Electronic Supplementary Information (ESI)

A Method for Determining the Uniquely High Molecular Weight of Chitin Extracted from Raw Shrimp Shells Using Ionic Liquids

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MATERIALS AND METHODS

Materials

Ionic liquid 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) was obtained from two suppliers, Iolitec USA (Tuscaloosa, AL, USA), Lot# P01633.4.3-IL-0189, water content 0.52%, 20 kg batch; and Proionic (Grambach, Austria), Lot# 17PI250_F3, water content 0.11%, 917 kg batch. The shrimp shell biomass was obtained from Mari Signum Dragon Drying facility (Vancleave, MS) and was dried in an oven overnight and ground to particle size <125 μm prior to use. PG-chitin was obtained from Sigma–Aldrich (St. Louis, MO), catalog number C-7170, Lot # SLBQ6580V and 68H7014, dried and sieved to <125 μm particle size prior to use.

Solvents *N,N*-Dimethylsulfoxide (DMSO) and *N*,*N*-Dimethylformamide (DMF), both ≥99.8% ACS, produced by VWR Chemicals BDH® were obtained from VWR Canada. *N*-Methyl-2-pyrrolidone (NMP), semi-grade for the electronics industry, supplied by VWR

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Chemicals BDH® was obtained from VWR Canada. Toluene for light scattering instrument calibration was of HPLC grade (purity 99.85%) manufactured by Acros Organics was obtained from VWR, West Chester, PA, catalog number 26837-0010, Lot# B0537317. Toluene was distilled prior to use, and kept under nitrogen, over 3Å molecular sieves dried at 300 °C and cooled to room temperature (RT) under Argon. *N*,*N*-Dimethylacetamide (DMAc, puriss. p.a., ≥99.5% (GC)) was obtained from Sigma–Aldrich (St. Louis, MO), and kept over 3Å-molecular sieves dried at 300 °C and cooled to RT under Argon. Anhydrous lithium chloride (LiCl, 99.5%) was purchased from Sigma–Aldrich (St. Louis, MO) and dried under vacuum at 60 °C, in an oil bath for 72 h before use.

Sodium hydroxide pellets (purity ≥95.0%) manufactured by Spectrum Chemical Mfg. Corp. were obtained from VWR Canada. Urea (purity 99.0-100%, crystallized ACS, VWR Chemicals BDH®) was obtained from VWR Canada. All aforementioned chemicals were used without additional purification unless otherwise noted.

DI water (DI) was obtained from two sources: a commercial Deionization Filter System (Culligan, Northbrook, IL) that provided water with specific resistivity of 16.82 MΩ cm at 25 °C, and an Evoqua Water Technologies' Purification System LDIRS03 (Richmond, VA).

Chitin Extraction

Exactly 4.001 g of ground biomass (<250 μm particle size) was added to 196.020 g $[C_2mim][OAc]$, in a 250 mL Erlenmeyer flask. The flask was covered with parafilm and microwaved in a domestic microwave for 6 min total, with 3 s pulses for the first minute, followed by 2 s pulses for the remainder of the time. After each set of pulses, the Erlenmeyer flask was taken out of the microwave and manually swirled in order to evenly distribute biomass, and allow even heat distribution. After 5 min, the sample was tested for crystallization by addition of a small amount (*ca*. 0.5 g) of solution into 50 mL of water. If the sample retained a fibrous structure upon crystallization, the solution was ready for the chitin coagulation step; if the sample's viscosity was too low to retain the fibrous structure, the microwaving was continued in the manner described above.

Before coagulation, the bulk of the solution was transferred into 6 x 50 mL plastic centrifuge test tubes and centrifuged from undissolved minerals using a Sorvall Legend XTR centrifuge (Thermo Electron LED Gmbh, Osterode am Harz, Germany). Three centrifugation cycles were

conducted, for 20 min each, at 3800 rpm. Between cycles the sample was reheated to 80 °C in a StableTemp gravity convention oven (Cole Parmer, Vermon Hills, IL) to keep the solution free-flowing for easier removal of any particulate matter.

Once centrifugation was complete, the solution was reheated again to 80 °C to keep it free flowing and then poured into a beaker of DI water (800 mL), for coagulation. When coagulation was complete, the water was decanted, and fresh DI water (800 mL) was added. The DI water was refreshed every hour, to constitute 14 washing cycles total. After washing was complete, the wet chitin was placed into a large crystallizing dish for drying in an 80 °C oven for 24 h until fully dried. The dried chitin was ground to ≤125 μm size particles and stored in a glass vial.

