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Kevin D. Clark, *University of Toledo*
Omprakash Nacham, *University of Toledo*
Honglian Yu, *University of Toledo*
Tianhao Li, *University of Toledo*
Melissa M. Yamsek, *University of Toledo*, et al.

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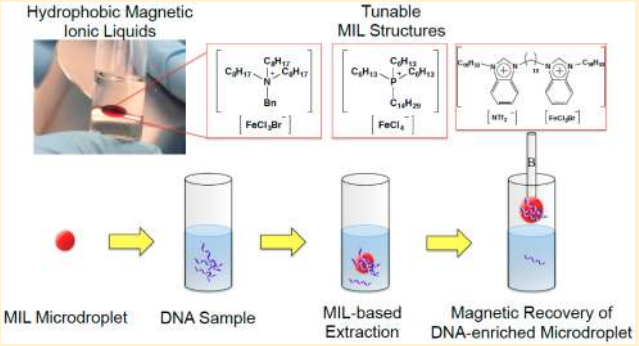
Extraction of DNA by Magnetic Ionic Liquids: Tunable Solvents for Rapid and Selective DNA Analysis

Kevin D. Clark, Omprakash Nacham, Honglian Yu, Tianhao Li, Melissa M. Yamsek, Donald R. Ronning, and Jared L. Anderson*

Department of Chemistry and Biochemistry, The University of Toledo, 2801 West Bancroft Street, MS 602, Toledo, Ohio 43606, United States

Supporting Information

ABSTRACT: DNA extraction represents a significant bottleneck in nucleic acid analysis. In this study, hydrophobic magnetic ionic liquids (MILs) were synthesized and employed as solvents for the rapid and efficient extraction of DNA from aqueous solution. The DNA-enriched microdroplets were manipulated by application of a magnetic field. The three MILs examined in this study exhibited unique DNA extraction capabilities when applied toward a variety of DNA samples and matrices. High extraction efficiencies were obtained for smaller single-stranded and double-stranded DNA using the benzyltrioctylammonium bromotrichloroferrate(III) ($[(C_8)_3BnN^+][FeCl_3Br^-]$) MIL, while the dicationic 1,12-di(3-hexadecylbenzimidazolium)dodecane bis[(trifluoromethyl)sulfonyl]imide bromotrichloroferrate(III) ($[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$) MIL produced higher extraction efficiencies for larger DNA molecules. The MIL-based method was also employed for the extraction of DNA from a complex matrix containing albumin, revealing a competitive extraction behavior for the trihexyl(tetradecyl)phosphonium tetrachloroferrate(III) ($[P_{6,6,6,14}^+][FeCl_4^-]$) MIL in contrast to the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL, which resulted in significantly less coextraction of albumin. The MIL-DNA method was employed for the extraction of plasmid DNA from bacterial cell lysate. DNA of sufficient quality and quantity for polymerase chain reaction (PCR) amplification was recovered from the MIL extraction phase, demonstrating the feasibility of MIL-based DNA sample preparation prior to downstream analysis.



Nucleic acids are biopolymers that have powerful and fundamental implications on the biochemical processes of every organism. Their applications in the life sciences have included the identification of DNA biomarkers in blood,[1] DNA-based therapeutics,[2] the study of ancient populations,[3,4] bioprospecting,[5] analysis of DNA from biopsies,[6] and understanding gene-disease relationships.[7] Research in these areas is fueled by the wealth of information made available through polymerase chain reaction (PCR), hybridization assays, and the various DNA sequencing methods. Unfortunately, the reliability of experimental results obtained from these techniques is limited by the complexity of isolating highly pure DNA from a cellular matrix or complex environmental samples. Proteins, small organic molecules, polysaccharides, and phospholipids are interfering agents that often challenge downstream applications.[8−10] Sensitive methods such as mass spectrometry or PCR that are preferred or necessary when only minute quantities of DNA are available are particularly affected by interfering compounds.[11−13] While numerous methodologies have been employed for the purification and preconcentration of DNA,[14] nucleic acid extraction remains a formidable bottleneck in many laboratories.

Traditionally, liquid−liquid extraction (LLE) with phenol-chloroform was used for the purification of DNA from biological samples.[15] Several adaptations to this method involving the addition of detergents to assist in the removal of proteins and polysaccharides have been made.[8,11] However, the dependence of these protocols on organic solvents and often time-consuming centrifugation steps has resulted in the development of more environmentally benign techniques that are capable of high sample throughput. In this realm, solid phase extraction (SPE) has been employed for the isolation of DNA prior to downstream analysis.[16−18] SPE technologies are primarily reliant upon the affinity of DNA toward a sorbent phase, commonly silica-based, in the presence of high ionic strength and/or chaotropic salts.[19] DNA retained on the SPE material is washed to remove interfering proteins, salts, and other cellular components and subsequently eluted with low ionic strength buffer. Several commercially available DNA extraction kits utilize SPE for DNA preconcentration and purification. While solvent consumption and analysis times are

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reduced in these approaches, they suffer from high cost per sample, particularly when the method involves the use of magnetic beads. Additionally, the recovery and purity of DNA is highly variable from kit to kit.[20] SPE approaches have also been incorporated into microfluidic domains for the purification of DNA.[21] Although reagent consumption is further reduced, these devices may require specialized equipment for fluid manipulation.[22]

