IONIC LIQUIDS AND CHITINOUS BIOMASS: MATERIALS, SYNTHESIS, AND APPLICATIONS FOR URANIUM SEQUESTRATION

by

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ABSTRACT

The mining of Uranium (U) ore, processing, and applications in weapons manufacturing and nuclear fuel have resulted in a legacy of contamination that require the creation of innovative methods that provide waste to volume minimization. Therefore a major objective of this research was to combine the reactivity of chitosan with the toughness and insolubility of chitin by surface modifications with UO$_2^{2+}$ selective moieties. Thus the IL platform consisting of the 1-ethyl-3-methyl-imidazolium cation [C$_2$C$_1$im]$^+$ paired with the acetate anion [OAc]$^-$ was selected and demonstrated to overcome solubility limitations of the biomass. This strategy was successful in producing the first report of electrospinning fibers directly from chitinous biomass in ILs. Concurrent to these results, supercritical carbon dioxide was explored as an alternative solvent to high boiling point coagulation baths to reduce economic and engineering challenges of using ILs at scale.

With the successful efforts producing high surface area fibers from chitinous biomass, both qualitative and quantitative analysis supported functionalization of fibers with the amidoxime functional group for aqueous uranyl ions. While these research efforts have demonstrated chitin as a versatile polymer back-bone for fiber applications for U recovery, complexity of the waste derived feedstock is challenging and other chemical components that remain in the processed shell waste are key variables.

Further characterization of the feedstock led to the discovery that metabolically inactive shrimp shell has the intrinsic ability to mineralize and reduce aqueous metal ions. This represents
a new alternative to promote stable secondary U(VI) phosphate U(VI) and insoluble U(IV) phases, providing an effective strategy for immobilizing U. In addition the rediscovery of the simple but understudied salt [NH$_3$OH][CH$_3$COO] not only demonstrates OAc$^-$ is an excellent ligand for U(VI) coordination, but its protic ionic liquid properties suggest a much broader application space. Therefore future work is warranted to determine the influence of carboxylic acids in the reduction of U(VI) and the speciation and stability of the U(IV) phase. However these results provide considerable improvements that address constraints of current bioremediation and abiotic precipitation techniques to provide a long term sink for one of the most abundant radionuclides released into the environment.
DEDICATION

This dissertation is dedicated to my daughter Gracie. May you continue to ask questions, observe, and explore.
## LIST OF ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AO</td>
<td>Amidoxime</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer Emmett Teller</td>
</tr>
<tr>
<td>bp</td>
<td>Boiling point</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>[C₂C₁im]</td>
<td>1-ethyl-3methyl-imidazolium cation</td>
</tr>
<tr>
<td>[C₂C₂im]</td>
<td>diethyl imidazolium cation</td>
</tr>
<tr>
<td>CN</td>
<td>Nitrile</td>
</tr>
<tr>
<td>Co</td>
<td>Cobalt</td>
</tr>
<tr>
<td>DA</td>
<td>Deacetylated</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DMAc</td>
<td>Dimethylacetamide</td>
</tr>
<tr>
<td>DU</td>
<td>Depleted uranium</td>
</tr>
<tr>
<td>Dw</td>
<td>Dry weight distribution</td>
</tr>
<tr>
<td>EDS</td>
<td>Electron dispersive spectroscopy</td>
</tr>
<tr>
<td>eV</td>
<td>Electron Volt</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>g/mol</td>
<td>Grams per mole</td>
</tr>
<tr>
<td>HAOAc</td>
<td>Hydroxyl ammonium acetate</td>
</tr>
<tr>
<td>HFIP</td>
<td>1,1,1,3,3,3-hexafluoro-2-propanol</td>
</tr>
</tbody>
</table>
HOAc  Acetic acid
IL    Ionic liquid
kV    Kilovolt
M     Molar
Mn    Manganese
m²/g  meter squared per gram
MDI   Materials Data Inc.
MeOH  Methanol
mL    milliliter
mPa   Megapascal
Mp    Melting Point
MSA   Methanesulfonic Acid
MW    Molecular weight
nm    Nanometer
OAc⁻  Acetate
PG    Practical Grade
PIL   Protic ionic liquid
PXRD  Powder x-ray diffraction
R_ac  Ratio of acetate
sc-CO₂ Supercritical carbon dioxide
SC-XRD Single crystal x-ray diffraction
SEM   Scanning Electron Microscopy
SS    Shrimp Shell
TEM Transmission Electron Microscopy
U Uranium
UV-vis Ultraviolet-visible
w/w Weight to weight
XPS X-ray-photon spectroscopy
YPG Yuma Proving Ground
< Less than
= Equal to
Å Angstrom
µ Micro
ACKNOWLEDGMENTS

I am grateful for this opportunity to foremost, thank my advisor, Prof. Robin D. Rogers for his guidance, and flexibility during my unique trajectory through graduate school. He has elevated the impact of my research and taught me invaluable lessons in communicating my research. I would also like to thank each member of my committee, Prof. Shane Street, Prof. Shanlin Pan, Prof. Paul Rupar, and Dr. Kim Lackey for their involvement in my PhD pursuit. My interactions with you in both the dissertation and academic progress have challenged me to become a better scientist.

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In addition I would like to acknowledge all members of the Rogers research group for guidance along the way. I would specifically like to thank Dr. Patrick Barber and Dr. Parker McCrary who have been collaborators, co-authors, and friends during this challenging experience.
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1 INTRODUCTION

1.1 Uranium in the Environment

Uranium (U) is the most abundant radionuclide released into the environment with high levels of contamination prevalent in soils and groundwater at sites associated with energy and defense applications.\textsuperscript{1} Both naturally occurring and anthropogenic sources of U are commonly dominated by the U(VI) uranyl cation ($\text{UO}_2^{2+}$) in oxic conditions ranging from soil to seawater.\textsuperscript{2} Although the rigid, linear geometry of $\text{UO}_2^{2+}$ is relatively inert due to strong covalent bonding, pH dependent ligand exchange readily occurs in the equatorial plane affecting solubility.\textsuperscript{3} This complex aqueous behavior represents a cause for environmental concern due to variable speciation in solution.\textsuperscript{4} Formation of strong complexes naturally occurring ligands makes recovery from solution challenging, thus understanding the aqueous behavior of $\text{UO}_2^{2+}$ is required for predicting potential migration and designing remediation strategies. Despite extensive study of U(VI) complexes there remains a need to gain fundamental insight of the chemistry required to further develop extractions and long term disposal technologies.\textsuperscript{5}

![Uranyl Cation CN=4 CN=5 CN=6](image)

**Figure 1.1** Structure of a) uranyl cation, b) Labile ligand exchange in coordination plane
Upon deposition of uraninite U(IV) in the environment from depleted uranium (DU) sources, the formation of potentially mobile U(VI) oxide hydrates such as meta-shoepite \((\text{UO}_2)_4\text{O}((\text{OH})_6\cdot 5\text{H}_2\text{O})\) and becquelerite \((\text{Ca(UO}_2)_6\text{O}_4\text{O}((\text{OH})_6\cdot 8\text{H}_2\text{O})\) is favored in oxic conditions. However, these initial oxidation products commonly undergo dissolution and precipitation with secondary oxide phases making \(\text{UO}_2^{2+}\) inaccessible to extraction solutions.

The current extraction chemistries to remove these environmental phases have commonly been based on the hard Lewis acid/base interactions of \(\text{UO}_2^{2+}\) and the carboxylate functionalities of citric acid \((\text{C}_6\text{H}_8\text{O}_7)\). However the chelation mode dictates the recovery; whereas bidentate \(\text{U(VI):citrate}\) complexes are readily precipitated, tridentate binuclear 2:2 \(\text{U(VI):citrate}\) complexes can be recalcitrant in solution. As removal of U from solution defines a major challenge for remediation, the utility of U extractions is greatly diminished if a secondary aqueous waste stream is generated requiring storage of high volumes of contaminated solutions.

Efforts to recover \(\text{UO}_2^{2+}\) from solution and mitigate mobility in the environment have primarily focused on mechanisms to alter solubility such as; reduction of U(VI) to U(IV), biological and chemical precipitation reactions, surface complexation, and mineralization of sparingly soluble U(VI) phases. Due to similarity in the redox couples of U(VI)/U(IV) and Fe(III)/Fe(II), Fe(III) reducing bacteria can utilize U(VI) as an alternative electron acceptor in anaerobic metabolisms inducing reduction to insoluble U(IV). However reduced phases can be re-oxidized to soluble U(VI) phases calling into question the long term stability. Furthermore biologically induced precipitation is hindered by toxicity and mass transfer limitations from U accumulation at cellular surfaces. Abiotic alternatives have been proposed based the addition of chemical phosphate \((\text{PO}_4^{3-})\) amendments or \(\text{PO}_4^{3-}\) bearing minerals such as apatite \([\text{Ca}_{10}(\text{PO}_4)_6\text{(OH)}_2]\) to precipitate sparingly soluble U(VI) phases. However these approaches are
limited to reversible surface complexation and secondary phosphate phases require saturated conditions not found in environmentally relevant conditions.\textsuperscript{14}

![Diagram of microorganisms](image)

Figure 1.2 Structure of a) Tridentate binuclear 2:2 U(VI): citric acid involving hydroxyl group can be recalcitrant to biodegradation, b) Bridged binuclear complex to soluble 1:2 U(IV): citric acid mononuclear complex

\section*{1.2 $U$(VI) Acetate System}

A primary goal of this work was to minimize the complexity observed in tri-carboxylic citric acid solutions, thus a mono-carboxylic based chemistry was explored to provide more predictive aqueous speciation in solution. Acetic acid (HOAc) is a rational choice as U(VI) oxide hydrates such as meta-shoepite ($\text{UO}_2\text{O}(\text{OH})_6\cdot5\text{H}_2\text{O}$) and becquelerite ($\text{Ca(UO}_2\text{)}_6\text{O}_4(\text{OH})_6\cdot8\text{H}_2\text{O}$) formed from the oxidation of DU are easily solubilized by acetic acid (HOAc).\textsuperscript{15} In addition, for bidentate ligands with reduced steric demands such as acetate (OAc\textsuperscript{−}), $\text{UO}_2^{2+}$ can accommodate six donor atoms and theoretical predictions indicate 1:3 U(VI) tris-acetate $\text{UO}_2(\text{CH}_3\text{COO})_3$ at pH 3.5.\textsuperscript{16} However, in many instances, temporal precipitation reactions alter solubility. Iron (Fe) and manganese (Mn) oxides have high charge densities and thus are important sinks for $\text{UO}_2^{2+}$.\textsuperscript{17} Fe(III) and Mn(IV) can occlude $\text{UO}_2^{2+}$ limiting access to
HOAc, and therefore reductive dissolution is required to liberate UO$_2^{2+}$ from secondary mineral phases.$^{18}$

To eliminate the need for multi-stage chemical extractions to solubilize both the primary oxides and secondary co-precipitates we discovered in this study that existing sequential extraction schemes consisting of acetic acid (CH$_3$COOH) and hydroxylamine (NH$_2$OH) could be modified to produce a protic ionic liquid (PIL), hydroxylammonium acetate, [NH$_3$OH][CH$_3$COO] (Figure 1.3).$^{19}$ We expected dissolution of [NH$_3$OH][CH$_3$COO] in acetic acid would provide a buffered solution which could maintain the required pH 3.5 to produce UO$_2$(CH$_3$COO)$_3^-$ during the dissolution of sedimentary CaCO$_3$ and release of CO$_3^{2-}$ while providing OAc$^-$ to chelate, solubilize, and allow removal of UO$_2^{2+}$ from the soil.$^{20}$

\[
\text{NH}_2\text{OH} + \text{CH}_3\text{COOH} \rightarrow \text{MeOH/H}_2\text{O} \quad \text{H}_3\text{N}^+\text{OH} \quad \text{O}^{-} \quad \text{CH}_3\text{COO}^{-}
\]

Figure 1.3. Reaction of 1:1 molar ratios of NH$_2$OH and HOAc

Thus an initial research goal of designing a simplified ligand system to provide a predictive system of coordination modes, and liberate U(VI) oxide hydrates and secondary Fe/Mn solid phases from environmentally relevant conditions into solution was accomplished. However the utility of leaching is diminished if a secondary aqueous waste stream is generated, so a second research objective focused on developing materials from chitinous biomass for recovering UO$_2^{2+}$ from solution.

### 1.3 Biomass and Ionic Liquids

Biopolymers such as cellulose, chitin, and chitosan (Fig. 1.4), have an array of characteristics that make them attractive materials for the production of nanofibers including; high molecular weights, biodegradability, and sourcing from renewable feedstocks. The
polysaccharide chitin is the second most abundant of Nature’s biopolymers, after cellulose, with versatile fiber-based applications including biocatalysis, energy storage, filtration, biosensing, and wound care.\textsuperscript{21} In addition, the application of chitin repurposes crustacean shells that would otherwise be land filled as bio-waste from the shellfish industry.

![Figure 1.4 Structure of a) Chitin, b) Chitosan, and c) Cellulose](image)

However, the potential of chitin has not yet been fully realized due to its recalcitrance to dissolution in most solvent systems.\textsuperscript{22} The poor solubility of chitin arises from a complex network consisting of both inter- and intramolecular hydrogen bonding. As a result, solvents employed for chitin dissolution include strong acids such as methanesulfonic acid (MSA), halogenated compounds such as hexafluoroisopropanol (HFIP), and lithium chloride (LiCl)/dimethylacetamide (DMAc).

The current industrial extraction of chitin from crustacean shells consists of demineralization by strong acid treatment and deproteinization by alkaline treatment.\textsuperscript{23} Further purification may also include MSA. Disadvantages of this approach beyond the chemical and energy intensity of the processes include the potential degradation of the polymer as a result of hydrolysis from corrosive environments. The latter is of particular concern because the utility of biopolymers is strongly dependent on the ability to process the natural polymer without loss of the intrinsic high molecular weight.
Ionic liquids (ILs, commonly defined as salts which melt below 100 °C) have been established as a viable alternative for the direct dissolution of biomass where organic and aqueous solvents are not effective\textsuperscript{25,26,27} The tunable aspect of ILs through selective ion choice can improve solvating power for dissolution of specific macromolecules through disruption of the hydrogen bonding network.\textsuperscript{28,29} Although structurally similar to cellulose, chitin requires a more basic anion such as acetate due to the increased number of hydrogen bond donors and acceptors. In 2010, we demonstrated the significance of the acetate anion through substantial improvements in chitin recoveries compared to chloride when coupled with the same 1-ethyl-3-methylimidazolium acetate, $[\text{C}_2\text{C}_1\text{Im}]^+$, cation (Fig. 1.5).\textsuperscript{30,31} Those results suggested higher molecular weight chitin could be obtained by extraction of dried shrimp shells and that the higher molecular weights are required to produce fibers by dry-jet wet spinning techniques.

![Figure 1.5 Structure of a) 1-ethyl-3-methylimidazolium $[\text{C}_2\text{C}_1\text{Im}]^+$ and b) 1,3-diethylimidazolium $[\text{C}_2\text{C}_2\text{Im}]^+$ cations.](image)

### 1.4 Material Considerations for Biomass

Appropriate chemistry can produce specific architecture of chitin derived fibers for UO$_2^{2+}$ recovery. In order to take full advantage of the range of potential uses for chitin materials, the ready preparation of reproducible, higher surface area, high porosity nanofibers would be desirable; such as those that might be obtained via electrospinning. Electrospinning uses an electric charge to pull micron and nano-sized fibers from a polymer solution. In order to electrospin, a solution of polymer is pushed through a charged spinneret where the electric potential must be increased above the surface tension of a polymer solution to form a Taylor
cone, from which is then ejected a charged viscous jet of polymer towards the collecting electrode.\textsuperscript{32} It is during this time that the solvent evaporates and concentrates the polymer solution and allows for the polymer entanglement to occur. The remaining solvent evaporates and the fibers are formed on the electrode. The system maintains balance by a combination of the polymer entanglement density, solution viscosity, and surface tension which allow for instabilities in the jet to be suppressed; preventing beads and creating smooth, continuous fibers.\textsuperscript{33}

The difficulty in nanofiber production via electrospinning arises from the difficulty in controlling several interrelated variables at the same time, such as viscosity, polymer concentration, and entanglement density. Entanglement density simply put is the concentration threshold which permits a physical interlocking between polymer chains which is critical to produce continuous fibers. This parameter is a combination of the molecular weight of the polymer and the concentration of the polymer, both of which are also directly related to solution viscosity. Too low of a polymer concentration would result in the formation of beads, whereas too high of a concentration leads to solutions too viscous for electrospinning.

