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David C. Simpson
Virginia Commonwealth University, dsimpson3@vcu.edu

Alexander J. Yates
University of Edinburgh

John H. Knox
University of Edinburgh

Pat R.R. Langridge-Smith
University of Edinburgh

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A novel two-laser interface for coupling capillary electrochromatography with ion-trap time-of-flight mass spectrometry

David C. Simpson*,[1], Alexander J. Yates, John H. Knox, and
Pat R.R. Langridge-Smith

University of Edinburgh, School of Chemistry, West Mains Road, Edinburgh, EH9 3JJ, Scotland

[1]Present address: Virginia Commonwealth University, Department of Chemistry, Richmond, VA 23284, USA

*To whom correspondence should be addressed:

David C. Simpson
Virginia Commonwealth University
Department of Chemistry
P.O. Box 842006
Richmond, VA 23284−2006, USA

Email: dsimpson3@vcu.edu
Tel: +1−804−617−7432

Keywords: capillary electrochromatography; two-step laser mass spectrometry; ion-trap; time-of-flight; mass spectrometry; polycyclic aromatic hydrocarbon

Abbreviations: CEC, capillary electrochromatography; EOF, electroosmotic flow; ESI, electrospray ionization; ID, inner diameter; L^2MS, two-step laser mass spectrometry; LC, liquid chromatography; MS, mass spectrometry; PAH, polycyclic aromatic hydrocarbon; REMPI, resonance-enhanced multi-photon ionization; SCX, strong cation exchange; TOF, time-of-flight

Abstract

An interface has been developed for the hyphenation of capillary electrochromatography (CEC) with mass spectrometry (MS). Chromatographic eluate vaporization and selective analyte ionization occur within a quadrupole ion-trap, which permits significant instrument simplification when compared with the atmospheric pressure interfaces typically used for CEC-MS. Vaporization is achieved using laser desorption at 1064 nm while ionization is accomplished through UV photoionization. This two-step approach, through ionization laser wavelength selection, can provide ultratrace analysis with high selectivity. The mass spectrometer is a hybrid ion-trap time-of-flight (TOF) instrument in which the ion-trap is used in radio frequency-only mode, with DC-pulse ejection, to provide decoupling of the different timescales required for CEC separation and TOF mass analysis. The ion-trap is capable of accumulating ions over multiple laser shots. The mass resolution of the demonstration instrument was circa 1500. Preliminary CEC-MS runs have been recorded for mixtures containing polycyclic aromatic hydrocarbons. A concentration detection limit of 500 nM, for naphthalene in acetonitrile, has been determined for the interface.

Introduction

Capillary electrochromatography (CEC) can be described as electroosmotic flow (EOF)-driven liquid chromatography (LC). EOF-drive has two significant advantages over pressure-drive. First, the plug flow profile found in EOF-driven LC results in reduced band dispersion when compared with the parabolic flow profile found in pressure-driven LC. Second, band dispersion-minimizing reductions in stationary phase support particle size, which would not place any additional demands on EOF-drive instrumentation, require increased applied pressures in pressure-drive LC [1]. However, these advantages have not yet resulted in widespread popularity [2,3], despite the reporting of numerous CEC applications [4–7]. A key CEC disadvantage is that mobile phase composition influences flow rate, thus adding an extra layer of complexity to method development. Furthermore, when interfacing to mass spectrometry (MS), finding a mobile phase that is ideal for both separation and interface is particularly challenging.

Gordon *et al*. were first to report the interfacing of CEC to MS, using a continuous flow-fast atom bombardment interface [8]. The earlier report of Verheij *et al*. is best regarded as describing a pressure-drive system in which an applied potential was used to add an electrophoretic component to the separation [9]. In contrast to the earliest work, most reported CEC-MS interfaces incorporate electrospray ionization (ESI). However, while ESI-based interfaces where eluate is sprayed directly into the mass spectrometer have been reported [10], the incompatibility of typical CEC mobile phases with ESI necessitates the addition of an intermediate step where column eluate is diluted in a compatible make-up flow. The most common means of adding make-up flow is the

sheath flow method [11]. Practical operation of such interfaces for CEC-MS has been described by Lane *et al*. [12,13]. However, since dilution reduces sensitivity and even careful mixing leads to at least some band dispersion, alternative interfacing strategies are clearly required.

Laser resonance-enhanced multi-photon ionization (REMPI) exploits resonant absorption to selectively ionize gas-phase molecules that absorb significantly at a chosen wavelength. Target analytes can be ionized in the presence of high concentrations of non-absorbing background species [14]. Naturally, REMPI must be paired with a method for producing gas-phase neutrals to complete an interface with a liquid-phase separation. The recently introduced atmospheric pressure laser ionization interface combines a heated nebulizer with REMPI at atmospheric pressure [15]; this interface, with the addition of ESI, has also been demonstrated for CEC-MS [16]. However, REMPI is most often used under vacuum with laser desorption, which can be used to provide plumes of vaporized neutrals. Long-wavelength laser desorption produces few ions directly, allowing independent optimization of desorption and ionization processes. This combination is known as two-step laser mass spectrometry (L^2MS) [17–19]. While it is possible for the same laser to be used for desorption and ionization, this can lead to complicated mass spectra as reactions occur in the resulting plume of desorbed neutrals and ions [20].

