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Chitosan: chain rigidity and mesophase formation

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ABSTRACT

Light-scattering measurements on samples of chitosan with degrees of acetylation (d.a.) of 42 and 15% have been performed in 0.1M acetic acid–0.2M sodium chloride. From the values of $R_G°$, a persistence length $q_t°$ of ~220 Å has been deduced, which is lower than that (~350 Å) of chitin and indicates a decrease of rigidity with decrease in d.a. The existence of a cholesteric mesophase above a critical value of [polymer] has been demonstrated.

INTRODUCTION

(1→4)-Linked copolymers of 2-acetamido-2-deoxy-β-D-glucose and 2-amino-2-deoxy-β-D-glucose, usually known as chitosan, show peculiar properties from both the macromolecular and applied points of view[1]. Poly-β-D-Glc*p*NAc (chitin) is an important natural polysaccharide and its macromolecular behavior has been studied only recently. Terbojevich *et al.*[2], using a non-degradative solvent (*N*,*N*-dimethylacetamide containing 5% of LiCl), showed that the main chain of chitin is rigid at room temperature, so that mesomorphic properties may be expected at a sufficiently high concentration of polymer. The persistence length, determined by light scattering, is ~350 Å, a value that is higher than those found[3,4] for cellulose in the same solvent where the existence of a cholesteric mesophase has been demonstrated[5]. However, the formation of gels[6] at concentrations of chitin as low as 1% has hampered similar observations.

Poly-β-D-Glc*p*N can be prepared by *N*-deacetylation of chitin, a process which generally reduces the molecular weight and gives a polymer that is strongly charged and soluble in acid media[1]. The rigidity of this fully deacetylated chain has not been determined hitherto.

Rinaudo and Domard[7] found a persistence length of 300 Å, in the absence of electrostatic contribution, for a sample of chitosan with a degree of acetylation (d.a.) of

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~15%. The value was obtained from viscosity data by applying a method based on Odijk's theory[8].

The viscosities of samples of chitosan with various d.a. have been examined[9] at different ionic strength (I) and treated according to the method of approximation suggested by Smidsrød and Haug[10]. The rigidity parameter (B) decreased with increase in d.a., indicating an opposite trend of the rigidity.

Ogura *et al.*[11] found that 30–90% solutions of chitosan (d.a. ~10%) in aqueous 10% acetic acid showed a swirl-like (finger print) pattern under the polarising microscope when sheared between glass plates. These results suggest that chitosan represents a family of rigid polymers regardless of d.a., and consequently they should form mesomorphic solutions.

The aim of this work was to determine the persistence length of chitosan having various d.a. and to confirm the existence of a mesophase at a sufficiently high concentration.

EXPERIMENTAL

Materials. — Samples of commercial chitosan (A and B, respectively) from Rybex Krill, *Euphasia superba* (Fisheries Central Board, Szczecin, Poland) and *Chionocetes japonicus* (Katakura Chikkarin, Tokyo, Japan), were used. The d.a. specified by the producers, 42% for A and 15% for B, were confirmed by ^{1}H-n.m.r. and u.v. spectroscopy[12].

In order to obtain samples with a lower d.p., samples A and B were hydrolysed with acid under different conditions of pH and temperature. The products were recovered by precipitation with M NaOH and their characteristics are reported in Table I. The d.a. was determined by u.v. analysis and the molecular weight by light scattering.

Phenyl isocyanate-modified chitosan. — Chitosan A (0.8 g), dispersed in *N,N*-dimethylacetamide (20 mL), was treated with pyridine (1 mL) and phenyl isocyanate (1

TABLE I

Characteristics of chitosan samples

Sample	*Hydrolysis conditions*	*D.a. (%)*	$\bar{M}_w \times 10^{-3}$	*D.p.*
A	—	42	1200	6718
A1	aqueous 1% HOAc, reflux, 20 min	42	800	4479
A2	0.6M HCl, 50°, 10 min	42	520	2911
A3	0.6M HCl, 25°, 367 h	40	330	1847
A4	0.6M HCl, 50°, 12 h	42	200	1120
A5	aqueous 10% HOAc, reflux, 29 h	46	170	952
B	—	15	630	3766
B1	0.6M HCl, 50°, 20 min	15	365	2182
B2	0.6M HCl, 50°, 1.5 h	15	320	1913
B3	0.6M HCl, 50°, 4.5 h	15	200	1195

mL) at 60°. The mixture was stirred for 3 h at 80°, the solid was collected and washed with water, and a solution in 0.1M acetic acid was dialysed against water and then lyophilised to give the product (0.4 g), with d.s. ~2% as determined from u.v. measurements (Fig. 4) using an extinction coefficient[13] of 15 500 cm^{-1} M^{-1} at 238 nm. The d.a. was unchanged.

