Doped amorphous silica nanoparticles as enhancing agents for surface-assisted time-of-flight mass spectrometry

Lim, Angelina Yimei; Gu, Feng; Ma, Zhun; Ma, Jan; Rowell, Frederick

2011

Lim, A. Y., Gu, F., Ma, Z., Ma, J. & Rowell, F. (2011). Doped amorphous silica nanoparticles as enhancing agents for surface-assisted time-of-flight mass spectrometry. *Analyst*, 136(13), 2775-2785.

<https://hdl.handle.net/10356/94338>

<https://doi.org/10.1039/C1AN15172J>

© 2011 The Royal Society of Chemistry. This is the author created version of a work that has been peer reviewed and accepted for publication by *Analyst*, The Royal Society of Chemistry. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [DOI: <http://dx.doi.org/10.1039/C1AN15172J>]

Downloaded on 26 Apr 2023 18:30:46 SGT

Doped amorphous silica nanoparticles as enhancing agents for surface-assisted time-of-flight mass spectrometry

Angelina Yimei Lim ^a, Feng Gu ^b, Zhun Ma ^b, Jan Ma ^a and Frederick Rowell ^c

^aSchool of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798. E-mail: alim@ntu.edu.sg; Fax: +65 6790 9081; Tel: +65 6513 8071

^bNanofrontier Pte Ltd, 50 Nanyang Drive, Research TechnoPlaza, Singapore 637553

^cRoar Particles plc, Netpark Incubator, Sedgefield, County Durham, TS21 3FD, UK

This article examines the use of doped amorphous silica nanoparticles for surface-assisted laser desorption/ionisation-time of flight-mass spectrometry (SALDI-TOF-MS) of hydrophilic and hydrophobic compounds. A range of particles with surface aliphatic carboxylic, aminophenyl, phenyl or aminopropyl groups have been produced and these have been doped with carbon black, polyaniline or graphite. The effects of surface groups and dopants on the laser desorption/ionisation process were studied. The key factor in effective LDI was the presence of carbon black dopant carrying carboxyphenyl or phenyl residues for positive and negative ion formation. The second key factor was the presence of hydrophilic surface functional groups for hydrophilic amino acid analytes for their detection in positive or negative mode as protonated or de-protonated species respectively whereas hydrophobic surfaces were needed for ionisation *via* cationisation for the hydrophobic analyte squalene. The mechanism for LDI of these particles appears to involve initial adsorption of the analyte onto the surface of the particle, formation of primary ions *via* adsorption of laser UV irradiation by carboxyphenyl residues attached to the carbon black network which act in an equivalent way to the matrix in matrix-assisted LDI. This is followed by reaction of the primary ions with neighbouring adsorbed analyte molecules. The latter are then released possibly *via* thermal desorption following proton donation or acceptance from/to *via* surface residues such as carboxylate groups associated with the carbon black within the dopant. Alternatively in the absence of such proton donor/acceptor residues as with hydrophobic particles, the primary ions are released from the particles during desorption and form cation adducts as sodiated and potassiated species in the gas phase above the surface.

Introduction

A variety of nanoparticles have been used to detect and characterise a wide range of compounds by employing mass spectrometry to detect ions formed on laser irradiation of

the compounds adsorbed onto the surface of the particles. This technique is termed surface-assisted laser desorption/ionisation mass spectrometry (SALDI-MS) and is often linked to time of flight detection as SALDI-TOF-MS. The most widely applied analytical method that preceded SALDI-TOF-MS generally employed as its first step, addition of a mixture of an external chemical agent (matrix) in excess and the analyte(s), both in solution, onto a metal target plate. On solvent evaporation, the matrix crystallised with analyte molecules spread throughout the resulting lattice of crystals. Laser irradiation onto the surface resulted in energy absorption by the matrix and the subsequent charge transfer to the analyte(s). The analyte(s) is then detected as a quasi-molecular ion. This approach is termed matrix assisted laser desorption/ionisation-TOF-MS (MALDI-TOF-MS).^{1,2}

The advantage of SALDI-TOF-MS stems from the absence of ions from the matrix which can severely interfere with the spectra from the target analytes, especially with those of low molecular mass since the nanoparticles used in SALDI-TOF-MS generally produce relatively simple background spectra.³ Types of nanoparticles used in SALDI-TOF-MS include metals and metal oxides,⁴⁻⁶ carbon including activated carbon,⁷ graphite,^{8,9} carbon nanotubes,^{10,11} silicon^{12,13} and silicate.¹⁴⁻¹⁶ In this process, analytes are generally deposited as a suspension of the analyte solution and the particles, onto the metal target plate. Following evaporation, the material on the plate is subjected to analysis by LDI-TOF-MS.¹⁷⁻²⁰

The use of hydrophobic sub-micron silica particles in SALDI-TOF-MS to identify drugs, nicotine and other residues and metabolites of both exogenous and endogenous origin within latent fingerprints has recently been reported.²¹⁻²⁵ To date, using this hydrophobic powder, highly polar constituents in latent fingerprints have not been identified within the SALDI-derived spectra. This is presumably due to the inability of polar constituents to adhere to hydrophobic silica particles resulting in ineffective energy absorption and charge transfer on laser irradiation. One of the aims of this study is to modify these hydrophobic silica nanoparticles to render them capable of detecting examples of common hydrophilic constituents such as those present in fingerprints using SALDI-TOF-MS. Amino acids will be used as an example of hydrophilic constituents and as an example of a major hydrophobic constituent, squalene will be examined. In addition, graphite and polyaniline will be studied as possible replacements for carbon black as UV absorbing agents for the SALDI process. Graphite as mentioned earlier, has been used in several studies as an aid to the ionisation process.^{7,8} The conducting polymer, polyaniline, was chosen since it has an absorption wavelength which peaks at 300 nm²⁶ similar to the wavelength of 337 nm for the nitrogen laser used for this study.

