*Carbohydrate Polymers* **22** (1993) 193–201

# Solution properties of chitosans: conformation and chain stiffness of chitosans with different degrees of *N*-acetylation

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(Received 23 February 1993; revised version received 4 May; accepted 6 May 1993)

Chitosans with degrees of *N*-acetylation, $F_A$, ranging from 0 to 0·6 were randomly degraded to different molecular weights and characterized by measuring intrinsic viscosities and osmotic pressure. In order to determine the stiffness parameter, $\underline{B}$, and the Mark–Houwink–Kuhn–Sakurada (MHKS) equations at different ionic strengths, intrinsic viscosities were measured at various ionic strengths. The Kuhn lengths at $\theta$-conditions, $A_{m\theta}$, were estimated from an interpolation utilizing the obtained stiffness parameters and a published relation between $\underline{B}$ and $A_{m\theta}$ for different polyelectrolytes. These values were then used to compute the characteristic ratios, $C_\infty$, which are defined at $\theta$-conditions. From the Flory–Fox viscosity relationship and a theory of Bloomfield and Zimm, the radii of gyration, $R_G$, and also the Kuhn lengths at different ionic strengths, $A_{mI}$, were calculated. In order to investigate the influence of the *N*-acetyl groups in contrast to the polyelectrolyte effect of the $NH_3^+$- group on the overall conformation, the intrinsic viscosity data were extrapolated to infinite ionic strength, and the resulting MHKS equations, the $R_{G\infty}$, and the $A_{m\infty}$ were estimated.

## INTRODUCTION

Chitosan is a linear polysaccharide that is composed of $\beta$-(1->4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc). Chitosan is produced in nature by microorganisms (e.g. fungi), or it is made by alkaline *N*-deacetylation of chitin, which is a structural polysaccharide in the outer shell of many crustaceans and insects. Chitin consists of the same sugar monomers, GlcNAc and GlcN, but in contrast to chitosan, it contains mainly GlcNAc. The amino group in chitosan has a $pK_a$-value of about 6·2–7 (Park & Choi, 1983; Rinaudo & Domard, 1989), which makes chitosan a polyelectrolyte at low pH-values. The solution properties of chitosan should mainly be governed by the degree of *N*-acetylation, $F_A$, the distribution of the acetyl groups, the pH and the ionic strength.

Due to the great potential for use of chitosan in several applications, particularly biomedical, it is of importance to obtain knowledge about how the chemical structure of chitosans is directly related to their conformational behaviour in solution, and to their physical and biological properties. This knowledge is needed in order to be able to choose the best chitosan for a particular application.

Hydrodynamic properties, such as the intrinsic viscosity and frictional coefficient, are closely related to polymer-chain conformations. The dependence of the intrinsic viscosity upon the molecular weight gives information about the conformation and the extension of the polymer. The Mark–Houwink–Kuhn–Sakurada (MHKS) equation expressing this fact is given by:

$$[\eta] = KM_V^{\underline{a}} \tag{1}$$

where $K$ and $\underline{a}$ are empirical constants that are valid for a specific polymer–solvent pair within one to two orders of magnitude of molecular weights (Bohdaneck'y & Kovár, 1982); and $M_V$ is the so-called viscosity average of the molecular weight. The exponent, $\underline{a}$, depends upon the conformation of the polymer.

It should be evident that the so-called salt tolerance is also a property that is related to the stiffness of polymer chains. The more flexible the chain, the higher the response, as observed from intrinsic viscosities, to changing ionic strengths. Based on this fact, Smidsrød

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and Haug (1971) suggested an empirical parameter, B̲, that provides a relative measure of chain stiffness of polyelectrolytes. A necessary and useful feature of B̲ is that it is independent of molecular weight, so that the comparison with polyelectrolytes of different chemical structure is possible. Also, B̲ is estimated without molecular weight determinations. Smidsrød and Haug concluded that within a relatively broad range of stochiometric charge densities, B̲ was solely dependent upon chain stiffness. In fact, Smidsrød and Christensen (1991) found a good correlation between B̲ and chain stiffness for several polyelectrolytes, as measured by the Kuhn statistical segment at $\theta$-conditions, $A_{m\theta}$. Such a correlation is also predicted by the theories of Flory (1953) and Fixman (1964).

From viscosity measurements, it is possible to estimate statistical parameters that represent the extension of the molecule, e.g. the radius of gyration, $R_G$. Such relations yielding these parameters basically rest upon the equivalent sphere model ('non-draining' theory) by Flory and Fox (1951):

$$[\eta] = \phi[(6 < R_G^2 >)^{3/2}]/M \tag{2}$$

where $\phi$ is a parameter which is a function of the spatial distribution form of the chain molecule units (Flory, 1969). Equation (2) is valid for random coils of high molecular weights. While Flory and Fox assumed a constant value of $\phi$, Bloomfield and Zimm (1966) calculated how $\phi$ decreased for chains that were expanded relative to random-walk Gaussian coils. As a quantitative measure of the chain extension, Bloomfield and Zimm used the exponent in the MHKS equation.

Another parameter that is frequently used for characterizing the stiffness of infinitely long, randomly coiled chains is the Kuhn length, $A_m$. The Kuhn length is a statistical segment that describes the extension of the chain in a given direction of the initial bond. It is defined by:

$$< h^2 >^{0.5} = (6 < R_G^2 >)^{0.5} = (A_m \cdot l \cdot DP)^{0.5} \tag{3}$$

where $< h^2 >^{0.5}$ is the root-mean-square of the end-to-end distance; $l$ is the virtual bond length per monomeric unit; and $DP$ is the degree of polymerization. For relatively stiff molecules (wormlike coils), $A_m = 2q$, where $q$ is the persistence length (Bloomfield *et al.*, 1974). Thus, by utilizing eqns (2) and (3), the conformation of polymers given by the statistical chain parameters radius of gyration, end-to-end distance, and the Kuhn length at different ionic strengths can be estimated from intrinsic viscosity and molecular weight data.

