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Hydrophobic Treatment Enabling Analysis of Wettable Surfaces Using a Liquid Microjunction Surface Sampling Probe/Electrospray Ionization-Mass Spectrometry System

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An aerosol application procedure involving one or more commercially available silicone-based products was developed to create hydrophobic surfaces that enable analysis of otherwise wettable, absorbent surfaces using a liquid microjunction surface sampling probe/electrospray ionization mass spectrometry system. The treatment process resulted in a hydrophobic surface that enabled formation of the requisite probe-to-surface liquid microjunction for sampling and allowed efficient extraction of the analytes from the surface, but did not contribute significant chemical background in the mass spectra. The utility of this treatment process was demonstrated with the treatment of wettable high-performance thin layer chromatography plates, post-plate development, and their subsequent analysis with the sampling probe. The surface treatment process for different surface types was described and explained and the effectiveness of the treatment and subsequent analysis was illustrated using alkaloids from goldenseal (*Hydrastis canadensis*) root separated on a normal phase silica gel 60 F_{254S} plate and peptides from protein tryptic digests separated on a ProteoChrom HPTLC Silica gel 60 F_{254S} plate and a ProteoChrom HPTLC Cellulose sheet. This simple surface treatment process significantly expands the analytical surfaces that can be analyzed with the liquid microjunction surface sampling probe, and therefore, also expands the analytical utility of this liquid extraction based surface sampling approach.

Sampling and ionizing materials present on surfaces under ambient conditions is an expanding area of research and application in mass spectrometry (MS).[1−7] Direct liquid extraction based surface sampling probes are one way to perform atmospheric pressure surface sampling and ionization.[1] One particular probe of this type, a continuous flow liquid microjunction surface sampling probe (LMJ-SSP),[8−18] reconstitutes or extracts an analyte from a surface by means of a wall-less liquid microjunction between the sampling end of the probe and the surface. The liquid extraction solvent is brought to the surface through the annular space between two coaxial tubes at the sampling end of the probe and is then carried on through the inner tube to the ionization source through a self-aspirating electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) emitter. This LMJ-SSP approach to surface sampling/ionization can be applied to all species that can be dissolved and conducted into the probe and subsequently ionized by the respective ionization method being used.

The LMJ-SSP has two general modes of operation, viz., a discrete spot sampling mode and a scanning (or imaging) mode,

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each of which can be used either manually or as an automated procedure.[15,19] The discrete sampling mode allows the operator to sample selected single spots from a surface for analysis by forming a liquid microjunction separately at each of those points. The imaging mode allows continuous sampling of material from a surface by maintaining a liquid microjunction with the surface as the sample is moved in both *x* and *y* coordinates relative to the stationary probe. Analytical applications of this continuous flow LMJ-SSP in these two different modes of operation have involved the sampling and analysis of dried drugs or proteins or solutions thereof from wells on microtiter plates,[8] drugs captured in solid-phase extraction cards,[10] a variety of dyes, inks, or pharmaceuticals on paper or separated on hydrophobic reversed-phase (C8 and C18) thin-layer chromatography plates,[11−14] exogenous compounds from thin tissue sections,[17] and surface-deposited and affinity-captured proteins.[16]

The requirement to form a liquid microjunction with the surface can limit the effectiveness of the coaxial tube LMJ-SSP when sampling from particular types of wettable, absorbent surfaces. This is particularly problematic in the scanning mode which requires a sustained, well controlled, liquid microjunction for an extended period of time (often many min). An important example of this limit is development lane scanning during the analysis of wettable and absorbant high-performance thin layer chromatography (HPTLC) plates. Not only can the solvent be lost into these surfaces but also delivery of the solvent at the point of sampling tends to develop analytes out from the vicinity of the probe before they can be sampled. As such, the application of the LMJ-SSP for the analysis of HPTLC plates has been limited to date to hydrophobic RP C8 and C18 plates.[11−14,18] Herein, we describe a simple, inexpensive surface treatment method, implemented post-plate development, that enables the LMJ-SSP to effectively sample from previously wettable HPTLC phases. Proper aerosol application of one or more silicone-based products to the developed plates is performed to create a hydrophobic surface that enables liquid microjunction formation, allows efficient extraction of the analytes from the plate, and does not contribute significant chemical background in the mass spectra. The surface treatment process is described and explained for different plate phases and the effectiveness of the treatment is illustrated by the LMJ-SSP/ESI-MS analysis of alkaloids from goldenseal (*Hydrastis canadensis*) root on a normal phase silica gel 60 F_{254S} plate and peptides from protein tryptic digests separated on a ProteoChrom HPTLC Silica gel 60 F_{254S} plate and a ProteoChrom HPTLC Cellulose sheet.

