

Dilute solution properties of carboxymethylchitins in high ionic-strength solvent

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The hydrodynamic characteristics in aqueous solution at ionic strength $I = 0.2$ M of carboxymethylchitins of different degrees of chemical substitution have been determined. Experimental values varied over the following ranges: the translational diffusion coefficient (at 25.0°C), $1.1 < 10^7 \times D < 2.9$ cm² s⁻¹; the sedimentation coefficient, $2.4 < s < 5.0$ S; the Gralen coefficient (sedimentation concentration-dependence parameter), $130 < k_s < 680$ mL g⁻¹; the intrinsic viscosity, $130 < [\eta] < 550$ mL g⁻¹. Combination of s with D using the Svedberg equation yielded 'sedimentation-diffusion' molecular weights in the range $40\,000 < M < 240\,000$ g mol⁻¹. The corresponding Mark-Houwink-Kuhn-Sakurada (MHKS) relationships between the molecular weight and s , D and $[\eta]$ were: $[\eta] = 5.58 \times 10^{-3} M^{0.94}$; $D = 1.87 \times 10^{-4} M^{-0.60}$; $s = 4.10 \times 10^{-15} M^{0.39}$. The equilibrium rigidity and hydrodynamic diameter of the carboxymethylchitin polymer chain is also investigated on the basis of wormlike coil theory without excluded volume effects. The significance of the Gralen k_s values for these substances is discussed. © 1998 Published by Elsevier Science Ltd. All rights reserved

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INTRODUCTION

In the last few years interest has increased considerably in the naturally occurring class of polymer known as the polysaccharides. This increase in interest has largely been due to (i) a steady decrease of the world's oil reserves for the production of synthetic polymers and (ii) a much wider appreciation that these natural polymers are ecologically pure. Polysaccharides—and their derivatives—are widely used in medicine, cosmetic, textile, paper, food and other branches of industry thanks to their unique collection of properties such as film-forming, gelling, thickening, hydrophobicity, low toxicity and biocompatibility¹. They may be used as drug and enzyme carriers, sorbents, encapsulation agents and structure-forming components. Among these polysaccharides there is a particularly growing interest in chitin, a material widely distributed in nature^{2,3}. The conversion of this polysaccharide into a water soluble form can be affected, for instance, by means of the carboxymethylation reaction^{4–9}. The chitin derivative produced in this way known as 'carboxymethylchitin' (CMCh) (Figure 1) and another derivative produced by deacetylation and known as 'chitosan' appear to be of considerable biotechnological potential¹. In contrast to chitosan, however, CMCh is soluble not only in acidic media but at any pH, making it an attractive

option in connection with its use in food products and cosmetics.

The information about the molecular characteristics of CMCh is unfortunately rather poor^{5,6}. In this paper we help to address this deficiency in our knowledge by providing a study on the hydrodynamic properties of CMCh samples and from these data we evaluate some useful molecular and conformational characteristics.

EXPERIMENTAL

Samples

CMCh samples were prepared by alkylation of chitin from crab and krill. Details of the procedure are as described in Vichoreva *et al.*^{8,9}. This procedure includes the alkylation of chitin with monochloroacetic acid (MCAA) in a water-isopropanol mixture containing NaOH after treatment of chitin also with NaOH in water-isopropanol media. With the aim of obtaining CMCh samples with various molecular weight we used different molar ratios of chitin and MCAA and also different sources of chitin (Table 1). The purification of CMCh from low-molecular-weight compounds was made by ultrafiltration of an aqueous solution through a selective membrane (retention point $\approx 1.5 \times 10^4$ Da). CMCh was then extracted from the pure solution by lyophilic drying.

The average degree of substitution of each CMCh preparation by carboxylic and *N*-acetyl groups was estimated by i.r. spectroscopy⁸, recorded under acidic (pH = 0.6) solution conditions in D₂O. Carboxylic and *N*-acetyl groups were calculated from the integrated intensity of the

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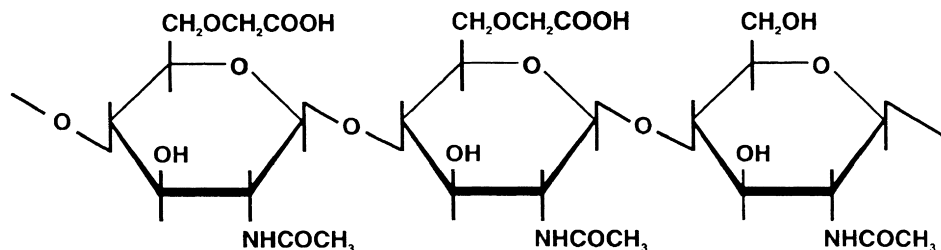


Figure 1 Three $\beta(1-4)$ linked residues in 6-*O*-carboxymethylchitin (CMCh) showing two residues carboxymethylated

Table 1 Composition of the 12 CMCh samples: $\{C_6H_7O_2(OH)_{2-x}(OCH_2COONa)_x(NHCOCH_3)_{1-z}(NH_2)_z\}$

CMCh sample	x	z	M_0
1	1.22	0.15	294
2	1.30	0.15	301
3	1.15	0.20	287
4	1.10	0.17	284
5	1.26	0.15	298
6	1.15	0.20	287
7	1.12	0.20	284
8	1.08	0.25	279
9	0.70	0.30	246
10	1.17	0.15	290
11	1.15	0.23	285
12	0.63	0.48	233

M_0 = molecular weight of the CMCh repeat unit, calculated from the chemical formula and the values of x and z .

absorption bands at wavenumber $\nu = 1728 \text{ cm}^{-1}$ (C=O) and $\nu = 1640 \text{ cm}^{-1}$ (amide I), respectively, and assuming respective molar absorption coefficients of 50 000 and 43 000 $\text{L mol}^{-1} \text{ cm}^{-2}$, respectively. Identical results were obtained by using the ^{13}C n.m.r. method^{10,11}, where it has been shown that nearly 90% of carboxymethyl substituents are situated at the C6 carbon atoms of each monosaccharide unit of the macromolecule.

