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Modified silica-containing matrices towards the MALDI-TOF-MS detection of small molecules

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ABSTRACT

The new silica-based matrices were designed to demonstrate the concept of using silica to immobilize classic MALDI matrix materials for the analysis of small molecules (<600 Da). The matrices were synthesized by covalently attaching a precursor of ionic liquids (a silylated amine) to the silica and then reacting it with the anion of a conventional MALDI matrix material such as α-cyano-4-hydroxy cinnamic acid (CHCA) to form an ionic macro-complex. This new matrix material displayed at least two advantages. First, the immobilization of the CHCA did not impact the chromophore region of CHCA molecule, which retained its original maximum absorption band at 328 nm. This made the new matrix material compatible with standard MALDI-TOF mass spectrometers equipped with nitrogen lasers. Second, by covalently immobilizing the cationic portion of the ionic liquid moiety on the silica, it was possible to avoid low-mass chemical noise arising from the matrix in the positive-ion mode. The new matrix materials were based on silica gel and mesoporous silica, SBA-15. Their small molecule utility was investigated using two neurotransmitters, dopamine (153.08 Da) and serotonin (176.09 Da). The MALDI-TOF-MS analysis of the neurotransmitters demonstrated that the matrix material based on the modified mesoporous silica ([SBA-15-Si-NH_3^+][CHC^-]) yielded spectra with minimal chemical noise and good analyte signal intensity. This was hypothesized to be due to the more efficient ionization facilitated by the larger surface area and spot uniformity of such a matrix material.

1. Introduction

Since the development of matrix assisted laser desorption ionization (MALDI) by Karas et al. [1] in 1985, and Tanaka et al. [2] in 1988, it has become an indispensable tool in the mass spectrometric analysis of a wide variety of bio-molecules and polymers. However, the utility of this technique does not extend to small analyte molecules because common MALDI matrices are low molecular weight organic acids, which produce a variety of matrix related ions that generate interfering signals in mass spectra. Rapid and effective structural analysis of small molecules has become important in a variety of chemistry related fields such as medicinal chemistry and metalobolmics [3]. A practical matrix or substrate for small molecules should be one that absorbs UV laser energy close to 337 nm (standard on most MALDI instruments) and does not produce peaks in the low-mass region.

The focus of the present work was on the development of a MALDI matrix for the analysis of small molecules using silica modified with ionic liquid precursors. Room-temperature ionic liquids have been used as matrices for MALDI [4–8] and have demonstrated very good analyte solubilization and dispersion, which resulted in more homogeneous sample targets. This, in turn, has translated into better shot to shot reproducibility. However, the background noise from the cationic part of ionic liquids dominated the low-mass region of the mass spectrum, thus limiting their use in the analysis of low-molecular weight species.

Silica based materials have been used for the matrix-free desorption/ionization on silica (DIOS) [9,10], desorption/ionization on silicon dioxide (DIOSD) [11], and silicon-nanoparticle-assisted laser desorption/ionization (SPALDI) [12]. These tend to have laborious preparation steps which involve caustic chemicals to clean the surface, and lack a long-term shelf-life without special care. They also involve special target plate substrates. Recently, 8-hydroxyquinoline-modified silica showed promise as a matrix for the analysis of low molecular weight analytes [13]. But, as mentioned above, the preparation is laborious.

We present a simple, convenient, and short synthesis of an efficient matrix for MALDI time-of-flight (TOF) analysis of small molecules of biological importance. The new matrix was designed as an ionic macro-complex between the classical MALDI matrix α-cyano-4-hydroxy cinnamic acid (CHCA), and a modified silica gel (silica gel-Si-NH_3^+) or mesoporous silica (SBA-15-Si-NH_3^+). The latter were synthesized by covalently linking a precursor of ionic liquids (a silylated amine) to the silica surface.

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The desorption/ionization properties of both the [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-] macro-complex matrix material were probed using two neurotransmitters, dopamine (153.08 Da) and serotonin (176.09 Da). These low molecular weight species are essential in many regulatory pathways in the central nervous system. Anomalous levels of dopamine have been linked with Parkinson's disease and Schizophrenia [14]. Serotonin is a major target for pharmaceutical treatments of depression [15]. They are also implicated in neuroimmune transmission [16]. The determination of dopamine and serotonin is important because they co-exist in biological systems and affect each other's release [17]. This manuscript presents a new MALDI-TOF mass spectrometric detection method for dopamine and serotonin, which complements the existing chromatographic [18,19], electrospray ionization mass spectrometric [20,21], and electrochemical methods [22,23].

