

Molecularly Imprinted TiO₂-Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry for Selectively Detecting α -Cyclodextrin

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This study describes a new means to conduct molecular recognition-based analysis using mass spectrometry. Taking advantage of the unique characteristic of the absorption capacity of the TiO₂ sol-gel material in the UV region, a TiO₂ sol-gel-deposited thin film was employed as the sample substrate to assist in UV laser desorption/ionization of analytes. Sol-gels are polymeric materials that are easy to prepare and modify at low temperatures. Molecularly imprinted TiO₂ sol-gels were generated for molecular recognition-based analysis. α -Cyclodextrin (CD) was selected as the template molecule and doped into TiO₂ in a sol-gel reaction. The molecularly imprinted TiO₂ sol was spin-coated on a glass slide, and appropriate template cavities in the TiO₂ sol-gel material were formed after the template molecules were removed. We demonstrate that this modified glass slide can be used to select α -CD from a sample solution containing equal amounts of α -, β -, and γ -CD (50 ppb each, 18 mL); α -CD was directly detected from the modified glass slide by matrix-assisted laser desorption/ionization mass spectrometry without the addition of extra matrix. This approach provides a new detection method for molecular recognition-based analysis.

Sol-gels are porous polymers that are produced by acid- or base-catalyzed hydrolysis, condensation, and polymerization of metal alkoxides.¹ Sol-gel reactions are performed generally at low temperatures. Several reports have described molecular imprinting of sol-gel materials by incorporating template molecules into sol-gel networks during the sol-gel process.^{2–8} The imprinting process typically involves the incorporation and sub-

sequent removal of target molecules from a polymeric network. The resulting material, now modified with template-shaped cavities, is then used to probe interactions with target molecules. Intermolecular noncovalent interactions, including hydrophobic interactions, electrostatic interactions, and hydrogen bonding, may be exploited in the binding of the target molecules.⁹ Modified sol-gels have been widely applied to the development of chemical sensors.^{10–12} Electrochemical analysis,^{3–5,8} quartz crystal microbalances,⁶ and optical spectroscopy⁷ are conventionally used as detection methods for molecular recognition-based analysis using sol-gels. These approaches, however, may provide false positive results because of unexpected interference associated with the matrix background.

Mass spectrometry is an alternative detection method that has the advantage of providing accurate data on molecular weights for analytes.^{13,14} Recently, we developed a new type of matrix system for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, using modified sol-gel materials as the sample substrate to assist the laser ($\lambda = 337$ nm) desorption/ionization of analytes.^{15–17} The sample substrate was prepared using tetraethoxysilane as the precursor; 2,5-dihydroxybenzoic acid, a common matrix-assisted laser desorption/ionization (MALDI) matrix, was doped into the sol-gel reaction. No extra MALDI matrix was added during MALDI analysis, since the modified sol-gel material had a molar absorption coefficient of ~ 1995 cm⁻¹ M⁻¹ at a wavelength of 337 nm and could assist the laser desorption/ionization of analytes. We have demonstrated this sol-gel matrix is suitable for analyzing small organic molecules, peptides, and small proteins by MALDI mass spectrometry.¹⁵ This paper describes an alternative sol-gel system, that of a deposited TiO₂ sol-gel thin film, that is an alternative MALDI matrix. A unique characteristic of TiO₂ sol-gel materials is their absorption capacity

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in the UV region.¹⁸ Additionally, TiO₂ sol–gels used for molecular recognition can be prepared simply by incorporating template molecules into the polymeric network during the sol–gel process. Cavities having the shape of the template are produced once the templates are removed. These two unique characteristics of TiO₂ sol–gel materials can be exploited to develop molecular recognition-based mass spectrometry, which uses molecularly imprinted TiO₂ films to probe target molecules and, subsequently, employs MALDI as the detection method.

Cyclodextrins (CDs) are enzymatically modified starches.¹⁹ The three major types of CDs are α -, β -, and γ -CD, which are composed of six, seven, and eight glucose units, respectively. Because they have hydrophobic cavities, CDs have been widely employed to study host–guest interactions that are involved in molecular recognition^{20–25} and have been applied to separation science.^{26,27} In this study, α -CD was selected as the template molecule to generate α -CD-imprinted TiO₂ sol–gel materials. The modified sol–gel material was prepared with the goal of specifically selecting α -CD from a solution of the three CDs.