EXPERIMENTAL SECTION

Materials and Reagents. Preparation of the standards and TLC plate development involving berberine chloride (Sigma Aldrich, St. Louis, MO) and goldenseal root (Botanical Liasions, Boulder, CO) used HPLC grade ethyl acetete and methanol from Fisher Scientific (Fair Lawn, NJ), 99%+ formic acid from Acros (Morris Planes, NJ), and HPLC grade water from Spectrum (Gardena, CA). HPTLC glass 20 × 10 cm Silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany) were used for the separation of these samples. Ammonium bicarbonate, trypsin, LiChrosolv methanol, and HPLC-grade water (Merck KGaA) were used for tryptic digest TLC. LC-MS grade Chromosolv solvents acetonitrile and water both with 0.1% formic acid (v/v) were obtained from Sigma Aldrich for use with the LMJ-SSP/ESI-MS analyses. Proteins bovine cytochrome *c*, equine myoglobin, β-casein from bovine milk, bovine serum albumin, and lysozyme from chicken egg white were obtained from Sigma Aldrich (Fluka, Buchs, Switzerland). ProteoChrom HPTLC Cellulose sheets and ProteoChrom HPTLC Silica gel 60 F_{254S} plates used for separations of the tryptically digested proteins were acquired from Merck KGaA. Trio Magic Carfa Silicone Oil aerosol spray was purchased from CAMAG (Muttenz, Switzerland). KIWI Camp Dry Heavy Duty Water Repellent spray was purchased over the counter locally.

Tryptic Digest. Tryptic digestion of model proteins was performed using ProteoExtract All-in-One Trypsin Digestion Kit (Merck KGaA). The protocol for digestion of proteins in solution was followed with an initial protein concentration of 2 μg μL^{-1} in the first step. The concentration of tryptic peptides recovered from the digestion was not determined using a BCA or UV/vis spectroscopy analysis. Trypsin was added to the protein buffer mixture such that the trypsin:protein ratio was 1:100. The mixture was incubated for 15 h at 37 °C.

Thin-Layer Chromatography. *Berberine and Goldenseal.* A 1.0 mg mL^{-1} berberine standard was prepared in methanol. This solution was serially diluted in methanol to make standards of 0.1−0.001 mg mL^{-1}. A 1.0 μL aliquot of each of these respective solutions was applied as a different 6 mm band on a HPTLC Silica Gel 60 F_{254S} plate and developed in ethyl acetate, water, and formic acid (80/10/10 v/v/v). To prepare a goldenseal root standard solution, 0.25 g of powdered sample was added to 5 mL of a methanol/water (80:20 v/v) solution and sonicated for 30 min. The solution was filtered twice with filter paper each time washing the filter with 2 mL of methanol. The filtrate and washings were combined and brought to a final volume of 20 mL with methanol. These standard solutions were applied in volumes from 0.5 to 10 μL to a HPTLC glass 20 × 10 cm Silica gel 60 F_{254S} plate as 8 mm long bands using a ATS 4 fully automated sample applicator (CAMAG, Muttenz, Switzerland). The samples were applied 20 mm from the bottom of the HPTLC plate and the distance between bands was 11.4 mm. Plates were developed vertically in a twin trough chamber with solvent-saturated atmosphere using ethyl acetate, water, and formic acid (80/10/10 v/v/v). The plates were air-dried and images acquired with a TLC visualizer (CAMAG). The plates were then treated by spraying a coating of Carfa Magic Silicone oil onto the surface after which the plate was left to dry in the vertical position for 12 h prior to mass spectrometric analysis (drying time can be reduced by drying a treated plate in a fume hood as is noted below).

Tryptic Digests. The tryptic protein digest separation was performed on ProteoChrom HPTLC Cellulose sheets or ProteoChrom HPTLC Silica gel 60 F_{254S} plates. Sample application was done using either an ATS 4 fully automated sample applicator or a Linomat V semiautomated sample applicator (CAMAG). A total sample volume of 7 μL was applied as 6 mm bands at a dosage speed of 50 nL s^{-1} equivalent to about 14 μg of the original protein per band. The samples were applied 10 mm

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from the bottom of the HPTLC plate and the distance between bands was 15 mm. The development of the HPTLC plates was carried out in a normal flat-bottomed chamber using 2-butanol/pyridine/ammonia/water (39:34:10:26, v/v/v/v) for the ProteoChrom Silica gel 60 F_{254S} plates and 2-butanol/pyridine/acetic acid/water (30:20:6:24, v/v/v/v) for the ProteoChrom HPTLC Cellulose sheets. The migration distance on the ProteoChrom Silica gel 60 F_{254S} plate and the ProteoChrom HPTLC Cellulose sheet used for the analysis was 50 mm achieved in 45−60 min.

