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Analysis of Organic Compounds by Particle Beam/Hollow Cathode Atomic Emission Spectroscopy: Determinations of Carbon and Hydrogen in Amino Acids

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A detailed evaluation of the analytical characteristics of a particle beam/hollow cathode glow discharge atomic emission spectroscopy (PB/HC-AES) system is described for applications in the area of organic sample analysis. The optimization of sample introduction, nebulization parameters, and glow discharge conditions was performed for the elemental analysis (focusing on C and H) of a group of amino acids. By use of a high-efficiency thermoconcentric nebulizer, analyte particles are introduced into a heated hollow cathode glow discharge source, in either flow injection or continuous-flow mode, for subsequent vaporization/atomization and excitation. Nebulization temperature, solvent composition, and liquid flow rate were studied to elucidate their roles in the ultimate analyte emission characteristics for organic compound analysis. The hollow cathode operating discharge current and gas pressure were optimized, with the general responses found to be similar to those for the case of metal analysis. Background interferences from solvent and additive media on carbon and hydrogen determinations were studied and substantially reduced. The analytical response curves for carbon and hydrogen present in amino acids were obtained using 200 μL injection volumes, showing less than 10% RSD for replicate injections over a concentration range of 10−250 ppm, with detection limits of 3 and 1 ppm, respectively, for C (I) and H (I) emission. Subsequent studies of the response of carbon and hydrogen emission signal intensities to differences in amino acid stoichiometries suggest a capability of the PB/HC-AES system for the determination of empirical formulas based on H (I)/C (I) intensity ratios.

Glow discharge (GD)-based techniques are gaining attention for the elemental analysis of metals and alloys by atomic absorption, emission, and mass spectrometric methods by virtue of their efficient atomization, excitation, and ionization processes.[1] The use of radio frequency (rf) powering has expanded the application of GD techniques for direct solids analysis of both conducting and nonconducting materials.[2] While the glow discharge has been developed and proven as a powerful spectrochemical source for various solid samples, the analysis of liquid samples has found only limited interest. A few publications have reported the use of hollow cathode glow discharges to sputter atomize solution residues.[3,4] In these applications, an aliquot (<200 μL) of the liquid sample is placed in the base of the hollow cathode, dried to a solid (residue) by direct or IR heating, and converted into gas-phase atoms through cathodic sputtering. Very impressive levels of detectability have been achieved by hollow cathode atomic emission spectrometry (HC-AES), with Chen and Williams[4] reporting limits of detection of 9 and 20 pg of P and Cl, respectively, in renal fluids. Even though the HC-AES is relatively straightforward and very sensitive, improvement in sample throughput to GD sources by use of some means of continuous sample introduction is strongly desired. This is particularly true when spectrochemical detection is employed in chromatographic systems. An important consideration in the development of chromatographic detectors is the maintenance of natural chromatographic characteristics such as retention/elution quality and solvent gradient compatibility. Therefore, in order to couple liquid chromatography with a low-pressure GD source, one must employ an appropriate interface to achieve high-efficiency solvent removal and analyte transport. Such an interface would ideally allow retention of both the chromatographic integrity and the analytical performance of the GD source.

The particle beam (PB) interface has found increasing use as a valuable sample introduction device for liquid chromatography/mass spectrometry because of its simplicity of operation and compatibility with a wide range of solvent polarities and flow rates.[5] The concept of the particle beam interface is to separate (and enrich) the analyte from a liquid sample through nebulization, desolvation, and momentum separation processes, eventually transferring the analyte into the detection source. In the nebulization process, the sample solution is converted into a fine aerosol to expedite analyte desolvation. A heated desolvation chamber provides further desolvation of the aerosol, producing a mixture of solvent vapor and analyte particles. A momentum separator performs analyte enrichment and solvent vapor removal simultaneously in two or three stages of differential pumping with corresponding skimmers. Differential pumping not only permits ready solvent vapor removal but also provides pressure reduction to match the pressure/vacuum requirement of the detection source. The resultant dry analyte particles, in the form of beam

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flow, enter the detection source for subsequent vaporization, excitation, or ionization.

The initial studies demonstrating the feasibility of coupling the particle beam interface with a glow discharge atomic emission source for aqueous sample analysis employed a planar cathode in the glow discharge source to collect analyte particles followed by atomization through cathodic sputtering.[6] However, unfavorable analyte particle loss and nonquantitative ablation in this diffuse cathode structure limited the analytical utility of the device. The implementation of a heated (200 °C) hollow cathode GD geometry greatly enhances the atomization process via efficient vaporization of analyte particles.[7] The analytical limits of detection (LOD) of the particle beam/hollow cathode glow discharge atomic emission spectroscopy (PB/HC-AES) system are thus improved from the ppm to ppb level for analysis of metallic elements due to the efficient HC atomization/excitation environment.

A detailed evaluation of the nebulization and desolvation characteristics of this PB/HC-AES system has been performed toward analytical applications for the analysis of transition metals.[8] The sample introduction conditions and particle beam operation parameters were optimized on the basis of variations of nebulizer temperature, capillary size, solvent composition, temperature, and absolute pressure of the desolvation chamber, as well as the addition of a supplemental helium gas flow. Systematic variations in instrumental response have been directly related to analyte transport through the particle beam interface.[9] The transport behavior and size distribution of the analyte particles were studied by collecting the particles, followed by determination of particle size and degree of hydration by scanning electron microscopy and quantification of the net transport efficiency by atomic absorption spectrophotometry. The results of those studies suggest that particle size distributions in the single-micrometer level are most efficiently transported across the particle beam interface. Finally, the carrier effect of enhanced signals through the addition of a modifier (6 M HCl) was verified to be the result of increased particle sizes relative to neat solutions. Each of these studies has involved the analysis of transition and alkali metal elements, suggesting a good deal of promise for the combination of the particle beam interface with the hollow cathode glow discharge atomic emission source as a valuable analytical technique for the analysis of volume-limited liquid samples.

