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Thin-layer chromatography–mass spectrometry using activated carbon, surface-assisted laser desorption/ionization

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Abstract

The analysis of compounds separated by thin-layer chromatography (TLC) by surface-assisted laser desorption ionization (SALDI) mass spectrometry has been demonstrated. The compounds are analyzed from the surface of the intact TLC plate, and the preparation of the TLC plate is rapid and robust: the gel surface is covered with 2 μm activated carbon particles, and glycerol is added. Analytes diffuse from the interior of the gel to the surface where they are adsorbed onto the activated carbon. A nitrogen laser is used to desorb analyte ions from the carbon particles in a time-of-flight mass spectrometer. A wide range of organic compounds, including peptides, can be detected, either as protonated or as cationized molecules. Interference with ''matrix peaks'' is limited since background TLC–SALDI mass spectra typically show only a few intense peaks at low mass. The detection limit for bradykinin from a developed plate is approximately 25 ng (calculated for $S/N=3$). The mass resolution (FWHM) varied from a high of about 500 to a low of about 100. This variability was likely due to surface charging. Methods to improve both mass resolution and sensitivity of TLC–SALDI are suggested.

Keywords: Surface-assisted laser desorption ionization; Mass spectrometry; Prometryn; Hydrochlorothiazide; Angiotensin; Bradykinin; Arginine

1. Introduction

Thin-layer chromatography (TLC) is an extensively used separation technique that offers distinct advantages over high-performance liquid chromatography (HPLC) for many applications [1,2]. It is true that TLC has a lower chromatographic resolution than HPLC and that HPLC offers greater flexibility through better control of experimental variables. However, TLC is rapid and cost-effective, requires minimal sample clean up, and offers the ability to run several samples in parallel. Thus, TLC is often the best choice for rapid screening of many samples. TLC is also well suited for analytes that have no chromophoric groups and thus require chemical methods of detection. Modern developments of TLC include automation and accurate quantitation [2].

A weakness of TLC is the difficulty of identifying unknowns. Common visualization methods using iodine vapor, ninhydrin and other reagents have low

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specificity. For this reason, there has been considerable interest in finding practical methods to obtain mass spectra on compounds separated by TLC. Several different approaches to this problem have been tried over many years [3,4]. The gel in a sample spot may be scraped off the plate and compounds analyzed with or without extraction from the gel. Alternatively, compounds may be analyzed directly from the intact TLC plate surface. In early efforts, using electron ionization (EI) or chemical ionization (CI), analytes were either volatilized into a gas stream that flowed into the ion source of a mass spectrometer, or volatilized directly inside the ion source using a direct insertion probe. These efforts have been reviewed [3,5]. More recently, fast atom bombardment (FAB) or secondary-ion mass spectrometry (SIMS) [6–9], plasma desorption [10,11], and laser desorption MS [12–14] have been used to analyze compounds separated by TLC.

Methods for mass spectrometric analysis of compounds directly from intact TLC plates are of particular interest because they could be used for fast and automated analysis as well as imaging of TLC chromatograms [3]. The laser microprobe was used by Novak and Hercules to obtain spectra of separated dyes [14]. Imaging with a good spatial resolution was achieved [12]. Infra-red (IR) laser desorption of analytes from TLC plates, followed by post-ionization, has been demonstrated by several groups [15–18]. Busch and co-workers [3,4,7,19–21] developed liquid SIMS (FAB) for TLC plate analysis and imaging. Other groups have also worked on this technique [8,22,23]. Recently, Hercules et al. demonstrated the use of MALDI for the analysis of intact TLC plates [24]. The method allowed for the imaging of analyte distributions in the plate [25]. Quantitation was demonstrated using an internal standard [26].

For sensitive mass spectrometric analysis of intact TLC plates, the analytes should be concentrated close to the surface of the gel. This is accomplished by extraction with a solvent; however, lateral diffusion of the analyte during extraction results in a loss of chromatographic resolution, and significant efforts have been devoted to reduce this problem [3,7,23,27].

