Determination of Manganese in Whole Blood by Cathodic Stripping Voltammetry with Indium Tin Oxide

*Cory A. Rusinek[a,b], Wenjing Kang[c], Keaton Nahan[a], Megan Hawkins[d], Cooper Quartermaine[d], Angela Stastny[a], Adam Bange[d], Ian Papautsky[c,e], and William R. Heineman[a**]*

[a] Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA
[b] Fraunhofer USA, Inc. Center for Coatings and Diamond Technologies, East Lansing, MI, 48824-1226
[c] Department of Electrical Engineering and Computing Systems, University of Cincinnati, Cincinnati, OH, 45221-0030, USA
[d] Department of Chemistry, Xavier University, Cincinnati, OH 45207-4221, USA
[e] Department of Bioengineering, University of Illinois at Chicago, Chicago, IL 60607-7161, USA
* e-mail: heinemwr@uc.edu

Received: ((will be filled in by the editorial sttaff))
Accepted: ((will be filled in by the editorial sttaff))

Abstract
Manganese (Mn) is a required trace metal in the body. In recent years however, it has garnered significant attention as a developmental neurotoxin in children with chronic exposure. It has been linked to complications such as memory loss with negatively associated IQ scores, attention deficit hyperactivity disorder (ADHD), and in extreme cases, the development of a Parkinson's disease analogue- manganism. Cathodic stripping voltammetry has proven to be a suitable method for electroanalytical determination of Mn. We have established Mn CSV using indium tin oxide (ITO) as the working electrode for the determination of Mn in bovine whole blood after an acid digestion. Reliable, accurate, and precise results were obtained as only 9% variation in the digested blood was observed. The CSV results were compared with graphite-furnace atomic absorption spectroscopy (GF-AAS) and inductively coupled plasma- mass spectrometry (ICP-MS) and favorable agreement across the methods was observed. Due to ITO's excellent positive potential window and stability under harsh environments, this method could be applied to other oxide-forming transition metals detectable by CSV.

Keywords: Indium Tin Oxide, Stripping Voltammetry, Whole Blood, Manganese

DOI: 10.1002/elan.((will be filled in by the editorial sttaff))

Manganese (Mn) has been identified as a developmental neurotoxin in children. [1] Though it is a required trace metal found within the body, chronic exposure to Mn in drinking water has been linked to a number of neurological complications. [2-5] Some of these complications include but are not limited to memory loss, learning disabilities in children, and the development of manganism - a disorder with symptoms similar to those of Parkinson's disease. [2-6] For these reasons among others, the Environmental Protection Agency has set the maximum contaminant level (MCL) in drinking water at a very low concentration of 50 ppb (0.9 μM). [6] A study completed in Bangladesh found that exposure to Mn in drinking water was directly correlated to deficiencies in mathematics scores in school. [1-8] In addition, the Harvard Medical School found that a 10-year old male exposed to levels of Mn as high as 1.2 ppm in drinking water scored well below average on memory tests. [7] Specifically, he scored no higher than the 19th percentile in general memory index, verbal memory, visual memory, and learning index tests. [7] Thus, trace detection of this neurotoxic metal in both environmental and biological samples has become very important.

Electroanalytical methods such as stripping voltammetry are becoming more prevalent for monitoring environmental contaminants such as metals and organic compounds (PAHs and PCBs) in both environmental and biological samples. [10-15] Due to inexpensive instrumentation and operating costs while maintaining low limits of detection and excellent selectivity, electroanalytical methods offer advantages over other analysis methods such as spectroscopy and spectrometry. [12,14] These advantages coupled with the ease of miniaturization make electroanalytical methods attractive. [14,15]

