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Denaturation and Polyelectrolyte Behavior of Gum Arabic and *Acacia Polyacantha* Gum Molecules.

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ABSTRACT

In this study, the protein denaturation, expansion properties, and polyelectrolyte properties of the gum arabic (*Acacia. seyal* and *Acacia. senegal*) and *Acacia. polyacantha* gum solution were determined. The protein content of *A. seyal*, *A. senegal* and *A. polyacantha* gum were found to be 2.3, 0.9, and 1.6 respectively. All gum samples showed an increase in viscosity with a decrease in salt concentration. In addition, *A. senegal* gum molecules demonstrated a large increase in viscosity with decreasing salt concentration. This led to the large expansion of *A. senegal* gum molecule comparison to A*. seyal* gum molecules. The expansion factors of *A. senegal, A. Seyal,* and *A. polyacantha* in water and sodium chloride solutions (0.5M) were found to be in the order of 5.8, 2.8, and 3.2 respectively. The solutions of *A. senegal* and *A. polyacantha* gum in water behaved as polyelectrolyte solutions, where as *A. seyal* gum solution behavior differed from polyelectrolyte solution. The results obtained show that urea was good a denaturation agent and the guanidine hydrochloride was not suitable as a denaturation agent for the gum molecules. Moreover, *A. senegal* was denatured more than *A. seyal* and *A. polyacantha*.

Keywords: Gum arabic, *Acacia polyacantha*, polyelectrolyte, denaturation.

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INTRODUCTION

Crud gum arabic, namely *Acacia senegal* (*A. senegal*) and *Acacia seyal* (*A. seyal*), is a biopolymer (polysaccharide) branched molecule with a high molecular mass and complex structure (Randall et al., 1989; Osman et al., 1994; Islam et al., 1997; Atgié et al., 2019). Gum Arabic that contains arabinose and galactose as major constituents are called arabinogalactan (AG)(Fincher et al., 1983). Chemically, all acacia gums are arabinogalactan-proteins (AGP). Moreover, gum arabic is present in mixed calcium, magnesium, and potassium salts of polysaccharidic acid (Elsheik, 2008; Osman et al., 1993). Gum arabic contains about 2.0 – 2.5%. protein. The nitrogen content for *A. senegal* and *A. seyal* 0.33%w/w, and 0.11%w/w respectively (Karamalla, 2000). In addition, amino acid composition of gum arabic is rich in hydroxyproline and serine while the aniline content is low (Elsheik, 2008). *A. polyacantha* gum is also a complex biopolymer (polysaccharide) with a molecular mass lower than gum arabic. *A. polyacantha* is based on a branched galactan core, to which uronic acid, rhamnose, and unusually short chains containing not more than three arabinose residues are attached (Omer, 2004). *A. polyacantha* contains about 1.8 – 2.6% w/w protein and 0.28 – 0.4%w/w nitrogen (FAO/WHO, 2012).

Denaturation is the alteration of a protein shape through some form of external stress (for example, by applying heat, acid, or alkali), in such a way that it will no longer be able to carry out its cellular function. Protein denaturation comprises any noncovalent change in the structure of a protein, which may alter the secondary, tertiary, or quaternary structure of the molecules (Bailey, 1990). It is initiated by by a sequence of conformational changes that produce associated volume changes. Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation.

Urea and guanidine hydrochloride are widely used as denaturation agents for different types of protein (Frensdorff et al., 1953). Guruaj Rao and Narasinga Rao were used urea and guanidinium hydrochloride to dissociate 12S proteins of mustard and rapeseed to 1.8 S proteins, demonstrating that the extent of dissociation depends on the concentration of the denaturant (Guruaj Rao and Narasinga Rao, 1983). The reagents denatured the protein, as evidenced by the resultant increase in viscosity. Different techniques and methods of protein denaturation have been used (Passos et al., 2014; Roche and Royer, 2018; Schon et al., 2017; laidis et al., 2017). In addition, recent studies of polyelectrolyte characters of arabinogalactans or glucuronomannans have been studied (da Silva et al., 2020).

