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Detection of Long Alkyl Esters of Succinic and Maleic Acid Using TLC-MALDI-MS

Jinhee Kim, Sang-pil Han, Jeongkwon Kim,* and Yeong-Joon Kim*

Department of Chemistry, Chungnam National University, Daejeon 305-764, Korea
**E-mail: jkkim48105@cnu.ac.kr; y2kim@cnu.ac.kr*
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Four esters of succinic and maleic acid were synthesized, separated by thin-layer chromatography (TLC), and identified using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). A comparison of matrix materials showed that 2,6-dihydroxybenzoic acid (2,6-DHB) yielded a greater ionization efficiency than 2,5-DHB prior to TLC separation. The location of each ester sample on the TLC plate was estimated by comparing the developed plate with a duplicate plate that had been visualized by immersion in a $KMnO_4$ solution. Generally, mass spectra obtained from the $KMnO_4$-visualized plate were relatively poor. Reproducible mass spectra with high peak abundance were difficult to obtain using the 2,6-DHB matrix from crude synthetic esters extracted from the TLC plates. Significant improvements in both reproducibility and sensitivity were realized by using pencil lead as the MALDI matrix. The current methodology will be beneficial to organic chemists since it can provide a guideline for simple and rapid characterization of small organic compounds.

Key Words : TLC, MALDI, Pencil lead, Esters, Mass spectrometry

Introduction

Succinic acid is an intermediate in the chemical transformation of several industrially useful chemicals including adipic acid, diols, tetrahydrofuran, N-methyl pyrrolidinone, and 2-pyrrolidinone. Succinic acid can be isolated as a metabolite from plants, animals, and microorganisms. Recently, many reviews have shown that fermentative production of succinic acid can be more cost-effective than petroleum-based production schemes.[1-3] This type of "green" technology is becoming more popular as a means of decreasing pollution caused by many petrochemical processes. Dialkyl esters of succinic acid are widely used in detergents, cosmetics, pharmaceuticals, and processed foods.[1-3] For example, dialkyl ester derivatives of diacids including succinic acid make excellent lubricants and can enhance the quality of diesel fuel when used as additives.[4-6] Similar approaches using fatty acid derivatives, commonly known as biodiesel, have recently been reported.[7-9]

Thin-layer chromatography (TLC) is a simple and convenient separation system that is widely used in tracking organic syntheses and separating natural product mixtures.[10] While the separation efficiency of TLC is not comparable to that of high-performance liquid chromatography (HPLC), TLC is inexpensive, simple, and fast. Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) is used extensively for identifying relatively large biomolecules.[11] Although protonation of neutral or anionic compounds is not favored in positive-ion-mode MALDI, they can be ionized using various cationization agents.[12] Compared with electrospray ionization, MALDI is fast and sensitive. Recently, TLC-MALDI has been used in analyses of peptides,[13] lipids,[14,15] oligosaccharides,[16] and medicines.[17] Additionally, matrix-free TLC-laser desorption/ionization (LDI) has been used in the analysis of alkaloids.[10]

Among the various matrices available for MALDI analyses, 2,5-dihydroxybenzoic acid (2,5-DHB) is one of the most common for analyzing proteins, peptides, and polyethylene glycol derivatives.[18] Recently, 2,6-dihydroxybenzoic acid (2,6-DHB) has also been used for the detection of oligonucleotides,[19] polysaccharides,[19] and lipids.[20] Graphite has also been introduced as an alternative matrix in MALDI-MS analyses of peptides, proteins, oligosaccharides, and synthetic polymers.[21-23] Recently wood charcoal was also utilized as an alternative matrix to analyze arginine, glucose, sucrose, and polyethylene glycols.[24] Applications using ultrafine particles, such as graphite, as matrix materials are often referred to as surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) to distinguish the technique from MALDI-MS, which employs organic liquid matrices.[25] In this article, "MALDI" will be used as a general term to describe various laser-induced desorption/ionization techniques using 2,5-DHB, 2,6-DHB, or pencil lead matrices. Graphite particles have been used as a matrix material in TLC-MALDI analyses of tetracyclines.[17] Because powdered graphite is not easy to handle, pencil lead has recently been used as a new matrix material.[26,27] Pencil lead has also been used in the TLC separation and MALDI analysis of porphines, where a line was pre-drawn with a pencil on the TLC plate where the sample was separated, followed by laser irradiation on the pencil line to obtain mass spectra.[28]

The current study describes, to our knowledge, the first reported detection of esters using TLC-MALDI-MS with a pencil lead matrix. Dialkyl succinates and maleates were synthesized, separated using TLC, and identified using

MALDI-MS. The relative performance of 2,5-DHB, 2,6-DHB, and pencil lead matrices was evaluated.