The understanding of the desorption/ionisation mechanism(s) of SALDI still remains contentious whereas those in MALDI-TOF-MS are much better understood.^{27,28} There is much research on new SALDI materials and its many uses but few attempt to understand the physical chemistry of these materials in the SALDI process. Through these experiments, we seek to not just introduce an effective SALDI nanomaterial for the detection of hydrophilic compounds but also to understand the desorption/ionisation mechanisms involving the use of these silica nanoparticles.

Experimental

Materials

Aqueous carbon black (CAB-O-JET® 300; provided as a 15% w/v pigment in water) was purchased from Cabot Carbon Ltd., Boston, Massachusetts, USA. The network of the carbon black particles carried 2-carboxyphenyl residues.²⁹ Tetraethyl orthosilicate (TEOS, 98%, Fluka), phenyltriethoxy orthosilicate (PTEOS 98%, GC), 3-aminopropyltriethoxysilane (APTES, 99%), aniline (ACS Reagent, ≥99.5%), ammonium persulfate (reagent grade, 98%), graphite (product number 78391), iron(II,III) oxide nanopowder and hydrochloric acid were obtained from Sigma-Aldrich, St Louis, Missouri, USA. *p*-Aminophenyltrimethoxysilane (APhTES), 3-(triethoxysilyl) propylsuccinic anhydride (TPSA), *N*-(trimethoxysilylpropyl) ethylene-diamine triacetic acid (EDTA) trisodium salts were purchased from Gelest Inc. (Morrisville, Pennsylvania, USA). Amino acids, squalene (minimum 98%), creatine, urocanic, palmitic, oleic acids and 2,5-dihydroxybenzoic acid (DHB) were from Sigma-Aldrich. For carbon black derivatisation, Cabot XC72R (pure carbon black) was from Cabot Carbon Ltd and aniline derivatives were from Sigma Aldrich. All reagents were used as received. All solvents used were of HPLC grade.

Instrumentation

SALDI experiments were carried out using a Shimadzu Biotech AXIMA TOF²™ time-of-flight mass spectrometer (Kratos Analytical Ltd., Manchester, UK) precalibrated with a calibration mix at positive reflectron mode. The calibration mix was prepared by mixing solutions of papaverine (1 mg ml⁻¹ in methanol), reserpine (1 mg ml⁻¹ in acetonitrile) and cesium iodide (10 mg ml⁻¹ in deionised [DI] water) in the respective volume ratio 1 : 1 : 2. Calibration was carried out by spotting 1 µl of calibration mix with 1 µl of 2,5-dihydroxybenzoic acid (10 mg ml⁻¹ in 20 : 80 v/v acetonitrile : water) onto a stainless steel MALDI plate and left to air-dry at ambient conditions. Mass charge values of 132.91 [Cs]⁺, 340.15 [papaverine + H]⁺, 392.72 [Cs₂I]⁺ and 607.27 [reduced reserpine + H]⁺ were used to calibrate the instrument in positive mode. For negative mode calibration, mass charge values of 153.02 [DHB-H]⁻, 386.71 [CsI₂]⁻ and 646.52 [Cs₂I₃]⁻ were used.

Field Emission Scanning Electron Microscopy (FESEM) was carried out on a JSM-6340F machine, JEOL Ltd., Akishima, Tokyo, Japan to measure the size and shape of the particles. Functional group analysis was carried out using Raman Spectrometry on an alpha300R instrument from WITec GmbH, Ulm, Germany using a 488 nm laser source and X-ray Photoelectron Spectroscopy (XPS) on an ESCA VG220i-XL from Thermo Scientific, Waltham, Massachusetts, USA. Gas adsorption was performed using an ASAP 2020 instrument, Micromeritics Instrument Corporation, Norcross, Georgia, USA for particle porosity calculations.

Synthesis of silica particles

Hydrophobic carbon black doped powder

(A) *PTEOS derived particles*. To a 50 ml tube, 35 ml of ethanol, 5 ml deionised water (DI water), 3 ml CAB-O-JET® 300 carbon black (CB) suspension, 2 ml TEOS, 2 ml PTEOS and 3.3 ml of ammonium hydroxide (28%) were added. The sealed tube was rotated at room temperature at 60 rpm overnight and then centrifuged at 4500 rpm for 5 minutes. The supernatant was decanted off and 40 ml ethanol : water (50 : 50) mixture was added to the residue. The particles were then re-vortexed until they were completely re-suspended. This washing step was repeated by using ethanol : water (70 : 30) mixture and pure ethanol as solvents, respectively. The final slurry was transferred to an evaporating basin and dried in the oven at 40 °C.

Hydrophilic carbon black doped powder

(B) *TEOS derived particles*. To a 50 ml tube, 35 ml of ethanol, 5 ml DI water, 3 ml CAB-O-JET® 300 carbon black suspension, 4 ml TEOS and 3.3 ml of ammonium hydroxide (28%) were added and sealed tube was rotated overnight. The washing and drying steps were the same as described for the synthesis of silica particles (A).

