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A novel approach of combining thin-layer chromatography with surface-assisted laser desorption/ionization (SALDI) time-of-flight mass spectrometry

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A novel means of combining thin-layer chromatography (TLC) with laser desorption/ionization mass spectrometry using a liquid matrix is proposed. Surface-assisted laser desorption/ionization (SALDI) mass spectrometry, which uses a mixture of a micrometer-sized carbon powder (graphite or activated carbon, the SALDI solid) and 15% sucrose/glycerol, dissolved in an equal volume of methanol (SALDI liquid) as a SALDI matrix, is used for laser desorption mass analysis. The ablation of carbon powder from a pencil drawing was used as an alternative to the SALDI solid. The liquid matrix resembled that used in a conventional SALDI matrix system. A line was drawn before separation with a pencil on the track of the sample developed on the TLC plate. After TLC separation, ~0.1 μl of SALDI liquid was directly applied to the chromatographic spots on the TLC plate. Porphyrins were used to demonstrate this combination owing to the visible colors of this type of compound. The analyte signal can be easily detected by irradiating the laser along the pencil line on the TLC plate. An additive, *p*-toluenesulfonic acid, is added to the SALDI liquid to enhance the signal's intensity. This additive dramatically improves the signal-to-noise ratio. A detection limit of ~500 pg is demonstrated for porphines, which is 50 times better than that corresponding to conventional TLC SALDI. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: thin-layer chromatography; surface-assisted laser desorption/ionization; liquid matrix; *p*-toluenesulfonic acid; porphyrins

INTRODUCTION

Thin-layer chromatography (TLC) is frequently used to separate and purify organic and small biochemical compounds. Mass spectrometry (MS) has been widely used as a detection method for TLC as it can provide information about the structures and molecular masses of analytes.[1,2] Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry combined with TLC has been thoroughly investigated.[3–9] Surface-assisted laser desorption/ionization (SALDI) is a laser desorption/ionization method that employs laser desorption/ionization with a carbon powder to couple the laser UV energy into a liquid solution.[10–16] The combination using conventional SALDI as an ionization method for TLC has been developed.[12,13] Very low interference from matrix ions allows SALDI analysis to be effectively combined with TLC. Furthermore, the SALDI liquid used in the SALDI matrix system reduces the interaction between analytes and silica gel on the TLC plate. Hence no extra extraction solvents are required, reducing the steps in the sample preparation and the chance of the sample spots spreading out on the TLC plate. However, the detection limit in TLC/SALDI is higher than that in TLC/MALDI.[12] The intensities of analyte signals in TLC/SALDI vary because of the inhomogeneous distribution of carbon particles on the gel surface.[12] Accordingly, this study was aimed at modifying the TLC/SALDI combination to lower the detection limit and obtain a uniform analyte signal distribution by preparing a homogeneous carbon powder distribution on the silica gel surface.

The detection limit in TLC/SALDI can be lowered by improving the extraction efficiency of analytes from the gel on the TLC plate, thereby raising the analyte molecules to the surface of the SALDI matrix. This method is effective since the laser only irradiates the surface of the matrix. The extraction efficiency relies on the extraction power of the extraction liquid used. Improving the desorption/ionization efficiency of analytes in mass spectrometry also helps to optimize the detection limit. Our recent study demonstrated that adding ionic surfactants such as *p*-toluenesulfonic acid (PTSA) to the SALDI liquid can significantly enhance the analyte signals in SALDI analysis.[16] The enhancement of the intensity of the analyte signal is suggested to be due both to the ionic

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interaction of surfactants with analytes and to the acidity of the additive.[16] Surfactant molecules tend to cover the surface of a liquid droplet, improving the detection limit by attracting charged analytes to the surface via ionic interaction. Using PTSA as an additive in the SALDI liquid has been proved to enhance effectively the signals of analytes such as dodecyltrimethylammonium bromide, methylephedrine and adenosine triphosphate (ATP).[16] Concentrations of PTSA between 0.1 and 0.5 M are added to the SALDI liquid to enhance the analyte signals.[16] Thus, adding appropriate amounts of PTSA to the extraction solvent, that is the SALDI liquid, is also expected to enhance the analyte signals and lower the detection limit in TLC/SALDI analysis.