Experimental

Materials. 2,5-DHB, 2,6-DHB, sodium trifluoroacetate (NaTFA), trifluoroacetic acid (TFA), maleic anhydride, 1-dodecanol, and 1-hexadecanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and succinic acid were purchased from DUKSAN (Seoul, Korea). P-toluenesulfonic acid monohydrate and sodium chloride (NaCl) were purchased from SAMCHUN (Seoul, Korea). Sodium sulfate was purchased from DAE JUNG (Kyunggi-Do, Korea). Aluminum-backed silica gel TLC plates (catalog No. 1.0555) were obtained from Merck (Darmstadt, Germany). Pencil leads (0.5 mm HB) were obtained from Dongapen (Deajeon, Korea).

Synthesis of Esters. Figure 1 shows the structures of the four dialkyl esters synthesized. A detailed synthesis and characteristics of each ester are given below.

Didodecyl Succinate (DDS): Succinic acid (300 mg, 2.54 mmol) and 1-dodecanol (1.35 mL, 7.62 mmol) were dissolved in 10 mL toluene. After adding p-toluenesulfonic acid (145 mg, 0.762 mmol) to the mixture as a catalyst, the mixture was refluxed at 120 °C for 1 h. The solution was diluted with water and diethyl ether and the aqueous layer was extracted several times with diethyl ether. The combined organic layers were dried with sodium sulfate and the products were recrystallized from a methanol solution. The product was obtained in 93% yield (1.07 g); mp 40 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (t, 4H, J = 7 Hz), 2.62 (s, 4H), 1.61 (m, 4H), 1.25 (m, 36H), 0.88 (t, 6H, J = 6 Hz).

Dihexadecyl Succinate (DHS): Succinic acid (300 mg, 2.54 mmol) and 1-hexadecanol (1.84 g, 7.62 mmol) were dissolved in 10 mL toluene. After adding p-toluenesulfonic acid (145 mg, 0.762 mmol) to the mixture as a catalyst, the mixture was refluxed at 120 °C for 1 h. The solution was diluted with water and diethyl ether and the aqueous layer was extracted several times with diethyl ether. The combined organic layers were dried with sodium sulfate and the products were recrystallized from a methanol solution. The product was obtained in 97% yield (1.39 g); mp 59 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (t, 4H, J = 7 Hz), 2.62 (s, 4H), 1.61 (m, 4H), 1.25 (m, 52H), 0.88 (t, 6H, J = 7 Hz).

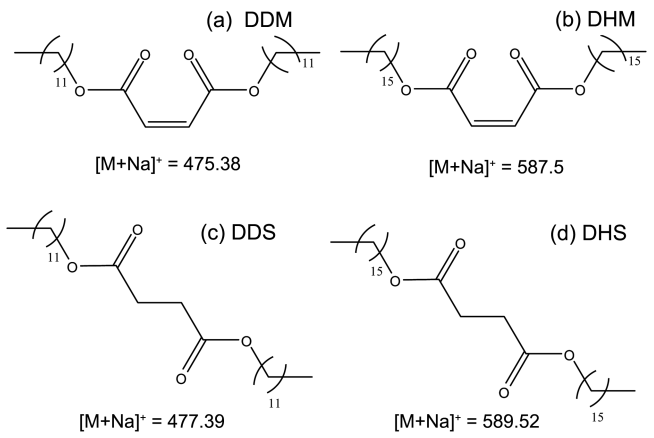


Figure 1. Structures of (a) didodecyl maleate (DDM), (b) dihexadecyl maleate (DHM), (c) didodecyl succinate (DDS), and (d) dihexadecyl succinate (DHS). The theoretical monoisotopic masses of sodium adducts for the esters are also shown.

Didodecyl Maleate (DDM): Maleic anhydride (249 mg, 2.54 mmol) and 1-dodecanol (1.35 mL, 7.62 mmol) were dissolved in 10 mL toluene. After adding *p*-toluenesulfonic acid (249 mg, 2.54 mmol) to the mixture, the mixture was refluxed at 130 °C for 6 h. The solution was diluted with 10 mL methanol. The volatile compounds were vacuumed off and the products were recrystallized from a methanol solution. The product was obtained in 81% yield; mp 31 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (s, 2H), 4.17 (t, 4H, J = 7 Hz), 1.67 (m, 4H), 1.26 (m, 36H), 0.88 (t, 6H, J = 7 Hz).

