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Extraction and Purification of DNA from Complex Biological Sample Matrices Using Solid-Phase Microextraction Coupled with Real-Time PCR

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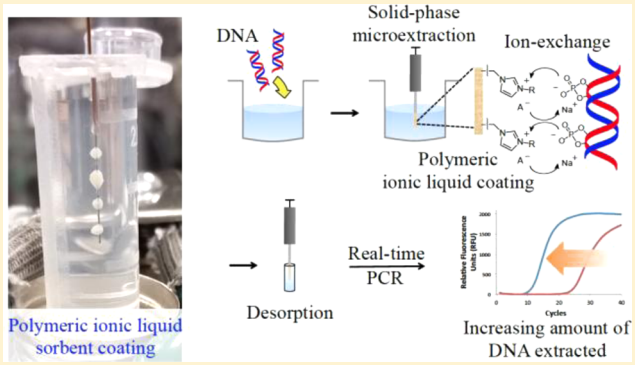
Supporting Information

ABSTRACT: The determination of extremely small quantities of DNA from complex biological sample matrices represents a significant bottleneck in nucleic acid analysis. In this study, polymeric ionic liquid (PIL)-based solid-phase microextraction (SPME) was applied for the extraction and purification of DNA from crude bacterial cell lysate with subsequent quantification by real-time PCR (qPCR) analysis. Using an on-fiber ultraviolet initiated polymerization technique, eight different PIL sorbent coatings were generated and their DNA extraction performance evaluated using qPCR. The PIL sorbent coating featuring halide anions and carboxylic acid groups in the cationic portion exhibited superior DNA extraction capabilities when compared to the other studied PILs and a commercial polyacrylate SPME fiber. Electrostatic interactions as well as an ion-exchange mechanism were identified as the driving forces in DNA extraction by the PIL sorbents. The selectivity of the PIL sorbent coating for DNA was demonstrated in the presence of PCR inhibitors at high concentration, where a quantifiable amount of template DNA was extracted from aqueous samples containing $CaCl_2$ and $FeCl_3$. Furthermore, the PIL-based SPME method was successfully applied for the extraction of DNA from crude bacterial cell lysate spiked with 1 pg mL^{-1} template DNA without requiring the use of organic solvents or centrifugation steps. Following PIL-based SPME of DNA from a dilute cell lysate, the qPCR amplification efficiency was determined to be 100.3%, demonstrating the feasibility of the developed method to extract high purity DNA from complex sample matrices.



Deoxyribonucleic acid (DNA) analysis holds great promise in a variety of applications including clinical diagnostics,[1] forensics,[2] genomics,[3] and food safety.[4] Valuable diagnostic information is often provided by subjecting DNA samples to the polymerase chain reaction (PCR) and sequencing methods. These sensitive bioanalytical techniques require the input of highly pure nucleic acids, particularly for the detection of extremely small quantities of target DNA.[5,6] Therefore, the isolation and preconcentration of DNA from complex cellular or environmental sample matrices is an important prerequisite for successful downstream analysis.

Conventional DNA purification methods have relied on phenol-chloroform liquid−liquid extraction (LLE) with modifications including surfactants,[7] sonication, fine grinding of the sample under liquid nitrogen using mortar and pestle,[8] and enzymatic degradation to assist in the removal of interfering agents.[9] However, these sample preparation procedures are often time-consuming, labor-intensive, and require multiple sample transfer steps that may result in the loss of target DNA. Furthermore, phenol-chloroform LLE methods often consume large volumes of organic solvent and are incompatible with automation. Solid phase extraction (SPE) techniques have reduced analysis times and organic solvent consumption in DNA purification by applying samples to a sorbent material that reversibly binds DNA.[10] However, SPE methods require tedious centrifugation steps, lack reusability, and suffer from high cost per sample.[11] In order to address the limitations associated with existing DNA purification methodologies, new sample preparation techniques that enable high-throughput analysis must be explored.

Solid-phase microextraction (SPME) is a solventless, nonexhaustive sample prepration technique developed by Pawliszyn and co-workers.[12] In practice, a thin layer of polymeric sorbent coating is immobilized on a solid support and exposed to either the headspace of the sample or directly immersed in the sample solution. Analytes that partition to the sorbent coating can then be desorbed and analyzed by an appropriate technique. SPME possesses a unique advantage over other methods because sampling and sample preparation are combined into a single step, often reducing the overall time required for analysis. SPME has been successfully applied in numerous areas of chemical research including environmental,[13] drug,[14] and biomolecule analyses.[15]

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Ionic liquid (IL)-based materials are becoming increasingly popular substrates in nucleic acid applications. ILs are molten organic salts possessing melting points below 100 °C. The physicochemical properties of ILs can be controlled by tailoring the structure of the IL cation and anion.[16] ILs have been applied as DNA preservation media,[17] solvents for stabilizing triplex DNA,[18] ion-conductive DNA films,[19] additives for enhancing the rate of DNA amplification in PCR,[20] and stationary phases for the separation of DNA in capillary electrophoresis.[21] Recently, our group and others have investigated various IL solvents in aqueous DNA extraction systems.[22−24] Hydrogen bonding, hydrophobic interactions, and electrostatic interactions between the cationic portion of the IL and the negatively charged phosphate groups of DNA are thought to be the driving forces for the extraction.