Twelve CMCh samples ('CMCh 1–12', Table 2) were thus prepared of differing estimated degrees of substitution by carboxymethyl groups (x) and acetyl groups ($1 - z$) according to the structural formula: $C_6H_7O_2(OH)_{2-x}(OCH_2COONa)_x(NHCOCH_3)_{1-z}(NH_2)_z$.

The mean values and standard deviations for x and $(1 - z)$ over the whole series of samples were 1.1 ± 0.1 and 0.78 ± 0.06 , respectively.

Solvent

In pure deionised water CMCh manifests classical polyelectrolyte behaviour^{12–14} (Figure 2). The CMCh chains may be considered as polyanions because the number of negative charges in the chain is considerably larger than the number of positive charges. Because the principal aim of this study is the evaluation of the molecular characteristics it is of course necessary to suppress the primary polyelectrolyte effects using the well-known procedure of addition of low molecular weight electrolyte. In our case the study was carried out in so-called 'Paley-buffer': ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$), 0.05 M, pH 7.0¹⁵, popularly used for protein work. The ionic strength can be changed by supplementary addition of NaCl (0.15 M in our case). The hydrodynamic studies were performed in 0.2 M solvent where the primary polyelectrolyte effects may be considered as suppressed. The solvent had the following characteristics at 25.0°C: density $\eta_0 = 1.0060 \text{ g mL}^{-1}$ and viscosity $\eta_0 = 0.91 \text{ cP}$.

Sedimentation

Velocity sedimentation was investigated using the Beckman modell-E analytical ultracentrifuge with a rotor speed of 47 660 rev min^{-1} in a single-sector cell of optical path 1.2 cm. Sedimentation coefficients, s , were calculated from the displacement of unimodal Schlieren curve maxima X as a function of time. Semi-automatic data capture was employed using a graphics digitising tablet¹⁶. Photographs were taken at 4 min intervals during 56 min, and negatives enlarged directly onto a graphics tablet interfaced to an IBM PC. The resulting records of the position of the sedimenting boundaries could then be analysed using the QUICKBASIC–NCMH algorithm MOD_EVEL to yield the sedimentation coefficient s_T values at temperature T .

Table 2 Hydrodynamic and molecular characteristics of carboxymethylchitin preparations (N = 1–12) in buffered solvent at 25.0°C

CMCh	$[\eta]$ (mL g^{-1})	k'	s_0 (S)	k_s (mL g^{-1})	$10^7 \times D_0$ ($\text{cm}^2 \text{ s}^{-1}$)	$\Delta n/\Delta c$ (mL g^{-1})	$10^{-3} \times M_{SD}$ (g mol^{-1})	$10^{10} \times A_0$	$10^{-7} \times \beta_s$
1	550	0.38	5.05	681	1.12	0.114	239	3.75	1.35
2	539	0.36	4.29	479	1.20	0.100	190	3.69	1.19
3	501	0.37	4.29	500	–	–	186 ^a /192 ^b	–	–
4	494	0.44	4.11	487	–	–	173 ^a /178 ^b	–	–
5	389	0.56	4.20	394	1.27	0.108	176	3.42	1.15
6	406	0.39	4.26	434	1.58	0.100	143	4.02	1.38
7	340	0.43	3.58	257	1.65	0.116	115	3.69	1.12
8	342	0.35	3.43	180	1.79	0.116	102	3.84	1.04
9	284	0.50	3.28	240	1.73	0.119	101	3.48	1.10
10	141	0.36	3.09	183	2.59	0.106	63	3.54	1.30
11	132	0.40	2.99	136	–	–	56 ^a /58 ^b	–	–
12	130	0.66	2.43	130	2.93	0.122	44	3.45	1.15

^afrom equation (8). ^bfrom equation (9).

