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Chitin fractionation and characterization in *N*,*N*-dimethylacetamide/lithium chloride solvent system

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Abstract

A detailed study of the interaction of chitin molecular species with the solvent system *N*,*N*-dimethylacetamide (DMAc)/lithium chloride (LiCl) allowed the development of a new method for chitin fractionation by coacervate extraction. The controlled increase of the extracting power of the solvent was carried out using slight modification of the solvent composition. Partial extractions of molecular species were done between coacervation and complete dissolution limits using different mixtures of DMAc/LiCl of increasing extracting power. Fractions were characterized in DMAc/LiCl 5% (w/w) by viscometry and size exclusion chromatography with refractive index and multi-angle laser light scattering detectors. Fractions obtained by coacervate extraction range from 80,000 to 710,000 g mol^{-1} with polydispersity index between 1.28 and 1.44. The Mark–Houwink–Sakurada equation constants *a* and *K* for chitin in DMAc/LiCl 5% (w/w) were found to be 0.95 and 7.6×10^{-5} dl g^{-1}, respectively.

Keywords: Chitin; Fractionation; Mark–Houwink–Sakurada equation; Light scattering; Size exclusion chromatography

1. Introduction

Chitin is a linear polysaccharide mainly composed of β-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucopyranose units. It occurs as an important constituent of the exoskeleton of many organisms (particularly crustaceans, insects and molluscs) and in the cell walls of most fungi and some algae.

N,*N*-dimethylacetamide (DMAc)/lithium chloride (LiCl) is one of the few non-degrading solvents of chitin (Austin, 1977). Some NMR spectroscopy studies have shown that DMAc/LiCl is a true (non-derivatizing) solvent of chitin (Gagnaire, St-Germain, & Vincendon, 1982). The dissolution mechanism can be described as follows: Li^+ ion is associated with the carbonyl oxygen of DMAc; this weak complex solvates the 'polyelectrolyte' formed by the association between Cl^- ions and labile proton groups (–OH and $-NHCOCH_3$) of the chitin chain, disrupting the extensive hydrogen bonds web of the crystalline sheet structure of chitin (Morgenstern & Kammer, 1996; Paner & Beste, 1976). Other similar mechanisms were also proposed, but the precise structure of the complex involved is still not well known (Morgenstern & Kammer, 1996). Like cellulose solutions in DMAc/LiCl, dissolution of chitin probably leads to kinetically stable rather than thermodynamically stable solutions. These solutions are likely to exhibit aging behavior, turning them into phase-separated systems (Morgenstern & Kammer, 1996).

LiCl concentration in DMAc obviously rules the extracting power of the solvent. The solubility of chitin is molecular weight dependent and increases with decreasing chain length. Each molecular species is dissolved, when its intrinsic dissolution limit is reached upon the addition of a DMAc/LiCl mixture of sufficient extracting power. A molecular weight dependent preferential adsorption phenomenon of the solvent components on the chitin chains possibly occurs in the present system (Termonia, 1999). It probably exerts a non-negligible effect on the intrinsic limit solubility of a given chain length.

The polymer industry recently showed a wide interest in the use of mixed solvent systems for polymer processing and isolation. Current industrial processes for polymer coagulation utilize mixed systems, which are designed to bring solution close to immiscibility (Termonia, 1999). The understanding of the DMAc/LiCl mixed solvent system is potentially interesting for such new developments.

Natural polymers usually present a distribution of molecular weights as a consequence of the polymer formation by an organism or the degradation processes suffered by the substance during isolation from the living tissues. During the molt cycle of shellfish, both synthesis of new chitin and partial recovery from the old cuticles occur (Espie & Roff,

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1995). The enzymes chitinase and chitobiase are involved in the recovery of chitin oligomers from the depolymerization of the old exoskeleton. Some depolymerization also occurs during the isolation of the chitin from the shrimp shells, principally during enzymatic deproteinization and acid demineralization. The molecular weight heterogeneity in macromolecular substance is a fundamental property described by several molecular weight averages. It exerts a permanent influence on all the properties of a substance, both in solution and in the solid state. Despite its importance, many studies did not considered the influence of this parameter on the properties of a specific system or product. The isolation of chitin molecular species of narrow distribution over a wide range of molecular weight will be obviously useful for the study of molecular weight dependent properties.

