Carbohydrate Polymers 74 (2008) 559–565

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chain stiffness and extension of chitosans and periodate oxidised chitosans studied by size-exclusion chromatography combined with light scattering and viscosity detectors

Bjørn E. Christensen *, Inger Mari Nygaard Vold [1], Kjell M. Vårum

NOBIPOL, Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Sem Saelands v. 6/8, NO-7491 Trondheim, Norway

ARTICLE INFO

Article history:
Received 9 November 2007
Received in revised form 3 March 2008
Accepted 7 April 2008
Available online 15 April 2008

Keywords:
Chitosan
SEC–MALS
Intrinsic viscosity
Mark–Houvink- equation
B-parameter
Periodate oxidation
Chain stiffness
Persistence length

ABSTRACT

Chitosans with different chemical compositions (F_A = 0.05–0.65) and different molecular weights (M_w = 36.000–460.000) were studied by size-exclusion chromatography combined with multi-angle laser light scattering (MALS) and viscosity detectors to provide R_G–M and $[\eta]$–M data as basis for further analysis using the wormlike chain model. In both cases intrinsic persistence lengths (q_0) in the range 5.1–7.6 nm were obtained, with little or no detectable dependence on F_A. These values are significantly lower than values obtained for alginates (12 nm), including homopolymeric mannuronan, using the same approach. This finding is also corroborated by differences in the Smidsrød *B*-parameter, confirming that chitosans are less extended than alginates, despite the similarity in basic chain geometry (cellulose type) and linear charge density. Partial periodate oxidation of chitosans led to a pronounced increase in the chain flexibility as shown by a gradual decrease in persistence length, approaching 2 nm for the most oxidised samples.

1. Introduction

Chitins are partially or fully de-*N*-acetylated chitins, usually obtained from shrimp shells. Its chemistry, properties and applications in a variety of fields have recently been reviewed (Vårum & Smidsrød, 2005). F_A denotes the fraction of remaining *N*-acetylated residues (*N*-acetyl-D-glucosamine), and is used as main parameter for describing the chemical composition.

Estimates of the chain stiffness and extension of chitosans with different chemical compositions have been reported by several investigators (Berth & Dautzenberg, 2002; Schatz, Viton, Delair, Pichot, & Domard, 2003, and references herein), primarily on the basis of analysis of the molecular weight dependence of the radius of gyration or the intrinsic viscosity. The classic approach involves conventional light scattering (obtaining M_w and $R_{G,z}$) and capillary viscometry (obtaining $[\eta]_w$), using polydisperse samples in different molecular weights ranges, typically produced by controlled degradation. In a few cases osmometry (obtaining M_n) has been employed (Anthonsen, Vårum, & Smidsrød, 1993). Early studies were restricted to a limited range of F_A, and molecular weight estimates based on light scattering suffered from the influence of aggregates. Multi-detector SEC studies of chitosans have later been introduced (Beri, Walker, Reese, & Rollings, 1993; Brugnerotto, Desbrières, Roberts, & Rinaudo, 2001; Fredheim & Christensen, 2003; Rinaudo, Milas, & Dung, 1993; Yanagisawa, Kato, Yoshida, & Isogai, 2006). It is today a widely used method for characterizing polymers in dilute solution.

Despite the large number of studies on chitosans, the detailed picture regarding chains stiffness estimates has varied. According to Berth and Dautzenberg (2002), 'there is a lot of contradiction in the literature'. Early estimates of the persistence lengths in the range 22 nm (Terbojevich, Cosani, Conio, Marsano, & Bianchi, 1991) to 30 nm (Rinaudo & Domard, 1983) were later shown to be overestimates ascribed to the influence of aggregation (Rinaudo et al., 1993). Studies based on apparently aggregate-free solutions have reported intrinsic persistence lengths in the range 5–7 nm (Berth & Dautzenberg, 2002; Rinaudo et al., 1993; Schatz et al., 2003). All values refer to pH around 4.5, where chitosans are fully charged.

The influence of *N*-acetyl groups on the chain stiffness has also been debated. In their study, Rinaudo et al. (1993) found that the intrinsic persistence length was independent of F_A for the range F_A = 0.02–0.21. Berth and Dautzenberg (2002) and Schatz et al. (2003) arrived at the same conclusion after considering F_A up to 0.60. The latter authors studied partially re-*N*-acetylated chitosans. In contrast, Brugnerotto et al. (2001) found on basis of SEC–MALS

* Corresponding author. Tel.: +47 73593327; fax: +47 73591283.
E-mail address: B.Christensen@biotech.ntnu.no (B.E. Christensen).
[1] Present address: Borregaard, P.O. Box 162, NO-1701 Sarpsborg, Norway.

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.04.012

(R_G-M data) that the persistence length of heterogeneously de-*N*-acetylated chitosan was 11 nm, independent of F_A in the range 0–0.25, whereas for homogeneously re-*N*-acetylated chitosan the persistence length increased from 11 to 15 nm when F_A increased up to 0.60. Fig. 3 in the referred article illustrates a somewhat limited quality of the R_G–M data which may be insufficient for precise estimates of the chain stiffness.

The present study provides results based on SEC with both light scattering and viscosity detectors, in addition to refractive index detector (SMV). Compared to R_G–M data, which are restricted to high molecular weights (typically >100.000 Da) due to the inherent limitation in light scattering, $[\eta]$–M data can be obtained for a wider range of molecular weights. Compared to previous studies, we report on a wider range of chemical compositions combined with a wider range of molecular weights. Samples in the M_w range 25–50.000 Da were especially prepared to allow studies of the $[\eta]$–M relationship in the wormlike chain region and for minimizing shear thinning effects in the viscosity detector. We also provide novel estimates of the refractive index increment as well as the *B*-parameter (Smidsrød & Haug, 1971) for the F_A-range 0.005–0.65. The latter allows extrapolation to infinite ionic strength to eliminate excluded volume effects caused by long range electrostatic repulsion (Vold, Kristiansen, & Christensen, 2006).

