Questions on this cume are drawn from the attached article, entitled “Chemical Reaction Interface Mass Spectrometry with High Efficiency Nebulization” by Jorabchi et al. (Anal. Chem. 2005, 77, 5402-5406). Where specific passages are relevant, they have been marked in the margin with the corresponding question number. The paper has been chosen in part for its relatively short length; it will probably be helpful for you to read quickly through the questions, then to read through the article, focusing on marked sections.

The questions are marked with point values, partly to provide an indication of the relative emphasis and expected level of detail. Keep your answers succinct and on-topic; points will be deducted for irrelevance. Many of these questions can be fully answered in 2-3 short sentences.

Please take pity on the grader, and strive to keep the answers in your blue book in order. Write clearly – what cannot be read cannot be graded. You can use the back of this or the other exams for scratch paper to help you organize your thoughts.
1. This paper is nominally a “Technical Note,” for which the Journal guidelines state:

Technical Notes (3–4 journal pages) are brief descriptions of novel apparatus or techniques. Author(s) must show ingenuity in describing the advantages of the new apparatus or techniques over those already available.

At 4.5 pages, this article is actually too long for a Note. Suppose you were a “hard-nosed” editor insisting that the paper be shortened. What sections or material do you feel could be cut from this paper without compromising its message? Why?

2. The authors state that “Among all techniques [for isotope labeling studies], elemental mass spectrometry offers a sensitive and relatively simple approach.” Describe briefly (including operating principles) at least one other method (not involving mass spectrometry) commonly used to monitor isotope labeling. Compare briefly its figures of merit (including cost and complexity as well as analytical FOM) with those of the techniques described in this paper. State whether the method you name is applicable to stable isotopes. If the method you chose is not applicable to stable isotopes, state principles on which stable isotope analysis can be performed without invoking mass spectrometry.

3. In the method described here, sample is converted to a particle beam, reacted with SO₂ in a microwave plasma, then ionized by electron ionization prior to mass analysis.
   a) Explain the rationale for each of these experimental choices. In other words, what purpose is served by the particle beam and the plasma? Why choose SO₂? As part of your answer, explain the purpose and operating principles of the “membrane desolvator” in Figure 1. Why is it so important to avoid solvent vapor?
   b) A common alternative to CRIMS is ICP-MS. Are any of these steps common in ICP? Why or why not?
   c) A common alternative to electron ionization (EI) is chemical ionization (CI). Explain how CI differs from EI, then discuss briefly its merits relative to EI for this application.

4. The authors extol the high efficiency nebulizer for its utility “over a wide range of microflow rates.” In most instances, such “strengths” in analytical methodologies are offset at least to some extent by parallel limitations. Are there fundamental limitations to the HEN that might offset the low-flow advantages outlined by the authors? If so, explain the limitations and their impact. If not, say why not.

5. The authors have used a quadrupole mass spectrometer, but assert that “The analytical performance of the system can also be dramatically enhanced by using state-of-the-art instrumentation on the MS part.” What are the strengths and limitations of the quadrupole analyzer for this application. What (if any) analyzer would you expect to provide the enhancement predicted by the authors? Explain why “dramatic” enhancements will (or will not) result from a change in analyzer.
6. The cited information about the SO₂ count rate seems inadequate to enable a reader to reproduce the optimum conditions, both because parameters other than the SO₂ flow rate probably affect the count, and in part because other parameters likely affect the efficiency of CRIMS. What other operating parameters (excluding those in Table 1) would you expect to be important? Why?

7. Near the top of page 5404 is the first and only mention of background counts. Compare the numbers cited with the experimental signal levels reported. Is the apparent signal:background ratio typical for instrumental analysis? What are the implications for the figures of merit? What likely accounts for the background?

8. The highlighted passage at the beginning of the R&D section is the first of several instances wherein it can be noted that the peak shapes (both for flow injection and for chromatography) are relatively asymmetric. What are possible reasons for the asymmetry in each case (FIA and chromatography)?

9. The first figure of merit (FOM) assessed is stability, which the authors report as %RSD, and compare with published values for ICPMS. Perhaps in the interest of saving space, the authors have not provided information about how the “short-term stability” was assessed. What other information would be needed to allow a reader to reproduce this measurement?

10. When authors provide sufficient information, it is sometimes possible for the reader to extend comparisons beyond those discussed by the authors. Considering the experiments of Figures 2-4, there is some overlap in conditions that might enable such comparisons. Where possible, use the data provided to make additional observations about data trends. Where information needed to make the extension is missing, tell what additional information you would need.