Various Solvent Systems

DMAc/LiCl Method 1: To prepare DMAc/LiCl (8.0 % w/v LiCl in DMAc), 2.008 g of LiCl was dissolved in 23.405 g (or 24.978 mL) of DMAc, stirred for 2 h and filtered using a syringe VWR Teflon 0.2 μm filter (Catalog # CA97035-174). Then 5.0 mL of this mixture was withdrawn and diluted with 5.4 mL and 3.4 mL of fresh DMAc, to prepare solutions of 4 and 5 % w/v LiCl in DMAc, respectively. After dilution, the solutions were again filtered using the syringe VWR Teflon but now using 0.45 μm filter. For the dissolution, 0.010 g of biopolymer was weighed in a culture vial. Then 5 mL of the DMAc-LiCl mixture (DMAc/4 wt% LiCl) was added and the mixture was stirred vigorously at 40 ^{o}C for 2 h. Since complete dissolution was not achieved, the stirring continued but the temperature was increased to 90 ^{o}C. The heating continued for 1 week, however, complete dissolution was never achieved. The results are presented in **Table S1**, **Entries 1** and **2**, for PG- and IL-extracted chitin, respectively.

The dissolution of chitins in DMAc/5 wt% LiCl (see **Table S1**, **Entries 3** and **4**, for PG and IL-extracted chitin respectively), and DMAc/8 wt% LiCl (see **Table S1**, **Entries 5** and **6**, for PG and IL-extracted chitin respectively) was conducted in a similar manner with the only exception being the amount of LiCl salt in DMAc/LiCl solution.

DMAc/LiCl Method 2: In another set of experiments, 0.010 g of sample was weighed in a beaker. Then 10 mL of DI water was added and the mixture was boiled for 2 h with occasional stirring using a glass rod. The solution was centrifuged and washed three times with 3 mL hot deionized water. The residue was collected in a beaker and 5 mL of methanol was added. The mixture was stirred with a magnetic stirrer for 30 min without heating. The solution was then

centrifuged and washed three times with 3 mL methanol each time. The residue was collected and soaked in fresh DMAc for 30 min with stirring. The resulting solution was centrifuged and washed three times with 3 mL DMAc each time. The residue was collected to be used later.

To prepare DMAc/LiCl (8.0 % w/v LiCl in DMAc), 2.008 g of LiCl was dissolved in 23.405 g (or 24.978 mL) of DMAc, stirred for 2 h and filtered using a syringe VWR Teflon 0.2 µm filter. To the chitin "residue", 5 mL of freshly prepared 8% w/v LiCl in DMAc (0.8 g LiCl in 10 mL DMAc) was added. The mixture was stirred continuously and heated at 40 C for 2 h. Since complete dissolution was not achieved within 2 h, the stirring was continued and the temperature increased to 90 ^{o}C. The heating continued for 1 week. The results are presented in **Table S1**, **Entries 7** and **8**, for PG- and IL-extracted chitin, respectively. The results are presented in **Table S1**.

NaOH/urea aqueous system 8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water: NaOH/urea aqueous systems of 8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water were prepared through dissolution of 8.004 g pelleted NaOH in 88.123 mL of DI water under stirring for 2 hours at room temperature, followed by addition of 4.006 g urea. Freshly-prepared NaOH/urea aqueous system was used to dissolve 0.5 wt% of each (PG- or IL-extracted) biopolymer by freeze/thaw (F/T) treatment as follows: exactly 0.010 g of biopolymer powder was added to 1.985 g of NaOH/urea aqueous system in a vial and the mixture was stirred at room temperature for 2 min. The mixture was then frozen by immersing the vial in a dry ice/ethanol cold bath (ca. -72 °C) for 3 min, followed by thawing at room temperature (ca. 8 min) and subsequent vigorous stirring (5 min). The F/T cycles were repeated several times to achieve full dissolution. The results are presented in **Table S1**, **Entries 9**, **10** and **11**, **12**, for PG- and IL-extracted chitin, respectively. If the biopolymer was not dissolved after 20 F/T cycles, it was then designated as insoluble.