2. Experimental

2.1. Reagents

The conventional MALDI matrix, α-cyano-4-hydroxy cinnamic acid (CHCA, [28166-41-8], catalog no. C2020) was purchased from Sigma–Aldrich (St. Louis, MO). Silica gel ([112926-00-8], catalog no. S733-1, grade 633, Type 60A, 200-425 MESH) was purchased from Fisher Scientific. SBA-15 particles (catalog no. 01-002) were purchased from Claytec Inc. According to the supplier's specifications, they had a framework structure of 1D-hexagonal SBA-15 type, the average framework pore size of 8.5 nm, BET surface area of 718 $m^2\ g^{-1}$, and framework pore volume of 0.90 $cm^3\ g^{-1}$. The 3-aminopropyltriethoxysilane (3-APTES, [919-30-2], catalog no. A0281306) was purchased from Acros Organics. Acetonitrile (MeCN) (UV, chromatography reagent grade) and methanol (MeOH) (UV, chromatography reagent grade) were from Burdick & Jackson. Deionized water was obtained from a Barnstead Nano-Pure cartridge system. The neurotransmitters dopamine ([62-31-7], catalog no. H8502) and serotonin ([153-98-0], catalog no. H9523) as well as angiotensin II ([4474-91-3], catalog no. A9525-1mg) were purchased from Sigma–Aldrich. Glacial acetic acid was purchased from Aldrich ([64-19-7], catalog no. 338826).

2.2. Preparation of [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-SI-NH_3^+][CHC^-] matrices

CHCA (190 mg, 1.0 mmol) and 3-APTES (200 μL, 1.0 mmol) were stirred together in 30.0 mL of methanol at room temperature for 1 h. Silica gel (2.0 g) or mesoporous SBA-15 silica (1.0 g) was added to the solution and the suspension was brought to 60 °C and stirred overnight. The suspension was cooled down to room temperature and the modified silica was separated by centrifugation and washed with methanol (5 × 12 mL). This yielded a pale yellow colored solid, which was dried at 70 °C for 5 h.

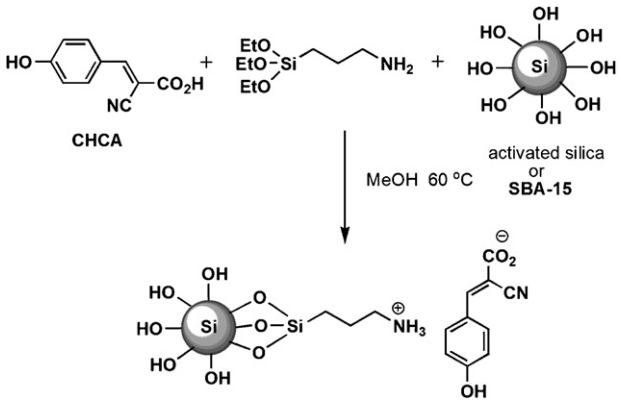
2.3. Spot preparation

The MALDI plate was spotted using the following protocol for all experiments. First, 6.0 μL of matrix, 4.0 μL of neurotransmitter (20.0 mM in 50:50 (v/v) MeOH/H_2O) and 1.0 μL of glacial acetic acid were mixed and vortexed for 30 s. Then, 1.0 μL of this solution was applied to MALDI target plate (stainless steel Scout 384 target plate, Bruker Daltonics, Billerica, MA) as a spot and dried for 15 min.

2.4. Instrumentation

All mass spectra were acquired on a Bruker REFLEX III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA) in reflected positive-ion mode. This instrument has been re-equipped with a 337 nm output nitrogen laser (Spectra Physics model 337ND, Newport Corp., Irvine, CA). The following instrument parameters were used for the positive-ion reflection mode: number of shots, 300; acceleration potential, ±20 kV. Data analysis was accomplished using Bruker Daltonics FlexAnalysis 2.4. The experiments were repeated at least three times. The ion intensities between replicates were reproducible within ±10% of each other. The spectra presented in the following discussion are representative of the results from each of the experiments. An average full-width at half maximum resolution for all spectra presented here was 1033. The mass spectrometer was externally calibrated using angiotensin II in a conventional CHCA (10.0 μL of a saturated solution in MeCN) matrix using 1.0 μL of a 1.0 mg mL^{-1} solution of angiotensin.

Infrared spectra were acquired on a Bruker Vector 22 FTIR using a Harrick Praying Mantis DRIFT accessory in KBr matrix. Trans-



Scheme 1. Synthetic route to ionic macro-complexes of [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-].