Laser-based mass spectrometry interfaces that operate under vacuum typically operate with solid samples. A notable example of an interface where laser irradiation is applied to a liquid is the laser induced liquid beam ionization/desorption approach [21]. Furthermore, matrix-assisted laser desorption ionization from the hyperbolic inner

surface of an ion-trap ring electrode has been demonstrated using a solid sample presented on a probe [22] and a flowing liquid probe [23]. Using this concept we have constructed an L^2MS CEC-MS interface that requires no make-up flow and that avoids the ion transfer losses associated with atmospheric pressure interfaces. The interface is selective for analytes with significant absorbance at the ionization laser wavelength. Desorption and ionization occurs within the quadrupole ion-trap, using cross-bored apertures in the ring electrode to allow laser access. One such aperture is used for presentation of chromatographic eluate. Mass spectra are obtained by ejecting product photoions from the trap into a time-of-flight (TOF) mass analyzer, a configuration chosen chiefly for speed (ejection into the TOF mass analyzer is faster than the scanning out process used in ion-trap mass analysis). Thus, no compromise is required between resolution and scanning speed, and flexibility is retained for options such as switching to higher repetition rate laser systems.

Instrument performance was evaluated with polycyclic aromatic hydrocarbons (PAHs) since they are important environmental pollutants and are amenable to laser ionization at 266 nm. Complex mixtures of PAHs have often been targeted using L^2MS [24]. Chromatographic efficiency was largely maintained, even though a transfer capillary in which pressure-drive conditions existed was used to connect the CEC column terminus to the interface. The use of a transfer capillary was beneficial because it allowed columns to be exchanged without opening the high-vacuum chamber. Expressed as a number of theoretical plates per meter, obtained from measurements of half-height peak widths and retention times, an average chromatographic efficiency of 94,000 was obtained with a test mixture that consisted of acenaphthene, biphenyl, fluorene,

naphthalene and phenanthrene. A concentration detection limit of 500 nM was obtained for naphthalene in acetonitrile.

Experimental Procedures

Materials

Acenaphthene, biphenyl, naphthalene, and phenanthrene were obtained from Sigma-Aldrich (Saint Louis, MO, USA); fluorene and ammonium acetate were supplied by BDH (Poole, UK). Acetone, acetonitrile, acetic acid, and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA); water was double distilled in house. Fused-silica capillaries were from Polymicro Technologies (Phoenix, AZ, USA). Strong cation exchange (SCX) combined with reversed-phase (mixed mode) chromatographic stationary phase (3 µm particles) was supplied by Hypersil (Runcorn, UK).

Capillary Electrochromatography

Capillaries were packed largely as described by Boughtflower *et al*. [25,26]. SCX combined with reversed-phase stationary phase was chosen over reversed-phase alone because the SCX character provides surface charges that increase EOF [27]. Briefly, a slurry of stationary phase particles suspended in acetone was driven rapidly into an empty fused-silica capillary using an applied pressure of 800 bar; a WellChrom K-1900 pneumatic LC pump (Knauer, Berlin, Germany) was used to quickly reach this operating pressure. Stationary phase particle suspension in the packing bomb was maintained using a Miniprobe 55T ultrasonic probe (Kerry Ultrasonics, Hitchin, UK). Particles were initially retained using a Valco (Houston, TX, USA) steel screen (thinner than a frit so reduced resistance to solvent flow). Packing was allowed to proceed for 15 min. After careful depressurization to avoid disturbing the newly packed bed, the system was

reconfigured without the packing bomb. Water was then passed through the new CEC column for 12 h, using an applied pressure of 800 bar, in an attempt to collapse any voids that formed during the packing process. An electric heating coil (Innovatech, Stevenage, UK) was then used to create a sintered frit at the high-pressure end of the column. The column was reversed for production of the second retaining frit. With this approach a number of short columns can be made simultaneously. The electric heating coil can also be used to create windows for absorbance detection.

A Waters (Milford, MA, USA) Capillary Ion Analyzer was used for stand-alone CEC measurements (214 nm absorbance detection module installed) and as a high-voltage power supply (−30 to +30 kV) for CEC-MS. The instrument was equipped with an autosampler. Electrokinetic sample injection was performed by applying a potential of 5 kV for 5 s while the column inlet was immersed in a vial containing the sample solution. The system required circa 3 s to ramp up to 5 kV, indicating that the injection might be better described as the application of 3.5 kV for 5 s. An Agilent (Palo Alto, CA, USA) 8453 spectrophotometer was used to obtain molar absorptivity constants in mobile phase at 214 nm. To simplify CEC-MS operation, a transfer capillary was used to carry column eluate to the MS-interface. For electrical isolation, a grounded junction was constructed using a Supelco (Bellefonte, PA, USA) Capillary Column Butt Connector, effectively a double-tapered ferrule in a compression unit; electrical connection was achieved by externally coating the inlet end of the transfer capillary with copper using an Edwards (Crawley, UK) E12E4 vacuum coater. Since EOF terminates at the grounded junction, the flow profile in the transfer capillary is parabolic and chromatographic efficiency will be reduced. To minimize this degradation in performance, the transfer

capillary was much narrower than the separation column [28]. In this work, a CEC column having an inner diameter (ID) of 100 µm was paired with a transfer capillary having an ID of 25 µm.

Mass Spectrometry

The mass spectrometer and associated power supplies were manufactured by R.M. Jordan (Grass Valley, CA, USA). Michael *et al*. have described the design and operation of this instrument [29]. For easy access, the source region of the mass spectrometer was housed in a cubic vacuum chamber (30 × 30 × 30 cm) equipped with three fused-silica windows for laser access (windows may be exchanged to allow lasers operating at λ > ~3 µm to enter the chamber). The source chamber was pumped with a Pfeiffer (Asslar, Germany) TPH2000 2,000 L s^{-1} turbomolecular pump, backed by an Edwards E2M40 two-stage rotary pump combined with an Edwards EH250 mechanical booster. This pumping system was chosen to ensure vacuum performance even when pumping relatively large volumes of solvent vapor. The source chamber is separated from the TOF chamber using a gate valve, allowing isolation of the ion-trap from the TOF analyzer. The flight tube of the TOF analyzer is equipped with a liner that allows it to be held at a specified electrical potential relative to ground. By holding the liner at a negative high potential, the positive high potentials that must be applied to other electrodes are reduced, minimizing the risk of arcing to electrically grounded parts of the instrument. The incorporation of a gate valve isolating the ion-trap from the TOF analyzer required some modification to the extraction optics and flight tube liner. The TOF analyzer is pumped with an Edwards EXT250 250 L s^{-1} turbomolecular pump backed by an Edwards E2M18 two stage rotary pump. The ultimate vacuum (no solvent

input) obtained in the source chamber was 5×10^{-8} mbar, while that obtained in the TOF chamber was 2×10^{-8} mbar. Typical working pressures (solvent flow at typical CEC volumetric flow rates) were 2×10^{-6} and 4×10^{-7} mbar, respectively. These pressures were measured using Penning gauges.

