Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 74 (2008) 10–15
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Characterization of chitin from *Illex argentinus* squid pen

M. Susana Cortizo [a], Carla F. Berghoff [a,b], José Luis Alessandrini [b,c,*]

[a] *Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), CC16 suc.4, 1900 La Plata, Argentina*
[b] *Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), 1900 La Plata, Buenos Aires, Argentina*
[c] *Departamento de Física, Facultad de Ciencias Exactas, UNLP and Instituto de Física de La Plata (IFLP), CC 67, 1900 La Plata, Argentina*

Received 24 July 2007; received in revised form 21 November 2007; accepted 8 January 2008
Available online 16 January 2008

Abstract

β-Chitin from *Illex argentinus* squid pens was isolated by using chemical methods and the pens composition determined. The structural characteristics of β-chitin were identified with infrared (IR), solid-state cross-polarization/magic-angle-spinning (CP-MAS) ^{13}C NMR spectroscopy and X-ray analysis. The viscosity average molecular weight M_v, calculated from the intrinsic viscosity was above 2×10^6 g mol^{-1}. The stiffness of the macromolecule was analyzed using the Yamakawa and Fujii theory for wormlike cylinders.

Keywords: Chitin; *Illex argentinus*; NMR; Molecular weight; Viscosity; Persistence length

1. Introduction

Short-finned squids of the genus *Illex* (Ommastrephidae, Illicinae) account for about 65% of the world's commercial fishery catches (Martínez, Pérez-Losada, Guerra, & Sanjuan, 2005). Among the four species, all distributed throughout the Atlantic Ocean, the Argentinian short-finned squid, *Illex argentinus* (Castellanos, 1960) is distributed along the continental shelf and slope of western South Atlantic Ocean, between latitudes 20°S and 55°S, but more abundantly in the 35–52°S area (Brunetti, 1988). This species made up 33% of the total catches in the Southwest Atlantic in 2001 (FAO, 2004). Annual catches attained are around of 500,000 ton (FAO, 1997). Argentina as the main iberoamerican squid producer between 1997 and 2001 (Goycoolea, Agulló, & Mato, 2004). A large quantity of processing by-products from the squid are discarded, some of them (i.e. squid pen) have been proposed as a source of valuable bioactive compounds, such as biopolymers for biotechnological and pharmaceutical applications (Kim & Mendis, 2006). In this context, the squid pen is a potential source of chitin, poly[β-(1 → 4)-*N*-acetyl-D-glucosamine], the most abundant biopolymer after cellulose. Although both polysaccharides are structurally similar, chitin differs in having an acetamide group at the C-2 position in place of the OH group.

Chitin has been identified in nature as the main supporting structure in a wide variety of organisms such as the exoskeleton or cuticle of different invertebrates (arthropods, mollusc, cnidarians, pogonophores) and in algae and fungi cellular wall (Kurita, 2001; Muzzarelli, Jeuniaux, & Gooday, 1986). According to its source chitin is found in three polymorphic forms, which differ in the packing of adjacent chains in successive sheets. While in the α-form chains are aligned in an antiparallel manner, held by strong intra- and inter-sheets hydrogen bonds (Minke & Blackwell, 1978). β-Chitin has a parallel arrangement, held by weak intra-sheets hydrogen bonds. The less well characterized γ-chitin form is a mixture of antiparallel and parallel chains that characterize the crystalline structure (Gardner & Blackwell, 1975; Rudall & Kenchington, 1973). In squid pen the chitin chains are orientated in the β conformation. Due to its structural differences β-chitin is more easily solubilized in known solvents such as dimethyl acetamide/LiCl, *N*-methyl-2-pyrrolidone/LiCl,

* Corresponding author. Address: Departamento de Física, Facultad de Ciencias Exactas (UNLP), CC 67, 1900 La Plata, Buenos Aires, Argentina. Tel.: +54 221 4247201; fax: +54 221 4250791.
E-mail address: alessan@fisica.unlp.edu.ar (J.L. Alessandrini).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.01.004

hexafluoroisopropanol (Kumar, 2000, Terbojevich, Carraro, Cosani, and Marsano (1988)). In addition, β-chitin is more reactive than α-chitin, an important property in regard to its chemical derivatization for different applications (Kurita, 2001; Rinaudo, 2006).

For *Illex argentinus* squid pen, there is information about the proportion of protein and chitin (Wu, Wu, & Chen, 2003), but no data is available about the macromolecular characterization of the polysaccharide. In the present work we describe the isolation and the macromolecular characterization of chitin from squid *Illex argentinus* pen.