EXPERIMENTAL SECTION

Reagents and Materials. Titanium(IV) *n*-butoxide was obtained from Acros, and ethanol was obtained from Showa (Tokyo, Japan). Nitric acid was purchased from J. T. Baker. Bradykinin, insulin, ubiquitin, cytochrome *c*, α -CD, β -CD, and γ -CD were purchased from Sigma (St. Louis, MO). Double-sided carbon tape was obtained from Ted Pella. Glycerol was purchased from Riedel-Haën (Deisenhofen, Germany).

Preparation of TiO₂ Thin Films. TiO₂ sol was prepared by stirring titanium(IV) *n*-butoxide (3.4 mL) and ethanol (1.5 mL) for 30 min at room temperature (~27 °C). Subsequently, a solution of ethanol (1.5 mL), water (0.18 mL), and 60% nitric acid (75 μ L) was slowly added into the titanium(IV) *n*-butoxide/ethanol solution, and the resultant mixture was stirred for 10 min in an ice bath. The TiO₂ sol solution was then stirred for 3 min at room temperature before being placed in a water bath whose temperature was maintained at 35 °C. The reaction mixture was stirred for 12 h. The TiO₂ sol (0.15 mL) was spin-coated on the surface of a microscope cover glass slide (1.8 cm \times 1.8 cm \times 0.15 mm). The glass slides were pretreated by soaking in a solution of hydrogen peroxide and sulfuric acid (1:2, v/v) for 30 min to remove impurities, and then they were washed with water and methanol. The TiO₂ sol solution was spin-coated on the surface of the glass slide using a spin coater at an initial speed at 700 rpm for 15 s and then at 1500 rpm for 10 s. The TiO₂ sol solution was slowly applied to the glass slide during spin coating. The

modified glass slide, coated with a TiO₂ thin film, was aged for 20 min at room temperature before use. The thickness of the film was measured using an electronic microscope to be ~400 nm.

Direct TiO₂-MALDI Analysis. Sample preparation for direct TiO₂-MALDI analysis was quite straightforward. To conveniently locate the sample spot from the glass slide during analysis, the sample deposition position was marked on the back of the glass slide using a color marker pen. The glass slide was then attached to a sample target using double-sided carbon tape. The sample solution (0.2 μ L) was applied to the mark position on the TiO₂ sol–gel-modified glass slide. After the volatile solvent had evaporated, the sample target was ready to be sent to the mass spectrometer for analysis.

Preparation of Molecularly Imprinted TiO₂ Sol–Gel Thin Films. The procedure for preparing molecularly imprinted TiO₂ sol–gel materials is similar to that described above. Titanium(IV) *n*-butoxide (3.4 mL) and ethanol (1.5 mL) were mixed and stirred for 30 min at room temperature. A solution of ethanol (1.5 mL), water (0.18 mL), and 60% nitric acid (75 μ L) was slowly added to the titanium(IV) *n*-butoxide/ethanol solution, which was then stirred for 10 min in an ice bath. α -CD (35.8 mg) was then added quickly to the sol solution, resulting in a molar ratio of 300:1 between titanium *n*-butoxide and α -CD. The solution was stirred for 3 min at room temperature before being placed in a water bath whose temperature was controlled at 35 °C; the mixture was stirred for 12 h. The molecularly imprinted TiO₂ sol (0.1 mL) was spin-coated on a glass slide (1.8 cm \times 1.8 cm \times 0.15 mm). The coating procedure was the same as that described above for preparing a pure TiO₂ thin film on a glass slide. The glass slides were then placed into water and stirred for 2 h to remove the template molecules.

Procedures for Probing α -CD. The modified glass slide, coated with a TiO₂ film imprinted with α -CD-shaped cavities, was used to probe traces of α -CD from a sample solution. After immersing it in the sample solution for a predetermined period, the glass slide was attached to a sample plate using double-sided carbon tape, and then the plate was sent into a mass spectrometer for MALDI analysis.

Instrument. The experiments were performed using a Biflex III (Bruker) linear time-of-flight mass spectrometer. The mass spectrometer was equipped with a 337-nm nitrogen laser, a 1.25-m flight tube, and a sample target with the capacity to load 384 samples simultaneously. The accelerating voltage was set to 19 kV. UV spectra were obtained using a UV spectroscopy (Agilent 8453).

