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Interfacing Capillary-Based Separations to Mass Spectrometry Using Desorption Electrospray Ionization

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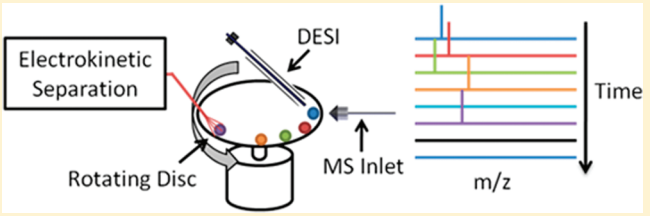
ABSTRACT: The powerful hybrid analysis method of capillary-based separations followed by mass spectrometric analysis gives substantial chemical identity and structural information. It is usually carried out using electrospray ionization. However, the salts and detergents used in the mobile phase for electrokinetic separations suppress ionization efficiencies and contaminate the inlet of the mass spectrometer. This report describes a new method that uses desorption electrospray ionization (DESI) to overcome these limitations. Effluent from capillary columns is deposited on a rotating Teflon disk that is covered with paper. As the surface rotates, the temporal separation of the eluting analytes (i.e., the electropherogram) is spatially encoded on the surface. Then, using DESI, surface-deposited analytes are preferentially ionized, reducing the effects of ion suppression and inlet contamination on signal. With the use of this novel approach, two capillary-based separations were performed: a mixture of the rhodamine dyes at milligram/milliliter levels in a 10 mM sodium borate solution was separated by capillary electrophoresis, and a mixture of three cardiac drugs at milligram/milliliter levels in a 12.5 mM sodium borate and 12.5 mM sodium dodecyl sulfate solution was separated by micellar electrokinetic chromatography. In both experiments, the negative effects of detergents and salts on the MS analyses were minimized.



Capillary electrophoresis (CE)[1−3] and micellar electrokinetic chromatography[4] (MEKC) are widely used electrokinetic separation methods that achieve rapid high-resolution separations. CE separates charged species, whereas MEKC separates both neutral and charged species. Electrokinetic separations have low sample consumption and do not need complex equipment, typically requiring only a capillary, electrodes, a power supply, injection system, buffer, and for MEKC, detergent. Both electrokinetic techniques offer higher resolution than conventional pressure-driven flow systems, largely caused by the plug flow profiles maintained in the capillary by the electroosmotic flow and the effective dissipation of heat by the capillary structure. Furthermore, electrokinetic separations are tunable by altering the solvent, buffer, and detergent composition.

Both separation methods can be directly coupled to various optical detection methods such as ultraviolet−visible (UV−vis) spectroscopy,[5] laser induced fluorescence,[6] and Raman spectroscopy.[7−9] Laser induced fluorescence has high sensitivity, a limit of detection (LOD) of 10^{-10} M or lower, but limited application because it requires molecules of interest to be natively fluorescent or labeled prior to analysis. Raman and UV−vis spectroscopy are more general techniques but have lower sensitivity, with LODs on the order of 10^{-5}−10^{-6} M.[5]

The speed, versatility, sensitivity, and specificity of mass spectrometry (MS) measurements make them an attractive alternative to optical spectroscopy for detection. Online coupling of electrokinetic separations to MS using electrospray ionization (ESI) has been previously demonstrated;[10] however, the presence of salts in separation buffers cause ion suppression and contamination of the mass spectrometer inlet. In MEKC, these problems are exacerbated because of the detergents used in the mobile phase.[11,12] Various strategies have been used to avoid these problems such as partial-filling MEKC−MS,[13] anodically migrating micelles,[14] and volatile surfactants.[15] These strategies can complicate separations. Furthermore, they are difficult to adapt to separations optimized using other detectors. Despite the difficulties, direct coupling of MEKC separations to ESI-MS has been accomplished for some systems but is not yet universal.[16,17] Matrix-assisted laser desorption/ionization (MALDI) has been interfaced to electrokinetic separations by depositing the effluent on a surface, encoding the temporal separations spatially allowing for subsequent MALDI-MS analysis.[18] This strategy was also used to couple surface enhanced Raman spectroscopy (SERS) by depositing effluent onto a SERS active surface.[9] CE-MALDI-MS has also been done online using a rotating ball interface, in which the column effluent is deposited on a rotating ball that transfers analyte into the vacuum region of the MALDI source.[19]

