

Original Research Article

Analytical data on acetone fractional precipitates of *Acacia polyacantha* gum.

Abstract

Three authentic *A. polyacantha* whole gum samples and five acetone fractions were analysed for nitrogen (protein), specific rotation, viscosity, pH, equivalent weight, and uronic acid content, differences were obtained between the mean values of these parameters for the three whole gum samples and the fractions on one hand and among the fractions themselves on the other hand. Fractions 1,2,3,4 and 5 shows nitrogen content of 0.32%, 0.35%, 0.40%, 0.39% and 0.37% and protein content of (2.11%, 2.31%, 2.64%, 2.57% and 2.44%) respectively. The mean values of specific optical rotation of the fractions was found -13.5°, -14.5°, -15.0°, -16.0° and -14.5° respectively. These data confirms the heterogeneity of *Acacia polyacantha* gum, and their fractions.

Keyword: Gum Arabic, *Acacia*, Fractional precipitates

1. Introduction

Gum arabic, sometimes known as the dried gummy exudation of *Acacia senegal* or closely related species of *Acacia* family *Leguminosae* (JECFA 1990). Defaye (1986) defines gum arabic as a highly branched uronic acid type heteropolysaccharide produced as exudates from trees of the genus *Acacia* maintained under unhealthy conditions. It was noted that gum was very heterogeneous and it was described as 'heteropolymolecular' i.e. having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer units, in addition to a distribution in molecular weight (Anderson 1966). Fractionation is one of the most important of analysis, the preparative technique for fractionating polydispersed polymer is the simplest, and it involves the addition of precipitants to unaqueous isolation of fractions having different solubility's. Coprecipitations may occur (Meer 1980).

Different fractions of different solubility's of the gum can be calculated, (fraction A), salt (fraction B), and the residual insoluble gel (fraction C) (Anderson 1968). The gum solution has been fractionated previously using ethanol, saturated sodium sulphate solutions (Meer 1980), ion exchange chromatography in DEAE-cellulose, phenyl sepharose CL-4B 9, anti-arabinogalactan protein (AGP) antibodies (Osman 1993), and electrophoresis. Fractionation of *A. senegal* by hydrophobic affinity chromatography reveals that it consists of at least three components: - Fraction 1 arabinogalactan (AG), fraction 2 (arabinogalactan protein) and fraction 3 glycoprotein (GP), (Randall 1989, Randall 1988). But even those contain a range of different molecular weight components revealing the polydispersity of the gum (Osman 1994). Fraction 1 containing 88% of the total has only small amount of protein. Fraction 2 represents 10% of the total with a protein of 12%. Fraction 3 resembles 1.24% of the total, but contains almost 50% protein, which is 25% of the total protein present in the whole gum. Arabinogalactan protein (AGP) is responsible for the emulsifying properties of gum arabic (Randall 1989, Randall 1988). The present study deals with some physical properties of five successive fractions obtained from sample of *A. polyacantha* gum by fractional precipitation using acetone.

2. Materials and methods

Authentic samples of *A. polyacantha* gum were collected as natural exudates in nodule forms from *A. polyacantha* tree with the help of Gum Arabic Company Ltd, Khartoum, Sudan. The gum samples were collected in season 2001 and 2002. The three samples were named as S₁, S₂ and S₃ respectively.

2.1. Methods

2.1.1. Fractionation

Solvent fractionation was carried out by a combination of the methods described by Tagar (1972). One liter of 10% clear homogenous of gum solution was placed into a three-necked round bottle flask connected with a mechanical stirrer and separating funnel. On addition of acetone 100ml portion precipitation resulted in fine filament of gum. The precipitated phase was obtained by adding one liter of acetone. On standing over night, the precipitate was separated and dried in a desiccator

(Fraction1). Different fractions (2 to5) were separated successively by successive addition of 200ml of acetone portion.

2.1.2. Nitrogen content

Nitrogen percentage was determined using semi micro Khjldahl method (A.O.A.C 1984), and the proteinaceous content was calculated as, protein % = nitrogen % X 6.6(Anderson 1986).

2.1.3. Specific rotation

The specific rotation for the three whole gums and the five fractions, aqueous solutions were determined using an optical activity Bellingham and Stanely (Ltd) polarimeter fitted with a sodium lamp with a cell pathlength 20cm. The gum solution (1%w/v) was passed through a No. 42 filter paper before carrying out measurements at room temperature (30C⁰). Triplicate readings were taken, averaged and the specific rotation was calculated. The specific rotation = $\alpha \times 100 / C \times L$ where α is observed optical rotation, C is concentration of the solution and L is length of the polarimeter tube in dm (Honig1964).

2.1.4. Viscosity

An aqueous solution (1%) was prepared from each sample of the whole gum samples and the five fractions and the rate of flow recorded for successive dilutions using a capillary viscometer (shott Gerate type 50120/11) in a constant temperature bath at 30⁰c (Billmeyer1971).

2.1.5. pH

The pH of the gum solutions was determined for the whole gum and the five fractions, using a Beckman Zeromatic IV pH meter at 30C⁰.

2.1.6. Equivalent weight

Apparent equivalent weight was determined according to the method reported in encyclopedia of chemical technologyvol.11 (Encyclopedia of chemical technology1966) with some modification.

2.1.7. Results and discussion

The present study, fractionation of authentic sample of *A. polyacantha* gum by solvent (acetone) has been carried out and some physicochemical properties have been determined and results compared between whole gum and fractions and among gum fractions themselves.