The aim of this work is to study gum arabic and *A. polyacantha* gum molecules in terms of protein denaturation, expansion properties and polyelectrolyte behavior in solutions.

EXPERIMENTAL

Ash content

To determine the ash content, a crucible was heated to 550 ˚C, cooled in a desiccators, and weighed (W_1). Two grams of gum samples were accurately weighed in a crucible (W_2), ignited at 550 ˚C in a muffle furnace until free from carbon, cooled in a desiccators, reweighed (W_3). The total ash % was calculated as follows

$$Total\ ash\ \% = 100\ \times (W2 - W1)$$

Cationic composition measurements

An atomic absorption spectrometer**,** (Perkin-Elmer, model Analyst 100) was used to determine seven elements, namely potassium, sodium, magnesium, calcium, iron, zinc, and lead. The dry ash method was used in sample preparation, where two grams of gum sample were placed in a well-glazed porcelain dish in a cold furnace and then heated to 550 ˚C**.** The temperature was maintained for 4 hours, then the samples were cooled and 10 ml of 3N HCl were added, covered with a watch glass, and boiled gently for 10 minutes. After cooling the samples were filtered into a 100 ml volumetric flask, and diluted with deionized water. The final dilution contained 1% lanthanum solution.

Total nitrogen and protein content measurements

The Kjeldahl method was used to determine the total nitrogen in *A. seyal*, *A. senegal*, and *A. polyacantha* samples. The procedure used was a two stage process in which the gum samples were digested in hot concentrated sulfuric acid and the ammonia which was released by using sodium hydroxide was neutralized with standard acid (Bradstreet, 1965).

0.5 g of each sample (in duplicate) was weighed and transferred to a Kjeldahl tablet (copper sulfate- potassium sulfate catalyst) and 10 mL of concentrated sulfuric acid (nitrogen-free) was added. The tube was then mounted in the digestion heating system which was preheated to 240 ˚C, and capped with an aerated manifold. The solution was subsequently heated at the above temperature until a clear pale yellowish-green color was observed, indicating the completion of the digestion. The tubes were then allowed to cool to room temperature. Their count was quantitatively transferred to a Kjeldahl distillation apparatus followed by the addition of distilled water and 30% w/v sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 mL of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02 M, hydrochloric acid using methyl red as an indicator. Blank titration was carried out in the same way.

Denaturation measurements and polyelectrolyte behaviour

Intrinsic viscosity was measured to study the expansion, polyelectrolyte behavior and denaturation of gum molecules using different salt concentrations, water, urea and guanidine hydrochloride as a solvents.

A 3% solution of gum was used as the starting concentration. About 0.6g of gum was dissolved in 20 mL of solvent (1M NaCl) and was hydrated overnight on a tumblemixer. The precise concentration was calculated based on the loss form drying and the solution was filtered using a 0.8µm nylon filter. The viscometer was immersed in a water bath adjusted to 25 ˚C ±1˚C and left to attain equilibrium. A glass pipette was used to insert the gum solution into the viscometer and a syringe was used to create a vacuum to bring the solution to the top demarcation line. The readings were taken using an accurate millisecond timer and the times were recorded in mm: ss: ms format (minutes, seconds, and milliseconds). The readings were taken in duplicate and time readings for the solvent were taken for each sample.

RESULTS AND DISCUSSIONS

Ash content

The mean values of the ash content of *A. seyal, A. senegal* and *A. polyacantha* were found to be 2.43%, 3.32% and 3.22% respectively. These values agree with results reported in the literature (Omer, 2004; Anderson and Anderson, 1963; Karamalla et al., 1998). The mean values also agree with the results obtained by Ibrahim (Ibrahim, 2006), but these mean values were lower than the mean values of *A. seyal* obtained by Hassan (Hassan, 2000).

Nitrogen and protein content

Table 1 shows the nitrogen and protein percentage obtained by the Kjeldahl method. A conversion factor of 6.25 was used to calculate the total protein percentage.