(C) *Aliphatic di-carboxylated silica from TEOS modified with TPSA*. To a 50 ml tube, 35 ml of ethanol, 5 ml DI water, 3 ml CAB-O-JET® 300 carbon black suspension, 4 ml TEOS and 3.3 ml of ammonium hydroxide (28%) were added and allowed to rotate for 4 hours. After which, the resulting silica colloidal dispersion was surface functionalized by adding 0.4 ml of TPSA. The mixture was rotated overnight to react when the contents were centrifuged at 4500 rpm for 5 minutes. The supernatant was decanted off and 40 ml DI water was added. This was followed by vortexing of contents to achieve a suspension state. The particles were collected following another centrifugation and the residue acidified by addition of HCl aqueous solution (0.1 M) until pH value reached 5. They were further washed with ethanol : water (70 : 30 by volume) then pure ethanol before drying in the oven at 40 °C.

(D) *Aliphatic tri-carboxylated silica from TEOS modified with EDTA*. The same protocol was followed according to preparation of (C) but TPSA was replaced by EDTA.

(E) *Aminopropylated-silica from TEOS modified with APTES*. The same protocol was followed as for preparation of (C) but TPSA was replaced by APTES.

(F) *p-Aminophenylated silica from TEOS modified with APhTES*. The same protocol was followed as for preparation of (C) but TPSA was replaced by APhTES.

Fig. 1a shows the functional groups attached to the surfaces of the resulting silica particles. Undoped particles for (A) to (F) were prepared according to the reactions above by replacing 3 ml CAB-O-JET® 300 carbon black suspension with 3 ml DI water. Similarly, graphite-doped particles for (A) to (F) were made by replacing 3 ml CAB-O-JET® 300 carbon black suspension with 1 g graphite.

Polyaniline powders. A mixture of 0.5 mol l⁻¹ aniline and 10 ml of 1.0 M HCl was sonicated for 30 minutes. After which, 5 ml of 1.1 mol l⁻¹ ammonium persulfate was added drop-wise over 30 minutes into the magnetically stirred mixture under ambient conditions. The contents were centrifuged and the residue was washed with deionised

water followed by acetone. The washing procedure was repeated 3 times and the residue transferred to an evaporating basin which was dried in the oven at 60 °C overnight. The dried residue of polyaniline was suspended in deionised water at a concentration of 200 g l⁻¹ and used in place of carbon black for the preparation of the following particles.

(G) *PTEOS polyaniline particles*. The same protocol was followed as for preparation of (A) but CAB-O-JET® 300 carbon black replaced by polyaniline suspension.

(H) *APTES polyaniline particles*. The same protocol was followed as for preparation of (E) but CAB-O-JET® 300 carbon black replaced by polyaniline suspension.

(I) *TPSA polyaniline particles*. The same protocol was followed as for preparation of (C) but CAB-O-JET® 300 carbon black replaced by polyaniline suspension.

Synthesis of phenylated carbon black derivatives

(1) Aniline

(2) 2-Aminobenzoic acid

(3) 3-Aminobenzoic acid

(4) 4-Aminobenzoic acid

APTES particles (E) and PTEOS particles (A) were prepared as described above but with four types of carbon black dopants. These dopants were prepared from diazotization of the (1) to (4) aniline derivatives listed above *via* diazotization in the presence of Cabot XC72R carbon black following an established method²⁹ to produce: (i) XC72R (underivatised carbon black), (ii) XC72R with phenyl substituent, (iii) XC72R with 2-carboxyphenyl substituent, (iv) XC72R with 3-carboxyphenyl substituent and (v) XC72R with 4-carboxyphenyl substituent. The final pH value was adjusted to 5 using DI water washes. Fig. 1b shows the structures of the resulting products with the aromatic residues covalently attached to the carbon black networks.

Particle characterisation

Hydrophobicity/hydrophilicity of powders from water wetting tests. A small portion (about 5 mg) of each of the powders A, B, C, D, E and F was placed onto a clean glass slide. A single drop of DI water was carefully dispensed onto the slide adjacent to the edge of the powder. The wetting of the powder and the time taken to achieve complete wetting of the spot was noted.

Size and shape analysis of powders by FESEM. 1 mg of each powder was suspended in 1 ml of ethanol and sonicated for 15 minutes. Immediately after sonication, 5 µl of suspension was placed onto an adhesive carbon tape, coated with a thin layer of platinum and examined under the FESEM at 15 kV.

Porosity of powders by nitrogen gas adsorption. The as-prepared powders were degassed at 100 °C for 4 hours prior to analysis. The pore size distribution was calculated according to the Barret–Joyner–Halenda (BJH) method.

Functional group modification verification by Raman, X-ray photoelectron spectroscopy and salicylaldehyde colour test. 10 mg of undoped powder sample was pressed onto a clean glass slide to form a smooth and flat surface. Raman spectrometry was carried out using a 488 nm laser, with spectral accumulation of 5 and integration time of 2 seconds. For XPS analysis, 1 mg of undoped powder sample was mounted onto a XPS stub using conductive carbon tape. A salicylaldehyde colour test was carried out by adding 20 times dilution of salicylaldehyde in acetonitrile to 0.1 g of undoped particles.^{30,31}

Effect of pH washes on LDI-MS for APTES particles (E). APTES-derived particles (DP), at pH 5 was washed with either 1 M HCl or NaOH solutions to obtain APTES particle suspensions of pH 1 and pH 10 respectively.