At $\theta$-conditions, the mean-square end-to-end distance in dilute solutions of polymer molecules is given by (Flory, 1953, 1969):

$$< h^2 >_0 = DP \cdot b_0^2 \tag{4}$$

where $b_0$ is the effective bond length. For real chains, $< h^2 >_0$ is a linear function of $DP$ only for infinitely long chains, where the segment distribution is Gaussian. The frequently used characteristic ratio, $C_\infty$, is defined as (Flory, 1969):

$$C_\infty = (< h^2 >_0)/(DP \cdot l^2) \tag{5}$$

and is generally $\neq 1$ for real chains. Thus, the value of $C_\infty$ reflects the preferred conformation of the polymer, and the local flexibility around the single bonds at $\theta$-conditions. It should be stressed that all of these statistical parameters given by eqns (2)–(5) are calculated for monodisperse samples. In the case of polydisperse samples, statistical parameters must only be compared if they originate from the same type of molecular weight average, and if the molecular weight distribution in the samples are about the same.

For chitosan, it is important to evaluate how the extension of the chain, and hence the solution properties, varies with the degree of acetylation, $F_A$. There are two different effects that must be taken into account. One is the effect caused by the bulky *N*-acetyl groups, which might be of, for example, steric origin or it might be due to intramolecular hydrogen bonding. This effect suggests that chitosans with the highest $F_A$ should hold the most expanded conformation and become the stiffest chain. Another effect is due to the positive charge of the free amino group in acidic solutions. If a repulsive polyelectrolyte effect is determining, chitosans with the lowest $F_A$ and thus the highest charge effects would exhibit the most expanded conformation (at low ionic strengths where the electrostatic effect is prevailing).

Terbojevich *et al.* (1991) measured $R_G$ by static light scattering in 0·17 M acetic acid and 0·2 M NaCl, and used these experimental data together with an iterative method of Manning (1981) and a theory of Benoit and Doty (1953) to calculate the persistence length at $\theta$-conditions, $q_\theta$. Terbojevich *et al.* calculated a value of 220 ± 20 Å for chitosans with $F_A$ of 0·4 and 0·15. Moreover, they calculated the electrostatic contribution to $q_\theta$, and found that this was negligible compared to the steric contribution.

From viscosity data in HAc/NaAc buffer pH 4·3, Rinaudo and Domard (1989) estimated the persistence length, $q_n$, of neutral chitosan with an $F_A$ of 0·15 to be 300 Å. They also assumed that the solution behaviour at $\theta$-conditions could be approximated by that at infinite ionic strength, and calculated $q_{n,\theta} = 42$ Å according to a theory of Yamakawa and Fuji (1974) for wormlike chains. No explanation was offered for the large difference between these calculated values.

Kienzle-Sterzer *et al.* (1984) also utilized the previously mentioned theory of Yamakawa and Fuji (1974) and computed the total persistence length, $q_t$ of a chitosan with $F_A = 0·2$. They found that $q_t$ varied from around 50 to 70 Å, dependent upon some calculated degree of ionization. Kienzle-Sterzer *et al.* did not demonstrate to which solvent conditions their calculated values referred.

Pogodina *et al.* (1986) determined the MHKS parameters for a chitosan with $F_A = 0{\cdot}2$ in 0·33 M HAc with 0·3 M NaCl from viscosity and sedimentation measurements. Also, from viscosity data and the mentioned theory of Yamakawa and Fuji, Pogodina *et al.* found that $A_m$ in this specific chitosan-buffer system was 180 Å.

A general criticism to all the above-mentioned work is the limited amount of experimental data that have been used for discriminating between the effects of polyelectrolyte, excluded volume and chain stiffness in relation to different degrees of *N*-acetylation. Different theories based on different models and approximations were needed to sort out and discriminate between the different effects.

None of the above-mentioned authors studied the effect of *varying* the $F_A$ on the values of the hydrodynamic properties of chitosan. However, Wang *et al.* (1991*a*) utilized static light scattering and viscosity measurements and evaluated the MHKS parameters for chitosans with $F_A$ ranging from 0 to 0·3. They found that the value of the exponent $\underline{a}$ increased with $F_A$. The range of $F_A$ studied in the above-mentioned works is 0–0·3, although it is possible to prepare chitosans that are soluble in water solutions having $F_A$ up to 0·6 (Sannan *et al.*, 1976).

Here the authors report on a systematic study of the variation in solution properties of partially *N*-acetylated chitosans as a function of $F_A$. In comparison to earlier publications related to solution properties of chitosans, the authors have investigated chitosans within a very broad range of $F_A$ and molecular weights. Moreover, from NMR-measurements it is known that the distribution of the *N*-acetyl groups along the chain in the utilized samples is random, that is, Bernoullian (Vårum *et al.*, 1991*a, b*). Such information about the monomer distribution is important when evaluating physical properties of heteropolymers. In addition, the samples that the authors have utilized are fully soluble both when performing the NMR-measurements for the determination of $F_A$, and also when investigating the solution properties. That is, the authors' $F_A$ are truly consistent with the obtained solution properties (in contrast to what might be the case if, for example, the solid-state method IR is used to determine $F_A$ and if the chitosan is only fractionally soluble at the conditions used for investigating the solution properties). Finally, the different molecular weights were prepared by random depolymerization of chitosans with relatively high molecular weights, implying that the molecular weight distribution should be a Kuhn distribution, that is, with $M_w/M_n$ close to 2. Indeed, recent GPC-LALLS experiments indicate that $M_w/M_n$ is close to 2 in the samples used in this study (Anthonsen, M.W., unpublished).

