"SAFE ALTERNATIVE SOLVENTS FOR ANTIBIOTICS EXTRACTION"

Final Progress Report

on

Center for Disease Control and Prevention Phase I
Grant No. 1 R43 OH07466-01

September 30, 2001 - March 31, 2002
Extended through September 30, 2002

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July 19, 2002

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LIST OF ABBREVIATIONS

^{1}H NMR	proton nuclear magnetic resonance spectroscopy
BMIM	1-*n*-butyl-3-methylimidazolium cation
Bu-Py	1-*n*-butylpyridinium cation
Cl^-	chloride
HCl	hydrochloric acid
HMIM	1-*n*-hexyl-3-methylimidazolium cation
imide	bis(trifluoromethanesulfonyl)imide anion
IR	infrared spectroscopy
KOAc	potassium acetate
MIBK	methylisobutylketone
NaOH	sodium hydroxide
Oct-Py	1-*n*-octylpyridinium cation
OMIM	1-*n*-octyl-3-methylimidazolium cation
PEEK	polyetheretherketone
PF_6	hexafluorophosphate anion
RTIL	room temperature ionic liquid
UV-Vis	ultraviolet-visible spectroscopy
VOC	volatile organic compound

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ABSTRACT

This research resulted in identification of non-volatile, non-flammable room temperature ionic liquids (RTIL's) to replace the toxic, flammable, volatile organic compound (VOC) solvents now used for extraction of antibiotics from fermentation broths. The solvents used for penicillin extraction include methyl isobutyl ketone, amyl acetate, butyl acetate, and chloroform. These solvents are toxic, irritants, volatile, and/or extremely flammable. RTIL's have attracted significant attention for green chemistry applications. They are ionic compounds that have very large liquidus ranges. Some are air and water stable and immiscible with water. They solubilize aromatics and carbonyl-containing compounds particularly well, and were found to be effective for penicillin-G extraction. RTIL's have previously been studied for liquid-liquid extraction and other separations. Being non-volatile, they are recyclable, and their large liquidus ranges allow efficient separations using temperature control. They are non-flammable, and are "tunable" solvents, as the cations can be substituted and/or paired with different anions to manipulate the properties of the liquids and tailor them for specific applications. The use of RTIL's instead of the toxic, flammable, VOC solvents now used would improve the health and safety of pharmaceutical workers, and reduce costs, since they are straightforward to synthesize. New processes or equipment would not be required.

SPECIFIC PHASE I AIMS

Engineering controls, used for managing worker exposure to occupational hazards, include the substitution of safer materials for hazardous ones. The proposed research has resulted in identification of non-volatile, non-flammable room temperature ionic liquid (RTIL) alternative solvents to replace the toxic and flammable volatile organic compound (VOC) solvents now used for extraction of penicillin and other antibiotics from their fermentation broths. The structure of the potassium salt of penicillin-G is shown in Figure 1. The solvents now commonly used for penicillin extraction include methyl isobutyl ketone (MIBK), amyl acetate, butyl acetate, and chloroform.[1] These solvents are toxic, irritants, volatile, and/or extremely flammable. RTIL solvents have attracted significant attention recently for green chemistry applications.[2] They are purely ionic compounds that have very large liquidus ranges and are non-volatile. Some are air and water stable and immiscible with water. Being ionic, they are very polar, and have substituted well for dipolar aprotic solvents in synthesis applications, even resulting in improved yields and selectivities.[3,4] MIBK is a dipolar, aprotic solvent. RTIL's have also been shown to solubilize aromatics and conjugated olefins particularly well,[5,6] as well as carbonyl compounds.[7,8] Penicillin-G and many antibiotics have aromatic and carbonyl functional groups and many have additional conjugated double bonds as well. RTIL's have already shown promise as solvents for liquid-liquid extraction[2b,5] and other types of separations.[6] Strong coulombic forces cause them to have no measurable vapor pressure, so a significant drawback to the use of molecular VOC solvents, loss of solvent by evaporation, is eliminated. Due to their lack of volatility they are recyclable, and their very large liquidus ranges allow efficient separations using temperature control. They are totally non-flammable and many are minimally toxic. They are "tunable" solvents, as the cations can be substituted and/or paired with different anions to manipulate the properties of the liquids and tailor them for specific applications. The use of RTIL's in place of the toxic, flammable, VOC solvents now used for extraction of antibiotics would improve the health and safety of pharmaceutical workers and those transporting the chemicals, as well as reducing costs, since many of these liquids are straightforward and relatively inexpensive to synthesize, and are recyclable. Their use would not require different processes or equipment from those currently in use.

The specific aims of the Phase I research included synthesis of four RTIL's, their use in extraction experiments to determine their effectiveness as extraction solvents for penicillin-G, analysis of the results of these extraction experiments, and development of a method for recovery of the penicillin from the RTIL's.

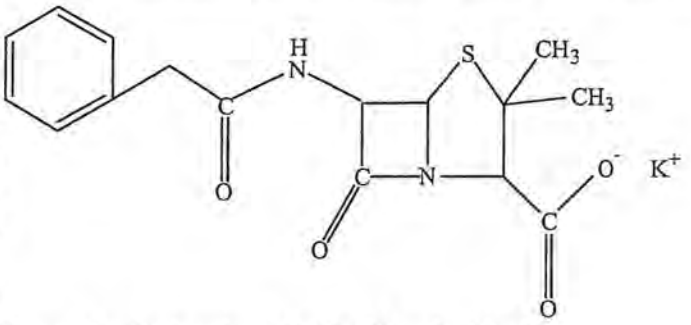

Figure 1. Potassium Salt of Penicillin-G.

1. *Synthesis and Characterization of RTIL's*

The first aim of this research was to synthesize and characterize four different RTIL's to be evaluated for extraction of penicillin-G from aqueous fermentation broth simulants. Ionic liquids are known to effectively solubilize compounds with aromatic and carbonyl functional groups, as well as those with conjugated double bonds. For liquid-liquid extraction, the RTIL must be immiscible

with water, and several have now been identified that are, including 1-*n*-butyl-3-methylimidazolium hexafluorophosphate (BMIM PF_6),[5] 1-*n*-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl) imide (BMIM imide), 1-*n*-butylpyridinium hexafluorophosphate (Bu-Py PF_6) and 1-*n*-butylpyridinium bis(trifluoromethanesulfonyl) imide (Bu-Py imide),[9] the RTIL's to be synthesized in this program. The structures of BMIM PF_6 and Bu-Py imide are shown in Figure 2. The syntheses of these RTIL's are straightforward.[5,9] The products were characterized by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.

