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Analysis of a complex polysaccharide (gum arabic) by multi-angle laser light scattering coupled on-line to size exclusion chromatography and flow field flow fractionation

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Abstract

The heterogeneous polysaccharide gum arabic has been characterized using size exclusion chromatography (SEC) and flow field flow fractionation (F4) coupled on-line to multi-angle laser light scattering (MALLS). Two distinct populations have been shown. About 80% of the material consist of highly branched arabinogalactan (AG) units. The rest is mainly composed of heterogeneous arabinogalactan–protein complex (AGP) of high molecular weight. The F4/MALLS method is shown to allow the complete analysis of gum arabic whereas SEC gave only partial information. From light-scattering-based radii of gyration (R_g) and F4-based hydrodynamic radii (R_h) we found that the ratio R_g/R_h is near 0.8 for AGP. This value is reasonably consistent with a globular conformation and gives further evidence in favour of the "wattle blossom model" proposed by Connolly et al. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Gum arabic; *Acacia senegal*; Size exclusion chromatography; Flow field flow fractionation; Light scattering; Separation

1. Introduction

Gum arabic is a natural polysaccharide exudated by tapped branches of *Acacia senegal* trees. This hydrocolloid is extensively used in a wide range of applications such as confectionery, beverage or liquid flavour emulsions, pharmaceuticals, cosmetic products, inks, etc. (Whistler & BeMiller, 1993).

The complex macromolecular structure of gum arabic is far from having been elucidated (Islam, Williams, Menzies & Phillips, 1997) even though much information about its composition and its physico-chemical properties is available in the literature. As proposed by Anderson and Stoddart (1966), the term of "heteropolymolecularity" adequately reflects the various causes of heterogeneity namely a variation in monomer composition and/or a variation in the mode of linking and branching of the monomers units, and broad molecular weight distribution.

Gum arabic is a highly branched arabinogalactan polysaccharide with rhamnose and glucuronic acid end units and containing a small proportion (ca. 2%) of protein (Akiyama, Eda & Kato, 1984; Anderson & Stoddart, 1966; Anderson, Hirst, Rahman & Stainsby, 1967; Churms, Merrifield & Stephen, 1983; Clarke, Anderson & Stone, 1979; Fincher, Stone & Clarke, 1983; Street & Anderson, 1983) composed mainly of hydroxyproline, serine and proline (Anderson, Hendrie & Munro, 1972; Anderson, Howlett & McNab, 1985; Anderson & Mc Dougall, 1987; Osman, Williams, Menzies & Phillips, 1993). Literature data reported that gum arabic is both heterogeneous and polydisperse (Anderson & Stoddart, 1966). First light scattering and osmometric measurements have resulted in, respectively, $\overline{M}_w = 580\,000$ g mol^{-1} and $\overline{M}_n = 240\,000$ g mol^{-1} (Mukherjee & Deb, 1962). More recently, characterisation by size exclusion chromatography (SEC), has given further information about the heterogeneity of this material. Vandevelde and Fenyo (1985) have shown that around 70–80% of the gum arabic is composed of a homogeneous polysaccharide (arabinogalactan AG) containing very little protein; the remainder consists of an arabinogalactan–protein complex (AGP). Later, Connolly, Fenyo and Vandevelde (1987, 1988) confirmed these results by degradation of this fraction using proteolytic enzyme (pronase). They have shown that the "wattle blossom model" could adequately describe the molecular structure of this polysaccharide (Fig. 1; from Connolly et al., 1987). In this model, a varying number of AG units of about $\overline{M}_n \sim 200\,000$ g mol^{-1}, are linked to a protein core to form the AGP complex. This complex represents about 20% of the sample, and shows a broad range of

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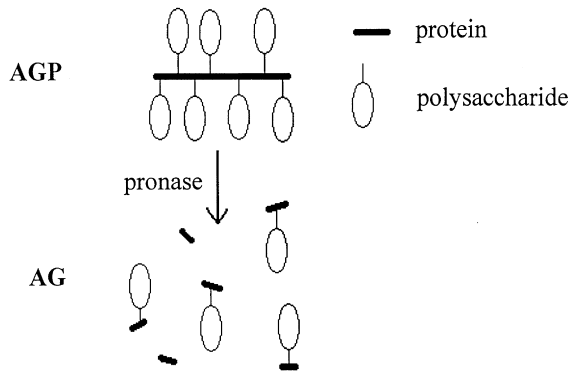

Fig. 1. Model for *A. senegal* gum before and after treatment with pronase reported by Connolly et al. (1987).

high molecular weight (Fincher et al., 1983). In contradiction with this model which predicts a compact conformation for both AG and AGP, it has been proposed that the structure of AGP is rather rod-like and has the shape of a "twisted hairy rope" (Qi, Fong & Lamport, 1991).