Center axes for the cross-bored, cylindrical apertures in the ion-trap ring electrode were located in the equatorial plane. The two axes make a perpendicular intersection at the center of the trap while the ring electrode was mounted such that one cross-aperture was horizontal and the other was vertical. The diameter of the bored apertures was 0.094 inches (2.4 mm) and the ID of the ring electrode at the equatorial plane was 0.785 inches (19.9 mm). A transfer capillary carrying eluate from the CEC column was terminated in the lower aperture of the vertical pair, the terminus flush with the ring electrode surface; a ceramic holder was used for centering and to ensure electrical isolation. An IR desorption laser was fired down the vertical axis to strike the transfer capillary terminus while a UV ionization laser was fired along the horizontal axis as illustrated schematically in Figure 1. The ion-trap was used in radio frequency-only mode as an ion storage and accumulation device; the amplitude of the trapping potential used was 1525 V (peak-peak). Tandem mass spectrometry was not employed. Collisional cooling of the product photoions results in a cloud of ions having a narrow range of kinetic energies, which is a requirement for effective TOF-MS. During ion ejection, the trapping potential was switched off and an extraction pulse of −193 V was applied to the ion-trap end cap electrode closest to the TOF mass analyzer.

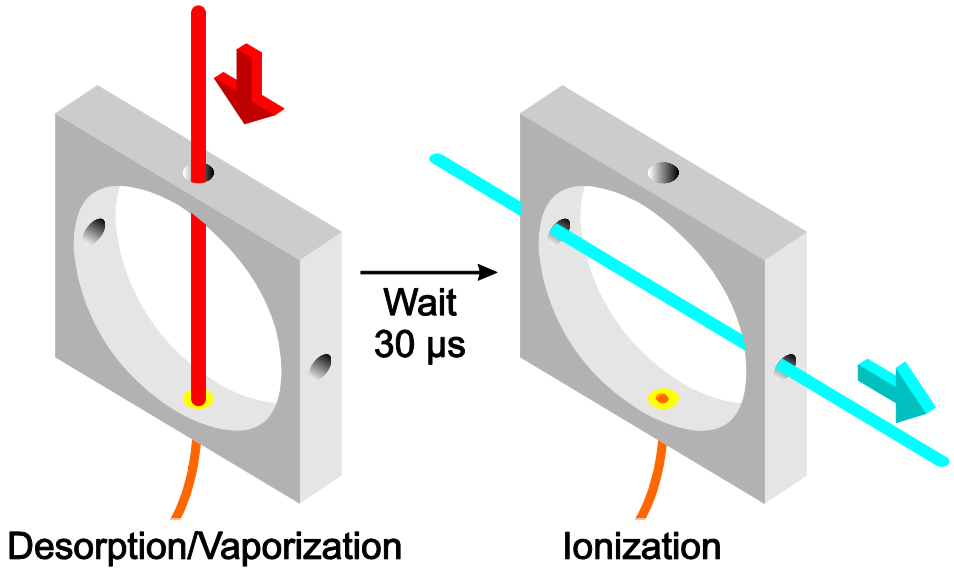

Figure 1: Schematic of the laser desorption laser ionization CEC-MS interface showing how the lasers are used within the ion-trap (IR laser beam for desorption; UV laser beam for ionization); the ring electrode shape has been simplified and the sample position is indicated by the capillary entering from below.

Laser powers were monitored using a Molectron (Portland, OR, USA) PM500A power meter in conjunction with a Molectron PM10V1 probe. Desorption was achieved using a Continuum (Santa Clara, CA, USA) Minilite Nd:YAG laser operating at 1064 nm; the laser was aligned using a quartz prism, followed by a lens and an iris before entering the vacuum chamber through a fused-silica window. The repetition rate was fixed at 10 Hz and laser power was tuned to maximize desorption while not providing sufficient power to effect non-resonant multi-photon ionization. With the meter placed below the trap and with the transfer capillary and holder removed, laser power was measured at circa 60 mW. Ionization was achieved using a Quantel (Les Ulis, France) Brilliant Nd:YAG laser equipped with harmonic generation modules to provide 266 nm laser pulses at a repetition rate of 10 Hz. Two quartz prisms were used to align the beam through a lens and a fused-silica window into the vacuum chamber. Laser power measured at the beam dump (a fused-silica window allowed the beam to exit the vacuum chamber after passing through the trap) was measured at circa 40 mW.

Experimental timing was controlled using an EG&G (Wokingham, UK) 9650A four-channel delay generator. The lasers fixed the experimental repetition rate at 10 Hz. Laser ionization occurred 30 μs after laser desorption to allow the plume of desorbed neutrals to expand into the space irradiated by the ionization laser. The duration of this delay was determined by tuning for maximum signal intensity. A second delay generator, a Stanford Research Systems (Sunnyvale, CA, USA) DG535, was added for ion accumulation over more than one laser cycle. For instrument tuning and characterization, a leak inlet was employed that consisted of a long length of fused-silica capillary terminated at the ion-trap ring electrode in the same way as the transfer

capillary; the length (564 mm) and ID (25 µm) of the leak inlet capillary were chosen to provide similar flow rates to the CEC system. Volumetric flow rates for the leak inlet were estimated using the Hagen–Poiseuille equation. Mass spectra were recorded using a LeCroy (Chestnut Ridge, NY, USA) 9350M 500 MHz digital oscilloscope. For collection of chromatographic data, the oscilloscope was controlled from a computer running LabVIEW (National Instruments, Austin, TX, USA) software. The time required for the transfer of a single mass spectrum (50,000 time points at 8-bit intensity resolution) from the oscilloscope to the computer was approximately 1.3 s. Due to the magnitude of this delay, during chromatographic runs, ten mass spectra were averaged before transfer to the computer, resulting in circa 27 mass spectra being recorded per minute.

