NaNO3)

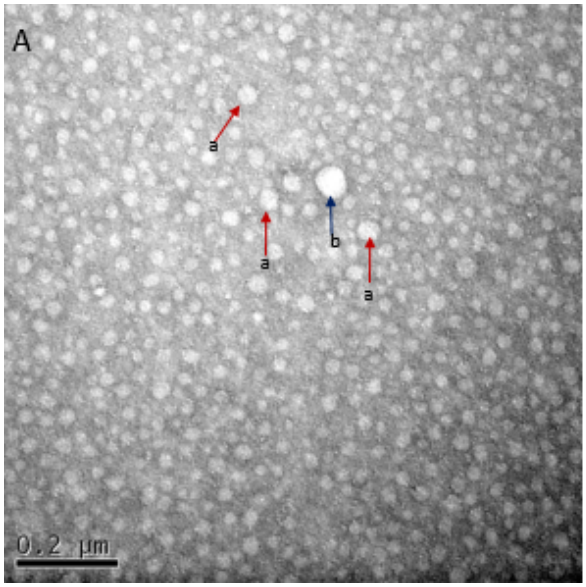
Figure 8. (a-b) - TEM Images of Polystyrene latex particles at 100K and 250K magnification; (c-d) TEM Images of Polystyrene latex particles in the presence of *Acacia senegal* gum 100K and 250K magnification. (e-f) - TEM Images of *Acacia seyal* adsorbed onto Polystyrene latex particles at 100K and 250K magnification

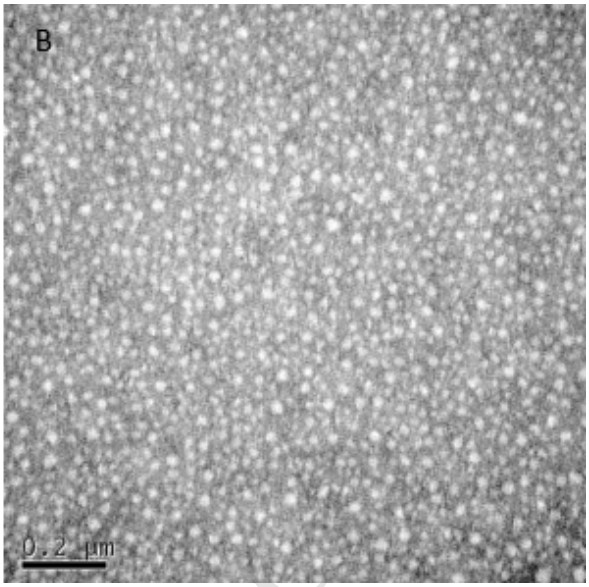
Figure 9a. Droplet size distribution of limonene oil-in-water emulsion prepared with *Acacia senegal* stored over 7 days period.

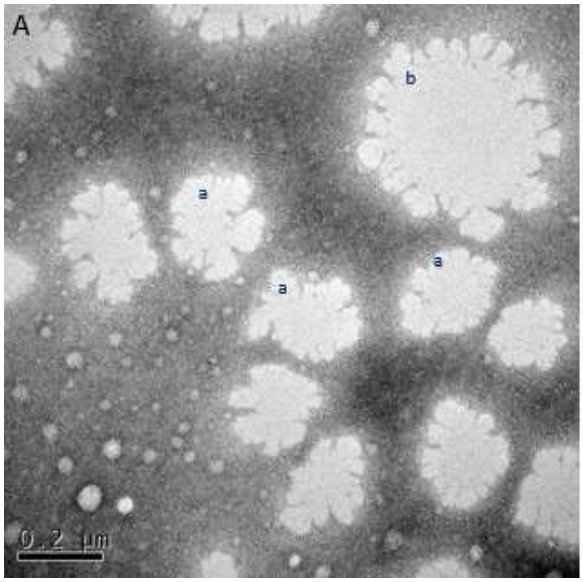
Figure 9b. Droplet size distribution of limonene oil-in-water emulsion prepared with *Acacia seyal* stored over 7 days period.