SALDI-TOF-MS of amino acids, squalene, fatty acids and metabolites

Solutions of 5 amino acids (alanine, lysine, glutamic acid, phenylalanine, tyrosine; 100 $\mu\text{g ml}^{-1}$ in deionised water), squalene (100 $\mu\text{g ml}^{-1}$ in acetone), 2 fatty acids (palmitic and oleic acids; 100 $\mu\text{g ml}^{-1}$ in ethanol) and 2 metabolites (creatine and urocanic acid; 100 $\mu\text{g ml}^{-1}$ in deionised water) were prepared. The silica particles were suspended in ethanol in a ratio of 1 : 2 (v/v) prior to use. The SALDI efficacies of silica matrices were determined out by mixing 1 μl of selected analyte (equivalent to 100 ng of each) with 1 μl of either A, B, C, D, E or F silica particle suspension onto a clean, 384-welled steel target plate. The resulting mixture was allowed to air-dry under ambient conditions. SALDI analysis was carried out at a fixed laser setting of 65 to 80 (power = 2.17–2.67 mW) with 100 accumulated profiles at 1 laser shot per profile in either positive or negative reflectron mode with the exception of fatty acids and metabolites which were only analysed in positive mode. All samples were prepared and analysed at least 4 times.

Limit of detection (LOD) of amino acids, squalene, fatty acids and metabolites

The prepared analyte concentrations of 100 $\mu\text{g ml}^{-1}$ were further diluted 1 : 10 v/v using the solvents in which the standards were dissolved as described above (deionised water, acetone or ethanol) to produce a final concentration of 100 ng ml^{-1} . These diluted standards (1 μl in each case, depositing 100, 10 and 1 and 0.1 ng of each analyte) were subjected to MS analysis in positive mode as described above using either APTES-DP–CB for amino acids and other polar metabolites or PTEOS-DP–CB for squalene and fatty acids. Tests were repeated using these diluted standards in the presence of the commonly used MALDI matrix, DHB (1 μl) added in place of the particle suspension. Following SALDI-MS and MALDI-MS, the intensities of the peaks were then compared. All samples were prepared and analysed at least 2 times.

Results

Characterization of particles

The wetting tests demonstrated that the TEOS-derived particles (DPs) (B) were highly hydrophilic (immediate wetting) and that the PTEOS-DPs (A) were highly hydrophobic (no wetting). For the undoped particles wetting was slower than for the (B) particles and wetting rates were EDTA-DPs (D) > APhTES-DPs (F) particles. APTES-DPs (E) and TPSA-DPs (C) particles were not completely wet after 10 minutes indicating that they were only slightly hydrophilic. The hydrophilicity of these particles deduced from the wetting profiles is B > D > F > E = C. For the carbon black embedded particles wetting was slightly faster presumably due to the presence of ionic carboxyphenyl surface groups on the carbon black particles. Complete wetting was seen for all the particles after 10 minutes except for (A) and the rate of wetting and hence order of hydrophilicity was the same as for the equivalent un-doped particles. These tests were not performed on the polyaniline-doped particles.

FESEM pictures (not shown) indicated that the size of all the hydrophilic nanoparticles is roughly spherical and approximately 50 nm in diameter. The particles have an average pore width of 10 nm according to calculations based on the BHJ method. With reference to a publication by the International Union of Pure and Applied Chemistry (IUPAC), solids containing pore diameters of between 2 and 50 nm are referred to as mesoporous structures.³² Fig. 2a shows the Raman spectra of 2 better MS-performing hydrophilic matrices, TPSA- and APTES-DPs compared with TEOS-DPs. TEOS-DPs are used for comparisons as TEOS is the common core particle used for subsequent surface modification in the formation of APTES- and TPSA-DPs. Peaks are observed at 3299.4 cm⁻¹ and 3455.7 cm⁻¹ for undoped APTES-DPs indicative of a primary amine group. These peaks are absent in the undoped TEOS-DPs. The prominent broad peak observed at 2920.9 cm⁻¹ only for TPSA-DPs indicates the presence of carboxylic acid C-H and O-H bonds. XPS was also carried out to compare the surface composition of TEOS- against APTES- and TPSA-DPs. Results were as expected with APTES-DPs having a much higher percentage of nitrogen than TPSA-DPs and which was absent in TEOS-DPs (Table 1). With reference to Table 1 and Fig. 2b, presence of carbonyl groups was detected in TPSA-DPs but was absent in both TEOS- and APTES-DPs. In addition, a qualitative colour test for amino groups was carried out using salicylaldehyde. Undoped APTES-DPs and APhTES-DPs modified particles turned yellow upon the addition of salicylaldehyde indicating the presence of amine groups whereas TPSA- and EDTA-DPs remained colourless even after 24 hours.

SALDI-TOF-MS of amino acids, squalene, fatty acids and metabolites

Positive ion reflectron mode for analysis of amino acids using particles A, C-I. In positive reflectron mode, best results were obtained for the 5 amino acids alanine (methyl R group), lysine (aminobutyl R group), tyrosine (phenoxy R group), phenylalanine (phenyl R group) and glutamic acid (carboxylate R group) when the hydrophilic particles from C, D, E and F were used as SALDI-agents and when carbon black carrying 2-carboxyphenyl residues was embedded within them (Table 2). These particles possess polar surface groups in addition to 2-substituted benzoic acid groups attached to the

carbon network within carbon black. This combination of hydrophilic residues and embedded carbon black powder generally produced $[M + H]^+$ and $[M + Na]^+$ peaks for the majority of the 5 amino acids. When the doping agent, carbon black (CB), was replaced by graphite, polyaniline or when no doping agent was present, then either no peak was observed or they were seen with low intensities demonstrating the fundamental role of this doping agent in the SALDI-TOF-MS process.