In recent years, there has been a steadily growing interest in the coupling of chromatographic separations with atomic spectroscopy sources to hopefully provide transition element speciation information, principally oxidation state assignments based on chromatographic characteristics. The demands for speciation information in environmental and biological areas has lead to an increased need for more comprehensive characterization of inorganic/organometallic species. It is not only important to detect the transition metal element, but also to identify the associated ligand species. The characterization of organic ligands/compounds, possibly through elemental analysis, is essential for the understanding of chemical form and related chemical behavior. The ideal situation for supplying analytical information is the combination of separation modes [gas chromatography (GC) or liquid chromatography (LC)] with compatible detection techniques that can provide complete compound or empirical formula information. If atomic spectrochemical (elemental analysis) methods are to be employed, empirical formula information can be obtained by computing the ratio of the responses of the individual component elements (e.g., Cu/C/H/O). At present, the coupling of capillary gas chromatography to a microwave-induced plasma atomic emission spectroscopy (GC/MIP-AES) source is the only commercially available option for providing this depth of information.[10−13] In the commercial implementation, the MIP source is optically sampled by a photodiode array for simultaneous multielement analysis, termed GC-AED for gas chromatography-atomic emission detector.[14] In general, the GC-AED approach has shown a wide analytical scope, supplying qualitative and quantitative information on the speciation analysis of petrochemicals and pesticide residues.[11,12] The ability of the GC/MIP-AES system to provide the elemental analysis and empirical formula information, which is traditionally obtained by various combustion methods, is expected to have significant impact in the speciation of volatile compounds. For example, Donais et al.[13] reported a solid/liquid extraction procedure with preparative gel permeation chromatography cleanup and GC/MIP-AES analysis for the quantification of methylmercury species in a variety of complex marine materials.

The ability to obtain information on the identity of ligand species for nonvolatile compounds that are separated by LC methods has not progressed as well as for volatile compounds. Ideally, one would look for a spectrochemical method wherein LC sample introduction is followed by sensitive non-metal analyses so that empirical formulas of organometallic species and the like can be deduced directly. Spectrochemical analysis of solution-phase samples is most often undertaken by use of atmospheric pressure flames and plasmas [i.e., the inductively coupled plasma (ICP)]. These high-temperature sources are very effective at dissociating molecular species down to atomic form for subsequent spectroscopic analysis. However, in the analysis of atmospheric elements such as N, O, H, C, and S, selectivity and sensitivity are sacrificed by very large amounts of continuous background signals. These signals are predominately due to solvent vapors and atmospheric gases within the excitation source. For example, the determination of O/H empirical ratios is impossible when aqueous solutions are introduced into the ICP because of the dissociation of water vapor. By the same token, atmospheric species (N_2, O_2, etc.) may be entrained into the plasma, producing a continuous spectral background in both optical emission and mass spectrometric sampling. Finally, in the case of atomic emission, many non-metals emit most strongly in the vacuum ultraviolet region of the optical spectrum; thus, absorption by atmospheric species in the optical path reduces levels of detectability.

While previous studies have indicated that the PB/HC-AES approach is useful for transition metal element analysis, the capability of this system in organic compound analysis, especially

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(14) HP G2350A Atomic Emission Detector for Gas Chromatography, Hewlett-Packard Co., Palo Alto, CA.

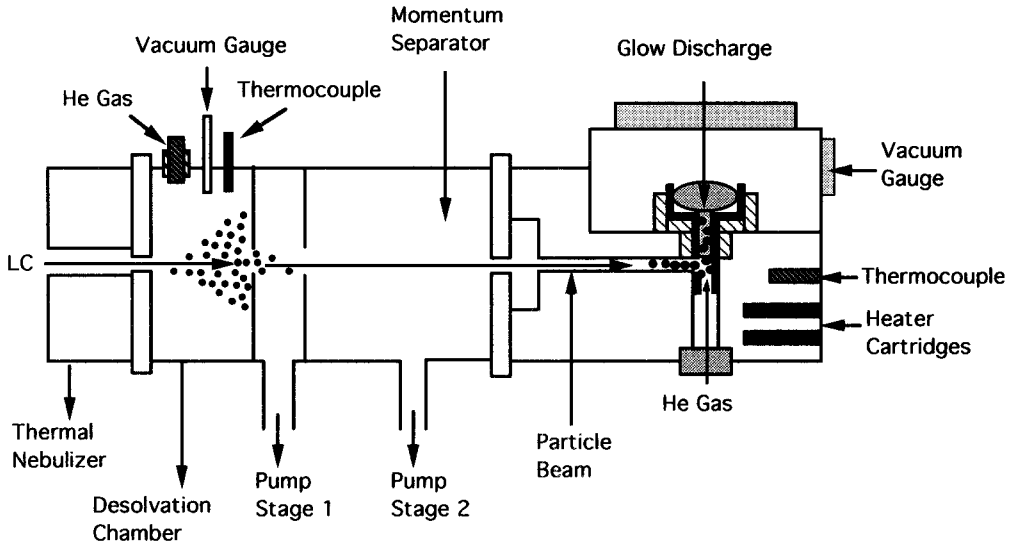

Figure 1. Diagrammatic representation of the PB/HC-AES source applied in these studies.