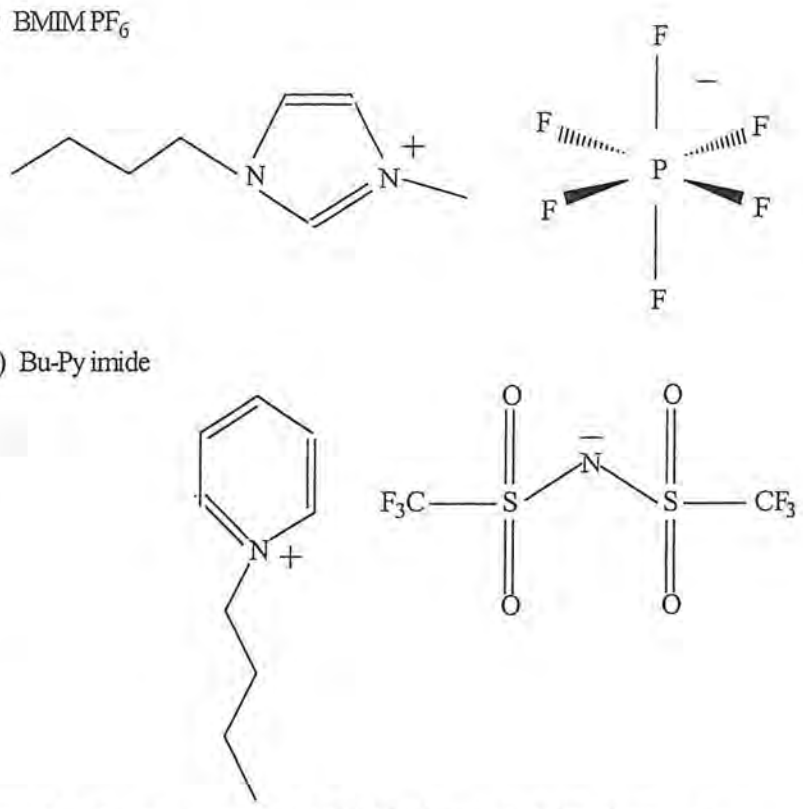

Figure 2. Structures of: (a) BMIM PF_6 (b) Bu-Py imide.

2. *Liquid-Liquid Extractions of Penicillin-G from Fermentation Broth Simulants*

The second aim of the research was to conduct a series of extractions of penicillin-G into the four RTIL's from a range of aqueous simulant solutions. The pH of the simulants and the available cations for ion-pairing with the penicillin were varied, and the effect of added lactose was evaluated. Industrially, penicillin is now extracted from broths that have been acidified to a pH of 2.5, ensuring that it is protonated. Control experiments were to be run using butyl acetate, a solvent that is now used industrially for penicillin extraction, but this was not possible, as explained in the Phase I Results section below. There are many other components of fermentation broths, including corn steep liquor, various salts, acids, and trace elements. The effects of these other components will be evaluated in the Phase II effort. The extraction of other antibiotics such as cephalosporin, streptomycin, and tetracyclines, will also be examined in Phase II.

3. *Analysis of Extraction Results*

The third aim of the project was to analyze the results of the extraction experiments to verify effectiveness of the new RTIL solvents as extractants for penicillin-G, and to make any indicated changes to the RTIL's or the extraction process based on the preliminary results. Quantification of penicillin in the two phases was performed using ultraviolet-visible (UV-Vis) and infrared (IR) spectroscopies. Both methods have been successfully used previously to quantify penicillin concentrations.[10] However, UV-Vis proved to be the best method for our purposes, as the penicillin was not concentrated enough in the aqueous phases for IR spectroscopy to be very useful. The aromatic and carbonyl functionalities of penicillin are chromophores and can be monitored by

UV-Vis spectroscopy. UV radiation is absorbed by the π electrons of double bonds, exciting them to antibonding π^* orbitals. Absorption by σ-bonds is at much lower wavelengths, below the useful range for spectroscopy, which ends at about 200 nm. Penicillin exhibits maxima in the UV region which can be used for its quantification.[11]

4. *Recovery of Extracted Penicillin from RTIL's and Their Reuse*

The fourth and final aim of the project was to verify that the penicillin can be recovered from the RTIL's in a facile manner, and that the RTIL's can be reused. Currently, the penicillin is often precipitated from the organic extractant phase as its potassium salt by addition of a concentrated solution of potassium acetate (KOAc). This did not cause precipitation of the penicillin from the RTIL's however. Another common approach to the recovery of penicillin is to extract it with alkali, and freeze-dry the extract. This approach was successfully explored in the Phase I. Significantly, it was later found that the penicillin can be effectively back-extracted from the RTIL's into plain, neutral water. Another factor to be considered in this step besides recovery of the penicillin is the suitability of the RTIL's for reuse. Recycling experiments were carried out with used RTIL phases.

Non-volatile, non-flammable RTIL solvents were identified and synthesized that can be substituted for the toxic, volatile, and flammable organic solvents now commonly used for liquid-liquid extraction of penicillin (and possibly other antibiotics) from their fermentation broths, using the same equipment now in use industrially. Examination of the effects of the various components of fermentation broths on the extractions, evaluation of RTIL's for extraction of other antibiotics from their fermentation broths, and optimization of the process will continue in the Phase II project, and a pilot study in a small pharmaceutical plant will be initiated. Substitution of these relatively non-hazardous solvents for the toxic, flammable, and volatile ones now being used will improve the health and safety of pharmaceutical workers, as well as those transporting the chemicals.

PHASE I RESULTS

Very significant progress has been made in achieving all four of the originally stated aims of the Phase I program. Suitable, water-immiscible RTIL's have been synthesized, characterized, and used in liquid-liquid extractions to remove up to 74% of the penicillin from dilute aqueous simulant solutions in one pass. Up to 77% of the extracted penicillin can then be back-extracted into neutral deionized water in one pass. In two passes, up to 96% of the extracted penicillin can be back-extracted into plain water. This is preferable to back-extraction with the basic solutions that are now used industrially. This water can then be freeze dried, as is now done commercially with the basic solutions, to recover the penicillin. Significantly, no base need be added as is now done commercially, and it will not be present in the recovered penicillin to contaminate it.

1. *Synthesis and Characterization of RTIL's*

The first aim of the Phase I project was to synthesize the RTIL's 1-butylpyridinium bis(trifluoromethanesulfonyl)imide (Bu-Py imide), 1-butyl,3-methylimidazolium bis(trifluoromethanesulfonyl)imide (BMIM imide), 1-butylpyridinium hexafluorophosphate (Bu-Py PF_6), and 1-butyl,3-methylimidazoium hexafluorophosphate (BMIM PF_6) for subsequent evaluation as liquid-liquid extractants for penicillin-G. Three of these were successfully prepared, as well as four additional ones.

RTIL's successfully synthesized and characterized include 1-hexyl,3-methylimidazoium bis(trifluoromethanesulfonyl)imide (HMIM imide), 1-octyl,3-methylimidazoium bis(trifluoromethanesulfonyl)imide (OMIM imide), BMIM PF_6, and 1-hexyl,3-methylimidazoium hexafluorophosphate (HMIM PF_6). Attempted syntheses of Bu-Py PF_6 and 1-hexylpyridinium hexafluorophosphate (Hex-Py PF_6) were not successful.

The chloride salts of the organic cations were first synthesized by treatment of the neutral pyridine or imidazole derivatives with the appropriate alkyl chloride. The resultant chloride salt of the now substituted cation was then treated with hexafluorophosphoric acid or lithium bis(trifluoromethanesulfonyl)imide to obtain the less viscous and water-immiscible RTIL's used for extractions. Yields of ~45% were typically obtained for the first step of the syntheses, and 85-90% for the second step.

