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Laser desorption ionization mass spectrometry of peptides on hybrid CHCA organic-inorganic matrix

Clément Fleith,[a] Sonia Cantel,[a] Gilles Subra,[a] Ahmad Mehdi,[b] Jeremie Ciccione,[a] Jean Martinez[a] and Christine Enjalbal*[a]

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We report applications of new hybrid organic-inorganic silica based materials as laser desorption/ionization (LDI)-promoting surfaces for high-throughput identification of peptides. The driving force of our work was to design a new material composed of conventional MALDI matrix covalently attached to silica with a high organic/inorganic ratio in order to improve the UV absorption by such LDI hybrid matrices. Amorphous CHCA-functionalized silica presenting an organic content up to 1.3 mmol.g^{-1} (around 40% in weight from TGA and elementary analyses measurements) gave very interesting LDI performances in terms of detection sensitivity as well as relative ionization discrepancy (spectral suppression) through the analyses of small synthetic peptides mixtures (550-1300 Da) taking CHCA and amorphous silica as model matrices for control experiments.

Introduction

A variety of inorganic materials have been used so far as inert matrix to detect and characterize a wide range of compounds in Laser Desorption Ionization Mass Spectrometry (LDI-MS). Ions are formed by laser irradiation of the molecules of interest directly deposited onto such non volatile substrates conditioned in various architectures (amorphous or nanostructured powders and surfaces).[1-5] This technique, also named Surface-Assisted Laser Desorption/Ionization Mass Spectrometry (SALDI-MS), was designed as an alternative to conventional Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) for small molecule analysis.[6,7] Indeed, organic matrices are typically mixed in great excess with the sample of interest to transfer energy from the incident UV laser beam to the analytes[8,9] leading to the formation of ions in the gas phase. The capability of MALDI-MS analysis of small molecules (less than 500 Da) is compromised due to the presence of abundant matrix background signals hampering efficient and sensitive low molecular weight compound detection. Although matrix signals can be suppressed by using appropriate matrix/analyte mixing ratio,[10,11] the use of inert materials that cannot be desorbed upon UV irradiation also provides an answer to solve this restriction and thus broadens the scope of laser desorption/ionization techniques from small molecule detection (SALDI-MS) to macromolecule analyses (MALDI-MS). However, the design of the LDI inert substrate is crucial to achieve both sensitive and reliable detection. Although most of the described LDI-MS analyses rely on UV-absorbing inert substrates presenting poor thermal conductivity in order to induce prompt vaporization of deposited compounds,[12-14] it occurred to us that combining the properties of organic and inorganic substrates could be very interesting to capitalize on all desorption/ionization processes that have been suggested to date[15-17] (photoionization, ion–molecule reactions, energy pooling, thermal ionization, and desorption of preformed ions). This motivated us to prepare hybrid materials for LDI-MS applications which present the specificity that the organic moieties are covalently anchored to the inert framework, and not just mixed with it as already investigated a decade ago,[6] preventing the deleterious formation of organic matrix ions.

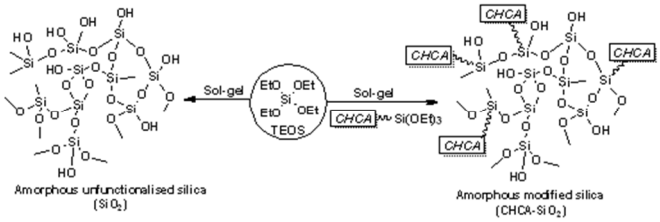
Based on our previous investigations of graphite, alumina and silica-based materials for sensitive and robust LDI-MS analyses of peptide and tryptic digest,[18-22] we focussed our attention on silica for two major reasons. First, such material has been widely investigatedin the literature as an LDI-MS substrate, either as amorphous powder or as nanostructure particles.[23-27] Moreover, functionalized silica can be obtained easily using post-functionalization of an existing silica network[28] or direct synthesis of modified organosilica[29] by hydrolysis and copolymerization of silica precursor (TEOS) with an trialkoxysilane RSi(OR')$_3$ bearing the organic moiety thanks to sol-gel process.[30] Second, standard experimental operating conditions can be employed for both synthesis and LDI analyses. No specific equipment is needed for material preparation and synthesis of hybrid silica that can be undertaken in any organic chemistry laboratory. In the same manner, silica powder can be handled as slurry and deposited onto a MALDI target using standard protocols (such as the dried droplet or thin film) suitable for analysis with any MALDI mass spectrometer. In order to increase the UV laser absorption capacity of silica, we decided to incorporate the most popular MALDI matrix used for peptide detection, α-cyano-4-hydroxycinnamic acid (CHCA). Several CHCA-modified hybrid materials were prepared using different silica supports (ordered, amorphous) and different approaches (direct synthesis and post modification on silica or pre-functionalized silica). Among all investigated synthetic methodologies, the preparation of amorphous hybrid silica by a direct synthesis approach using a trialkoxysilane derivative of CHCA was found to exhibit the expected high organic loading. The prepared hybrid material, noted CHCA-SiO_2, was engaged in various LDI-MS analyses of synthetic peptide mixtures. We report the peptide behaviours that were compared with reference experiments performed with CHCA and amorphous SiO_2 to probe in parallel the corresponding organic and inert matrices.

