

Submission ID #: 61055

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Project Page Link: <https://www.jove.com/account/file-uploader?src=18621823>

Title: Setup of Capillary Electrophoresis-Inductively Coupled Plasma Mass Spectrometry (CE-ICP-MS) for Quantification of Iron Redox Species (Fe(II), Fe(III))

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Author Questionnaire

1. **Microscopy:** Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **no**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **no**
3. **Filming location:** Will the filming need to take place in multiple locations? **no**

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Vivek Venkataramani**: Excessive iron results in free redox-active Fe(II) and can cause devastating effects within the cell such as oxidative stress and death by lipid peroxidation known as ferroptosis. Quantitative measurement of ferrous and ferric iron is the key for closer insight into these detrimental processes.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

- 1.2. **Bernhard Michalke**: This CE-ICP-MS technique provides both fast analysis and low quantification limits, which are necessary for quantifying iron redox species.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. CE-ICP-MS Interface Setup

- 2.1. Begin by selecting a nebulizer with low self-aspirating volume [1] and preparing the appropriate parts to mount the home-made interface [2].
 - 2.1.1. Talent picking up and demonstrating the appropriate nebulizer.
 - 2.1.2. LAB MEDIA: Table 2.
- 2.2. Connect two 3-way female Luer connectors with a male cone Luer connector [1] by connecting the left end of the lower 3-way Luer bar to the male connector [2] and the male connector to the middle connection of the upper 3-way Luer [3].
 - 2.2.1. Talent holding parts 3 and 4. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset.*
 - 2.2.2. Talent connecting the left end of the lower 3-way Luer bar to the male connector. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize the connection between part 3 (the one at the bottom) and 4 (just the one that connects the two 3's).*
 - 2.2.3. Talent connecting the male connector to the middle connection of the upper 3-way Luer. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize the connection between part 3 (the one at the top) and 4.*
- 2.3. Next, put a 1-centimeter tube over the Platinum wire [1] and use a 1-centimeter silicone tube to connect the first tube and the nozzle of a male Luer connector [2]. Fix the assembly to the middle connection of the lower 3-way Luer connector by screw rotation [3]. *Videographer: This step is important!*
 - 2.3.1. Talent putting the tube over the wire. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize parts 1 and 7, just the bottom part 1.*
 - 2.3.2. Talent putting the silicone tube over the tube and the nozzle of the Luer connector. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize parts 5 and 2, just at the middle connection (where the outlet electrode is located).*
 - 2.3.3. Talent fixing the assembly to the Luer connector.
- 2.4. Push a 1-centimeter tube over the outlet of the CE capillary and position it 8 to 9 centimeters from the end [1], then connect it to the nozzle of the male Luer connector with a silicone tube [2]. *Videographer: This step is difficult and important!*

- 2.4.1. Talent pushing the tube over the outlet of the capillary. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize all part 1 and the CE capillary, just the top part 1.*
- 2.4.2. Talent putting the silicone tube over the first tube and the nozzle of the connector. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize parts 5 and 2, just at the top connection.*
- 2.5. Put the whole assembly through the bar of the upper 3-way T-connector and fix the male Luer connector and left end of the female 3-way Luer connector by screw rotation [1].
 - 2.5.1. Talent putting the assembly through the bar of the upper 3-way T-connector and fixing the Luer connectors by screw rotation.
- 2.6. Then, fix the 25-centimeter silicone tube at the nozzle of a male Luer connector [1] and fix the whole assembly at the bottom of the bar from the lower 3-way Luer connector [2].
 - 2.6.1. Talent fixing the silicone tube at the nozzle. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize parts 5 and 2, just at the bottom (outlet electrolyte flow).*
 - 2.6.2. Talent fixing the whole assembly. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize the connection between parts 3, 5 and 2, just at the bottom (outlet electrolyte flow).*
- 2.7. Tightly push the 1-centimeter silicone tube 5 millimeters over the end of the nebulizer while the second male Luer cone connector is plugged into the protruding part of the tube [1].
 - 2.7.1. Talent pushing the silicone tube over the end of the nebulizer. *Video Editor: Show the schematic in Figure 1 (not the photo) as an inset and emphasize the connection between parts 4, 6, and the nebulizer.*
- 2.8. Move the interface part with the protruding CE capillary to the male cone at the nebulizer [1], then carefully insert the capillary through the male cone and the wider part of the nebulizer capillary [2]. *Videographer: This step is difficult and important!*
 - 2.8.1. Talent moving the mounted interface part to the male cone at the nebulizer.
 - 2.8.2. Talent inserting the male capillary through the male cone.
- 2.9. If necessary, adjust the length of the protruding capillary by unscrewing the left-hand male Luer, moving the Luer with attached tubes into the required position, and re-installing it [1]. The upper female 3-way Luer connector should fit tightly to the male cone [2]. When finished with the setup, proceed with ICP-MS [added]. *Videographer: This step is difficult and important!*

- 2.9.1. Talent unscrewing the left-hand male Luer, moving the Luer with attached tubes into the required position, and re-installing it.
- 2.9.2. Proper connection between the upper female 3-way Luer connector and the male cone.

Added shot: Talent on the computer showing the Mass-spec results with the two iron peaks.

Results

3. Results: Analysis of SH-SY5Y Cell Lysate

- 3.1. Calibration curves were created for iron-three and iron-two redox species. The limits of detection were 3.1 micrograms per liter for iron-two and 3.2 micrograms per liter for iron-three [1], and linearity was demonstrated up to 150 micrograms per liter [2].
 - 3.1.1. LAB MEDIA: Figure 2.
 - 3.1.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the first 4 data points on both graphs, up to 150 µg/L.*
- 3.2. The analysis of the SH-SY5Y cell lysate showed a slightly slower migration for iron redox species due to the somewhat higher conductivity. The measured concentrations of iron-three and -two were 330 micrograms per liter and 84 micrograms per liter, respectively, resulting in an iron two to three ratio of 0.25 [1].
 - 3.2.1. LAB MEDIA: Figure 3.

Conclusion

4. Conclusion Interview Statements

- 4.1. **Bernhard Michalke:** The most important step of this protocol is the careful insertion of the protruding capillary through the mounted interface and its suitable positioning related to the nebulizer capillary.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.9.1 and 2.9.2.*

- 4.2. **Vivek Venkataramani:** The presented method may become a promising tool to deepen the insight into iron-dependent molecular processes. Carefully designed studies are needed to validate if Fe(II)/Fe(III) quantification can serve as a potential biomarker for neurodegenerative disease or cancer progression and to pinpoint tissues at risk for Fe(II)-mediated lethal damage.

4.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