[C_2mim][OAc] – DMAc 1:1 v/v, 1:0.50 v/v, 1:0.25 v/v, and 1:0.1 v/v were only used on IL-extracted chitin as follows: 0.010 g of polymer was dissolved in [C_2mim][OAc] as follows. For direct dissolution, IL (~5 mL) followed by IL-extracted chitin (0.010 g) were weighed with a microbalance (Secura 125-1S, Sartorius Lab Instruments GMBH, Goettingen, Germany) into 4-dram screw top vials (VWR supplies, model number V2757C-FM-SP, catalog number 470146-668), equipped with a Teflon-coated stir bar, capped, and protected from water absorption with parafilm wrapped around the top. The vials were placed onto a hotplate stirrer (Talboys-

Troemner, Thorofare, NJ) and stirred in a heated (90 °C) oil bath for 48 h, until complete dissolution of the polymer was obtained. After that, solutions were allowed to cool to RT, and while continuing to be stirred, 5.0, 1.7, 0.88 mL, and 0.26 mL of DMAc were added at once, to prepare solutions IL/DMAc 50:50 v/v, 75:25 v/v, 85:15 v/v, and 95:5 v/v. The results are presented in **Table S1** (**Entries 13**, **14**, **15**, **16**) and **Figures S1 - S2**.

[C_2mim][OAc] – DMF (**Table 1, Entries 17 – 20**); **[C_2mim][OAc] – NMP** (**Table 1**, **Entries 21 – 24**); **[C2mim][OAc] – DMSO** (**Table 1**, **Entries 25 – 28**) in a ratio 50:50 v/v, 75:25 v/v, 85:15 v/v, and 95:5 v/v were prepared and tested as described above for the **[C_2mim][OAc] – DMAc** solvent system.

Table S1. Solvent Systems Attempted for Dissolution of Chitin

#	Solvent System	Chitin Type	Chitin Load, wt% (mg/mL)	Dissolution Conditions	Result
1	DMAc/4 wt% LiCl	PG	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
2	DMAc/4 wt% LiCl	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
3	DMAc/5 wt% LiCl	PG	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
4	DMAc/5 wt% LiCl	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
5	DMAc/8 wt% LiCl	PG	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
6	DMAc/8 wt% LiCl	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
7	DMAc/8 wt% LiCl	PG	0.5 wt% (~5 mg/mL)	Chitin activated in DMAc by stirring 2 days followed by dissolution in the selected solvent for up to 1 week, at 90 °C	Solubilized
8	DMAc/8 wt% LiCl	IL-extracted	0.5 wt% (~5 mg/mL)	Chitin activated in DMAc by stirring 2 days followed by dissolution in the selected solvent for up to 1 week, at 90 °C	Not solubilized
9	8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water	PG	0.5 wt% (5 mg/g)	5 freeze-thaw cycles	Not solubilized
10	8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water	PG	0.5 wt% (5 mg/g)	10 freeze-thaw cycles	Solubilized

11	8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water	IL-extracted	0.5 wt% (5 mg/g)	10 freeze-thaw cycles	Not solubilized
12	8 wt%/4 wt%/88 wt% NaOH/urea/deionized (DI) water	IL-extracted	0.5 wt% (5 mg/g)	20 freeze-thaw cycles	Not solubilized
13	[C_2mim][OAc] – DMAc 50:50 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
14	[C_2mim][OAc] – DMAc 75:25 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
15	[C_2mim][OAc] – DMAc 85:15 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
16	[C_2mim][OAc] – DMAc 95:5 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
17	[C_2mim][OAc] – DMF 50:50 v/v	IL-extracted	0.5 wt% (5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
18	[C_2mim][OAc] – DMF 75:25 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
19	[C_2mim][OAc] – DMF 85:15 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
20	[C_2mim][OAc] – DMF 95:5 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
21	[C_2mim][OAc] – NMP 50:50 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
22	[C_2mim][OAc] – NMP 75:25 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
23	[C_2mim][OAc] – NMP 85:15 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
24	[C_2mim][OAc] – NMP 95:5 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
25	[C_2mim][OAc] – DMSO 50:50 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
26	[C_2mim][OAc] – DMSO 75:25 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
27	[C_2mim][OAc] – DMSO 85:15 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution
28	[C_2mim][OAc] – DMSO 95:5 v/v	IL-extracted	0.5 wt% (~5 mg/mL)	Dissolution in the IL, followed by addition of a cosolvent	Polymer precipitated from solution

