ON-CHIP SEPARATION AND DETECTION OF NON-FLUORESCENT TOXINS IN WATER USING FLUORESCENT MOBILITY MARKERS

Tarun Khurana and Juan G. Santiago
Department of Mechanical Engineering, Stanford University, USA

ABSTRACT

We present an indirect fluorescence detection technique to detect unlabled/untreated toxins such as phenol, cresol and their derivatives present in water. We leverage isotachophoresis and fluorescent species termed as mobility markers to preconcentrate, separate and indirectly detect the nonfluorescent toxins using standard fluorescence detection system. We easily achieve ~ 1 μM detection sensitivity with high reproducibility with this technique.

KEYWORDS: Indirect detection, Mobility Markers, Isotachophoresis, Spacers

INTRODUCTION

Release of compounds of phenol and their derivatives are a persistent and high-impact health issue associated with industrial waste water. Phenol compounds are identified by the Environmental Protection Agency (EPA)[1] as toxic and their concentration in waste water is regulated and monitored to reduce their environmental impact. Traditional detection techniques such as colorimetry, chromatography and capillary electrophoresis (CE) either lack sufficient sensitivity or require time-consuming derivitization and sample preparation.[2] We here present a novel technique which combines isotachophoresis and fluorescent mobility markers for to detect non-fluorescent phenol and cresol and their derivatives..

THEORY

We recently described a method to detect non-fluorescent analytes on standard microfluidic chip platforms equipped with fluorescence detection.[3] We leverage isotachophoresis (ITP) to electrophoretically segregate both analytes and fluorescent species termed mobility markers into distinct zones. We employ a high mobility leading electrolyte (LE) and a low mobility trailing electrolyte (TE), as is done in standard ITP. As shown in Figure 1, we inject a mixture of non-fluorescent analytes and fluorescent markers between the LE and TE in a microfluidic channel. Under the influence of the applied electric field, the unlabeled analytes and fluorescent makers segregate into contiguous zones arranged in order of reducing mobility and move at identical speed. The non-fluorescent analyte zones are detected as dark regions or "gaps" between the fluorescent marker zones. The mobilities of markers on either side of the analyte zone provide upper and lower bound of that analyte's mobility. Also, the width of the "gap" between adjacent fluorescent marker zones is a linear measure of initial concentration of the unlabeled analyte(s) sandwiched between them.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

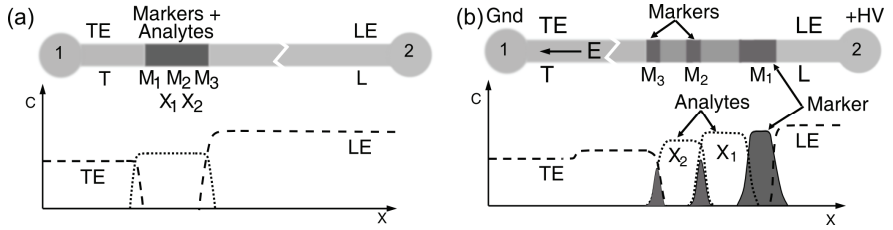

Figure 1: Schematic of the ITP-fluorescent marker assay for indirect fluorescence detection of unlabeled analytes. A plug of mixture of unlabeled analyte and fluorescent mobility markers is injected between LE and TE. Upon application of an electric field, markers and analyte ions self-segregate into distinct zones. Fluorescent markers are directly detectable, and a gap in the marker signal unambiguously marks the presence of one or more analytes whose mobilities are bounded by the two adjacent fluorescent markers.

EXPERIMENTAL

We have performed extensive ITP/mobility marker separation and detection with a variety of non-fluorescent analytes. For the detection of phenol and p-cresol we used 250mM Bis-tris-propane (BTP) and 25 mM acetic acid (pH 8.1) as the LE and the TE contained 100 mM BTP 100 mM EACA (epsilon-amino caproic acid) and 5 mM barium hydroxide. For the detection of nitro and chlorophenols, we changed the LE to 20 mM BTP -10 mM HCl and added 2% PVP to dynamically suppress electroosmotic flow. We used an NS-95 microchip (Caliper, Mountain View, CA) with a simple cross pattern and 34 μm wide and 12 μm deep channels and used standard epifluorescent microscope setup for imaging We applied ~600 V across the microchannel using a high voltage power supply (Labsmith, HVS-300D) to initiate ITP focusing of analyte and mobility marker zones. We chose commercially available fluorophores, AlexaFluor 488, Oregon Green Caroxylic acid, Fluorescein, Bodipy and dextran-AlexaFluor (MW-10,000) and green fluorescent protein (sgGFP) as the mobility markers to finely bracket the toxin zones.