Chitin nanofibers have been spun from available sources including; practical grade (PG) chitin\textsuperscript{34,35}, analytical grade chitin, and low molecular weight chitin powder using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), a corrosive and potentially hazardous fluorinated solvent from both an environmental and human health perspective.\textsuperscript{36,37} Not only was the solvent highly toxic, but depolymerization of the chitin by Co\textsuperscript{60} $\gamma$ radiation was required to enhance solubility when working with this grade of chitin. The MW of the pure chitin had to be reduced by an order of magnitude (from 910,000 to 91,000 g/mol) for this process to work. It is interesting to note that with a decrease in molecular weight, the concentration required for successful electrospinning
increases due to the need for greater polymer density. Attempts to electrospin the related chitosan in HFIP have been unsuccessful due to its insolubility, but solvents such as acetic acid, trifluoroacetic acid, and acidic aqueous solutions of these acid have shown promise.\textsuperscript{38,39,40} 

Although we previously demonstrated fibers by dry-jet/ wet-spinning, higher surface area is desirable for surface functionalization. Our early electrospinning experiments with chitin extracted directly from shrimp shells in \([\text{C}_2\text{C}_2\text{Im}][\text{OAc}]\) indicated phase transformations were occurring in the spin dope. This is easy to rationalize due to the above ambient melting point of \([\text{C}_2\text{C}_2\text{Im}][\text{OAc}]\) (30 °C) when compared to the very similar \([\text{C}_2\text{C}_1\text{Im}][\text{OAc}]\) (-20 °C). Therefore continuing research was focused on \([\text{C}_2\text{C}_1\text{Im}][\text{OAc}]\) due to the lower melting point to decrease the possibility of a phase transformation. Experimental efforts to optimize nanofiber production determined 2.0 % w/w shrimp shell \([\text{C}_2\text{C}_1\text{Im}][\text{OAc}]\) provided optimal viscosity for a high molecular weight (MW) spin dope to suppress instabilities with smooth, continuous fibers at 20kV.\textsuperscript{41} 

Electrospinning of chitin fibers directly from a solution of raw shrimp shell in ILs was demonstrated for the first time and found to depend on key parameters such as viscosity and concentration of the solutions only available with ILs. However the use of ILs presents economic issues and the cost of fiber production is a key driver for developing sustainable materials. Distillation can be used to remove the antisolvents from the IL, however, the energy intensive process presents further economic and engineering challenges at scale. Alternatives to high-boiling liquid antisolvents, was explored through the use of super-critical carbon dioxide (sc-CO\(_2\)) as a coagulation solvent for chitin in the IL. Based on our groups previously reported chemisorption of CO\(_2\) we exploited the identified mechanism to precipitate chitin from the IL as a potential recycling scheme.\textsuperscript{42}
1.5 Fiber Applications and Surface Modification

The properties of chitin and chitosan are quite different and depend significantly on two main structural features, degree of deacetylation (%DDA) and molecular weight. With increased %DDA, the polymer becomes more soluble in acidic solutions, as well as becoming more easily modified chemically due to the amine functional groups. While this processing provides routes to new materials, the decreased molecular weight affects properties such as strength, solubility, and biodegradability which can change significantly. For applications using chitosan, the modification of the entire polymer before making the final material degrades strength and includes unnecessary or wasted chemicals from unnecessary derivatization. The harsh initial processing conditions to obtain chitin combined with the degradation of desired properties upon deacetylation of the entire polymer, severely limits the resulting material properties and potential applications.

We have sought ways to combine the favorable properties of both materials, such as the reactivity of chitosan with the toughness and insolubility of chitin, while preserving their biodegradability. Because chitin is insoluble in virtually every solvent, it is especially suited to surface-selective functionalization. Surface functionalization has the potential to preserve many of the good properties that are lost by completely converting chitin to chitosan. Because only a small fraction of the chitin would need to be converted, the physical properties should correspond to those of bulk chitin. However, for applications such as metal adsorption where the reactivity occurs at the surface, both the capacity and reactivity of surface-functionalized chitin should be indistinguishable from those where the entire material has been converted. Further, by controlling the surface area, one has control over the capacity of each fiber for chemical modification.
The solubility of high MW chitin in ILs provides a ready route to these materials. The chitin can be extracted directly from crustacean shells in a single step and directly spun into fibers, cast as films, or coagulated as beads. The insolubility of these chitin materials in aqueous media allows direct surface functionalization via deacetylation with base followed by reaction at the exposed amine. Such a strategy should greatly reduce the amount of time, energy, and chemicals needed to prepare functionalized chitin materials. The combination of IL-based forming of chitin followed by surface functionalization leads to a platform for making a plethora of new functional materials which overcome the disadvantages of both chitin (lack of solubility and lack of reactive amines) and chitosan (lack of strength and lack of durability).

1.6 Biomimetic Mineralization

The previous research efforts have demonstrated chitin as a versatile polymer back-bone for fiber applications for U recovery. But potential variability from using a waste derived feedstock is challenging and other chemical components such as CaCO₃ and proteins that remain in the processed shell waste are key variables for chemical and engineering applications. Extensive research has been directed at elucidating the structural and chemical components present in crustacean exoskeletons in relation to biomineralization with simple inorganic precursors on a chitin scaffold.⁴⁴

Mechanistic insight into the precise assembly of biominerals by macromolecules remains elusive.⁴⁵ Living organisms such as crustaceans have been described as “the champions of mineral mobilization and deposition” and are capable of the cyclic transport of large amounts of ions to form highly sophisticated crystalline materials from simple inorganic precursors.⁴⁶ Many of these biominerals are deposited in multiphase organic-inorganic composites within a macromolecular structural framework of chitin or collagen.⁴⁷ Of the inorganic mineral
components, the divalent calcium cation (Ca$^{2+}$) is the most commonly utilized in biominerals as carbonates and phosphates in shell and bone. However the source of ions varies and can involve mechanisms such as uptake from the external environment or transport from temporal storage within the organism. This ion supply can either be transported by active metabolic pumping or proceed by passive diffusion with anions to maintain electroneutrality.

Biological control of the ion source is not required for the formation of the mineral, as biologically induced mineralization is common even in heterogeneous conditions. From a chemistry perspective, this makes both the source of ions and the choice of cation trivial, provided the ion solution chemistry can impart chemical activity to a spatial template. Therefore, if a biomaterial possesses active functional groups within an organized framework, the only remaining requirement for biogenic mineral formation should be the delivery of ions. Chemically controlled nucleation and growth of crystals on polysaccharide polymer templates has been demonstrated by *in vitro* studies to probe biomineralization. Such studies have indicated a major role of phosphorylated components of the acidic protein matrix in controlling mineralization. Of particular significance are the phosphate ester metabolites phosphoenolpyruvate (PEP), 3-phosphoglycerate (3PG), and phosphoserine (the phosphorylated amino acid serine, SerP) (Fig. 1.6).

![Figure 1.6 Structure of a) phosphoenolpyruvate (PEP), b) 3-phosphoglycerate (3PG), and c) phosphoserine (SerP).](image-url)
1.7 Research Plan and Purpose of the Project

A major objective of this research was to provide considerable improvements that address constraints of current uranium bioremediation and abiotic precipitation techniques to provide a long term sink for one of the most abundant radionuclides released into the environment. Fundamental to addressing this major environmental challenge is recovering U from high volumes of waste that are pervasive on a national scale. As both naturally occurring and anthropogenic sources of U are dominated by the uranyl cation (UO$_2^{2+}$) in oxic conditions, recovering U from contaminated soils and resulting aqueous waste streams can be viewed as a logical extension of ongoing research for extraction of UO$_2^{2+}$ from seawater with chitinous biomass. Thus an initial goal of this study was to combine the reactivity of chitosan with the toughness and insolubility of chitin by surface modifications with UO$_2^{2+}$ selective moieties. Here it will be demonstrated that ionic liquids (ILs; salts with melting points below 100 °C) offer unprecedented control of chitinous biomass enabling material applications for advanced fibers for recovering U from solution.

Chapter 2 will introduce the first reported instance of the electrospinning of chitin fibers directly from a solution of raw shrimp shell in ILs. This material application of chitinous biomass was demonstrated for the first time and yielded critical parameters such as viscosity and concentration of the solutions only available with ILs. However the use of ILs presents economic issues and the cost of fiber production is a key driver for developing sustainable materials. Distillation can be used to remove the antisolvents from the IL, however, the energy intensive process presents further economic and engineering challenges at scale. As a result in Chapter 3 alternatives to high-boiling liquid antisolvents was explored through the use of super-critical carbon dioxide (sc-CO$_2$) as a coagulation solvent for chitin in the IL. Upon depressurization of the sc-CO$_2$ reactor, a phase boundary was observed across the fluid interface from the release of
CO₂ (g) from the IL-rich phase and the solid chitin film was physically removed from the IL surface providing a potential route for an economical and energy efficient IL recycling scheme. Next, Chapter 4 addresses a major objective of this research to combine the reactivity of chitosan with the toughness and insolubility of chitin by surface modifications with UO₂²⁺ selective moieties. Both qualitative and quantitative analysis supported surface functionalization commensurate with the known affinity of the amidoxime functional group for aqueous uranyl ions and with the hardness of the coordinating/functional group as a Lewis base.

The previous chapters demonstrated chitin as a versatile polymer back-bone for fiber applications for U recovery. But potential variability from the contribution of CaCO₃ and proteins in the waste derived feedstock is challenging to characterize. Therefore Chapter 5 marks a departure from the alkaline conditions of seawater which produce the triscarbonato species UO₂(CO₃)₃⁴⁺, solution chemistry developed to extract U(VI) oxide hydrates (UO₂)₄O(OH)₆·5(H₂O) provided an opportunity to dictate speciation and chelation modes. Chapter 5 also denotes a transition from room temperature ionic liquids such as [C₂C₇mim][OAc] to the use of protic ionic liquids. This revealed that there are significant synthetic advantages to using a biogenic framework of chitinous biomass at the nanometer to micrometer length scale if functional groups from the matrix retain activity. Indeed the intrinsic chemical composition of CaCO₃ in the shrimp shell matrix proved an ideal in situ reaction template upon exposure to an acidified and chelated UO₂²⁺ ion precursor, releasing calcium and providing the UO₂²⁺ ions rapid access to phosphate (PO₄³⁻). Synchrotron-based µ-XRD confirmed ligand exchange from OH⁻ the parent phase to the PO₄³⁻ ligand product phase reduces solubility by 30 orders of magnitude (log Kₛₚ 5.52 to 26.50). Specifically, we have demonstrated
that metabolically inactive shrimp shell has the intrinsic ability to mineralize aqueous metal ions, including anthropogenic metals like uranium with rapid kinetics, in unsaturated conditions.

Finally, Chapter 6 represents the application of the biomimetic mineralization mechanism and protic ionic liquid chemistry discovered in this dissertation toward the ultimate goal of U sequestration. The protic ionic liquid (PIL) Hydroxyl Ammonium Acetate (HAOac) was developed as a ligand to provide a pH controlled U(VI) acetate system, with an acidic composition to solubilize primary U(VI) oxide hydrate soil contaminants such as meta-shoepite \((\text{UO}_2)_4\text{O}(	ext{OH})_6\cdot5\text{H}_2\text{O}\) and becquelerite \((\text{Ca(UO}_2)_6\text{O}_4\text{O}_4\cdot8\text{H}_2\text{O})\), and a reducer to provide reductive dissolution of secondary Fe(III) and Mn(IV) oxide sinks. The resulting uranyl tris-acetato precursor species in solution was coupled with a solid matrix of waste shrimp shell for nucleation in the carboxylic-acid rich system. A stable product phase was revealed by rapid formation of a crystalline secondary phosphate phase in unsaturated conditions at the solution-solid interface.

Further XANES characterization of the feedstock led to the discovery that metabolically inactive shrimp shell has the intrinsic ability to mineralize and reduce aqueous metal ions. This represents a new alternative to promote stable secondary U(VI) phosphate U(VI) and insoluble U(IV) phases, providing an effective strategy for immobilizing U. Future work is warranted to determine the influence of carboxylic acids in the reduction of U(VI) and the speciation and stability of the U(IV) phase. However these results provide considerable improvements that address constraints of current bioremediation and abiotic precipitation techniques to provide a long term sink for one of the most abundant radionuclides released into the environment.
1.8 Novel Contributions in this Study

1. We electrospun, for the first time, nanofibers directly from an ionic liquid extract of shrimp shells. (US Provisional, Appl. No. 13/949,501, July 24, 2012)

2. The use of sc-CO\textsubscript{2} chemisorption as an alternative to high boiling point antisolvents for potential recycling of IL after chitin extraction. (US Provisional Application No. 61/764,770 02/14/13)

3. Surface modification of chitin fibers with the uranyl selective ligand amidoxime

4. The transformation of uranyl oxide ((UO\textsubscript{2})\textsubscript{8}O\textsubscript{2}(OH)\textsubscript{12}·12(H\textsubscript{2}O)) into uramphite NH\textsubscript{4}(UO\textsubscript{2})(PO\textsubscript{4})·3H\textsubscript{2}O with metabolically-inactive shrimp shell using the physical structural and chemical reservoir of the biogenic platform.

5. We synthesized the protic ionic liquid hydroxylammonium acetate [NH\textsubscript{3}OH][CH\textsubscript{3}COO] for separation applications. (US Patent Application 62/042,392 filed on 08/27/14)

6. Hydroxylammonium acetate [NH\textsubscript{3}OH][CH\textsubscript{3}COO] as a protic ionic liquid for U(VI) oxide hydrates complexation.

7. Rationally designed engineered mechanisms of UO\textsubscript{2}\textsuperscript{2+} uptake by biogenic phosphates.

1.9 References


2 ELECTROSPINNING OF CHITIN NANOFIBERS DIRECTLY FROM AN IONIC LIQUID SOLUTION OF SHRIMP SHELLS

Taken as part of a published manuscript: Barber, P. S.; Griggs, C. S.; Bonner, J. R.; Rogers, R. D. Green Chem. 2013, 15, 601–607

2.1 Introduction

A major motivation of this work was to produce a high surface area material such as fibers that might be obtained via electrospinning. The biopolymer chitin represents an inexpensive and renewable source to facilitate a high waste-to-volume, immobilized form of U. Yet, only recently have ionic liquid platforms been suggested and demonstrated as a viable option for electrospinning biopolymers.\(^1,2,3,4\) In addition, even with a suitable IL platform which provides suitable solubility for a given biopolymer, this is only one challenge in producing high surface area materials such as nanofibers. Electrospinning in ILs is difficult to control due to both the typical high viscosity of IL solutions and the lack of IL volatility as indicated in recent efforts with biopolymers and composites in ILs. Lack of volatility requires that a coagulation bath be present to remove the nonvolatile IL. This allows the ionic liquid to wash away from the fibers as they are traveling towards the electrode (placed under the bath). IL platforms for electrospinning biopolymers have been primarily limited to cellulose or cellulose fiber composites, but even here they may require an additional co-solvent such as dimethyl sulfoxide (DMSO).\(^5\) For example, Xu \textit{et al.} utilized a combination of a co-solvent, DMSO, and a rotating drum within the collection bath to address the viscosity and non-volatility of 1-allyl-3-methylimidazolium chloride ([AMIM][Cl]) to electrospin cellulose.\(^6\)
Therefore fiber formation is not just about solubility of the polymer, it is contingent on the concentration of polymer to achieve sufficient entanglement density while maintaining an attainable viscosity threshold. To use ILs for electrospinning of chitin, one must find an IL which provides the necessary solubility of a high molecular weight chitin that at low concentration will provide sufficient entanglement density at low enough viscosity to successfully electrospin chitin nanofibers.

Here we discuss our attempts to combine the solvating ability of ILs and the high MW chitin accessible by direct IL extraction from shrimp shells to demonstrate electrospinning of high MW, chitin nanofibers. We investigated the electrospinning of chitin fibers from chitin solutions with two ILs, \([C_2C_1Im][OAc]\) and \([C_2C_2Im][OAc]\), and studied the effects of varying the electrospinning conditions through five separate trials.