2. Experimental

2.1. Materials and isolation of pen

Squid *Illex argentinus* were collected from local marine food industries, which was caught on the continental shelf and slope of western South Atlantic Ocean from latitudes 35° to 40°S during the fishing season (March to August). The squid was transported and maintained completely frozen until pen isolation. The extracted pens were treated with tap water to wash off residual materials and frozen until use.

Prior to use the pens were washed with distilled water and dried at room temperature to constant weight.

Afterwards, the sample was dried in vacuum oven until constant weight in order to determine water content.

2.2. Ash content

The ash content was estimated by weight difference after thermal treatment of the product in an oven at 700 °C. The spectroscopic study of energy dispersion was carried out in a scanning electron microscope (Philips, SEM 505 model) using energy-dispersive X-ray (EDX) with a SUTW-Sapphire detector (EDAX).

2.3. Lipids–lipoprotein extraction

The isolation and purification of the polysaccharide was carried out in a similar way to Chaussard and Domard (2004). Squid pens were cut into approximately 2 cm pieces, suspended in acetone and mechanically ground in an Ultra Turrax T25 equipment at 70 rpm for 30 s, maintaining the sample temperature at 0 °C. Then, centrifuged and dried until constant weight. The powder was sieved and the fraction between 0.45 and 0.85 μm was used.

The lipid–lipoprotein content was determined by extraction (1 g/100 ml) with chloroform/methanol (2/1, vol/vol) at 40 °C for 3 h and subsequent gravimetric analysis. The obtained soluble product was characterized by infrared spectrometry after solvent evaporation.

2.4. Deproteinization

The sample was treated with 1 M aqueous solutions of NaOH (10 ml/g) at room temperature for 24 h (Chaussard & Domard, 2004). The product was filtered in Buchner funnel, washing several times with deionised water to remove the excess of NaOH until reaching neutral pH. The supernatant was reserved for protein quantification by means of UV spectrometry using a Bradford method (Bradford, 1976). The chitin sample was lyophilised and kept in a freezer until use.

2.5. Chitin characterization

Chitin structural analysis was carried out by infrared spectrometry (Shimadzu IR435 equipment), in KBr pellets and by nuclear magnetic resonance spectroscopy, ^{13}C NMR with CP-MAS technique (cross-polarization, magic-angle-spinning) using a BRUKER-AVANCE II instrument working at 75.47 MHz. The contact time was 1 ms, the repetition time was 4 s and 4096 scans were accumulated. The condition of magnetization transfer between proton and carbon was optimized using a sample of adamantane.

The degree of *N*-acetylation (DA) was determined according to Heux, Brugnerotto, Desbrières, Versali, and Rinaudo (2000).

The X-ray diffraction patterns was obtained using a Philips PW1710 wide angle X-ray powder diffractometer provided with a diffracted bean monochromator and CuK_α radiation ($\lambda = 1.5406$ Å). The voltage was 40 kV and the intensity 30 mA. The 2θ angle was scanned between 4° and 40°, and the counting time was 2.0 s at each angle step (0.02°).

The determination of chitin's intrinsic viscosity ($[\eta]$) was carried out by dissolving at about 0.2 mg/ml in *N*,*N*-dimethylacetamide containing 5% (vol %) lithium chloride under mechanical agitation during 48 h at room temperature. Dilutions were prepared from the stock solution such that $0.3 < \eta_{sp} < 0.8$. (η_{sp} being the specific viscosity). Capillary viscosities were measured at 25.00 (±0.05) °C with Ostwald viscometer with flow time for the solvent $t_0 > 120$ s and a mean shear rate of 240 s^{-1}.

An Haake Rotovisco RV2 rotational rheometer was used in order to test the shear rate dependence of the chitin solution. The measurement was carried out with a NV coaxial cylinder sensor system and a MK 50 measuring heads at 25 °C.

3. Results and discussion

3.1. Squid pen composition

The extracted pen represents 0.2% w/w of the squid *Illex argentinus*. It was found that the squid pen contain 1.0 wt% (base dry) of ash, 2.3 wt% of lipids and lipoproteins, 64 wt% of protein and 31 wt% of chitin. The difference is attributed to water percent. Similar results were found by (Kurita et al, 1993) using *Ommastrephes bartrami* squid pen (58 wt% and 35–40 wt% of protein and chitin, respectively). EDX analysis gives information about the main ele-

ments present in the squid pen ashes. The weight percent of the main elements are shown in Table 1. The low amount of inorganic matter indicates that the demineralization step can be avoided during the extraction process, in contrast to chitin extracted from crustacean shells.