Recently, SEC coupled on-line to multi-angle laser light scattering (MALLS), has been demonstrated to be a very powerful method for characterisation and analysis of highly polydisperse polymer systems. This technique is based on the absolute detection of light scattered from all the eluted fractions of the polymer after SEC separation (Wyatt, 1993). Consequently, in addition to the average molecular weight ($\overline{M}_w$ and/or $\overline{M}_n$) and the radius of gyration ($\overline{R}_g$), it is possible to obtain the M_w or R_g distribution along the chromatogram (Capron, Grisel & Muller, 1995; Capron, Yvon & Muller, 1996; Picton, Mocanu, Mihai, Carpov & Muller, 1995; Picton, Merle & Muller, 1996). Moreover, from the power law describing the M_w dependence of R_g ($R_g \propto M_w^x$) useful information about polymer conformation is available through the value of x (0.3 for globular shape, 0.5 for flexible coil and ~1 for rod-like chain).

Literature data and our own experience show that SEC/MALLS gave useful information concerning the polydispersity of gum arabic. However some problems still exist, mainly the separation between AG and AGP is not totally satisfactory and the largest fractions of AGP are partly eluted in the void volume of the chromatographic columns.

A novel analytical technique, called flow field flow fractionation (F4), used on-line with MALLS seems promising for sterical separation of water soluble polymers. Based on the diffusive physico-chemical properties of the sample, the separation by F4 could cover a large range of particle sizes, contrary to the SEC fractionation (Giddings, 1966, 1993). The F4 method offers many advantages: the surface area (membrane) is about 100 times smaller than packed columns therefore limiting problems due to adsorption; shear degradation is limited due to less tortuous flow than in SEC systems; and finally, with F4, the conditions of elution can be controlled and optimised by the operator to improve resolution.

The present paper concerns the molecular characterisation of gum arabic using on-line fractionation by F4 or SEC with MALLS detection. Its main objective is to compare the two techniques with respect to the molecular characteristics of this complex polysaccharide.

2. Experimental

2.1. Solution preparation

Purified exudate from *A. senegal* (gum arabic) was kindly supplied by CNI (Rouen, France). The powdered sample was dispersed at about 7, 5 or 3.5 g l^{-1} and gently stirred for a few hours at ambient temperature in 0.1 M $LiNO_3$ which is used as eluent for the separation.

Solutions were clarified through a 0.22 μm filter unit (Millex GS). Water was from a Milli-Q water reagent system.

2.2. Analytical line

The absolute determination of molecular weight and size distributions was performed by coupling on-line either a SEC or a F4, a MALLS and a differential refractive index detector (DRI). A four-way valve fitted upstream to the MALLS detector largely facilitated the choice of F4 or SEC method.

2.2.1. Size exclusion chromatography

$LiNO_3$ (0.1 M) was filtered through 0.1 μm filter unit (Millipore), carefully degassed (ERC-413), eluted at 0.6 ml min^{-1} flow rate (Kontron HPLC pump 420), and clarified through a 0.45 μm filter unit upstream columns. The sample was injected through a 100 μl full loop.

The 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.

2.2.2. Flow field flow fractionation

The first description of the technique was published at the end of the 1960s (Giddings, 1966). The separation of particles is achieved in a separation channel ("the fractionator") by the application of a force field, which acts across the channel thickness whereas the carried liquid (linear flow L_F) flows in the direction of the longitudinal axis. In F4, the transverse force is generated by an external cross flow (C_F). The transverse field causes the transport of the particles to the lower wall of the channel (the "accumulation wall"). In the normal mode, particles below 0.5 μm in size diffuse by Brownian motion against the applied field. As a result, for a given applied cross-field, the larger the particles are (the smaller the diffusion coefficient) the closest is the equilibrium distance from the accumulation wall. As the linear flow stream shows a parabolic velocity profile, the layers flow significantly more slowly close to the

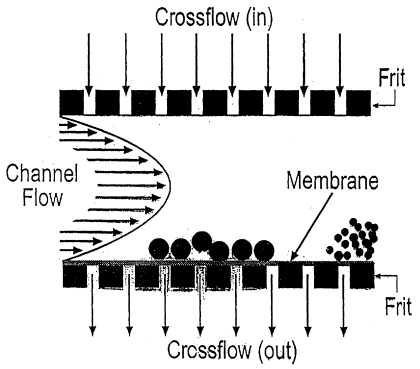

Fig. 2. Illustration of the F4 separation process.

wall than in the centre of the channel (Fig. 2). As a consequence, the smaller particles leave the channel before the larger ones contrary to what happens in SEC. The separated components are then detected and analysed by MALLS at different times after elution from the channel (Adolphi & Kulicke, 1997; Roesner & Kulicke, 1994; Wittgren, Walhund, Derand & Wesslen, 1996; Wittgren & Walhund, 1997a,b).

