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Complete List of Authors:	Hofstetter, Josephine; Access Sensor Technologies Wydallis, John; Access Sensor Technologies Neymark, Gabe; Access Sensor Technologies Reilly, Thomas; Access Sensor Technologies Harrington, James; Alexco Resources Henry, Charles; Colorado State University, Chemistry;

Quantitative Colorimetric Paper Analytical Devices Based on Radial Distance Measurements for Aqueous Metal Determination

Josephine C. Hofstetter,[a,*] John B. Wydallis,[a] Gabe Neymark,[b] Thomas H. Reilly,[a] Jim Harrington,[c] and Charles Henry,[a,d,*]

[a]Access Sensor Technologies LLC, 430 N College Ave, Fort Collins, CO 80524 USA

[b]Department of Mechanical Engineering, Colorado State University, 400 Isotope Dr., Fort Collins, CO, 80521 USA

[c]Alexco Environmental Group, 171 N. College Ave, Fort Collins, CO 80524

[d]Department of Chemistry, Colorado State University, Center Ave, Fort Collins, CO 80523 USA

*To whom correspondence should be addressed.

Email: jhofstetter@accsensors.com; Tel: 303-810-3390

Email: chuck.henry@colostate.edu; Tel: 970-491-2852

Abstract

Here we report a new microfluidic paper-based analytical device (mPAD) for quantifying metals in water. Metals represent an important class of water contaminants that come from a variety of sources including mining, transportation, manufacturing, waste management, and energy production. Current technologies for quantifying aquatic metals in water are expensive, relatively slow, tedious, provide inadequate performance, and difficult to use in a field setting. As a result, a need exists for simple, portable, power-free measurement tools that enable rapid in-field quantification of aquatic metals. The reported metal test cards, referred to as the On-Target Water Chemistry test cards, represent a major improvement over previously reported linear distance-based detection systems comprised of paper. With the On-Target approach, the sample flows outwards radially and reacts with colorimetric complexing agents, significantly reducing assay time. The diameter of the resulting color formation is directly proportional to analyte concentration. The On-Target cards were used for detecting copper, iron, and zinc with detection limits as low as 0.1 ppm in ~ 3 min and single ppb in combination with a membrane pre-concentration system.

Introduction

Aqueous metals, such as Fe, Ni, and Cu, are regulated by numerous State and Federal agencies because they are recognized as significant threats to human and ecosystem health.[1] Cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), and zinc (Zn) are also essential nutrients for humans as they participate in important biochemical and physiological functions.[2] However, overexposure to these metals can be carcinogenic and cause cellular and tissue damage thereby leading to a variety of adverse health effects and human diseases.[2,3] Other possible health effects from over exposure to metals include gastrointestinal illness, reproductive problems, and neurological disorders.[4] Many industries, such as mining, energy production, waste management, and transportation contribute to pollutant release. Greater than 60% of rivers, lakes, and estuaries in the US are presently ‘impaired’ with levels of metals, pathogens, and organic matter above EPA limits due to the release of pollutants.[5] Operators in the environmental remediation sector and those handling wastewater disposal are required to monitor and control the release of metals.[6] Many legacy mines and industrial sites continue to release metals into the environment at levels significantly exceeding EPA limits; these sites frequently perform analytical testing to verify remediation but the frequency of testing is limited by the cost of current analytical methods. There are also approximately 36,000 abandoned mines in the western United States that are in need of reclamation.[7] The federal Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) – also known as ‘Superfund’ – was passed in 1980 to fund the cleanup of such uncontrolled or abandoned hazardous-waste sites, accidents, spills, etc.[8] As a result, tens of thousands of samples are analyzed for metals each day in the US alone.

Dissolved aquatic metals are presently measured using two distinct methods: in-field or laboratory. Common laboratory methods include atomic absorption spectroscopy (AAS) and inductively coupled plasma (ICP) coupled with either optical emission spectroscopy (OES) or mass spectrometry (MS).[9,10] These methods are sensitive and selective but require large complicated instruments that delay the time between sample and results. Consequently, in-field assays that are not as sensitive or selective are frequently used.

