1. [20 points] There are many different voltammetric techniques that are frequently applied to analytical problems. These include, among others, differential pulse polarography, cyclic voltammetry, chronoamperometry, chronocoulometry, rotating disk electrode voltammetry and ac voltammetry.

There are two questions that are often asked about an electrode reaction: Is it reversible (in the electron-transfer sense)? Is it diffusion controlled?

For any TWO voltammetric methods, describe as completely as you can the criteria for reversibility and diffusion control.
CUMULATIVE EXAMINATION IN ANALYTICAL CHEMISTRY

May 8, 1999

2. [20 points] In her seminar of March 26, "Science for the Sake of Art", Janice Carlson of Winterthur discussed applications of analytical chemistry for the identification or authentication of art objects.

In general what are some of the special requirements on analytical methods as applied to the characterization or identification of art objects?

As one of her examples she described the use of X-ray fluorescence for authentication of silver objects. Briefly describe the theory and instrumentation of X-ray fluorescence. Briefly explain why the technique is particularly suitable for these analyses. What data were obtained on the silver artifacts and how were these data used for authentication? Is X-ray fluorescence useful for the analyses of other types of objects? Explain.

Another technique that is often used in museum or conservation laboratories is IR analysis. Give examples of problems in the characterization of art objects where IR analyses would be useful. What are some of the associated difficulties?
3. [20 points] This question concerns the analytical method known as flow injection analysis (FIA).

The basic elements of FIA quantitation consist of a continuous flow pump, an injector, a mixing coil and a suitable detector.

(a) FIA typically involves the dispersion-controlled reaction of a reagent in the flowing stream (the carrier) with the injected analyte solution. Dispersion, $D$, is given by the ratio of the injected analyte concentration, $c_0$, to the observed concentration, $c$, at the detector ($D = c_0/c$). In FIA, mixing occurs under dispersion control, and $D$ often is near 2. Explain what "dispersion control" means by considering the profiles expected for a plug of colored liquid injected into a flowing, uncolored sample of the same liquid. What would happen over time with NO flow? What happens to the plug shape over time with axial convection along the direction of the tube? What would the plug shape be over time with radial convection in the direction perpendicular to the flow?

(b) In flow injection, the reaction between the analyte and the detection reagent is generally controlled by dispersion and is often incomplete. Why is it that we can use an incomplete reaction to calibrate analyte to the measured color?

(c) The volume of the analyte sample can strongly influence the signal in FIA. Unlike chromatographic separations, no internal standard is included to determine sample volume in FIA. Use the figure below to explain why no internal calibration is needed in FIA.

![Graphs showing absorbance and dispersion over time and length](image)

Effect of sample volume and length of tubing on dispersion. (a) Tube length: 20 cm; flow rate: 1.5 mL/min; injected volumes are in µL. (b) Sample volume: 60 µL; flow rate: 1.5 mL/min.
(d) The diagram below shows a flow injection determination of Ca(II) in water by formation of a complex with o-cresolphthalein complexone at pH 10. From the figure, determine the average concentration of the analyte in the samples whose signals appear between 2 and 3 minutes.

(a) Flow-injection apparatus for determining calcium in water by formation of a colored complex with o-cresolphthalein complexone at pH 10. All tubing had an inside diameter of 0.5 mm. A and B are reaction coils having the indicated lengths. (b) Recorder output. The four sets of curves at the left are for duplicate injections of standards containing 5, 10, 15, and 20 ppm calcium.
CUMULATIVE EXAMINATION IN ANALYTICAL CHEMISTRY

May 8, 1999

4. [20 points] This question is based upon the attached article entitled "How to Interface a Liquid Chromatograph to an Inductively Coupled Plasma - Mass Spectrometer for Elemental Speciation Studies" by Mary Kate Donais.

a) [5 points] Write a balanced equation for the generation of AsH₃ from AsO₄³⁻ in a hydride reactor. Refer to the highlighted section in the third column on page 34 of the article for the reagents used in the experiment.

b) [5 points] Explain how the various arsenic species are separated in the experiment shown in Figure 3. Refer to the highlighted section at the bottom of the second column on page 34 of the article for the chromatographic conditions used in the experiment.