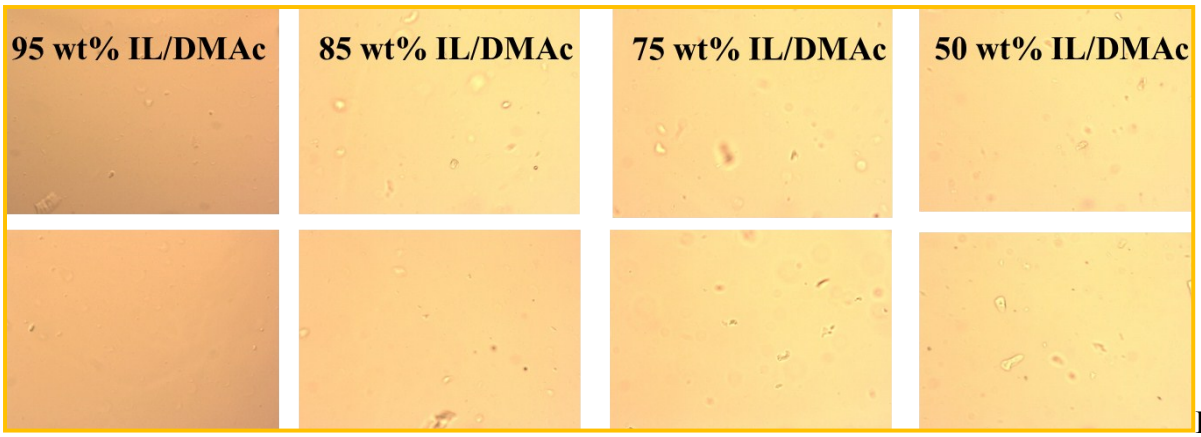


Figure S1. Microscopy images of the IL-extracted chitin in IL/DMAc showing undissolved polymer particles

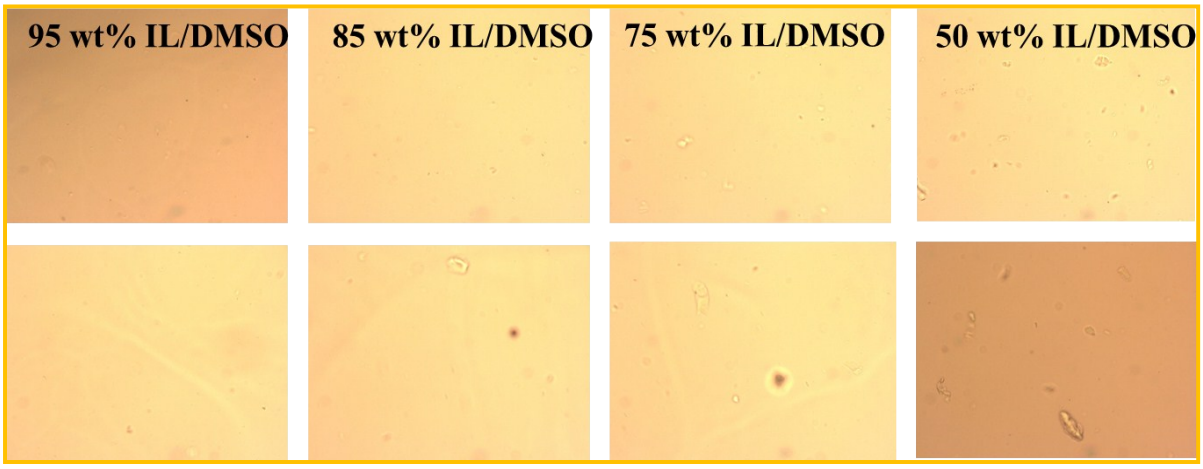


Figure S2. Microscopy images of the IL-extracted chitin in IL/DMSO showing undissolved polymer particles

Light Scattering

Solution Preparation

Method 1: Heating of Individual Chitin Samples in the IL

Each set of sample solutions was prepared using either direct dissolution of chitin at each concentration or consecutive dilutions of stock chitin solution. For direct dissolution, IL (~5 g) followed by IL-extracted or PG-chitin (0.0004 to 0.0040 g/g solution) was weighed with a microbalance (Secura 125-1S, Sartorius Lab Instruments GMBH, Goettingen, Germany) into 4-dram screw top vials (VWR supplies, model number V2757C-FM-SP, catalog number 470146-668). One 5 g sample of the neat IL (not loaded with chitin) was likewise weighed into an empty vial. All vials were equipped with a Teflon-coated stir bar, capped, and protected from water absorption from air with parafilm wrapped around the top. The vials were placed onto a hotplate stirrer (Talboys - Troemner, Thorofare, NJ) and stirred for 30 min, to ensure good mixing and to prevent any fine solids from falling to the bottom of the vial. The vials were then placed into a preheated (90 ^{o}C) in-house made aluminum heating block with 9 or 12 cylindrical holes (27 mm diameter) deep enough to surround all liquid inside the vials; one 3 mm-diameter hole was dedicated for positioning of a thermocouple. The samples were heated at 90 ^{o}C for 48 h while stirring, until complete dissolution of the polymer was obtained. Adequate stirring of the samples during dissolution appeared to be critical to eliminate polymer gelation and ensure even dissolution between vials. Chitin sample concentration (c) was calculated as mass of chitin divided by total mass of solution and expressed in g/g. The example of these samples is presented in **Table S2**, below.