The nitrogen and protein content of *A. seyal* was in the range obtained by Hassan *et al* (Hassan et al., 2005). using the Kjeldahl method from 0.11% to 0,19% for the nitrogen content and from 0.73% to 1.12% for the protein content. The results of *A. seyal* and *A. senegal* also agreed with comparative analytical data for *A. senegal* and *A. seyal* gums reported by Karamallah and Osman *et al* (Karamalla, 2000; Osman et al., 1993a). For *A. polyacantha* gum, the results obtained were less than the range reported by Omer (Omer, 2004).

Cationic composition

The mineral composition of the samples was determined (using atomic atomic absorption spectrophotometer) the average values of cationic composition obtained in Table 2 were in agreement with the results reported by Buffo *et al* (Buffo et al., 2001). The results obtained for potassium, calcium, and magnesium of *A. polyacantha* gum differed from results recorded by Omer (Omer, 2004). The concentrations of calcium and potassium, recorded in this study were near the range of the results recorded by Siddig (Siddig, 2003), while the concentrations of magnesium and iron both differed from it (Siddig, 2003). Measured values of potassium, calcium, and magnesium were relatively high, indicating that the gum is a salt primarily comprised of these minerals. Moreover, *A. senegal* and *A. polyacantha* contained high levels of potassium while *A. seyal* contain high levels of calcium.

Intrinsic viscosity, denaturation and polyelectrolyte behaviour

Dextran and casein were used to compare the denaturation effect of solvents on the samples. Dextran was used as a pure polysaccharide and casein was used as a pure protein. Gums are polysaccharides that contain a small portion of protein (arabinogalactan protein complexes).

Table 3, Figures 1,2, and 3 show an increase in viscosity with a decrease in salt concentration with different gum samples. This was due to the polar groups being sufficiently neutralized with counter ions (salt ions) such that the repelling effect was decreased in more concentrated salts, whereas with more diluted salt concentrations the gum molecule expanded due to the repelling effect. This occurs despite the gum samples molecule being highly branched and may be due to the greater rotational freedom of the 1-6 linkages. A smaller increase in viscosity and less expansion was observed in *A. polyacantha* gum molecules than in those of *A. senegal* and *A. seyal*. This may be due to the comparatively lower molecular weight of *A. polyacantha* gum .*A. senegal* gum molecules showed a large increase in viscosity with decreasing salt concentration, leading to larger expansion of *A. senegal* gum molecules than *A. seyal* gum molecules, despite *A. seyal* gum having a larger molar mass. This is indicative of differences in molecular structure and branching which may lead to differences in expansion.

Figures 2 and 3 show the behavior of *A. senegal* and *A. polyacantha* gum in water. They behaved as polyelectrolyte solutions as evidenced by the curvetted line. Figures 1 shows the behavior of *A. seyal* gum in water. It did not act as a polyelectrolyte solution. Table 3 shows the effect of different concentrations of urea and guanidine hydrochloride on the viscosity of different gum solutions. The results indicate that urea is a good denaturation agent and the guanidine hydrochloride is not suitable as a denaturation agent for the gums molecule. *A. senegal* was denatured more than *A. seyal* and *A. polyacantha* because *A. senegal* contains a higher ratio of protein than *A. seyal* and *A. polyacantha*. This is substantiated by the large expansion of *A. senegal* in water. The expansion factors of *A. senegal*, *A. seyal* and *A. polyacantha* in water and sodium chloride solutions (0.5M) were calculated from Table 3. The expansion factors were found to be 5.8, 2.8 and 3.2 respectively (Figures 4). This indicates that *A. Senegal* expanded more than *A. seyal* and *A. polyacantha*. Moreover, the expansion factor of *A. Senegal* was about twice the expansion factor of *A. seyal* and *A. polyacantha*. This can be attributed to the difference in fine structure expected between the molecules in different *Acacia* gum species.

