Final Report

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Project Title: Development of a Microfluidic Paper Analytical Device (uPAD) for Airborne Metals

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List of Terms and Abbreviations

DTT - Dithiothreitol

ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy

LOD – Limit of Detection, defined as 3-times signal to noise ratio

LOQ – Limit of Quantification, defined as 10-times signal to noise ratio

mPAD – Microfluidic paper-based analytical devices

OEL - Occupational exposure limit

PAD – Paper-based analytic devices

PEL – Permissible exposure limit

PM – Particulate Matter

Abstract

Morbidity and mortality from occupational respiratory disease are estimated to cost ten billion dollars each year in the U.S. alone. World-wide, such diseases are associated with approximately 425,000 annual occupationally-related mortalities. Yet, despite the high incidence and prevalence of occupational respiratory disease, the paradigm for assessing exposure to occupational aerosol hazards has remained largely unchanged over the last 25 years. This paradigm, designed to support monitoring for regulatory compliance, is both cost and time intensive, as collected samples must be shipped or transported to a laboratory for subsequent gravimetric or chemical analysis, taking from one to two weeks for a typical turnaround. To exacerbate the issue, current exposure assessment methods for many of these hazards lack sufficient detection sensitivity, especially when assessing exposures for task-based activities over a period of minutes to hours. Such limitations can prevent the practicing industrial hygienist from determining the source of a particular hazard, or even whether or not a hazard even exists. Consequently, there is a critical need to improve the sensitivity and timeliness of aerosol exposure assessment methods in occupational settings.

This project developed a new technology to quantify exposure to airborne metals in the workplace. The new technology is based upon *microfluidic paper analytical devices* (mPADs). An mPAD consists of a miniature capillary circuit printed onto ordinary filter paper, allowing for precise analytical chemistry to be conducted directly on the collected sample. We developed mPADs for in-situ analysis of the following metals: Pb, Cu, Mn, Ni, Fe, Cr, Zn, and Cd. This new form of analytic chemistry costs less than $1 per assay and yet was sensitive and specific enough to provide results comparable with the current state-of-the-art (inductively coupled plasma) at a fraction of the cost. Furthermore, mPADs are capable of same-day, in-field use. The mPAD has the potential to be analogous to a gas detector tube, or ‘Draeger Tube’ for aerosols. Thus, the mPAD shows promise to enable industrial hygienists to conduct exposure assessment faster, cheaper, and simpler than ever before. By increasing the efficiency and economy of hazard monitoring, industrial hygienists will find it easier to recognize, evaluate, and control workplace hazards and thus, improve worker health.

Section 1

Significant (Key) Findings.

Key Finding #1: Paper-based microfluidic technology holds tremendous promise to advance the state of the art in occupational exposure assessment for metal aerosols. This research led to the development of a simple, low-cost assay to quantify metals on air sampling filters (at levels relevant to and below published OELs).

Key Finding #2: Paper-based microfluidic technologies provide sensitive and specific detection of metals (Pb, Cu, Mn, Ni, Fe, Cr, Zn) within 25% of accepted reference methods.

Key Finding #3: Distance-based detection using paper-based microfluidic devices can quantify levels of metals on air sampling filters using a filter, a pipette, and hole punch, and a commercial microwave.

Translation of Findings.

This new form of analytic chemistry costs less than $1 per assay and yet was sensitive and specific enough to provide results comparable with the current state-of-the-art (inductively coupled plasma) at a fraction of the cost. Furthermore, mPADs are capable of same-day, in-field use. The mPAD has the potential to be analogous to a gas detector tube, or 'Draeger Tube' for aerosols. Thus, the mPAD can enable industrial hygienists to conduct exposure assessment faster, cheaper, and simpler than ever before. By increasing the efficiency and economy of hazard monitoring, industrial hygienists will find it easier to recognize, evaluate, and control workplace hazards and thus, improve worker health.

In the developing world (where occupational hazards are all the more prevalent and severe), the mPAD technology has the potential to be revolutionary. Because the mPAD costs less than 1$ to deploy, this technology can enable hazard assessment (and abatement) in resource-poor environments. To date, this work has generated eight manuscripts published in the peer-review literature and dozens of presentations to scientific, public-sector, and private-sector audiences.

Outcomes/ Impact.

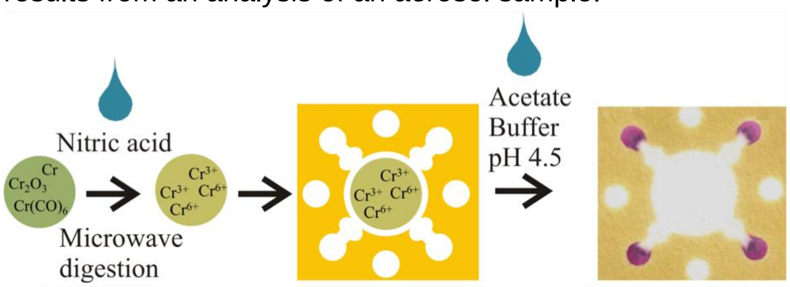
In this project we developed mPADs for in-situ analysis of the following metals: Pb, Cu, Mn, Ni, Fe, Cr, Zn, and Cd. A potential end outcome from this work is that these assays will enable same-day, in-field exposure assessment for metals, whereas, before, the time from sampling to results could last from days to weeks. Once this technology is productized/commercialized, it will enable low-cost, high-throughput screening of exposures at much larger scale (and much reduced cost). More efficient (and timely) monitoring will lead to improved hazard detection which will naturally lead to improved hazard control and worker health.