The study includes a series of partially periodate oxidised chitosans (Vold & Christensen, 2005) in order to observe changes in chain stiffness as previously observed for alginates (Vold et al., 2006). Because of the extensive depolymerisation occurring during oxidation of chitosans, R_G–M data are not obtainable except for the lowest degrees of oxidation. Thus, the analysis depends on $[\eta]$–M data.

2. Materials and methods

2.1. Materials

Chitosans with $F_A = 0.16$ and 0.52 were obtained from FMC NovaMatrix (Sandvika, Norway). Remaining samples were prepared in the laboratory using homogeneous de-*N*-acetylation. Partial depolymerisation was obtained by degradation with nitrous acid (Anthonsen et al., 1993) or by acid hydrolysis (Vårum, Ottøy, & Smidsrød, 2001). Typically, chitosan (1.5 mg/ml) with $F_A = 0.16$ and $M_w = 270.000$ Da was depolymerised in 0.8 M HCl at 60 °C for 20 h to obtain $M_w = 90.000$. A plot of M_w^{-1} versus t (not shown) yielded a straight line with slope 3.4×10^{-7} h^{-1}, corresponding to a *pseudo* first order rate constant of 2.9×10^{-4} h^{-1}.

Periodate oxidised chitosans with $F_A = 0.01$, 0.16 and 0.52 were prepared and characterised with respect to the degree of oxidation (F_{ox}) as described previously (Vold & Christensen, 2005).

2.2. Analyses

Refractive index increments were determined using an Optilab DSP differential refractometer (Wyatt, U.S.A.) operating at $\lambda_0 = 633$ nm as described previously (Vold et al., 2006).

Size-exclusion chromatography (SEC) with online multi-angle laser light scattering (MALS) and viscometry (VISC) measurements were performed as described previously (Vold et al., 2006). The eluent was 0.2 M ammonium acetate adjusted to pH 4.5. This gives stable measurements in SEC–MALS. 0.2 M Na-acetate (pH 4.5) was tried in a few cases, but did not produce better results (some low M_w samples tended to aggregate slightly). Since $A_2c << M^{-1}$ all calculations are basically unaffected by the choice of A_2 provided A_2 does not largely exceed typical values found in the literature (around 2–6 10^{-3}). This was conformed by systematically varying A_2 in the data processing.

The *B*-parameter was determined by conventional capillary viscometry as described earlier (Anthonsen et al., 1993).

3. Results

3.1. Refractive index increment

Four high molecular weight chitosans with $F_A = 0.01$, 0.16, 0.33 and 60, respectively, were analysed by refractometry using the Optilab DSP RI detector (offline mode). To obtain $(dn/dc)_\mu$ samples were initially dialysed extensively against the solvent. Because the refractive index of the solution increased strictly linearly with the sample concentration subsequent measurements were based on a single concentration (0.50 mg/ml, $n = 3$). With 0.2 M ammonium acetate (pH 4.5) as solvent RI measurements were not perfectly stable, and some scatter in the data was observed. This is attributed to the volatility of ammonium acetate, which may lead to small and variable – but easily detectable – differences in the refractive index of the solvent itself. With 0.2 M Na-acetate (adjusted to pH 4.5) (non-volatile) more stable measurements were obtained. Results are given in Fig. 1. When the concentration of chitosan was expressed in terms of the free amine form, $(dn/dc)_\mu$ increased with decreasing F_A. However, when expressed as chitosan acetate, $(dn/dc)_\mu$ was essentially independent of F_A, equalling 0.142. This simplifies data evaluation based on SEC–MALS, and we have chosen to express all calculated molecular weights as the corresponding acetate form.

3.2. B-parameter

The *B*-parameter for chitosans ranging in F_A from <0.002 to 0.66 was determined at pH 4.5, varying the ionic strength with NaCl. Results are given in Fig. 2. The *B*-parameter decreases marginally from $F_A < 0.002$ to $F_A = 0.4$, whereas a steeper decrease is observed for higher values of F_A.

3.3. SMV analysis acid degraded chitosans

Chitosans with $F_A = 0.005$, 0.16, 0.44 and 0.65 were degraded in acid to obtain M_w ranging from about 450.000 to about 20.000 Da. Each sample was analysed by SMV using varying sample concentrations and columns, each optimised for the molecular weight range studied. Fig. 3a shows data obtained for two chitosans with $F_A = 0.05$ and $M_w = 240.000$ Da and 38.000 Da, respectively, using two columns (TSK G6000PWXL and 4000PWXL, serially connected. Fig. 3b shows results for the 38.000 Da sample using a single col-

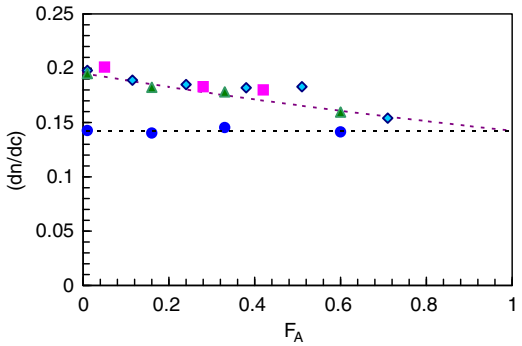

Fig. 1. Refractive index increments $(dn/dc)_\mu$ for chitosans, with different degrees of acetylation (F_A) dissolved in 0.2 M Na-acetate, pH 4.5. (●) Present study, concentration of chitosan expressed as acetate, (▲) present study, concentration of chitosan expressed as free amine, (◇) Data from Schatz et al. (2003) (free amine), (■) Data from Terbojevich et al. (1992) (free amine).