11. Consider the discussion of linearity and limits of detection near the end of page 5405.
   a) What constitutes “acceptable linearity,” and how important a FOM is this? Is R² a good measure of linearity? Why or why not?
   b) The authors cite 3 LOD’s: one at m/z 44, one at m/z 45, and one for “fully ¹³C-labeled glucose.” What causes the differences among the numbers cited? Are the differences reasonable? Why or why not?
Chemical Reaction Interface Mass Spectrometry with High Efficiency Nebulization

Kaveh Jorabchi,† Kaveh Kahan,‡ Paolo Lecchi,‡ and Akbar Montaser*,†


A high efficiency nebulizer (HEN) coupled to a heated spray chamber and a membrane desolvator is used for liquid sample introduction in chemical reaction interface mass spectrometry (CRIMS). Compared to the conventional thermospray nebulizer operated at solvent flow rate of 1 mL/min, the HEN provides small droplets at lower flow rates (10–100 µL/min), improving the desolvation and analyte transport efficiency. As a result, the sensitivity for carbon detection by CRIMS is improved by a factor of 4. The new arrangement offers an easy-to-use and robust interface, facilitating the availability of a variety of liquid chromatographic techniques to the CRIMS. Separation interface, facilitating the availability of a variety of liquid chromatographic techniques to the CRIMS. Separation and detection of labeled peptides in a mixture of unlabeled biopolymers is illustrated at a solvent flow rate of 45 µL/min as an example of new possibilities offered by the improved liquid introduction interface.

Isotope ratio analysis has become an increasingly popular technique in many research areas such as geochemistry, nutrition, physiology, and pharmacology. For example, the isotopic pattern may be used as a signature to identify the geographic origin of a sample. Also, stable isotope labeling is widely used in drug metabolism studies. Among all techniques, elemental mass spectrometry offers a sensitive and relatively simple approach for such analysis by breaking the molecules into their constituent atoms, thereby simplifying the mass spectra and isotope ratio calculations. In this regard, inductively coupled plasma mass spectrometry (ICPMS) has become the method of choice for the analysis of the majority of elements. However, the elemental and isotopic analysis of organic compounds is challenging partly because of poor ionization efficiency of the most abundant elements (i.e., H, N, and C) in these compounds in Ar ICP and elevated background levels due to air entrainment in atmospheric pressure ICPMS. Although recent advances highlight the potential to overcome such problems, the applicability of ICPMS as a viable method for the isotopic analysis of the cited elements requires further investigations.

In an alternative approach, the sample is directed into a CuO furnace for complete combustion, and the extracted reaction products (such as CO2) are detected by an isotope ratio mass spectrometer (IRMS) with an electron impact ion source. Normally, gas chromatography is used for sample introduction in IRMS, limiting the method to low molecular weight (MW) and thermally stable compounds. In one study, a moving belt interface was applied to transfer the analytes eluting from a liquid chromatography (LC) column to an IRMS. This approach, however, is not easy to use and compromises the chromatographic resolution. Such shortcomings along with the increasing demand for the analysis of large MW biopolymers, e.g. DNA and proteins, have led to the development of chemical reaction interface mass spectrometry (CRIMS) with a particle beam interface for liquid sample introduction. In CRIMS, a low-pressure microwave induced plasma (LP-MIP) is utilized as a reaction cell. The chemical environment of the plasma may be altered by addition of the reactant gases. The analytes introduced into the MIP cavity react with the reactant gas, and the stable products are detected by a mass spectrometer after electron impact ionization. This approach is in fact a generalization to IRMS, offering a variety of reactions suitable for detection of different elements. Note that IRMS is limited to combustion by oxygen.

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Liquid sample introduction in CRIMS requires an interface that produces small analyte particles devoid of solvent and entrained by a carrier gas free from solvent vapor. The solvent removal efficiency is particularly important in CRIMS. Conventional, the liquid sample introduction in CRIMS consists of a thermospray nebulizer (TSN) operated at 1 mL/min, coupled to a heated spray chamber and a membrane desolvator. Droplet generation by TSN is pneumatic in nature, with the nebulizer gas provided by vaporizing a fraction (60–90%) of the solvent in the TSN capillary. Thus, the nebulization energy (or the amount of the solvent vapor) decreases at lower solvent flow rates, increasing the droplet sizes (Sauter mean diameter >8 µm below 0.6 mL/min water in a 127-µm-i.d. capillary), and reducing the analyte transport efficiency. Although narrower capillaries (50-µm i.d.) reduce the minimum solvent flow requirement, the nebulization mechanism in TSN hinders the development of nebulizers for practical use at flow rates below 100 µL/min, limiting the compatibility of CRIMS with a variety of low-flow LC techniques. Pneumatic micronebulizers, however, offer efficient nebulization at low solvent flow rates because the nebulization energy is provided by a high-speed flow of an inert gas independent of the solvent flow rate. Despite numerous advances in the nebulization and desolvation methods in plasma spectrometries only one report has addressed the liquid sample introduction in CRIMS and only at high solvent flow rates (>1 mL/min).