In a separate experiment, solvent vaporization at the capillary terminus was observed using a video camera equipped with a macro lens. For viewing, the capillary terminus was moved just inside one of the fused-silica windows ordinarily used to allow lasers to enter the vacuum chamber. Desorption laser optics were adjusted to align the beam to the new target and vacuum chamber pressure was increased to circa 10^{-3} mbar to better represent conditions found inside the enclosed ion-trap. Targeting an object placed just inside a vacuum chamber window would be expected to result in scattered laser light exiting the vacuum chamber. Therefore, the area around the window and video camera was carefully enclosed.

Results

CEC Test Separation

Characterization of the interface required a test separation. Operating at 266 nm, the ionization laser was suitable for REMPI of PAHs. Naphthalene, biphenyl, fluorene, acenaphthene and phenanthrene were chosen as test mixture components. CEC separation was optimized without the mass spectrometer, using absorbance detection at 214 nm. Figure 2 shows an example chromatogram. Baseline separation was achieved for all five peaks, with the close spacing of fluorene and acenaphthene providing a quick indication of resolution. Peak identities were determined by sample spiking. Chromatographic efficiencies for each peak, expressed as plate numbers obtained from measurements of half-height peak width and retention time, are listed in Table 1. The baseline disturbance at circa 6.5 min represents the solvent front, giving the linear mobile phase velocity as 1.1 mm s^{-1}. Assuming that the flowing volume of the column is 50% of the open tube volume, the volumetric flow rate is estimated at 260 nL min^{-1}. Using this flow rate along with the peak area for naphthalene (0.00207 min), the molar absorption coefficient for naphthalene at 214 nm in mobile phase (57,800 M^{-1} cm^{-1}), and the detector path length (100 µm), the amount of naphthalene injected can be determined to be approximately 1 picomole. Naphthalene was present in the injected solution at 500 µM, so the injection volume is 2 nL. Alternatively, the injection volume can be obtained by multiplying the volumetric flow rate by the injection time and the injection potential as a proportion of the running potential. Using the averaged value of 3.5 kV over 5 s, a similar result for the injection volume, 2.5 nL, is obtained.

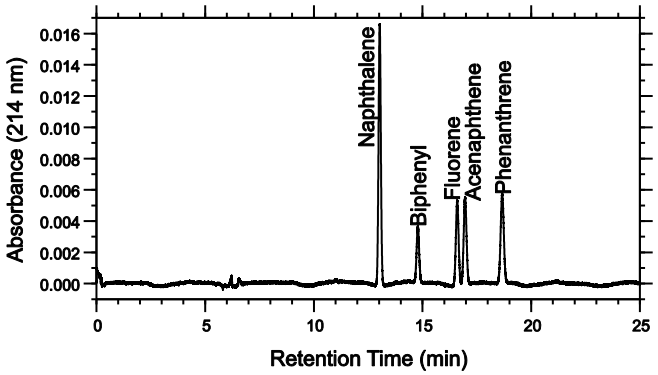

Figure 2: Chromatogram showing the CEC separation of a test mixture containing PAHs. Injection was electrokinetic, achieved by applying a potential of 5 kV for 5 s while the column inlet was immersed in a vial containing each sample component at 500 µM in acetonitrile. The running potential was 30 kV and the mobile phase was 75% acetonitrile, 25% 50 mM aqueous ammonium acetate adjusted to pH 6.0 with acetic acid. Stationary phase was SCX combined with reversed-phase (mixed mode) material. Column ID was 100 µm and length from inlet to detection window was 432 mm; total length was 624 mm. Chromatographic efficiencies of at least 70,000 theoretical plates were achieved for all five peaks.

Table 1: Comparison of chromatographic efficiencies obtained using on-column absorbance detection (Figure 2) with those obtained using the hyphenated CEC-MS system (Figure 5a). Chromatographic efficiencies are expressed as plate numbers obtained from measurements of half-height peak widths and retention times. Plate counts per meter are provided to aid comparison between columns of different lengths.

Analyte (listed in elution order)	Chromatographic Efficiency for CEC with Absorbance Detection at 214 nm (thousands of plates/plates per meter)	Chromatographic Efficiency for the Hyphenated CEC-MS System (thousands of plates/plates per meter)
Naphthalene	70/162	55/100
Biphenyl	73/168	66/121
Fluorene	76/176	32/58
Acenaphthene	73/170	80/147
Phenanthrene	71/162	24/44

Behavior of Liquids and Solutions Entering the Ion-Trap

Desorption in L^2MS is most commonly performed using 10.6 μm laser irradiation. However, using this wavelength resulted in damage to the fused-silica capillary terminus. Therefore, desorption was carried out at 1064 nm, a wavelength at which fused-silica is transparent and at which no damage to the capillary terminus was observed. However, non-resonant multi-photon ionization becomes more likely at shorter wavelengths, resulting in the imposition of laser power limits when only desorption is required. Such power limits can readily be determined by ramping intensity until ions are detected without the ionization laser. Since the aim of this work was to devise a selective MS-interface, the use of REMPI was essential. Therefore, the ability of the desorption laser to vaporize solvent at lower, L^2MS-compatible, powers (60 mW) was investigated. Furthermore, a major concern with the introduction of liquids through a narrow capillary into a high-vacuum chamber is that rapid evaporation may lead to flow stoppage through freezing. A flowing MALDI probe has been reported that required an infrared heater to avoid solvent freezing [23]. Therefore, solvent outflow from the capillary terminus was observed under vacuum using a video camera. Without laser irradiation, solvent accumulation was observed (a droplet formed at the tip and excess solvent ran down the outside of the capillary). This behavior was seen in the case of acetonitrile, water and methanol, as well as a solution of naphthalene at 5 mM in acetonitrile. When the 1064 nm laser was focused onto the end of the capillary at typical experimental power output settings, no solvent accumulation was observed. Figure 3 shows the case where an acetonitrile droplet was allowed to form by using a shutter to block the laser path, followed by the shutter being opened to permit the laser to strike capillary terminus.