This paper reports the results of fractionation of chitin by coacervate extraction. The basic principle of this technique is the subdivision of a chitin sample in several fractions using solubility differences between molecular species of different molecular weight. A coacervate is a polymer-rich liquid–gel phase, which separates upon the addition of a non-solvent to a polymer solution. Conditions for the coacervate formation must guarantee a liquid coacervate (polymer precipitation must be avoid). The non-solvent (DMAc) is added to the solution (chitin/DMAc/LiCl 5%), until essentially all of the chitin is in the coacervate (the gel phase). The chitin-poor liquid phase, containing the lowest molecular weight fraction, is removed and the chitin isolated. The coacervate is then extracted with a mixture having a slightly greater solvent power. This procedure is repeated, until the fractionation is complete. The fractionation by coacervate extraction can be simply described as a liquid–liquid extraction. This technique allows fast phase equilibrium and suppression of low molecular weight contamination in higher fractions (Francuskiewicz, 1994). In contrast with some fragmentation techniques (chemical hydrolysis, sonication), fractionation is more susceptible to give fractions without chemical (end-groups changing) and crystalline structure modifications (Sharples, 1971; Terbojevich, Cosani, Conio, Ciferri, & Bianchi, 1985; Terbojevich, Cosani, Bianchi, & Marsano, 1996). From our knowledge, no fractionation method applied to chitin has been reported.

The intrinsic viscosity $[\eta]$ of a polymer solution is related to the molecular weight M by the Mark–Houwink–Sakurada (MHS) equation

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

where K and a are empirical constants valid for a specific polymer–solvent system at a given temperature (Flory, 1953). The equation strictly holds for monodisperse samples. If the polymer is polydisperse, $[\eta]$ is related to a viscometric average molecular weight (M_v):

$$[\eta] = KM_v^a \tag{2}$$

The exponent a is a polymer conformation parameter, which decreases with increasing molecular compactness: extended rod-like molecules have a value greater than 1, random coil molecules have a value between 0.8 in a good solvent and 0.5 in a poor solvent.

The aim of this work is to understand the molecular weight dependent comportment of chitin in DMAc/LiCl and develop a fractionation method allowing the efficient separation of chitin molecular species found in the natural distribution. Fractions will be characterized in DMAc/LiCl 5% (w/w) by viscometry and size exclusion chromatography (HPSEC) with refractive index (RI) and multi-angle laser light scattering (MALLS) detectors. These methods will ensure complete mass characterization of the fractions: intrinsic viscosity, weight (M_w) and number (M_n) average molecular weight, radius of gyration ($\langle r^{1/2}\rangle^2$) and distribution curve. The use of an absolute method to determine the molecular weight of chitin (MALLS) allowed us to overcome the lack of suitable standards. The MHS equation constants will be determined from viscometry and size exclusion chromatography data. Several fractions of narrow distribution spread over a wide range of molecular weight will be needed for that purpose.

2. Experimental

2.1. Materials

Chitin (98.5% purity; 1.2% proteins, 0.3% minerals) produced from northern shrimp shells (*Pandalus Borealis*) was obtained from ABK-Gaspésie Inc. (Matane, Que., Canada). DMAc (99.8%, anhydrous) and LiCl (99.0%, anhydrous) were purchased from Sigma-Aldrich Canada Ltd. LiCl was vacuum dried overnight at 100 °C before use.

The final chitin purification was achieved by dissolution in DMAc/LiCl 5% (w/w) and centrifugation (insoluble chitin chains were precipitated and discarded). Soluble chitin was precipitated with DMAc and distilled water, goosh filtrated, washed with water and vacuum dried at 80 °C. The degree of acetylation was evaluated to be 91% by infrared spectroscopy on a Nicolet Magna IR 560 spectrometer (Shigemasa, Matsuura, Sashiwa, & Saimoto, 1996) and elemental analysis (N/C ratio, Carlo-Erba 1106 elemental analyzer).