Bu-Py imide was synthesized using the following procedure. Pyridine and 1-chlorobutane were combined in a 1:1 molar ratio and were refluxed at 100°C for 72 hours. 1-Butylpyridinium chloride was isolated as a white crystalline powder and washed 3 times with ethyl acetate. Residual ethyl acetate was removed under vacuum. 1-Butylpyridinium chloride was then treated with lithium imide in the following manner. 1-Butylpyridinium chloride was dissolved in deionized H_2O. A molar equivalent of lithium imide was dissolved in deionized H_2O and added dropwise to the rapidly stirred aqueous 1-butylpyridinium chloride solution. A separate, light yellow ionic liquid phase formed upon addition of the 1-butylpyridinium chloride solution. The phases were separated and the RTIL was washed with deionized H_2O and dried under vacuum for 72 hr. Light orange 1-hexylpyridinium bis(trifluoromethanesulfonyl)imide was synthesized in the same manner using 1-chlorohexane as the starting material.

The imide salts of the imidazolium derivatives were prepared in an analogous manner, by treatment of the commercially available 1-methylimidazole with the appropriate *n*-alkyl chloride to yield chloride (Cl^-) salts of the now substituted cations. Equimolar amounts of the starting materials were combined in a flask and heated with stirring at 70 °C for 72 hr. The resultant viscous Cl^- salt was washed with ethyl acetate and dried under vacuum at 70 °C for at least 12 hr, and then treated with lithium imide as were the pyridinium derivatives. Aqueous hexafluorophosphoric acid was reacted with the Cl^- salts of the substituted cations in the same way as lithium imide to yield the PF_6-based RTIL's.

All RTIL's were characterized and shown to be pure by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, performed at the University of Colorado in Boulder. Representative spectra of BMIM imide and Bu-Py imide, with peak assignments and integration results, are shown in Figures 3 and 4. The Bu-Py imide had not been dried for the full 72 hr, and a peak resulting from a small amount of water appears in its spectrum.

The first aim of the program was successfully completed. Three of the four proposed RTIL's were synthesized, and synthesis was attempted for the one that was not successfully produced. In addition, several other appropriate RTIL's were synthesized. All were characterized and shown to be pure by ^{1}H NMR. They were evaluated as liquid-liquid extractant phases for penicillin-G in the following task.

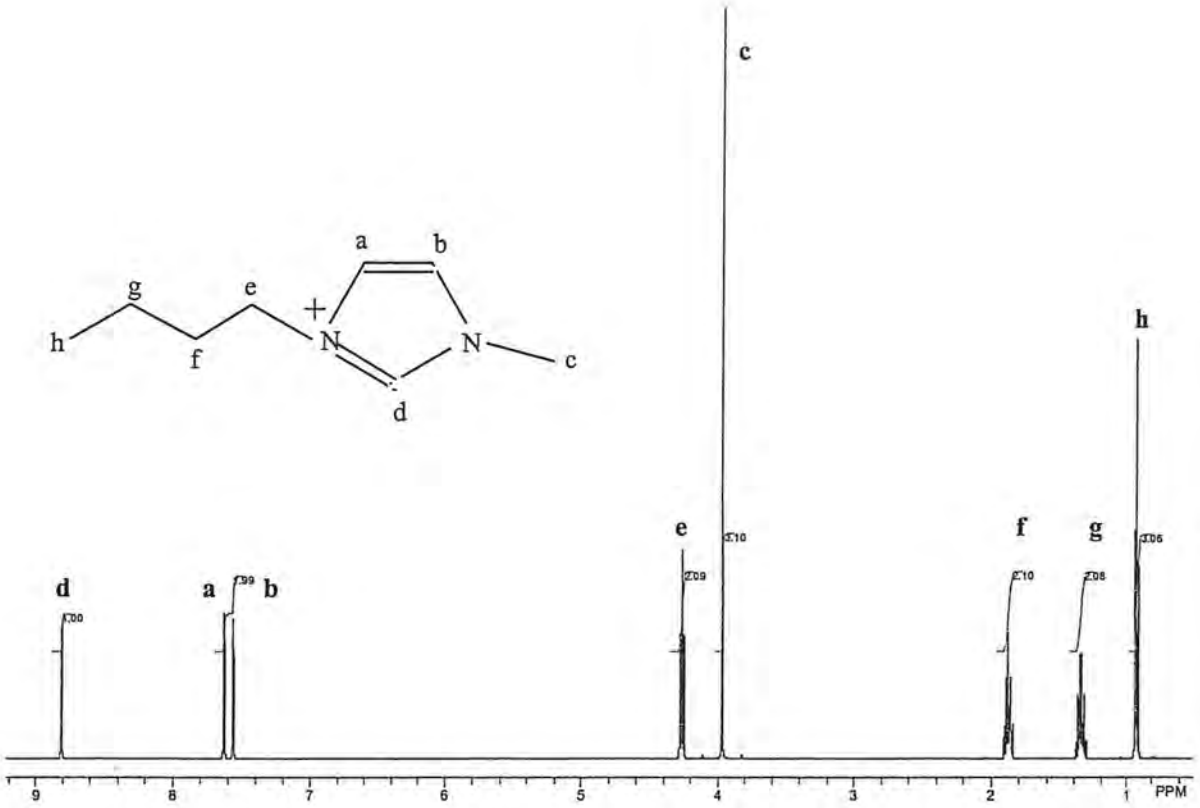

Figure 3. ^{1}H NMR spectrum of BMIM imide.