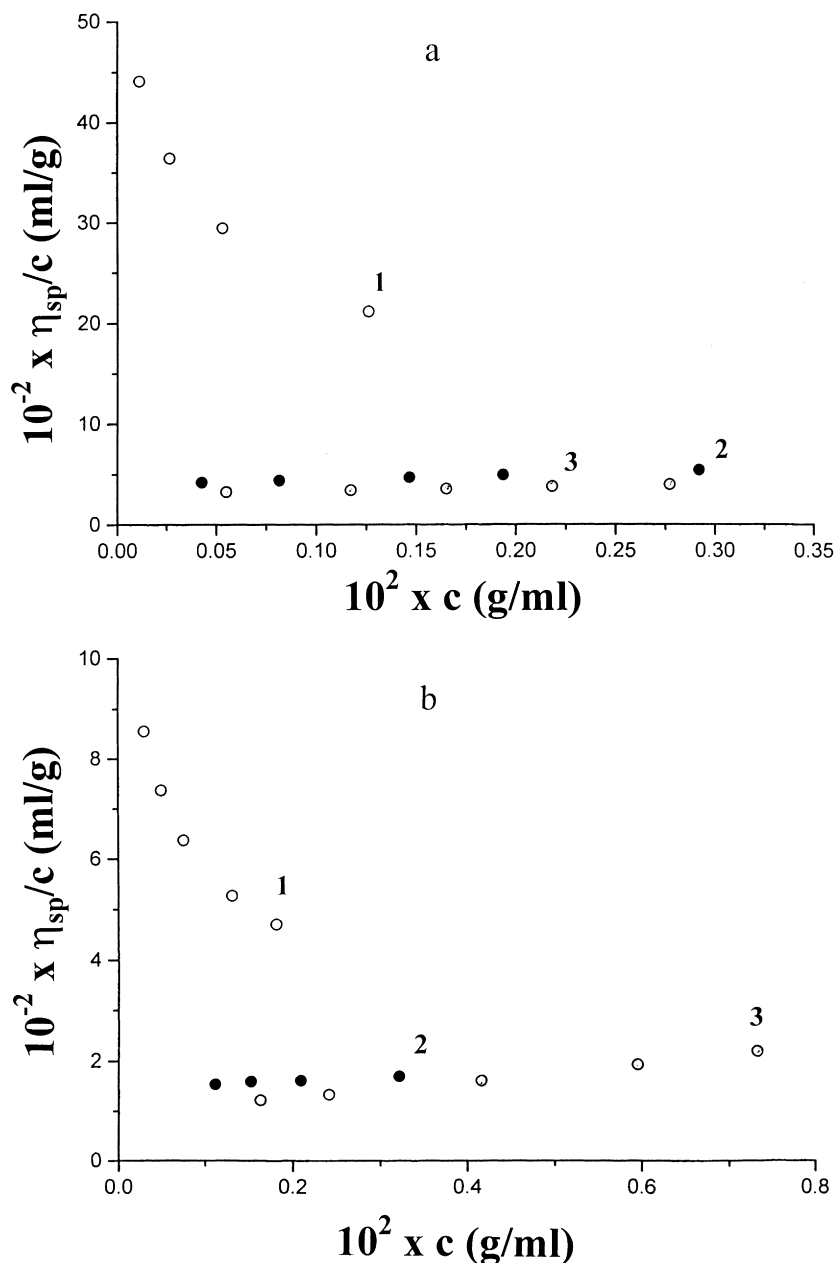


Figure 2 Plots of ρ_{sp}/c versus c for two CMChs in: (1) pure deionised water; (2) $I = 0.05$ M; (3) $I = 0.2$ M. (a) CMCh-8, (b) CMCh-10

s_T values were corrected to the solvent density and viscosity at 25.0°C (s_{25}) using the standard procedure described in 12,17,18. The dependence of s_{25} , subsequently referred to as 's' on concentration, c (g mL^{-1}) as described by the relation $s^{-1} = s_0^{-1}(1 + k_S c)$ was studied in the concentration range $(0.03\text{--}0.47) \times 10^{-2} \text{ g mL}^{-1}$ (Figure 3). The 'infinite dilution' s_0 and the 'Gralen coefficient' k_S obtained in this way are presented in Table 2.

Translational diffusion

The translational diffusion coefficients were determined from the time-dependence of dispersion of the diffusion boundary formed in a glass cell of optical path length, 3.0 cm at an average solution concentration $c = 0.03 \times 10^{-2} \text{ g mL}^{-1}$. D_T values obtained at temperature T were corrected to 25.0°C values using the standard procedure^{12,17}. The values of D_{25} (hitherto referred to as 'D' values) obtained at these low concentrations were assumed to be the values extrapolated to zero concentration, D_0 . This is reasonable since the concentration dependence of D is

generally smaller than for other hydrodynamic coefficients such as the sedimentation coefficient, s , or the reduced specific viscosity, h_{sp}/c ¹⁹. The optical system for recording the solution–solvent boundary in diffusion analyses was a Lebedev's polarizing interferometer¹⁷. The refractive index increment $\Delta n/\Delta c$ was determined from the area spanned by the interference curve, and its average value ($\Delta n/\Delta c$) was $0.111 \pm 0.007 \text{ mL g}^{-1}$ at a wavelength $\lambda = 550 \text{ nm}$.

Intrinsic viscosity

Measurements of viscosity were made at 25.0°C using a 2 mL Schott–Geraete automatic Ostwald glass capillary viscometer. The flow time of the solvent $\tau_0 = 84.5 \text{ s}$. The Huggins relationship^{12,17} was employed for calculating the values of intrinsic viscosity $[\eta]$ and Huggins' parameter K_H : $\eta_{sp}/c = [\eta] + K_H[\eta]^2 c + \dots$ (The average value of K_H was 0.43 ± 0.07).