Dihexadecyl Maleate (DHM): Maleic anhydride (249 mg, 2.54 mmol) and 1-hexadecanol (1.84 g, 7.62 mmol) were dissolved in 10 mL toluene. After adding *p*-toluenesulfonic acid (249 mg, 2.54 mmol), the mixture was refluxed at 130 °C for 6 h. The solution was diluted with 10 mL methanol. The volatile compounds were pumped off and the products were recrystallized from a methanol solution. The product was obtained in 77% yield; mp 52 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (s, 2H), 4.17 (t, 4H, J = 7Hz), 1.66 (m, 4H), 1.26 (m, 52H), 0.87 (t, 6H, J = 7 Hz).

Purification of Esters. Each of the crude synthetic esters was first dissolved in ethyl acetate to a concentration of 10 mg/mL and recrystallized three times from methanol. The recrystallized product was then dissolved in ethyl acetate to 10 mg/mL.

MALDI of Purified Esters. A three-layer preparation method was used to deposit the purified esters onto the MALDI plate. The first layer consisted of 1 μL of the DHB matrix (2,5-DHB or 2,6-DHB at 10 mg/mL in pure ACN with 0.1% TFA). The first layer was allowed to dry and a second layer, consisting of a 1-μL aliquot of a mixture containing 0.5 μL ester and 0.5 μL matrix, was added. Finally, 1 μL of the cationization agent (10 mM NaTFA in water) was added to the top of the sample plate.

Combination of TLC with MALDI-MS. Each of the synthesized esters was dissolved in ethyl acetate to a concentration of 10 mg/mL. The dissolved ester was spotted onto a TLC plate (1 cm × 4 cm) using a capillary tube. The TLC plate was then developed in an ethyl acetate/hexane (1:4) mixture to a distance of 5 cm. To visualize the spots, the TLC plate was placed immediately into a 66-mg/mL $KMnO_4$ solution, followed by heating at 150 °C for 30 s in an oven.[29,30] R_f values for each product were calculated using the visualized spot for each product. These values were then used to estimate the location of samples on the TLC plate that had not been exposed to $KMnO_4$. The TLC plates were placed on a stainless steel MALDI plate with double-sided, conductive, adhesive tape.

Caution. The TLC plates must be firmly attached to the MALDI plate. Loosely attached TLC plates may come off of

the MALDI plate and severely damage the MALDI-MS system.

To obtain MALDI mass spectra, one drop (~20 μL) of 10 mg/mL 2,6-DHB solution was first deposited onto the TLC sample spot and allowed to dry. This was followed by a drop (~20 μL) of 10 mM NaTFA. When evaluating the pencil lead as an alternative matrix, the pencil lead was scribbled onto the TLC sample spot, followed by one drop (~20 μL) of aqueous, saturated NaCl. All mass spectra were obtained using a Kratos Axima CFR (Shimadzu, Japan) time-of-flight mass spectrometer equipped with a 337-nm nitrogen laser in a positive linear mode.

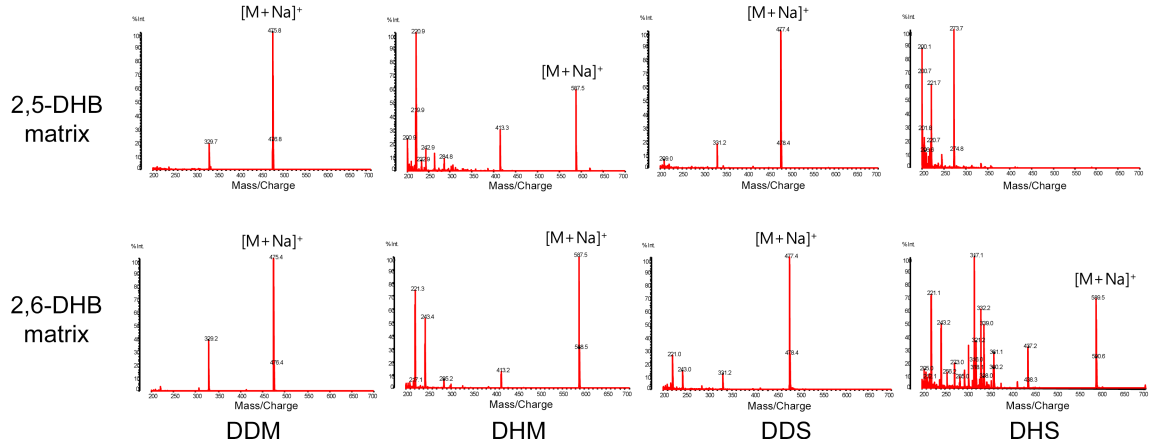


Figure 2. MALDI mass spectra of DDM, DHM, DDS, and DHS using a 2,5-DHB matrix (upper spectra) and a 2,6-DHB matrix (lower spectra). No peak from DHS was observed with the 2,5-DHB matrix.

