

# Using the Inherent Electrochemistry in a Microfabricated Nanospray Emitter System for Analytical Advantage

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## OVERVIEW

- Electrochemistry is inherent to NanoMate® nanospray emitter system as with all electrospray ion sources.
- The inherent electrochemistry can be used to advantage by using non-coated, carbon-impregnated, "echem" spray tips when efficient oxidation is required.
  - Electrochemical oxidation products of metalloporphyrins were produced using the nanospray emitter system. The oxidation efficiency depended on the electrochemical properties of the different porphyrins and whether coated (standard) or non-coated ("echem") carbon-impregnated nanospray tips were used.
  - Efficient oxidation in positive ion mode was also utilized to tag a cysteine-containing peptide on-line with electrochemically generated benzoquinone in order to improve peptide identification.

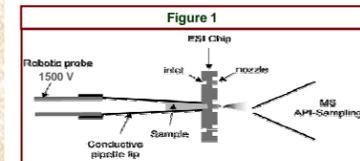
## INTRODUCTION

- This research is focused on understanding, controlling and utilizing inherent electrochemical reactions in a NanoMate® nanospray emitter system [1].
  - To-date investigations focused on eliminating unwanted electrochemical oxidation in the NanoMate® nanospray emitter system have used redox buffers or have prevented direct contact between the sample and the electrode by using coated tips [2].
  - Special, non-coated ("echem") tips (direct contact of analyte and the electrode) exhibits very efficient electrochemical activity towards analyte oxidation in positive ion mode.
  - Electrochemistry inherent to the electrospray technique has been studied in detail and can be used for analytical advantage [3-4].
  - No utilization of the effective electrochemistry coupled to this automated, high-throughput nanospray system has been accomplished for analytical advantage until now.
- Electrochemical oxidation products of metalloporphyrins were identified using coated and uncoated nanospray tips. The oxidation efficiency depended on:
  - The electrochemical properties of the different porphyrins.
  - Whether coated or non-coated carbon-impregnated nanospray tips were used.
- Efficient oxidation was utilized to tag a cysteine-containing peptide on-line with electrochemically generated benzoquinone.
  - Hydroquinone is effectively oxidized in the nanospray tip in positive ion mode and the benzoquinone generated underwent a 1,4-Michael addition with the free cysteines on a peptide in the same solution to effectively tag each cysteine with a quinone [4-6].
- A novel strategy is proposed to eliminate unwanted analyte electrochemistry for cases where analytes have low oxidation potentials.

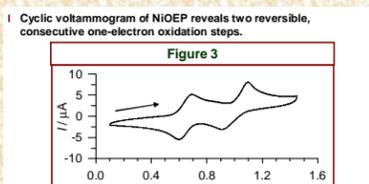
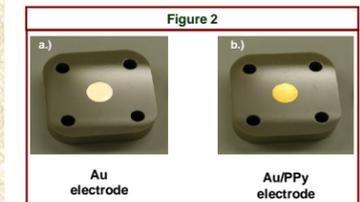
## EXPERIMENTAL

**Samples and Reagents.** 5 mM reserpine (Aldrich, Milwaukee, WI), 5 mM peptide NRCSGGSCWN (Aldrich) and 5 mM hydroquinone (Aldrich) solutions were prepared as a 50/50 (v/v) mixture of water (Milli-Q, Bedford, MA) and acetonitrile or methanol (Burdick and Jackson, Muskegon, MI) containing 5.0 mM ammonium acetate (99.999%, Aldrich) and 0.75% (v/v) acetic acid (PPB/Teflon grade, Aldrich). The 2 mM solutions of 2,3,7,8,12,13,17,18-octaethyl-21H,23H-porphine nickel(II) (NIOEP, Aldrich), 2,3,7,8,12,13,17,18-octaethyl-21H,23H-porphine cobalt(II) (CoOEP, Aldrich) were prepared as a 75/25 (v/v) mixture of acetonitrile and methylene chloride (Burdick and Jackson) containing 250 mM trifluoromethanesulfonate (lithium triflate, 99.995%, Aldrich). Pyrrole (Aldrich) was used as received.