Recently, we presented a new laser desorption ionization method that uses a carbon powder to couple the laser UV energy into a liquid solution [28]. We call this method surface-assisted laser desorption ionization, or SALDI. The development of SALDI was inspired by Tanaka et al.'s early work, in which 300 Å diameter cobalt particles were used to couple the laser energy into a glycerol solution [29]. However, evidence is that the governing processes in SALDI require μm-size particles [30]. Originally, a graphite powder was used for SALDI, but it was later found that a μm-sized activated carbon powder was more reliable in yielding mass spectra [31]. It was realized that SALDI had several advantages that were potentially useful for TLC interfacing. First, SALDI is well suited for the analysis of a wide range of organic compounds [28,32]. One matrix system, such as activated carbon and glycerol, works for most analytes. In contrast to matrix-assisted laser desorption ionization (MALDI), only a few SALDI background ions appear at low mass. The sensitivity of SALDI was originally reported to be approximately 10 pmol (10^{-5} *M* bradykinin in 1 μl) [28]. However, subsequent experiments showed that by using a mixed solvent, where the volatile component quickly evaporates from the sample, and a smaller amount of carbon powder about 0.1 pmol of bradykinin can be detected. In this report, it is shown that analysis of compounds directly from intact TLC plates by SALDI–MS is straightforward with easy sample preparation. Preliminary results were reported at a conference [31].

2. Experimental

2.1. Chemicals

Glycerol (99.5%, ACS reagent) was obtained from Fisher and the activated carbon powder (Darco G-60, 2 μm) from Aldrich. Hydrochlorothiazide and promethryn were obtained from Supelco, hydroflumethiazide from Sigma, and reagent grade ninhydrin from Baker. The pencil lead was Pental (HB, ''Hi polymer''). All other chemicals and solvents used in this study were obtained commercially and used without further purification.

2.2. Thin-layer chromatography

Silica gel 60 TLC plates (Merck, Germany), with a plastic backing and with a fluorescent indicator, were used. In experiments aimed at optimizing the procedures for preparing the TLC plate for SALDI–MS, analyte solutions were ''spotted'' onto a TLC plate by pipetting 1.0 μl of an analyte solution onto the dry plate, and no TLC separations were performed. In experiments where the TLC plate was to be developed, about 0.5 μl of the analyte solution was applied to the plate. Analyte concentrations and the solvents used are given in the text. For peptide and amino acid separations, a developing solvent of water–methanol–acetic acid (44:50:6 v/v/v) was used [21]. A developing solvent consisting of acetone–methanol (1:1, v/v) was used for separating diuretics. The TLC separations were carried out at room temperature. About 10 lanes were used on each plate. Each separation was run in two TLC lanes; one lane was used for visualization and the other for MS. After development, the plates were dried and the lanes separated by cutting.

A 0.20% ninhydrin in ethanol solution was used to visualize amino acids and peptides on the TLC plate. The ninhydrin solution was added to the dry plate using a pipette and the plate was dried with a hot air gun. Other organic analytes were visualized using iodine vapor.

2.3. Mass spectrometry

SALDI mass spectra were obtained on a Voyager RP time-of-flight (TOF) mass spectrometer (PerSeptive Biosystems, Framingham, MA, USA). The instrument had a 1.4 m linear flight tube, a 4 ns pulsed 337 nm nitrogen laser, a single stage reflector and a dual stage ion source with continuous ion extraction. A 2 1/4 in.×2 1/4 in. stainless steel sample plate was inserted into the ion source by means of a pneumatic system (1 in.=2.54 cm). Excised pieces of TLC plates were attached to the stainless steel sample plate using double-sided sticky tape. Inside the source, the sample plate was attached to a computer-controlled XY stage that allowed precise manipulation of the sample relative to the fixed laser focus position. The samples were viewed by a TV camera and monitor that allowed the desorption site to be selected by visual inspection. All samples were run in linear mode with 28 kV accelerating voltage, 16.8 kV secondary grid voltage, and 84 V guide wire voltage. Approximately 40 single shot mass spectra were averaged on a 500 MHz digitizing oscilloscope, TDS 520A (Tektronix, Wilsonville, OR, USA). Spectra were downloaded to a personal computer and mass-calibrated using PerSeptive and GRAMS (Galactic Software, Salem, NH, USA) software. In this report, mass resolution is quoted as FWHM values. Internal mass calibration was used throughout this work.