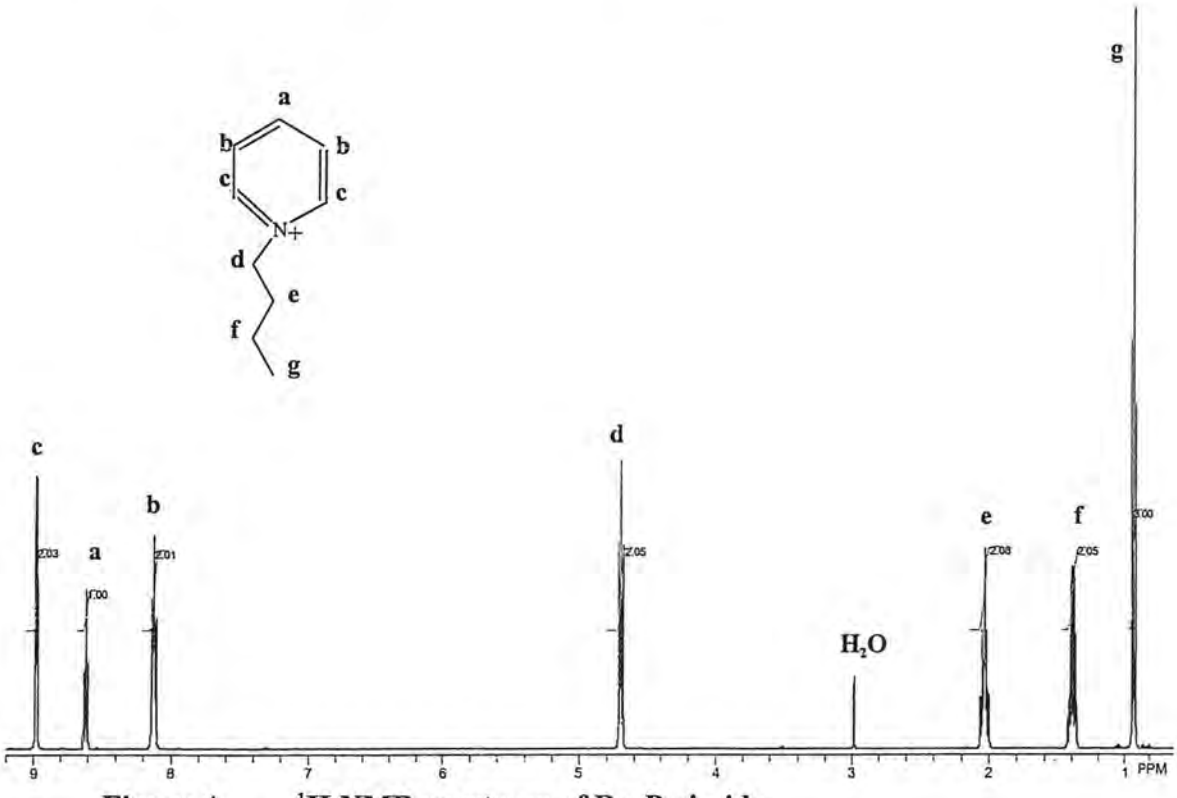

Figure 4. ^{1}H NMR spectrum of Bu-Py imide.

2. *Liquid-Liquid Extractions of Penicillin-G from Fermentation Broth Simulants*

The second aim of the Phase I program was to evaluate the synthesized RTIL's as extractant phases for removal of penicillin-G from aqueous fermentation broth simulant solutions. VOC solvents are now used industrially, and their replacement with non-volatile, non-flammable RTIL's would be desirable for health, safety, and environmental reasons.

Extractions were performed from acidic, basic, and neutral aqueous solutions, with and without citrate buffer and lactose. The potassium salt of penicillin-G was usually used, but other more lipophilic cations were evaluated in some experiments in attempts to improve solubility of the ion-paired penicillin in the RTIL's and to enhance its extraction from the aqueous simulant solutions.

In a typical extraction experiment a 0.2-2 wt % penicillin solution was prepared, initially in a citric acid buffer. Using the buffer allowed quantification of the penicillin-G UV peak at 264 nm. Without it, there was too much interference in the region. This solution (5 mL) was combined with 5 mL of the desired RTIL. Initially, this mixture was stirred 1 hr to allow equilibration. However, it was soon determined that only 5 min or less of shaking is necessary for the extraction to reach equilibrium. The solutions were allowed to settle 10 min before UV-Vis spectra were taken. It was found that Bu-Py imide and Hex-Py imide produced significant interference in the 230 - 300 nm region of the UV spectrum, in which we were interested. We were therefore not able to quantify penicillin extraction using these ionic liquids. BMIM imide and HMIM imide did not produce significant interference, and were used thereafter.

UV-Vis analysis of the aqueous phases initially showed no removal of penicillin from the citrate buffer solution. It was first hypothesized that penicillin was not extracted because a suitable cation was not present in the aqueous phase. In order to retain charge balance, both an anion and a cation must be extracted into the RTIL. It was initially expected that potassium would be extracted with the penicillin anion into the ionic liquid phase, but potassium is very well coordinated by water and perhaps could not be readily extracted into the RTIL's. Secondly, at pH 5, citrate is a doubly charged anion. This doubly charged anion may be extracted preferentially by the RTIL instead of the singly charged penicillin anion. RTIL's solubilize charged species particularly well.

The solution to the first scenario was to add an additional cation to the aqueous penicillin solution to ion pair with the penicillin and promote its extraction. A large organic cation is expected to be extracted with the penicillin more readily than potassium. After experimenting with several larger cations, the dye Azure A ($CH_{14}H_{14}N_3SCl$) was chosen as a candidate. This is the chloride salt of a large organic cation, and it has an intense blue color in solution that makes its presence readily detectable visually. It contains aromatic rings, which increase solubility in RTIL's. Its structure is shown in Figure 5. The solution to the second scenario was to lower the pH of the aqueous simulant solution, which is already done in industrial extraction processes as well. At pH 3 or 4 citrate is a singly charged anion which should have less affinity for the RTIL, thereby allowing the penicillin anion to be more readily extracted.

To examine the effect of Azure A on penicillin extraction, a molar equivalent of Azure A was added to a 0.2% penicillin solution containing citrate buffer. After 5 min of shaking with BMIM imide the blue color had been completely extracted into the RTIL phase. However, UV-Vis indicated that the penicillin anion had not been extracted with the Azure A cation. Azure A had clearly been extracted by the ionic liquid, but an anion other than

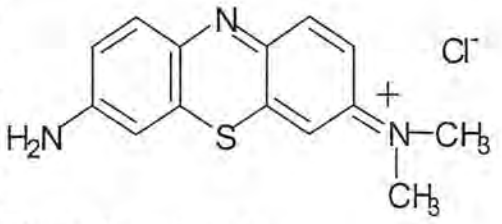

Figure 5. Azure A.

penicillin had apparently been extracted with it.

Azure A was again added to an aqueous penicillin solution, and the pH of the citrate buffer was reduced to 4 by addition of citric acid. A UV-Vis spectrum was taken of the aqueous phase, which indicated significant extraction based on a decrease in intensity of the 264 nm penicillin peak. Several extractions were performed at different pH values, with and without Azure A. As an example, the UV-Vis spectrum of a 0.2% penicillin solution at pH 3 with Azure A before and after extraction with HMIM imide is shown in Figure 6.

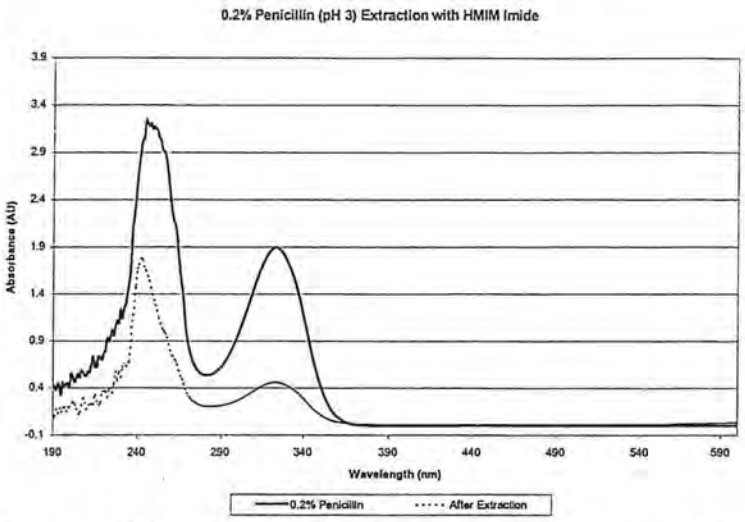

Figure 6. 0.2% Penicillin before and after extraction with HMIM Imide.