Desorption electrospray ionization (DESI) is an MS ionization source that allows chemicals on a surface to be analyzed at ambient pressure with either minimal or no sample preparation.[20] In DESI, charged microdroplets are directed toward a surface near the inlet of a mass spectrometer. Species on the surface are desorbed, ionized, and transferred into the instrument. DESI has

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been used to detect a wide range of analytes such as explosives,[21] illicit drugs,[22] and pharmaceuticals.[23,24] DESI also allows imaging of the spatial distribution of chemicals on various surfaces[25] and in complex tissues.[26,27] DESI has high sensitivity (femtomoles)[28] and high salt tolerance. Previous reports show that in the presence of 2% salt by weight in a deposited solution, analytes can be detected at hundreds of nanograms/milliliter.[29] This high salt tolerance makes DESI an attractive method for coupling electrokinetic separations to MS because the composition of the separation buffer will not interfere with the sampling. Furthermore, signal increases have been shown by doping the DESI spray solvents with small quantities of surfactants[30] and changing solvents depending on the nature of the analytes of interest.[31] By interfacing the separation to MS using DESI, analyte signals can be increased by varying the composition of the reagents in the DESI spray.

This report demonstrates the interfacing of electrokinetic separations, both CE and MEKC, to MS using DESI. It relies on edge sampling, disk-based systems similar to those previously published.[32,33] In these experiments, analytes are separated in a column and the effluent is sprayed onto a rotating paper disk to spatially record the electropherogram or chromatogram. After separation and deposition, the surface is analyzed using DESI-MS. The method takes advantage of DESI's high salt tolerance to allow for the use of nonvolatile CE and MEKC buffers without source contamination and ion suppression. The sample preparation in this technique is inherent to the electrokinetic separations. Other than deposition, the effluent need not be prepared specifically for MS analysis. The possibility of analysis using this novel technique is demonstrated using a mixture of three rhodamine dyes for CE and a mixture of three cardiac drugs for MEKC.

EXPERIMENTAL SECTION

Chemicals. HPLC grade methanol, acetone, ultrafiltered water, glacial acetic acid (Fischer Scientific, Hampton, NH), Rhodamine 6G (Eastman Kodak Company, Rochester, NY), Rhodamine B (The British Drug House LTD, Poole, U.K.), Rhodamine 575 (Exciton, Dayton, OH), sodium borate (J.T. Baker, Phillipsburg, NJ), sodium dodecyl sulfate (Bio Rad, Hercules, CA), diltiazem, verapamil, and nicardipine hydrochloride (Sigma Aldrich, St. Louis, MO) were used without further purification.

Capillary Electrophoresis. A fused silica capillary that has a polyimide coating was used as the column (60 cm in length; 360 μm outer diameter; 75 μm internal diameter; purchased from Polymicro Technologies, Phoenix, AZ). The beginning of the column was kept in a vial containing separation buffer and a platinum electrode. A separation potential of 27 kV was provided by a high-voltage power supply (Series EL, Glassman High Voltage Inc., High Bridge, NJ). Approximately 1.5 cm of the polyimide coating was removed from the column 50 cm from the buffer vial, creating a transparent section that allowed online monitoring of the separations by UV−vis spectroscopy (HyperQuan Inc., Colorado Springs, CO). Analyte solutions were injected by inserting one end of the capillary column in a vial of analyte elevated 10 cm above the other end for 2 s. The exterior of the capillary was wiped clean before it was reinserted into the buffer vial. With measurement of the time width of solvent peaks in the electropherograms and chromatograms, the upper-limit of the injection volumes were calculated to be 100 nL or lower. For CE, the separation buffer was a solution containing 10 mM sodium borate, water, and 30% acetone. The injected solution contained the rhodamine dyes (1 mg/mL), 42.5% water, 42.5% methanol, and 15% acetone. For MEKC, the separation buffer was a solution of 12.5 mM sodium borate, 12.5 mM sodium dodecyl sulfate (SDS), water, and 15% acetone. The MEKC separation conditions were similar to previously published separations of the same analytes.[34] The injected solution contained the cardiac drugs (1 mg/mL), 57% water, 18% acetone, 12.5% methanol, and 12.5% acetonitrile. For both experiments, the upper-limit injection volume calculations yield approximately 200 pmol of each analyte as all analytes have similar molecular weights.