2.4. SALDI sample preparation

The method to prepare the TLC gel surface for SALDI–MS that consistently gave the best results will here be referred to as the ''standard method''. This method was used unless otherwise noted. If required, the analyte was first localized on the TLC plate. The plate was dried, and the sample spot was excised using scissors or a sharp knife. The excised gel was structurally intact on its plastic support. The size of the cut-out piece varied from 2 mm×2 mm to 1 cm×1 cm. Larger pieces of the TLC plate required very long pump-down times in the vacuum lock. The TLC plate cut-out was attached to the sample plate, and a 0.5 μl volume of suspension C (see below) was pipetted onto the analyte TLC spot. This left a 2 mm diameter dark spot on the gel surface. The spot was inspected to ensure that the black carbon powder was uniformly distributed. The gel surface should not be completely covered by the carbon; a surface coverage of about 50% was found to give the best results. Approximately 0.5 μl of pure glycerol was pipetted on top of the carbon-covered sample spot. Initially, the glycerol formed a droplet on the top of the plate, but after about 10 s the glycerol had been absorbed by the silica gel. The activated carbon powder remained essentially in place. To reduce pump-down time, the TLC plate was left in room air for 5 to 10 min to allow for most of the methanol and water to evaporate.

Three activated carbon suspensions of different composition were used in this work. Each suspension contained activated carbon (AC), glycerol (Gl), sucrose (S), methanol (M) and water (W). Suspension A had the following composition by mass: AC

(6.6%), Gl (8.0%), S (2.3%), M (57%), and W (26.1%). It was prepared by adding 25 mg activated carbon to 0.10 ml of sucrose–glycerol–methanol (5:28:67, w/w/v). A 0.10-ml volume of 5% (w/v) sucrose in water and a 0.20 ml volume of methanol were added. Suspension B had the following composition by mass: AC (5.8%), Gl (14.6%), S (3.4%), M (9.1%), and W (67.2%). It was prepared by combining 0.10 ml glycerol–methanol (1:1, v/v) with 0.30 ml of 5% (w/v) sucrose in water and adding 25 mg activated carbon. Suspension C had the following composition by mass: AC (2.7%), Gl (6.7%), S (4.1%), M (4.2%) and W (82.3%). It was prepared by adding 25 mg of activated carbon powder to 0.10 ml of a mixed glycerol–methanol solvent (1:1, v/v). This suspension was mixed with a solution of 5% (w/v) sucrose in water in a 1:8 volume ratio.

3. Results and discussion

3.1. Background ions at low mass

Since TLC is used primarily for separation of low-mass organic compounds, it is essential that a mass spectrometric analysis method has a low ion background in the low mass region. Fig. 1 shows a SALDI mass spectrum of a blank TLC plate. The mass spectrum is seen to be relatively ''clean'' and dominated by a few mass peaks. The most abundant ions are Na^+, K^+, the adduct between Na^+ and glycerol ($m/z=115$), and the adduct between Na^+ and sucrose ($m/z=365$). A minor peak at $m/z=203$ is assigned to a sodium ion adduct with a monosaccharide fragment. A few, low-intensity mass peaks are seen in the mass range below 350, see inset in Fig. 1. These peaks were not identified, but most of