Table S2. Solution Preparation for Light Scattering by Heating of Individual Chitin Samples in the IL

#	Chitin	Weight of Chitin Powder (g)	Weight of Chitin Powder + IL (g)	Concentration Chitin in the IL (g/g)
1	PG	0.0016	5.0006	0.000318
2	PG	0.0027	5.0068	0.000539
3	PG	0.0030	5.0095	0.000589
4	PG	0.0047	5.0135	0.000931
5	PG	0.0151	4.9969	0.003018
6	PG	0.0197	5.0155	0.003932

Method 2: Solution Preparation by Serial Dilution of 'Stock' Chitin Sample

A stock solution of concentration 0.020 g/g (PG-chitin and IL-Extracted Chitin) was prepared by direct dissolution of chitin in the IL. For the PG-chitin stock solution, 0.200 g chitin was heated in 9.801 g of the IL, in a 100 ^{o}C oil bath while stirring for 48 h. For the IL-chitin stock solution, 0.200 g chitin was heated in 9.803 g of the IL. Simultaneously, neat IL for use when diluting the stock concentration was heated in the same oil bath while stirring for consistency. The heated IL was weighed directly into the 4-dram vials, followed by increasing increments of the stock solution to obtain solutions of concentration 0.0009 to 0.009 g/g solution. Chitin solution concentration (c) was calculated as the multiplication of the concentration of the stock solution (in g/g) and the mass of stock solution divided by total mass of solution and expressed in g/g.

Table S3. Solution Preparation for Light Scattering Measurements

#	Chitin	Weight of Stock Solution[a] or Solid Chitin[c] (g)	Total Weight of Solution (g)	Concentration Chitin in the IL (g/g)	SLS Measurement
PG Trial 1					
1	PG[a]	0.14032	3.15299	0.00093	
2	PG[a]	0.23060	3.23321	0.00153	Excluded from SLS measurement[b]
3	PG[a]	0.39061	3.39748	0.00258	
4	PG[a]	0.45152	3.44984	0.00300	Excluded from SLS measurement[b]
5	PG[a]	0.50578	3.50897	0.00335	
6	PG[a]	0.74710	3.75606	0.00493	
PG Trial 2					
7	PG[c]	0.01062	10.09473	0.001052	Excluded from SLS measurement[b]
8	PG[c]	0.01517	10.12073	0.001499	
9	PG[c]	0.02029	10.16188	0.001997	
10	PG[c]	0.03051	10.14172	0.003008	
11	PG[c]	0.04082	10.13092	0.004029	
PG Trial 3					
12	PG[c]	0.01084	10.06539	0.001076	
13	PG[c]	0.01611	10.18359	0.001581	Excluded from SLS measurement[b]
14	PG[c]	0.02103	10.18503	0.002064	
15	PG[c]	0.02948	10.25663	0.002874	
16	PG[c]	0.04280	10.07039	0.004250	
17	PG[c]	0.05194	10.31694	0.005034	Excluded from SLS measurement[b]
18	PG[c]	0.06289	10.44675	0.006020	No measurement taken [d]
IL-Extracted Trial 1					
20	IL-Extracted[c]	0.01079	10.24308	0.001053	
21	IL-Extracted[c]	0.01537	10.09789	0.001522	Excluded from SLS measurement[b]
22	IL-Extracted[c]	0.02031	10.17426	0.001996	
23	IL-Extracted[c]	0.03018	10.18241	0.002964	
24	IL-Extracted[c]	0.04032	10.14278	0.003975	
IL-Extracted Trial 2					
25	IL-Extracted[c]	0.01047	10.22778	0.001025	
26	IL-Extracted[c]	0.02082	10.22408	0.002036	
27	IL-Extracted[c]	0.02540	10.26518	0.002474	Excluded from SLS measurement[b]
28	IL-Extracted[c]	0.03015	10.14863	0.002971	
29	IL-Extracted[c]	0.03528	10.27731	0.003433	

[a]Samples prepared *via* serial dilution of stock solution; [b]Excluded from SLS measurements due to significant color difference from the rest of the samples; [c]Samples prepared *via* direct dissolution of chitin into IL in separate vials; [d]High viscosity solutions cannot be measured accurately.

