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Use of Gel Permeation Chromatography in Studies of *Acacia Polyacantha* Gum

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ABSTRACT

Fractionation of *Acacia polyacantha* gums(Kakamut) using a gel permeation column coupled to a multi – detector system comprising light scattering, refractive index and UV detector gave an insight in the molecular mass of the gum molecules. The resultant chromatograms showed a high molecular weight (Mw) fraction associated with much of the protein present in the polymer molecule, a low molecular mass fraction of much lesser amount of protein and low molecular mass proteinaceous fraction. The molar mass distribution pattern produced for *A. polyacantha* differed from that obtained for *A. senegal* gum. However the patterns of the two types of gums differed in the proportion of each fraction. Comparison of three *A. polyacantha* gum samples revealed that the Layoon soil samples (Layoon forest) showed, a higher Mw fraction (32%) than the samples of the clay soil (Abugarin and Gargadah) 30% and 20%, respectively.

INTRODUCTION

Gel permeation chromatography (GPC) is a well-established technique for the characterization of polymers. The column fractionation is based on the molecular sieve

effect “gel filtration”. The polymer is passed through a column packed with gel particles which contain pores of various sizes.

GPC coupled on line with an absolute molecular weight determined device such as a lesser light scattering photometer and a concentration sensitive detectors such as refractive index or ultraviolet are currently the best available techniques for a quick and indirect determination of polymer molecular weights and their distribution. The light scattering detector utilizes the principle that the intensity of light scattered elastically b a molecule is directly proportional to Mw. Using the refractive index detector connected directly after the light scattering detector, it was possible to measure the molecular weight of each fraction as it elutes from the GPC column. In addition, an ultraviolet (UV) was used detector at 214 nm which specifically estimates the amount of protein in the fractionated material. A typical GPC elution profile of *A. polyacantha* gum reveals that the light scattering response shows two distinctive peaks. The first peak has a high response since it corresponds to the high molecular weight material arabinogalactan protein (AGP) content .The second peak is broader with lower response and it accounts for the rest of the gum (90%). The refractive index (RI) response also shows two peaks but the response is opposite to that in light scattering. This is because it is a concentration detector and since the AGP is only 10% of the total its peaks is smaller than that of the arabinogalactan (AG) and glucoprotein (GP). The UV response shows three peaks. The first peak is for AGP, which has the protein core, and the carbohydrate attached to it. The second peak appears as a shoulder immediately after the AGP and corresponds to AG. Finally the third peak elutes just before the total volume and it corresponds to the GP. The GP peak is not detected on the light scattering (mass detector), since it has low molecular weight, also it cannot be seen on the refractive index detector (Siddig2003). AGP for *A.senegal* (gum arabic) could be degraded by

proteolysis enzymes, to give molecules with molecular mass similar to the bulk of the gum, and hence, it has been suggested that this fraction has a Wattle- blossom structure where, approximately, five blocks of carbohydrate are attached to a common polypeptide chain (Connolly et al 1988, Osman et al (1993). Qi et al (1991) isolated the molecular species of *A.Senegal*, corresponding to AGP by GPC fractionations, and following hydrogen fluoride deglycosylation, they concluded that the polypeptide chain consisted of ca 400 amino acid residues with a simple empirical formula [hyp4, ser2, thr, Gly, leu, his]. This finding was also consistent with Williams et al (2000), findings. Qi (1991) suggested that the molecules resembled a twisted hairy rope with small blocks of polysaccharide ca30 residues attached to the peptide chain. The aim of this work is to fractionate the *A.polyacantha* gum using gel permeation column coupled to a multi-detector system comprising light scattering, RI and UV detectors gave insight of the distribution of the molecular fractions of the gum molecule.

MATERIAL AND METHODS

The *A. polyacantha* (*syn campylacantha*) gum samples one, two and three (AP1,AP2 and AP3) ,respectively, were collected from Abugarin, Gargadah (Blue Nile State), and Layoon forest (South Kordofan) as the first collection of the 2001\2002 gum season.

