Eur Biophys J (2003) 32: 457–464
DOI 10.1007/s00249-003-0317-8

ARTICLE

**Monica Fee · Neil Errington · Kornelia Jumel
Lisbeth Illum · Alan Smith · Stephen E. Harding**

Correlation of SEC/MALLS with ultracentrifuge and viscometric data for chitosans

Received: 7 January 2003 / Revised: 28 March 2003 / Accepted: 8 April 2003 / Published online: 14 June 2003
© EBSA 2003

Abstract Attempts have been made to correlate estimates of molecular weight for a group of cationic polysaccharides known as chitosans between the highly popular technique of size-exclusion chromatography coupled to multi-angle laser light scattering, "SEC-MALLS", and the less convenient but more established technique of sedimentation equilibrium in the analytical ultracentrifuge. Four pharmaceutical grade chitosans of various molecular weights and degrees of acetylation (4–30%) were chosen. Better correlation than previous was achieved, although some batch variability was observed. Despite the broad spectrum in degree of acetylation, a $\log s^{\circ}_{20,w}$ versus $\log M_w$ scaling plot appeared to fit a straight line with power-law exponent $b = 0.25 \pm 0.04$, i.e. between the limits of rod (0.15) and coil (0.4–0.5), although this may be the average of a lower b value at low M_w and higher b at high M_w. With regard to viscosity, a $\log[\eta]$ versus $\log M_w$ scaling plot appeared to also fit a straight line with power-law exponent $a = 0.96 \pm 0.10$, again between the coil (0.5–0.7) and rod (1.8) limits.

Keywords Chitosan · Conformation · Molecular weight · SEC-MALLS · Sedimentation equilibrium

Presented at the conference for Advances in Analytical Ultracentrifugation and Hydrodynamics, 8–11 June 2002, Grenoble, France

M. Fee · N. Errington · K. Jumel · S. E. Harding (✉)
NCMH Physical Biochemistry Laboratory,
School of Biosciences,
University of Nottingham,
Sutton Bonington, LE12 5RD, UK
E-mail: steve.harding@nottingham.ac.uk

L. Illum · A. Smith
West Pharmaceutical Services,
Drug Delivery and Clinical Research Centre Ltd,
Albert Einstein Centre,
Nottingham Science and Technology Park,
Nottingham, NG7 2TN, UK

Introduction

Despite their increasing importance in the pharmaceutical and healthcare industries, chitosans [cationic polysaccharides consisting of *N*-acetyl-D-glucosamine units and D-glucosamine units in varying proportions (see Fig. 1)] have proved difficult to characterize with regard to molecular weight owing to their polycationic nature. This is primarily because of the failure of results from SEC-MALLS (size-exclusion chromatography coupled on-line to a multi-angle laser light-scattering detector) to agree or show compatibility with other techniques, such as sedimentation equilibrium in the ultracentrifuge, or measurements from viscometry. These difficulties have largely been attributed to anomalous behaviour on the size-exclusion columns that have been available, and discrepancies of over an order of magnitude in the weight average molecular weight have been observed.

When dissolved in aqueous acidic medium, chitosan becomes a cationic polyelectrolyte, making it a potential excipient for many applications in the food and pharmaceutical industries. These include, for example, controlled drug and flavour release from tablets or using microencapsulation techniques, film formation or as fillers in dietary foods, etc. (Illum 1998; Skaugrud et al. 1999). Chitosan is of particular interest to the pharmaceutical industry since it possesses mucoadhesive properties (see, for example, Harding et al. 1999), which result in increased contact time with a mucosal surface leading to enhanced drug absorption, and has also been demonstrated to enhance the delivery of proteins and peptides across mucosal membranes (Artursson et al. 1994; Illum et al. 1994, 2001; Natsume et al. 1999; Tengamnuay et al. 2000; Davis 2001). The mucoadhesive properties have been attributed to interactions between the $-NH_3^+$ groups left after deacetylation of the chitin and the terminal $-COO^-$ (sialic acid) on mucosal surfaces, reinforced through hydrophobic interaction between groups of un-deacetylated residues on chitosan with fucose on mucins (Deacon et al. 1999).

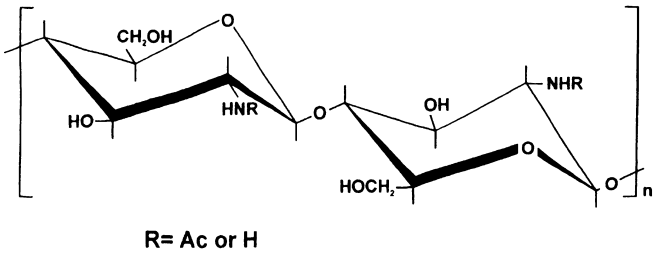

Fig. 1 Schematic representation of chitosan; R = acetyl or H, depending on the degree of acetylation

Chitosan has also been shown to cause a transient widening of the tight junctions of the epithelial cells in vitro, which is thought to contribute to enhanced drug permeability (Illum and Davis 2001; Illum et al. 2001).

For many applications of chitosan, and especially in the pharmaceutical industry, the molecular weight and degree of acetylation dictate the properties and thereby the effectiveness of the chitosan for a particular application. For example, Schipper et al. (1996) have reported that both these properties are very important for its absorption enhancement of hydrophilic drugs across mucosal surfaces. They found that a low degree of acetylation and/or high molecular weight appeared to be necessary for chitosans to increase epithelial permeability.