Evaluation of the Refractive Index (n)

An Abbemat 500 refractometer (Anton Paar, Ashland, USA) was used to measure refractive index. The refractometer was allowed to equilibrate to 25 °C after the sample cell was rinsed with acetone, and wiped with KIMTECH Kimwipes® Low-Lint Wipers (4.4 x 8.4") supplied by ULINE. The refractometer was then calibrated at 25 °C using DI water. To measure refractive index, enough of each solution was added to cover the optics. The solutions were allowed to sit for 120 s to equilibrate at 25 °C, and the refractive index was measured once per minute until a stable value of n was obtained.

Determination of the Refractive Index Increment (dn/dc)

Plots of n as a function of chitin concentration (c) were constructed. The slopes of these lines were calculated and recorded as dn/dc.

Viscosity Measurements

Viscosity measurements were conducted on a ViscoLab-4000 viscometer (PAC LP, Houston, TX) at 25 °C, using the 50-1000 cP piston. For heating the viscometer to 25 °C, an electric heating pad was wrapped around the steel chamber of the viscometer.

Static Light Scattering (SLS)

Materials

Rectangular glass cuvettes (World Precision Instruments, standard optical glass cuvettes, open-top type, Teflon lid, thickness 1.25 mm x 2, light path 10 mm, volume 3.5 mL, outside dimensions 45 mm × 12.5 mm × 12.5 mm; spectral range 350-2500 nm) were used for all measurements.

Method

The molecular weights of chitin samples were determined by the SLS technique using a Zetasizer Nano ZS (Malvern Instruments, Inc., Westborough, MA) at 25 °C with a laser wavelength of 633 nm and a scattering angle of 173°. Prior to the measurements the power performance (laser power) of the instrument was checked with a toluene scattering standard with

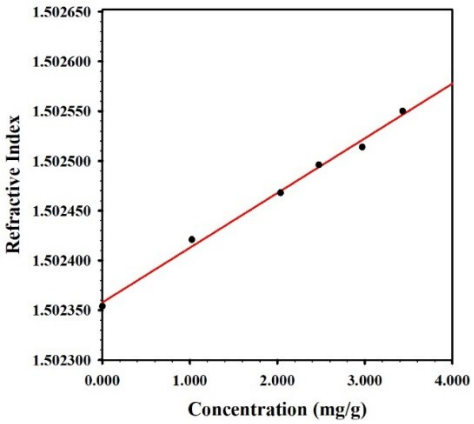
known Rayleigh ratio to allow the system to be characterized at all attenuation levels and normalize sample scattering accordingly. For that, anhydrous, distilled toluene was placed into a glass cuvette and the scattering was measured in back scattering mode with the attenuator's setting consecutively changed from 1 to 11. (Note: The Rayleigh ratio of toluene is known to be R_{Tol}: 1.35 x 10^{-5} cm^{-1}, and the refractive index n_{Tol} is 1.4909.) The results are presented below in **Table S4**.

Table S4. Calculation of Attenuation Factor

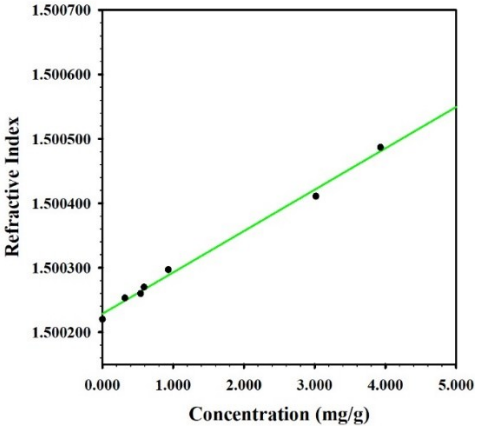
Attenuation	Counts/s	Attenuation Factor
11	218.40	1
10	67.00	0.3067
9	23.00	0.1060
8	10.00	0.0436
7	2.45	0.0126

The standard operation procedures of the instrument were set to prompt the operator to enter sample viscosity first, and then specify the measurement temperature (25 ^{o}C). Measurement type was set to manual, with 10 runs/measurement, 10 s run duration, and 10 total measurements. The delay between measurements was set to 10 s. The following parameters were used for the IL dispersant: Solvent: [C_2mim][OAc]; T = 25 ^{o}C (120 s equilibration time at this temperature prior to measurement); Dispersant's viscosity: 151 cP; Dispersant's refractive index (n) 1.502.