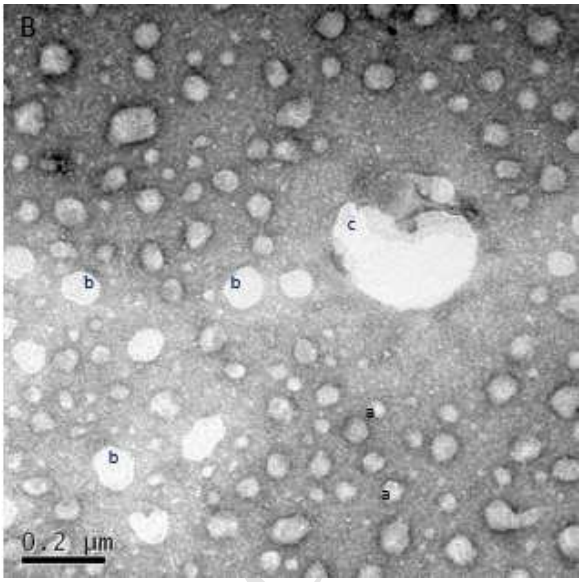
Figure 10. Adsorption isotherms for *A. senegal* and *A. seyal* adsorbing onto limonene oil (Adapted from Padala, Williams, & Phillips, (2009). Adsorption of Gum Arabic, Egg White Protein and Their Mixtures at the Oil–Water Interface in Limonene Oil-in-Water Emulsions. Copyright (2009) American Chemical Society.

