Dissolution or extraction of crustacean shells using ionic liquids to obtain high molecular weight purified chitin and direct production of chitin films and fibers†

Ying Qin,‡ Xingmei Lu,§ Ning Sun and Robin D. Rogers*

Received 25th February 2010, Accepted 12th April 2010
First published as an Advance Article on the web 22nd April 2010
DOI: 10.1039/c003583a

1-Ethyl-3-methyl-imidazolium acetate can completely dissolve raw crustacean shells, leading to recovery of a high purity, high molecular weight chitin powder and to fibers and films which can be spun directly from the extract solution.

Chitin, a linear amino polysaccharide composed of β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucose units (Fig. 1a) found in the outer skeleton of arthropods, is the second most plentiful natural polymer after cellulose.[1] Its bioactivity, biocompatibility, and low toxicity make it suitable for controlled drug release formulations, cosmetics, food preservation, fertilizers, or biodegradable packaging materials,[2] while its ability to absorb both metal ions and hydrophobic organic compounds make it useful in waste water processing and other industrial applications.[3] However, due to its high density of hydrogen bonds, it is completely insoluble in water, most organic solvents, and dilute acidic or basic solutions, and thus the application of chitin has not been fully exploited. Various chemical modifications have been applied to make chitin more easily soluble,[4] the most important of which is *N*-deacetylation to form chitosan (Fig. 1b).[2,3]

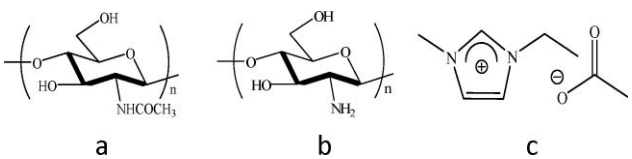

Fig. 1 Structure of a) chitin, b) chitosan, and c) [C_2mim]OAc.

Chitin can be obtained commercially in pure grade or practical grade (PG-chitin). PG-chitin is primarily produced from crustacean shells by a chemical method that involves acid demineralization of the shell, followed by removal of shell proteins by alkali treatment, and then decolorization.[5] It can be further purified by methanesulfonic acid treatment[6] to obtain pure chitin. Our analyses of the commercial samples we obtained (ESI, Table S1†), indicated chitin contents of 81.8% chitin in pure grade and 78.9% in PG-chitin (compared to 27.2% chitin in shrimp shells). In addition, the mineral (ash) content in PG-chitin is higher than in pure chitin.

Even though the current industrialized chemical process isolates chitin efficiently, the chitin molecular weight (MW) is reduced during processing.[2] A less chemical- and energy-intensive process for obtaining the chitin, and a purer, higher molecular weight chitin product would be desirable for many applications including fiber spinning.

Chitin is known to form microfibrillar arrangements in living organisms, and the presence of microfibrils suggests that chitin should be a good candidate for fiber spinning.[7] However, only a few papers describing the spinning of chitin fibers have been reported, mainly due to the limited number of solvent systems which can readily dissolved chitin in sufficient quantity and with appropriate rheology for spinning. Thus, producing chitin fibers or even films continues to be a challenge in chitin research.

In those cases where chitin fibers have been produced, commercial chitin powder has been used with solvent systems such as (1) halogenated solvents (*e.g.*, trichloroacetic acid (TCA), dichloroacetic acid (DCA),[7] or formic acid–DCA mixtures,[8] or (2) amide–LiCl systems (*e.g.*, *N*,*N*-dimethylacetamide (DMAc)–5% LiCl).[9] The drawbacks of these methods include the use of corrosive chemicals which can degrade the polymer upon even short exposures and difficulties in the complete removal and recovery of the solvent from the fiber. An environmentally-benign solvent which could readily solubilize chitin or even crustacean shells without derivatization would be greatly beneficial in this arena.