Of the hydrophilic powders tested, TPSA-DPs with carboxylated carbon black (CB) dopant recorded the highest analyte intensity readings for analysis of amino acids. Overall, TPSA-DPs–CB gave excellent signals for all 5 amino acids as positive quasimolecular ions, appearing with high intensities as both $[M + H]^+$ and $[M + Na]^+$. APTES-DPs–CB was a close second with all 5 amino acids recording intense readings as $[M + H]^+$. Using the most hydrophobic powder, PTEOS-DPs–CB, tyrosine was detected as the dimer ion $[2M]^+$. Alanine, glutamic acid, lysine and phenylalanine were largely detected as $[M + Na]^+$. All 3 polyaniline-containing particles gave poor or no results.

Although TPSA-DPs–CB gave higher intensities for the amino acids analysed, the spectral quality of APTES-DPs–CB was far better with low background noise and fewer background peaks, producing a clean spectrum, as shown in Fig. 3a–c. APTES-DPs–CB reported much higher *S/N* ratios than compared to TPSA-DPs–CB. Thus, APTES-DPs–CB was the best performer among the powders tested for detection of the amino acids in positive ion reflectron mode.

In positive mode, pH 1 washed APTES-DPs–CB gave the highest intensity reading for phenylalanine $[M + H]^+$ at *m/z* 166 whilst pH 10 washed particles produced the highest intensity reading for $[M + Na]^+$ at *m/z* 188. This was to be expected since at low pH (<3), all carboxylate and amine groups will be fully protonated across the surface of the particles whilst at high pH the carboxylate groups will all be present as the sodium salts. However APTES-DPs–CB washed at pH 5 produced better quality spectra compared to both pH 1 and 10, with minimal background noise. Spectra blanks for APTES-DPs–CB at pH 1 gave significant noise between *m/z* 250 and 450 whilst APTES-DPs–CB at pH 10 gave many background peaks below *m/z* 300. Spectra blanks for APTES-DPs–CB at pH 5 were relatively clean with just a few low intensity background peaks. The observed increase in background at pH 1 and pH 10 at specific *m/z* regions may be due to chemical degradation of adsorbed analytes and/or surface residues on the particles on laser irradiation. Fig. 4 shows the signal intensity results for silica particles with (i) underivatized carbon black dopant, (ii) carbon black dopant carrying phenyl residues, (iii) 2-carboxyphenyl residues, (iv) 3-carboxyphenyl residues and (v) 4-carboxyphenyl residues. Again, intensities of $[M + H]^+$ and total ion intensities are greater for the hydrophilic APTES-derived-2-carboxyphenyl-CB particles (E(ii)) than the equivalent hydrophobic PTEOS-DP–CB particles. It is interesting to note that for the hydrophobic PTEOS-DPs (A), 3-carboxyphenylated carbon black particles produced the highest signal intensities whilst for the hydrophilic particles (E) this was seen with the 2-carboxyphenylated particles.

Positive ion reflectron mode for analysis of squalene, using particles A, C–I.
Sodiated squalene was clearly detected using PTEOS-derived CB analysed in positive ion reflectron mode. TEOS-derived CB gave good signals for squalene as well. This was

followed by TPSA-derived CB and EDTA-derived CB which gave weak intensities at $[M + Na]^+$. The other powders had insignificant detection values. Referring to Fig. 3d, PTEOS-derived CB produced the best results, giving a clean spectrum with the peak of interest, m/z 433 ($S/N = 22$), clearly shown with. Squalene was not detected as $[M + H]^+$. Other studies have shown that non-polar compounds, like squalene, are not easily detected in the $[M + H]^+$ form by SALDI-MS.³³

Negative ion reflectron mode for analysis of amino acids and squalene, using particles A, C–I. As shown in Table 2, for all powders tested, no signal was detected for squalene. The five amino acids were mainly detected as $[M - H]^-$ ion. TPSA-DPs–CB gave the highest intensity values for amino acid detection. However, after taking into account spectrum quality and background noise, EDTA-DPs–CB would be the overall preferred particle matrix for detection of these amino acids in negative mode. This is followed by TEOS-, APhTES- and APTES-DPs–CB. The carboxylic acid-containing particles performed better than the amine modified particles for the detection of amino acids. This is expected since particles were used following washing to pH of 5 where the majority of carboxylate groups likely to be in the present as anions and proton acceptors, assuming the pK_a of the aliphatic carboxylate group is approximately 4 on the surface of the particles. At this pH, the aliphatic and aromatic amino groups will still be mainly protonated and hence poorer proton acceptors. Although not faring as well when compared to the hydrophilic matrices, PTEOS-DPs–CB was able to produce relatively good peak intensities for amino acid detection as $[M - H]^-$. Un-doped, graphite-doped or polyaniline-doped particles were unable to act as enhancing agents in negative mode, producing insignificant signal detection.

In negative mode, no $(M - H)^-$ peak was seen with pH 1-washed APTES-DPs–CB. This was expected since the fully protonated amino groups will not act as proton acceptors. Highest $[M - H]^-$ intensity reading was observed at pH 10 again as expected since the primary amino groups are now able to function as proton acceptors. As before the quality of the negative spectrum at pH 5 was better than that at pH 10, exhibiting minimal background interference peaks. Similar results (not shown) were also observed for APTES-DPs–CB at pH 1, 5 and 10 with the four other amino acids.

SALDI-TOF-MS of fatty acids and metabolites. Using APTES-DPs–CB and PTEOS-DPs–CB, additional tests were carried out on fatty acids: palmitic and oleic acid and the endogenous polar metabolites creatine and urocanic acid. Referring to Fig. 5a and b, PTEOS-DPs–CB gave better results for both fatty acids with higher S/N ratios as $[M + Na]^+$ and $[M + K]^+$ ions compared to APTES-DPs–CB. Conversely, as shown in Fig. 5c and d respectively for the hydrophilic metabolites creatine and urocanic acid, APTES-DPs–CB was the preferred matrix with significantly higher intensity signals as $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$ ions.