Polymeric ionic liquids (PILs) are a subclass of ILs generated by incorporating polymerizable groups within the cation/anion of the IL structure. Owing to their structural tunability, enhanced thermal stability, and resistance toward harsh matrix conditions, PILs have been applied as sorbent coatings in SPME. A core advantage of PILs in SPME is the ability to modify the chemical composition of the PIL sorbent to facilitate the extraction of a broad range of analytes from complex sample matrices.[25] Careful design and modification of the PIL chemical structure through imparting functional groups that engage in favorable interactions with DNA may provide enhanced DNA extraction efficiency and selectivity. Previously, Wang and co-workers investigated the application of PIL-based microspheres for the extraction of DNA from aqueous solution and demonstrated the feasibility of utilizing the PIL scaffold for DNA enrichment.[26] Until now, the PIL-based SPME platform has not been exploited extensively in the sample preparation of nucleic acids from biological samples. In a recent study, our group demonstrated the application of PIL-based SPME for the analysis of bacterial plasmid DNA (pDNA) from aqueous solution.[27] In this approach, pDNA was extracted using a PIL-based SPME device and subjected to end-point PCR amplification followed by digital imaging densitometry. Although semiquantitative end-point PCR amplification methods are useful in many cases, the post-PCR steps required for amplicon detection (e.g., gel electrophoresis) are time-consuming and require numerous sample transfer steps. Moreover, accurate and reliable quantification of nucleic acids at low concentrations is not achievable using end-point methods.[28] Real-time quantitative PCR (qPCR) amplification is a technique wherein the accumulating amplicon is detected after each cycle using fluorescent DNA probes. In addition to reducing analysis time by circumventing the need for gel electrophoresis, qPCR addresses the limitations associated with quantification in end-point PCR.[29,30]

This study constitutes the first report in the application of SPME sorbent coatings interfaced with qPCR for the extraction of bacterial pDNA from crude cell lysate. In this study, eight different PIL-based sorbent coatings were prepared and their DNA extraction performance evaluated using qPCR. By systematically modifying the PIL sorbent coating composition and observing the resulting DNA extraction performance, electrostatic interactions as well as exchangeable anions were found to play important roles in DNA extraction. The binding capacity and selectivity of the PIL-based sorbent coating for template DNA was investigated in the presence of PCR inhibitors. Under optimal conditions, the developed PIL-based SPME approach was capable of preconcentrating pDNA from crude bacterial cell lysate with sufficient quality and quantity for qPCR analysis. The fiber-based DNA extraction platform provides a simple, rapid, and automatable technique that is highly suitable for laboratory or field sampling applications.

EXPERIMENTAL SECTION

Materials and Measurements. NEB 5-alpha Competent *Escherichia coli* cells, 50 mM magnesium chloride solution, and dimethyl sulfoxide (100%) were obtained from New England Biolabs (Ipswich, MA, U.S.A.). The QIAquick Gel Extraction Kit and QIAprep Spin Miniprep Kit were purchased from QIAgen (Valencia, CA, U.S.A.). 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, U.S.A.). SsoAdvanced universal SYBR Green supermix for real-time PCR assays was obtained from Bio-Rad Laboratories (Hercules, CA, U.S.A.). DNA LoBind polypropylene micro-centrifuge tubes were purchased from Fisher Scientific. An 85 μm polyacrylate (PA) SPME fiber was obtained from Supelco (Bellefonte, PA, U.S.A.). ^{1}H NMR spectra were recorded using Bruker 500 MHz nuclear magnetic resonance spectrometer. The film thickness of the PIL sorbent coatings was determined using a JEOL JSM-6060 LV low vacuum scanning electron microscope (SEM).

Preparation of DNA Samples. A modified plasmid with a 135 bp DNA insert was used for the transformation of competent *E. coli* cells by heat shock. The transformed cells were cultured in 120 mL of Luria−Bertani (LB) media with 100 μg mL^{-1} ampicillin at 37 °C for 24 h. pDNA was then purified from the cell culture using a QIAprep Spin Miniprep kit by following the manufacturer's instructions. The concentration of the pDNA was measured using a NanoDrop 2000c spectrophotometer from Thermo Scientific (Wilmington, DE, U.S.A.). Purified pDNA containing the 135 bp target sequence was amplified by PCR using primers with sequences of 5′-CAC GCT TAC ATT CAC GCC CT-3′ and 5′-CGA GCG TCC CAA AAC CTT CT-3′. Following amplification, the amplicon was subjected to agarose gel electrophoresis using a BRL H4 Horizontal Gel Electrophoresis System from Life Technologies with a Neo/Sci dual output power supply (Rochester, NY, U.S.A.). The 135 bp amplicon was excised from the gel and purified using a QIAquick Gel Extraction kit by following the manufacturer's instructions. The concentration of DNA was measured using a NanoDrop 2000c spectrophotometer. A standard solution of 2.89 ng μL^{-1} of DNA was prepared in 1× TE buffer (pH 8.0) and serially diluted with 1× TE buffer (pH 8.0). All DNA standard solutions used in the study were stored at −80 °C and thawed on ice prior to use.