The gel permeation chromatography was determined according to method reported by Siddig(2003). The GPC system comprising a high precision HPLC pump (water, USA), an injector (Rheodyne 7125, Rheodyne, UK), a GPC column (Sepharose 6, Pharmacia, Sweden) attached to a multi-angle laser light scattering system (DAWN DSP, Wyatt Technology, USA), and also attached to a differential refractometer (Optilap DSP,

Wyatt Technology USA), and UV detector (Pye Unicam, UK). The software used was Astra 4.5 for window Wyatt Technology, USA.

The system is switched on for two hours to equilibrate before carrying out any analysis. Gum samples were accurately weighed 0.02g in a small vial to which 5ml of 0.2 M aqueous NaCl were then added. The vials were stoppered and kept on a roller shaker for two hours. 100-microlitre solutions were injected into the GPC system via 0.45-micron filter (water Millipore) into the injector fitted with 100-microlitre loop. Elution buffer, 0.2 M NaCl, was passed at flow rate of 0.5 ml /minute at ambient temperature. The gum was fractionated while passing through the Sepharose 6 GPC column and fractions were detected via multi - angle light laser system (MALLS), RI and UV detectors. The responses were collected and analyzed in real time by the computer software.

RESULTS AND DISCUSSION

Acacia polyacantha gum samples were fractionated using a GPC. The fractionation profiles were followed by multi - detector system comprising a multi-angle laser light scattering (MALLS) detector, a refractive index detector and ultraviolet detector. The main advantages of such system is that it provides qualitative and quantities information simultaneously. MALLS provide molecular mass elution volume profile, while refractive index detector provides fraction's concentration. elution volume profile, where as the UV detector provides volume profile, where as the UV detector provides a profile that follows, the protein distribution in the fractions. The computer software of the system accurately calculates the molecular parameters such as weight average molecular weight(

Mw) and number average molecular weight (Mn) parameters, and plots of all detectors responses in real time.

Fig.**1** showed the MALLS profiles obtained for *A. polyacantha* sample one AP1.

The figure shows three peeks. In this respect *A. polyacantha* is unique compared to *A. senegal* and *A. seyal* that showed only two peaks for similar profiles (Siddig2003). The UV detector shows more than two peaks, and the RI detector shows only two peaks, fraction 1 of high molecular weight low protein and low concentration. Fraction 2 molecular weight is lower than fraction 1 and has highest protein content but low concentration, where as fraction 3 which has the lowest molecular weight, lowest protein but present higher concentration.

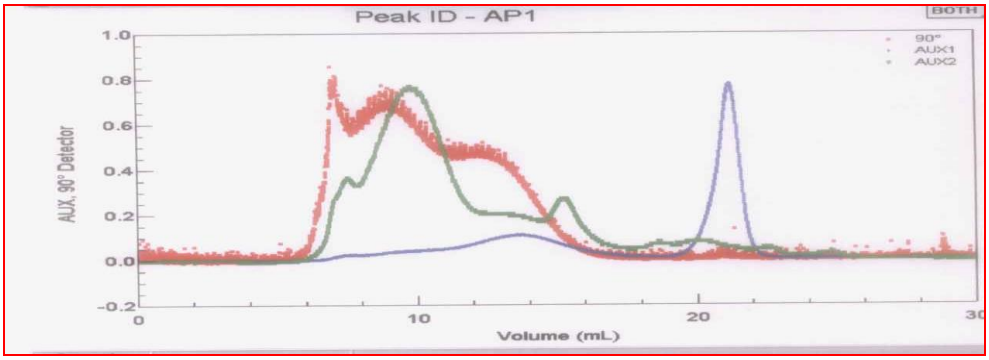

Fig.1. Detectors' response vs. elution volume chromatogram for *A. polyacantha* gum test sample(AP1)
Green line = UV, Blue line =RI and Red line = MALLS