The length of the column from the vial to the UV−vis detector was surrounded by plastic tubing allowing the capillary to be cooled by a countercurrent flow of water. Upchurch Scientific (Oak Harbor, WA) manufactured all fittings described below and model numbers refer to their catalogue, unless otherwise noted. After passing through the UV−vis instrument, the capillary was fed through the 180° openings of the PEEK tee (P-727, diameter of the bore was 500 μm) and 0.020 in i.d. PEEK tubing. Acetone was infused into the 90° opening of the tee at a flow rate of 10 μL/min using a syringe pump (Harvard Apparatus, Holliston, MA). This auxiliary flow was introduced to counteract the suction generated by spraying the effluent onto the surface. In particular, acetone was chosen because of its high evaporation rate which helped minimize the wetting of the surface and subsequent spreading of deposited species. The PEEK tubing and capillary column terminate at a stainless steel female adapter (model no. 1583), which was grounded through a 11 kΩ resistor. The voltage drop across the resistor allows the current flowing through the capillary to be monitored with an op-amp follower. The column effluent and acetone mix at the female adapter. At this fitting, the endings of the fused silica capillary and the acetone-containing PEEK tubing should align. If the fused silica capillary extends beyond the end of the PEEK tubing, the suction is substantially increased. If the fused silica capillary ends a few millimeters recessed in the PEEK tubing, the deposited bands are much broader. A fused silica capillary (150 μm i.d.) transports this mixture into a fused silica makeup adapter (FSMUA1.5M, VICI, Houston, TX) which supplies a sheath flow of dry nitrogen at 0.6 L/min. The spray was directed on to a rotating surface. The flow rates of N_2 and the auxiliary solvent must be properly balanced to obtain a stable spray without creating pockets of air in the capillary column. At these gas and auxiliary solvent flow rates, the deposition spray diameter was approximately 1.5 mm. Minimizing both the gas and acetone flow rates can reduce the physical size of the deposited analyte spots but has the detrimental effect of increasing the time delay between elution from the column and deposition on the surface. The parameters detailed above were a compromise between elution time, deposition spot size, and spray quality. Figure 1 shows the experimental setup. Alternate delivery strategies were attempted that did not use any makeup flow or gas. These strategies produced minimal success. Comparatively, the system described offered substantial robustness.

The rotating Teflon surface was circular, having a diameter of 8 cm. The surface was covered with Whatman 3MM CHR chromatography paper (GE Healthcare, Little Chalfont, U.K.) or manila envelope paper. For the rhodamine dye separation, deposited spots were visible to the eye against the white surface of the chromatography paper, facilitating optimization of fittings, flow rates, and tubing sizes. Manila envelope paper covered surfaces were used exclusively for MS analysis as they produced

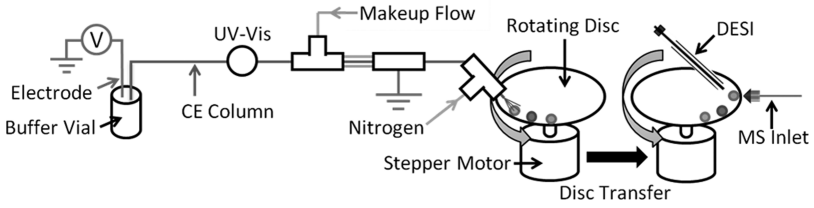

Figure 1. Experimental setup used to interface capillary electrophoresis to a mass spectrometer using desorption electrospray ionization.