RESULTS AND DISCUSSION

Figure 1 displays the UV absorption spectrum of the TiO₂ film. The absorbance of the TiO₂ sol–gel-deposited thin film at a wavelength of 337 nm is $\sim 2.5 \times 10^3$ cm⁻¹. Such an absorption capacity suggests that it can be employed directly as the sample substrate for MALDI analysis. Analytes such as bradykinin, insulin, and ubiquitin could be observed in the MALDI mass spectra by using the TiO₂ sol–gel thin film as the assisted substrate without the addition of organic matrix. When trace glycerol was added on the TiO₂ sol–gel film for TiO₂-MALDI analysis, better mass spectral quality could be obtained. Panels a–c of Figure 2 show the TiO₂-sol–gel MALDI mass spectrum of bradykinin (M_bH⁺), insulin (M_iH⁺), and cytochrome *c* (M_cH⁺),

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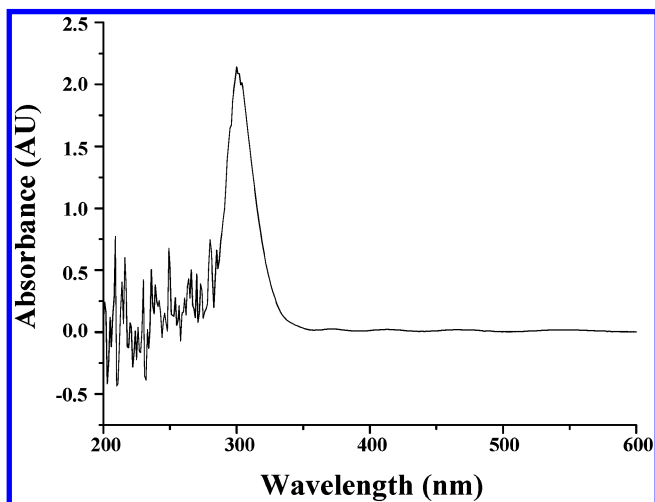


Figure 1. UV absorption spectrum of the TiO₂ film.

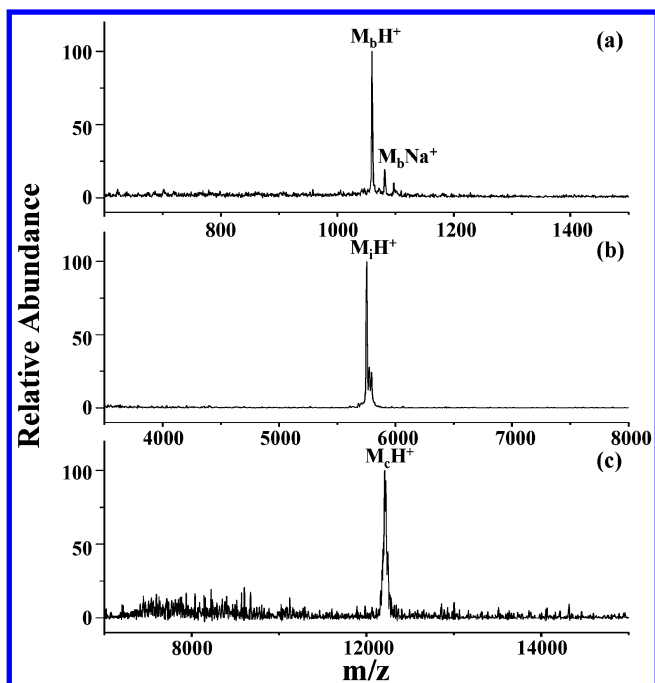


Figure 2. TiO₂-MALDI mass spectra of (a) bradykinin (188 pmol) (b) insulin (34 pmol), and (c) cytochrome *c* (80 pmol) with the addition of glycerol (15% glycerol/methanol, 0.2 μL) on the TiO₂ sol-gel film.

respectively, with the addition of glycerol on the TiO₂-sol-gel film. The results are similar to those obtained by Schürenberg et al. in their previous report,²⁸ which employed TiN nanoparticles/glycerol as the assisted material for protein analysis. However, inorganic particles may contaminate the ion source. Using TiO₂-sol-gel-deposited thin film as the assisted material can avoid this problem. Some background ions are contributed directly from the TiO₂ film (results not shown). A series of ion peaks corresponding to TiO⁺, TiO₂H⁺, Ti₂O₃⁺, Ti₂O₄H⁺, Ti₃O₅⁺, Ti₃O₆H⁺, and Ti₄O₇⁺ were observed in the mass spectrum. Thus, TiO₂-MALDI analysis should be applied only for analytes with molecular masses greater than 500 Da to avoid matrix interference from the TiO₂ film.