Hydrophobic coating procedures for the silica gel plates and cellulose plates differed. In both cases, the plates were placed in a fume hood, laying flat, and spray coated with the Trio Magic Carfa Silicone Oil. Plates were coated evenly until the silicone layer visibly oversaturated the stationary phase. A Kimwipe tissue was hand-pressed down onto the HPTLC plate using a glass plate for 5 s to remove the excess silicone. This blotting procedure was then performed again using a new Kimwipe. At this point, Silica gel 60 HPTLC plates were allowed to dry in open air for 7−10 h. In the case of the HPTLC cellulose plates, after the second Kimwipe blotting, the plates were sprayed for 2 s with a light coat of KIWI Camp Dry Heavy Duty Water Repellent. Immediately following this application, excess silicone resting on top of the cellulose stationary phase was removed using the double blotting procedure described above. The cellulose plates were allowed to dry in a fume hood for 7−10 h after coating.

LMJ-SSP/ESI-MS. An LTQ XL linear ion trap mass spectrometer (Thermo Electron, San Jose, CA) with Xcalibur software version 2.0 was used in this work. The particular LMJ-SSP probe system used was exactly the same as that which has been described previously[8,11] with only the mounting system modified to accommodate the particular mass spectrometer. The self-aspirating LMJ-SSP probe was built using a stainless steel tee, a 10 cm long inner sampling/emitter capillary with a 254 μm o.d. and a 127 μm i.d., an outer tube on the sampling end with 635 μm o.d. and 327 μm i.d., and a nebulizer tube on the spray side. Photographs of the current setup can be found in the Supporting Information (Figure S1). The nebulizing gas flow was used to adjust the probe aspiration rate to be in balance with the pumped flow of eluting/spray solvent (10 μL min^{-1} using a 1 mL syringe attached to a syringe pump) into the probe. An approximately 27 cm long section of PEEK tubing (127 μm inner diameter and 1/16 in. outer diameter) with an upstream ground point was used to supply the elution solvent to the probe/emitter. The ESI voltage applied to the probe and the capillary and tube lens voltages were independently optimized for the goldenseal alkaloids and peptides by infusing micromolar level standards of each through the probe.

An MS2000 robotic *x*, *y*, *z* platform (Applied Scientific Instrumentation, Inc., Eugene, OR) was used to hold and maneuver the TLC plates in a perpendicular position relative to the stationary LMJ-SSP for analysis. As described elsewhere,[11] the original microscope slide holder supplied with the stage was replaced with a home-built TLC plate holder made from rigid, nonconductive polymer. The MS2000 platform could be controlled manually by use of a joystick in the *x*- and *y*-directions and by use of a jog wheel for *z*-direction control for initial alignment and LMJ formation. A camera used to observe the liquid microjunction during operation was equipped with an Optem 70 XL zoom lens (Thales Optem Inc., Fairport, NY). All TLC plate lane scans were enabled by using HandsFree TLC/MS, software written in-house to control the ASI 2000 stage. Before a lane was scanned, a LMJ was created at a position along the development lane below the spotting point by manual adjustment of the jog wheel and joystick via the ASI 2000 control system. After the LMJ was made, the mass spectrometer data acquisition process was initiated simultaneous with the beginning of the lane scan (100 μm s^{-1}). When the scan and data collection processes were finished, the LMJ was broken by moving the stage away from the probe in the *z*-direction.

Full scan mass spectra were acquired with surface scans of all the goldenseal-related plates. During surface scans of the tryptic digest separations lanes, automatic gain control was used with MS/MS product ion mass spectra acquired in data dependent mode with the three most abundant peaks within each full scan mass spectrum subjected to dissociation. The normalized collision energy was set to 35% and three microscans were acquired for each spectrum over a product ion range of 200−2000 Da. Dynamic exclusion was set to three so MS/MS would be performed on a certain m/z peak a maximum of three times. To identify the peptides observed, the MS/MS spectra were extracted from raw data files and converted to MS2 file format.[20] The MS2 files were searched using the DBDigger[21] proteomics database search program which used the MASPIC[22] scoring scheme and the DTASelect[23] algorithm for filtering the MS2 files. The DTA Select algorithm used a ΔCN of at least 0.08 and cross correlation (X_{corr}) scores of 20 (+1), 25 (+2), and 40 (+3).

RESULTS AND DISCUSSION

Surface Treatment and Analysis Concept. To expand the use of the LMJ-SSP probe to include analysis of wettable or absorbent surfaces, such as normal-phase HPTLC plates, simple, inexpensive surface treatment methods were explored. We found that proper aerosol application and curing of one or more silicone-based products could create a hydrophobic surface that enabled liquid microjunction formation, allowed extraction of the analyte, but did not contribute significant chemical background in the mass spectra. It is worth noting that the modification of planar chromatographic surfaces with nonpolar, hydrophobic materials like paraffin and silicone oil has a rich history as a means to create hydrophobic reversed-phase TLC plates.[24] These procedures were developed before the wide availability of bonded phases.[25] Although the focus of the results and discussion here is on