Dextran, a pure polysaccharide, showed no significant change in its intrinsic viscosity in different solvents, namely 0.2M NaCl, water, and 2M urea because there is no protein in its structure (Figures 5). Casein protein molecules showed different intrinsic viscosity in different solvents due to denaturation effects by water and 2 M urea. The intrinsic viscosity of Casein in 0.2M NaCl was found to be 12.04 ml/g, while in water it was 23.72 ml/g, and in 2M urea, it was 18.29 ml/g (Figures 6).

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Table 1: Average value of nitrogen and Protein content of the samples

Sample	Nitrogen %	Protein%
Acacia senegal	0.368	2.3
Acacia seyal	0.1440	0.9
Acacia polyacantha	0.2560	1.6

Each reading is average of three reading

Table 2: Averages values of elements composition of the gums

Element	Gum sample					
	A. seyal		*A. senegal*		*A. polyacantha*	
	w/w %	µg/g	w/w %	µg/g	w/w %	µg/g
Fe	0.004	43.98	0.0037	37.04	0.0035	34.64
Na	0.011	111.1	0.0067	67.13	0.0065	64.82
K	0.280	2802	0.9459	9459	0.7818	7810
Ca	0.942	9417	0.7092	7092	0.4113	4114
Mg	0.123	1229	0.2159	2160	0.0100	112.5
Zn	0.001	7.863	0.0021	20.51	0.0005	4.615
Pb	0.001	7.576	0.0008	7.576	0.0008	7.576

Each reading is average of three reading

Table 3: Intrinsic viscosity of the gums samples in different solvents

Solvent	Intrinsic viscosity		
	A. seyal	*A. senegal*	*A. polyacantha*
0.5 NaCl	14.51	14.51	10.52
0.2 NaCl	15.48	20.02	11.72
0.1 NaCl	15.95	20.42	13.22
0.05 NaCl	18.25	22.05	13.42
0.01 NaCl	22.77	28.18	14.26
0.001 NaCl	31.80	59.54	29.04
water	39.95	84.02	33.48
2M urea	47.07	87.90	31.42
4M urea	48.06	80.85	29.65
6M urea	47.47	83.41	31.61
8M urea	45.08	81.58	31.04
6M GHCl	14.47	17.56	10.36

Each reading is average of three reading

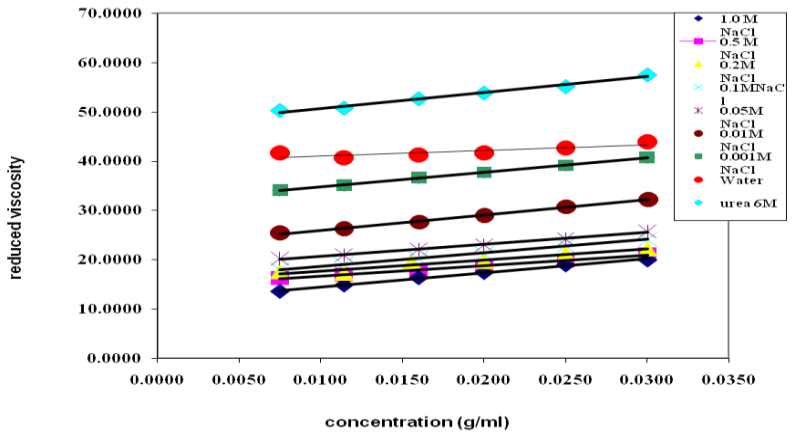

Figure 1: Viscosity of *A. seyal* gum in different solvents.