c) [5 points] Suggest a reason why the peaks in the "hydride" chromatogram of Figure 3 are broader than the peaks in the "nonhydride" chromatogram.

d) [5 points] The chromatogram in Figure 4 is an example of an "over-separation". In other words, the chromatographic conditions could be changed so that the two components are still baseline-resolved but the total analysis time is decreased. How would you change the chromatographic conditions in this experiment to decrease the total analysis time? Refer to the highlighted text on page 35 of the article for the chromatographic conditions used in the experiment.
How to Interface a Liquid Chromatograph to an Inductively Coupled Plasma–Mass Spectrometer for Elemental Speciation Studies

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Elemental speciation is of growing importance in many scientific fields including geology, biochemistry, medicine, and environmental science and is increasingly moving beyond university research into practical application. Speciation studies can be easily implemented through the interfacing of a liquid chromatograph to an element-specific instrument such as an inductively coupled plasma–mass spectrometer.

Inductively coupled plasma–mass spectrometry (ICP-MS) is a sensitive analytical technique used to quantify total trace-level concentrations of elements in a variety of sample types including environmental, clinical, geological, semiconductor, nuclear, and specialty chemical. However, the measurement of total elemental concentrations in a sample provides no information about the chemical form in which the element exists in the sample. Each elemental form, whether it be an inorganic salt, organometallic compound, protein–metal complex, or other, has its own unique physical properties such as solubility, boiling point, toxicity, and metabolic pathways in living organisms. The quantitation of individual elemental forms in a sample, referred to as elemental speciation, is therefore very important to accurate data evaluation. Elemental speciation is a growing field of analytical chemistry and is of increasing importance to a variety of industries.

One common approach to elemental speciation is the interfacing of a liquid chromatograph with a spectrometric instrument such as an ICP-MS system. Although liquid chromatography (LC) is more commonly used to separate organic molecules, it also can be used to separate metal-containing species. A mixture of elemental species is introduced into the chromatograph and is separated into individual components. Under the proper LC conditions, the individual species elute one by one from the LC column directly into the ICP-MS system where they are detected and quantified. The resulting data are in the form of element-specific chromatograms. Figure 1 illustrates this process.

A variety of LC methods can be used with ICP-MS to separate mixtures of elemental species. Ion-exchange chromatography and ion-pairing, reverse-phase chromatography can be used to separate species based on their charge. These two techniques are the most common types of LC used with ICP-MS for element-specific chromatography. For example, separations of either arsenic species or mercury species in environmental samples can be achieved using ion-exchange or ion-pairing, reverse-phase chromatography. Another LC method, size-exclusion chromatography, can be used to separate high-molecular-weight species according to their size and shape. The binding of lead by proteins can be studied using size-exclusion chromatography as well as the uptake of isotopically enriched elements by large biomolecules. With proper considerations specific to the LC

![Figure 1. Block diagram of LC-ICP-MS system.](image-url)
mobile phase and column type, most LC separations can be interfaced with an ICP-MS system.

CONSIDERATIONS OF INTERFACING LC WITH ICP-MS

Mobile phase. The first consideration involved in interfacing LC and ICP-MS is the mobile phase used for the separation. The LC mobile phase will be introduced continuously while acquiring the LC-ICP-MS data and is analogous to the LC-MS rinse solution. Because exposure to the LC mobile phase is continuous and can be for many hours, a stable ICP-MS signal is necessary to ensure reproducible integration of the chromatographic data. Matrix suppression and cone buildup (even with platinum cones) can occur with high dissolved solids (>8% approximately) and a high organic (>10% approximately) content. Some mobile phases with higher dissolved solids can be used with a nebulizer more tolerant to dissolved solids, such as a V-groove nebulizer. Also, organic content in the mobile phase can be increased through the use of a direct-injection nebulizer (DIN) and/or the addition of oxygen into the argon plasma to minimize carbon buildup on the cones. In general, low dissolved solids and a low organic content in the mobile phase will reduce the deposition of material on the ICP-MS sampling cone, consequently reducing the likelihood of drift in the ICP-MS signal over time. Table I summarizes some mobile phases that have been used previously for LC-ICP-MS applications.