2.2 Experimental

2.2.1 Chemicals

All materials were used as purchased. The ionic liquids used for chitin dissolution were 1,3-diethylimidazolium acetate \([C_2C_2Im][OAc]\) from BASF (Florham Park, NJ) with 90% purity, and 1-ethyl-3-methylimidazolium acetate \([C_2C_1Im][OAc]\) from IoLiTec Ionic Liquids Technologies Inc (Heilbronn, Germany). Practical grade chitin (PG chitin) obtained from crab shells \(C7170\) was purchased from Sigma (St. Louis, MO) and used directly without further purification. Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, AL and ground to a < 125 µm particle before use.

2.2.2 Viscosity Measurements

Viscosity measurements were taken at 30.0(1) °C with a Cambridge Viscosity (Medford, MA) Viscometer, VISCOlab 3000. Approximately 2-3 mL of each solution was placed in the
sample chamber. The correct sized piston corresponding to the expected viscosity range was added and the measurement was taken. The reported values were taken as an average of three measurements per reading as computed by the instrument.

2.2.3 PXRD and FT-IR Spectroscopy

Samples and reference materials were characterized by powder X-ray diffraction (PXRD) and infrared (IR) spectroscopy. The PXRD was collected on a Bruker D2 Phaser powder X-ray diffractometer with Ni-filtered Cu Kα radiation. (Madison, WI) Infrared (IR) analyses were obtained by direct measurement of the neat samples by utilizing a Bruker Alpha FT-IR instrument, Bruker Optics Inc. (Billerica, MA) featuring an attenuated total reflection (ATR) sampler, and spectra were obtained in the range of $\nu_{\text{max}} = 400$–4000 cm$^{-1}$.

2.2.4 Ionic Liquid Extract of Shrimp Shells

Small scale dissolution of shrimp shell was performed using a domestic microwave oven (SHARP Carousel R-209KK, Mahwah, NJ) at full power. All solutions were made in a similar fashion with an appropriate mass of chitinous sample being added to a mass of ionic liquid corresponding to a final weight percent (wt%). The mixture was heated in the microwave for a total of two min using 2-3 sec pulses. A detailed example follows:

0.200 g ground shrimp shell was dissolved in 9.80 g [C$_2$C$_1$Im][OAc] to give a total mass of 10 g to constitute 2 wt%. The mixture was heated for a total of 2 min using 2-3 sec pulses with mechanical stirring between pulses with a glass stirring rod. The solution slightly darkened with time during the heating, most likely due to slight decomposition of the IL. After dissolution, the undissolved particles were removed through centrifugation and the supernatant was used for electrospinning experiments.
2.2.5 Electrospinning Techniques and Apparatus

The chitin solution was subjected to electrospinning, which is schematically represented in Figure 2.1. All electrospinning experiments were conducted in a similar fashion. A 3 mL sample of the chitin solution was transferred to a syringe with a needle. A voltage was applied to the needle electrode with a grounded charge in the form of a stainless steel plate beneath the glass collection bath. The nozzle-to-ground target distance was fixed at certain distance. The chitin solution was delivered to the spinner head using air pressure from a syringe pump (New Era Pump Systems Inc., Farmingdale, NY) with a set flow rate. The coagulation solvent was water. A detailed example follows:

A 3 mL sample of 2 wt% shrimp shells in [C$_2$C$_1$Im][OAc] was transferred to a syringe with a 18.5 G needle. A voltage of 20 kV was applied to the needle electrode with the needle-to-ground target distance fixed at 10 cm. The syringe pump was set to a flow rate of 0.5 mL/min. The coagulation bath was filled with water. The solution was then subjected to electrospinning. Upon complete electrospinning of the 3 mL of solution, the resulting fibers were kept in the water bath overnight to ensure the complete removal of the ionic liquid. The fibers were then removed, washed with water, and dried upon the electron microscopy stubs for analysis.

2.2.6 Optical and Electron Microscopy

Fibers were characterized by optical and electron microscopy. An Olympus CH30 Light Microscope (Center Valley, PA) with attached camera was used for visualization of the fibers at 40X and 100X magnification. Electron microscopy was performed using a Hitachi S-2500 scanning electron microscope (Tokyo, Japan) with an accelerating voltage 10 kV. The dried samples were prepared by sputter coating with a gold/platinum alloy using a Technics Hummer Sputter Coater (Alexandria, VA). The coated samples were then placed on a pin-mount into the
sample chamber where they were brought under high vacuum for the microscopy. Transmission electron microscopy samples were prepared by drying samples and loading onto a copper sample holder. The sample was loaded into the instrument's vacuum chamber, then once stable inserted into the chamber for viewing.

2.3 Results and Discussion

2.3.1 Electrospinning with Practical Grade Chitin

Our electrospinning apparatus consists of a high-voltage power supply connecting a needle and a grounding electrode plate under a coagulation bath. The needle is attached to a syringe which delivers the polymer solution through air pressure delivered from a syringe pump. Due to the solubility of the ionic liquid and insolubility of chitin, water was used as the coagulation solvent. After loading the syringe with the appropriate solution, voltage was applied to the system by computer-controlled software.
The preparation of all the chitin extract solutions for electrospinning followed previously reported procedures. A certain mass of chitinous sample was extracted in a certain mass of IL in a domestic microwave using 2-3 second pulses with stirring for a total of 2 minutes. Once the extraction of the chitinous sample was completed, the sample was centrifuged to remove the insoluble materials. The extracted chitin solution was then loaded into a syringe and was subjected to electrospinning conditions described from here out.

Due to its low cost, availability, and previous success with electrospinning our initial electrospinning attempts used PG-chitin. PG-chitin was dissolved in crystalline [C$_2$C$_2$Im][OAc] using the microwave dissolution described above to give a 1.5 wt% solution which was subjected to electrospinning at 28 kV, with an 18.5 G needle, and at a needle-to-ground distance of 13 cm.
(Trial 1). The images in Figure 2.2 show the typical results of the electrospinning experiments. During the experiment an electrospun mat was formed on the surface of the coagulation bath (Figure 2.2A) and could be pulled off with a simple transfer pipet in one piece (Figure. 2.2B). This is significant as it indicates a tangled mass of chitinous material representative of the expected non-woven mat typical of electrospinning; however, optical microscopy (Figures. 2.2C and 2.2D) indicated initial problems with the electrospinning. At 100 X magnification the image indicates that under these conditions the production of beads and beaded fibers are present. Electron microscopy (Figure 2.3) provides more detailed material images showing beads and beaded fibers. The presence of beads and beaded fibers are indicative of two potential problems: inadequate chitin concentration would not provide sufficient entanglement density necessary for the electrospinning of continuous fibers and too high of a voltage would lead to electrospraying of beads.
Figure 2.2 Standard images and optical micrographs of chitin materials from trial 1.
To overcome these problems, we initially chose to increase the flow rate of the polymer solution by increasing the diameter of the needle, which allowed a faster rate of solution flow. With the large needle size and with the applied air pressure from the syringe pump, the rate was too great for the system and the electrospinning was unsuccessful. Allowing the solution to flow by gravity, instead of by air pressure, resulted in an optimal rate of flow.

Materials collected through this method (Trial 2) are shown in Figure 2.4 and show electrospun fibers at the low micron range. This indicates that the instabilities of the ejected jet is more suppressed than the previous trial and result in improved fibers. Beads are generally no longer produced which is representative of an improved entanglement density. This is most
likely due to the increased flow providing access to more polymer for the entanglement to occur. However, the lack of smooth, continuous fibers indicates that fiber production is occurring inconsistently due to a lack of sufficient concentration or excess voltage which could provide instabilities in the jet formation.\textsuperscript{9}

![Figure 2.4 Electron microscopy of chitin materials from Trial 2.](image)

Though the electrospinning had not been optimized, our goal of developing a method to utilize high molecular chitin directly from biomass required us to switch sources of chitin. With chitin extracted directly from shrimp shells, the molecular weight will be higher than PG-chitin and therefore the concentration of polymer for sufficient entanglement density is lower. It was shown previously that with increased molecular weight at a given concentration chain entanglements were increased and led to successful fiber formation.\textsuperscript{10,11,12} Therefore switching to
a higher molecular weight, as extracted directly from shrimp shells, could lead to a different set of conditions for the generation of consistent and smooth nanofibers.

2.3.2 Influence of Chitin Source and Ionic Liquid

Our experiments continued by preparing a solution from dried shrimp shells in [C$_2$C$_2$Im][OAc] at a 3 wt% concentration by microwave dissolution followed by centrifugation. The supernatant was removed and subjected to electrospinning. Though this solution was free flowing, once under electrospinning conditions the solution coagulated within the needle and stopped flowing. Phase transformations were observed during this event and indicative that the ionic liquid was solidifying under these conditions. Though we had not seen this in previous attempts it is easy to rationalize the solid nature of the ionic liquid due to its relativity high melting point (30 °C) when compared to the very similar 1-ethyl-3-methylimidazolium acetate [C$_2$C$_1$Im][OAc] (-20 °C). Based on the applied charge of the system, this could be a separation of the polymer from the ionic liquid similar to that of electrophoresis, which produces a pure ionic liquid that crystallizes at room temperature. Continuing on required us to look for an IL with a lower melting point to try and decrease the possibility of a phase transformation.

Preparing a solution of shrimp shell extract and [C$_2$C$_1$Im][OAc] at 3 wt% produced not only a more viscous solution than with [C$_2$C$_2$Im][OAc], but was too viscous to flow through our electrospinning setup. Decreasing the viscosity was achieved by lowering the concentration to 2 wt% and the resulting solution was subjected to electrospinning. Figure 2.5 shows fibers mostly in the micron range, but closer views indicate that there is nanofibers. The nanofibers show smooth surfaces indicative that optimal conditions are met; however this is only a small percentage of the material actually spun. Most fibers appear to be composed of smaller fibers.
braided together or are asymmetric in their cross-section shape indicating that instabilities in the jet formation necessary in the electrospinning are not being suppressed.

Figure 2.5 Electron microscopy of chitin materials from Trial 4.

To try and optimize the consistency of nanofiber production we decreased the size of the needle to 18.5 G to provide a smaller surface area for the Taylor cone to form. With this smaller needle an increase in the flow was required to feed the polymer solution through the needle. The 2 wt% shrimp shell extract solution in [C$_2$C$_1$Im][OAc] was subjected to electrospinning and the resulting fibers can be seen in Figure 2.6.
From these images it is clear that optimal electrospinning conditions have been reached as there are smooth and continuous fibers in the micro and nano ranges. Figure 2.6 (left) shows the non-woven mat of electrospun fibers, (middle) shows the close up image highlighting the smoothness and consistency of the fibers, and the image (right) shows an isolated single fiber. Through pixel correlation, the diameter of this fiber is 670 nm. To our knowledge this is the first instance of a one-pot extraction and electrospinning of high molecular weight chitin nanofibers.

To further investigate this system we determine the optimal viscosity of the solution by preparing a series of solutions at different shrimp shell extract concentrations in [C$_2$C$_1$Im][OAc] and subjected them to electrospinning. Table 2.1 summarizes the viscosity and results from the electrospinning experiment. Beginning at 1.5 wt% shrimp shell loading the viscosity approximately doubles from 549 to 972 cP with the addition of 0.5 wt% shrimp shells. With the addition of another 1 wt% the viscosity approximately doubles again to 2297 cP. We previously mentioned the excellent fibers produced by the 2 wt% solution above. Micro and nanofibers were produced from the 1.5 wt% solution, however the fibers lacked the structural support given
by the 2 wt% solution. This insufficient concentration of the polymer or lack of optimal voltage have been determined to be unable to suppress the instabilities of the jet needed for successful electrospinning. Increasing the loading to 3 wt% produced a solution too viscous to flow through the needle without extremely high pressure. An alternative to decrease the viscosity would be to increase the temperature, however, our setup would not allow for temperature control and we were unable to test this solution. Modifications are underway to allow for high-throughput electrospinning, with the addition of temperature control.

<table>
<thead>
<tr>
<th>Shrimp shell loading (wt%)</th>
<th>Viscosity (cP) [30 °C]</th>
<th>Electrospinning Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>92(5)</td>
<td>N/A</td>
</tr>
<tr>
<td>1.5</td>
<td>549(3)</td>
<td>Fibers produced were weak and structurally degraded.</td>
</tr>
<tr>
<td>2.0</td>
<td>972(3)</td>
<td>Fibers produced were smooth and continuous</td>
</tr>
<tr>
<td>3.0</td>
<td>1664(43)</td>
<td>Too viscous for spinneret/needle</td>
</tr>
<tr>
<td>3.0 ([C\textsubscript{2}C\textsubscript{1}Im][OAc])</td>
<td>880(8)</td>
<td>Coagulated in needle with application of charge</td>
</tr>
</tbody>
</table>

Table 2.1 Viscosity and electrospinning results from a series of solutions of shrimp shells in [C\textsubscript{2}C\textsubscript{1}Im][OAc].

Further investigation into the differences of viscosity between [C\textsubscript{2}C\textsubscript{2}Im][OAc] and [C\textsubscript{2}C\textsubscript{1}Im][OAc] led to very interesting results. Table 2 compares the viscosities of solutions shrimp shells at 3 wt% with both ILs. At 3 wt% shrimp shell, both ILs had solids remaining, however, the [C\textsubscript{2}C\textsubscript{2}Im][OAc] mixtures were much darker in color with more solids remaining undissolved than for the [C\textsubscript{2}C\textsubscript{1}Im][OAc] mixtures which retained the ILs lighter color with less solids. These results suggests that [C\textsubscript{2}C\textsubscript{2}Im][OAc] extracts less chitin from the SS and that either the IL or any chitin extracted is more degraded during the process. The viscosity of the 3 wt% solution with [C\textsubscript{2}C\textsubscript{1}Im][OAc] is approximately double the viscosity of the same solution with [C\textsubscript{2}C\textsubscript{2}Im][OAc]. This is quite surprising if one considers the melting points of the two ILs.

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However, as indicated previously the 2 wt% solution of shrimp shells and [C\textsubscript{2}C\textsubscript{1}Im][OAc] was the solution that produced the best electrospinning results and this solution has a very similar viscosity to that of the 3 wt% solution with [C\textsubscript{2}C\textsubscript{2}Im][OAc]. This suggests that the viscosity is mostly dependent on the amount of dissolved chitin and that [C\textsubscript{2}C\textsubscript{2}Im][OAc] does not dissolve chitin as well as [C\textsubscript{2}C\textsubscript{1}Im][OAc].

### 2.4 Conclusions

In summary, chitin nanofibers were produced by electrospinning chitin directly from a solution of 2.0 wt% shrimp shell in [C\textsubscript{2}C\textsubscript{1}Im][OAc]. The results from this study demonstrate a simple method for producing high surface area chitin fibers directly from biomass in a one pot system. This is the first instance of electrospinning chitin directly from an ionic liquid solution with or without auxiliary solvents, demonstrating a viable process from a renewable feedstock. This capability is attributed to the higher molecular weight chitin produced through the direct dissolution of shrimp shells in [C\textsubscript{2}C\textsubscript{1}Im][OAc] which provided the optimal viscosity, concentration, and necessary entanglement density require for the electrospinning of smooth, continuous chitin nanofibers.

### 2.5 Acknowledgments

The authors would like to thank the DOE Office of Nuclear Energy’s Nuclear Energy University Programs (Sub-Contract - #120427, Project - #3123) for project funding and Dr. Kim Lackey and the Optical Analysis Facility at The University of Alabama for microscopy support.

### 2.6 References


3 COAGULATION OF CHITIN AND CELLULOSE FROM 1-ETHYL-3-METHYLLIMIDAZOLIUM ACETATE IONIC LIQUID SOLUTIONS USING CARBON DIOXIDE


3.1 Introduction

Interest in using ILs within a lignocellulosic biorefinery is due in part to their ability to dissolve biopolymers such as cellulose, as well as raw biomass. In Chapter 2 chitin was demonstrated as a high surface area platform for fiber applications. However due to the high cost of the ILs, it was determined making the chitin materials would be more economical if the IL could be recovered. We and others have proposed extending the biorefinery concept to ocean-based biopolymers using ILs for the dissolution, extraction, and electrospinning of chitin from crustacean shells. However, one key processing step needing improvement is recycling of the IL after an antisolvent (e.g., water or ethanol) is added to coagulate the dissolved biopolymers by solvating the IL. Distillation can be used to remove the antisolvents from the IL, however, the energy intensive process presents economic and engineering challenges at large scale. We have been searching for alternatives to high-boiling liquid antisolvents that would promote facile separation from the IL.