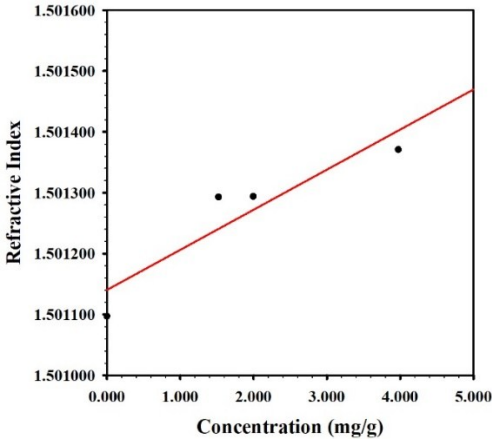
After all samples were recorded, the IL scattering was also determined using the same treatment and measurement conditions that were used for the IL-chitin samples. The IL was heated together with the samples, placed into a glass cuvette, and the scattering was measured in the back scattering mode with the attenuator set at 11 (no attenuation) so that IL scattering could be subtracted from overall value.



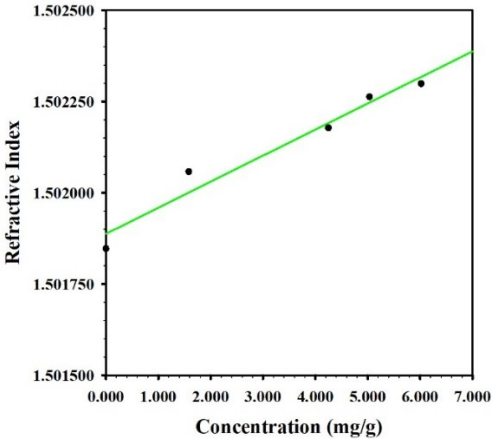
dn/dc 0.055 (R^2 0.995)



dn/dc 0.064 (R^2 0.994)

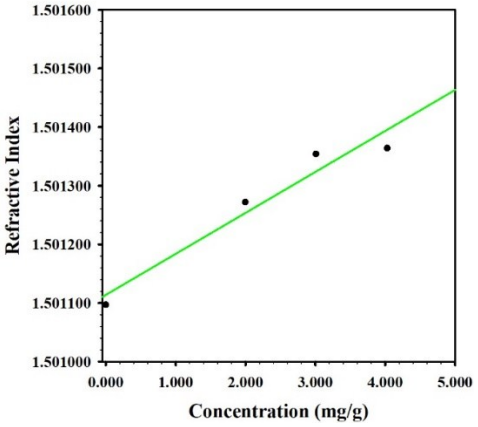


dn/dc 0.066 (R^2 0.852)



dn/dc 0.071 (R^2 0.957)

ND



dn/dc 0.070 (R^2 0.945)

Figure S3. Refractive index measurements. The slope of the plot defines dn/dc; IL-extracted chitin on the left (red) and PG-chitin on the right (green).

Table S5. Laser Parameters and Toluene Standard

Parameter	Values
Wavelength (nm):	633.0
T (^{o}C):	25.0
Solvent n_o:	1.5024
R_{Tol} (cm^{-1}):	1.35×10^{-5}
n_{Tol}:	1.4909
Optical Constant Toluene (K):	1.07×10^{-8}
Tol/Solv Int Ratio:	0.3

Table S6. Refractive Index Increment (dn/dc)

Chitin type	dn/dc
IL-extracted	0.055
IL-extracted	0.066
	0.061 ± 0.006
PG	0.071
PG	0.070
PG	0.064
	0.069 ± 0.003