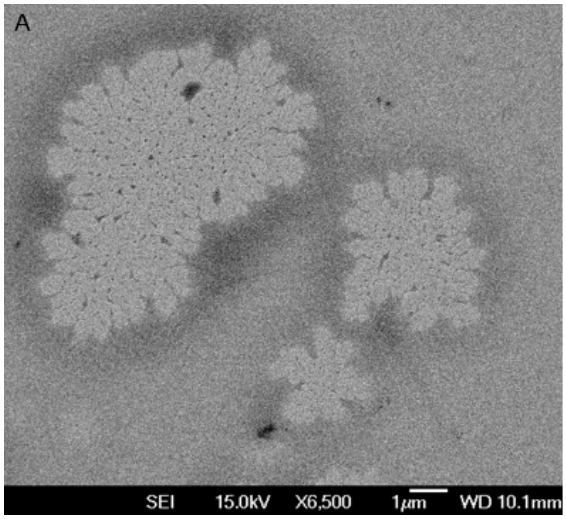


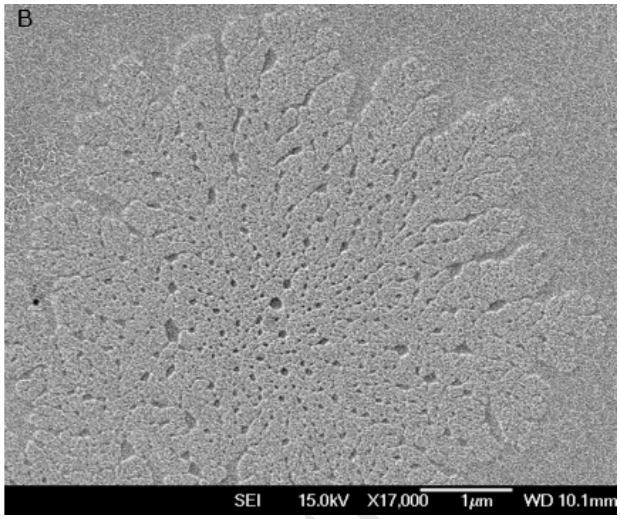


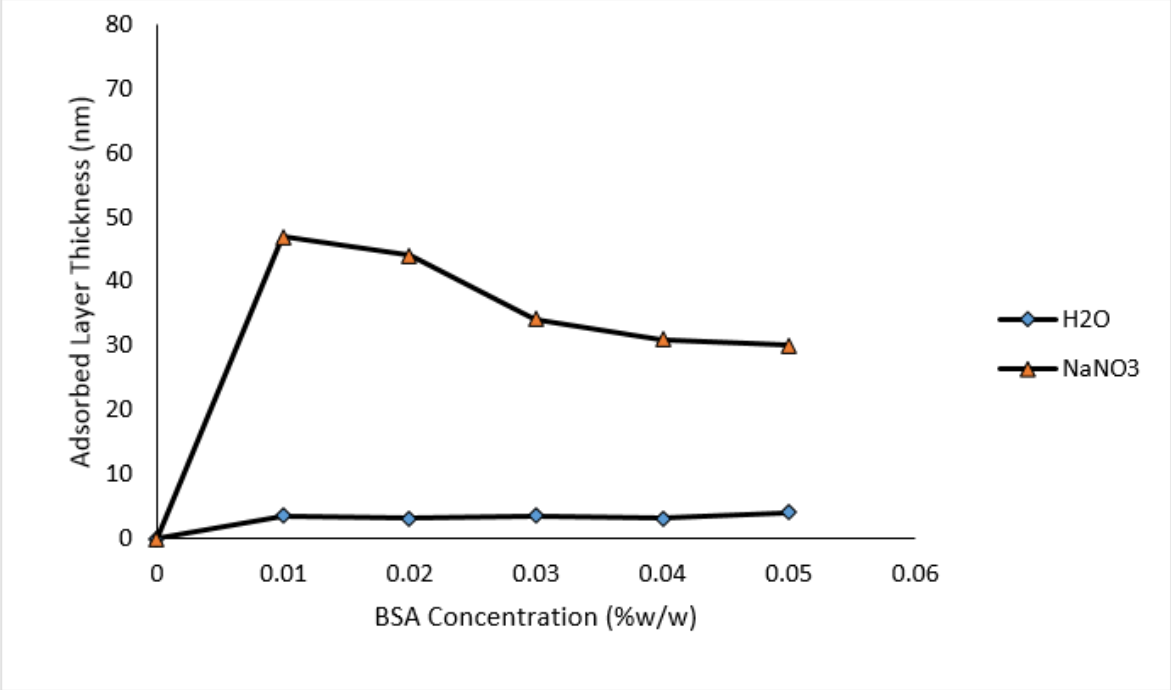


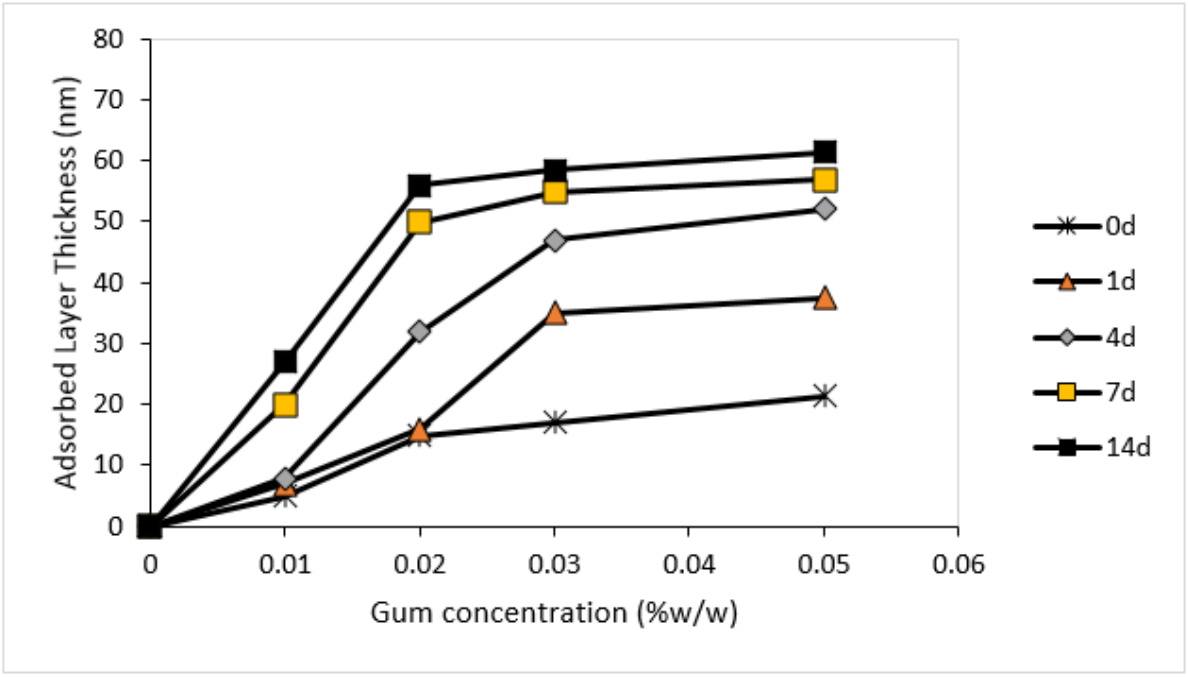