Recently, ionic liquids (ILs) have been explored as solvents in nucleic acid applications. ILs are organic molten salts that possess melting points at or below 100 °C. Owing to the broad range of potential cation and anion combinations, ILs may be tailored to interact with a variety of important biomolecules.[23,24] Careful engineering of the IL structure has given rise to innovative DNA extraction systems,[25,26] ion conductive DNA films,[27] and DNA preservation media.[28] Wang and co-workers described a LLE method in which DNA was extracted from aqueous solution using the 1-butyl-3-methylimidazolium hexafluorophosphate ($[BMIM^+][PF_6^-]$) IL.[25] They suggested that the extraction was driven by electrostatic interactions between the IL cation and the negatively charged phosphate backbone of DNA. In a more recent study, our group investigated the extraction performance of several ILs using an *in situ* dispersive liquid−liquid microextraction (DLLME) technique.[26] Several important structural features of the cation were found to promote hydrophobic and hydrogen bonding interactions with DNA. For example, the 1-(1,2-dihydroxypropyl)-3-hexadecylimidazolium bromide ($[C_{16}POHIM^+][Br^-]$) IL exhibited high extraction efficiencies for duplex DNA using very small volumes of the IL extraction solvent. Although structural engineering of the ILs offered high extraction efficiencies, manipulation of the resulting DNA-enriched IL microdroplet proved to be a challenge.

Magnetic extraction phases have been employed in nucleic acid analysis as mobile substrates for the rapid extraction of DNA. In magnet-based approaches, the DNA-enriched extraction medium is readily isolated and controlled by application of an external magnetic field. Functionalized magnetic beads are commonly used in forensics and drug discovery applications to increase sample throughput by eliminating the need for tedious centrifugation steps.[29,30] Although magnet-based extractions are capable of recovering highly pure nucleic acids, variable extraction efficiencies ranging from as low as 40% to 70% have been reported when using DNA IQ paramagnetic beads.[29] The development of magnetic IL solvents for analytical extractions has the potential to profoundly impact nucleic acid analysis by combining the tunability of the IL with the magnetic nature of the solvent. Compared to existing methodologies, there are several benefits to a magnetic IL-based DNA extraction approach. The ability to tailor the IL structure to achieve favorable electrostatic interactions with the phosphate backbone of DNA can provide enhanced extraction efficiency. Additionally, recovery of the DNA-enriched extraction phase by the application of a magnetic field has the potential to significantly reduce the time required for sample preparation. The ability to magnetically manipulate the IL can also be exploited in downstream analysis, such as injection into microfluidic devices.

Magnetic ionic liquids (MILs) are a special subclass of ILs that retain the unique, tunable physicochemical properties of traditional ILs while also exhibiting a strong susceptibility to external magnetic fields. Several magnetoactive ILs have been previously reported in the literature containing high-spin transition metals such as iron(III), gadolinium(III), and dysprosium(III).[31−33] Recently, Deng and co-workers employed the trihexyl(tetradecyl)phosphonium tetrachloroferrate(III) MIL for the extraction of phenolic compounds from aqueous solution.[34] However, the use of MILs as solvents in the preconcentration and purification of biomolecules has never been explored. This is likely due to the challenge of designing a MIL extraction medium that exhibits both magnetic susceptibility and sufficient hydrophobic character to achieve phase separation in an aqueous sample environment upon exposure to an applied magnetic field.

This study constitutes the first report involving the extraction of DNA using hydrophobic MILs. In total, three hydrophobic MILs, namely 1,12-bis[*N*-(*N*′-hexadecylbenzimidazolium)-dodecane bis[(trifluoromethyl)sulfonyl]imide bromotrichloferrate(III) ($[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$), benzyltrioctylammonium bromotrichloroferrate(III) ($[(C_8)_3BnN^+][FeCl_3Br^-]$), and trihexyl(tetradecyl)-phosphonium tetrachloroferrate(III) ($[P_{6,6,6,14}^+][FeCl_4^-]$), were employed for the direct extraction of DNA from an aqueous solution. Isolation of the extraction phase was achieved by applying an external magnetic field, thereby circumventing time-consuming centrifugation steps. The optimized MIL-based extraction procedures are capable of performing rapid and highly efficient extraction of double-stranded and single-stranded DNA from a matrix containing metal ions and protein. Plasmid DNA (pDNA) extracted from a bacterial cell lysate using the MIL-based method was shown to be a high quality template for PCR.

EXPERIMENTAL SECTION

Reagents. Benzimidazole, trioctylamine, 1,12-dibromododecane, and guanidine hydrochloride (GuHCl) were purchased from Acros Organics (NJ, USA). Trihexyl(tetradecyl)-phosphonium chloride was purchased from Strem Chemicals (Newburyport, MA, USA). Deuterated chloroform was obtained through Cambridge Isotope Laboratories (Andover, MA, USA). Iron(III) chloride hexahydrate ($FeCl_3 \bullet 6H_2O$), 1-bromohexadecane, benzyl bromide, sodium dodecyl sulfate (SDS), albumin from chicken egg white, and DNA sodium salt from salmon testes (stDNA, approximately 20 kbp) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride, sodium hydroxide, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, potassium acetate, silica gel sorbent (230−400 mesh), and tris-(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Synthetic oligonucleotides including duplex (20 bp, molecular weight = 12 232 Da), single-stranded DNA oligonucleotides (33 mer, molecular weight = 10 075 Da), and primers were purchased from IDT (Coralville, IA, USA). The pET-32 plasmid was obtained from EMD Millipore (Billerica, MA, USA). NEB 5-alpha Competent *Escherichia coli* cells and Phusion High-Fidelity DNA Polymerase were obtained from New England Biolabs (Ipswich, MA, USA). Agarose and tris(hydroxymethyl)aminomethane (Tris) were obtained from P212121 (Ypsilanti, MI, USA). A 1 Kb Plus DNA Ladder (250−25,000 bp) was obtained from Gold Biotechnology, Inc. (St. Louis, MO, USA) with SYBR Safe DNA gel stain and bromophenol blue being supplied by Life Technologies (Carlsbad, CA, USA) and Santa Cruz Biotech (Dallas, TX, USA), respectively. QIAquick Gel Extraction and QIAamp DNA Mini Kits were purchased from QIAgen (Valencia, CA,