## EXPERIMENTAL

### Materials

The chitosans with $F_A$ of 0·6 and 0·15 were kindly provided by Pronova Biopolymers (Drammen, Norway). Homogenous *N*-deacetylation were performed when preparing the chitosan with $F_A$ of 0·6 (Vårum *et al.* 1991*a*). The chitosan with an $F_A$ of 0 was made in the authors' laboratory by heterogeneous *N*-deacetylation as described by Vårum *et al.* (1991*a*), except that the time and temperature used was 4 h and 95°C, respectively. The preparation of chitosan with an $F_A$ of 0 was done from a starting material with $F_A = 0{\cdot}15$. The $F_A$ and the statistical distribution of the monomers along the chain were determined by 500 MHz $^1$H and 125 MHz $^{13}$C NMR spectroscopy.

### Preparation of chitosan fractions

In order to obtain samples of different molecular weights with approximately the same type of molecular weight distribution, random degradation (resulting in a Kuhn distribution) with nitrous acid was performed by the method of Allan and Peyron (1989). Thereafter, the chitosans were reduced with $NaBH_4$. In order to transform the chitosan to the chloride salt, the solutions were adjusted to pH 5 at which pH the ionization degree of chitosan according to its p$K_a$-value is roughly 97% (without considering ion pair condensation), dialysed against NaCl (0·2 M) and distilled water, filtered, lyophilized, and stored in a freezer at −20°C. Chitosans not degraded with nitrous acid were converted to the chloride salt in the same manner.

### Apparatus and measurements

The viscosity measurements were performed in a Schott-Gerate Ubbelohde viscosimeter as previously described (Draget *et al.*, 1992). In order to obtain the most accurate values, the intrinsic viscosities were determined as the average of extrapolating both log $(\eta_{sp}/c)$ and log $[\ln(\eta_r/c)]$ to zero concentration (Stokke *et al.*, 1992). Determination of osmotic pressure of the solutions was carried out using a Knauer Membran Osmometer no. 01.00 fitted with a Satorius SM 11736 cellulose acetate membrane with a cut off of 30 kg/mol. The buffer used was 0·02 M HAc/NaAc, pH 4·5 with different concentrations of NaCl. The polymer solutions were filtered through 0·8 μm Millipore-AA filters. The concentration of chitosan chloride used for the osmotic pressure measurements ranged from 0·2 to 0·6 g/100 ml, and the concentration of NaCl was 0·1 M. For the viscosity determinations, the chitosan chloride concentrations used were such that the relative viscosity, $\eta_r$, of the most concentrated solution was about 3–4. The chitosan chloride concentrations were not corrected for water contents.

Viscosity measurements were performed at 20°C, whereas the osmotic pressure measurements were done at 22°C.

## RESULTS AND DISCUSSION

### Osmometry and MHKS equations

The number averages of the molecular weight, $M_n$, and the second virial coefficients, $A_2$, as determined from osmotic pressure measurements are given in Table 1. An example of a plot of the experimental data is given in Fig. 1. The results demonstrate the expected solution behaviour of a polyelectrolyte, showing large and positive $A_2$. In particular, the values of $A_2$ clearly reflect the polyelectrolyte character of the chitosans as it is in the order of $10^{-3}$ ml mol/g$^2$ which is typical of polyelectrolytes and stiff coils in aqueous solvents (Harding *et al.*, 1991). The second virial coefficient of polyelectrolytes has contributions both from the conventional excluded volume and from the charge effects. It is seen from Table 1 that the value of $A_2$ is an overall increasing function as $F_A$ decreases, indicating that at 0·1 M ionic strength the charge effect contributes relatively more than the excluded volume effect. This is in agreement with suggestions made by Tanford (1961) and Odijk (1979). From the values of $A_2$ it is not possible to observe the dependence upon molecular weight as would be expected for neutral polymers (Tanford, 1961), again suggesting that the charge effects are predominating.

**Table 1. Intrinsic viscosities at 0·1 M ionic strength and results from osmotic pressure measurements**

| $F_A$ | $[\eta]_{0.1}$$^a$ (ml/g) | $M_n$$^a$ (g/mol) | $A_2 \times 10^3$ (ml mol/g$^2$) |
|---|---|---|---|
| 0·6 | 26 | 15 000 | 2·4 |
| | 67 | 19 000 | 3·0 |
| | 112 | 27 000 | 2·9 |
| | 119 | 32 000 | 3·3 |
| | 148 | 37 000 | 3·5 |
| | 282 | 69 000 | 2·9 |
| | 307 | 61 000 | 3·9 |
| | 370 | 102 000 | 3·4 |
| | 282 | 69 000 | 2·9 |
| | 813 | 164 000 | 1·5 |
| 0·15 | 185 | 35 000 | 6·6 |
| | 208 | 42 000 | 5·3 |
| | 322 | 62 000 | 4·6 |
| | 393 | 78 000 | 6·0 |
| | 439 | 82 000 | 5·8 |
| | 499 | 98 000 | 5·5 |
| | 567 | 125 000 | 5·8 |
| | 780 | 245 000 | 5·8 |
| 0 | 146 | 15 000 | 6·1 |
| | 274 | 42 000 | 6·0 |
| | 295 | 48 000 | 5·7 |
| | 379 | 64 000 | 7·6 |
| | 487 | 117 000 | 5·5 |
| | 665 | 210 000 | 7·7 |
| | 931 | 310 000 | 7·2 |

$^a$Chitosan chloride.