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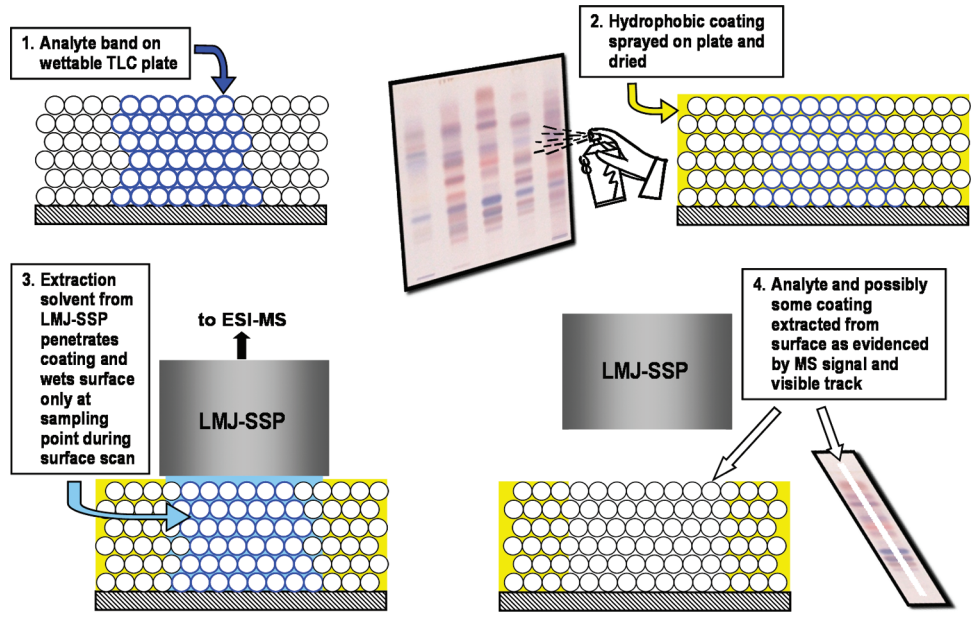

Figure 1. Surface treatment process developed and the subsequent surface sampling procedure schematically illustrated for a wettable HPTLC plate.

HPTLC, these same treatment and analysis methods were successfully applied to other types of wettable or absorbent surface like frosted glass slides, kimwipes, and dried blood spot paper (Figures S2 and S3 in the Supporting Information).

The surface treatment process developed and the subsequent surface sampling procedure used here are illustrated schematically in Figure 1. In this case, shown for a developed HPTLC plate, the analyte of interest was dispersed in a band on the surface of the stationary phase particles. By aerosol application, the complete phase, including the analyte, was impregnated with a hydrophobic silicone material. Depending on the type of surface, one or more silicone products were applied in proper order and amount to achieve the desired surface hydrophobicity and analyte extractability (see Experimental Section). During the analysis, the extraction solvent from the LMJ-SSP penetrated the hydrophobic coating, wetted the stationary phase at the sampling point, and dissolved the analyte that was then aspirated into the probe, electrosprayed, and detected in the mass spectrometer.

After sampling of the treated surfaces, whether in spot sampling or scanning mode, the sampled region had a different visual appearance than the rest of the plate. In offline studies it was found that neither silicone product showed significant solubility in methanol, acetonitrile, or methanol/water or acetonitrile/water mixtures. Rather, the silicone material was immiscible with these solvents being dispersed therein as microdroplets or forming a colloidal- or emulsion-like phase. Thus, during the LMJ-SSP analysis of the surface it is possible that some immiscible silicone material may be removed in the extraction solvent as microdroplets or pushed out from the immediate vicinity of the probe by the extraction solvent. It is also possible that the solvent simply penetrates into the silicone layer, possibly by a swelling mechanism known to occur from the interaction of organic solvents and silicone polymer networks.[26] In any case, no significant mass spectral signal was observed when sampling from an appropriately cured treated surface. Silicone polymer ion signals were sometimes observed if the curing procedures were not followed (see Figure S4 in the Supporting Information). Large polymer chains without sufficient charging if present would be outside the m/z range of the mass analyzer. However, the successful analyses illustrated by the data presented below indicate that little if any signal suppression from the coating took place.

Goldenseal Alkaloids on Silica Gel 60 HPTLC Plates. Authentic goldenseal extracts contain four primary alkaloids, viz., berberine, tetrahydroberberine, hydrastine, and often hydrastinine, a degradation product of hydrastine.[27] Other alkaloids are known to also be present, but at much lower amounts. Figure 2a shows the image of the goldenseal extract development lane acquired using 366 nm UV light. Figure 2b–h shows the extracted ion chronograms for the two major alkaloids and several minor abundant alkaloids expected in an authentic goldenseal extract obtained during a surface sampling lane scan with the LMJ-SSP/ESI-MS system. The most intense signals extracted from full scan (m/z 100–1000) mass spectral data were observed for m/z 384 (retention factor (R_f) = 0.32) and m/z 336 (R_f = 0.46, relative abundance 100 = 2.03×10^7 counts) which are assigned as hydrastine and berberine, respectively. Hydrastinine, which decomposes during ESI-MS analysis,[28] was observed at m/z 190 (R_f = 0.12), berberastine at m/z 352 (R_f = 0.41), canadaline at m/z 370 (R_f = 0.51), and tetrahydroberberine at m/z 340 (R_f = 0.60).