Since analytes require the assistance of carbon powder for ionization in SALDI, inhomogeneous carbon deposition on the TLC plate may result in some difficulties in searching analyte signals in TLC/SALDI analysis. In this study, carbon powder ablated from a pencil drawing was used as an alternative to the SALDI solid in the SALDI matrix compositions, to obtain a homogeneous distribution of carbon particles on the gel surface. Preparing the pencil drawing after TLC separation may cause the silica gel with analytes to be scratched out or the analyte molecules to be covered by carbon powder. Therefore, a line was drawn with a pencil on the path of the developing sample before TLC separation to deposit the carbon powder without causing these problems. Carbon deposition using this method of pencil drawing was much more homogeneous than by applying a suspension of carbon powder mixed with SALDI liquid on the gel surface. The analyte signal can be easily found by just scanning along the black line. The search area is minimized, saving time the taken required by the analysis.

EXPERIMENTAL

Reagents

Glycerol, tetrahydrofuran (THF) and ethanol were purchased from Riedel-de Haën (Deisenhofen, Germany), sucrose from J. T. Baker (Phillipsburg, NJ, USA), acetone, dimethylformamide (DMF), methanol and chloroform from Mallinkrodt (Phillipsburg, NJ, USA), PTSA from Sigma (St. Louis, MO, USA) and 4,4′,4″,4‴-(21*H*, 23*H*-Porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) (PTB) and 5,10,15,20-tetrakis(4-hydroxyphenyl)-2*H*,23*H*-porphine (THP) from Aldrich (Milwaukee, WI, USA). A 2B pencil was purchased from Bensia (USA).

SALDI sample preparation

A line was drawn with a 2B pencil on the silica gel surface of a TLC plate. Approximately 0.05 µl of porphine solution with a given concentration was first applied to the pencil line to simulate the sample preparation conditions on the TLC plate. Subsequently, 0.1 µl of SALDI liquid (15% sucrose/glycerol–methanol (1 : 1, v/v)) was applied on the sample spot. The drop of liquid matrix dispersed as a circular spot with a diameter of 1 mm. A 1 × 1 cm piece of the TLC plate including this sample deposit was cut out using scissors and fixed to a sample tray using double-sided tape. The sample was left to stand at room temperature for 5 min to allow the volatile organics to evaporate, and was then loaded into the mass spectrometer for analysis.

Sample preparation for TLC/SALDI analysis

A line was pre-drawn with a 2B pencil on the TLC plate (Merck, Darmstadt, Germany) on the track of the developing sample. Porphines dissolved in DMF were employed as samples in the TLC separation. Approximately 0.05 µl of sample solution was applied to the initial position on the TLC plate. Developing solvent A was chloroform–ethanol–THF (2 : 1 : 1, v/v). The R_f values for PTB and THP were 0.38 and 0.86, respectively. Developing solvent B consisted of acetone and methanol. The R_f value for PTB was 0.88. After TLC separation, 0.1 µl of SALDI liquid, 15% sucrose/glycerol–methanol (1 : 1, v/v) including various amounts of PTSA, was added to the individual sample spot. The sample spot was cut out with scissors. The excised gel was intact on its aluminum support. The size of the cut-out piece was about 1 × 1 cm. This piece was attached to a sample plate using double-sided tape. The sample plate was then ready to load into the mass spectrometer for analysis. The sample signal was searched along the black pencil line on the TLC plate. Figure 1 displays the sample preparation scheme.

Mass spectrometer

Experiments were carried out using a PerSeptive (Framington, MA, USA) LaserTec Benchtop linear instrument equipped with a 1.2 m vertical flight tube and a 337 nm nitrogen laser. The sample plate, used as a horizontal plate, could load 100 samples simultaneously. The acceleration voltage was set to 20 kV. We fixed no more than two sample spots on the sample tray at a time considering the high vacuum requirement in the mass spectrometer.

