

NOTES

Degree of Polymerization of Sodium Polygalacturonate by Membrane Osmometry

INTRODUCTION

Customarily membrane osmometry is the method of choice for measuring number-average degree of polymerization (\overline{DP}_n) between 100 and 1000.¹ Recently,² we have observed that sodium polygalacturonate (NaPG) with a \overline{DP}_n of only 35 by end-group analysis had a radius of gyration R_g in solution between 60 and 90 Å. These results indicated that NaPG should be sufficiently membrane impermeable that \overline{DP}_n can be measured by membrane osmometry. Moreover, because of the relatively low \overline{DP}_n , NaPG would give a large change of osmotic pressure π with concentration. Thus it would be a good reference compound for membrane osmometry, since NaPG is readily available and lyophilizes to a fluffy white powder. Furthermore, with the \overline{DP}_n value obtained by osmometry, we compare size and molecular weight measurements on NaPG by several techniques.

EXPERIMENTAL

Preparation of Sodium Polygalacturonate

Polygalacturonic acid (PG) (>98% pure from Sigma Chemical Co.*) was dissolved in distilled H₂O and centrifuged for 1/2 h at 8000g. The supernatant was lyophilized and stored for further use. Later, 1 g of PG was dissolved in 60 mL of 0.05M Na phosphate buffer, pH 7.0, titrated with 0.1M NaOH to pH 7.0, and dialyzed against four changes of distilled H₂O over 48 h. Dialysis bags were Spectrapor with a molecular weight cutoff of 12,000. Retentate from dialysis was lyophilized and stored for further use. This material had a pH of 6.2 when dissolved in 0.05M NaCl.

Osmometry

Osmotic pressures were measured in a Knauer type 1.00 membrane osmometer equipped with a thermostatted cell (Utopia Instrument Co., Joliet, IL). Solvent was 0.05M NaCl. Semipermeable membranes (Schleicher and Schuell, AC 62) were cellulose acetate with pore size diameters between 50 and 100 Å. The osmometer cell was maintained at $35 \pm 0.1^\circ\text{C}$. The output from the electronic pressure transducer in the osmometric cell was monitored continuously by a potentiometric recorder. Recorder traces of π against time, which were flat and parallel to the baseline, indicated no tendency for NaPG to permeate the membrane. Generally, equilibrium was obtained 5–10 min after the third time the cell had been rinsed through with the polymer solution. Samples were measured in increasing order of concentration.

RESULTS AND DISCUSSION

Reduced osmotic pressure π/C (in cm H₂O dL/g) was plotted against concentration C (in g/dL) (see Fig. 1) according to

$$\pi/C = RT/\overline{M}_n + RT/\overline{B}C$$

the modified van't Hoff equation.³ Here R is the universal gas-law constant, 848 cm (H₂O dL/mole deg, and T is the absolute temperature in degrees Kelvin. Linear least-squares analysis gave 33.81 ± 0.56 for the intercept RT/\overline{M}_n , and 27.7 ± 1.7 for the slope RT/\overline{B} . A \overline{DP}_n of 39.0 ± 0.6 was obtained by dividing the product of R and T by the product of the intercept and 198.1, the molecular weight of a NaPG residue. Interestingly, for PG, Powell et al.⁴ have reported a \overline{DP}_n of 35 by end-group titration if galacturonic acid is assumed to be the end group and a \overline{DP}_n of 25 if L-rhamnose is assumed to be the end group. From a combination of x-ray diffraction and NMR data,^{5,6} it has been suggested

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

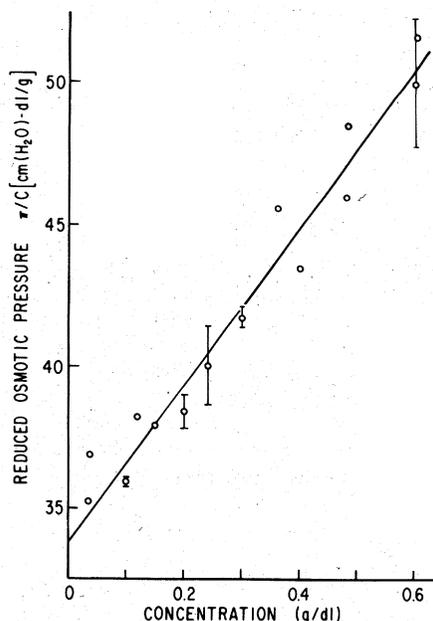


Fig. 1. Modified van't Hoff plot for Na polygalacturonate in 0.05M NaCl. Error bars were obtained by calculation of the standard deviation of π for concentrations made up in duplicate or triplicate.

that sodium polygalacturonate can be represented in the solid state by a flat ribbonlike helix and that the length h of a monomer unit projected along the x axis is between 4 and 6 Å. Taking a median values of 5 Å for h and 37.0 for \overline{DP}_n , the average length of a NaPG molecule in the solid state is 37.0×5 or 185 Å. If one assumes about 6% uncertainty in \overline{DP}_n and 20% uncertainty in h , there would be a 26% or about 48 Å uncertainty in the chain length. Thus the radius of gyration R_g calculated from the long dimension in the solid state would be $185/(12)^{1/2}$ or 54 ± 14 Å.⁷ Interestingly, we have found by size-exclusion chromatography² (SEC) values of 60 and 90 Å for the radius of gyration R_g of NaPG in phosphate buffer at pH 7.3 and concentrations of 0.08 and 0.16M, respectively. In 0.08M phosphate buffer and pH 3.7, a value of 77 Å was found for R_g . These values correspond to dextrans with a weight-average molecular weight between 42×10^3 and 96×10^3 daltons, and \overline{DP}_n between 155 and 355.

In conclusion, evidence from several sources indicates that NaPG is highly asymmetrical in solution, even in 0.05M NaCl where charge-charge repulsions should be fairly well shielded, particularly when compared to dextran, which is a compact slightly branched random coil in solution.⁸ Thus the molecular weight of NaPG would be grossly overestimated if one attempted to obtain molecular weight distributions for NaPG by calibrating SEC or gel filtration columns with dextran reference compounds. In addition, we have found that the R_g of NaPG in solution and those calculated from the long dimension in the solid state are comparable (see Table I). Finally, it has been demonstrated that NaPG, by virtue of a relatively large R_g in combination with a relatively low \overline{DP}_n , is ideally suited as a reference compound for aqueous membrane osmometry.

TABLE I
Summary of Properties for Sodium Polygalacturonate

Method	R_g (Å)	\overline{DP}_n	Reference(s)
SEC	60-90		2
Titration		35	2,4
Osmometry	≥ 50	39	This work
X-Ray Diffraction ^a	54		5,6

^a Obtained from h (length of monomer unit) $\times \overline{DP}_n / (12)^{1/2}$.

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