Figure 3a displays the TiO₂-MALDI mass spectrum of a mixture comprising equal amounts (10 ng) of α-, β-, and γ-CD

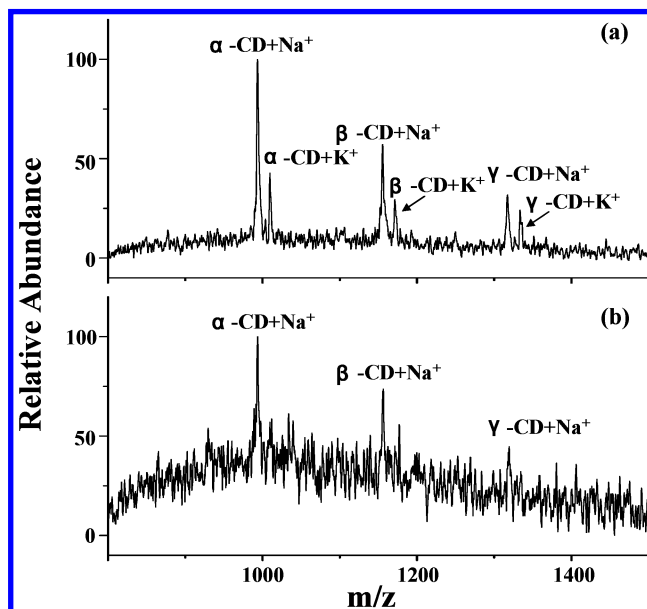


Figure 3. TiO₂-MALDI mass spectra of equal amounts of α-, β-, and γ-CD: (a) 10 ng; (b) 2 ng without the addition of glycerol on the TiO₂ sol-gel film.

using a TiO₂ sol-gel-deposited thin film as the sample substrate. The alkali cation adducts of CDs still dominate the MALDI mass spectrum. The peaks at *m/z* 996, 1158, and 1320 correspond to the sodiated ions of α-, β-, and γ-CD, respectively, while those at *m/z* 1012, 1174, and 1336 represent their corresponding potassium ion adducts. When the amount of each CD in the mixture was reduced to 2 ng, however, only the sodium and potassium adducts of α-CD, a weak sodiated β-CD, and γ-CD peak were observed in the TiO₂-MALDI mass spectrum (Figure 3b).

A molecularly imprinted TiO₂ sol-gel-deposited thin film was prepared for molecular recognition-based analysis. α-CD was selected as the template to be doped into the TiO₂ sol-gel during the sol-gel process. TiO₂ thin films were prepared by spin casting TiO₂ sol onto the surface of a glass slide. Figure 4a displays the direct laser desorption mass spectrum of the TiO₂ thin film doped with α-CDs. The sodium and potassium adduct ions of α-CD appear at *m/z* 996 and 1012, respectively. The α-CD molecules were then removed from the modified glass slide by simply placing the slide in stirred water at 35 °C for 2 h. To examine whether any trace of α-CD molecules remained on the glass slide, we employed a laser to irradiate the modified glass slide for direct laser desorption mass spectrometric analysis. Figure 4b displays the MALDI mass spectrum of the TiO₂ sol-gel film after washing. No ions arising from α-CD remain on the film after this washing procedure.