Ionic liquids (ILs, now defined as salts which melt below 100 °C), have received attention recently for the ability to efficiently dissolve cellulose and other natural biomaterials such as wood which contains hemicelluloses and lignin, beside cellulose.[10–12] Chitin can be described as cellulose with one hydroxyl group on each monomer replaced by an acetylamine group. This difference allows for increased hydrogen bonding between adjacent chains, and thus one might expect that chitin would be even more difficult to dissolve than cellulose.[13] Indeed, only limited reports of pure chitin dissolved in ILs have appeared. Xie *et al.*[14] reported that 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) can dissolve pure chitin and chitosan with solubilities of *ca.* 10 wt% in 5 h at 110 °C, and Yamazaki *et al.* obtained similar solubilities with 1-allyl-3-methylimidazolium bromide [Amim]Br at 100 °C for 24 h.[15] Wu *et al.*[13] reported

Center for Green Manufacturing and Department of Chemistry, The University of Alabama, Tuscaloosa, AL, 35487, USA.
E-mail: rdrogers@as.ua.edu

† Electronic supplementary information (ESI) available: Tables S1–S4. See DOI: 10.1039/c003583a

‡ Current address: Alabama Innovation and Mentoring of Entrepreneurs, The University of Alabama, Tuscaloosa, AL 35487, USA.
§ Current address: Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China.

using the acetate salt [C_4mim]OAc to dissolve 'native' chitin with 3–7 wt% solubility at 110 °C. According to the chemical provider of the chitin used in this study, the native chitin can also be classified as 'pure' chitin with chitin content of 94.7–96.4%.

Crustacean shells (*e.g.*, shrimp shells) contain not only chitin, but also large amounts of protein, mineral salts, and a small amount of lipids, and we expected these to be even harder to dissolve than either PG-chitin or pure chitin. To the best of our knowledge, no one has reported either the direct dissolution of crustacean shells (or even PG-chitin) or the extraction of chitin from crustacean shells using ILs.[16]

We have found that as a result of the basicity of the acetate anion, 1-ethyl-3-methylimidazolium acetate ([C_2mim]OAc, Fig. 1c) can not only readily dissolve PG-chitin, but raw crustacean shells as well. The chitin recovered from the latter exhibits higher purity and higher MW than chitin obtained from current processes. *In addition, chitin fibers can be spun directly from the solution obtained by dissolution of raw crustacean shells, and these fibers are as strong as those prepared using the same method with cellulose pulp with degree of polymerization (DP) 1056.*

We first compared the ability of [C_2mim]OAc, [C_4mim]Cl, and [C_2mim]Cl to dissolve a given mass of pure chitin *vs.* PG-chitin. Chitin samples were added to the IL (1 g PG-chitin to 10 g IL or 0.5 g pure chitin to 2 g IL) and the mixtures heated with magnetic stirring at 100 °C for 19 h. The results (Table 1) indicated that much more of the pure chitin sample (80.0%) could be dissolved in [C_2mim]OAc than either [C_4mim]Cl (24.4%) or [C_2mim]Cl (13.9%). Even with the lower loading (1 part to 10) chosen for PG-chitin, much less (15.2%) can be dissolved than pure chitin in [C_2mim]OAc, presumably because of the higher mineral content of the former. Nonetheless, [C_2mim]OAc could dissolve much more of the PG-chitin samples than either [C_2mim]Cl (4.2%) or [C_4mim]Cl (6.8%).

For cellulose and other carbohydrates, research has shown that the primary mechanism of solvation by an IL is the anion's ability to break the extensive hydrogen-bonding networks by specific interactions with hydroxyl groups,[17] and it has been shown that improved dissolution of biopolymers is possible by increasing the hydrogen bond basicity of the anion.[18] The much higher solubilities of chitin of any grade in [C_2mim]OAc *vs.* [$C_n$mim]Cl observed here (Table 1) support this concept.

Table 1 also shows that [C_4mim]Cl gives better dissolution for all the chitinous samples than [C_2mim]Cl. This is consistent with a recently published result,[19] where [C_4mim]Cl is reported to exhibit better cellulose dissolution than [C_2mim]Cl and other ILs bearing longer alkyl chains, although the reason is still uncertain.

Microwave heating (3 s pulses at full power for 2 min in a domestic microwave oven with vigorous stirring between pulses) is more efficient than oil-bath heating in dissolving chitinous biomass. Pure and PG-chitin (*ca.* 0.4 g) were found to be completely dissolved in 10 g of [C_2mim]OAc with total irradiation time under 2 min.

Table 1 Percent chitin load dissolved in selected ILs at 100 °C for 19 h

	Percent load mass dissolved (%)		
	[C_2mim]Cl	[C_4mim]Cl	[C_2mim]OAc
Pure chitin[a]	13.9	24.4	80.0
PG-chitin[b]	4.2	6.8	15.2
Shrimp shells[b]	9.7	10.0	46.0

[a] Load = 0.5 g pure chitin per 2 g IL; [b] Load = 1 g chitinous sample per 10 g IL.