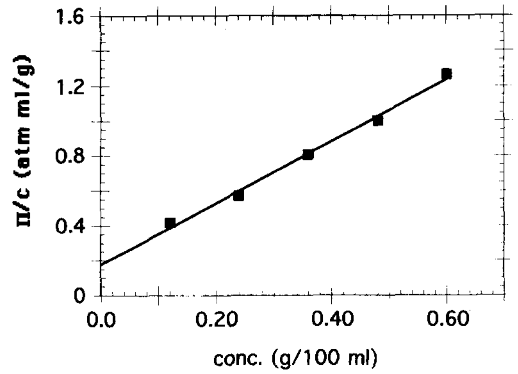

**Fig. 1.** Reduced osmotic pressure, $\pi/c$, versus chitosan chloride concentration ($F_A = 0$, $[\eta]_{0.1} = 665$ ml/g).

Figure 2 displays the MHKS equations for the three chitosans at 0·1 M ionic strength, where $M_n$ and intrinsic viscosities (in ml/g) from Table 1 are plotted in a double logarithmic plot. The regression lines are:

| $F_A$ | *Equation* | *Correlation coefficient* |
|---|---|---|
| 0·6 | $\log [\eta]_{0.1} = 2.6590 + 1.06 \log M_n$ | $R = 0.983$ |
| 0·15 | $\log [\eta]_{0.1} = 1.2329 + 0.778 \log M_n$ | $R = 0.978$ |
| 0 | $\log [\eta]_{0.1} = 0.2524 + 0.583 \log M_n$ | $R = 0.995$ |

The value of the exponents, a, in the MHKS equation increases with increasing $F_A$, indicating that it is the influence of the *N*-acetyl groups rather than the electrostatic effect of the charged amino groups that is determining the extension of the chitosan chain at 0·1 M ionic strength. A discussion of the influence of charge effects on the conformation of polyelectrolytes is given by Odijk (1977) and Odijk and Houwaart (1978). The specific value of a is of course dependent upon the solvent, and especially upon the ionic strength. Therefore, it is not possible directly to compare values in the literature given for chitosans even if they have equal contents of $F_A$. Wei Wang *et al.* (1991*a*, *b*) used viscosity

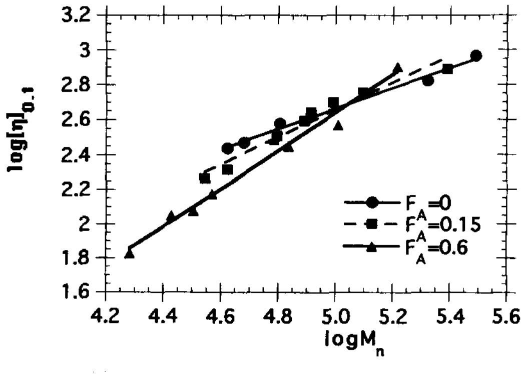

**Fig. 2.** Log $[\eta]_{0.1}$ versus log $M_n$ at 0·1 M ionic strength (MHKS equations).

and light scattering measurements and found that the $\underline{a}$-values in 0·2 M HAc with 0·1 M NaAc at 30°C increased with $F_A$ for the chitosans having $F_A$ ranging from 0 to 0·3, which is consistent with the results reported in this work. The constant, $K$, in the MHKS equation shows an opposite effect, since it decreases with increasing $F_A$. The dependencies of $\underline{a}$ and $K$ upon $F_A$ at 0·1 M ionic strength (pH 4·5) are plotted in Fig. 3. The regression lines are:

| *Equation* | *Correlation coefficient* |
|---|---|
| $\log K_{0 \cdot 1} = -0{\cdot}427 - 3{\cdot}821 F_A$ | $R = 0{\cdot}985$ |
| $a_{0 \cdot 1} = 0{\cdot}6169 + 0{\cdot}7590 F_A$ | $R = 0{\cdot}983$ |

These relations are of practical importance since they can be used for estimating $\underline{a}$ and $K$ for chitosans with different $F_A$ meeting the same conditions, and with approximately the same molecular weight distribution (Kuhn distribution) as the fractions used for determining the equations. The MHKS equations can then be used for estimating the viscosity average of the molecular weight from viscosity measurements, again provided that the chitosans have approximately the same molecular weight distribution as the fractions used for determining the equations.

Basically, due to the mediation of the long-range electrostatic effects of the charged amino groups, the value of $\underline{a}$ varies with ionic strength. Therefore, it is important to study the dependence of $\underline{a}$ upon ionic strength. Comparison of $\underline{a}$-values at infinite ionic strength is especially interesting, since these values reflect the conformation when the electrostatic interaction is suppressed. Hence, the results at infinite ionic strength demonstrate the influence of the $N$-acetyl groups. The plots in Fig. 4 show linear dependencies of the intrinsic viscosity upon the inverse square root of the ionic strength, as expected for linear polyelectrolytes (Fixman, 1964). Since the measurements at different ionic strengths were carried out at pH 4·5, which differs from the pK$_a$-value of chitosan by roughly 2 pH units, one should not expect that the degree of ionization at this pH should change significantly with varying ionic strengths. The parameters from MHKS equations at varying ionic strengths as determined from intrinsic viscosities are given in Table 2. Values at infinite and 0·005 M ionic strength were obtained by extrapolations from the plots in Fig. 4. From Table 2 it can be seen that the value of $\underline{a}$ is consistently higher for the chitosan with the highest $F_A$, irrespective of ionic strength. Further-