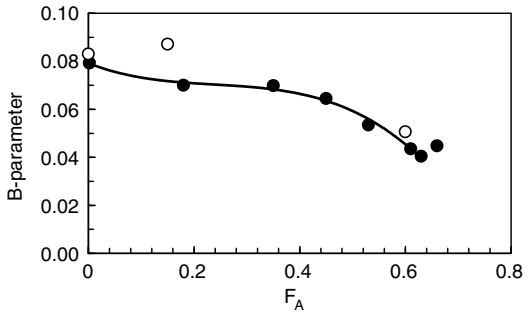

Fig. 2. *B*-parameter of chitosans with different degrees of acetylation (F_A). (●) Present study. (○) Recalculated (see text) data reported by Anthonsen et al. (1993).

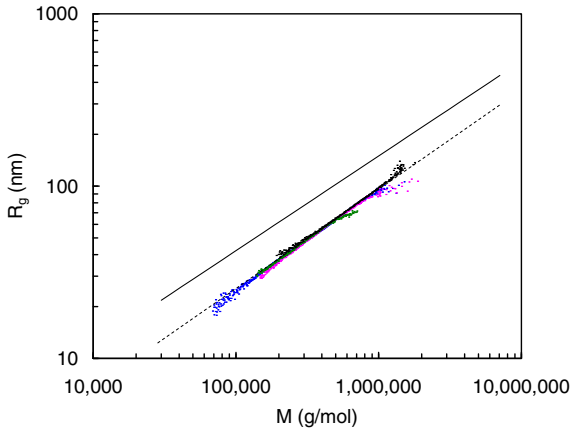

Fig. 4. Molecular weight dependence of the radius of gyration (R_G) for chitosans with F_A = 0.05 (blue), F_A = 0.16 (pink), F_A = 0.44 (green) and F_A = 0.64 (black), obtained by SMV. Dotted line: All data fitted to Eq. (1). Solid line: Recalculated literature data (Berth & Dautzenberg, 2002). All molecular weights refer to the acetate form of chitosans. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

umn (TSK G4000PWXL). Signal enhancement (MALS) of the low M_w sample was obtained by injecting a larger amount of sample, but without exceeding the concentration where peak distortion occurred (Vold et al., 2006). Data for the other chitosans were qualitatively similar and are therefore not shown.Data for R_G were generally restricted to high molecular weight (undegraded) samples due to the inherent limitation in light scattering (R_G > ca. λ/20). Result obtained for chitosans with F_A = 0.05, 0.16, 0.44 and 0.65 are shown in Fig. 4. Data cover the M range 100.000–1.000.000 Da, and R_G range 20–120 nm. All chitosans seem, within experimental error, to fall on the same line. Regression analysis yielded (as average values):

$$R_G = 2.99 \cdot 10^{-2} M^{0.59} \quad (1)$$

Results obtained for F_A = 0.05, 0.16, 0.44 and 0.65 are given in Fig. 5. Using samples in both a high M_w range (250–450.000 Da) and low M_w range (30–40.000 Da) for each F_A the data span almost two orders of magnitude of molecular weights. As for R_G the data are essentially independent of F_A. However, a pronounced curvature is observed in the data. For practical reasons the data were fitted to two different exponential equations, similar to what has been reported earlier for hyaluronan (Mendichi, Šoltés, & Schieroni, 2003) and alginates (Vold et al., 2006):

$$[\eta] = 4.50 \cdot 10^{-4} M^{1.15} \quad (M < 90.000) \quad (2a)$$

$$[\eta] = 2.30 \cdot 10^{-2} M^{0.80} \quad (M > 90.000) \quad (2b)$$

3.4. SMV analysis of periodate oxidised chitosans (POC)

Results obtained for three chitosans (F_A = 0.01, 0.16 and 0.52) and different degrees of oxidation are summarised in Table 1. The extensive depolymerisation observed previously (Vold et al., 2006) is confirmed (see Table 2).

The effective degree of oxidation, defined as the experimentally determined fraction of oxidised GlcN residues (F_{ox} = [$GlcN_{ox}$]/[$GlcN]_0$), is much lower than the theoretical value (stoichiometric amount of added periodate: P_0 = [IO_4^-]/[$GlcN]_0$), primarily because of depolymerisation and overconsumption of periodate at chain termini (Vold et al., 2006). The molecular weight dependence of the intrinsic viscosity of POC is shown in Fig. 6. As for alginates (Vold et al., 2006), chitosans show a pronounced decrease in [η] (at constant M) with increasing degree of oxidation, indicating a progressive increase in chain flexibility. Because of the chitosan-specific degradation occurring during the oxidation (Vold et al., 2006) the most oxidised chitosans (F_{ox} > 0.1) has M_w in the range of 4.000–20.000. The corresponding R_G values are far too low for detection with light scattering.

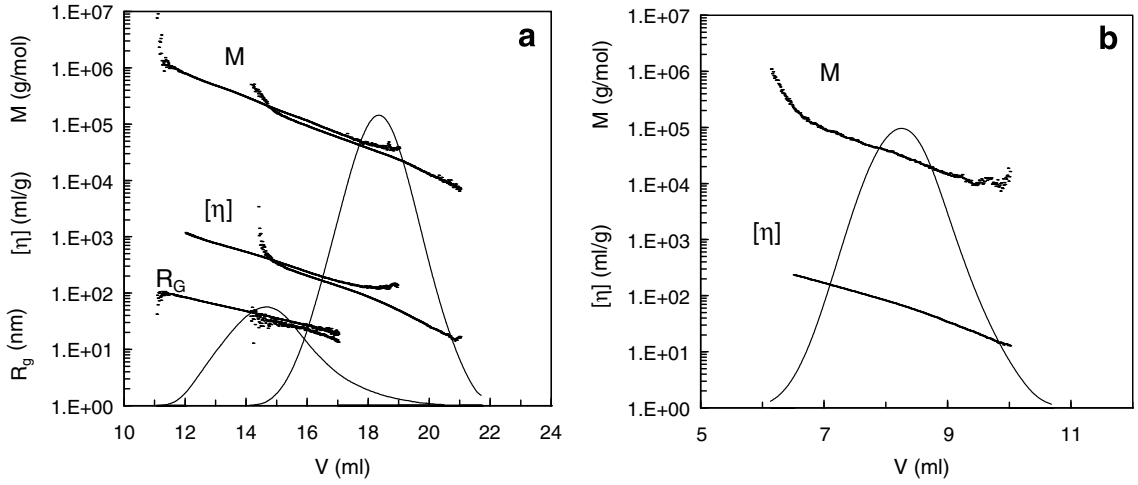

Fig. 3. SMV (SEC–MALS–VISC) analysis of a high and low M_w chitosan using 3 serially connected columns (TSK G6000 + 5000 + 4000 PWXL) (a) and a low M_w chitosan using a single column (TSK G4000 PWXL) (b).