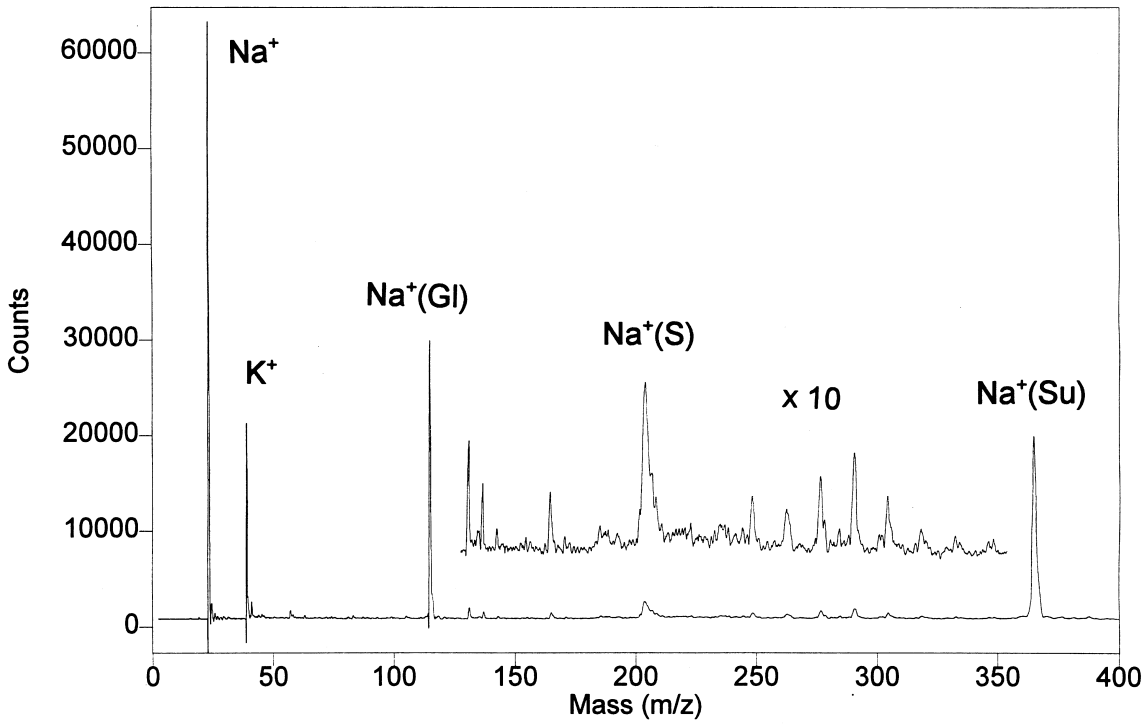

Fig. 1. A typical TLC–SALDI background mass spectrum. One μl of activated carbon suspension A was spotted onto the plate surface, with no additional glycerol added. Background spectra obtained with several different plate preparation methods, including the ''standard'' method, and with different activated carbon suspensions were all very similar to this spectrum.

them only appeared in the presence of the silica gel. They are presumably due to additives to the silica gel, such as gel binder and fluorescence indicator. At increased laser pulse energy, the intensities of these ''silica gel peaks'' were increased, and a series of carbon clusters, C_n^+, also appeared [28]. Essentially no protonated glycerol ions are observed in Fig. 1, as the SALDI ionization was dominated by sodium ion cationization. This was typical of ''background'' mass spectra in positive ion mode. In negative ion mode, there were a larger number of background ion peaks than in positive ion mode.

3.2. Extraction of analyte from TLC gel and peptide mass spectra

For optimum sensitivity in the analysis of intact TLC plates, analytes must be concentrated close to the gel surface. The problem of extracting analytes from the bulk of the gel is encountered whether using FAB, SIMS, or MALDI MS [3,25]. In this work, initial efforts were made to extract analytes from the interior of the gel by the gradual addition of an organic solvent, such as ethanol or methanol. A SALDI carbon suspension was added before the organic solvent had dried out, and SALDI mass spectra of the analytes were obtained (spectra not shown). However, it was difficult consistently to obtain good-quality mass spectra with this method, and the lateral spreading of analytes was considerable.

It has been shown that analytes in TLC gels can be concentrated by volatile organic solvents that flow through the gel toward areas of rapid evaporation [21,23]. Efforts were made to use this process to concentrate the analytes in the gel surface, before adding the activated carbon suspension, by supplying organic solvents through small holes punctured in the TLC backing. However, this approach was found to have the same problems of reproducibility and analyte spreading as application of organic solvent to the front of the TLC plate. Efforts to apply the organic solvent onto the gel using a fine spray were also largely unsuccessful because of analyte spreading. A different approach tried was to scrape off the silica gel in the sample spot. The resulting silica powder was mixed with an activated carbon suspension and subjected to activated carbon-SALDI mass analysis. Mass spectra were easily obtained by this procedure, but they were generally of lower quality than those later obtained from intact TLC plates.

Because both FAB and SALDI use a low-volatility liquid, such as glycerol, analyte extraction and TLC plate preparation procedures developed for TLC-FAB [3] is expected to be applicable, with minor modifications, also to TLC–SALDI. Analyte spreading is less extensive with glycerol, than with more typical organic solvents. The reason is that analyte diffusion, as well as liquid flow, is much slower with the high-viscosity glycerol liquid. Also, vacuum pump-down times tend to be shorter with glycerol than with the high-vapor pressure solvents, because the latter must be removed from the silica gel during pump-down. For these reasons, glycerol was used both for analyte extraction and for SALDI desorption throughout this work.

In initial glycerol extraction experiments, a 0.5 μl volume of glycerol was added to the dried sample spot, followed within a few seconds by 0.5 μl of a SALDI suspension. The plate was then air-dried. Typical mass spectra are shown in Fig. 2. The TLC–SALDI mass spectrum of angiotensin in Fig. 2a was obtained using SALDI suspension A, and that in Fig. 2b using SALDI suspension B. It is seen that sodiated peptide ions dominate the upper mass spectrum, whereas the lower mass spectrum is dominated by the protonated peptide. The reason for this difference is that suspension A had a higher ratio of activated carbon to glycerol (0.83:1, w/w) than suspension B (0.40:1, w/w). In experiments preceding the TLC work described here, we have often observed this effect. Fig. 2 illustrates that it is possible to choose between protonation and cationization in TLC–SALDI by varying the composition of the activated carbon suspension. However, care must be taken to use reproducible sample handling and preparation procedures to minimize sample-to-sample variations in the relative intensities of cationized and protonated analyte peaks.