Experimental

All reagents were purchased from Aldrich Chemical and used without further purification. All solvents were of analytical grade. Methanol was purchased from Fisher and acetonitrile from Carlo Erba. The deionized water used in all experiments was obtained using a Milli-Q system (Millipore, Milford, USA). A peptide calibration standard kit and CHCA matrix was purchased from BrukerDaltonics. Diammonium hydrogen citrate, diammoniumsulphate, trifluoroacetic acid, and potassium perfluorooctanesulfonate were obtained from Merck (VWR, France). ^{1}H and ^{13}C NMR analyses were performed with Bruker Avance DPX 200 MHz and Bruker Avance AM 300 MHz mass spectrometers using residual undeuterated solvents as an internal reference for calibration ($CDCl_3$, d = 7.26 ppm for ^{1}H NMR). Mass spectrometric experiments were conducted on a Z-Q mass spectrometer (Waters, Milford, USA) fitted with an electrospray ion source operated in the positive mode (ESI+) and a quadripole mass analyzer.

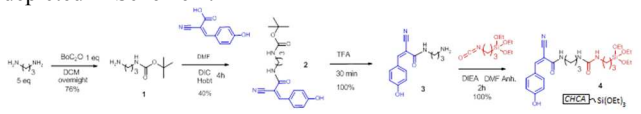
Synthesis of amorphous silica: SiO_2 and CHCA-SiO_2

Amorphous silica (noted SiO_2) was prepared by hydrolysis and polycondensation of tetra-ethoxysilane (TEOS) under acidic conditions according to standard sol-gel chemistry.[31]



Scheme 1. Amorphous silica synthesis strategies

As depicted in Scheme 1, CHCA-modified silica was prepared in the same way except that 20 % molar of trialkoxysilyl derivative of CHCA, noted CHCA-$Si(OEt)_3$, were added to TEOS during hydrolytic and condensation process. The key derivative CHCA-$Si(OEt)_3$ was prepared according to the following 4 steps protocol depicted in Scheme 2.