The solubility of PG-chitin in [C_2mim]OAc suggested that this IL might dissolve (or extract) chitin directly from chitinous biomass such as crustacean shells. To test this hypothesis, frozen shrimp were thawed, carefully peeled to make sure no obvious meat was left, and the backs and tails retained. These were washed three times with tap water, dried in an oven (80 °C) for 2 days, and then ground (1 min) using a mill and sieved into particle sizes of 0.125–0.5 mm. The ground shells were added to the ILs (1 g to 10 g) and heated in an oil bath as above. [C_2mim]OAc dissolved much more of the shrimp shell samples (46.0%) than [C_2mim]Cl (9.7%) or [C_4mim]Cl (10.0%). In fact, [C_2mim]OAc could dissolve 10.2% of the shrimp shells by stirring at ambient temperature over a period of 4 months. Using the microwave method at a loading of 0.4 g per 10 g IL, 73.5% shrimp shell sample (at least 94% of the available chitin, see below) can be dissolved in [C_2mim]OAc with total irradiation time of 2 min.

The dissolved chitins were reconstituted using water which solubilizes the IL, resulting in precipitation of the chitin as a white floc (as observed for IL cellulose solutions[10]). The flocs were collected, repeatedly washed with water, and dried (80 °C, 20 h). Elemental analyses (ESI, Table S2†) indicated that the reconstituted chitins were of higher purity than the undissolved chitins, while energy-dispersive X-ray spectroscopy (ESI, Table S3†) confirmed the presence of trace and relatively large amounts of minerals in PG-chitin and shrimp shells, respectively, but not in the reconstituted samples. Importantly, the reconstituted chitin from direct extraction of shrimp shells is of higher purity than the commercially available pure chitin used in this study.

It is interesting to compare the amount of chitin available in the three dissolved chitinous materials to the chitin recovered after dissolution (microwave heating), where one finds a high recovered chitin yield (ESI, eq. 2†) from shrimp shells (94%), but lower yields from PG-chitin (87.4%) and pure chitin (40.2%) (Table 2). We surmised that the presence of lower MW chitin polymers, as might be expected from commercial processing of chitin, might be more water-soluble, and were washed out during the IL processing.

To test this hypothesis, we measured the relative viscosities (η_{rel}) at 35 °C of [C_2mim]OAc and solutions of identical concentrations (0.21 g chitin in 10 g IL) of each chitin source as received and after dissolution, coagulation, and redissolution. A decrease in viscosity was observed (Table 2, and ESI, Table S4†) from the original chitin solutions to the reconstituted chitin solutions; a small decrease for pure chitin (η_{rel} = 1.13 *vs.* 1.10) and shrimp shells (1.55 *vs.* 1.43), but a dramatic decrease for PG-chitin (2.02 *vs.* 1.28). More interesting from our perspective are the results for shrimp shell extracts, where the relative viscosity for redissolved reconstituted shrimp shell chitin (1.43) is higher than either reconstituted PG-chitin or pure chitin. *This suggests that the chitin extracted directly from shrimp shells is of higher MW, and would be in keeping with the reduced processing and less harsh conditions in the IL extraction than in the industrial processes described earlier.*

Table 2 Data related to dissolution and recovery of chitin from IL solution and resulting fiber properties

Samples	Available chitin recovered (%)[a]	Relative viscosity (η_{rel})[b]	Conc. to spin fiber (wt%)	Fiber thickness (mm)[c]	Ultimate stress (MPa)[d]	Failure strain (%)[d]	Young's modulus (GPa)[d]
Pure chitin	40.2	1.13	—[e]	—	—	—	—
PG-chitin	87.4	2.02	3.4	0.13(3)	80(9)	5.0(9)	4.7(8)
Shrimp shells	94.0	1.55	3.8	0.06(1)	133(13)	3.3(3)	8.6(2)
Rec. pure chitin[f]	—	1.10	—[e]	—	—	—	—
Rec. PG-chitin[f]	—	1.28	4.0	0.09(1)	132(10)	6.9(4)	5.3(5)
Rec. shrimp shells[f]	—	1.43	2.1	0.07(1)	237(26)	13(4)	10(1)
Cellulose MCC[g]	—	—	11.5	0.15(3)	129(17)	24(3)	—
Cellulose pulp[h]	—	—	4.3	0.07(1)	217(17)	13(0)	—