In this report, a pneumatic high efficiency nebulizer (HEN) is explored for sample introduction in CRIMS over a wide range of microflow rates (10–1000 µL/min). Such an arrangement reduces the solvent load and is beneficial for an efficient coupling of LC techniques with CRIMS, as illustrated in this study by the detection of labeled proteins and peptides using a small-bore column.

EXPERIMENTAL SECTION

CRIMS Instrumentation. The instrumental setup is shown in Figure 1 and is described in detail elsewhere. Briefly, the solution is nebulized and the solvent is removed by desolvation via a heated spray chamber and membrane desolvator (Vestec–Universal Interface). Analyte particles then pass through a momentum separator into a low-pressure MIP (∼6 Torr, 70 W forward power, <2 W reflected power, Optos, Rockville, MD). To quantitatively convert the analyte carbon content into CO₂, sulfur dioxide is added to the plasma as an oxidizing agent using a microliter-flow valve (Series 203, Granville-Phillips, Boulder, CO). Reaction products of interest (CO₂ in this study) are analyzed by ICP-MS.

EXPERIMENTAL SECTION

Figure 1. Schematic diagram of the instrumental setup for chemical reaction interface mass spectrometry (CRIMS).
a quadrupole MS (model 5971 HP MSD, Agilent Technologies, Foster City, CA) equipped with an electron impact ion source connected to the chemical reaction interface through a heated fused-silica capillary (320-μm i.d.). The operating conditions are optimized for the best signal-to-noise ratio for m/z 44 (CO₂⁻) by adjusting the SO₂ flow (≈2 × 10⁶ counts/s for ³²S¹⁴O₂⁻ signal). Although the background values vary in a relatively large range (2000–10000 counts/s) in day-to-day optimizations, no appreciable deterioration in signal-to-noise ratio for carbon detection is observed.

Sample Introduction. Two nebulizers are used in this study: (1) the conventional thermospray nebulizer (Scientific Instrument Services, Ringoes, NJ) operated at 1 mL/min; (2) a high efficiency nebulizer (HEN-170-A0.1, Meinhard Glass Products, Analytical Reference Materials International Corp., Golden, CO) operated at 10 and 100 μL/min. A 75-μm-i.d. (320-μm-o.d.) fused-silica capillary (Polymicro Technologies, Phoenix, AZ) is inserted in the HEN’s solution capillary to reduce the dead volume. The operating conditions for the sample introduction methods are summarized in Table 1.

Analytical performance of the CRIMS with two nebulization methods is studied by flow injection analysis (FIA) of glucose, using a 50:50 water/acetonitrile solvent delivered by a syringe pump (model KDS1, KD Scientific, Holliston, MA). A 2 μL PEEK sample loop (Upchurch Scientific, Oak Harbor, WA) is used at a solvent flow rate of 10 μL/min. A 5-μL loop is utilized for all other solvent flow rates. For the signal stability studies (see Results and Discussion), a 50-μL loop is utilized to provide a flattop peak.

A linear gradient from 25 to 65% acetonitrile in water containing 0.1% trifluoro acetic acid (TFA) is delivered at 45 μL/min by an HPLC pump (Series IV, LabAlliance, State College, PA) and a flow splitter (Acurate, LC Packing, San Francisco, CA) for the separation of peptides and proteins in a 1-mm polymeric column (PRP-1, Hamilton Co., Reno, NV). The identity of the peptides is confirmed by matrix-assisted laser desorption ionization equipped with a time-of-flight analyzer (MALDI-TOF). For this purpose, dried peptides are dissolved in water containing 0.1% TFA at a concentration of ~1 μM, and 0.5 μL of the peptide solution is mixed directly on the MALDI probe with 1 μL of matrix (4-hydroxy-α-cyanocinnamic acid). MALDI analysis is performed in linear mode with the acquisition of positive ions using an Axima CFR instrument (Kratos Analytical, Chestnut Ridge, NY).

Solvents and Materials. Standard purity helium and nitrogen (99.995%, Roberts Oxygen Company Inc., Gaithersburg, MD) are used for plasma and nebulization. The analyses are performed using HPLC grade solvents (OmniSolve, EMD Chemicals Inc., Gibbstown, NJ). With the exception of chicken lysozyme (Worthington Biomedical Corp., Lakewood, NJ) and ¹³C-iodoacetic acid (Cambridge Isotopes, Andover, MA), all the standards and reagents are purchased from Sigma-Aldrich Corp. (St. Louis, MO).

