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Acacia macracantha gum as a possible source of arabinogalactan–protein

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Abstract

Acacia macracantha, a Venezuelan *Gummiferae* species, exudes a soluble gum which has a very high polydispersity index (Mw/Mn = 3.75). It consists of carbohydrate predominantly and a proteinaceous material as a minor component. The relatively high nitrogen content (4.98%) led to study the latter material. Fractionation of the gum by atmospheric pressure size exclusion chromatography (APSEC) was monitored by determination of total protein and carbohydrate contents. High pressure exclusion chromatography (HPSEC), coupling on-line with multi-angle light scattering (MALLS) and differential refractive index (DRI) detectors were also used. The elution profiles of the original gum and those of the gum after basic and enzymatic hydrolyses showed a heterogeneous system. Studies of the highest molecular weight fraction, that contain carbohydrate and protein, revealed the presence of hydroxyproline, serine, and lysine. On the other hand, sequential acid hydrolysis study of this fraction demonstrated the presence of rhamnose and arabinose as terminal and internal residues, respectively. The findings suggest that the gum from *A. macracantha* contains probably AG and AGP.

Keywords: Gum exudates; *Acacia macracantha*; Arabinogalactan–protein; Elution profile

1. Introduction

Acacia macracantha gum, a Venezuelan *Gummiferae* sp., exudes a light brown gum very soluble in water. Analytical data of this gum have been published (Martínez, León de Pinto, & Rivas, 1992; Martínez, León de Pinto, Rivas, & Ocando, 1996). The polysaccharide contains D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid, and 4-*O*-methylglucuronic acid as has been reported for other *Gummiferae* gums (Churms & Stephen, 1984; Gamon, Churms, & Stephen, 1986; Gamon & Stephen, 1986; Islam, Phillips, Sljivo, Snowden, & Williams, 1997) and for *Acacia senegal* gum (Islam et al., 1997; Street & Anderson, 1983). This gum contains very high nitrogen content (4.98%), this feature is unusual for *Gummiferae* gums (Beltrán, León de Pinto, Martínez, & Rincón, 2005b; Martínez et al., 1992, 1996). A combination of chemical methods and ^{13}C NMR spectroscopy demonstrated that the polysaccharide gum is a highly branched structure. The core consists of a β-1,3-galactan with β-1,6-D-galactose branch points. L-Arabinose and D-glucuronic acid were also present which were difficult to remove after Smith-degradation process. The branches of the polysaccharide structure are constituted by galactose, arabinose, rhamnose, and uronic acids' residues (Martínez et al., 1996).

The molecular weight distribution of *Gummiferae* and *Vulgares Acacia* gums has been reported. The *Gummiferae* gums have higher average molecular weight than the *Vulgares* ones (Al-Assaf, Phillips, & Williams, 2005).

Many studies of the well-known gum from *A. senegal* (gum arabic) have shown the structural complexity of this

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gum. It is a mixture of an arabinogalactan (AG) and an arabinogalactan–protein (AGP) (Akiyama, Eda, & Kato, 1984; Connolly, Fenyo, & Vandevelde, 1987; Picton, Bataille, & Muller, 2000; Qi, Fong, & Lamport, 1991; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005).

The complex arabinogalactan–proteins are proteoglycans found in higher plants, their function is not clearly elucidated; however, they seem to be implicated in diverse developmental roles such as differentiation, cell–cell recognition, embryogenesis, and programmed cell death (Gaspar, Johson, McKenna, Bacic, & Schultz, 2001; Nothnagel, 1997). The behavior of gum arabic in aqueous solutions seems to be related to the presence of AGP, its interfacial properties appeared to be strongly influenced by their arabinogalactan–protein complex (Aoki et al., 2007; Fauconnier et al., 2000).

This work deals with the fractionation of *A. macracantha* gum and partial characterization of the fractions that contain probably AG and AGP.