Scheme 2. Synthesis of CHCA-$Si(OEt)_3$

The synthesis of N-tert-butoxycarbonylaminopropylamine (compound 1 in Scheme 2) was achieved from a 2 g (9 mmol) aliquot of di-tert-butyl dicarbonate (Boc_2O) dissolved in dichloromethane (CH_2Cl_2) (20 mL) that was added dropwise to a solution of propyldiamine (3.78 mL, 45 mmol) in CH_2Cl_2 (40 mL) at 0°C. The reaction was stirred at 0°C for 3 h and for 16 h at room temperature to reach completion. The solvent was removed by vacuum distillation and the residue was washed with water. Then the white powder (di-substituted Boc-propyldiamine-Boc) was filtered. NaCl was added to the filtrate until the solution was saturated and mono-Boc-protected propyldiamine was extracted by ethyl acetate three times. A colourless oil was obtained after ethyl acetate removal by vacuum distillation. The crude mixture was purified by column chromatography on silica gel with mixtures of CH_2Cl_2/CH_3OH as the eluent. Compound 1 was obtained as a white powder (1.16 g, 76% yield). ^{1}H NMR (200 MHz, $CDCl_3$, 25°C, TMS): 1.42 (s, 9H, $3CH_3$), 1.59 (m, 2H, CH_2), 2.75 (t, 2H, CH_2NH_2), 3.17 (dt, 2H, CH_2NH). MS (ESI+): $[M+H]^+$ = 175.1 Da. For the synthesis of tert-butyl-3-CHCA-propyldiamine (compound 2 in Scheme 2), 1.16 g of compound 1 (6.7 mmol) was dissolved with 20 mL of dimethylformamide (DMF), 1.25 mL of N,N'-diisopropylcarbodiimide (DIC) (8.0 mmol), 1.23 g of hydroxybenzotriazole (HOBt) (8.0 mmol) and 1.51 g of CHCA were added into the mixture. The reaction was stirred for 4h. The crude mixture was purified by column chromatography on silica gel with mixtures of ethyl acetate/cychlohexane as the eluent. Compound 2 was obtained as yellow crystals (0.92 mg, 40% yield). ^{1}H NMR (200 MHz, $CDCl_3$, 25°C, TMS): 1.42 (s, 9H, $3CH_3$), 1.59 (dt, 2H, CH_2), 3.10 (dt, 2H, CH_2NH_2), 3.29 (dt, 2H, CH_2NH), 5.55 (br, 1H, NH), 6.97 (d, 2H, aromatic), 7.20 (br, 1H, NH), 7.91 (d, 2H, aromatic), 8.12 (s, 1H, aliphatic). MS (ESI+): $[M+H]^+$ = 346.2 Da. For the preparation of HCCA-propyldiamine (compound 3 in Scheme 2), 400 mg (1.16 mmol) of compound 2 was dissolved in trifluoroacetic acid (TFA) (4 mL), the mixture was stirred at room temperature for 30min. TFA was then removed by vacuum distillation. Compound 3 was obtained as a yellow powder (100% yield). ^{1}H NMR (200 MHz, D_2O, 25°C, TMS): 1.88 (dt, 2H, CH_2), 3.03 (dt, 2H, CH_2NH_2), 3.38 (dt, 2H, CH_2NH), 6.92 (d, 2H, aromatic), 7.82 (d, 2H, aromatic), 7.98 (s, 1H, aliphatic). MS (ESI+): $[M+H]^+$ = 246. 2 Da. The key intermediate (compound 4 in Scheme 2) was obtained from 20 mmol of compound 3 (49 mg) dissolved in 2 ml of anhydrous DMF. 20 mmol (49 µL) of triethoxy(3-isocyanatopropyl)silane was added into this solution. Diisopropylethylamine (DIEA) was then added dropwise to keep the mixture under basic condition. The reaction was stirred for 2h at room temperature under argon atmosphere. MS (ESI+): $[M+H]^+$ = 409.3 Da. After removal of the solvent, the water sensitive product 4 was directly introduced into the next step by reaction with TEOS as depicted in Scheme 1 to produce the hybrid material HCCA-SiO_2.

The reference SiO_2 was obtained as a white powder whereas the hybrid material HCCA-SiO_2 is obtained as a yellow powder. These amorphous silica were characterized for two different batches by TGA and elementary analysis. From the elemental analyses of C and N values, the organic loading was estimated using amorphous silica SiO_2 as a reference. It was found to be 1.402 $mmol.g^{-1}$ (σ = 0.008) and 1.375 $mmol.g^{-1}$ (σ = 0.009) from C and N measurements, respectively. The organic % mass is around 40%, value very close to this obtained by TGA. The long-term shelf stability of the hybrid material CHCA-$SiO_2$was evaluated by thoroughly washing a sample that was stored for one month at room temperature. After drying, an aliquot was subjected to elemental analysis providing C and N measurements of 1.399 $mmol.g^{-1}$ and 1.352 $mmol.g^{-1}$, respectively, indicating that no significant loss of organic content was detectable upon storage.