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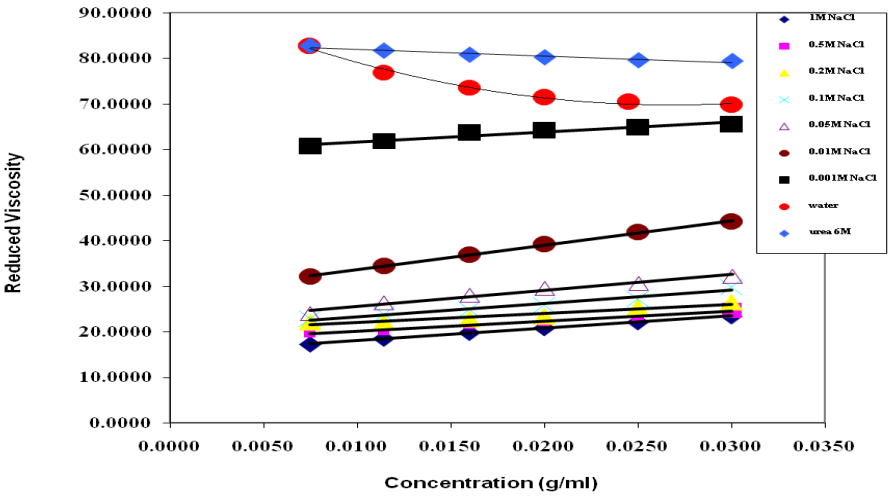

Figure 2: Viscosity of *A. senegal* gum in different solvents.

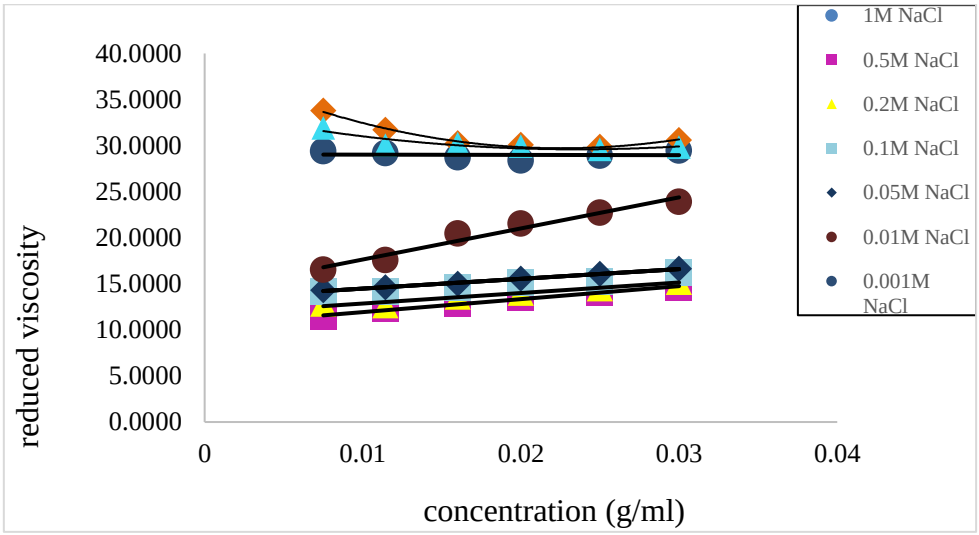

Figure 3: Viscosity of *A. polyacantha* gum in different solvents.

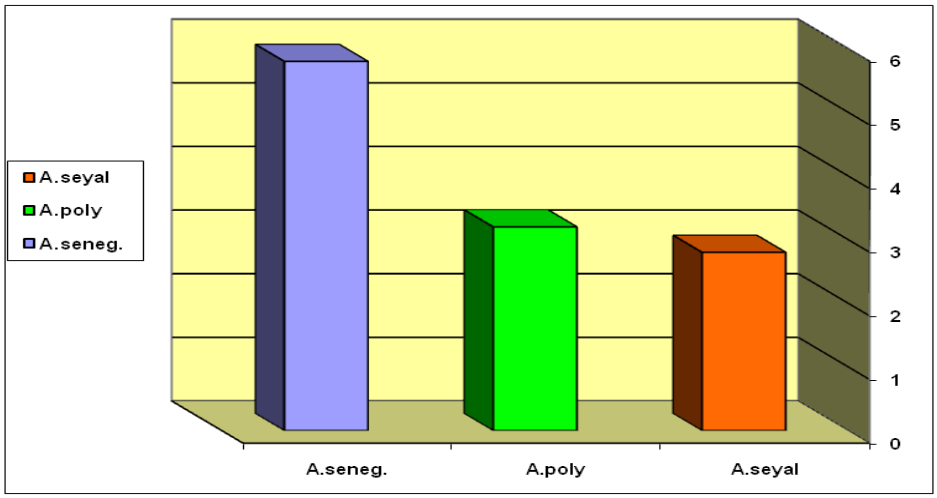

Figure 4: Expansion factors of the gums.