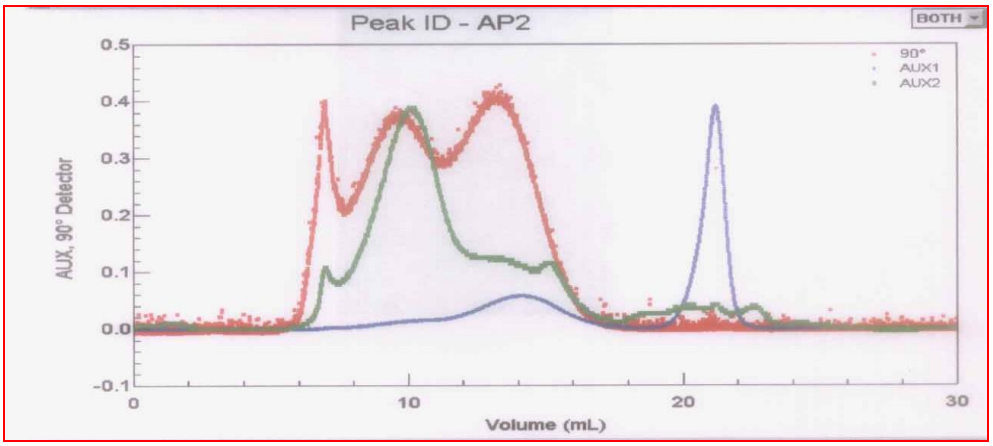

Fig.2.Detectors' response vs. elution volume chromatogram for *A. polyacantha* gum test sample (AP2)Green line = UV, Blue line =RI and Red line = MALLS

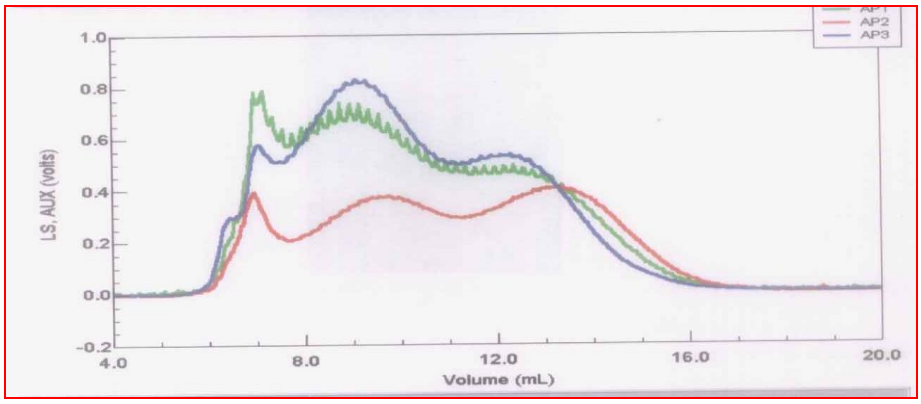

Fig. 4. Light scattering response vs. elution volume chromatogram for *A.polyacantha* gum test samples (AP1, AP2 and AP3)
Green line = AP1, Blue line =AP3 and Red line =AP2

Fig.**2** illustrated the molecular weight distribution of sample AP2. The light scattering detector explains three peaks; UV detector shows more than three peaks where as RI detector showed two peaks. Fraction one(F1) and Fraction three F3 showed approximately same molecular weight distribution and same protein

co

ncentration but F3 has the highest concentration compared to the other two fractions. Fraction two (F2) showed lower molecular weight compared to F1 and F3 but highest protein and low concentration.

