

# Hydrodynamic characterization of chitosans varying in degree of acetylation

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*The molecular weights and gross aqueous solution conformation of two chitosans of different degrees of acetylation, 'Sea Cure + 210' (11% acetylated) and KN50 (58% acetylated) were characterized by viscometry, analytical ultracentrifugation and dynamic light scattering. The hydrodynamic parameters obtained were used to determine both the molecular weights and the gross solution conformation of the two chitosans. Using the Wales–Van Holde ratio of sedimentation coefficient concentration regression coefficient ( $k_s$ ) to intrinsic viscosity  $[\eta]$ , the Sea Cure + 210 chitosan, which is much less acetylated than the KN50, is highly asymmetric in conformation. This, in conjunction with the charge on the molecule, would suggest a rod-like conformation in solution. The two largest KN50 chitosans have widely differing values for the Wales–Van Holde ratio, suggesting different solution conformation. However, when the series is examined as a whole using the Mark–Houwink–Kuhn–Sakurada relationships relating molecular weight to both intrinsic viscosity and translational diffusion coefficient, then a more spheroidal structure, approximating to a random coil, is predicted.*

**Keywords:** Chitosan; conformation; molecular weight; physicochemical properties

## Introduction

Over the past 15 years the interest in chitosan has increased very rapidly in both the academic and industrial fields. Five international conferences on chitin and chitosan have been convened in the USA (1977 and 1991), Japan (1982), Italy (1985) and Norway (1988). Many potential commercial applications have been found for chitosan, in areas ranging from cosmetics<sup>1</sup> to agriculture<sup>2</sup>, to medicine<sup>3</sup>, to waste-water treatment<sup>4</sup>. However, in comparison to many anionic polysaccharides, there is still relatively little known about chitosan in terms of its physicochemical properties.

The aim of this study is to characterize two different chitosans (Sea Cure + 210 and KN50) in terms of estimates of their gross solution conformations.

## Experimental

### Materials

**Chitosans.** The Sea Cure + 210 chitosan (SC + 210) was supplied by Protan Inc., Drammen, Norway. It was supplied as the glutamate salt, was of the medium viscosity variety and was 11% acetylated as determined by <sup>1</sup>H-n.m.r. spectroscopy<sup>5</sup>. The KN50-1 chitosan was prepared by homogeneous deacetylation using the method of Sannan *et al.*<sup>6</sup>. It was converted to the chloride

salt and was 58% acetylated. The lower molecular weight KN50 (KN50-2,3,4) was prepared by degradation with NaNO<sub>2</sub> at low pH (see Ref. 7).

**Solvents.** The main solvent used in this study was an aqueous 0.2 M acetate buffer (pH = 4.3), prepared using equimolar amounts of anhydrous sodium acetate and glacial acetic acid (see e.g. Ref. 8).

All solutions for sedimentation equilibrium experiments were dialysed against this buffer overnight and the dialysate used as the reference solution. All chemicals used were of the highest available grade (i.e. Fisons Analar or equivalent).

### Methods

**Partial specific volume determination.** An Anton-Paar DMA 02C precision density meter (Anton Paar KG, Graz, Austria) coupled to a thermostatically controlled, refrigerated water bath was used to measure solution and solvent densities. The instrument was calibrated using CsCl solutions<sup>9</sup> and deionized distilled water was used as a reference standard as its density is known over a wide range of temperatures.

The arithmetical mean of 10 consistent values of density for each concentration of solution was taken and plotted against the concentration. Concentration was

adjusted by dilution of a stock solution of accurately known concentration using the dry weight of the polymer. The partial specific volume was calculated from this using the following equation<sup>10</sup>

$$\bar{v} = \frac{1 - d\rho/dc}{\rho_0} \quad (1)$$

where  $\rho_0$  is the density at infinite dilution and  $d\rho/dc$  (the density increment) is obtained from the slope of the plot.

**Sedimentation coefficient measurements.** An MSE Centriscan-75 (MSE Instruments, Crawley, UK) analytical ultracentrifuge was used, equipped with an aluminium rotor and a scanning schlieren optical system using a wavelength of 546 nm. The instrument produces scans showing the position of the sedimenting boundary at set time intervals. Between five and eight consecutive scans were used to determine each sedimentation coefficient.