Buoyancy factor

The buoyancy factor or 'density increment' $(1 - \nu\rho_0) =$

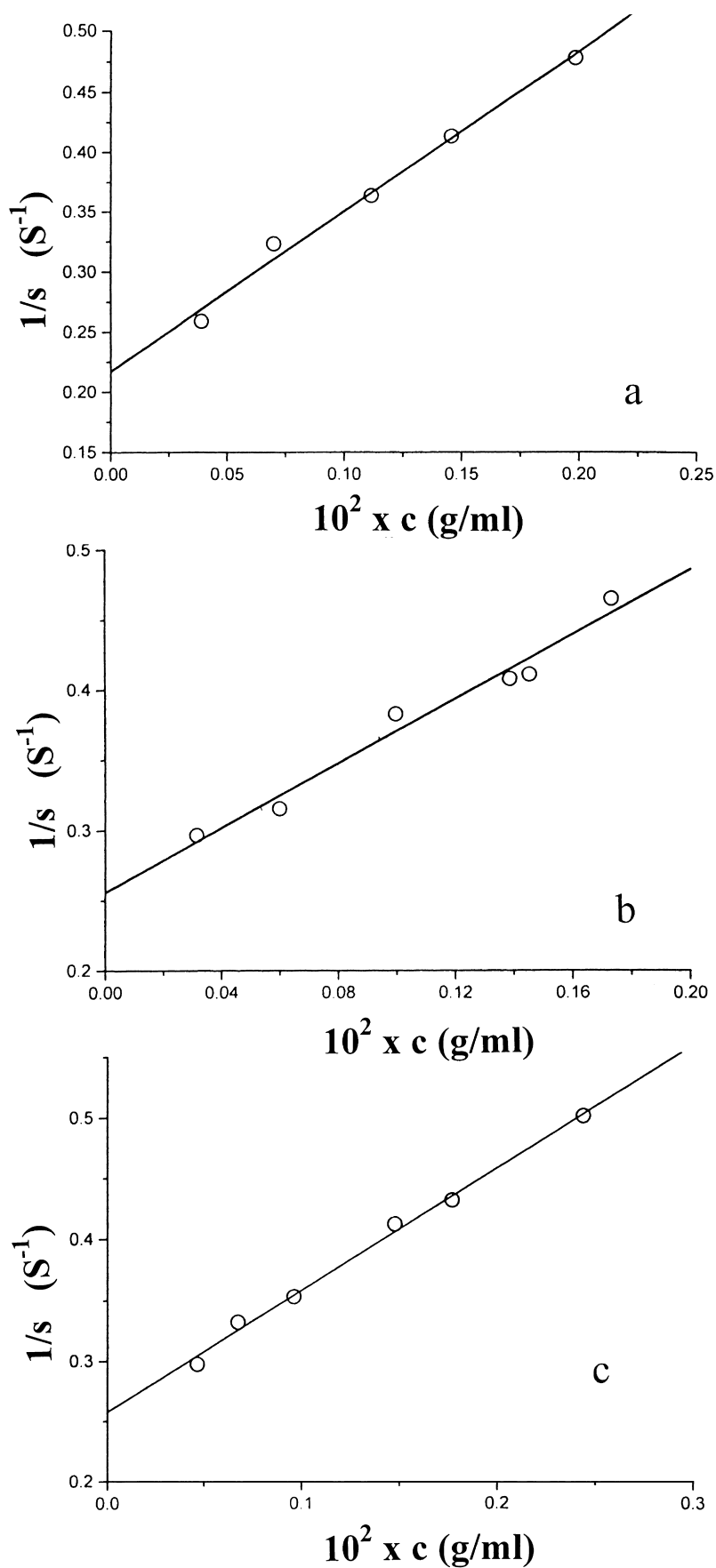


Figure 3 Concentration dependence of the (reciprocal) sedimentation coefficient s^{-1} for three CMChs in $I = 0.2$ m solvent: (a) CMCh-1; (b) CMCh-3; (c) CMCh-6

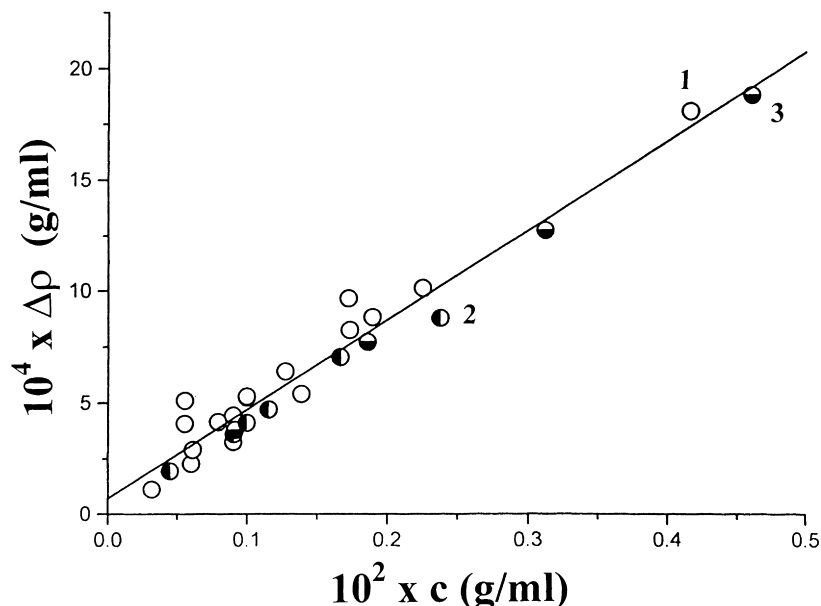


Figure 4 Dependence of density increment $\Delta\rho = \rho - \rho_0$ on concentration, where ρ and ρ_0 are the density of solution and solvent respectively for CMCh in the following solvents: (1) pure water; (2) $I = 0.05$ M; (3) $I = 0.2$ M. The slope of this dependence is the buoyancy factor $(1 - v\rho_0)$

$\Delta\rho/\Delta c = 0.467$, where v is the partial specific volume of polymer, was measured with an Anton Paar (Graz, Austria) density meter accordingly to the procedure of Kratky *et al.*²⁰ (Figure 4). The same value of $\Delta\rho/\Delta c$ was obtained for CMChs in both pure water and in the $I = 0.2$ solvent.

RESULTS AND DISCUSSION

Matrix of hydrodynamic values

The set of hydrodynamic data $[\eta]$; D_0 ; s_0 and k_S obtained for different samples may be considered as a primary 'experimental matrix' (4 columns and 12 rows; Table 3) describing the hydrodynamic properties of the CMChs. It is possible to transform this 'experimental matrix' into a new one composed of molecular weights and hydrodynamic parameters. The ability to perform this transformation followed by the *subsequent* extraction of molecular information is based on the fundamental relationships which exist that relate each of these parameters with the molecular weight polymeric chain size (in terms of the mean-square end-to-end distance of the chain, $\langle h^2 \rangle$)^{12,17,21–23}:

$$[\eta] = \Phi_0 \langle h^2 \rangle^{3/2} \times M^{-1} \quad (1)$$

$$D_0 = k_B T / f = k_B T (P_0 \eta_0 \langle h^2 \rangle^{1/2})^{-1} \quad (2)$$

$$s_0 = M(1 - v\rho_0) f^{-1} N_A^{-1} \quad (3)$$

$$k_S = B \langle h^2 \rangle^{3/2} \times M^{-1} \quad (4)$$

In these relations k_B is Boltzmann's constant, f is the translational frictional coefficient, and N_A is Avogadro's number. Φ_0 and P_0 are the Flory²⁴ parameters²⁴ and B is another dimensionless parameter.

The well-known Svedberg formula for molecular weight determination follows from equations (2) and (3):

$$M_{SD} = [RT/(1 - v\rho_0)] s_0 / D_0 = R[s]/[D] \quad (5)$$

where $[s] = s_0 \eta_0 / (1 - v\rho_0)$, $[D] = D_0 \eta_0 / T$, R is the gas constant and the subscript 'SD' means from the sedimentation and diffusion coefficients. Elimination of $\langle h^2 \rangle$ from equations (1)–(3) give the expression for the Mandelkern–Flory–Tsvetkov–Klenin parameter A_0 ^{25,26}:

$$\text{or} \quad A_0 = R[s][\eta]^{1/3} M^{-2/3} \quad (6a)$$

$$A_0 = R[D]^2 [s][\eta]/100 \quad (6b)$$

where $[\eta]$ is in mL g⁻¹ (NB some workers still prefer to use dL g⁻¹: in this case $[\eta]/100$ in equation (6) should be replaced by $[\eta]$). The elimination of $\langle h^2 \rangle$ from equations (2)–(4) provides us with the possibility of calculating the sedimentation parameter^{23,27}

$$\text{or} \quad \beta_S = N_A [s] k_S^{1/3} M^{-2/3} \quad (7a)$$

$$\beta_S = N_A \{ R^{-2} [D]^2 [s] k_S \}^{1/3} \quad (7b)$$

Table 3 Scaling relation parameters between the hydrodynamic values and molecular weight for CMCh in 0.2 M buffer

