
Thermodynamic non-ideality of dilute solutions of sodium alginate studied by sedimentation equilibrium ultracentrifugation

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Abstract. The molecular weight of sodium alginate derived from *Laminaria hyperborea* has been measured by sedimentation equilibrium ultracentrifugation. In addition, values for the second and third virial coefficients have also been obtained.

Introduction

Polysaccharide molecular weights are most frequently determined by light scattering. This technique is difficult to apply in many cases because of the presence of supramolecular structures which scatter more heavily than single chains. This distortion of the data can lead to curved Zimm plots and severe overestimates of the molecular weight.

In principle, an attractive alternative is analytical ultracentrifugation and, unless the effects of thermodynamic non-ideality are severe, measurement at a single finite, but low, concentration (~ 0.2 mg/ml) yields a value for the 'apparent' molecular weight which is within a few per cent of the true value. Alginates are one class of polysaccharide for which non-ideality effects (through high exclusion volumes, or unsuppressed charge or 'Donnan' effects) are known to be particularly severe (1). In this study, we briefly investigate this non-ideality behaviour for two similar alginates from *Laminaria hyperborea* in a phosphate chloride buffer of ionic strength, $I = 0.3$ mol/dm³ and show that even at moderately low solute concentrations (~ 1 mg/ml), a virial expansion with a single non-ideal term is insufficient to describe this non-ideality. The problems of working in a buffer of too low an ionic strength are also indicated.

Materials and methods

Two samples of sodium alginate were used. The first was supplied by G.Sjåk-Bræk, of NTH, Trondheim (sample 1) and this had a mannuronate/gulonate (M/G) ratio of 0.39. Sample 2 was a very similar alginate to sample 1 and was obtained from Protan of Drammen, Norway. This latter sample is known commercially as 'Pronova'. Solutions of the alginates were made up in phosphate chloride buffer (of pH 6.8 and made up to ionic strength of 0.3 according to Green (2)) and extensively dialysed against pure buffer. Above this ionic strength, no significant further suppression of the Donnan contribution to the non-ideality for a similar alginate (manucol DM) has been observed (3).

The partial specific volume was taken to be 0.44 ml/g (4). Apparent molecular weights ($M_{w,app}$) of solutions with finite concentrations (between 0.25 mg/ml and 2.5 mg/ml) were measured at 20°C using the 'low speed' sedimentation

equilibrium technique (5) in an analytical ultracentrifuge fitted with a 5 mW He-Ne laser light source and interference optics. Equilibrium of the fringe pattern (and hence of the solution's concentration gradient) was reached in about a day. The fringes were recorded photographically, and successive frames on the negative were compared on a micro-comparator until no further change was observed. This was used to find when equilibrium had been reached. The equilibrium fringes were digitized with a 'gel-scanner' controlled from a microcomputer. The data were then transferred to a mainframe computer where they were analysed via an 'operational' point average molecular weight, M^* (5), to obtain a value of $M_{w,app}$. This type of procedure has been described elsewhere (6).

Results

The concentrations of the solutions were corrected to allow for the moisture content of the alginate. This was measured by drying to constant weight and found to be 10.8 (± 0.2)%. Plots of $M_{w,app}$ as a function of the corrected (loading) concentration (c) are shown in Figure 1.

Discussion

The data have been fitted in two ways. First, to the quadratic approximation of the virial expansion (7) in c with a weighted least squares analysis (8):

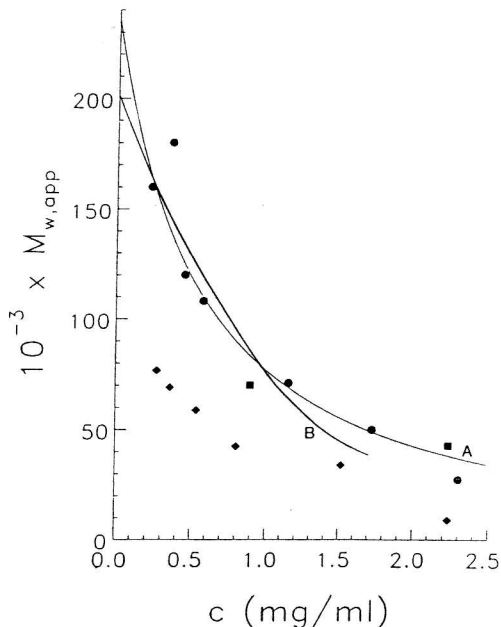


Fig. 1. Apparent molecular weight ($M_{w,app}$) plotted against corrected concentration (c). ● = sample 1 at $I = 0.3$; ■ = sample 2 at $I = 0.3$; ◆ = sample 2 at $I = 0.1$. The lines are quadratic fits to all the $I = 0.3$ points: (A) fit in $1/M_{w,app}$; (B) fit in $M_{w,app}$. Typical errors in $M_{w,app}$ are 10%.

$$(M_{w,\text{app}})^{-1} = (M_{w,0})^{-1} + 2Bc + 3Cc^2$$

The constants B and C are the second and third thermodynamic (or 'osmotic') virial coefficients respectively. The intercept, $M_{w,0}$, is the value of $M_{w,\text{app}}$ extrapolated to zero concentration—it is hence independent of non-ideality. The least squares analysis showed $M_{w,0}$ to be $240 (\pm 50) \times 10^3$ g/mol as shown by line A in Figure 1.

Secondly, the data were fitted to a quadratic equation of the form:

$$M_{w,\text{app}} = M_{w,0} (1 + a_1c + a_2c^2)$$

This is shown as line B on Figure 1. This procedure gives $M_{w,0}$ to be $201 (\pm 11) \times 10^3$ g/mol—a significantly smaller error. The physical relevance of the coefficients a_1 and a_2 is, however, not immediately obvious compared to B , which has been well considered (see e.g. ref. 9).

Limited data at $I = 0.1$ are included in Figure 1. It is clear that the ionic strength charge effects have not been adequately suppressed, and because of the unknown form of the extrapolation below ~ 0.2 mg/ml these data would lead to a severe underestimate of $M_{w,0}$.

Martinsen *et al.* (11) have used a wide variety of other techniques to measure the molecular weight of sample 1 as described above and found it to be $217 (\pm 10) \times 10^3$ g/mol and $214 (\pm 10) \times 10^6$ g/mol from wide-angle and small-angle laser light scattering respectively, in good agreement with the present finding. We can also compare our value of $B = 4 (\pm 1) \times 10^{-3}$ ml mol/g² with their values of 7×10^{-3} ml mol/g² and 4.7×10^{-3} ml mol/g². The least squares analysis also gives the value of C to be $0.3 (\pm 0.3)$ ml² mol/g³. Although this means that, statistically, C may be zero, allowing for a non-zero C does allow considerable variation in the value of $M_{w,0}$.

Finally, it should be pointed out that different preparations of sodium alginate have given a range of molecular weights. For instance, Wedlock *et al.* (10), using an alginate with a mannuronate/guluronate (M/G) ratio of 0.63, quote a molecular weight of 6.45×10^5 g/mol.

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