The in-field analysis technologies can be broken down into three categories: non-instrumented analysis (i.e., dipstick style tests), portable instrument supported analysis, and indirect ‘point and shoot’ analysis. Non-instrumented tests include basic visual comparative colorimetric products such as the Hach Colorwheel™ or Color Discs, Millipore Mquant™ and MColortest™, and CHEMetrics Colorimetrics and Titrimetrics.[11–14] These solution chemistry kits currently require up to 19 steps which can introduce human error, have a narrow dynamic range, and can be inherently subjective since the result is based on human observation of visual color comparison. Test subjectivity can be reduced through electronic ‘reading’ of the colorimetric reaction with a fluorimeter or spectrophotometer but these external instruments add hundreds to thousands of dollars in cost.[15,16] The CHEMetrics Instrumental Colorimeter and Hach SL1000 Portable Parallel Analyzer can streamline analysis by removing the sample preparation and interference blocking steps via disposable Vacu-vial® and Chemkey® consumables, respectively, but require a relatively high upfront investment.[17,18]

Point and shoot methods, such as handheld X-Ray Fluorescence (XRF) instruments are an alternative for solution-phase measurements.[19] XRF instruments are often mentioned synonymously with aquatic testing, but can actually only analyze solids/soil for total elemental components and cannot differentiate between dissolved contaminants (mobile or leachable) and sequestered immobile ones. As many firms now remediate metals via chemical sequestration (i.e., converting the metals to an immobile state and leaving them in the ground), there is a critical need to differentiate between mobile and immobile metals, a major drawback of the XRF method.[20,21] The relatively low sample throughput from third-party analyses limits on-site decision making, slows the time-to-completion, and increases overall project costs. In the academic realm, researchers have also made advancements towards fieldable detection systems for detection of metals, including paper-based platforms.[22–34]

Here we report a microfluidic paper-based analytical device, named On-Target Chemistry cards, for rapid quantification of metals in water. The cards are a modified version of the previously reported linear distance-based paper analytical devices where the analyte concentration is read as the length of color formation along a test channel.[24,33–35] The distance-based approach requires no external instrumentation and eliminates the need to differentiate hues and intensities common to other colorimetric paper-based analytical devices. Linear distance-based paper analytical devices have been demonstrated for a variety of analytes, including metals.[24,33–35] While the distance-based detection has proven effective, the analysis can take up to 30 min based on the slow liquid transport down the channel. The On-Target Water Chemistry test cards still possess the advantages of the linear distance-based paper analytical devices (portable, low-cost, easy-to-use, and quantitative) with the additional benefit of results being generated in 2-3 min. The reduced analysis time is achieved using a circular flow path where the sample flows radially outward from the tests central inlet (**Figure 1**, label 5) and, in the presence of the target analyte, creates a circular color change whose diameter correlates with analyte concentration (**Figure 1**, label 7). The flow rate is faster because the increasing diameter results in higher capillary pressure to offset the increasing viscous drag. Without preconcentration, low ppm (mg/L) detection limits have been achieved. To address lower analyte levels, a membrane pre-concentration system is also reported as a system that integrates with the On-Target test cards. Using the combined systems, it was possible to achieve single ppb detection limits of metal ions within 9 min. The new radial detection technology will ultimately allow sampling to occur more frequently and in more settings to ultimately permit better monitoring and mitigation of environmental pollutants than can be achieved at present.

Experimental Section

Chemicals and materials. All solutions were prepared using deionized (DI) water. Ascorbic acid, bathophenanthroline, cadion, cetylpyridinium bromide hydrate, L-ascorbic acid, nitric acid, potassium acetate, sodium hydroxide, zincon, Whatman Grade 4 Chromatography Paper, and Empore Extraction Disks (Model 2271) were all purchased from Sigma-Aldrich (St. Louis, MO). Laminationers (3 Mil, 9” x 11.5”) were purchased from Amazon (Seattle, WA) and mylar bags (3” x 5”) were purchased from Uline (Seattle, WA). The real-world water samples were collected from the Animas River at the Gold King Mine (Silverton, CO) and the Argo Tunnel Water Treatment Plant (Idaho Spring, CO).

