MICROFLUIDIC CAPILLARY ELECTROPHORESIS SYSTEM FOR ORGANOCHLORIDE DETECTION AND SPECIATION

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ABSTRACT

We present a novel labeling technique for detection and speciation of organochloride compounds using microcapillary zone electrophoresis coupled with laser induced fluorescence detection. Organochloride compounds in an aqueous mixture are conjugated with glutathione using 1,5 Diazabicyclo [4.3.0] non-5-ene as a catalyst. The conjugated species are then labeled with Pacific Blue succinimidyl ester and analyzed using a commercially available microcapillary electrophoresis chip. This approach was used to separate and identify trichloroethylene and dichloromethane in an aqueous mixture. In addition, we present a method for programmable, automated sample processing of commercially available CE microchips using Lifting Gate microvalve technology.

KEYWORDS: Microvalve, Trichloroethylene, Dichloromethane, Capillary Electrophoresis.

INTRODUCTION

Due to their extensive use as industrial solvents and metal degreasers, chlorinated organic solvents, such as trichloroethylene (TCE) and dichloromethane (DCM), have become persistent contaminants in certain groundwater environments. Currently, the most common methods for detection and analysis of chlorinated organic compound are gas chromatography (GC) coupled to a mass spectrometer (MS), electron capture detector (ECD), Hall's electrolytic conductivity detector (HECD), or a flame-ionization detector (FID). Although the limit of detection is in the sub-ppb range for HECD and in the low-ppb range for MS,[1] samples have to be transported to the laboratory, with the risk of analyte volatilisation or degradation. Although several portable sensors with TCE detection capabilities have been developed such as a fiber-optic-based TCE sensors,[2] microbial TCE sensors,[3] and flow injection TCE sensors,[4] each have significant drawbacks including instability, high cost, long response time, lack of specificity, and low detection sensitivity. Existing technologies therefore lack the capability to perform *in situ* organochloride speciation despite the fact that on-site measurement capability can significantly reduce time and cost, as well as enable real-time data for better decision making.

Microcapillary zone electrophoresis (μCZE) coupled with laser induced fluorescence (LIF) detection has enabled high sensitivity separation and analysis of a broad range of compounds within complex sample types. Furthermore, this technology is readily miniaturized into a portable format for field analysis, and has been coupled with automated microfluidic sample processing technology.[5] Despite these advantages, this approach has not been used for analysis of organochloride contaminants in groundwater environments since many of these compounds cannot be readily "tagged" with any commercial fluorophores.

Here we demonstrate a novel approach for fluorescently labeling organochoride compounds within a mixture and separation via capillary zone electrophoresis (Figure 1). Glutathione has a thiol group that can form conjugates with a variety of species including organochloride compounds. To our knowledge, this property has not previously been used to fluorescently label organochloride compounds for μCZE analysis. We used Diazabicyclo [4.3.0] non-5-ene (DBN) as a catalyst for the conjugation of TCE and DCM with glutathione (GSH) to form S-(1,2-dichlorovinyl) glutathione (DCVG) and S-(chloromethyl) glutathione (SCMG), respectively.[6]

The use of PDMS, "Lifting Gate" microfluidic technology enables direct, and facile integration of microfluidic processors with glass microcapillary electrophoresis chips.[5] Here we demonstrate an application of this technology to automate sample processing for commercially available μCZE chips.

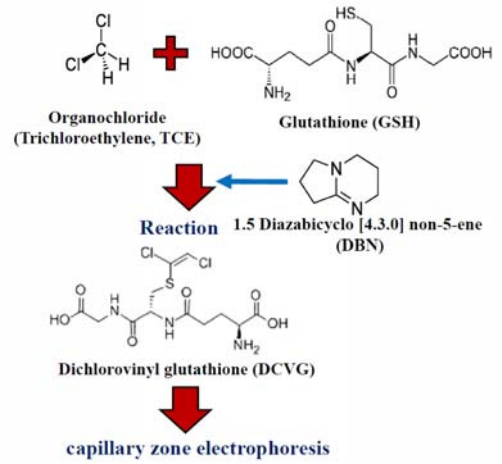

Figure 1: Organochloride labeling reactions.

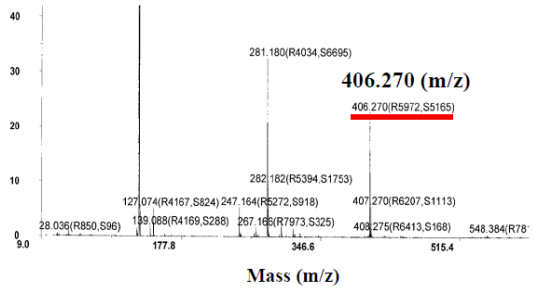

Figure 2: Confirmation of organochloride conjugated with GSH by mass spectrometry, 406 m/z.

