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Solution properties of capsular polysaccharides from *Streptococcus pneumoniae*

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ABSTRACT

Capsular polysaccharides from ten different serotypes of *Streptococcus pneumoniae* have been studied with regards their hydrodynamic properties in solution, namely their sedimentation coefficient and molar mass distributions, solution conformations and flexibilities (persistence lengths L_p), important properties for the construction of polysaccharide and glycoconjugate vaccines. Sedimentation and molar mass distributions (obtained by sedimentation velocity and equilibrium analysis in the analytical ultracentrifuge supported by size exclusion chromatography coupled to multi-angle light scattering measurements) were generally unimodal, with weight (mass) average molar masses ranging from 100×10^3 to 1300×10^3 g/mol. Estimates of chain flexibilities from three different procedures applied to intrinsic viscosity, sedimentation coefficient and molar mass data, showed that the polysaccharides from all the serotypes studied had semi-flexible structures in solution with persistence lengths in the range from ~4 to 9 nm.

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1. Introduction

Streptococcus pneumoniae, a major cause of human disease, was one of the first pathogens to be isolated and characterised (see e.g. Gray & Musher, 2008). It is responsible for a range of serious illnesses such as pneumoniae, meningitis, bacteraemia and conjunctivitis. *S. pneumoniae* cells are Gram-positive (Gram, 1884), lancet-shaped cocci (i.e. elongated cocci with a slightly pointed outer curvature). They are usually seen as pairs of cocci (diplococci), but they may also occur singly and in short chains (Gray & Musher, 2008). Of particular importance are the capsular polysaccharides from these microorganisms. Capsular polysaccharides are polysaccharides which are covalently linked to the peptidoglycan backbone of the bacterial cell wall. It has long been known that capsular polysaccharides from *S. pneumoniae* are immunogenic and when used as vaccines, provide type-specific protection against pneumococcal infections (MacLeod, Hodges, Heidelberger, & Bernhard, 1945). The capsular polysaccharide coat is also what differentiates more than 90 serotypes of *S. pneumoniae*, with only a relatively small number of those serotypes being associated with disease (Tuomanen, Austrian, & Masure, 1995). They are known to have short tandem repeat units of 2–8 sugar residues (see e.g. Jones, 2005). The repeat units can either be linear or branched, and may contain non-carbohydrate substituents such as o-acetyl, glycerol phosphate, or pyruvate ketals. Most are polyanionic; exceptions include serotypes 7F and 14, which are neutral, and some unusual monosaccharide residues can be found, such as pneumosamine (Jones, 2005).

A variety of vaccine possibilities against *S. pneumoniae* are currently being researched to improve upon the existing capsular polysaccharide and glycoconjugate vaccines: glycoconjugate vaccines are constructs where the capsular polysaccharide is conjugated with a carrier protein, such as tetanus toxoid protein, which improves the effectiveness and longevity of the vaccine (see e.g. Astronomo & Burton, 2010). Vaccine treatment against *S. pneumoniae* is not new. It was firstly introduced in 1918 by Rosenow and further developed by Heffron (1979), where the method used was one of whole cell administration to patients. In the 1940s, whole-cell vaccines were supplanted by the next generation of purified capsular polysaccharides (PS). Interest in these vaccines diminished, as a result of the availability of new wave of antimicrobial drugs (e.g. penicillin and sulphonamides) and their efficacy for the treatment of pneumococcal infections; whole-cell vaccines were withdrawn from the market in 1954 (Mavroidi et al., 2007). A renewed interest in vaccines for the prevention of pneumococcal infections during the 1960s and 1970s resulted in further development of carbohydrate vaccines against *S. pneumoniae* and led to the current 23-valent capsular polysaccharide vaccine (Pneumovax23™) now available (Mäkelä & Butler, 2008; Mavroidi et al., 2007) and the new hepta-valent pneumococcal

