Anal. Chem. **2002,** *74,* 6216−6223

Thin-Layer Chromatography and Electrospray Mass Spectrometry Coupled Using a Surface Sampling Probe

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A combined surface sampling probe/electrospray emitter was used for the direct readout of thin-layer chromatography plates by electrospray mass spectrometry. The technique was demonstrated with reversed-phase C18 plates using a three-dye mixture composed of methylene blue, crystal violet, and rhodamine 6G for positive ion mode detection and a separate dye mixture containing fluorescein, naphthol blue black, and fast green FCF for negative ion mode detection. Acquisition of mass spectra of components of individual bands on the plate was shown by manual stepping to and sampling from specific locations within the bands. Computer-controlled scanning of development lanes on the plate was illustrated by using multiple ion monitoring in both positive and negative ion modes. Commercial TLC plates were used and no postseparation processing other than drying of the plates was required prior to mass spectrometric analysis. Readout resolution, the limits of scan speed, detection levels, TLC phase, and eluting solvents were investigated and discussed.

Thin-layer chromatography (TLC) is a largely mature, but very important general laboratory tool.[1,2] In comparison to modern column chromatography alternatives such as high-performance liquid chromatography, TLC is a relatively inexpensive, flexible, and portable method that requires minimal equipment and little method development time. Although not particularly mainstream, much TLC research continues in the areas of separation media or development methodologies as well as in the areas of detection, identification, and quantification methods.[3,4] In the latter three areas, the direct coupling of TLC and mass spectrometry (MS) and tandem mass spectrometry (MS/MS or MS^n) are of particular interest because of the low detection levels, molecular identification capabilities, and detection specificity the techniques provide.[5−10]

The literature is replete with examples of the use of mass spectrometry to analyze fractions separated on TLC plates by the tedious, time-consuming off-line method of "scrape and elute". There are also numerous examples over the last two decades of attempts to couple directly TLC and MS. In those attempts, most of the inlet/ionization systems directly amenable to surface analysis, such as secondary ion mass spectrometry, have been used with varying success.[5−10] Matrix-assisted laser desorption/ionization (MALDI) has had the most recent successes in TLC/MS with several notable advances coming from the laboratory of Hercules and co-workers.[11−15] Major obstacles or limitations in using MALDI-MS to couple TLC and MS have been the need for extensive postseparation preparation of the TLC plates prior to analysis, the need for specialized plates, the low-mass spectral noise from the MALDI matrix, and the requirement that the analysis be carried out in the vacuum chamber of the instrument.

Electrospray (ES)[16] provides an atmospheric pressure inlet/ionization system alternative for TLC/MS coupling that might not be subject to many of the limitations of MALDI-MS. The challenge with directly coupling TLC and ES-MS is to provide a means to sample a surface with this liquid solution introduction/ionization technique. Anderson and Busch[17] have described a microcapillary

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10.1021/ac020540+ CCC: $22.00
Published on Web 11/19/2002

extraction device to remove sample from TLC plates for subsequent analysis using ES-MS. Their method was not a direct TLC/ES-MS coupling, but rather a clever variation of the off-line method of "scrape and elute". However, two more recent reports provide a suggestion as to how on-line surface sampling for TLC/MS might be done with ES-MS.

Lev and co-workers[18] described a scanning capillary that could be used to sample spatially the products of electrode reactions above an electrode immersed in a liquid solution and to direct those products to an ES-MS system. Wachs and Henion[19] described an integrated capillary sampling probe/ES emitter system for sampling liquid sample solutions from wells of a multiwell plate. In both reports, the concept of a coaxial tube sampling probe was used, wherein the space between the outer and inner capillary tubes facing the sample was used to deliver solvent to the surface. The inner tube was used to draw liquid from the surface to the ES source. The results in the report of Wachs and Henion[19] included the reconstitution in a well of dried berberine and cytochrome *c* and the sampling of the resultant solution with the capillary sampling probe. They extrapolated from these results that their sampling probe/emitter system could be used to sample analytes from any variety of surfaces, including the surface of TLC plates.

Using the basic sampling/ES emitter probe design of Wachs and Henion,[19] we indeed demonstrate in this paper a directly coupled TLC/ES-MS system. The performance of the system is illustrated using commercially available reversed-phase (RP) C18 plates with a variety of dyes using positive or negative ion mode detection. No postseparation processing of the plates, other than drying, is required prior to analysis. Mass spectra are acquired by manual stepping to and sampling from individual spots on the plate. Computer-controlled scanning of development lanes on the plate is also demonstrated. Readout resolution and the limits of scan speed, detection levels, TLC phase, and eluting solvents are discussed.

EXPERIMENTAL SECTION

Samples and Reagents. A test dye mixture for use with positive ion mode ES-MS was prepared in methanol (J. T. Baker, Phillipsburg, NJ) to provide 850 ng/μL rhodamine 6G (Eastman Kodak, Rochester, NY), 620 ng/μL crystal violet (95%, Aldrich, Milwaukee, WI), and 540 ng/μL methylene blue (83%, Aldrich) (Figure 1). Solutions of these individual dyes were prepared in methanol at 830, 720, and 680 ng/μL, respectively, for spotting standards. Solutions of the individual dyes were also prepared in a 60:40 (v/v) mixture of methanol (Baker) and water (Milli-RO 12 Plus, Bedford, MA) containing ~1 vol % acetic acid (HOAc, PPB/Teflon grade, Aldrich) at a concentration of 10 μM for optimizing the ES-MS system. A commercial dye mixture designed for the testing of RP plates (Test Dye Mixture III, Analtech, Newark, DE) was used in negative ion mode. This methanolic solution contained nominally 750 ng/μL each of fast green FCF, fluorescein, and naphthol blue black and 1000 ng/μL rhodamine B (Figure 1). Aliquots of this mixture were separated on and the resultant bands isolated from glass-backed 5 × 20 cm RP-C18 plates with a preadsorbant zone (Whatman, Maidstone, U.K.) developing the plates with 60:40 (v/v) methanol/water containing 15 mM ammonium acetate (NH_4OAc, 99.999%, Aldrich). These isolates were used to prepare roughly 10 μM solutions of each component in 60:40 (v/v) methanol/water for optimizing ES-MS detection.