CMCh	Correlation parameters	b_i	Δb_i	K_i	r_i
1	$s_0 - D_0$	-0.63	0.09	2.08×10^{-17}	-0.9385
2	$D_0 - [\eta]$	-0.61	0.06	5.61×10^{-6}	-0.9681
3	$s_0 - [\eta]$	0.34	0.05	5.18×10^{-14}	0.9150
4	$k_S - s_0$	2.88	0.33	7.0	0.9555
5	$[\eta] - M$	0.94	0.08	5.58×10^{-3}	0.9672
6	$D_0 - M$	-0.60	0.03	1.87×10^{-4}	-0.9908
7	$s_0 - M$	0.39	0.03	4.10×10^{-15}	0.9752

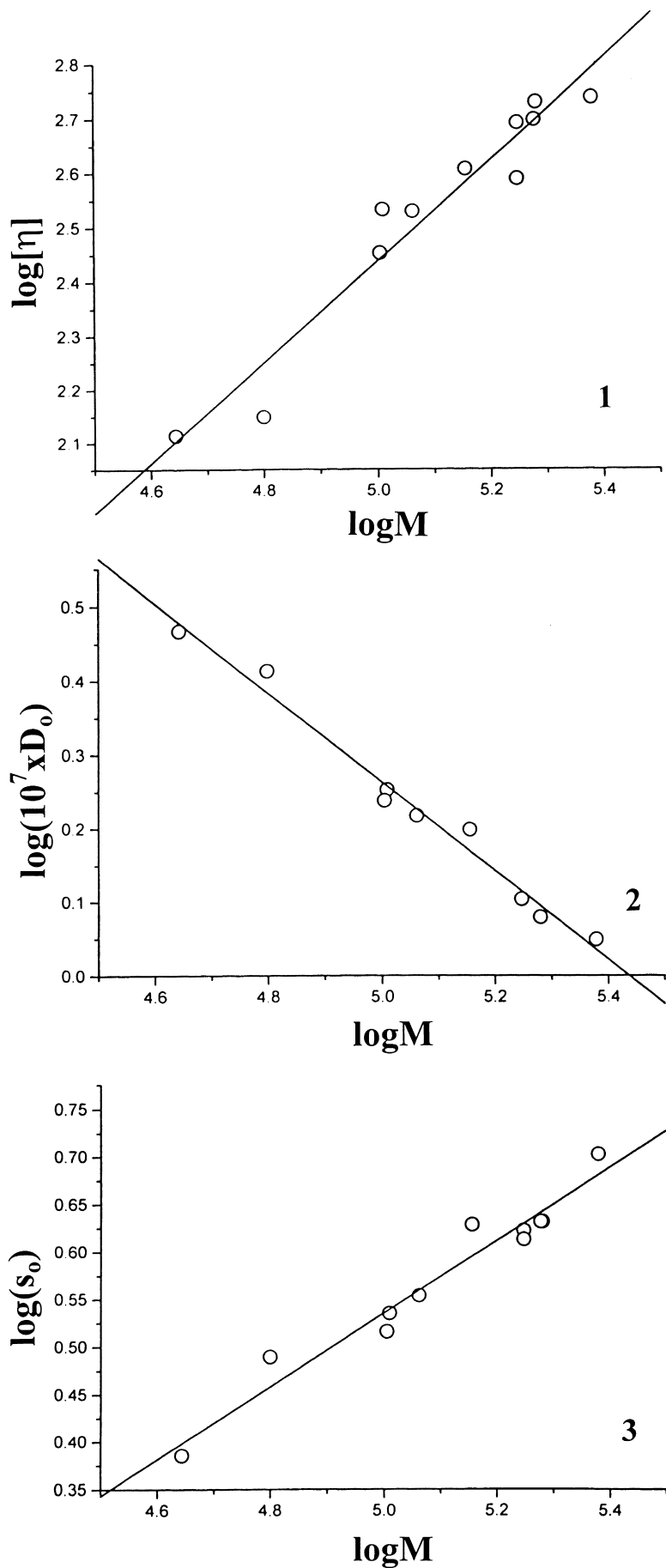


Figure 5 MHKS double logarithmic 'scaling' plots for CMCh samples at 25°C in $I = 0.2$ M solvent. (1) viscosity. (2) diffusion. (3) sedimentation

Values for all these parameters are presented in Table 2. The mean value (over all the CMChs) of the hydrodynamic parameters A_0 and β_s are, respectively, $(3.65 \pm 0.08) \times 10^{-10} \text{ erg K}^{-1} \text{ mol}^{1/3}$ and $(1.20 \pm 0.04) \times 10^7 \text{ mol}^{1/3}$, values which are characteristic of linear polymers^{23,27,28}. These values of A_0 and β_s were then utilised for calculation of the polymer molecular weight from both s_0 and $[\eta]$ and also from s_0 and k_s for samples 3, 4 and 11 by using the following formulae^{29,30}:

$$M_{s\eta} = (R/A_0)^{3/2} [s]^{3/2} [\eta]^{1/2} \quad (8)$$

$$M_{ks} = (N_A/\beta_s)^{3/2} [s]^{3/2} k_s^{1/2} \quad (9)$$

For a homologous series of polymers the ‘scaling relations’ between the hydrodynamic parameters and molecular weight can be investigated: These are the well known Mark–Houwink–Kuhn–Sakurada type relations: $Pr_i =$

$K_{ij} Pr_j^{b_{ij}}$, where $Pr_i, Pr_j \equiv [\eta], s_0, D_0, k_s$ ($i \neq j$) and Pr_j may also be the molecular weight M (Figure 5). ‘Pr’ simply stands for ‘property’, and the indices b_{ij} are presented in Table 3. Taking into account experimental error, a good correlation is observed between these scaling indices. This correlation follows also from the fundamental relationships equations (1)–(4):

$$b_1 = b_7/b_6; \quad b_2 = b_6/b_5; \quad b_3 = b_7/b_5; \quad (10)$$

$$b_4 = (2 - 3b_7)/b_7; \quad |b_6| = 1/3(1 + b_5); \quad |b_6| + b_7 = 1$$

and is a the characteristic feature of homologous series of polymers.