Mixture	Sequence	Entry	M (g.mol^{-1})	$(M+H)^+$	$(M+Na)^+$	$(M+K)^+$
	PICAK	P1	530.29	531.30	553.28	569.25
	DIGLVR	P2	671.40	672.40	694.39	710.36
A	WLIAGDR	P3	829.44	830.45	852.43	868.41
	LMYVHWVK	P4	1074.57	1075.58	1097.56	1113.53
	WGVYAPLFDK	P5	1194.61	1195.62	1217.60	1233.57
	SEGFK	P6	566.27	567.28	589.26	605.23
	SWAMVK	P7	720.36	721.37	743.35	759.33
B	YICPADR	P8	836.39	837.39	859.37	875.35
	NGFLMCSALK	P9	1082.53	1083.30	1105.28	1121.25
	ADPNYHIGETK	P10	1243.58	1244.59	1266.57	1282.55
	VTEFK	P11	622.33	623.24	645.22	661.19
	TPSSVLK	P12	730.42	731.43	753.41	769.38
C	LGHAPEVR	P13	877.48	878.49	900.47	916.44
	LMYVHWVR	P14	1102.58	1103.59	1125.57	1141.54
	MLQYGMFVER	P15	1272.60	1273.61	1295.59	1311.56
	FAQIR	P16	633.36	634.37	656.35	672.32
	SWAMVR	P17	748.37	749.38	771.36	787.33
D	YDTSIVQK	P18	952.49	953.50	975.48	991.45
	PGAHIWEAGAK	P19	1135.58	1136.59	1158.57	1174.54
	LESMGVDFNANK	P20	1323.61	1324.62	1346.50	1362.57
	AGVDGPK	P21	642.33	643.34	665.32	681.29
	AVGKKKK	P22	757.52	758.53	780.51	796.48
E	YDTSIVQR	P23	980.49	981.50	1003.48	1019.45
	VEYLASITLK	P24	1135.65	1136.66	1158.64	1174.61
	AEQVPLGSYDTGK	P25	1363.66	1364.67	1386.65	1402.62
	VTEFR	P26	650.34	651.35	673.33	689.30
	MWAENK	P27	777.35	778.36	800.34	816.31
F	AFISVGPLAR	P28	1029.60	1030.61	1052.59	1068.56
	GNGQIVFHAAR	P29	1168.61	1169.62	1191.60	1207.57
	QLDFGEVLANTGR	P30	1418.72	1419.73	1441.71	1457.68
	AGVDGPR	P31	670.34	671.35	693.33	709.30
	YICPADK	P32	808.38	809.39	831.37	847.34
G	CASHTFLVR	P33	1032.52	1033.53	1055.51	1071.48
	GHLPTVSENAR	P34	1179.60	1180.61	1202.59	1218.56
	IWDPSNLIAQPTK	P35	1481.79	1482.80	1504.78	1520.75

Table 1. Peptide mixtures and corresponding protonated and metal-cationed ions

Peptide Synthesis

Peptides were synthesized on the IBMM Synbio3 platform using Fmoc/tBu microwave assisted solid-phase peptide synthesis on 2-chlorochorotrityl resin.[32] After cleavage, peptides were purified by RP-preparative LC/MS (Autopurification system, Waters Milford, CA). All peptides were prepared as C-terminal carboxylic acids and their sequences were designed to mimic proteolytic digests performed with either trypsin or Lys-N. Prior to use, peptides were dissolved in deionized water at a 10^{-3}M concentration. These stock solutions were then diluted in deionized water in order to have 10^{-4}M, 10^{-5}M, 10^{-6}M and 10^{-7}M final peptide concentrations.Six peptide mixtures have been chosen according to their molecular mass and amino acids composition as depicted in Table 1.