It has been demonstrated that extraction with a chloroform/methanol 2/1 (vol/vol) mixture is an efficient solvent system for separating lipids and lipoproteins (Chaussard & Domard, 2004). In fact, the presence of the following characteristic absorption bands from the IR spectrum (Fig. 1): 3400 cm^{-1} (O—H), 1730 cm^{-1} (C=O), 1640 cm^{-1} (amide I), 1600 cm^{-1} (RHC=CHR cis) confirms the presence of both compounds.

3.2. Chitin characterization

Fig. 2 shows the IR spectra of isolated chitin. The β-chitin polymorphic form was confirmed by an unique band at 1660 cm^{-1} assigned to the stretching vibration of C=O group (amide I) hydrogen bonded to N—H of the neighbouring intra-sheet chain (Kurita et al., 1993). Furthermore, the existence of following bands allows to characterize the polysaccharide (Cardenas, Cabrera, Taboada, & Miranda, 2004): 3420 cm^{-1} (O—H) a broad band which is attributed to the intra molecular hydrogen bond —O(3)—H—O(5) from the ring, 3280 cm^{-1} (N—H), 1560 cm^{-1} (N—H, amide II), 1370 cm^{-1} (C—H, CH_3), 1315 cm^{-1} (C—N and N—H, amide III), 1160 cm^{-1}, 1105 cm^{-1}, 1060 cm^{-1} and 1025 cm^{-1}, are assigned to the C—O—C and C—O stretching vibrations modes.

Table 1
Metal content in *Illex argentinus* squid pen

Element	Percentage
Na	0.045
Mg	0.01
P	0.051
K	0.021
Ca	0.017

The crystalline structure of the purified chitin was analyzed by X-ray diffraction. Fig. 3 shows the corresponding diffractogram which exhibits two broad peaks at $2\theta = 8.10°$ (inter-sheet distance: 10.91 Å) and $2\theta = 19.4°$ (4.57 Å). The inter-sheet distance depends on the degree of hydration of the sample (from 9.3 to 11.1 Å) (Jaworska, Sakurai, Gaudon, & Guibal, 2003). The higher the value of the inter-sheet distance along the *c* axis, the higher the degree of hydration. A value of 10.91 Å was found in the present case, which is corresponds to a β-chitin structure.

The crystallinity index (CI, %) was calculated using the following expression (Focher, Beltrame, Naggi, & Torri, 1990):

$$\mathrm{CI}(\%) = [(I_{110} - I_{\mathrm{am}})/I_{110}] \times 100 \quad (1)$$

where I_{110} is the maximum intensity of the diffraction peak (1 1 0) at $2\theta = 19.4°$ and I_{am} correspond to the amorphous diffraction intensity at $2\theta = 12.6°$. Indeed, a value of 74.9% was found in the present case, which is consistent with the results found by other researches (Focher et al., 1990; Jaworska et al., 2003).

The crystallites size (D) was estimated by means of the Scherrer equation:

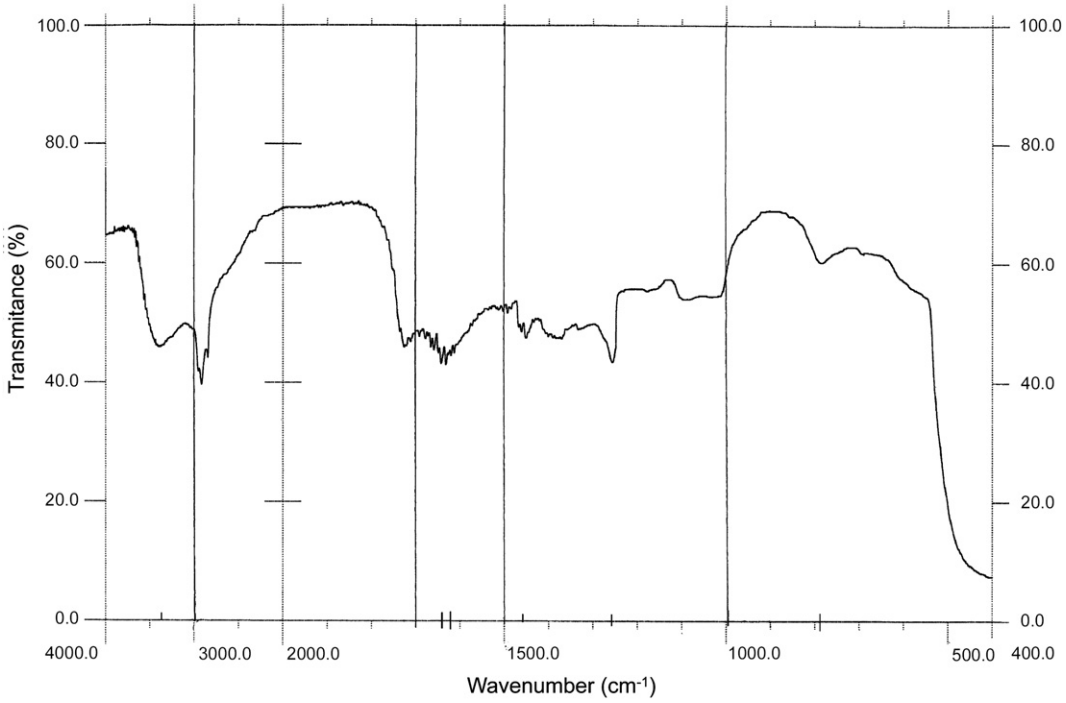

Fig. 1. IR spectrum of lipids and lipoproteins extracted from *Illex argentinus* squid pen.

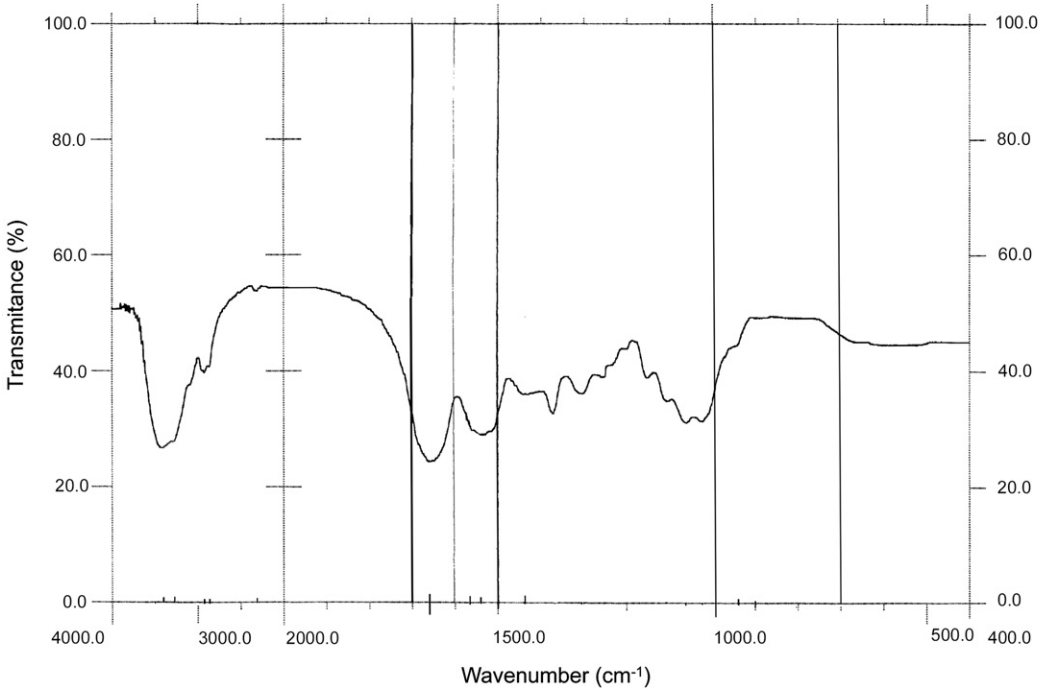

Fig. 2. IR spectrum of β-chitin from *Illex argentinus* squid pen.