Section II. Scientific Report

The goal of this project was to develop a new, low-cost method for measuring metal exposure in occupational health settings that was faster, amenable to use at the point of measurement, and could be used with personal sampling filters. Our approach combined a new analytic technique, microfluidic paper-based analytical devices (mPADs), with improvements in sampler technology to achieve our goals. Our proposed focus was Pb, Cu, Mn, and Ni as detectable metals; during the project, we measured all of these metals as well as Fe, Cr, Zn, and Cd. We also demonstrated use of the mPAD platform to measure aerosol reactivity as an indirect measure of metal content and long-term health effect. The project had three specific aims; progress from each aim is discussed below.

Aim 1: Construct a single-analyte microfluidic paper analytical device (μPAD) for metal analysis that is compatible with existing, size-selective personal aerosol samplers.

Aim 1 sought to develop single-analyte tests for metal species using mPADs. Chemistry was to be based on a variety of published literature with adaptations for the mPAD analytic technique. We initially proposed four metals, Pb, Cu, Mn, and Ni as the target analytes but have expanded this to include Fe, Cr, Zn, and Cd. From this aim, we published a single paper on Cr analysis (Rattanrat et al., 2013) that demonstrated we could measure this important metal using a simple test. The figure below shows the device operation and typical results from an analysis of an aerosol sample.



Punch	Actual level (μg)*	Color product on the device	Measured level(μg)**
2 punches	0.41		0.47±0.01
3 punches	0.61		0.64±0.01

Figure 1
Top) Schematic showing overall analysis protocol starting from a filter sample (left). Acid digestion is accomplished directly on the filter using nitric acid and a microwave. The filter is transferred to the mPAD and acetate buffer added to elute Cr as a mixture of oxidation states to the mPAD. As Cr travels from the center to the outside spots, it reacts with Ce(IV) to oxidize all Cr to Cr(VI). Dark purple color corresponds to reaction of Cr(VI) with colorimetric indicator. Bottom) Analysis of filter samples collected from industrial incineration ash. To improve detection limits, multiple punches were used. Cr concentration was also measured with ICP and the levels were found to correlate.

In addition to our work with metals, we used the platform to add assays for additional toxic compounds in air and water that were not originally proposed. Beyond the concentration of specific metals in aerosols, there is also an interest in their oxidative reactivity as an indicator for oxidative stress potential (in the human body). Metals are often linked to this process through Fenton chemistry. Using the mPAD platform originally developed for metals analysis, we developed a simple assay for measuring aerosol oxidative load based on the established dithiothreitol (DTT) assay.[1] For mPAD-based analysis, we created a device that contained four detection zones, three with the detection dye and one as a control. To perform the assay, we first added DTT to the sample and allowed it to react. Next, the filter sample was transferred to the mPAD and buffer was added to elute the remaining DTT from the filter to the device. The more reactive the sample, the lower the resulting color as shown in the figure below (left panel). Once demonstrating viability and validating against traditional methods, we carried out a personal exposure study where volunteers collected personal samplers and collected filters with PM2.5 and PM10 over a 24-hr period. Three different environmental conditions were tested, one where the volunteers spent 8 hours outside on a clean air day, one where the student spent 8 hours outside when the local air was impacted by the High Park fire, and one where the volunteer spent 8 hours working in a commercial kitchen. Reactivity followed expected trends with regards to mass-normalized reactivity. The results are novel not only because we demonstrated that different aerosols posess different levels of oxidative reactivity but also because our mPAD method was successful at detecting DTT consumption from a personal sampling filter. The traditional DTT method requires hundreds of micrograms of PM mass and, therefore, is only applicable for relatively high-volume sampling.