The sedimentation coefficients obtained ( $s_{T,b}$  corresponding to the buffer,  $b$ , at temperature  $T$ ) were then corrected to standard conditions ( $s_{20,w}$ , corresponding to a temperature of 20°C and water as the solvent). The equation below is used for this purpose<sup>11</sup>:

$$s_{20,w} = \frac{\eta_{20,w}(1 - \bar{v}\rho)_{T,b}}{\eta_{T,b}(1 - \bar{v}\rho)_{20,w}} \times s_{T,b} \quad (2)$$

These  $s_{20,w}$  values are then extrapolated to infinite dilution in order to obtain  $s_{20,w}^\circ$ .

**Sedimentation equilibrium.** For measurement of molecular weight, low-speed sedimentation equilibrium analysis was employed. A Beckman model E analytical ultracentrifuge with an electronically controlled drive was used. Rayleigh interference optics were employed as the method of recording the distribution of solute at sedimentation equilibrium. A 5 mW, He-Ne laser was used as the light source. Double sector cells with optical path lengths of 30 mm and 12 mm were used. An Ultrosan XL enhanced laser densitometer (LKB Instruments, Bromma, Sweden) with appropriately modified software was used to capture the fringe data. A Fourier series PASCAL algorithm 'ANALYSER' was used to produce the fringe concentration (relative to the meniscus) versus radial displacement from the data<sup>12</sup>. These data were then transferred to the mainframe IBM 3081/Q computer at Cambridge for full molecular weight analysis<sup>13</sup>.

The concentration, in fringe displacement units, of the chitosan at the meniscus was finite at the rotor speeds used, and was obtained by mathematical manipulation of the fringe data as described in Ref. 13. (Apparent) whole cell weight-average molecular weights,  $M_{w(app)}^\circ$ , were obtained from the limiting value at the cell base of a particularly useful operational point average,  $M^*$  (Ref. 13). The thermodynamic or 'osmotic pressure' second virial coefficient,  $B$ , and the 'ideal' weight average molecular weight,  $M_w$ , were estimated from plots of  $1/M_{w(app)}^\circ$  versus concentration according to equation 3 (see e.g. Ref. 14).

$$1/M_{w(app)}^\circ = 1/M_w(1 + 2BM_w c) \quad (3)$$

**Determination of intrinsic viscosity.** The intrinsic viscosities of the chitosans were determined using an Ostwald-type viscometer of 2 ml capacity.

The viscometer was suspended in a thermostatically controlled water bath (Schott-Geräte) maintained at 25.00 ( $\pm 0.05$ )°C. The flow times were recorded electronically using photoreceptors mounted on the viscometer stand capable of detecting the passage of the solution meniscus. From the solution: solvent flow time ratio the kinematic relative viscosity was obtained. Because of the low concentrations used (between 0.2 and 1.2 mg/ml), the density corrections for the different solutions were assumed to be very small and the kinetic viscosities were assumed to be approximately equal to the dynamic viscosities<sup>15</sup>. The intrinsic viscosity was found by extrapolation to infinite dilution of the reduced specific viscosities in the standard way<sup>11</sup>.

**Dynamic light scattering.** The translational diffusion coefficient for each of the chitosans was measured using a Malvern Instruments (Malvern, UK) System 4700c light scattering photometer. This system comprises a 40 mW He-Ne laser, a water bath maintained at 25.00 ( $\pm 0.05$ )°C, a filter and pump system for cleaning the water bath, a variable angle photomultiplier and a 64 channel correlator. An IPC personal computer using Malvern Automeasure software (version 5.3) was used to process the data and produce diffusion coefficients.

To remove dust and other supramolecular particles the samples were passed through a 0.45  $\mu$ m Millipore filter. Where the solution could not pass through this filter then the sample was prepared using buffer that had been filtered several times previously. This did not leave serious dust contamination in the sample. Light scattering cells were kept dust-free according to a procedure similar to that given by Sanders and Connell<sup>16</sup>.

A mean value for diffusion coefficient was calculated from up to 30 repeats of each measurement and several concentrations of each sample were used. The scattered light was measured at an angle of 90° to that of the incident light.

The diffusion coefficients obtained were for the sample in buffer at 25°C and were converted to standard conditions (20°C and water as solvent) using the following equation (see e.g. Ref. 17)

$$D_{20,w} = \frac{293.1\eta_{T,b}}{T\eta_{20,w}} \times D_{T,b} \quad (4)$$

The resulting corrected diffusion coefficients were plotted against concentration and linear regression analysis performed to obtain the 'infinite dilution'  $D_{20,w}^\circ$  value for each sample using the following equation, where  $k_D$  is the regression coefficient

$$D_{20,w} = D_{20,w}^\circ(1 + k_D c) \quad (5)$$

## Results and discussion

Table 1 gives a summary of the hydrodynamic, light scattering and viscometric data for both chitosans.

