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Gel Permeation Chromatography of the Xanthan Gum Using a Light Scattering Detector

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Summary

This work gives the first gel permeation chromatogramms obtained on native xanthan using a refractometric and a light scattering detectors. Results of molecular weights distribution analysis give a polydispersity 1.2 for the native polymer and 1.7 for enzymic partially degraded polymer. The experimental conditions and chromatogramms interpretation are briefly discussed.

INTRODUCTION

Up to now, the gel permeation chromatography of random polyelectrolytes such as polystyrene sulfonic or polyacrylic sodium salts has been developed (SPATORICO and BEYER, 1975 ; COOPER and MATZINGER, 1979 ; DESBRIERES et al, 1982). In presence of excess of external salt (eluent over 5.10^{-2} M) to screen electrostatic exclusion process, the universal calibration $[\eta]$ M(V) proposed by BENOIT (BENOIT et al, 1966) for neutral polymersis valid (DOMARD et al, 1979 ; RINAUDO et al, 1980, 1981 ; ROCHAS et al, 1980).

In this work, the gel permeation chromatography of Xanthan, a rigid rodlike molecule is investigated in aqueous solvent. A light scattering detector, a differential refractometer and a viscosimeter are adapted on line to describe the molecular weight distribution. The interpretation of the chromatogramms is discussed taking into account the conformation of the polymer.

EXPERIMENTAL

The xanthan is a bacterial polysaccharide produced by Xanthomonas campestris ; the initial sample is from Rhône-Poulenc Industries. It is purified as described previously (RINAUDO and MILAS, 1978) and characterized in 0.1 M NaCl by intrinsic viscosity $[\eta]$ determined in a Ubbelohde viscosimeter (ϕ = 0.5 mm) and M_w given by light scattering using a Fica photogoniodiffusometer. Samples partially hydrolyzed by enzymic treatment are tested too (RINAUDO and MILAS, 1980).

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The gel permeation chromatography (GPC) is performed on Silica gels Spherosil from Rhône-Poulenc (average pore diameters 150, 300, 500, 1250, 3000 Å) and Fractosil from Merck (average pore diameters 10.000, 25.000 Å). The gels in equal weight except for 25.000 Å filled five glass columns (height = 120 cm ; inner diameter 1.1 cm). The calibration of the set of columns is impossible due to the large pores for which no standard is available. The radius pore distributions are obtained by mercury porosimetry as first approach to control the average pore diameters and the porous volumes. The detectors are a Iota differential refractometer, a capillary viscosimeter (ϕ = 0.5 mm) adapted to a Fica counting unit and a light scattering diffusometer Chromatix KMX 6 working at θ = 6-7° and λ = 633 nm. The eluent is 0.1 M $NaNO_3$ with 1 % ethyleneglycol to avoid adsorption ; the xanthan solutions are prepared directly in the same solvent and filtered on 0.2µm membrane to take off microgels and cell debris.

RESULTS AND DISCUSSION

As pointed out previously, when the ionic concentration of the eluent is over 5.10^{-2} M, the exclusion process in gel permeation chromatography is controlled by the hydrodynamic volume. In fact with xanthan, a rigid rod like molecule (MARET et al, 1981) in presence of external salt, problems exist to interprate the chromatogramms. First, the chromatogramm depends on the polymer concentration injected (which must be lower than 1 g. l^{-1} on native polymer) to avoid tailing due to the high viscosity and on the flow rate adopted ; even for Cp $<$ 1 g.l^{-1}, a displacement of the peak to larger elution volume when the flow rate decreases from 48 to 16 ml/hour was observed. It is necessary to adopt the lower flow rate available such as to avoid polymer orientation and/or deformation. In our experimental conditions when the flow rate is 40 ml/h, $\dot{\gamma} \simeq 400\ s^{-1}$. These experimental constraints lead to much difficulties for detection and interpretation of experimental results. At end, the molecular dimensions of xanthan (presumed to be a cylinder of 6 to 10.000 Å length) implies the use of large pores radius for which no standard exists.

On partially hydrolysed polymer in the range where standards exist , the use of the calibration $[\eta]$ M (V) can be discussed using the viscosimetric detector. In fact, the intrinsic viscosity $[\eta]$ obtained with a capillary detector is far from that obtained for low rate of shear (RINAUDO et al, 1979) and no convenient experimental conditions allow to work on $\dot{\gamma} < 1\ s^{-1}$ corresponding to the newtonian regime (CHAUVETEAU and ZAITOUN, 1981). Coupling of a viscosimeter as detector becomes of no use in this problem even for low molecular weight samples in the range where standards exist and where viscosity is less dependent on $\dot{\gamma}$.

Considering the $[\eta]$ M calibration established with random polymers, the average molecular weights determined are much overestimated. In our opinion, a second specific effect due to the polymer conformation has to be considered : equilibrium distribu-

tion in the porous materials for rigid rod like molecules (GIDDINGS et al, 1968) is quite different that for spheres or coils (LAURENT and KILLANDER, 1964). This point was recently discussed by AUVRAY (1981) and AUBERT and TIRRELL (1982) who give the expression of the partition coefficient as a function of the pore diameters and the length of the rod like molecules.