higher signal than the chromatography paper. The paper was attached to the Teflon surface with double-sided tape (3M, St. Paul, MN). For both deposition and sampling by DESI, the surfaces were rotated using a stepper motor (23MD, Anaheim Automation, Anaheim, CA) controlled by a programmable function generator (DS345, Stanford Research Systems, Sunnyvale, CA). Each step was approximately 160 μm of displacement at the edge of the surface where analysis is performed. During deposition of the effluent from the capillary column, the surface was rotated 160 μm/s for CE and 160 μm every 2 s for MEKC. For all separations, time zero was denoted using a blue ink marker which has a strong signal at m/z 478. In all experiments, the disks were analyzed no later than 2 h after deposition.

Desorption Electrospray Ionization Mass Spectrometry. An LCQ Classic quadrupole ion trap mass spectrometer (ThermoFisher Scientific, San Jose, CA) was used for all experiments. For all experiments, the capillary was held at 15 V and 150 °C. The automatic gain control was set at 10^8. For the CE experiments, spectra were acquired using a scan range of m/z 150−800 and 3 microscans per spectrum. For the MEKC experiments, the MS was scanned in the range m/z 400−500 with 5 microscans per spectrum. The DESI source including the rotating sampling stage was previously described in detail.[33] Briefly, a DESI source and stepper motor were mounted on x,y and z manipulators. The DESI emitter was held at +5 kV. It was positioned 2 mm above the surface at an angle of 60°. Dry nitrogen (Praxair, Danbury, CT) was used as the nebulizing gas at a flow rate of 0.5 L/min and a pressure of 50 psi. The spray solvent, methanol, was infused at a rate of 10 μL/min using the syringe pump on the mass spectrometer. These parameters produced a DESI spray diameter of approximately 2 mm at the surface. Prior to analyzing surface tracks, the DESI source angle and distances are optimized by maximizing the ion intensity produced by the dye spot used to mark time zero on the surface. During MS analysis, the surface rotated 313 μm/s, equating to a full rotation time of 13 min. At this rate, a 30 s wide MEKC peak, 2.3 mm wide on the disk, is analyzed in 7.3 s.

RESULTS AND DISCUSSION

Jackson, Talaty, Cooks, and Van Berkel[29] first showed that DESI is capable of analyzing analytes in the presence of large amounts of salt in the deposited sample. Their work motivated us to pursue DESI's use as a bridge to allow for MS analysis of electrokinetic separations. We suggest that the reason DESI is less sensitive to salts is because of their lower solubility in the methanol spray solvent.

When a mixture of Rhodamine B, Rhodamine 6G, and Rhodamine 575 is separated using CE, two peaks are observed in the electropherogram (Figure 2a). The first peak corresponds to Rhodamine 6G (retention time (RT) = 3.6 min) and the second peak to Rhodamine B and Rhodamine 575 (RT = 4.6 min), which coelute. The order was verified by measuring the retention