Preparation of solutions. — Dilute solutions for light scattering and d*n*/d*c* measurements were prepared by stirring chitosan with a known amount of 0.1M acetic acid. When dissolution was complete, sodium chloride was added to 0.2M. Sample A was only partially soluble, and pretreatment with aqueous 1% acetic acid (overnight at room temperature), followed by neutralisation with alkali, then washing with water to neutral pH, and lyophilisation, was necessary in order to obtain completely soluble material.

Concentrated solutions were prepared from weighed amounts of aqueous 10% acetic acid and polymer (C_p is given as w/w), which were stirred together for 3 days at room temperature. The solutions were then examined by optical microscopy.

Light scattering. — Measurements were performed at 25°, using a Sofica Model 42 000 photometer with cylindrical cells immersed in toluene. Non-polarised laser light (λ 633 nm) was used, covering scattering angles (θ) between 30° and 150°. A Rayleigh ratio $R_{90} = 8.96 \times 10^{-6}cm^{-1}$ was used for calibration of the instrument with benzene[14]. Solution and solvents were clarified by centrifugation at 25 000*g* for 3 h. Molecular weights ($\bar{M}_w$), second virial coefficients (A_2), and radii of gyration (R_G) were calculated[3] by Zimm plots.

The values of d*n*/d*c*, determined using a Cromatix CMX-16 refractometer at λ 633 nm, were 0.180 ±0.005 and 0.191 mL/g for A and B, respectively. Light-scattering and d*n*/d*c* measurements before and after extensive dialysis of the polymer solution against the solvent showed negligible differences. Therefore, the effect of a non-equilibration of the chemical potential does not significantly modify the results.

Optical microscopy. — The critical concentration for the appearance of the anisotropic phase was determined, at room temperature, by observing a small amount of solution placed between a slide and a cover slide with a polarising microscope (Reichert Polivar).

Circular dichroism. — C.d. spectra were recorded at room temperature for solutions of samples of chitosan derivatives in aqueous 10% acetic acid, using a JASCO J-500 A spectropolarimeter.

RESULTS AND DISCUSSION

Light scattering. — The range of concentrations investigated was 0.1–1.5 × 10^{-3}g/mL. The data gave linear angular plots and there was a high positive dependence on concentration, which ruled out multimerisation phenomena of chitosan chains[15]. Fig. 1 shows, as examples, the Zimm plots for samples A2 and B. Table II contains the molecular parameters $\bar{M}_W$, R_G, and A_2, together with the expansion coefficient (α_t) and the total persistence length (q_t°) obtained as discussed below.

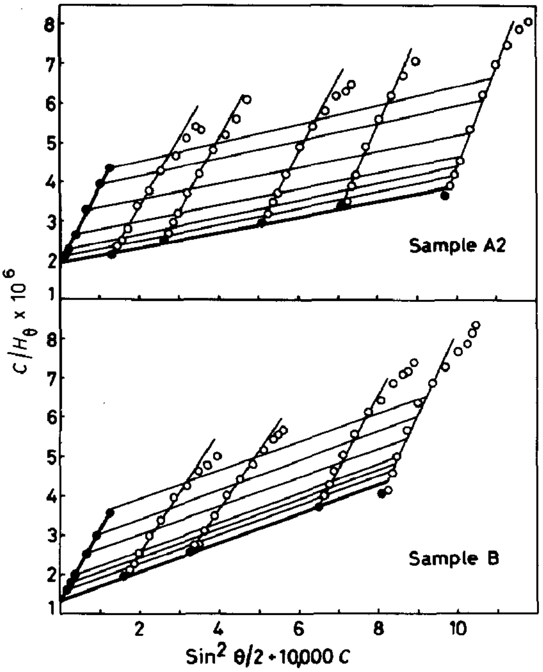

Fig. 1. Zimm plots for samples A2 and B (C expressed in g/mL).