In this communication, we report that a bare, uncoated-ITO electrode can be used for accurate sample analysis for the determination of Mn^{2+} in bovine whole blood. Though ITO is not typically used for such measurements, we have already shown the material's superior performance in Mn^{2+} CSV over other electrode materials such as glassy carbon, boron-doped diamond, and mercury. [5] The intrinsic selectivity of CSV itself removes several interferences and the only significant interference observed was iron (Fe^{2+}) at a 20:1 concentration ratio of Fe^{2+}:Mn^{2+}. [5] This positive experience with its application to environmental samples caused us to investigate its suitability for biological samples. ITO's stability under the harsh environment used in this work proved to be a crucial factor in its ability to quantitatively determine Mn^{2+} in bovine blood samples. CSV with ITO yielded similar results to other commonly used techniques for analysis of biological samples such as graphite furnace-atomic absorption spectroscopy (GF-AAS) and inductively coupled plasma-mass spectrometry (ICP-MS).

Osteryoung square-wave voltammetry (OSWV) was used for the electrochemical stripping step because of its ability to significantly minimize non-faradaic current, allowing it to achieve low limits of detection. In OSWV, the voltage is scanned in a step-wise fashion with forward and reverse current pulses. [14,15] The current pulses are executed in a way that allows the electrode charging current to decay, allowing for measurement of faradaic current only. [14,15] In order for sufficient pre-concentration to occur at the electrode surface, the proper deposition potential and time must be used. In CSV, the deposition potential is typically positive relative to the oxidation potential to oxidize analyte onto the electrode surface, which in this case is the oxidation of Mn^{2+} to insoluble MnO_2. As stated above, ITO has advantages over other electrode materials because of its excellent positive potential window. We investigated this and found Current response increased and reached a maximum at +1200 mV (data not shown). This is the same potential that we found to be optimal for Mn^{2+} CSV in buffer and water samples. [5]

Deposition time can affect current response considering that as deposition time increases, more analyte can deposit at the electrode surface. All analyte can be effectively depleted from the solution onto the electrode surface if a sufficiently long deposition time is used. However, these long deposition times are generally not needed to obtain a voltammogram that can be quantitatively measured nor to achieve sufficient detection limits. It can be seen from Fig. 1 that even at a deposition time of 1 min, a quantifiable voltammogram is obtained for 100 ppb Mn^{2+}. The peak current continues to increase with deposition time until at 10 min a more symmetrically shaped voltammogram is obtained.

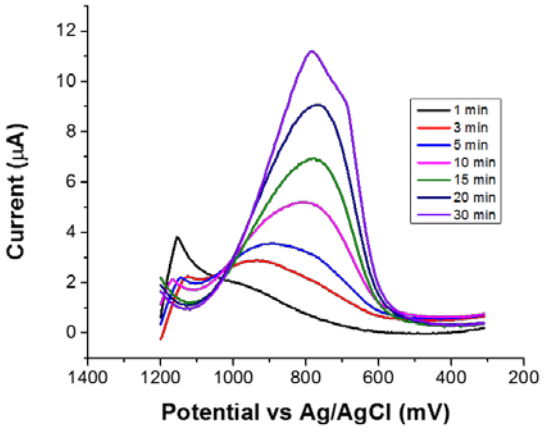


Fig. 1. Deposition time optimization. Deposition potential: + 1200 mV. [Mn^{2+}]: digested bovine blood + 100 ppb Mn^{2+} (1.8 μM).

However, since minimal negative effect (resolution from background, symmetrical peak shape) was seen using a deposition time of only 3 min, all subsequent experiments were completed using 3 min deposition.

