Dissolution or Extraction of Crustacean Shells Using Ionic Liquids to Obtain High Molecular Weight Purified Chitin and Direct Production of Chitin Films and Fibers

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Supporting Information

Materials. [C_2mim]Cl, [C_4mim]Cl, and [C_2mim]OAc (purity ≥90%) were obtained from BASF (Ludwigshafen, Germany) and dried in a vacuum oven at ~70 °C for 20 h before use. Deionized (DI) water was obtained from a commercial deionizer (Culligan: Northbrook, IL) with specific resistivity of 17.25 MΩ cm at 25 °C. Dimethyl sulfoxide (DMSO) (≥99.6%) was purchased from Aldrich (St. Louis, MO) and used as received. Pure chitin (from crab shells, C9752) and practical grade chitin (PG-chitin; from crab shells, C7170) were purchased from Sigma (St. Louis, MO) and used directly without further purification.

Frozen shrimp were obtained from Cox's Wholesale Seafood, Inc. (Tampa, FL). The shrimp were thawed, carefully peeled to make sure no obvious shrimp meat was left, and the back and tail of the shells were retained. The shells were washed three times with tap water and then dried in oven (Precision Econotherm Laboratory, Winchester, VA) at 80 °C for 2 days. The dried shells were ground for 1 min using a Janke & Kunkel mill (Ika Labortechnik, Wilmington, NC) and separated using brass sieves (Ika Labortechnik, Wilmington, NC) with pore sizes ranging from 0.125 mm to 1 mm, into particle sizes of 0.125 - 0.5 mm.

Characterization of chitin and crustacean shells. The moisture content of chitinous samples was determined gravimetrically by mass loss after drying the chitin or crustacean shells in an oven at 105 °C for 24 h.[1] Ash content was determined as the final mass obtained after heating the dry sample in a dish in a muffle furnace at *ca.* 525 °C until constant weight was obtained.[2] Chitin content was determined according to the Black and Schwartz methodology.[3]

Comparison of pure chitin, PG-chitin, and crustacean shell dissolution under a set of standard conditions using oil bath heating method. Approximately 1 g of PG-chitin, or dried, ground shrimp shells was mixed with 10 g of dried IL in a 50 mL Pyrex® glass beaker with a magnetic stir bar. For dissolution of pure chitin, 0.5 g of pure chitin was mixed with 2 g of dried IL in a 20 mL glass vial with a magnetic stir bar. The mixture was vigorously stirred (~700 rpm) and heated in an oil bath at 100 °C in air using a digital stirring hotplate (Isotemp®, Fisher Scientific, Dubuque, IA). After heating for 19 h, the mixture was transferred

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to a 20 mL glass vial, and *ca.* 2-10 mL of DMSO were added to the mixture to reduce the viscosity and remove any dissolved substances adsorbed or trapped within the solid texture. The mixture was vortexed by a Thermolyne type 37600 mixer (Dubuque, IA) for 30 s and centrifuged (Clay Adams® Brand DYNAC centrifuge, Sparks, MD) at $100 \times g$ for 10 min. (Dilution of dissolution mixture by DMSO was carried out at room temperature and finished within 30 min. We have tested and found that chitin is not soluble in DMSO under these conditions.) The undissolved residue was then separated, washed with DI water (3 × 20 mL) and dried in oven at 80 °C for 20 h. The dried residue was weighed and the dry mass (m_R) was used to calculate the percent dissolved by eq. 1:

$$Percent\ Dissolved = \frac{m_I - m_R}{m_I} \times 100 \tag{1}$$

where m_I is the initial mass of dried chitinous sample.

Study of shrimp shell dissolution at room temperature. Approximately 1 g of dried, ground shrimp shells (as above) was mixed with 10 g of dried [C_2mim]OAc in a 20 mL glass vial. The mixture was left at room temperature with occasional stirring for 4 months in air. After that, the viscous mixture was diluted by DMSO (10 mL) and the undissolved residue was separated, washed, and dried by the same method as above. The percent dissolved was calculated using eq. 1.

Study of microwave irradiation on chitin dissolution. Approximately 0.2 - 0.4 g of chitinous sample was mixed with 10 g of dried IL in a 20 mL glass vial. The mixture was heated in a domestic microwave oven (SHARP Carousel R-209KK, Mahwah, NJ) using 3 s pulses at full power (**CAUTION: care must be taken to avoid overheating the IL**). Between each pulse, the vial was removed, the mixture was manually stirred by a glass rod and then replaced in the microwave. Complete dissolution was monitored by removing a drop of the mixture and placing it in between two pieces of closely contacted glass slides for observation of any undissolved residue using an optical microscope (Reichert Stereo Star Zoom 580, Depew, NY).