For the mass spectra in Fig. 2, pure glycerol for extraction was added first to the TLC plate, followed by the carbon suspension. However, with this method, the carbon particles were displaced from the spot where they were originally applied and formed a

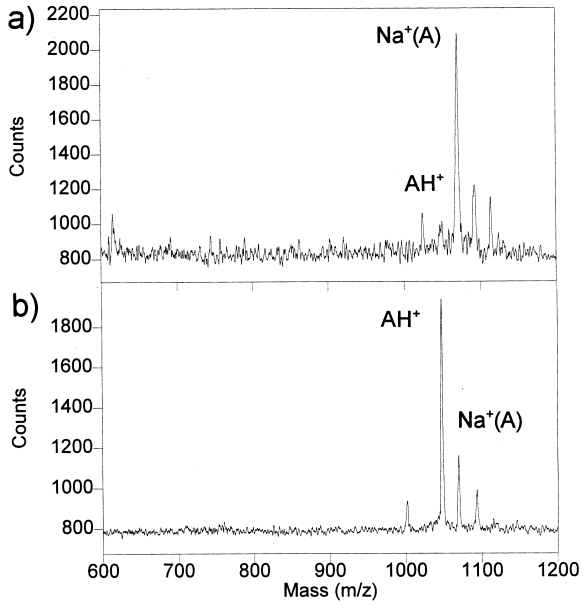

Fig. 2. (a) TLC–SALDI mass spectra of angiotensin II; 1 μl of a $5 \cdot 10^{-4}$ *M* (0.5 nmol) peptide in aqueous solution was spotted onto the TLC plate. The peptide was extracted from the plate by adding 0.5 μl glycerol to the sample spot and, subsequently, 0.5 μl of activated carbon suspension A. (b) Same as above, but suspension B with a lower ratio of activated carbon to glycerol was used.

(black) ring. In the center of the ring, over the original sample spot, where only a few carbon particles remained, the analyte ion signals were weak or absent. The best analyte mass spectra were obtained along the inner edge of the ring of activated carbon. Further out, where the carbon particles totally covered the silica surface, no analyte mass spectra were obtained. This observation is consistent with peptide adsorption onto the activated carbon particles being essentially irreversible. Analytes that diffuse up to the gel surface become adsorbed on those carbon particles that were closest to the gel surface. If the layer of carbon particles was thick, the analytes remained inaccessible to laser desorption.

In order to further study the desorption process, we examined the transport of dyes (Victoria Blue and Crystal Violet) in TLC plates prepared for activated carbon-SALDI analysis using a light microscope. The TLC plate was illuminated from below such that the dye concentration throughout the thickness of the gel was observed. The diffusion of the dye through the silica gel, and the adsorption onto activated carbon, was followed through the changes in color. The dye was seen to become depleted around areas covered by activated carbon particles, and this confirms that the adsorption was essentially irreversible.

3.3. Dispersion of carbon particles and potential for imaging

In order to obtain a SALDI ion signal that reflected the concentration of analyte over the TLC plate, a method to distribute the carbon particles more uniformly over the sample surface had to be found. With practice, we learned to achieve a relatively uniform distribution of the particles by spotting a 0.5 μl volume of suspension C onto the dried sample spot. As described in Section 2.4, suspension C contained less activated carbon than suspensions A or B. The particles covered a circular area that was approximately 5 mm in diameter. During the subsequent addition of about 0.5 μl of glycerol onto the same spot, there was no noticeable displacement of the activated carbon particles. This method is described as the ''standard'' procedure in Section 2.4. Fig. 3a shows a resulting TLC–SALDI mass spectrum of bradykinin and Fig. 3b a spectrum of angiotensin II. In these mass spectra, the protonated peptides dominate strongly over sodiated peptides. This was typically observed when using suspension C, due to the low ratio of activated carbon to glycerol.