Other discernible peaks were observed above a signal level arbitrarily set at 1×10^5 counts, including m/z 365 (R_f = 0.02), m/z 503 (R_f = 0.35), m/z 368 (R_f = 0.46), and m/z 342 (R_f = 0.48). These species were not definitively identified. Peaks corresponding to primary alkaloids of adulterants or admixtures

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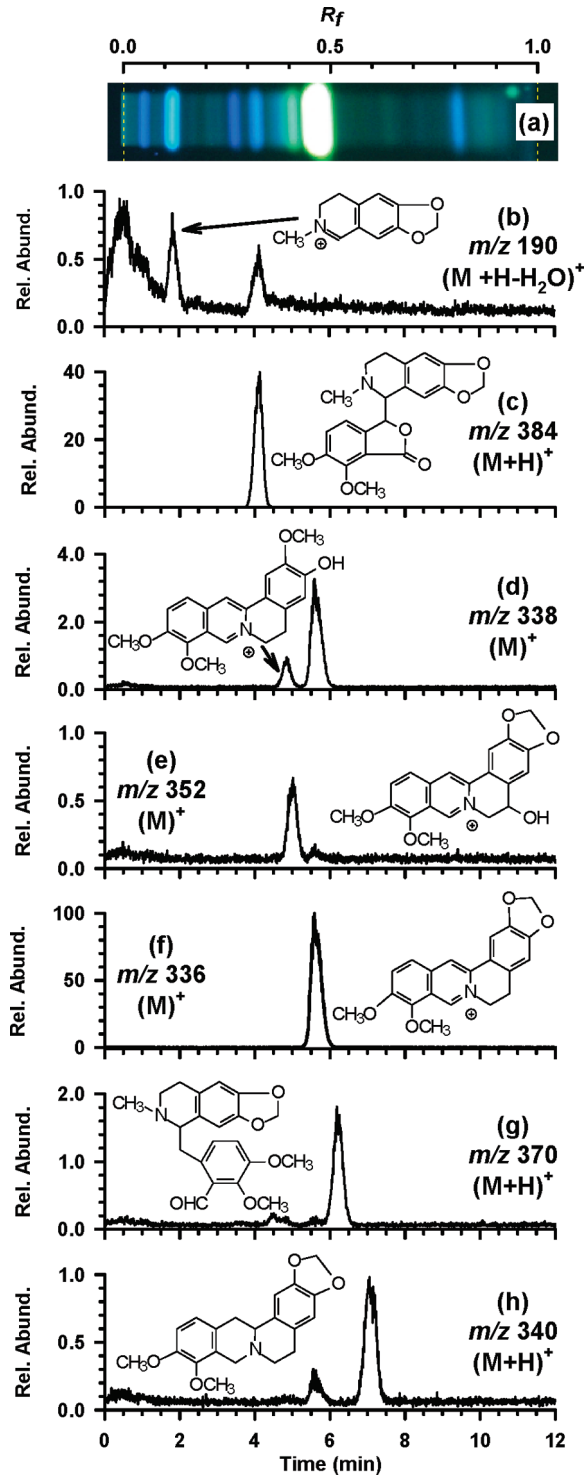

Figure 2. (a) UV (366 nm) image of goldenseal root extract developed on a HPTLC glass-backed Silica gel 60 F254s plate using 80/10/10 ethyl acetate/water/formic acid (v/v/v). The extracted ion current profiles for the major and several minor goldenseal alkaloids are plotted in panels (b) *m/z* 190, hydrastinine (observed as *m/z* consistent with water loss during sampling/ionization),[28] (c) *m/z* 384, hydrastine, (d) *m/z* 338, jatrorrhizine, (e) *m/z* 352, berberastine, (f) *m/z* 336, berberine, (g) *m/z* 370, canadaline, and (h) *m/z* 340, tetrahydroberberine as indicated. The signal intensity in each panel was normalized to the signal from the most intense alkaloid berberine (*m/z* 336 in panel (f), relative abundance 100 = 2.03×10^7 counts). The development lane was scanned relative to the LMJ-SSP from low to high R_f at 100 μm s^{-1} using an extraction/ESI solvent composed of 40/60 methanol/water (v/v) flowing at 10 μL min^{-1}.