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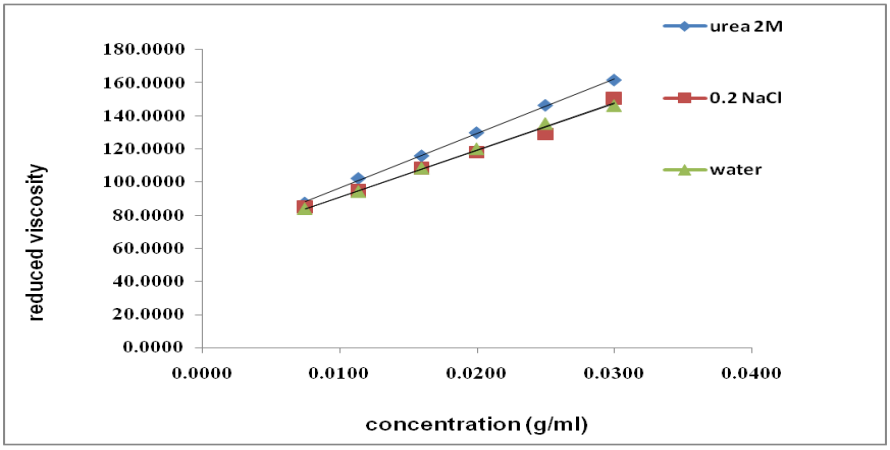

Figure 5: Viscosity of dextran in different solvents.

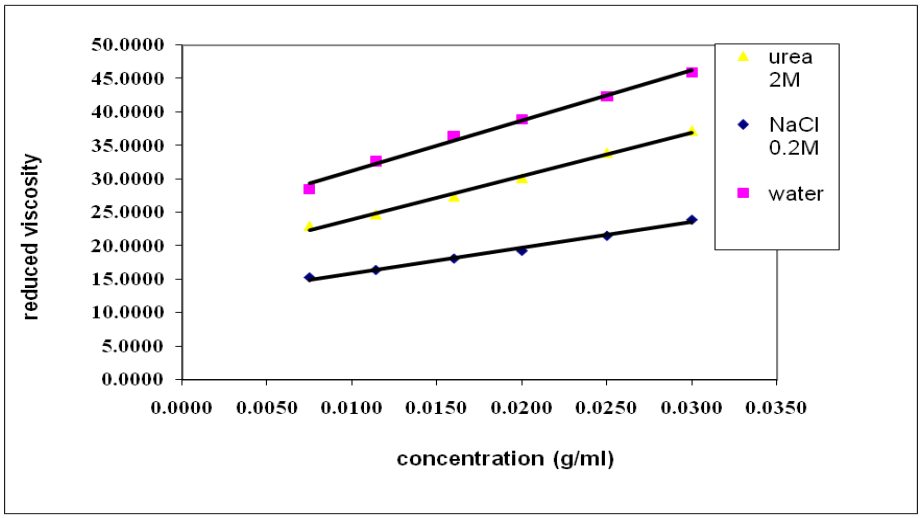

Figure 6: Viscosity of sodium caseinate in different solvents.

CONCLUSIONS

The results showed that urea is a good denaturation agent for *Acacia* gum molecules. Further, *A. senegal* and *A. polyacantha* gum behaved as polyelectrolyte solutions in water, while *A. seyal* gum did not. *A. senegal* expanded in salt solutions more than *A. seyal* and *A. polyacantha* and their expansion factors were 2.8, 5.8, and 3.2 respectively, indicating a difference in the fine structure between the molecules in different *Acacia* gums. In addition, *A. senegal* molecules were concluded to be longer and exhibit less branching than *A. seyal* molecules.

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