Column size. The second consideration regarding interfacing LC with ICP-MS is the size of the LC column. LC column flow rate and sample size are controlled by the length and internal diameter of the column chosen for a particular application. Likewise, the nebulizer type and spray chamber type used in the ICP-MS instrument depend on the LC column flow rate and sample size. Table II lists typical LC column dimensions and sample sizes along with possible nebulizer and spray chamber types. The column flow rates provided in the table are approximate and should be optimized for each LC separation. LC columns greater than 4.6 mm i.d. can be interfaced with an ICP-MS system through the use of a flow splitter to reduce the column flow rate.

Column nebulizer interface. The final consideration is the interface between the LC column and the nebulizer. The tubing length between the column output and the nebulizer input should be minimized to reduce postcolumn peak broadening. If possible, one piece of tubing should be

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Type of Chromatography</th>
<th>Elemental Species Studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM ammonium formate in water at pH 5.0</td>
<td>Size-exclusion</td>
<td>Boron-containing polysaccharides</td>
<td>This paper</td>
</tr>
<tr>
<td>40 mM sodium phosphate (6.5 mM octyrylammonium phosphate, 2% methanol) in water at pH 9.0</td>
<td>Reverse-phase</td>
<td>Arsenic species</td>
<td>This paper</td>
</tr>
<tr>
<td>10-40% methanol (gradient elution)</td>
<td>Size-exclusion</td>
<td>Cobalamine analogues</td>
<td>(1)</td>
</tr>
<tr>
<td>10 mM ammonium acetate (0.1 M acetonitrile and 0.1 M methanol)</td>
<td>Reverse-phase</td>
<td>Methylmercury</td>
<td>(2)</td>
</tr>
<tr>
<td>5 mM nitric acid and 25 mM ammonium nitrate</td>
<td>Ion-exchange</td>
<td>Bromate</td>
<td>(3)</td>
</tr>
<tr>
<td>8 mM sodium pentane sulfonate at pH 3 with methanol gradient from 40-90%</td>
<td>Ion-pairing, reverse-phase</td>
<td>Organics</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Table II. Column, nebulizer, and spray chamber configurations for LC-ICP-MS system.

<table>
<thead>
<tr>
<th>Column I.d. (mm)</th>
<th>Column flow rate</th>
<th>Sample size (µL)</th>
<th>Nebulizer</th>
<th>Spray chamber</th>
<th>Recommended?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>50 µL/min</td>
<td>0.25-1.0</td>
<td>Direct-injection</td>
<td>None required</td>
<td>Yes</td>
</tr>
<tr>
<td>4.6</td>
<td>1 mL/min</td>
<td>20-100</td>
<td>Concentric</td>
<td>Quartz low-volume impact bead</td>
<td>Yes, but increased peak broadening compared with low-volume impact beads</td>
</tr>
<tr>
<td>4.6</td>
<td>1 mL/min</td>
<td>20-100</td>
<td>V-groove</td>
<td>Quartz double-pass</td>
<td>Yes, but slightly worse short-term stability compared with concentric nebulizer</td>
</tr>
<tr>
<td>4.6</td>
<td>1 mL/min</td>
<td>20-100</td>
<td>Direct-injection</td>
<td>None required</td>
<td>No, column flow rate too high for DIN</td>
</tr>
<tr>
<td>4.6</td>
<td>1 mL/min</td>
<td>20-100</td>
<td>Microconcentric</td>
<td>Quartz double-pass</td>
<td>No, column flow rate too high for MCN</td>
</tr>
</tbody>
</table>
used for this connection. If the column output cannot be directly interfaced with the nebulizer input, a zero-dead-volume fitting should be used as the union between two pieces of tubing. Again, this will minimize peak broadening. The minimization of interface volume (tubing and/or fittings) is especially vital to small internal diameter LC columns (<1.0 mm i.d.) because of the low flow rate and small amount of sample being detected for these separations.

EXAMPLES

LC–ICP-MS methods have been developed for many sample types and elemental species, and its use is now extending beyond the university research lab and into industrial application. Below are two examples that serve as a good introduction to the technique and its potential application to real-world samples. All data were collected on PlasmaQuad ICP-MS instrumentation (VG Elemental, Franklin, MA). Many excellent resources on speciation and element-specific chromatographic analysis are available in the literature.