On-Target device fabrication. The On-Target test cards are comprised of three material layers. The central layer is made of Whatman Grade 4 filter paper. Wax designs are printed onto the central layer to contain lateral fluid movement (**Figure 1**) using a Xerox ColorQube 8570DN. The wax is melted using a Puhui T-962C infrared oven at 150 °C for 4 min. Inkjet printing (Epson ET-2550) was used to add a QR code and details on the specific assay. Finally, inkjet printing was used to deposit assay reagents that both generate colors in the presence of analyte and control selectivity in the 3.8 cm test zone. The top and bottom layers of the card are commercial lamination sheets, which are impermeable to fluid, and control evaporation. A sample introduction orifice (i.e., test inlet) is laser cut in the top lamination layer using a Trotec Speedy100$^{\mathrm{TM}}$ laser system for sample addition. The lamination and porous layers are assembled, precisely aligned with a custom fixture, and then laminated together at 145 °C (Akiles Prolam). The three combined layers are then laser cut around their perimeter to yield individual test cards, each having the dimensions of a standard business card (3.5” × 2.0”). The test cards are stored in Mylar bags at ambient conditions (22 ± 2 °C) until used. Adobe Illustrator CS6 (version 19.2.1) was used for the design of the On-Target cards printed features and laser cutting pattern. The diameter to concentration relationship was plotted using Origin Pro8 SR4 v8.0951 (Northampton, MA).

Results and Discussion

Diameter-based colorimetric detection method. The On-Target test cards can be used to test water from mine water runoff, storm water, pool/spa, industrial wastewater, household water supplies, or recreational bodies of water (**Figure 2a**). To use an On-

Target test card, 100 µL of sample is collected (**Figure 2b**) and added to a vial containing 10 µL of buffer (**Figure 2c**) that adjusts the sample pH to an acceptable range for the assay, ensures the analyte is in the correct oxidation state, masks interferences, and, depending on the test type, assists with immobilization or color change enhancement. After mixing by briefly shaking the vial, the contents of the vial are added to the sample inlet port of an On-Target card using a disposable pipet (**Figure 2d**). After 3 min, the metal concentration is determined by using a ruler to measure the diameter of the circular color change area (**Figure 2f**). The resulting diameter is compared to a calibration curve or conversion table to convert the diameter to analyte concentration. The test cards allow for quantitative readout without needing expensive external analysis equipment. This is accomplished using the unique diameter-to-concentration relationship that results from the target ion being trapped and depleted from the bulk sample upon reacting with the pre-printed colorimetric chemistries.

On-Target Water Chemistry test cards have been developed and thoroughly tested for 3 metal ions: copper (Cu^{2+}), total iron (Fe^{2+} and Fe^{3+}), and zinc (Zn^{2+}). We adapted assays for the three metals using previously reported mPAD methods[23,24,29,34,35] along with the following colorimetric reagents and their referenced procedures: zincon for Cu,[36–39] bathophenanthroline for Fe,[40,41] and dithizone for Zn.[42,43]