Mass spectrometry

Mass spectrometry analyses were performed on instrument located in the IBMM platform of instrumentation, Laboratoire de Mesures Physiques of University Montpellier 2, Montpellier. MALDI mass spectra were recorded on an Ultraflex III TOF/TOF instrument (Bruker Daltonics, Wissembourg, France) equipped with LIFT capability. A pulsed Nd:YAG laser at a wavelength of 355 nm was operated at a 100 Hz (MS data including images) or 200 Hz (MS/MS data) frequency with a laser focus of 53%. The source was operated in the positive mode with a delayed extraction time of 30 ns. In conventional MALDI experiments, a solution of the selected CHCA matrix was mixed with the peptide sample in equal amount and 0.5 µL of this solution was deposited onto the MALDI target according to the dried droplet procedure. After evaporation of the solvent, the MALDI target was introduced into the mass spectrometer ion source. To perform LDI analyses on amorphous silica, the above mentioned standard MALDI plate (ground steel/polished) was used. 1 mL of acetonitrile (CH_3CN/H_2O) (3/7 or 5/5, v/v) was added to the SiO_2 and CHCA-SiO_2 substrates (quantities of 5 mg of inert material). This heterogeneous mixture was sonicated for 5 minutes, vortexed and 0.5 µL of such suspension was then aliquoted by a pipetting device and spotted onto the MALDI target defining a deposit position (spot). After solvent evaporation, a drop of the peptide aqueous sample (0.5 µL) was placed onto the CHCA-SiO_2 deposit and allowed to dry at room temperature. Data were acquired with the Flex Control software under the following conditions. For MS analyses and images acquisition, an acceleration voltage of 25.0 kV (IS1) was applied for a final acceleration of 21.95 kV (IS2). The reflectron mode was used for the Tof analyzer (voltages of 26.3 kV and 13.8 kV). For MS/MS analyses, an acceleration voltage of 8.0 kV (IS1) was applied for a final acceleration of 7.25 kV (IS2). The reflectron mode was used for the Tof analyzer (voltages of 29.5 kV and 13.9 kV). Fragmentation experiments were performed under laser induced dissociation (LID) conditions with the LIFT cell voltage parameters set at 19.0 kV (LIFT 1) and 3.2 kV (LIFT 2) for a final acceleration of 29.5 kV (reflector voltage) and a pressure in the LIFT cell around 4 x 10^{-7} mbar. The precursor ion selector was set manually to the first monoisotopic peak of the molecular ion pattern for all analyses. MS and MS/MS data were processed with the Flex Analysis software. Mass lists were generated according to the following parameters: centroid as peak detection algorithm, S/N threshold 3, peak width 0.5, and peak height 80%. External calibration was performed with commercial peptide mixture (Calibration peptide standard 2, Bruker Daltonics, Wissembourg, France). Images were recorded with the Flex Imaging software. The selected surfaces (5 mm^2) were irradiated with a laser fluence of 40%. The chosen resolution of 50 µm allowed imaging each deposit within 10 minutes.

Results and Discussion

Hybrid material preparation

It is well known to MALDI users that organic matrices must be present in large excess compare to the analytes (1000:1) to trigger the desorption/ionization processes. Provided that a high loading of CHCA onto silica is achieved, the resulting hybrid material should combine LDI properties from both organic and silica moieties. Our first attempts to post-functionalize silica nanoparticles with CHCA according to various chemistries provided the expected materials but with an organic content that was quite low (<<0.5 $mmol.g^{-1}$) (data not shown). Facing such disappointing results, we aimed to increase the organic loading onto silica by changing the synthetic strategy. A direct synthesis approach was undertaken, using hybrid trialkoxysilane derivative of CHCA copolymerized with tetraethoxysilane (TEOS). TEOS constitutes the 'elementary building block' of the hybrid silica, yielding amorphous organic-inorganic material bearing the expected CHCA pending chains. The preparation of unfunctionalized amorphous silica obtained only from TEOS condensation was done in parallel. This amorphous silica was used as reference in this article.

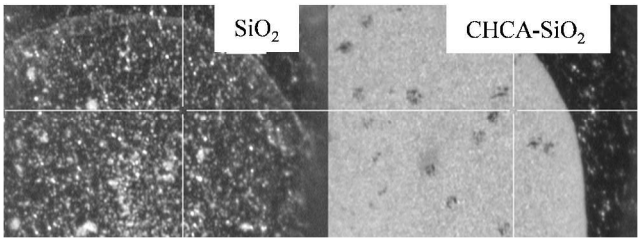


Figure 1. MALDI's camera pictures for SiO_2 and CHCA-SiO_2 deposits after laser irradiation