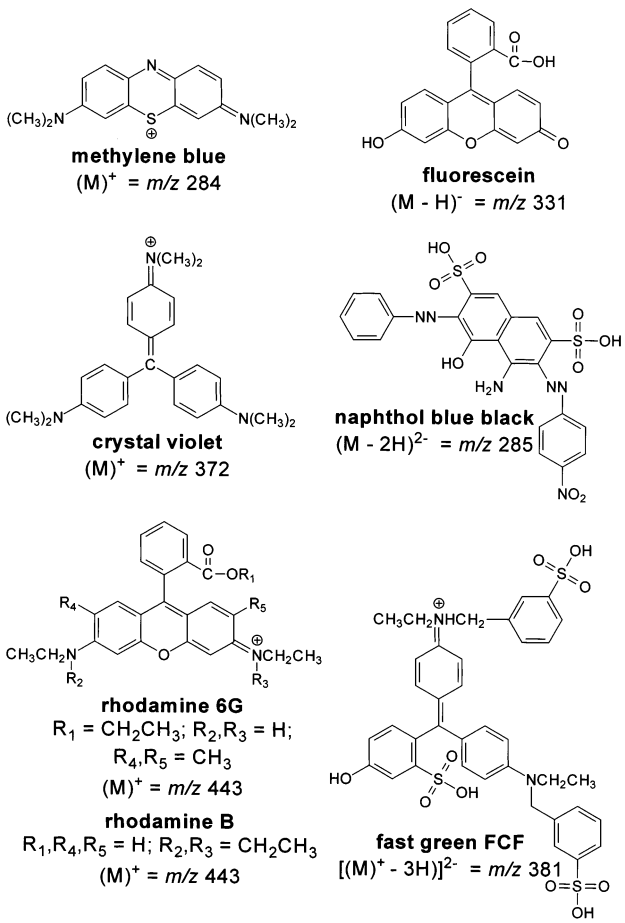

Figure 1. Dyes used in these experiments and the mass-to-charge ratio of the major ion from each that was observed or monitored.

TLC. The plates used for the TLC/ES-MS coupling were glass-backed 10 × 10 cm HPTLC RP-C18 plates (P/N EM-13724-5, EM Science, Gibbstown, NJ). The sorbant material on these plates is based on silica gel type 60, and the surface of this silica gel is chemically modified with an octadecylmethylsilane to the highest possible degree. This treatment makes the plates particularly hydrophobic relative to many other commercially available RP-C18 plates. Hydrophobicity of the plate is a critical parameter in the current sampling scheme (vide infra). The positive ion test mixture was separated by developing with 60:40 (v/v) methanol/tetrahydrofuran (Aldrich) containing 50−100 mM NH_4OAc. The negative ion test mixture was separated by developing with 70:30 (v/v) methanol/water. Following development, plates were air-dried for several hours or dried in an oven at 110 °C for 30 min before mass spectrometric analysis. Color photographs of the developed plates were taken with a Coolpix 990 digital camera (Nikon, Tokyo, Japan).

ES-MS. ES-MS experiments were performed on a PE Sciex API165 single quadrupole mass spectrometer (MDS Sciex, Concord, ON, Canada). Figure 2 shows a schematic illustration of

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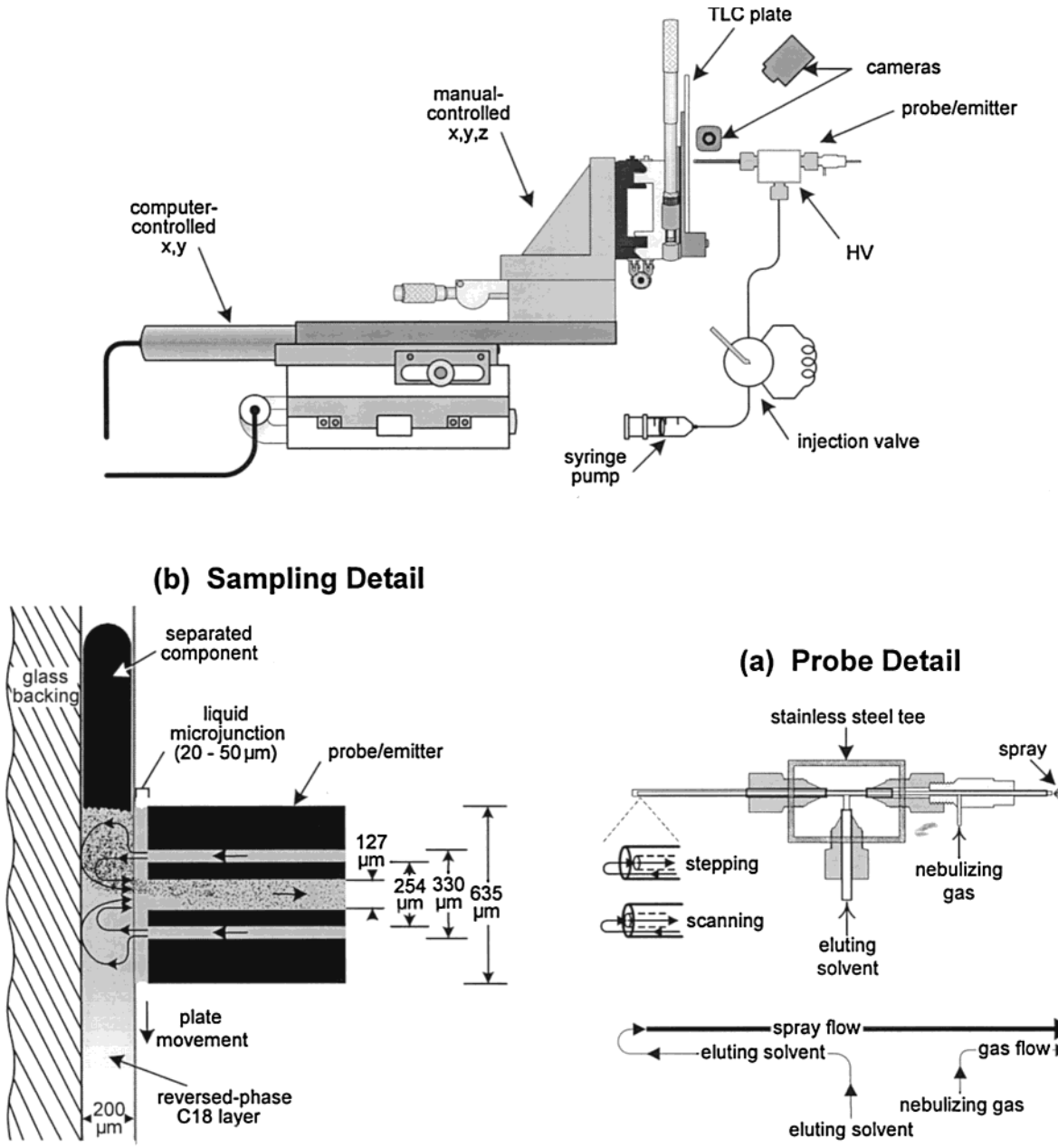