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conjugate vaccine PCV7 (Gray & Musher, 2008). The first pneumococcal conjugate vaccine contained seven serotypes (Prevenar^{TM}) and was licensed in 2000 (Wyeth Vaccines, Maidenhead, Berkshire, UK). The new conjugate vaccine greatly reduced the frequency of disease due to the seven vaccine serotypes 4, 6B, 9V, 14, 18C, 19F and 23F (Long, 2005). Capsular polysaccharides of *S. pneumoniae* have been coupled to different proteins, e.g. tetanus toxoid, diphtheria toxin or its genetic mutant form CRM197 (a non-toxic form of diphtheria toxoid), the outer membrane protein complex [OMPC] of *Neisseria meningitidis* and more recently, protein D derived from *Haemophilus influenza* (Goldblatt, Assari, & Snapper, 2008; Mavroidi et al., 2007). Of these, the tetanus and diphtheria toxoid conjugates have been the most successful.

The importance of understanding the important molecular properties underpinning the optimal development of carbohydrate polymer based vaccines against serious disease was emphasised in a recent paper by Astronomo and Burton (2010). The molecular weight (molar mass) and molecular weight or molar mass distributions are two of the most important physical parameters relevant to the efficacy and immunogenicity of capsular polysaccharides (see for example Jennings, 1983; Kabat & Bezer, 1958), although conformation and conformational flexibility (as represented by the persistence length L_p) are also important. In this study the solution molecular probes of analytical ultracentrifugation (AUC) and the independent method of size exclusion chromatography coupled with multi-angle laser light scattering (SEC–MALS) and viscometry were used to comprehensively characterise a representative range of ten purified native capsular polysaccharides of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F to examine the hydrodynamic commonality or diversity of these materials.

2. Materials and methods

2.1. Materials

Native capsular polysaccharides were extracted and purified from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F, hereafter referred to in this study as SP (1), SP (4), SP (5), SP (6B), SP (7F), SP (9V), SP (14), SP (18C), SP (19F) and SP (23F) respectively. Eight of the ten polysaccharides are acidic, whereas SP (7F) and SP 14 are neutral ones. The purification of the polysaccharides consists of broth centrifugation and the precipitation of the polysaccharide (PS) with a quaternary ammonium salt, such as cetyltrimethylammonium bromide (CTAB). This cationic detergent has the property to form insoluble complexes with several polyanions: such as polysaccharides, nucleic acids and proteins. The precipitation occurs below their critical electrolytic concentration (CEC), a parameter which is function of the pK_a or the pI (proteins can only precipitate above their pI, where they are negatively charged), and also of the concentration and of the molecular size of the PS (for the same PS: the lower the size the lower will be the CEC). This property can be used either to precipitate impurities and leaving the PS in solution (negative mode) at a conductivity above the CEC or either to precipitate the PS (positive mode) at a conductivity below the CEC, leaving other impurities in solution. The CEC is not easily determined in a complex medium such as the broth. Nevertheless, a clear image can be obtained by relating the electrolytic concentration to the conductivity. Each acidic polysaccharide has a specific insolubility curve as a function of the salt concentration. If this concentration is expressed as conductivity, most curves are overlaying. The [PS-CTA] complex can be redissolved by dissociation at increasing the salt concentration which allows a selective removal of lipopolysaccharide (LPS). The neutral 7F and 14 PS cannot be purified by a CTAB precipitation in positive mode. In this case the negatively charged CPS is fixed on an anion exchange chromatography column while the PS is recovered in the flow through. Finally the PS are precipitated by addition of alcohol which allows us to obtain the PS as a powder and to eliminate the salts from the mother liquid. The purified polysaccharides were dissolved in phosphate buffer (Green, 1933) pH ~ 6.8, I = 0.1 M, at 20.0 °C, with the exception of SP (7F) and SP (14) which were dissolved in 2 M NaCl solution (optimal conditions for solvation and future conjugation studies). Solutions were mixed by magnetic stirring at the ambient temperature for 24 h.