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USA). Deionized water (18.2 MΩ cm) obtained from a Milli-Q water purification system was used for the preparation of all solutions (Millipore, Bedford, MA, USA).

Synthesis and Characterization of Hydrophobic Magnetic Ionic Liquids. Chemical structures of the three MILs investigated in this study are shown in Figure 1. The

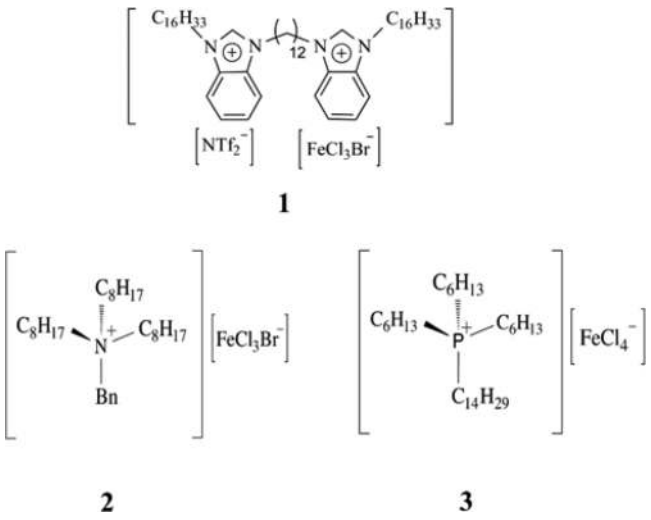

Figure 1. Structures of the three hydrophobic MILs examined in this study: (1) $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$, (2) $[(C_8)_3BnN^+][FeCl_3Br^-]$, and (3) $[P_{6,6,6,14}^+][FeCl_4^-]$.

$[P_{6,6,6,14}^+][FeCl_4^-]$ MIL was prepared using a previously reported procedure.[32] The synthesis of two hydrophobic MILs, namely, $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ (**1**) and $[(C_8)_3BnN^+][FeCl_3Br^-]$ (**2**), was carried out as described in our recent work[35] and is shown in Figure S1. A detailed synthetic procedure is available in the Supporting Information. ^1H NMR, ^{13}C NMR, ESI-MS, and UV−vis were used to characterize the three MILs, as shown in Figures S2−S9. To illustrate the hydrophobic and paramagnetic behavior exhibited by the MILs, two videos are provided in the Supporting Information that show microdroplets of the benzyltrioctylammonium bromotrichloroferrate(III) MIL being magnetically manipulated in an aqueous sample.

Instrumentation. High performance liquid chromatography with UV detection was performed on a LC-20A liquid chromatograph (Shimadzu, Japan) consisting of two LC-20AT pumps, a SPD-20 UV/vis detector, and a DGU-20A_3 degasser. Chromatographic separations were performed on a 35 mm × 4.6 mm i.d. × 2.5 μm TSKgel DEAE-NPR anion exchange column with a 5 mm × 4.6 mm i.d. × 5 μm TSKgel DEAE-NPR guard column from Tosoh Bioscience (King of Prussia, PA). The column was equilibrated with a mobile phase composition of 50:50 (A) 20 mM Tris-HCl (pH 8) and (B) 1 M NaCl/20 mM Tris-HCl (pH 8). For stDNA analysis, gradient elution was performed beginning with 50% mobile phase B and increased to 100% B over 10 min. In the separation of ssDNA as well as DNA and albumin, the column was first equilibrated with 20 mM Tris-HCl followed by gradient elution from 0% to 50% B over 10 min and then 50% to 100% B over 5 min. A flow rate of 1 mL min^{-1} was used for all HPLC separations. DNA and albumin were detected at 260 and 280 nm, respectively.

All extractions were performed in 4 mL screw cap vials. Isolation of the magnetic ionic liquid extraction phase was achieved using a cylinder magnet (B = 0.9 T) or rod magnet (B = 0.66 T) obtained from K&J Magnetics (Pipersville, PA). A Techne FTgene2D thermal cycler (Burlington, NJ, USA) was used for all PCR experiments. Agarose gel electrophoresis was performed in a Neo/Sci (Rochester, NY) electrophoresis chamber with a dual output power supply. Gels were visualized at 468 nm on a Pearl Blue Transilluminator (Pearl Biotech, San Francisco, CA).

MIL-Based Single Droplet Extraction. The procedure for the MIL-based static single droplet extraction (SDE) method was performed as shown in Figure S10. Briefly, a 20 μL droplet of MIL was suspended from a magnetic rod (B = 0.66 T) and lowered into a 4.17 nM solution of stDNA buffered by 20 mM Tris-HCl (pH 8). After 5−120 min, the MIL droplet was removed from the sample and a portion of the aqueous phase subjected to HPLC analysis to determine the concentration of DNA remaining after extraction.