Figure 2. Schematic illustration showing the sampling probe and its components and the combined manual and computer-controlled manipulator stages used to position the TLC plate relative to the sampling probe. Inset a shows the detail of the sampling/emitter probe. Inset b shows the sampling probe at the surface of a TLC with the formation of a liquid microjunction.

the sampling probe/ES emitter and *x*, *y*, *z* manipulator system used to position the TLC surface relative to the sampling probe. The probe was modeled after that described by Wachs and Henion[19] with only slight variation (Figure 2, inset a). The main body of the probe system is a stainless steel tee (Alltech, Deerfield, IL) that accepts $^1/_{16}$-in.-o.d. tubing using $^1/_4$-28 fittings. The metal sampling/sprayer tube that extends the length of the device is a 10-cm-long, 31-gauge stainless steel tube (1.27-μL volume, Unimetics, Folsom, CA). This tube projects through the tee and is held in place from the spray side of the tee with a short piece of $^1/_{16}$-in.-o.d. Teflon tubing of the appropriate inside diameter and a modified Kel-F nut tapped to accept the threads of a nebulizer fitting from the Sciex TurboIonSpray source. Slipped over the spray end of the sampling tube was a concentric 23-gauge stainless steel tube that was held in place with a short piece of $^1/_{16}$-in.-o.d. Teflon tubing of the appropriate inside diameter and a Kel-F nut. This arrangement provided a path for a continuous flow of eluting solvent for the sampling end as well as adjustable pneumatic nebulization of the ES process at the spray end. The eluting solvent was pumped through the base of the tee at a flow rate of 10 μL/min using a syringe pump. The local vacuum created by pneumatic nebulization along with the electrostatic field created by applying the high ES voltage (±4.5−5.0 kV) to the tee aspirates the liquid from the surface end of the probe through the inner tube toward the mass spectrometer interface. The sampling end of the sampling/spray tube was positioned flush with the end of the 23-gauge tube for scanning the plate laterally relative to the stationary probe. When the plate was stepped to sample different positions on the plate, the inner tube was preferably positioned about 50−100 μm inside the outer tube. Sample to tune the

instrument was introduced through the probe system by using a variable-volume loop injector (Rheodyne model 7125, Cotati, CA) placed upstream.

The sampling probe/ES emitter assembly was mounted on a plexiglass extension from an *x*, *y*, *z* manipulator mounted on the frame of the mass spectrometer source housing. This allowed the probe to be positioned for optimum ES performance. A TLC plate was mounted vertically at 90° to the probe using an insulating L-shaped cleat mount and double-sided tape. This mount was fastened on the vertical stage of a manual *x*, *y*, *z* manipulator, which in turn was mounted on top of a computer-controlled *x*, *y* manipulator from a scanning electrochemical microscope (CH Instruments, Austin, TX). This arrangement allowed for both manual stepping of the surface relative to the probe to sample a particular spot on the plate or computer-controlled scanning of a development lane on the plate. Two black and white CCD cameras (Protana A/S, Odense, Denmark) focused on the sampling end of the probe from different angles facilitated positioning of the surface relative to the probe.

Safety Precautions. The sampler/sprayer floats at the high ES voltage and appropriate shields should be used to avoid accidental contact with this device or with the TLC plate being investigated. The holder mounting for the TLC plate should be isolated from ground potential and from the manipulator supporting it, which should be grounded to prevent shock.

RESULTS AND DISCUSSION

Figure 2 shows a schematic illustration of the sampling probe/ES emitter and manipulator system used to position the TLC surface relative to the sampling probe. Also included in the figure are insets showing the detail of the probe and the solvent flow paths (inset a) and detail of the sampling and elution of material from the TLC plate surface (inset b). The TLC plate to be examined was mounted to the vertical stage of the manual manipulator, and the plate was positioned at 90° relative to the sampling probe. The TLC plate rather than the sampling/emitter probe was moved to the appropriate position for sampling from the plate. Two basic modes of sampling were investigated, a manual "stepping sampling mode" and a computer-controlled "scanning sampling mode", each making use of a liquid microjunction between the probe and the TLC plate surface to sample material from the surface.