RESULTS AND DISCUSSION

Our previous study demonstrated that using surfactants can enhance the signals of analytes such as methylephedrine and ATP.[16] Porphines were employed here as samples to demonstrate the combination of TLC with SALDI. The effects of surfactants on the analyte signal of porphines were also first examined. Additionally, the carbon ablation from the pencil drawing was used as an alternative to carbon powder in the SALDI matrix. The Experimental section describes the sample preparation.

Figure 2(a) shows the negative SALDI mass spectrum of PTB directly deposited on a pencil line on the TLC plate without separation. The pseudomolecular PTB ion ($[M - H]^-$, M = PTB) is shown at m/z 790. PTB can be

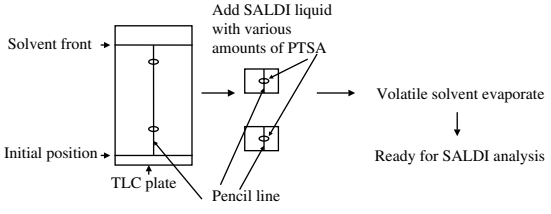

Figure 1. TLC/SALDI sample preparation.

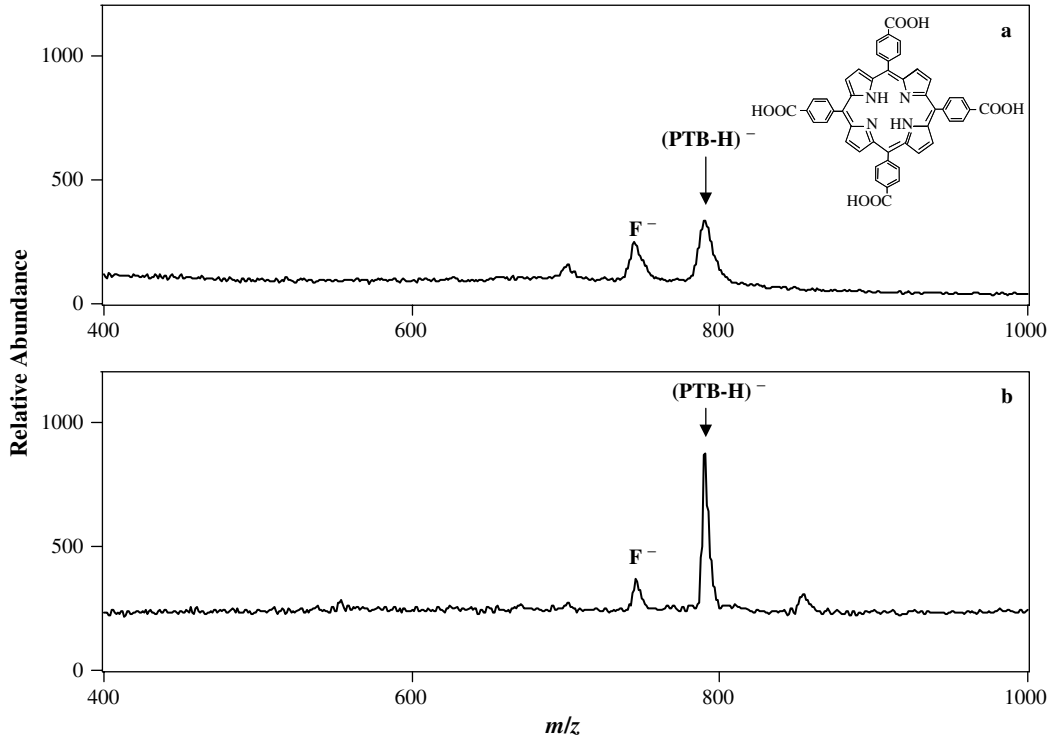

Figure 2. Negative SALDI mass spectra of PTB (50 ng), (a) without PTSA in the SALDI liquid and (b) with 0.1 M PTSA in the SALDI liquid.

observed in both positive and negative ion mass spectra, the signal intensity being higher in the negative than in the positive ion mode. The ion peak at m/z 745 (F^-) corresponds to the fragment of PTB formed losing a carboxylic group. Figure 2(b) shows the negative SALDI mass spectrum of PTB directly deposited on a pencil line on the TLC plate with 0.1 M PTSA in the SALDI liquid matrix. Obviously, the PTB signal is enhanced and the mass resolution is improved in Fig. 2(b) owing to the effects of PTSA. Furthermore, the ratio of the relative signal intensity of PTB to the fragment ion at m/z 745 in Fig. 2(b) exceeded that in Fig. 2(a). Adding PTSA to the SALDI liquid reduces the fragmentation. The ion peak is more intense in Fig. 2(b) than in Fig. 2(a), indicating that the ionization efficiency is enhanced.