[a] See ESI eq. 2. [b] $\eta_{rel} = t_s/t_0$ (t_s: Flow time of solution, t_0: flow time of IL (35 °C; same concentrations). [c] Avg. five samples. [d] Avg. three fibers. [e] Fibers could not be formed. [f] Second dissolution. [g] Microcrystalline cellulose of DP = 270.[18] [h] Kraft pulp of degree of polymerization (DP) = 1056.[20]

If indeed the IL dissolution process provides purified chitin of higher average MW, we believed it should be possible to prepare chitin fibers directly from IL solution. While this has been achieved for dissolution of cellulose, to our knowledge no-one has been able to spin a pure chitin fiber from an IL solution.

For each chitin source (pure, PG, shrimp shells, and chitin regenerated from each type after IL dissolution), 0.2–0.4 g was dissolved in 10 g IL in a 20 mL vial using a domestic microwave oven. From each solution, an attempt was made to spin a chitin fiber using the dry-jet wet-spinning method we have successfully employed for producing cellulose fibers from IL solution.[20] DI water was used as the coagulant bath and any fibers produced were soaked in warm DI water for 1–2 days to remove any residual IL and then air dried.

It was not possible to spin fibers from either a solution of pure chitin or reconstituted pure chitin, suggesting the MW of the chitin polymers was low. Chitin fibers could be spun from solutions of as-received or reconstituted PG-chitin and shrimp shells, in agreement with the relative viscosity results indicating these chitins are of higher MW. Interestingly, *chitin fibers could be spun from a solution resulting from the direct dissolution of shrimp shells in a one-pot process.*

Further evidence for the relative MWs of the chitin samples was obtained by testing the tensile strengths of each fiber using an MTS Q-Test 25 machine with a specially designed pneumatic grip. Three fibers of uniform cross-section from each type were tested using a load cell of 22.4 N capacity and a cross-head speed maintained at 1.27 mm min^{-1}.

The results (Table 2) indicate that chitin fibers made from the second dissolution of PG-chitin or reconstituted shrimp shells are stronger than those made from the first dissolution of either chitin source (132.2 *vs.* 80.1 MPa and 237.2 *vs.* 133.8 MPa, respectively). These results, along with the values for relative viscosities of the IL solutions, strongly suggest that the reconstituted chitins have higher average MW than the original chitin samples. In addition, the fiber produced from reconstituted shrimp shell chitin is much stronger than the fiber produced from reconstituted PG-chitin (237.2 *vs.* 132.2 MPa), again supporting the viscosity data and suggesting the MW of the chitin recovered from the direct extraction from shrimp shells is much higher than that of PG-chitin. Interestingly, the chitin fibers are as strong as cellulose fibers (DP = 1056) prepared from ILs in a similar process.[20]

The morphologies of the chitin fibers were analyzed by scanning electron microscopy (SEM) and are shown in Fig. 2. Different chitin materials give different fiber morphologies. Fig. 2a shows a chitin fiber made from shrimp shells with a belt shape indicating chitin solidified at a relatively slow rate, thus the chitin fiber was fully stretched on the godets and resulted in a belt-shaped fiber. Fig. 2b shows a fiber made from PG-chitin; this has a cubic cross-section composed of multilayers, which might come from a differential coagulation rate from surface to the interior.

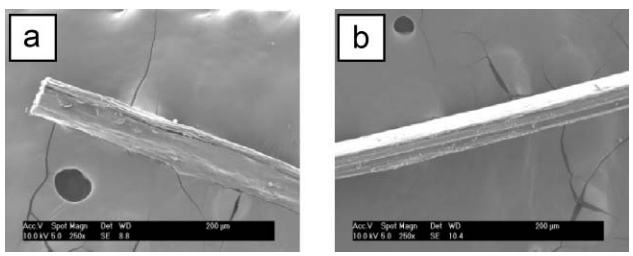

Fig. 2 SEM micrographs of the chitin fibers: (a) from shrimp shells and (b) from PG-chitin.

Taken as a whole, several important observations and conclusions can be drawn from this work.