Stepping Sampling Mode. In the stepping sampling mode, the plate surface was made to physically contact the sampling probe to establish a liquid microjunction between the surface and probe at that particular point on the plate and to sample from that point. Immediately after the initial contact with the probe, the surface was pulled back slightly (<100 μm) to eliminate direct physical contact of the probe and surface. Tight physical contact between the probe and surface, when sampling, affected solution flow rates and often led to signal spikes when the surface was pulled back from the probe. The soluble components in the separation layer dissolved in the eluting solvent and were swept into the sampling capillary (Figure 2, inset b). The dissolved components were sprayed from the opposite end toward the mass spectrometer. When sampling at that position was complete, the surface was pulled back from the probe by usually several hundred micrometers, severing the liquid junction, and moved to a new lateral position and the process repeated. In this sampling mode, it was preferred to have the sampling end of the inner sampling/spray capillary positioned ~100 μm inside the outer capillary (Figure 2, inset a). This mitigated any tendency for the eluting solvent to spray out of the back of the capillary toward the TLC plate when the surface was positioned a short distance back from the probe during transition to a new sampling position.

The hydrophobicity of the separation layer on the RP plates used in this study was crucial for this sampling mode (as well as the scanning mode, vide infra). The very small gap between the probe and the surface (<100 μm) and the surface tension of the liquid, combined with the hydrophobic nature of the RP-C18 layer, confined the liquid junction and prevented the solvent from flowing beyond the width of the probe into the layer or out onto the layer surface. Normal-phase plates and wettable reversed-phase plates were not successfully sampled with this sampling approach. With those phases, a substantial fraction of the eluting solvent flowed radially from the probe out into the thin-layer phase rather than back into the sampling capillary. This effectively developed the material at the sampling spots out from the vicinity of the probe, making the sampling ineffective. Successful sampling, even with the hydrophobic RP-C18 plates, required a compromise in solvent system that balanced analyte solubility (needed for efficient extraction) and phase wettability. We found that a maximum of 60 vol % methanol (or acetonitrile, data not shown) in water could be used before plate wetting became an issue.

The data in Figure 3 are illustrative of the stepping sampling mode. The picture in panel a shows four lanes on the developed TLC plate. Spotted in lanes 1−3 were the dyes methylene blue, crystal violet, and rhodamine 6G, respectively. A mixture of the three dyes was spotted in lane 4. Visual comparison indicates that band 1 is methylene blue, band 2 is crystal violet, and band 3 is rhodamine 6G. The full-scan, background-subtracted mass spectra obtained while using the probe to sample from a location within each of the three bands confirmed this identification (Figure 3, panels b−d). In each of the three positive ion mass spectra shown, the base peak was that expected of the singly charged dye molecule (see Figure 1). In the spectra for both methylene blue and crystal violet, a substantial peak was observed at 14 m/z units less than the base peak (m/z 270 and 358, respectively). These peaks corresponded in mass to the N-demethylated analogues of the respective compounds. Tertiary amines undergo facile oxidative demethylation,[20] and this process might occur directly on the TLC plate or in the spray capillary of the probe by involvement in the inherent oxidative processes of positive ion mode ES.[21] The ES mass spectra of 10 μM solutions of the individual dyes each showed a peak for the demethylation product, but at a significantly lower abundance than that observed in the spectra in Figure 3.

The stepping mode of sampling has at least three major utilities. The first use is being able to select discrete regions on the plate to interrogate with a spatial resolution approximately equivalent to the sampling probe outer diameter (~635 μm). The white spots that are visible in each band in lane 4 (indicated with arrows) are due to the sampling process and are each approximately the same diameter as the probe. Efficient extraction of the dyes from the plate with the probe reveals the white RP-

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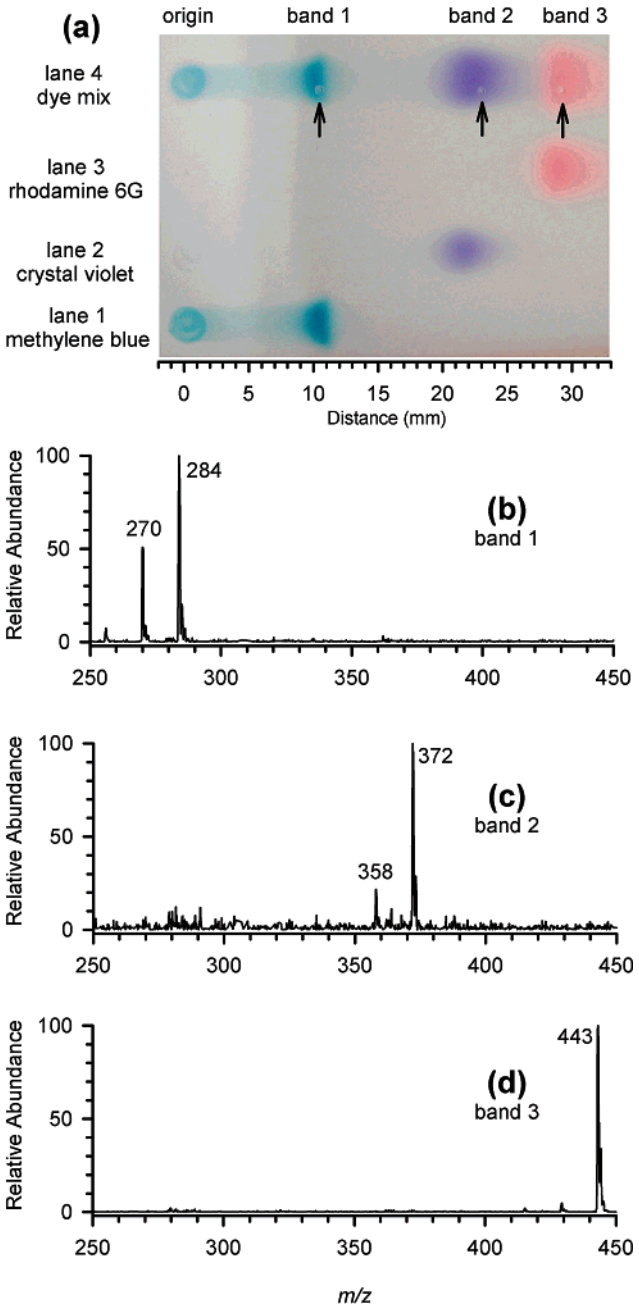

Figure 3. (a) Picture of four lanes on TLC plate showing complete development of three individual dyes spotted (2 μL each), viz., 1.13 μg of methylene blue (lane 1), 1.37 μg of crystal violet (lane 2), and 1.66 μg of rhodamine 6G (lane 3), as well as a mixture (2 μL) of these same three dyes (1.07, 1.25, and 1.70 μg, respectively, lane 4). Arrows indicate sampling position in each band in lane 4. Background-subtracted ES mass spectra acquired in positive ion mode in lane 4 from (b) band 1 (methylene blue), (c) band 2 (crystal violet), and (d) band 3 (rhodamine 6G) are shown. The background spectrum was acquired by sampling a region between bands 1 and 2 in lane 4. Sampling time at each band was 60 s. Scan range *m*/*z* 250−450, dwell time 1.0 ms, and 0.1 *m*/*z* step size.