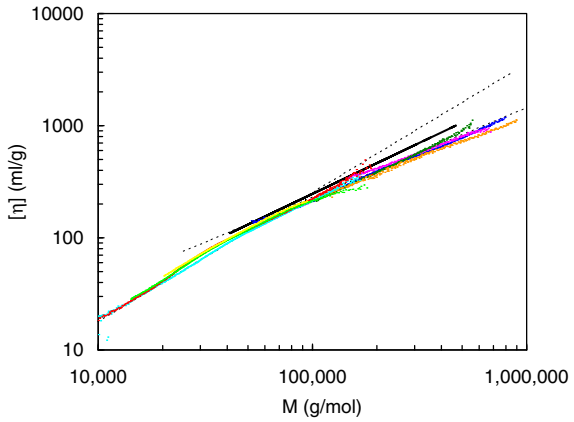

Fig. 5. Molecular weight dependence of the intrinsic viscosity [η] for chitosans with F_A = 0.05 (blue, light blue), F_A = 0.16 (pink, red) F_A = 0.44 (green, light green) and F_A = 0.64 (yellow, dark yellow), obtained with SMV. Dotted lines: Data fitted according to Eqs. (2a) and (2b). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Molecular characteristics of periodate oxidised chitosans

F_A	P_0	F_{ox}	D_{ox}	M_w	$[\eta]_w$	q (nm)
0.01	0	0	0	190,000	840	8.2
	0.05	0.02	0.02	33,000	108	6.1
	0.10	0.04	0.04	21,000	58	4.8
	0.30	0.09	0.09	8000	18	3.4
	0.60	0.16	0.16	7000	9	2.4
0.16	0	0	0	360,000	779	7.3
	0.05	0.03	0.03	50,000	156	6.3
	0.10	0.06	0.05	31,000	84	5.2
	0.30	0.13	0.11	12,000	31	4.4
0.52	0	0	0	330,000	746	7.5
	0.05	0.04	0.02	99,000	259	6.3
	0.10	0.07	0.03	79,000	166	5.1
	0.30	0.14	0.07	33,000	63	4.2
	1.00	0.40	0.19	9000	17	2.2

Abbreviations: P_0, moles IO_4^- added per mole GlcN (theoretical degree of oxidation), F_{ox}, Fraction of oxidised GlcN residues determined experimentally, D_{ox}, Fraction of oxidised residues (=$F_{ox}(1 - F_A)$), M_w, weight average molecular weight, $[\eta]_w$, weight average intrinsic viscosity; q, persistence length.

Table 2
Estimated persistence lengths (nm) of chitosans based on the wormlike chain model for R_G–M and [η]–M

F_A	M_L (acetate)	B-parameter	R_G–M data	[η]–M data	
			q_0	q (I = 0.2)	q_0 ($I \to \infty$)
0.05	427	0.0788	4.0	8.1	5.1
0.16	424	0.0715	4.2	7.9	5.4
0.44	414	0.0643	5.1	7.5	5.9
0.65	406	0.0351	7.0	6.6	5.8

4. Discussion

The square of the refractive index increment $(dn/dc)^2_\mu$ enters calculations of molecular weight based on light scattering data, and accurate estimates are therefore crucial. The literature provides some inconsistencies in the dependence of F_A on $(dn/dc)_\mu$. Our results (Fig. 1) are quantitatively in reasonably good agreement with those obtained by both Terbojevich, Cosani, Focher, Naggi, and Torri (1992), and Schatz et al. (2003). In contrast, Beri et al. (1993) report a value of 0.181, independent of F_A. We note that when the concentration of chitosan includes the acetate counterion, $(dn/dc)_\mu$ becomes independent of F_A, equalling 0.142. We adopt this practice because it which simplifies calculations based on SEC–MALS. Thus, all calculated molecular weights refer to the acetate salt form.

The B-parameters given in Fig. 2 are in fair agreement with those reported by Anthonsen et al. (1993). A re-examination of the literature data shows that the samples with the lowest molecular weights (M_n < 40.000) tend to deviate from trends from higher molecular weights, besides having the largest uncertainty. By omitting such samples from the analysis, the B-parameter are in better agreement with data in Fig. 2, with re-calculated values 0.083, 0.087 and 0.051 for F_A equalling 0, 0.15 and 0.60, respectively.

The R_G–M relation determined above confirms the well established fact that chitosans behave as random coils in a good solvent. The data conform reasonably well with the literature data compiled by Berth and Dautzenberg (2002) (included in Fig. 4). The vertical shift in the R_G–M line (factor of 1.5–2) is primarily ascribed to the fact that the literature data refer to polydisperse samples, where $R_{G,z}$ and M_w are determined, whereas slice results from SEC–MALS are considered being monodisperse. Moreover, the literature data show some scatter, which influences the two-parameter fit used to determine both the pre-exponential factor and the exponent. Most importantly, we are unable to detect significant differences in the R_G–M data for chitosans with different F_A.

