

# Hydrodynamic evidence for an extended conformation for citrus pectins in dilute solution

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## ABSTRACT

Data from hydrodynamic measurements (sedimentation velocity, sedimentation equilibrium and viscometry) are suggestive of an extended rather than a spheroidal conformation for citrus pectins in dilute solution. Evidence is presented from 1. the Wales-van Holde parameter  $k_s/[\eta]$  for a series of pectin fractions and unfractionated material, and 2. the slopes of Mark-Houwink sedimentation and viscosity plots for the same series of fractions.

## INTRODUCTION

Pectins have a wide variety of uses as gelling and thickening agents in the food industry. Their properties will be influenced by their conformation under the particular conditions in which they are used. A study using sedimentation equilibrium, sedimentation velocity and viscosity measurements on fractionated citrus pectin was undertaken to investigate the gross conformation of citrus pectin (sphere, rod, random coil etc.) under dilute aqueous solution conditions.

## EXPERIMENTAL

The initial fractionation and viscometric characterisation had been carried out earlier by Berth and co-workers (1,2). The sample was a citrus pectin (Koch-Light Ltd.) with a degree of esterification of ~60% and a galacturonate content of ~70%. Concentrations (w/v) were determined by dry weight of pectin. Chromatographic separations had been carried out on GPC columns of Sepharose 2B/Sepharose 4B, in a phosphate buffer at pH 6.5 with 1mM Na<sub>2</sub>EDTA and 0.3mM NaN<sub>3</sub> added. 10ml fractions were taken from the column and their intrinsic viscosities determined in a Viscomatic (FICA, France) viscometer at 25.0°C (1,2).

For the present study, consecutive fractions were taken,

combined in pairs, and the resultant 20ml fractions freeze dried prior to analysis. The solvent used for sedimentation analysis of the 20ml fractions was a phosphate chloride buffer of  $I=0.3$ ,  $pH=6.5$ ). For sedimentation equilibrium,  $1mM Na_2EDTA$  and  $2mM NaN_3$  were added, and the sample was exhaustively dialysed against the solvent before use.

Sedimentation equilibrium was performed using a Beckman Model E analytical ultracentrifuge equipped with Rayleigh interference optics, a 5 mW He-Ne laser light source and an RTIC temperature control system, following low speed sedimentation equilibrium procedures (3) to determine weight average molecular weights ( $M_w$ ). Initial concentrations were of the order of 0.7 mg/ml. Sedimentation velocity experiments were carried out using an MSE Centriscan 75 analytical ultracentrifuge at  $20.0^\circ C$ , with scanning schlieren optics set at 546nm. Sedimentation coefficients at a series of concentrations ( $s_c$ ) were determined, corrected for solvent density to conditions of water at  $20.0^\circ C$  and extrapolated to 'infinite dilution' against concentration (corrected for radial dilution in the ultracentrifuge cell), to determine the infinite dilution sedimentation coefficient ( $s$ ) using the expression:

$$1/s_c = (1/s)(1 + k'_s c)$$

where  $k'_s$  is the concentration dependence parameter of the sedimentation coefficient for  $s_c$  values corrected for solvent density. This is further corrected for solution density using (4):

$$k_s = k'_s - \bar{v}$$

where  $\bar{v}$  is the solute partial specific volume [determined here by precision densimetry using an Anton Paar (Graz) DMA 02C precision density meter as  $(0.57 \pm 0.01)$  ml/g].

In the paper of Berth *et al* (2), values for the intrinsic viscosity are given for fractions of 10ml bandwidth. The equivalent intrinsic viscosity values for combined adjacent fractions (i.e. of 20ml bandwidth) were calculated from the weighted average of each set of two 10ml values.

#### CONFORMATIONAL PROBES

Knowing the molecular weights, sedimentation coefficients and intrinsic viscosities of the pectin fractions, it is possible to examine their possible conformations by two approaches:

1. Use of the "Wales-van Holde" parameter. The value of the ratio  $k_s/[\eta]$  is characteristically  $\sim 1.6$  for spheres and random coils, and substantially lower for asymmetric molecules (5-7).

2. Mark-Houwink approach. Two of the Mark-Houwink type equations are relevant here:

$$[\eta] = K' M^a ; \quad s = K'' M^b$$

The values of  $a$  and  $b$  can be estimated from the slopes of log-log plots, and have characteristic values for the three main conformational groups (Table 1).

Table 1. Values of  $a$  and  $b$  for different classes of macromolecular shape (from Ref. 8)

	$a$	$b$
sphere	0	0.67
random coil	0.5-0.8	0.4-0.5
rod	1.8	0.15

## RESULTS

Table 2. Results for fractionated pectins.