TABLE II

Chain parameters of chitosan samples

Sample	$\bar{M}_w \times 10^{-3}$	R_G (Å)	α_t	q_t° (Å)	$A_2 \times 10^3$ (*mol. mL.* g^{-2})
A	1200	1720	1.08	224	1.2
A1	800	1350	1.07	213	0.9
A2	520	1040	1.06	200	1.0
A3	330	850	1.04	226	1.5
A4	200	670	1.02	229	2.0
A5	170	580	1.02	229	0.3
B	630	1170	1.05	189	2.0
B1	365	890	1.04	203	1.4
B2	320	840	1.03	211	1.2
B3	200	650	1.03	216	1.0

Figure 2 shows a plot of log R_G *vs.* log d.p. for the samples of the series A and B. Although the R_G values of the latter are systematically lower than those of the former, the difference is within experimental error (±10%).

In order to calculate q_t°, it is necessary to know α_t, which takes into account the steric and electrostatic effects of the excluded volume on the dimensions of the chain.

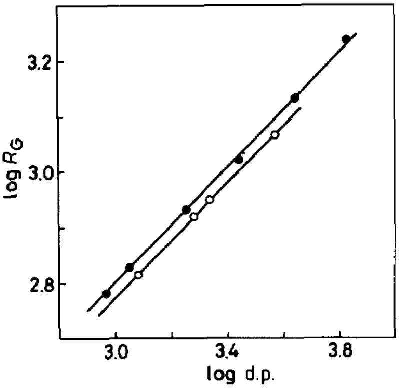

Fig. 2. Logarithmic plots of $<R_G>$ *vs.* d.p. for chitosan of the series A (●) and B (o).

The unperturbed dimension $R_G{}^\circ$ (equation *1*) can then be obtained,

$$R_G{}^\circ = R_G/\alpha_t \tag{1}$$

and, by using Benoit–Doty's equation[16],

$$<R_G{}^{\circ 2}> = L^2\left(\frac{1}{6L'} - \frac{1}{4L'^2} + \frac{1}{4L'^3} - \frac{1-e^{-2L}}{8L'^4}\right), \tag{2}$$

which is valid for worm-like chains, $q_t{}^\circ$ can be deduced. In equation *2*, L and $L' = L/2q_t{}^\circ$ are the contour length and the reduced contour length of the chain, respectively.

For cellulose and its derivatives[17], $q_t{}^\circ$ ranges approximately between 40 and 200 Å, depending on the kind of substituent and d.s., the nature of the solvent, and the temperature. For the majority of cellulosic polymers[17], α_t is ~ 1, and therefore $<R_G{}^2>$ has often been used instead of $<R_G{}^{\circ 2}>$ in equation *2*.

However, when ionic groups are distributed along the chain, the electrostatic field affects the values of α_t and $q_t{}^\circ$. The total effect depends not only on the number of charges per unit length, but also on the ionic strength (*I*) of the solvent, and vanishes as *I* increases.

In order to estimate α_t, the iterative method suggested by Manning[18] was used, which is based on equations[19] that are valid for polymers with non-asymptotic molecular weights, after the introduction of the dependence of the diameter of the chain on the ionic strength. The suggested method involves choosing a reasonable value of $q_t{}^\circ$, and calculating $<R_G{}^{\circ 2}>$ from equation *2* and α_t from equations 4–8 of ref. 18. R_G can then be obtained from equation *1* and compared with the experimental value. If the value of R_G calculated is larger (lower) than the experimental value, a new lower (larger) value of $q_t{}^\circ$ is assumed and the calculation is repeated until an acceptable agreement is obtained. Table II contains the values of α_t, R_G, and $q_t{}^\circ$.

The total persistence length can be considered as the sum of two contributions,

$$q_t^\circ = q_{st}^\circ + q_e \tag{3}$$

where q_{st}° represents that part of q_t° due to the steric properties of the uncharged polymer, and q_e is the contribution deriving from all of the electrostatic interactions. This second term can be obtained from the equation[20]

$$q_e = f^2\, Q/4k^2\beta^2, \tag{4}$$

where Q ($\equiv e^2/DKT$) is the Bjerrum length[20] (7.14 Å in water at 25°), K^{-1} is the Debye screening length (3.04 $I^{-\frac{1}{2}}$ Å in water), β is the contour length between two adjacent charges, and f is the effective dissociation constant (the fraction of the charge not compensated from associated or condensed counter-ions; $f = 1$ if $\beta/Q > 1$, $f = \beta/Q$ if $\beta/Q < 1$).