1) Practical grade chitin with its higher mineral content and presumed higher molecular weight is much more difficult to dissolve in ILs than pure chitin.

2) Replacing the chloride anion with the more basic acetate anion in dialkylimidazolium ILs, not only allows the ready dissolution of PG-chitin, but even the dissolution or extraction of chitin in raw shrimp shells.

3) Using [C_2mim]OAc, 94% of the available chitin in shrimp shells can be extracted in a single step and recovered in a form of higher MW and purity than the current industrial multistep chemical process.

4) Chitin fibers can be spun from IL solutions of dissolved shrimp shells, although if the chitin is first reconstituted and redissolved prior to spinning, the fibers are much stronger.

Although further work is needed to improve the energy cost of IL recycling, which is currently carried out by evaporation of the aqueous wash, our results have implications both in reducing the environmental burden of the current pure chitin/chitosan process chemistries, and in opening up new markets for chitin where either the polymer itself or products from the polymer

might be developed. Our own work in this area will investigate homogenous solution chemistry on chitin, the development of composite materials, as well as the investigation of other interesting applications in pharmaceuticals, drug delivery, and medical devices.

Acknowledgements

We thank BASF Corporation for ILs and initial financial support, Cox's Wholesale Seafood, Inc. for donation of shrimp, and the Li Foundation for a fellowship (XL).

Notes and references

1 D. H. Bartlett and F. Azam, *Science*, 2005, **310**, 1775–1777.
2 J. Synowiecki and N. A. Al-Khateeb, *Crit. Rev. Food Sci. Nutr.*, 2003, **43**, 145–171.
3 M. N. V. R. Kumar, *React. Funct. Polym.*, 2000, **46**, 1–27.
4 H. Sashiwa and Y. Shigemasa, *Carbohydr. Polym.*, 1999, **39**, 127–138.
5 A. Percot, C. Viton and A. Domard, *Biomacromolecules*, 2003, **4**, 12–18.
6 S. Hirano and N. Nagao, *Agric. Biol. Chem.*, 1988, **52**, 2111–2112.
7 P. R. Austin, *US Pat.* 3892731, 1975.
8 S. Tokura, N. Nishi and J. Noguchi, *Polym. J.*, 1979, **11**, 781–786.
9 F. A. Rutherford and P. R. Austin, in *Proceedings of the First International Conference on Chitin/ Chitosan*, ed. R. A. A. Muzzarelli and E. R. Pariser, MIT Sea Grant Report, MIT SG 78-7, 1978, pp. 182–192.
10 R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *J. Am. Chem. Soc.*, 2002, **124**, 4974–4975.
11 D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna and R. D. Rogers, *Green Chem.*, 2007, **9**, 63–69.
12 N. Sun, M. Rahman, Y. Qin, M. L. Maxim, H. Rodríguez and R. D. Rogers, *Green Chem.*, 2009, **11**, 646–655.
13 Y. Wu, T. Sasaki, S. Irie and K. Sakurai, *Polymer*, 2008, **49**, 2321–2327.
14 H. Xie, S. Zhang and S. Li, *Green Chem.*, 2006, **8**, 630–633.
15 S. Yamazaki, A. Takegawa, Y. Kaneko, J. Kadokawa, M. Yamagata and M. Ishikawa, *Electrochem. Commun.*, 2009, **11**, 68–70.
16 S. E. Hecht, R. L. Niehoff, K. Narasimhan, C. W. Neal, P. A. Forshey, D. V. Phan, A. D. M. Brooker and K. H. Combs, *World Pat.* WO 2006/116126 A2. The extraction of chitin from biomass using ILs is claimed; however, no examples are provided.
17 R. C. Remsing, R. P. Swatloski, R. D. Rogers and G. Moyna, *Chem. Commun.*, 2006, 1271–1273.
18 Y. Fukaya, A. Sugimoto and H. Ohno, *Biomacromolecules*, 2006, **7**, 3295–3297.
19 T. Erdmenger, C. Haensch, R. Hoogenboom and U. S. Schubert, *Macromol. Biosci.*, 2007, **7**, 440–445.
20 N. Sun, R. P. Swatloski, M. L. Maxim, M. Rahman, A. G. Harland, A. Haque, S. K. Spear, D. T. Dalyand and R. D. Rogers, *J. Mater. Chem.*, 2008, **18**, 283–290.