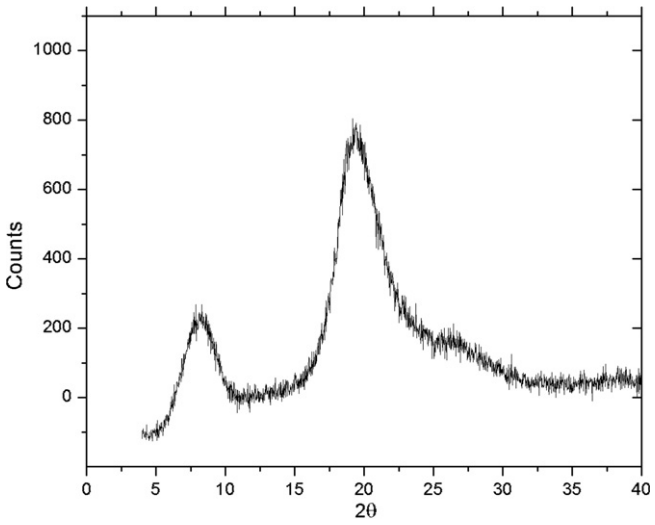

Fig. 3. X-ray diffractogram of β-chitin from *Illex argentinus* squid pen.

$$\Delta(2\theta) = 0.9\lambda/(D\cos\theta) \quad (2)$$

where $\Delta(2\theta)$ is the full width at half maximum of the peak at $2\theta = 19.4°$ and λ is the radiation wavelength. In our case, a 2.32 nm crystallite size value was found, according to the results found by other researchers for similar kind of samples (Jaworska et al., 2003).

The β-chitin CP-MAS ^{13}C NMR spectrum is shown in Fig. 4. Seven signals are assigned to the eight carbon atoms of the *N*-acethylglucosamine repetitive unit which appear at the following chemical shifts (ppm) (Tanner, Chanzy, Vincendon, Roux, & Gaill, 1990): $\delta = 172.6$ (C═O),

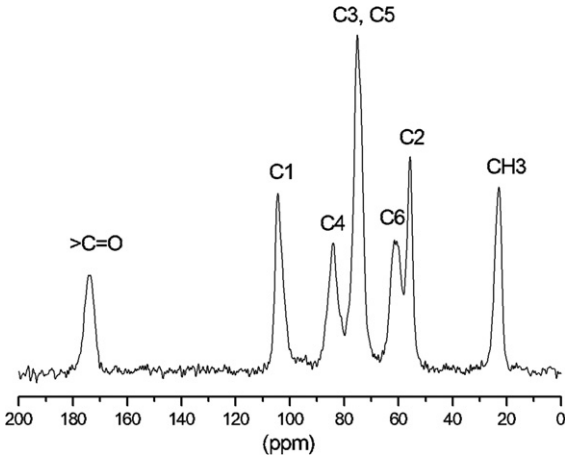

Fig. 4. ^{13}C CP/MAS solid-state spectrum of β-chitin from *Illex argentinus* squid pen.

104.4 (C^1), 84.1 (C^4), 75.0 (C^3 and C^5), 61.4 (C^6), 55.7 (C^2) and 22.8 (CH_3). The C═O signal appears as a sharp and symmetric profile, indicating a unique conformational state, typical of a β-chitin structure. The signals of C^3 and C^5 merge into single resonance centered at 75.0 ppm, while in the α-chitin spectra that signals were reported to appear as a doublet (Cardenas et al., 2004; Focher et al., 1990). The differences between the two polymorphous were attributed to different C^3 and C^5 configurations resulting from the hydrogen bonds established.

The degree of acetylation (DA) was determined from the relative intensities of the resonance of the ring carbon (I_{C1}, I_{C2}, I_{C3}, I_{C4}, I_{C5}, I_{C6}) and CH_3 group (I_{CH_3}) according to the following equation (Ottøy, Vårum, & Smidrød, 1996):

$$\mathrm{DA}(\%) = \frac{I_{\mathrm{CH_3}} \times 100}{[I_{\mathrm{C1}} + I_{\mathrm{C2}} + I_{\mathrm{C3}} + I_{\mathrm{C4}} + I_{\mathrm{C5}} + I_{\mathrm{C6}}]/6} \quad (3)$$

A DA of 96% was found for the β-chitin isolated of the squid *Illex argentinus* pen. Different DA values from chitin extracted of other sources: 92% for pens of *Ommastrephes bartrami* squid (Kurita et al., 1993), 100% for pens of *Loligo vulgaris* squid (Tolaimate et al., 2000), and 79% for pens of *Loligo formosana* squid (Methacanon, Prasitsilp, Pothsree, & Pattaraarchachai, 2003) have been reported. These differences in DA could be attributed to the different isolation conditions of chitin, due to the demineralization step in which acidic conditions are necessary or due to the deproteinization step under different alkaline conditions. In our case, because of the very low ash content, the former step appears to be irrelevant and was avoided in this work. The deproteinization step was carried out under mild conditions (1 M NaOH aqueous solution at room temperature during 24 h), as suggested by Chaussard (Chaussard & Domard, 2004) in order to avoid the possible degradation and deacetylation of native chitin. It is expected that the present chitin isolation conditions allows the original structure of the polysaccharide to be preserved avoiding the degradation of the macrostructure.