The modified glass slide containing α-CD-shaped cavities was used to extract traces of α-CD from a sample solution (10 ppb, 18 mL). The extraction time was 2–3 h. Figure 5a shows the TiO₂-MALDI mass spectrum obtained using this approach and shows the sodiated α-CD peak at *m/z* 996 in the mass spectrum. No such analyte ion is observed in the corresponding mass spectrum when a glass slide modified with TiO₂ sol-gel material lacking CD-shaped cavities was used as an extraction probe for the same sample (results not shown). This result demonstrates that the presence of the α-CD-shaped cavities in the TiO₂ sol-gel film is essential for the extraction process. Extra glycerol was also added

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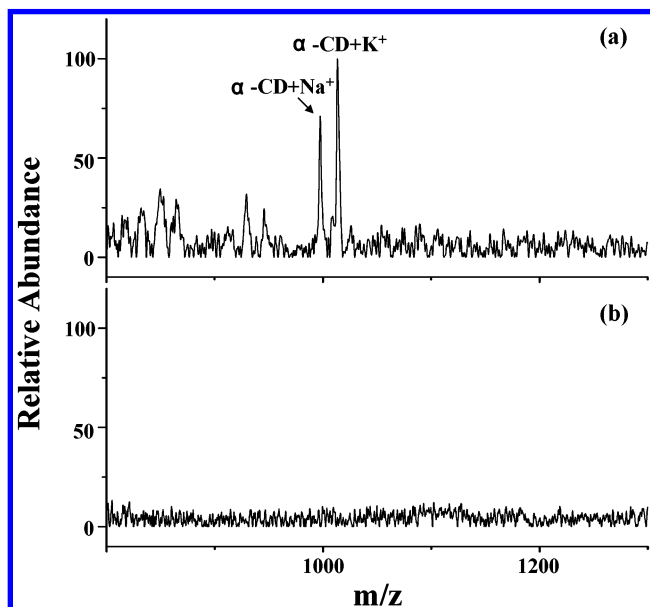


Figure 4. (a) Direct laser desorption mass spectrum of the TiO_2 thin film doped with α -CDs. (b) Direct laser desorption mass spectrum of the TiO_2 -sol-gel film after removing the α -CDs by washing in water for 2 h.

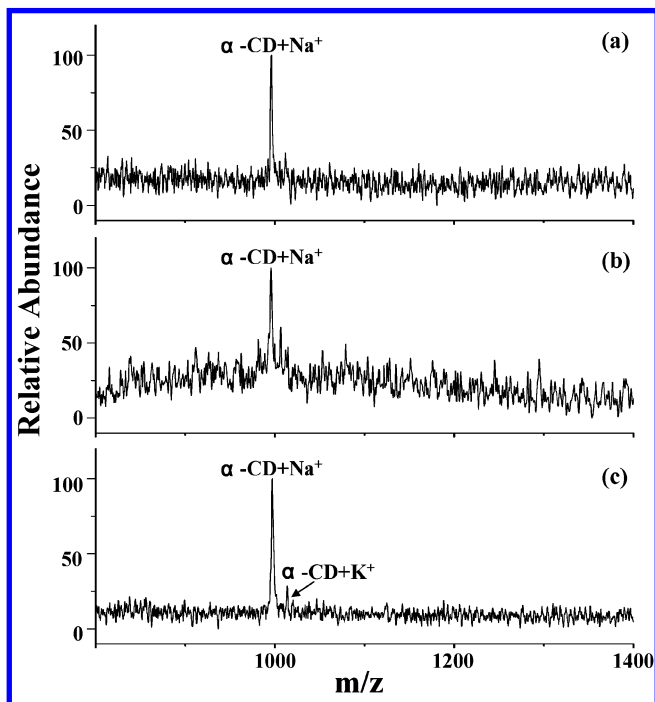


Figure 5. TiO_2 -MALDI mass spectra probing the sensitivity and selectivity for α -CD from a solution containing α -CD (10 ppb, 18 mL) using a glass slide coated with an α -CD-imprinted TiO_2 film as the probe. (a) Direct laser desorption analysis; (b) extra glycerol (0.2 μL of 15% glycerol/methanol) was added on the TiO_2 -sol-gel film before laser desorption analysis; (c) TiO_2 -MALDI mass spectrum probing the sensitivity and selectivity for α -CD from a solution containing equal amounts of α -, β -, and γ -CD (50 ppb, 18 mL) using a glass slide coated with an α -CD-imprinted TiO_2 film as the probe.

on the TiO_2 film after extraction for TiO_2 -MALDI analysis to investigate whether the α -CD analyte signal was enhanced because of the presence of glycerol. The sodiated α -CD ions still dominate the mass spectrum, and the ion intensity is similar to that in Figure 5a (see Figure 5b). The addition of glycerol on the