for the determination of non-metal elements, warrants further study in order to extend the analytical applications. By taking advantage of the low-pressure environment to eliminate the atmospheric background, one could expect that the analysis of atmospheric elements such as carbon, hydrogen, oxygen, and nitrogen would be possible with the glow discharge device. We present here further exploration of the PB/HC-AES system for the analytical characterization of organic ligands/compounds. Specifically, amino acids are detected on the basis of their elemental carbon and hydrogen emission characteristics. In addition to the obvious importance in biological systems, amino acids have been chosen here as a test case because they are representative of small organic molecules, and to a lesser extent hydrocarbon ligands. The compatibility of this system with different solvent compositions and liquid flow rates is a primary concern in the analysis of organic compounds. The C (I) 193.0 nm emission intensity is used to compare the responses of non-metal elements introduced in organic molecule form with those of aqueous metal ions to variations in discharge current and discharge gas pressure. Calibration quality in the analysis of amino acids was evaluated on the basis of both C (I) and H (I) monitoring, with limits of detection calculated to be on the single ppm level. The relationship between the respective elemental responses and the number of C and H atoms in a given amino acid (for fixed molar quantities) was assessed. As desired, there is a direct relationship between the elemental composition of the individual amino acid and the analytical response. The ultimate extension of these studies to the determination of empirical formulas is demonstrated by correlating the measured H (I)/C (I) emission intensity ratios to their true values in a range of amino acids. The analytical merits of PB/HC-AES seen in the analysis of amino acids reveal a promising future for application in both inorganic and organic analyses, as is needed for comprehensive speciation assessment.

EXPERIMENTAL SECTION

Glow Discharge Source. The instrumentation of the particle beam/hollow cathode glow discharge atomic emission source is basically the same as applied in previous studies.[7] The hollow cathode is mounted at the center of a stainless-steel "thermoblock" that ensures easy access to the plasma via optical monitoring. The source vacuum port, discharge gas inlet, pressure gauge, electrical feedthroughs, and heating components are also placed on the thermoblock. The entire glow discharge source is heated for more efficient sample vaporization and atomization by a pair of cartridge heaters (Model SC 2515, Scientific Instrument Services, Ringoes, NJ), with the block temperature monitored by a W−Re thermocouple. The overall gas pressure of the glow discharge source (a combination of helium discharge gas and other gaseous sources from the particle beam interface) is monitored by a vacuum gauge (DV-24, Teledyne Hastings-Raydist, Hampton, VA).

The hollow cathode discharge is powered by a Kepco (Flushing, NY) Model BHK 2000 supply, operating in a current-controlled mode, in the range of 0−100 mA. One significant change in the design of the hollow cathode for this particular system has been made on the basis of recent particle transport studies.[9] As shown in Figure 1, the hollow cathode has been extended in length (now 24 mm) such that it intersects the incoming particle beam. In this way, the analyte material is vaporized directly into the hollow cathode, as opposed to the previous design, which relied on a perpendicular He gas flow to sweep the material into the excitation region. Therefore, the new geometry is capable of enhancing particle capture and improving analytical performance.

Particle Beam Interface. The interface to combine a liquid delivery system and the hollow cathode glow discharge source is the Thermabeam (Extrel Corp., Pittsburgh, PA) LC/MS particle beam interface. The interface consists of a thermoconcentric nebulizer, a heated spray chamber, and a two-stage momentum separator.[5−8] The liquid sample is introduced via a HPLC pump into a fused-silica capillary (110 μm i.d.) mounted within a stainless steel tube (1.6 mm o.d.) A dc potential difference across the stainless steel tubing results in resistive heating along the tube, producing a thermal component to the aerosol formation. Helium gas flows (~360 mL/min) through the gap between the central fused-silica capillary and outer stainless steel tubing as a sheath gas to improve heat conduction and to breakup the liquid at the capillary tip (i.e., pneumatic nebulization). The resulting fine aerosol is directed into a steel spray chamber wrapped in heating tape (150−200 °C). An additional 6 mm i.d. inlet allows addition of a supplemental flow (250 mL/min) of He gas.[8] The separation

of the aerosol mixture is achieved in the subsequent two-stage momentum separator that skims out the relatively low mass nebulizer gas and solvent molecules, effectively enriching the analyte beam in the differential pumping regions. After passing through the momentum separator, the resultant beam of dry particles enters the hollow cathode glow discharge volume for subsequent vaporization, atomization, and excitation.

Sample Preparation and Solution Delivery. The solubility of "organic" analytes in different solvents and the effects of those solvents on the resultant emission spectra are important considerations in the sample preparation process. All amino acid samples were prepared with deionized, distilled water from reagent grade amino acids (Sigma Chemical Co., St. Louis, MO). The solvent and analyte solutions are delivered in a continuous mode from a solvent reservoir to the nebulizer by a Waters (Division of Millipore, Milford, MA) Model 510 high-performance liquid chromatography pump. When operated in the flow injection mode, the sample is introduced to the liquid flow through a manual sample injector (Model 7520, Rheodyne Inc., Cotati, CA) with a 200 μL sample loop volume. No chromatographic separations are performed in this study. Based on previous studies,[9] 6 M HCl was added in a 1:5 ratio to each sample aliquot, as the chloride has been identified as a "carrier" that promotes the formation of particles which are more efficiently transported through the PB interface ($\sim$2$-$8 μm diameter).