For poly-β-D-GlcN, β is ~5.1 Å and β/Q is <1. A value of q_e of ~1 Å (for I = 0.2–0.3) is deduced from equation *4*. For d.a. 50%, β/Q is >1 and a value of q_e of <1 Å is obtained. Therefore q_e is negligible with respect to q_{st}° for the values of d.a. examined. Moreover, a medium value q_t° of 220 ±20 Å is realistic for the range of d.a. considered.

A value for q_t of 350 Å has been found[2] for chitin in *N,N*-dimethylacetamide–5% LiCl. Even if a small effect of the nature of the solvent on the unperturbed dimensions cannot be excluded, the value of q_t for chitin is higher than that of chitosan with d.a. 15–42%, and there is no doubt that the rigidity of the main chain increases with increase in d.a.

This behaviour can be explained in terms of a molecular model according to Minke *et al.*[21]. The formation of hydrogen bonds between the CH_2OH of one residue and an OAc group of an adjacent residue could stabilise the extended conformation around the glucosidic linkage. Therefore, as the d.a. increases, the rigidity of the chain should also increase.

At variance with this concept is cellulose acetate, since acetylation decreases the persistence length[22]. Random acetylation could lower the number of inter-unit hydrogen bonds and increase the flexibility.

Finally, with respect to the value of q_t of 300 Å found by Rinaudo *et al.*[7] through the dependence of the intrinsic viscosity on the ionic strength of a chitosan with d.a. 15%, the results now reported are comparable, as the persistence length depends markedly on the experimental method used[17].

Concentrated solutions. — By using a sample of chitosan with molecular weight 200 000 and d.a. ~10%, Ogura *et al.*[11] observed fingerprint-like patterns in highly concentrated solutions (C_p 30–90%) in acetic acid–water (10:90). This effect was interpreted as due to a cholesteric organisation of the anisotropic phase of the polymer. No other evidence of the existence of the mesophase has been reported, nor has the solubility limit been discussed.

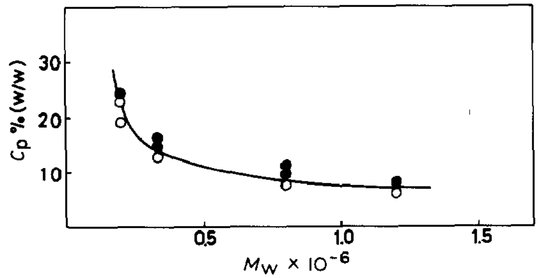

Fig. 3. Border line between isotropic (●) and anisotropic (○) solutions for chitosan of A series as a function of $\bar{M}_w$.

In order to clarify the conditions under which the liquid crystalline phase appears, samples of increasing concentration were prepared (see Experimental) and a plot of C_p *vs.* $\bar{M}_w$ is given in Fig. 3 for chitosan samples with d.a. 42%. The continuous line, which represents the boundary between monophasic and biphasic regions, shows a pronounced upturn at low $\bar{M}_w$. As in aqueous 10% acetic acid, some degradation of the polymer could occur, and the position of the observed upturn could be modified substantially even at room temperature, but the value of C_p at the plateau cannot change. The plateau value (7%) corresponds to a volume fraction (v_p') of 0.054, assuming additivity of the specific volumes (V_{sp}) of the polymer (0.76) and the solvent (1). A similar situation has been discussed for hydroxypropylcellulose in dichloroacetic acid[22]. Moreover, upturns at low d.p. have been observed for cellulose derivatives in different solvents and for other rigid and semi-rigid uncharged polymers[23,24].

