Polymer Bulletin 11, 397-400 (1984)

Polymer Bulletin

Gel Permeation Chromatography of Starch and Other Uncharged Polysaccharides

Ph. Salemis and M. Rinaudo

Centre de Recherches sur les Macromolécules Végétales, CNRS*, B.P. 68, 38402 Saint Martin d'Heres cedex, France

Summary

The experimental results given in this paper concern the gel permeation chromatography of starch and other uncharged polysaccharides (dextrans, levan, scleroglucan,...) in organic solvent. The experimental conditions (solvent, columns) are proposed to get after direct solubilization the molecular weight distribution of the polymers using a refractometric and a light scattering detectors on line.

INTRODUCTION

Molecular weight distribution of uncharged polysaccharides, is generally not available due to the need of a direct solvent which avoids derivative preparation and decrease of the molecular weight. Gel permeation chromatography (G.P.C.) is the most convenient method to obtain this molecular weight distribution, but it needs standards for gel calibration in the solvent used, gels chemically and physically stable and without polymer adsorption in the experimental conditions. Our contribution gives the conditions for G.P.C. and molecular weight distribution of starch and other uncharged polysaccharides. Previous results were obtained on maize starch in perchloric acid with Sepharose 2B (YAMADA & TAKI, 1976) on amylose Avebe in DMSO with desactivated silicagel (Porasil) at 30° C (VAN DIJK et al., 1976) and in DMSO at 80°C on µ styragel (WATERS Ass. Comm.).

EXPERIMENTAL

For G.P.C., dimethyl sulfoxide (DMSO) was used in the presence of methanol (15 % v/v) and an external salt (ammonium acetate). It is well known that DMSO is a good solvent for much uncharged polysaccharides (LANGLOIS & WAGONER, 1976).

Amylose (Avebe-Holland) is characterized by a β-amylolysis limit of 70 % ; amylose (Sigma Type III) is from potato ; amylopectin (Snow, Société Française du Mais) is a waxy maîze corn starch with an average distance between two branch points of 21 monomeric residues (HEYRAUD & SALEMIS, 1982) ; starches A, Amilo and Globe are commercial samples from potato, tapioca and corn respectively ; scleroglucan (CECA France) is a fungial branched polysaccharide (VINCENDON & RINAUDO, 1982) with one lateral monomer per three monomeric residues ; levan is a bacterial polysaccharide.

Each sample of starch is defatted by stirring in DMSO (24 hours) recovered by precipitation in methanol, dried under reduced pressure at 60° C, and then solubilized in DMSO before use. The other polysaccharides are dissolved in DMSO with no further treatment.

All the solubilisations are made under nitrogen atmospher. Moisture content of the sample is determined with a SETARAM thermobalance type G70.

---

*Laboratoire Propre du CNRS associé à l'Université Scientifique et Medicale de Grenoble

The standards are dextrans from Pharmacia Sweeden with reference DT (Table 1) and dextrans fractions (less polydispersed) kindly given by Pharmacia.
H.P.L.C. chromatograph from WATERS (M 6000 A pump and U 6 K injector) is used with three coupled diol silica porous gel columns from MERCK (granulometry 10 µm, porosity 2 x 1 000 A, 1 x 100 A, ref : 250-4-RT). The columns are thermostated (at chosen temperature) with a water jacket.
The detectors are a light scattering apparatus KM X 6 from Chromatix (λ = 633 nm) and a Iota differential refractometer from JOBIN YVON (λ = 940 nm) both on line.