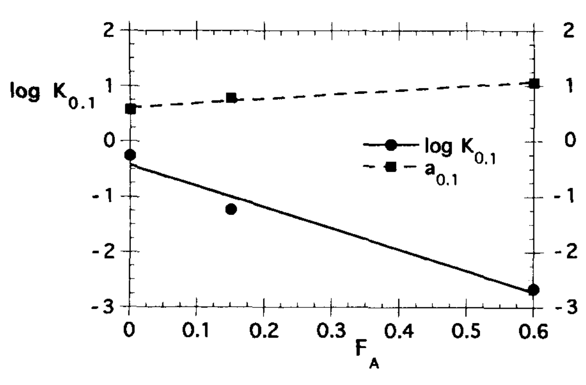

**Fig. 3.** Log $K_{0 \cdot 1}$ and $a_{0 \cdot 1}$ versus $F_A$.

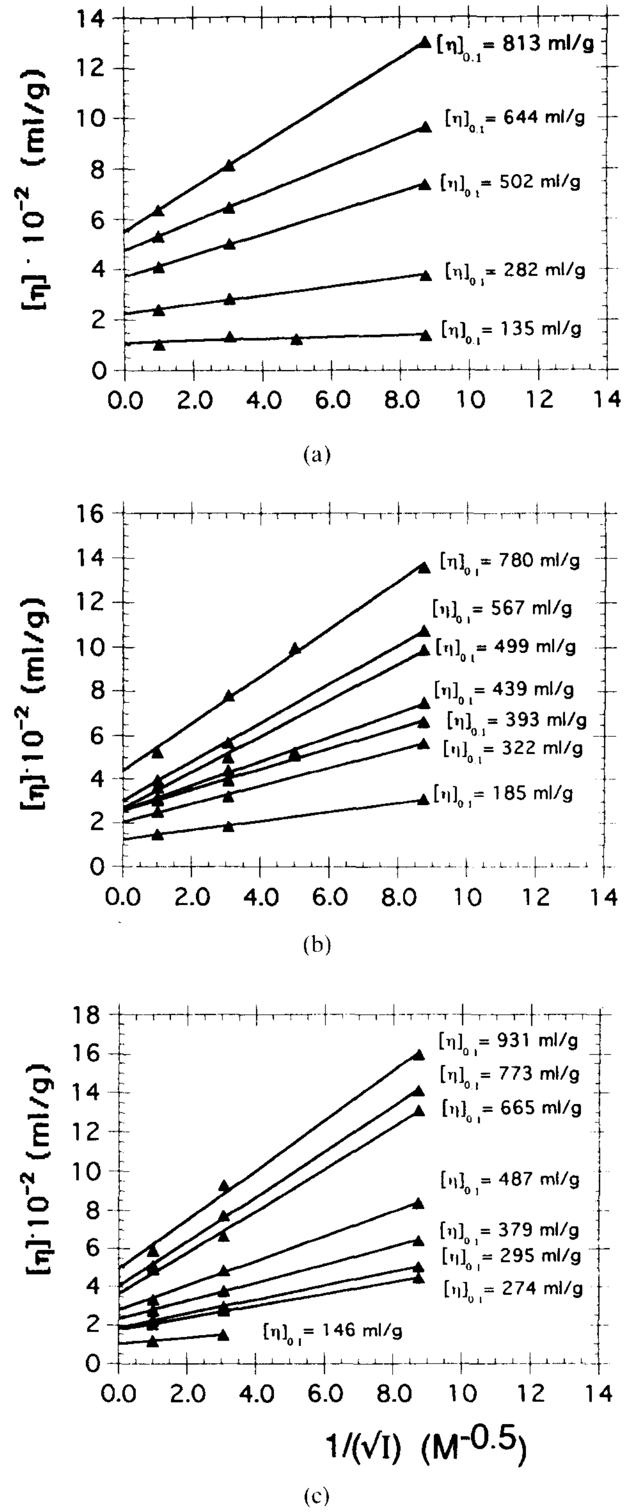

**Fig. 4.** Intrinsic viscosities as functions of ionic strength: (a) $F_A = 0{\cdot}6$; (b) $F_A = 0{\cdot}15$; and (c) $F_A = 0$.

**Table 2. Values of *a* and $K^a$ from MHKS equations at different ionic strengths**

| *I* (M) | $F_A$ = 0·6 | | | $F_A$ = 0·15 | | | $F_A$ = 0 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *a* | $K^a$ | $R^b$ | *a* | $K^a$ | $R^b$ | *a* | $K^a$ | $R^b$ |
| $\infty^c$ | 0·95 | 6·22e-03 | 0·999 | 0·62 | 2·09e-01 | 0·979 | 0·47 | 1·11 | 0·996 |
| 1·007 | 1·06 | 1·81e-03 | 0·999 | 0·66 | 1·75e-01 | 0·981 | 0·52 | 8·36e-01 | 0·997 |
| 0·107 | 1·06 | 2·18e-03 | 0·983 | 0·78 | 5·85e-02 | 0·978 | 0·58 | 5·59e-01 | 0·995 |
| 0·0131 | 1·29 | 2·31e-04 | 0·998 | 0·79 | 9·69e-02 | 0·960 | 0·64 | 5·20e-01 | 0·998 |
| 0·005$^c$ | 1·37 | 1·14e-04 | 0·998 | 0·81 | 1·01e-01 | 0·956 | 0·66 | 5·44e-01 | 0·998 |

[a] Valid for [η] in ml/g.
[b] Correlation coefficient from linear regression analysis.
[c] Extrapolated values.

more, the increase in a̲ with increasing $F_A$ at infinite ionic strength demonstrates the influence of the *N*-acetyl groups when all the electrostatic interactions are partly eliminated. In conclusion, the hydrodynamic behaviour of chitosans is dominated by the *N*-acetyl groups.