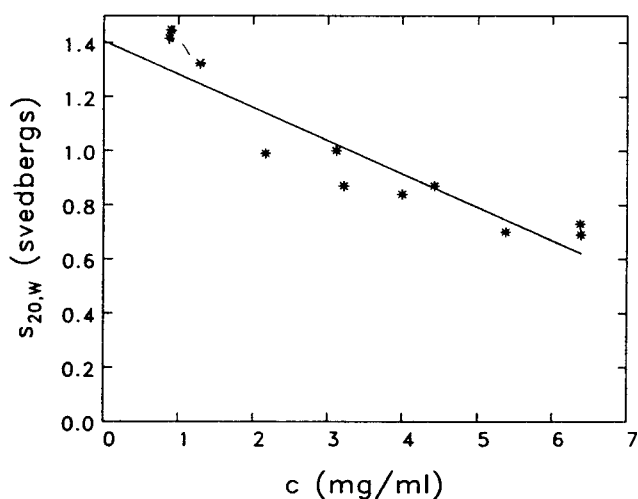
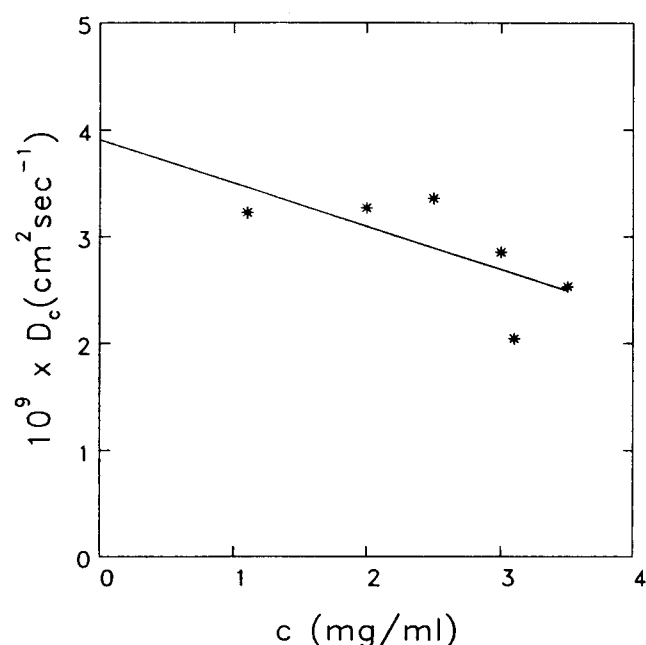
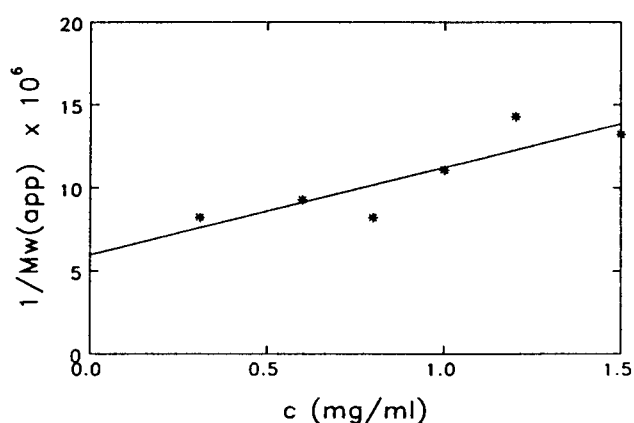
### Sea Cure + 210

Using equation 1 the partial specific volume was calculated to be  $(0.580 \pm 0.011)$  ml/g. This value was used in molecular weight calculations from sedimentation equilibrium and in all corrections for sedimentation coefficient.

