Influence of Emitter Electrode Material on the Nature and Abundance of Ions in Electrospray Mass Spectra

Keiji G. Asano and Gary J. Van Berkel

Organic and Biological Mass Spectrometry Group
Chemical Sciences Division
Oak Ridge National Laboratory, Oak Ridge, TN

We previously demonstrated that a thin-channel electrode emitter device can be used to control the electrochemical oxidation of an electroactive analyte, such as *N*-phenyl-1,4-phenylenediamine [1]. For non-electroactive analytes, such as bovine insulin, the thin-channel electrode emitter behaves similarly to the standard stainless steel tubular electrodes. However, changing the nature of the electrode material can change the electrochemical reactions taking place in a system. Because electrochemical reactions alter solution composition, this compositional change might be reflected in the electrospray mass spectra, particularly at low flow rates. We have found that the electrode material, especially at low flow rates (2.5 μL/min), in conjunction with the solvent system, influence the nature and abundance of ions in electrospray mass spectra for both electroactive and non-electroactive analytes.

All mass spectra were obtained on a PE SCIEX API165 quadrupole mass spectrometer with a thin-channel, planar electrode emitter system replacing the standard TurboIonSpray™ source. This device easily allows the electrode to be changed while maintaining identical settings from experiment to experiment that assures only one parameter is being varied, i.e. the electrode material. Six millimeter diameter disk electrodes were fabricated from glassy carbon (GC), stainless steel (SS), platinum (Pt), silver (Ag), gold (Au), copper (Cu), nickel (Ni), and zinc (Zn). The main solvent systems for these experiments have been 1/1 (v/v) water/methanol or water/acetonitrile containing only 5 mM ammonium acetate (ca. pH 7) or also acetic acid (ca. pH 4).

Reserpine - Reserpine is a fairly easy to oxidize compound (E_p ~ 0.75 V vs. Ag/AgCl) by a nominal 1-proton, 1-electron mechanism. The oxidized species that appears at *m/z* 607 contains a quaternary nitrogen. We have found at low flow rates (2.5 μL/min) that significant oxidation occurs using the GC, Pt, and SS electrodes, and to a lesser extent with Ni (**Figure 1**). Ag, Zn, and Cu do not produce the oxidized species of reserpine because they are acting as redox buffers. In addition, although the ion at *m/z* 607 was the predominant molecular species at low flow rates in acidic methanol solvent with GC, no oxidation occurred with the Pt electrode. At higher flow rates (20 μL/min) the oxidation efficiency is decreased and very little if any *m/z* 607 is observed even though electrospray currents are higher. Fairly abundant chemical follow-up reaction products also were observed at *m/z* 625 and 641 (+ OH and + 2 OH) for acetonitrile solutions and *m/z* 625 and 639 (+ OH and + OCH_3) for the methanol solutions.

Cytochrome *c* – Spectra acquired with the different electrodes showed similar charge state distributions but with differences in relative abundances. With respect to absolute signal levels, the SS electrode performed the best. Oxidation of the Ag and Au electrode yielded adducts in the acidic acetonitrile solution, forming $[M+xH+nAg]^{(x+n)+}$ and $[M+XH]^{X+}$ ions with AuCN attachment, respectively (**Figure 2**). Although Ag adducts were observed in the acidic methanol solution, no Au adducts were observed.

Angiotensin – Some minor differences in ion abundances of the doubly charged molecular species with GC, Pt, and SS electrodes. The oxidation of Ag and Cu electrodes yielded $[M+H+Ag]^{2+}$ and $[M+H+Cu]^{2+}$, respectively, as well in both acidic solvent systems. Singly charged ions and their corresponding adducts were formed as well. In the acidic acetonitrile solution, we observed an ion corresponding to the (b_3+Ag) adduct, but not in the acidic methanol solution.