Another interesting result, especially from a practical point of view, is the crossing of the lines given by the MHKS equations (Fig. 2). At lower molecular weights the intrinsic viscosity of the completely *N*-deacetylated chitosan surpasses that of the chitosan with $F_A$ = 0·6. This is partly caused by differences in mass per unit length for different $F_A$, which implies that the chain contour lengths are different for equal molecular weights. However, as can be seen from Fig. 5 where the intrinsic viscosities at 0·1 M ionic strength are plotted as functions of number average degree of polymerization, $DP_n$, instead of molecular weight, the lines still cross. This is again partly due to differences in mass per unit length, and also to the fact that the intrinsic viscosity is per gram sample. In addition, the electrostatic effect has an impact. At longer chain lengths, the stiffness of the chitosan with $F_A$ = 0·6 increases enough to cancel out the effect caused by the electrostatic effect and the origin of the units of intrinsic viscosity.

It is of interest to consider which chitosan gives the highest viscosity at different ionic strengths. An evaluation of this is given by observing the chain length at which the intrinsic viscosity of chitosan with $F_A$ = 0·6 exceeds that of $F_A = 0$. This is shown in Fig. 6 for varying ionic strengths. It is seen that the higher the ionic strength, the lower the $DP_n$ at which the intrinsic viscosity of the chitosan with $F_A$ = 0·6 exceeds that for the chitosan with $F_A = 0$. This illustrates that the polyelectrolyte effect exerts a higher influence on the conformation at lower ionic strengths.

## Parameters characterizing chain extension

In order to obtain an additional, independent measure of chain stiffness and conformation, the stiffness parameter, B̲, was determined from viscosity measurements (Smidsrød & Haug, 1971). An important advantage of using B̲ as an estimate of chain conformation of polysaccharide is that molecular weight determination, which is often difficult and time-consuming, is not included in its determination. The value of B̲ is determined by extrapolating to 0 in a logarithmic plot of the salt tolerance, *S*, versus $[\eta]_{0\cdot1}$, the values of *S* and $[\eta]_{0\cdot1}$ being given in terms of dl/g. The value of *S* is determined from the slope of the lines in Fig. 4. Plots showing the determination of the B̲-values are given in Fig. 7, and the corresponding regression lines are:

| $F_A$ | *Equation* | *Correlation coefficient* |
|---|---|---|
| 0·6 | $\log S = -1{\cdot}616 + 1{\cdot}728 \log [\eta]_{0\cdot1}$ | $R = 0{\cdot}995$ |
| 0·15 | $\log S = -1{\cdot}011 + 1{\cdot}217 \log [\eta]_{0\cdot1}$ | $R = 0{\cdot}988$ |
| 0 | $\log S = -1{\cdot}026 + 1{\cdot}219 \log [\eta]_{0\cdot1}$ | $R = 0{\cdot}996$ |

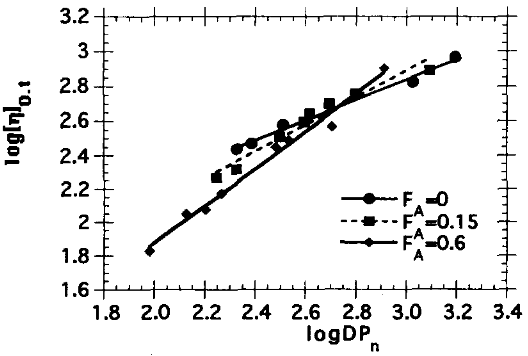

**Fig. 5.** Log $[\eta]_{0\cdot1}$ versus log $DP_n$ at 0·1 M ionic strength.

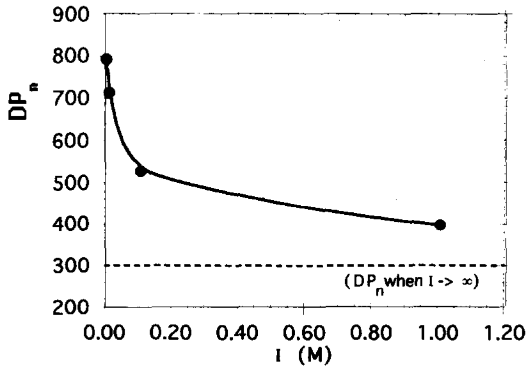

**Fig. 6.** Number average degrees of polymerization, $DP_n$ at which $[\eta]_I$ is equal for chitosans with $F_A$ = 0·6 and 0 versus ionic strength.

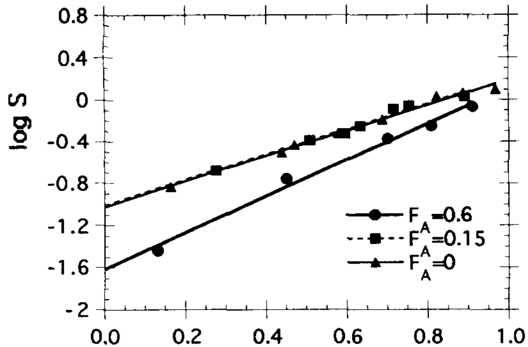

**Fig. 7.** Log $S$ as a function of log $[\eta]_{0 \cdot 1}$.

The resulting $\underline{B}$-values are presented in Table 3. The value of $\underline{B}$ is considerably smaller for the chitosan with $F_A = 0 \cdot 6$ than for the chitosans with lower $F_A$, again indicating that a higher *N*-acetyl content increases the stiffness of the chain.