2. Materials and methods

2.1. Origin and purification of gum samples

Gum from *A. macracantha* (Gummiferae Series) was collected, by the authors, in March–April 2004 (no rainy season), from trees located in Zulia State, Venezuela, South America. The identification of the voucher specimens was confirmed by Prof. Lourdes Cardenas de Guevara, Botanic taxonomist of the Universidad Central de Venezuela.

The gum exudates, collected 2 weeks after the exudation, were purified. Sample solutions (3%) were prepared and filtered through muslin and then through Whatman No. 1 and No. 41 filter papers, dialyzed for 2 days against tap water, and freeze-dried.

2.2. General methods

2.2.1. Amino acid determination

The sample (5 mg) was hydrolyzed (6 M HCl; 1 mL) in a nitrogen atmosphere, for 12 h. The solution was filtered through Whatman No. 1 and reduced in volume to dryness. The residue was dissolved in a buffer solution (sodium citrate, pH 2.20) and injected to a post column derivatizer (Pickering Laboratories), adapted to a cation exchange column (Na^+, 8 μm, 3 mm ID × 250 mm) and a Perkin-Elmer 785 UV/VIS detector (570 nm).

2.2.2. Partial basic hydrolysis

The original gum (5 g) was hydrolyzed with a saturated barium hydroxide solution (200 mL) at 100 °C for 8 h. The hydrolyzed gum was neutralized with sulfuric acid (1 M), filtered, and freeze-dried (Gamon et al., 1986).

2.2.3. Elution profiles of the original gum and the gum after basic treatment

2.2.3.1. Atmospheric pressure size exclusion chromatography (APSEC).

The pure gum samples (150 mg) were dissolved in deionized water (10 mL). The solution was added to a glass column (50 × 1.5 cm) packed with Sephacryl S-400; NaCl 0.1 M solution was used as eluent. The elution profile was monitored by the measurement of carbohydrate and protein contents in each collected fraction (2 mL). The carbohydrate content was determinated by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) at 490 nm, while the protein content was measured directly in the collected fractions at 220 nm.

2.2.3.2. Hydrolysis with trifluoracetic acid (TFA).

Sequential acid hydrolysis was done on the highest molecular weight fraction, observed in the elution profile of *A. macracantha* gum by APSEC. The sample (100 mg) was hydrolyzed with trifluoracetic acid (0.1 M, 100 °C, 1 h). The acid concentration was increased (up to 2 M), after 1 h. Aliquots withdrawn at different intervals (1, 6, 15, and 24 h) were analyzed by p.c. with the following solvent system (v/v): ethyl acetate:acetic acid:formic acid:water (18:3:1:4).

2.2.4. Enzymatic treatment

The gum sample (250 mg) was dissolved in deionized water (25 mL) and pH was adjusted (7.5). Pronase (3.4%, 1 mL) was added to the gum solution (9 mL). This reaction system was incubated overnight at 37 °C (Connolly, Fenyo, & Vandelvelde, 1988).

2.3. Analytical techniques

The average molecular weight, high molecular weight component, and molecular weight distribution were performed by coupling on-line HPSEC to a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI). The light scattering signal is proportional to the product of concentration and molecular weight, and the DRI signal is only proportional to the concentration.

2.3.1. High pressure size exclusion chromatography (HPSEC)

The size exclusion chromatography (SEC) line consisted of an OHPAK SB-G guard column as protection and two OHPAK SB 804 and 806 HQ columns (Shodex) in series. The column packing is a polyhydroxymethylmethacrylate gel. The flow carrier (0.1 M $LiNO_3$ solution) was degassed (ERC 413), filtered (0.1-μm filter, Millipore), eluted with flow rate (0.5 mL min^{-1}) (Flom HPLC pump 301), and clarified through 0.45-μm filter unit upstream columns. The sample (100 μl, full loop) was injected.