MS experimental set-up

Experimental conditions for sample and substrate deposition were first optimized in order to achieve an even peptide distribution upon air-drying. In contrast, high confinement of the deposited sample on a restricted area led to 'hot spots' where very abundant analyte signals were recorded. However, irradiation of the close surroundings only produced very tiny ion intensities affecting the detection response. As is it shown in Figure 1, amorphous SiO_2 gave heterogeneous deposits inducing 'hot spots' while we were pleased to see that CHCA-SiO_2 provided much more homogeneous deposits. Holes made by the laser upon irradiation (Figure 1) shown that dry CHCA-SiO_2 spots that looked like thin films behaved similarly as the CHCA organic matrix deposits. Practically, at the end of the LDI-MS experiments, the CHCA-SiO_2 deposits was very easily washed

with water/methanol after use as a conventional CHCA deposit making the MALDI plate re-usable for any purposes. Any user could thus perform MALDI and LDI experiments with the same equipment. Peptide mixtures that have been previously studied in the laboratory[22] were subjected to MALDI and LDI experiments in order to probe the performances of the new hybrid material in terms of sensitivity and sample coverage, *i.e.* number of detected peptides due to ionization discrimination. All analyses were carried in triplicate to validate the reproducibility and repeatability of the method. The results recorded for all studied mixtures (A to G) deposited at 10^{-5} M with CHCA-SiO_2 and CHCA as matrix are provided as supplementary information: one set of LDI-MS spectra is displayed in Figures S1 to S7 and Table S1 gathers the mean relative abundances of the detected peptide ions and the signal variations from the triplicated analyses. Since this new hybrid material was designed to prevent spectra pollution by matrix ions in the low mass range, particular attention was devoted to the potent production of CHCA ions which could occur upon in-source fragmentation of the organic pendant chains. Although some very low mass ions were observed with weak abundances in all conducted experiments, there were never attributed to CHCA but to surface and/or sample contaminations.

LDI-MS performance of the hybrid material

We first compare the desorption/ionization behavior of the peptide mixture B (Table 1) from CHCA-SiO_2 and from CHCA and amorphous SiO_2 taken as reference matrices (Figure 2).

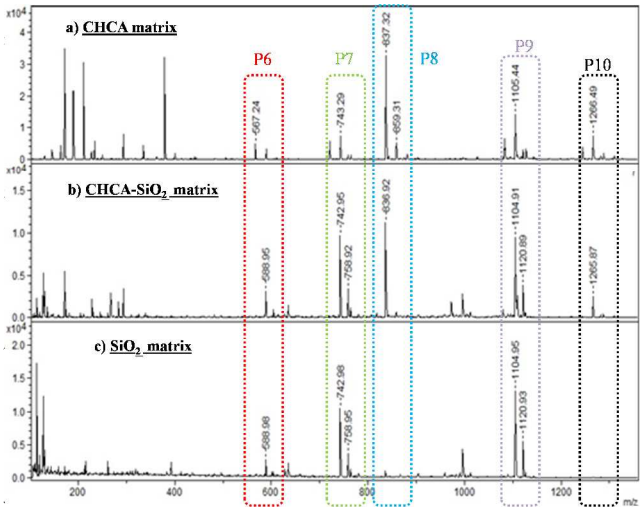


Figure 2. Peptide mixture B analysed from CHCA, CHCA-SiO_2 and SiO_2 as LDI-MS matrix

For each peptide, signal over noise ratios (S/N) of all produced ions corresponding to protonated and cationized species ($[M+H]^+$, $[M+Na]^+$, $[M+K]^+$) were cumulated to establish the overall signal intensity (see Table S1 of the supplemental material). From there, we determined the relative abundance for all peptides and then compared the LDI-MS performances for all tested substrates. On the spectrum **a)** we can see a classic spectrum for a peptide mixture deposited with CHCA, the low mass range of the mass spectrum being polluted by ions from the matrix, reaching intensity higher than the peptide ones. We can also notice discrimination for the low mass peptides (P6 and P7) in the studied mixture. For the same sample, in **b)** we used hybrid CHCA-SiO_2 as matrix. We noticed less pollution into the low masse range. The two signals around 1000 Da are due to the fragmentation of higher mass peptides (not detailed in this work). We can also notice less discrimination between the five peptides. Notably, peptides P6 and P7 were clearly detected exhibiting abundant molecular ions. LDI-MS/MS experiments were then carried out to sequence the detected peptides. For instance, the MS/MS spectrum of one peptide (P8, Mixture B) is displayed in Figure S8 of the supplementary data information. In **c)** is displayed the spectrum using amorphous SiO_2 as reference LDI matrix. As expected, hybrid material CHCA-SiO_2 shows a chimerical behavior in between an organic and an inert matrix; the spectrum recorded from CHCA is seemingly added onto the SiO_2 one.

