

# Enhancing the Dynamic Range for Proteomics Applications Using Electrochemical/Electrospray Ionization (ECI/ESI) Mass Spectrometry

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## Objective

To apply novel electrochemical ESI to proteomics to enhance the dynamic range

## Introduction

The electrochemical aspects of electrospray ionization are well documented but there are virtually no applications of ECI/ESI MS to proteomics and very few applications under RP-HPLC conditions. The paucity of redox active labeling compounds and the lack of understanding of what ion source hardware and conditions are required to obtain electrochemical ionization at an analytically useful level in an ESI ion source may contribute to this limited applicability. However, the unsurpassed sensitivity of electrochemical detection and the selectivity of mass spectrometric detection makes this a very interesting analytical target, particularly in proteomics applications. Therefore, we provide our initial efforts at establishing ECI/ESI MS as a sensitive and selective proteomics tool with increased dynamic range.

## Experimental Design



Figure 1. Standard AP/ESI ion source



Figure 2. Corona discharge observed under ECI/ESI conditions: at an electrode with a high positive potential and a sharp edge electrons will be transferred into the gas phase creating a plasma.

In this initial study, a standard, commercial AP/ESI ion source (Fig. 1) was mounted on a Waters LCT TOF mass spectrometer. Using ferrocene-based label we developed conditions to obtain ECI sensitivities equal to or greater than ESI. We determined one critical objective to be the maximization of the corona discharge (Fig. 2) present at the end of the ESI capillary. Capillary voltage, desolvation gas temperature and capillary position were all adjusted to increase the amount of observed corona discharge and subsequently the observed signal from the ferrocene labeled compounds.

## Results: New ECI/ESI mechanism

We propose that the corona discharge present in the ion source provided an escape pathway for the removed electrons from the tip of the metal capillary. With a stainless steel capillary, the electrons with high positive energy were removed from an Fe atom located at the capillary's tip by a neutral N<sub>2</sub> thus oxidizing Fe to Fe<sup>+</sup> (Fig.1).

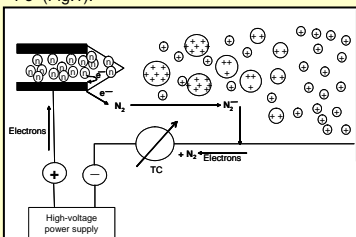


Figure 1: Overview ECI/ESI mechanism

If the analyte has an ionization potential lower than Fe, a one electron oxidation of the analyte occurred on the surface of the capillary. The oxidized analyte molecule in solution was then sprayed and desolvated by the normal ES process. To complete the electrical circuit, the corona discharge generated N<sub>2</sub><sup>+</sup> eventually traveled to a surface at ground potential and released its electron.

## Results: derivatized sulfhydryl groups

To demonstrate the efficiency of ECI versus ESI we derivatized glutathione with N-ferrocenyl iodoacetamide (Fig. 4). The resulting S-ferrocenyl labelled peptide is ionizable by both ESI and ECI ionization.

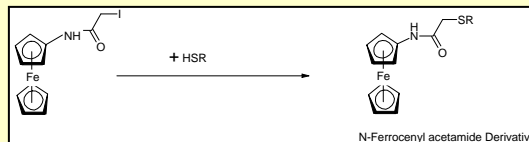


Figure 4: Reaction of N-ferrocenyl iodoacetamide with sulfhydryl groups

Using standard ESI conditions (100 femtomoles of derivatized glutathione) the ESI [M+H]<sup>+</sup> ion at m/z 549 had an intensity of 100 arbitrary units (abu) while the ECI M<sup>+</sup> ion at m/z 548 had an intensity of 50 abu. To maximize the amount of observable corona discharge and thus increase the ECI/ESI to 300 abu, we then altered the ionization conditions: max. capillary potential voltage 5kV, max. desolvation gas temp 500 °C and extended the capillary as far as possible (ca 2 mm).