Limit of detection (LOD) of analytes. The results for the LOD study are shown in Table 3. The LOD was taken as the mass of analyte that produced a signal to noise ratio of above 3 : 1 for the chosen spectral peak. The LOD of analytes adsorbed onto the particles was around 10 ng (range 1–100 ng). For the amino acids and other polar metabolites tested, DHB performed better than APTES-DP–CB for 4 of the 7 tested,

while glutamic acid had a lower LOD using these particles, with urocanic acid and lysine giving equivalent results. The LOD results with the three non-polar analytes showed that PTEOS-DP-CB gave a lower LOD with oleic acid while DHB was better with squalene. It was also observed that the particles resulted in cleaner background spectra compared to DHB over the spectral range of interest. In Fig. 6a, only the peaks relevant to creatine (m/z 132, 154, 170) were observed when APTES-DPs-CB was used as a particle matrix. However when DHB was used, several other peaks other than the relevant creatine peaks were detected as well. Similarly in Fig. 6b, when PTEOS-DP-CB was used, peaks relevant to oleic acid (m/z 305, 321) were clearly detected with minimal background interference. When DHB was used, peaks other than those specific for oleic acid were also observed.

Discussion

The results indicate that carbon black plays a key role in the ionisation process. It is well known that carbon can act as a SALDI-enhancing agent in the form of graphite,^{8,9} fullerenes, carbon nanotubes,^{10,11} diamond and as carbon black itself as activated carbon.⁷ The carbon atoms in these isomorphs exist as either planar networks (sp^2 hybridized; first three examples) or as three dimensional networks (sp^3 hybridized, last two examples). The reasons for this ionizing capacity possibly stem from its conductivity, both thermal and electronic, and its ability to adsorb UV radiation at 337 nm, the wavelength of the nitrogen laser used in this study. It has also been noted that carboxylated forms of carbon nanomaterials, including diamond, graphite, carbon nanotubes and fullerenes formed on acid oxidation, showed improved SALDI-ionisation properties with peptides although the mechanism for this has not been fully elucidated.^{34–36}

When graphite was embedded into the silica particles the ionisation properties of the resulting particles for the analytes tested were poor compared with those containing carbon black. Also, it was not possible to replace carbon black with polyaniline as the resulting particles produced weak or no signals although it is also a conducting polymer and UV adsorbing agent. The effect of carbon black possibly stems from the fact that the carbon black was supplied as a carboxylated product formed from interaction of diazotised anthranilic acid with carbon black which results in covalent incorporation of ortho-substituted benzoic acid residues into the carbon network.²⁹ The ability of 2-substituted aromatic compounds to act as matrix enhancing agents in MALDI-TOF-MS is well established and appears to stem from their ability to adsorb energy in the UV region and transfer this energy to analyte molecules leading to their protonation in the primary stage of the MALDI-processes.³⁷ To test this, unmodified carbon black was grafted with carboxyl side groups at either the 2-, 3- or 4-carboxyphenyl positions using the appropriate diazotised amino-benzoic acid. This modified carbon black was then used to synthesize APTES (E) and hydrophobic PTEOS particles (A). APTES particles containing the carbon black with carboxyl side groups at the 2-position produced the best results when tested with the amino acids. It may be inferred therefore that the presence of 2-carboxylated phenyl residues on the carbon black dopant within the silica nanoparticles greatly assisted ionisation of analyte molecules that are adsorbed onto the surface of the particle.

If analyte adsorption is also a key prerequisite for influencing the LDI-ionisation process then it will be expected that the nature of the other surface groups on the particle will play a major influence on the initial adsorption process. This was found in practice since hydrophilic particles having flexible alkylcarboxylic (C, D) and alkylamino (E, F) residues gave the best $[M + 1]^+$ ionisation for amino acids and hydrophilic metabolites but not hydrophobic squalene or fatty acids. However when hydrophobic particles with surface phenyl groups (A) were used, the situation was reversed with $[M + Na]^+$ ions mainly produced for the analytes.

The results observed from this series of experiments may provide a better understanding of the SALDI process taking place on the silica particles studied herein. It appears that two steps may be required for successful detection of analytes; firstly it is necessary that the analyte molecules are bound to the surface of the particle and hence particles must be chosen which possess suitable surface functional groups which are compatible with those of the target analyte. Secondly, ionisation-enhancing agents must be embedded within the particles to achieve ionisation of the adsorbed analyte during laser irradiation. This doping agent appears to act in a similar manner to a conventional chemical matrix such as 2,5-dihydroxybenzoic acid in the MALDI-TOF-MS process serving to assist in the ionisation process. Carbon black acts as both a UV absorbing agent and a conducting medium to transfer heat and charges within the particle. In addition, a carboxyphenyl residue attached to the carbon black is required to effect analyte ionisation following UV irradiation. The presence of such surface carboxylic acid groups is implied from the faster wetting times observed for carboxylated carbon black-doped particles compared to those seen when untreated carbon black was used. This is to be expected since carboxylated carbon black is used commercially as a wetting agent in inks. This represents the primary ionisation step also seen in MALDI-TOF-MS. These findings support the pseudo proton transfer process suggested by Chang *et al.*³⁸ whereby a proton from the acidic matrix (the 2-carboxyphenyl group in our particles) is shared with a basic site of the analyte. Using an amino acid as an example, as illustrated in Fig. 7a, ionic interactions can occur between aminoalkyl silyl groups and carboxyphenyl groups attached to the carbon black dopant and an adsorbed amino acid molecule. The presence of electron or proton accepting residues on surface groups adjacent to the carboxyphenyl matrix-like residue and the adsorbed analyte, may result in charge delocalization which may lower the ionisation potential of the surface-attached matrix-like residue (carboxyphenyl group) resulting in enhanced multi-photon ionisation of the matrix-like complex on the surface.³⁹

