REGULAR PAPER

Modifications of TLC-MALDI-TOFMS for Lower Detection Limits Comparable with Conventional MALDI-TOFMS

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A TLC-MALDI-TOFMS system has been developed for rapid identification of compounds in a mixture produced in an organic synthetic reaction. In this method, only main compounds were only identified. In the present work, some modifications were added to the TLC-MALDI-TOFMS system in order to detect minor components existing in the mixture at a much lower level. Detection limits attained by this modified system were comparable with that of a conventional MALDI-TOFMS system.

1. Introduction

Thin layer chromatography (TLC) has been widely used, especially in organic synthetic laboratories, to optimize the reaction conditions, by which their target compounds are efficiently produced. The main role of TLC is separation and purification of samples. The identification of samples can be conducted only by the comparison of the *R_f*-values. On the other hand, mass spectrometry (MS) can unequivocally estimate the samples. A combination system of TLC-FABMS has been developed and applied to identify unknown pure compounds separated on TLC plate.¹⁾ Recently, Hercules *et al.* reported a TLC-MALDI-TOFMS system, in which large molecular weight compounds were detected in sub-nanogram range.^{2), 3)} We have proposed a TLC-MALDI-TOFMS system for the identification of unknown oligomeric compounds produced in our laboratory.⁴⁾ However, its detection limit has not yet been discussed. In this work, we added some modifications to the TLC-MALDI-TOFMS system to improve the detection limit. Metal acetylacetonates (acac) were used as test samples, which have been used for analysis of metals. Detection limits observed by the new TLC-MALDI-TOFMS system were comparable with that found in a conventional MALDI-TOFMS system.

2. Experimental

2.1 MALDI-TOFMS

A MALDI-TOFMS instrument used in this study was a Voyager Elite (PE Biosystem, Japan). The MS was operated under the following conditions: accelerating voltage of 20 kV, delayed extraction (DE) time of 200 ns, a UV laser at 337 nm and pulse accumulation of 256. A liner mode was used for low detection limits. The UV laser intensity was adjusted to 2400 (arbitrary unit). The pressure in the ion source and the flight tube were held less than 5×10^{-7} and 5×10^{-8} Torr, respec-

tively.

2.2 TLC plate

TLC plates were purchased from Merck Co. (Germany). A silica gel layer of 0.2 mm thickness was coated on an aluminium plate of 0.1 mm thickness (Silica gel-60). A TLC plate of a rectangle (about 1 cm $\times$ 5 cm) was cut out by scissors. Aluminum back of about 1 mm width appeared by scrubbing the silica gel layer of one side on the TLC plate. Then, a part of the silica gel layer was scrubbed out for making tapered silica gel thin edges as in the previous paper.⁴⁾ Additionally, the tapered silica gel thin edges were shaped like the teeth of a saw (Fig. 1) under a stereoscopic microscope. The distance between the wedges was about 1 mm.

2.3 Reagents

Samples used were chromium(III) acetylacetonate [Cr(III)(acac)₃] and cobalt(III) acetylacetonate [Co(III)(acac)₃] of analytical grade (Wako Chemicals, Japan), which were diluted with acetone at the concentrations of 1 μ g/mL. Dithranol (Merck Co., Germany) dissolved in acetone (10 μ g/mL) was used as a matrix for MALDI. Solvents of acetone, methanol, and methylisobutylketone (MIBK) were of analytical grade (Wako Chemicals, Japan). The reagents were used without further purification.

Fig. 1. Schematic diagram of modified TLC plate for TLC-MALDI-TOFMS.