RESULTS AND DISCUSSION

Signal Stability and Optimization. Typical signal stability for HEN–CRIMS is measured by monitoring a 5-min steady signal at m/z 44 (¹³C¹⁴O₂⁻) obtained by 50-μL flow injection of 1 μg/μL glucose solution at 10 μL/min and helium nebulizer gas flow rate of 1 L/min. The wash-in time is 10 s while it takes ~50 s for the signal to drop to 10% of the steady value during the wash-out period. The short-term signal stability during the 5-min interval is ~6% RSD, which is higher than the typical values (<3%) reported for pneumatic nebulizers commonly used in ICPMS.²³ This observation reveals that a large portion of the signal variation in CRIMS is caused by fluctuations in plasma properties or the extraction process from the plasma into the ion source.

Helium and nitrogen are used to study the effect of the nebulizer gas type and gas flow rate on the sensitivity of the carbon detection by CRIMS. The results are illustrated in Figure 2 for the flow injection of 5 μg of glucose at 10 μL/min. The first five peaks are obtained using helium at gas flow rates of 0.3, 0.5, 0.7, 0.9, and 1.2 L/min. The three subsequent peaks are obtained using nitrogen at flow rates of 0.5, 0.7, and 1.0 L/min. Excluding the first injection at 0.3 L/min for helium, the peak areas for seven subsequent injections vary within 8%. Although this value may seem slightly larger than the typical signal RSD, it implies that a minimum gas flow is required for the formation of an acceptable spray (~0.3 L/min for helium). Above this threshold, the spray characteristics satisfy the need for the optimum desolvation of the aerosol and the sensitivity is relatively independent of the nebulizer gas type and gas flow rate. Thus, the rest of the

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Table 1. Operating Conditions for the Sample Introduction Systems

<table>
<thead>
<tr>
<th>Thermospray Nebulizer</th>
<th>High Efficiency Nebulizer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capillary i.d. (μm)</strong></td>
<td>75, 70</td>
</tr>
<tr>
<td><strong>Capillary temperature (°C)</strong></td>
<td>140</td>
</tr>
<tr>
<td><strong>Solution flow rate (μL/min)</strong></td>
<td>1000</td>
</tr>
<tr>
<td><strong>Gas annulus i.d. (μm)</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>Gas orifice i.d. (μm)</strong></td>
<td>240</td>
</tr>
<tr>
<td><strong>Gas flow rate (L/min)</strong></td>
<td>helium, 0.3–1.2; nitrogen, 0.5–1.0</td>
</tr>
<tr>
<td><strong>Solution flow rate (μL/min)</strong></td>
<td>10–100</td>
</tr>
</tbody>
</table>

**Heated Spray Chamber and Membrane Separator**

| **Heated chamber temperature (°C)** | 60 |
| **Membrane temperature (°C)** | 60 |
| **Carrier gas flow rate (L/min)** | 7 |
| **Sweep gas flow rate (L/min)** | 18 |
measurements are performed using nitrogen as the nebulizer gas at a flow rate of 0.7 L/min.

The sensitivity of CRIMS using HEN is compared to that with TSN in Figure 3 for the double injections of 5 µg of glucose. The higher sensitivity obtained for HEN at 45 µL/min compared to TSN at 1 mL/min solvent flow rate reveals that the reduction of solvent load and generation of fine aerosol at low solution flow rates significantly improves the analyte transport efficiency through the heated spray chamber and membrane interface, increasing the overall sensitivity. Note that a higher sensitivity is reported for TSN compared to pneumatic nebulizers using ICP spectrometries, in contrast to the trend observed in CRIMS.17-23 Thermospray nebulizer provides good transport efficiency (up to 50%) at high flow rates (>1 mL/min) and, thus, a large analyte transport rate. Whereas, for transient signals such as those encountered in chromatography and FIA, the only important parameter is the analyte transport efficiency realized by pneumatic nebulizers at microliter per minute flows. The wider peaks obtained by HEN are due to ~20 times lower solvent flow rate for HEN compared to TSN, sweeping the same sample loop size for both nebulizers.