Sedimentation velocity experiments yielded a plot of corrected sedimentation coefficient versus concentration

**Table 1** Summary of results of chitosan characterization

Property	Units	Fraction				
		SeaCure +210	KN50-1	KN50-2	KN50-3	KN50-4
$\bar{v}$	(ml/g)	0.580( $\pm 0.011$ )	0.565( $\pm 0.038$ )	0.565( $\pm 0.038$ )	0.565( $\pm 0.038$ )	0.565( $\pm 0.038$ )
$DA$	(wt%)	11	42	42	42	42
$M_w$	(g/mol)	162( $\pm 10$ ) $\times 10^3$	64.1( $\pm 5.0$ ) $\times 10^3$	28.9( $\pm 2.0$ ) $\times 10^3$	8.8( $\pm 0.5$ ) $\times 10^3$	4.3( $\pm 0.2$ ) $\times 10^3$
$B$	(ml mol g $^{-2}$ )	2.75 $\times 10^{-2}$	1.696 $\times 10^{-3}$	2.691 $\times 10^{-3}$	—	—
$BM$	(ml g $^{-1}$ )	4455	108.7	77.8	—	—
$s_{20,w}^0$	(S)	1.41( $\pm 0.05$ )	1.32( $\pm 0.02$ )	1.27( $\pm 0.11$ )	—	—
$k_s$		88.6( $\pm 10.8$ )	64.5( $\pm 2.6$ )	75.7( $\pm 20.5$ )	—	—
$[\eta]$	(ml/g)	540( $\pm 20$ )	800( $\pm 24$ )	112( $\pm 5$ )	67( $\pm$ )	26( $\pm$ )
$Dz_{20,w}$	(cm $^2$ s $^{-1}$ )	3.9( $\pm 0.6$ ) $\times 10^{-9}$	7.7( $\pm 0.7$ ) $\times 10^{-9}$	1.96( $\pm 0.06$ ) $\times 10^{-8}$	20.5( $\pm 0.04$ ) $\times 10^{-8}$	2.58( $\pm 0.05$ ) $\times 10^{-8}$

**Figure 1** Graph of corrected sedimentation coefficient ( $s_{20,w}$ ) versus concentration for Sea Cure +210 chitosan**Figure 3** Corrected translational diffusion coefficient,  $D_{20,w}^0$  (cm $^2$  s $^{-1}$ ) of Sea Cure +210 as a function of concentration**Figure 2** Graph of inverse apparent weight-average molecular weight versus concentration for Sea Cure +210 chitosan

(Figure 1). Upon extrapolation to infinite dilution this gave  $s_{20,w}^0$  to be  $(1.41 \pm 0.05)\text{S}$  ( $1\text{S} = 1 \times 10^{-13}\text{s}$ ) with a sedimentation coefficient concentration regression coefficient,  $k_s$ , of  $(88.6 \pm 10.8)\text{ml/g}$ .

Molecular weight measurements from sedimentation equilibrium experiments were also extrapolated to infinite dilution as a plot of  $1/M_{w(\text{app})}$  versus concentration (Figure 2). From this plot the thermodynamic or 'osmotic pressure' second virial coefficient,

$B$  ( $2.75 \times 10^{-2}\text{ml mol g}^{-2}$ ) and  $M_w^0$  ( $162\,000 \pm 10\,000\text{g/mol}$ ) were obtained.

Figure 3 shows the plot of diffusion coefficient versus concentration for the Sea Cure +210 chitosan, the intercept giving  $D_{20,w}^0 = (3.90 \pm 0.57 \times 10^{-9}\text{cm}^2\text{s}^{-1})$ .

In order to estimate the 'overall cross' conformation of the chitosans in solution the Wales–Van Holde ratio of  $k_s/[\eta]$  was used (see Ref. 18). This ratio is known to have a value of 1.6 for compact spheres and lower values for more extended particles<sup>19,20</sup>. Rowe and colleagues<sup>12,20</sup> have shown that it is possible to estimate the axial ratio of the equivalent rigid prolate ellipsoid of revolution. This makes the ratio useful in order to obtain quantitative information concerning the asymmetry of the particle.

For Sea Cure +210 the value for  $k_s/[\eta]$  is  $0.164 \pm 0.027$  which indicates a very extended conformation, well outside the limits for random coil or sphere, with an axial ratio in the region of 200.