Many studies have reported the evidence of covalent bonds between chitin and proteins (Hackman, 1960; Hackman & Goldberg, 1958; Rudall & Kenchington, 1973). It is highly probable that these covalent bonded proteins were the last to be removed of the chitin chains in a purification process. Elemental analysis, total kjeldahl nitrogen and minerals determination were done on the chitin before and after the last step of purification (dissolution in DMAc/LiCl 5% w/w and centrifugation). The comparison of the results with theoretical values ($N/C = 0.150$ for chitin with degree of acetylation of 91%) taking from literature (Roberts,

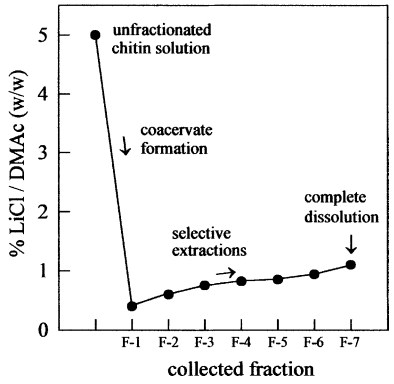

Fig. 1. Scheme of the fractionation method by coacervate fractionation.

1992) clearly shows that the chitin obtained has been completely purified. Some studies have proposed bonding arrangements between proteins and chitin in which the *N*-acetyl group of the chitin is involved through an *N*-acyl structure in the chitin–protein link (Hackman, 1960; Rudall & Kenchington, 1973). Therefore, it is logical that the chitin chains associated with proteins are insoluble (or less soluble) in DMAc/LiCl 5%, since the *N*-acetyl group is involved in the mechanism of dissolution. No analysis was realized on the insoluble chitin, but the hypothesis that covalent bonded proteins were removed by dissolution in DMAc/LiCl 5% w/w and centrifugation is highly plausible. No specific chromatographic analysis has been realized to verify for trace quantities of peptides or amino acids attached to the soluble purified chitin chains.

2.2. Fractionation by coacervate extraction

The first step was the investigation of chitin solubility behavior in DMAc/LiCl. The limits of complete coacervate formation and complete dissolution were found experimentally. The coacervate was formed by the addition of a precise volume of DMAc to a solution of chitin (1%, w/v) in DMAc/LiCl 5% (w/w). The addition of DMAc was the only way to obtain phase separation of the system without dramatic precipitation of the solution and loss of dissolution power. The coacervation limit was evaluated by observation of the chitin state in solution after the addition of a volume of DMAc. The LiCl concentration in the solution rules the extracting power of the solvent and consequently the solubility of chitin. For a chitin solution of ~0.1% (w/v), the chitin was completely precipitated (not coacervated), when LiCl concentration was below 0.25%. Subsequent extractions on this precipitated chitin with mixtures of weak extracting power were not possible with short extraction period (no dissolution occurred). Under the specific conditions used, the most advantageous LiCl concentration for a good coacervate formation was evaluated to be 0.40% (as discussed later in Section 3).

To ensure an efficient coacervate/liquid separation by centrifugation in subsequent extractions, it was necessary to adjust the coacervate: solvent ratio properly. The 1:10 ratio was found to be appropriate for an easy phase separation and a good chitin distribution in each fraction. Slight variation of this ratio often led to the impossibility of phase separation (solution completely liquid or gel-like). The 1:10 ratio was kept for all the extractions. Under these conditions, the complete dissolution limit was evaluated using different mixtures of DMAc/LiCl of increasing extracting power for the extraction of the coacervate. The chitin in the coacervate was completely dissolved (in one extraction), when the LiCl concentration in the solution reached 1.3%. Therefore, the range of effective LiCl concentration for selective extractions (for these specific conditions) holds between 0.4 and 1.3%.

