

# Analyte Reduction in APCI and APPI

Gary J. Van Berkel and Vilmos Kertesz

Organic and Biological Mass Spectrometry Group  
Chemical and Analytical Sciences Division, Oak Ridge National Laboratory  
Oak Ridge, Tennessee 37831-6365, USA

In a recent report, Karancsi and Si gel [1] showed that nitroaromatic compounds are reduced to the corresponding amines in an atmospheric pressure chemical ionization source (APCI). The extent of reduction, from a few percent to complete reduction, was found to be dependent on the compound and on the nature of the solvent system. Reduction was promoted with protic solvents like water and methanol, but diminished or eliminated in dried acetonitrile or methylene chloride. The occurrence of this reduction was likened to that observed for nitroaromatics in a traditional chemical ionization (CI) source [e.g., 2], but no details of the possible mechanisms were discussed. Our interest in such reduction processes lies in the possible use of APCI, or the new related technique, atmospheric pressure photoionization (APPI) [3], as interfaces for on-line electrochemistry mass spectrometry (EC/MS) experiments. Electrospray (ES) has proven to be very suitable for EC/MS and means have been developed to avoid the influence of the inherent electrochemical process of ES on the EC occurring in the on-line cell [4,5]. The possibility of using APCI or APPI for EC/MS experiments might expand the range of compounds amenable to study. In this work we investigated the reduction of imines ( $RR'C=NH$ ) to the corresponding amines ( $RR'CNH_2$ ) during APCI-MS and APPI-MS analysis. We compared the ES mass spectra of select imines with the APCI and APPI mass spectra of these same imines obtained as a function of: (1) heated nebulizer probe temperature, (2) heated nebulizer cleanliness, (3) corona discharge current, and (4) the solvent system.

A SCIEX API365 triple quadrupole equipped with pneumatically-assisted ES, APCI and APPI sources was used. The stainless steel capillary ES emitter was held at 4.5 kV with  $N_2$  used for nebulization. The APCI source was used without modification with  $N_2$  as the auxiliary and nebulizer gas. Details of the prototype APPI source are the same as those described elsewhere [3]. APPI lamp current was 0.7 mA with an acetone (distilled) dopant flow of 10 L/min. *N*-phenyl-1,4-phenylenediamine, **1**, and thionin, **2**, were prepared in aqueous methanol (or acetonitrile) containing 5.0 mM ammonium acetate (pH 7). Acetic acid or formic acid were added to achieve pH 4 and pH 3, respectively. Solutions were either infused or flow injected at 50 L/min. **1** was oxidized on-line to *N*-phenyl-1,4-phenylenediimine, **1a**, in a thin-layer flow-by electrode electrochemical cell [4,5].

Our studies indicate that imines may be reduced to the corresponding amines in APCI and APPI by means of a surface enhanced process that involves reactive species generated in the ionization plasma. This complicates any use of APCI or APPI for the general analysis of these compounds or for use with EC/ES. However, with proper control of solvent conditions and heated nebulizer temperature, the reduction can be completely avoided or minimized. The reduction of imines to amines appears to be a general phenomenon for this compound class (Figures 1 and 2). The extent of reduction observed increased with decreasing heated nebulizer probe temperature indicating that a surface process was involved (Figure 2). The character of the surface within the heated nebulizer probe (presence of hydrocarbon residue) was also found to play a role in the reduction process. Water (and possibly other protic solvents and additives) promoted the reduction process whereas it was diminished with nonprotic solvents (Figure 3). We believe that reactive hydrogen radicals produced in the ionization plasma may be involved in the reduction process.

Our results show that reduction of imines to amines in APCI and APPI can be avoided or minimized through the use of nonprotic solvents (acetonitrile), high heated nebulizer probe temperatures (500 °C), and a clean heated nebulizer probe liner. Conversely, reduction of imines to amines in APCI and APPI can be enhanced by using water as a solvent (>50% v/v), by using low heated nebulizer probe temperatures (<400 °C), and by using a dirty heated nebulizer probe liner.