2.2. Sedimentation velocity analytical ultracentrifugation

Sedimentation velocity experiments were performed using a Beckman (Palo Alto, CA, USA) Optima XL-I analytical ultracentrifuge equipped with Rayleigh interference optics and an automatic on-line data capture system. Conventional 12.0 mm double-sector epoxy cells with sapphire windows were loaded with 400 μL of different concentrations (0.1–2.0 mg/mL) of each sample and a matching amount of the corresponding reference buffer in appropriate channels. Samples were centrifuged at 45,000 rpm. Concentration profiles in the analytical ultracentrifuge cell were recorded using the Rayleigh interference optical system and converted to concentration (see Harding, 2005a) (in units of fringe displacement relative to the meniscus) versus radial position, r at particular times t. Data were analysed using the least squares modelling of the concentration profile referred to as the $ls - g^*(s)$ model incorporated into the SEDFIT algorithm (Schuck, 1998). SEDFIT generates an apparent distribution (based on a Fredholm integral and numerical solution of the differential equation describing the sedimentation process – known as the Lamm equation) of sedimentation coefficients in the form of $g^*(s)$ versus $s_{T,b}$, where the (*) indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects (see e.g. Harding, 2005a). This was followed by a correction to standard solvent conditions – namely the density and viscosity of water at 20.0 °C – to yield $s_{20,w}$ using the utility program SEDNTERP (Laue, Shah, Ridgeway, & Pelletier, 1992). To account for non-ideality (co-exclusion and backflow effects), the apparent sedimentation coefficients ($s_{20,w}$) was calculated at a series of different cell loading concentration and extrapolated to infinite dilution using the Gralén (1944) relation:

$$\frac{1}{s_{20,w}} = \frac{1}{s^0_{20,w}}(1 + k_s c) \quad (1)$$

where k_s is the Gralén or concentration dependence coefficient.

2.3. Sedimentation equilibrium in the analytical ultracentrifuge

Samples were prepared at a concentration of 0.3 mg/mL sufficiently low to minimise the effects of thermodynamic non-ideality. A volume of 1.0 mL of each sample was dialysed for 48 h at the ambient temperature, each against 300 mL of solvent. Sedimentation equilibrium experiments were performed using the Beckman (Palo Alto, CA, USA) Optima XL-I analytical ultracentrifuge equipped with Rayleigh interference optics and an automatic on-line data capture system. The modified long (20.0 mm) optical path length double-sector titanium cells with sapphire windows were loaded with 0.7 mL of dialysed sample and a matching amount of reference buffer dialysate in appropriate channels. Samples were centrifuged at rotor speeds selected to give a sufficient fringe increment from meniscus to base (Harding, 2005b). Using the Rayleigh interference optical system, scans were taken every hour and equilibrium was reached after approximately 48 h. Records of the relative concentration distribution of the solute at equilibrium were analysed to give the weight (mass) average apparent molar mass $M_{w,app}$ using the *MSTARI* algorithm, implemented by K. Schilling (Nanolytics

Ltd., Germany) for ORIGIN software (version 6.0) (Originlab Corporation, Northampton, USA) based on the algorithm of Cölfen and Harding (1997) and the M^* function of Creeth and Harding (1982). The use of the long path length cells (20 mm) meant that low loading concentrations could be used to give a sufficient signal (~0.3 mg/mL). At such low concentrations, non-ideality effects will be small and hence the apparent weight average molecular weight will be approximately equal to the true weight average molecular weight M_w (Harding, 2005a). The *z*-average molar mass M_z was calculated using the *MFIT* procedure (Ang & Rowe, 2010).

2.4. Size exclusion chromatography coupled to multi-angle light scattering (SEC–MALS) and to a differential pressure viscometer