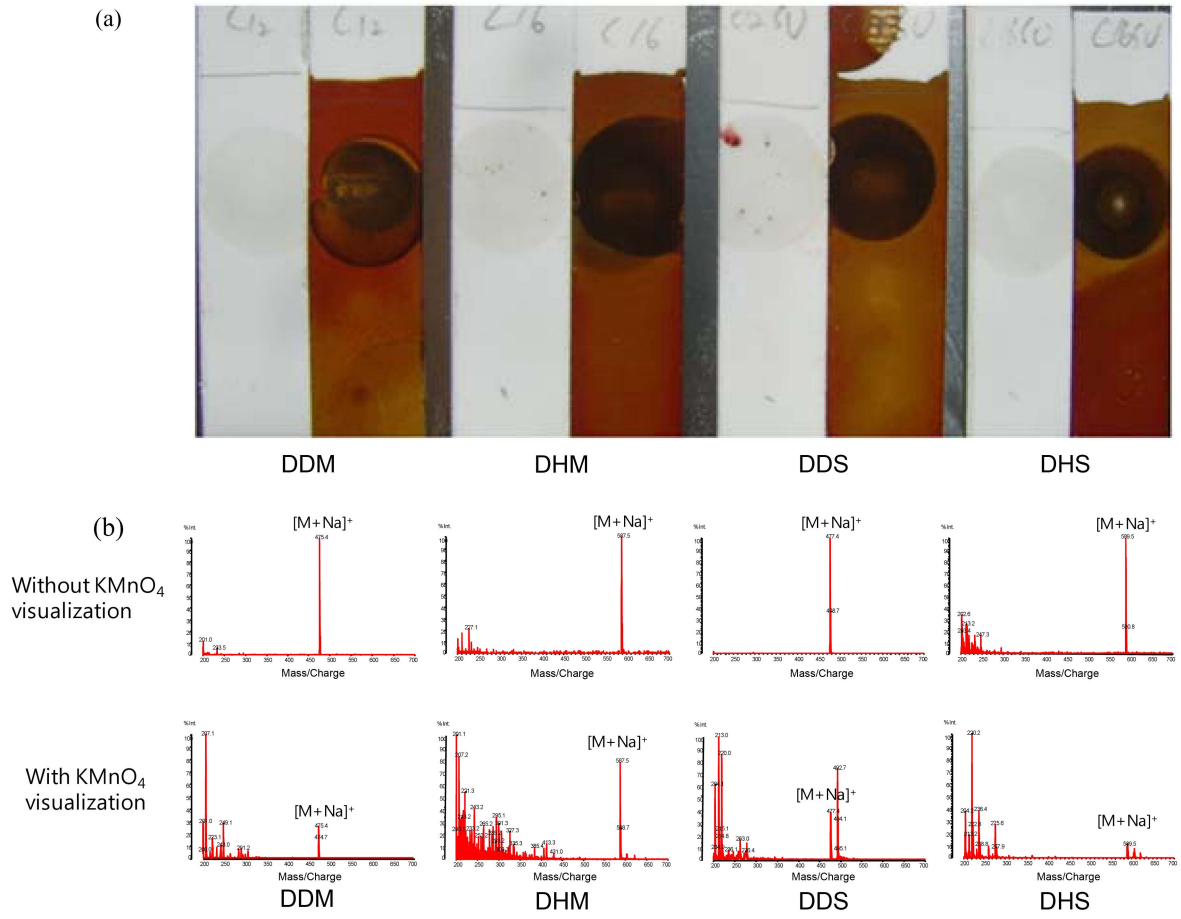


Figure 3. MALDI analyses of purified synthetic esters with a 2,6-DHB matrix. (a) TLC plates with (right) and without (left) $KMnO_4$ visualization. The circle in the middle of each TLC plate is due to the addition of the 2,6-DHB solution. (b) MALDI mass spectra were obtained directly from the TLC plate with (lower spectra) and without (upper spectra) $KMnO_4$ visualization. The locations of sample spots on the TLC plates without $KMnO_4$ visualization were estimated from plates that had been exposed to $KMnO_4$.

Results and Discussion

Matrix Selection. Generally, 50% ACN in acidified water (0.1% TFA) is used to prepare matrix solutions. However, in the current study, we used a matrix solution containing 0.1% TFA in pure ACN to prevent potential hydrolysis of the esters during sample preparation. The relative performance of 2,5-DHB and 2,6-DHB matrices for analyses of the purified synthetic esters is shown in Figure 2. Similar performance was observed for both matrices and with all esters, with the exception of DHS. The 2,6-DHB matrix exhibited significantly better performance for DHS than did 2,5-DHB. No peak characteristic of DHS was detected using the 2,5-DHB matrix. Thus, 2,6-DHB was used in all further analyses.