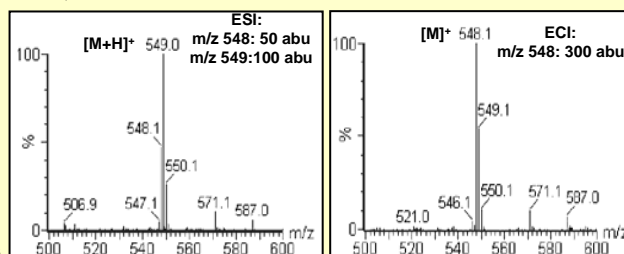


Figure 5. ESI mass spectrum of derivatized GSH

Figure 6. ECI mass spectrum of derivatized GSH

## Results: FBA-derivatized glucose

When glucose was derivatized with ferrocene boronic acid (FBA) (Fig. 7) and analyzed by ECI/ESI, an impressive 2,500 fold gain in sensitivity was observed indicating the potential of this technique (Fig. 8 and 9).

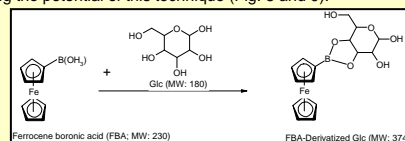


Figure 7: Reaction of ferrocene boronic acid with glucose.

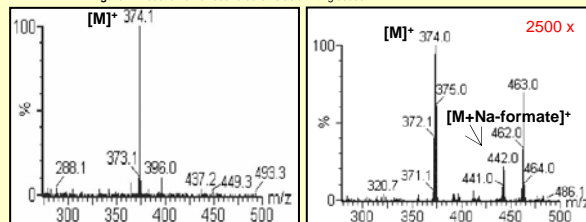


Figure 8: Residual ECI spectrum of FBA derivatized glucose under ESI conditions

Figure 9: ECI spectrum of FBA derivatized glucose

## Results: UV-activity of ferrocene label

We further explored an often overlooked feature of ferrocene based labelling compounds: their UV activity. This is useful when derivatizing a compound in a mixture and the RP-HPLC retention time of the product is unknown. To demonstrate this UV activity, we first reduced the disulfide bond of somatostatin-14 with TCEP and derivatized the free cysteines with N-ferrocenyl iodoacetamide. The resulting ferrocene labelled compound was easily located in the UV/VIS (380nm) trace of the RP-HPLC (Fig. 10, top panel) and the ECI/ESI spectrum showed intense doubly and triply charged ions (Fig. 11).

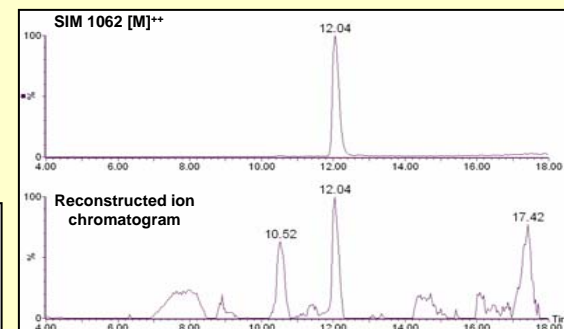


Figure 10: RP-HPLC chromatogram of TCEP and N-Ferrocenyl iodoacetamide treated Somatostatin-14

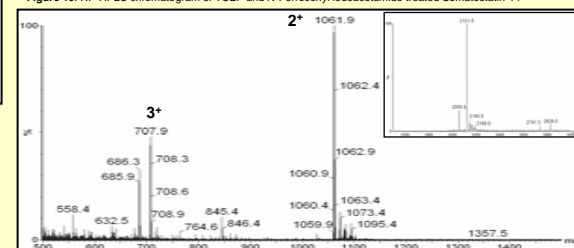


Figure 11: ECI mass spectrum of N-Ferrocenyl iodoacetamide treated Somatostatin-14; inset shows deconvoluted spectrum

## Conclusions

Our initial efforts in using a RP HPLC-ECI/ESI-MS for proteomics applications are presented. After labeling with ferrocene labels, sulfhydryl groups and glucose derivatives can be detected by ECI at high sensitivity. The UV activity of the label can additionally be used. Taking together, we conclude that our new ECI/ESI MS method can be used as a new, sensitive and selective proteomics tool.

## Acknowledgements

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