2.3.2. Multi-angle laser light scattering (MALLS)

The SEC–MALLS technique has been described previously (Picton et al., 2000). The MALLS photometer, a DAWN–Enhanced Optical System (EOS) from Wyatt Technology Inc. (Santa Barbara, CA, USA), is fitted to a

K5 cell with 18 photodiodes and InGasAs, 30 mW laser ($\lambda = 690$ nm). The QELS detector, from Wyatt Technology is connected to 115° of the MALLS detector. The collected data were analyzed using the Astra V-4.85 software package. The concentrations of each eluted fraction have been determined with the DRI (ERC 7515A) according to a classical value used for polysaccharides of dn/dc (0.15 mL g^{-1}). The samples (5 g L^{-1}) were dissolved in the 0.1-μm filtered carrier ($LiNO_3$ 0.1 M + NaN_3 0.02%, water from Milli-Q water reagent system), stirred gently (5 h), and filtered (0.45 μm type membrane, Millipore).

3. Results and discussion

Acacia macracantha gum contains high nitrogen content (4.98%) in comparison with the values reported for other *Gummiferae* spp. gums (Beltrán et al., 2005b; Churms & Stephen, 1984; Gamon et al., 1986; Islam et al., 1997), but lower than the value for *Acacia erioloba* gum (Gamon & Stephen, 1986). The unusual high nitrogen content of the gum from *A. macracantha* suggests that it would be a good sample to study the proteinaceous material. Amino acid composition of this gum, Table 1, showed high proportion of hydroxyproline, proline, lysine, and serine, as has been reported for other *Acacia* gums, i.e., *A. tortilis*, *A. robusta,* and *A. seyal* gum (Anderson & Yin, 1987; Churms & Stephen, 1984; Gamon et al., 1986; Underwood & Cheetam, 1994) and for *A. senegal* gum (Vulgares series) (Akiyama et al., 1984; Islam et al., 1997).

The elution profile of *A. macracantha* gum, Fig. 1, obtained by atmospheric pressure size exclusion chromatography (APSEC), showed many fractions which have their characteristic range of elution volume (mL). The first fraction, constituted by carbohydrate and protein, may contain the population of the highest molecular weight. The second fraction of higher elution volume may correspond to oligosaccharides linked to peptides. The fractions 3 and 4 of relatively low molecular weight contain only peptide and carbohydrate, respectively.

Table 1
Amino acid composition of the original gum from *Acacia macracantha*

Amino acid (%)	
Hydroxyproline	25
Threonine	0.4
Serine	18
Glutamine	0.3
Proline	10
Glycine	6
Alanine	5
Valine	4
Isoleucine	0.1
Leucine	7
Tyrosine	0.1
Phenylalanine	0.2
Lysine	19
Histidine	6
Total	100

Aspartic acid, cystine, methionine, tryptophan and arginine were not detected.

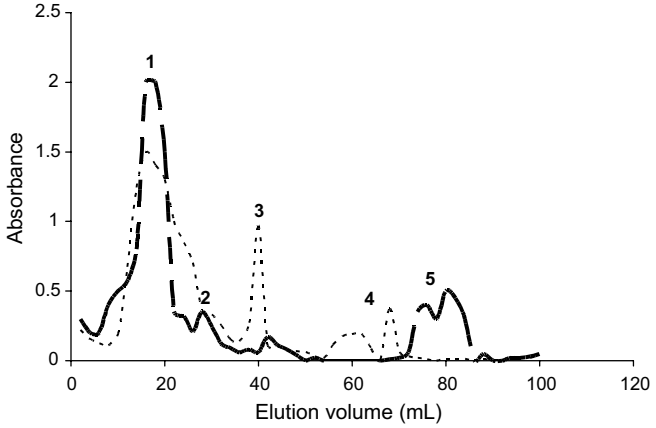

Fig. 1. Size exclusion chromatography of the original gum from *A. macracantha* on Sephacryl S-400. Injection sample 10 mL (15 mg/mL), column (50 × 1.5 cm); NaCl 0.1 M solution as eluent. The absorbances measured at 220 (···) and 490 nm (---). The elution volume of the main fraction (1) is 12–22 mL.