Since SEC elution slices can be considered as monodisperse, at least as a first approximation, further analysis of R_G–M data does not require further correction for polydispersity. Data can be directly entered into the wormlike chain model as described previously by (among others) Berth, Dautzenberg, and Peter (1998), which incorporates a procedure to obtain estimates of the intrinsic persistence length (q_0) corresponding to θ-conditions. The main corrections stems from the electrostatic contribution to chain expansion, which depends strongly on the ionic strength and the charge density. The mass per unit length (M_L) was calculated from the monomer weight according to Eq. (3):

$$M_L = \frac{F_A M_{GlcNAc} + (1 - F_A) M_{GlcN}}{b} \quad (3)$$

where M_{GlcNAc} and M_{GlcN} are the monomer weights of intrachain N-acetyl glucosamine (203 g/mol) and glucosamine (acetate salt, 221 g/mol) residues, respectively, and b is the average bond length (0.515 nm). Using the fitted line in Fig. 5 as basis for further analysis, restricted to experimental R_G values between 20 and 120 nm, we obtain intrinsic persistence lengths (q_0) between 7.0 nm (F_A = 0.65) and 4.0 nm (F_A = 0.05) (Table 1). The dependence on F_A simply reflects the corresponding variation in charge density, which is a major parameter in calculating the electrostatic contribution to chain expansion. In comparison, Berth et al. (1998) obtained q_0 of 6.5 nm, 12.5 nm and 6.0 nm for chitosans with F_A = 0.25, 0.225 and 0.07, respectively. Except for the value 12.5 nm our data agree reasonably well with these values, as well as a value of 5.0 nm obtained by Rinaudo et al. (1993). Values in the range 22 nm (Terbojevich et al., 1991) and 30 nm (Rinaudo & Domard, 1983) have been ascribed to the influence of aggregation (Rinaudo et al., 1993).

Our data may suggest a stiffening of chitosan with increasing F_A, provided all chitosans have exactly the same R_G–M relationship at I = 0.2 M. However, the precision in the fitted data is not sufficiently good to unequivocally support this conclusion. A minor change in the fitting parameters (typically 5%) in the R_G–M equation above affects the calculated q_0 by as much as 14–20%. Thus, additional data and analyses are required. In our case this is possible because of independent measurements of the intrinsic viscosity.

Intrinsic viscosity data obtained by SMV were compared to data obtained independently using SEC–LALLS (M_w) and conventional

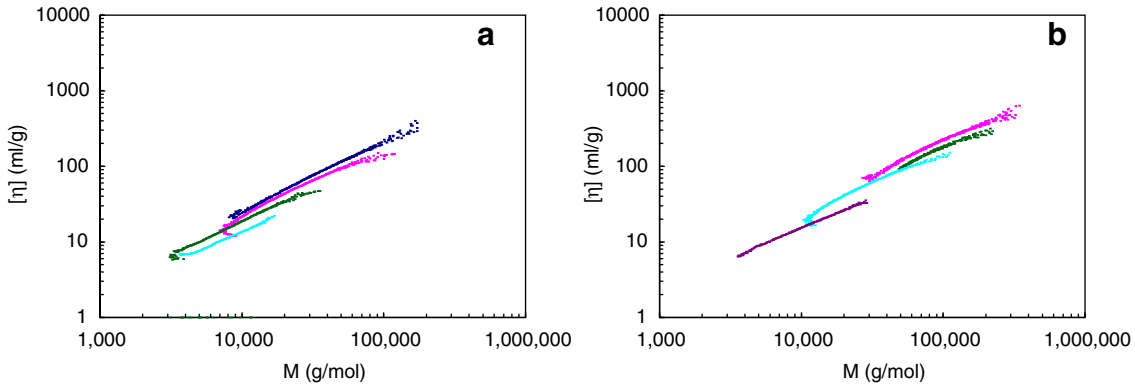

Fig. 6. Molecular weight dependence of the intrinsic viscosity [η] for periodate oxidised chitosans with F_A = 0.05 (Top–down: F_{ox} = 5%, 10%, 30% and 60%) (a), and F_A = 0.52 (Top–down: F_{ox} = 5%, 10%, 30% and 100%) (b), obtained with SMV.

capillary viscometry (Fig. 5). In this case we could use data published earlier (Ottøy, Vårum, Christensen, Anthonsen, and Smidsrød (1996)), and which have been shown to be in good agreement with data reported by other groups (Fig. 10 of Berth & Dautzenberg, 2002). For our purpose the reported M_w and [η] values were re-calculated from the free amine form to the acetate form, which leads to a considerable shift of the curve. SMV and offline data almost coincide in the M_w range 50–100.000 Da. At higher molecular weights the data appear to diverge as SMV data show a smaller slope. This may possibly be an effect caused by shear thinning due to the high shear rate (about 3000 s^{-1}) in the online viscometer, but due to the scatter in the literature data this cannot be assessed conclusively. A qualitatively similar, but far less pronounced effect was observed for alginates (Vold et al., 2006). The [η]–M curve becomes steeper at low molecular weights. The possibility for independent verification on the basis of the literature data is limited as few data in this M_w range have been published. Nevertheless, this trend is indeed expected as randomly coiled chitosans approach the stiff coil region and, eventually the stiff rod limit, with decreasing molecular weight.