Typical extraction results from this series of experiments, shown in Table 1, indicate several trends. First, there was no difference between the extraction ability of BMIM imide and HMIM imide. These two ionic liquids extract similar amounts of penicillin at pH 3 or 4. Secondly, both the pH of the initial penicillin aqueous phase and the presence of Azure A affect the extraction of penicillin. It is clear that lower pH values improve the extraction of penicillin as with conventional industrial systems. Using HMIM Imide, only 38% of the penicillin was extracted at pH ~4. At pH ~3, 67% penicillin was extracted, and at pH ~2, 72% penicillin was extracted in one pass.

Table 1.
Penicillin Extraction Results

Ionic Liquid	~ pH of Penicillin Solution	264 nm abs. Initial	Penicillin Conc. Initial	264 nm abs. Final	Penicillin Conc. Final	Percent Extraction
BMIM Imide	4	2.449	0.013	1.41	0.007	42.45
BMIM Imide	3	1.015	0.005	0.297	0.002	70.82
HMIM Imide	4	1.197	0.006	0.739	0.004	38.30
HMIM Imide	3	2.021	0.011	0.660	0.003	67.38
HMIM Imide	3 no Azure A	0.964	0.005	0.535	0.003	44.56
HMIM Imide	2	1.943	0.010	0.548	0.003	71.84
HMIM Imide	2 no Azure A	2.178	0.011	0.515	0.003	76.40

Preliminary results indicate that the presence of Azure A affects penicillin extraction differently depending upon the pH of the simulant solution. At pH ~3 with Azure A, HMIM imide extracted 67% penicillin. At pH ~3 without Azure A, HMIM imide only extracted 45% of the penicillin. Therefore, at pH ~3, Azure A improves penicillin extraction. At pH ~2 however, there was no significant difference in HMIM imide extraction with and without Azure A. With Azure A, 72% of the penicillin was extracted, and 76% of the penicillin was extracted without Azure A.

These results clearly demonstrate that penicillin can be effectively extracted from aqueous solutions using RTIL's. Both the pH of the penicillin solution and the presence of additional anions and cations in that solution affect its extraction into the RTIL. Acidic solutions were necessary for significant extraction, as with conventional extraction processes, and additional cations may enhance penicillin extraction depending on the pH. There is a large variety of cations in the actual fermentation broths for the penicillin to be paired with for extraction.

However, when RTIL phases were reused for a second extraction, very little penicillin was extracted, and it was hypothesized that the citrate anion was preferentially migrating into the RTIL, ion-exchanging with penicillin already extracted and inhibiting the further removal of penicillin. To eliminate competition for removal of penicillin from aqueous simulant solutions by citrate, extractions were carried out from more concentrated (0.5 - 2.0 wt %) aqueous penicillin solutions acidified with HCl, and the simulants were then diluted with citrate buffer prior to analysis by UV-Vis. This procedure allowed quantification of the penicillin peak at 240 nm without inhibition of penicillin extraction by citrate, and provided much better results. The results of representative extractions performed without citrate buffer are shown in Table 2 and discussed below.

A penicillin concentration of 1.0 wt% in a pH ~3 HCl solution was initially used as the simulant solution, resulting in 11.4% extraction in one pass by OMIM imide. This seems rather low, but actually is similar to the total amount of penicillin extracted from the lower concentration simulants. A similar extraction from a simulant with 0.5% penicillin concentration resulted in 13.2% extraction. No Azure A was used in these extractions, and its presence, or the presence of other similar cations, would have resulted in improved extraction at this pH, as discussed above.

Table 2.
Penicillin Extraction Results - No Citrate Buffer, OMIM Imide, 2% Penicillin

~ pH of Aqueous Solution	264 nm abs. Initial	Penicillin Conc. Initial (M)	264 nm abs. Final	Penicillin Conc. Final (M)	Percent Extraction
3	0.74065	0.00388 (1%)	0.65606	0.00343	11.4
3	0.96884	0.00507 (0.5%)	0.84075	0.00440	13.2
3 (w/ lactose)	0.92940	0.00486	0.75470	0.00395	18.8

However, Azure A is very difficult to work with at these higher concentrations, and its effect was already demonstrated.

Similar extraction experiments were carried out from a simulant solution containing 0.5 wt% penicillin and 0.75 wt% lactose. Lactose is a primary component of fermentation broths, and this is a realistic ratio of lactose to penicillin. OMIM imide was used as the extractant phase, and the simulant was acidified to pH ~3 with HCl as in the above extractions. The added lactose actually enhanced penicillin extraction, as it was now 18.9%, up from 13.2% without the lactose. The results of control experiments using butyl acetate could not be quantified due to formation of a precipitate during extractions.

The second aim of the project was successfully completed. The RTIL's synthesized and characterized in the first aim were evaluated as liquid-liquid extractants for penicillin, and they did rapidly remove up to 76% of the penicillin from acidified aqueous simulant solutions in one pass. The presence of lactose enhanced penicillin extraction into the RTIL's. It was demonstrated that significant amounts of penicillin can be extracted from these aqueous simulant solutions into non-volatile, non-flammable RTIL's in a very short time.

3. Analysis of Extraction Results

The objective of this task was to quantify penicillin extraction from the aqueous simulant solutions into the RTIL's. It was proposed that this would be done by UV-Vis and/or IR, and UV-Vis has been successfully used, monitoring the absorbance at 264 nm.

Penicillin-G exhibits characteristic peaks in the UV region that follow Beer's Law and have been shown to give linear calibration curves. A spectrum of 0.2% penicillin-G (potassium salt) is shown in Figure 7. However, initial attempts to obtain linear calibration curves were unsuccessful due to numerous interferences in the region of the spectrum that is of interest. Some of these interferences were found to be due to the pyridinium-based RTIL's, which apparently remained in the aqueous phases to some extent, perhaps as emulsions. Thereafter, only the imidazolium-based RTIL's were used.