SEC–MALS was used as an independent method for obtaining estimates for molar mass. This was first used to successfully characterise molar mass and molar mass distribution of polysaccharides in 1991 (Horton, Harding, & Mitchell, 1991) and has since become the method of choice for most polymeric systems (see e.g. Wyatt, 2012). The present apparatus comprised an X-Act 4 channel degassing unit (Jour Research, Onsala, Sweden), a Jasco Intelligent HPLC Pump – PU-1580 (Jasco Corporation, Great Dunmow, UK), fitted with a Spark-Holland Marathon Basic autosampler (Spark Holland, Emmen, The Netherlands) combined with a guard column and TSK Gel G6000, 5000, and 4000 columns connected in series (Tosoh Biosciences, Tokyo, Japan), together with a column temperature regulator (Anachem, Luton, UK). Light scattering intensity was detected using a DAWN® HELEOS™ II, an 18-angle light scattering detector (Wyatt Technology Corporation, Santa Barbara, USA). Also coupled on-line to the system (between the photometer and the concentration detector) was a ViscoStar™ II on-line differential viscometer, for the measurement of relative viscosities η_r, and an Optilab® rEX refractive index detector for registering concentration. A solution of each Streptococcal polysaccharide prepared at a concentration of ~1.5 mg/mL and then filtered through a 0.45 μm syringe filter (Whatman, Maidstone, England) – to remove any insoluble material or dust and then injected into the autosampler. 100 μL of each solution was delivered onto the columns at ambient temperature. The eluent employed was phosphate chloride buffer pH ~ 6.8; I = 0.1 M, pumped at a flow rate of 0.8 mL/min. ASTRA™ (Version 5.1.9.1) software (Wyatt Technology Corporation) was used which utilises a proprietary band-broadening correction algorithm. Refractive increment dn/dc values of 0.130–0.139 mL/g were used (Bednar & Hennessey, 1993). Intrinsic viscosities $[\eta(V)]$ as a function of elution volume V were estimated using the Solomon–Ciuta equation (1962):

$$[\eta] \approx \frac{(2\eta_{sp} - 2\ln(\eta_{rel}))^{1/2}}{c} \quad (2)$$

where $\eta_{sp} = \eta_{rel} - 1$ and c is the corresponding concentration.

3. Results and discussion

3.1. Analytical ultracentrifugation

Streptococcal polysaccharides were first of all characterised by sedimentation velocity in the analytical ultracentrifuge to establish their molecular integrity. Unimodal plots were seen in all cases for the apparent sedimentation coefficient distributions (Fig. 1). Under these conditions pneumococcal polysaccharides have apparent weight average sedimentation coefficients ranging from 3.6 S to 8.4 S as reported in Table 1. All showed classical dependencies of

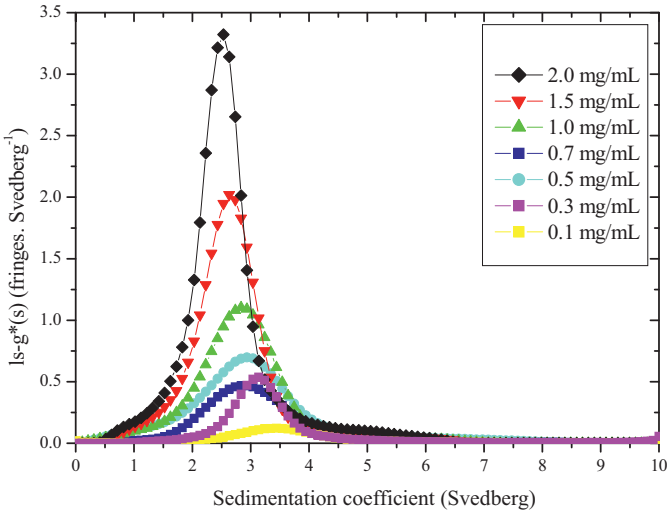

Fig. 1. Example of a sedimentation coefficient distribution $g^*(s)$ profile for capsular *Streptoccocal* polysaccharides. SP (5) at different concentrations.

$s_{20,w}$ on c, indicative of non-ideality and the absence of associative effects (Fig. 2).

Molar mass values estimated from sedimentation equilibrium using the *MSTARI* and *MFIT* algorithms for weight-average molar masses and *z*-average molar masses respectively are also reported in Table 1.

3.2. Size exclusion chromatography coupled to multi-angle laser light scattering (SEC–MALS)

Values for the weight average molar mass, M_w and *z*-average molar mass M_z – for native capsular polysaccharides of *S. pneumoniae* – obtained from SEC–MALS are also reported in Table 1 and the values obtained appear to be in good agreement with those obtained from sedimentation equilibrium in the analytical ultracentrifuge. Table 1 also reports the weight average intrinsic viscosity for each serotype studied and the power law exponent *a*.