All the extraction steps were realized under nitrogen at constant temperature (25 ± 0.1 °C). The fractionation temperature was set close to room temperature to minimize fluctuation between each step of the method. Efficient coacervate dispersion in the whole solution was ensured by magnetic stirring. Optical microscopy observations (Zeiss Axioskop, Leika lenses, 3 CCD camera) showed that the coacervate was homogeneously dispersed in the solution after 45 min of intense magnetic stirring. The stirring period for phase equilibrium was then fixed to 90 min for each extraction including the coacervate formation. Phase separation was carried out by centrifugation (Sorval centrifuge, 8000 rpm, 25 ± 0.1 °C, 60 min) in four 250 ml polypropylene bottles. Slow aspiration of the liquid phase was used for the final separation.

Quantitative fractionation was performed as follows: 920 ml of DMAc were added to 80 ml of a 1.08% chitin solution in DMAc/LiCl 5%, resulting in a 0.41% LiCl coacervate. The stirring period was 90 min. Phase separation was done by centrifugation and the liquid phase (containing the first fraction) was collected. After this step, different mixtures of DMAc/LiCl were used (with an increasing extracting power gradient) for the coacervate extraction. The extracting power gradient was relatively weak to ensure the complete development of phase equilibrium and the sharpness of the separation. The LiCl concentration of these solutions was 0.60, 0.75, 0.82, 0.85, 0.94, and 1.10%, respectively. The fractionation method steps are resumed in Fig. 1. Each fraction collected was precipitated with DMAc and water (16 h of stirring), goosh filtrated (fine porosity) and vacuum dried at 80 °C. For the calculation of LiCl concentration in solution, the proportions of LiCl and DMAc were assumed to be identical in both phases.

2.3. Viscometry

Chitin solutions (0.03–0.05 g dl^{-1}) were prepared in DMAc/LiCl 5% (w/w). Solutions were filtered through dried and pre-weighted 0.45 μm nylon filters. After filtration, filters were washed with water (for removing trace of DMAc and LiCl), dried at 100 °C and weighted. This procedure ensures that all the insoluble particles were removed

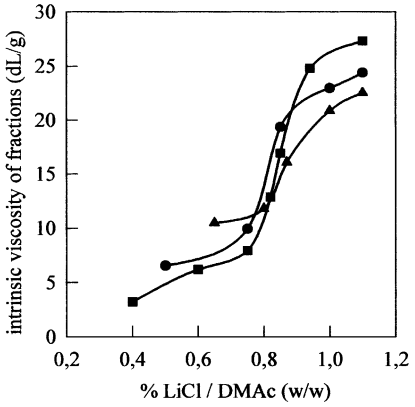

Fig. 2. Influence of coacervate formation on fractionation. 0.35 (▲), 0.40 (■), and 0.50 (●) %LiCl coacervation.

and allows an efficient evaluation of chitin concentration. Intrinsic viscosity data were determined with a suspended-level Ubbelohde viscometer (no. 1, B-917) at 30 ± 0.01 °C. The viscometer used had a negligible kinetic energy correction for solvent flow time over 150 s. Four dilutions with the solvent were done for each sample of chitin. Dilutions were made directly in the viscometer and relative viscosity values were between 1.1 and 1.6. Three flow time measurements (within 0.1 s) were done for each dilution. The flow time data were used to calculate the reduced viscosity and then the intrinsic viscosity by a linear regression of the reduced viscosity versus concentration. Considering the uncertainty on flow time measurement, concentration and temperature fluctuation, the overall error on reduced viscosity was evaluated to be ± 0.4 dl g^{-1}.

2.4. Specific refractive index increment

In a mixed solvent system, there is a preferential adsorption of the solvent component on the polymer chain segments (Huglin, 1989; Sedlák, 1996; Vink & Dahlström, 1967). To obtain accurate molecular weight values by light scattering analysis in DMAc/LiCl, the measurements must be done at a constant chemical potential of LiCl (Donnan equilibrium pressure). If this phenomenon of preferential adsorption is not considered, light scattering analysis will yield only apparent molecular weight values. It is therefore necessary to dialyze the chitin solutions against the solvent prior to perform the measurements (Sedlák, 1996). The specific RI increment (dn/dc) of the chitin in DMAc/LiCl 5% (w/w) must also be determined with dialyzed samples. All the chitin solutions were prepared as described in Section 2.3 and dialyzed 5 days against DMAc/LiCl 5% (w/w) in stainless steel cells in which two reservoirs were separated by a 0.2 μm nylon membrane supported on PTFE joints. Five solutions of concentrations between 0.0095 and 0.1145 g dl^{-1} were used for the dn/dc determination. The measurements were performed using a RI detector (Optilab 903, Wyatt Technology Corporation) with incident light of 632.8 nm at 65 °C. Thermal equilibrium was reached after a few hours. The dn/dc value obtained and used for light scattering calculation was 0.091 ± 0.006 ml g^{-1}.