The chemisorption of CO$_2$ in 1-ethyl-3-methylimidazolium acetate [C$_2$C$_1$Im][OAc] was previously reported through chemical reaction of an in situ carbene with CO$_2$. Formation of the zwitterion produces one mole of acetic acid, which hydrogen bonds with the strongest acceptor, any remaining acetate anion. Since super-critical carbon dioxide (scCO$_2$) is inexpensive, non-
explosive, highly available, easy to remove from extracted products, and is considered to be the most suitable fluid in supercritical processes, we explored whether scCO$_2$ (or even CO$_2$(g)) could be used as a coagulation solvent for biopolymer IL solutions. We hypothesized that if CO$_2$ reacted with [C$_2$C$_1$Im][OAc] even when a biopolymer was dissolved in it, the IL would react to form the adduct, the biopolymer would precipitate, and the IL could be recycled easily through the stoichiometric addition of water. To test our hypothesis, we chose to focus first on the coagulation of chitin extracted from dried shrimp shells with [C$_2$C$_1$Im][OAc], due to its higher molecular weight than commercially available practical grade or pure chitin which we anticipated would be more easily coagulated because of its lower solubility.

3.2 Experimental

3.2.1 Chemicals

Microcrystalline cellulose and other reagents were used as obtained from commercial sources (Sigma-Aldrich, Milwaukee, WI) unless otherwise noted. All solvents were ‘solvent grade’ and used as received without additional purification. The ILs 1-ethyl-3-methylimidazolium acetate [C$_2$C$_1$Im][OAc] was purchase from IoLiTec, Ionic Liquids Technologies Inc (Tuscaloosa, Al) and used for the super-critical CO$_2$ studies. Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, Al and ground to a < 125 µm particle before use.

3.2.2 Dissolution of Shrimp Shells into Ionic Liquids

For dissolution experiments 0.600 g Shrimp shells were dissolved in 29.400 g [C$_2$C$_1$Im][OAc] through a microwave dissolution process in a domestic microwave oven (SHARP Carousel R-209KK, Mahwah, NJ) at full power. The solution was heated for a total of two minutes, utilizing 2-3 second pulses and mechanical stirring between pulses. After two
minutes there remained undissolved particles typical of this type of dissolution and likely composed of proteins and minerals, such as CaCO₃. Upon cooling to room temperature the [C₂C₇Im][OAc] solution was dark brown after dissolution and was highly viscous.

3.2.3 Coagulation of Chitin using sc-CO₂

To evaluate the CO₂ coagulation, 0.600 g of dried shrimp shell was extracted with 29.400 g of [C₂C₇Im][OAc] and centrifuged to separate the insoluble material. Solutions of 5–6 mL solutions were then loaded into a high pressure windowless reactor and the reactor was purged and filled with CO₂(ℓ) and then sealed. The reactor was heated with a water bath to 35–40 °C and the pressure was increased to between 1100–1500 psi (supercritical conditions for CO₂ are 31.5 C and 1070 psi). The solutions from above were centrifuged to remove all undissolved particles and 5 mL of each solution was decanted into 8 mL centrifuge tubes for the sc-CO₂ experiment. Performed individually, the solutions were introduced into the high-pressure apparatus (Denton DCP-1 Critical Point Dryer). The system was degassed by purging with CO₂ at room temperature. After 1 minute, the system was sealed and the temperature was increased by use of a water bath (50 °C). The pressure was maintained between 1100-1500 psi, for the duration of the experiment, after which the system pressure was released and the sample was removed. The chitinous material was removed from the top of the solution upon which is was washed with minimal water. Upon placing the material in water, the evolution of gas was observed. The material was dried to constant weight at 80 C and the mass was recorded.

3.3 Results and Discussion

3.3.1 Coagulation of Chitin in a Batch Reactor

A solution of chitin extracted from dried shrimp shell (0.6 g) with [C₂mim][OAc] (29.4 g) was prepared using a microwave process described previously. Aliquots of the extract solution
(5-6 g) were then loaded into a high pressure windowless reactor at room temperature, the reactor purged and filled with CO$_2$(l) to 6.2 MPa, and then sealed. The batch reactor was heated to 35–40 °C increasing the pressure to 7.6–10.3 MPa, above the critical pressure. Separate samples were contacted with scCO$_2$ for 1, 2, or 4 h. After depressurization, a phase boundary was observed across the fluid interface (Figure 3.1b). The film initially inhibited the release of CO$_2$ from the IL-rich phase until overcome by the gas pressure (Figure 3.1c). The solid film was then physically removed from the IL surface using forceps.

The adhering IL was easily removed from the chitin by minimal water addition during which CO$_2$ effervescence was observed (Figure 3.1d). (The addition of water as a purification step was employed only to remove IL for measurement of recovery yields and could be exchanged for thermal or physical separation in the process design.) The chitin (Figure 3.1e) was dried to constant weight. The yields based on the mass recovered and the available chitin in the shrimp shells (22 ± 1%) were 19 ± 4% (1 h contact), 21 ± 6% (2 h), and 20 ± 7% (4 h). We previously reported that using water as the coagulation solvent, up to 94% of the available chitin could be recovered. The low yield here and the observation of gas trapped in the IL-rich phase of the solution beneath the film led us to hypothesize that further coagulation was prevented by limited mass transfer and reaction only at the fluid interface.

Figure 3.1 The coagulation of chitin from a solution of shrimp shell extract in [C2mim][OAc]. The extract solution (a), the coagulated chitin film (b), the coagulated chitin film being lifted by
the pressure of the gas (c), the film once placed in water (d), and the chitin film removed from water (e).

### 3.3.2 Sequential Batch Reactions

We then attempted to determine if increased chitin recovery could be obtained with a sequential batch system at 1 h contact times followed by film removal after each contact. Two different chitin solutions were compared, one from direct extraction of 2 wt% dried shrimp shell and a second by dissolution of 1.75 wt% of regenerated chitin (previously extracted and coagulated). Approximately 5-6 g samples of each were loaded into the reactor and pressurized with CO$_2$ for 1 h as described above. The samples were then weighed to measure the amount of CO$_2$ absorbed, followed by removal of the surface film.

This entire process was repeated until the entire solution was solidified, which depending on the solution was 5-7 times. Each film was washed with a minimal volume of water and dried to constant weight for yield determination. Figure 3.2 summarizes the cumulative chitin recovery and the mass of chitin coagulated for each sequential 1 h contact time (Table S1). The mass of chitin recovered after each 1 h contact was 5.1 ± 0.9 mg and 10 ± 2 mg for the shrimp shell extract and regenerated chitin solutions, respectively, indicating that coagulation in this batch reactor was indeed limited to the fluid interface. Nonetheless, 95% of the available chitin in the shrimp shells was recovered from the extract solution (ca. 0.45% chitin in solution) and 57% of the chitin in the much more concentrated regenerated chitin solution (1.75%) after 5 x 1 h contacts. We believe the higher recoveries from the extract solution are due to the presence of other dissolved material from the shrimp shells (e.g., CaCO$_3$) which would reduce the number of free acetate anions available to dissolve the chitin.
3.3.3 Recovery of the IL

While contact with scCO$_2$ does provide a method for the coagulation of chitin from [C$_2$C$_1$Im][OAc] solution, a potential purification problem remains in finding a low energy method to remove and recycle any residual IL from the biopolymer. We made several attempts to remove the IL completely from the chitin films using only CO$_2$ (without using water or other antisolvent). Coagulated chitin films with residual IL were placed in a porous metal basket and contacted with scCO$_2$ within the static high-pressure reactor for several hours. Although the residual IL had adsorbed some CO$_2$, IL remained on the film. The same result was obtained when the samples were continually purged in the reactor with liquid CO$_2$ at 6.2 MPa for 1 h.

We also considered more conventional techniques such as pressing or heating. Some of the residual IL could be removed by simply suspending and heating the films to decrease the viscosity of the IL and allowing it to drip off. In one experiment, after the suspended film was heated for 12 h at 100 °C, up to 82% of the IL was removed; however, at these temperatures, there is no recycling advantage over using water or ethanol as the antisolvent.
In order for IL recycling to provide a cost-advantage, minimal energy must be used in the recovery process of the IL from the coagulation solvent. Though we have greatly decreased the amount of water used in the coagulation process by being able to concentrate the chitin from the chitin/IL solution using CO$_2$, a purification step is required through use of water or heat to remove the residual IL. We can envision an optimized process that would coagulate the biopolymer using scCO$_2$ in a continuous flow reactor where the biopolymer material would then be stripped of the majority of the residual IL through physical separation.

### 3.4 Conclusions

The use of CO$_2$ chemisorption as an alternative coagulating process has the potential to provide an economical and energy efficient method for recycling the IL by eliminating the need to distill higher boiling coagulation solvents from the IL, or at least reducing the amount of antisolvent which must be removed. While perhaps not the final answer, and with many engineering parameters to be determined, this coagulation route should be considered when [C$_2$ mim][OAc] or closely related ILs are chosen as the biopolymer dissolution solvent.

### 3.5 Acknowledgments

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### 3.6 References


4 SURFACE MODIFICATION OF IONIC LIQUID-SPUN CHITIN FIBERS FOR THE EXTRATION OF URANIUM FROM SEAWATER: SEEKING THE STRENGTH OF CHITIN AND THE CHEMICAL FUNCTIONALITY OF CHITOSAN


4.1 Introduction

Sequestration of UO$_2^{2+}$ from the environment mirrors extraction from seawater, thus the strategy of amidoxime-functionalized chitin fibers offered a logical proof of concept as a high surface area platform to minimize waste-to-volume for immobilizing UO$_2^{2+}$ on a solid phase. In this paper, we have selected one possible application for these fibers from a U.S. Department of Energy project aimed at developing high capacity adsorbents for the extraction of uranium from seawater$^1$ as a proof-of-concept demonstration. Successful implementation would require a fiber strong enough to withstand deployment in the sea, with the chemical functionality required to append the uranium selective extractant to the fiber surface. The current benchmark process is to use a sorbent made by grafting uranyl-selective amidoxime functional groups$^2,3$ onto synthetic polyethylene polymers using high energy irradiation techniques.$^4$ A recent cost analysis indicated that the adsorbent comprises 43% of the total cost for extracting uranium from seawater,$^5$ and research has focused on lowering the cost through increasing adsorbent capacity and recycling by increasing the surface area$^{68}$ or developing alternative functional groups to amidoxime.$^{9,10}$ Even though polyethylene polymers are a major source of anthropogenic marine pollution$^{11,12,13}$ and are made from nonrenewable petroleum, there has been little emphasis on
lowering the cost or environmental impact of the process by changing the sorbent backbone to a more suitable material.

The strength, durability, and water resistance of polyethylene materials are sufficient for long term marine deployment, however, in our opinion, the main reason for the use of polyethylene is simply that it was the first backbone polymer used and it worked. We believe that the material which will ultimately be used in this application must address the environmental concerns about polyethylene while meeting or exceeding its performance. We have thus proposed an alternative strategy to use a naturally available waste material, shrimp shells, to obtain a high molecular weight chitin which could be used to prepare strong, high surface area, biodegradable sorbents. Here, we demonstrate the preparation, characterization, and utilization of chitin fibers via dry-jet wet spinning of a solution of chitin extracted from shrimp shells in 1-ethyl-3-methylimidazolium acetate \([\text{C}_2\text{C}_1\text{Im}]\text{[OAc]}\), and the surface functionalization of the fibers with the uranyl-selective amidoxime functional group.

4.2 Experimental

4.2.1 Chemicals

Reagents were used as obtained from Sigma-Aldrich, Milwaukee, WI, unless otherwise noted. All solvents were ‘solvent grade’ and used as received without additional purification. 1-ethyl-3-methylimidazolium acetate \([\text{C}_2\text{C}_1\text{Im}]\text{[OAc]}\) was purchased from Iolitec (Ionic Liquids Technologies, Inc., Tuscaloosa, AL). Deionized (DI) water was acquired from an in-house system (Culligan Water Systems, Chattanooga, TN) with a measured resistivity of 17.4 MΩ. Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, AL where the shrimp shells were dried at a specialized facility by pressing with a screw press to eliminate the majority of the water, followed by heating at up to 160 °C in a
fluidized bed dryer until the material had a final moisture content of less than 5 wt%. The material received from the drying facility was ground using an IKA Works Universal Grinding Mill (Wilmington, NC) and sieved to give a powder with a particle size of <250 µm. Ground shrimp shells were further dried in a Precision Scientific Econotherm Laboratory Oven, Model 1025 (Winchester, VA) at 80 ºC for 24 h. ‘Regenerated and purified chitin’ refers to chitin that has been extracted from shrimp shells using [C$_2$C$_1$Im][OAc], coagulated, washed, sonicated in water, and then dried in the oven at 80 ºC for 24 h.

4.2.2 Preparation of Chitin Fibers from Shrimp Shells (SS Fibers)

Extraction of chitin from dried shrimp shell was performed using a domestic microwave oven (SHARP Carousel R-209KK, Mahwah, NJ) using a previously reported procedure. **CAUTION!** Care must be taken when using microwave heating because [C$_2$C$_1$Im][OAc] is an efficient microwave absorber and heating occurs rapidly, which can easily lead to degradation of the ILs, and/or chitin, or even explosions of sealed systems.

All solutions were made in a similar fashion with an appropriate mass of shrimp shell being added to a mass of IL corresponding to a final weight percent (wt%). For example, a 6 wt% solution was prepared by mixing 19.290 g ground shrimp shell in 301.044 g [C$_2$C$_1$Im][OAc] to give a total mass of 320.330 g. The mixture was heated at full power for six 10 s long pulses, followed by 48 5 s long pulses for a total heating time of 5 min. The solution was stirred by hand with a glass stirring rod between pulses and was observed to darken slightly with time during the heating. After dissolution, the solution was centrifuged to remove insoluble materials, loaded into four 60 mL syringe tubes, and degassed in an oven at 80 ºC overnight resulting in approximately 60 mL of solution per syringe.
The four syringes filled with chitin-IL solution were used to prepare chitin fibers following a dry-jet wet spinning method as described previously for producing cellulose and chitin fibers from IL solution.\textsuperscript{14} The spinning setup consisted of a syringe pump (for controlled rate of extrusion), a water bath (for coagulation of chitin and dissolution of IL), godets within the water bath (for fiber stretching), and a spool (for collection of spun fibers). After degassing, each syringe was attached to the syringe pump (Model No. NE-1010, New Era Pump Systems, Inc., Farmingdale, NY) with a temperature jacket and controller set to 80 °C. Each solution was extruded into a 0.6 m long water bath after a small air gap (~1.5 cm). The regenerated chitin filament was led through the pair of godets twice, to provide adequate fiber stretching and elongation, and then collected onto a spool. The syringe pump was set at an extrusion rate of 1.5 mL/min. The voltage setting for the motor spinning the godets was 4.2 V. DI water was used as the coagulant. Each spool of fibers was then placed in a 600 mL beaker and soaked in 500 mL DI water for 2 days, exchanging the water several times to remove the residual IL. Four spools containing approximately 1 g (dried weight) of fibers each were obtained. One set of fibers (SS fibers) was air dried for analysis, while the remaining three spools of fibers were stored in water until the desired surface modification was complete.

4.2.3 Deacetylation of Chitin Fibers (DA Fibers)

Three of the four spools of chitin fibers were treated in the standard method for preparing chitosan. The amounts of reagents were chosen to ensure an excess. About 1 g SS of fibers was removed from each of three spools and placed in separate 400 mL beakers. To each beaker was added 200 mL of 1.25 M NaOH and a magnetic stir bar. The beakers were covered with watch glasses and the mixtures were heated at 80 °C for 8 h with stirring. After 8 h, the fibers appeared to lighten in color, and the liquids were decanted. Each set of DA fibers was washed with DI
water a total of three times (3 x 100 mL) with swirling. One set of DA fibers was air dried for analysis while the remaining two sets of fibers were carried through immediately to the next modification without drying. No visible changes were observed during this process.