Use of a citrate buffer in the simulant solutions eliminated other interferences. Initially the buffer was added to the simulants prior to extraction, but, as described in the previous task, citrate apparently competed with penicillin for migration into the RTIL's, limiting penicillin removal. More concentrated penicillin solutions were later used for the extractions, and they were then diluted with the buffer after extraction and prior to UV-Vis analysis. This procedure improved penicillin extraction and still allowed quantification of its UV peak at 264 nm. A typical calibration curve is shown in Figure 8. The equation of the line

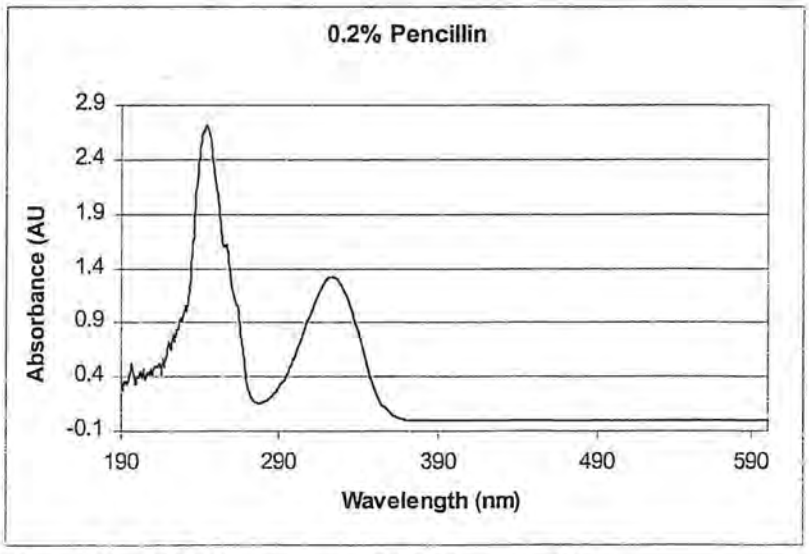

Figure 7. UV-Vis spectra of 0.2% penicillin.

obtained from the data points was then used to calculate penicillin concentrations.

Choice of the blank used for the UV-Vis spectrum was another important factor in eliminating spectral interferences. At first just the citrate buffer simulant solution was used (without penicillin), and interferences were present that prevented attainment of good calibration curves. Later, this blank solution was first subjected to parallel extraction by the RTIL along with the penicillin-containing simulant solution, and was then used as the blank. This further reduced the interferences.

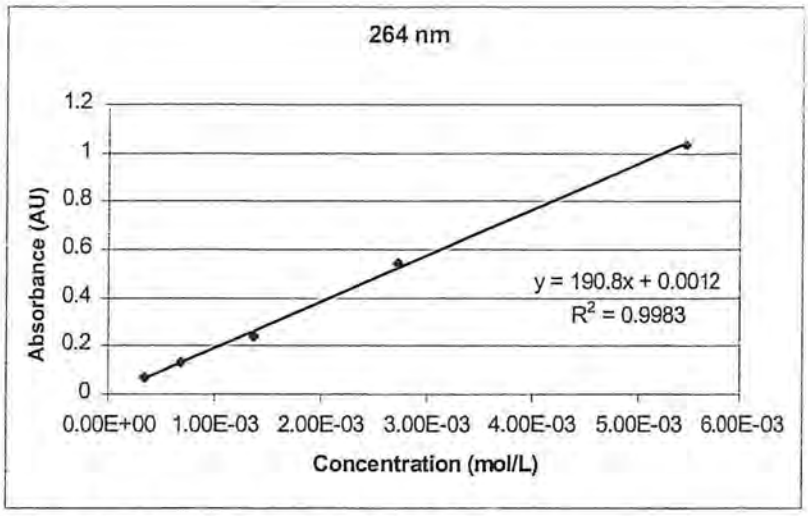

Figure 8. Calibration curve for penicillin.

After back-extraction of penicillin from an OMIM imide RTIL phase into deionized water, the RTIL was analyzed for purity by ^{1}H NMR. The spectrum looked almost exactly like that of the pure RTIL except for a few extremely small new peaks that were near the noise level, indicating possible trace contaminants. The RTIL phase was washed with water a second time and the RTIL was again analyzed by ^{1}H NMR. These small impurity peaks were now absent, and the spectrum looked just like that of the unused RTIL. No significant concentration of any impurities that are discernable by ^{1}H NMR remained in the RTIL.

After initial difficulty, Aim 3 was successfully completed, and the aqueous penicillin solutions were quantified.

4. Recovery of Extracted Penicillin from RTIL's, and Their Reuse

The objective of this aim was to develop a method to recover the extracted penicillin from the RTIL's, and then to evaluate their suitability for reuse. Industrially, penicillin is generally either back-extracted into a sodium carbonate solution and then freeze-dried, or precipitated as its potassium salt by addition of potassium acetate to the VOC extractant solvents used. Significantly, in this program, we have demonstrated that the penicillin can be very effectively back-extracted from the RTIL's into pure, neutral water, meaning that it can be recovered in a more pure form while creating less waste, improving worker safety, and cutting chemical costs.

Initially, attempts were made to precipitate the penicillin out of the RTIL simply by chilling it, but this was not successful. Then attempts were made to precipitate it as its potassium salt with addition of potassium acetate, but even with heating, the potassium acetate was not soluble in the RTIL's. Other more soluble potassium salts were tried, but no penicillin precipitated out.

Next, a series of experiments was carried out to evaluate the effect of pH on back-extractions into the citrate buffer solutions. The results are shown in Tables 3, 4, and 5. In all cases, the penicillin had been extracted into the RTIL from a 0.2 wt% penicillin, pH ~2 citrate buffer solution. In these back-extraction experiments, the phases were shaken together 5 min and allowed to settle 10-15 min before analysis of the aqueous phase. The penicillin was first back-extracted into pH ~5 and 7 citrate buffer solutions from HMIM imide extractant phases. Of the extracted penicillin,

51.5% was recovered into the pH 5 buffer in one pass, and 75.8%.into the pH 7 buffer. Under these conditions, a higher pH seemed to promote better back-extraction of the penicillin, but there may be other factors involved. Only 53.9% of the initial penicillin concentration had been extracted into the HMIM imide that was subsequently back-extracted with the pH 7 buffer, while 74.4% was in the HMIM imide that was back-extracted with the pH 5 buffer. It could be that when there is a lower concentration of penicillin in the RTIL phase, a higher percentage of it can be back-extracted.

Table 3.
Penicillin Recovery into pH 5 and 7 Buffer Solutions from HMIM Imide.

Procedure	~pH of Aqueous Penicillin Solution	264 nm abs. Initial	Penicillin Conc. Initial (M)	264 nm abs. Final	Penicillin Conc. Final (M)	Percent Extraction
extraction	2	1.7279	0.00905	0.44372	0.00232	74.4
back-extraction (recovery)	5	1.2842	0.00672	0.66175	0.00346	51.5
extraction (new RTIL phase)	2	3.5832	0.01877	1.6533	0.00866	53.9
back-extraction (recovery)	7	1.9299	0.01011	1.4638	0.00767	75.8

Table 4.
Penicillin Recovery into pH 8 Buffer Solution from OMIM Imide.

Procedure	~pH of Aqueous Buffer Solution	264 nm abs. Initial	Penicillin Conc. Initial (M)	264 nm abs. Final	Penicillin Conc. Final (M)	Percent Extraction
extraction	2	1.7318	0.00907	0.5633	0.00295	67.5
back-extraction (recovery)	8	1.1685	0.00612	0.6484	0.00339	55.5
extraction (same RTIL phase)	2	3.7499	0.01965	3.4097	0.01786	9.08
back-extraction (recovery)	8	0.8603	0.00450	0.5825	0.00305	67.7

Table 5.
Penicillin Recovery into pH 9 Buffer Solution from OMIM Imide.