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2.4 Procedure

With a micro-syringe (1 μL full scale), sample solutions were spotted on the TLC plates. The shape of the silica gel layer was formed like the teeth of a saw as shown in Fig. 1. Samples were developed and separated by migration of the solvent (MIBK) in a tank as the conventional TLC method. After developing, the plates were taken out from the tank, and dried. The plates were rotated 90 degree and put into the other tank containing methanol which could easily dissolve the samples, and the samples were eluted onto the top of the teeth of a saw. The plates were dried and most silica gel layer was scrubbed out except for a part of the layer containing the samples. Then, the matrix solution was deposited by a micro-syringe (10 μL). After being dried, the TLC plate was adhered on a sample holder of the MS by adhesive tape (Nichiban Co., Japan). The sample holder was inserted into the ion source for obtaining the mass spectra.

3. Results and Discussion

Figure 2 shows the MALDI-TOF mass spectra obtained by a conventional method for Cr(III)(acac)_3 , Co(III)(acac)_3 , and their mixture. In the mass spectrum of Cr(III)(acac)_3 (Fig. 2(a)), many cluster peaks appear at m/z 519 $[\text{MatCr}_3(\text{acac})\text{K}]^+$, m/z 599 $[\text{Cr}_2(\text{acac})_5]^+$, m/z 721 $[\text{Cr}_2(\text{acac})_5\text{Na}]^+$, and m/z 838 $[\text{Mat}_2\text{Cr}(\text{acac})_3\text{K}]^+$ in addition to the peaks at m/z 226 $[\text{Mat}]^+$, m/z 250 $[\text{Cr}(\text{acac})_2]^+$, m/z 349 $[\text{Cr}(\text{acac})_3]^+$, m/z 372 $[\text{Cr}(\text{acac})_3\text{Na}]^+$, and m/z 388 $[\text{Cr}(\text{acac})_3\text{K}]^+$, where $[\text{Mat}]$ is an abbreviation of the matrix (Dithranol, $\text{MW}=226$, $[\text{Mat}]=225$). The peak at m/z 599 is used for the calculation of S/N ratio, because the peak is stronger than the molecular peak at m/z 349. The S/N ratio of 505 was obtained. The detection limit calculated was about 3.0 ng for the sample at $\text{S/N}=3$. In the mass spectrum of Co(III)(acac)_3 , the peak of $[\text{Co}_2(\text{acac})_4]^+$ at m/z 514 appears as a base peak (Fig. 2(b)) and the molecular peak of $[\text{Co}(\text{acac})_3]^+$ at m/z 356 is observed with a small intensity.

Fig. 2(a)

Fig. 2(b)

Fig. 2(c)

Fig. 2. Mass spectra of 500 ng samples by conventional MALDI-TOFMS. a): Cr(III)(acac)₃, b): Co(III)(acac)₃, c): mixture of Cr(III)(acac)₃ and Co(III)(acac)₃. Mat: abbreviation of the matrix (Dithranol, MW = 226, [Mat] = 225).

ty, and the peak at m/z 415 is assigned to $[\text{Co}_2(\text{acac})_3]^+$. Additionally, the peaks of $[\text{MatCo}(\text{acac})]^-$ at m/z 382 and $[\text{MatCo}_2(\text{acac})_2]^+$ at m/z 541 are recorded with small intensities. The S/N ratio was 287 for the peak at m/z 514. The detection limit of the sample was about 5.2 ng at S/N = 3, which was higher than that of Cr(III)(acac)₃. The detection limit is dependent upon the characteristics of samples. In the mass spectrum of the mixture of Cr(III)(acac)₃ and Co(III)(acac)₃ (Fig. 2(c)), a characteristic peak appears at m/z 507 $[\text{CoCr}(\text{acac})_4]^-$ as a base peak, which might be resulted from a exchange reaction. The S/N ratio was 740 and the detection limit of about 2 ng was calculated for the peak.