4.2.4 Nitrile Functionalization of Deacetylated Fibers (CN Fibers)

Two of the three sets of DA fibers were treated to append a nitrile to the primary amine. The amounts of reagents were chosen to ensure an excess. 200 mL of ethyl acetate was added to each portion of ~1.00 g DA fibers. 293 µL (0.321 g, 3.10 mmol, 0.5 eq.) 4-chlorobutyronitrile and 441 µL (0.320 g, 3.20 mmol, 0.51 eq.) triethylamine were then added and the mixtures were covered with a watch glass and heated at 50 °C overnight. After completion, the liquids were decanted and 50 mL ethyl acetate was added to the fibers and swirled to wash the fibers of residual reagents. The CN fibers were washed a total of three times with 50 mL ethyl acetate each. One set of CN fibers was carried through to the final step, while one set was dried in air for analysis. No visible changes were observed.

4.2.5 Amidoxime Functionalization of Nitrile Fibers (AO Fibers)

The chitin content of the dried shrimp shell and fibers were measured by the Black and Schwartz method. Infrared (IR) spectra were obtained in the range $\nu_{\text{max}} = 400$–4000 cm$^{-1}$ on a Bruker Alpha FT-IR instrument, Bruker Optics Inc. (Billerica, MA) with an attenuated total reflection (ATR) sampler by pressing solid samples directly against the ATR diamond. IR spectra were recorded on whole fibers, as well as fibers ground by hand into a powder.

X-ray photoelectron spectroscopy (XPS) was performed on a Kratos AXIS 165 Multitechnique Electron Spectrometer (Kratos Analytical Ltd, Manchester, U.K.). Samples were loaded on copper tape. Monochromated Al-Kα radiation was used as the excitation source. Thermogravimetric analyses (TGA) were conducted with a Mettler-Toledo (Columbus, OH)
DSC/TGA 1. The instrument’s internal temperature was calibrated by observing the melting points of Au, Zn, and In. Samples of 5–10 mg were analyzed in 70 μL alumina pans under an air atmosphere. All samples were heated from room temperature to 75 °C with a 30 min isotherm at 75 °C in order to ensure excess volatiles or residual solvents were removed. Following the isotherm, samples were heated to 1000 °C at 5 °C/min, then held at 1000 °C for 30 min. Decompositions temperatures were recorded as the onset to 5% weight mass loss (T_{5\%dec}).

The tensile properties of the fibers were determined using a MTS Q-Test 25 machine (Eden Prairie, MN) equipped with a specially designed pneumatic grip suitable for thin and flexible fiber testing. A load cell of 22.4 N capacity was used for load measurement. The cross head speed was maintained at 1.27 mm min^{-1} from an initial cross head distance of 15.24 cm. For this measurement, additional fibers of each type were prepared following the exact protocol from above. Before drying, the fibers were cut into approximately 38 cm pieces, laid on the bench top, and then air dried. Fibers were inspected and those with no visible defects were chosen for testing. Cross-sectional areas were measured using digital calipers with 0.0254 cm precision at seven points along the fiber spaced approximately 2.5-5 cm apart and averaged. The tensile strength of the fibers was measured using single fibers and at least seven fibers of uniform cross-section from each type were tested.

4.2.6 Radiotracer Distribution Experiments

CAUTION! 233U is a radioactive alpha and gamma emitter and appropriate training and laboratory safety methods should be observed. Minimize handling time and activity used to reduce exposure. Consult and comply with all local regulations regarding storage and disposal of radioactive materials.
Distribution ratios were determined by measuring activities in counts per minute (cpm) using a Packard Cobra-II gamma counter (Meriden, CT). All dry weight distribution ratios were determined radiometrically by batch contacts of the fibers with the desired solutions at 25(1) °C. The dry weight distribution ratios were calculated as in eq. 1:

\[
D_w = \left( \frac{A_o - A_f}{A_f} \right) \left( \frac{V_{eq}}{m_R \text{ (dwcf)}} \right)
\]

where \(A_o\) is the count rate in solution prior to contact with the fiber, \(A_f\) is the count rate in solution after contact with the fiber, \(V\) is the volume (mL) of solution in contact with the fiber, \(m_R\) is the mass (g) of wet fiber, and the dry weight conversion factor (dwcf) allows conversion to the dry mass of fiber.

To determine the dry weight conversion factor for each type of fiber, clean one dram (3.70 mL) borosilicate glass shell vials were dried to constant weight in the oven at 110 °C and their tare weights were recorded. Approximately 4-5 mg of each of the four fibers were placed in the tared vials and the fibers were then dried to constant weight in the oven at 110 °C. The mass of the dried fibers was divided by the mass of the wet fibers to determine the dry weight conversion factor (dwcf). The dwcf's were measured in triplicate for each fiber and were determined to be: 0.93(1), 0.964(3), 0.96(1), and 0.963(7) for SS, DA, CN, and AO fibers, respectively. The averaged values were used for all calculations using eq. 1.

Each batch uptake experiment was performed by adding 5 μL of 1 μCi/1 μL aqueous \(^{233}\text{UO}_2\text{Cl}_2\) to 1.3 mL of DI water, gently mixing, and removing a 150 μL aliquot for gamma counting (\(A_o\)). A total of 1 mL of the remaining solution (\(V\)) was added to a known mass (~2.5 mg) of wet fibers (\(m_R\)). The mixture was then shaken at 75 rpm on a New Brunswick Scientific C25 Incubator shaker table (Edison, NJ) at 25 °C. Aliquots of each solution were taken for counting at 1.5, 4, 22, 44, and 144 h. To remove aliquots, the solutions were centrifuged for 2
min at 2000xg (3800 rpm) to separate the phases and a 150 µL aliquot \( (A_i) \) of the supernatant was taken for gamma counting. Dry weight distribution ratios \( (D_{w}) \) for each fiber were calculated from the change in activity at 144 h using eq. 1.

### 4.3 Results and Discussion

#### 4.3.1 Preparation and Surface Modification of Fibers

Since both cost and biodegradability were motivating factors\(^5\) for using chitin, a decision was made to use the raw extracted chitin in a one-pot process, rather than extracting the chitin, purifying it \( \text{via} \) coagulation in water, and then re-dissolving the chitin prior to use. From prior work, we anticipated that this could lead to higher amounts of impurities in the fibers and some sacrifice in overall strength. Nonetheless, the simplicity of this process or some small variation of it would keep chemical and energy usage to a minimum.

While the four types of chitin fibers were prepared several times to conduct all experiments, when comparing the four types within this study and for each type of experiment, all fibers were prepared from the same batch of shrimp shell extract spinning solution. The spinning solution was prepared by extraction of ca. 19 g dried shrimp shell (6 wt\%) in \([\text{C}_2\text{mim}][\text{OAc}]\) followed by centrifugation to remove any insoluble residue. The extract solution was then loaded into four 60 mL syringes which were degassed in an oven at 80 °C overnight. Each syringe was used to dry-jet wet spin a spool of about 1 g of chitin fibers using techniques and equipment we have previously reported for chitin and cellulose.\(^14\) Each spool of chitin fibers was washed with DI water and further soaked for 1-2 days to remove residual IL. One spool of the chitin fibers (designated SS fibers) was removed and dried in air, while the remaining spools were kept in water until used in the next step.
Surface modification of the chitin fibers followed the reaction pathways noted in Scheme 1 taking advantage of the insoluble nature of chitin. First, SS fibers were taken off the three spools, placed in separate beakers, and each stirred in 400 mL of 1.25 M aqueous NaOH at 80 °C for 8 h to deacetylate the surface. The solutions were decanted from the fibers which were then washed three times with 100 mL DI water. Approximately 1 g of the fibers (designated as DA fibers) were set aside for analysis and the remaining fibers were carried on to the next step.

To attach the uranyl-selective amidoxime ligand to the now free amine groups on the chitin fiber surface, a two-step reaction was conducted. Two ~1 g portions of the DA fibers in separate beakers were stirred in an ethyl acetate solution containing 0.0155 M 4-chlorobutyronitrile and 0.0160 M triethylamine at 50 °C overnight. The reaction solutions were then decanted and each set of fibers was washed three times with 50 mL ethyl acetate resulting in the nitrile-functionalized fibers (designated CN fibers). An approximately 1 g portion of the CN fibers were air dried and kept for analysis. The other ~1 g portion of CN fibers was then stirred in 0.047 M aqueous hydroxylamine at 80 °C overnight. The solution was decanted and the fibers were washed three times with DI water to provide the amidoxime-functionalized (AO) fibers.

Figure 4.1 Synthetic scheme for the surface modification of chitin fibers using a typical method for deacetylation of chitin and a chemical route previously used by our group to append amidoxime groups onto imidazolium cations.
In total ~1 g each of SS, DA, CN, and AO fibers were prepared and air dried before characterization and analysis. The fibers were flexible but brittle, with no visible degradation as a result of any of the surface treatments.

4.3.2 Fiber Characterization

The chitin content of the dried shrimp shell starting material and each type of fiber were measured by the Black and Schwartz method\textsuperscript{15} revealing chitin contents of 22(1), 58.3(5), 64(3), 61.8(2), and 63.2(8)% for shrimp shell and SS, DA, CN, and AO fibers, respectively (Table 4.1). The large increase in chitin content for the fibers when compared to the initial chitin content of the dried shrimp shells was expected since the IL extracts chitin while leaving most of the shell matrix behind as previously reported. The apparent increase of chitin content from the SS fibers to DA fibers suggests that the caustic treatment removes additional non-chitin material from the fibers, whereas further treatment to append the ligand does not. The highly basic conditions of the deacetylation treatment could remove residual proteins in a manner similar to the caustic wash used to purify chitin in the industrial process.
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<th>Shrimp Shells</th>
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<th>DA Fibers</th>
<th>CN Fibers</th>
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</tr>
<tr>
<td><strong>Residual</strong> (%)</td>
<td>47</td>
<td>17(1)</td>
<td>9(1)</td>
<td>3(3)</td>
<td>5(1)</td>
</tr>
<tr>
<td><strong>Decomposition (T₅₀% onset, °C)</strong></td>
<td>-</td>
<td>274(4)</td>
<td>226(2)</td>
<td>229(8)</td>
<td>265(1)</td>
</tr>
<tr>
<td><strong>Fiber Diameter (mm)</strong></td>
<td>-</td>
<td>0.20(5)</td>
<td>0.20(3)</td>
<td>0.25(3)</td>
<td>0.24(2)</td>
</tr>
<tr>
<td><strong>Break Stress (MPa)</strong></td>
<td>-</td>
<td>9(2)</td>
<td>9(3)</td>
<td>9(3)</td>
<td>7(1)</td>
</tr>
<tr>
<td><strong>Break Elongation (%)</strong></td>
<td>-</td>
<td>6(3)</td>
<td>6(2)</td>
<td>7(2)</td>
<td>5(2)</td>
</tr>
<tr>
<td><strong>Yield Stress (MPa)</strong></td>
<td>-</td>
<td>7(2)</td>
<td>5(2)</td>
<td>4(3)</td>
<td>4(1)</td>
</tr>
<tr>
<td><strong>Yield Elongation (%)</strong></td>
<td>-</td>
<td>2(1)</td>
<td>2(1)</td>
<td>2(1)</td>
<td>2(1)</td>
</tr>
<tr>
<td><strong>Young’s modulus (MPa)</strong></td>
<td>-</td>
<td>3(1)</td>
<td>2(1)</td>
<td>2(1)</td>
<td>2(1)</td>
</tr>
</tbody>
</table>

Table 4.1 Composition and physical properties of shrimp shells and modified fibers.

Further analyses were conducted using thermogravimetric analyses (TGA, Figure. 4.2) on two independent samples of each material. The results for all four fibers are relatively similar with three major mass losses; the initial loss of water during the isotherm at 75 °C, a large mass loss at ~275 °C, attributed to the decomposition of the chitin material, and a third mass loss at 700 °C, characteristic of CaCO₃ decomposition to CaO and CO₂. Moisture content appears to decrease slightly upon treatment from 7% in SS fibers to 4% in DA, CN, and AO fibers.
Figure 4.2 Thermogravimetric analysis of SS (black), DA (blue), CN (pink), and AO (red) fibers, as well as regenerated and purified chitin (grey).

All of the fibers have relatively similar thermal decomposition temperatures ($T_{5\%onset}$) with values of 274(4), 226(3), 229(8), and 265(1) °C for SS, DA, CN, and AO fibers, respectively. SS fibers show the most deviation between the other fibers, showing a much smaller residual ash content (25% compared to ~40% for the other fibers). We believe this is due to a higher protein content in the SS fibers. Treatment with NaOH is the common method for removal of proteins when extracting chitin from shrimp shells. A decrease in thermal decomposition temperature between chitin (SS fibers) and chitosan (DA fibers) as seen here has been reported before for chitosan and chitin.\textsuperscript{16}

The CaCO$_3$ content of each fiber was determined using the decomposition of CaCO$_3$ at 700 °C by calculating mass loss as CO$_2$. Values of 16.9(2), 23.6(1), 29(1), and 26(2)% CaCO$_3$ were determined for SS, DA, CN, and AO fibers, respectively (Table 1). The relative increase in CaCO$_3$ content for the surface treated fibers is most likely due to the removal of proteins by the
NaOH treatment as discussed above. The fiber residuals were calculated through mass balance and the trend follows a general decrease in residual with increasing treatment to the fibers.

Attenuated total reflectance infrared (ATR-IR) spectroscopy was used to study the surface of the fibers by placing them directly on the surface of the ATR sample window, as well as to study the bulk material by grinding the fibers prior to measurement. A portion of the normalized spectra of the unground fibers are presented in Figure 4.3. All unground fibers show similar spectra characteristic of chitin with subtle, yet significant, differences. The spectra contain bands common with chitin including $\nu_{\text{OH}} = 3443 \text{ cm}^{-1}$, $\nu_{\text{NH}} = 3275 \text{ cm}^{-1}$, $\nu_{\text{CH}} = 2930 \text{ cm}^{-1}$, and characteristic amide bands at $\nu_{\text{CO}} = 1650 \text{ cm}^{-1}$, $\nu_{\text{CN}} = 1631 \text{ cm}^{-1}$, $\nu_{\text{NH}} = 1561 \text{ cm}^{-1}$, and $\nu_{(\text{amide band III})} = 1315 \text{ cm}^{-1}$.

Figure 4.3 A portion of the normalized IR spectra for unground SS (black), DA (blue), CN (pink), and AO (red) fibers.
A few changes which occur in the spectra upon treatment of SS fibers are noteworthy. The bands at 3443, 3275, and 2930 cm\(^{-1}\) change in intensity. The change in the higher wavenumber bands could be due to increased moisture content and therefore increased hydrogen bonding as has been shown before.\(^{38}\) The band at 2930 cm\(^{-1}\) undergoes a significant change in intensity though we have been unable to identify the cause. Within the lower range of 1200-1800 cm\(^{-1}\), the most significant changes are from the decrease and sharpening of the bands at 1650 and 1631 cm\(^{-1}\), as well as the increase in intensity of the band at 1420 cm\(^{-1}\). Both changes are indicative of a change in the deacetylation of the material and the most significant changes occur with the treatment to prepare the DA fibers which, as indicated in the chitin content measurement (Table 1), resulted in a relatively significant composition change.

To characterize the interior part of the fiber which was not exposed to the treatment baths, the fibers were dried and ground to a fine particle size and the IR spectra were recorded using the same parameters as the unground fibers. A portion of the spectra of the ground fibers are shown in Figure. 4.4, along with the spectra of ground shrimp shells and CaCO\(_3\) for comparison.
Overall, the spectra of the ground SS fibers are similar to the spectra of the unground fibers indicating the bulk material and surface are similar and characteristic of chitin as expected. However, significant differences between ground and unground DA, CN, and AO fibers were observed with a large increase in the band at ~1400 cm\(^{-1}\). When compared to the overlaid spectrum of CaCO\(_3\), the data suggests an increase in the relative CaCO\(_3\) concentration with the first treatment of fibers (to make DA fibers), which is also consistent with the removal of some protein material in the deacetylation step. This correlates well with the TGA and chitin content measurement data presented above, which also indicates that the deacetylation treatment removes protein and perhaps a small amount of chitin.