#### KN50 chitosan

The partial specific volume ( $0.565 \pm 0.038$ ) ml/g was

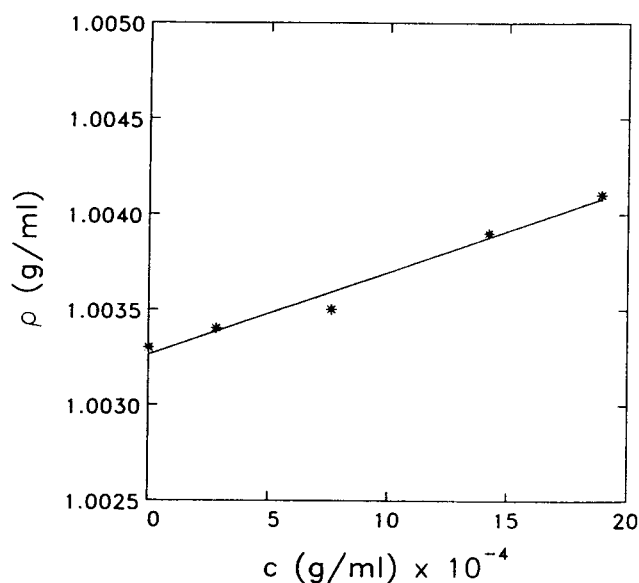


Figure 4 Graph of solution density versus concentration for chitosan KN50-1

obtained from a plot of solution density versus concentration for the undegraded KN50 fraction (Figure 4). This was taken as the value for all KN50 fractions as it is a thermodynamic property and all fractions have the same approximate composition. The value is in good agreement with that obtained for Sea Cure +210, the difference due to the greater degree of acetylation for KN50.

Sedimentation equilibrium experiments gave the molecular weights as shown in Table 1 along with the second virial coefficients ( $B$  ml mol g<sup>-2</sup>). The values of  $BM$  for the KN50 chitosans (see Table 1) are considerably lower than for Sea Cure +210, indicating that the KN50 chitosans are considerably less non-ideal than the Sea Cure. This is not unexpected when the charges on the molecules are considered.

The intrinsic viscosity of the heaviest fraction is much greater than that for the Sea Cure +210 even though the molecular weight is less than half. This indicates that the chain in the KN50 chitosans is much stiffer than that of the Sea Cure +210. The  $z$ -average diffusion coefficients of the KN50 chitosans follow, as expected, an inverse relationship to molecular weight and intrinsic viscosity. The KN50 chitosans have higher diffusion coefficients than the Sea Cure +210.

The values of the Wales-Van Holde ratio for the KN50-1 and KN50-2 chitosans are  $(0.081 \pm 0.004)$  and  $(0.677 \pm 0.152)$  respectively. These values and the axial ratios for the 'equivalent' rigid structures predicted from them ( $>200$  and approximately 10 respectively) are very diverse. KN50-1 is predicted to have a very extended rod-like conformation whereas KN50-2 is predicted to be more compact. This observation was quite unexpected, since the samples differ only in molecular weight, not in chemical composition. This indicates that the use of this method is not strictly reliable for small polysaccharides of this type. Also the ratio could not be calculated for all of the KN50 chitosans as the KN50-3 and KN50-4 fractions were too slow moving for sedimentation velocity analysis.

If, however, the whole series of KN50 chitosans is considered as part of a homologous series<sup>11</sup> then the

gross solution conformation can also be probed using two of the Mark-Houwink-Kuhn-Sakurada (MHKS) equations. The two equations used here relate molecular weight to intrinsic viscosity (equation 6) and to translational diffusion coefficient (equation 7). Others, not used here, relate molecular weight to sedimentation coefficient and radius of gyration (see e.g. Ref. 21).

$$[\eta] = K'M^a \quad (6)$$

$$D_{20,w}^0 = K''M^{-\epsilon} \quad (7)$$

The parameters  $K'$ ,  $K''$ ,  $a$  and  $\epsilon$  are empirical constants for given solute-solvent systems at given temperatures. The  $a$  and  $\epsilon$  parameters can be related to the gross solution conformation of the polymer by using the 'Haug's triangle' representation of conformation (see Figure 5). Here the three extremes of conformation – rod, random coil and compact sphere – are placed at the apexes of an equilateral triangle (see e.g. Refs 21, 22). A given conformation is represented by a locus along the sides of the triangle and the values of  $a$  and  $\epsilon$  help to define the locus. Double logarithmic plots of the experimental results (Figure 6a and 6b) provide the values of  $a$  and  $\epsilon$ . We obtain values of  $a$  and  $\epsilon$  from these plots of  $1.14 (\pm 0.27)$  and  $0.521 (\pm 0.16)$  respectively, where the errors given are those due to the fitting of a line to the data. These values approximate to a conformation between the extremes of random coil and rigid rod.

In summary the moderately acetylated KN50 chitosans approximate to a coiled molecule with low flexibility, whereas the highly deacetylated Sea Cure +210 chitosan appears to have an extended conformation with a more flexible chain.