Procedure	~pH of Aqueous Buffer Solution	264 nm abs. Initial	Penicillin Conc. Initial (M)	264 nm abs. Final	Penicillin Conc. Final (M)	Percent Extraction
extraction	2	2.4554	0.01286	0.9804	0.00513	60.1
back-extraction (recovery)	9	1.4751	0.00772	0.9298	0.00487	63.0
2nd back-extraction (recovery) same RTIL phase	9	0.5453	0.00285	0.0632	0.00032	11.4
3rd back-extraction (recovery) same RTIL phase	9	0.4821	0.00252	0	0	0
2nd extraction same RTIL phase	2	3.6693	0.01922	3.3550	0.01758	8.6
back-extraction (recovery)	9	0.7964	0.00417	0.5442	0.00285	68.28

In another similar experiment, penicillin was back-extracted from OMIM imide into buffer solutions of pH ~8 and 9. Of the penicillin that was extracted into the RTIL, 55.5% was back-extracted into the pH 8 buffer solution in one pass, and 63.0% into the pH 9 solution.. These values are very close, and the difference could be explained by the pH's of the buffer solutions and/or by the fact that again, slightly more of the original penicillin concentration was in the RTIL that was back-extracted with the ph 8 buffer (67.5%) than in the RTIL that was back-extracted with the pH 9 buffer (60.1%). The RTIL phase that had been back-extracted with the pH 9 buffer was back-extracted with fresh pH 9 buffer a second time, and 11.39% of the remaining penicillin was recovered, giving a total of 67.2% recovery of penicillin from the RTIL in two passes. When the experiment was repeated, 47.5% of the penicillin was initially extracted, and 76.1% was back-extracted in one pass.

A second extraction was performed using the same RTIL phase that had been back-extracted with the pH 8 buffer, and it was again back-extracted with the buffer solution. This time, 67.7% of the extracted penicillin was back-extracted in one pass. In a parallel experiment using a pH 9 buffer solution for back-extraction, 63.0% of the extracted penicillin was recovered in one pass. A second back-extraction was done into pH 9 buffer, and 11.4% of the remaining penicillin was recovered, for a total 67.2% recovery of the originally extracted penicillin in the two passes. A second extraction was performed using this same RTIL phase and it was again back-extracted with ph 9 buffer solution, giving 68.3% back-extraction. Back-extraction with acidic solutions was not successful.

These results were already quite promising, but we have since discovered that the extracted penicillin can be back-extracted even more effectively from these RTIL's into plain, neutral water. When use of citrate buffer for extractions was discontinued, it was also discontinued for the back-extractions. More concentrated penicillin simulant solutions were used and they were later diluted with the buffer prior to analysis, as described in the results for the third aim above. In these experiments, the penicillin was extracted into OMIM imide from water acidified with HCl to pH ~3. The results are shown in Table 2. When a 1% penicillin solution was used, the OMIM imide extracted 11.4% of it, as described in the results for the second aim, above. It was back-extracted as described above, except that plain, neutral water was used instead of citrate buffer solution. In one pass, 70.2% of the extracted penicillin was recovered into the water. A second back-extraction removed 92.7% of what remained in the RTIL, for total recovery of 96.5% of the penicillin in only two passes. The experiment was repeated starting with a 0.5% penicillin solution. The OMIM imide extracted 13.2% of it. In one pass, 76.8% of this was back-extracted into the water. Use of plain water for the recovery is a significant improvement over use of the basic solutions now employed in industry. It reduces chemical costs, improves worker safety, and the recovered penicillin is not contaminated with base.

A recycling experiment was carried out using this same OMIM imide phase, from which 76.8% of the extracted penicillin had been back-extracted already. This time only 6.68% of the penicillin was extracted. However, 13.2% of the penicillin that was originally extracted was still in the RTIL. Also, it was estimated that perhaps 1/6 of the volume of the RTIL phase was lost between the two extractions, so this result is actually quite promising. When this phase was back-extracted, 73.6% of the penicillin was recovered in one pass. Recycling experiments will be proposed for the Phase II project using much larger volumes of RTIL so that the volume loss will not be so significant, and with the RTIL phase being thoroughly purified of penicillin between extractions by successive back-extractions with water.

The fourth aim was successfully completed. A superior, non-polluting, and very effective method of recovery of the extracted penicillin was demonstrated, rapid back-extraction into plain, neutral water. Very good penicillin recovery, up to 76.8% in one pass and up to 96.5% in two passes, was demonstrated. The results of the recycling experiments were promising, and more work remains to be done in this area in the Phase II program.

5. *Extra Aim: Extraction of Penicillin Using a Redox-Recyclable Extractant Immobilized in an RTIL.*

An additional approach to recovery of penicillin from fermentation broth simulants was evaluated and found to be effective. The PI has previous experience with redox-active extraction and recovery of ionic species from aqueous waste streams. In this approach to ion-exchange extraction, the extractant is oxidized to its cationic form while being supplied with a suitable counterion for ion-exchange with the target ion. It is then contacted with the aqueous waste stream and, in liquid-liquid extraction, the target ion is replaced in the aqueous phase by the counterion of the extractant while the target ion preferentially migrates into the extractant phase, based on the relative solvation energies of the exchanging ions in the two phases. Significantly, recovery of the extracted ion in a small volume results when the extractant is reduced to its neutral form and the target ion is immediately and completely released. This is a major improvement over conventional ion-exchange materials, which typically must be regenerated by contact with large volumes of a high-salt, acidic, or basic "strip solution" which must then be disposed of. The target ion is

contained in this strip solution in a low concentration and cannot be recovered.

An electrochemical flow cell was used for these experiments. The cell body was composed of two halves that were compressed together with three 1/4-20 throughbolts, with a DARAMIC® microporous separator between them. Offset o-ring design allowed for ample compression strength and easily repeated sealing on the membrane. The cell body was fabricated of polyetheretherketone (PEEK), 2 1/4" round stock. Platinized titanium was used for the electrodes. Electrolytes were pumped through each half at 10 mL/min using a peristaltic pump.

In these experiments, it was verified that penicillin can be effectively extracted in this way. There was not enough time to carry out recovery experiments in the Phase I program. Either n-butylferrocene or *t*-butylferrocene was dissolved in the RTIL at a concentration of 0.2 M. This solution was circulated through the anode section of the cell for 1-3 hours while being supplied with chloride as a counterion. The catholyte was 1 M HCl. Color change indicating oxidation of the ferrocene derivative to cationic ferricenium could be seen almost immediately. After 1 hour, it was determined by UV-Vis that most of the extractant was oxidized. The extractant phase was then contacted with penicillin-containing acidic simulant solutions (acidified with HCl) as was the plain RTIL in Aim 2 results above, and chloride and penicillin exchanged rapidly based on their relative solvation energies in the two phases. The aqueous phase was analyzed by UV-Vis as described in Aim 3 results to determine the degree of penicillin extraction from the simulants. Results are shown in Table 6.

Table 6.
Redox-Recyclable Extractions.