Fraction	$k_s$ (ml/g)	$[\eta]$ (ml/g)	$k_s/[\eta]$
9-10	81.6±21.9	809.3	0.10
13-14	92.8±14.0	624.5	0.15
15-16	70.6±10.2	479.4	0.15
17-18	62.2±32.8	341.3	0.18
19-20	27.0±5.8	217.5	0.12
21-22	119.3±52.0	160.5	0.74

The values of  $k_s/[\eta]$  for all the pectin fractions (Table 2) can be seen to be substantially lower than 1.6, indicating an extended, rod like conformation in all cases. This is confirmed by the plot of  $\log s$  versus  $\log M_w$  (Figure 1a), which has a slope from linear regression of  $0.17 \pm 0.10$ , very close to the value of 0.15 for rods, but clearly not consistent with a spherical or random coil conformation.

The initial slope of  $(2.0 \pm 0.3)$ , [estimated by linear regression of the first five data points] of the  $\log [\eta]$  versus  $\log M_w$  plot (Figure 1b) appears to support this rod like classification. It is however significant to note that there is a decrease in slope with increasing molecular weight which could be an indication of a change in shape to a more flexible form as the molecular weight increases. Not shown in Fig 1b are two points of higher molecular weight which have anomalously low values for the intrinsic viscosity. We feel this is due to the presence of appreciable amounts of compact high molecular weight spherical aggregates contaminating the sample (1,2). If these species are also present (in proportionally lesser quantities) in the lower molecular weight fractions, this could provide an alternative explanation for the observed change in slope as molecular weight increases. Such effects would not be observed

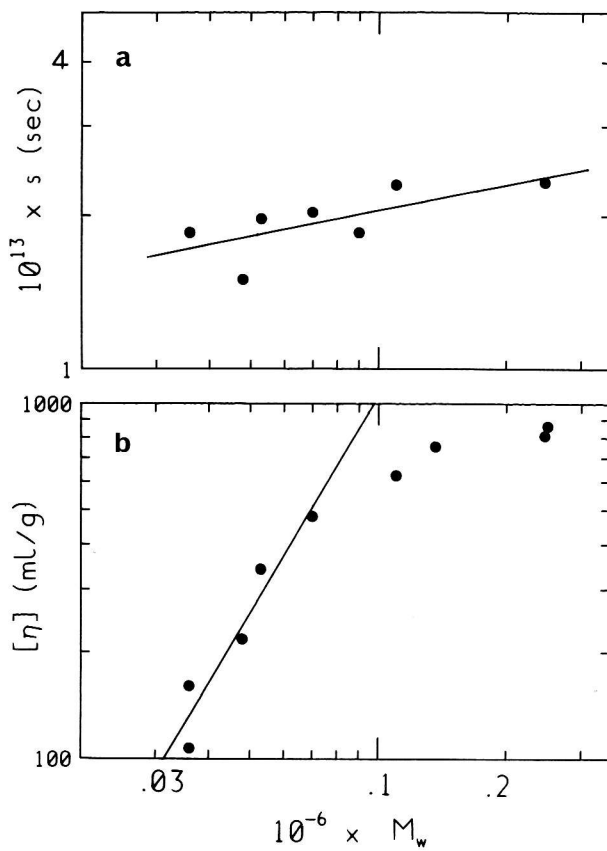


Figure 1. "Double Log Plots" of (a) Sedimentation Coefficient and (b) Intrinsic Viscosity Data versus Molecular Weight for Citrus Pectin Fractions

in the  $\log s$  versus  $\log M_w$  plot (Fig 1a), since the sedimentation coefficient  $s_w$  will not be affected (higher molecular weight species removed by the centrifugal field).

#### DISCUSSION

Our results appear in all cases to support an extended rod-like conformation for citrus pectins in dilute aqueous solution. This is in agreement with evidence from theoretical calculations on polygalacturonate chains (9). Independent light scattering work on pectins has also indicated an extended conformation (10), although the wormlike chain model was considered more appropriate.

If true, the extended conformation found for citrus pectin, in comparison with many other polysaccharides, is probably due to: (i) The severe steric hindrance of the  $\alpha$ -1-4 galacturonate linkage, which is biaxial, and (ii) The relatively low molecular weight.

Further, the results we have found suggest that it is inappropriate to estimate pectin molecular weights from measurement of intrinsic viscosity using a single Mark-Houwink relation. This, and other related phenomena will be considered in more detail in a future publication.

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