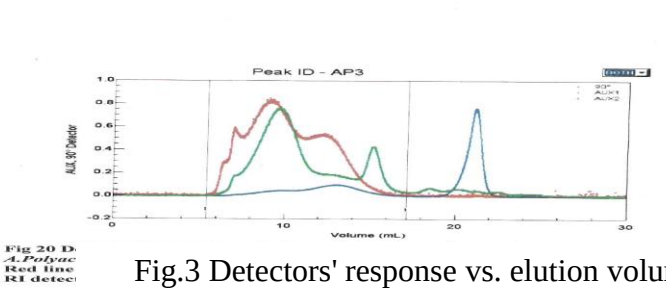

Fig.3 Detectors' response vs. elution volume chromatogram for *A.rotyacantha* gum test sample (AP3).
Red hine = light scattering dectot, Green line= UV detector and Blue line = RI detector

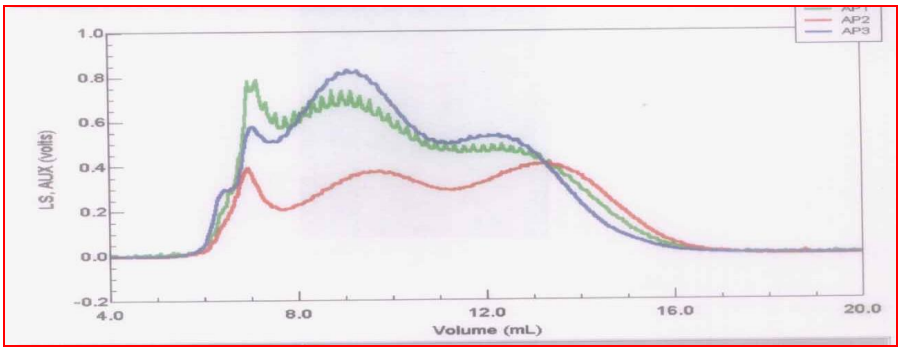

Fig. 4. Light scattering response vs. elution volume chromatogram for *A.polyacantha* gum test samples (AP1, AP2 and AP3).

Green line = AP1, Blue line =AP3 and Red line =AP2

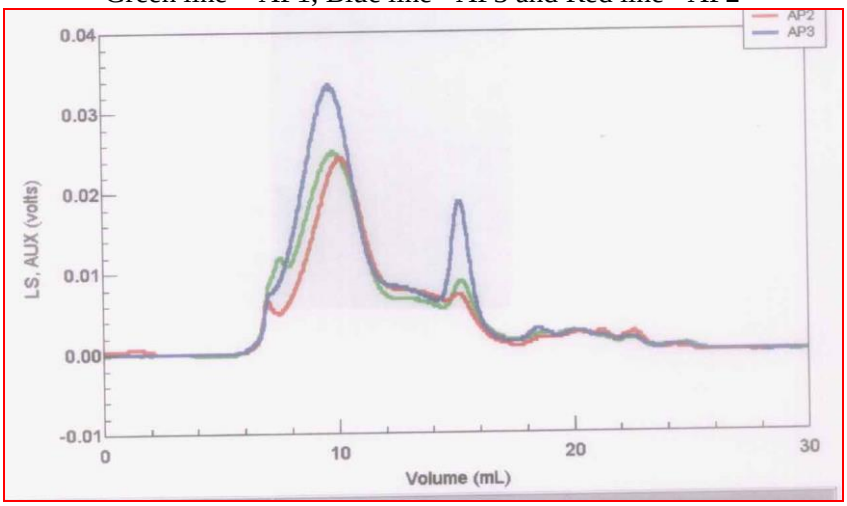

Fig. 5. UV response vs. elution volume chromatogram for *A.polyacantha* gum test samples (AP1, AP2 and AP3)