Examples of Raw Data

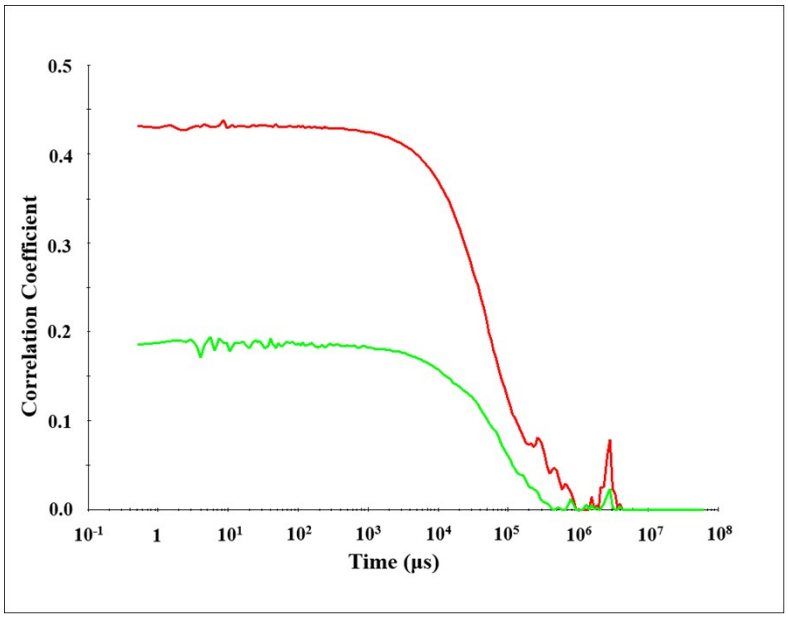


Figure S4: Comparison of raw correlation data and correlation coefficient for randomly chosen samples of the same concentration of IL-extracted chitin (red line) and PG-chitin (green line). Samples are the same as shown in **Figure 1 (main text)**: IL-extracted (1.0534 mg/g; red) and PG-chitin (1.0520 mg/g; green)

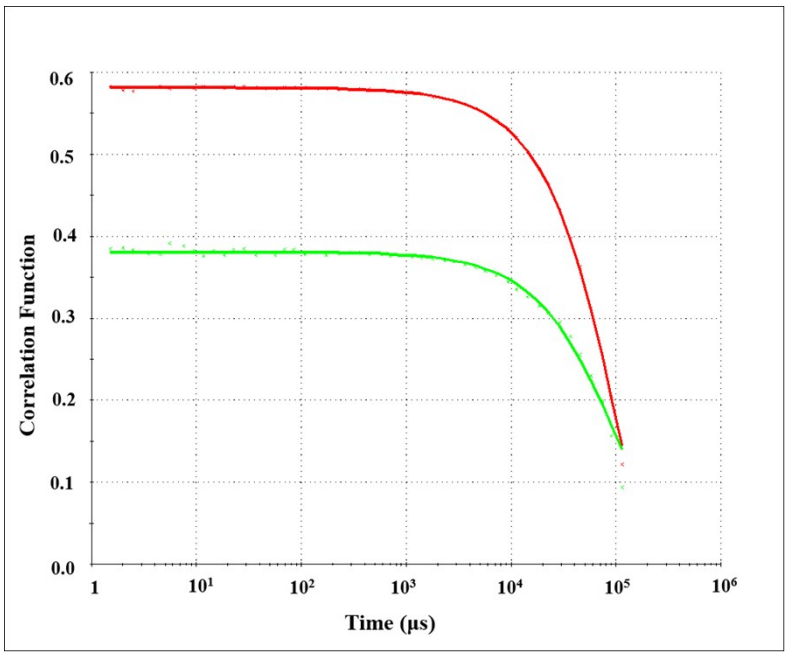


Figure S5: Cumulant fit for randomly chosen samples of the same concentration of IL-extracted chitin (red line) and PG-chitin (green line). Samples are the same as shown in **Figure 1 (main text)**: IL-extracted (1.0534 mg/g; red) and PG-chitin (1.0520 mg/g; green)

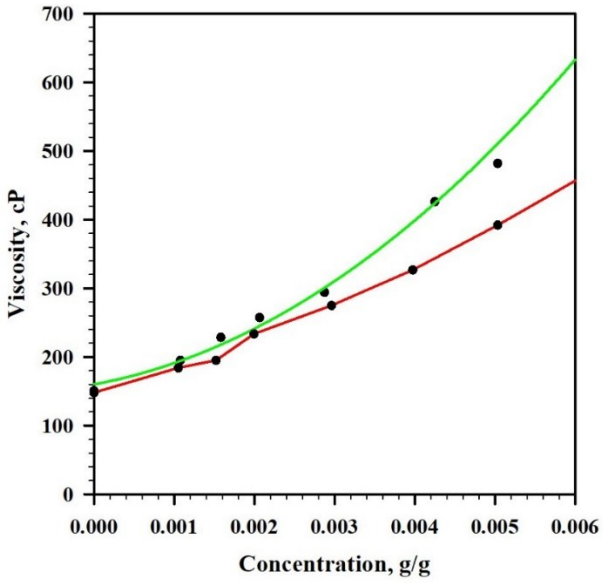


Figure S6. Viscosity for chitin IL-extracted (red) and PG-chitin (green) at 25 ^{o}C