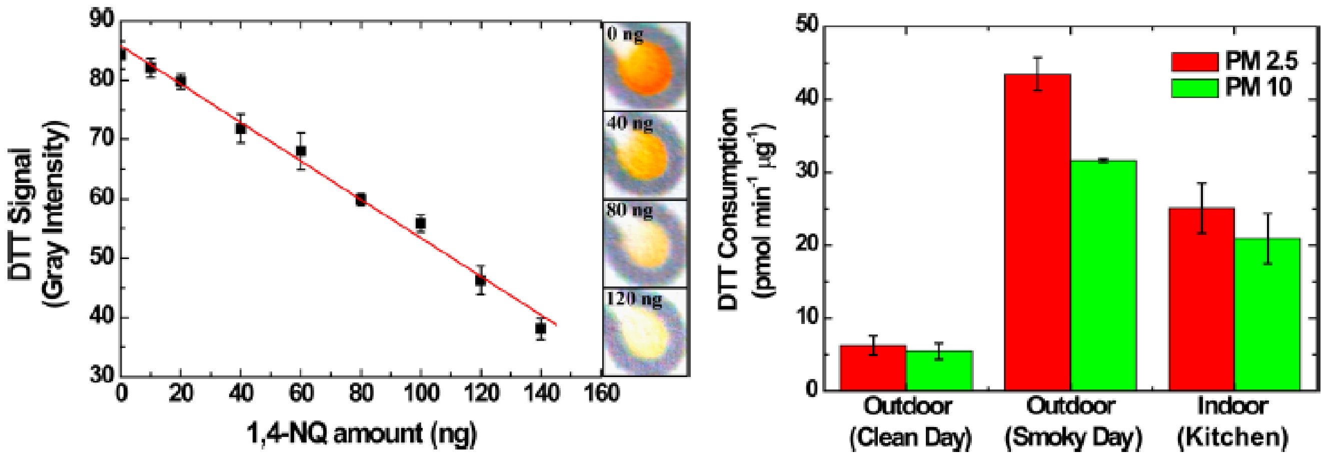


Figure 2: Left) calibration curve for the detection of ROS activity using DTT as the model. Right) Results from personal aerosol exposure for volunteers exposed to three different settings, outdoors on a clean day, outdoors on a day during the CO Highpark fire, and a day spent working in a commercial kitchen. Samples for both PM2.5 and PM10 were collected and evaluated.

A final major development of Aim 1 was the realization of a new detection mode for mPADs we refer to as distance-based detection or the *Chemometer*. The method was developed to remove any need for instrumentation for quantitative analysis (i.e., a scanner or camera). Figure 3 below shows the concept behind distance-based detection. First, an mPAD containing a long flow channel is created using wax printing. Second, the long channel is modified by printing colorimetric reagents directly onto the channel. Third, on addition of sample at the base of the channel, the analyte flows upward via capillary action and reacts with the indicator, causing a color change along the channel. As long as the product is insoluble, a colored band formed whose length corresponds to the concentration in the original sample. We demonstrated the viability of this method for detection of Ni, ROS, and glucose using three different types of colorimetric indicator assays. Representative results are shown in Figure 4 below. While the initial work was performed using single analytes, we are currently optimizing a three analyte system capable of multiplexed analysis. The manuscript describing this work is in preparation.

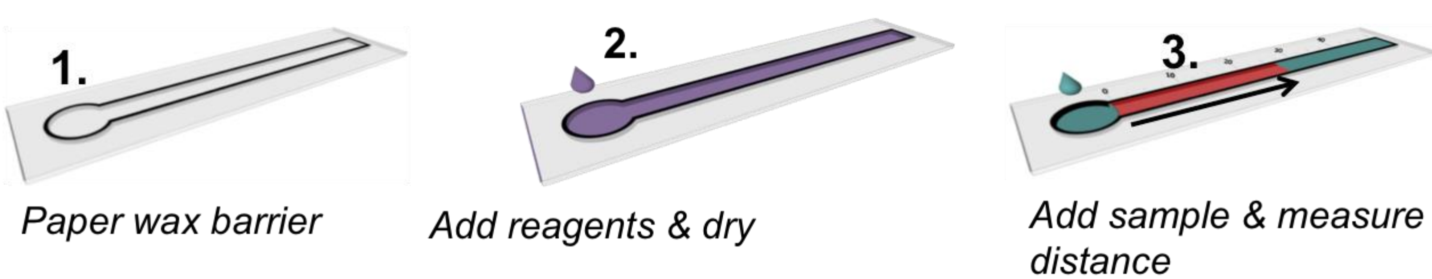


Figure 3: Left to right) schematic drawing of the methods used for chemometer operation. (1) A channel structure is printed using a wax printer. (2) Reagents are printed onto the chemometer using an inkjet printer. (3) Sample is added causing color formation along the channel.