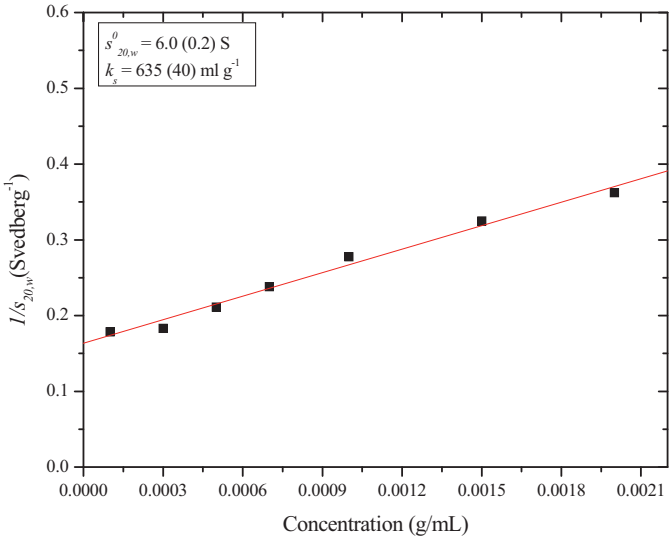

Fig. 2. Example of a concentration dependence (reciprocal) of sedimentation coefficient plot for capsular polysaccharide SP (1) at different concentrations.

Table 1
Hydrodynamic properties of capsular polysaccharides from *Streptococcus pneumoniae*.

Serotype	$s^0_{20,w}$ (S)	$10^{-3} \times M_w$ (g/mol)[a]	$10^{-3} \times M_z$ (g/mol)[b]	$10^{-3} \times M_w$ (g/mol)[c]	$10^{-3} \times M_z$ (g/mol)[c]	$[\eta]$ (mL/g)	a	k_s (mL/g)	k_s $[\eta]$
SP (1)	6.0 ± 0.2	555 ± 30	580 ± 30	600 ± 30	670 ± 35	712 ± 3	0.8	635 ± 40	0.9
SP (4)	3.8 ± 0.4	445 ± 20	415 ± 15	475 ± 25	575 ± 30	680 ± 2	0.7	490 ± 110	0.7
SP (5)	3.6 ± 0.1	220 ± 10	315 ± 15	240 ± 15	375 ± 20	233 ± 2	0.6	290 ± 35	1.3
SP (6B)	8.4 ± 0.2	960 ± 50	820 ± 40	910 ± 45	950 ± 50	457 ± 2	0.8	500 ± 25	1.0
SP (7F)	6.3 ± 0.2	1100 ± 55	985 ± 30	1150 ± 60	1230 ± 60	261 ± 2	0.8	240 ± 25	0.9
SP (9V)	7.4 ± 0.1	1250 ± 60	1190 ± 35	1100 ± 55	1125 ± 55	484 ± 2	0.8	365 ± 15	0.8
SP (14)	6.6 ± 0.1	1175 ± 60	1060 ± 30	1000 ± 50	1060 ± 55	283 ± 2	0.7	250 ± 20	0.9
SP (18C)	5.3 ± 0.1	610 ± 30	575 ± 15	600 ± 30	640 ± 30	835 ± 3	0.9	670 ± 35	0.8
SP (19F)	6.5 ± 0.2	640 ± 30	585 ± 15	650 ± 35	720 ± 35	574 ± 2	0.8	640 ± 40	1.0
SP (23F)	7.4 ± 0.2	820 ± 40	910 ± 10	960 ± 50	1040 ± 50	618 ± 3	0.8	635 ± 30	1.0

[a] Sedimentation equilibrium MSTAR analysis.
[b] Sedimentation equilibrium MFIT analysis.
[c] SEC–MALs.