It is clear that the pharmaceutically important properties of chitosan (including solubility) are not only dependent on their chemical nature (dictated by the degree of acetylation), but also their physical properties (molecular weight and conformation). Although the degree of acetylation can be measured with considerable confidence [e.g. by NMR (Vårum et al. 1991)], it is also important to be able to attach some degree of confidence to the SEC/MALLS determination method for molecular weight. Successful molecular weight determinations of chitosan by SEC/MALLS have been reported by Beri et al. (1993). However, these authors did not compare their results with those from other independent techniques. Attempts at molecular weight determinations by SEC/MALLS in our own laboratories have previously proved difficult due to the anomalous behaviour of chitosan on our SEC columns, and results were generally incompatible with those obtained by sedimentation equilibrium in the analytical ultracentrifuge. The cationic nature of chitosan is responsible for its wide range of desirable interactions; however, it may also cause interactions with the SEC column packing material and/or residues from other previously run samples. A dedicated column system for chitosan was therefore used for the re-examination of the use of SEC/MALLS which was considered important due to:

1. The ease of obtaining absolute molecular weight data from SEC-MALLS compared with the use of other methods. Since 1991 (Horton et al. 1991; Rollings 1991, 1992), this method has become the most popular for characterizing polysaccharide molecular weights in solution, and, since 1996, also glycoconjugates such as mucus glycoproteins (Jumel et al. 1996, 1997).
2. The belief that there is a clear link between the functional properties of chitosans and their mucoadhesive and epithelial absorption enhancement capabilities for drug delivery and film forming potential for healthcare products (e.g. shampoos).

This paper explores the use of SEC/MALLS for four polysaccharides of pharmaceutical grade covering a range of degree of acetylation, DA (4–30%), and molecular weight. Comparative molecular weight data were obtained by sedimentation equilibrium in the analytical ultracentrifuge. A correlation of the Mark–Houwink–Kuhn–Sakurada (MHKS) power-law type is also attempted between weight average molecular weight, M_w, and both the intrinsic viscosity or the sedimentation coefficient to check for consistency between the data and to see if these particular chitosans of different DA and M_w approximate a homologous series.

Materials and methods

Materials

Four chitosans were provided by Pronova Biomedical, Oslo, Norway (G112, G114, G213 and G214). These were glutamate salts with DA values ranging from 4 to 30% and of reputedly different molecular weight, as indicated by the dynamic viscosity values (of 1% solutions) provided by Pronova (Table 1). A separate batch of G213 was also supplied.

For all experiments, the chitosan samples were dissolved in a 0.2 M acetate buffer (pH 4.3). For sedimentation equilibrium experiments, samples were dialysed against the buffer solution overnight so that the chemical potential of the solvent was equal in both the sample solution and the reference solvent (dialysate).

Methods

Molecular weight: sedimentation equilibrium

Low-speed sedimentation equilibrium was used to determine the molecular weights (weight averages) of the various chitosans. Two analytical ultracentrifuges (AUCs) were employed. Firstly, a Beckman XL-I Analytical Ultracentrifuge was used for chitosan solutions in the range of concentrations 0.5–1.0 mg/mL, at a rotor speed of 14,000 rpm (sample G112) or 8000 rpm (the other samples) and a temperature of 20.0 °C. The data collected from the Rayleigh interference optical system were analysed using the MSTARI algorithm (Cölfen and Harding 1997) to give an apparent weight average molecular weight, $M_{w,app}$. A low concentration sample (0.3 mg/mL) was also analysed using a Beckman Model E

Table 1 Commercial manufacturer's data (courtesy of Pronova, Drammen, Norway)

Chitosan*	G112	G114	G213[(a)]	G213[(b)]	G214
Degree of acetylation (%)	30	7	17	20	4
Viscosity of 1% solution (mPa s)	5	18	79	153	59

*G213[(a)], batch no. 610-583-09; G213[(b)], batch no. FP1-A-708-01

Analytical Ultracentrifuge (at rotor speeds between 10,000 and 20,000 rpm and a temperature of 20.0 °C) equipped with Schlieren optics, adapted with a CCD camera system (Clewlow et al. 1997) for automatic capture of the optical data. Use was made of the long optical path cells (30 mm), impossible to use in the XL-I. The data were analysed by fitting to the Lamm equation (see, for example, Clewlow et al. 1997):

$$\mathrm{d}\ln\left(\frac{1}{r}\frac{\mathrm{d}n}{\mathrm{d}r}\right)/\mathrm{d}\left(r^2\right) = M_{\mathrm{z,app}}(1-\bar{v}\rho)\omega^2/2RT \quad (1)$$

where $\bar{v}$ is the partial specific volume (mL/g) determined by precision densimetry for each chitosan (Kratky et al. 1973), ρ the solvent density (ml/g) R is the gas constant and T the temperature (K). A plot of $\ln(\frac{1}{r}\frac{\mathrm{d}n}{\mathrm{d}r})$ versus r^2 has a slope proportional to the apparent z-average molecular weight ($M_{\mathrm{z,app}}$). The data set was then transformed by integrating with respect to r (distance from the centre of rotation in cm) and analysed using MSTARI (Cölfen and Harding 1997) to obtain $M_{\mathrm{w,app}}$.