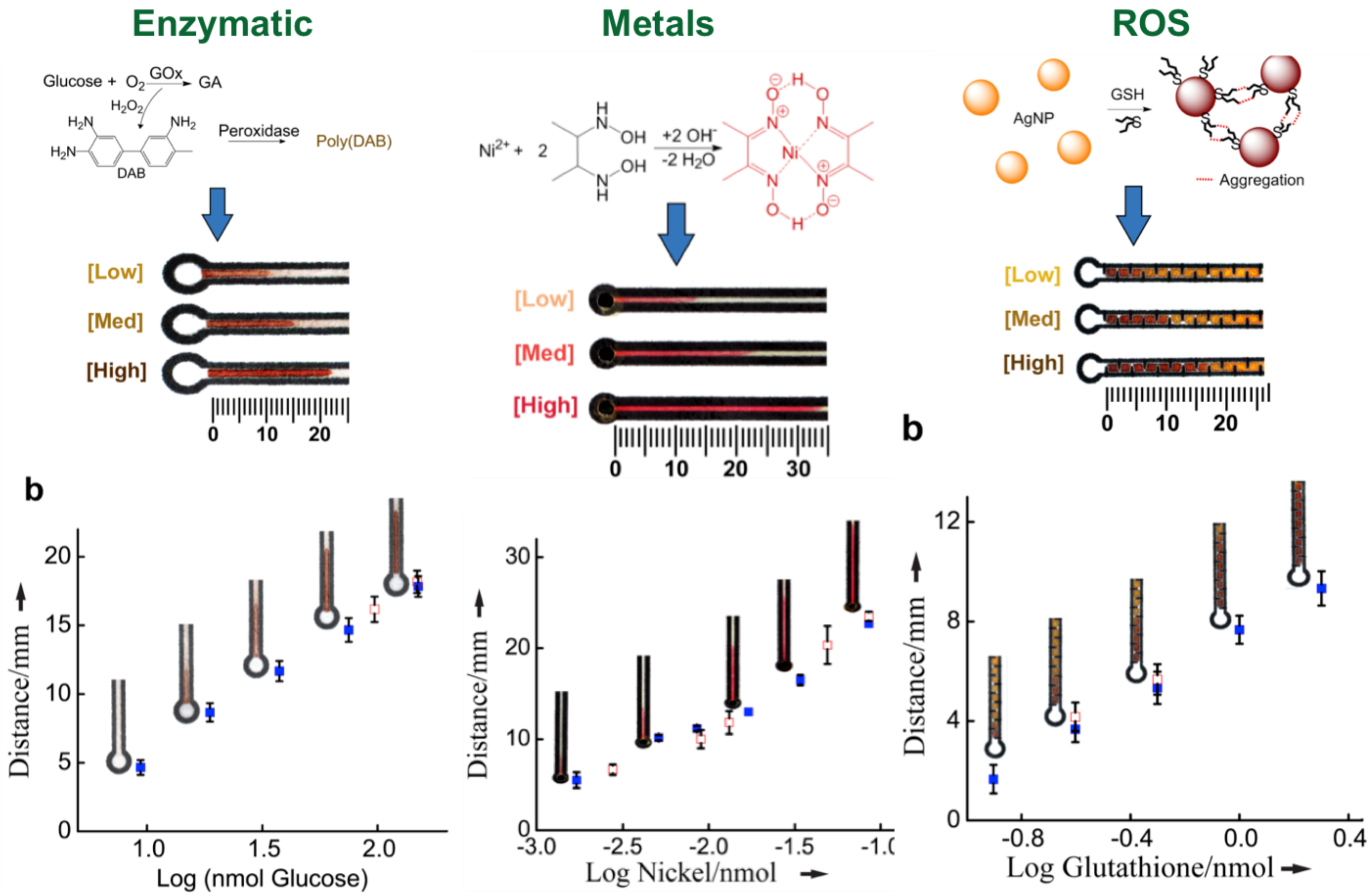


Figure 4: Left to right) Example applications of the chemometer technology for detection of Ni (left), Glucose (middle) and ROS (right).

Aim 2: Construct a multi-analyte μPAD for analysis of Pb, Cu, Mn, and Ni concentrations in air.

After establishing the initial colorimetric chemistry, we developed a multiplexed assay for measuring Fe, Ni, and Cu. We then validated this method using aerosolized ash samples.[2] The figure below shows the device layout and typical results in a photograph. To accomplish multiplexed analysis, we had to develop methods for pretreating the sample before it reached the detection zone because each chemistry required a unique pH condition as well as a specific metal oxidation state. With the functional chemistries in hand, we evaluated a series of validated reference standards collected on filter membranes. The samples were complex, containing Ag, Al, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn, Co, and V, and yet, our measured values matched the validated levels with no statistical difference in values. To date, this paper (Mentele et al.) has been cited 47 times since publication in 2012 suggesting its importance to the field.

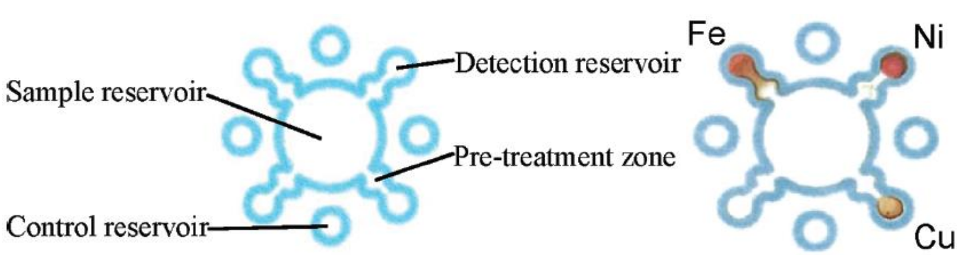


Figure 5: Left) Schematic drawing of the mPAD device used for multiplexed analysis of metals. Right) photograph of a completed device after reaction with an aerosol sample.

While this chemistry allowed us to profile three important metals, there was a desire to extend the mPAD approach to additional metals, including Pb and Cd as well as adding the colorimetric chemistry developed subsequently and described in the results section of Aim 1. To accomplish our goal, we created a three-dimensional device and added electrochemistry as a detection motif in addition to the colorimetric methods described above. Electrochemical detection is useful because it can give very low detection limits (nanograms) for many metals using anodic stripping voltammetry. A schematic of our device and the fabrication method is shown in Figure 6 below. The device is very simple and still costs just pennies to make. In this device, the top layer measures Ni, Fe, Cu, and Cr by colorimetric methods and Pb and Cd using electrochemistry in the bottom layer. Operation of the device is straightforward; a sample containing liberated metals on a filter is added to the center of the device and metals are eluted out, first for electrochemical detection and then for colorimetric detection (Middle figure). Using this device, we were able to achieve detection limits below 1 μg for colorimetric methods and 0.05 ng for electrochemical methods. Selectivity was demonstrated by testing with samples containing Ag, Al, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn, Co, and V. Accurate quantification of metals from resuspended baghouse dust as a validated laboratory control.