As seen in Fig. 7a, ionic interactions between surface groups and the amino acid serve to hold the amino acid close to the surface of the particles. This may prevent early desorption of the analyte through thermal effects on laser irradiation enabling more efficient proton donation or electron donation to occur between the ionised surface “matrix” groups and analyte molecules on the surface during secondary processes. It is assumed that such secondary processes producing protonated or deprotonated species occur on the surface of the particles. The ionised amino acid molecules then leave the surface due to thermal effects and electrostatic attraction towards the detector.

This model assumes that unlike MALDI-TOF-MS, little or no secondary ionisation occurs between activated carboxylated aromatic residues and analyte molecules in the desorbed state above the surface on which the particles are immobilised since these “matrix-like” residues are covalently anchored to the carbon black backbone embedded into the particles’ surface. Spectra of the doped particles washed at pH 5 are relatively simple indicating that little chemical decomposition or ablation of the surface occurs. At pH 5, the phenyl-carboxylate group on carbon black of APTES-DP-CB and PTEOS-DP-CB (pK_a of about 4 in benzoic acid derivatives) will be about 90% protonated. Since the pK_a values for the α -carboxylic acid groups of the amino acids are between 1.8 and 2.6, these groups are over 99% de-protonated at pH 5. For the α -amino groups the pK_a values are between 8.8 and 10.6, hence in all cases at pH 5 these amino groups will be fully protonated. For glutamic acid and aspartic acids with ionisable side chains, the carboxylic acid groups (pK_a around 4 for both) will be about 10% ionized. For lysine (pK_a 10.53) and tyrosine (pK_a 10.07) the side chain groups will be fully protonated at pH 5. The aminopropyl residues on the surface of APTES-DPs will also be fully protonated. Hence at a pH of 5 the ionisation of the residues on the surface of the particles and on the adsorbed amino acids will favour the proposed SALDI mechanism model shown in Fig. 7a.

When APTES-DPs-CB was tested with various adrenocorticotrophic hormone peptides, good spectra were obtained for molecules with masses below about m/z 2000 (results not shown). The cause of this limitation possibly lies in the strong adsorption of analyte onto the particles due to multiple interactions which may prevent desorption of ionised species on laser irradiation.

In MALDI-TOF-MS secondary ionisation is thought to occur between the ionised matrix molecules present in excess in a plasma plume above the surface, and desorbed analyte molecules. Such secondary ionisation produces proton transfer and is thought to be the main source of cationisation of analyte molecules.⁴⁰ Hence if a similar mechanism occurs on the doped silica particles, it would be expected that in the positive ion mode, analyte protonation would result from surface-assisted ionisation events whereas sodiation of analyte would result from secondary ionisation events in the gas phase between cations and analyte molecules such as squalene.

Taking squalene as an example, the proposed process for ionisation of hydrophobic molecules is shown in Fig. 7b. It assumes that hydrophobic interactions such as those between the phenyl group of silica particle A and hydrocarbon squalene hold the analyte close to the surface of the particle. The energy adsorbed by the carbon black residues within the particles on UV laser irradiation, energises squalene which is then thermally desorbed and becomes cationised in the desorbed plume above the target plate on collision with energised metal cations. This explanation can be similarly applied to the detection of fatty acids using type A silica particles.

It has been noted that when carbon nanotubes and other carbon nanoparticles are used directly as enhancing agents in SALDI-MS that ablation from the surface can occur which can seriously affect the instrument.⁴¹ In contrast to the carbon black-embedded silica particles used in this study there have been no observed ill effects resulting in ion source contamination. Routine cleaning and maintenance of the machine has sufficed.

When the particles were used with latent fingerprints deposited on metal surfaces only fatty acids and squalene were detected in the resulting MS. This has been shown to be due to the masking effect of the major hydrophobic constituents which block signals due to the minor hydrophilic ones (results not shown). A fuller discussion of this phenomenon and of a simple method for enabling analysis of both polar and non-polar constituents in fingerprints using a modified version of the particles described herein is the subject of a further publication.

In conclusion, it has been shown that silica nanoparticles modified with appropriate surface groups and with appropriate doping agents can be used as effective surface-assisting agents in laser desorption/ionisation time-of-flight mass spectrometry for analysis of standards of amino acids, other examples of polar and non-polar endogenous metabolites are reported to be found in fingerprints. The sensitivity obtained using these particles is equivalent to that using a conventional MALDI-TOF-MS matrix enhancing agent (DHB) although the on-particle SALDI-TOF-MS process is simpler to perform and provides cleaner background spectra. The SALDI mechanisms associated with the use of these silica nanoparticles with such analytes have also been proposed.