For additional surface characterization, X-ray photoelectron spectroscopy (XPS) was performed on the unground fibers. All fibers were first analyzed through a survey scan (over a
binding energy range of 0 to 1000 eV) to determine the elements present within the top 1-12 nm of the surface of the samples. Carbon, N, and O were found as expected for chitin, with additional peaks for Ca and Na for certain fibers. Both DA and CN fibers appear to contain Na which would indicate the fibers contain residual NaOH after the deacetylation treatment. The AO fibers do not contain Na, indicating the final treatment within water was significant enough to remove the final traces of Na.

As all steps of the treatment are expected to involve changes to the surface nitrogen atoms, high resolution scans of the N 1s region of the spectrum (390-410 eV, shown in Figure. 4.5) were done to investigate these changes. In contrast to the slight changes observed in the IR data, which indicated the similarities of the bulk composition of the fibers, the major changes in the XPS spectra of different fibers offer unequivocal evidence for surface modification. The differences also correlate with the expected chemical changes. The N 1s peak for DA fibers is sharper and at slightly lower binding energy than the N 1s peak for DA fibers. As the natural chitin in SS fibers typically contains a certain amount of free amine, the broader N 1s peak probably contains contributions from two types of nitrogen atoms, acetylated and deacetylated (or amide and amine nitrogen atoms). Upon deacetylation, the N 1s peak sharpened and moved slightly to lower binding energy, indicating that there is now one type of nitrogen atom, and it is more reduced than the nitrogen atoms in natural chitin. Both of these observations are consistent with deacetylation. Further treatment to the CN fiber did not significantly alter the peak present in the DA fibers, though it does appear to broaden slightly which would indicate two nitrogen types are present, a second amine and a nitrile. The AO fibers show a severely broadened N 1s peak, which might be expected considering this fiber surface now has three nitrogen atom types;
a secondary amine, a primary amine, and oxime. The XPS shifts also correlate with those that have been observed in XPS spectra of amidoxime grafted mesoporous carbon materials.\textsuperscript{18}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.5.png}
\caption{High resolution XPS scans of the N 1s region for SS (black), DA (blue), CN (pink), and AO (red) fibers.}
\end{figure}

### 4.3.3 Fiber Physical Properties

The tensile strengths of the fibers were measured using single fibers of ~38 cm length with an initial crosshead distance of 15.24 cm. Seven fibers of each type with uniform cross-sections and no visible flaws were examined for break stress and yield stress (Fig. 4.6 and Table 1). Figure 4.6 shows stress-strain curves for all fibers, each of which display a small linear elastic region with similar slopes, followed by a period of strain hardening that ends in fiber failure. Comparison of the diameter, yield stress, and break stress of the four fiber types revealed that all were similar within measurement error. The tensile strengths are low (9±2 MPa) when compared to the higher tensile strength (237±26 MPa) measured for chitin fibers prepared from IL solutions of chitin which was first extracted, reconstituted and then re-dissolved before spinning.
The brittleness previously observed while drying the fibers was confirmed through the similarities in the values for the break stress and yield stress and is most likely attributed to the significant amount of CaCO$_3$ present within the fibers. This is supported by the low values for the determined Young’s modulus (Table 1), which are three orders of magnitude lower than the fibers prepared from the redissolution of regenerated chitin.

![Figure 4.6 Stress–strain curves for SS (black), DA (blue), CN (pink), and AO (red) fibers.](image)

Though the fibers prepared here display significantly weaker tensile strengths, the values suggest that surface modification does not seem to significantly alter the strength and integrity of the bulk chitin fiber. The data suggest that even though the method used here might produce the most cost effective fibers for bulk use, it is likely that the intended application involving deployment in the sea would require much stronger fibers. If this indeed is the case, the extracted chitin could be easily purified by first reconstituting it after extraction followed by redissolution of the now purified chitin material prior to spinning.
4.3.4 Uranium Extraction

Each of the four types of fibers was tested for their ability to remove UO$_2$Cl$_2$ from very dilute aqueous solution. Dry weight distribution ratios were determined radiometrically at 25(1) °C by batch contacts of ca. 2.5 mg of each fiber with 1 mL of DI water spiked with ca. 0.007 µCi of $^{233}$UO$_2$Cl$_2$ and shaken for 144 h. Aliquots of the solution were taken for counting at certain intervals to compare uptake kinetics, and dry weight distribution ratios ($D_w$) for each fiber were calculated from the change in activity at 144 h using eq. 1.

The differences in the $D_w$ values (Fig. 4.7) at 144 h support surface functionalization by showing that each treatment affected uranium uptake. The AO fibers show the highest affinity for UO$_2^{2+}$, commensurate with the known affinity of the amidoxime functional group for aqueous uranyl ions. The distribution ratios for the other fibers correlate with the hardness of the coordinating/functional group as a Lewis base: SS (amide) > CN (nitrile) > DA (amine).
Figure 4.7 $D_n$ values for extraction of $^{233}$UO$_2$Cl$_2$ from water by SS (black), DA (blue), CN (pink), and AO (red) fibers.

4.4 Conclusions

By exploiting the insolubility of natural chitin, we have developed a platform for surface modification of chitin materials. Building upon traditional methods for deacetylation, which provides access to the primary amine, we have modified the surface of chitin fibers with a functional extractant (here, amidoxime for the extraction of uranium from seawater), leaving an inner core of chitin that represents the bulk material. The complete compositional analysis and physical properties suggest that surface modification was successful in imparting chemical functionality without significantly altering the bulk properties of the material.

We believe the overall platform demonstrated here (i.e., materials with the internal properties of chitin, but the surface functionality of chitosan) has broad application in separations and environmental remediation. However a deeper understanding of residual CaCO$_3$ and proteins
is warranted based on the TGA and IR spectra of the bulk material. Subsequent efforts in this work are targeted at chemical reservoirs in waste shrimp shell to provide insight on how the bulk material can be adapted through simple chemical manipulation.

4.5 Acknowledgments

The authors would like to thank the DOE Office of Nuclear Energy Nuclear Energy University Programs (Sub-Contract – #120427, Project – #3123) for support of this work. We also thank Rob Holler and The University of Alabama Central Analytical Facility for the collection of the XPS data.

4.6 References


5 BIOMIMETIC MINERALIZATION OF URANIUM BY METABOLICALLY INACTIVE SHRIMP SHELL


5.1 Introduction

The previous chapters in this work have demonstrated chitin as a versatile polymer backbone for fiber applications for U recovery. But potential variability in other chemical components that remain in the processed shell waste are key variables for chemical and engineering applications. Therefore we chose to evaluate waste shrimp shells without perturbation of the inherent organic-inorganic matrix to provide not only a template, but an active reaction environment for assembly of inorganic crystalline phases. Although nature does not use heavy metals for structural design, biominerals such as fishbone apatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\), can be employed to immobilize heavy metal cations such as lead(II) (\(\text{Pb}^{2+}\)) and uranyl (\(\text{UO}_2^{2+}\)) as insoluble phosphates.\(^1\) However, this process is dominated by surface complexation on the mesoporous bone structure, and secondary mineral phases are limited to precipitation in localized surface regions of saturation.\(^2\) In contrast to a mineralized surface such as hydroxyapatite, the inorganic phase of shrimp shell contains labile amorphous calcium carbonate (ACC) and thus provides a convenient spatial template for ion flux upon removal of \(\text{Ca}^{2+}\) and infiltration of externally loaded ions into the shell framework. In our study, \(\text{UO}_2^{2+}\) is advantageous as it is easily distinguished from background ions in microscopy due to high electron density and biological non-occurrence.
5.2 Experimental

5.2.1 Chemicals

CAUTION! Hydroxylamine can become explosive if heated and/or concentrated above 50 wt%. Please refer to Material Safety Data Sheet (MSDS) for further details (CAS # 7803-49-8). All reagents were used as obtained. Acetic acid (CH₃COOH) was purchased from VWR (Radnor, PA, USA) and manufactured by BDH Merck Ltd. (Poole Dorset, UK). Hydroxylamine was purchased from Alfa Aesar (Ward Hill, MA). All solvents were ‘solvent grade’ and used as received without additional purification. Deionized (DI) water was acquired from an in-house system Culligan Water Systems (Rosemont, IL) with a typical resistivity of 17.4 MΩ·cm (resistivity not measured during these experiments).

5.2.2 Chitinous Biomass Source

Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, AL where the chitinous biomass was dried at a specialized facility by first pressing with a screw press to eliminate the majority of the water, followed by heating at up to 160 ºC in a fluidized bed dryer until the material had a final moisture content of less than 5 wt%. The material received from the drying facility was ground using an IKA Works Universal Grinding Mill (Wilmington, NC) and sieved to give a powder with a particle size of <125 µm. Ground shrimp shells were further dried in a Precision Scientific Econotherm Laboratory Oven, Model 1025 (Winchester, VA) at 80 ºC for 24 h.

5.2.3 Surface Area Determination

Surface area was determined utilizing the Quanta chrome Instruments high speed Nova e-series 3200 BET (Brunauer, Emmett, and Teller) surface area and pore analyzer (Boynton Beach,
Florida) with nitrogen adsorption. The BET analysis determined specific surface area of bone samples in the powder form through the process of gas sorption. Results provide critical data for understanding the way surface area of samples react and change over the treatments conditions. Samples (n=3) were degassed for a minimum of 5 hours at 105°C under vacuum flow prior to analysis; results are expressed in meter square per gram (m²/g).

5.2.4 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

Total metal and dissolved metal concentrations were determined by SW-846 Method 6010B (USEPA, 1999) for inductively coupled plasma atomic absorption spectrometry (ICP-AES) on a Perkin-Elmer Optima 4300 Dual View (Waltham, MA).

5.2.5 Synthesis of Protic Ionic Liquid

Hydroxylammonium acetate ([NH₃OH][CH₃COO]): 276.7 g (8.376 mol) NH₂OH, as a 50% (w/w) solution of NH₂OH in water (total solution mass = 553.3 g) was cooled and stirred with magnetic stirring in a 1 L round-bottom flask submersed in a ice bath. To the stirring solution was added 528.2 g (8.795 mol, 1.05 eq.) CH₃COOH dropwise over the course of several hours. Upon the completed addition, the mixture was stirred overnight and allowed to warm to room temperature. The mixture was heated to 40 °C with stirring while being sparged with air to evaporate the water. Upon concentration of the solution, large amounts of a white crystalline solid precipitated. The solid was filtered, washed with a small amount of cold methanol and diethyl ether to remove any residual acetic acid, and dried under reduced pressure for 1 h. 619.243 g white solid remained (Yield = 79.5%). ¹H NMR (360 MHz, DMSO-d₆) δ 8.58 (s, 4H), 1.86 (s, 3H). ¹H NMR (360 MHz, D₂O) δ 1.68 (s, 3H). ¹³C NMR (360 MHz, DMSO-d₆) δ 175.3, 22.7.
5.2.6 Uranium Leaching from Soil

The Geotechnical and Structures Laboratory (GSL) at the ERDC-Vicksburg site performed sieve analysis tests used to evaluate the physical structure of the soils and provide the soil classification. Brown (SP) soil (53% sand, 35% gravel) with highly visible U(VI) oxy-hydroxides was obtained from Yuma Proving Ground (YPG) (Yuma, AZ) as part of Environmental Security Technology Certification Program ESTCP ER-201321. PXRD confirmed metaschoepite as being a major mineral phase. Soil was initially physically separated to exclude ≥ 4.76 mm size fractions. The soil was then ground at 650 rpm for 10 minutes in a Fritsch Pulverisette (Idar-Oberstein, Germany) in order to achieve particle size reduction as a means to ensure representative sub-samples. The ground soil was poured into a plastic bag and homogenized by kneading by hand vigorously for 2 minutes. After mixing, the total concentration of uranium in soil was determined to be 9330 mg/kg. 10g of soil were then placed in 100 mL of a buffered acidic leach solution of 0.3 M [NH$_3$OH][CH$_3$COO] in 4.16 M acetic acid (pH = 2.61) in a 250 mL Nalgene sample bottle and agitated for 1 h on a Eberbach reciprocal shaker (Ann Arbor, MI) table at low speed and allowed to equilibrate for 24 h. This produced an aqueous solution uranium concentration of 1420 mg/L U(VI) (pH=3.46).

5.2.7 Reaction of U with Shrimp Shell

The buffered uranium leach solution (1420 mg/L) (pH=3.46) was used as the solution chemistry to passively supply ions to the dried and ground shrimp shell. To simulate exogenous uptake, 7 g of dried shrimp shell was placed in 500 mL of the buffered uranium leach solution for 1 h at ambient temperature and pressure in a 1 L Nalgene sample bottle. A sub-sample was removed, filtered with a 0.45-μm syringe filter, and analyzed by ICP-AES. The relationship of
the uranium concentration remaining in the aqueous solution was then calculated with respect to
the mass of the shrimp shell in grams (g) of uranium per kilogram (kg) of shrimp shell.

5.2.8 Nuclear Magnetic Resonance (NMR)

The $^1$H, $^{13}$C, $^{31}$P NMR spectra were recorded using a Bruker AV-500 (Karlsruhe, Germany) spectrometer operating at 500, 125 MHz, and 202.5 MHz, respectively. For $^{31}$P NMR, the sample was prepared by mixing 0.145 g dried ground shrimp shell with 7.2 g [NH3OH][CH3COO] in a round-bottom flask and heating to 100 °C for 1 h. The supernatant was separated through centrifugation and dissolved in deuterium oxide. $^{31}$P NMR (D$_2$O, 202.5 MHz): δ 0.9 (s).

5.2.9 Powder X-Ray Diffraction (P-XRD)

Data were collected on a Bruker (Madison, WI) D2 Phaser powder X-ray diffractometer with Ni-filtered Cu Kα radiation.

5.2.10 Synchrotron-based micro-X-ray Diffraction (µ-XRD)

Synchrotron-based micro-X-ray Diffraction (µ-XRD) was performed at beamline X27A at the National Synchrotron Light Source at Brookhaven National Laboratory in Upton, NY. XRD data was collected using Bruker SMART 1500 CCD area detector at 0.7093λ (Mo-Kα). Aluminum oxide ($\alpha$-Al$_2$O$_3$) and silver behenate (AgC$_{22}$H$_{43}$O$_2$) were used as calibration standards. The beam size on the sample was approximately 7 μm x 10 μm using Rh-coated Kirkpatrick-Baez focusing optics. X-rays were selected using a water-cooled channel-cut Si(111) monochromator. Uramphite spectra in the XRD diagram was calculated using the crystallographic information file (.cif) for that mineral using Materials Data, Inc (MDI) (Livermore, CA)-Jade 2010, XRD processing software package.
5.2.11 Scanning Electron Microscopy (SEM)

High-resolution imaging was performed using an FEI Nova Nano SEM 630 field emission environmental scanning electron microscope (Hillsboro, OR). All images were obtained in low vacuum mode (pressure of 0.3 to 0.5 mbar) to minimize charging and the need for applying conductive coatings for imaging. All images were obtained using a backscattered electron detector to improve phase contrast between the lower molecular weight organic material biomineral phases and the higher molecular weight uranium rich phases. Samples for SEM imaging were prepared by depositing approximately 25 mg of material onto carbon tape affixed to an SEM stub. Following deposition, the loose material was removed by lightly dusting with compressed zero air.

5.2.12 Energy Dispersive X-Ray Spectrometer (EDS)

Chemical analysis was performed using an integral Bruker Quantax AXS energy-dispersive x-ray spectrometer (EDS) system (Berlin, Gemany). Analysis of specific objects of interest was performed along with elemental mapping to observed the spatial distribution in elements and, in particular, the distribution in uranium rich phases (i.e., were they just a uniform coating or were they present as discrete particles).

5.3 Results and Discussion

5.3.1 Solid Phase Characterization of Chitinous Biomass

Dried shrimp shells were obtained from a drying facility operated by the Gulf Coast Agricultural and Seafood Cooperative (Bayou Le Batre, AL) where the shell was dried using a screw press, followed by heating (at up to 160 °C) in a fluidized bed dryer, and subsequently ground to a particle size of < 125 µm. The measured surface area of the ground shell was
determined to be 1.59 m$^2$/g, which is low compared to the 92.3 m$^2$/g that can be achieved with mesoporous fish bones upon collagen removal by chemical treatment. This suggests that if surface dominated mechanisms associated with apatite occur with shrimp shell, the capacity will be much lower.