## STRUCTURES

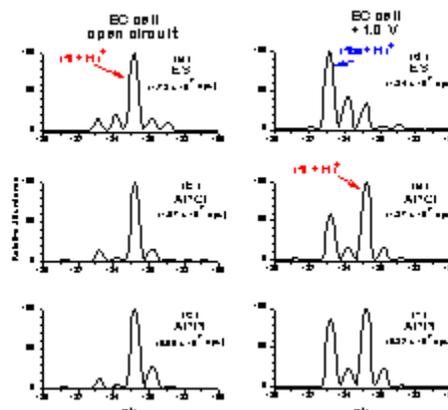
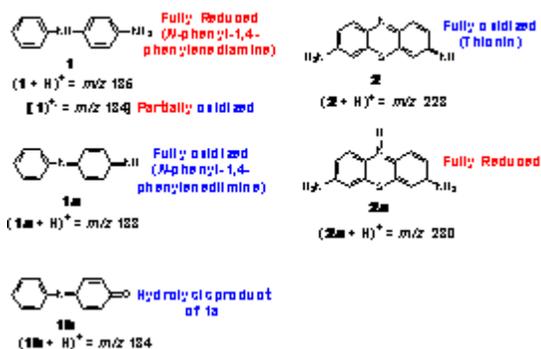


Figure 1. (a) ES, (b) APCI and (c) APPI mass spectrum of 20 M *N*-phenyl-1,4-phenylenediamine, **1**. (d) ES, (e) APCI and (f) APPI mass spectrum of nominally 20 M *N*-phenyl-1,4-phenylenedimine, **1a**, produced on-line by electrochemical oxidation of *N*-phenyl-1,4-phenylenediamine, **1**. For both APCI and APPI the heated nebulizer temperature was 400 °C, solvent flow rate was 50 L/min, and the solvent was water/methanol (1/1 v/v) with 5.0 mM ammonium acetate (pH 4). APCI corona discharge current was 2 A. APPI lamp current was 0.7 mA with an acetone dopant flow of 10 L/min.

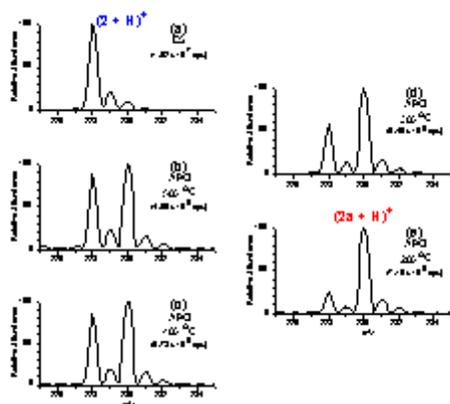


Figure 2. (a) ES mass spectrum and (b, c, d, and e) APCI mass spectra of 20 M thionin, **2** (water/methanol, 1/1 v/v, 5.0 mM ammonium acetate, pH 3). APCI spectra were obtained at heated nebulizer probe temperatures of (b) 499 °C, (c) 400 °C, (d) 300 °C, and (e) 200 °C. Solvent flow rate was 50 L/min. APCI corona discharge current was 2 A.

[1] Karancsi, T.; SI gel, P. *J. Mass Spectrom.* **1999**, *34*, 975-977. [2] Budzikiewicz, H. *Org. Mass Spectrom.* **1988**, *23*, 561-565. [3] Robb, D. B.; Covey, T. R.; Bruins, A. P. *Anal. Chem.* **2000**, *72*, 3653-3659. [4] Deng, H.; Van Berkel, G. J. *Anal. Chem.* **1999**, *71*, 4284-4293. [5] Deng, H.; Van Berkel, G. J. *Electroanalysis* **1999**, *11*, 857-865.

Vilmos Kertesz acknowledges support through an appointment to the Oak Ridge National Laboratory (ORNL) Postdoctoral Research Associates Program administered jointly by the Oak Ridge Institute for Science and Education and ORNL. APCI-, APPI- and ES-MS instrumentation was provided through a Cooperative Research and Development Agreement with SCIEX (CRADA No. ORNL96-0458). This work was supported by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, United States Department of Energy under Contract DE-AC05-00OR22725 with ORNL, managed and operated UT-Battelle, LLC.

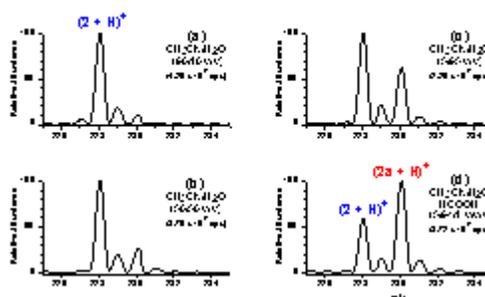


Figure 3. APCI mass spectra of 20 M thionin, **2** prepared in different aqueous acetonitrile solutions. (a) 90/10 v/v acetonitrile/water, (b) 50/50 v/v acetonitrile/water, (c) 5/95 v/v acetonitrile/water and (d) 5/94/1 v/v acetonitrile/water/formic acid. Heated nebulizer probe temperature was 500 °C, the solvent flow rate was 50 L/min, and the corona discharge current was 5 A.