The main difficulty in carrying solution properties studies of chitin is related with its insolubility in commonly used solvents. Nowadays, DMAc/LiCl 5% is the most widely used system, in which the dissolution proceeds via strong interaction of the LiCl with intermolecularly hydrogen-bonded hydroxyl and acetamido groups (Vincendon, 1985). This interaction destroys the intermolecular hydrogen bond and allows chitin to swell and then dissolve in the solvent.

Mark–Houwink–Sakurada (MHS) parameters (K, a) relating intrinsic viscosity $[\eta]$ and weight average molecular weight M_w in this solvent were determined at 25 °C in a restricted range of molecular weights (i.e. below 5.1×10^5 g mol^{-1} corresponding to $[\eta] \approx 2200$ cm^3 g^{-1}) by Terbojevich et al. (1988) (T88) as

$$[\eta](\mathrm{cm^3\ g^{-1}}) = 0.24 M_w^{0.69} \quad (4)$$

The Huggins and Kramer viscosity relations (η_{sp}/c and ln η_r/c versus c, respectively) for the present β-chitin in DMAc/LiCl 5% at 25 °C are represented on the viscosity–concentration plot in Fig. 5. The plots are linear within the concentration range for the viscometric measurements ($0.02 < c < 0.1$ mg ml^{-1}). The 0.1 mg ml^{-1} solution of β-chitin in DMAc/LiCl 5% was analyzed as a function of shear rate in the range 200–950 s^{-1}, displaying Newtonian behavior (data not shown). The intrinsic viscosity $[\eta]$ was estimated as the average of the ordinate intercepts from the two extrapolations as $[\eta] = (72 \pm 2)\ 10^2$ cm^3 g^{-1}. Huggins and Kramer coefficients were $k_H = 0.34$, $k_K = -0.15$, respectively. Assuming that the power law from T88 Eq. (4) is valid up to higher intrinsic viscosities, the molecular weight was estimated as $M_v = 3.07 \times 10^6$ g mol^{-1}.

The stiffness of the chain was then analyzed in terms of the Yamakawa and Fujii (1974) (YF) theory for the intrinsic viscosity of wormlike cylinders with contour length L and diameter d. Here, the intrinsic viscosity is expressed in terms of the reduced diameter $d' = d.\ q^{-1}$ and the reduced contour length $L' = M \cdot M_L{}^{-1}$, where M_L is the molar mass per unit length, both sizes been measured in units of the persistence length q. Choosing the same structural parameters used by Terbojevich et al. (1988): $M_L = 39.50$ g mol^{-1} Å^{-1}, $d = 6.63$ Å, plots of the intrinsic viscosity calculated for molecular weights around 3×10^6 g mol^{-1} against the persistence length q cross the experimental $[\eta]$ value at $q = 145 \pm 5$ Å, in excellent agreement with the viscometry persistence length obtained by these authors with lower molecular weights.

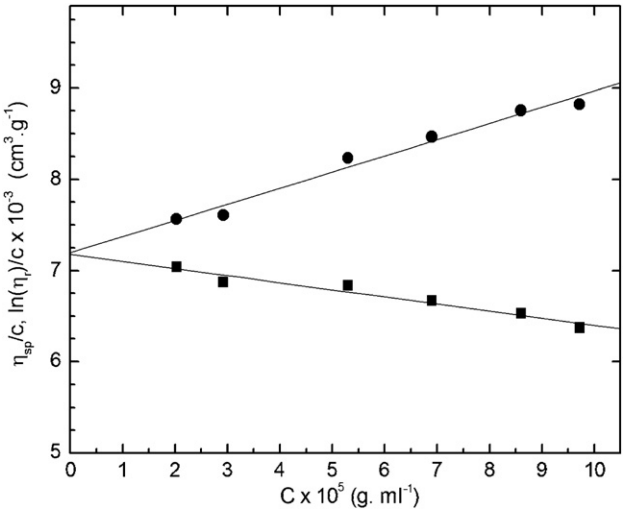

Fig. 5. Viscosity of β-chitin in LiCl (5%)/DMAc at 25 °C as a function of concentration. (●) η_{sp}/c, (■) ln $(\eta_r)/c$.