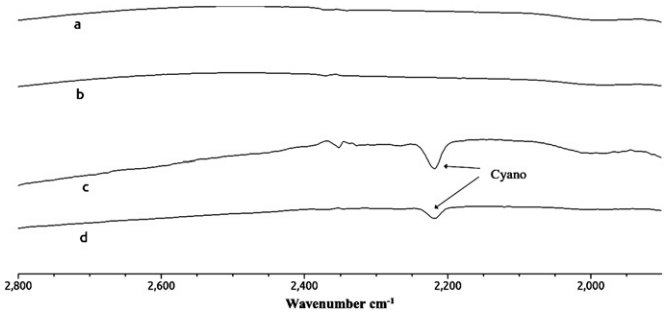


Fig. 1. FTIR spectra of (a) unmodified activated silica, (b) unmodified SBA-15 mesoporous silica, (c) [silica gel-Si-NH_3^+][CHC^-], and (d) [SBA-15-Si-NH_3^+][CHC^-].

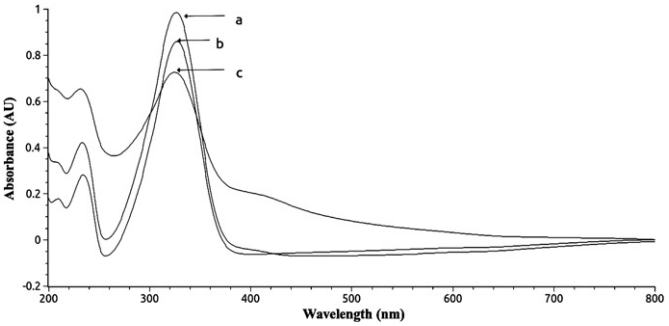


Fig. 2. UV–visible absorption spectra of aqueous solutions of free (a) CHCA, and suspensions of (b) [silica gel-Si-NH_3^+][CHC^-], and (c) [SBA-15-Si-NH_3^+][CHC^-].

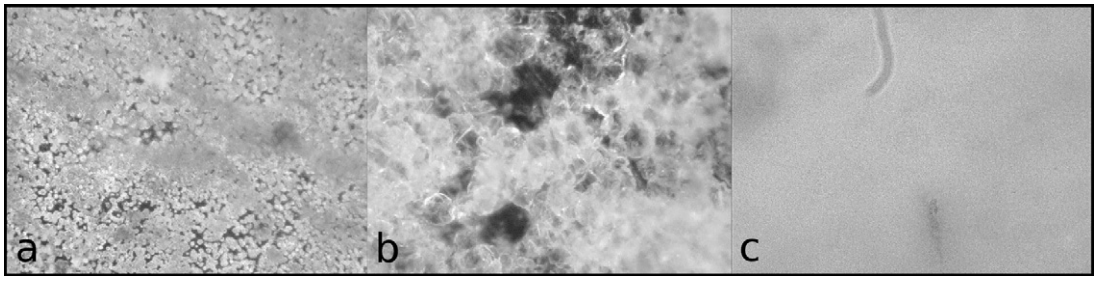


Fig. 3. Microscope images of dried drop deposits of (a) CHCA, (b) [silica gel-Si-NH_3^+][CHC^-], and (c) [SBA-15-Si-NH_3^+][CHC^-].

mission UV–visible spectra were acquired on a Varian-Cary 5000 UV–visible spectrophotometer in aqueous solution.

3. Results and discussion

3.1. Synthesis of [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-] matrix materials

The silica substrates (silica gel and mesoporous silica SBA-15) were modified with a molecular precursor of ionic liquids (3-APTES) according to Scheme 1.

The modification involved the hydrolysis of 3-APTES, followed by its condensation on the silica surface. This yielded a silica surface that was covalently modified with terminal primary amines. The terminal primary amine was subsequently reacted with CHCA. This resulted in the formation of the CHC salt of the amine, the new ionic macro-complexes [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-]. This scenario was supported by FT-IR (Fig. 1) and UV–visible (Fig. 2) measurements of the modified and unmodified silica substrates. The immobilization of CHCA was confirmed by the presence of an absorption band at 2205 cm^{-1} in the spectrum of the [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-] matrix materials. The 2205 cm^{-1} stretching band is characteristic for the cyano group present in the CHCA structure.