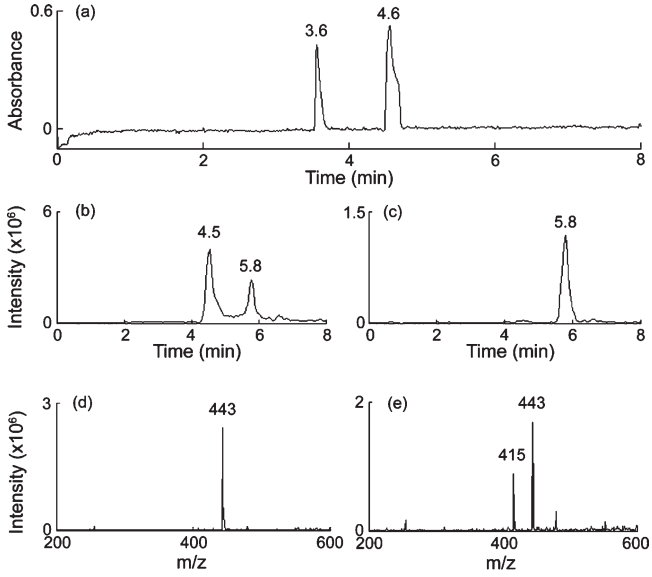

Figure 2. CE separation of a mixture of three rhodamine dyes: (a) electropherogram as measured by UV−vis; (b) ion chromatogram for m/z 443; (c) ion chromatogram for m/z 415; (d) mass spectrum for a retention time of 4.5 min; and (e) mass spectrum for a retention time of 5.8 min. The selected retention times correspond to the peaks in the electropherogram.

times for individual analytes. After depositing the effluent, the surface is transferred to the DESI source. In MS analysis, Rhodamine B and Rhodamine 6G are both observed at m/z 443 and Rhodamine 575 at m/z 415. Rhodamine 6G fragmentation was a concern. Individual solutions of each dye were analyzed by DESI-MS and compared to every combinatorial mixture of dyes at equal concentration. The results showed that Rhodamine 6G fragmentation to produce a m/z 415 peak was minimal. Furthermore, Rhodamine 6G and Rhodamine B were distinguishable at equal concentration depositions by the intensity of the m/z 443 peak; Rhodamine 6G produces a much more intense peak than Rhodamine B. Figure 2b,c shows the ion intensities versus equivalent separation time as measured by DESI-MS for the two m/z values of interest. The traces clearly show the two peaks seen in the electropherogram but at a later time as the UV−vis detector is not at the end of column. Average mass spectra across each peak are shown in Figure 2d,e. There is a single peak at m/z 443 in Figure 2d which is identified as Rhodamine 6G based on the electropherogram retention time. The coelution of Rhodamine B and Rhodamine 575 is elucidated in Figure 2e as peaks at both m/z 415 and m/z 443 are observed.

When the pharmaceutical drugs verapamil, nicardipine, and diltiazem are separated using MEKC, three peaks are observed in the UV−vis chromatogram (Figure 3a). The order of elution is

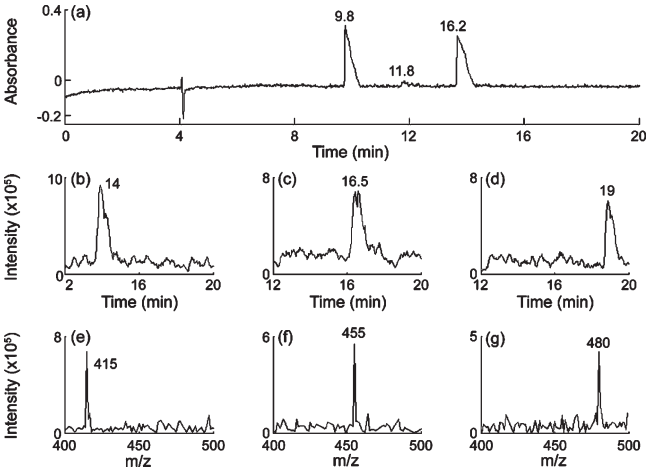

Figure 3. MEKC separation of a mixture of three cardiac drugs: (a) chromatogram as measured by UV−vis; (b) ion chromatogram for m/z 415; (c) ion chromatogram for m/z 455; (d) ion chromatogram for m/z 480; (e) mass spectrum for a retention time of 14 min; (f) mass spectrum for a retention time of 16.5 min; and (g) mass spectrum for a retention time of 19 min. The selected retention times correspond to the peaks in the chromatogram.