To account for non-ideality, the reciprocal of the apparent weight average molecular weights $M_{\mathrm{w,app}}$ determined over a range of concentrations, c, were extrapolated to zero concentration. The thermodynamic or "osmotic pressure" second virial coefficient, B, and the "ideal" or "true" weight average molecular weight, M_{w}, were estimated from plots of $1/M_{\mathrm{w,app}}$ versus concentration according to (see, for example, Harding 1995a):

$$1/M_{\mathrm{w,app}} = (1/M_{\mathrm{w}})(1+2BM_{\mathrm{w}}c) \quad (2)$$

Molecular weight: SEC-MALLS

The molecular weights (weight averages) of the chitosan samples were then also determined by using SEC-MALLS. Acetate buffer, pH 4.3, was pumped at a flow rate of 0.8 mL/min through a column system consisting of TSK-G5000PW, TSK-G4000PW and TSK-G3000PW analytical columns protected by a guard column. Solutions of each chitosan sample were prepared in acetate buffer, pH 4.3, at a concentration of 5 mg/mL and 100 μL was injected onto the columns at ambient temperature (sample filtered through 0.45 μm filter to remove any insoluble material or dust prior to injection). The eluting fractions were monitored using a Dawn DSP multi-angle light-scattering photometer (Wyatt Technology, Santa Barbara, USA) fitted with a 5 mW He-Ne laser and a differential interferometric refractometer (Optilab 903, Wyatt Technology, Santa Barbara, USA). Apparent weight average molecular weights, $M_{\mathrm{w,app}}$, were obtained using the so-called Debye plot method (see, e.g. Wyatt 1992, 1993), where a plot of $R(\theta)/Kc$ versus $\sin^2\theta/2$ yields $M_{\mathrm{w,app}}$ from the intercept, θ being the scattering angle, $R(\theta)$ the Rayleigh excess scattering ratio and K, the optical constant, is $4\pi^2\mathrm{n}^2(\mathrm{d}n/\mathrm{d}c)^2/(\lambda_0{}^4\mathrm{N_A})$; n is the solvent refractive index, $\mathrm{d}n/\mathrm{d}c$ is the specific refractive index increment (mL/g), N_{A} is Avogadro's number and λ_0 is the wavelength of the scattered light in vacuo (cm). The specific refractive index increment was calculated from the DA using the equation of Anthonsen et al. (1994):

$$\mathrm{d}n/\mathrm{d}c = 0.201 - 0.056(\mathrm{DA}) \quad (3)$$

Because of the low concentrations after dilution from the column, the contribution from the $2\mathrm{BM_w}c$ term in Eq. (2) can be reasonably assumed to be ~0, so to a good approximation $M_{\mathrm{w}} \approx M_{\mathrm{w,app}}$.

Sedimentation coefficient measurements

A Beckman XLI Analytical Ultracentrifuge was used for the determination of the sedimentation coefficients at 20.0 °C and a rotor speed of 45,000 rpm. Sedimentation coefficients (s_{obs}) were obtained by analysing 10 consecutive scans using the "time derivative" algorithm DCDT+ (Philo 2000). These were corrected to standard solvent conditions (the density and viscosity of water at 20.0 °C) using the equation (see Schachman 1959):

$$s_{20,\mathrm{w}} = \left\{\left(1-\bar{v}\rho_{20,\mathrm{w}}\right)/\left(1-\bar{v}\rho_{T,\mathrm{b}}\right)\right\}\left\{\eta_{T,\mathrm{b}}/\eta_{20,\mathrm{w}}\right\}s_{\mathrm{obs}} \quad (4)$$

where $s_{20,\mathrm{w}}$ is the sedimentation coefficient in terms of the standard solvent water at 20 °C; s_{obs} is the measured sedimentation coefficient in the experimental solvent/buffer at temperature T; $\rho_{20,\mathrm{w}}$ and $\eta_{20,\mathrm{w}}$ are the density and viscosity of water at 20 °C and $\rho_{T,\mathrm{b}}$ is the density and viscosity of the buffer used for the experiments at temperature T. Measurements were made at a series of low concentrations (0.3–1.0 mg/mL) to minimize non-ideality; nonetheless, $s_{20,\mathrm{w}}$ values (or reciprocals thereof) were then extrapolated to infinite dilution to yield $s^{\circ}_{20,\mathrm{w}}$.

Determination of intrinsic viscosity

Intrinsic viscosities were determined using an Ostwald-type viscometer of 2 mL capacity over a concentration range of 0.2–2.0 mg/mL. The flow times were recorded at 20.00 ± 0.01 °C. From the solution/solvent flow-time ratio the kinematic relative viscosity was obtained. Because of the low concentrations used (< 1 mg/mL), the density corrections were assumed to be negligible and the kinematic viscosities were assumed to be approximately equal to the dynamic viscosities (Tanford 1955). The intrinsic viscosity was found by extrapolation to infinite dilution of the reduced viscosities using the Huggins equation (see, for example, Harding 1997).

Results and discussion

Molecular weight

For four of the chitosans, much better agreement than previous is seen between the two totally independent

Table 2 Thermodynamic and hydrodynamic data

Property*	Units	Fraction				
		G112	G114	G213[(a)]	G213[(b)]	G214
M_{w} (sed. eqm.)	g mol^{-1}	57,000 ± 800	82,000 ± 9000	161,000 ± 18000	–	132,000 ± 15000
M_{w} (SEC-MALLS)	g mol^{-1}	42,400 ± 3000	129,000 ± 6000	203,000 ± 8000	207,500 ± 8000	172,000 ± 6000
M_{z} (sed. eqm.)	g mol^{-1}	90,000 ± 11,000	386,000 ± 30,000	442,000 ± 30,000	417,000 ± 41,000	246,500 ± 18,000
$M_{\mathrm{w}}/M_{\mathrm{n}} = M_{\mathrm{z}}/M_{\mathrm{n}}$	–	2.2	3.4	2.5	2.9	1.5
$2BM$ (sed. eqm.)	mL g^{-1}	–	2200	4600	–	4100
$s^{\circ}_{20,\mathrm{w}}$	S	1.76 ± 0.04	1.99 ± 0.05	2.71 ± 0.22	2.69 ± 0.07	2.74 ± 0.12
$[\eta]$	mL g^{-1}	100 ± 8	310 ± 15	420 ± 20	610 ± 30	550 ± 20