Real-Time Quantitative PCR Assays. Real-time quantitative PCR (qPCR) assays were performed on a CFX96 Touch Real-Time PCR Detection System from Bio-Rad Laboratories (Hercules, CA, U.S.A.) according to the following thermal cycling protocol: initial denaturation step of 3 min at 95.0 °C, followed by 40 cycles of 10 s at 95.0 °C and 30 s at 64.0 °C. All amplification reactions were performed in triplicate. For each reaction, 1 μL template DNA was mixed with 19 μL reaction mix containing 10 μL of SsoAdvanced universal SYBR Green supermix (2×), 2.6 μL of 50 mM $MgCl_2$, 1 μL of DMSO, 0.8 μL of 10 μM forward and reverse primers, and 3.8 μL of deionized water. For the external calibration curve, an additional 10 mM NaCl was also included in the reaction mixture. Quantification cycle (Cq) values were used to calculate the amount of DNA that was extracted using the SPME fibers.

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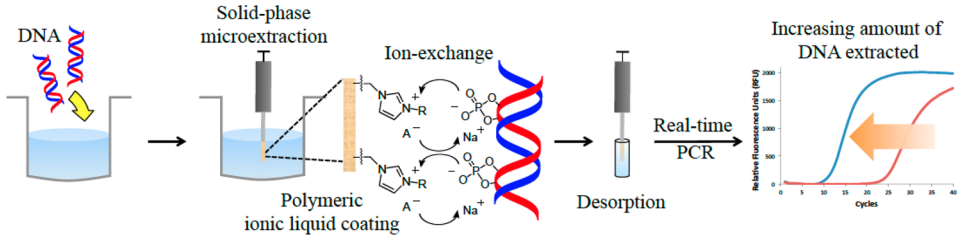

Figure 1. Schematic representation of template DNA extraction using PIL-based SPME approach and subsequent quantification utilizing a real-time PCR assay.

As shown in Figure S1, in order to evaluate the amplification efficiency, a four point calibration curve with a 10-fold dilution series was developed. The amplification efficiency was calculated using eq 1, where the slope is the slope of the linear regression in the calibration curve.

$$\text{efficiency} = [10^{(-1/\text{slope})} - 1] \times 100 \quad (1)$$

The qPCR amplification efficiency was found to be 98.3% under the conditions employed in this study.

Extraction of DNA Using SPME Devices. A schematic for the general procedure employed in DNA extraction using PIL-based SPME followed by qPCR assay is depicted in Figure 1. A 10 pg mL^{-1} solution of DNA was prepared in 10 mL of 1× TE buffer (pH 8) immediately prior to extraction. DNA was then extracted by immersing a SPME sorbent in the sample solution under agitation at 650 rpm. Following SPME, DNA was desorbed from the sorbent coating by immersion in 50 μL of 1 M NaCl desorption solution for 30 min. To relieve the inhibition caused from 1 M NaCl, 20 μL of a desorption solution was diluted five-fold with deionized water prior to the qPCR assay. Finally, a 1 μL aliquot of the diluted desorption solution was subjected to qPCR amplification, resulting in a final NaCl concentration of 10 mM. Prior to the next extraction, the SPME sorbent was washed using 2 × 10 mL of 1× TE buffer for 25 min.

RESULTS AND DISCUSSION

Structural Design of PIL Sorbent Coatings to Enhance DNA Extraction. In this study, eight different PIL sorbent coatings were prepared by combination of different IL monomers and cross-linkers, as shown in Table 1. IL monomers and cross-linkers were produced according to the reported procedures from the literature.[31−33] ^{1}H NMR was used to characterize the IL monomers and cross-linkers, as shown in Figures S2−S11 of the Supporting Information. Materials and detailed procedures employed for the fabrication of PIL-based SPME devices are described in the Supporting Information. The approximate film thickness values of all PIL sorbent coatings are shown in Table 1 and a representative SEM micrograph of a PIL-based sorbent coating (fiber **4**) is shown in Figure S12.

In SPME, the chemical composition of the sorbent coating has a profound influence on the extraction of target analytes from a sample matrix. An optimal sorbent coating can enhance the extraction performance as well as improve the sensitivity of the developed extraction procedure. To investigate the effect of the PIL sorbent coating composition on DNA extraction, the structures of IL monomers and cross-linkers were modified with different anions and a variety of functional groups appended to the IL cations. The extraction performance of the PIL sorbent coatings was examined under similar experimental conditions using qPCR as the method of analysis. The amount of DNA extracted by the sorbent coating is correlated to a Cq value obtained from qPCR analysis. A lower Cq value indicates a greater quantity of extracted template DNA. A difference in one Cq value corresponds to a 2-fold difference in the amount of extracted DNA when the amplification efficiency is 100%.