TLC-MALDI of Purified Synthetic Esters. Purified synthetic esters were first separated by TLC. Figure 3(a) shows the TLC plates before and after $KMnO_4$ visualization followed by the addition of a drop of 2,6-DHB matrix and a drop of 10 mM NaTFA for DDM, DHM, DDS, and DHS. Because the esters are colorless and do not exhibit any intrinsic fluorescence, it was not possible to locate the spots prior to visualization. However, the visualized spots were used to estimate the location of sample spots on an identical TLC plate that had not been exposed to $KMnO_4$. Figure 3(b) shows MALDI mass spectra of the purified synthetic esters following TLC separation with and without $KMnO_4$ visualization. TLC plates that had not been exposed to $KMnO_4$ exhibited higher signal-to-noise ratios and lower background interference. Also note that the quality of spectra obtained following TLC separation without $KMnO_4$ visualization was higher than those obtained without prior separation (Fig. 2).

TLC-MALDI of Crude Synthetic Esters. Crude synthetic esters were analyzed using TLC-MALDI in the same way as the purified synthetic esters. Figure 4(a) shows the TLC plates with and without $KMnO_4$ visualization for the crude synthetic esters, followed by the addition of a drop of 2,6-DHB matrix and a drop of 10 mM NaTFA. Figure 4(b) shows MALDI mass spectra of these crude synthetic esters after separation on a TLC plate. As above, $KMnO_4$ visualization resulted in higher background noise and poorer reproducibility in the MALDI-MS spectra. No peak for DHM or DDS was detected with $KMnO_4$ visualization. The poor performance following $KMnO_4$ visualization is likely due to chemical modification of the esters by $KMnO_4$ or interference by the high salt concentration of the $KMnO_4$