The gum, after basic hydrolysis, showed a modified elution profile, Fig. 2, in comparison with that of the original gum, Fig. 1. The absence of the fractions 3 and 4 that have higher elution volume indicates the vulnerability to basic hydrolysis of some linkages present in the molecular populations. The peptidic linkages are generally vulnerable to basic hydrolysis, except those hydroxyproline-*O*-glycosidic linkages, that are stable in base, in contrast to other *O*-glycosylated hydroxyamino acids such as serine and threonine which may undergo β-elimination (Goodrum, Patel, Leykam, & Kieliszewski, 2000; Lamport & Miller, 1971). The lowest molecular weight fractions (5), Fig. 1, remains after basic treatment (2), Fig. 2, which may indicate the presence

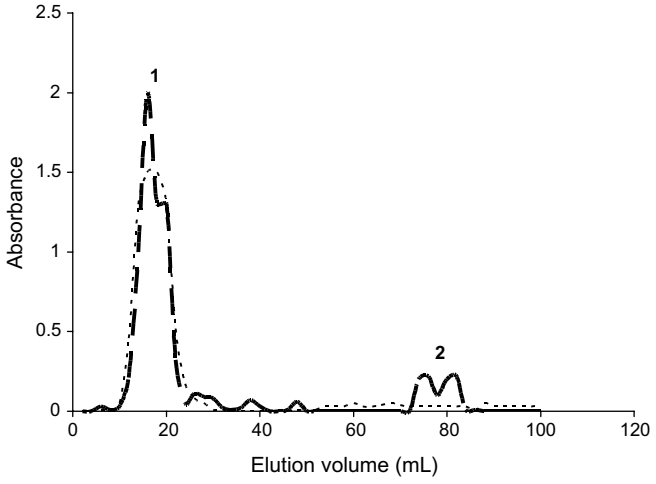

Fig. 2. Size exclusion chromatography of the gum from *A. macracantha*, after basic treatment, on Sephacryl S-400. Injection sample 10 mL (15 mg/mL), column (50 × 1.5 cm), NaCl 0.1 M solution as eluent. The absorbances measured at 220 (···) and 490 nm (---). The elution volume of the main fraction (1) is 14–24 mL.

of oligosaccharides. Much variation was not observed in the main fraction that may contain the highest molecular weight component, Figs. 1 and 2. This little variation may be attributed to partial alkaline hydrolysis and the APSEC experimental conditions were not suitable to separate the populations that have very close molecular weight. Although, the alkali conditions used and the APSEC technique led to isolation and further characterization of this main fraction, that contains carbohydrate and proteinaceous material.

Study of the main fraction of high molecular weight, isolated after basic hydrolysis, showed the presence of hydroxyproline, serine, and lysine. These amino acids, specially hydroxyproline, have been reported previously for *A. robusta* and *A. tortilis* gum (Churms & Stephen, 1984; Gamon et al., 1986). On the other hand, sequential hydrolysis of this fraction with trifluoracetic acid, Table 2, demonstrated that rhamnose is present as terminal residues in the carbohydrate structure of the main fraction; meanwhile, the relative difficulty to remove arabinose residues may suggest their position as internal residues. It is important to note that hydroxyproline, serine, and arabinose have been involved in the carbohydrate–protein linkage, in the structure of many gums (Akiyama et al., 1984; Kieliszewski, Kamyab, Leykam, & Lamport, 1992; Lamport & Miller, 1971; Nothnagel, 1997; Qin, Yamauchi, Aizawa, Inakuma, & Kato, 2001).