C18 phase of the plate. A comparison of the band size to the size of the sampling spot shows that at most only 10% of any one of the sample bands in Figure 3 (e.g., band 1) was extracted from the plate and sprayed. Thus, the second utility is relatively low detection levels that must be ~100 ng spotted or less.

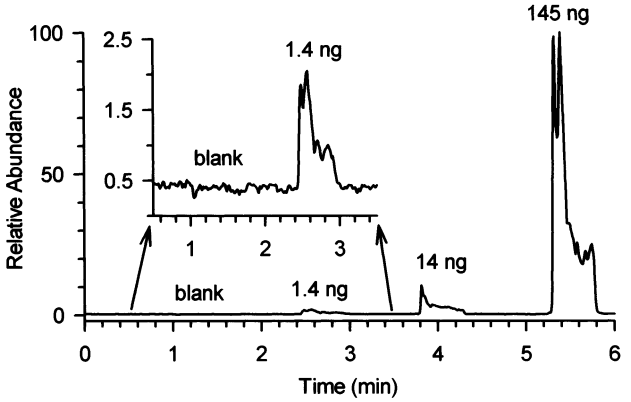

Figure 4. Single ion monitoring mass chromatogram (*m*/*z* 443, dwell time 500 ms) obtained in positive ion mode during the sequential step sampling (30-s sampling time) of four separate bands from developed spots (0.4 μL each) of differing amounts of rhodamine 6G, viz., 0 (blank solvent), 1.4, 14, and 145 ng.

For more accurate evaluation of detection levels in the stepping mode, an experiment was performed in the single ion monitoring mode using rhodamine 6G. Stock solutions of different concentrations of this dye were prepared, and 0.4 μL of each was spotted in different lanes and the plate developed. Each spot was then sampled for 30 s from lowest to highest concentration. Figure 4 is the single ion monitoring mass chromatogram for *m*/*z* 443 obtained during the sequential step sampling of each developed band. The insert in the figure shows the signal from the 1.4-ng sample clearly visible, indicating detection levels near the 1-ng level spotted. This level was achieved because the diameter of this band was comparable to the diameter of the probe. Above ~14 ng spotted, the band size became significantly larger in diameter than the probe. As such, one would not expect a linear increase in signal with amount spotted once spot diameter is larger than the probe diameter.

The detection level data in Figure 4 also demonstrate the third utility of the stepping sampling mode, viz., prolonged signal that is useful when full scan mass spectra are obtained. These data show a spike in the signal level immediately after the probe starts to sample the surface. Following the signal spike, the signal rapidly drops to a plateau level that lasts until the surface is pulled back from the probe. This behavior was due to the fact that the extraction of most of the material from the plate surface happened very rapidly. However, the prolonged signal plateau allows additional time for acquisition of the more time-consuming full-scan mass spectra.

Scanning Sampling Mode. In the scanning sampling mode, the plate surface was made to physically contact the sampling probe to establish a liquid microjunction between the surface and probe. Then the surface was pulled back to form a liquid junction approximately 20−50 μm wide. A liquid junction any wider had a tendency to either trail the probe or actually break off, severing contact with the surface, as the scan took place. Just as in the case of stepping sampling, the soluble components in the separation layer were dissolved in the eluting solvent, swept into the sampling capillary, and sprayed (Figure 2, inset b). In this case, the surface was continually moving relative to the probe. It was preferred in this sampling mode to have the sampling end of the inner sampling/spray capillary positioned flush with the outer