Reconstitution of chitin from IL solution. Each clear IL solution was poured slowly into a beaker containing 200 mL of coagulating solvent (DI water) and white flocs formed immediately. Each mixture was then stirred at room temperature for 3 h and poured into four 50 mL glass vials for centrifugation. After centrifugation at $100 \times g$ for 10 min, the settled flocs were triply washed with DI water (3x 100 mL), and dried at 80 °C for 20 h in the oven. The yield of reconstituted chitin was calculated using eq. 2:

$$Chitin\ Reconstitution\ Yield\ (wt\%) = \frac{m_{RC} \times C_{RC}}{m_D \times C_{DC}} \times 100 \tag{2}$$

where m_{RC} is the mass of dried reconstituted chitin and m_D is the mass of dissolved chitinous material. In partial dissolution, m_D = $m_I - m_R$; and in complete dissolution, m_D is the total amount of chitinous sample dissolved in IL. C_{RC} is the chitin content (wt%) of the reconstituted chitin, C_{DC} is the chitin content (wt%) of the dissolved chitinous sample. Both C_{RC} and C_{DC} were determined

according to the Black and Schwartz methodology.[3] For shrimp shell, the chitin reconstitution yield calculation is based on an assumption that the dissolution of the components is not selective.

Viscosity study on chitin-IL solutions. 0.0252 g chitinous sample was mixed with 12 g IL in a 20 mL vial. The mixture was heated in a domestic microwave oven using 3 s pulses at full power for up to 2 min till complete dissolution was reached. Between each pulse, the vial was removed; the mixture was manually stirred by a glass rod and then replaced in the microwave. The chitin-IL solution was then transferred to an Ubelohde viscometer, and the flow time was measured at 35 $^{\circ}$C by keeping the viscometer in a water bath. The flow time was measured at least 3 times and the differences were less than 0.2 sec. The reported results are the average of the three readings. The relative viscosities were calculated using eq. 3:

$$\eta_{rel} = \frac{\eta_s}{\eta_0} = \frac{t_s}{t_0} \tag{3}$$

where η_{rel} is the relative viscosity, η_s or t_s is the viscosity or flow time of the solution, and η_0 or t_0 is the viscosity or flow time of the solvent.

Chitin fiber preparation. 0.2 – 0.4 g chitin (PG-chitin, shrimp shells or chitin regenerated from them) was mixed with 10 g IL in a 20 mL vial. The mixture was heated in a domestic microwave oven using 3 s pulses at full power for 2 min till complete dissolution was reached. Between each pulse, the vial was removed; the mixture was manually stirred by a glass rod and then replaced in the microwave. The chitin-IL solutions were used to produce chitin fibers using a dry-jet wet spinning method as described for producing cellulose fibers from IL solution.[4] DI water was used as the coagulant. After centrifugation, where gas bubbles were completely removed, each chitin-IL sample was carefully loaded into a 10-mL syringe which was then attached to a syringe pump (Model No. NE-1010, New Era Pump Systems, Inc, Farmingdale, NY, USA) with a temperature controller set at 60 $^{\circ}$C. Each solution was extruded into a 0.6 m long water bath after a 5 cm air gap. Each regenerated chitin filament was led through the first two steps of the godets, and then wound onto the take-up spool. An extrusion rate of 0.7 mL/min was used. The voltage settings for godets and take-up spool were 4.2 V and 2.9 V, respectively. The produced fibers were soaked in warm DI water for 1-2 days to remove the residual IL and then air dried.

Characterization. Elemental analyses (CHNO) for chitin or reconstituted chitin samples were obtained from Galbraith Laboratories, Inc. (Knoxville, TN). EDS spectra and SEM images were recorded on a Philips XL30 SEM-EDS (Eindhoven, The Netherlands) where the SEM was equipped with elemental analysis capabilities. The SEM images were taken by first sputter-coating the samples with gold to improve the conductivity. The tensile properties of the fibers were determined using a MTS Q-Test 25 machine attached with a specially designed pneumatic grip suitable for thin and flexible fiber testing. A load cell of 22.4 newton capacity was used for load measurement. The cross head speed was maintained at 1.27 mm min^{-1}.