The F4 is a universal fractionator model F-1000, from Fractionation, LLC (Salt Lake City, USA). The channel dimensions are the following: length (l) = 27.7 cm, breadth (b) = 2 cm and thickness (w) = 254 μm. The "accumulation wall" is coated with a cellulose regenerated membrane (M_w cut off = 10 000 g mol^{-1}). The linear channel flow stream (L_F) is regulated with an intelligent pump HPLC 301, while the cross flow is generated by a P-500 (Pharmacia Biotech) dual piston syringe pump. The sample-injected volume consists of a 100 μl full loop. When the sample reaches the head of the channel, the linear flow is diverted to keep particles under the cross flow during the time needed for equilibrium. This relaxation time is proportional to the applied cross flow (about 3-sweep time).

For well-retained species, a good approximation of the diffusion coefficient (D_t) can be obtained from the retention time (t_r), the channel thickness (w) and the flow rates (L_F and C_F) using the following relationship (Adolphi & Kulicke, 1997):

$$D_t = w^2 \cdot C_F / 6 t_r \cdot L_F. \quad (1)$$

The Stokes radius R_h is simply related to D_t by:

$$R_h = k_B T / 6\pi\eta D_t \quad (2)$$

where $k_B T$ is the thermal energy and η the solvent viscosity (0.9 mPa s).

2.2.3. Multi-angle laser light scattering

The MALLS photometer, a DAWN-F from Wyatt Technology Inc. (Santa Barbara, USA) is fitted with a K5 cell and a He–Ne laser (λ = 633 nm). After the separation in the fractionator or the SEC, the light scattered from each eluted fraction (V_i) of very low concentration (C_i measured by DRI-ERC 7515A according to the known value of dn/dc) is detected simultaneously at 15 angles (between 15 and 150°). The Astra V-4.5 software package was used for calculating the molecular weight (M_{wi}) and the radius of gyration (R_{gi}) from the extrapolation of the light scattered to zero-angle at each slice according to the following relation:

$$(KC/\Delta R_\theta)_i = 1/P_\theta (1/M_{wi} + 2A_2 C + \cdots) \quad (3)$$

where

$$K = 2\pi n_0 (\mathrm{d}n/\mathrm{d}c)/\lambda^4 N_a \quad (4)$$

where n_0 is the refractive index of the solvent, dn/dc the refractive index increment of the polymer in solution: 0.141 ml g^{-1} for gum arabic (Randall, Phillips & Williams, 1989), λ the laser light wavelength (633 nm), ΔR_θ the excess Rayleigh factor (reduced scattering intensity at the angle θ). P_θ (the form factor) describes the scattered light angular dependence from which R_{gi} is obtained:

$$P_\theta = 1 + 16\pi^2 \langle R_{gi} \rangle^2 \sin^2(\theta/2)/3\lambda^2 \quad (5)$$

A_2 is the second Virial coefficient, which reflects the polymer–solvent interaction.

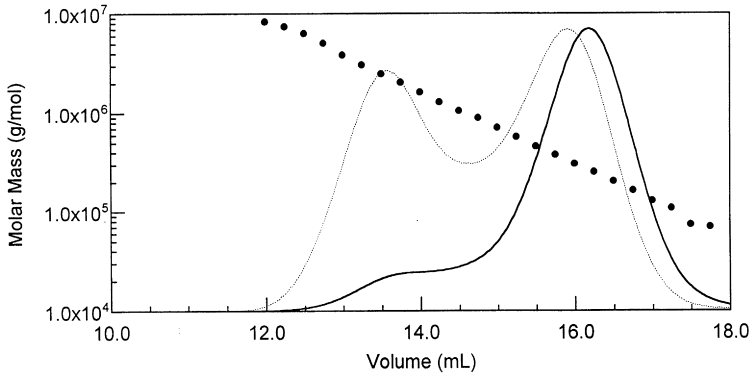

Fig. 3. SEC/MALLS: M_w as a function of elution volume for gum arabic (C_p = 3.5 g l^{-1}) flow rate of 0.6 ml min^{-1}, refractive index (filled line) and light scattering (dotted line).

Table 1
Physico-chemical characteristics of gum arabic obtained by SEC/MALLS experiments

	First fraction (1) (from 12 to 14.6 ml)	Second fraction (2) (from 14.6 to 18 ml)	Whole gum (1 + 2) (from 12 to 18 ml)
$\overline{M}_w$ (g mol^{-1}) ± 7%	2 100 000	325 000	540 000 530 000[a]
$\overline{M}_n$ (g mol^{-1}) ± 7%	1 700 000[b]	250 000	273 000
$\overline{R}_g$ (nm)	–	–	–
Polymer recovery	11%	80%	91%
$I = \overline{M}_w/\overline{M}_n$	1.2 ± 0.1[b]	1.3 ± 0.1	2 ± 0.2

[a] LALLS result.
[b] See text.

MALLS and DRI detectors were calibrated by using filtered toluene and glycerol, respectively. The MALLS instrument was normalised using standard pullulan P-100 (isotropic light scattering).