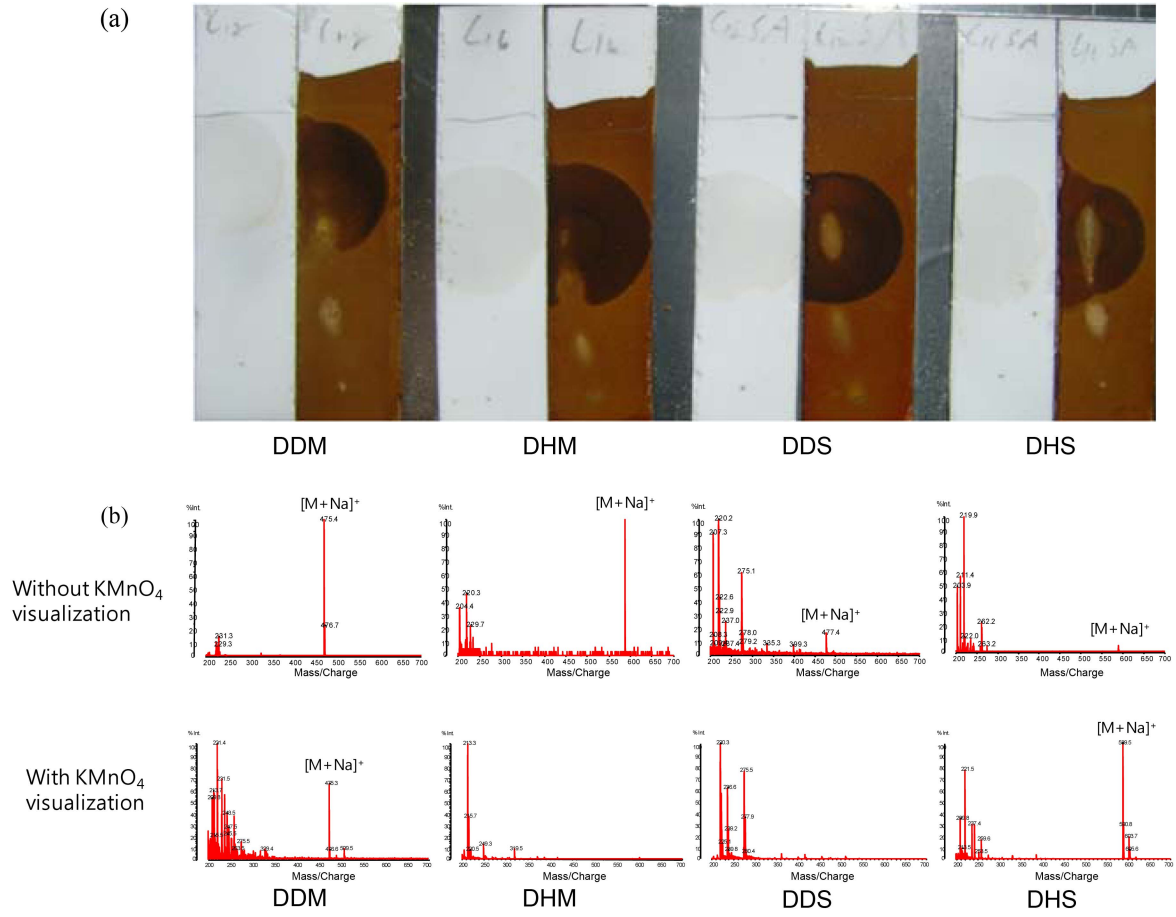


Figure 4. MALDI analyses of crude synthetic esters with a 2,6-DHB matrix. (a) TLC plates with (right) and without (left) $KMnO_4$ visualization. The circle in the middle of each TLC plate is due to the addition of the 2,6-DHB solution. (b) MALDI mass spectra were obtained directly from the TLC plate with (lower spectra) and without (upper spectra) $KMnO_4$ visualization. The locations of sample spots on the TLC plates without $KMnO_4$ visualization were estimated from plates that had been exposed to $KMnO_4$.

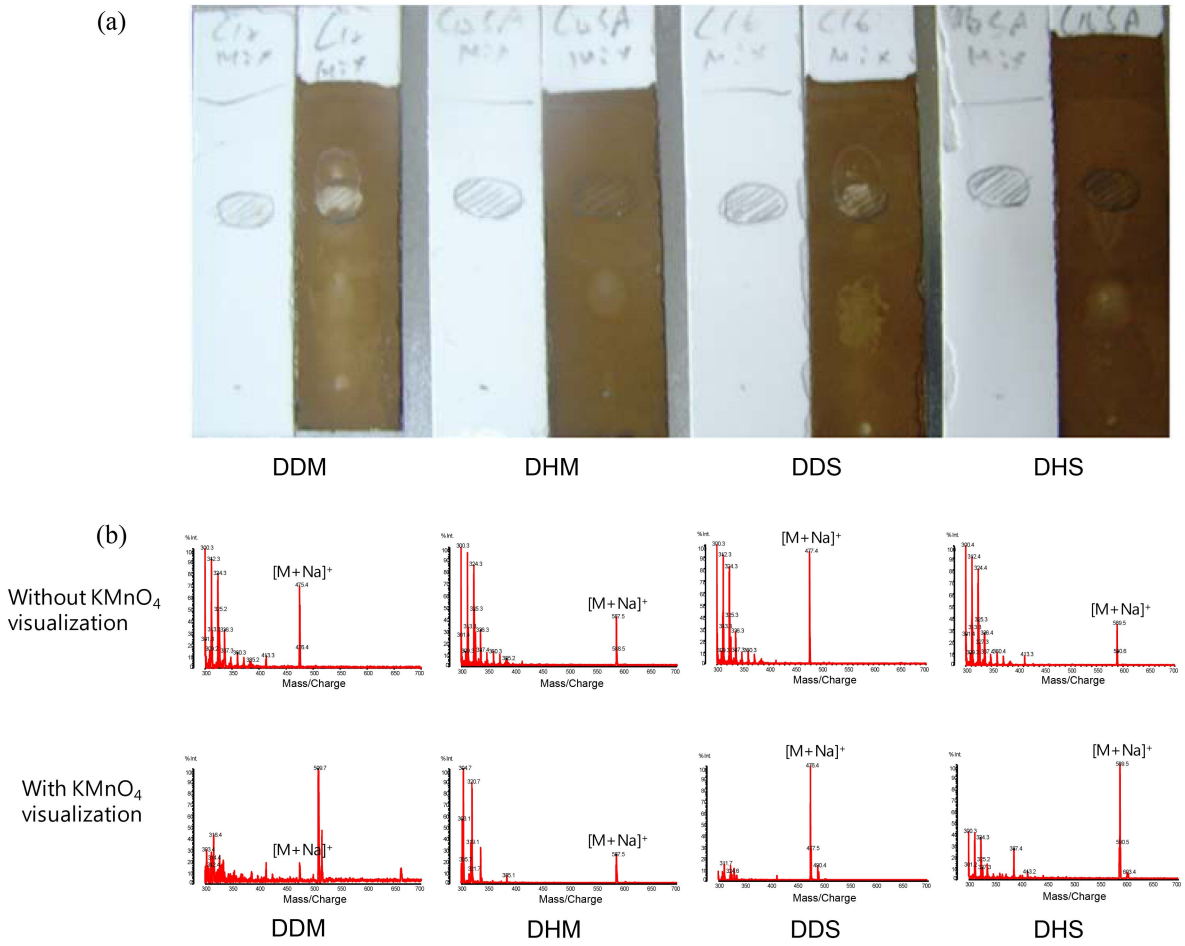


Figure 5. MALDI analyses with a pencil lead matrix. (a) TLC plates with (right) and without (left) $KMnO_4$ visualization. The photos were taken after scribbling with pencil lead. (b) MALDI mass spectra were obtained directly from the TLC plate with (lower spectra) and without (upper spectra) $KMnO_4$ visualization. The locations of sample spots on the TLC plates without $KMnO_4$ visualization were estimated from plates that had been exposed to $KMnO_4$.