2.5. Size exclusion chromatography with RI and MALLS detectors

The system consisted of a HPLC pump (Waters, HPLC 515), two columns (Polymer Labs linear PL-gel 10 μm and Shodex KF804), a RI detector (Optilab 903, Wyatt Technology Corporation) and a MALLS detector (DAWN DSP-F, Wyatt Technology Corporation). Data acquisition and calculation were performed using the software Astra (version 4.70.07, Wyatt Technology Corporation). The mobile phase/solvent used for the analysis was DMAc/LiCl 5% (w/w). The samples were prepared as described in Section 2.3 and dialyzed 5 days against DMAc/LiCl 5% (w/w). Mobile phase and samples were filtered through nylon 0.45 μm.

Light scattering measurements were carried out with vertically polarized incident light at 632.8 nm and the photometer was calibrated with toluene. Scattered intensities were measured in the angular range of 15–160°. The system was operated at 65 °C using a column heater (Waters) and a circulating bath (Polyscience) for the RI detector. The MALLS detector temperature was controlled by an internal system. Thermal equilibrium was reached after a few hours. The flow rate was 0.600 ml min^{-1} and the injection volume was 500 μl. The limit chitin concentration to avoid columns overloading was approximately 0.12 g dl^{-1}. From the absence of bimodal distribution in the light scattering signal, we concluded that no chitin aggregation occurred in the solvent system used (Ottøy, Vårum, Christensen, Anthonsen, & Smidsrød, 1996).

3. Results and discussion

3.1. Fractionation

The influence of coacervate formation on the fractionation curve is shown in Fig. 2. With a 0.35% LiCl coacervation, the chitin-rich phase was too compact and seemed to be slightly precipitated, not completely coacervated. Subsequent extractions on this coacervate with mixtures of weak extracting power were not possible when using short extraction period (no dissolution occurred). In contrast, massive extraction and selectivity loss occurred with higher LiCl concentrations. The 0.50% LiCl coacervation allowed the extraction of smaller chains when using weak dissolution power mixtures, but the selectivity was lower than the 0.4% LiCl coacervation for higher extracting power mixtures. The lower intrinsic viscosities for higher fractions obtained in 0.5% LiCl coacervation (in comparison with 0.4% LiCl coacervation) were probably related to the sharper LiCl concentration gradient around 0.8% LiCl. With a 0.4% LiCl coacervation, subsequent extractions

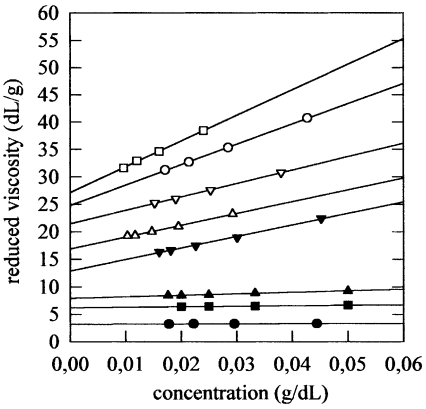

Fig. 3. Viscosity–concentration plots of chitin fractions in DMAc/LiCl 5% (w/w). F-1 (●), F-2 (■), F-3 (▲), F-4 (▼), F-5 (△), F-6(○), F-7(□), and unfractionated (▽).

showed good mass selectivity and efficient extractions were possible with weak extracting power mixtures. From these observations, the most advantageous LiCl concentration for coacervate formation seems to be around 0.40%.

Some factors have not been studied here and should be considered for a complete understanding of the fractionation mechanism: coacervate re-dissolution kinetics, steric hindrance effect in the coacervate physical structure and molecular weight dependant adsorption phenomenon.