The polyelectrolyte character of chitosan increases with increasing degree of deacetylation, which will lead to increased expansion of the more highly deacetylated chitosans at low and medium ionic strengths. On the other hand, by increasing the degree of acetylation, the rather large acetyl group would probably lead to

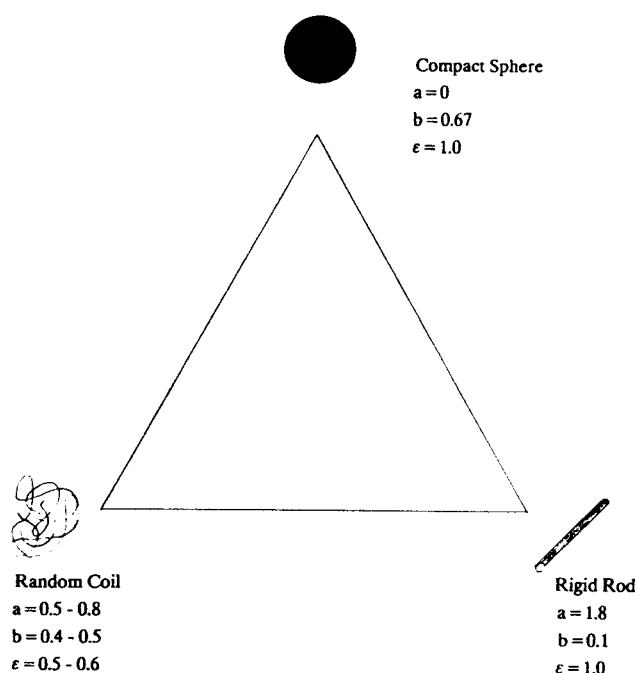
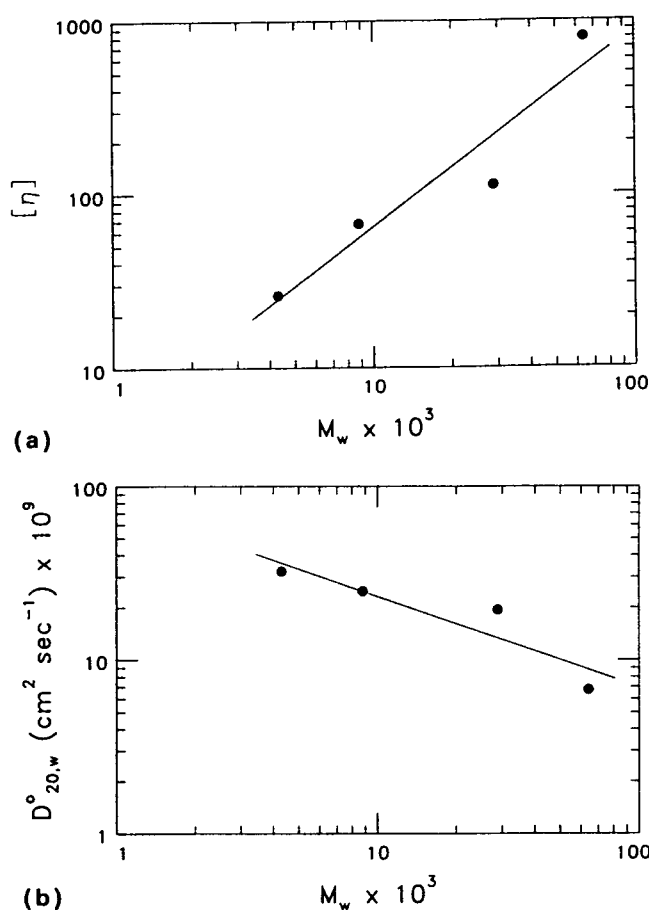


Figure 5 The 'Haug triangle' representation of macromolecular gross solution conformation



**Figure 6** Double logarithmic graphs of (a) intrinsic viscosity versus molecular weight for the KN50 chitosans, and (b) translational diffusion coefficient versus molecular weight

restricted rotation around the glycosidic linkage. This may be due to the size of the acetyl group and/or to intramolecular hydrogen bonds. It is not possible to distinguish between these effects from our experimental data. The findings of Wang *et al.*<sup>23</sup> using the MHKS  $\alpha$  parameter, show that as the degree of deacetylation of the chitosan increases the  $\alpha$  parameter decreases indicating that the chitosan chain becomes less extended.

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