At very low C_i, the term $2A_2C_i$ is quite negligible. This has been verified from low angle laser light scattering (LALLS) (KMX-6, Chromatix) measurements, A_2 has been measured to be equal to $A_2 = 5 \times 10^{-5}$ ml mol g^{-2}. A plot of $KC/\Delta R_\theta$ vs. $\sin^2(\theta/2)$ yields a line whose intercept and slope give M_w and R_g, respectively. The extent of polymer is determined from the ratio of the calculated eluted mass (integration of the refractometer signal through the known d*n*/d*c*) and the known injected mass.

3. Results and discussion

Fig. 3 shows the evolution of the weight average molecular weight (M_w) as a function of elution volume. The filled line shows the refractive index (or concentration signal) whereas the dotted line shows the light scattering response. The latter is a function of both polymer concentration and M_w. Two distinct populations are clearly shown even if they are not separated satisfactory. The first peak reflects a small fraction of the sample as suggested by the very low refractive index signal. Nevertheless, this fraction scatters the light with a high intensity, which indicates the presence of very large molecular weight species. On the contrary, the second peak represents the major part of the sample. However, the scattered light intensity is relatively smaller than for the first peak. This clearly denotes that the second population is composed of smaller molecular weight species. We have summarised in the Table 1, the results for the whole gum and each population. The second fraction (2) has a M_w of about 325 000 g mol^{-1} and represents about 80% of the sample.

The average size ($\overline{R}_g$) of fraction 2 is probably too small with regard to the light wavelength (633 nm) to be measured. The scattered light is quite isotropic and no angular dependency is detected. The first fraction (1) which represents about 10% of the sample, has a high $\overline{M}_w$ of about 2 000 000s g mol^{-1}. The whole sample molecular weight is about 550 000 g mol^{-1}. This value has been verified by low angle laser light scattering measurements. All those results agree well with the literature (Connolly et al., 1988; Fenyo & Vandevelde, 1990; Mukherjee & Deb, 1962; Randall, Phillips & Williams, 1988; Randall et al., 1989; Williams & Langdon, 1995) and they confirm the "wattle blossom model". Fractions 1 and 2 are representative of the AGP complex and the AG units, respectively. Nevertheless

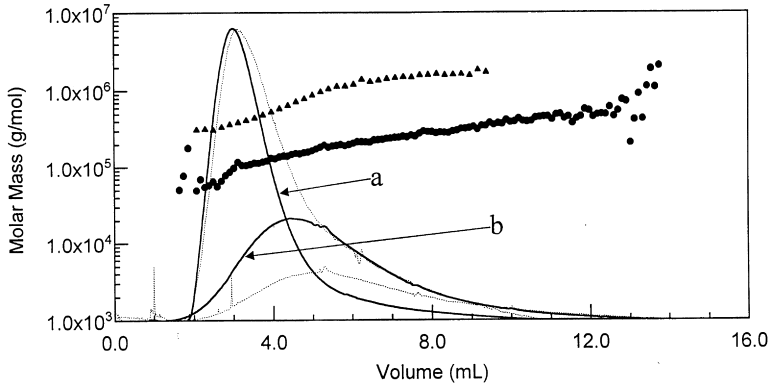

Fig. 4. F4/MALLS: M_w as a function of elution volume of gum arabic ($C_p = 3.5$g l^{-1}), refractive index (filled line) and light scattering (dotted line). (a) ▲: $F_L = 0.25$ ml min^{-1} and $F_C = 0.4$ ml min^{-1}. (b) ●: $F_L = 0.5$ ml min^{-1} and $F_C = 1$ ml min^{-1}.

Table 2
Physico-chemical characteristics of gum arabic obtained by F4/MALLS experiments for two different cross flows (C_F)

	$L_F = 0.25$ ml min^{-1}; $C_F = 0.4$ ml min^{-1}	$L_F = 0.5$ ml min^{-1}; $C_F = 1$ ml min^{-1}
$\overline{M}_w$ (g mol^{-1}) ± 7%	534 000	250 000
$\overline{M}_n$ (g mol^{-1}) ± 7%	444 000[a]	223 000
$\overline{R}_g$ (nm)	–	–
Polymer recovery	95%	82%
$I = \overline{M}_w/\overline{M}_n$	1.2 ± 0.1[a]	1.1 ± 0.1

[a] See text.

the separation of both fractions is not fully satisfactory. It is probably the reason why the $\overline{M}_w$ of the second fraction is overestimated in comparison with certain published results or predictions (Islam et al., 1997). Besides, the probable presence of AG units in the integration of fraction 1 makes it difficult to find reliable estimation for $\overline{R}_g$ of AGP. Moreover for this fraction of AGP, a poor separation certainly occurs for the first elution volumes (void volume). It could explain the apparent narrow polydispersity that we obtained (~1.2). This result is questionable with regards to the wattle blossom model for which it is expected to observe a very broad range of AGP complex. As a consequence, the SEC separation leads to unreliable values of reported M_n and polydispersity for the AGP fraction. In summary, SEC/MALLS gives some very useful information on the heteropolydispersity of gum arabic, but the separation is not totally efficient. The main objective of this work was to check if better separation and further information could be obtained using F4/MALLS.