**NanoMate®/MS.** The experiments were accomplished using a NanoMate® nanospray emitter system (Figure 1, Advion BioSystems, Ithaca, NY) coupled to a 4000 QTrap hybrid triple quadrupole/linear ion trap mass spectrometer (MDS Sciex, Concord, Ontario, Canada). The inherent electrochemistry took place in the coated and non-coated carbon-impregnated nanospray tips and the electrochemical oxidation products were monitored by their mass spectrometric signal: in the metalloporphyrin study, the electrochemically produced radical cations and the double-charge cations of metalloporphyrins were monitored, while in the peptide tagging experiments, a mixture of a cysteine-containing peptide and hydroquinone was sprayed and the untagged and benzoquinone (generated electrochemically from hydroquinone) tagged peptides were monitored.



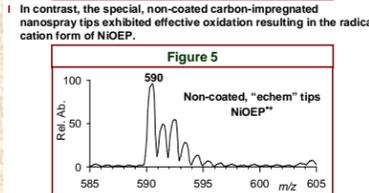
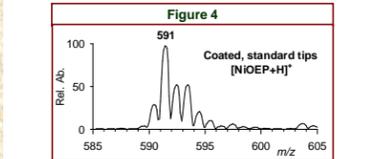
**Off-line Electrochemistry.** The off-line electrochemical experiments were controlled using a CH Instruments model 660 Electrochemical Workstation (Austin, TX). A Pt wire was used as the auxiliary electrode and a Ag/AgCl/3 M KCl electrode (model RE-6, Bioanalytical Systems, West Lafayette, IN) served as the reference electrode. Poly(pyrrole) (PPy) was electropolymerized onto a 6 mm dia. Au thin-layer flow cell electrode (model MF-1015, Bioanalytical Systems) from a solution containing 50 mM pyrrole and 0.3 M ammonium acetate in water by consecutively holding the potential at 1.2 V and 1.3 V, each for 5 minutes:



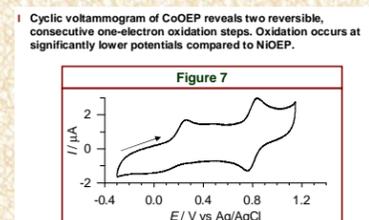
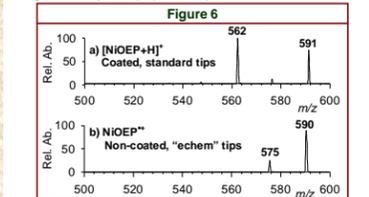
**Oxidation scheme of NIOEP:**

$$[NIOEP] \xrightarrow{-0.65 V} [NIOEP]^{\cdot+} + e^- \xrightarrow{-1.1 V} [NIOEP]^{2+} + e^-$$

$[NIOEP+H]^{\cdot+} = m/z 591$



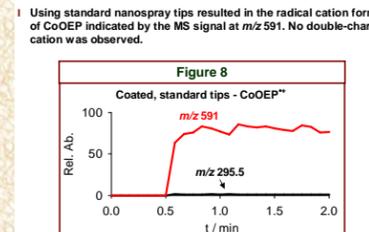
Protonated and radical cation forms of NIOEP have different fragmentation pathways (CE=40 eV).



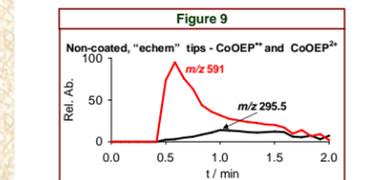
**Oxidation scheme of CoOEP:**

$$[CoOEP] \xrightarrow{-0.25 V} [CoOEP]^{\cdot+} + e^- \xrightarrow{-0.85 V} [CoOEP]^{2+} + e^-$$