In the plate preparation procedure just described, the analyte spot typically spread from an initial diameter of about 2 mm to about 3 mm. Good-quality TLC–SALDI mass spectra were reliably obtained over the whole sample spot area; however, the intensity of the analyte ion signal varied significantly as the laser focus was moved over the sample spot. Moreover, in some spots, the analyte ion signal lasted for several minutes, whereas it was short-lived in other spots. We believe that the variability of the SALDI analyte ion signal is explained as follows: the highest-intensity UV laser focus had a diameter of about 10 μm, as determined by measuring the sizes of burnmarks on polyvinylidene difluoride (PVDF) polymers using a scanning electron microscope. Though the activated carbon particles seemed to be uniformly distributed by the unaided

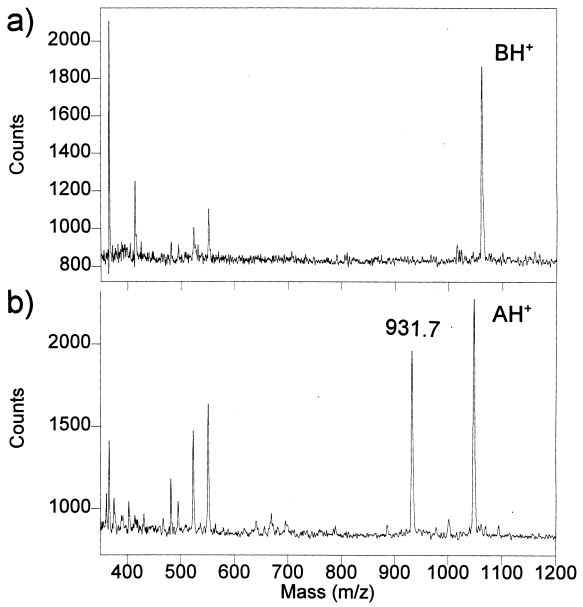

Fig. 3. (a) TLC–SALDI mass spectrum of (a) bradykinin (2.5 nmol) and (b) angiotensin II (0.5 nmol). Activated carbon suspension C (0.5 μl) followed by 0.5 μl glycerol was pipetted onto the sample spot, see ''standard'' method in Section 2.4. The peak at m/z=931.7 may be due to angiotensin II that has lost a terminal aspartic acid.

eye, inspection under a light microscope revealed that the μm-sized particles aggregated together on the TLC surface, resulting in an non-uniform particle distribution. Thus, it is not surprising that there should be a significant statistical variation in the availability of SALDI desorption sites within the laser focus, resulting in a variable ion signal intensity. For TLC imaging, the distribution of carbon particles on the gel surface should ideally be more uniform than what is obtained with the ''standard'' method. Averaging the ion signal over several focal points, as has been done for imaging in TLC–MALDI [24], would also be helpful.

3.4. Alternate SALDI liquids and the role of sucrose

Liquids other then glycerol, such as threitol, thiothreitol, thioglycerol, 3-nitrobenzyl alcohol (NOBA), ''magic bullet'' and poly(ethylene glycols) (PEGs), were successfully used in this work to extract analytes and to obtain TLC–SALDI mass spectra. The mass spectra were similar to those obtained with glycerol, except that the intensities of the cationized (Na^+) analytes in the spectra were usually higher than when glycerol was used. It was shown by Didonato and Busch [7] that threitol can be used as a matrix in TLC-FAB to reduce lateral analyte spreading and to improve analyte extraction efficiency. In our TLC–SALDI experiments, threitol was gently heated before being applied to the TLC plate. This matrix reduced pump-down time and gave TLC–SALDI mass spectra that were very similar to those obtained with glycerol.

Sucrose was always added to the carbon suspensions in the TLC–SALDI experiments. The sole purpose was to reduce ion source contamination. In our early graphite SALDI experiments (prior to the TLC work described here), it was found that carbon particles dislodged from the sample as the glycerol evaporated and the sample dried. The ion source became contaminated by the particles and occasionally had to be cleaned. In an effort to reduce this problem, many compounds were evaluated as additives to the SALDI suspension. It was found that mono- or disaccharides, added to the glycerol, greatly reduced contamination of the ion source. The saccharide acts as an ''adhesive'', making it more difficult for carbon particles to detach from the sample. A few new peaks due to sodiated saccharides appeared in the SALDI mass spectra, see for example, Fig. 1. Otherwise, the mass spectra were essentially unaffected, as long as the mass ratio of saccharides to glycerol in the sample was less than about 0.1. At higher saccharide concentrations, analyte mass spectra started to degrade. To evaluate the effect of added sucrose on TLC–SALDI mass spectra, varying amounts of sucrose were added also to the pure glycerol used for extraction. It was found that the effects on the mass spectra were small up to a 15% (w/w) sucrose concentration. At higher concentrations, the intensity of the analyte ion signal decreased significantly. An alternate approach toward reducing contamination by carbon particles is to apply a very small amount of carbon. For example, a pencil was used to draw a line over the sample spot, and pure glycerol was added. Peptide mass spectra were easily obtained by this method. However, the quality of the spectra was not as good as with the carbon suspensions.