3.3. Wales–van Holde ratio

Wales–van Holde (1954) ratio, $R = k_s/[\eta]$ is perhaps the simplest guidance/indicator of a molecule's conformational flexibility. The limits appear to be ~1.6 for a compact sphere or a non-draining random coil, and ~0.1 for a stiff rod (Creeth & Knight, 1965, 1967). From Table 1 it appears that the values for all serotypes are consistent with semi-flexible structures ($R \sim 0.7$–1.0). Interpretation may be affected by complications though draining although these are likely to be small compared with the strength of the hydrodynamic interaction between the segments of the macromolecule (see e.g. Tanford, 1961).

3.4. Mark–Houwink–Kuhn–Sakurada power law relationship

We can take advantage of the particular feature of the SEC–MALs-viscostar system in providing an on-line record of $[\eta](V)$ versus $M(V)$ where V is the SEC elution volume facilitating the use of the "Mark–Houwink–Kuhn–Sakurada" – (MHKS) power law relation linking $[\eta]_w$ (Viscostar^{TM}) M_w (from SEC–MALS)

$$[\eta] \sim M^a \tag{3}$$

The MHKS or "Mark–Houwink" exponent a is obtained using a double logarithmic plot of intrinsic viscosity versus molar mass (see e.g. Harding, Vårum, Stokke, & Smidsrød, 1991 and references cited therein) and Fig. 3 gives an example. The values obtained (Table 1) are in good agreement with those of Bednar and Hennessey (1993) and are again indicative of semi-flexible coil type molecules.

3.5. Estimation of the persistence length L_p

For a more quantitative estimate of chain flexibility we can use the persistence length L_p, which has theoretical limits of 0 for a random coil and ∞ for a stiff rod. Practically the limits are ~1–2 nm for a random coil and ~200–300 nm for a very stiff rod shaped macromolecule. Persistence lengths, L_p can be estimated using several different approaches using either intrinsic viscosity (Bohdanecky, 1983; Bushin, Tsvetkov, Lysenko, & Emelianov, 1981; Harding, 1997) or sedimentation coefficient (Yamakawa & Fujii, 1973) measurements. For example the relation (Bohdanecky, 1983; Bushin et al., 1981):

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2} \tag{4}$$

where Φ is the Flory-Fox coefficient (2.86×10^{23} mol^{-1}) and A_0 and B_0 are tabulated coefficients, and the Yamakawa–Fujii equation (Yamakawa & Fujii, 1973):

$$s^0 = \frac{M_L(1-\bar{\nu}\rho_0)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \cdots\right] \tag{5}$$

Yamakawa and Fujii showed that A_2 can be considered as $-\ln(d/2L_p)$ and $A_3 = 0.1382$ if the L_p is much higher than the chain diameter, d (see also Freire & Garcia de la Torre, 1992). Difficulties arise if the mass per unit length is not known, although both relations have now been built into an algorithm Multi-HYDFIT (Ortega & Garcia de la Torre, 2007) which estimates the best estimates – or best range of values of L_p and M_L based on minimisation of a target function Δ. An estimate for the chain diameter d is also required but extensive simulations have shown that the results returned for L_p are relatively insensitive to the value chosen for d which was fixed at an average of ~0.8 nm (see e.g. Kök, Abdelhameed, Ang, Morris, & Harding, 2009; Morris, Garcia de la Torre et al., 2008; Morris, Patel et al., 2008; Patel et al., 2008).