More recently, Poirier and Charlet (2002) reported an alternative set of MHS parameters: $K = 7.6 \times 10^{-3}$ cm^3 g^{-1}, $a = 0.95$ (± 0.02) valid up to 710×10^3 g mol^{-1} (corresponding to $[\eta] \approx 2730$ cm^3 g^{-1}), obtained by coacervate extraction in DMAc/LiCl 5% at 30 °C with a narrower polydispersity index. Now, with these parameters the molecular weight of squid pen β-chitin reduces to $M_v = 1.95 \times 10^6$ g mol^{-1} and the persistence length increases to $q = 190 \pm 5$ Å. Almost identical results for both M_v and q were obtained by a second set of MHS parameters from Terbojevich, Cosani, Bianchi, and Marsano (1996) (T96) (i.e. $K = 2.1 \times 10^{-2}$ cm^3 g^{-1}, $a = 0.88$), determined with more samples than in (T88) and obtained from different fragmentation techniques.

β-Chitin was also analyzed by Lamarque, Viton, and Domard (2004) and two different molecular weights were reported from viscometric measurements: $M_v = 1.34 \times 10^6$ g mol^{-1} and $M_v = 1.01 \times 10^6$ g mol^{-1} when using T88 or T96 data, respectively. By application of YF theory to their measurements, persistence lengths $q = 145$ Å for the first set and $q = 170$ Å were obtained from the second one, showing the same tendency found in the present work. (MKS parameters from Poirier et al. give $M_v = 1.07 \times 10^6$ g mol^{-1} and $q = 165$ Å, close to the predictions for T96).

Finally, it is worth mentioning that Mazeau and Rinaudo (2004) have performed molecular modeling of polysaccharides to predict the behavior of polymers in solution as well as in solid state. From their calculation of the characteristic ratio of 100% acetylated pure chitin they found a limiting value of 125 Å for the persistence length. This upper bound is not in agreement with the values obtained from viscometric measurements analyzed with the existing MHS parameters and the application of the YF theory to extract the persistence length. Much work remains to be done in the field of chitin characterization.

4. Conclusion

The chitin isolated from *Illex argentinus* squid pen have been characterized with several techniques. The polysaccharide exhibited a β-chitin structure according to IR and ^{13}C NMR spectra, characterized both by high degree of crystallinity (74.9%) and degree of acetylation (96%), in good agreement to other β-chitin isolated from squid species.

High average viscosity molecular weight (above 2×10^6 g mol^{-1}) were estimated from extrapolation of the existing intrinsic viscosity–molecular weight relationships for β-chitin in DMAc/LiCl 5%. The persistence length obtained with YF theory is in the range 145–190 Å, depending of the MHS parameters used. These discrepancies were also found in viscometric data from other authors.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) under PIP No. 5994 and Universidad Nacional de La Plata (Project X403), Argentina.

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72*(1–2), 248–254.

Brunetti, N. E. (1988). Contribución al conocimiento biológico-pesquero del calamar argentino (Cephalopoda, Ommastrephidae, *Illex argentinus*) Ph.D. Thesis, Universidad Nacional de La Plata, La Plata.

Cardenas, G., Cabrera, G., Taboada, E., & Miranda, S. P. (2004). Chitin characterization by SEM, FTIR, XRD, and ^{13}C cross polarization/mass angle spinning NMR. *Journal of Applied Polymer Science, 93*(4), 1876–1885.

Chaussard, G., & Domard, A. (2004). New aspects of the extraction of chitin from squid pens. *Biomacromolecules, 5*, 559–564.

FAO. (1997). *Yearbook of fishery statistics. Catches and landings 1995* (Vol. 80). Rome, 714 pp.

FAO. (2004). *The state of world fisheries and aquaculture. Part 1: World review of fisheries and aquaculture*. Fisheries department.

Focher, B., Beltrame, P. L., Naggi, A., & Torri, G. (1990). Alkaline *N*-deacetylation of chitin enhanced by flash treatments reaction kinetics and structure modifications. *Carbohydrate Polymer, 12*(4), 405–418.

Gardner, K. H., & Blackwell, J. (1975). Refinement of the structure of b-chitin. *Biopolymers, 14*(8), 1581–1595.