3.5. Applications to organic compounds and developed plates

An advantage of SALDI is that mass spectra are easily obtained from a wide range of organic compounds, using a single sample preparation technique [28,32]. The main requirement for obtaining mass spectra is that the analyte, An, has a high enough proton affinity to form AnH^+ gas-phase ions or can complex with a metal ion, M^+, to form $M^+(An)$ gas-phase ions. The application of TLC–SALDI to organic compounds is illustrated in Fig. 4a with a mass spectrum of prometryn. The protonated analyte is detected at $m/z=242$. The negative ion mode TLC–SALDI mass spectrum of hydrochlorothiazide, a diuretic, is seen in Fig. 4b. The two chlorine isotope peaks are seen at $m/z=296$ and 298. A mass peak at $m/z=294$ is due either to an impurity or to fragmentation. In this work, TLC–SALDI mass spectra were obtained of porphyrins, phospholipids, hydroflumethiazide, and ergosterol, in addition to several amino acids and peptides. The ability to obtain mass spectra from wide range of compounds is a characteristic that SALDI shares with FAB and is a main reason that SALDI may be a suitable ionization method for the analysis of organic compounds separated by TLC.

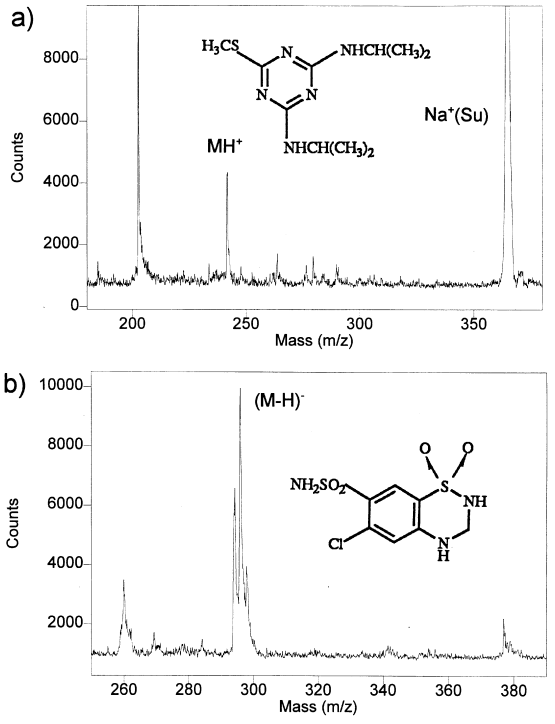

Fig. 4. (a) TLC–SALDI mass spectrum of the herbicide prometryn. The protonated analyte [MH^+] is detected. One μl of a 10 μg/μl solution in methanol was applied to the gel and the ''standard'' method was used to prepare the plate. (b) Negative ion mode TLC–SALDI mass spectrum of the diuretic hydrochlorothiazide. One μl of a 10 μg/μl solution in methanol was applied to the gel, and the ''standard'' method was used to prepare the TLC plate. The two chlorine isotope peaks of the $[M-H]^-$ ion are seen, as is a lower mass impurity or fragment ion.

The procedures used to prepare TLC plates for SALDI were optimized using plates onto which the analytes had been spotted, i.e., the plates were not developed. However, in most cases it was straightforward to obtained mass spectra also from developed TLC plates. This is illustrated in Fig. 5 which shows a TLC–SALDI mass spectrum of arginine. A mixture of 0.5 nmol bradykinin and 50 nmol arginine had been spotted onto the TLC plate, and the plate was developed. The mass spectra obtained from the bradykinin spot were similar to that in Fig. 3a.