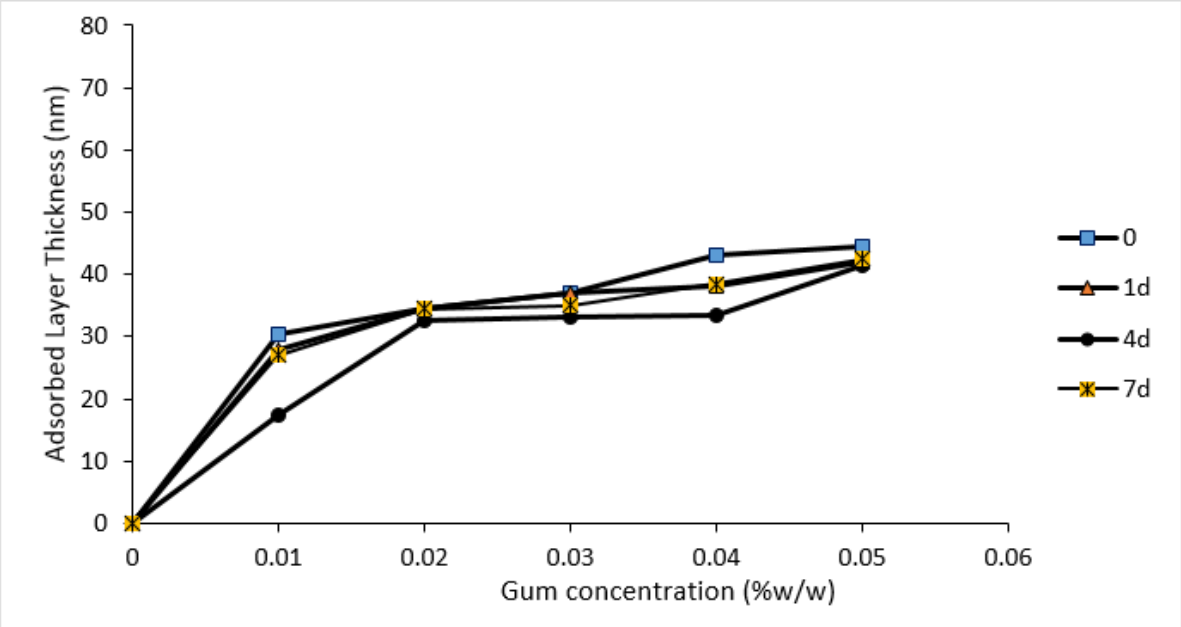


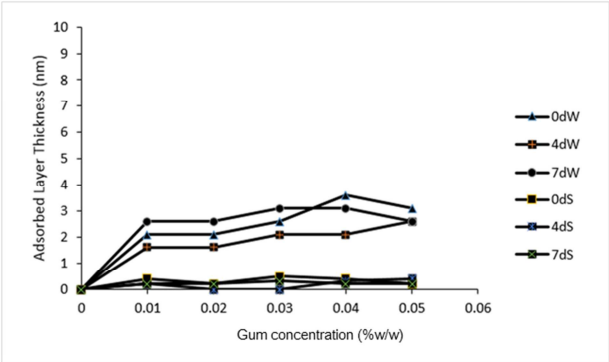


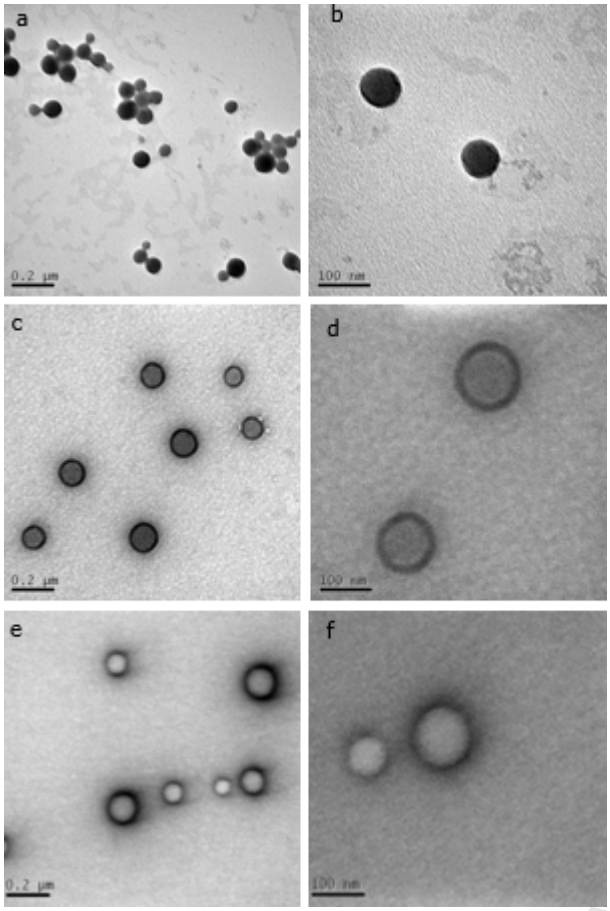