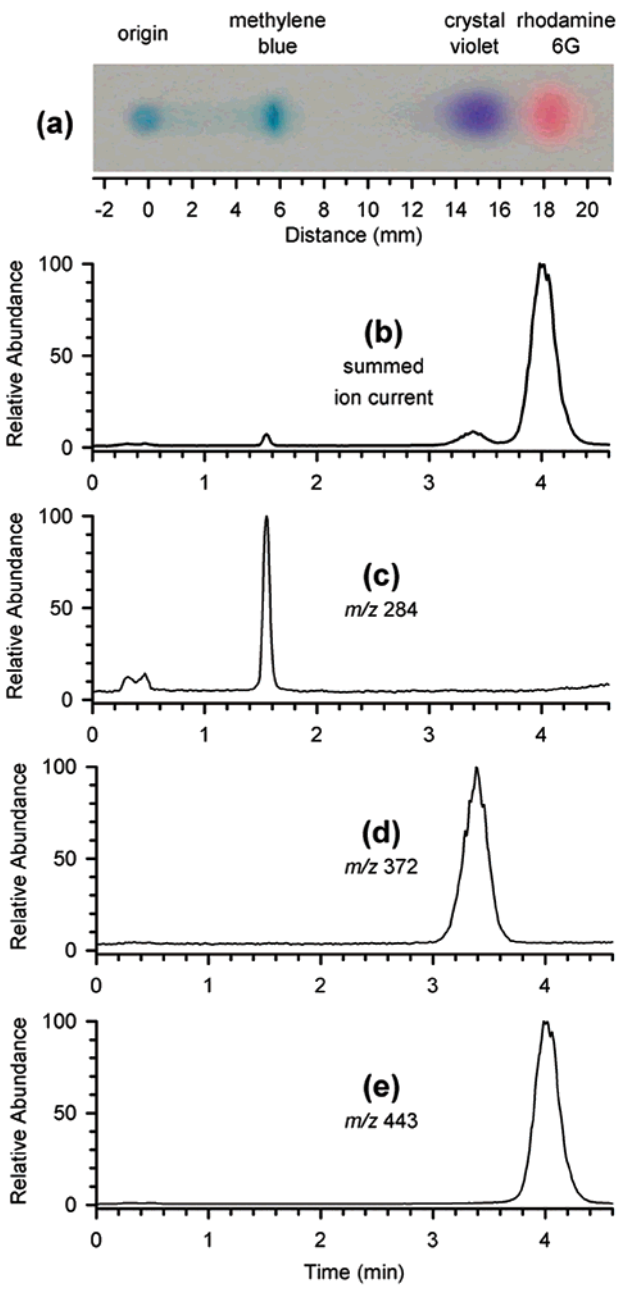

Figure 5. (a) Picture of complete development lane showing the separated components of a spotted mixture (0.4 μL) of 215 ng of methylene blue, 250 ng of crystal violet, and 340 ng of rhodamine 6G. The positive ion mode ion current chromatograms obtained while scanning the probe across the lane are (b) the summed ion current from the single ion monitoring mass chromatograms for each of the dyes and the single ion monitoring mass chromatograms for (c) *m*/*z* 284 (methylene blue), (d) 372 (crystal violet), and (e) 443 (rhodamine 6G). TLC plate was scanned relative to the probe along the development lane at 90 μm/s. Single ion monitoring dwell time, 150 ms for each *m*/*z*.

capillary of the probe/emitter (Figure 2, inset a). This made it easier to establish and maintain a relatively wide yet stable liquid microjunction between the probe and surface. The wider junction relaxed the precise prescan orthogonal positioning of the probe and surface over the distance to be scanned. If the width of the liquid junction tended toward being too narrow (<20 μm) or too wide (>100 μm) during a scan, it was easily adjusted in real time to maintain optimum sampling.

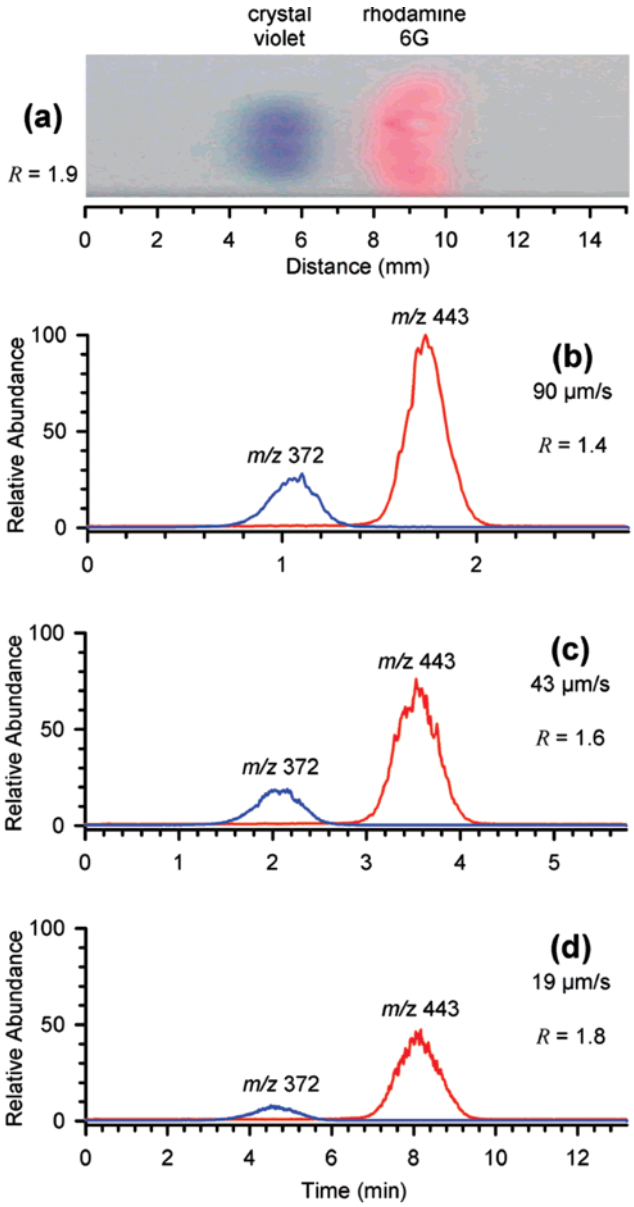

Figure 6. (a) Picture of a section of a development lane showing the separated components of a spotted mixture (0.4 μL) of 250 ng of crystal violet and 340 ng of rhodamine 6G following mass spectrometric analysis. The overlaid single ion monitoring chromatograms obtained in positive ion mode for *m*/*z* 372 (blue curve, crystal violet) and 443 (red curve, rhodamine 6G) obtained while scanning the plate surface relative to the probe along the development lane at (a) 90, (b) 43, and (c) 19 μm/s are shown. Signal abundances were normalized relative to the signal level for *m*/*z* 443 in panel b. Calculated chromatographic resolution, *R* (eq 1), is shown in each panel. Single ion monitoring dwell time, 150 ms for each *m*/*z*.