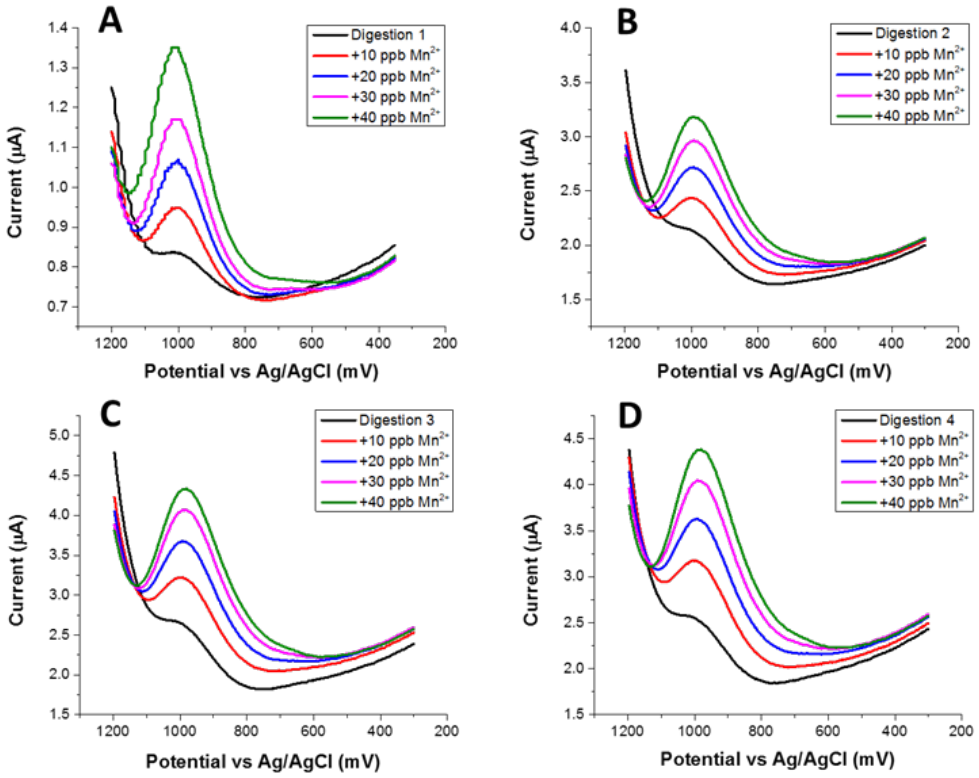
In order to investigate ITO as a working electrode for the determination of Mn^{2+} in biological samples, bovine whole blood samples were analyzed. For the CSV analysis, the standard addition method was used. After measuring 3 replicates of the acid-digested whole blood, 4 successive additions of 10 ppb Mn^{2+} were added to the digested blood samples. The resulting cathodic stripping voltammograms are shown in Fig. 2.

It can be seen in Fig. 2 that both the digestion and analyses are reproducible albeit, with slight changes in the background current. Each set of voltammograms shows quantifiable MnO_2-Mn^{2+} reduction peaks from the digested blood sample as well as each subsequent addition of Mn^{2+}. The standard addition curves constructed from the data obtained in Fig. 2 were linear as R^2 values of 0.998, 0.988, 0.993, and 0.996 were obtained for digestions 1, 2, 3, and 4, respectively.

The calculated Mn^{2+} concentrations from the data in Fig. 2 are shown in Table 1 below. The results from digestion 1 were compared with those obtained with ICP-MS and GF-AAS. For the calculations of Mn^{2+} using CSV, the y-intercept obtained from the standard addition curve was divided by the slope and then multiplied by the dilution factor (14.3, 11.7, 11.1, and 11.2 for each separate digestion and neutralization procedure). For digestion 1, the calculated Mn^{2+} concentrations using CSV and GF-AAS agree at the 95% confidence level

whereas between CSV and ICP-MS the confidence level agreement decreases to 80%.

Fig. 2. Cathodic stripping voltammograms of acid-digested bovine whole blood. (A) Digestion completed on 01/09/16, (B) digestion completed on 02/09/16, (C) digestion completed 02/26/16, and (D) digestion completed 03/21/16. Each digestion sample



was from a fresh batch of Na-Heparin bovine whole blood. Deposition potential: +1200 mV; deposition time: 3 min

The calculated Mn^{2+} concentrations varied by 9% using CSV across all 4 acid-digested bovine blood samples. These results show that CSV with ITO can be used for quantitative determination of Mn^{2+} in whole blood; comparable Mn^{2+} concentrations are obtained as well as similar accuracy and precision to that of ICP-MS and GF-AAS.