Fig. 4 shows the M_w distribution of gum arabic using two different conditions of F4/MALLS experiments with a constant applied cross flow. In run (a) a linear flow of 0.25 ml min^{-1} and a cross flow of 0.4 ml min^{-1} were applied while run (b) was carried out with a linear flow of 0.5 ml min^{-1} and a cross flow of 1 ml min^{-1}. Under both conditions only one peak is detected therefore indicating that the two populations of gum arabic were not separated. Nevertheless, the distribution of M_w for both runs is very broad. The order of separation totally agrees with the F4 theory. The peak obtained for the (a) run (DRI or LS) is clearly narrower than the peak obtained for the (b) run. At the same time, the measured molecular weights are much higher in run (a) than in run (b). The experimental results are reported in Table 2. The $\overline{M}_w$ value of 530 000 g mol^{-1} obtained for run (a), agrees well with the $\overline{M}_w$ of the whole gum as measured by SEC/MALLS and by LALLS. As a confirmation, nearly all the gum (95%) is recovered. On the contrary, run (b) seems to represent only the population of AG units according to the results obtained by SEC/MALLS for the second fraction, i.e. an average M_w of 250 000 g mol^{-1} and about 80% of the gum recovered. The differences can be explained by considering the magnitude of the applied cross flow. If the cross flow is too small, most of particles are too far from the accumulation wall and the fractionation does not occur satisfactorily. It is probably what occurred in run (a); both fractions of gum arabic leave the channel without being separated. This could explain the low value of the polydispersity (1.2), which probably has no real meaning as no separation was achieved. On the other hand, a good separation is observed in run (b) because the elution is extended over a broader range of volume. Probably, this can be explained by a sufficient magnitude of the applied cross flow (1 ml min^{-1}). Thus, the polydispersity near unity, is, this time more reliable than in the case of run (a). This result could indicate that the major part of the gum, composed of AG units, is very homogeneous in size and in composition. Moreover, at this cross flow of 1 ml min^{-1}, the first fraction (AGP complex) is focused near the accumulation wall. As a result the AGP complex is not eluted even after 12 ml of eluted solution. As a consequence, the first fraction stays in the fractionator while the second fraction is totally eluted in about 10 ml. This situation is very favourable for a good separation of the two species.

In the following experiment, two distinct cross flows were applied. A first cross flow (C_{F1}) of 1 ml min^{-1} was used to

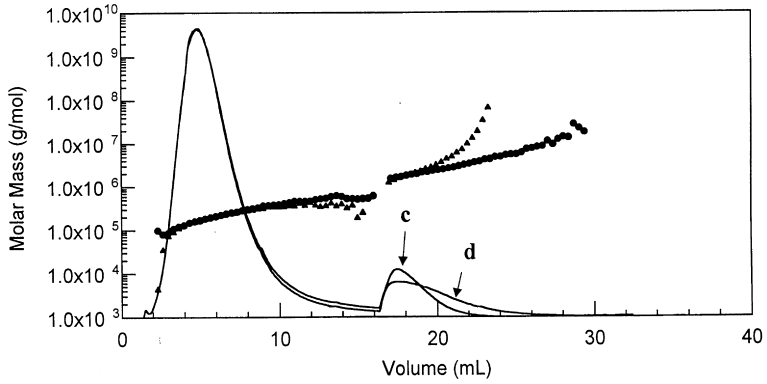

Fig. 5. F4/MALLS: M_w as a function of elution volume of gum arabic ($C_p = 3.5$ g l^{-1}), refractive index (filled line) and light scattering (dotted line). (c) ▲: $F_L = 0.5$ ml min^{-1} and $F_{C1} = 1$ ml min^{-1} (30 min) and $F_{C2} = 0.2$ ml min^{-1}. (d) ●: $F_L = 0.5$ ml min^{-1} and $F_{C1} = 1$ ml min^{-1} (30 min) and $F_{C2} = 0.3$ ml min^{-1}.

Table 3
Physico-chemical characteristics of gum arabic obtained by F4/MALLS experiments: $L_F = 0.5$ ml min^{-1}, $C_{F1} = 1$ ml min^{-1} (during 30 min) following by $C_{F2} = 0.3$ ml min^{-1} (during 30 min)

	First fraction (1) (from 2.2 to 16.1 ml) according to $C_{F1} = 1$ ml min^{-1}	Second fraction (2) (from 16.4 to 32.2 ml) according to $C_{F2} = 0.3$ ml min^{-1}	Whole gum (1 + 2) (from 2.2 to 32.2 ml)
$\overline{M}_w$ (g mol^{-1}) ± 7%	243 000	2 600 000	520 000
$\overline{M}_n$ (g mol^{-1}) ± 7%	200 000	2 200 000[a]	226 000
$\overline{R}_g$(nm)	–	26 ± 6	–
Polymer recovery	80%	11%	91%
$I = \overline{M}_w/\overline{M}_n$	1.2 ± 0.1	1.2 ± 0.1[a]	2.3 ± 0.2