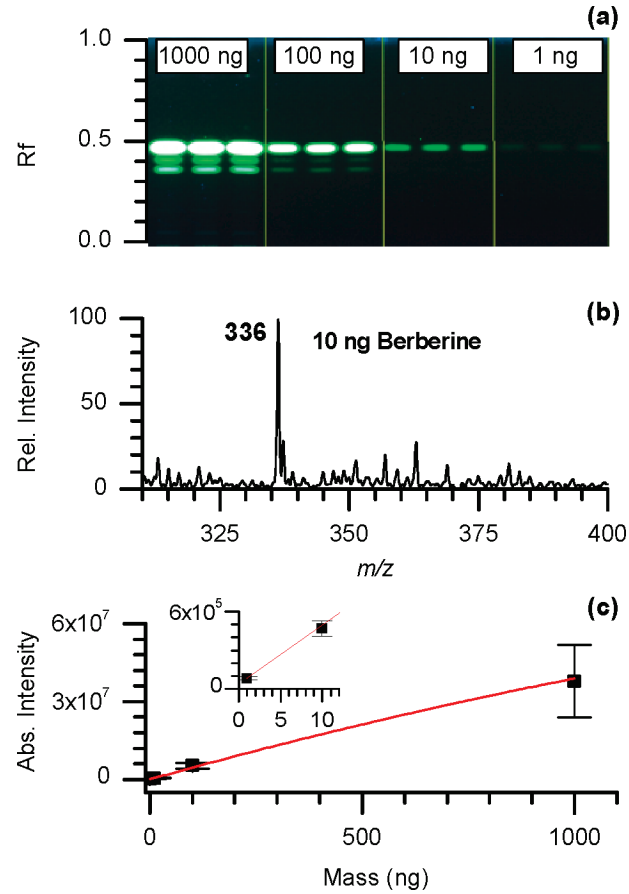

Figure 3. (a) UV (366 nm) image of berberine standard developed on a HPTLC glass-backed Silica gel 60 F_{254S} plate using 80/10/10 ethyl acetate/water/formic acid (v/v/v). The development lane was scanned relative to the LMJ-SSP in a direction perpendicular to the development direction across the berberine bands at R_f = 0.48 at 100 μm s^{-1} using an extraction/ESI solvent composed of 40/60 acetonitrile/water/formic acid (v/v/v) flowing at 10 μL min^{-1}. (b) Full scan mass spectrum from one of the 10 ng band replicates. (c) Calibration curve based on integrated peak areas from the *m/z* 336 extracted ion current.

commonly substituted for goldenseal, such as coptisine (*m/z* 320), were not detected. However, the *m/z* 338 peak at R_f = 0.39 would be consistent with the presence of jatorrhizine, but this alkaloid is not a reported component of goldenseal.[27] The more abundant *m/z* 338 peak (R_f = 0.46) is the (M + 2) isotope peak from berberine. Some bands obvious in the image of the plate (e.g., bands at R_f = 0.27 and 0.80) did not give detectable mass spectral response under the conditions used. The species in these bands may not be easily extracted using the current extraction solvent composition, may not be effectively ionized in positive ion mode ESI, or may be outside of the *m/z* range scanned. There is no direct evidence to indicate that the lack of signal from these bands was caused in any way by the silicone treatment.

To test detection levels, a berberine reference standard was applied at six different concentrations in triplicate, the plate was developed and silicone treated, and read out using the LMJ-SSP/ESI-MS system. The plate was scanned at fixed R_f across the replicate bands rather than up each development lane (Figure 3a). Additional bands other than the characteristic light green fluorescent zone of berberine at R_f = 0.48 were visible on the plate, indicating the reference standard was not pure. A

representative mass spectrum is shown in Figure 3b for one of the 10 ng berberine band scans which shows berberine ion as the base peak in the spectrum. The calibration ion curve in Figure 3c is based on the integrated peak area values of the extracted ion current for m/z 336. The data was best fitted with a second-order polynomial due to the signal "roll over" for the 1000 ng band, which was attributed to detector saturation. The readout of the 100, 10, and 1 ng bands had acceptable reproducibility with RSD values of 20.5%, 13.2%, and 15.0%. The low nanogram detection levels observed here are similar to those levels reported for similar small molecules using the LMJ-SSP to analyze hydrophobic reversed-phase TLC plates[13] and in the case of another ambient surface sampling methods, desorption electrospray ionization (DESI)-MS, when analyzing this same compound from a normal phase plate.[28]

Tryptic Peptides on ProteoChrom HPTLC Silica Gel 60 Plates and Cellulose Sheets. The separation of peptides from the tryptic digestion of proteins was performed on both ProteoChrom HPTLC Silica gel 60 plates and Cellulose sheets. After development and drying, the plates were treated with the aerosol silicone sprays as described in the Experimental Section. The different nature of the two stationary phases required a different coating procedure for each plate type to achieve the desired surface properties for LMJ-SSP analysis. Each development lane was scanned separately in data-dependent MS/MS mode. To illustrate the quality of the read out and the mass spectral signal achieved, the extracted ion current profiles for all peptides identified by LMJ-SSP/ESI-MS/MS of a BSA tryptic digest separated on a ProteoChrom HPTLC Silica gel 60 F_{254S} plate are shown in Figure 4. The photo in Figure 4a is that of a ninhydrin-stained plate of the separated digest similar to the plate actually analyzed. Averaged full scan mass spectra over the indicated regions along the scanned development lane are presented Figure 4, c, d, and e. The identity of the peptides ions observed in these spectra is indicated by the number annotation which can be correlated with the complete tabulation of identified peptides for this protein in Table 1. Low R_f bands were not identified and may be the result of irreversible binding of peptides onto the ProteoChrom HPTLC Silica gel 60 F_{254S} stationary phase. Of course, the peptide identifications were based on database matching of the product ion spectra of the peptide precursor ions not on peptide mass alone.