Equilibrium rigidity of CMCh chains

The interpretation of hydrodynamic data requires the evaluation of the role of volume interactions and their effect

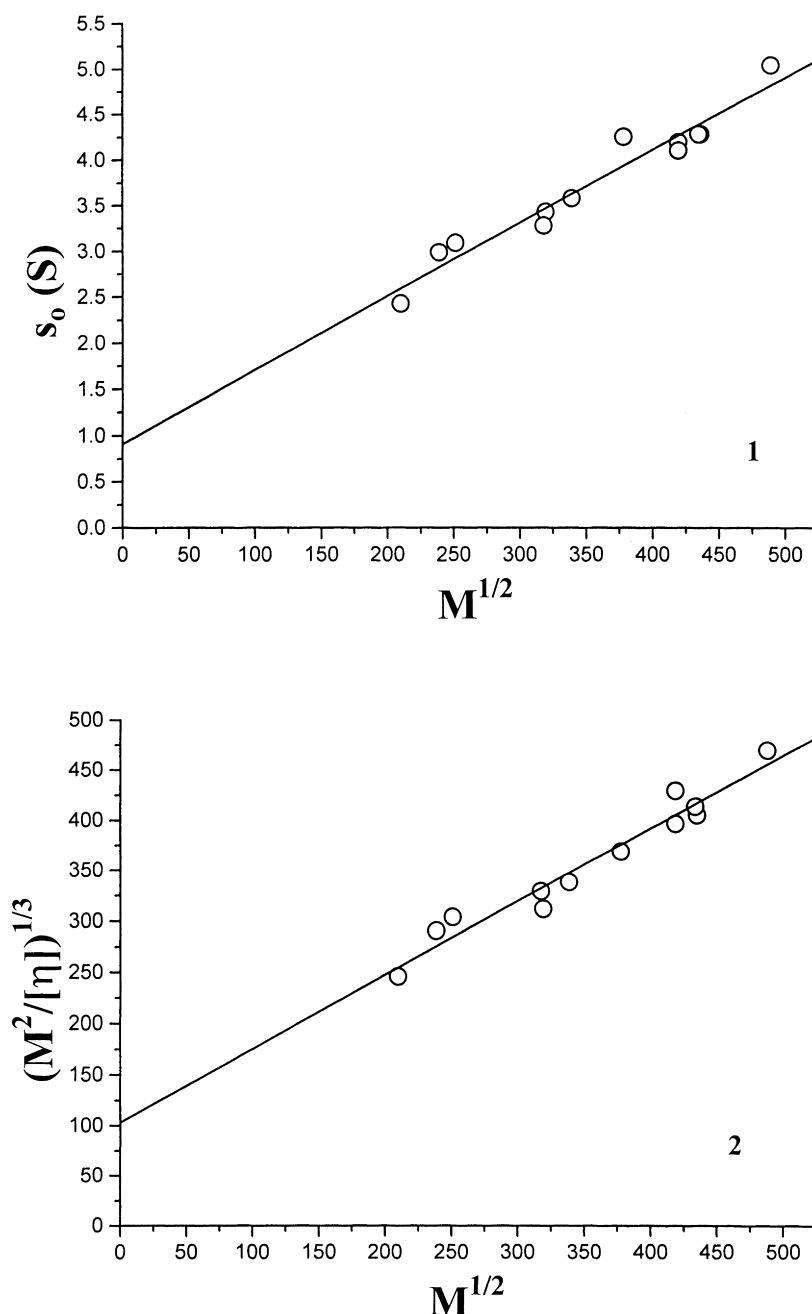


Figure 6 s versus $M^{1/2}$ (a) and $\{M^2/[\eta]\}^{1/3}$ versus $M^{1/2}$ plots for CMChs

on the dimensions of the macromolecules being studied. On each occasion when a specific polymer–solvent system is studied, this problem is tackled by taking into account the chemical structure of the polymer chain together with any additional information from other sources. Using hydrodynamic data alone, it is possible to evaluate the predominant influence of either volume effects or the competing effects of intrachain draining only if the hydrodynamic data are available over a very large range of molecular weight, M ($M_{max}/M_{min} \approx 10^2-10^3$): one can then decide on the basis of change in the Mark–Houwink–Kuhn–Sakurada exponents, and in particular the most sensitive one from the dependence of $[\eta]$ on M . In our case this is not possible because only a fivefold range of M is available. A more sensitive assay for establishing which type of interaction predominates is Hearst’s plot of $M/[\eta]$ versus $M^{1/2}$.^{31–33} In our case such a plot is characteristic of relatively short linear chains with the predominance of draining effects over volume effects. This is also indicated by the fact that the plot of Burchard–Stockmayer–Fixman^{34,35} which is used to remove the volume effects in linear polymer chains leads for CMCh chains to a result (negative intercept on the ordinate) which has no physical meaning. This behaviour also implies that the intramolecular draining effects do indeed prevail and volume effects may be neglected to a first approximation. Further interpretation will now be carried out on the basis of theories describing chain behaviour without volume interactions.

Translational friction

Data on translational friction will now be considered in the framework of the Hearst–Stockmayer and/or Yamakawa–Fujii theories describing the behaviour of a wormlike necklace³⁶ or a wormlike cylinder³⁷. For chains fulfilling the condition $L/A > 2.28$, where L is the contour length of the macromolecule and A is the length of the Kuhn segment, the following analytical expression is obeyed:

$$[s]N_A P_0 \equiv M[D]P_0 k^{-1} = (M_L/A)^{1/2} M^{1/2} + (P_0 M_L/3\pi) \times [\ln(A/d) - \varphi(0)] \dots \dots \quad (11)$$

where $M_L = M/L$ and d is the hydrodynamic chain diameter. The coefficient $\varphi(0)$ has been evaluated as either 1.431³⁶ or 1.056³⁷. Figure 6 describes the dependence of s_0 on $M^{1/2}$. The slope of this curve may be used to evaluate the Kuhn segment length, A (or the ‘persistence length’, $L_p = A/2$ ³⁸) which characterizes equilibrium chain rigidity, whereas the intercept is a manifestation of the hydrodynamic diameter d . As a result, the following values are obtained: $A_f = 240 \times 10^{-8}$ cm and $d_f = 13.6 \times 10^{-8}$ cm where the subscript ‘f’ refers to the fact that these coefficients have been calculated from the translational frictional based parameters (s or D). These values for A_f and d_f have been obtained by using the value of Flory hydrodynamic parameter $P_0 = 5.11$ ²⁰.