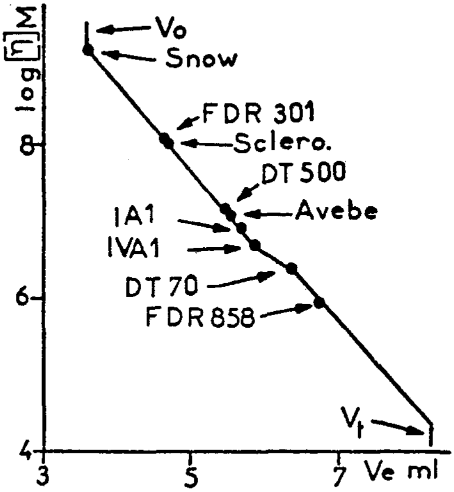
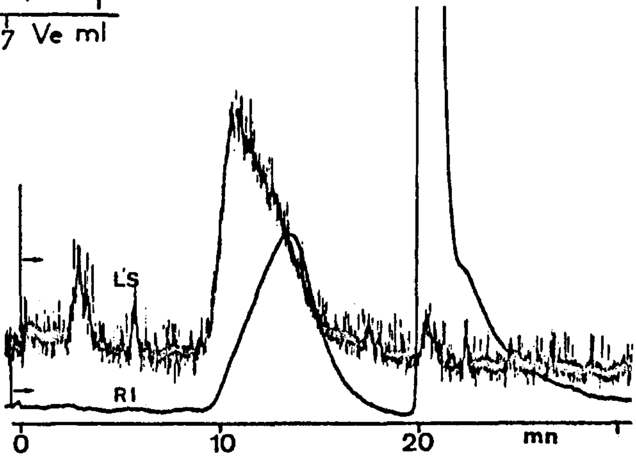


Fig. 1 - Universal calibration curve log [η] M = f(Ve)

Fig. 2 - Amylose Avebe chromatogram (for conditions see text).



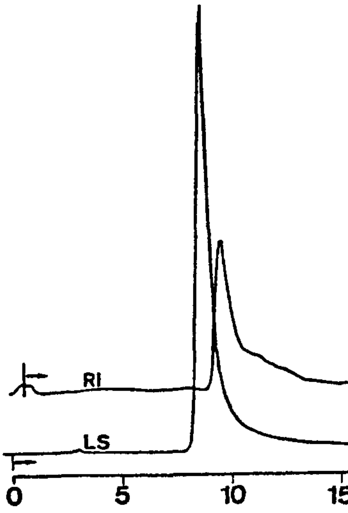


Fig. 3 - Amylopectin Snow chromatogram (for conditions see text).
LS = light scattering trace
RI = differential refractometer trace.

The intrinsic viscosity $[\eta]$ of the samples were determined with automatic viscosimeter from FICA (France) equipped with an Ubbelohde cappillary (inner diameter 0.58) and a system avoiding water absorption by the solvent.

Considering the relation between the light scattering signal and the molecular weight, as the dn/dc is unknown in that solvent at 633 nm, one takes three samples (dextrans 1A1, DT 500 and IVA1) as reference of molecular weight (Mw is used) ; so, it allows to determine an average value of a constant K' which includes the dn/dc, the value of the refractive index of the solvent (n) and the light scattering apparatus characteristics (transmittance, cell length,...) ; this K' value allows to determine the characteristics for the other standards from the light scattering signal. The values are given in Table 1.

In our opinion, the determination of the value of K' by this method is preferable because it is not easy to determine the dn/dc due to the water absorption in this very hygroscopic solvent. Now, taking into account data of the litterature from which we assume that the dn/dc is nearly constant for the glucose polymers (Polymer Handbook, 1975), it becomes possible to determine the molecular weight of the different neutral polysaccharides with the same K' value.

TABLE 1 : Molecular weights of the dextran standards (DT) and of the uncharged polysaccharides.

	This work		Literature		
	Mw	Mn	Mw		Mn
DT 500	376300	165600	378000	➔	183000
DT 70	71700	48900	64000	➔	43500
FDR 301 FR3	$1.13\cdot10^6$	616000	1368000	➔	654000
IA1	223500	98000	221000	*	117000
IVA1	116700	71200	118000	*	88000
FDR 858	42700	25000	41000	*	26000
SCLEROGLUCAN	540000	231000			
LEVAN	$2.64\cdot10^6$	$1.15\cdot10^6$			
STARCH A	$7.11\cdot10^6$	$1.35\cdot10^6$			
GLOBE 03400	$13.9\cdot10^6$	$2.99\cdot10^6$			
AMILO 3005	$18.54\cdot10^6$	$3.62\cdot10^6$			
AMYLOPECTIN SNOW	$15.96\cdot10^6$	$8.5\cdot10^6$			
AMYLOSE AVEBE	283000	153000	287000	+	110000

➔ DOMARD and RINAUDO (in press).
* Pharmacia
+ VAN DIJK et al. (1976).