Scanning Electron Microscopy (SEM) analysis revealed that the processed shell retains its hierarchical structure. It is apparent that grinding and fracturing of the exoskeleton exposes pore canals associated with the 3-D ion transport framework, and yet the integral layers of the epicuticle, exocuticle, and endocuticle remain discrete (Figure 5.1). This suggests that grinding may enhance access to chemical reservoirs without compromising the spatial orientation.

Figure 5.1 SEM images of the waste shrimp shell: a) top view of pore canals associated with ion transport; b) side view of epicuticle, exocuticle, and endocuticle associated with amorphous
calcium carbonate (ACC), chitin, and molecularly dispersed PO$_4^{3-}$; c) chemical components of the cuticle.

### 5.3.2 Energy-dispersive x-ray Spectrometer (EDS)

With confirmation that the multiphase structure remained intact, we confirmed the presence of a phosphorous-rich region co-located with calcium using Energy Dispersive Spectroscopy (EDS) (Figure 5.2). This suggests small molecular weight glycolytic intermediate residues such as PEP, 3PG, and SerP remain associated with Ca$^{2+}$ in the exocuticle and could provide a source biogenic phosphate esters.$^4$ Although not expected to function metabolically as part of the non-living carapace, the presence of molecular energy and material transfer in the biological scaffold is a major biosynthetic and kinetic advantage for crystal growth. Fixed amino acid residues can lower activation energy by providing a template for increased collision probability.$^5$ Thus the only remaining requirement for manipulation of this system is the solution chemistry to passively diffuse ions into the structural confinement of the shell interior.

![Figure 5.2](image)

Figure 5.2 a) Side view SEM of exocuticle with Ca (red) and P (blue) above non-calcified tissues and b) EDS showing co-located Ca and P.
**5.3.3 Reaction of Shrimp Shell with U**

Based upon our characterization of the biogenic phosphate source of the ground shrimp shell and previously determined chemical and kinetic constraints for apatite as sorbents for uranyl (UO$_2^{2+}$) ions, we postulated that the CaCO$_3$ in the shrimp shell matrix could act as an *in situ* template upon exposure to an acidified and chelated UO$_2^{2+}$ ion precursor, releasing calcium and providing the UO$_2^{2+}$ ions rapid access to phosphate (PO$_4^{3-}$) for sequestration within the composite biomaterial. Uranyl minerals formed as secondary phases can be important sinks for uranium in the environment, with uranyl phosphate mineral phases being particularly useful for controlling uranium mobility in the environment due to their extremely low solubility.

Therefore, the underlying hypothesis was that upon removal of the labile ACC into solution, the pre-organized architecture of the shell should open, offering space for assembly of crystalline uranyl phosphate. However, we expected that carefully moderated chemical conditions would be required to promote dissolution of the inorganic phase without further degradation of biomolecular residues.

Mann *et al*. 2009 reported the ionic liquid (IL; salts that are liquid at temperatures below 100 °C) ethanolammonium formate (EtAF) stabilized proteins and enhanced enzymatic activity through favorable hydrogen bond interactions with the alcohol group on the cation. We realized that common selective leaching solutions consisting of acetic acid (CH$_3$COOH) and hydroxylamine (NH$_2$OH) employed to extract oxides such as schoepite ((UO$_2$)$_8$O$_2$(OH)$_{12}$·12(H$_2$O)) from contaminated soil sources, could be modified to produce a protic ionic liquid (PIL), hydroxylammonium acetate, [NH$_3$OH][CH$_3$COO], similar in composition to EtAF. We expected dissolution of [NH$_3$OH][CH$_3$COO] in acetic acid would provide a buffered solution which could maintain acidity during the dissolution of CaCO$_3$ and
release of $\text{CO}_3^{2-}$ while providing acetate ions to chelate, solubilize, and allow electroneutral transport of $\text{UO}_2^+$ to the shell substrate.$^{10}$

To test our proposed mechanism, a solution of $[\text{NH}_3\text{OH}][\text{CH}_3\text{COO}]$ in acetic acid (pH = 2.61) was used to dissolve schoepite. The resulting acidic $\text{UO}_2^{2+}$ solution at pH 3.46 was exposed to the dried, ground shrimp shell to simulate exogenous uptake from the external environment in a batch reactor at ambient temperature. The solubility of the inorganic fraction was immediately evident with the release of $\text{CO}_2(g)$ bubbles for approximately 5 min and the release of $\text{Ca}^{2+}$ as measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). (Sugawara, et al. reported similar rapid reaction rates in real time observations of crystalline $\text{CaCO}_3$ formation in the presence of calcification-associated peptide (CAP-1).$^{11}$) After visible release of $\text{CO}_2$ stopped, the acidic supernatant (pH 3.78) was analyzed for $\text{UO}_2^{2+}$ removal from solution, and solids were removed and dried for solid state characterization. $^{31}\text{P}$-NMR spectra of the crude extract obtained from decalcification of the shrimp shell was found to be consistent with the release of phosphoric acid esters.

5.3.4 PXRD confirmation of Secondary Crystalline Phase

Powder X-ray diffraction (PXRD) analysis of the reacted shell confirmed both the removal of the $\text{CaCO}_3$ (calcite) peaks and additional sharp peaks confirming the presence of a new highly crystalline phase. Despite having a very low measured BET surface area (1.59 $\text{m}^2/\text{g}$), the shrimp shell had a substantial $\text{UO}_2^{2+}$ uptake capacity of 27 g/kg sorbent (compared to 34 g $\text{UO}_2^{2+}$/kg sorbent for treated mesoporous fish bones) suggesting diffusion of the solution into the shell interior in concert with the removal of the calcite mineral phase from the shell matrix.
5.3.1 Soil Collection and Chemical Extractions

Soils samples collected from the arid environment test site Yuma Proving Ground (YPG) were dominated with CaCO₃ with trace ferro-manganese oxide sinks. The source of U was DU garden soil in which intact penetrators were buried for >5 years at a depth of 12 in. Visible shoepite was observed in the soil profile from 22.9-30.5 cm and just below the penetrator from 33.5 to 38.1 cm. Sequential extractions of these 2 soil lifts from a previous study showed high U concentrations associated with the Carbonate fraction from 22.9 to 38.1 cm and with the Fe-Mn Oxide fraction below the penetrator (33.5 to 38.1 cm).¹² These results were consistent with dissolution of uranyl oxide hydrates and precipitation of authigenic associations with Fe (III)/Mn (IV) oxide sinks.¹³ Working with a previously well characterized soil system provided an opportunity to tune the leaching chemistry to dictate speciation and chelation modes. Thus we explored a simple organic acid scheme to provide controlled aqueous chemistry to liberate anthropogenic U(VI) and authigenic solid phases from environmentally relevant conditions and subsequently remove UO₂²⁺ from solution with a biogenic phosphate. To minimize the complexity observed in tri-carboxylic citric acid solutions, a mono-carboxylic acid based chemistry was explored to provide more predictive aqueous speciation in solution.

5.3.2 Solid State Characterization of U Reacted Solids

Further analysis of the material with SEM provided more evidence of crystallization. Square tabular crystals with a very high contrast compared to the substrate were found originating from the shell interior (Figure.5.3). EDS provided a quantitative analysis of this new crystalline material indicating a composition of 48.6% U, 21.3% O, 6.45% Ca, and 5.8% P.
Figure 5.3 a) Crystalline microstructures at 10,000X magnification; b) crystalline growth observed beneath the surface of the structure at 40,000X magnification; and c) tabular crystals at 60,000X magnification.

5.3.3 Synchrotron-based Micro-X-ray diffraction (µ-XRD)

To confirm the identity of the mineral phase, synchrotron-based micro-X-ray diffraction (µ-XRD) was performed on the material and compared to calculated patterns from the literature using the MDI-Jade software package (Figure 5.4). The experimental XRD pattern was identified as uramphite, \([\text{NH}_4][\text{UO}_2\text{PO}_4]\text{•}3\text{H}_2\text{O}\). These findings are consistent with the composition determined by EDS and indicate quick, spontaneous mineralization using only the shrimp shell and an ion delivery solution.
Figure 5.4 a) Shrimp shell with P-rich exocuticle; b) synchrotron-based micro-X-ray diffraction confirming uramphite; and c) SEM of crystalline uramphite formation concentrated in the region of the exocuticle.

These results show that the rapid mineralization of uranyl in shrimp shell is accessible through a biomimetic pathway using structures and chemicals which remain active in the shrimp shell matrix after the expiration of the organism and even processing of the shell. The rapid crystallization of $\text{UO}_2^{2+}$ in the form of uramphite occurred when the shrimp shell was exposed to a solution containing a protic ionic liquid which was able to supply the inorganic building block and meet the solution chemistry criteria for ion flux. The schematic of the overall reaction scheme with the amorphous precursor and crystalline product is shown in Figure 5.4. Not unexpectedly, the mineralization of $\text{UO}_2^{2+}$ into shrimp shell also shows differences compared to the mineralization of $\text{Ca}^{2+}$. Previous $\text{Pb}^{2+}$ and $\text{Cd}^{2+}$ studies on shellfish waste have observed carbonate phases such as cerussite and otavite attributed to cation exchange with $\text{Ca}^{2+}$. However, in our system, phosphate is incorporated into the inorganic phase rather than carbonate, and the crystallites which form do not resemble the original calcite crystallites in size or shape. This
hints that the partial functionality of a biomineralization system, such as the one studied here, may also be a route to new inorganic compounds and structures when taken outside of its natural conditions. Specifically, we have demonstrated that metabolically inactive shrimp shell has the intrinsic ability to mineralize aqueous metal ions, including anthropogenic metals like uranium with rapid kinetics, in unsaturated conditions.

5.4 Conclusions

In this study, we have demonstrated the importance of a metabolically inactive system’s physical structure and chemical reservoirs in rapid, bottom-up self-assembly without life or active cellular control. We have developed a chemistry-based strategy for harnessing biomineralization functionality utilizing the key elements of a complex yet metabolically inactive biological system found in an abundant waste product and have further shown that it can be extended beyond elements used by nature. These results provide valuable insight on how biological systems in non-living organisms can be adapted through simple chemical manipulation. Furthermore, we have also shown that a natural biomineralization pathway has considerable, though modified, activity towards a non-essential ion. We anticipate this study will contribute to the understanding of biomineralization and provide a novel chemistry perspective for extending natural design principles towards technological applications with other metal cations of interest. Certainly in regard to uranium, this represents a new alternative to promote stable meta-autunite phases and provide a long term sink for one of the most abundant radionuclides released into the environment.

5.5 Acknowledgments

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6 CHITINOUS BIOMASS-MEDIATED PRECIPITATION AND REDUCTION OF U(VI): THE DUAL ROLE OF HYDROXILAMMONIUM ACETATE


6.1 Introduction

After investigating several aspects of making materials for uranium sequestration, the findings in the previous chapter provide a new motivation to use solution chemistry from Chapter 5 to target anthropogenic depleted uranium in the soil. The structural dependence of uranium complexed with organic ligands makes recovery from solution challenging, thus understanding the pH dependent chelation modes of UO$_2$$^{2+}$ with carboxylate functionalities is required for predicting potential migration and designing remediation strategies. We recently reported a novel protic ionic liquid (PIL) based approach for “tuning” U(VI) acetate leach chemistry and promotion of a thermodynamically stable secondary product phase as demonstrated by formation of the meta-autunite [NH$_4$][UO$_2$PO$_4$]$\cdot$3H$_2$O from an aqueous acidic leach solution derived from an environmental shoeepite ((UO$_2$)$_8$O$_2$(OH)$_{12}$$\cdot$12(H$_2$O)) upon contact with waste shrimp shell.$^1$ The protic ionic liquid (PIL), hydroxylammonium acetate (HAOac) buffered in acetic acid, can serve as both a chelating ligand and acidic media to liberate calcite providing access to biogenic phosphates in the chitinous biomass. Although biomass has been proposed for remediation and recovery of uranium from solution, surface mediated mechanisms commonly require chemically intensive treatment to enhance surface interactions.$^2$ However chitinous biomass from waste crustacean shells is intrinsically well suited as a solid interface for the formation of U(VI) crystalline phases from a chemistry perspective based on labile CaCO$_3$
and controlled access to phospholipid components of waste crustacean shells on the chitin scaffold.

For any sequestration-based remediation strategy to be successful, detailed chemical information connecting the parent phase at the source to the desired product endpoint is required. Hence the objectives of this study were to first evaluate amphiptotic HAOac as a ligand for dissolution of U(VI) oxide hydrates with predictive, bidentate, CN 6, \((\text{UO}_2\text{OAc}_3^-)\) aqueous speciation in solution. Secondly, removal of \(\text{UO}_2^{2+}\) from solution with chitinous biomass was benchmarked by comparison of \(\text{PO}_4^{3-}\) bearing minerals such as fish-bone apatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\) with respect to formation of secondary phosphate phases indicative of long stability.

### 6.2 Experimental

#### 6.2.1 Chemicals

**CAUTION!** Hydroxylamine can become explosive if heated and/or concentrated above 50 wt%. Please refer to Material Safety Data Sheet (MSDS) for further details (CAS # 7803-49-8). All reagents were used as obtained. Acetic acid (CH\(_3\)COOH) was purchased from VWR (Radnor, PA, USA) and manufactured by BDH Merck Ltd. (Poole Dorset, UK). Hydroxylamine was purchased from Alfa Aesar (Ward Hill, MA). Uranyl oxide (UO\(_3\)) was obtained from Strem Chemicals, Inc. (Boston, MA) and uranyl carbonate (UO\(_2\)CO\(_3\)) was purchased from International Bio-Analytical Industries Labs, Inc. (Boca Raton, FL). All solvents were ‘solvent grade’ and used as received without additional purification. Deionized (DI) water was acquired from an in-house system Culligan Water Systems(Rosemont, IL) with a typical resistivity of 17.4 M\(\Omega\)·cm (resistivity not measured during these experiments).
6.2.2 Chitinous Biomass Source

Dried shrimp shells were received from the Gulf Coast Agricultural and Seafood Cooperative in Bayou La Batre, AL where the chitinous biomass was dried at a specialized facility by first pressing with a screw press to eliminate the majority of the water, followed by heating at up to 160 °C in a fluidized bed dryer until the material had a final moisture content of less than 5 wt%. The material received from the drying facility was ground using an IKA Works Universal Grinding Mill (Wilmington, NC) and sieved to give a powder with a particle size of <125 µm. Ground shrimp shells were further dried in a Precision Scientific Econotherm Laboratory Oven, Model 1025 (Winchester, VA) at 80 °C for 24 h.

6.2.3 Synthesis of Protic Ionic Liquid

Hydroxylammonium acetate ([NH$_2$OH][CH$_3$COO]): 276.7 g (8.376 mol) NH$_2$OH, as a 50% (w/w) solution of NH$_2$OH in water (total solution mass = 553.3 g) was cooled and stirred with magnetic stirring in a 1 L round-bottom flask submersed in a ice bath. To the stirring solution was added 528.2 g (8.795 mol, 1.05 eq.) CH$_3$COOH dropwise over the course of several hours. Upon the completed addition, the mixture was stirred overnight and allowed to warm to room temperature. The mixture was heated to 40 °C with stirring while being sparged with air to evaporate the water. Upon concentration of the solution, large amounts of a white crystalline solid precipitated. The solid was filtered, washed with a small amount of cold methanol and diethyl ether to remove any residual acetic acid, and dried under reduced pressure for 1 h. 619.243 g white solid remained (Yield = 79.5%). $^1$H NMR (360 MHz, DMSO-d$_6$) δ 8.58 (s, 4H), 1.86 (s, 3H). $^1$H NMR (360 MHz, D$_2$O) δ 1.68 (s, 3H). $^{13}$C NMR (360 MHz, DMSO-d$_6$) δ 175.3, 22.7.
6.2.4 Soil Samples

The Geotechnical and Structures Laboratory (GSL) at the ERDC-Vicksburg site performed sieve analysis tests used to evaluate the physical structure of the soils and provide the soil classification. Brown (SP) soil (53% sand, 35% gravel) with highly visible U(VI) oxy-hydroxides was obtained from Yuma Proving Ground (YPG) (Yuma, AZ) as part of Environmental Security Technology Certification Program ESTCP ER-201321. PXRD confirmed metaschoepite as being a major mineral phase. Soil was initially physically separated to exclude ≥ 4.76 mm size fractions. The soil was then ground at 650 rpm for 10 minutes in a Fritsch Pulverisette (Idar-Oberstein, Germany) in order to achieve particle size reduction as a means to ensure representative sub-samples. The ground soil was poured into a plastic bag and homogenized by kneading by hand vigorously for 2 minutes. After mixing, the total concentration of uranium in soil was determined to be 9330 mg/kg.