Table 1 shows some physical data for three authentic samples of *A. polyacantha* gum and its fractions. It illustrated that the mean value for nitrogen content for the three whole gum samples is 0.35% which is in agreement with that (0.35%) reported by Karamalla(1965) and within the range (0.33-0.36%) given in recent studies (Siddig 2003) . It is also in a close agreement with the mean value of nitrogen 0.34% reported by Ishag (1977).

Also from Table 1, fractions F₃, F₄, and F₅ are richer in nitrogen than the whole gum. It is evident from the protein content in *polyacantha* gum that the high amount of protein in the molecules is associated with the high molecular weight fraction. This finding of protein content in gum fractions agrees with the observation in *A.senegal* and *A. seyal* (Hassan2000).

According to Dickinson(1990)Fractionation of gum arabic using gel filtration gave only two major fractions when monitored by UV absorbency at 220 nm and a specific uronic acid assay .The first fraction (10%of the total mass) contained 10%protein and 90% carbohydrate, the hydrogenous fraction (90% of the total mass) contained only a small 0.3 – 1%) protein.

The above conclusions support the heterogeneity of *A. polyacantha* gum with regard to nitrogen (protein) content. Recently, it has been confirmed that protein rich high molecular weight component arabinogalactan protein (AGP) provides the functionality of gum arabic as an emulsion stabilizer (Karamalla1965).

Table. 1. The nitrogen and protein content of three authentic samples (S₁ , S₂ ,S₃) of *A. polyacantha* gum and its fractions (F₁ –F₅).

Sample	Nitrogen %	Protein %	Viscosity (ml/gm)	pH	Equivalent weight	Uronic acid %	Specific rotation
S ₁	0.35	2.31	10.60	4.8	1273.34	12.54	-16.0 ⁰
S ₂	0.36	2.38	10.20	5.0	1173.71	16.53	-20.5 ⁰
S ₃	0.34	2.24	10.20	4.9	1619.87	11.98	-20.5 ⁰
F ₁	0.32	2.11	11.07	4.7	1515.15	12.80	-13.5 ⁰
F ₂	0.35	2.31	11.07	4.7	1595.74	12.16	-14.5 ⁰
F ₃	0.40	2.64	11.17	4.7	1578.95	12.30	-15.0 ⁰
F ₄	0.39	2.57	10.31	4.6	1595.74	12.16	-16.0 ⁰
F ₅	0.37	2.44	11.26	4.6	1630.43	11.90	-14.5 ⁰

As can be seen from Table 1 the mean value of specific rotation of the three authentic *A. polyacantha* gum samples is (-19.0^0) which is higher than that (-10.3^0) reported by Biswas (2000), and also greater than the range (-7^0 to -13.0^0) showed by Siddig (2003). This value was less than the range (-24.5^0 to -36.7^0) for *A. senegal* reported by Siddig (1996). The specific optical rotation of the fractions ranged from (-13.5^0 to -16.0^0) but the values are generally less than those for unfractionated whole gum (-16.0^0 to -20.5^0). This results show increasing in the values of specific optical rotation for the first four fractions -13.5^0 , -14.5^0 -15.0^0 and -16.0^0 . In addition it seems that the mean value for the specific optical rotation for the five fractions (calculated as -14.7^0) is less than the mean value for the whole gum (19.0^0).

Table 1 represents that the mean value of intrinsic viscosities of the crude gums (10.3ml/gm) was significantly less than those (10.3ml/g) of the fractions (10.99ml/g). However, the values of intrinsic viscosities of *A. polyacantha* gum was closer to the results obtained by Siddig (2003) (12.7ml/g), and less than (14ml/g) for *A. seyal* reported by Elkhatim (2001). The variation in the intrinsic viscosity values of the fractions indicate the heterogeneity of *polyacantha* gum, such variation may be due to the variation in molecular size, branching and the degree of polymerization of monosaccharide in the gum polymer system.

It is clear from Table 1 that the pH value of whole gum ranges from 4.8 to 5.0. The mean values for the fractions ranges from 4.6 to 4.7. The mean value for the whole gum (4.9) is greater than that for the fractions (4.7). The mean value of pH of the whole gum samples was found to be (4.9) which was in agreement with that value of *senegal* gum (3.19-5.61) (Siddig 1996), and in agreement to the range of (4.9) reported by Siddig (2003).

Table 1 represented the mean value of equivalent weight of the whole gum samples was (1355.64). The value is less than that of the fractions mean value (1583.20). However the above result is in agreement with that obtained by Siddig (1996).

Table 1 illustrated that all *polyacantha* whole gum samples and fractions have acidic sugar (glucuronic acids), uronic acid content of the whole gum and fractions from 11.98 to 16.53, 11.90 to 12.80 respectively. It is clear that the mean value of uronic acid content of the whole gum is 14.58) which is higher than that for the fractions (12.26). The mean value (14.58) for the whole gum samples which is in the range of *senegal* gum (10.34% to 123.32%) reported by Siddig (1996) and is in close agreement with the mean value of *Anogesissus Leiocarpus* gum reported by Eltayab (1999). Successively we have obtained different compounds by fractional precipitation of *A. polyacantha* gum-using acetone; such results were obtained by Anderson and Stoddard (1966) for *A. senegal*. Successively we have obtained different compounds by fractional precipitation of *A. polyacantha* gum-using acetone; such results were obtained by Anderson and Stoddard (1966) for *A. senegal*.

The above fractionation of *polyacantha* gum confirmed the hetero polymolecular polymer, nature consisting of molecules that differ in their sugar composition and their mode of linkage as well as in molecular size. All data presented here from physicochemical analysis, indicated that the fractionated gums differed in several aspects from crude gum samples.

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