EXPERIMENTAL

The glutathione labeling reaction was performed for a mixture of TCE and DCM with the following conditions: 111 µM DBN, 26 µM GSH, 6 µM TCE, 6 µM DCM. These reactions are performed in aqueous solution at room temperature for 30 minutes, and then stored at 2-8 °C until further analysis. In a separate reactions containing only TCE or DCM, the presence of DCVG and SCMG were confirmed by mass spectrometry. Figure 2 shows the mass spectrum for the TCE conjugation reaction.

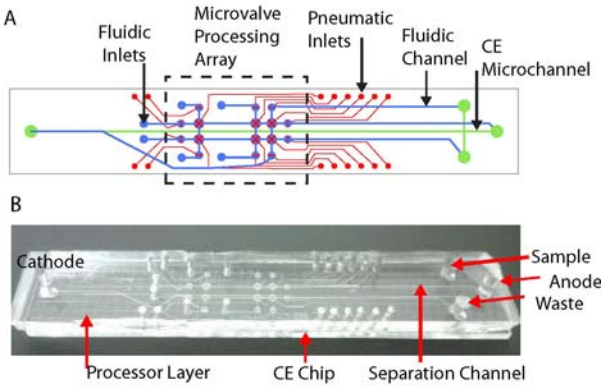

Figure 3: (A) Layout of the Lifting Gate microfluidic processor integrated with a commercial CE microchip. Electrophoretic, fluidic, and pneumatic channel features are shown in green, blue and red respectively. The pneumatic and fluid layers are formed in PDMS and bonded to the glass CE microchip. (B) Photograph of the assembled device.

The resulting glutathione conjugates, which contain free primary amines, were fluorescently labeled with Pacific Blue succinimidyl ester using a previously described procedure.[5] Samples labeled with Pacific Blue were diluted in pH 9 borate buffer and then analyzed using a commercially available µCZE chip (Micralyne, Inc.) This glass chip contains a cross injection structure and a separation channel that is 80 mm long, 50 µm wide and 20 µm deep. Confocal fluorescence detection was performed using a 405 nm laser excitation source and a PMT detector.

The automated sample processor for commercial µCZE chips, which uses a Lifting Gate microvalve design,[5] is shown in Figure 3. Six punched holes in the processing array serve as reagent inlets and reaction reservoirs. Microvalves are actuated by vacuum and pressure pulses applied to the pneumatic inlets using computer controlled solenoid valves (The Lee Company). The 2D microvalve array in the center of the chip enables automated sample processing operations and transfer to the sample inlet of the µCZE chip. As a preliminary test of our sample processor, we developed a program for automated dilution of a sample and transfer to the sample inlet of the µCZE chip. This was followed by electrophoretic separation and LIF analysis.

RESULTS AND DISCUSSION

Figure 4 shows the μCZE results for a mixture containing TCE and DCM, labeled according to the method described above. A DCVG standard was used to confirm the presence and concentration of DCVG in the reaction. The DCVG peak corresponds to approximately 100 nM, which indicates a 1.2% yield for the TCE/glutathone conjugation. The additional peak resulting from the reaction of DCE with glutathione was attributed to SCMG. Figure 5 shows results from an automated program for dilution and transfer of Pacific Blue labeled glutathione to the μCZE sample reservoir. Compared to manual sample processing and loading, the on-chip program resulted in higher overall signal and peak efficiency.

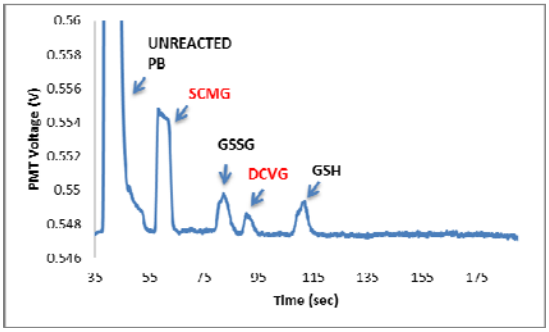

Figure 4: Electropherogram of organochloride speciation. Glutathione resulted in two peaks due to the presence of oxidized (GSSG) and reduced (GSH) forms.

CONCLUSION

The results presented here demonstrate an effective method for detection and quantification of organochloride species in an aqueous mixture. We are currently developing automated programs for glutathione conjugation and Pacific Blue labeling to enable fully autonomous sample processing.

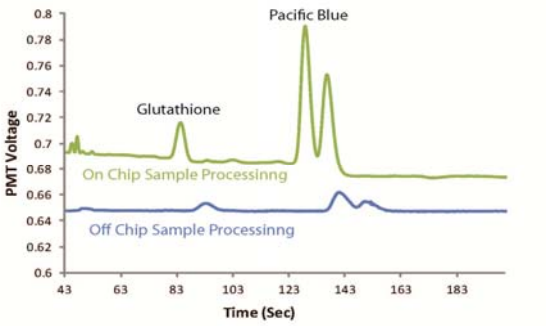

Figure 5: Results of an automated sample processing program for dilution and sample loading of Pacific Blue labeled glutathione.

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