Table 1. Analysis method comparison for acid-digested bovine whole blood

Analysis Method (Digestion/Sample #)	[Mn^{2+}], ppb (µM)
ICP-MS (1)	60 ± 11 (1.1 ± 0.2)
GF-AAS (1)	48 ± 4 (0.9 ± 0.1)
CSV (1)	46 ± 10 (0.8 ± 0.2)
CSV (2)	58 ± 13 (1.1 ± 0.2)
CSV (3)	48 ± 11 (0.9 ± 0.2)
CSV (4)	54 ± 11 (1.0 ± 0.2)

An accurate, precise CSV method for the determination of Mn^{2+} in bovine whole blood using an ITO working electrode was shown to give comparable results to those obtained with other commonly used analytical methods (GF-AAS, ICP-MS). After optimization of the CSV method, accurate Mn^{2+} determination with ITO was obtained with 95% and 80% confidence level agreement with GF-AAS and ICP-MS, respectively. Though we recently reported ITO as an ultrasensitive working electrode for Mn^{2+} in water samples, ITO has never been used for metals determination in biological samples like whole blood. The potential applied in the CSV method is one where few other metal ions electrolyze, giving the method excellent selectivity as no interferences were observed from other components in the bovine whole blood samples. The advantage of ITO's excellent sensitivity to Mn^{2+} under harsh conditions coupled with its potential to be miniaturized into a microfabricated sensor make this method an attractive one for point-of-care applications.

This method is a potential alternative to traditional expensive and time-consuming blood-metal measurements without compromising sensitivity and/or selectivity. The low cost of instrumentation and ease of use make this technique potentially applicable to hospital

laboratories where result turn-around time could be significantly reduced. To achieve an applicability such as this, future directions involve miniaturizing and expediting the digestion process for rapid, accurate analysis.

Experimental

All CSV measurements were made using a BASi Epsilon Electrochemical Analyzer (Bioanalytical Systems, West Lafayette, IN). Measurements were made in a 10 mL cell consisting of ITO coated glass slides (Corning 1737F, 11-50 Ω/sq, 135 nm, Thin Film Devices, Anaheim, CA) with 10 x 40 mm dimensions as the working electrode, a Ag/AgCl reference electrode, and a platinum (Pt) wire auxiliary electrode. The basic parameters for OSWV that were used for the stripping step were: square wave amplitude = 25 mV, step potential = 5 mV and frequency = 25 Hz. The extrapolated baseline current method described by Kissinger and Heineman was used to measure peak currents (i_p). [14]

For the bovine blood digestion, 0.350 mL of Na-Heparin bovine blood was added to the 10 mL digestion vials. For blank solutions, 0.350 mL of deionized water was added. 1.00 mL of concentrated trace metal grade HNO_3 was then added to all digestion vials (sample and blank). The vials were then placed in the hotblock and pre-digested at 90 °C for 30 min; the temperature was then increased to 120 °C for 90 min. After this, the vials were removed from the hotblock and allowed to cool for 10 min before adding 0.250 mL of 30% H_2O_2. The vials were placed back in the hotblock for an additional 90 min at 120 °C before removing and cooling once more. 0.150 mL of H_2O_2 was added and the vials were placed back on the hotblock at 120 °C for 45 min. The samples were then cooled and placed back into the 4 °C refrigerator until neutralized for analysis.

For the neutralization, 5.0 M NaOH was added dropwise to each digested vial (sample and blank) until the pH was raised to 5.0. A pH of 5.0 was previously found to be the optimal pH for Mn^{2+} CSV on ITO. [5] After neutralization, the dilution factor varied from 11-12x. The samples were analyzed by CSV, GF-AAS, and/or ICP-MS.