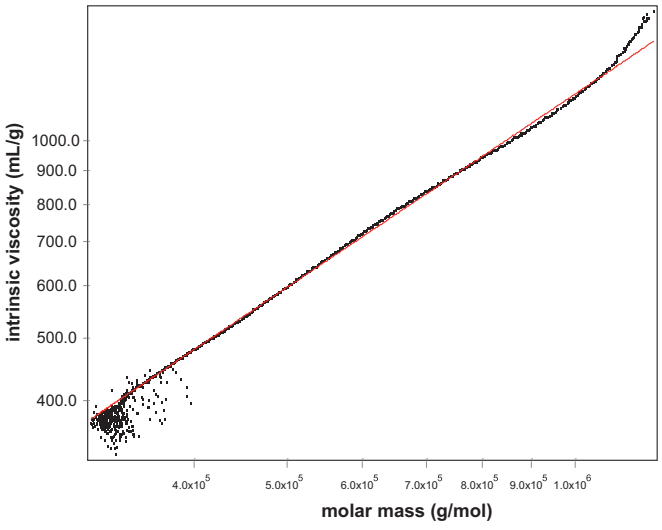

Fig. 3. Example of a Mark–Houwink–Kuhn–Sakurada (MHKS) plot for capsular polysaccharide SP (1) from the differential pressure viscometer coupled on-line to a size exclusion chromatography column and a multi-angle laser light scattering detector. The plot is of intrinsic viscosity $[\eta]$ as a function of elution volume (ordinate) versus molar mass M as a function of elution volume. The red line represents the fit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

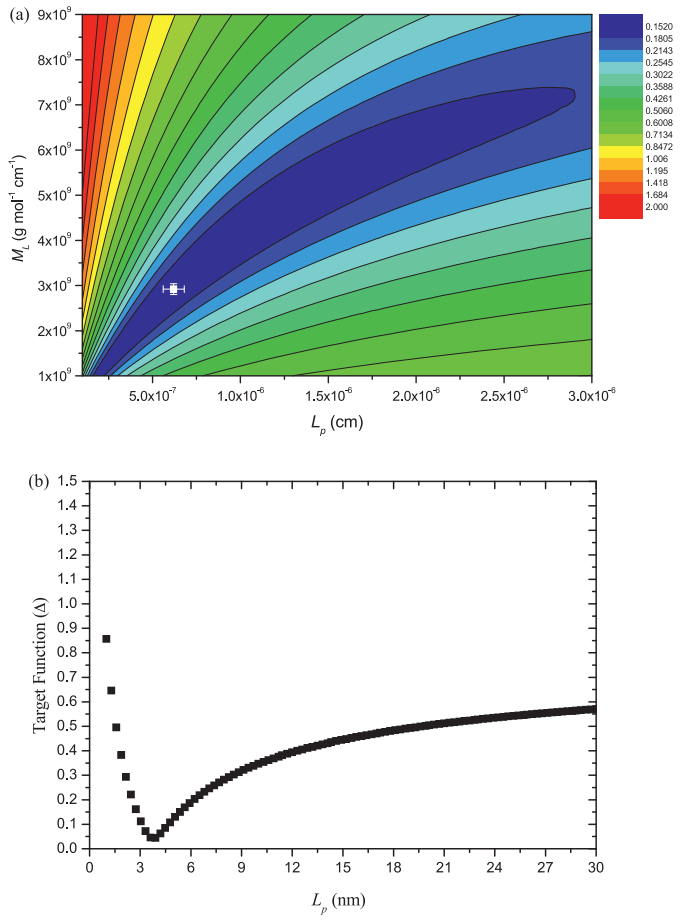

Fig. 4. (a) Plot of mass per unit length M_L versus persistence length L_p for capsular polysaccharide SP (4). The plot yields $L_p \sim 6.2$ (nm) and $M_L \sim 2.92 \times 10^9$ (g mol^{-1} cm^{-1}) at the minimum target (error) function value of 0.15. (b) Plot of the target function (Δ) versus persistence length L_P for SP (6B) where $L_p \sim (3.9 \pm 0.2)$ nm.

Firstly M_L and L_p were treated as variables and the minimum value of the target function Δ was estimated on a 2D contour plot for each polysaccharide: an example is given in Fig. 4a, and the values estimated for all the serotypes given in Table 2. Second, the M_L was estimated separately from the average mass and length of the sugar residues: an example of a "1D" minimisation plot is given in Fig. 4b. Values for all the serotypes are given in Table 2. All the values are consistent with semi-flexible coil structures.

Table 2
Values for mass per unit length M_L and persistence length L_P obtained from HYDFIT analysis, for capsular *Streptococcal* polysaccharides.