Goycoolea, F., Agulló, E., & Mato, R. (2004). Fuentes y procesos de obtención. In A. Pastor de Abram (Ed.), *Quitina y quitosano: Obtención, caracterización y aplicaciones* (pp. 105–154). Fondo editorial de la Pontificia Universidad Católica del Perú.

Heux, L., Brugnerotto, J., Desbrières, J., Versali, M. F., & Rinaudo, M. (2000). Solid state NMR for determination of degree of acetylation of chitin and chitosan. *Biomacromolecules, 1*(4), 746–751.

Jaworska, M., Sakurai, K., Gaudon, P., & Guibal, E. (2003). Influence of chitosan characteristics on polymer properties. I: Crystallographic properties. *Polymer International, 52*(2), 198–205.

Kim, S., & Mendis, E. (2006). Bioactive compounds from marine processing byproducts – A review. *Food Research International, 39*(4), 383–393.

Kumar, M. N. V. R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymer, 46*(1), 1–27.

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progress in Polymer Science, 26*(9), 1921–1971.

Kurita, K., Tomita, K., Tada, T., Ishii, S., Nishimura, S., & Shimida, K. (1993). Squid chitin as a potential alternative chitin source: Deacetylation behavior and characteristic properties. *Journal of Polymer Science: Part A: Polymer Chemistry, 31*(2), 485–491.

Lamarque, G., Viton, C., & Domard, A. (2004). Comparative study of the first heterogeneous deacetylation of a- and b-chitins in a multistep process. *Biomacromolecules, 5*(3), 992–1001.

Martínez, P., Pérez-Losada, M., Guerra, A., & Sanjuan, A. (2005). First genetic validation and diagnosis of the short-finned squid species of the genus *Illex* (Cephalopoda: Ommastrephidae). *Marine Biology, 148*, 97–108.

Mazeau, K., & Rinaudo, M. (2004). The prediction of the characteristics of some polysaccharides from molecular modeling. Comparison with effective behavior. *Food Hydrocolloids, 18*(6), 885–898.

Methacanon, P., Prasitsilp, M., Pothsree, T., & Pattaraarchachai, J. (2003). Heterogeneous *N*-deacetylation of squid chitin in alkaline solution. *Carbohydrate Polymer, 52*(2), 119–123.

Minke, R., & Blackwell, J. (1978). The structure of b-chitin. *Journal of Molecular Biology, 120*(2), 167–181.

Muzzarelli, R. A. A., Jeuniaux, C., & Gooday, G. W. (1986). *Chitin in nature and technology*. New York: Plenum Press.

Ottøy, M. H., Vårum, K. M., & Smidrød, O. (1996). Compositional heterogeneity of heterogeneously deacetylated chitosans. *Carbohydrate Polymer, 29*(1), 17–24.

Poirier, M., & Charlet, G. (2002). Chitin fractionation and characterization in *N*,*N*-dimethylacetamide/lithium chloride solvent system. *Carbohydrate Polymers, 50*(4), 363–370.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*(7), 603–632.

Rudall, K. M., & Kenchington, W. (1973). The chitin system. *Biological Reviews, 49*, 597–636.

Tanner, S. F., Chanzy, H., Vincendon, M., Roux, J.-C., & Gaill, F. (1990). High-resolution solid-state carbon-13 nuclear magnetic resonance study of chitin. *Macromolecules, 23*(15), 3576–3583.

Terbojevich, M., Cosani, A., Bianchi, E., & Marsano, E. (1996). In A. Domard, C. Jeuniaux, R. Muzzarelli, & R. Roberts (Eds.). *Advances in chitin science* (Vol. 1, pp. 333–339). Lyon: Jacques André.

Terbojevich, M., Carraro, C., Cosani, A., & Marsano, E. (1988). Solution studies of the chitin–lithium chloride-*N*,*N*-di-methylacetamide system. *Carbohydrate Research, 180*(1), 73–86.

Tolaimate, A., Desbrières, J., Rhazi, M., Alagui, A., Vincendon, M., & Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer, 41*(7), 2463–2469.

Vincendon, M. (1985). ^1H NMR study of the chitin dissolution mechanism. *Die Makromolekulare Chemie, 186*(9), 1787–1795.

Wu, S. D., Wu, C. S., & Chen, H. C. (2003). Cuticle structure of squid *Illex argentinus* pen. *Fisheries Science, 69*(4), 849–855.

Yamakawa, H., & Fujii, M. (1974). Intrinsic viscosity of wormlike chains. Determination of the Shift Factor. *Macromolecules, 7*(1), 128–135.