Table S1. Compositional analysis (wt%) of chitin and crustacean shells

	Pure chitin	Practical grade chitin	Shrimp shells	Crustacean shells[5]
Moisture content	5.5	5.3	8.7	N/A
Ash content	0.3	2.2	34.5	30-50
Chitin content	81.8	78.9	27.2	13-41
Chitin content of the reconstituted chitin from dissolution of each chitin source	87.1	84.7	81.1	N/A

Table S2. Elemental analyses for four chitin sources before and after dissolution and coagulation

			C, %	H, %	N, %	O, %
Entry	Theoretical value for chitin $(C_8H_{13}NO_5)_n$		47.29	6.40	6.90	39.41
1	Pure chitin	Result	42.47	6.89	6.16	42.81
		% deviation from theory	*10.19*	*7.66*	*10.72*	*8.63*
2	Reconstituted pure chitin	Result	46.52	6.64	6.54	38.82
		% deviation from theory	*1.63*	*3.75*	*5.22*	*1.50*
3	PG-chitin	Result	44.25	6.89	5.89	39.28
		% deviation from theory	*6.43*	*7.66*	*14.64*	*0.33*
4	Reconstituted PG-chitin	Result	45.51	6.50	6.17	39.47
		% deviation from theory	*3.76*	*1.56*	*10.58*	*0.15*
5	Reconstituted shrimp shell chitin	Result	44.18	6.72	6.64	41.61
		% deviation from theory	*6.58*	*5.00*	*3.77*	*5.58*

Table S3. Comparison of the chemical composition observed in the EDS analysis for chitinous samples and the chitin reconstituted from them

Pure chitin			Reconstituted pure chitin		
Element	Weight%	Atomic%	Element	Weight%	Atomic%
C K	30.06 ±1.06	35.18±1.12	C K	29.72±0.47	34.78±0.51
N K	27.09±0.08	27.18±0.01	N K	27.80±0.42	27.90±0.45
O K	42.86±1.12	37.65±1.12	O K	42.49±0.04	37.33±0.06
Total	100.00	100.00	Total	100.00	100.00
Practical grade chitin			**Reconstituted practical grade chitin**		
Element	Weight%	Atomic%	Element	Weight%	Atomic%
C K	31.01±1.06	36.56±1.17	C K	31.08±0.59	36.36±0.65
N K	26.63±0.79	26.93±0.87	N K	26.13±0.76	26.21±0.78
O K	40.34±0.23	35.71±0.29	O K	42.36±0.30	37.21±0.21
Si K	0.43±0.01	0.22±0.01	Si K	0.44±0.20	0.22±0.10
Cl K	0.75±0.04	0.30±0.01	Total	100.00	100.00
Ca K	0.85±0.06	0.30±0.02			
Total	100.01	100.00			
Shrimp shells			**Reconstituted shrimp shell chitin**		
Element	Weight%	Atomic%	Element	Weight%	Atomic%
C K	22.55±0.79	28.95±0.74	C K	30.22±0.15	35.54±0.13
N K	27.31±0.54	30.06±0.31	N K	25.14±0.50	25.35±0.47
O K	37.20±0.25	35.85±0.57	O K	43.82±0.57	38.20±0.15
Si K	0.23±0.04	0.13±0.02	Si K	0.83±0.07	0.42±0.04
Ca K	12.19±0.98	4.69±0.42	Total	100.00	99.50
Mg K	0.53±0.06	0.33±0.04			
Total	99.99	100.00			

Table S4. Relative viscosities of chitin in [C$_2$mim]OAc at 35 $^{\circ}$C

Samples	Conc. of chitin in [C$_2$mim]OAc (%)	Total heating time (sec)[a]	Relative Viscosity(η_{rel})[b]
[C$_2$mim]OAc	0	N/A	1
Pure chitin	0.21	120	1.13
PG-chitin	0.21	120	2.02
Shrimp shells	0.21	120	1.55
Rec. pure chitin	0.21	60	1.10
Rec. PG-chitin	0.21	60	1.28
Rec. shrimp shell chitin	0.21	60	1.43

[a]Reconstitued chitin samples are easier to be completely dissolved than unprocessed chitin, thus less heating time was used.
[b]η_{rel} = flow time of the solution/flow time of the solvent ([C$_2$mim]OAc).

References

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