So we adopt a light scattering detector which allows to decrease much the polymer concentration injected (to 2.10^{-4} g. injected) and determines the molecular weight distributions without use of any calibration. On figures 1 and 2 the chromatogramms obtained on native and partially degraded xanthans using differential refractometer and light scattering detectors are given. The analysis of chromatogramms is realized as generally by combination of both set of experimental data without corrections for virial coefficient ; the data are respectively :

$$M_w = 3 \times 10^6 \qquad M_w/M_n = 1.2 \qquad \text{and } M_w = 220.000 \qquad M_w/M_n = 1.7$$

The conclusions of this work are that :

- the agreement between static determination of $[\eta]$ and $\overline{M_w}$ and values obtained from GPC is very good when $[\eta]$ is obtained in the same range of $\dot{\gamma}$.

- the only way to get the molecular weight distribution on these rigid rod like molecules is the coupling with a light scattering detector which avoid any calibration. The polydispersity obtained on the native xanthan is 1.2 to compare with 1.4 obtained by WELLINGTON (1981) from electronic microscopy.

- the use of viscosimeter coupling and of the $[\eta]$ M calibration for this type of molecule is no more convenient.

In addition, it is necessary to mention a last effect previously discussed (DESBRIERES et al 1982) ; even in a range where flow rate has no effect on the position of the peak, the maximum of the chromatogramm moves to lower elution volume when polymer concentration injected decreases. Nevertheless the average molecular weight deduced from light scattering detection remains unchanged. At last, it must be pointed out that the native sample is partially excluded from the gel whose maximum porosity is lower than announced (an average pores diameter of 8 500 Å determined by porosimetry has to be compared with the values of 25 000 Å given by Merck). Following, the polydispersity seems to be under-estimated.

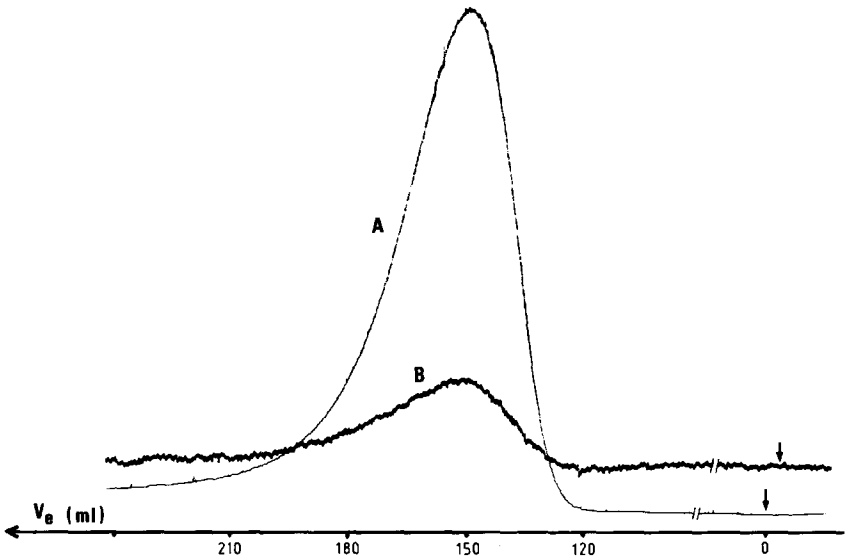
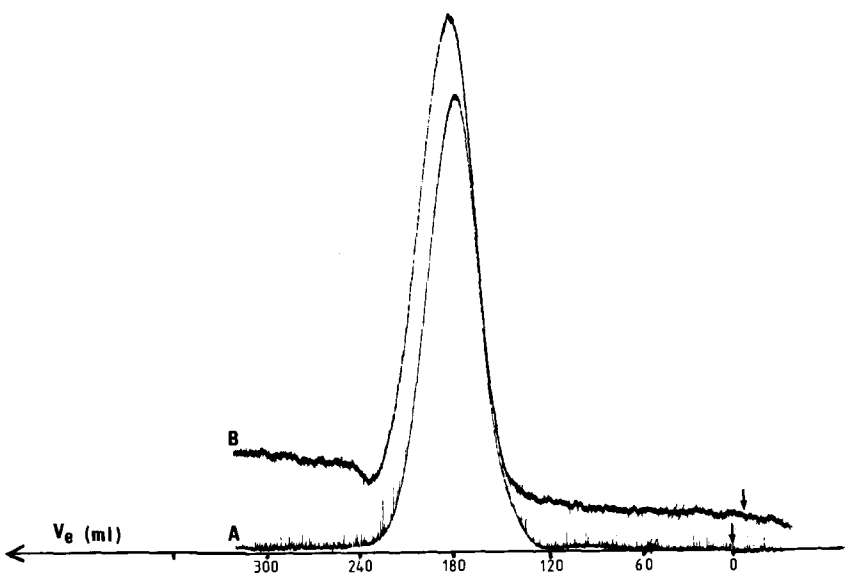


Figure 1. Gel permeation chromatogramm for a native xanthan sample
A - Light scattering trace
B - Differential refractometric trace
(500 µl sample injected ; polymer concentration 1 g/l ; flow rate 30 ml/hour ; Δn sensitivity x 1/4).

Figure 2. Gel permation chromatogramm for a partially hydrolysed xanthan (500 µl injected ; polymer concentration ≈5g/l; flow rate 45 ml/hour ; Δn sensitivity x 1/4).



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