The mass spectral data in Figure 5 were acquired by scanning along the center line of a complete development lane shown in the picture in panel a from below the original spotted sample up to the solvent front. The same dye mixture used to acquire the data in Figure 3 was spotted on the plate. The summed ion current chromatogram and the single ion monitoring chromatograms for *m*/*z* 284 (methylene blue), 372 (crystal violet), and 443 (rhodamine 6G) are shown. There was good correspondence between the location of the bands in the picture of the development lane and the location of the bands as determined from the mass chromato-

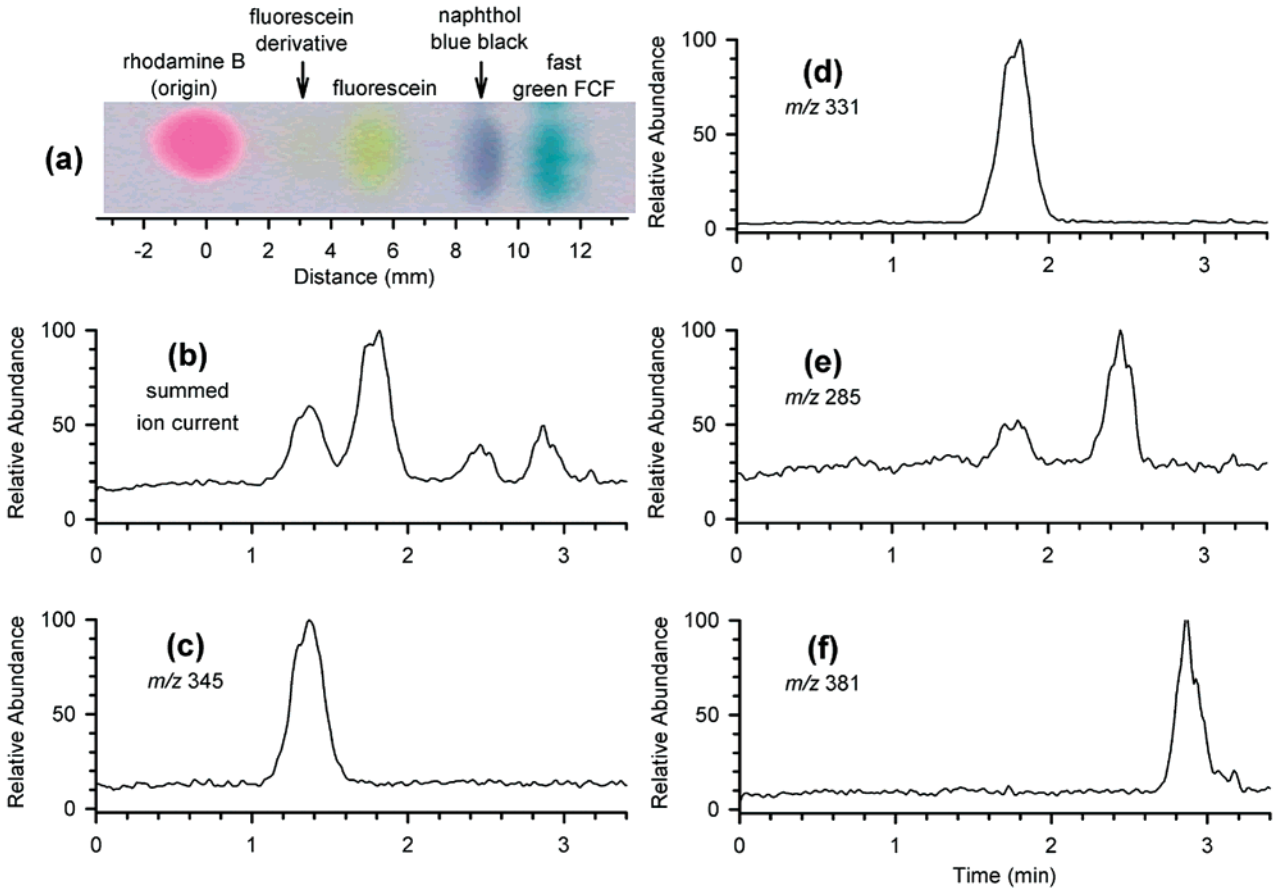

Figure 7. (a) Picture of complete development lane taken with simultaneous exposure to visible and UV light showing the separated components from a spotted mixture (0.4 μL) of 400 ng of rhodamine B, 300 ng of fluorescein, 300 ng of naphthol blue black, and 300 ng of fast green FCF. A suspected derivative of fluorescein was also present in an unknown amount. The negative ion mode ion current chromatograms obtained while scanning the probe across the lane are (b) the summed ion current from the single ion monitoring mass chromatograms for each of the dyes, and the single ion monitoring mass chromatograms for (c) *m*/*z* 345 (unknown fluorescein derivative), (d) 331 (fluorescein), (e) 285 (naphthol blue black), and (f) 381 (fast green FCF). TLC plate was scanned relative to the probe along the development lane at 90 μm/s. Single ion monitoring dwell time, 200 ms for each *m*/*z*.

grams. The mass spectral data also revealed that some of the methylene blue remained at the origin. This observation was consistent with the chromatographic data shown in panel a of Figure 3. Although nearly the same amount of each dye was spotted, the mass spectral signals were very different, which was not unexpected as each dye has an inherently different ES response, a different bandwidth, and probably a different extraction efficiency. The data in Figure 5 further indicate that chromatographic resolution is well preserved during the readout. For example, the well-separated bands of crystal violet and rhodamine 6G (panel a) are baseline resolved in the mass chromatograms (panels d and e).

The data shown in Figure 6 were acquired to investigate more quantitatively the effect of scan speed on readout resolution in the scanning mode and on signal levels. The same three-dye mixture as used previously was separated, and the region of the chromatogram containing crystal violet and rhodamine 6G (Figure 6, panel a) was scanned at three different scan rates. For each different scan, the probe was placed to travel through the bands at a slightly different horizontal position. The mass chromatograms in panels b−d are plotted as a function of time, and this time corresponds in each case to that time required to scan the full 1.5 cm of the development lane at a scan rate of 90, 43, and 19 μm/s, respectively. The more intense signal achieved at the fastest scan rate was immediately apparent in these data. This behavior resulted from the very rapid extraction of most of the material from the plate surface coupled with the rapid sampling of material from new locations at the higher scan rate. This interpretation is supported by the data in Figure 3 that show a spike in the signal level in the step sampling mode immediately after the probe starts to sample the surface. Following this spike, the signal rapidly dropped to a plateau because most of the material that was easy to extract had been removed from the spot. At the lowest scan rate, the maximum signal was diminished by as much as 50%. However, the time window over which the signal maximum was observed was much wider. This was advantageous when full-scan mass spectra in the scanning sampling mode were acquired (data not shown).