Acknowledgements

The authors gratefully acknowledge funding provided by NIEHS Grant R01 ES022933. We also thank Dr. Necati Kaval, Dr. Daoli Zhao, and Dr. Stephen Mills for helpful discussion.

References

[1] Grandjean, P; Landrigan, P.J. *The Lancet Neurology*. **2014**, *13(3)*, 330-338

[2] Jiang, Y; Zheng, W; Long, L; Zhao, W; Li, X; Mo, X; Lu, J; Fu, X; Li, W; Liu, S; Long, Q; Huang, J; Pira, E. *Neurotoxicology* **2007**, *28*, 126-135

[3] Yokel, R.A. *Neurol. Med.* **2009**, *11*, 297-310

[4] Baker, M; Simpson, C.D; Stover, B; Sheppard, L; Checkoway, H; Racette, B.A; Seixus, N.S. *J. of Occup. Hyg.* **2014**, *11*, 210-217.

[5] Rusinek, C.A; Bange, A; Warren, M; Kang, W; Nahan, K; Papautsky, I; Heineman, W.R. *Anal. Chem.* **2016**, *88(8)*, 4221-4228.

[6] United States Environmental Protection Agency, Manganese TEACH Chemical Summary. http://www.epa.gov/teach/chem_summ/manganese_summary.pdf (Accessed March 2015).

[7] Woolf, A; Wright, R; Amarasiriwardena, C; Bellinger, D. **2002**, *Environmental Health Perspectives*. **2002**, *110(6)*, 613-616.

[8] Haynes, E.N; Ryan, P; Chen, A; Brown, D; Roda, S; Kuhnell, P; Wittberg, D; Terrell, M; Reponen, T. *Science of the Total Envirnoment.* **2012**, *427*, 19-25.

[9] 2014 TRI Factsheet: City- Marietta, OH. https://iaspub.epa.gov/triexplorer/tri_factsheet.factsheet?&pstate=OH&pcity=marietta&pyear=2014&pDataSet=TRIQ1. (Accessed March 2016).

[10] Pinyayev, T; Seliskar, C.J; Heineman, W.R. *Anal. Chem.* **2010**, *82*, 9743-9748.

[11] Rusinek, C.A; Bange, A; Papautsky, I; Heineman, W.R. *Anal. Chem.* **2015**, *87(12)*, 6133-6140.

[12] Kang, W; Pei, X; Bange, A; Haynes, E.N; Heineman, W.R; Papautsky, I. *Anal. Chem.* **2014**, *86*, 12070-12077.

[13] Pei, X; Kang, W; Yue, W; Bange, A; Heineman, W.R; Papautsky, I. *Anal. Chem.* **2014**, *86*, 4893-4900.

[14] Kissinger, P.T; Heineman, W.R. *Laboratory Techniques in Electroanalytical Chemistry*, 2nd. Ed,; Marcel Dekker: New York, 1996.

[15] Strobel, H.A; Heineman, W.R. *Chemical Instrumentation: A Systematic Approach*, 3rd. ed.; Wiley: New York, 1989.

[16] El-Desoky, H.S; Ismail, I.M; Ghoneim, M.M. *J. Solid State Electrochem.* **2013**, *17(12)*, 3153-3167.

[17] Scollary, G.R; Nan Chen, G; Cardwell, T.J; Vincente-Beckett, V.A. *Electroanalysis*. **1995**, *7*, 386-389.

[18] Zudans, I., Paddock, J.R., Kuramitz, H., Maghasi, A.T., Wansapura, C.M., Conklin, S.D., Kaval, N., Shtoyko, T., Monk, D.J., Bryan, S.A., Hubler, T.L., Richardson, J.N., Seliskar, C.J., Heineman, W.R. *J. Electroanal. Chem.* **2004**, *565*, 311-320.