[a] See text.

elute completely the fraction of smaller size, i.e. 80% of AG units. Thereafter a second cross flow (C_{F2}) was applied to elute the AGP species. Two runs have been conducted, at the same value of C_{F1} (1 ml min^{-1} for 30 min), but differing by the magnitude of C_{F2} (0.2 ml min^{-1}—run (c) and 0.3 ml min^{-1}—run (d)). The distributions of M_w along the chromatograms for runs (c) and (d) are shown in Fig. 5. In both cases, a separation of gum arabic into two very distinct fractions was achieved. Obviously, the first peak is the same in both runs, but the run (d) affords the best results for the second peak. Indeed, for run (d) we have obtained a more linear distribution of M_w and a fractionation on a broader range of volumes than for run (c). Thus, the experimental results from run (d) were detailed. The results for each population and for the whole gum are reported in Table 3. They agree with the previous SEC/MALLS observations. We have obtained 80% of homogeneous AG units with $\overline{M}_w$ of 250 000 g mol^{-1} while about 10% of the sample, probably AGP complex, consist of very high $\overline{M}_w$. The whole gum (91%), which has been found to have a $\overline{M}_w$ of 520 000 g mol^{-1}, also corresponds to our previous results. The broad range of eluted fraction of AGP (second fraction) makes it possible to calculate an average radius of gyration of about 25 nm with reasonable precision. This value of $\overline{R}_g$ agrees rather well with the literature data (Islam et al., 1997; Williams & Langdon, 1995). However, this experiment is not totally satisfactory because the second peak is not really gaussian. This could be due to the fact that the fractionation of the AGP complex is not complete as shown by the doubtfully low value of the polydispersity (1.2). This value is unexpected, for the same reasons as explained above when analysing the SEC/MALLS data.

Although the resolution seems better with the SEC method, the fractionation of the whole sample is more efficient with the F4 method. This becomes obvious in the determination of the R_g distribution of the AGP population (Fig. 6).

Using relations (1) and (2), one could attempt to estimate hydrodynamic radii (R_h) distribution for both AG and AGP fractions. For each fraction $R_{h1(i)}$ of the first population, the diffusion coefficient is given simply by:

$$1/D_{t1}(i) = 6t_{r1(i)}L_F/w^2 \cdot C_{F1}, \quad (6)$$

where $t_{r1(i)} = t_{r(i)} - t_0$ (void volume).

For the second fraction, we have considered that during the 30 min (t_{r2}) of the first applied cross flow (C_{F1}), AGP molecules were not eluted. Under the application of the second cross flow (C_{F2}), we suppose in a first simplifying approach that the diffusion coefficient of each fraction ($R_{h2(i)}$) of the second population could be given by:

$$1/D_{t2}(i) = 6L_F/w^2[(t_{r2}/C_{F1}) + (t_{r2(i)}/C_{F2})] \quad (7)$$

and $t_{r2(i)} = t_{r(i)} - t'_0$ (end of the applied C_{F1}).

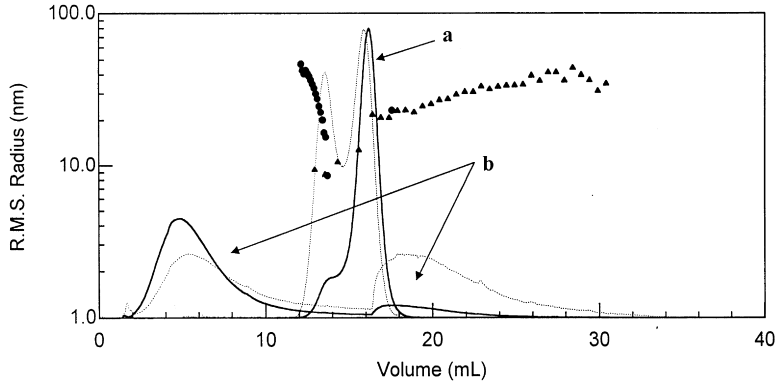

Fig. 6. R_g separation and elution profiles of SEC (a) ● and F4 (b) ▲ gum arabic ($C_p = 3.5$ g l^{-1}).