Serotype	M_L (g mol nm^{-1})	L_p (nm)	M_L (g mol nm^{-1})[a]	L_p (nm)[a]
SP (1)	660 ± 30	6.5 ± 0.6	500	7.4 ± 0.8
SP (4)	290 ± 10	6.2 ± 0.6	598	9.5 ± 0.5
SP (5)	400 ± 20	5.2 ± 0.2	491	7.9 ± 0.4
SP (6B)	630 ± 30	6.2 ± 0.8	414	3.9 ± 0.2
SP (7F)	440 ± 20	7.0 ± 0.5	464	7.4 ± 0.3
SP (9V)	520 ± 20	6.8 ± 0.4	548	7.1 ± 0.2
SP (14)	480 ± 20	6.5 ± 0.5	472	6.2 ± 0.3
SP (18C)	390 ± 20	6.5 ± 1.0	478	8.5 ± 1.0
SP (19F)	500 ± 20	6.2 ± 0.7	498	6.2 ± 0.2
SP (23F)	530 ± 20	6.8 ± 0.4	438	4.0 ± 0.3

[a] M_L estimated from chemical structure and L_p evaluated from HYDFIT with this M_L fixed at this value.

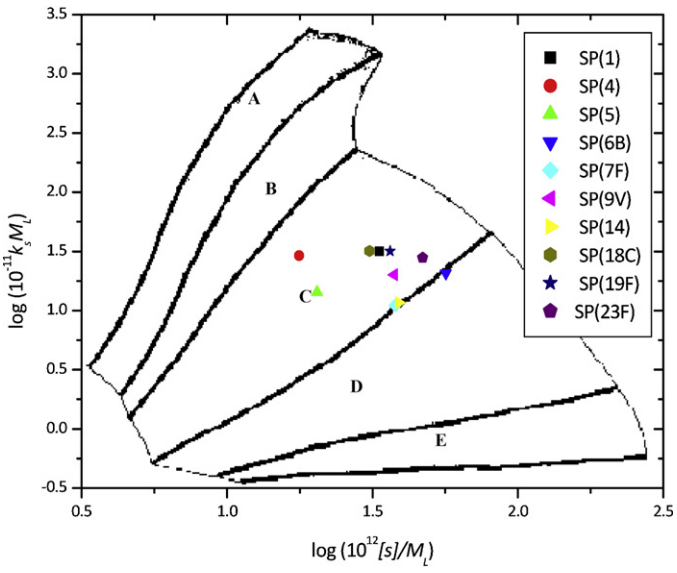

Fig. 5. Conformation zoning plot. k_s is the concentration dependence sedimentation coefficient (mL/g), M_L is the mass per unit length and [s] is the intrinsic sedimentation coefficient. All 10 *Streptococcal* capsular polysaccharides have conformations in Zone C (semi-flexible coil) or near the boundary with Zone D (highly flexible).

3.6. Sedimentation conformation zoning

A check for consistency of the above results can be obtained from a sedimentation conformation zone plot of $k_s M_L$ versus [s]/M_L where [s] (Pavlov, Harding, & Rowe, 1999; Pavlov, Rowe, & Harding, 1997) is given by:

$$[s] = \frac{s^0_{20,w}\eta_{20,w}}{1 - \bar{\nu}\rho_{20,w}} \quad (6)$$

Fig. 5 shows that all the capsular polysaccharides studied here appear to behave as semi-flexible chains.

4. Concluding remarks

In this study we have characterised the molar mass and molar mass distributions of a representative selection of capsular polysaccharides from *S. pneumoniae* and shown them to have well defined properties with relatively narrow molar mass distributions as confirmed by two independent techniques – analytical ultracentrifugation (sedimentation velocity and sedimentation equilibrium) and size-exclusion chromatography coupled to multi-angle total-intensity light. They all have similar conformational flexibilities as shown by three different methods based on analytical ultracentrifugation, light and dilute solution intrinsic viscosity – and all point to semi-flexible coil shapes with persistence lengths ~7 nm. This knowledge may have importance in understanding the behaviour of these molecules both as polysaccharide vaccines and as part of glycoconjugate constructs (Astronomo & Burton, 2010).

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