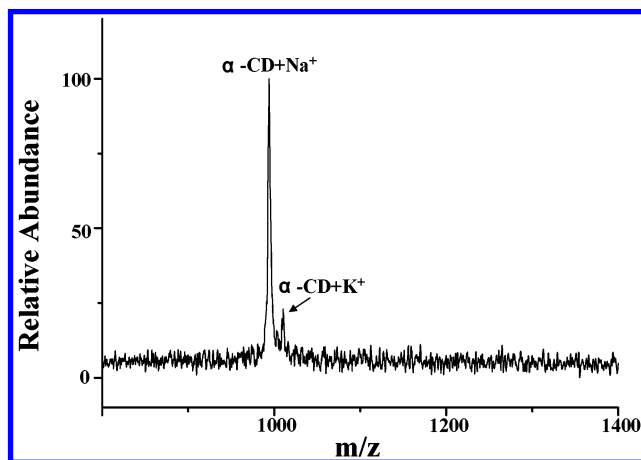


Figure 6. TiO_2 -MALDI mass spectrum probing the sensitivity and selectivity for α -CD from a solution (18 mL) containing α - (50 ppb), β - (100 ppb), and γ -CD (100 ppb) using a glass slide coated with a α -CD-imprinted TiO_2 film as the probe.

TiO_2 film does not help in improving the ion intensity. It might be because α -CD molecules prefer to form sodium adducts. Thus, the extra proton source that provided by glycerol does not affect the results. To prove that the cavities were specific for α -CD, both β - and γ -CD were added to the sample solution. The selectivity of the modified glass slide containing α -CD-shaped cavities was tested in the extraction of CDs from a sample solution (18 mL) containing equal amounts (50 ppb) of α -, β -, and γ -CD. Figure 5c displays the TiO_2 -MALDI mass spectrum that results from this extraction process. Only α -CD's sodium and potassium adducts appear, at m/z 996 and 1012, respectively, which indicates that a molecularly imprinted TiO_2 film with template-shaped cavities can be used to selectively bind target molecules and that such a molecularly imprinted TiO_2 film can be directly analyzed using TiO_2 -MALDI-MS. No analyte ions were observed in the corresponding mass spectrum when a glass slide modified with TiO_2 sol-gel material lacking CD-shaped cavities was used as an extraction probe for the same sample.

Additionally, different ratios of these three CDs were also used as the sample to demonstrate the feasibility of this approach. The selectivity of the modified glass slide containing α -CD-shaped cavities was tested in the extraction of CDs from a sample solution (18 mL) containing α - (50 ppb), β - (100 ppb), and γ -CD (100 ppb). Figure 6 shows the TiO_2 -MALDI mass spectrum of the extraction result. Still, only the sodium and potassium adducts of α -CD are observed in the mass spectrum. These results demonstrate that this approach is an effective one for molecular recognition-based analysis. In addition to size complementarity between α -CD and the cavities imprinted in the TiO_2 film, we believe that the hydroxyl groups located around the binding pocket are involved in discriminating between analytes through significant hydrogen-bonding interactions.

CONCLUSIONS

A pure inorganic TiO_2 sol-gel thin film with or without glycerol addition has been demonstrated to be effective as the assisted material in MALDI analysis. The largest molecule observed in the TiO_2 -MALDI mass spectrum is ubiquitin, while the largest molecule obtained in the TiO_2 -MALDI analysis is cytochrome *c*

with the addition of glycerol on the TiO₂ sol-gel film. The detection limit for the direct TiO₂-MALDI analysis of α -CD is in the nanogram regime, which is much higher than that of conventional MALDI mass spectrometry. Additionally, molecularly imprinted TiO₂ films are useful for probing target molecules selectively. The combination of these two functions of TiO₂ film represents a new way to undertake molecular recognition-based analysis using mass spectrometry. Incorporating templates into TiO₂ sol-gels is a very straightforward process, which makes this current approach a highly promising one for widespread use. The long extraction time (~2–3 h) required for analysis using this approach, however, may limit its applications in the rapid

identification of analytes. Thus, further studies, such as using electrochemical methods to speed up the probing process and, hence, reduce the probing time, may be necessary to improve this technique.

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