It should be noted that the use of $\underline{B}$ as a stiffness parameter is restricted to relatively strong polyelectrolytes, and to polymers that do not undergo conformational transitions with varying salt concentrations (Smidsrød & Haug, 1971; Urbani & Brant, 1989). Chitosan showed a linear dependence of intrinsic viscosity upon the inverse square root of ionic strength (Fig. 4), indicating that the molecule does not undergo any order–disorder transitions within the salt concentrations investigated.

Also, the charge density of the polymers for which the $\underline{B}$-values are being compared should not differ significantly. Regarding the polyelectrolyte character of chitosan, we have no experimental data to quantify this specific property of the polymer. However, since the viscosity measurements were performed at pH 4·5 which is sufficiently low for all the amino groups to be charged, and since the *N*-acetyl groups are randomly distributed along the chain, the charge density should increase linearly with decreasing $F_A$. However, the *effective* charge density should be rather constant due to ion pair condensation (Imai, 1961; Manning, 1969). Smidsrød and Haug (1971) demonstrated that for pectins with degrees of esterification below about 80%, and also for the flexible molecule polyacrylate with degrees of dissociation exceeding 10%, the value of $\underline{B}$ was independent of stochiometric charge density. The chitosans studied in this work have stochiometric charge densities ranging from 40 to 100%. Following the above discussion, the use of $\underline{B}$ as a parameter for characterizing chain stiffness of chitosans should be valid.

Radii of gyration and Kuhn lengths at different ionic strengths as calculated from eqns (2) and (3) are given in Table 4. The value of $A_{ml}$ decreases with decreasing $F_A$, again indicating that chains with higher $F_A$ are more extended. However, the *exact* numbers of these parameters should be regarded as tentative, since their evaluation involves several steps that rely on theories, and not solely on experimental data. In particular, three aspects that need additional comments are: the value of the Flory–Fox constant, $\phi$, to be used in eqn (2); the validity of using eqns (2) and (3) since these equations are evolved for monodisperse samples; and finally the

**Table 3. Intrinsic viscosities at different ionic strengths, salt tolerances, molecular weights and stiffness parameters**

| $F_A$ | $[\eta]_{1 \cdot 007}$[c] | $[\eta]_{0 \cdot 107}$[c] | $[\eta]_{0 \cdot 013}$[c] | $S$[b] (dl $M^{0 \cdot 5}$/g) | $M_n$[c] (g/mol) | $\underline{B}$ |
|---|---|---|---|---|---|---|
| | | (dl/g)[a] | | | | |
| 0·6 | 1·03 | 1·35 | 1·38 | 0·036 | 30 000 | 0·02 |
| | 2·39 | 2·82 | 3·76 | 0·175 | 69 000 | |
| | 4·10 | 5·02 | 7·39 | 0·423 | 115 000 | |
| | 5·33 | 6·44 | 9·63 | 0·557 | 145 000 | |
| | 6·36 | 8·13 | 13·02 | 0·861 | 164 000 | |
| 0·15 | 1·49 | 1·85 | 3·09 | 0·209 | 35 000 | 0·10 |
| | 2·53 | 3·22 | 5·66 | 0·410 | 62 000 | |
| | 3·03 | 3·93 | 6·64 | 0·473 | 78 000 | |
| | 3·13 | 4·39 | 7·50 | 0·558 | 82 000 | |
| | 3·65 | 4·99 | 9·90 | 0·820 | 98 000 | |
| | 3·95 | 5·67 | 10·76 | 0·883 | 125 000 | |
| | 5·24 | 7·80 | 13·58 | 1·070 | 245 000 | |
| 0 | 1·16 | 1·46 | — | 0·146 | 15 000 | 0·09 |
| | 2·02 | 2·74 | 4·45 | 0·311 | 42 000 | |
| | 2·24 | 2·95 | 5·07 | 0·367 | 48 000 | |
| | 2·75 | 3·79 | 6·45 | 0·476 | 64 000 | |
| | 3·33 | 4·87 | 8·39 | 0·646 | 117 000 | |
| | 4·90 | 6·65 | 13·12 | 1·079 | 210 000 | |
| | 5·12 | 7·73 | 14·14 | 1·158 | 260 000 | |
| | 5·90 | 9·31 | 15·96 | 1·272 | 310 000 | |

[a] Due to the definition of $\underline{B}$, $[\eta]$ and $S$ are given in terms of dl/g.
[b] From Fig. 4.
[c] Chitosan chloride.