The SMV-based [η]–M relationships shown in Fig. 5 are within experimental uncertainty the same for all chitosans in the F_A-range 0.05–0.65. Since the same was observed for the R_G–M data, we conclude that the chain stiffness and extension of chitosans is basically independent of the chemical composition under the conditions used here (pH 4.5, I = 0.2 M). For estimates of the persistence length (q) based on [η]–M we apply the so-called Bohdanecký approach (Bohdanecký, 1983), which is a simplified and more user-friendly version of the wormlike chain model (Patel, Picout, Ross-Murphy, & Harding, 2006). We follow the procedure described previously for alginates (Vold et al., 2006 and references herein) by plotting $(M^2/[\eta])^{1/3}$ as a function of $M^{1/2}$ (Fig. 7). Despite some scatter in the data, which is mainly attributed to weak chromatographic detector signals in the beginning and in the end of the peaks, data covering the M range 10.000 Da to about 600.000 Da fall on a single, straight line. In contrast, the literature data (adapted from Fig. 5) results in some non-linearity. From estimates of the slopes and intercepts of the fitted lines, combined with M_L values calculated according Eq. (3), we obtain persistence lengths ranging from 8.1 nm (F_A = 0.005) to 6.6 nm (F_A = 0.65). Fitting all data to a single line (dotted line in Fig. 7) yielded an average value of 7.4 nm, using an average M_L of 418 nm^{-1}. Contrary to the direct estimate of q_0 based on R_G–M analysis, the estimate obtained from intrinsic viscosity measurements include an electrostatic contribution, which varies with F_A. To obtain estimates of q_0 we used the method introduced by Vold et al. (2006). In brief, intrinsic viscosities corresponding to infinite ionic strength ($[\eta]_\infty$) were calculated from values obtained at I = 0.2 M (experimental data) by means of

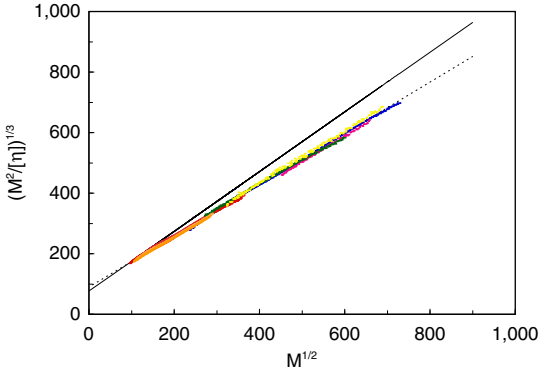

Fig. 7. Bohdanecký plots for chitosans with F_A = 0.05 (blue, light blue), F_A = 0.16 (pink, red) F_A = 0.44 (green, light green) and F_A = 0.64 (yellow, dark yellow). Dotted line: Average fit. Solid line: Average fit using $[\eta]_\infty$ instead of $[\eta]_I$ (see text). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

the Smidsrød B-parameter for chitosans (Fig. 2) using Eq. (4) (Vold et al. 2006):

$$[\eta]_I = [\eta]_\infty + B[\eta]_{0.1}^{1.3}(I^{-1/2}) \quad (4)$$

Results so obtained (q_0) range from 5.1 nm (F_A = 0.05) to 5.8 nm (F_A = 0.65) (Table 1). Reversal of the order compared to values obtained at I = 0.2 M is attributed by the pronounced influence of F_A on the B-parameter, reflecting the large variation in linear charge density. Compared to the q_0 values obtained from R_G–M analysis, the corresponding estimates obtained from viscosity data are in the same range, but with less dependence on F_A. The latter is probably more correct because [η]–M data cover a larger molecular weight interval than for R_G–M (and with generally less noise). Fitting all data to a single line (solid line in Fig. 7) yielded an average value of 5.4 nm, based on an average M_L of 418 nm^{-1} (from Eq. (3)).

An alternative method for obtaining M_L is to use the partial specific volume (Bohdanecký, 1983). Using an average value of 0.55 ml/g (Cölfen, Berth, & Dautzenberg, 2001) and the solid line in Fig. 7 we obtain M_L = 480 nm^{-1} and q_0 = 6.3 nm. The value 480 nm^{-1} is higher than the value based on physical dimensions of the monosaccharides (418 nm^{-1}). A quite similar result has also observed for hyaluronan (Mendichi et al., 2003). The discrepancy remains to be explained, but emphasizes the fact that comparison of stiffness estimates between different polymers should be based on exactly the same methodologies.

Despite the variation in intrinsic persistence length estimates obtained here, data are well within the range of other investigators as discussed above. Compared to other polysaccharides, however,

some interesting differences are noted. Using the same methodologies and almost the same experimental conditions (buffer and pH was different due to restricted solubility of chitosans near pH 6) Vold et al. (2006) studied a range of alginates, including homopolymeric mannuronan. In this case the persistence length was much higher (15 nm at $I = 0.17$ M and 12 nm extrapolated to infinite ionic strength), and independent of the chemical composition. Interestingly, the semi-empirical B-parameter, which in itself is an independent stiffness parameter, is also markedly different for mannuronan and fully de-*N*-acetylated chitosan. Dentini, Rinaldi, Risica, Barbetta, and Skjåk-Bræk (2005) report $B = 0.033$ for mannuronan, whereas chitosan (Fig. 2) has $B = 0.079$ ($F_A \rightarrow 0$). The B-parameter is calculated from the ionic strength dependence of the intrinsic viscosity, and shows empirical correlation to the unperturbed dimensions of a series of polyelectrolytes with different charge densities (Smidsrød & Haug, 1971), at least in the range found for chitosans and alginates. Given that chitosans and mannuronan have nearly the same polymer backbone (cellulose type), as well as identical charge densities (for low F_A) such differences are rather unexpected and cannot readily be explained. In terms of polyelectrolyte theories, the major parameter governing internal charge repulsion is the linear charge density, which is the same in the two extreme cases. Alginates differ from chitosans by having charges (carboxylic group) at C6, whereas in chitosans the charges are found at C2. As pointed out by Vårum and Smidsrød (2005), the empirical data which form the basis for correlation of the B-parameter with unperturbed dimensions does not include polysaccharides with charge at C2. To clarify the picture future work should therefore focus on a broader, comparative study of polysaccharides, which can be analysed under exactly the same experimental methods, and analysed by the same models.