Green line = AP1, blue line = AP3 and red line = AP2

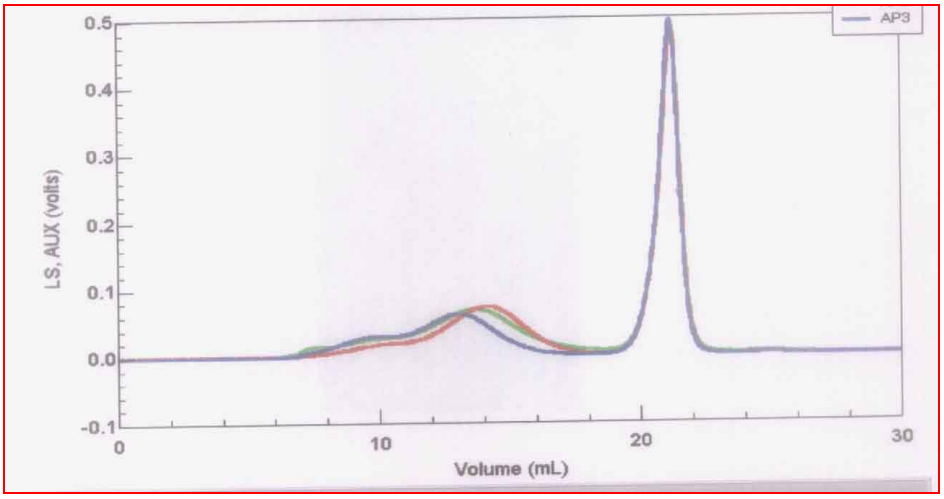

Fig. 6. RI response vs. elution volume chromatogram for *A. polyacantha* gum test samples (AP1, AP2 and AP3)

Green line = AP1, blue line = AP3 and red line = AP2

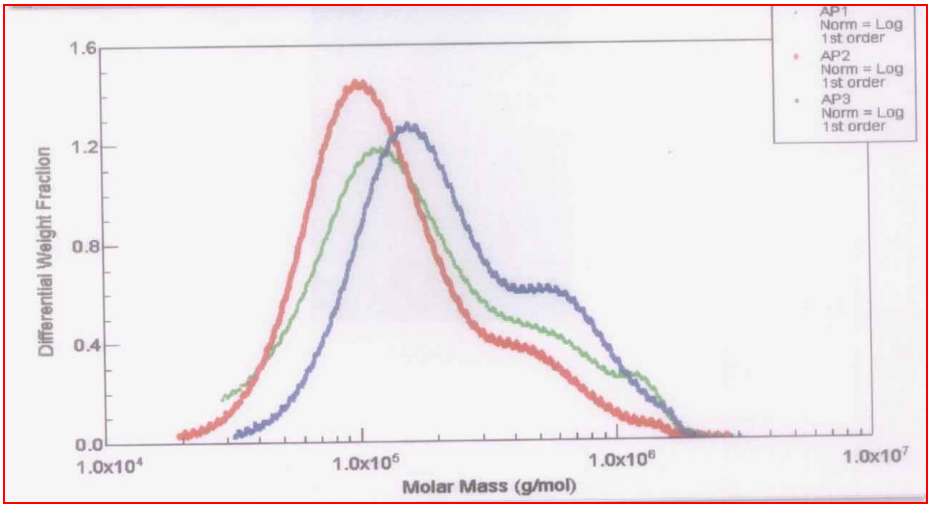

Fig.7. Differential weight fraction vs. molar mass (g/mol) chromatogram for *A.polyacantha* gum test samples (AP1, AP2 and AP3)