RESULTS AND DISCUSSION

We first show separation and detection of phenol and cresol using three mobility markers. In Figure 2, we show separation and detection of phenol (30 μM – 50μM) and cresol (50 μM) using an additional mobility marker. In these experiments, we used optimized LE composition to achieve rapid separation these two analytes whose fully-ionized electrophoretic mobilities differ by <3%.

In Figure 3, we demonstrate detection and separation of 2-nitrophenol (NP), 2-chlorophenol (CP), 2,4,6 trichlorophenol (TCP) and of p-Cresol (Cre). We here imp roved detection sensitivity by using three different strategies (i) lowering the LE concentration (10 mM BTP-HCl) (ii) adding barium hydroxide to the TE to prevent zone widening due to carbonic acid from dissolved CO_2 (iii) adding 2% polyvinyl pyrrolidone (PVP) to the solutions to dynamically suppress EOF. The mobility marker peak dispersion is significantly reduced as seen in Figure 3(a)-(c).

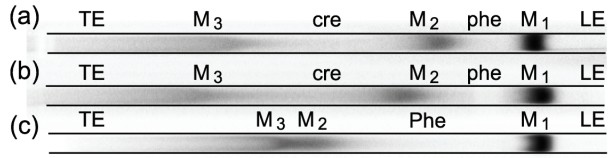

Figure 2. Inverted intensity images of fluorescent markers demonstrating separation and detection of phenol (phe) and cresol (cre). In case (a) and (b), the initial cresol concentration was 50 µM and initial phenol concentration was 30 µM and 40 µM respectively. In case (c), only 50 µM phenol was present and markers M_2 and M_3 adjoined. Here M_1: Fluorescein, M_2: Bodipy, M_3: Dextran Alexa Fluor 488.

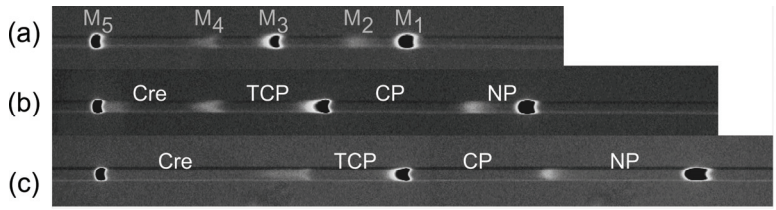

Figure 3. False color images of five fluorescent markers for indirect detection of four phenolic toxins. The three images are experiment results from three different initial concentrations of the toxins. The concentrations of nitrophenol (NP), chlorophenol (CP), trichlorophenol (TCP) and cresol (Cre) are respectively (i)10 µM, 15 µM, 15 µM and 20 µM in case a (ii) 10 µM,30 µM, 30 µM and 20 µM in case b and (iiii) 30 µM, 30 µM, 30 µM and 40 µM in case c. The mobility markers are as follows M1: Alexa Fluor-488, M2: Oregon Green carboxylic acid, M3: Fluorescein, M4: Bodipy, M5: green fluorescent protein (GFP).

CONCLUSIONS

We have demonstrated the application of our isotachophoretic mobility marker based indirect fluorescent detection technique towards detection of non-fluorescent toxins such as phenols and their derivatives that pose threat to the environment. We have also achieved improvements in detection sensitivity and robustness of the assay by optimizing the experiment conditions.

REFERENCES

[1] Keith, L. H., 1991, "Compilation of Sampling Analysis Methods," US Environmental Protection Agency, Boca Raton, FL, pp. 389-486.

[2] Edgerton, T. R., Moseman, R. F., Lores, E. M., and Wright, L. H., 1980, "Determination of Trace Amounts of Chlorinated Phenols in Human-Urine by Gas-Chromatography," Analytical Chemistry, 52(11), pp. 1774-1777.

[3] Khurana, T. K., and Santiago, J. G., 2008, "Preconcentration, separation, and indirect detection of nonfluorescent analytes using fluorescent mobility markers," Analytical chemistry, 80(1), pp. 279-286.