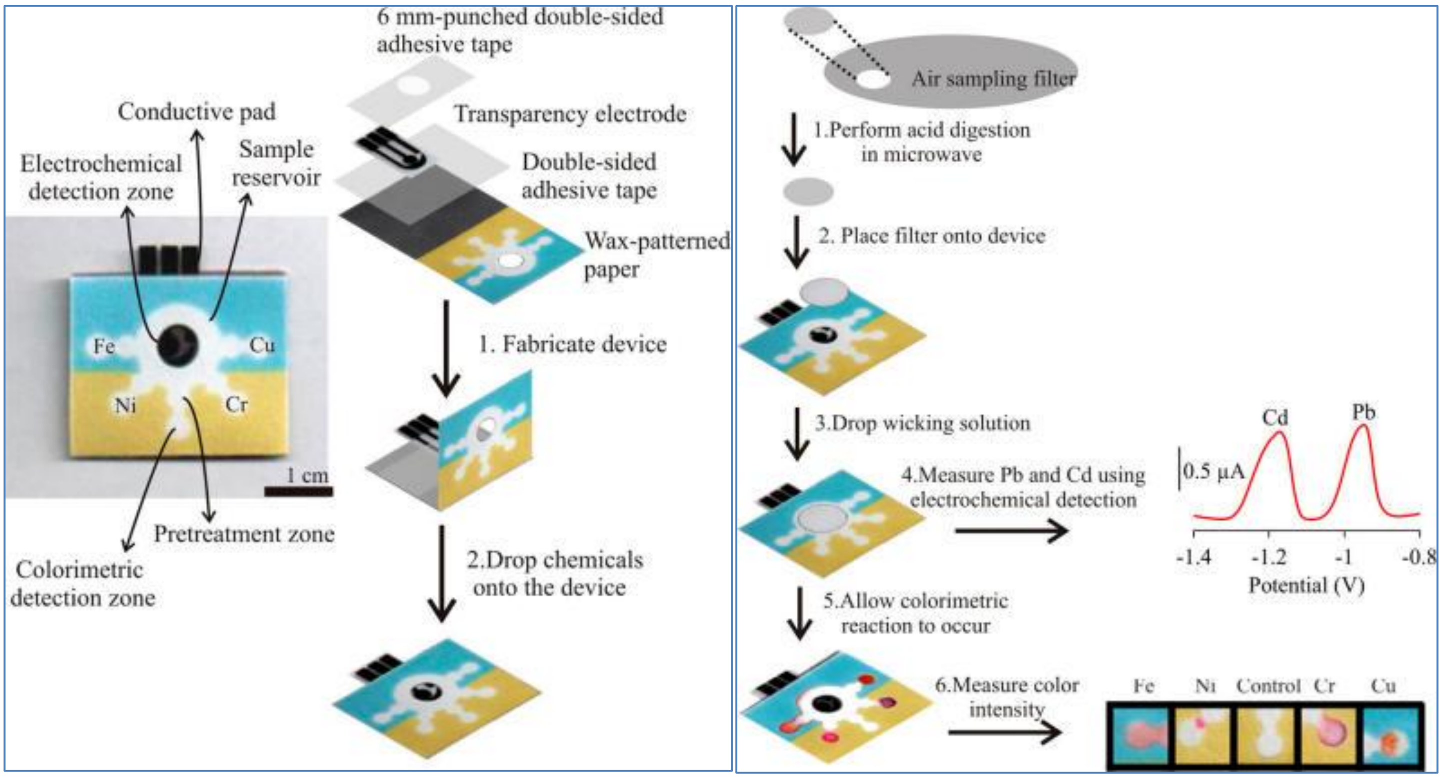


Figure 6) Left) photograph of a functional device as well as the fabrication scheme. Right) Schematic showing the analysis procedures as well as colorimetric and electrochemical results for analysis of all six metals in a single sample.

Aim 3: Validate the performance of the multi-analyte μPAD in the field.
Our last step of this project focused on validating the mPAD technology with real-world samples relevant to occupational exposure. For this study, we elected to sample from a group of welders using representative MIG, TIG and Arc welding techniques.[3] For these studies, we only measured Fe, Cu, Ni, and Cr using colorimetric methods; the analytic protocol is shown in the figure below. Welding fume samples were collected from all three sources and analyzed by both the mPAD method and traditional ICP-OES (gold standard). A comparison plot of the results for these samples is shown below. Over the full range of values, a correlation slope of 0.99 was obtained while at lower concentrations a correlation slope of 0.78 was obtained. The under prediction of concentrations at low levels was likely due to the use of mild digestion conditions relative to the ICP methods.