For cellulosic chains, which possess chiral centers in each repeating unit, the cholestericity of the anisotropic phase has been confirmed by studying the c.d. spectra. For homeotropic texture (*i.e.*, the axis of the cholesteric helix is perpendicular to the surface of the sample), these spectra show a peculiar and strong dichroic band, the λ_{max} of which is related to the pitch (P) of the helix[25]. In the work now reported, even the most concentrated and anisotropic solutions did not show c.d. bands. Thus, either the anisotropic phase did not have a cholesteric nature, or P, and consequently λ_{max}, was out of the range of λ of the instrument used. The second hypothesis can be supported by an experiment which is widely used for biological systems[26]. When an optically inactive compound is complexed to a helical structure, optical asymmetry is induced and a strong c.d. band appears. There is no effect if the binding involves a random conformation.

The PhNHCO group was used as a marker and the d.s. was limited in order to avoid variations of the properties of the polymer. Figure 4 shows the u.v. spectra of chitosan and the PhNHCO derivative, which has a new λ_{max} at 238 nm. At C_p ~10%, but not ~4%, the PhNHCO derivative had a strong c.d. peak (Fig. 5) at the same λ_{max} as in Fig. 4. These results confirm the presence of a cholesteric texture which is responsible for the observed anisotropy.

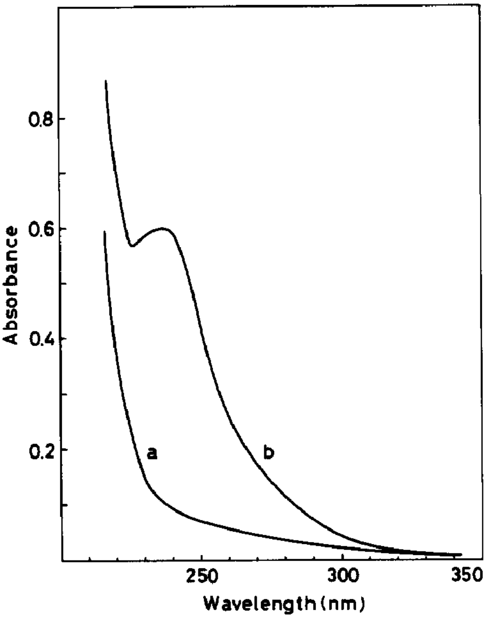

Fig. 4. U.v. spectra of (*a*) chitosan and (*b*) its PhNHCO derivative. Polymer concentration, 1 g/L; optical path, 1 cm.

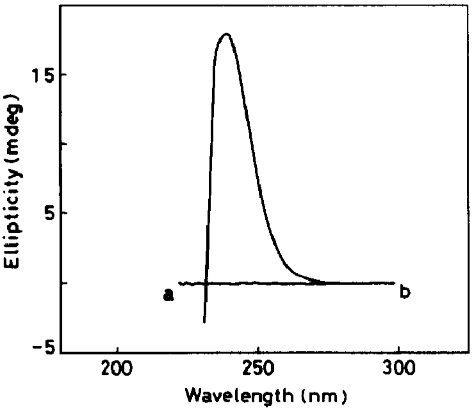

Fig. 5. C.d. spectra of chitosan derivative: (*a*) C_p ~4%; (*b*) C_p ~10%.

A comparison between v_p' determined experimentally and calculated from q_t° can be performed by using theories developed for conventional uncharged polymers. In fact, the ionic strength of 7% chitosan in aqueous 10% acetic acid derives mainly from the charges distributed along the chain. Since the K_0 of the amino group is 6.5[7] and the pH of aqueous 10% acetic acid is 2.7, a value for I of 0.3–0.4 can be deduced. At this relatively high value of I, the electrostatic interactions are low[27], as already observed from the estimated value of q_t°.

According to Flory's lattice theory[28],

$$v_{p\,th}' = \frac{8}{(2q/d)}\left(1 - \frac{2}{2q/d}\right), \tag{5}$$

where d is the diameter of the chain, whereas Onsager's virial theory[27] states that

$$v_{p\,th}' = \frac{3.25}{(2q/d)} \tag{6}$$

Assuming a value for d of 6.6 Å^2 and the maximum value of q_t° of 240 Å, then values of $v_{p\,th}'$ of 0.11 and 0.045 are evaluated, respectively, from equations *5* and *6*. It is evident that the experimental value of v_p' is closer to that predicted from equation *6*. Equation *5* was developed in the absence of any kind of interactions which, if present, could lower the value of v_p' considerably[28].

A general discussion on this subject for uncharged polymers has been reported[29].

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