diltiazem (RT = 10 min), verapamil (RT = 12 min), and nicardipine (RT = 16 min), which agrees with previously published results.[32] The relative standard deviations for the retention times is ∼2% for triplicate measurements. All three compounds exhibit tailing. The tailing could be caused by analyte absorption on the walls of the capillary or varying analyte migration rates between the buffer and injected solution. Parts b, c, and d of Figure 3 show extracted ion chromatograms, with time axes in the separation frame, for diltiazem (m/z 415), verapamil (m/z 455), and nicardipine (m/z 480), respectively. The average mass spectra across each peak are shown in parts e, f, and g of Figure 3. The spectra do not contain any adduct or contaminant peaks from the salt or detergent. In a reference experiment, one solution containing sodium borate (2.5 mg/mL), SDS (2 mg/mL), and the MEKC analytes (0.5 mg/mL) and a second solution containing only the analytes (0.5 mg/mL) were made and analyzed by DESI-MS, 5 μL of each analyte solution was spotted over approximately 1 cm^2. The results of the experiment are seen in parts a and b of Figure 4. Both spectra are similar in intensity for the analyte peaks and no major adduct peaks were observed. Inlet contamination was not observed in either the reference experiment or any of the separation experiments. In sharp contrast, we attempted direct infusion ESI-MS but severe inlet contamination problems prevented us from pursuing this approach.

For both experiments, the peaks in extracted ion chromatograms are 2−3 times wider than the corresponding peaks in UV−vis electropherograms and chromatograms. The rotation rates of the disks during deposition and the time widths of the peaks as measured by UV−vis would yield approximately 2.5−3 mm spots. However, deposited analyte spots were 4−5 mm wide. Some of the difference could be the convolution of the eluting peak width with the deposition spray diameter (approxmately 1.5 mm). This effect can be minimized by increasing the rotation rate of the surface or minimizing the diameter of the deposition spray, but both strategies have drawbacks. In the presented work, the rotation rates were chosen to yield spots concentrated enough to effectively measure. Increasing the rotation rate reduces the density of analyte on the surface which increases

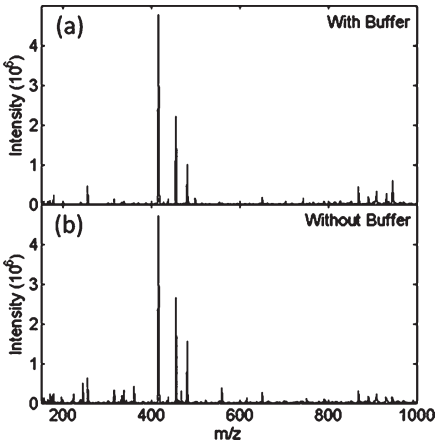

Figure 4. DESI-MS of a mixture of three cardiac drugs: (a) deposited with sodium borate and SDS and (b) deposited without sodium borate and SDS.

the difficulty of DESI-MS analysis, and decreasing the deposition spray diameter through decreasing the gas and auxiliary solvent flow rates led to nonrobust separations and deposition sprays. Broadening occurring prior to deposition could be the result of poor mixing of the makeup solvent (acetone) and effluent from the capillary column. Further refinement of the mixing section by changing the fitting or using a micromixer could reduce the broadening prior to deposition. A potential source of postdeposition broadening could be that the DESI spray pushes the analytes laterally along the surface as the disk rotates. The high sensitivity of DESI offers the possibility to detect femtomole quantities of analytes[28] eluting from columns which could match the limits of detection of most capillary-based detectors, aside from fluorescence, for all but the lowest injection volumes.

CONCLUSIONS

This report describes a method to monitor electrokinetic separations with MS by depositing the column effluent on to a rotating disk for DESI analysis. This was demonstrated using mixtures of rhodamine dyes and pharmaceutical drugs. Monitoring separations such as MEKC with MS using DESI offers a simple and rapid analysis. The salt tolerance of DESI enables direct analysis of separations using buffers traditionally viewed as incompatible with ESI-MS due to ion suppression, instrument contamination, and/or adduct formation. The strategy presented here is suitable as a general strategy for interfacing capillary-based separations to MS using DESI. The decoupling of separation buffers from MS sampling solvents allows MS monitoring of standard separation methods regardless of the detector originally employed.

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