Figure 3(a) is the SALDI mass spectrum of another porphine, THP ($MH^+ = 680$, M = THP) for a sample directly irradiated from the pencil line on the TLC plate, with the same sample prepared in the same way as for Fig. 2. The ion peak at m/z 115 is the sodiated glycerol ion, while the peaks at m/z 203 and 277 correspond to the sodiated fragment ions of sucrose and the peak at m/z 365 corresponds to the sodiated sucrose ion. The fragment ions of THP are shown at m/z 301 and 414. The signal intensity of the sodium adduct ion of THP at m/z 702 ($THPNa^+$) exceeds that of the pseudomolecular ion at m/z 680. However, when extra PTSA (0.5 M) was added to the SALDI matrix, the pseudomolecular ion of THP dominated the SALDI mass spectrum [Fig. 3(b)] while the signal intensity of the sodiated THP ion decreased. The fragmentation of THP at m/z 301 and 414 was also reduced. In addition, the mass resolution was also improved.

The TLC/SALDI combination was also examined with the assistance of PTSA additive. Figure 4(a) shows the negative TLC/SALDI mass spectrum of PTB. The sample preparation is described in the Experimental section. The ion peak at m/z 790 corresponds to the pseudomolecular ion of PTB, while the peak at m/z 745 (F^-) is the fragment ion of PTB formed by losing a carboxylic group. Figure 4 shows the negative TLC/SALDI mass spectrum of PTB for a SALDI liquid containing 0.1 M PTSA. The pseudomolecular ion at m/z 790 dominates the mass spectrum. Furthermore, the ion peak is much more intense than in Fig. 4(a), indicating that the desorption/ionization efficiency is enhanced by adding PTSA. The ratio of the relative ion signal intensity of the pseudomolecular PTB ion to the fragment at m/z 745 is also increased. The PTB signal increases as the concentration of PTSA increases in the SALDI liquid. Additionally, the mass resolution of the analyte peak is obviously improved. However, the ion abundance of PTB started to drop as the concentration of PTSA was increased to a certain level. Figure 4(c) shows the TLC/SALDI mass spectrum of PTB, the SALDI liquid matrix containing 1.0 M PTSA. The signal intensity of the pseudomolecular PTB ion is lower than that in Fig. 4(b). A high PTSA density on the matrix surface effectively suppressed the intensity of the analyte ion peaks when the PTSA in the SALDI liquid was increased to a certain concentration.

Figure 5(a) shows the TLC/SALDI mass spectrum of THP. The peak at m/z 680 corresponds to the pseudomolecular ion of THP. The sodiated THP ion is observed at m/z 702, while the alkaline adduct ion of THP ($[[M - H]^-Na^+K^+]^+$) is observed at m/z 740. As the concentrations of PTSA in the SALDI liquid matrix increase, the signal intensity of the pseudomolecular ion of THP (MH^+) was enhanced. The THP dimer appears at m/z 1359 when the SALDI liquid matrix