The Huggins viscosity relation is represented on the viscosity–concentration plots in Fig. 3. Extrapolation yields the intrinsic viscosity for each fraction. Fractions 1–4 were found to have lower intrinsic viscosity than the unfractionated sample. In contrast, fractions 6 and 7 have higher intrinsic viscosity values. From this observation, it seems that the method is more selective for smaller chains extraction. The degree of acetylation is 90–92% for all the fractions.

Fig. 4 shows the size exclusion chromatogram of the unfractionated chitin sample. The chitin obtained from ABK-Gaspésie has been purified by enzymatic and chemical processes. The first step of deproteinization was achieved by enzymatic hydrolysis using a commercial

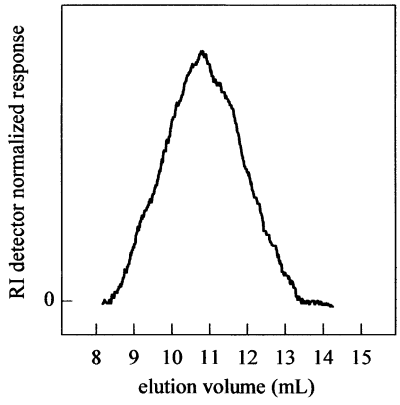

Fig. 4. Size exclusion chromatogram of the unfractionated chitin sample.

mixture. During this operation, residual enzymes from the shrimp shells (such as chitinase and chitobiase) still have activity and induce depolymerization on high molecular weight chitin chains leading to an unfractionated chitin with a low polydispersity index of 1.45 (for an unfractionated biopolymer, the polydispersity index is usually around 1.8–2).

Fig. 5 shows the size exclusion chromatograms of fractions 1, 3, and 5–7. Fractions 2 and 4 are not shown to avoid figure overloading. The distribution curve shifts from fraction 1 to 7, showing that distinct molecular species were separated by fractionation. The distribution pattern of fractions 1–4 are only slightly sharper than the unfractionated sample. The narrow distribution patterns of fractions 5 and 7 fit well with the calculated polydispersity index (M_w/M_n) of 1.33 and 1.28 (Table 1). Fraction 6 contains sensibly the same higher chains than fraction 7, but more mid-length chains. Accordingly, M_w decreases and the polydispersity index increases. These results are detailed in Table 1.

The judgment of fractionation quality and efficiency was monitored by the analysis of some criteria:

1. *Molecular weight average*. The increasing of M_w from fraction 1–7 denotes the obvious separation of species with distinct molecular weight by fractionation.
2. *Polydispersity index decreasing*. The polydispersity index calculated for each fraction is lower than that of the unfractionated sample. This confirms that the polydisperse initial chitin have been separated into fractions with different molecular weight of more narrow molecular weight distribution. The shape of the distribution curve allows a visual countercheck of the calculated polydispersity index values.
3. *Mass balance*. The summation of weights for all the fractions obtained is not equal to the initial weight of chitin. The weight recovery of 80% is essentially related to the loss of higher chains during the phase separation process by centrifugation. In each step of centrifugation, some coacervate stuck to the centrifuge tubes walls. Because the higher chains remained for longer time in the coacervate, they were subjected to more loss than the smaller ones. Some other loss probably occurred during the goosh filtration of the precipitated fractions. The weight of each dried fraction was between 5.5 and 21.5% of the initial sample weight. The fraction weight increased with the increasing of the extracting power of the mixture used for the extraction. The selectivity of the method was therefore higher, when lower extracting power mixtures were used. The breakpoint for massive dissolution seems to be around 0.85% LiCl. The mass selectivity could be eventually improved by the use of higher coacervate: solvent ratio near this breakpoint.
4. *Average of intrinsic viscosity*. Intrinsic viscosity is a weight average of the values of individual components

$$[\eta] = \sum [\eta_i] w_i \quad (3)$$

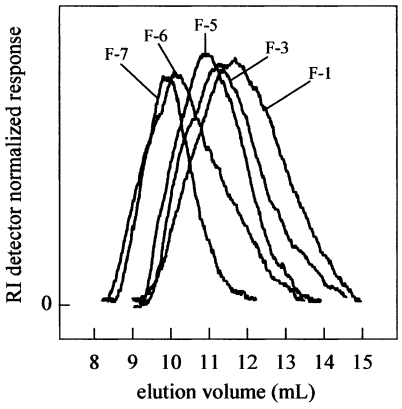

Fig. 5. Size exclusion chromatograms of some fractions.