MIL-Based Dispersive Droplet Extraction. The general MIL-based dispersive droplet extraction (DDE) approach employed in this study is depicted in Figure S11. A 4.17 nM solution of stDNA was prepared in 20 mM Tris-HCl (pH 8). An optimized volume of MIL (typically 20 μL) was added to the aqueous DNA solution and manually shaken for 5−60 s, resulting in a dispersion of the hydrophobic MIL in the aqueous phase. In the case of the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL, it was gently heated prior to extraction. The vial was then placed in a 0.9 T magnetic field to facilitate the rapid isolation of MIL followed by HPLC analysis of a 20 μL aliquot of the aqueous phase.

Extraction of Synthetic Oligonucleotides and Duplex DNA. Solutions of synthetic oligonucleotides and duplex DNA were prepared such that the mass of DNA in aqueous solution was consistent with the experiments involving stDNA (100 μg of stDNA in 2 mL of Tris-HCl). For extractions of ssDNA, a 33 base oligonucleotide with sequence 5′-CAC CAT GAC AGT GGT CCC GGA GAA TTT CGT CCC-3′ was dissolved in 20 mM Tris-HCl (pH 8) resulting in a final concentration of 1499 nM. In the case of synthetic dsDNA, an aqueous solution containing 1224 nM of 20 bp duplex (sequence: 5′-ATG CCT ACA GTT ACT GAC TT-3′ and its complementary strand) was prepared in 20 mM Tris-HCl (pH 8). Solutions containing single-stranded oligonucleotides or duplex DNA were subjected to MIL-based DDE with a 20 μL portion of the aqueous phase being analyzed by HPLC.

Extraction of DNA from a Complex Matrix. Sample matrices containing either metal ions or protein (albumin) were prepared from stock solutions. A sample solution containing 388 mM NaCl, 153 mM KCl, 38.1 mM $CaCl_2·2H_2O$, 28.3 mM $MgCl_2·6H_2O$, and 4.17 nM stDNA was extracted in triplicate using MIL-based DDE for all three MILs. For experiments involving protein as a matrix component, the samples were prepared at an albumin concentration of 3.4 μM and stDNA concentration of 4.17 nM with the pH varied from 3.5 to 8.

PCR and DNA Sequence Analysis. For DNA sequence analysis, a modified pET-32 plasmid containing an 879 bp gene encoding human 5′-methylthioadenosine phosphorylase (MTAP) was extracted using the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL in the DDE approach. The pDNA-enriched MIL microdroplet was removed from solution using a 0.66 T rod magnet and stored at room temperature for 24 h. Recovery of the pDNA was achieved by dispersion of the MIL microdroplet in 200 μL of 20 mM Tris-HCl (pH 8) for 2 min. A 2 μL aliquot of the aqueous phase was subjected to PCR using primers for

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the MTAP gene. The PCR products were separated by agarose gel electrophoresis, and the band containing the MTAP gene was extracted from the gel using a QIAquick Gel Extraction Kit. An external DNA sequencing service (Eurofins Genomics, Huntsville, AL) performed sequence analysis of the MTAP gene amplified from the pDNA recovered from the MIL extraction phase.

Amplification of the MTAP gene was performed using the primers 5′-TGC TGT TCC AGG GAC CT-3′ (molecular weight = 5,177.4 Da) and 5′-GAA TTC GGA TCC GGA CGC-3′ (molecular weight = 5,524.6 Da). A 2 μL aliquot of aqueous solution containing pDNA recovered from the MIL extraction phase was added to a PCR tube with 34.5 μL of DI H_2O and 10 μL of 5X Phusion HF buffer. Primers and dNTPs were added to achieve a final concentration of 0.2 μM and 200 μM, respectively. Finally, 1 unit of Phusion High Fidelity DNA polymerase was added to the reaction mixture. The total reaction volume was 50 μL. The following temperature program was used for amplification of MTAP: 5 min initial denaturation at 95 °C and 30 cycles comprised of a 30 s denaturation step at 95 °C, a 45 s hold at 54 °C for annealing, and a 45 s elongation step at 72 °C.

Recovery of DNA from the MIL Extraction Phase. Following MIL-based DDE of a 4.17 nM solution of stDNA, the DNA-enriched MIL microdroplet was first transferred into a microcentrifuge tube containing 1 mL of 3 M potassium acetate (pH 4.8) and vortexed for 2 min, ensuring a homogeneous solution. A silica sorbent column was constructed by measuring 750 mg of silica particles into a Pasteur pipet with the exit end blocked by a glass wool frit. The column was conditioned with 2 mL of 6 M GuHCl, and the sample was subsequently loaded at approximately 1 mL min^{-1}. The sorbent was flushed with 1 mL of isopropanol and the first fraction collected. Next, 750 μL of ethanol was added, and the turbid solution was centrifuged at 16,200*g* for 15 min. The pellet was washed with 80% ethanol for 1 min. The sample was centrifuged once more at 16,200*g* for 10 min, and the supernatant was decanted. The pellet was dried under an air stream and reconstituted in 100 μL of Tris-HCl (pH 8), and a 20 μL aliquot was removed for HPLC analysis.

As an alternative, a rapid approach to DNA recovery was employed. After MIL-based DDE, the DNA-enriched MIL microdroplet was collected from aqueous solution using a 0.66 T rod magnet and immersed in 200 μL of Tris-HCl (pH 8) for 2 min. The microdroplet was then removed from solution and the aliquot subjected to PCR amplification.