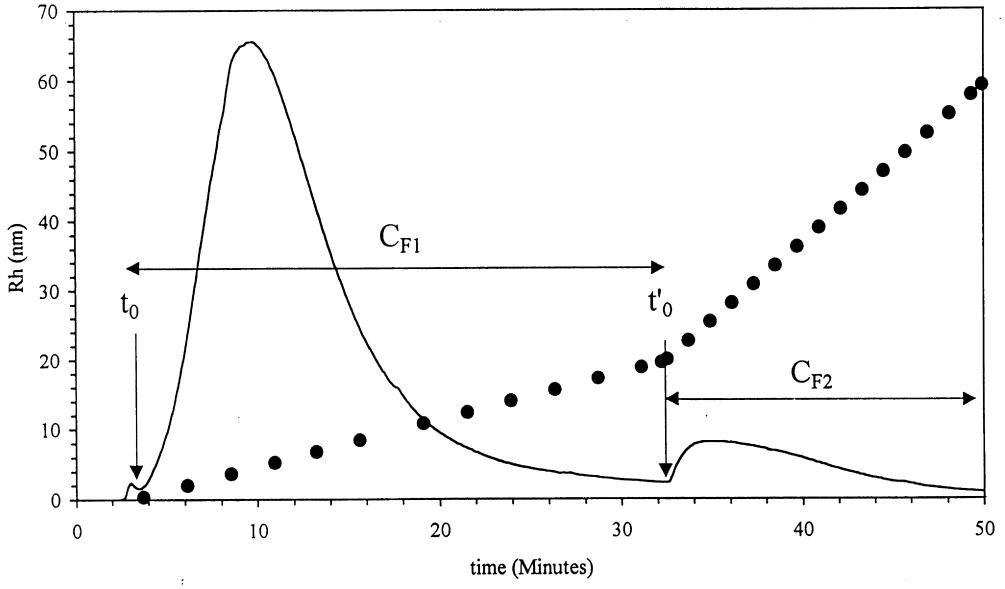

Fig. 7. Chromatograms and evolution of calculated hydrodynamic radii (R_h) (from relations (2), (6) and (7)) for gum arabic: $F_L = 0.5$ ml min^{-1}, $F_{C1} =$ 1 ml min^{-1} (30 min) and $F_{C2} = 0.3$ ml min^{-1}.

Fig. 7 shows the distribution of hydrodynamic radii (R_h) versus time. The first population, corresponding to the AG units, exhibits very low R_h (about 5 nm for the maximum of concentration) according to its low molecular weight and its highly branched structure. Moreover, R_h are distributed in a narrow range, therefore indicating a low polydispersity as already mentioned. On the contrary, the sizes of the second population (AGP complex) are larger (R_h from 25 to more than 50 nm) and the distribution is broader. This result agrees with the expected polydispersity of this fraction

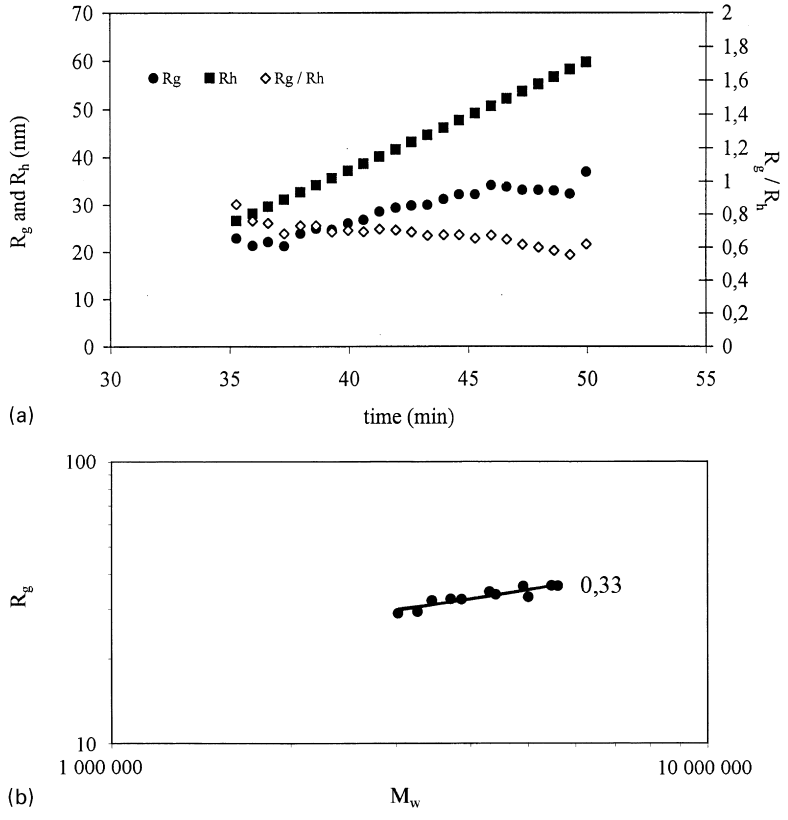

Fig. 8. (a) R_g, R_h and ratio R_g/R_h of AGP species as a function of elution time. (b) Distribution of R_g vs. M_w for AGP species.