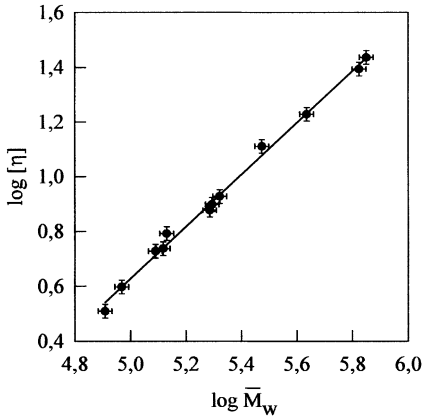

Fig. 6. MHS constants determination: double logarithmic plot of $[\eta]$ versus M_w.

where $[\eta]$ is the intrinsic viscosity and w the weight fraction of the fraction. From this equation, the intrinsic viscosity average of the fractions was found to be lower than the intrinsic viscosity of the starting material (obviously because the recovery was 80%). Assuming that all the lost material was supposed to be found in fraction 7, the value obtained from this equation is 20.3 dl g^{-1}, a value close to the 21.5 dl g^{-1} initial value.

5. *Reproducibility*. It was evaluated for the first three fractions of five fractionation experiments. The intrinsic viscosities averages for fractions 1–3 were 3.1, 5.9, and 8.0 dl g^{-1} with standard deviation of 0.3, 0.4 and 0.3 dl g^{-1}, respectively. Considering the experimental error on intrinsic viscosity determination, the fractionation method developed here is quite reproducible.

3.2. Determination of the Mark–Houwink–Sakurada equation constants

The constant a (Eq. (2)) was obtained from the slope of a double logarithmic plot of $[\eta]$ versus M_w, while the value log K was obtained from the extrapolation (Fig. 6). Seven fractions (F-1 to F-7) and five others from different fractionation experiments were used for the MHS constants determination. The polydispersity index of these fractions was between 1.28 and 1.44. The MHS equation constants a and K for chitin in DMAc/LiCl 5% (w/w) were found to be 0.95 (± 0.02) and 7.6×10^{-5} ($\pm 0.2 \times 10^{-5}$ dl g^{-1}), respectively. The uncertainties were evaluated from viscometry and HPSEC-MALLS-RI analysis standard deviations. The correlation coefficient of 0.9952 for the curve in Fig. 6 denotes the excellent linearity of the MHS equation obtained:

$$[\eta] = 7.6 \times 10^{-5} M_w^{0.95} \quad (4)$$

The MHS constants for chitin in DMAc/LiCl 5% (w/w) have been reported by few workers. The published values from these works are presented in Table 2. The first value of 0.69 for the constant a, determined with few samples only (Terbojevich, Carraro, Cosani, & Marsano, 1988), has been finally replaced by 0.88 in 1996 (Terbojevich et al., 1996). This later value was determined with more samples obtained from different fragmentation techniques.

The value of constant a is generally high for polymer with stiff chain or particular high draining properties (Elias, 1977). Since cellulose is known for these special draining properties, it is likely that chitin (similar in structure to cellulose) has a similar behavior (Elias, 1977). A value of

Table 1
Mass characterization of the fractions

Fraction number	% LiCl/DMAc	Weight (g)	Weight fraction (w_i)[a]	$[\eta]$ (dl g^{-1})	$\bar{M}_w$ ($\times 10^{-3}$ g mol^{-1})	$\langle r_g^2 \rangle^{1/2}$ (nm)	$\bar{M}_w/\bar{M}_n$
F-1	0.41	0.048	0.06	3.2	81	26.7	1.42
F-2	0.60	0.055	0.06	6.2	135	36.7	1.39
F-3	0.75	0.058	0.07	7.9	197	48.0	1.41
F-4	0.82	0.074	0.09	12.9	298	61.7	1.37
F-5	0.85	0.106	0.12	16.9	432	75.1	1.33
F-6	0.94	0.186	0.21	24.8	627	102.1	1.44
F-7	1.10	0.161	0.19	27.3	708	108.2	1.28
Initial sample	–	0.864	1.00	21.5	570	92.1	1.45