*M_{w} (sed. eqm.): weight average molecular weight obtained from sedimentation equilibrium. M_{w} (SEC-MALLS): weight average molecular weight obtained from SEC-MALLS. M_{z} (sed. eqm.): z-average molecular weight obtained from sedimentation equilibrium. $M_{\mathrm{w}}/M_{\mathrm{n}} = M_{\mathrm{z}}/M_{\mathrm{w}}$: identity holds for a log-normal distribution (Fujita 1962). BM: product of second virial coefficient (B) and molecular weight

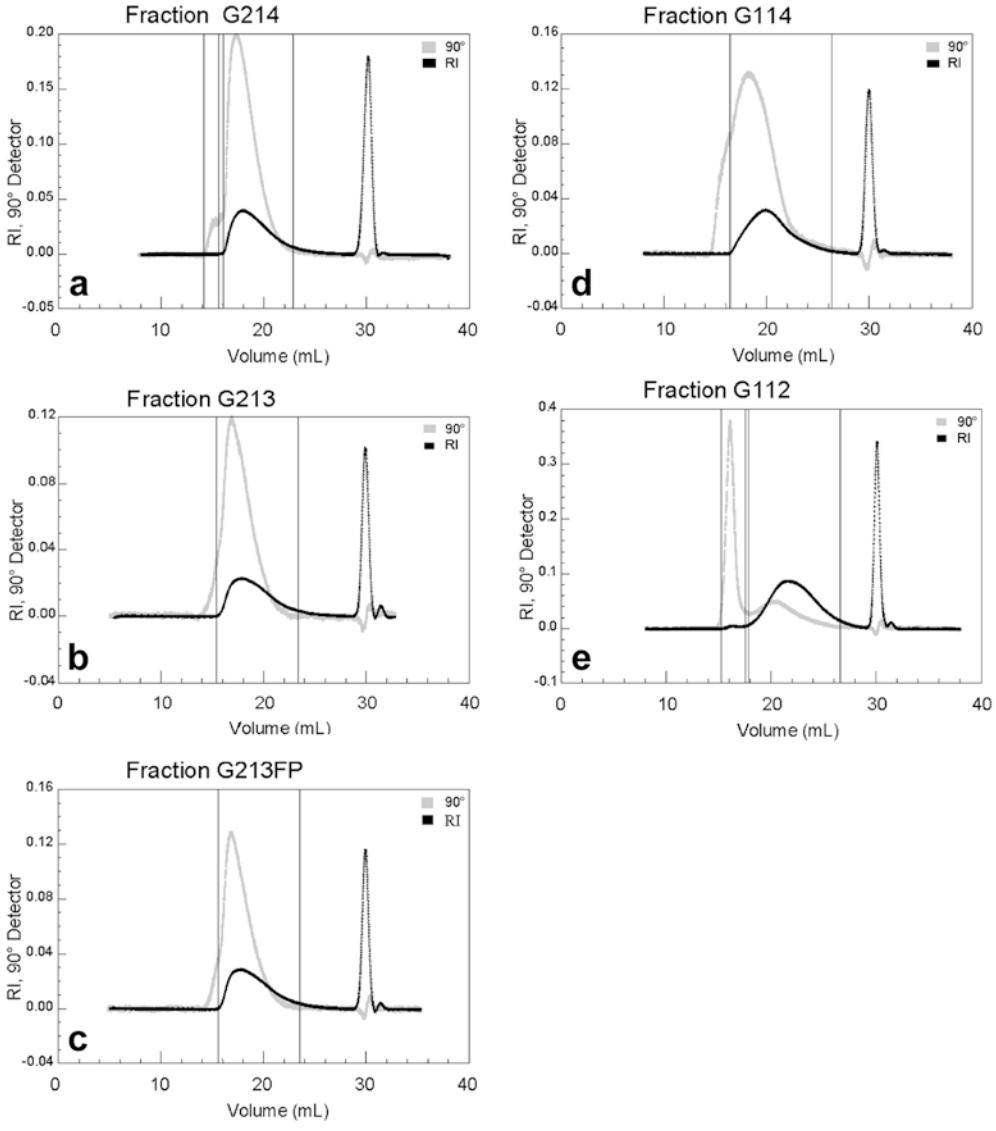

Fig. 2a–e SEC-MALLs elution volume for each chitosan fraction. Signals from the refractive index or RI (concentration) and 90° angle light-scattering detectors are shown in each case. The *vertical lines* indicate the regions of the two traces chosen to calculate the weight average molecular weight corresponding to Table 2. The *additional pair of vertical lines* on the *left* for chitosans G214 and G112 at low elution volume correspond to the peaks for the trace amounts of supramolecular aggregate. The RI peaks at high elution volume correspond to salt elution

techniques of sedimentation equilibrium and SEC-MALLS (Table 2) for the weight average molecular weights, M_w, although, with the exception of G112, sedimentation equilibrium molecular weights still come out somewhat lower.