Figure 2. Separation of arsenic species with a 1.0-mm-i.d. column and a 1.0-mm-i.d. column.

Figure 3. Detection of arsenic species with and without postcolumn hydride generation.
graphic detection are also available and provide a more detailed description of hydride generation techniques along with specific applications for environmental, clinical, food science, medicine, biochemistry, and other sample types (6-8).

Comparison of LC-ICP-MS configurations for arsenic speciation in environmental samples (9).

Many forms of arsenic can exist in the environment including arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC). The toxicities of these species vary considerably, with inorganic As(III) and As(V) the most toxic, the methylarsenic acids less toxic, and the biological detoxification products AsB and AsC the least toxic. The wide range in toxicity of these species necessitates measurement of elemental species in environmental samples for accurate pollution monitoring and risk assessment. Arsenic speciation is also challenging to the analytical chemist because of the different forms that can all be found in one sample.

Ion-pairing, reverse-phase chromatography with a standard bare column and a microbore column was investigated for the separation of the six arsenic species mentioned above. A synthetic water sample containing approximately 100 ppb each of the six species was studied. Chromatogram A in Figure 2 (9) was collected using a 4.6 mm-i.d., reversed-phase LC column (RP-2 C18, 250 mm × 4.6 mm-i.d., 50 µL injection loop; Hamilton, Reno, NV). Chromatogram B was collected using a reversed-phase Columbus C18 column (Phenomenex, Torrance, CA). 150 mm × 1.0 mm-i.d., with a 0.5-µL injection loop. The mobile phase for both separations consisted of 4 mM sodium phosphate buffer adjusted to pH 9.0 1% (v/v) methanol, and 0.5 mM tetraethylammonium phosphate as the ion-pairing reagent. The 1.0 mL/min flow rate for the 4.6 mm-i.d. column permitted the use of a standard concentric nebulizer and quartz double-pass spray chamber. The separation using the 1.0 mm-i.d. column had an optimized flow rate of only 60 µL/min, however, which required the use of a direct-injection nebulizer (Cetac Technologies, Omaha, NE). 75As was monitored by ICP-MS for both separations. Sharper peaks, improved resolution, and a shortened separation time were achieved with the 1.0 mm-i.d. micro-LC column. These improvements were most likely due to the direct intro-

duction of column effluent into the plasma with the DIN as compared to the need for a spray chamber with the concentric nebulizer. The micro-LC column also uses less mobile phase and less sample volume — benefits to most laboratories. Changes in the mobile phase and ion-pairing reagent are currently being investigated to further improve this separation; however, the primary toxic species are completely resolved under the described conditions.

Arsenic sensitivity can be increased through the formation of hydrides as a mode of sample introduction. Hydride generation is especially challenging for LC-ICP-MS because the hydride species must be formed online as they elute from the LC column but before detection by ICP-MS. This post-column hydride formation must be quick and have a short retention time in the hydride apparatus so as to retain the separation achieved on the LC column. Slow hydride formation or long residence times in the hydride apparatus will cause broadening of the arsenic peaks and loss of the chromatographic separation.

A comparison of signal intensity with and without the use of hydride generation for LC-ICP-MS is provided in Figure 3 (10). Separation of As(III), As(V), MMA, and DMA was achieved using a reversed-phase Resolve C18 column, 150 mm × 4.6 mm-i.d., 5 µm spherical particles, 50 µL injection loop (Waters, Milford, MA). The mobile phase was 6 mM potassium dihydrogen phosphate adjusted to pH 5.7, 0.2% (v/v) methanol, and 2 mM tetrabutyl-
ammonium hydroxide as the ion-pairing reagent at a flow rate of 1 mL/min.

The separation was first performed using a concentric nebulizer and a low-volume spray chamber without the online hydride generator. The hydride generator was then added on-line after the LC column to study its effect on signal intensity. The hydrides were introduced directly into the ICP-MS torch without the use of a nebulizer or spray chamber. The hydrides were produced using a 0.6% (w/v) NaBH4 solution containing 0.1% (w/v) NaOH and a 10% (v/v) HCl solution. An argon flow of approximately 150 mL/min was used to sweep the hydrides from the generator into the ICP torch.