Sample coverage

We decided to further evaluate the potency of hybrid CHCA-SiO_2 material to diminish the ionization discrimination by analysing the peptide mixture at decreasing concentrations. We choose to compare the cumulated relative signal to noise (S/N) ratio of all detected ions for each peptide from the studied mixture deposited onto CHCA-SiO_2, CHCA, and amorphous SiO_2. Here we present the results for quantities of deposited peptides of 50 pmol, 5 pmol, 500 fmol and 50 fmol of mixture B (Figure 3).

For 50 pmol, we noticed that only three peptides in the mixture were detected from amorphous SiO_2, whereas CHCA gave the five peptide ions but with severe ionization discrepancy. CHCA-SiO_2 gave hybrid results between both previous matrices, the three most intense peptide ions being those detected by amorphous SiO_2. The two other peptides are also desorbed with good S/N.

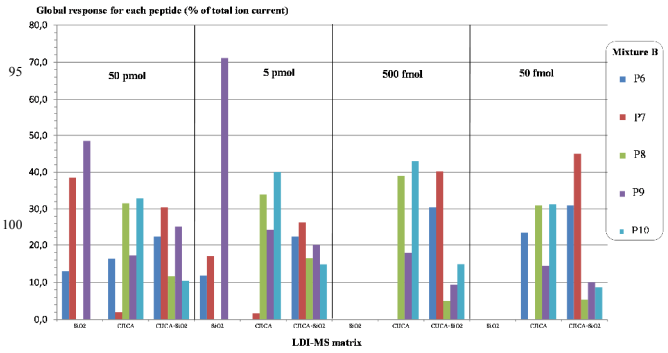


Figure 3. Ionization efficiency of the five peptides constituting mixture B at different deposited quantities.

At 5pmol, only three ions from the peptide mixture were seen with SiO_2 as matrix. With CHCA, the three heavier peptides were detected, the lighter peptide (P6) disappeared from the spectrum and the second one (P7) was largely discriminated. With CHCA-SiO_2, the five peptides are homogenously detected, showing that this new material is a better matrix in matter of lower ionization discrimination. At 500 fmol, the CHCA matrix gave only the three heavier peptides, while the hybrid material enabled to detect the five peptides. At 50 fmol, four peptides were now observed

while CHCA-SiO_2 was reaching its limit in term of sensitivity (S/N = 3-13), the five peptides from the mixture being nevertheless still detectable. The six others mixtures depicted in Table 1 were analysed at decreasing concentrations showing the same behaviors. Interestingly, the new material give hybrids results between SiO_2 and CHCA, indicating that probably many mechanisms of desorption, based on photoionization and thermal processes, must be involved. Moreover, when the quantities of deposited analytes were decreased, the LDI response observed from the inert substrate (SiO_2) seems to take over the CHCA one. At last but not least, imaging studies were carried out to assess the peptide localization within the amorphous CHCA-SiO_2 deposits. Whatever the targeted peptides, even if thin films were recovered after dryness, they were found to be exclusively concentrated at the outskirt of the deposit. The images of the protonated ion as well as metal-cationed species with sodium and potassium salts are shown in Figure 4a for one peptide (P7) of mixture B. This result was quite different from what we observed in our previous studies conducted with SiO_2 conditioned as nanoparticles that led also to thin film but that provided very homogenously distributed peptides all over the deposit.[21] The repeatability of ion measurements from the hybrid material was as good as the one observed with CHCA (CV < 30%) provided that laser shots were fired at the spot periphery.

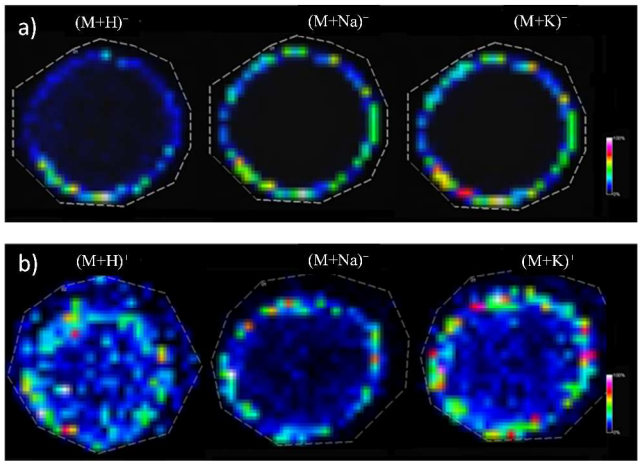


Figure 4. Images of protonated and cationized ions of peptide P7 (Mixture B) deposited at 10^{-5} M with a) CHCA-SiO_2 matrix and b) CHCA-SiO_2 supplemented with diammonium sulphate.