RTIL	Extractant	Oxidation Time	Simulant Solution	D Value	% Extraction
BMIM imide	n-butyl	1 hr.	pH = 4	1.44	59
HMIM imide	t-butyl	1 hr. 20 min.	water	0.20	17
HMIM imide	n-butyl	1 hr. 20 min.	water	0.22	18
HMIM imide	n-butyl	1 hr. 20 min.	pH = 10	0.17	15
HMIM imide	n-butyl	1 hr. 25 min.	pH = 4	0.35	26
OMIM imide	t-butyl	2 hrs.	water	0.37	27
OMIM imide	n-butyl	2 hrs.	water	0.39	28
OMIM imide	n-butyl	2 hrs.	pH = 4	1.29	56
OMIM imide	n-butyl	2 hrs.	pH = 2	1.02	50
OMIM imide	n-butyl	2 hrs.	pH = 2 (0.1% penicillin)	1.64	62
Oct-Py imide	n-butyl	2 hrs.	pH = 2	3.12	76

Extraction time = 1 hr.; 0.2% penicillin; extractant concentration = 0.2 M.

Up to 76% penicillin extraction was realized, which is very promising. Extractions were first carried out from a pH 4 simulant solution using *n*-butylferrocene in BMIM imide, resulting in 59% extraction. Parallel extractions were then carried out from neutral deionized water using *t*-butylferrocene and *n*-butylferrocene. Less penicillin was extracted, 17% and 18% respectively. The two ferrocene derivatives appear to extract penicillin equally well. Two more extractions parallel to the one using *n*-butylferrocene were carried out, from simulant solutions of pH 10 (NaOH added) and 4. The results were slightly poorer with the basic solution, 15% extraction. With the acidic solution, 26% extraction was obtained, somewhat better than from neutral water.

OMIM imide was evaluated next as the extractant diluent. Parallel extractions were performed from neutral water using *t*-butylferrocene and *n*-butylferrocene. The results were again almost identical, 27% and 28% extraction respectively. This is better than the results obtained using HMIM imide, but the extractant was oxidized for longer, 2 hours instead of 1 hour 20 min. Two more extractions parallel to the one using *n*-butylferrocene were carried out, from simulant solutions of pH 2 and 4. From the pH 4 simulant, 56% of the penicillin was extracted; while from the pH 2 simulant, 50% was extracted. The optimum pH appears to be higher than 2. Another similar extraction was performed from a pH 2 simulant containing half the concentration of penicillin, and the results were somewhat better, 62% extraction. The best results to date were obtained using the RTIL Oct-Py imide, 76% extraction. The simulant in this experiment was acidified to pH 2, so based on the above results, presumably even more penicillin would have been extracted if the pH was higher.

These results are very promising. It has been demonstrated at Eltron by the PI that when a redox-active extractant is electrochemically reduced, anions paired with it are immediately and completely released, allowing recovery in a very small volume. This unique approach to penicillin recovery can be further explored in the Phase II program, as well as the originally proposed approach.

Significant Findings

The specific aims of the Phase I project have been met. Suitable, water-immiscible RTIL's were successfully synthesized in good yield, and were characterized and shown to be pure by ^{1}H NMR spectroscopy. It was found that these non-volatile, non-flammable RTIL's can be substituted for the VOC solvents now in use industrially with very promising results. In liquid-liquid extractions, using the RTIL's instead of the VOC solvents now used, up to 76% of the penicillin initially present in the simulant solutions was extracted in one pass. More work remains to be done with recycling experiments, but the initial results are promising. After initial difficulties with interferences, a method for accurate quantification of penicillin in the aqueous solutions by UV-Vis was developed and subsequently used successfully to demonstrate that these RTIL's can extract significant amounts of penicillin-G from aqueous simulant solutions, in a very short time. Significantly, it was also demonstrated that the extracted penicillin can be effectively recovered into plain water in a short time. In an extra task, it was also shown that penicillin can be successfully extracted into RTIL's using a redox-recyclable extractant, which also allows recovery of the penicillin in a small volume, with electrochemical regeneration of the extractant. Results of initial recycling experiments were promising, and more work remains to be done in this area. The fundamental concepts have been proven, and a significant start has been made on optimization of the proposed process for expansion in the Phase II project.

Usefulness of Findings

Substitution of non-volatile, non-flammable RTIL's for VOC solvents in extraction of penicillin and other antibiotics from their fermentation broths would be very desirable. It would improve worker safety, reduce chemical costs, and limit chemical emissions to the environment. The results of this program indicate that such substitution is indeed feasible. In addition, the fact that the penicillin can be recovered from the RTIL's into plain water rather than the basic solutions now used industrially is an added bonus. This also would decrease chemical costs, improve worker safety, and would allow recovery of pure penicillin not contaminated by base. Redox-recyclable extraction of penicillin into RTIL's is particularly appealing, since the secondary waste produced in recovery of the penicillin can be greatly reduced. The processes being explored will be applicable for extraction and recovery of other antibiotics as well, and should have broad industrial applications.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Centers for Disease Control
and Prevention (CDC)

Memorandum

Date: January 14, 2003

From: Lee M. Sanderson, Ph.D., Program Official
Office of Extramural Programs, NIOSH, E-74

Subject: Final Report Submitted for Entry into NTIS for Grant 5 R43 OH007466-02.

To: William D. Bennett
Data Systems Team, Information Resources Branch, EID, NIOSH, P03/C18

The attached final report has been received from the principal investigator on the subject NIOSH grant. If this document is forwarded to the National Technical Information Service, please let us know when a document number is known so that we can inform anyone who inquires about this final report.

Any publications that are included with this report are highlighted on the list below.

Attachment
cc: Sherri Diana, EID, P03/C13

List of Publications

Title:	Safe Alternative Solvents for Antibiotics Extraction
Investigator:	Jennifer F. Clark, Ph.D.
Affiliation:	Eltron Research, Inc.
City & State:	Boulder, CO
Telephone:	(303) 530-0263
Award Number:	5 R43 OH007466-02
Start & End Date:	9/30/2001–9/29/2002
Total Project Cost:	$99,998
Program Area:	Control Technology
Key Words:	solvents

Final Report Abstract:
This research resulted in identification of non-volatile, non-flammable room temperature ionic liquids (RTIL's) to replace the toxic, flammable, volatile organic compound (VOC) solvents now used for extraction of antibiotics from fermentation broths. The solvents used for penicillin extraction include methyl isobutyl ketone, amyl acetate, butyl acetate, and chloroform. These solvents are toxic, irritants, volatile, and/or extremely flammable. RTIL's have attracted significant attention for green chemistry applications. They are ionic compounds that have very large liquidus ranges. Some are air and water stable and immiscible with water. They solubilize aromatics and carbonyl-containing compounds particularly well, and were found to be effective for penicillin-G extraction. RTIL's have previously been studied for liquid-liquid extraction and other separations. Being non-volatile, they are recyclable, and their large liquidus ranges allow efficient separations using temperature control. They are non-flammable, and are "tunable" solvents, as the cations can be substituted and/or paired with different anions to manipulate the properties of the liquids and tailor them for specific applications. The use of RTIL's instead of the toxic, flammable, VOC solvents now used would improve the health and safety of pharmaceutical workers, and reduce costs, since they are straightforward to synthesize. New processes or equipment would not be required.

Publications
No publications to date.