***On-Target* device performance.** As part of the rigorous testing to understand the performance of each metal test, we completed calibration curves and interference testing for Cu, Fe, and Zn. The calibration curves were collected following the procedure outlined in **Figure 2** with standard metal solutions at concentrations ranging from 0 ppm to 100 ppm. The calibration curves in **Figure 3** show the relationship between test diameter and metal concentration for each test along with representative test cards images for 1, 10, 25, 50, and 100 ppm. The working range of the test cards is 0.1 to 20 ppm and the full range calibration curves follow a second order polynomial. The associated fit equations for the Cu, Fe, and Zn curves are $y=-0.26x^2+4.85x+1.99$ ($R^2=0.98$), $y=-0.02x^2+1.39x+2.01$ ($R^2=0.99$), and $y=-0.01x^2+1.26x+2.6$ ($R^2=0.99$), respectively. The calibration curves are non-linear due to evaporation through the inlet port and the device being mass transfer limited. In other words, the metal ions within the sample front are depleted (i.e., complexed with the colorimetric reagent) before reaching the outer edges of the device along with evaporation preventing the unbound metal ions in the last plug of fluid from flowing outward. The lowest concentration discernible by eye for the Cu, Fe, and Zn test cards is 100 ppb. The average percent coefficient of variation is 1.4%, 2.0%, and 2.1% (n=8) for Cu, Fe, and Zn, respectively. The test cards are stable for at least 8 months at room temperature (22 ± 2 °C) in Mylar bags. To determine the stability of the test cards we have an ongoing stability study where the test cards are tested in quadruplicate every month. After 10 months of testing, there has been no change (results are all within error) in signal diameter.

The specificities of all tests were determined through a series of interference screening experiments.[44] Thirteen different potential interferents were tested both individually and combined with the target metal using the On-Target format. All results were compared to a positive (standard solution of target metal) and negative control (blank of deionized water) to determine whether a non-specific interaction occurred. For example, an aqueous mixture of 10 ppm Zn and 10 ppm Cd was tested on a Zn On-Target card and compared to that of an aqueous solution of 10 ppm Zn. The Zn and Cd mixture produced the expected pink color change but with a diameter of 25 mm, which is 11 mm larger than the Zn solution alone (14 mm). The tolerance ratio, defined as the ratio of interferent to analyte that causes a change of 10% or more,[24] is equal to 1 for Cd in the Zn test. This means that the cadmium interferes with the chemistry of the Zn On-Target card and is therefore classified as an interfering species at or above 10 ppm. Tests that included the interfering species alone (i.e., no target metal present) and yielded a color change not matching the expected color (**Table 1**) were considered to not interfere so long as in the presence of the target metal, the color formation was of the expected diameter and color. By following this approach, the interferents in **Table 1** were identified and their tolerance ratio was determined.

Membrane pre-concentration system. While ppm detection limits are sufficient for some metals, lower levels are needed to assess environmentally relevant levels of many metals. We expanded the range of detectable concentrations on the On-Target Water Chemistry test cards down to single ppb levels through the development of a new membrane pre-concentration system. The pre-concentrator (depicted in **Figure 4**) housing was designed as an assembly of 2-dimensional acrylic pieces. The base pieces are 1/4 inch acrylic to increase rigidity and the upper housing is 1/8 inch acrylic. These pieces were cut on a laser cutter to allow for rapid design iterations. The reported pre-concentrator takes 1 L of aqueous sample and concentrates 95% of the contained metals onto an Empore^{TM} Chelation Disk (160.0 ± 8.0 mL/min or 5.7 ± 0.3 min, 18.8 Watts, n=24) located in the filter cassette on the pre-concentrator outlet. The metals are then eluted off the disk using 500 μL of nitric acid (pre-loaded and sealed in a syringe) that's pushed through the filter cassette and into a buffer vial specific to the metal test of interest. The buffer vial is pre-loaded with 3 M potassium hydroxide to neutralize the acid and a concentrated buffer to set the pH for the specific test. The resulting solution is 1000X more concentrated and can be analyzed directly on an On-Target test card. **Figure 5** is a plot of the diameter of color change vs. the concentration of aqueous Zn (**Figure 5a**) and Cu (**Figure 5b**) detected on the On-Target cards with and without use of the pre-concentration system. When the pre-concentration system is used in combination with On-Target test cards, the limit of detection is 2.5 ppb for Zn and 1 ppb for Cu and the percent coefficient of variation is 18.5% and 6.5% for Zn and Cu, respectively. While these values are higher than some traditional assays, the short assay time combined with the simplicity of the system still make these systems of interest, particularly in screening experiments.