Figure 2 shows the light-scattering and refractive index (RI, measurement of concentration) traces obtained from SEC-MALLS for each chitosan sample. From the light-scattering traces it can be seen that in each case the peaks from the macromolecular component are skewed and not symmetrical. There was also evidence of persistently occurring trace amounts of high molecular weight aggregate from the 90° angle light-scattering traces, as indicated by the shoulders on the low elution volume or high molecular weight side of the light-scattering peaks. However, except for sample G112, these light-scattering signals are not accompanied by corresponding RI (concentration) signals, indicating that only negligible amounts ($<1\%$) are present in the samples. No such supramolecular particles were observed from the sedimentation velocity $g^*(s)$ distributions obtained using time-derivative DCDT+ analysis, which showed only a single, approximately symmetrical, peak for in each case, including G112 (Fig. 3), and no fast moving supramolecular boundary was observed.

Significance of non-ideality: advantage of SEC-MALLS

Both the SEC-MALLS and the sedimentation equilibrium methods allow direct evaluation of the absolute molecular weight of the macromolecule. However, the time scale for each method varies greatly, with the SEC-MALLS measurement taking approximately 60 minutes (after appropriate equilibration of the SEC columns). The sedimentation equilibrium method, on the other hand, can require up to 3 days for equilibrium to be reached per sample. Further, since higher concentrations are required (absolute minimum is 0.2 mg/mL),

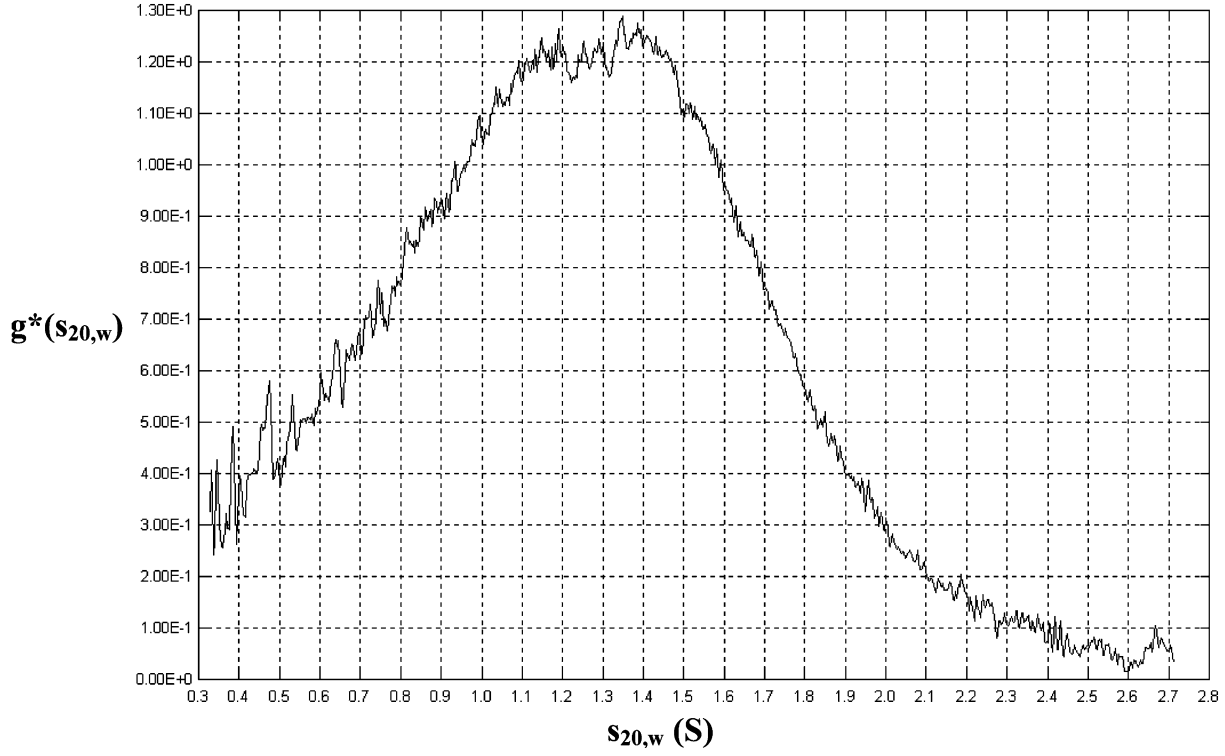

Fig. 3 $g^*(s_{20,w})$ versus $s_{20,w}$ plot for chitosan G112 from a sedimentation velocity experiment (45,000 rpm, 20.0 °C) and DCDT+ analysis

thermodynamic non-ideality effects cannot be neglected and measurements at several concentrations (and extrapolation to infinite dilution) are normally required. The problem is particularly acute for chitosans because suppression of one of the two main causes of solution non-ideality – polyelectrolyte behaviour – by increasing ionic strength is only partially possible because of solubility problems.

We explain further: non-ideality causes the molecular weight for chitosan calculated from AUC data to be lower than the true molecular weight. There are two major contributing factors to this phenomenon: the excluded volume and the polyelectrolye effect. The excluded volume arises from one molecule excluding another from the space occupied by it. For a sphere the excluded volume is eight times the molecular volume: the more extended the molecule (e.g. through intramolecular charge repulsion effects if the molecule is charged), the greater the excluded volume. Another contribution to exclusion volume comes from the swelling of the molecule through ''hydration'' or time-averaged association with water molecules. For a polysaccharide molecule in solution, the swollen volume can be 100 times its volume in the dry state (see, for example, Harding 1995b). The second contribution to solution thermodynamic non-ideality comes from polyelectrolyte phenomena through intermolecular repulsions. Such effects will be large for chitosan owing to the high charge density on the molecule (e.g. for a molecule of ~10% DA, there will be ~90 charges per 100 monomer residues) when in aqueous acidic solution. Such effects can normally be suppressed by charge shielding simply by increasing the ionic strength of the solution medium. Unfortunately for chitosans, large increases in ionic strength lead to reduced solubility and salting out can occur.