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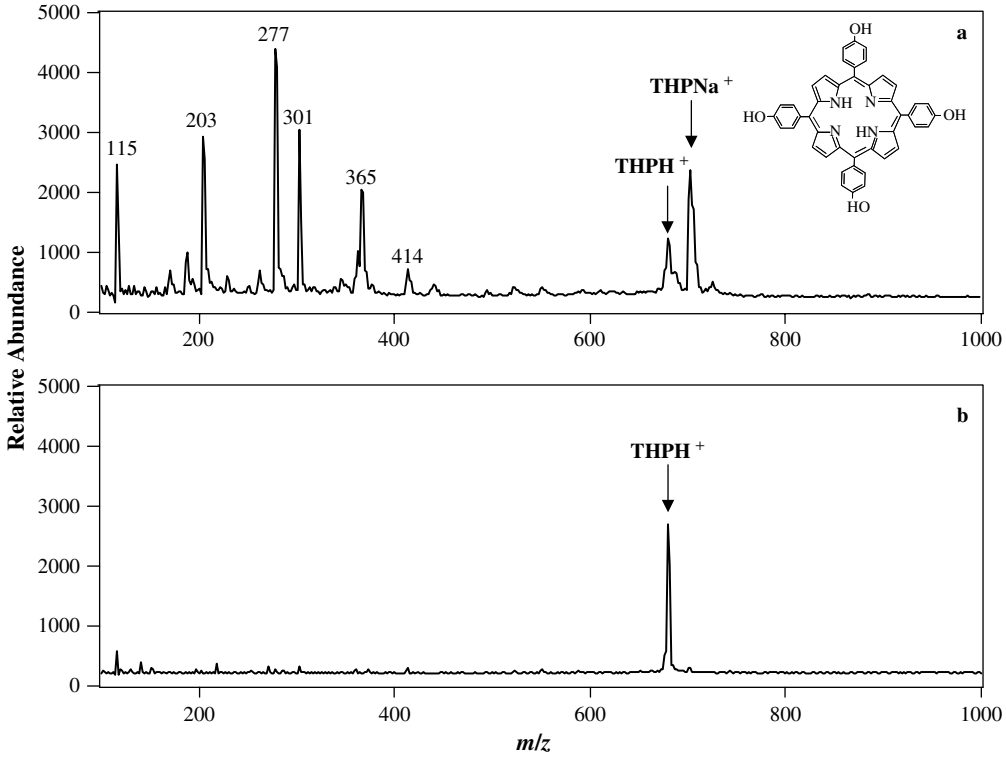

Figure 3. SALDI mass spectra of THP (5 ng), (a) without PTSA in the SALDI liquid and (b) with 0.5 M PTSA in the SALDI liquid.