$[CoOEP+H]^{\cdot+} = m/z 592$

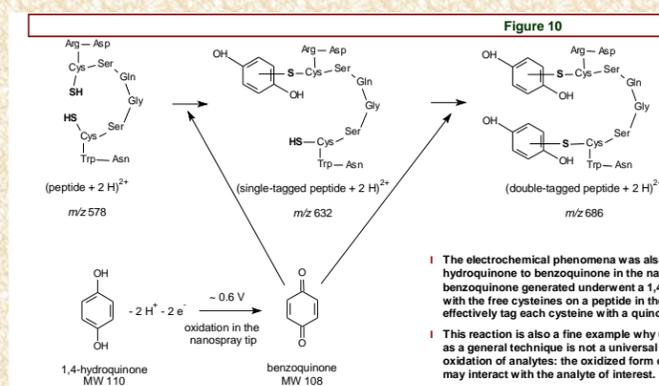


The carbon-impregnated "echem" nanospray tips oxidized CoOEP to its radical cation form at the very beginning (m/z 591), followed by the slow disappearance of the signal of the radical cation and the appearance of the double-charge cation at m/z 295.5.



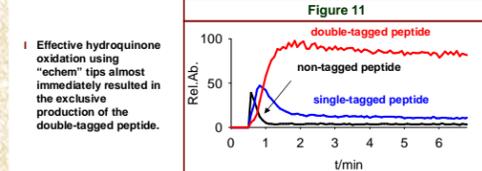
The potential window accessible is not as positive when using coated nanospray tips, but CoOEP (an analyte with low oxidation potential) could still be effectively oxidized.

## RESULTS AND DISCUSSIONS

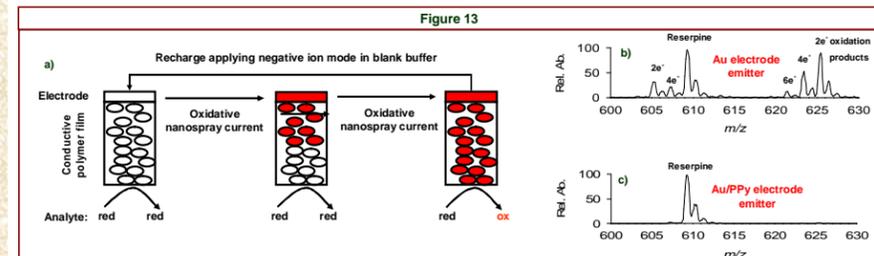


The electrochemical phenomena was also utilized to oxidize hydroquinone to benzoquinone in the nanospray tip. The benzoquinone generated underwent a 1,4-Michael addition with the free cysteines on a peptide in the same solution to effectively tag each cysteine with a quinone moiety.

This reaction is also a fine example why use of redox buffers as a general technique is not a universal solution to prevent oxidation of analytes: the oxidized form of the redox buffer may interact with the analyte of interest.



Effective hydroquinone oxidation using "echem" tips almost immediately resulted in the exclusive production of the double-tagged peptide.



These preliminary experiments were accomplished with a thin-layer electrode emitter system [6]. Mass spectra of reserpine was recorded first using a bare Au electrode emitter, followed by coating the electrode with electropolymerized poly(pyrrole) and recording the reserpine mass spectrum again.

## CONCLUSIONS

- Inherent electrochemistry in nanospray ionization can be utilized for analytical advantage.
  - Change the molecular ionic species observed for metalloporphyrins. Different species have different fragmentation pathways providing complementary information.
  - Cysteine tagging by electrochemically generated reactive species (benzoquinone) could improve peptide assignments for protein identification.
- Inherent electrochemical oxidation of analytes can be suppressed by coating the tips with electrochemically deposited redox polymers.
  - Coating limits the diffusion of analyte to the electrode surface.
  - The electrospray current is used for oxidation of the redox polymerfilm, not for the oxidation of analyte. The redox polymerfilm buffers the interfacial potential.

## NEAR FUTURE

- Study different polymerfilm-modified electrodes for possible use to either enhance (via catalysis) or suppress (via blocking analyte diffusion and/or redox buffering) analyte electrochemistry inherent to electro/nanospray devices.
- Prepare and characterize polymer-coated nanospray tips for use in NanoMate® system.

## REFERENCES

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