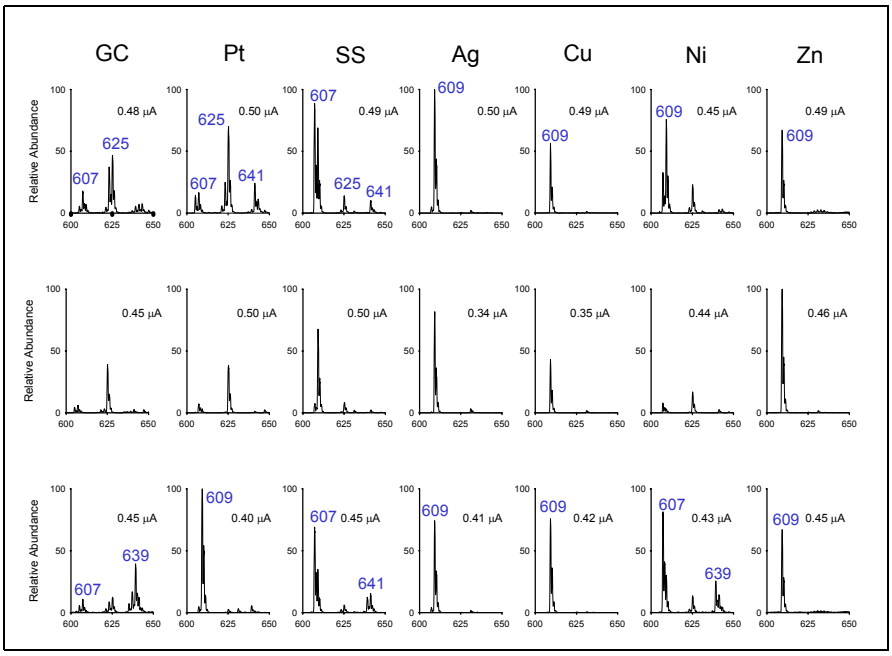

Figure 1 - Electrospray mass spectra for reserpine with various electrodes in different solvent systems. (top) 1/1 (v/v) H_2O/ACN, 5 mM NH_4OAc, 0.75% HOAc, (middle) 1/1 (v/v) H_2O/ACN, 5 mM NH_4OAc, (bottom) 1/1 (v/v) H_2O/MEOH, 5 mM NH_4OAc, 0.75% HOAc. Flow = 2.5 µL/min, 4.5 kV

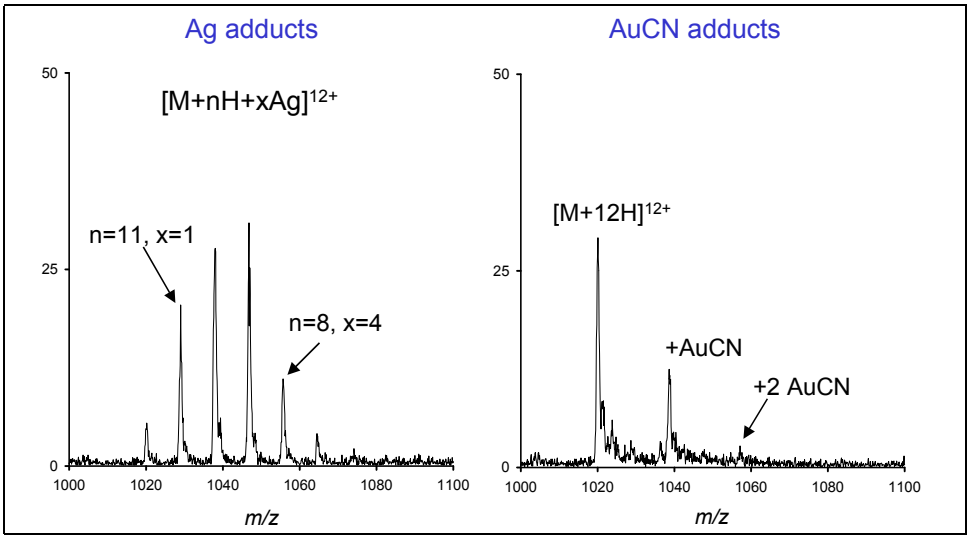

Figure 2 - +12 charge state of cytochrome c (in acidic acetonitrile soluton) showing Ag and AuCN adduct ions

[1] Van Berkel, G.J. et. al. *Anal. Chem*. **2002**, *74*, 5047-5056.

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