Green line = AP1, blue line = AP3 and red line = AP2

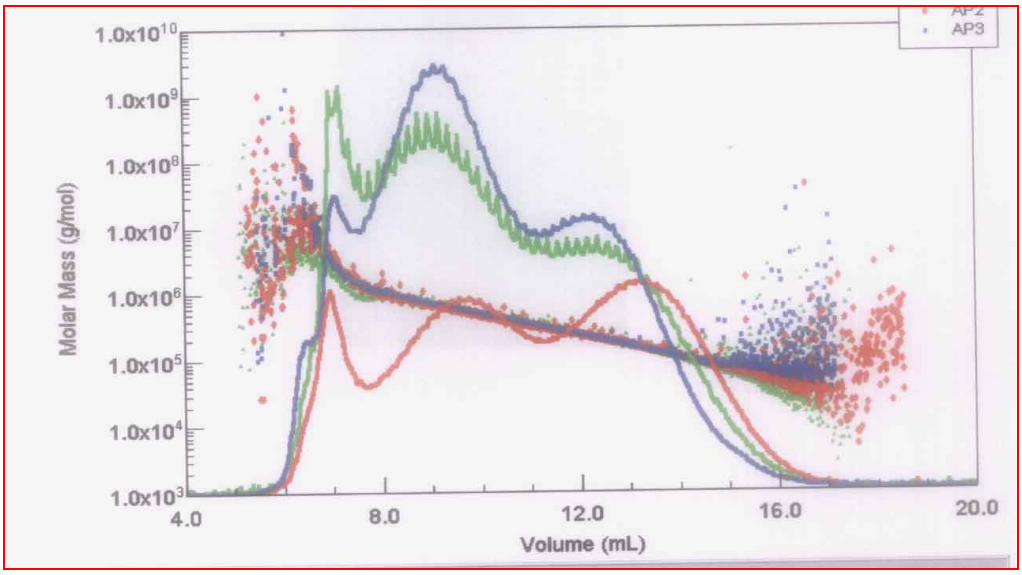

Fig.8. Molar mass – elution volume GPC profile for *A.polyacantha* gum test samples (AP1, AP2 and AP3)

Green line = AP1, blue line = AP3 and red line = AP2

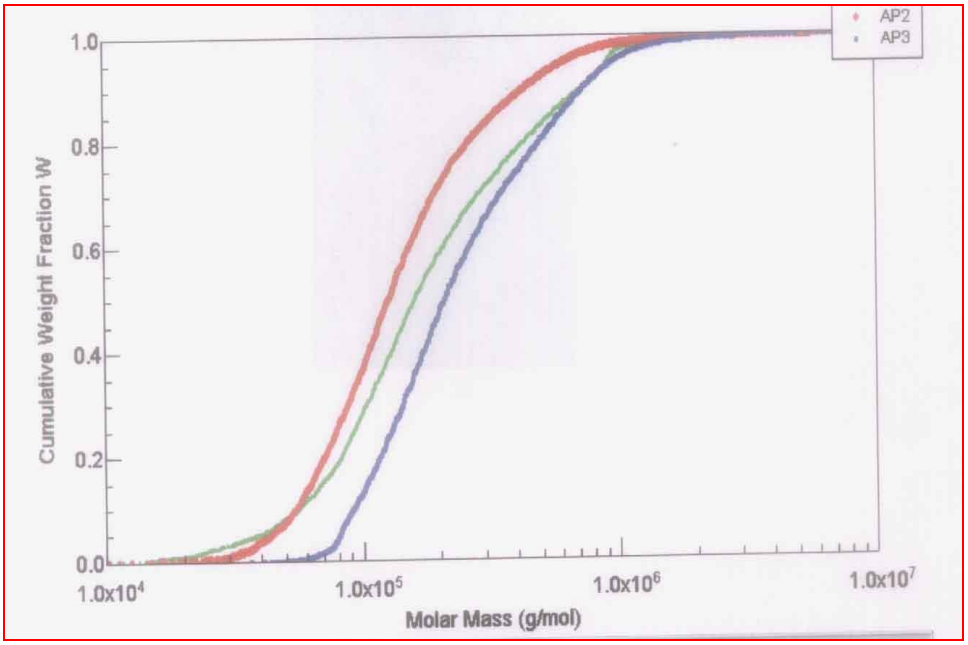

Fig. 9. Cumulative molar mass fractions for *A. polyacantha* gum test samples (AP1, AP2 and AP3)

Green line = AP1, blue line = AP3 and red line = AP2

Fig.**3** presented the molecular weight distribution of sample AP3. The light scattering detector showed three peaks, F2 showed highest molecular weight, highest protein and low concentration, F1 showed lower molecular weight, lowest protein and the lowest concentration, F3 showed the lowest molecular weight, low protein compared to F2 and highest concentration.