Real-world detection of the *On-Target System*. The Cu, Fe, and Zn On-Target Water Chemistry test cards were tested with three water samples: Gold King Mine water before and after remediation and untreated water from the Argo Tunnel. The samples were also analyzed by Flame Atomic Adsorption Spectroscopy (FAAS) analysis for validation. The performance of the On-Target cards relative to the traditional FAAS method are shown in **Figure 6**. The concentration of metal ions in the three water samples ranged from 0.1 ppm to 34.8 ppm. At concentrations between 1 ppm and 30 ppm, the two methods agree at the 95% confidence interval. At concentrations above 30 ppm the results deviate for the two methods due to the On-Target cards calibration curves plateauing from saturation of the color formation diameter as explained above.

The EPA levels range from 0.1 to 500 ppm,[45] depending on the metal, which is within the working range of the reported system. Concentrations detected above the EPA cutoff are considered ‘impaired’ and of serious health concern. The On-Target test cards allow for quantitative aqueous metal analytics to be generated on-site for emergency response and on-going monitoring and remediation. Looking to the future, the test card will be expanded for more analytes and sample types.

Conclusions

In conclusion, a new form of distance-based detection using radius or diameter instead of length is reported. The approach achieves similar or better detection limits in one-tenth the time of linear devices enabling results in 2-3 minutes and represents a significant step forward in this field. To demonstrate the utility of the approach, Cu, Zn, and Fe assays were developed and used to measure levels in mining water samples. To improve detection limits and enable measurement of ppb concentrations, a simple pre-concentration system was developed that uses a portable pump to concentrate metals onto an ion-exchange membrane. After elution and neutralization, the final system could achieve 1000-fold sample enrichment. When the distance-based test cards were used for real samples, the method agreed with the results from AAS from 0.1 to 30 ppm but did deviate at higher concentrations due to color saturation.

Acknowledgments

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Figure Captions

Figure 1. Photograph of an On-Target test card for Fe with labels on the different device features: inkjet printed labeling, hydrophobic wax barrier defining test zone, hydrophobic wax barrier around the perimeter of the card, bare hydrophilic chromatography paper, laser cut test inlet, printed colorimetric reagent for aqueous Fe, and color change resulting from 100 ppm of Fe.

Figure 2. Photographs of an Fe On-Target test card being used to test a Colorado River, including (a) the sampling location, (b) water being collected from river, (c) 100 μL aliquot of collected sample being added and mixed with buffer in a vial, (d) addition of the buffered sample to the test card inlet, (e) sample flowing radially outward with color change occurring from Fe complexation, and (f) measuring of the resulting color change diameter to determine concentration.

Figure 3. Calibration curves for Fe, Zn, and Cu On-Target cards with representative images of test zones for 1, 10, 25, 50, and 100 ppm on each test type. Each data point represents the average of four independent test card results and the error bars are the standard deviations of each concentrations average diameter.

Table 1. The expected color changes and known interfering species for each test type.

Figure 4. Photograph of the pre-concentration system with labels on the key components.

Figure 5. On-Target calibration curves for (a) Zn and (b) Cu with and without the pre-concentration system. The calibration curves are plotted on a logarithmic scale. Each data point represents the average diameter measured from four independent samples and test cards, and the error bars correspond to the standard deviation.

Figure 6. Performance of the On-Target test cards for Cu, Fe, and Zn relative to traditional flame atomic absorption spectroscopy laboratory-based testing. The solid line represents perfect agreement between the two methods.

TOC Graphic. The On-Target test cards are a rapid testing method for untrained users to quantitatively assess contaminants in aqueous samples.