Extraction of DNA from Bacterial Cell Lysate. The conditions used to culture NEB 5-alpha Competent *E. coli* cells containing pDNA are described in the Supporting Information. A 10 mL aliquot of an overnight *E. coli* cell culture was centrifuged at 16,200*g* for 5 min and resuspended in 300 μL of 20 mM Tris buffer containing 10 mM EDTA (pH 8). Lysozyme (200 μg) was added to the solution, which was then incubated for 5 min at room temperature, followed by the addition of 600 μL of 0.2 N NaOH, 1% SDS (w/v). After gentle mixing of the solution, 400 μL of 3 M potassium acetate (pH 4.8) was added. The contents were thoroughly mixed and centrifuged at 16,200*g* for 10 min. A 400 μL aliquot of the supernatant was transferred to a clean vial, and the solution was extracted using the MIL-based DDE approach. The pDNA was then recovered using either the aforementioned silica-based or the rapid immersion procedure prior to PCR amplification.

RESULTS AND DISCUSSION

Structural Design of Hydrophobic MILs for DNA Extraction. The selection of MILs as solvents for the rapid extraction of nucleic acids from aqueous solutions requires compounds that are highly hydrophobic while also possessing sufficient magnetic susceptibility. A recent study of the $[BMIM^+][FeCl_4^-]$ MIL in aqueous solution (less than 20% (v/v) MIL) showed that phase separation did not occur upon application of a 1 T magnetic field.[36] Consequently, relatively hydrophilic MILs have limited utility in aqueous extraction systems due to the high phase ratio required to avoid complete miscibility of the MIL.

Common strategies for imparting hydrophobicity to ILs involve selection of a noncoordinating, hydrophobic anion and/or functionalization of the IL cation. The incorporation of anions such as $[NTf_2^-]$ generally not only reduces the solubility of ILs in water but also precludes the use of paramagnetic anions, such as $[FeCl_4^-]$. In an effort to develop sufficiently hydrophobic MILs that still possess paramagnetic behavior, a dicationic platform with $[NTf_2^-]/[FeCl_3Br^-]$ heteroanions was chosen. As shown in Figure 1, the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL takes advantage of this approach and is comprised of both hydrophobic and paramagnetic anions. Although a greater magnetic moment can be achieved by employing two $[FeCl_3Br^-]$ anions in a dicationic MIL, increased water-miscibility is also observed.[37] The cationic portions of the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ and $[(C_8)_3BnN^+][FeCl_3Br^-]$ MILs are functionalized with long alkyl chains and benzyl moieties, which significantly increases their overall hydrophobicity.

Optimization of DNA Extraction Mode. The amount of DNA extracted by the hydrophobic MIL extraction phases was evaluated indirectly by subjecting an aliquot of the post-extraction aqueous phase to HPLC analysis. An external calibration curve for both dsDNA and ssDNA was established and used to calculate the DNA concentration in aqueous solution. Values of extraction efficiency (E) were obtained using the relationship between the DNA concentration in the aqueous phase following extraction (C_{aq}) and the concentration of DNA in the standard solution (C_{st}), as shown in eq 1.

$$E = \left[1 - \frac{C_{aq}}{C_{st}}\right] \times 100 \quad (1)$$

Time-consuming centrifugation steps in extraction and purification protocols represent a major bottleneck in nucleic acid sample preparation. In the development of MIL-based DNA extraction methods, considerable attention was given to the compromise between extraction time and efficiency. Identical volumes of MIL were used to extract DNA from an aqueous solution using both SDE and DDE modes. An obvious advantage of DDE over SDE is the dynamic mixing of the MIL extraction solvent with the aqueous medium, which allows for rapid distribution of DNA between the two phases. This is illustrated in Table 1 where the extraction efficiency of stDNA is shown for the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL using both SDE and DDE modes. The relatively low extraction efficiencies observed for the SDE technique, particularly at short extraction times, are likely due to less available MIL surface area for interaction with DNA when compared to DDE. The precision of each extraction mode ranged from 1.6 to 8.7% and 0.4 to 3.4% for SDE and DDE, respectively, using triplicate extractions. While the SDE mode required 2 h to achieve an

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Table 1. Comparison of Single Droplet and Dispersive Droplet Extraction Modes for the Extraction of stDNA from an Aqueous Solution Using the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL

single droplet extraction[a]		
time (min)	% extraction efficiency (n = 3)	%RSD
10	5.5	1.6
20	33.3	3.0
30	40.5	8.7
60	60.3	3.3
90	61.6	8.6
120	63.1	4.1
dispersive droplet extraction[b]		
time (s)[c]	% extraction efficiency (n = 3)	%RSD
5	76.8	3.4
30	75.6	0.4
60	79.3	2.3
120	76.5	2.1
300	77.0	1.2

[a]Conditions: DNA concentration: 4.17 nM; volume of MIL: 20 μL; total solution volume: 2 mL; pH 8. [b]Conditions: Manual agitation time: 30 s; all other experimental parameters unchanged. [c]Refers to duration of applied magnetic field.

extraction efficiency of 63.1%, the DDE mode provided efficiencies greater than 76% after just 30 s of dynamic mixing and 5 s of phase isolation by exposure to a magnetic field. No appreciable gain in extraction efficiency was observed when the magnetic field was applied at time points greater than 5 s. Therefore, DDE was selected as the optimum extraction mode for subsequent DNA extractions using the three hydrophobic MILs.