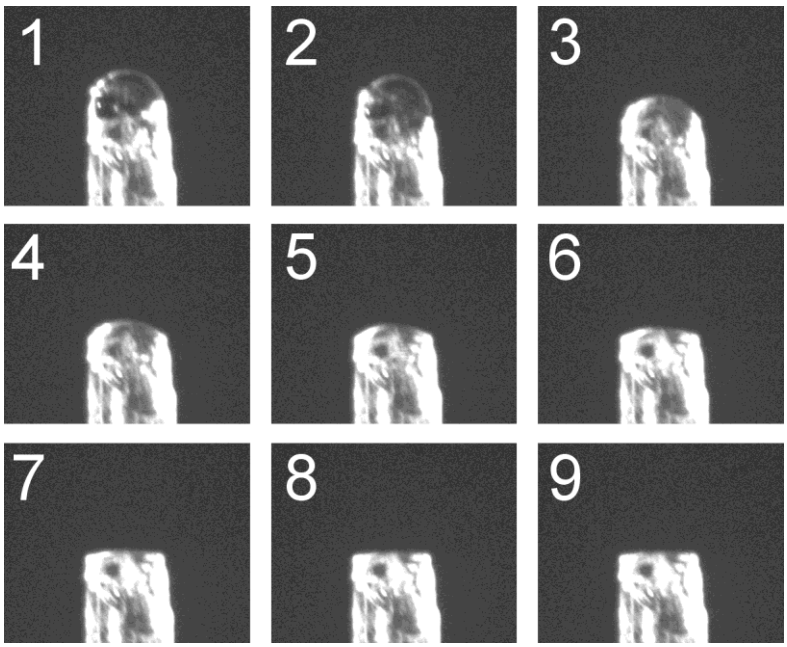

Figure 3: Filmstrip, each frame separated by 0.2 s, showing the effect of laser irradiation (1064 nm) on an acetonitrile droplet at an ambient pressure of circa 10^{-3} mbar (typical ion-trap operating pressure). The laser path was blocked to allow the droplet shown in Frame 1 to form. For scale, the width of the capillary is circa 400 µm.

Interface Characterization using the Leak Inlet

Characterization of the MS-interface using eluting peaks from CEC separations was difficult due to the transient nature of the signal, resulting in the adoption of a leak interface capable of providing a constant stream of analyte. Naphthalene in acetonitrile at various concentrations was used as test solution, flowing to the ion-trap at a calculated flow rate of 280 nL min^{-1}. First, trapping time was investigated. 268 µs were required from initializing the cycle through to the ionization laser pulse and a further 100 µs were required for recording a TOF mass spectrum, so trapping times close to the full laser cycle time of 100 ms were available. Figure 4a illustrates the variation in signal intensity (expressed as peak area for a fixed integration window centered on the molecular ion) observed for naphthalene at 500 µM in acetonitrile when using trapping times ranging from 10 µs to 90 ms. Similarly, Figure 4b shows the trend for 5 mM naphthalene in acetonitrile. At both concentrations, using very short trapping times results in poor signals. Longer trapping times result in increased signal until a plateau is reached, with the plateau achieved at a shorter trapping time for the higher concentration. However, a drop in molecular ion signal for the longest trapping times is seen for the higher concentration.