Figures

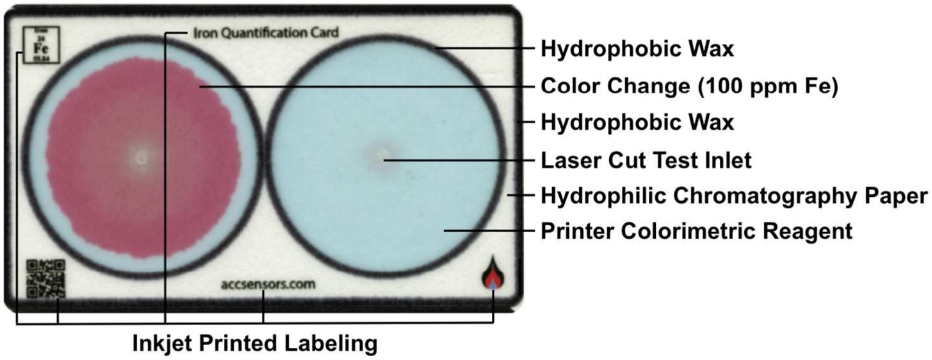
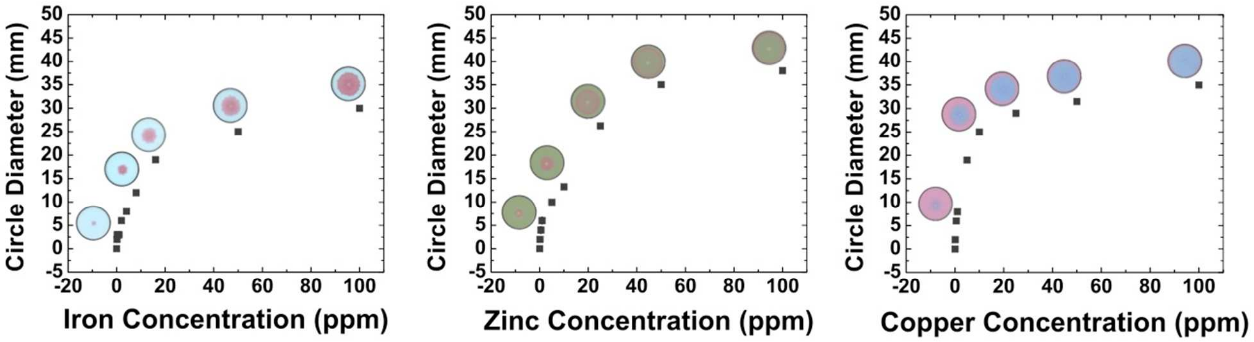


Figure 1 / Hofstetter et al.

Figure 2 / Hofstetter et al.



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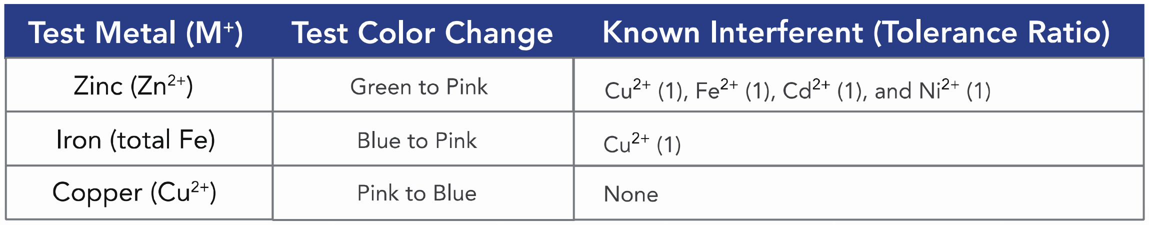
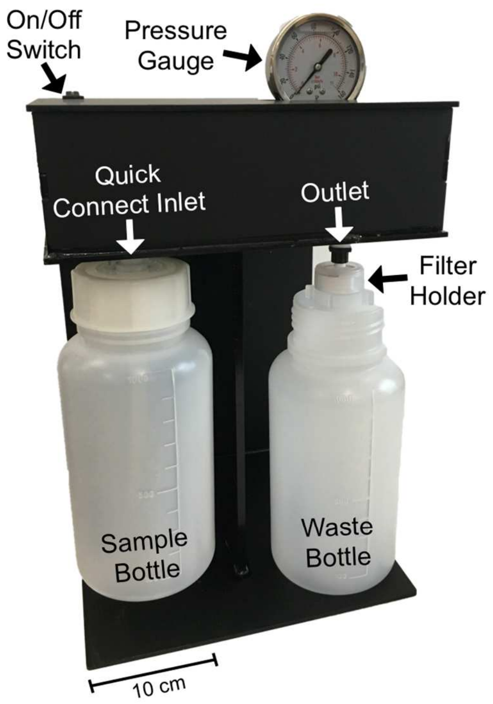