Oxidation of chitosans with periodate clearly reduces the chain flexibility as observed in Fig. 6. $[\eta]$–M data could further be analysed according to the wormlike chain model. The corresponding Bohdanecký plot is shown in Fig. 8, and the calculated persistence lengths (obtained at $I = 0.2$ M) are plotted in Fig. 9. The B-parameter has not been determined for POCs, and intrinsic persistence lengths (q_0) are currently not obtainable. For comparison between different POCs at different F_A values the persistence lengths are plotted as a function of the fraction of oxidised residues ($D_{ox} = [GlcN_{ox}]/([GlcN]_0 + [GlcNAc]_0) = F_{ox}(1 - F_A)$) rather that F_{ox}, which is the fraction of oxidised GlcN residues (Vold et al., 2006). According to Fig. 9, the persistence length drops quickly and reaches about 2 nm at $D_{ox} \rightarrow 0.3$. This means that periodate oxidised chitosans become almost as flexible as pullulan (Buliga & Brant, 1987). The figure includes a comparison to corresponding data reported for periodate oxidised alginates (Vold et al., 2006), obtained at approximately the same ionic strength ($I = 0.17$ M). The general trend is that persistence lengths for oxidised alginates are 2–3 times higher than for chitosans with the same degree of oxidation. Data may possibly converge at extremely high degrees of oxidation. However, this range cannot be studied by the current method because of the extensive depolymerisation occurring during oxidation, as well as problems in determining the correct D_{ox}. Results for $F_A = 0.01$ and $F_A = 0.52$ overlap completely except at $D_{ox} = 0$, whereas data for chitosans with $F_A = 0.16$ lie generally above those of $F_A = 0.01$ and 0.52. These observations appear somewhat non-intuitive. The overall chain expansion is assumed to is governed primarily by two factors, namely the introduction of flexible linkages (expressed through D_{ox}) and the electrostatic expansion since data refer to $I = 0.2$ M. The former should to a first approximation be independent of F_A, whereas the latter factor should depend on F_A since the linear charge density is proportional to $(1 - F_A - D_{ox})$ (neglecting possible contributions from counterion condensation at low F_A). Hence, the persistence length at a given D_{ox} should increase in the order q ($F_A = 0.01$) > q ($F_A = 0.16$) > q ($F_A = 0.52$), which

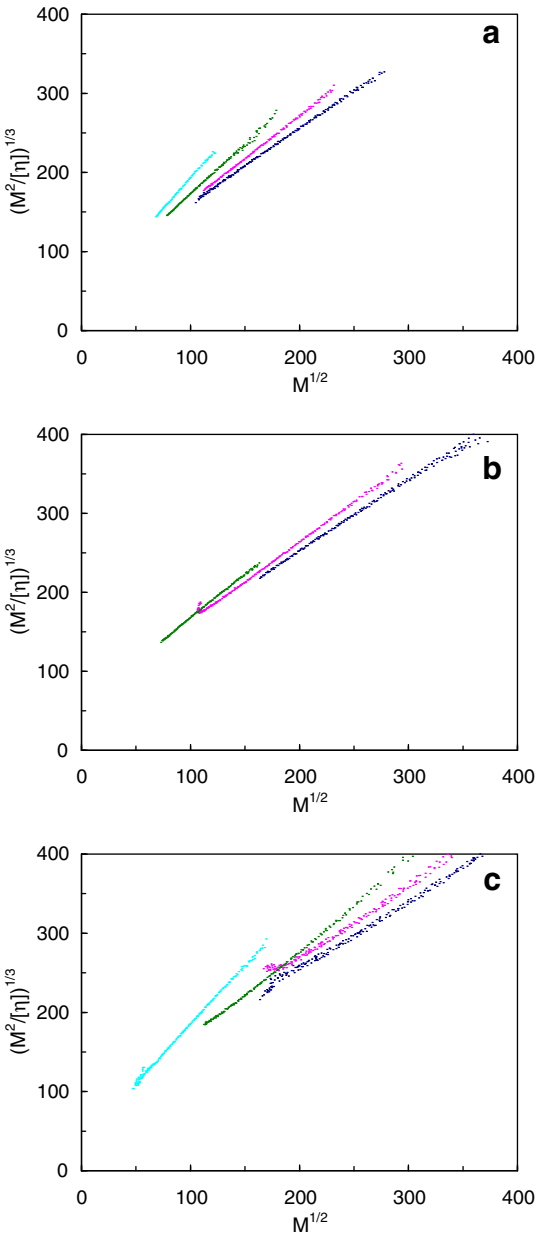

Fig. 8. Bohdanecký plots for periodate oxidised chitosans with $F_A = 0.05$ (Right–left: $P_0 = 5\%$, 10%, 30% and 60%) (a), $F_A = 0.16$ (Right–left: $P_0 = 5\%$, 10% and 30%) (b), $F_A = 0.52$ (Right–left: $P_0 = 5\%$, 10%, 30% and 100%).

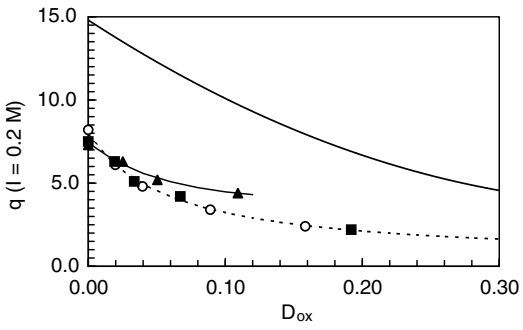

Fig. 9. Dependence of the degree of oxidation (D_{ox}) on the persistence lengths (obtained at $I = 0.2$ M) for periodate oxidised chitosans with $F_A = 0.05$ (○), $F_A = 0.16$ (▲) (b) and $F_A = 0.52$ (■). Solid line: Corresponding data for periodate oxidised alginate (adapted from Vold et al., 2006).

is not in accordance with Fig. 9. The reasons remain currently unclear, but a few possible factors may be pointed out. First, the exact determination of F_{ox} is uncertain as it depends on the method used (Vold et al., 2006). Secondly, any form of aggregation could in principle influence the $[\eta]$–M data and hence, the estimates of persistence lengths. As mentioned above, R_G–M data, which could provide additional information, were unavailable because of the extensive depolymerisation occurring during oxidation.