Optical Spectrometer and Data Acquisition System. The hollow cathode emission is sampled optically by use of a plano-convex fused-silica lens (45 mm diameter, 10 cm focal length) such that the image of the excitation region is focused $\sim$1:1 on the entrance slit of the monochromator. A 0.24 m Czerny$-$Turner spectrometer equipped with a 2400 grate/mm holographic grating (Digikrom 240, CVI Laser Corp., Albuquerque, NM) is applied for optical monitoring, with the scanning range, slit width, spectral calibration, and wavelength selection adjusted via the monochromator control interface. The atomic emission signals detected by a photomultiplier tube (Hamamatsu Model R955, Bridgewater, NJ) are converted into voltage signals with an analog current meter. A Macintosh IIsi computer is employed to record the output of the current meter via a National Instruments (Austin, TX) NB-MIO-16X interface board. For this particular application, an $X-Y$ recorder-type program within the National Instruments LabView 2 software environment has been developed to record the data. Finally, the obtained digital data are processed and managed in the form of Microsoft (Seattle, WA) Excel files.

RESULTS AND DISCUSSION

Effects of Solvent Composition on Spectral Background. As noted previously, the effect of solvent composition on the steady-state spectral features is an important consideration, particularly when the analysis of non-metals is concerned. This is especially true when the solvent and the target analytes have elements in common, such as in the case of amino acid analyses where aqueous solvents could contribute H (I) emission and organic solvents could contribute a continuous C (I) background. The effect of solvent composition on the background emission was evaluated at the C (I) 193.0 nm transition across a range of methanol/water compositions (0$-$100%) while only the solvents (i.e., no analytes) are nebulized in a continuous-flow fashion. In order to assess any gross spectral background level shifts, a second wavelength [nominally the Cu (I) 324.7 nm transition] was also monitored as the solvent composition was changed. This

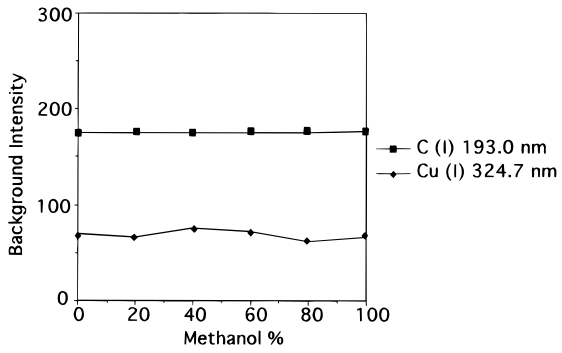

Figure 2. Effect of solvent (blank) composition on spectroscopic background levels at the C (I) 193.0 nm and Cu (I) 324.7 nm wavelengths.

wavelength was chosen to illustrate the changes in continuum contributions (reflective of plasma energetics) for typical analytes. Solvent carryover would also be evident in this region due to emission from radical species (e.g., OH and CH). As shown in Figure 2, changes in the solvent composition from pure water to pure methanol do not result in any significant changes in the spectral background levels. One might have expected that the C (I) levels would increase gradually with the percentage of methanol. The fact that the intensity does not change proves that the particle beam interface very effectively prohibits solvent vapors from entering the hollow cathode source region. The higher level of C (I) emission at 193.0 nm than at 324.7 nm is likely due to organic impurities in the discharge gas lines and vacuum pump vapors, which do represent a constant background at this point.

Effects of Discharge Conditions and Solvent Composition on Analyte Response. Once inside the HC volume, the desolvated particles of the nebulized amino acids must be vaporized and dissociated to atomic form, and the component atoms excited to yield the resultant atomic emission. The vaporization of particles is initiated by the elevated temperature (220 °C) of the cathode walls. Dissociation may be effected in the vaporization process or though collisions with electrons, ions, and atoms in the gas phase. Therefore, the respective discharge current and gas pressure may contribute to the production of free atoms. These parameters are well-known to control the extent of analyte atomization (through sputtering) and excitation in all glow discharge devices.[1] In terms of delivering analyte particles to the HC itself, the nature of the solvent will impact the nebulization characteristics as well as the desolvation efficiencies of the respective droplets. Therefore, while there do not appear to be appreciable spectral matrix effects induced by changes in solvent composition (Figure 2), there will most certainly be effects due to differences in nebulization/desolvation characteristics.

Figure 3 depicts the response of the C (I) 193.0 nm transition emission intensity on the applied discharge current for 200 μL injections of 100 ppm DL-valine. In this set of experiments, a 150 °C nebulization temperature was employed with a solution flow rate of 1.5 mL/min. As would be expected for a case where discharge current simply controls the extent of excitation, the emission intensity values are seen to increase almost monotonically with increases in current.[7] One exception to the normal behavior is seen in the case of using 100% methanol solvent where a complex response is seen. No straightforward explanation can be given for this behavior at present. Clearly observed at the high operating currents (>60 mA) is an enhancement in analyte

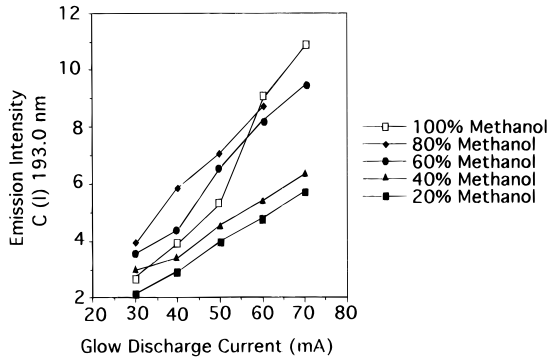

Figure 3. Effects of solvent composition and glow discharge current on C (I) 193.0 nm emission intensity: 200 μL injections of 100 ppm DL-valine; source pressure 4 Torr He; nebulizer temperature 150 °C.