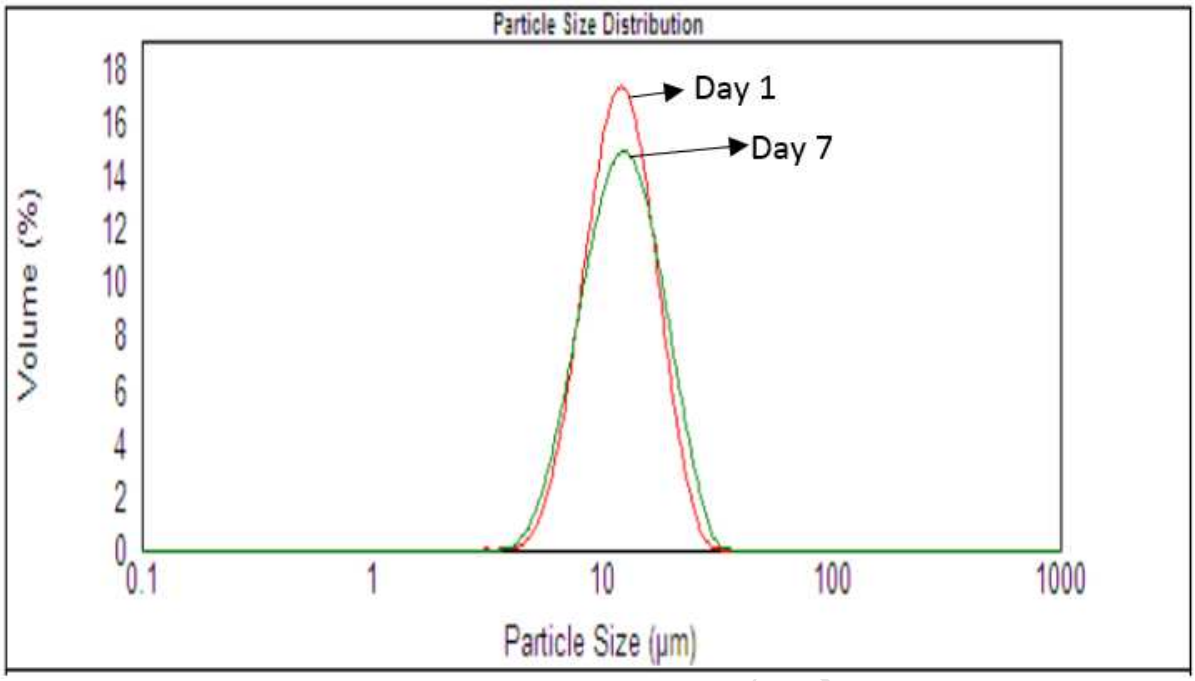


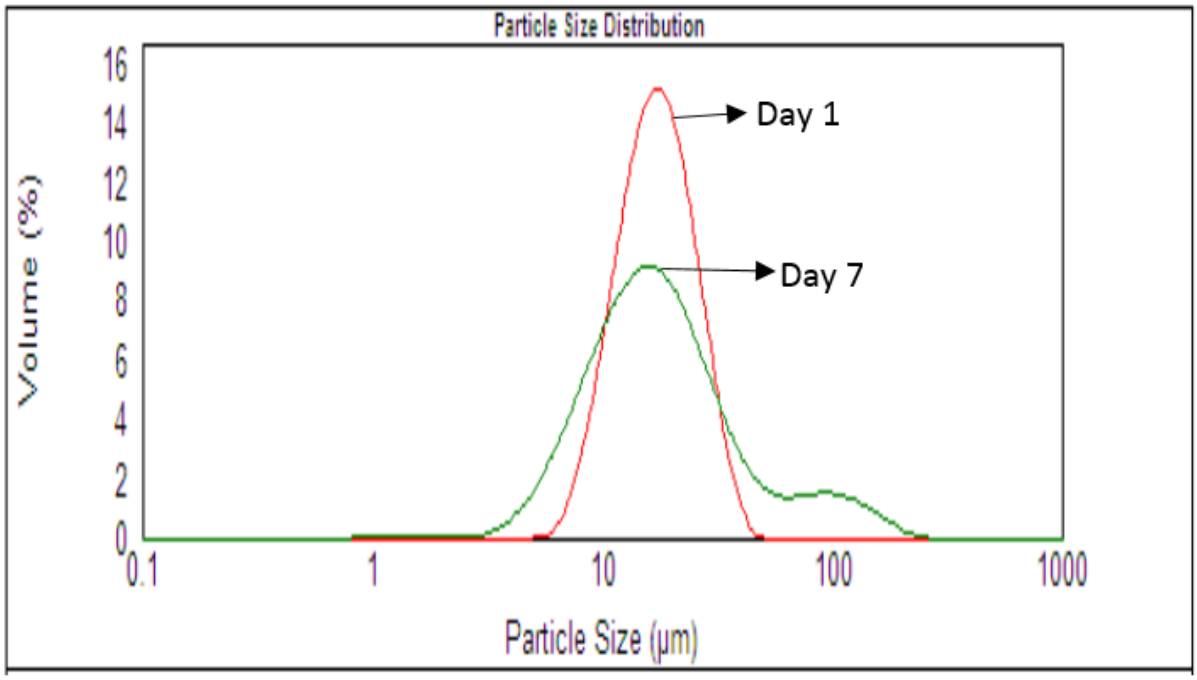


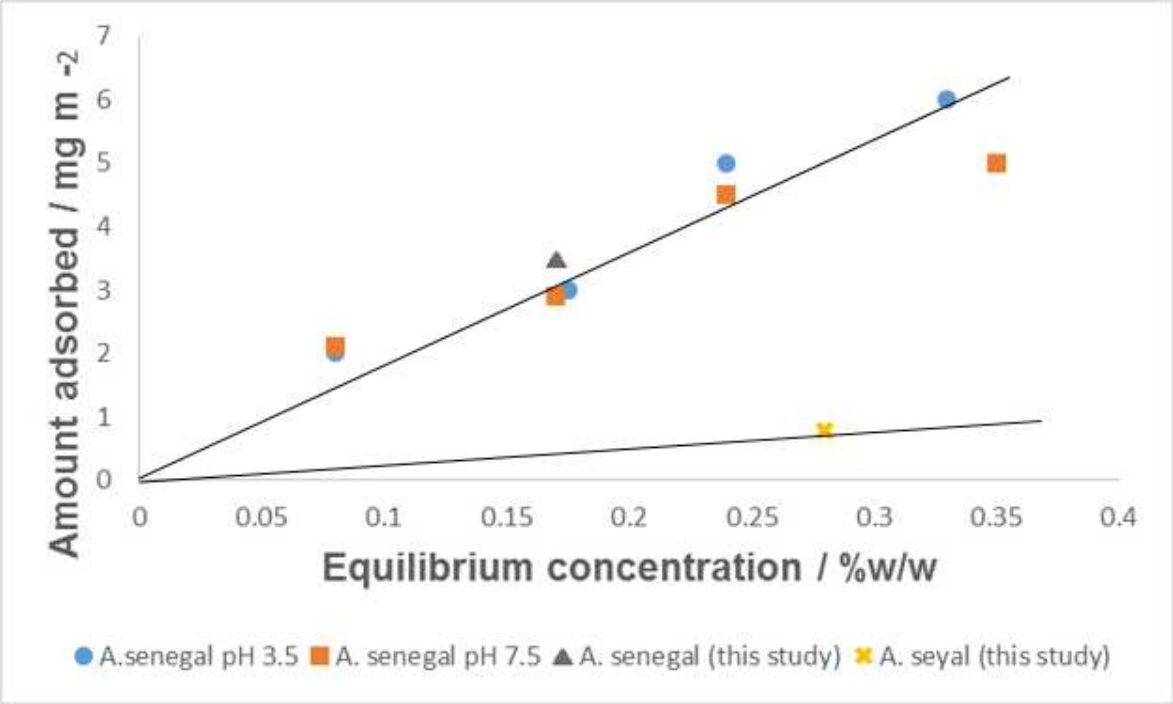












Highlights

- *Acacia* gums in solution display molecular association
- *A. senegal* gum forms multilayers at the solid / liquid interface
- *Acacia* gum composition observed using TEM and STEM.