The protein sequence coverages achieved in these experiments are reported in Table 2 for all five proteins with both ProteoChrom HPTLC plate types. Amino acid sequences for each tryptic protein analyzed, indicating identified peptides, can be found in Figure S5 in the Supporting Information. The sequence coverage from each plate type was similar for all the protein digests examined except for bovine serum albumin (BSA). The BSA analysis on the ProteoChrom HPTLC cellulose sheet resulted in a 40.5% sequence coverage whereas a substantially higher coverage, 60.5%, was achieved with the ProteoChrom HPTLC Silica gel 60 F_{254S} plate. Twenty-nine BSA tryptic peptides were identified through their respective MS/MS spectra in a single cellulose lane scan, while 41 BSA tryptic peptides were identified in a single BSA lane scan on the ProteoChrom HPTLC Silica gel 60 F_{254S} plate.

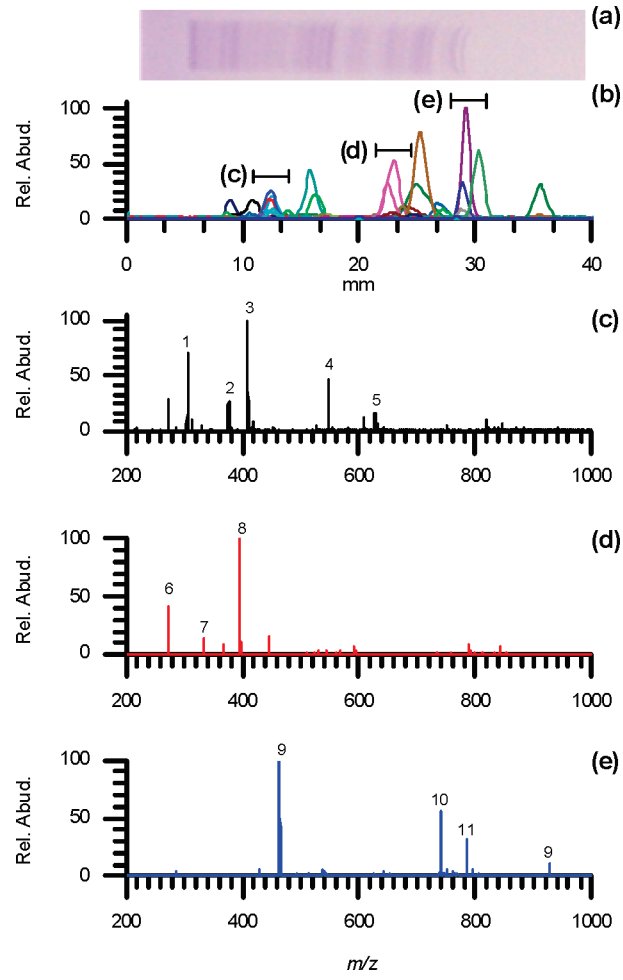

Figure 4. (a) Image of BSA tryptic peptides developed on ProteoChrom HPTLC Silica gel 60 F_{254S} using 2-butanol/pyridine/ammonia/water (39:34:10:26, v/v/v/v) and stained using ninhydrin. (b) Extracted ion current profiles for all peptide precursor ions that were identified by the LMJ-SSP/ESI-MS/MS scan of the development lane in an unstained duplicate lane to that shown in (a). Representive full scan, averaged mass spectra over the areas indicated in panel b are shown in panels c, d, and e, respectively. Ion signals labeled numerically 1−11 that were identified as tryptic peptides using MS/MS spectra are reported with numbered superscripts in Table 2 with all other identified peptides. The development lane was scanned relative to the LMJ-SSP from low to high R_f at 100 μm s^{-1} using an extraction/ESI solvent composed of 40/60 acetonitrile/water/formic acid (v/v/v) flowing at 10 μL min^{-1}.