Effect of MIL Volume on Extraction Efficiency. The effect of MIL volume on extraction efficiency was investigated for the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ and $[P_{6,6,6,14}^+][FeCl_4^-]$ MILs. A 2 mL solution of 4.17 nM stDNA was extracted using MIL volumes ranging from 10 to 25 μL. As shown in Figure S12, larger volumes of extraction solvent provided improved DNA extraction efficiencies for both MILs. Higher extraction efficiencies were obtained using the monocationic $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL compared to the dicationic $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL, even at larger droplet volumes. A significant increase in extraction efficiency was observed for the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL when the MIL microdroplet volume was increased from 15 to 20 μL, suggesting a saturation effect at lower volumes of extraction solvent.[25,26] However, the enhancement of extraction efficiency for the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL was much less pronounced. Because 20 and 25 μL showed similar extraction efficiencies, the smaller microdroplet volume was used in subsequent studies. It is important to note that all three MILs examined in this study retained their hydrophobic character and exhibited phase separation when subjected to the external magnetic field, even at very low microdroplet volumes (e.g., 10 μL).

Effect of pH on Extraction Efficiency. The pH of environmental or biological DNA sample solutions is often variable and may have implications on the extraction behavior of interfering matrix components. As pH adjustments are often employed in sample pretreatment steps to minimize the coextraction of contaminants,[38,39] it is important to examine its effect on the MIL-based extraction of DNA. To investigate the effect of pH on extraction efficiency, solutions of stDNA ranging from pH 2.5−10.9 were prepared and subjected to MIL-based DDE. The phosphate groups of DNA molecules possess pK_a values below the studied pH range. Therefore, it is expected that they bear negative charges capable of favorable electrostatic interactions with the MIL cation.[40] As shown in Figure 2, the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL exhibited extraction

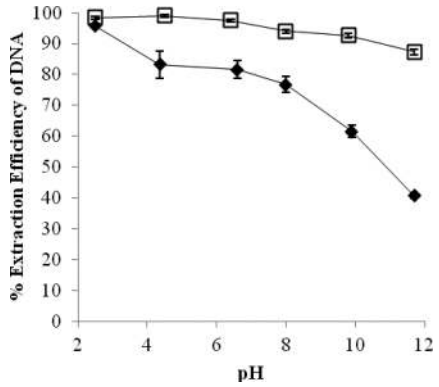

Figure 2. Effect of aqueous solution pH on the extraction efficiency of stDNA using MIL-based dispersive droplet extraction. Open squares (□) represent the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL, while diamonds (◆) indicate the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL.

efficiencies greater than 87% across the pH range studied. Furthermore, the extraction efficiency of stDNA for the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL showed little dependence on the pH of the solution. In contrast, a considerable decrease in extraction efficiency was observed when the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL was used to extract stDNA from increasingly basic solutions. In an effort to maintain high extraction efficiency while avoiding the harsh pH extremes that may compromise the structural integrity of DNA, pH 8 was selected for subsequent extractions.

Extraction of Single-Stranded Oligonucleotides and Duplex DNA. Short length nucleic acids play a central role in molecular recognition and hybridization applications.[2] To investigate the feasibility of extracting smaller DNA molecules, MIL-based extraction was applied to short length single-stranded oligonucleotides and duplex DNA. As shown in Table 2, the extraction of low molecular weight dsDNA and ssDNA appears to be MIL-dependent. In the case of the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL, extraction efficiencies of 69.3% and 57.6%

Table 2. Extraction Efficiencies of dsDNA and ssDNA Using the Three Hydrophobic MILs

MIL	% extraction efficiency of 20 kbp stDNA[a] (n = 3)	% extraction efficiency of 20 bp dsDNA[b] (n = 3)	% extraction efficiency of 33-mer ssDNA[c] (n = 3)
$[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$	76.8 ± 2.6	64.0 ± 1.1	67.7 ± 3.0
$[(C_8)_3BnN^+][FeCl_3Br^-]$	41.0 ± 0.9	69.3 ± 4.4	57.6 ± 5.0
$[P_{6,6,6,14}^+][FeCl_4^-]$	93.8 ± 0.6	91.4 ± 0.3	94.0 ± 0.2

[a]Conditions: 4.17 nM; total solution volume: 2 mL; pH 8; volume of MIL: 20 μL; manual agitation time: 30 s. [b]Conditions: 1224 nM; other conditions held constant. [c]Conditions: 1499 nM; other conditions held constant.

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were observed for 20 bp DNA and 33-mer ssDNA, respectively. However, the same MIL produced an extraction efficiency of only 41.0% for stDNA indicating that it appears to preferentially extract smaller oligonucleotides. In contrast, the dicationic MIL exhibited higher extraction efficiency values for stDNA than the 20 bp dsDNA, while the $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MIL provided extraction efficiencies exceeding 91% for stDNA, 20 bp dsDNA, and ssDNA. These preliminary data indicate that it may be possible to design MILs that are selective for particular sizes of oligonucleotides or duplex DNA.

Extraction of DNA from a Complex Matrix. Components of biological samples, such as metal ions and proteins, are known to diminish the sensitivity and reproducibility of nucleic acid analysis.[9] In some cases, the viability of downstream experiments may be compromised if the sample is not sufficiently purified from contaminants.[10,11] Thus, it is important to determine the effect of biologically relevant impurities on MIL-based DNA extraction. To study this, a complex matrix was simulated through the addition of metal ions or proteins (albumin) to an aqueous solution of DNA.