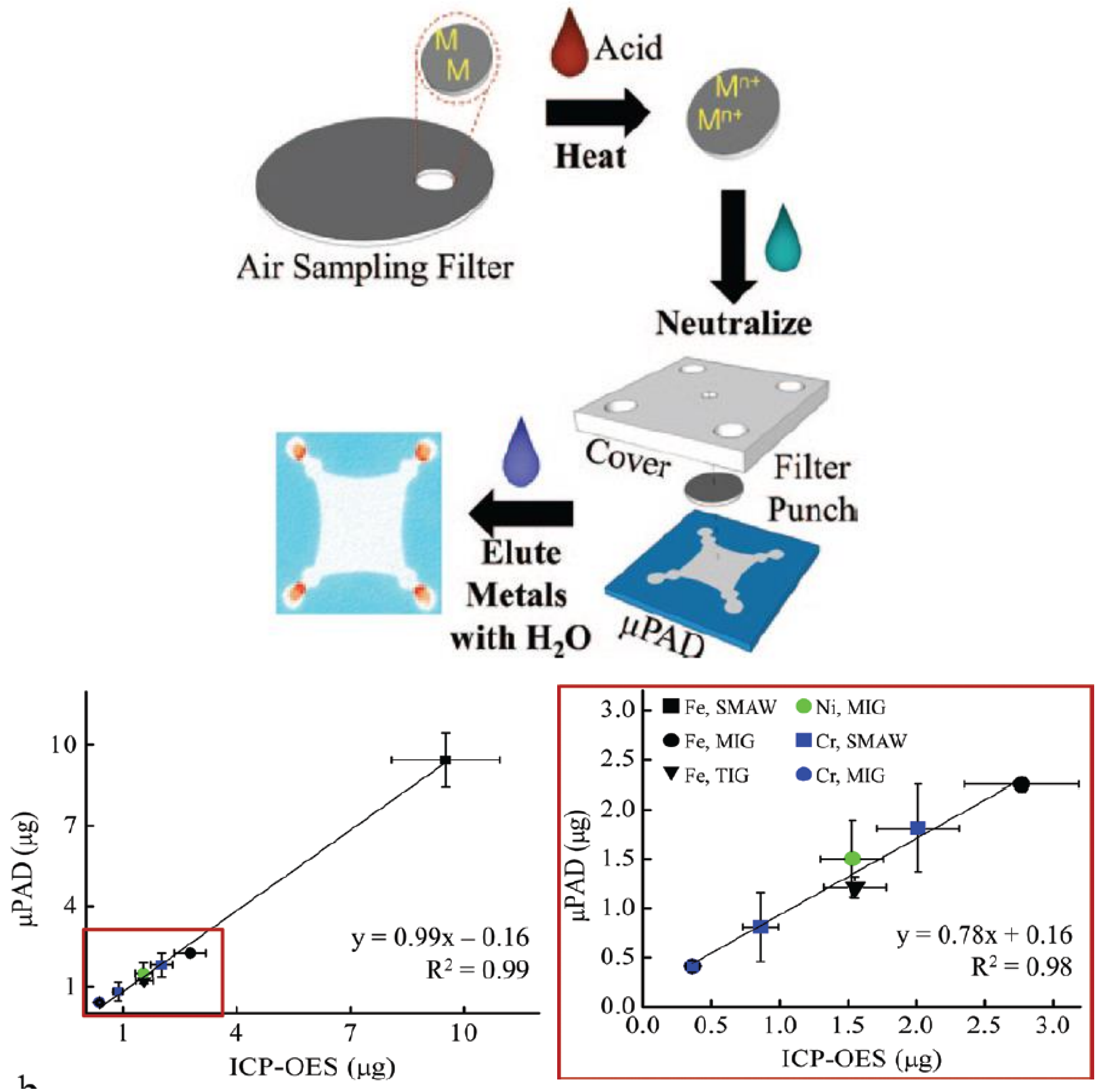
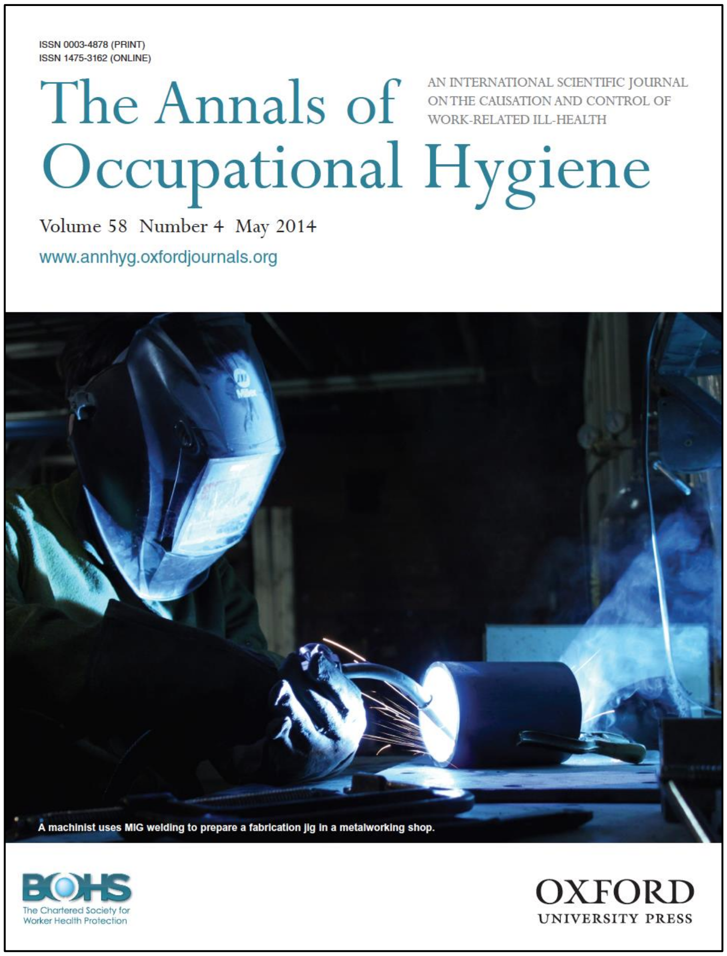