[a] $\sum w_{(1-7)} = 0.80 \rightarrow [\eta] = \sum_{1-7} w_i[\eta_i] = 14.7$ dl g^{-1}. If assuming all lost material in F-7 ($w_7 = 0.39$) $\rightarrow \sum w_{(1-7)} = 1.00 \rightarrow [\eta] = \sum_{1-7} w_i[\eta_i] =$ 20.3 dl g^{-1}.

Table 2
MHS constants for chitin in DMAc/LiCl 5% (w/w)

Research group	a	K (dl g^{-1})	M_w range ($\times 10^{-3}$ g mol^{-1})
Terbojevich et al. (1988)	0.69	2.4×10^{-3}	90–510
Terbojevich et al. (1996)	0.88	2.1×10^{-4}	120–1200
This work	0.95 ($\pm$0.02)	7.6×10^{-5} ($\pm 0.2 \times 10^{-5}$)	80–710

1.19 for the constant a of the system cellulose/DMAc/LiCl 9% has been reported by McCormick, Callais, and Hutchinson (1985). This high LiCl concentration in DMAc possibly enhance the expansion of the cellulose chains in solution. Vincendon (1985) demonstrated by ^{1}H NMR the persistence in DMAc/LiCl 5% of intramolecular hydrogen bonds (such as they exist in the solid state) between C(3)OH and O(5′), the ring oxygen atom of the neighboring unit. The presence of these hydrogen bonds would be expected to hinder rotation about the glycosidic bond and hence impart considerable rigidity to the chitin chain in this solvent system. The relatively high radius of gyration $\langle r_g^2 \rangle^{1/2}$ values obtained by light scattering (Table 1) suggest a high rigidity of chitin chains in solution in DMAc/LiCl 5%. The value of 0.95 ± 0.02 for the constant a is also consistent with this hypothesis. The difference with the value obtained by Terbojevich et al. (1996) can be explained from the fact they used samples with unspecified polydispersity index in a different molecular weight range.

The MHS equation obtained in this work allows the determination of M_w for chitin sample with similar polydispersity index (1.3–1.4) by viscometry in DMAc/LiCl 5% under the same conditions. For samples with different polydispersity, this equation will give a viscometric average molecular weight (M_v). Since M_w is close to M_v when the constant $a \sim 1$, variations in molecular weight distribution between different samples may be tolerated (Ottøy et al., 1996). The equation obtained is valid from 80,000 to 710,000 g mol^{-1} for samples with degree of acetylation close to 91%.

4. Conclusion

A new method for chitin fractionation by coacervate extraction in the mixed solvent system DMAc/LiCl has been developed. The controlled increase of the extracting power of the solvent was successfully carried out using slight modification of the solvent composition. Partial extractions of molecular species were done between coacervation and complete dissolution limits using different mixtures of DMAc/LiCl of increasing extracting power. Fractions obtained by coacervate extraction range from 80,000 to 710,000 g mol^{-1} with polydispersity index between 1.28 and 1.44. An interesting reduction of the polydispersity has been observed for some of these fractions. Continuous chitin fractionation could be potentially automated using counter-current chromatography fractionation (Francuskiewicz, 1994). Complete mass characterization of the fractions has been realized in DMAc/LiCl 5% (w/w) by viscometry and HPSEC-MALLS-RI. The MHS equation constants a and K for chitin in DMAc/LiCl 5% (w/w) were found to be 0.95 ($\pm$0.02) and 7.6×10^{-5} ($\pm 0.2 \times 10^{-5}$) dl g^{-1} respectively. This study improves the understanding of the mixed solvent system DMAc/LiCl. It could be potentially interesting for new polymer processing developments.

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