3.6. Mass resolution and sensitivity

The mass resolution observed in TLC–SALDI mass spectra varied significantly from sample to sample. In the best mass spectra, the mass resolution was similar to that obtained in experiments where the

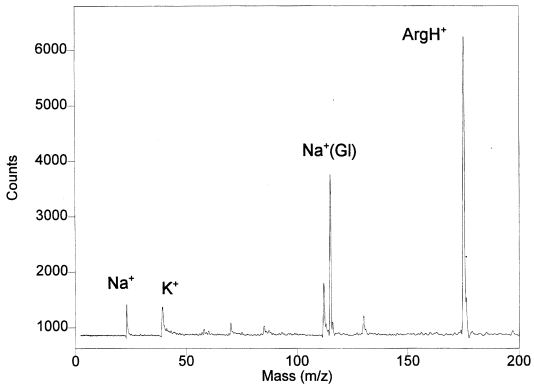

Fig. 5. TLC–SALDI mass spectrum of arginine separated on the TLC plate; 100 nmol peptide was applied to the TLC plate prior to the plate being developed; ''standard'' method was used to prepare the TLC plate.

carbon powder suspension is deposited directly on the metal sample plate [28]. Thus, unit mass resolution up to m/z about 300, and a mass resolution of 500 for bradykinin at $m/z=1061$, was occasionally attained. In other cases, the mass resolution was below unity at m/z larger than about 100. For example, in mass spectra of hydrochlorothiazide from developed plates, the three peaks in the molecular ion region, seen in Fig. 4b, were not resolved. Mass resolution was also degraded when the laser power was increased. In SALDI experiments on frozen samples, we have found that inadequate electrical contact between the SALDI particles and the metal sample plate [30] will result in low mass resolution. That surface charging was a factor in the TLC–SALDI experiments is supported by the observation that ion flight times was sometimes seen to vary from sample to sample.

It is of interest to compare the sensitivity of TLC–SALDI with that of TLC–MALDI since either method can be used on a TOF mass spectrometer equipped with a UV laser. The sensitivity of TLC–SALDI varies depending on the analyte. Only for small peptides can we compare with published MALDI results. Hercules et al. reported a 2 ng detection limit for small peptides [24], calculated as the quantity of analyte required for a signal-to-noise (S/N) ratio of 3. A mass spectrum with a sample loading of about 100 ng was used for this calculation [24]. In TLC–SALDI, it was found that with 500 ng bradykinin spotted onto the TLC plate, the S/N ratio was 50–100. The calculated detection limit in TLC–SALDI is thus about 20 ng. When the plate was developed, the bradykinin detection limit was slightly higher, about 25 ng. Thus, TLC–SALDI is presently about one-order of magnitude less sensitive than TLC–MALDI for bradykinin. On the other hand, TLC–SALDI may well be more sensitive than TLC–MALDI for many lower-mass organic compounds, because of the congestion of intense matrix peaks in MALDI below mass 400.

4. Conclusions

There are two main advantages of TLC–SALDI: a single sample preparation method, that is also simple-to-use and reliable, can be used to obtain ions from a wide range of organic compounds, including small peptides. Few background ions are present that can interfere with analyte ions. In addition, TOF mass spectrometers are widely available and relatively affordable. In many such instruments, an intact TLC plate can be inserted into the ion source. However, a higher-capacity pumping system would typically be required if glycerol is to be applied over a larger area. Moderate cooling of the TLC plate inside the ion source, or replacing the glycerol with a lower vapor pressure compound, should decrease pumping requirements considerably.

Presently, both the mass resolution and the sensitivity of TLC–SALDI are lower than that of TLC–MALDI. To achieve an improved mass resolution, the problem of surface charging must be solved. We are presently working on simple methods to ensure electrical contact between the glycerol in the TLC sample spot and the metal sample plate. Using a time-of-flight instrument with delayed ion extraction should also significantly improve mass resolution. With respect to sensitivity, it must be noted that the present work was undertaken to demonstrate the applicability of TLC–SALDI to peptides and lower molecular-mass organic compounds. Achieving maximum sensitivity was not a priority. Still, the sensitivity of TLC–SALDI, shown here, should be sufficient for most TLC separations of organic compounds.

These are good reasons to believe that the sensitivity of TLC–SALDI can be significantly improved over what was observed in this work. In the light microscope studies of the extraction of dyes using the ‘‘standard’’ method, it was seen that much dye remained in the TLC gel after the extraction. It should be possible to find procedures with a much higher extraction efficiency. In addition, the fact that the adsorption of analyte onto the activated carbon is essentially irreversible in principle makes it possible to concentrate analytes onto a small mass of carbon, and this also should result in an improved sensitivity.

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