Fig.**4** showed the Light scattering signal for the gum samples, which showed different molecular weight distribution, it reveals that sample AP1 (Layoon) has highest molecular weight followed by sample AP3 (Gargadah) and then AP2 (Abugarin), it is clear that there is some difference between samples. The sandy soil sample (AP1) illustrates higher Mw fractions than the clay soil samples (AP1, AP2) eluted at retention volume 6.9 ml. High molecular weight fraction for sample AP1 (Layoon) obtained from sandy soil was found to be 32%, whereas the high molecular weight fraction for sample AP2 (Abugarin) was found to be 30% and the sample AP3 (Gargadah) shows high molecular weight fraction as 20%. It reveals that the sandy soil of *A. polyacantha* possess high proportion of high molecular weight fraction in comparison with the gum from clay soil.

Fig.**5** showed the UV response at 214 nm of the gum samples. The distribution pattern of UV signal for the three samples are, similar while the amount of protein associated with each fraction in all samples varies. This is reflected in the total amount of protein in each sample (AP1=0.28%, AP2=0.35% and AP3=0.40%) (Omer2004). 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 distribution of

protein agrees with the observation of *A. senegal* and *A. seyal* (Osman1993, Randall et al 1988 and Hassan2000).

Fig.**6** showed the refractive index response for the three samples of *A. polyacantha* gum. From studying this response, it observed that low molecular weight fractions are same and equal, and the high molecular weight fractions are similar and vary in amount in each sample.

Fig.**7** showed differential weight fraction and the molar mass for the *polyacantha* gum samples AP1, AP2 and AP3, the differential weight fraction illustrates the greater similarity in the details of the molecular structure with differences in polydispersity in each sample.

Fig.**8** showed the molar mass distribution, according to this distribution the higher molecular mass fractions eluted first from the GPC column than the others lower molecular mass fractions.

Fig.**9** explained the difference in properties of the three samples AP1, AP2 and AP3 associated with natural products (Flory1953). However the percentage of *polyacantha* is unique compared to *A. senegal and A. seyal* that showed only two peaks for similar profiles. The UV detector shows more than two peaks, and the RI detector shows only two peaks, fraction 1 of high molecular weight low protein and low concentration. Fraction 2 molecular weight is lower than fraction 1 and has highest protein content but low concentration, whereas Fraction 3 which has the lowest molecular weight, lowest protein but present higher concentration.

CONCLUSIONS

In this paper we presented a range of a molecular weight distribution for *polyacantha* gum samples drawn from season 2001 using multi angle laser light scattering system. The observation obtained from this study can be used for setting specification of this gum. The anticipated results, can serve as a bench mark for other workers to similarly characterize their own gum product.

Acknowledgment

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تجزئة صمغ الكاكموت باستخدام عمود نفاذية الهلام المدمج ضمن نظام متعدد المكشاف

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الملخص

اجريت دراسة تحليلة علي عينات نقية من صمغ الكاكموت من السودان (موسم2002/2001) لتوضيح تجزئة صمغ الكاكموت باستخدام عمود نفاذية الهلام المدمج ضمن نظام متعدد المكشاف والتي شملت مكشاف التشتت الضوئي،مكشاف معامل النكسار ومكاشف الأشعة فوق البنفسجية. نمط توزيع الاجزاء الجزيئية لصمغ الكاكموت أوضح الكروماتوغرام الناتج وجود جزءا جزئيا عالي الوزن الجزيئ مرتبطا بالقسم الاكبر من بروتين صمغ الكاكموت ، وجزءا بروتينيا له وزن جزيئيا صغيرا. ويختلف نمط توزيع الاجزاء الجزئية لهذا الصمغ عن نمط توزيع صمغ الهشاب. اظهرت مقارنة ثلاثة انواع من صمغ الكاكموت باستخدام نفس النظام متعدد المكشاف ، ان الصمغ المنتج من التربة الرملية (غابة الليونة - جنوب كردفان) قد اظهر جزيئات ذات وزن جزيئي عالي مقارنة بالوزن الجزيئي لجزيئات الصمغ المنتج من التربة الطينية (ابوقرن و قرقدة- جنوب النيل الأزرق)، الذي أعطي (20-32%) علي التتابع.