Figure 2 illustrates the DNA extraction performance of different PIL fibers and a commercial PA fiber. For PIL fibers **3** and **8** as well as the commercial PA fiber, the mass of template DNA extracted was not determined, as the high Cq values obtained were out of the calibration range, indicating the poor extraction efficiency of these fibers. As shown in Figure 2, the incorporation of benzyl moieties within the cross-linker of fiber **2** resulted in a diminished DNA extraction efficiency compared to fiber **1**. Further tailoring of the PIL sorbent coatings by incorporating polar functional groups into the cationic structure, such as a carboxylic acid (fiber **4**) or alcohol moieties (fiber **7**), resulted in higher DNA extraction than PIL sorbents lacking polar moieties. For example, the average Cq value obtained from extractions using fiber **1** was 30.42 ± 0.22, whereas fibers **4** and **7** exhibited superior DNA extraction capabilities with Cq values of 27.89 ± 0.73 and 29.25 ± 0.16, respectively. Intraday fiber-to-fiber extraction reproducibility was determined using a set of three fibers for PIL fibers **1** and **4**, resulting in Cq values with relative standard deviations (RSD) of 1.8% and 4.6%, respectively.

The anionic component of the PIL-based sorbent coating was observed to have a major influence on DNA extraction. As shown in Figure 2, substitution of halide-based anions (fiber **1**) with NTf_2^- anions (fiber **3**) within the PIL sorbent coatings of similar cationic composition resulted in a 4.5 cycle increase in the Cq value, indicating a dramatic decrease in DNA extraction performance. With a cation composition identical to fiber **4**, two sorbent coatings were prepared by replacing the halide anions with NTf_2^- anions in either the monomer (fiber **5**) or the monomer and cross-linker (fiber **6**). From Figure 2, the amount of DNA extracted using fibers **5** and **6** was lower than that observed for fiber **4**. The diminished extraction performance of fibers **5** and **6** suggests that the halide anions in fiber **4** play a crucial role in extracting higher amounts of DNA from aqueous solutions.

Optimization of Extraction Parameters for PIL Fiber 4. On the basis of its superior DNA extraction performance in comparison to the other six PIL fibers studied, fiber **4** was selected for optimization of the PIL-based SPME method. Previous investigations of DNA extraction using PIL sorbents established that a desorption solution containing 1 M NaCl was essential for recovering DNA from the sorptive phase.[27] However, when a similar concentration of NaCl was employed for desorption of DNA in PIL-based SPME interfaced with

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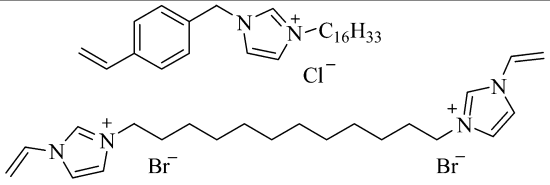
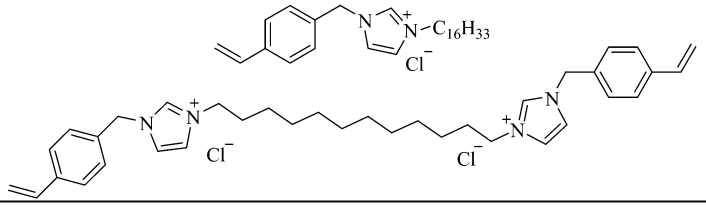
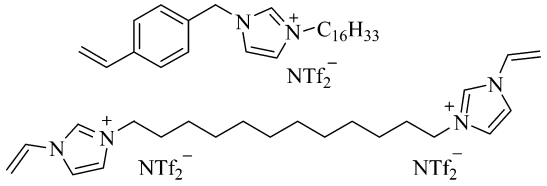
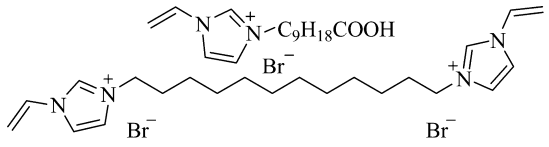
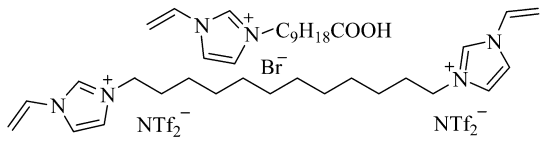
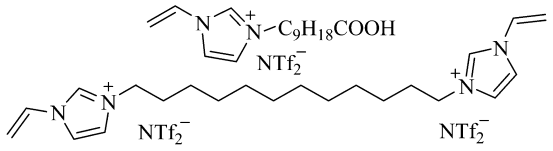
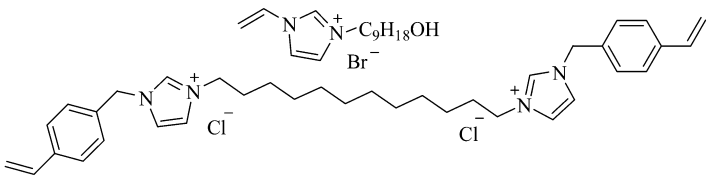
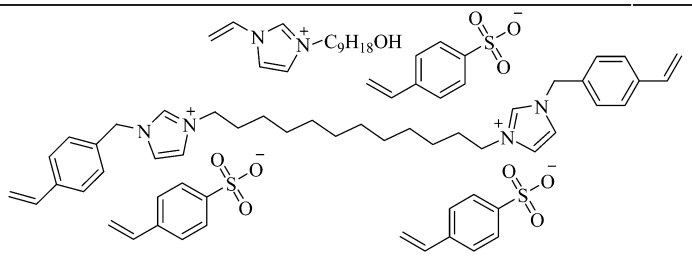
Table 1. Chemical Composition of all PIL-Based SPME Sorbent Coatings Examined in This Study for the Extraction of DNA