The extraction performance of the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$, $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$, and $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MILs was evaluated for 20 kbp stDNA in the presence of NaCl, KCl, $CaCl_2{\cdot}2H_2O$, and $MgCl_2{\cdot}6H_2O$. Figure S13 shows that the extraction efficiency for the dicationic $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$ MIL was somewhat diminished by the addition of the mono- and divalent metal ions, in contrast to what was observed for monocationic imidazolium-based ILs.[25] A very small to negligible variation in extraction efficiencies was observed for the $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$ and $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MILs.

The effect of protein on the extraction efficiency of DNA was studied by preparing aqueous 20 kbp stDNA solutions containing albumin as a model protein. The extraction efficiencies of both stDNA and albumin were monitored over a pH range from 3.5 to 8. As shown in Figure 3, each of the three studied MILs exhibited unique extraction behavior in the presence of stDNA and albumin. Figure 3A shows that high extraction efficiencies for both stDNA and albumin were obtained using the dicationic $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$ MIL at pH 8. Interestingly, a comparison of Figure 2 and Figure 3A reveals that the extraction efficiencies of stDNA in the absence of albumin were similar to those observed after albumin had been spiked into the aqueous solution. However, Figure 2 and Figure 3B show that the extraction efficiency of stDNA for the $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MIL was decreased by 46% in the presence of albumin at pH 8. As shown in Figure 3C, the $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$ MIL provided relatively lower extraction efficiencies of stDNA across the pH range studied.

With an isoelectric point of 4.6, albumin possesses an overall negative charge at higher pH and may compete with DNA by also engaging in electrostatic interactions with the MIL cation.[41] To examine this effect, the pH of the sample solution was lowered which resulted in a corresponding decrease in the amount of extracted albumin for the $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ and $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$ MILs. Furthermore, lowering of the sample pH significantly enhanced the extraction efficiency of stDNA for the $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MIL. Although these results seem to suggest that electrostatic interactions between the MIL and albumin are diminished at low pH, coextraction of albumin was still observed for all three MILs investigated. This may be due to interactions between the

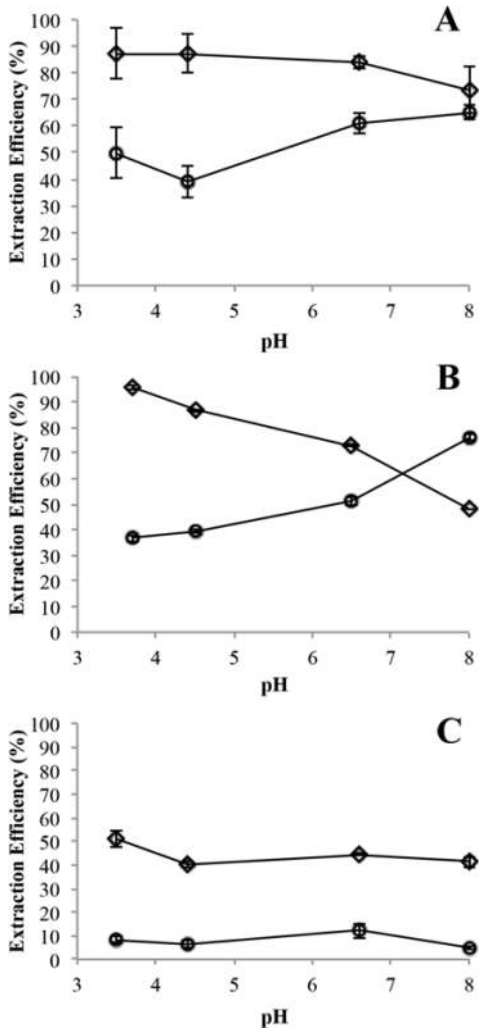

Figure 3. Effect of hydrophobic MIL type, pH, and albumin on the extraction efficiency of 20 kbp stDNA: (A) $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$, (B) $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$, and (C) $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$. Diamonds (◊) represent extraction efficiency values of stDNA and circles (○) denote extraction efficiencies of protein.

hydrophobic amino acid side chains of albumin and the long alkyl groups of the MIL cations that promote the extraction of protein, regardless of solution pH.[41] As shown in Figure 3C, the coextraction of albumin was less pronounced when employing the $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$ MIL. Although it extracted less stDNA compared to the other two MILs, the $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$ MIL exhibited an albumin extraction efficiency of just 5.0% at pH 4.4, while the $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^{-}]$, $FeCl_3Br^{-}]$ and $[P_{6,6,6,14}^{+}][FeCl_4^{-}]$ MILs produced extraction efficiencies nearing 40% at the same pH. These findings suggest that DNA extracted by the $[(C_8)_3BnN^{+}][FeCl_3Br^{-}]$ MIL microdroplet may have less protein contamination than DNA extracted by the other two MILs under the same conditions. Though not fully understood, the extraction behavior of the three MILs investigated in this study suggests that it may be possible to design MIL-based solvents capable of enhancing the selectivity toward DNA in the presence of proteins.

Recovery of DNA from the MIL Extraction Phase. The recovery of high quality DNA following an extraction step is important for accurate downstream analysis, especially in PCR

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and DNA sequencing experiments.[3,4] To ensure that DNA extraction performed by the MIL solvent did not alter any portion of the DNA sequence, pDNA extracted by the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL was subjected to sequence analysis. The MTAP gene sequence obtained from pDNA extracted by the MIL and the sequence of a pDNA standard are shown in Figures S14 and S15, respectively. The pDNA extracted by the MIL was shown to contain a MTAP gene identical to the standard, indicating that the pDNA was not altered during the MIL extraction step or that the amount of any alterations to the integrity of the biomolecule are sufficiently low to be detected.