Rotational friction/intrinsic viscosity

Direct application of the dependence of $M/[\eta]$ on $M^{1/2}$ following from Hearst’s theory does not make it possible to evaluate equilibrium rigidity from viscometric data. This impossibility is a consequence of the narrow range of molecular weight M and of the fact that our data are available in the region of change in the slope in such a representation. Therefore, this plot is very sensitive to the peculiarities of hydrodynamic interaction on chains of these relative lengths.

In this case Bushin’s plot^{39,40} can be used for the quantitative interpretation of viscometric data. This plot has repeatedly been used to interpret data for rigid-chain polymers including $\beta(1-4)$ glucans and is analogous to the plot corresponding to equation (11) in the interpretation of viscometric data. This analogy is based on the widely used assumption that the chain sizes in the same macromolecules are equivalent in the phenomena of translational and rotational friction ($\langle h^2 \rangle_f = \langle h^2 \rangle_r$), which can be expressed in the analytical form as follows:

$$[s]P_0 N_A = (M^2 \Phi_0 / [\eta])^{1/3} \quad (12)$$

Hence, viscometric data (essentially a manifestation of rotational frictional properties) can be processed according to equations (11) and (12) in a system of coordinated $(M^2/[\eta])^{1/3}$ versus $M^{1/2}$. This plot is also shown in Figure 6. The slope of this dependence is used to determine the Kuhn segment length ($A_\eta = 240 \times 10^{-8}$ cm) and the intercept serves to determine the hydrodynamic diameter ($d_\eta = 8.6 \times 10^{-8}$ cm). These values are obtained by using the value of Flory hydrodynamic parameter $\Phi_0 = 2.87 \times 10^{23(20)}$.

Equation (12) can be rearranged as follows:

$$[s] = (M^2 \Phi_0 / [\eta])^{1/3} / P_0 N_A = (M^2 \Phi_0 / [\eta])^{1/3} (A_0 / R) \quad (13)$$

When experimental values of A_0 and M_{SD} are used, equation (13) degenerates into an identity and, hence the plot of $(M^2/[\eta])^{1/3}$ versus $M^{1/2}$ in this case is not independent. The use of theoretical values of P_0 and Φ_0 implies that the corresponding theoretical value of A_0 is used:

$$A_0 \equiv k P_0^{-1} \Phi_0^{1/3} = 3.87 \times 10^{-10}$$

Equilibrium rigidity in comparison with other $\beta(1-4)$ glucans

Good agreement exists between the translational frictional and viscosity values of the Kuhn length A and the diameter d . Table 4 gives the values of A and d obtained in this work in comparison with the corresponding values for chitosan^{41,42} and methylcellulose^{43,44}. The equilibrium rigidity values of CMCh and chitosan molecules (which are obtained in solutions at high ionic strengths) are comparable, and consistent with a semi-flexible polymer

Table 4 Values of equilibrium rigidity A and hydrodynamic diameter d of CMCh, chitosan and methylcellulose. A_f , d_f : determined from translational frictional properties; A_h , d_h : determined from viscometric properties

Polymer	$10^8 \times A_f$ (cm)	$10^8 \times d_f$ (cm)	$10^8 \times A_h$ (cm)	$10^8 \times d_h$ (cm)	Refs
CMCh	240	13.6	240	8.6	This work
Chitosan	240	7.0	180	3.6	41,42
Methyl-cellulose	180	7.4	130	4.8	43,44

chain (for a more extensive comparison with other polysaccharides, in terms of the persistence length $L_p = A/2$, see Table 1.3 of Ref. 1). The hydrodynamic diameter is greater in the case of CMCh, which seems reasonable because the length of substituents in CMCh is greater than that in chitosan. However, the rigidity of both these chitin derivatives is greater than that for chains of water-soluble methylcellulose: this difference is possibly caused by the incompletely suppressed electrostatic interactions in the chains of the CMCh and chitosan.

Concentration coefficients of sedimentation

The values of k_s may be compared to those of $[\eta]$ ^{45,46}. Both characteristics have the same dimensionality. This

comparison is shown in *Figure 7* which may be used to evaluate the dimensionless parameter $k_s/[\eta]$ obtained from the slope of this figure. For the CMCh–0.2 M buffer solvent system the value of $k_s/[\eta]$ is unity. This value is smaller than $\approx 1.6\text{--}1.7$ ⁴⁴ which is usually obtained for flexible-chain macromolecules and is characteristic of draining macromolecules^{46,47}. As repeatedly mentioned in theoretical papers^{48,49} and from discussions deriving from the experimental results of velocity ultracentrifugation^{22,50,51}, a comparison of s_0 and k_s values obtained in one series of experiments can provide molecular information on the macromolecular system being studied. This is possible if the concept of the sedimentation parameter β_s is used and the molecular weight of samples may be evaluated from the