Fiber	IL monomer and crosslinker composition[a]	Approximate film thickness (μm)
Fiber 1		81
Fiber 2		80
Fiber 3		82
Fiber 4		69
Fiber 5		100
Fiber 6		80
Fiber 7		72
Fiber 8		81

[a]The dicationic IL cross-linker employed within each sorbent coating was 50% by weight with respect to the weight of IL monomer.

qPCR analysis, amplification was inhibited due to the high salt concentration within the qPCR solution (data not shown). qPCR amplification was recovered by performing a 5-fold dilution of the desorption solution prior to analysis. To reduce

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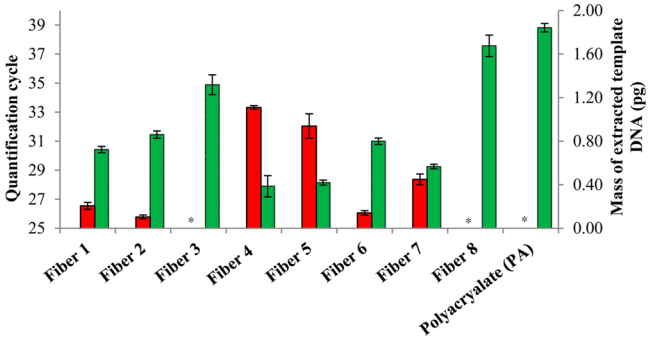

Figure 2. Comparison of extraction performance of different PIL-based sorbent coatings. All experiments were performed in triplicate (n = 3); concentration of 135 bp DNA: 10 pg mL^{-1}; sample solution: 1× TE buffer (pH 8.0); extraction time: 10 min; desorption time: 30 min; desorption solvent: 1 M NaCl; desorption solvent volume: 50 μL; (green bar) represents quantification cycles; (red bar) denotes mass of extracted template DNA. *Mass of template DNA extracted by PIL fibers **3** and **8** and a commercial polyacrylate fiber was not determined as the Cq values obtained were out of the calibration range.

carry-over effects as well as minimize the number of washing steps prior to the subsequent extraction, a desorption time of 30 min was chosen for all extractions. Figure S13 shows typical qPCR amplification plots obtained from a diluted desorption solution following PIL-based SPME (fiber **4**) and a washing fraction prior to a subsequent extraction. This demonstrates good DNA recovery and reusability of the PIL sorbent coating with no detectable carry-over.

The effect of extraction time on the amount of DNA extracted from an aqueous solution containing 10 pg mL^{-1} DNA was examined from 5 to 90 min using fiber **4**. As shown in Figure S14, the amount of DNA extracted increased with an increase of the extraction time from 5 to 60 min. However, beyond an extraction time of 60 min, no significant increase in the amount of DNA extracted was observed. In an effort to maintain a compromise between overall analysis time and the amount of DNA extracted, an extraction time of 30 min was chosen for all subsequent experiments.

The extraction of biological samples is heavily influenced by the pH of the sample solution. Careful optimization of sample solution pH often minimizes the coextraction of interfering components and enhances the extraction of target analytes.[34] In this study, the effect of pH on DNA extraction was examined by varying the pH from 2 to 10. An aqueous solution containing 10 pg mL^{-1} of DNA was employed to investigate the effect of pH on PIL-based SPME using fiber **4**. The desired sample solution pH was obtained by adjustment with either HCl or NaOH. As shown in Figure S15, higher amounts of DNA were extracted when performing extractions at pH 8 and 10 compared to pH 4 and 6. The amount of DNA extracted did not vary significantly from pH 4 to 6. However, a very high Cq value was obtained at pH 2 and may be due to depurination of the template DNA in acidic solutions.[35,36] In order to achieve high extraction efficiency while minimizing the deleterious effects of acidic pH, sample solutions were adjusted to pH 8 in all extractions.