To assess the total quantity of DNA recovered after MIL-based DNA extraction, a 4.17 nM solution of stDNA was extracted using the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL. After dissolution of the stDNA-enriched MIL microdroplet in 3 M potassium acetate (pH 4.8), the sample was loaded onto silica sorbent. The sorbent was flushed with 1 mL of isopropanol, and the first fraction was collected, which contained stDNA and excess salt. The stDNA was precipitated with cold ethanol, and the excess salt was removed by washing the pellet with 80% ethanol. In this approach, HPLC analysis determined the recovery of stDNA from the MIL microdroplet to be 57 ± 6%. The yield of the MIL-based DDE method was 23.5 μg of stDNA. Comparatively, a QIAamp DNA Mini Kit was capable of recovering 84 ± 5% of the stDNA from a 4.17 nM solution with a yield of 84.4 μg.

Extraction of DNA from Bacterial Cell Lysate. To test the applicability of the MIL-based DNA extraction method, pDNA in an *E. coli* cell lysate was extracted using the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL and subjected to PCR. This MIL was chosen to minimize protein coextraction (*vide supra*). The following two methods were employed for the isolation of DNA from the MIL extraction phase: an approach targeting greater quantities of high purity DNA and a rapid approach for recovering a sufficient quantity of high quality template DNA for PCR. In order to assess whether each recovery procedure was capable of isolating PCR-amplifiable DNA from *E. coli*, pDNA was extracted from a bacterial cell lysate using the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL and subjected to both the silica-based and the rapid immersion method. As shown in Figure 4, the silica-based method provided a more intense PCR product band (Lane 3) than did the rapid immersion approach (Lane 2). Nonetheless, immersion of the pDNA-enriched MIL microdroplet in Tris-HCl for just 2 min was capable of transferring sufficient pDNA for PCR amplification and visual detection of the MTAP gene on an agarose gel. This method has great potential for high throughput nucleic acid analyses such as the rapid screening of an environmental sample for microorganisms or identification of DNA biomarkers in virtually any sample.[1,5]

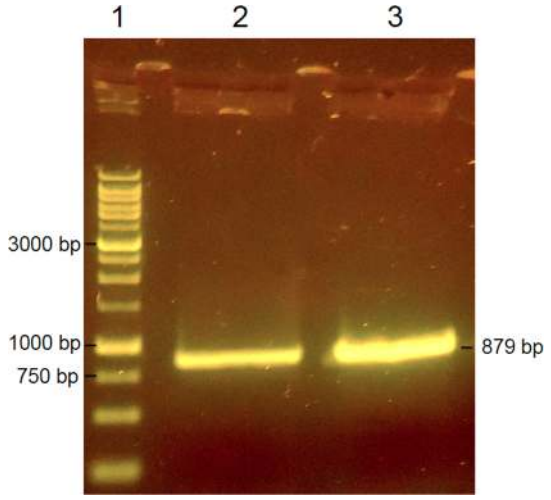

Figure 4. Agarose gel electrophoresis of the MTAP gene after PCR amplification from pDNA recovered from the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL extraction phase. Lane 1 shows a 250−25,000 bp DNA ladder, Lane 2 represents PCR products from pDNA recovered by rapid immersion of the DNA-enriched microdroplet in Tris-HCl, and Lane 3 shows the PCR products obtained from pDNA recovered by semiexhaustive DNA recovery.

CONCLUSIONS

As the demand for high-throughput nucleic acid analysis continues to grow, so does the need for developing DNA extraction methods capable of addressing the time-consuming barriers encountered during traditional extraction procedures. In this study, hydrophobic MILs were employed for the first time as solvents for the extraction of DNA from aqueous solution. The MIL-based method allows for rapid, highly efficient extractions providing a DNA-enriched microdroplet that is easily manipulated in aqueous solution by application of a magnetic field. Higher extraction efficiencies were obtained for shorter oligonucleotides and DNA duplexes with the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL, while the dicationic $[(C_{16}BnIM)_2C_{12}^{2+}][NTf_2^-, FeCl_3Br^-]$ MIL afforded higher extraction efficiencies for the much longer stDNA. MIL-based extraction of stDNA from a complex matrix containing albumin further highlighted the unique extraction profiles for the MILs, revealing competitive extraction behavior for the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL and less pronounced coextraction for the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL. These results provide a basis for the structural customization of MILs to achieve enhanced selectivity toward a variety of DNA samples. Key to the broad applicability of this method is the recovery of DNA from the MIL extraction phase which was determined to be 57 ± 6%. Furthermore, sequence analysis demonstrated that the DNA recovered from the MIL extraction phase was intact and the sequence unmodified. Plasmid DNA from a bacterial cell lysate was extracted using MIL-based DDE and shown to provide sufficient pDNA quantity and quality for PCR. These materials may serve as interesting solvent systems in many applications. A particularly intriguing application is in microfluidic devices where their paramagnetic properties can be exploited for precise control of sample movement.

ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedure, Figures S1−S15, and two videos. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 4195301508. Fax: 4195304033. E-mail: Jared.Anderson@UToledo.edu.

Notes

The authors declare no competing financial interest.

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