**Table 4. Conformational parameters of non-degraded chitosans calculated from number average molecular weights and intrinsic viscosities at different ionic strengths**

| $F_A$ | $M_0$[a] (g/mol) | $I$ (M) | $[\eta]_I$[a] (dl/g) | $\phi$[b] $\times 10^{21}$[b] (1/mol) | $M_n$[a] (g/mol) | $DP_n$ | $<R_G^2>^{0·5}$[c] (Å) | $A_{mI}$[d] (Å) | $A_{m\theta}$[e] (Å) | $C_\infty$[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| 0·6 | 200·8 | ∞[g] | 5·50 | 1·23 | 164 000 | 815 | 368 | 195 | 180 | 34 |
| | | 1·007 | 6·36 | 1·00 | | | 414 | 247 | | |
| | | 0·107 | 8·13 | 1·00 | | | 449 | 291 | | |
| | | 0·013 | 13·02 | 0·65 | | | 607 | 532 | | |
| | | 0·005[g] | 17·67 | 0·58 | | | 698 | 703 | | |
| 0·15 | 198·3 | ∞[g] | 4·39 | 2·25 | 245 000 | 1236 | 319 | 97 | 50 | 9 |
| | | 1·007 | 5·24 | 2·10 | | | 346 | 114 | | |
| | | 0·107 | 7·80 | 1·66 | | | 428 | 174 | | |
| | | 0·013 | 13·58 | 1·67 | | | 514 | 251 | | |
| | | 0·005[g] | 19·53 | 1·60 | | | 588 | 329 | | |
| 0 | 197·5 | ∞[g] | 4·96 | 2·80 | 310 000 | 1559 | 334 | 84 | 50 | 9 |
| | | 1·007 | 5·90 | 2·70 | | | 358 | 97 | | |
| | | 0·107 | 9·31 | 2·48 | | | 428 | 139 | | |
| | | 0·013 | 15·96 | 2·20 | | | 534 | 215 | | |
| | | 0·005[g] | 22·95 | 2·10 | | | 612 | 283 | | |

[a] Chitosan chloride.
[b] From Table 5, valid for $[\eta]$ in dl/g and $R_G$ in cm.
[c] From eqn (2).
[d] From eqn (3).
[e] Interpolated from results of Smidsrød and Christensen (1991).
[f] From eqns (3) and (5).
[g] Extrapolated values.

dependency of the statistical parameters upon the molecular weight distribution of the samples.

In addition to dissimilarities in chain parameters arising from theoretical considerations, differences also arise when evaluating the parameters from different experimental methods, e.g. viscosity and light scattering (Burchard, 1968; Kamide & Saito, 1987). Utilizing the number average of the molecular weight results in higher values of the statistical chain parameters than those obtained if the weight average was used. However, for the purpose of this work, the comparison of statistical parameters that are evaluated in the same way from the same type of experimental data is applicable. Because of the above-mentioned aspects, comparison with other published results is of secondary importance.

**Table 5. Values of the MHKS exponent, *a*, and ε and $\phi$ at different ionic strengths**

| $F_A$ | $I$ (M) | $a$ | $\varepsilon$[a] | $\phi \times 10^{21}$[a] (1/mol) |
|---|---|---|---|---|
| 0·6 | ∞[b] | 0·95 | 0·300 | 1·32 |
| | 1·007 | 1·06 | 0·373 | 1·00 |
| | 0·107 | 1·06 | 0·373 | 1·00 |
| | 0·013 | 1·29 | 0·527 | 0·68 |
| | 0·005[b] | 1·37 | 0·580 | 0·61 |
| 0·15 | ∞[b] | 0·62 | 0·080 | 2·13 |
| | 1·007 | 0·66 | 0·107 | 2·05 |
| | 0·107 | 0·78 | 0·187 | 1·66 |
| | 0·013 | 0·79 | 0·193 | 1·55 |
| | 0·005[b] | 0·81 | 0·207 | 1·45 |
| 0 | ∞[b] | 0·47 | 0 | 2·80 |
| | 1·007 | 0·52 | 0·013 | 2·68 |
| | 0·107 | 0·58 | 0·053 | 2·48 |
| | 0·013 | 0·64 | 0·093 | 2·13 |
| | 0·005[b] | 0·66 | 0·107 | 2·01 |

[a] From Bloomfield and Zimm (1966).
[b] Extrapolated values.

Also shown in Table 4 are the Kuhn lengths at $\theta$-conditions, $A_{m\theta}$, and the characteristic ratios, $C_\infty$. The $A_{m\theta}$ are interpolated from the previously mentioned correlation obtained by Smidsrød and Christensen (1991). From this relationship, the chitosans with $F_A$ of 0·15 and 0 have chain stiffnesses which are comparable to those of CM-celluloses, whereas the chain of chitosan with $F_A = 0·6$ is slightly stiffer than an alginate with approximately 40% of L-guluronate residues. Hence, the chain stiffness parameters seem reasonable in comparison with other polysaccharides. As seen from the $A_{m\theta}$ (which should mainly arise from the 'intrinsic' chain stiffness and not from charge effects), the *N*-acetyl group in chitosan seems to expand the molecule and have a similar effect to that which the diaxial glycosidic linkages exert in homopolymeric guluronate sequences in alginates. However, the values of neither $A_{m\theta}$ nor $C_\infty$ should be taken as exact, since the relationship from Smidsrød and Christensen (1991) used for obtaining $A_{m\theta}$ is purely empirical ($C_\infty$ being calculated from $A_{m\theta}$).

Traditionally, many authors have used the approach that $\theta$-conditions for the polymer in question can be approximated by the conditions at infinite ionic strength. This is not actually correct, since even at $\theta$-conditions the concentration of the added supporting electrolyte influences the conformation of the polymer (Skolnick & Fixman, 1977). This is also corroborated from the data in Table 2, which show that the $\underline{a}$-values from the MHKS equations for the chitosans with $F_{\mathrm{A}}$ of 0·15 and 0·6 have $\underline{a}$-values at infinite ionic strength which are higher than 0·5.

## ACKNOWLEDGEMENTS

This work has been financed by the University of Trondheim (research grant to M.W.A.), the Royal Norwegian Council for Scientific and Industrial Research (NTNF), the Norwegian Fisheries Research Council (NFFR) and Pronova Biopolymers A/S, Drammen.

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