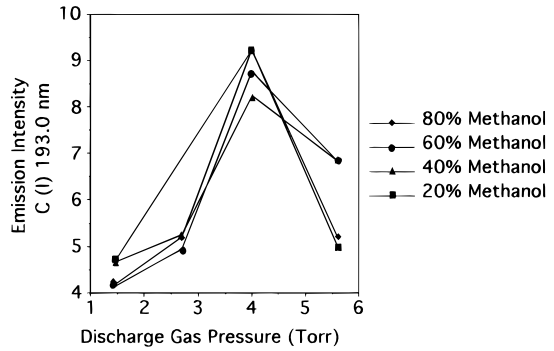

Figure 4. Effects of solvent composition and discharge gas pressure on C (I) 193.0 nm emission intensity: 200 μL injections of 100 ppm DL-valine; source pressure 4 Torr He; nebulizer temperature 200 °C.

response with increasing fractions of methanol content. On the basis of the higher volatility of methanol (bp = 65 °C) than water (bp = 100 °C), one can assume that the observed effect is the direct result of enhanced desolvation of the analyte particles from the methanol solution.

In an attempt to possibly compensate for the likely differences in nebulization/desolvation characteristics seen in Figure 3, the temperature of the Thermabeam nebulizer tip was increased from 150 to 200 °C. Figure 4 illustrates the mitigating effects of the use of the higher nebulizer temperature while also demonstrating the effects of the He discharge gas pressure on the carbon analyte emission. As seen in previous studies for metal analytes,[7] the C (I) emission intensities are optimized in a narrow pressure range, maximizing at a value of ~4 Torr in this case. While the presence of a pressure maximum is not surprising, the more important aspect of these data is the fact that the higher nebulizer temperature seems to have nullified the solvent matrix effects seen in Figure 3, with the intensity value differences at the optimum pressure being ~10% here vs 100% in the previous figure. The data presented in Figures 3 and 4 suggest a situation where enhanced volatility, via greater methanol content or higher nebulization temperatures, produces drier particles which in turn are more effectively transported to the HC source.[9]

The final point of evaluation pertaining to sample introduction/excitation is the role of the solution flow rate. In the case of discrete sample flow injection, increases in solution delivery rate would be expected to increase the transient peak height through temporal compression, though integrated intensities should not be affected. On the other hand, solution flow rates greatly affect the solvent loading in the desolvation chamber, its temperature, and the gas dynamics/pressure in the interface. Each of these

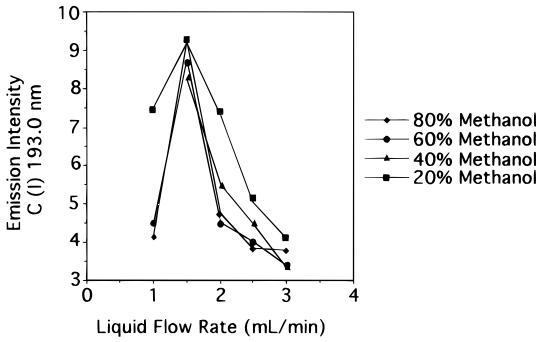

Figure 5. Effects of solvent composition and solvent flow rate on C (I) 193.0 nm emission intensity: 200 μL injections of 100 ppm DL-valine; discharge current 30 mA; nebulizer temperature 200 °C.

deleterious effects increase with increasing solvent flow rates.[8] Figure 5 illustrates the competing effects of increased transient peak heights with increases in flow rate up to 1.5 mL/min, followed by a steady decrease in analyte intensities at higher flow rates. The responses seen here are the same as those for metal analysis by PB/HC-AES.[8] As was the case for changes in discharge pressure (Figure 4), the use of the 200 °C nebulizer tip temperature produces fairly uniform responses for the range of solvent compositions.

Analytical Characterization. The previously described studies confirm that the monitored C (I) emission intensities for DL-valine sample injections behave very much the same in response to variations in discharge conditions as metals introduced in aqueous solution and that matrix effects based on the use of different methanol/water solvent mixtures are relatively minor. Having studied the basic operational aspects of amino acid analysis, an evaluation of the analytical performance of the PB/HC-AES technique was undertaken. A number of key aspects must be investigated in terms of the ability of this approach to analyze organic molecules/ligands and provide both quantitative and qualitative information. In the case of "inorganic" analyses, the determination of a particular metal species' concentration is the primary objective. In "organic" analyses, elemental concentrations must be related to the concentration of the molecule/ligand via the empirical formula, or vice versa. Thus calibration quality must be assessed in terms of both "elemental" quantification and "empirical" responses. These aspects are demonstrated below in the determinations of both carbon and hydrogen in concentration and molecular composition terms. Ultimately, it is desirable to relate the analytical responses of component elements to the empirical formula of the analyte molecule. Simple amino acids are employed here to demonstrate these characteristics.