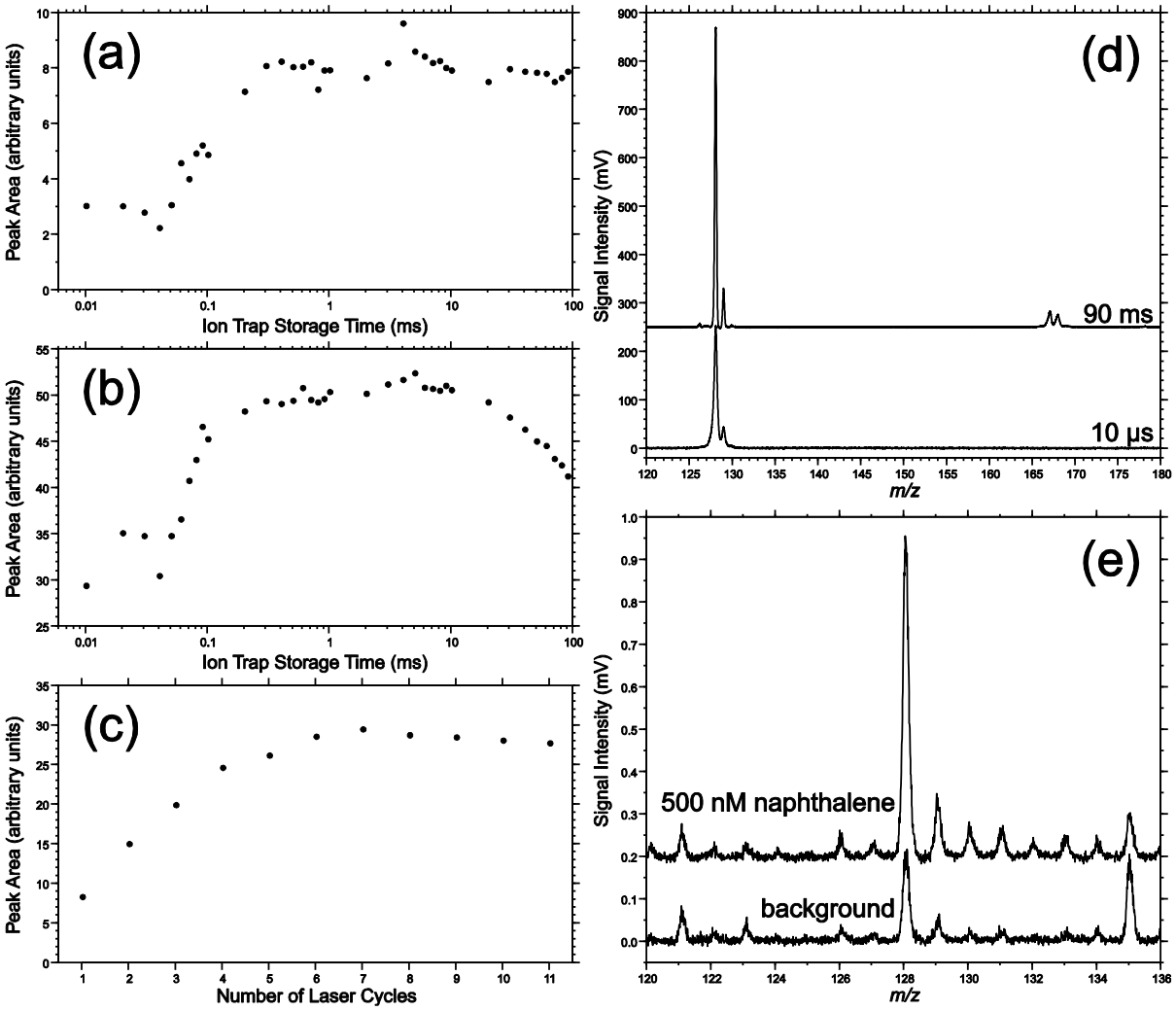

Figure 4: Interface performance characteristics obtained using the leak inlet operating at a flow rate of 280 nL min^{-1}. Each mass spectrum and data point results from the combination of 100 mass spectra. First, the effect of varying ion-trap storage time on molecular ion peak area is shown for 500 µM (a) and 5 mM (b) naphthalene in acetonitrile. Second, the impact on molecular ion peak area of storing ions over a number of laser cycles, with the delay between the final laser pulse and ion ejection fixed at 10 ms, is displayed for 500 µM naphthalene in acetonitrile (c). Mass spectra for 10 µs and 90 ms ion-trapping times for 5 mM naphthalene in acetonitrile, with the 90 ms trapping time data displaced +250 mV for clarity, are also provided (d). Finally, a mass spectrum obtained for 500 nM naphthalene in acetonitrile, with the data displaced +0.2 mV for clarity, is compared with that for acetonitrile alone (e).

Signal increase over that found at the shortest trapping times is due to collisional cooling. Ions will be produced along the laser path from side to side of the trapping space, resulting in some time being required to concentrate ions toward the center of the trap for efficient extraction to the mass analyzer. Initially, helium was added, as is normal in ion-trap instruments, but on tuning it was found that helium was unnecessary. Solvent vapor alone appeared to provide sufficient cooling. However, Figure 4d illustrates the disadvantage of using solvent vapor for ion cooling, indicating why the naphthalene molecular ion signal decreases at the longest trapping times in Figure 4b. It can be seen that at 90 ms trapping time a new peak has formed at *m/z* = 167, consistent with the formation of an acetonitrile adduct. In contrast, the 10 µs trapping time spectrum shows no adduct, but the naphthalene peak is much wider and smaller due to insufficient time having been allowed for cooling and concentration of the ion cloud. Since splitting analyte signal between two peaks is not beneficial, but reducing peak widths and increasing intensities is advantageous, a fixed trapping time of 10 ms was selected for CEC-MS experiments.

The leak inlet analyte introduction system was also used to estimate a concentration detection limit for the system. As illustrated in Figure 4e, this was found to be in the region of 500 nM for naphthalene in acetonitrile. Sample consumption at this concentration was 0.23 femtomoles per laser shot (100 laser shots were averaged to produce Figure 4e). Accumulation of ions over a number of laser cycles is an obvious way to improve interface sensitivity. Using 500 µM naphthalene in acetonitrile entering through the leak inlet at 280 nL min^{-1}, the effect of increasing cycle count on molecular ion peak area was observed. Storage time between the final laser pulse and ion ejection

was fixed at 10 ms. In Figure 4c, peak area can be seen to rise steadily up to circa seven cycles and then stay approximately constant for higher numbers of laser cycles. Maximum peak area of approximately 30 arbitrary units at seven cycles (versus 8 arbitrary units for one cycle) does not simply indicate that the trap was full; larger peak areas were found when 5 mM naphthalene in acetonitrile was used in the leak inlet as shown in Figure 4b (peak area units are arbitrary because they are calculated from micro-channel plate detector output voltages and flight times, but they can be compared). However, although at first sight this gain in sensitivity appears useful, ions created by earlier shots and concentrated into the center of the trap will be repeatedly irradiated by both laser beams. Such heating of these ions can result in dissociation, complicating the mass spectra produced. Since retaining simple mass spectra dominated by molecular ions was an advantage for CEC-MS, multiple accumulation cycles were not used.