Detection sensitivity

In order to improve the detection response of the newly prepared hybrid material, organic additives including surfactants such as diammoniumhydrogen citrate, diammonium sulphate, trifluoroacetic acid, potassium perfluorooctanesulphonate, were added to the deposited solutions as commonly performed in reported LDI methods.[18,31] Only diammoniumhydrogen citrate and diammoniumsulphate showed some improvements with increased ion abundancies of the heaviest peptides at 10^{-6} M (500 fmol deposited) as showed in Figure S9 and Table S2 of supplemental information.

However, the images produced for the same peptide deposited at the same concentration with the best additives (diammonium hydrogen citrate and diammonium sulphate) showed that the analytes were more homogeneously spread all over the spot as displayed in Figure 4b. This behavior was observed for most of the studied peptides but some of them remained localized at the spot outskirt. The high content of organic pendant chains (>40%) anchored to amorphous silica were thus found to affect both the material physical properties (deposits recovered upon dryness as aggregates *vs* thin film) but also the peptide repartition within the film (with or without additives). To achieve efficient peptide detection, the laser shots must thus be concentrated at the edge of the spot without the recourse to an additive or it can be fired at random provided that diammonium hydrogen citrate and diammonium sulphate were added during the sample preparation protocol.

Conclusions

We have demonstrated that hybrid organic-inorganic amorphous CHCA-silica, easily prepared by direct sol-gel synthesis from CHCA derivative, can be used as a new target for LDI-MS analysis. This hybrid substrate allowed the efficient desorption/ionisation of peptides with masses ranging from 550 to 1400 Da. As expected this material provided also hybrid results between organic and inert matrices. The evaluation of the material has been undertaken with a large number of peptides to probe the detection efficiency including both sensitivity (limit of detection) and ionization discrepancy (sample coverage). Complementary studies have been performed to evaluate structural characterisation capacity of the CHCA-SiO_2 by MS/MS profiling. The described LDI-MS method was efficient as conventional MALDI experiment and produces complementary data in the low mass range.

Notes and references

[a]*Institut des Biomolécules Max Mousseron, UMR 5247, Universités Montpellier 1 et 2, Bâtiment Chimie (17), Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France*
[b]*Institut Charles Gerhardt, UMR 5253, CMOS, CC1701,Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France*

† Electronic Supplementary Information (ESI) available:
Figures S1. LDI-MS spectra of the studied peptide mixture A at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S2. LDI-MS spectra of the studied peptide mixture B at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S3. LDI-MS spectra of the studied peptide mixture C at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S4. LDI-MS spectra of the studied peptide mixture D at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S5. LDI-MS spectra of the studied peptide mixture E at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S6. LDI-MS spectra of the studied peptide mixture F at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Figures S7. LDI-MS spectra of the studied peptide mixture G at 10^{-5} M recorded from CHCA matrix (top) and from CHCA-SiO_2 matrix (bottom)
Table S1. Triplicate analyses of peptide mixtures A to G deposited at 10^{-5} M with CHCA and CHCA-SiO_2 as matrix
Figure S8. MS/MS spectrum of peptide P8 (Mixture B) deposited with CHCA-SiO_2 matrix.
Figure S9. Peptide mixture B deposited with different matrix: CHCA, CHCA-SiO_2, SiO_2 and CHCA-SiO_2 supplemented with diammonium citrate and diammonium sulphate.
Table S2. Relative ion abundances for peptide mixture B deposited on CHCA-SiO_2 with different additives.

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Laser desorption ionization mass spectrometry of peptides on hybrid CHCA organic-inorganic matrix

Clément Fleith,[a] Sonia Cantel,[a] Gilles Subra,[a] Ahmad Mehdi,[b] Jeremie Ciccione,[a] Jean Martinez[a] and Christine Enjalbal*[a]

Graphical abstract