Figure 3 shows mass spectra of Cr(III)(acac)₃ and Co(III)(acac)₃ obtained by the newly developed TLC-MALDI-TOFMS system. The samples were developed and separated by MIBK⁵⁾ as a conventional TLC method and eluted by methanol on the top of the silica gel layer shaped like the teeth of a saw. The mass spectra in Figs. 3(a, b, c, and d) were recorded at R_f = 0.45, 0.47, 0.50, and 0.53 on the TLC plate, respectively. The mass spectrum at R_f = 0.47 is similar to that of Cr(III)(acac)₃ (Fig. 2(a)) and gives a S/N ratio of 1241 for the peak at m/z 599. The calculated detection limit for the peak is 1.2 ng at S/N = 3. The S/N ratios of the both sides at R_f = 0.45 and 0.50 are 29 and 292, respectively. No peak except matrix peak is recorded at R_f = 0.53. These observations imply that the sample is Cr(III)(acac)₃, and mainly concentrated on the wedge at R_f = 0.47. Figs. 3 (e, f, and g) shows the mass spectra obtained at R_f = 0.29, 0.32, and 0.35, respectively. The sample at R_f = 0.32 is assigned to Co(III)(acac)₃ by comparison of the mass spectra in Fig. 2(b) and Fig. 3(f). The S/N ratio of the mass spectrum in Fig. 3(f) is 737 for the peak at m/z 514. The detection limit of 2.0 ng is obtained for the peak. The S/N ratios in Figs 3(e) and

(g) are 78 and 239. The peak at m/z 514 was not recorded at R_f = 0.38 (not shown). These observations indicate that Co(III)(acac)₃ was eluted by methanol and concentrated mainly on the wedge of R_f = 0.32.

We have reported a TLC-MALDI-TOFMS system in an early paper,⁴⁾ in which tapered TLC plates without the wedge were used. Detection limits were also measured for the tapered TLC plates. We obtained the S/N ratios of 532 and 206 for Cr(III)(acac)₃ and Co(III)(acac)₃, respectively. These values were comparable to the values obtained by conventional MALDI-TOFMS (Figs. 2(a), (b)).

In the mass spectrum of a mixture of Cr(III)(acac)₃ and Co(acac)₃ (Fig. 2(c)), an adduct peak is observed at m/z 507 $[\text{CoCr}(\text{acac})_4]^-$. The species, however, could not be detected by NMR for a mixture of dithranol (used as the matrix), Cr(III)(acac)₃, and Co(III)(acac)₃ in acetone-*d*₆. These observations imply that the adduct ion may be resulted from the reaction of the samples and the matrix in the plume formed after irradiation of the laser light. Therefore, identification of a compound in a mixture by MALDI-TOFMS would be more difficult by EI and FAB-MS. However, the present TLC-MALDI-TOFMS system would give more simple mass spectra, because a mixture is separated and concentrated on the wedges and no exchange reaction between samples occurs

4. Conclusion

A TLC plate was modified for obtaining a low detection limit in a TLC-MALDI-TOFMS system. The Silica gel layer on the TLC plate was tapered and shaped like the teeth of a saw (wedge) under a stereoscopic microscope. Then, samples were concentrated on the wedges of the silica gel layer. Lower detection limits were attained by the TLC-MALDI-TOFMS system developed in this study compared to that found by the

Fig. 3(a)

Fig. 3(b)

Fig. 3(c)

(Fig. 3(a), 3(b), and 3(c) captions on p. 132)

Fig. 3(d)

Fig. 3(e)

Fig. 3(f)

(Fig. 3(d), 3(e), and 3(f) captions on p. 132)

S/N = 239

Fig. 3(g)

Fig. 3. Mass spectra by developed TLC-MALDI-TOFMS. a): at $R_f=0.45$, b): at $R_f=0.47$, c): at $R_f=0.50$, d): at $R_f=0.53$, e): at $R_f=0.29$, f): at $R_f=0.32$, g): at $R_f=0.35$.

conventional MALDI-TOFMS system. The detection limits measured by the early reported TLC-MALDI-TOFMS method, which had no wedge on the plates, were comparative to that obtained by the conventional MALDI-TOFMS system.

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