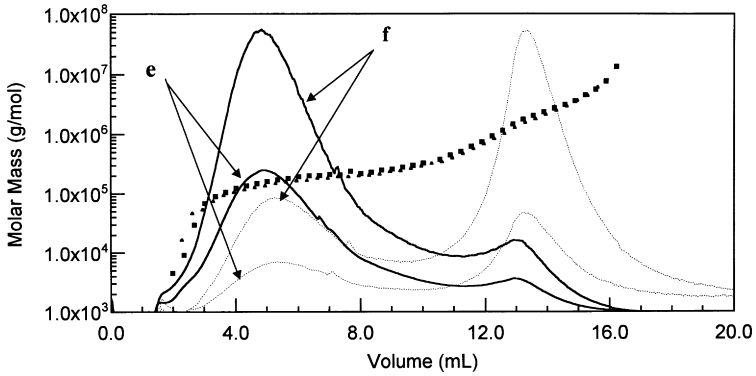

Fig. 9. F4/MALLS: M_w as a function of elution volume: (e)—$C_p = 3.5$ g l^{-1} (▲), (f)—$C_p = 7$ g l^{-1} (■), refractive index (filled line) and light scattering (dotted line). F4 separation: $F_L = 0.5$ ml min^{-1}, $F_{C,initial} = 1$ ml min^{-1} (13 min) followed by a linear decrease (10 min) down to $F_{C,final} = 0.1$ ml min^{-1}.

and agrees with literature data (Randall et al., 1989; Williams & Langdon, 1995).

The shape of the AGP molecules could be elucidated by comparing the R_g and the corresponding values of R_h with those theoretically predicted for spheres, ellipsoids and rods, respectively. As shown in Fig. 8a the value of the "shape factor" R_g/R_h (~0.7) is in good agreement with the predicted value for a spherical shape and is incompatible with a rod-like structure ($R_g/R_h \sim 2$). This is consistent with the "wattle blossom model". A confirmation of the highly branched and compact conformation of AGP can be seen in the value of the slope of R_g vs. M_w reported in Fig. 8b. Williams and Langdon (1995) have shown a similar tendency from on-line SEC/Quasi-Elastic Light Scattering measurements.

The above reported data illustrate how efficient F4/MALLS could be with respect to the analysis of polydisperse polysaccharides. Nevertheless, as shown in Fig. 7, the chromatogram of the second population of gum arabic is not really satisfactory, notably because of its non-gaussian shape. To improve the separation a new protocol was used which consisted in programming a cross flow linearly decreasing between C_{F1} and C_{F2}. A C_{F1} of 1 ml min^{-1} was applied for 13 min followed by application of a linear decrease for 10 min down to a C_{F2} of 0.1 ml min^{-1} (L_F has been set to 0.5 ml min^{-1}). Two samples of gum arabic were analysed at two different concentrations: 3.5 g l^{-1} (run (e)) and 7 g l^{-1} (run (f)), respectively (Fig. 9). Both runs give quite similar profiles. Two distinct populations are clearly observed but the separation is not fully achieved as was also observed using SEC/MALLS. The second population leaves the channel before the elution of the first population is complete. Despite this fact, results obtained are in agreement with those previously reported (Table 4). As in the case of the SEC experiment, the determination of $\overline{R}_g$ for the AGP complex is difficult. On the other hand, we can notice that the second peak is better resolved. As a consequence, the value found for the polydispersity ($I = 2$ for AGP and $I = 3$ for the whole gum) and the M_n are more reliable.

4. Conclusion

To sum up, very encouraging results have been obtained for elucidating the molecular characteristics of gum arabic. By this way, the F4/MALLS seems to be a novel interesting method of fractionation. Therefore, a question remains about either regular or random structure for the AG units. The very low polydispersity we found for this population

Table 4
Physico-chemical characteristics of gum arabic obtained by F4/MALLS experiments: $L_F = 0.5$ ml min^{-1}, $C_{F1} = 1$ ml min^{-1} (during 13 min) followed by a linear decrease (during 10 min) of C_F down to $C_{F2} = 0.1$ ml min^{-1} (during 20 min). Polymer concentration (C_p) of gum arabic: 3.5 and 7 g l^{-1}

	First fraction (1) (from 2.2 to 10 ml)		Second fraction (2) (from 10.2 to 17.2 ml)		Total gum (1 + 2)	
	$C_p = 3.5$ g l^{-1}	$C_p = 7$ g l^{-1}	$C_p = 3.5$ g l^{-1}	$C_p = 7$ g l^{-1}	$C_p = 3.5$ g l^{-1}	$C_p = 7$ g l^{-1}
$\overline{M}_w$ (g mol^{-1}) ± 7%	150 000	170 000	1 800 000	1 800 000	400 000	500 000
$\overline{M}_n$ (g mol^{-1}) ± 7%	130 000	140 000	900 000	1 000 000	150 000	170 000
$\overline{R}_g$ (nm)	–	–	5 ± 50[a]	12 ± 16[a]	–	–
Polymer recovery	84%	80%	15%	16%	100%	96%
$I = \overline{M}_w/\overline{M}_n$	1.2 ± 0.1	1.2 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	2.7 ± 0.3	2.8 ± 0.3

[a] See text.

seems to argue in favour of a regular structure. Otherwise, further works are in progress to test the ability of F4 in the study of polysaccharides (e.g. xanthan) for which SEC/MALLS separation appears very difficult due to adsorption, shear degradation and size limitation.

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