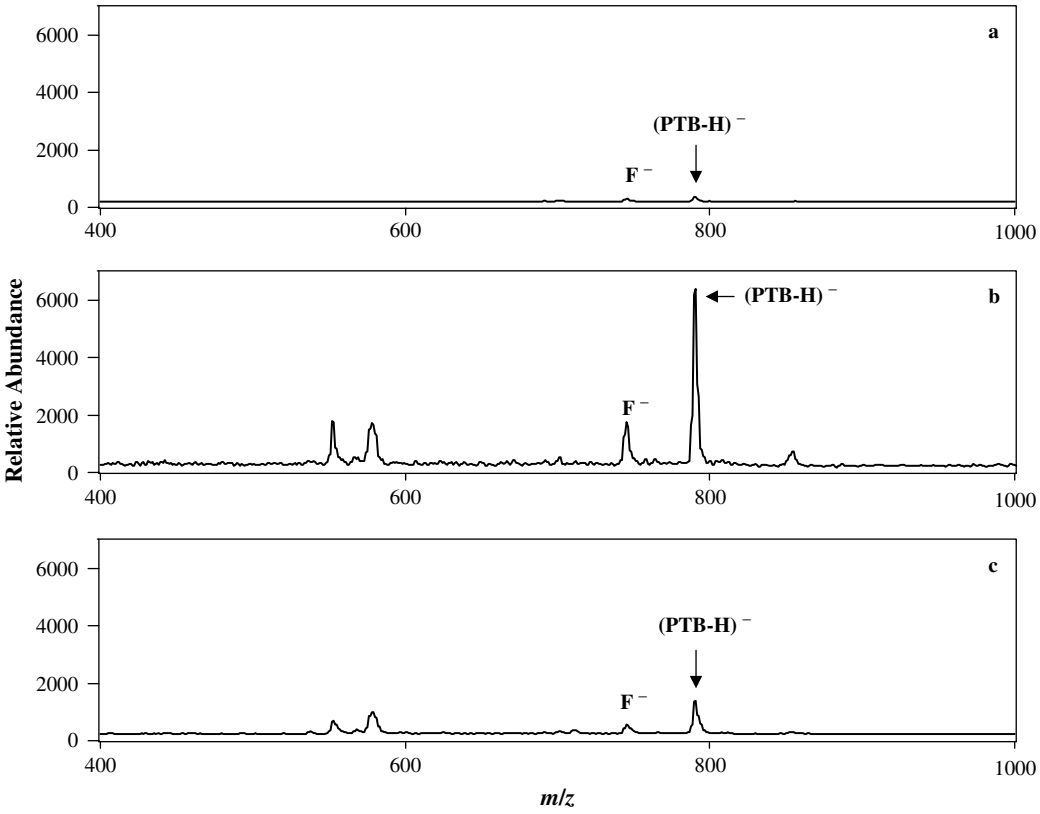

Figure 4. Negative TLC/SALDI mass spectra of PTB (100 ng), (a) without PTSA in the SALDI liquid, (b) with 0.1 M PTSA in the SALDI liquid and (c) with 1.0 M PTSA in the SALDI liquid. Developing solvent B was used in TLC separation.

contained more than 1 M PTSA [Fig. 5(b)]. Furthermore, the ratio of the signal intensity of the pseudomolecular THP ion to the noise intensity was improved as the concentration of PTSA was increased. However, similar to the results in Fig. 4, the intensity of the pseudomolecular THP ion started to decline when the SALDI liquid was spiked with an excess of PTSA. Figure 5(c) shows the TLC/SALDI mass spectra of THP with the SALDI liquid containing 3.0 M PTSA. The

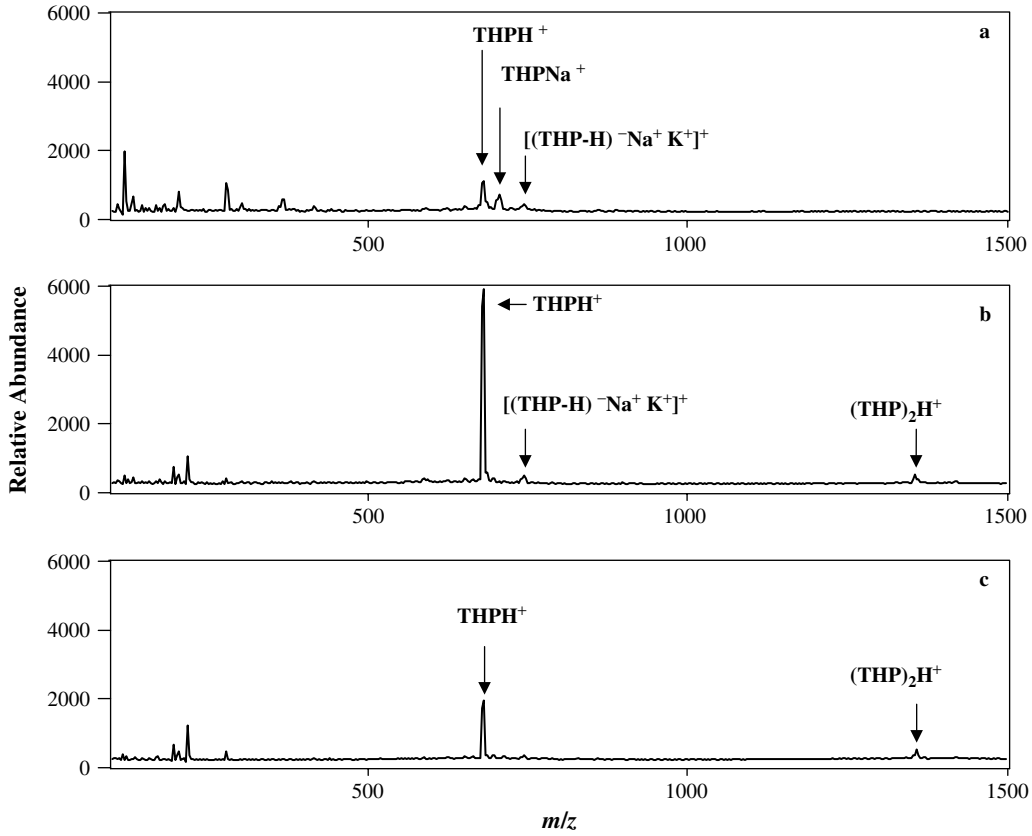

Figure 5. TLC/SALDI mass spectra of THP (50 ng), (a) without PTSA in the SALDI liquid, (b) with 1 M PTSA in the SALDI liquid and (c) with 3 M PTSA in the SALDI liquid. Developing solvent A was used in the TLC separation.