Figure 7: Top) Schematic showing analytical protocol for measurement of metals on an mPAD. Bottom left) Correlation plot for mPAD versus ICP values for entire concentration range tested. Bottom Right) Correlation plot for mPAD versus ICP values over the low concentration range.

Published Papers

1. Santhiago, M., C.S. Henry, and L.T. Kubota, *Low cost, simple three dimensional electrochemical paper-based analytical device for determination of p-nitrophenol.* Electrochimica Acta, 2014. **130**: p. 771-777.
2. Rattanarat, P., et al., *Multilayer paper-based device for colorimetric and electrochemical quantification of metals.* Anal Chem, 2014. **86**(7): p. 3555-62.
3. Cate, D.M., et al., *Rapid Detection of Transition Metals in Welding Fumes Using Paper-Based Analytical Devices.* Annals of Occupational Hygiene, 2014. **58**(4): p. 413-423.

This paper was featured on the Cover of the Annals of Occupational Hygiene:



4. Santhiago, M., et al., *Construction and electrochemical characterization of microelectrodes for improved sensitivity in paper-based analytical devices.* Anal Chem, 2013. **85**(10): p. 5233-9.
5. Sameenoi, Y., et al., *Microfluidic Paper-Based Analytical Device for Aerosol Oxidative Activity.* Environ Sci Tech, 2013. **47**: p. 932-940.
6. Rattanarat, P., et al., *A microfluidic paper-based analytical device for rapid quantification of particulate chromium.* Anal Chim Acta, 2013. **800**: p. 50-5.
7. Dungchai, W., et al., *Determination of aerosol oxidative activity using silver nanoparticle aggregation on paper-based analytical devices.* Analyst, 2013. **138**(22): p. 6766-73.
8. Cate, D.M., et al., *Simple, distance-based measurement for paper analytical devices.* Lab Chip, 2013. **13**(12): p. 2397-404.

Papers in Submitted or in Preparation

1. Cate, D. M., et al., *Multi-channel Distanced Based Detection for Multiplexed Metals Analysis.* Analytical Chemistry, 2014, in preparation.
2. Cate, D. M., et al., *Recent Developments in Paper-Based Microfluidic Devices*. Analytical Chemistry, 2014, submitted (invited review).

Conference Presentations (co-PI Henry)