Hyphenated CEC-MS System Performance

Figure 5a shows CEC-MS selected ion chromatograms for the separation of a test mixture consisting of naphthalene, biphenyl, fluorene, acenaphthene and phenanthrene (biphenyl and acenaphthene are isobaric and hence share the same chromatogram), each present at 5 mM. A comparison of chromatographic efficiencies obtained from the data illustrated in Figure 5a with those achieved using on-column absorbance detection (illustrated in Figure 2) is presented in Table 1. All plate counts were calculated from measurements of half-height peak widths and retention times. While chromatographic efficiencies were fairly constant for on-column detection, those for MS vary considerably and were on average about 55% of those for absorbance detection. Efficiencies are

especially low for fluorene and phenanthrene due to increased tailing when compared with the other test mixture components. Similar CEC-MS peak asymmetry and resolution were seen when each test mixture component was present at 500 µM, as illustrated in Figure 5b. Retention times are somewhat shorter when compared with the separation in Figure 2 because the running potential of 30 kV was applied from the column inlet to the grounded junction (546 mm) in the CEC-MS case versus across both the packed bed and the open section of tubing beyond the detection window (combined length 624 mm) in the absorbance detection case.

Since the aim of this work was to create a selective analytical system for the detection of targeted compounds from complex matrices, conditions were sought that resulted mostly in the production of molecular ions and hence simple mass spectra. However, while PAH radical cations are resilient toward decomposition, fragmentation through acetylene and hydrogen atom neutral losses does occur [30]. Figure 5c shows the mass spectrum obtained at the selected ion chromatogram peak maximum for phenanthrene from the same CEC-MS run used to construct Figure 5a. Fragmentation through the neutral loss of acetylene, to produce the biphenylene radical cation [31], is prominent. Acetylene loss was also seen for naphthalene, while hydrogen atom neutral losses were observed for fluorene and acenaphthene. Since fragmentation could result in misleading mass spectra for co-eluting species, the benefits of efficient chromatography are clear.

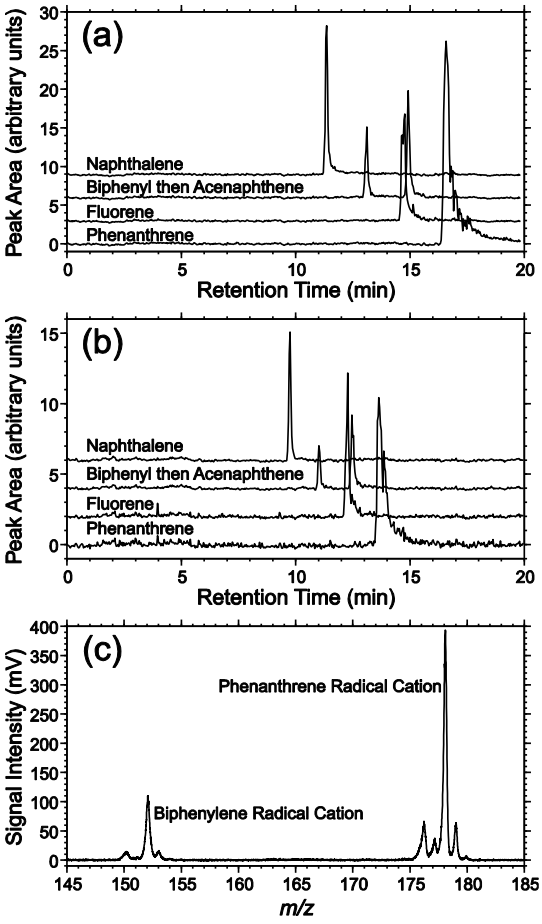

Figure 5: Selected ion chromatograms, displaced vertically in 3 (a) or 2 (b) arbitrary unit steps for clarity and with isobaric biphenyl and acenaphthene sharing the same chromatogram, for the CEC-MS separation of a test mixture containing each sample component at 5 mM (a) or 500 µM (b) in acetonitrile. Electrokinetic injection was achieved by applying a potential of 5 kV for 5 s while the column inlet was immersed in the sample vial. The running potential was 30 kV and the mobile phase was 75% acetonitrile, 25% 50 mM aqueous ammonium acetate adjusted to pH 6.0 with acetic acid. Stationary phase was SCX combined with reversed-phase (mixed mode) material. Column dimensions were 100 µm ID × 546 mm, while transfer capillary dimensions were 25 µm ID × 540 mm. Chromatographic resolution is lower than that found for absorbance detection, but fluorene and acenaphthene are still distinct. Acetylene and hydrogen atom neutral losses were observed for some test mixture components, with the fragmentation of phenanthrene to produce biphenylene being particularly prominent (c).

Discussion

A new CEC-MS interface based on L^2MS has been demonstrated using the separation of a small number of PAHs. Due to the isocratic chromatography and small column volumes employed, maximum injection volumes compatible with high chromatographic performance were in the low nL range. Naturally, such small injection volumes limit sensitivity (except for cases, such as single cell analyses, where only small volumes are available). The much larger injection volumes (often in the low-µL range) typically encountered in pressure-drive LC, using similarly sized columns, are made possible by gradient elution. While gradient elution-CEC was first reported in the mid-1990s [32,33], intricate equipment and the challenges of working with a system where EOF rates change with mobile phase composition through the course of a run discourage widespread adoption. While mobile phases designed to generate constant flow rates under gradient conditions have recently been reported [34], further limiting flexibility in method development is not desirable. In general, developing an effective separation in a system where mobile phase composition directly influences flow rate is perhaps the greatest drawback of CEC.