The fast scanning rate was also found to offset systematically the location of the bands in the picture and the location of the bands in the mass spectral data by about a probe width. The time for solution to flow from the TLC plate surface through the probe and to be sprayed was ~7.6 s at 10 μL/min. At 90 μm/s, the probe position relative to the plate surface shifted laterally almost 690 μm, or just over 1 probe diameter (635 μm). At 19 μm/s, the shift was only 140 μm. This systematic spectral "position skewing" was

also accompanied by a degradation in chromatographic readout resolution. Resolution (R) of two chromatographic bands in TLC has been defined as the distance between the band centers (d) divided by the average of the widths (W_1 and W_2) of the bands (eq 1).[2] The resolution of the bands in the picture in Figure 6

$$R = d/[(W_1 + W_2)/2] \quad (1)$$

was calculated as 1.9. The resolution calculated from the mass spectral data obtained at a scan rate of 90 μm/s was 1.4 (panel a). The resolution improved to 1.6 at 43 μm/s (panel b) and to 1.8 at 19 μm/s (panel c). However, there was a significant tradeoff between optimal readout resolution and the time of analysis. The ~21% gain in readout resolution obtained by slowing the scan rate from 90 to 19 μm/s was obtained at the expense of a 460% increase in analysis time. Resolution would be further degraded at any scan speed if full-scan mass spectra were acquired with a low duty cycle instrument such as the quadrupole mass analyzer used in this study. However, this added degradation would be largely avoided by using a high duty cycle ion trap or time-of-flight mass analyzer. Unless the mass spectral data are needed for precise imaging of the development lane or if differentiation of separated isomers or other isobaric compounds is required, the need for optimum readout resolution can probably in many cases be compensated for by the mass-selective nature of the detection.

The data in Figure 7 demonstrate that the TLC plates can also be sampled and analyzed for species that form negative ions. For this experiment, a commercial dye mixture composed of rhodamine B, naphthol blue black, fluorescein, and fast green FCF was used (Figure 1). A prior interrogation of the isolated components of the mixture (see Experimental Section) showed that rhodamine B gave no signal in the negative ion mode and that a fifth component was present in the mixture that had a molecular mass of 346 Da (observed at m/z 345). This component is most likely a methoxy or methyl ester derivative of fluorescein. As the data in Figure 7 show, the chromatographic integrity of the separation was well maintained by the mass spectral readout.

CONCLUSIONS

The results presented here have demonstrated that a surface sampling/ES emitter system can be used to read out commercial unmodified TLC plates with positive and negative ion mode ES-MS. Detection levels for developed plates were shown to be in the low-nanogram range spotted. Sampling of the plate surface was accomplished via a manual spot selection "stepping sampling mode" or via a computer-controlled "scanning sampling mode" of a development lane. Complete "hands-off" automation of both modes would be expected to be possible with integration of a spot or lane locator system and guide or feedback mechanism that would precisely position the surface and probe for sampling. Among other commercial instrumentation, for example, many of these same features are already incorporated into robotic gel spot cutters used in the field of protein analysis.[22]

Spatial sampling resolution in the stepping mode was essentially the width of the sampling probe. Chromatographic resolution was maintained within 26% when scanning at 90 μm/s and was improved to within 5% when scan speed was slowed to 19 μm/s. It should be possible to improve readout resolution at fast scan rates by decreasing the volume of sampling/spray capillary though use of a smaller diameter or shorter capillary. These same changes might also improve detection levels by limiting dilution of the components dissolved from the TLC plate. Higher flow rates might be used to improve readout resolution, but at the expense of higher sample dilution and the associated lower signal levels. In any case, better resolution readout may not be needed for most types of TLC. To improve detection, other probe designs might be investigated that actually sample more of a separated band. Most developed bands are not round, but elongated ovals. Thus, a probe that samples a larger fraction of the band width, while maintaining a narrow sampling width along the direction of the development lane, might be of advantage.

With the current sampling scheme, we were limited to the use of hydrophobic RP-C18 TLC plates. This is not as limiting as it might seem, because many of the types of analytes best separated using RP plates; viz., polar and ionic analytes, including biomolecules, are ideal for ES-MS detection. Nevertheless, the ability to sample from TLC plates with other, wettable, phases would be highly desirable. A surface contact probe that isolates that portion of the surface to be sampled or a probe that samples the eluting solvent from the edges of the probe might solve the wettability issue.

(22) Information on the features and capabilities of one commercial gel spot cutter can be downloaded from the Internet at http://www.bio-rad.com.

ACKNOWLEDGMENT

A.D.S. acknowledges an appointment to the Energy Research Undergraduate Laboratory Fellowships sponsored by the U.S. Department of Energy Office of Science and administered by the Oak Ridge Institute for Science and Education for Oak Ridge National Laboratory (ORNL). Dr. Timothy Wachs (Cornell University) is thanked for helpful discussions regarding the construction and operation of the sampling/emitter probe. Dr. Heinz E. Hauck (Merck, Darmstadt, Germany) and Dr. Fred Rabel (EM Science, Gibbstown, NJ) are acknowledged for helpful discussions regarding the TLC plates used in this work. Dr. Peixin He (CH Instruments, Inc., Austin, TX) is thanked for software modifications to the SECM system that facilitated its use in these experiments. Becky R. Maggard (ORNL) is thanked for creation of Figure 2. The API165 mass spectrometer was provided through a Cooperative Research and Development Agreement with Sciex (CRADA No. ORNL96-0458). This research was sponsored by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC, for the U.S. DOE under Contract DE-AC05-00OR22725.

Received for review August 20, 2002. Accepted September 26, 2002.

AC020540+