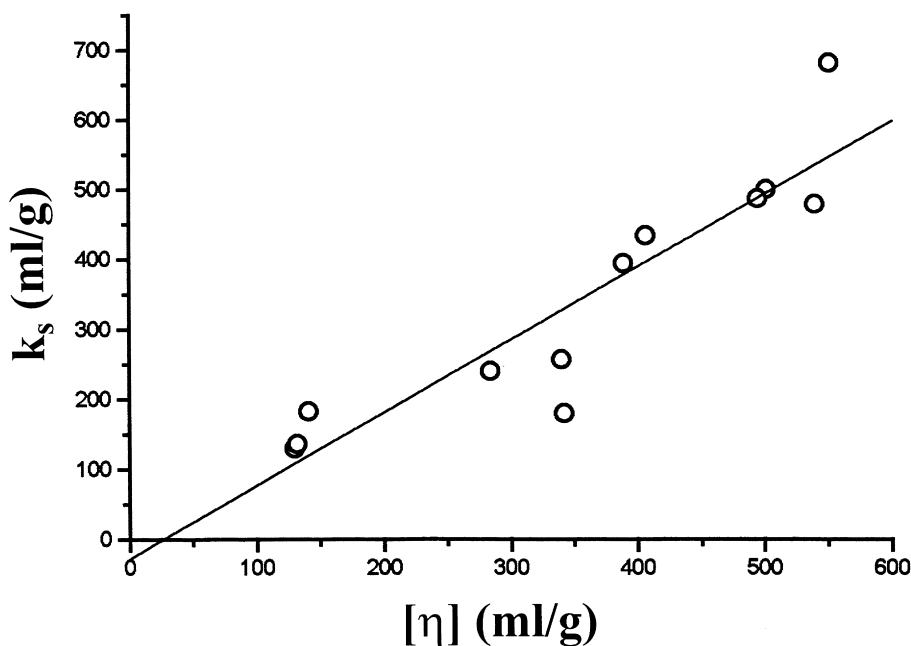


Figure 7 Dependence of the Gralen sedimentation concentration-dependence parameter k_s on intrinsic viscosity $[\eta]$ for CMChs. The slope of this plot $k_s/[\eta] = 1.0$, with a linear correlation coefficient $r = 0.9156$

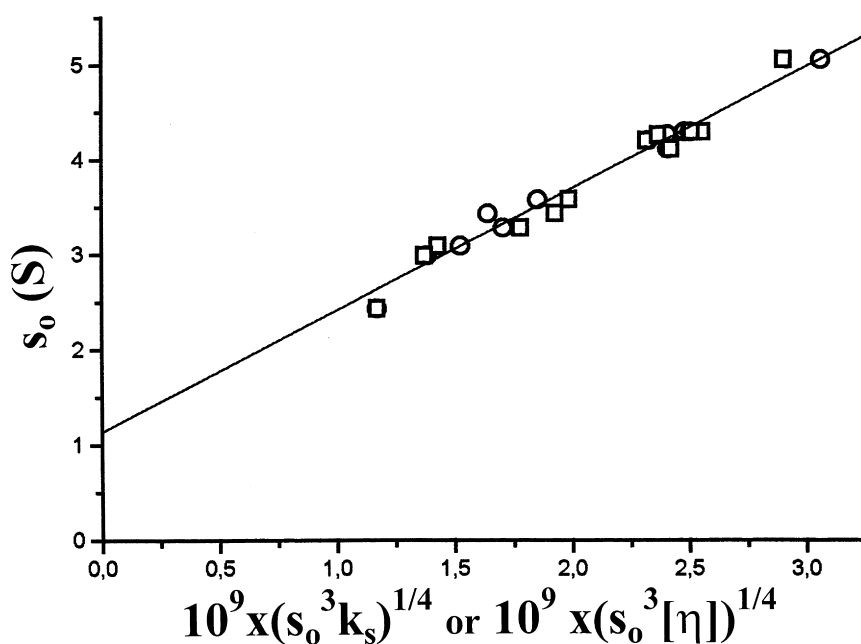


Figure 8 Dependence of s_0 on $(s_0^3 k_s)^{1/4}$ (circles) and $(s_0^3 [\eta])^{1/4}$ (squares)

generalized Wales–van Holde–Rowe equation:

$$M_{KS} = (N_A/\beta_s)^{3/2} [s]^{3/2} k_s^{1/2} \quad (14)$$

where ‘KS’ means from the sedimentation coefficient and its concentration dependence or ‘Gralen’ parameter. Thus, an analogue of equation (11) is the plot of s_0 versus $(s_0^3 k_s)^{1/4}$ (Figure 8), which can also be applied to assess the equilibrium rigidity and estimate the hydrodynamic diameter. Using the mean experimental value of β_s obtained in this work, we obtain $A_{KS} = 280 \times 10^{-8}$ cm from the slope of Figure 8 and $d_{KS} = 10 \times 10^{-8}$ cm from the intercept ($r = 0.9901$). The values which have thus been obtained on the basis of sedimentation data alone are in satisfactory agreement with the values of A_f , A_η and d_f , d_η considered above. It is worth remarking that since the average experimental value of the dimensionless parameter $k_s/[\eta]$ is ~ 1.0 for the polymer–solvent system being investigated, the plot of s_0 on $(s_0^3 k_s)^{1/4}$ must virtually coincide with that of s_0 on $(s_0^3 [\eta])^{1/4}$ (Figure 8). This coincidence also implies that the sedimentation parameter β_s and the hydrodynamic parameter $\beta \equiv A_0/k$ expressed in corresponding units also virtually coincide: $\beta \equiv A_0/k = (1.23 \pm 0.02) \times 10^{-10}$ mol^{1/3}. All the above reflects the fact that the hydrodynamic parameters Φ_0 and B in equations (1) and (4) are virtually identical for the CMChs. It is also worth stressing that this conclusion is a strong one because it is supported by three fundamentally independent approaches.

Polydispersity effects

$\beta(1-4)$ glucans are usually relatively monodisperse polymers compared to other polysaccharides. The polydispersity indices, M_w/M_n for these substances and their derivatives are generally < 1.2 ^{52–54}. Calculations show⁵⁵ that undisturbed chain dimensions do not significantly change across the range $1 < M_w/M_n < 1.5$ and more recent work^{56,57} has shown that the estimation of equilibrium rigidity is also not significantly affected.

CONCLUDING REMARKS

This study has thus demonstrated some important molecular characteristics underpinning the functional properties of carboxymethylchitins. It has been shown that the hydrodynamic behaviour can be adequately interpreted in terms of a wormlike coil in the absence of volume effects to a first approximation. The effect of different charge density on the equilibrium rigidity for carboxymethylchitins possessing a net negative charge does not appear to be significant, and the equilibrium rigidity of the carboxymethylchitin chain has been shown to be comparable to those for chitosans (deacetylated chitin) at high ionic strength, which, unlike carboxymethylchitins are only soluble in acidic conditions. Further development of this investigation could involve:

- (1) the study of the effect of different ionic strengths on the equilibrium rigidity;
- (2) the behaviour of truly polyampholytic material ($x = z$); and
- (3) in work on progress, on the properties of CMCh film-sand would be part of a general programme to add to our general understanding of polyelectrolyte materials differing in nature and also of self-organizing molecular systems.

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