To illustrate the significance of the non-ideality, we have included in Table 2 values for the term $2BM_w$ (2×second virial coefficient×weight average molecular weight) in Eq. (2). For a spherical, uncharged molecule this would have a value of approximately 5–10 mL/g, and the value increases as the system deviates from ideality. From Table 2 it can be seen that values calculated for this term are in the range 1000–2000 mL/g, indicating that chitosan is a highly non-ideal macromolecule [a comparative table for polysaccharides is given in Harding (1995b)].

At very low solute concentrations, ideal behaviour is approached (Van Holde 1998) and the effects of non-ideality should be small. For many systems of biological macromolecules, particularly globular proteins, measurement at a single finite, but low (0.2–0.5 mg/mL) concentration can give an estimate of the apparent molecular weight within a few percent of the true molecular weight. However, even with loading concentrations as low as 0.2 mg/mL there can be non-negligible underestimates of the true molecular weight (Harding 1992) for those polysaccharides yielding solutions of high $2BM_w$ values; chitosan appears to be in that category (Harding 1995b). The problem is compounded since the modern commercial instrument (XL-I) permits a minimum concentration of not 0.2 but 0.5 mg/mL.

With SEC-MALLS, however, there is normally no need to correct for non-ideality in the system so long as the sample is diluted in the columns to such an extent that the concentrations of the volume "slices" passing through the light-scattering detector are much lower than the initial loading concentration. These concentrations are usually 0.1 mg/mL or less (depending on the molecular weight of the scatterer), and corrections arising from thermodynamic non-ideality are not significant (Wyatt 1993).

Inertness of the SEC column

Although the SEC-MALLS method of determining the molecular weight of chitosan is very quick and simple, there may still remain some uncertainties in terms of the "inertness" of the column packing materials and in the extrapolation of the angular intensity functions to zero angle, as highlighted by Harding (1995b). The former appears to be particularly important in the case of cationic polyelectrolytes; however, it seems that the use of a dedicated column system can eliminate most of these uncertainties. Nevertheless, it is still advisable to determine the molecular weight using the AUC to obtain a reliable and independent verification of the results.

Conformation

The conformation of chitosans has been the subject of considerable interest over the last decade, particularly with the regard to the effect of the degree of acetylation of the molecule, the molecular weight and the solvent conditions in which it is studied (see, e.g. Wang et al. 1991; Anthonsen et al. 1993; Errington et al. 1993; Ottøy et al. 1996; Berth et al. 1998; Cölfen et al. 2001; Berth and Dautzenberg 2002).

Many methods for assaying the conformation of chitosan and other polysaccharides in solution appear to be based on the dependence of a solution parameter (such as intrinsic viscosity, radius of gyration or sedimentation coefficient) with molecular weight. The simplest are the Mark–Houwink–Kuhn–Sakurada type of scaling or power-law plots of log(parameter) versus log(M_w) (see, e.g. Smidsrød and Andresen 1979; Tombs and Harding 1997). Figures 4 and 5 shows such plots for the four chitosans of this study in terms of log$s^\circ_{20,w}$ versus logM_w and log[η] versus logM_w, respectively. The SEC-MALLs values for M_w have been included, and an additional data point has been included corresponding to a separate batch of G213, called G213[(b)]. A linear fit to the log$s^\circ_{20,w}$ versus logM_w data yielded an MHKS exponent $b = 0.25 \pm 0.04$, i.e. between the limits of rod (0.15) and coil (0.4–0.5). Similarly, a linear fit to the log[η] versus logM_w scaling data yielded an MHKS exponent $a = 0.96 \pm 0.10$, again between the coil (0.5–0.6) and rod (1.8) limits. However, interpretation of such data should be done with caution because of claims by others of a dependence of conformation on the degree of acetylation (see, e.g. Anthonsen et al. 1993). Despite this reservation, the values obtained appear similar to those for three chitosans of DA in the range 22–31% (Cölfen et al. 2001): $a \approx 1$ and $b \approx 0.24$. Furthermore, the data reassuringly show consistency between the sedimentation coefficient and viscosity data. More advanced analyses, such as involving normalized scaling relations (Pavlov et al. 1997, 1999), can be used to take into account the differences in DA, but these await knowledge of the mass per unit length of chitosans as a function of DA. It should also be pointed out that if the sedimentation equilibrium values for M_w are used instead, different values of a and b are returned ($\sim$1.5 and 0.5, respectively), although these values are based on only four data points.