Reduced chromatographic resolution for CEC-MS when compared with on-column absorbance detection CEC could have multiple causes. Additional band dispersion originating from the pressure-drive conditions found between the column terminus and the ion-trap and imperfect assembly of the grounded junction would be expected to impact all test mixture components. Shortening the transfer capillary would be expected to result in improved resolution, but vacuum chamber size and the desire to keep the

grounded junction accessible, to allow columns to be exchanged without opening the chamber, precluded this simple solution. However, these causes of band dispersion cannot explain the differential peak tailing observed for the heaviest test mixture components, fluorene and phenanthrene. Overloading of fluorene and phenanthrene seems unlikely as similar chromatographic peak shapes are observed at 5 mM (Figure 5a) and 500 µM (Figure 5b), but adsorption to the bare fused-silica inside walls and terminus of the transfer capillary could be important since the larger molecules, having greater polarizability, would be more likely to interact with the surface. Therefore, tailing for the heavier test mixture components might best be investigated by using coated capillaries to reduce adsorption, although sufficient robustness to survive laser irradiation at the terminus could prove challenging.

Non-chromatographic sources of peak asymmetry must also be considered. Persistence of desorbed neutrals in the ion-trap seems unlikely given the supersonic rate at which the plume of desorbed neutrals expands. Desorbed neutrals not ionized when intended are expected to be dispersed before the next laser shot. However, incomplete laser desorption is possible. Any material not desorbed would remain at the transfer capillary terminus, carrying over to the next laser desorption event. The lighter test mixture components would more readily evaporate, explaining the enhanced tailing observed for heavier fluorene and phenanthrene. Moving to 10.6 µm laser desorption could perhaps be beneficial, but a carefully designed assembly would be required to protect the fused-silica capillary terminus while avoiding the introduction of dead volumes detrimental to separation performance.

While the interface was designed around the compatibility of CEC flow rates with the

vacuum pumping capacity of the instrument, pressure-drive LC systems that can reliably supply similar flow rates have recently become commonplace. There is no reason why the interface could not be combined with these systems or indeed with any separation method having appropriate flow rate and eluate composition. The key advantage of the interface is the selectivity that arises from ionization laser wavelength choice, which offers the possibility of detecting a target analyte among high concentrations of non-absorbing species. Furthermore, being able to analyze target compounds directly in complex matrices offers the possibility of simplified sample preparation procedures, thus reducing total analysis time and the chance of incorporating biases into methods. In contrast, for most other interfaces, little can be done to target particular analytes.

The instrumental system also offers the possibility of enhanced sensitivity through achieving an effective 100% duty cycle. Most separations provide a continuous stream of eluate and are coupled with continuous ion sources, such as ESI, while most MS instruments can only accept ions for a small proportion of the total cycle time. For example, instruments based on orthogonal-acceleration TOF mass analyzers typically have duty cycles in the range from 5% to 30% [35]. The L^2MS interface reported here is capable of an effective 100% duty cycle because eluate simply collects at the capillary terminus between laser shots. No material should be lost, although volatile compounds might be depleted by evaporation. Sensitivity is also enhanced by creating ions inside the trap, thus avoiding transmission losses from a distant source. However, sensitivity will be lost due to the ionization laser beam only irradiating part of the trap volume.

Interface characterization using the chromatographic system was difficult due to the transient nature of eluting peaks, so performance was assessed using a leak inlet that

was designed to provide a continuous stream of analyte at flow rates mimicking those of the separation. Generally, the interface performed as expected: collisional cooling time was required to focus ions toward the center of the trap and sensitivity could be improved by accumulating ions over a number of laser cycles. What was not originally anticipated was the role of solvent vapor. It was quickly determined that helium was not required for trapping, presumably due to sufficient cooling being achieved with solvent vapor alone. The simplest way to regain control of ion-trap operating pressure would be to lower the flow rate sufficiently so that helium would be required to augment solvent vapor. The amount of helium added could then be tuned for optimum performance. Furthermore, reducing the amount of solvent vapor present would reduce adduction, improving the viability of accumulation over multiple laser cycles. However, maintaining chromatographic performance while reducing flow rate would require a reduction in column ID, which would lead to a concomitant reduction in injection volume and thus sensitivity.

In conclusion, an interface between liquid phase separations and mass spectrometry has been demonstrated that is designed around the concept of selective laser ionization of target compounds in the presence of high concentrations of non-absorbing species. Many performance-enhancing improvements could be envisioned, such as a data system fast enough to remove the need for spectrum averaging and adjustments to the vacuum chamber to allow the use of shorter transfer capillaries. Tailing, which was observed for the heaviest test mixture components and was presumably due to adsorption to fused-silica surfaces or incomplete desorption from the transfer capillary terminus, is more problematic; however, switching to an appropriately coated transfer

capillary and careful selection of desorption laser wavelength and power for each system is likely to be beneficial.

Acknowledgements

We especially thank Robert J. Boughtflower for assistance with various aspects of CEC, David P.A. Kilgour for LabVIEW programming, and Robert R.J. Maier for his help in the design and construction of the mass spectrometer. This work was supported by the Engineering and Physical Sciences Research Council [grant number GR/L32460/01]. D.C.S. received a Collaborative Awards in Science and Engineering studentship sponsored by Imperial Chemical Industries.

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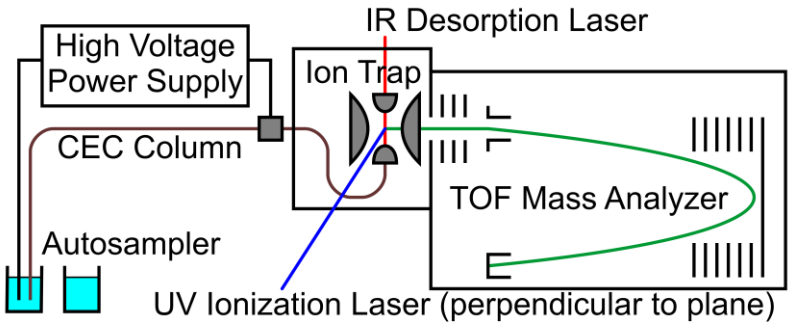
Table of Contents Graphic



Development of a novel instrument for the hyphenation of capillary electrochromatography with ion-trap accumulation time-of-flight mass spectrometry via a temporally and spatially separated laser desorption laser ionization interface.