(a) Carbon Determinations. The choice of the analytical wavelength to be monitored is a very important aspect in atomic emission spectroscopy. In the case of carbon, the C (I) 193.0 nm transition is the most intense in the HC-AES spectrum, even though the sensitivity of the present photomultiplier tube is diminished here (down by 20−30%) relative to the visible region of the spectrum. The analytical response curve for C (I) emission of DL-valine (plotted as the integrated intensity of the signal transient) is shown in Figure 6. The data represent triplicate 200 μL injections over the concentration range of 10−250 ppm. The transient peak areas are calculated within Microsoft Excel by integrating the total signal from the peak starting point, over a fixed peak width, after subtracting the background for an analogous time scale. As indicated in previous studies, the

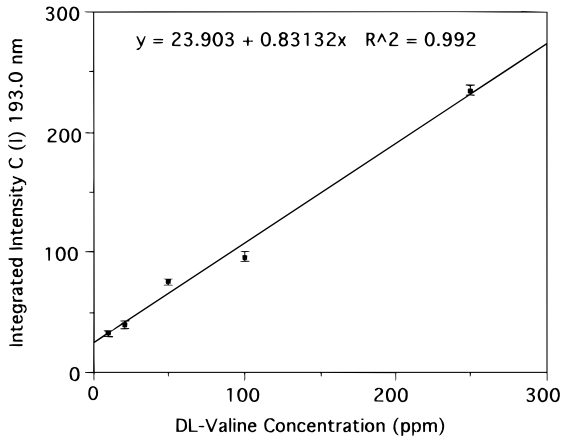

Figure 6. Analytical response curve obtained for integrated C (I) 193.0 nm emission intensities. Error bars represent the range of values for triplicate 200 μL injections of DL-valine. Discharge current 30 mA; source pressure 4 Torr He; nebulizer temperature 200 °C.

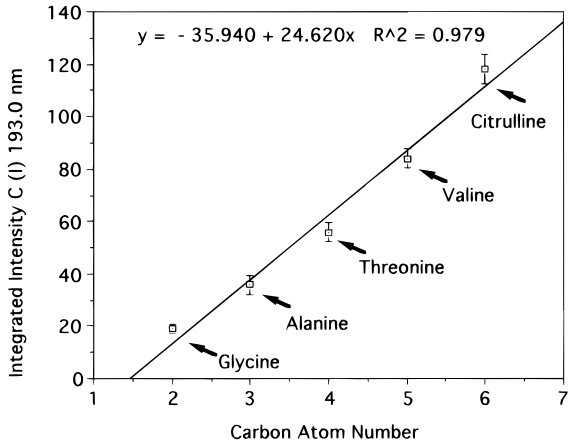

Figure 7. Response curve obtained for integrated C (I) 193.0 nm emission intensities as a function of number of carbon atoms per mole of amino acid. Error bars represent the range of values for triplicate 200 μL injections of 10^{-3} M solutions. Discharge current 30 mA; source pressure 4 Torr He; nebulizer temperature 200 °C.

addition of 6 M HCl in a 1:5 volume ratio (acid/sample) is employed here to improve the analyte particle transport efficiency.[7–9] The variability for the triplicate injections at each analyte concentration are seen to be less than 10% RSD. Based on the linear regression statistics of the calibration curve and the use of triplicate solvent blank injections, a limit of detection of 3 ppm (5.3×10^{-9} mol) DL-valine (2 ppm elemental carbon) is calculated. This value compares well with the established method of indirect chemiluminenscence detection of amino acids (2×10^{-9} mol).[15] UV−visible absorbance of precolumn BZTC and PTC derivatives (3.9×10^{-12} mol),[16] and UV−visible absorbance of postcolumn ninhydrin complexes (1.5×10^{-11} mol)[17] are more sensitive methods than the PB/HC-AES at this stage of development, though they require chemical modification steps. The quantification range and linearity depicted in this calibration curve (Figure 6) is generally better than these established methods, demonstrating the feasibility of the practical application of the PB/HC-AES technique for the basic elemental analysis of carbon in organic compounds and ligands and the detection of amino acids under HPLC separation conditions.

The second level of quantification is the determination of possible relationships between molecular formula and analyte (carbon) response. Ideally, the C (I) emission intensities would be proportional to the absolute number of carbon atoms (elemental concentration) in the injected sample but would also be proportional to the number of carbon atoms in the pure analyte molecule in the case of equimolar sample injections. As a first level of evaluation, a series of 10^{-3} M DL-amino acids were analyzed. In this case, DL-type glycine, alanine, threonine, valine, and citrulline, having carbon atom numbers ranging from 2 to 6, were chosen as analytes. The results of this study are shown in Figure 7, revealing a clear proportional relationship between emission signal (expressed in terms of peak area) and the number of carbon atoms in the individual molecules. It is believed that the high level of atomization and excitation efficiency within the HC environment produces analyte responses that can indeed be correlated with both absolute carbon concentrations and/or the molecular formula of the analyte molecule. Further evaluations of these sorts of responses for the cases where aromatic amino acids are injected must be undertaken to fully characterize the freedom of the C (I) response from effects of the structure of the molecule.