solution. However, $KMnO_4$ visualization was necessary to locate the samples on the TLC plates.

The MALDI mass spectra of the separated crude synthetic esters exhibited much lower intensity peaks, particularly for DDS and DHS, and higher background noise levels relative to those obtained with the purified synthetic esters. It was difficult to identify sections of each sample that would provide abundant and reproducible signals even without $KMnO_4$ visualization. This may be due to interference from side-products in the crude preparation.

TLC-MALDI of Crude Synthetic Esters Using a Pencil Lead Matrix. In the absence of $KMnO_4$ visualization, which decreased system performance, sample spots on the TLC plate were circled with pencil prior to loading the TLC-MALDI plate into the mass spectrometer. High-intensity signals were repeatedly obtained on or near the pencil line despite the fact that the esters should have been in the center of the circled area, based on comparisons with $KMnO_4$-visualized plates. It is possible that the esters spread out as a result of adding the 2,6-DHB matrix solution because lateral spreading of analytes, caused by using a liquid matrix, has been observed.[10,17,31] However, no ester signal was observed at the edges of the sample spots unless coincident with the pencil line. This suggested that the pencil lead was in fact a more suitable matrix than 2,6-DHB.

Thus, in place of the 2,6-DHB matrix solution, pencil lead was scribbled over the sample spot ensuring that the whole sample surface was covered, as shown in Figure 5(a). Figure 5(b) shows mass spectra of the crude synthetic esters obtained from TLC-MALDI analyses using the pencil lead matrix. Strong ester signals were obtained from nearly all locations within the sample spot. Signals were significantly higher than those obtained with the 2,6-DHB matrix (Fig. 4(b)). Signal intensity was further enhanced by substituting a drop of saturated NaCl in place of the 10 mM NaTFA as a cationization agent (data not shown). Figure 5(b) shows that plates that had been visualized with $KMnO_4$ yielded similar quality spectra as TLC plates that had not been exposed to $KMnO_4$, indicating that the pencil lead matrix apparently overcame the interference from $KMnO_4$. Background signal levels from the pencil lead were observed in the low *m/z* region at *m/z* 300, 312, 324, and 336 (Fig. 5). These background signals did not overlap with the signals from the synthetic esters.

Advantages of TLC-MALDI Using Pencil Lead. TLC is a relatively simple separation technique, relative to LC or GC. Additionally, samples can be stored on TLC plates for further analyses, which is not possible in other chromatography techniques.[17] Although the use of pencil lead as a MALDI matrix has been reported,[26,27] it is far from common. To our knowledge, only one article describes the use of pencil lead as a TLC-MALDI matrix, where a pencil line was pre-drawn on the TLC plate for the analysis of porphines.[28] Because liquid matrices are known to cause lateral spreading on TLC plates,[10,17,31] the use of a solid matrix such as pencil lead is advantageous. Scribbling the pencil lead over the TLC plate may also provide a simpler and more homogeneous means of distributing the solid matrix than applications of carbon powder suspensions.[28] In the current study, pencil lead was an effective matrix in analyses of esters using TLC-MALDI-MS.

Conclusions

Four dialkyl esters were synthesized and systematically separated and analyzed by TLC-MALDI-MS. Each of the four esters was synthesized via combinatorial reactions of succinic acid or maleic anhydride with 1-dodecanol or 1-hexadecanol, and separated from side-products or residual reactants using TLC. Among the various matrices used in this study (2,5-DHB, 2,6-DHB, and pencil lead), pencil lead yielded the best performance. MALDI samples were prepared by scribbling the pencil lead over the top layer of the sample area on the TLC plate, followed by the addition of a drop of saturated NaCl as a cationization agent. Laser irradiation of sample spots with pencil lead yielded high-abundance signals from each of the esters. Because no lateral spreading was observed with the pencil lead matrix, signal strength was homogeneous throughout the sample spots.