Fig. 2 shows the UV–visible spectra that were recorded for the free CHCA molecules (Fig. 2a), and CHCA molecules that were immobilized on the silica gel (Fig. 2b), and mesoporous SBA-15 particles (Fig. 2c). All three spectra showed a characteristic absorption band of CHCA at 328 nm. Apparently, our CHCA immobilization protocol did not affect the position of the UV absorption maximum, which indicated that the chromophore region of CHCA molecule reminded largely intact. This is a critical aspect of the proposed synthetic method because it allows for the use of new MALDI matrices with the standard MALDI-TOF mass spectrometers.

Fig. 3 shows the microscopic images obtained at a magnification of 150× of (a) CHCA matrix, (b) [silica gel-Si-NH_3^+][CHC^-] and (c) [SBA-15-Si-NH_3^+][CHC^-] macro-complex dispersed on the stainless steel plate. The dispersions were prepared by evaporating solvent from 1.0 mg mL^{-1} CHCA solution or suspensions of the [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-] in ethanol. The images in Fig. 3 show that the matrix spots made of the [SBA-15-Si-NH_3^+][CHC^-] macro-complexes were significantly more homogeneous than those made of the conventional CHCA or activated silica particles. The SBA-15 based deposit was virtually featureless at this magnification. This could be attributed to the lesser particle agglomeration in the case of modified mesoporous silica due to the surface charges that were introduced in the course of surface modification as well as the smaller particle size. Such increased matrix homogeneity is very beneficial for the analytical performance (reproducibility and signal intensity) of the MALDI-TOF.

3.2. MALDI-TOF MS analysis of dopamine and serotonin using new matrix materials

A series of MALDI-TOF mass spectra were obtained using dopamine and serotonin with a conventional CHCA matrix and the two new matrix materials, [silica gel-Si-NH_3^+][CHC^-] and [SBA-15-Si-NH_3^+][CHC^-]. The goal was to determine the relative utility of each of the matrices with respect to reducing the observable chemical noise, i.e., reducing the matrix peaks due to the low-mass cations, in MALDI spectra of these neurotransmitters.

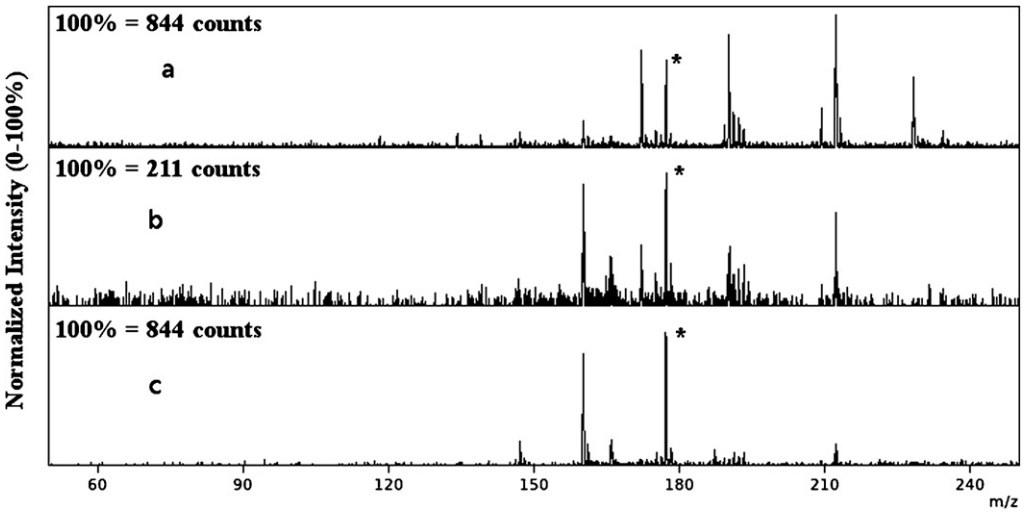


Fig. 4. MALDI-TOF MS mass spectra of 7.3 nmol/spot dopamine (*) recorded at different matrices: (a) conventional CHCA, (b) [silica gel-Si-NH_3^+][CHC^-], and (c) [SBA-15-Si-NH_3^+][CHC^-]. All spectra were normalized to highest intensity peak in the mass range.