5. Conclusions

SMV analysis of chitosans with F_A between 0.05 and 0.65 and covering a broad molecular weight wide range demonstrates that chitosans are considerably more flexible than alginates. The influence of F_A is marginal and mainly of academic interest. Estimates of the intrinsic persistence (5.1–7.6 nm) are in good agreement with recent literature data obtained by other approaches. Periodate oxidation leads to both depolymerisation as well as a significant drop in persistence lengths, approaching 2 nm (at $I = 0.2$ M) for D_{ox} of 0.3.

Acknowledgements

This work was financially supported by the Research Council of Norway (Contracts 134674/I10 and 145945/I10). Financial contribution from the CARAPAX project (5th European Framework Program 'Quality of Life and Management of Living Resources') is acknowledged. Ann-Sissel Ulset and the late Mildrid H. Myhr are thanked for technical assistance. Professor Kjetill Østgaard and Professor emeritus Olav Smidsrød are thanked for stimulating and fruitful discussions.

References

Anthonsen, M. W., Vårum, K. M., & Smidsrød, O. (1993). Solution properties of chitosans: Conformation and chain stiffness of chitosans with different degrees of *N*-acetylation. *Carbohydrate Polymers, 22*, 193–201.

Beri, R. G., Walker, J., Reese, E. T., & Rollings, J. E. (1993). Characterization of chitosans via coupled size-exclusion chromatography and multiple-angle laser light-scattering technique. *Carbohydrate Research, 238*, 11–26.

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

Berth, G., Dautzenberg, H., & Peter, M. (1998). Physico-chemical characterization of chitosans varying in degree of acetylation. *Carbohydrate Polymers, 36*, 205–216.

Bohdanecký, M. (1983). New method for estimating the parameters of the wormlike chain model from the intrinsic viscosity of stiff-chain polymers. *Macromolecules, 16*, 1483–1492.

Brugnerotto, J., Desbrières, J., Roberts, G., & Rinaudo, M. (2001). Characterization of chitosan by steric exclusion chromatography. *Polymer, 42*, 9921–9927.

Buliga, G. S., & Brant, D. A. (1987). Temperature and molecular weight dependence of the unperturbed dimensions of aqueous pullulan. *International Journal of Biological Macromolecules, 9*, 71–76.

Cölfen, H., Berth, G., & Dautzenberg, H. (2001). Hydrodynamic studies on chitosans in aqueous solution. *Carbohydrate Polymers, 45*, 373–383.

Dentini, M., Rinaldi, G., Risica, D., Barbetta, A., & Skjåk-Bræk, G. (2005). *Carbohydrate Polymers, 59*, 489.

Fredheim, G., & Christensen, B. E. (2003). Polyelectrolyte complexes: Interactions between lignosulfonate and chitosan. *Biomacromolecules, 4*, 232–239.

Mendichi, R., Šoltés, L., & Schieroni, A. G. (2003). Evaluation of radius of gyration and intrinsic viscosity molar mass dependence and stiffness of hyaluronan. *Biomacromolecules, 4*, 1805–1810.

Ottøy, M. H., Vårum, K. M., Christensen, B. E., Anthonsen, M. W., & Smidsrød, O. (1996). Preparative and analytical size exclusion chromatography of chitosans. *Carbohydrate Polymers, 31*, 253–261.

Patel, T. R., Picout, D. R., Ross-Murphy, S. B., & Harding, S. E. (2006). Pressure cell assisted solution characterization of galactomannans. 3. Application of analytical ultracentrifugation techniques. *Biomacromolecules, 7*, 3513–3520.

Rinaudo, M., & Domard, A. (1983). Preparation and characterization of fully deacetylated chitosan. *International Journal of Biological Macromolecules, 5*, 49–52.

Rinaudo, M., Milas, M., & Dung, P. L. (1993). Characterization of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. *International Journal of Biological Macromolecules, 15*, 281–285.

Schatz, C., Viton, C., Delair, T., Pichot, C., & Domard, A. (2003). Typical physicochemical behaviors of chitosan in aqueous solution. *Biomacromolecules, 4*, 641–648.

Smidsrød, O., & Haug, A. (1971). Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity of different ionic strengths. *Biopolymers, 10*, 1213–1227.

Terbojevich, M., Cosani, A., Conio, G., Marsano, E., & Bianchi, E. (1991). Chitosan: Chain rigidity and mesophase formation. *Carbohydrate Research, 209*, 251–260.

Terbojevich, M., Cosani, A., Focher, B., Naggi, A., & Torri, G. (1992). Chitosans from Euphausia superba. 1: Solution properties. *Carbohydrate Polymers, 18*, 35–42.

Vårum, K. M., Ottøy, M. H., & Smidsrød, O. (2001). Acid hydrolysis of chitosans. *Carbohydrate Polymers, 46*, 65–70.

Vårum, K. M., & Smidsrød, O. (2005). Structure–property relationship in chitosans. In S. Dumitriu (Ed.), *Polysaccharides. Structural diversity and functional versatility* (pp. 625–642). Marcel Dekker.

Vold, I. M. N., & Christensen, B. E. (2005). Periodate oxidation of chitosans with different chemical compositions. *Carbohydrate Research, 340*, 679–684.

Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2006). A study of the chain stiffness and extension of alginates, *in vitro* epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules, 7*, 2136–2146 [Corrections: Biomacromolecules (2007), 8, 2627].

Yanagisawa, M., Kato, Y., Yoshida, Y., & Isogai, A. (2006). SEC–MALS study on aggregates of chitosan molecules in aqueous solvents: Influence of residual *N*-acetyl groups. *Carbohydrate Polymers, 66*, 192–198.