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References

1. Takahashi, K.; Sakano, H.; Numata, N.; Kuroda, S.; Mizuno, N. *Drug Dev. Ind. Pharm.* **2002**, *28*, 1285.
2. Zeikus, J. G.; Jain, M. K.; Elankovan, P. *Appl. Microbiol. and Biot.* **1999**, *51*, 545.
3. Song, H.; Lee, S. Y. *Enzyme Microb. Technol.* **2006**, *39*, 352.
4. Anastopoulos, G.; Lois, E.; Zannikos, F.; Kalligeros, S.; Teas, C. *Tribol. Int.* **2001**, *34*, 749.
5. Trofimov, V. A.; Spirkin, V. G.; Bocharov, A. A.; Trofimova, M. V. *Chem. Tech. Fuels Oils* **1999**, *35*, 18.
6. Trofimov, V. A.; Spirkin, V. G.; Kozhekina, E. A.; Bocharov, A. A. *Chem. Tech. Fuels Oils* **1996**, *32*, 30.
7. Anastopoulos, G.; Lois, E.; Karonis, D.; Zanikos, F.; Kalligeros, S. *Ind. End. Chem. Res.* **2000**, *40*, 452.
8. Anastopoulos, G.; Lois, E.; Serdari, A.; Zanikos, F.; Stournas, S.; Kalligeros, S. *Energ. Fuel.* **2000**, *15*, 106.
9. Drown, D. C.; Harper, K.; Frame, E. *J. Am. Oil Chem. Soc.* **2001**, *78*, 579.
10. Shariatgorji, M.; Spacil, Z.; Maddalo, G.; Cardenas, L. B.; Ilag, L. L. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3655.
11. Hong, J. M.; Lee, A.; Han, H.; Kim, J. *Anal. Biochem.* **2009**, *384*, 368.
12. Cvacka, J.; Svatos, A. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2203.
13. Panchagnula, V.; Mikulskis, A.; Song, L.; Wang, Y.; Wang, M.; Knubovets, T.; Scrivener, E.; Golenko, E.; Krull, I. S.; Schulz, M.; Heinz Emil, H.; Patton, W. F. *J. Chromatogr. A* **2007**, *1155*, 112.
14. Muller, M.; Schiller, J.; Petkovic, M.; Oehrl, W.; Heinze, R.; Wetzker, R.; Arnold, K.; Arnhold, J. *Chem. Phys. Lipids* **2001**, *110*, 151.
15. Fuchs, B.; Schiller, J.; Suess, R.; Zscharnack, M.; Bader, A.; Mueller, P.; Schuerenberg, M.; Becker, M.; Suckau, D. *Anal. Bioanal. Chem.* **2008**, *392*, 849.
16. Nimptsch, K.; Suss, R.; Riemer, T.; Nimptsch, A.; Schnabelrauch, M.; Schiller, J. *J. Chromatogr. A* **2010**, *1217*, 3711.
17. Crecelius, A.; Clench, M. R.; Richards, D. S.; Parr, V. *J. Chromatogr. A* **2002**, *958*, 249.
18. Lee, A.; Yang, H. J.; Kim, Y.; Kim, J. *Bull. Korean Chem. Soc.* **2009**, *30*, 1127.
19. Jessome, L.; Hsu, N. Y.; Wang, Y. S.; Chen, C. H. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 130.
20. Schiller, J.; Suss, R.; Fuchs, B.; Muller, M.; Petkovic, M.; Zschornig, O.; Waschipky, H. *Eur. Biophys. J. Biophys. Lett.* **2007**, *36*, 517.
21. Harvey, D. J. *Mass Spectrom. Rev.* **1999**, *18*, 349.
22. Nielen, M. W. F. *Mass Spectrom. Rev.* **1999**, *18*, 309.
23. Zhu, Y. F.; Lee, K. L.; Tang, K.; Allman, S. L.; Taranencko, N. I.; Chen, C. H. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1315.
24. Lee, S. Y.; Kim, J.; Yang, H. J.; Shin, S.; Hong, J. M.; Kim, J. *Mass Spectrom. Lett.* **2010**, *1*, 33.
25. Sunner, J.; Dratz, E.; Chen, Y. C. *Anal. Chem.* **1995**, *67*, 4335.
26. Black, C.; Poile, C.; Langley, J.; Herniman, J. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1053.
27. Langley, G. J.; Herniman, J. M.; Townell, M. S. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 180.
28. Wu, J. Y.; Chen, Y. C. *J. Mass Spectrom.* **2002**, *37*, 85.
29. Gumieniczek, A.; Przyborowski, L. *Acta Pol. Pharm.* **1994**, *51*, 429.
30. Volf, K.; Pitr, K. *Soud Lek* **1992**, *37*, 25.
31. Peng, S.; Edler, M.; Ahlmann, N.; Hoffmann, T.; Franzke, J. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2789.