RESULTS AND DISCUSSION

A. UNIVERSAL CALIBRATION

The set of columns was calibrated using dextran standards in the chosen solvent. First one demonstrates that at a given temperature, the composition of the solvent is determinant to avoid adsorption on silica gel ; the best composition found is : 0.5 M ammonium acetate, 15 % v/v methanol. In addition, the temperature is also very important ; best conditions adopted was 62° C. To show that no adsorption occurs for the different polymers (glucose polymers) one determines the area of the chromatograms proportionnal to the quantities of polymers injected and the position of the peak.

It must be point out that adsorption of dextrans on diol silica gel is always loose, but it increases drastically with starch and particulary with amylopectin for which must difficulties were encountered. In a second step, one established the universal calibration curve $\log[\eta]M = f(Ve)$ in which the hydrodynamic volume $[\eta]M$ is plotted as a function of the elution volume Ve. (with $[\eta]$ = intrinsic viscosity determined in the same thermodynamic conditions and $M = (Mn \times Mw)^{1/2}$). Experimental results of this calibration are given in Figure 1. Average molecular weights of the samples were determined and compared with value given by Pharmacia (Table 1) and those obtained in our laboratory in aqueous G.P.C. (DOMARD & RINAUDO, in press).

B. MOLECULAR WEIGHT DISTRIBUTION OF NEUTRAL POLYSACCHARIDES

First amylose and amylopectin where chromatographied in the chosen conditions (figures 2 and 3). On the linear polymer amylose the molecular weights are in good agreement with those given by VAN DIJK *et al.*, (1976). Then the hydrodynamic volume is calculated as $[\eta](MnMw)^{1/2}$ and plotted on the calibration curve ; it agrees with the universal calibration.
The amylopectin chromatogram is given in Figure 3 ; the larger molecular weight are partially excluded due to their large hydrodynamic volume ; the characteristic Mn and Mw are given in Table 1. Commercial samples of starch are then used. Two distributions seem to appear which weight fractions are in agreement with the yield of the both components (amylose and amylopectin) determined from iodine titration. At end one determines the molecular weights of the other polysaccharides which agree with the universal calibration curve ; the scleroglucan and the levan are well characterized in these experiments and the molecular weights are easily obtained (Table 1).

CONCLUSION

This paper described the experimental conditions for gel permeation chromatography of starch and other uncharged polysaccharides. It is the first time that the molecular weight distributions of these polysaccharides are obtained ; the solvent used avoids adsorption on the gel and allows direct solubilization of the polymers.

Acknwoledgment : The authors thank M. DULONG from MERCK company for his technical assistance.

REFERENCES

W. BANKS and C.T. GREENWOOD. "Starch and its components",(1975) Edinburg University press, Edinburg.

A. DOMARD and M. RINAUDO. Polymer, (in press).

A. HEYRAUD and Ph. SALEMIS. Carbohydr. Res. 107, 123 (1982).

D.P. LANGLOIS and J.A. WAGONER. In "Starch chemistry and technology", R. L. WHISTLER and E.F. PASCHALL ed., Academic Press, N.Y., volume II, 469 (1967).

Polymer Handbook. J. BRANDRUP and E.H. IMMERGUT ed. JOHN WILEY and Sons, Inc. ; 2nd ed., (1975).

J.A.P.P. VAN DIJK, W.C.M. HENKENS and J.A. SMIT. J. Polym. Sci. 14, 1485 (1976).

M. VINCENDON and M. RINAUDO. Carbohydr. Polymers. 2, 135 (1982).

WATERS Associate (communication).

T. YAMADA and M. TAKI. Die Stärke 28, 374 (1976).

Accepted February 15, 1984