The elution profile, Fig. 1, discussed above, is according to the presence of a complex heterogeneous system as has been observed in some *Gummiferae* and *Vulgares Acacia* gums (Akiyama et al., 1984; Al-Assaf et al., 2005; Fauconnier et al., 2000; Underwood & Cheetam, 1994).

Pure *A. macracantha* gum has also been analyzed, by coupling on-line SEC/MALLS/QELS/DRI. Differential refractive index (DRI) and light scattering (LS) profiles, together with molar masses (MD) and hydrodynamic radii distributions (HRD), are shown in Fig. 3 and Table 3. The gum is a very polydisperse system (Mw/Mn = 3.75). The values of average molecular weight ($Mw = 9.2 \times 10^5$) and number molar masses ($Mn = 2.5 \times 10^5$ g mol^{-1}) are in the range reported for *A. senegal* gum, but lower than other *Gummiferae* gums, i.e., *A. karroo* and *A. sieberana* gums

Table 2
Sugars removed from highest molecular fraction* isolated from *Acacia macracantha* gum by acid hydrolysis in different conditions

Hydrolysis time** (h)	Removal of monosaccharide
1[a]	Rha[a]
1	Gal/GluA[b]
6	Gal/GluA/Ara[b]
15	Gal/GluA/Ara[b]
24	Gal/GluA/Ara[b]

* The fraction, containing carbohydrate and protein, was isolated by APSEC.
** Acid hydrolysis was done with trifluoracetic acid at different conditions: [a,b](0.1 and 2 M, successively).

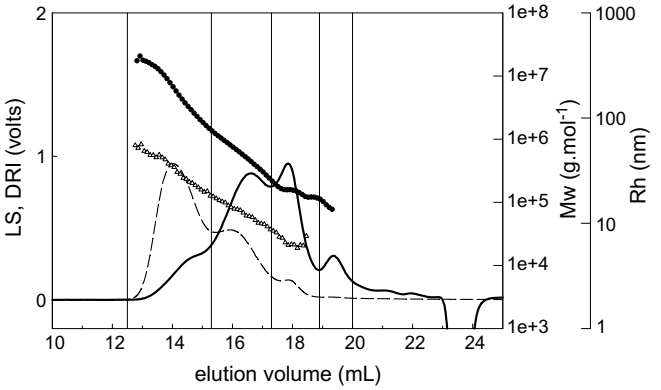

Fig. 3. SEC/MALLS elution profile of the gum from *A. macracantha* (LS: dotted line; DRI: full line). Molar masses distribution: full circle; hydrodynamic radii distribution: open triangle.

which have very high average molecular weight (2.99 and 7.10×10^6, respectively) (Al-Assaf et al., 2005).

Four fractions were observed and analyzed in a range of elution volume (mL), Fig. 3, Table 3, although, separation between these fractions is not absolute. The first fraction (12–15) showed the typical behavior of small amount (low DRI response) and very large molar masses (high LS response). This fraction, about 13% of the whole gum, contains the highest molecular weight populations ($Mw = 4.55 \times 10^6$; $Mn = 3 \times 10^6$). The other three fractions, 87% of the whole gum, contain lower molar masses, Table 3. The molar distribution of the last fraction (19–20) showed poor LS response due to the very low molar masses. These characteristics are not far from other Acacia gums (Al-Assaf et al., 2005; Picton et al., 2000; Siddig et al., 2005).