6.2.5 U Leaching from Soil

10g of soil were then placed in 100 mL of a buffered acidic leach solution of 0.3 M [NH₃OH][CH₃COO] in 4.16 M acetic acid (pH = 2.61) in a 250 mL Nalgene sample bottle and agitated for 1 h on a Eberbach reciprocal shaker (Ann Arbor, MI) table at low speed and allowed to equilibrate for 24 h. This produced an aqueous solution uranium concentration of 1420 mg/L U(VI) (pH=3.46).

6.2.6 Reaction of Uranium with Solid Phases

The buffered uranium leach solution (1420 mg/L) (pH=3.46) was used as the solution chemistry to passively supply ions to the dried and ground shrimp shell. To simulate exogenous uptake, 7 g of dried shrimp shell was placed in 500 mL of the buffered uranium leach solution for 1 h at ambient temperature and pressure in a 1 L Nalgene sample bottle. A sub-sample was
removed, filtered with a 0.45-µm syringe filter, and analyzed by ICP-AES. The relationship of the uranium concentration remaining in the aqueous solution was then calculated with respect to the mass of the shrimp shell in grams (g) of uranium per kilogram (kg) of shrimp shell.

6.2.7 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

Total metal and dissolved metal concentrations were determined by SW-846 Method 6010B (USEPA, 1999) for inductively coupled plasma atomic absorption spectrometry (ICP-AES) on a Perkin-Elmer Optima 4300 Dual View (Waltham, MA).

6.2.8 Powder X-ray Diffraction

Data were collected on a Bruker (Madison, WI) D2 Phaser powder X-ray diffractometer with Ni-filtered Cu Kα radiation.

6.2.9 Scanning Electron Microscopy (SEM)

High-resolution imaging was performed using an FEI Nova Nano SEM 630 field emission environmental scanning electron microscope (Hillsboro, OR). All images were obtained in low vacuum mode (pressure of 0.3 to 0.5 mbar) to minimize charging and the need for applying conductive coatings for imaging. All images were obtained using a backscattered electron detector to improve phase contrast between the lower molecular weight organic material biomineral phases and the higher molecular weight uranium rich phases. Samples for SEM imaging were prepared by depositing approximately 25 mg of material onto carbon tape affixed to an SEM stub. Following deposition, the loose material was removed by lightly dusting with compressed zero air.
6.2.10 Energy Dispersive X-Ray Spectrometer (EDS)

Chemical analysis was performed using an integral Bruker Quantax AXS energy-dispersive x-ray spectrometer (EDS) system (Berlin, Gemany). Analysis of specific objects of interest was performed along with elemental mapping to observed the spatial distribution in elements and, in particular, the distribution in uranium rich phases (i.e., were they just a uniform coating or were they present as discrete particles).

6.2.11 Single crystal X-ray diffraction.

Coordination chemistry of hydroxylammonium Acetate (HAOac) with uranium salts was characterized by its single crystal X-ray structure. The X-ray data was collected on a Bruker SMART diffractometer equipped with a CCD area detector using graphite monochromated Mo-Kα (λ = 0.71073 Å) radiation. A single crystal was mounted on a glass fiber and transferred to the goniometer for data collection. The crystal was cooled to -100 °C under a cold nitrogen gas stream. The structure was solved using the SHELXTL software package\(^3\) and the absorption corrections were made with SADABS.\(^4\) The structure was refined by full matrix least squares on \(F^2\).

6.2.12 X-ray Absorption Near Edge Structure (XANES)

Synchrotron-based micro-X-ray absorption near edge structure (XANES) spectroscopy was performed at beamline X27A, and bulk XANES data was collected at beamline X11A at the National Synchrotron Light Source at Brookhaven National Laboratory in Upton, NY. Known U minerals were analyzed and used as standards to aid in the identification of unknown U species. The beamlines were calibrated using a zirconium metal foil (Kα of 17998 eV). For μ-XRF and XANES analysis at X27A, samples were dried on mylar film or Kapton tape in order to establish
a single/thin layer on the film. The Mylar film was used to minimize the effects of beam-induced species reduction. Samples were prepared directly before arrival at the synchrotron facility. The beam size on the sample was approximately 7 µm x 10 µm using Rh-coated Kirkpatrick-Baez focusing optics. XANES data were collected using a Vortex ME4-multichannel spectrometer solid-state detector. The X-rays were selected using a water-cooled channel-cut Si(111) monochromator. Sample holders were sealed with Mylar film to minimize the effects of beam-induced species reduction. Samples were prepared directly before arrival at the synchrotron facility. The beam size on the sample was approximately 1 cm x 4 cm. XANES data were collected using a 13-element Ge detector. The X-rays were selected using a water-cooled double crystal monochromator with Si(111) crystals. All XANES data were analyzed using the X27A Plot and Athena (Newville, 2001) programs where appropriate. Linear combination fitting (LCF) was conducted from -20 to 30 eV around E0.

6.3 Results and Discussion

6.3.1 U(VI) Acetate Leaching Chemistry

Based on previous experimental and theoretical studies, the OAc ligand is a logical choice for leaching UO$_2^{2+}$ due to small steric demands and the solubility of U(VI) oxide hydrates in HOAc.$^5$ Investigations of the coordination of U(VI) with carboxylic acids have indicated pH dependent 1:3 U(VI) tris-acetate UO$_2$(CH$_3$COO)$_3$ at pH 3.5.$^6$ However, Fe and Mn oxides have high charge densities and thus are important sinks for UO$_2^{2+}$. Fe(III) and Mn(IV) can occlude UO$_2^{2+}$ limiting access to HOAc and therefore reductive dissolution is required to liberate UO$_2^{2+}$ from secondary mineral phases.$^7$

To eliminate the need for multi-stage chemical extractions to solubilize both the primary oxides and secondary co-precipitates we incorporated reductive dissolution with acetic acid
(CH$_3$COOH) with addition of hydroxylamine (NH$_2$OH) to produce the protic ionic liquid (PIL) hydroxylammonium acetate, [NH$_3$OH][CH$_3$COO] (Figure 6.1). We expected dissolution of [NH$_3$OH][CH$_3$COO] in acetic acid would provide a buffered solution which could maintain the required pH 3.5 to produce UO$_2$(CH$_3$COO)$_3^-$ during the dissolution of sedimentary CaCO$_3$ and release of CO$_3^{2-}$, provide OAc$^-$ to chelate UO$_2^{2+}$ from the soil, and access any occluded U from trivalent and tetravalent ferro-manganese nodules.

Figure 6.1 (a) Reaction of 1:1 molar ratios of NH$_2$OH and HOAc; (b) Proposed tris-acetato leaching system

Reaction of 1:1 molar ratios of NH$_2$OH and HOAc led to the formation of a crystalline [NH$_3$OH][CH$_3$COO] confirmed as a PIL (mp = 87 °C). The salt was then buffered to produce a solution consisting of 0.3M [NH$_3$OH] [OAc] in dilute 4M HOAc at pH 3.34. The solution was then used to leach UO$_2^{2+}$ from soil contaminated with highly visible U(VI) oxy-hydroxides was obtained from Yuma Proving Ground (YPG). The reaction of the tuned U(VI)-acetate system with the contaminated soil had a resulting pH of 3.46 and removed 90% of uranium by targeting U(VI) oxides and providing reductive dissolution of Fe (III) /Mn(IV) secondary phases in a single step extraction. To further evaluate the coordination chemistry and reaction mechanisms of [NH$_3$OH][CH$_3$COO] with environmental solid phases, solid state crystallography experiments were performed with commercially available salts U(VI) oxide (UO$_3$), and U(VI)
carbonate UO$_2$(CO$_3$). Single crystals produced from both species via slow evaporation indicated consistent UO$_2$OAc$_3$ ($R_{Ac}=3$) coordination chemistry with six O donors and bidendate chelation (Figure 6.2).

![Figure 6.2](image)

Figure 6.2 a) ([H$_3$O][UO$_2$(OAc)$_3$]) produced via slow evaporation with UO$_2$(CO$_3$), b) ([NH$_3$OH][UO$_2$(OAc)$_3$]) produced via slow evaporation with UO$_3$

Although the observed solid phases are not necessarily representative of the aqueous speciation, the experimental results support aforementioned density functional theory calculations (DFT) for pH 3.5 with an excess of OAc$.^9$ In addition, ultraviolet-visible (UV-vis) spectrum of the aqueous phase shows maxima at 445nm and 460nm consistent with UO$_2$OAc$_3$.^9 Thus we accomplished our initial research goal of enhanced extraction with 90% removal of UO$_2^{2+}$ and predictive aqueous speciation. However the utility of leaching is diminished if a secondary aqueous waste stream is generated, so our second research objective focused on developing materials from chitinous biomass for recovering UO$_2^{2+}$ from solution.

6.3.2 U(VI) Reacted Solids

Phosphates represent an important class of ligands for actinides due to their prominent role in separations and formation of insoluble actinide phosphate minerals.$^{10}$ Thus in aerobic near-surface environments phosphate barriers composed of materials such as hydroxyapatite (HAP) have been employed to immobilize U.$^{11}$ Fish-bone apatite [Ca$_{10}$(PO$_4$)$_6$(OH)$_2$] derived from fin-fish waste has been evaluated as a permeable reactive barrier (PRB) due to the
mesoporous structure and high surface areas that can be achieved through chemical pretreatment.\textsuperscript{12} The goal of this phosphate application is the removal of U from solution through precipitation of stable uranyl phosphates. However, environmental concentrations may be under saturated with respect to precipitation and reversible sorption mechanisms require long term monitoring.\textsuperscript{13} Chitinous biomass from shellfish waste represents an overlooked source of phosphate as the chemical reservoirs in crustacean shells contain 20-40\% protein with 6 to 8 million tonnes produced globally.\textsuperscript{14}

Based on our previous observation of biomimetic mineralization of U with shrimp shell we elected to further investigate mechanisms of removal from solution and formation of secondary phosphate phases with respect to U sequestration applications.\textsuperscript{1} Both biogenic phosphate sources were challenged with the acidic uranyl acetate solution leached from the YPG soil under saturated with respect to precipitation (1400 mg/L). Scanning Electron Microscopy-Energy Dispersive X-Ray Spectrometer (SEM-EDS) revealed uniform distribution of U on the fishbone apatite and localized regions of U associated with residual phosphate in the waste crustacean shell (Figure 6.3). Although both materials produce the desired net effect of U removal from solution, clearly the apatite is dominated by surface phenomena, yet it is postulated that waste crustacean has microenvironments in which P is present at higher concentrations relative to the bulk material.
In agreement with previous HAP studies, no secondary crystalline phases were detected in apatite with sorbed U(VI) from aqueous solution of 1400 mg/L by Powder X-ray Diffraction (PXRD). In contrast, PXRD of the U(VI) reacted shrimps shell indicated the formation of secondary crystalline phases from contact of DU leaching solution with shrimp shells (Figure 6.4).
To determine the influence of other potential soil constituents leach from the YPG soil, UO$_2$CO$_3$ was spiked into the leaching solution and reacted with the shrimp shell. Similar crystalline peaks were observed indicating other dissolved particles from soil are not part of the spontaneous crystallization. The increases in crystallinity observed in the shrimp shell upon removal of calcite in the absence of U do not match the patterns observed in the U(VI) reacted solids and are attributed to chitin post demineralization.

### 6.4 XANES Speciation

X-ray Absorption Near Edge Structure (XANES) was performed to determine U oxidation state in the solid phase. Although not unprecedented, speciation showed mixed valence states U(VI) and U(IV) on the shrimp shell indicating that reduction had occurred on the solid phase as well as precipitation of a secondary crystalline phase (Figure 6.5). Kushwaha et al. 2012
reported mixed valence states in reduction coupled with adsorption of UO$_2^{2+}$ from acidic solutions on palm-shell agrowaste.$^{15}$

Bulk XANES data show that the majority (77%) of the U species in the shrimp shell is reduced uranium, U(IV). The $\mu$-XANES data shows that some spots or locations within the shrimp shell are mostly U(IV), whereas some spots are a mixture of U(IV)-U(VI) with an overall average of 72% U(IV).

In other proposed biomass sorbents such as alfalfa, carboxyl functionalities have been indicated as the primary functional group responsible for binding UO$_2^{2+}$. However carboxylic acids are also known to lower interfacial barriers for biomineralization, as well as energetic
barriers to reduction. Therefore this engineered pathway for immobilization of U with chitinous biomass exhibits dual functionality with both precipitation of secondary U(VI) crystalline phase as a minority phase and a reduced non-crystalline U(IV) at the solution-solid interface attributed to adsorption coupled reduction or association with carboxylates on the solid phase.\textsuperscript{17}

\textbf{6.5 Conclusions}

Although further research is required to assess the long term stability, waste crustacean shells are capable of producing the two desired transformations associated with U remediation, a crystalline secondary uranyl phosphate and reduction to U(IV). Based on the previously identified precipitation of uramphite $[\text{NH}_4][\text{UO}_2\text{PO}_4]\cdot3\text{H}_2\text{O}$, ligand exchange from $\text{OH}^-$ to $\text{PO}_4^{3-}$ reduces solubility by 30 orders of magnitude (log $K_{sp}$ 5.52 to -26.50). However the XANES speciation indicates that reduction is occurring in the solid phase which suggests that a U(IV) mineralogical endpoint such as $[\text{CaU(PO}_4)_2\cdot\text{H}_2\text{O}]$ may also be forming. Although the mechanism is yet to be determined, the dual functionality for \textit{in-situ} stabilization of U from waste crustacean shells is an effective strategy for removing U from solution. Furthermore, using a true waste product with 6 to 8 million pounds produced globally represents a functional biomaterial that does not require land use for biomass growth or extensive chemical pre-treatment.

\textbf{6.6 Acknowledgments}

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Laboratory. X27A is supported in part by the U.S. Department of Energy (DOE) - Geosciences (DE-FG02-92ER14244 to The University of Chicago - CARS). Use of the NSLS was supported by the DOE, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02- 98CH10886.

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7 CONCLUSIONS AND FUTURE WORK

Both aprotic and protic ILs present a wide range of opportunities to overcome challenges in developing chitinous materials. Ionic liquids such as [C$_2$C$_1$mim][OAc] offer unprecedented control of chitinous biomass enabling material applications for advanced fibers. Through a rationally designed series of research objectives we were able to report the first instance of electrospinning chitin fibers from directly from biomass in ILs, develop a recycling strategy for the IL with the alternative solvent sc-CO$_2$, and demonstrate surface modification of chitin fibers for enhanced recovery of uranium. In addition the rediscovery of the simple but understudied salt [NH$_3$OH][CH$_3$COO] not only demonstrates OAc$^-$ is an excellent ligand for U(VI)coordination, but its protic ionic liquid properties suggest a much broader application space.

Through our exploration of the structural and chemical reservoirs in waste shrimp shell we provided valuable insight on how biological systems in non-living organisms can be adapted through simple chemical manipulation. Specifically, we have demonstrated that metabolically inactive shrimp shell has the intrinsic ability to mineralize aqueous metal ions, including anthropogenic metals like uranium with rapid kinetics, in unsaturated conditions. We anticipate this discovery will contribute to the understanding of biomineralization and provide a novel chemistry perspective for extending natural design principles towards technological applications with other metal cations of interest. This represents a new alternative to promote stable secondary U(VI) phosphate U(VI) and insoluble U(IV) phases, providing an effective strategy for immobilizing U.
Future work is warranted to understand the reduction mechanism of the U(IV) majority phase and the P concentrations in the microenvironments resulting in the crystalline U(VI) minority phase. Specific future objectives include probing the influence of pH in the proposed adsorption-coupled reduction using carboxylic acid chemistry to elucidate the adsorption mechanism and identity of the U(IV) phase through XPS and EXAFS techniques. In addition, quantitative relationships of U and P in specific regions of the shell matrix need to be determined via EDS to understand the precipitation of the U(VI) minority phase uranphite.