- David Cate, Yupaporn Sameenoi, Mallory Mentele, John Volckens, Charles Henry, "Microfluidic tools for personal exposure assessment," *invited lecture*, Lab-on-a-Chip World Congress, San Diego, CA, September, 2012.
- Yupaporn Sameenoi, John Volckens, Charles Henry, "Fast measurements of inherent and catalytic aerosol oxidative activity," oral presentation, AAAR, October 2012, Minneapolis, MN
- David Cate, Yupaporn Sameenoi, Mallory Mentele, John Volckens, Charles Henry, "A low-cost method for quantifying metal aerosol in the field: Microfluidic paper-based analytical devices (μPAD)," oral presentation, AAAR, October, 2012, Minneapolis, MN.
- David Cate, Yupaporn Sameenoi, Mallory Mentele, John Volckens, Charles Henry, "Microfluidic tools for personal exposure assessment," *invited lecture*, Latin American Capillary Electrophoresis society meeting, Buenos Aires, Argentina, December, 2012.
- Yupaporn Sameenoi, Scott Noblitt, John Volckens, Jeff Collett, Susanne Hering, Charles Henry, "Microfluidic approaches to the analysis of ambient aerosols," *invited lecture*, Nanyang Technical University, Singapore, January, 2013.
- David Cate, Yupaporn Sameenoi, Poomrat Natanrat, John Volckens, Charles Henry, "Personal Exposure Assessment using Paper-Based Microfluidic Devices," *invited lecture*, Pure and Applied Chemistry Conference, Bang Saen, Thailand, January, 2013.
- David Cate, Yupaporn Sameenoi, Mallory Mentele, Jana Jokerst, Larry Goodridge, John Volckens, Charles Henry, "Analytical Chemistry using paper, pencils, and crayons," *invited lecture*, Center for Environmental Medicine, Colorado State University, Jan 2013.
- David Cate, Yupaporn Sameenoi, John Volckens, Charles Henry, "Personal exposure assessment to particulate metals using a paper-based analytical device," *invited talk*, SPIE Photonics West, San Francisco, February, 2013.
- David Cate, Yupaporn Sameenoi, John Volckens, Charles Henry, "Microfluidic Tools for Personal Exposure Assessment," *Invited Talk*, Medical Device Research Center, MIT, May, 2013.
- David Cate, Murilo Santhiago, John Volckens, Charles Henry, "Assessing Exposure to Environmental Pollutants using Paper-Based Analytical Devices," *Invited Talk*, Gordon Research Conference on the Chemistry and Physics of Microfluidics," Pisa, Italy, June 2013.
- David Cate, Yong Shin Kim, John Volckens, Charles Henry, "Microfluidic Paper-Based Analytical Devices for Personal Exposure Assessment," *Invited Talk*, Department of Chemistry, University of Oviedo, Oviedo, Spain, November, 2013.
- David Cate, Jaclyn Adkins, Yong Kim, John Volckens, Charles Henry, "Electrochemical Paper-Based Analytical Devices," Electrochemistry Gordon Conference, *Invited talk*, Ventura, CA, January, 2014.
- David Cate, Jaclyn Adkins, Yong Kim, John Volckens, Charles Henry, "Personal Exposure Assessment using Paper Microfluidic Devices," Pittcon, Invited Talk, Chicago, IL, March, 2014.
- Charles Henry, "Advances in Microfluidics," University of Tasmania, Hobart Tasmania, April, 2014, Invited lecture.
- Charles Henry, David Cate, Jaclyn Adkins, "Paper-Based Microfluidic Devices," Australia-New Zealand Microfluidics Conference, Hobart, Tasmania, Australia, Plenary Lecture, April, 2014.
- Charles Henry, Yong Kim, David Cate, John Volckens,, "Mulitfunctional Microfluidic Paper-Based Devices for Environmental Analysis," Invited Keynote, MicroTAS 2014, San Antontio, TX, October, 2014.

Conference Presentations (co-PI Volckens)

- Invited Speaker: "Arts and Crafts for the 21st Century Industrial Hygienist: How Crayons, Paper, and Pencils Can Help Revolutionize Occupational and Environmental Health." AIHA Rocky Mountain Section Fall Techniocal Conference. Arvada, CO, September, 2014.
- Invited Keynote Speaker. "19th century innovations for 21st century exposure science: how crayons, paper and citizen-based science can revolutionize our field." National Environmental Monitoring Conference. Washington, DC, August 2014.
- Invited Plenary Speaker. "The 8th International Symposium on Modern Principles for Air Monitoring and Biomonitoring. Marseille, France, June 2014.

- “Inexpensive Microfluidic Devices for Multiplexed Metal Measurement in Particulate Matter,” Poster Presentation, American Association for Aerosol Research. Portland, OR. October 2013.
- Invited Speaker. “19th Century Innovations for 21st Century Exposure Science: How Crayons, Paper, and Heat Can Revolutionize Industrial Hygiene,” National Institute for Occupational Health and Safety, Cincinnati, OH, April 2013.
- Invited Lecture. “Microfluidic approaches to the analysis of ambient aerosols,” Nanyang Technical University, Singapore, January, 2013.
- Invited Lecture. “Personal Exposure Assessment using Paper-Based Microfluidic Devices,” Pure and Applied Chemistry Conference, BangSaen, Thailand, January, 2013.
- Invited Speaker. “Personal exposure assessment to particulate metals using a paper-based analytical device,” SPIE Photonics West, San Francisco, February, 2013.
- Invited Lecture. “Electrochemistry of Atmospheric Micro- and Nanoparticles,” Pittsburg Conference on Analytical Chemistry, Orlando, FL, March, 2012.
- Invited Plenary Lecture. “A Small Solution to a Big Problem: Lab-on-a-Chip Technology for Characterizing Atmospheric Aerosols,” Wyoming meeting of the ACS, April, 2012.
- Invited Lecture. “Microfluidic tools for personal exposure assessment,” invited lecture, Lab-on-a-Chip World Congress, San Diego, CA, September, 2012.
- “A low-cost method for quantifying metal aerosol in the field: Microfluidic paper-based analytical devices (μPAD),” oral presentation, Lab-on-a-Chip World Congress, October, 2012, Minneapolis, MN.
- Invited Lecture. “Microfluidic tools for personal exposure assessment,” Latin American Capillary Electrophoresis society meeting, Buenos Aires, Argentina, December, 2012.
- “Paper-Based Microfluidic Devices for Aerosol Exposure Assessment.” Platform Presentation. International Society for Exposure Science. November, 2012. Seattle, WA.
- “Paper-Based Microfluidic Devices for Aerosol Exposure Assessment.” Platform Presentation. American Association for Aerosol Research. October, 2012. Minneapolis, MN.
- "A Low-Cost Method for Quantifying Metal Aerosol in the Field: Microfluidic Paper-Based Analytic Devices (mPADs)." Platform presentation. American Association for Aerosol Research. October, 2011. Portland, OR.