Comparison of the elution profile (DRI and LS), molar masses, and HRD distributions of the original gum from *A. macracantha*, Fig. 3, and that exhibited by the gum after partial basic hydrolysis, Fig. 4, shows some interesting modifications. A sensible decrease of DRI response of the first fraction from the pure gum (13%) to the gum after alkaline hydrolysis (3%) is shown in Table 4. The distribu-

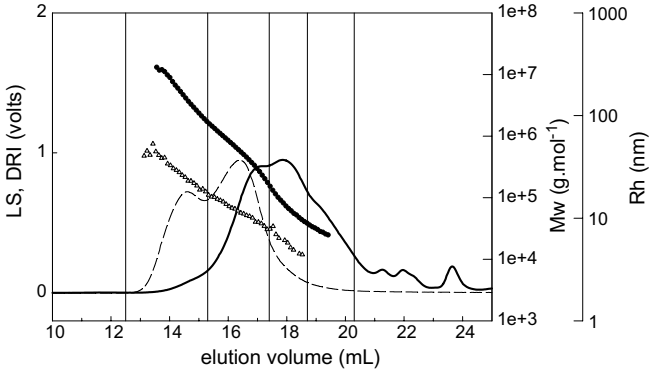

Fig. 4. SEC/MALLS elution profile of the gum from *A. macracantha* after basic hydrolysis (LS: dotted line; DRI: full line). Molar masses distribution: full circle; hydrodynamic radii distribution: open triangle.

Table 3
Physico-chemical characteristics of *Acacia macracantha* original gum obtained by SEC/MALLS experiments

	Fraction (elution volume, mL)				Whole gum (11.8–20.0)
	1 (11.8–15.2)	2 (15.2–17.1)	3 (17.1–18.9)	4 (18.6–20.0)	
Mw (g mol^{-1})	4.5×10^6	6.6×10^5	1.65×10^5	Poor LS signal	9.2×10^5
Mn (g mol^{-1})	3×10^6	5.4×10^5	1.6×10^5		2.5×10^5
Polymer recovery (%)	13	42	36	9	87

Polydispersity index: 3.75.

Table 4
Physico-chemical characteristics of *Acacia macracantha* gum, after basic hydrolysis, obtained by SEC/MALLS experiments

	Fraction (elution volume, mL)				Whole gum (11.8–20.0)
	1 (11.8–15.2)	2 (15.2–16.9)	3 (16.9–18.6)	4 (18.6–20.0)	
Mw (g mol^{-1})	4.3×10^6	6.8×10^5	1.3×10^5	Poor LS signal	3.8×10^5
Mn (g mol^{-1})	3.2×10^6	5.5×10^5	9×10^4		7×10^4
Polymer recovery (%)	3	22	42	21	94

tion of smaller fractions in size (i.e., fractions 2–4) is also modified after alkaline hydrolysis. This behavior may be related to the removal of some *O*-glycosylated hydroxyamino acids, vulnerable to alkaline hydrolysis (Goodrum et al., 2000; Lamport and Miller, 1971).

The effects of enzymatic treatment (pronase) on the SEC/MALLS are presented in Fig. 5 and Table 5, the proportion of the first fraction decreases from 13% to 3%, Table 5, as was observed after the alkaline hydrolysis. However, the other fractions (i.e., 2, 3, and 4) do not experiment much modifications by pronase treatment. The enzymatic specific reaction supports also the removal of some *O*-glycosylated hydroxyamino acids. Unfortunately, the presence of pronase, as traces, overlaps the light scattering response of the smallest fractions in size (fractions 3 and 4).

Studies of gums *A. senegal* (gum arabic) and *Acacia seyal* (gum talha) have demonstrated that the high molar mass fraction corresponds to an arabinogalactan–protein complex (AGP) (Al-Assaf et al., 2005; Picton et al., 2000; Siddig et al., 2005). A way to evidence AGP consists in a pronase treatment leading to destruction of such species (Connolly et al., 1987, 1988). The findings described above may indicate that fraction 1 is probably the complex arabinogalactan–protein (AGP) and the other fractions (2, 3, and 4) correspond to arabinogalactan units (AG). Apparently, the polydispersity index is the main difference between *A. macracantha* and *A. senegal* gums. There are two main fractions in *A. senegal* gum (Picton et al., 2000; Siddig et al., 2005), while four fractions are observed in *A. macracantha* gum according to SEC/DRI response.