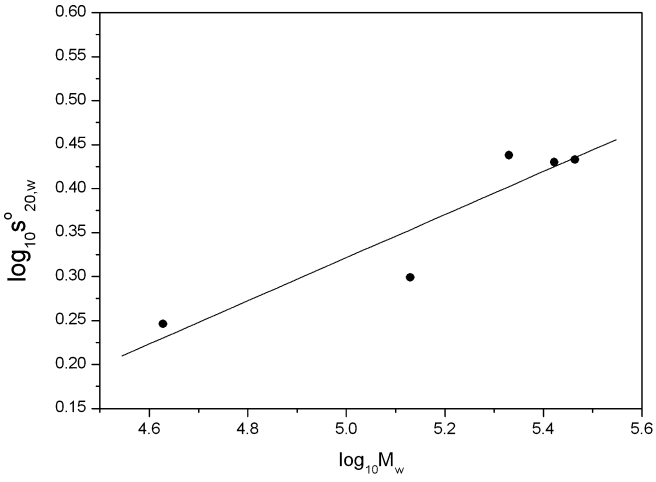

Fig. 4 Double logarithmic plot of sedimentation coefficient versus weight average molecular weight (SEC-MALLs values)

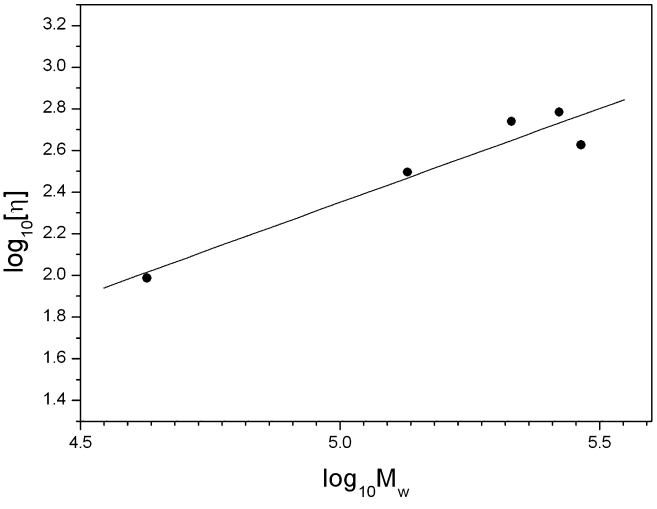

Fig. 5 Double logarithmic plot of intrinsic viscosity versus weight average molecular weight (SEC-MALLs values)

Conclusions

Confidence in our ability to measure molecular weight and conformation accurately is of particular relevance to the use of chitosans for the delivery of substances across the mucosal membrane through bioadhesion and absorption enhancement through transient widening of tight junctions (Kotzé et al. 1997). Larger molecular weights and more extended conformations would appear to offer more opportunities for interaction.

In conclusion, however, although much better agreement is now possible between SEC-MALLs and sedimentation equilibrium, some caution still needs to be exercised when interpreting molecular weight data for these materials, particularly with regards to the trace amounts of high molecular weight material appearing in the SEC-MALLs profiles. The use of two independent methods for determining the molecular weights for these substances is still highly recommended.

Acknowledgements We thank Professor Arthur Rowe for helpful comments and advice.

References

Anthonsen MW, Vårum KM, Smidsrød O (1993) Solution properties of chitosans: conformation and chain stiffness of chitosans with different degrees of N-acetylation. Carbohydr Polym 22:193–201

Anthonsen MW, Vårum KM, Hermansson AM, Smidsrød O, Brant DA (1994) Aggregates in acidic solutions of chitosans detected by static laser light scattering. Carbohydr Polym 25:13–23

Artursson P, Lindmark T, Davis SS, Illum L (1994) Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (caco-2). Pharm Res 11:1358–1361

Beri RG, Walker J, Reese ET, Rollings JE (1993) Characterization of chitosans via coupled size-exclusion chromatography and multiple-angle laser light-scattering technique. Carbohydr Res 238:11–26

Berth G, Dautzenberg H (2002) The degree of acetylation of chitosans and its effect on the chain conformation in aqueous solution. Carbohydr Polym 47:39–51

Berth G, Dautzenberg H, Peter MG (1998) Physico-chemical characterization of chitosans varying in degree of acetylation. Carbohydr Polym 36:205–216

Clewlow AC, Errington N, Rowe AJ (1997) Analysis of data captured by an on-line image capture system from an analytical ultracentrifuge using Schlieren optics. Eur J Biophys 25:311–317

Cölfen H, Harding SE (1997) MSTARA and MSTARI: interactive PC algorithms for simple, model independent evaluation of sedimentation equilibrium data. Eur J Biophys 25:333–346

Cölfen H, Berth G, Dautzenberg H (2001) Hydrodynamic studies on chitosans in aqueous solution. Carbohydr Polym 45:373–383

Davis SS (2001) Nasal vaccines. Adv Drug Delivery Rev 51:21–42

Deacon MP, Davis SS, White RJ, Nordman H, Carlstedt I, Errington N, Rowe AJ, Harding SE (1999) Are chitosan-mucin interactions specific to different regions of the stomach? Velocity ultracentrifugation offers a clue. Carbohydr Polym 38:235–238

Errington N, Harding SE, Vårum KM, Illum L (1993) Hydrodynamic characterisation of chitosans varying in degree of acetylation. Int J Biol Macromol 15:113–117

Fujita H (1962) Mathematical theory of sedimentation analysis. Academic Press, New York, p 297

Harding SE (1992) Sedimentation analysis of polysaccharides. In: Harding SE, Rowe AJ, Horton JC (eds) Analytical ultracentrifugation in biochemistry and polymer science. Royal Society of Chemistry, Cambridge, UK

Harding SE (1995a) On the hydrodynamic analysis of macromolecular conformation. Biophys Chem 55:69–93

Harding SE (1995b) Some recent developments in the size and shape analysis of industrial polysaccharides in solution using sedimentation analysis in the analytical ultracentrifuge. Carbohydr Polym 28:227–237