Table 1. Protein Sequence Coverages Obtained by LMJ-SSP/ESI-MS/MS Analysis of Tryptic Digests Separated on ProteoChrom HPTLC Silica Gel 60 F_{254S} Plates and ProteoChrom HPTLC Cellulose Sheets

	% sequence coverage obtained with each HPTLC plate type	
protein	ProteoChrom HPTLC Cellulose	ProteoChrom HPTLC Silica gel 60
bovine serum albumin	40.5	60.5
β-casein	22.3	29.5
cyctochrome c	90.4	89.4
myoglobin	98.0	85.6
lysozyme	75.2	88.4

Table 2. MS/MS Identified Peptides from the LMJ-SSP/ESI-MS/MS Analysis of BSA Tryptic Digests Separated on a ProteoChrom HPTLC Silica gel 60 F254s Plate[a]

peptide	charge state 1	2	3	R_f
SEIAHR		*		0.29
FKDLGEEHFK		*	*	0.24
DLGEEHFK		*		0.21
GLVLIAFSQYLQQC″PFDEHVK			*	0.48
LVNELTEFAK	*	*		0.42
VASLR[6]		*		0.45
DDSPDLPK		*		0.19
LKPDPNTLC″DEFK			*	0.22
FW+G:KYLYEIAR		*		0.48
YLYEIAR[9]	*	*		0.53
GAC″LLPK		*		0.48
VLTSSAR		*		0.42
FGER	*			0.42
AW+SVAR		*		0.48
AEFVEVTK	*	*		0.29
LVTDLTK[8]	*	*		0.47
EC″C″HGDLLEC″ADDR			*	0.22
ADLAK	*	*		0.29
YIC″DNQDTISSK		*		0.21
SHC″IAEVEK			*	0.22
DAIPENLPPLTADFAEDK		*		0.22
NYQEAK[2]		*		0.23
DAFLGSFLYEYSR[11]		*		0.52
RHPEYAVSVLLR			*	0.24
HPEYAVSVLLR		*	*	0.49
DDPHAC″YSTVFDK			*	0.22
HLVDEPQNLIK		*	*	0.30
QNC″DQFEK		*		0.21
LGEYGFQNALIVR[10]		*	*	0.55
KVPQVSTPTLVEVSR[4]			*	0.23
VPQVSTPTLVEVSR		*		0.48
M*PC″TEDYLSLILNR		*		0.49
TPVSEK	*	*		0.22
C″C″TESLVNR		*		0.34
RPC″FSALTPDETYVPK[5]			*	0.23
AFDEK[1]	*	*		0.22
LFTFHADIC″TLPDTEK			*	0.36
ATEEQLK[3]		*		0.21
TVM*ENFVAFVDK		*		0.41
LVVSTQTALA.-		*		0.65
IETMR[7]		*		0.19

[a] (1) Modifications: G (+42.0106), *M (+15.9949), ″C (+57.0215), + W (+31.9898). (2) Superscripts on some peptides are those peptides indicated by number in Figure 4, c, d, and e.

The higher sequence coverage, as it relates to the BSA digest, can be attributed to the greater separation efficiency afforded of peptides on the ProteoChrom HPTLC Silica gel 60 F_{254S} media versus ProteoChrom cellulose under the reported development conditions. β-Casein gave the lowest sequence coverage of the examined proteins on both ProteoChrom HPTLC Silica gel 60 F_{254S} and cellulose HPTLC plates. When the base peak chromatograms from β-casein lane scans on both HPTLC plate types were examined closely, they revealed that many significant ion signals, corresponding to the visualized bands, were not being identified as β-casein tryptic peptides. Further investigation revealed the possibility that the digested β-casein contained phosphorylated serine residues which precluded their identification using DBdigger.[21] Collision-induced dissociation (CID) can be used to identify the presence of phosphorylation; however, because of H_3PO_4 (98 Da) and/or HPO_3 (80 Da) losses the fragmentation spectra are complex. The use of electron capture dissociation (ECD), for example, would provide more sequence data and unequivocal determination of phosphorylated peptides.[29]

In any case, the protein sequence coverage we achieved here was better in all cases than the coverage we have reported for the same protein digests using either the LMJ-SSP and hydrophobic reversed-phase HPTLC plates[18] or DESI-MS and these same normal phase (untreated) plates.[30] The higher sequence coverage reported may be due in part to the use of a newer more sensitive ion trap in the present studies. However, the quality of the data still attests to the effectiveness of the surface treatment and the subsequent LMJ-SSP readout of these surfaces that otherwise could not have been analyzed by this type of sampling probe.

CONCLUSIONS

We demonstrated in this paper that a simple, inexpensive surface treatment process using commercially available silicone aerosol sprays could be used to create the proper surface characteristics for effective LMJ-SSP analysis of wettable surfaces that have been previously inaccessible to this surface sampling approach. The treatment process and subsequent analysis presented here emphasized wettable HPTLC phases, using examples of separated small molecule natural products and peptides from protein tryptic digests on three different stationary phase types. As we also mentioned, providing data in the Supporting Information, other wettable surfaces like dried blood spot paper and Kimwipes can be made amenable to analysis with this treatment. Thus, this simple surface treatment process significantly expands the analytical surfaces that can be analyzed with the LMJ-SSP, and therefore, also expands the analytical utility of this liquid extraction based surface sampling approach.

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on December 15, 2010, with an error in Figure 1. The corrected version was reposted on December 20, 2010.

SUPPORTING INFORMATION AVAILABLE

Photograph of the LMJ-SSP setup on LTQ mass spectrometer, mass spectra, and peptide ID information. This material is available free of charge via the Internet at http://pubs.acs.org.

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