(b) Hydrogen Determinations. In order for the PB/HC-AES approach to be truly useful in the determination of empirical formulas of species such as amino acids, relationships of the sorts depicted in Figures 6 and 7 must also be realized for other elements. The determination of hydrogen is always challenging to analytical chemists because of the ubiquitous nature of hydrogen in the atmosphere, solvents, and gas supplies. In the first case, the reduced-pressure HC makes atmospheric contributions to the signals negligible. As shown in Figure 2 for C (I) emission, the PB interface is very effective in removing solvent vapors from the particle stream; therefore, solvent contributions to H (I) emission would be expected to be minimal here. For an isolated HC glow discharge source, hydrogen contributions can originate from two primary sources: the discharge gas inlet and the source vacuum port. These sources are clearly evident in spectral scans in the region of the H (I) 656.3 nm transitions. To ensure the best possible analytical features, zeolite-type gas purifiers were placed between the HC-AES source and He gas tank and in the vacuum pump line. The background emission spectra before and after the addition of purifiers were scanned through the entire range of the monochromator. The strong hydrogen background emission was in fact dramatically reduced (∼80%) after implementation of the purifiers. As one final means of possibly reducing the contributions to background hydrogen, KCl was used as a carrier additive to the solutions instead of the 6 M HCl, as the mechanism for the enhanced particle growth might also include trapping of hydrogen. In practice, the addition of the 4.6 M KCl to each sample produced H (I) responses that were ∼20% below those using HCl with the DL-valine but appreciably more than if no chloride was added at all.

Figure 8 depicts the analytical response curve for H (I) 656.3 nm emission for 200 μL injections of DL-valine over the concentration range of 10−250 ppm. As was the case for carbon, the hydrogen response curve shows very good linearity, with the variations for triplicate injections being less than 10% RSD at each of the concentration values. The quality of the calibration data for hydrogen, however, is not as good as seen for carbon. The most obvious source of the deteriorated performance is the high

(15) Koerner, P. J.; Nieman, T. A. *Mikrochim. Acta* **1987**, *11*, 79−90.
(16) Woo, K.-L.; Ahan, Y.-K. *J. Chromatogr., A* **1996**, *740*, 41−50.
(17) Biochrom 20 Amino Acid Analyser, Pharmacia Biotech, Cambridge, UK.

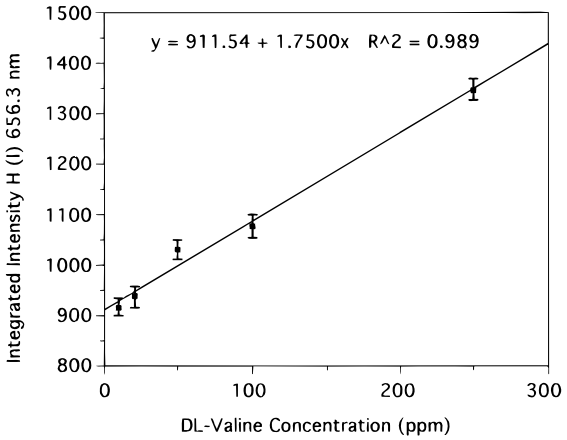

Figure 8. Analytical response curve obtained for integrated H (I) 656.3 nm emission intensities. Error bars represent the range of values for triplicate 200 μL injections of DL-valine. Discharge current 30 mA; source pressure 4 Torr He; nebulizer temperature 200 °C.

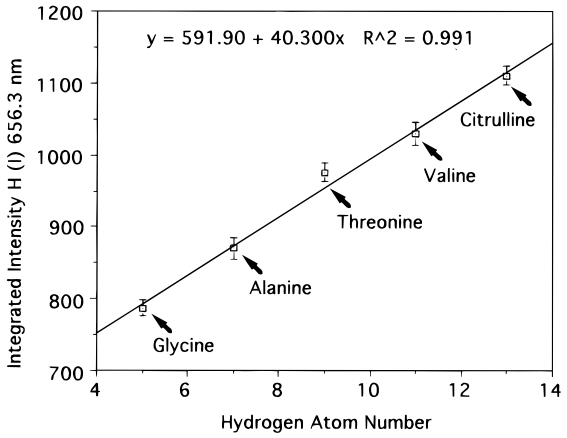

Figure 9. Response curve obtained for integrated H (I) 656.3 nm emission intensities as a function of number of hydrogen atoms per mole of amino acid. Error bars represent the range of values for triplicate 200 μL injections of 10^{-3} M solutions. Discharge current 30 mA; source pressure 4 Torr He; nebulizer temperature 200 °C.

level of background hydrogen, which is not completely removed with the present purifier system, leading to high background equivalent concentrations (BEC) and greater sample-to-sample variations. It is believed that the high BEC is likely due to solvent transported to the source volume within the analyte particle, which would not be accounted for in the background correction step. In any case, the linear regression data yield a limit of detection of 1 ppm DL-valine based on the hydrogen emission, which is equivalent to 0.1 ppm elemental hydrogen, equating to a limit of detection of 2.2×10^{-9} mol of DL-valine. Again, given the early stage of development of this methodology, this is an encouraging level of detectability.