Test Metal (M^+)	Test Color Change	Known Interferent (Tolerance Ratio)
Zinc (Zn^{2+})	Green to Pink	Cu^{2+} (1), Fe^{2+} (1), Cd^{2+} (1), and Ni^{2+} (1)
Iron (total Fe)	Blue to Pink	Cu^{2+} (1)
Copper (Cu^{2+})	Pink to Blue	None

Table 1 / Hofstetter et al.



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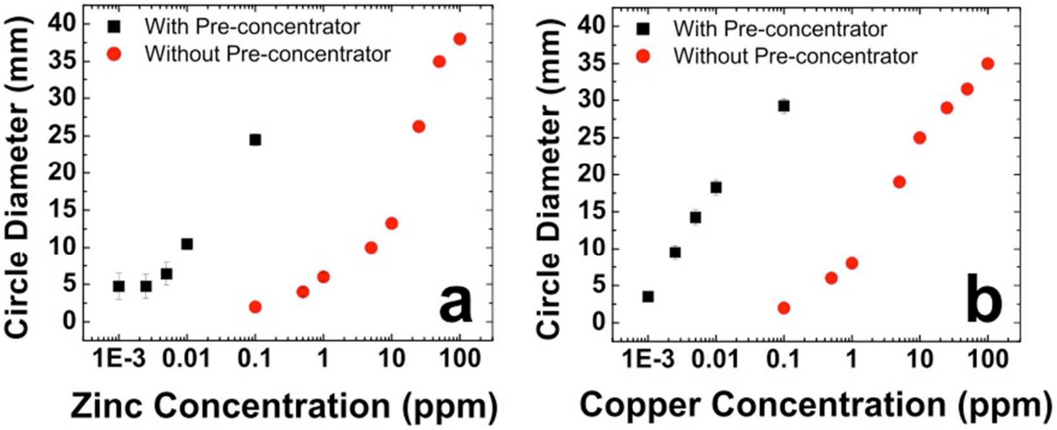

Figure 5 / Hofstetter et al.

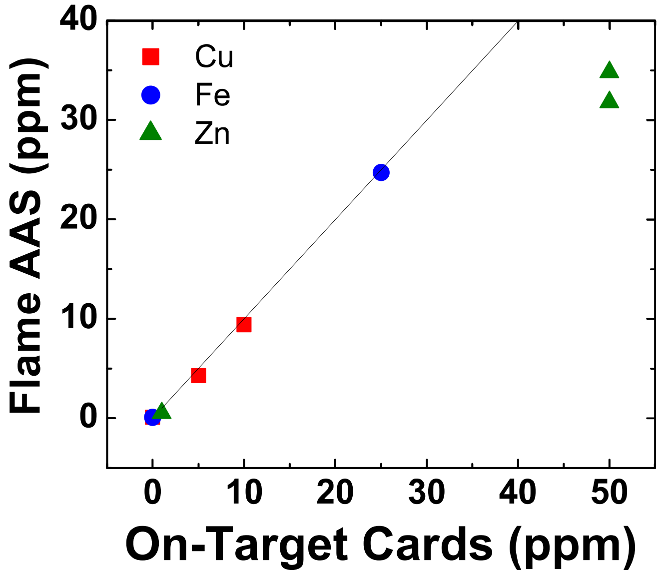

Figure 6 / Hofstetter et al.

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