relative ion abundance of THP is lower than in Fig. 5(b). The dimer of THP remains in the mass spectrum. The presence of dimeric ions may be due to the high density of THP ions that formed in the matrix–analyte plasma during laser irradiation. These results account for the finding that the addition of PTSA to the SALDI liquid indeed increases the desorption/ionization efficiency in the SALDI analysis. The detection limit of THP in this TLC/SALDI combination is about 500 pg according to the experimental results. This detection limit is more than 50 times better than that in the conventional TLC/SALDI combination,[12] and is comparable to that of TLC/MALDI.[3,9]

A previous report discussed a possible mechanism by which PTSA enhances analyte signals in SALDI analysis.[16] The characteristics of PTSA including surface activity and acidity enhance the analyte signals in the SALDI analysis. PTSA is especially helpful in enhancing the analyte signal in TLC/SALDI analysis. The surface activity of PTSA allows its molecules to remain on the surface of the liquid matrix and attract charged ions to the surface by ionic interaction. The population of analyte molecules on the surface of the liquid matrix is therefore increased, and the desorption/ionization efficiency of analyte molecules from the matrix surface during laser irradiation is improved. A better desorption/ionization efficiency of analytes, which improves the mass resolution, may result from the analyte molecules remaining on the very top of the surface of the matrix under the ionic attraction of PTSA. Furthermore, the ability of PTSA to provide protons to surrounding analytes in the SALDI matrix promotes ionization toward the analytes. A red shift in the UV absorption spectra of PTSA dissolved in the SALDI liquid was observed as the concentration of PTSA was increased. The red shift occurred because the polarity of the solvent (15% sucrose/glycerol–methanol (1 : 1, v/v) was increased as the PTSA concentration increased in the SALDI liquid owing to the acidity of PTSA. The maximum absorption wavelength of PTSA in the UV spectrum was shifted from 284 to 310 nm as the PTSA concentration changed from 0.01 to 3.0 M. Furthermore, the absorbance at wavelength 337 nm increased from 0.0024 to 0.46 as the concentration varied from 0.01 to 3.0 M. The findings indicate that the energy absorbed and transferred by the SALDI matrix including the PTSA additive is more efficient because the capacity of the SALDI liquid to absorb energy from the laser is improved with increasing PTSA concentration.

These results together demonstrate that the TLC/SALDI combination is a more sensitive method than conventional TLC/SALDI. Furthermore, the operation for implementing this TLC/SALDI combination is very simple. A more homogeneous deposition of carbon powder can be simply achieved by drawing a line with a 2B pencil on the a TLC plate. Thus, searching of analyte signals on the TLC plate was greatly facilitated since the analyte signal appeared only in the presence of both carbon powder and SALDI liquid. Only a very small volume of liquid matrix was deposited on the pencil line and the spot spread in a limited area (diameter 1 mm). Accordingly, the laser was required only

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to irradiate a spot along 1 mm of the pencil line, reducing the time required for the analysis.

CONCLUSIONS

A simplified combination of TLC with SALDI is presented. The approach does not involve too many complicated procedures and special techniques. The ablation of carbon powder from a 2B pencil can be directly employed as a medium for transferring energy. Using the liquid matrix as the matrix system simplifies the preparation of the sample for extraction from the silica gel. Such a matrix can be easily modified by spiking with additives such as PTSA to improve the sensitivity and mass resolution for the analysis. A low detection limit (~500 pg) for porphines is thus achieved. Surfactants with similar characteristics to PTSA should have the same effect as PTSA in improving the analyte signals in TLC/SALDI. Thus, analogous compounds for rendering analyte signals in SALDI are currently under investigation in our laboratory.

Furthermore, the low matrix interference from the SALDI matrix also facilitates analysis. This TLC/SALDI combination can be applied to various kinds of small organics. Further studies for extending the applications are being explored.

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