Insight into the Extraction Mechanism of PIL Sorbent Coatings. Previous studies have postulated that hydrophobic and electrostatic interactions between ILs and DNA play a significant role in extracting DNA from aqueous solutions. In order to gain insight toward the mechanism of DNA extraction by the PIL-based sorbent coatings, the DNA extraction performance of the PIL sorbent coatings were first compared to a commercial PA sorbent phase. As shown in Figure 2, the PIL-based sorbent coatings extracted a higher amount of the template DNA compared to the PA fiber under similar experimental conditions. These results are in good agreement with a previous comparison of PIL and PA sorbents for DNA extraction.[27] Unlike the PA fiber, PIL sorbent coating possesses a cross-linked cationic framework that is capable of engaging in electrostatic interactions with the negatively charged phosphate backbone of the template DNA.

Apart from electrostatic interactions playing a significant role in extracting DNA, it is conceivable that an ion-exchange mechanism between the anions of the PIL sorbent coating and negatively charged DNA molecules may also occur. To investigate the possibility of ion-exchange, a nonexchangeable anion component was incorporated into the PIL sorbent. Previously, Feng and co-workers investigated anion exchange between a PIL sorbent coating containing p-styrenesulfonate anions and high ionic strength solutions (30% w/v NaCl) over an extended period of time. By copolymerizing p-styrenesulfonate anions with the cationic moieties of the PIL sorbent, exposure of the PIL to salt solutions for 3 h resulted in no significant variation in the sulfur composition of the PIL as determined by elemental analysis, indicating that the PIL anions were not exchanged with the salt solution.[33] In order to evaluate the ion-exchange interactions between the PIL sorbent coating and template DNA molecules, a similar approach was applied in which the halide-based anions of fiber **7** were substituted with polymerizable p-styrenesulfonate anions to yield fiber **8**.

Although the fibers possessed identical cationic composition, Figure 2 shows a dramatic decrease in the amount of DNA extracted by fiber **8** compared to fiber **7**. The diminished extraction performance observed for fiber **8** may be due to the lack of exchangeable anions within the PIL sorbent coating due to copolymerization of the anion with the cationic framework.[33] The results indicate that a combination of electrostatic interactions and ion-exchange comprise the driving forces for the extraction of DNA by PIL-based sorbent coatings. Since ion-exchange processes occur rapidly, the data in Figure S14 seem to indicate that the electrostatic interactions between the PIL and DNA may contribute to longer extraction times.

Effect of PCR Inhibitors on DNA Extraction Using PIL-Based SPME. qPCR is an extremely powerful, rapid, and sensitive diagnostic tool for the analysis of nucleic acids. However, when applied to biological samples the sensitivity and reproducibility of the technique can be drastically reduced, owing to the presence of PCR inhibitory components such as metal ions.[6,37] Since the ion-exchange mechanism between the PIL sorbent coating and DNA may also be influenced by the presence of metal salts, the DNA extraction capability of fiber **4** was evaluated in the presence of $FeCl_3$ and $CaCl_2$. A complex sample matrix was simulated by the addition of varying amounts of metal salts to a 10 pg mL^{-1} aqueous DNA solution. Under the studied qPCR conditions, the minimum inhibitory concentrations of $FeCl_3$ and $CaCl_2$ were determined to be 30 μM and 5 mM, respectively (data not shown), which also represent metal ion concentrations found within clinically relevant samples[6] and food samples.[37] However, to evaluate the DNA extraction performance of the PIL sorbent coating (fiber **4**) in rigorous sample environments, higher concentrations of

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$FeCl_3 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$ were employed in aqueous DNA solutions.

As shown in Figure 3A, by increasing the concentration of $CaCl_2 \cdot 2H_2O$ from 0 to 100 mM, the amount of template DNA

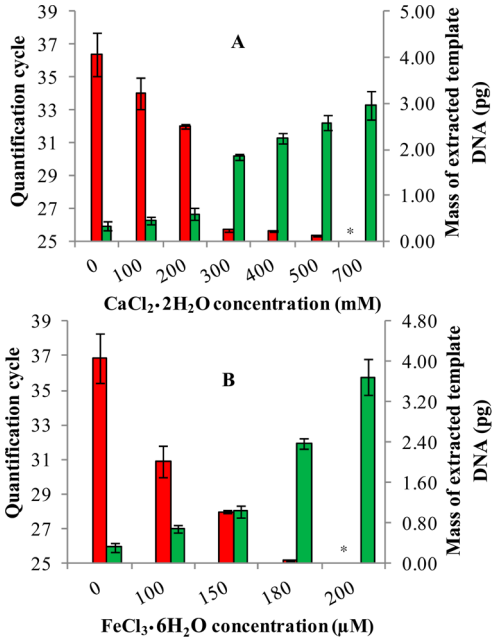

Figure 3. DNA extraction using fiber **4** in the presence of two know PCR inhibitors (A) $CaCl_2 \cdot 2H_2O$ and (B) $FeCl_3 \cdot 6H_2O$. All experiments were performed in triplicate (n = 3); concentration of 135 bp DNA: 10 pg mL^{-1}; sample solution: 1× TE buffer (pH 8.0); extraction time: 30 min; desorption time: 30 min; desorption solvent: 1 M NaCl; desorption solvent volume: 50 μL. *Mass of extracted template DNA was not determined as the Cq value obtained was out of the calibration range: (red bars) mass of extracted DNA; (green bars) denotes quantification cycle.