Note the change in the relative intensities of the four species. The As(III) and As(V) signals increased significantly, but the MMA and DMA signals increased only slightly. The shift in relative intensities is a result of the differing reactivities of arsenic species to hydride formation.

Boron in plant cells (11). Boron is essential for the normal growth of plants. Its function as a plant micronutrient has been shown to be associated with the pectic polysaccharide Rhamnogalacturonan II (RG-II), which can exist as either a monomer or dimer in plant cell walls. The boron in RG-II exists as a borate ester that cross-links two chains of RG-II to form a dimer. The cross-linking of this polysaccharide is believed to be related to the pore size and mechanical strength of cell walls. The uptake of boron by plant cells can be studied by the exposure of growing plant cells, Chenopodium album,
to $^{10}$B-enriched boric acid (cells grown by A. Fleischer and R Ehwald, Humboldt Universität zu Berlin) followed by analysis of the extracted polysaccharide fraction for the $^{10}$B/$^{11}$B isotope ratio. Boron contamination from water and glassware makes the direct measurement of the $^{10}$B/$^{11}$B isotope ratio in RG-II difficult, however. Size-exclusion chromatography solved this problem by separation of RG-II from free borate as seen in Figure 4. A Superdex-75 HR 10/30 size-exclusion column (Pharmacia Biotech, Piscataway, NJ) was used for the separation with $^{10}$B and $^{11}$B monitored simultaneously by the ICP-MS system. The mobile phase was 10 mM ammonium formate at pH 5 with a flow rate of 1 mL/min. Approximately 250 μg of sample was injected onto the column. Integration of the peaks yielded the natural abundances of the boron isotopes for the borate peak (20% $^{10}$B, 80% $^{11}$B), whereas the $^{10}$B abundance was enhanced for RG-II (70% $^{10}$B, 30% $^{11}$B).

CONCLUSIONS

LC–ICP-MS can be a valuable tool for studying elemental speciation in a variety of sample types. The interface between the liquid chromatograph and the ICP-MS system is simple and inexpensive to assemble. With proper consideration to the LC mobile phase, the LC column, and the ICP-MS sample introduction system, most LC separations can be easily converted to element-specific detection. Multiple isotopes can be measured simultaneously by the ICP-MS, thus permitting monitoring of all elements of interest together with possible interferences and contaminants throughout the LC separation. The wide applicability of LC–ICP-MS and its benefits for complex matrix analysis make it a technique that will certainly grow in use during the coming years.

ACKNOWLEDGMENTS

The author would like to thank Peter Uden, Julian Tyson, Hakan Gurleyuk, Steve Long, and Malcolm O'Neil for collaboration on the speciation examples used in this article. The author also gratefully acknowledges Steve Hassan at Thermo Separation Products for the HPLC used for speciation work at VG Elemental.

REFERENCES


(9) Data for Figure 2 were collected in January 1997 by the author and S.E. Long (National Institute of Standards and Technology, Gaithersburg, MD).

(10) Data for Figure 3 were collected in January 1998 by the author and H. Gurleyuk (University of Massachusetts, Amherst). H. Gurleyuk’s research is directed by PC, Uden and J.F. Tyson.

(11) Data were collected in February 1998 by the author and M. O'Neil (University of Georgia, Athens). Background information provided by M. O'Neil.

Mary Kate Donals, PhD, is a senior application scientist at VG Elemental, Franklin, Massachusetts. She received a PhD in analytical chemistry from the University of Massachusetts, Amherst, in 1995. She was a National Research Council Postdoctoral Associate at the National Institute of Standards and Technology in Gaithersburg, Maryland, from 1995 to 1997.
5. [20 points] Compare and contrast the features of the most common types of infrared absorption and Raman scattering measurements.
   (a) Draw schematic diagrams of typical instruments used for each type of measurement. Label each diagram component with a specific example of the component, e.g. the Nernst glower is a common IR source.
   (b) List the advantages of each measurement over the other.