**Analytical Performance of HEN–CRIMS.** The analytical figures of merit for CRIMS are investigated using FIA of 0.5–25 µg of glucose at 10 and 100 µL/min. Figure 4 shows typical results at 100 µL/min. The reproducibility of the peaks areas is 2–10% based on three measurements. Generally, better precision is observed at higher solvent flow rates and concentrations. The calibration curves are shown in Figure 5A and B for 10 and 100 µL/min, respectively. An acceptable linearity ($R^2 > 0.995$) is observed over a wide range of solvent flow rates. At the solvent flow rates used in this study, the limit of detection for unlabeled glucose falls in the range 0.2–0.3 µg at m/z 44 ($^{12}\text{C}^{16}\text{O}_2^+$) or 0.6–1 µg at m/z 45 ($^{13}\text{C}^{16}\text{O}_2^{+}$). Note that this detection limit translates to picomoles of proteins in terms of the carbon content. However, CRIMS is mainly used for detection of isotopic tags in biological molecules (e.g., $^{13}\text{C}$, $^{15}\text{N}$, and $^{34}\text{S}$), resulting in lower detection limits via improving signal-to-noise ratio. For example, the detection limit for a fully $^{13}\text{C}$-labeled glucose is ~1 ng with the current setup, an improvement of more than 2 orders of magnitude compared to the natural glucose. The analytical performance of the system can also be dramatically enhanced by using state-of-the-art instrumentation on the MS part.

**Peptide and Protein Separation.** The results above indicate that HEN–CRIMS arrangement can be utilized as a universal detector for a variety of liquid chromatographic methods with minimum adjustments. Prior to this study, the CRIMS was operable only at solvent flow rates of 1 mL/min and above. This

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**Table 2. Peptide and Protein Mixture**

<table>
<thead>
<tr>
<th>name</th>
<th>sequence</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>bradykinin</td>
<td>RPPGFSFER</td>
<td>1060</td>
</tr>
<tr>
<td>angiotensin II</td>
<td>DRRVYIHPC</td>
<td>1046</td>
</tr>
<tr>
<td>angiotensin II analogue</td>
<td>Sar-RVYIHPC</td>
<td>912</td>
</tr>
<tr>
<td>insulin</td>
<td>51 amino acids</td>
<td>5732</td>
</tr>
<tr>
<td>horse apomyoglobin</td>
<td>152 amino acids</td>
<td>16952</td>
</tr>
<tr>
<td>peptide A$^*$</td>
<td>GYSGLNWVCWAK</td>
<td>1328</td>
</tr>
<tr>
<td>peptide B$^*$</td>
<td>NLGNFCSALSSDITASVNCAK</td>
<td>2512</td>
</tr>
</tbody>
</table>

$^*$ Peptides A and B are obtained from proteolytic digestion of chicken lysozyme. 13C centers are at cysteine residues indicated as C.
requirement hinders the use of low-flow separations and necessitates postcolumn solvent addition for techniques such as size exclusion chromatography.\textsuperscript{10,12}

To demonstrate the applicability of HEN–CRIMS at low flow rates, a mixture of peptides and proteins (Table 2) is separated using a 1-mm column at 45 \( \mu \text{L/min} \) to identify the \(^{13}\text{C}\)-labeled components. The mixture includes 25 \( \mu \text{g} \) of each of the unlabeled peptides and is spiked with labeled peptides A and B obtained from the proteolytic digestion of chicken lysozyme previously reduced and alkylated with \(^{13}\text{C}\)-iodoacetic acid.

The HPLC column outlet is directly connected to the HEN, and CRIMS is operated under the conditions described above for glucose with no further adjustments. The chromatograms obtained by monitoring \(^{12}\text{C}\text{O}_2\text{}^+\) and \(^{13}\text{C}\text{O}_2\text{}^+\) are shown in Figure 6A. The presence of \(^{13}\text{C}\)-labeled components is evidenced in a specific “enrichment trace” obtained by subtracting the natural \(^{13}\text{C}\) abundance (1.12\%) from the \(m/z\) 45 trace as illustrated in Figure 6B. Note that peptides A and B have only one and two \(^{13}\text{C}\) centers in their sequences, respectively.

**CONCLUSIONS**

A pneumatic high efficiency nebulizer is explored for liquid sample introduction in chemical reaction interface mass spectrometry. This arrangement allows CRIMS to operate over a much lower range of solvent flow rates (1–100 \( \mu \text{L/min} \)) with minimum adjustments on the conventional operating conditions. The new arrangement offers linear analytical response \((R^2 > 0.995)\) and improved sensitivity for carbon detection over the conventional thermospray nebulizer by providing higher analyte transport efficiency and significantly reduced solvent load. In addition, the flexible solvent flow rate for HEN opens new horizons in the application of CRIMS as a detector for liquid chromatographic methods.

**ACKNOWLEDGMENT**

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