extracted by fiber **4** decreased slightly and produced a 0.35 unit increase in Cq value. A gradual decrease in the extraction performance of the fiber was observed when the concentration was increased to 700 mM; unfortunately, at concentrations higher than 700 mM, no amplicon was detected. This may be attributed to either the lower amount of DNA extracted by the sorbent coating or coextraction of calcium ions and subsequent inhibition of qPCR amplification. Figure 3B illustrates the effect of $FeCl_3 \cdot 6H_2O$ concentration on DNA extraction using fiber **4**. By increasing the concentration of $FeCl_3 \cdot 6H_2O$ to 100 μM, approximately 50% less DNA was extracted when compared to an aqueous DNA solution without metal ions. Nevertheless, a quantifiable amount of template DNA was extracted by the fiber even at concentrations as high as 180 μM $FeCl_3 \cdot 6H_2O$. Further addition of $FeCl_3 \cdot 6H_2O$ resulted in a drop of the sample solution pH (pH < 3) and no amplicon was detected following PIL-based SPME. These results demonstrate that the PIL-based SPME platform can function as a viable sample preparation tool in selectively extracting DNA from metal rich biological samples.

Extraction of DNA from Bacterial Cell Lysate. The extraction and purification of extremely small quantities of DNA from complex biological or environmental samples represents a significant bottleneck in nucleic acid analysis. The PIL-based SPME platform provides a simple and rapid technique for DNA extraction that eliminates the tedious, multistep sample pretreatment procedures required for conventional methods. Because interfering constituents within complex sample matrices may influence the DNA binding capacity of the SPME sorbent coating and alter extraction performance, the effects of a bacterial cell lysate sample matrix on the PIL-based SPME method were investigated. Initially, the DNA binding capacity of fiber **4** was studied in 1× TE buffer. As shown in Figure S16, an increase in the amount of DNA extracted was observed upon increasing the concentration of template DNA from 0 to 6 pg mL^{-1}, after which no significant change in Cq value was detected. This suggests that fiber **4** reached a saturation point in extracting template DNA from buffered aqueous solution at around 6 pg mL^{-1}.

To evaluate the effect of matrix components on DNA extraction using the PIL-based SPME approach, a complex sample matrix consisting of crude bacterial cell lysate was prepared by subjecting *E. coli* cells (approximately 8.12×10^8 cells) to alkaline lysis. Detailed procedures for cell cultures and cell lysis conditions are described in the Supporting Information. Template DNA was spiked at three concentration levels in crude cell lysate, dilute cell lysate, and an aqueous sample matrix, and subsequently extracted using fiber **4**. As shown in Table S1, DNA concentrations of 1, 4, and 8 pg mL^{-1} in crude cell lysate samples yielded Cq values that were significantly higher than the corresponding aqueous sample solutions, indicating a strong matrix effect on DNA extraction. The DNA extraction performance of fiber **4** was compared to a commercially available SPE kit, which provided Cq values of 18.31 ± 0.32 following the DNA extraction protocol described by the manufacturer. Nevertheless, a quantifiable amount of template DNA was extracted using SPME with good reproducibility in a process that did not require centrifugation or the use of organic solvent to extract DNA. In an effort to minimize the effect of the sample matrix, crude bacterial cell lysate samples were diluted with 1× TE buffer in a 1:0.5 ratio prior to DNA extraction. Figure 4 shows a comparison of the qPCR amplification plots following PIL-based SPME of the three studied sample matrices spiked with 8 pg mL^{-1} template

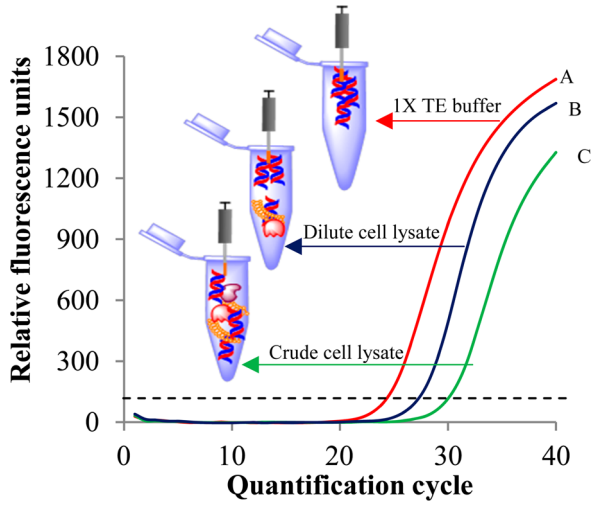

Figure 4. Representative qPCR amplification plot following PIL-based SPME using fiber **4** from different sample matrices: (A) 1× TE buffer (pH 8); (B) dilute *E. coli* cell lysate with 1× TE buffer in 1:0.5 ratio; (C) *E. coli* crude cell lysate. Spiked 135 bp DNA concentration: 8 pg mL^{-1}; extraction time: 30 min; desorption time: 30 min; desorption solvent: 1 M NaCl; desorption solvent volume: 50 μL.