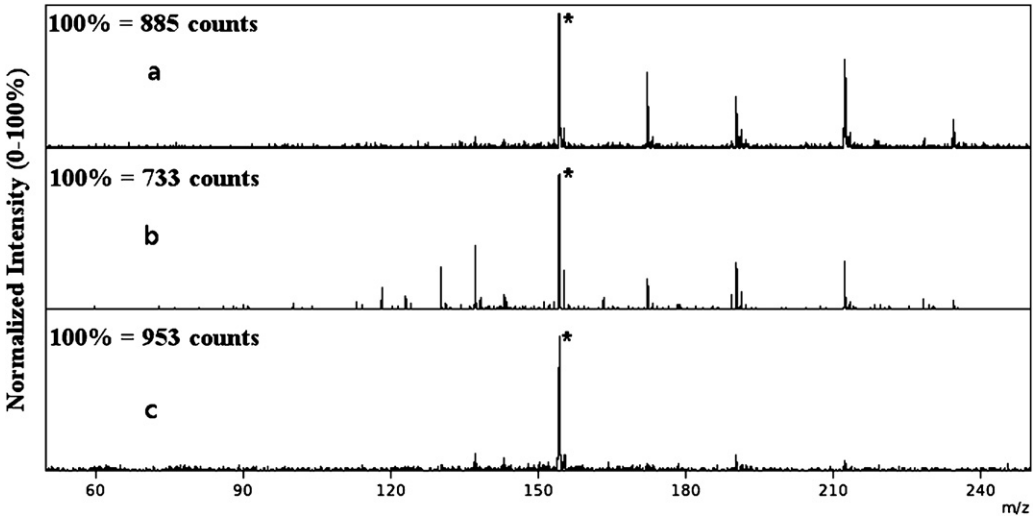


Fig. 5. MALDI-TOF MS mass spectra of 7.3 nmol/spot serotonin (*) recorded at different matrices: (a) conventional CHCA, (b) [silica gel-Si-NH3$^+$][CHC$^-$], and (c) [SBA-15-Si-NH_3^+][CHC$^-$]. All spectra were normalized to highest intensity peak in the mass range.

Fig. 4 shows the mass spectra observed using dopamine in the MALDI experiments with the two new matrix materials and the standard CHCA matrix. The mass spectrum observed from a standard CHCA matrix (Fig. 4a) had significant cationic peaks due to the matrix at *m*/*z* 171.9, 190.1, and 212.1 along with the peak from the protonated dopamine molecule at *m*/*z* equal to 154.1. These low mass cationic peaks are fairly typical results for a CHCA matrix [24]. The mass spectrum from the [silica gel-Si-NH_3^+][CHC$^-$] matrix was similar (Fig. 4b), although it displayed a few lower intensity peaks. The immobilization of CHCA on the modified SBA-15 particles ([SBA-15-Si-NH_3^+][CHC$^-$]) was the most successful because it yielded almost no background cationic matrix peaks in the low-mass region relative to the analyte signal intensity while generating an intense peak for dopamine at *m*/*z* equal to 154.1 (Fig. 4c).

A comparable picture emerged from the MALDI-TOF experiments with serotonin. The mass spectrum taken with the standard CHCA matrix (Fig. 5a) yielded the peaks at *m*/*z* 171.9, 190.1 and 212.1 along with a protonated serotonin peak at *m*/*z* equal to 177.1. Compared with the standard CHCA matrix, the modified matrix [silica gel-Si-NH_3^+][CHC$^-$] (Fig. 5b) showed similar peaks from the matrix along with a strong parent-ion peak from the serotonin at *m*/*z* equal to 177.1. Again, the immobilization of CHCA on the modified SBA-15 particles ([SBA-15-Si-NH_3^+][CHC$^-$]) was the most successful because it yielded very low relative background noise resulting from cationic matrix peaks in the low-mass region and an intense peak for the analyte serotonin at *m*/*z* 177.1 (Fig. 5c).

The lower relative chemical noise in the case of the ([SBA-15-Si-NH_3^+][CHC$^-$] matrix material (Figs. 4c and 5c) can be ascribed to the higher surface area of the mesoporous SBA-15 substrate. Apparently, the higher surface area of mesoporous silica substrate was conducive to more efficient laser desorption and ionization processes for the analyte [9,25].

4. Conclusions

The MALDI-TOF mass spectrometric detection of small neurotransmitters (dopamine and serotonin) is shown to be free of interferences from a new matrix made of an ionic macro-complex based on a mesoporous silica ([SBA-15-Si-NH_3^+][CHC$^-$]. This new matrix is based on a commercially available silica substrate, is easy to synthesize under mild conditions (60 °C, methanol), and can be used on a regular MALDI plate with no requirements for special setups. Such matrix has a potential to be useful also for the MALDI-TOF MS detection of other small molecules including those belonging to the classes of environmental pollutants and warfare agents.

Acknowledgements

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