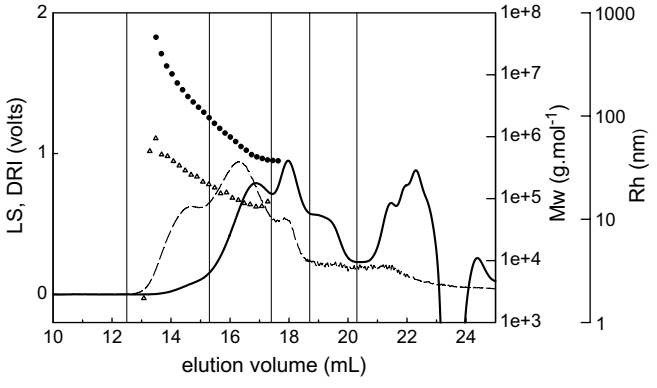

Fig. 5. SEC/MALLS elution profile of the gum from *A. macracantha* after pronase treatments LS: dotted line; DRI: full line. Molar masses distribution: full circle; hydrodynamic radii distribution: open triangle.

Comparison of the DRI elution profiles of the pure gum from *A. macracantha* with those after enzymatic and basic hydrolyses, Fig. 6, showed that both reactions lead to fraction 1 degradation, which contains probably AG and AGP. The other three fractions, observed in the elution profile of the original gum, do not experiment variation after pronase treatment, Fig. 6, but they are modified after alkaline hydrolysis. This observation leads to envisage a different process of AGP degradation occurring during the two treatments, as has been established for *Acacia tortuosa* gum (Beltrán et al., 2005a).

The fraction that has the lowest elution volume observed by APSEC corresponds to the highest molecular weight population according to HPSEC, Figs. 1 and 3, respectively. The isolation and further analytical characterization of this high molecular weight fraction demonstrated the presence of carbohydrate and protein. Acid hydrolysis led to the removal of rhamnose and arabinose from the polysaccharide gum and amino acid composition showed hydroxyproline serine and lysine. The behavior of the gum after basic and enzymatic hydrolyses and the physicochemical characteristics, Tables 3–5, were similar to those reported for *A. senegal* gum (Picton et al., 2000; Siddig et al., 2005). The analytical data support the possible pres-

Table 5
Physico-chemical characteristics of *Acacia macracantha* gum, after enzymatic treatment, obtained by SEC/MALLS experiments

	Fraction (elution volume, mL)				Whole gum (11.8–20.0)
	1 (11.8–15.2)	2 (15.2–17.4)	3 (17.4–18.9)	4 (18.6–20.0)	
Mw (g mol^{-1})	4.9×10^6	7.15×10^5	Molar masses polluted by residual pronase light scattering		7.4×10^5
Mn (g mol^{-1})	3.7×10^6	6×10^5			5×10^5
Polymer recovery (%)	3	41	34	22	65

Polydispersity index: 1.48.

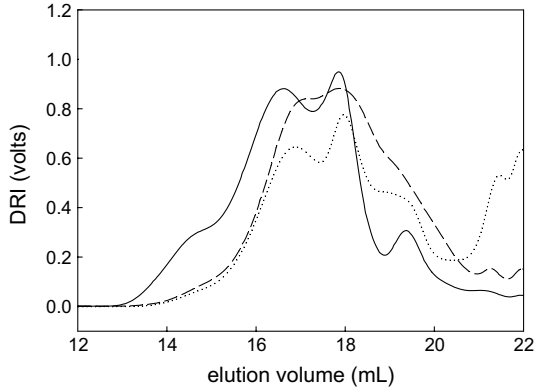

Fig. 6. Comparison of SEC DRI elution profiles of original gum from *A. macracantha* corresponding to the gum after basic and enzymatic hydrolyses (—) with the corresponding to the gum after basic (---) and enzymatic (···) hydrolyses.

ence of the complex arabinogalactan–protein in *A. macracantha* gum.

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