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Table 2. Features of PIL-Based SPME and Commercial SPE DNA Extraction Methods

method	quantification	preparative applications	organic solvent	vacuum manifold/ centrifugation	reusability[a]	sorbent
PIL-based SPME	yes	no	not required	not required	~50 extractions	tunable polymeric ionic liquid
SPE kit	yes	yes	ethanol/isopropanol	required	not recommended	silica

[a]Extraction of DNA from bacterial cell lysate.

DNA. A significant increase in the amount of amplifiable DNA was extracted following dilution of the crude bacterial cell lysate. Table S1 shows a similar increase in the amount of amplifiable DNA for all spike concentrations upon diluting the crude cell lysate with 1× TE buffer. Interday extraction reproducibility was evaluated at a spiked template DNA concentration of 8 pg mL^{-1} in dilute cell lysate samples, resulting in Cq values with RSD of 2.9% (n = 6). As shown in Figure S17, to evaluate the quality of template DNA extracted from dilute cell lysate sample (spiked template DNA concentration of 8 pg mL^{-1}), a four point calibration curve with 2-fold dilution series was developed. The amplification efficiency and linearity of the calibration curve were found to be 100.2% and R^2 = 0.98, respectively. Furthermore, as shown in Figure S18, when a melting point analysis was performed on the qPCR amplified product following PIL-based SPME on the dilute cell lysate sample (spiked template DNA concentration 8 pg mL^{-1}), no significant variation in melting point was detected from the standard (1 pg mL^{-1} in 1× TE buffer), indicating the quality of template DNA extracted from dilute cell lysate remains identical to the standard sample.

Encouraged by these results, the PIL-based SPME method was applied for the extraction of 6 kb pDNA containing a 135 bp DNA insert from the crude lysate of approximately 5.12 × 10^7 *E. coli* cells. Detailed procedures for cell culture and lysis are described in the Supporting Information. It is worthwhile to mention that no amplification was observed after direct analysis of 1 μL of the crude cell lysate sample by qPCR. However, amplification was detected following PIL-based SPME of the bacterial cell lysate with a Cq value of 23.22 ± 0.32 (n = 3), as determined from the qPCR amplification plot, demonstrating the suitability of this technique for the extraction of DNA from complex samples.

After performing the aforementioned experiments, as described in the previous sections (approximately 85 extractions), fiber reusability was investigated by determining the DNA extraction capability in aqueous solution. The RSD of the Cq values obtained for triplicate extractions using the PIL-based SPME approach was 2.7%. Because of its simplicity, reusability, and portability, PIL-based SPME represents an alternative to existing technologies that require organic solvents and time-consuming centrifugation steps for DNA extraction, particularly in resource limited settings and field sampling applications (Table 2).

CONCLUSIONS

As the nucleic acid field trends toward faster, more selective, and more portable analysis methods, a major bottleneck is represented by the lack of sample preparation techniques that satisfy these criteria. This study demonstrates, for the first time, the coupling of SPME with real-time qPCR for the analysis of DNA. By modifying the chemical composition of the PIL-based sorbent coating to include halide anions and polar groups in the cation component, the DNA extraction performance of the PIL-based method was enhanced relative to six other PILs and a commercial sorbent. The DNA extraction mechanism of the PIL sorbent coatings was investigated by incorporating a polymerizable anion component incapable of undergoing ion-exchange into the PIL fiber. The diminished DNA extraction performance of the PIL fiber bearing polymerizable anions compared to PIL fibers with exchangeable anions established the importance of ion-exchange in the mechanism of DNA extraction by PIL sorbents. To evaluate the selectivity of the best performing PIL fiber **4**, DNA was extracted from aqueous solutions spiked with metal salts at concentrations higher than the qPCR minimum inhibitory concentration. Amplification was detectable for $FeCl_3{\cdot}6H_2O$ and $CaCl_2{\cdot}2H_2O$ concentrations as high as 180 μM and 700 mM, respectively, demonstrating the applicability of the PIL-based SPME method for DNA extraction from samples with high metal salt content (e.g., blood or milk samples). Furthermore, PIL-based SPME was applied for the extraction of DNA from bacterial cell lysate, where the isolated DNA was of sufficient quantity and purity for high efficiency amplification in qPCR. The results demonstrate that PIL-based SPME constitutes a selective and portable nucleic acid extraction technique that can be readily interfaced with qPCR for the rapid and sensitive analysis of DNA.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01861.

Figures S1−S17, Table S1, materials and fabrication procedures for preparing PIL fibers, conditions for cell cultures, and lysis protocols (PDF).

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Notes

The authors declare no competing financial interest.

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