Using the same series of amino acids applied in the study of the role of molecular formulas on the carbon atomic emission intensities, the effect of the number of hydrogen atoms (ranging from 5 to 13 here) on the observed response was investigated. As seen in Figure 9, there is indeed a direct relationship between the H (I) intensity and number of hydrogen atoms per molecule, for 200 μL injections of the 10^{-3} M solutions. The presence of background hydrogen is seen in these data through the elevated *y*-intercept. Even so, it is clear that the H (I) emission originating from the analyte compounds is quite well behaved. The obtained hydrogen calibration characteristics show promising sensitivity

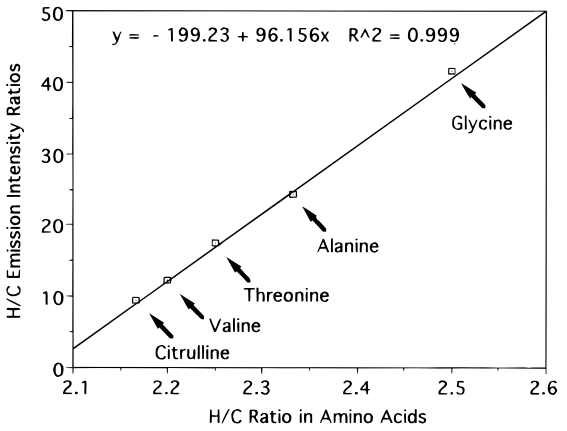

Figure 10. Comparison of experimentally obtained H (I) 656.3 nm/C (I) 193.0 nm emission intensity ratios to the actual atom ratios (H/C) for the range of amino acids.

and quantification capabilities, suggesting the further application of this PB/HC-AES system to other "atmospheric" elements such as O, N, and S present in amino acids or other organic compounds, so long as the atmospheric background is effectively eliminated.

(c) Empirical Formula Determinations. The ultimate goal of these studies was the evaluation of the PB/HC-AES technique as a means of determining the empirical formula of an organic compound. Specifically, as in the case of the GC-AED technique, the basic approach is envisioned to involve using the ratio of the emission intensities of the component elements as a measure of the ratio of those elements in a given compound. As was shown in Figures 7 and 9, the responses of both the carbon and hydrogen atomic emissions are reflective of the molecular formulas of the respective amino acids. Of course these relationships in themselves are meaningless, as the analysis of equimolar compounds is purely idealistic. The analytically relevant approach is to use the intensity ratios of the composite elements and translate them to empirical formulas. Figure 10 illustrates this strategy for the very simple case of combining the data plotted in Figures 7 and 9. Shown in the figure is the relationship between the ratio of the H (I)/C (I) integrated emission intensities to the actual ratio of those elements in the suite of amino acids. As can be seen in the plot and the corresponding regression data, the anticipated linear dependence holds true with the highest correlation coefficient ($R^2 = 0.999$) of any of the data presented here. This correlation is all the more impressive considering the fact that these data were taken on different days! It should be mentioned that the measured H (I)/C (I) intensity values are not expected to be equal to the actual atom ratio densities, as the respective transitions differ substantially in their sensitivities. While the group of amino acids used in the test case here may not be considered to be representative of a very wide array of organic molecules/ligands, it is clear at this level that the PB/HC-AES approach holds promise for further investigations in its use as a means of determining empirical formulas of simple organic compounds.

CONCLUSIONS

The studies described here demonstrate the feasibility of the particle beam/hollow cathode atomic emission spectroscopy (PB/HC-AES) technique for the determination of carbon and hydrogen in organic compounds. In particular, the approach has been used successfully in the determination of those elements in simple

amino acids. The desire to analyze organic compounds in solution requires capability in terms of operation with a range of solvent types. The combination of the thermoconcentric nebulizer and the momentum separator is shown to be effective at removing remnants of most solvent species, thus alleviating much of the possible spectral interference. In addition, operation of the nebulizer tip at high temperatures (200 °C) eliminates differences in sample introduction efficiencies as solvent identity is varied. In this way, solvent matrix effects in gradient elution situations are likely to be minimal.

The analytical performance of the PB/HC-AES has been characterized for the elements carbon and hydrogen. Quantification has been undertaken with respect to the C (I) 193.0 nm and H (I) 656.3 nm responses as a function of analyte compound (DL-valine) composition and as a function of the molecular formulas for a range of amino acids. Linear calibration curves were produced for both elements over a DL-valine concentration range of 10−250 ppm. On the basis of the regression statistics, the calculated detection limit for DL-valine based on carbon and hydrogen emission is found to be 3 and 1 ppm, respectively. These values correlate with 2 and 0.1 ppm of carbon and hydrogen, respectively, for the 200 μL injections, 320 and 24 pg in absolute terms. It is difficult to find comparable figures of merit for other atomic spectroscopy techniques which rely on solution sample introduction. The analytical responses of both elements are shown to be directly related to the number of the atoms of each in the respective molecular formulas for a range of aliphatic amino acids. Finally, the feasibility of using the PB/HC-AES technique to generate empirical formula information based on the H (I)/C (I) emission intensity ratios has been demonstrated for this group of amino acids.

While the use of simple amino acids is far from representative of the possible types of organic compounds/ligands encountered in liquid environmental or biological samples, it is believed that further evaluation of the PB/HC-AES technique in these areas is warranted. The previously demonstrated ability to detect transition metals to concentration levels of 1−10 ppb is complemented well by the ability to sensitively detect non-metals such as C, H, and S. To this end, much work remains in studying the role of molecular structure on the responses of the various analyte elements. For example, aromatic species may respond differently than aliphatic compounds. The choice of analytical transitions for additional elements will also be important in method development. In the ideal case, it is envisioned that this approach could be employed in an analogous fashion to the GC-AED, though for liquid samples with nonvolatile analyte molecules. As such, the PB/HC-AES may find a range of applications as a liquid chromatography detector in both environmental and biological systems.

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