Harding SE (1997) The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. Prog Biophys Mol Biol 68:207–262

Harding SE, Davis SS, Deacon MP, Fiebrig I (1999) Biopolymer mucoadhesives. Biotechnol Genet Eng Rev 16:41–85

Horton JC, Harding SE, Mitchell JR (1991) Gel permeation chromatography-multi angle laser light scattering characterisation of the molecular mass distribution of ''Pro-nova'' sodium alginate. Biochem Soc Trans 19: 510–511

Illum L (1998) Chitosan and its use as a pharmaceutical excipient. Pharm Res 15:1326–1331

Illum L, Davis SS (2001) Nasal vaccination: a non-invasive vaccine delivery method that holds great promise for the future. Adv Drug Delivery Rev 51:1–3

Illum L, Farraj NF, Davis SS (1994) Chitosans as a novel nasal delivery system for peptide drugs. Pharm Res 11:1186–1189

Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS (2001) Chitosan as a novel nasal delivery system for vaccines. Adv Drug Delivery Rev 51:81–96

Jumel K, Fiebrig I, Harding SE (1996) Rapid size distribution and purity analysis of gastric mucus glycoproteins by size exclusion chromatography/multi angle laser light scattering. Int J Biol Macromol 18:133–139

Jumel K, Fogg FJJ, Hutton DA, Pearson JP, Allen A, Harding SE (1997) A polydisperse linear random coil model for the quaternary structure of pig colonic mucin. Eur Biophys J 25:477–480

Kotze AF, de Leeuw BJ, Luessen HL, de Boer AG, Verhoef JC, Junginger HE (1997) Chitosans for enhanced delivery of therapeutic peptides across intestinal epithelia: in vitro evaluation in Caco-2 cell monolayers. Int J Pharm 159:243–253

Kratky D, Leopold A, Stabinger H (1973) The determination of the partial specific volume of proteins by the mechanical oscillator technique. Methods Enzymol 27D:98–110

Natsume H, Iwata S, Ohtake K, Miyamoto M, Yamaguchi M, Hosoya K, Kobayashi D, Sugibayashi K, Morimoto Y (1999) Screening of cationic compounds as an absorption enhancer for nasal drug delivery. Int J Pharm 185:1–12

Ottøy MH, Vårum KM, Smidsrød O (1996) Compositional heterogeneity of heterogeneously deacetylated chitosans. Carbohydr Polym 29:17–24

Pavlov GM, Rowe AJ, Harding SE (1997) Conformation zoning of large molecules using the analytical ultracentrifuge. Trends Anal Chem 16:401–405

Pavlov GM, Harding SE, Rowe AJ (1999) Normalized scaling relations as a natural classification of linear macromolecules according to size. Prog Colloid Polym Sci 113:76–80

Philo JS (2000) A method for directly fitting the time derivative of sedimentation velocity data and an alternative algorithm for calculating sedimentation coefficient distribution functions. Anal Biochem 279:151–163

Rollings JE (1991) Use of online laser light-scattering photometry coupled to chromatographic separations for determination of biopolymer molecular mass, branching, size and shape distributions. Biochem Soc Trans 19:493–494

Rollings JE (1992) Use of on-line laser light scattering coupled to chromatographic separations for the determination of molecular weight, branching, size and shape distributions of polysaccharides. In: Harding SE, Sattelle DB, Bloomfield VA (eds) Laser light scattering in biochemistry. Royal Society of Chemistry, Cambridge, UK

Schachman HK (1959) Ultracentrifugation in biochemistry. Academic Press, New York

Schipper NGM, Vårum KM, Artursson P (1996) Chitosans as absorption enhancers for poorly absorbable drugs. 1. Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelium (Caco-2) cells. Pharm Res 13:1686–1692

Skaugrud Ø, Hagen A, Borgersen B, Dornish M (1999) Biomedical and pharmaceutical applications of alginate and chitosan. Biotechnol Genet Eng Rev 16:23–40

Smidsrød O, Andresen L (1979) Biopolymerkjemi. Tapir, Trondheim, Norway

Tanford C (1955) Intrinsic viscosity and kinematic viscosity. J Phys Chem 59:798–799

Tengamnuay P, Sahamethapat A, Sailasuta A, Mitra AK (2000) Chitosans as nasal absorption enhancers of peptides: comparison between free amine chitosans and soluble salts. Int J Pharm 197:53–67

Tombs MP, Harding SE (1998) An introduction to polysaccharide biotechnology. Taylor and Francis, London, p 15

Van Holde KE (1998) Principles of physical biochemistry. Prentice-Hall, Englewood Cliffs, NJ, USA

Vårum KM, Anthonsen MW, Grasdalen H, Smidsrød O (1991) High-field NMR spectroscopy of partially N-deacetylated chitins (chitosans). 1. Determination of the degree of N-acetylation and the distribution of N-acetyl groups in partially N-deacetylated chitins. Carbohydr Res 211:17–23

Wang W, Bo S, Li S, Qin W (1991) Determination of the Mark-Houwink equation for chitosan with different degrees of deacetylation. Int J Biol Macromol 13:281–285

Wyatt PJ (1992) Combined differential light scattering with various liquid chromatography separation techniques. In: Harding SE, Sattelle DB, Bloomfield VA (eds) Laser light scattering in biochemistry. Royal Society of Chemistry, Cambridge, UK, pp 35–58

Wyatt PJ (1993) Light scattering and the absolute characterization of macromolecules. Anal Chim Acta 272:1–40