References

- [1] K. Dreisewerd, *Chem. Rev.*, 2003, **103**, 395.
- [2] M. Karas and R. Kruger, *Chem. Rev.*, 2003, **103**, 427.
- [3] C. K. Chiang, W. T. Chen and H. T. Chang, *Chem. Soc. Rev.*, 2011, DOI: 10.1039/c0cs00050g.
- [4] K. Tomoya, S. Takumi, T. Mitsuo and N. Haruki, *J. Mass Spectrom.*, 2000, **35**, 417.
- [5] E. P. C. Lai, S. Owega and R. Kulczycki, *J. Mass Spectrom.*, 1998, **33**, 554.
- [6] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida and T. Matsuo, *Rapid Commun. Mass Spectrom.*, 1988, **2**, 151.
- [7] M. Han and J. Sunner, *J. Am. Soc. Mass Spectrom.*, 2000, **11**, 644.
- [8] W. Kang, J. Kim, K. Paek and K. Shin, *Rapid Commun. Mass Spectrom.*, 2001, **15**, 941.
- [9] S. Cha and E. S. Yeung, *Anal. Chem.*, 2007, **79**, 2373.
- [10] S. Xu, Y. Li, H. Zou, J. Qiu, Z. Guo and B. Guo, *Anal. Chem.*, 2003, **75**, 6191.
- [11] S. Ren and Y. Guo, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 255.
- [12] J. Wei, J. M. Buriak and G. Siuzdak, *Nature*, 1999, **399**, 243.
- [13] X. Wen, S. Dagan and V. H. Wysocki, *Anal. Chem.*, 2006, **79**, 434.
- [14] Q. Zhang, H. Zhou, Z. Guo, Q. Zhang, X. Chen and J. Ni, *Rapid Commun. Mass Spectrom.*, 2001, **15**, 217.
- [15] M. A. Hashir, G. Stecher, R. Bakry, S. Kasemsook, B. Blassnig, I. Feuerstein, G. Abel, M. Popp, O. Bobleter and G. K. Bonn, *Rapid Commun. Mass Spectrom.*, 2007, **21**, 2759.
- [16] C. Lee, K. Kang, J. Kim, Y. Kim, H. Shim, T. Hwang, H. Rhee and B. Kim, *Microporous Mesoporous Mater.*, 2007, **98**, 200.
- [17] J. Kim, K. Paek and W. Kang, *Bull. Korean Chem. Soc.*, 2002, **23**, 315.
- [18] M. Dattelbaum and S. Iyer, *Expert Rev. Proteomics*, 2006, **3**, 153.
- [19] L. Cheng, L. Zhou, L. Tao, M. Zhang, J. Cui and Y. Li, *J. Cancer Res. Clin. Oncol.*, 2008, **134**, 769.
- [20] V. Subramanian, G. Balarajah and R. C. Pollock, *Gastroenterology*, 2008, **134**, A-196.
- [21] B. J. Theaker, K. E. Hudson and F. J. Rowell, *Forensic Sci. Int.*, 2008, **174**, 26.
- [22] F. J. Rowell and B. J. Theaker, Sunderland University, W.I.P.O. WO/ 2007/017701, 2007.
- [23] F. J. Rowell, K. E. Hudson and J. Seviour, *Analyst*, 2009, **134**, 701.
- [24] M. Benton, F. Rowell, L. Sundar and J. Ma, *Surf. Interface Anal.*, 2009, **42**, 378.
- [25] M. Benton, M. J. Chua, F. Gu, F. Rowell and J. Ma, *Forensic Sci. Int.*, 2010, **200**, 28.
- [26] H. Xia and Q. Wang, *J. Nanopart. Res.*, 2001, **3**, 401.
- [27] H. W. Tang, K. M. Ng, W. Lu and C. M. Che, *Anal. Chem.*, 2009, **81**, 4720.
- [28] R. Arakawa and H. Kawasaki, *Anal. Sci.*, 2010, **26**, 1229.
- [29] J. A. Belmont, *US pat.*, 5554739, September 1996.
- [30] Y. An, M. Chen, Q. Xue and W. Liu, *J. Colloid Interface Sci.*, 2007, **311**, 507.
- [31] V. Mahalingam, S. Onclin, M. Peter, B. J. Ravoo, J. Huskens and D. N. Reinhoudt, *Langmuir*, 2004, **20**, 11756.

- [32] J. Rouquerol, D. Avnir, C. W. Fairbridge, D. H. Everett, J. H. Haynes, N. Pernicone, J. D. F. Ramsay, K. S. W. Sing and K. K. Unger, *Pure Appl. Chem.*, 1994, **66**, 1739.
- [33] X. Dong, J. Cheng, J. Li and Y. Wang, *Anal. Chem.*, 2010, **82**, 6208.
- [34] N. Amini, M. Shariatgorji and G. Thorsen, *J. Am. Soc. Mass Spectrom.*, 2009, **20**, 1207.
- [35] C. Pan, S. Xu, L. Hu, X. Su, J. Ou, H. Zou, Z. Guo, Y. Zhang and B. Guo, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 883.
- [36] M. Najam-ul-Haq, M. Rainer, Z. Szabó, R. Vallant, C. W. Huck and G. K. Bonn, *J. Biochem. Biophys. Methods*, 2007, **70**, 319.
- [37] J. Krause, M. Stoeckli and U. P. Schlunegger, *Rapid Commun. Mass Spectrom.*, 1996, **10**, 1927–1933.
- [38] W. C. Chang, L. C. L. Huang, Y. S. Wang, W. P. Peng, H. C. Chang, N. Y. Hsu, W. B. Yang and C. H. Chen, *Anal. Chim. Acta*, 2007, **582**, 1.
- [39] G. R. Kinsel, Q. Zhao, J. Narayanasamy, F. Yassin, H. V. R. Dias, B. Niesner, K. Prater, C. St. Marie, L. Ly and D. S. Marynick, *J. Phys. Chem.*, 2004, **108**, 3153.
- [40] J. Zhang and R. Zenobi, *J. Mass Spectrom.*, 2004, **39**, 808.
- [41] S. F. Ren, L. Zhang, Z. H. Cheng and Y. L. Guo, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 333.

List of Tables

Table 1	Surface composition of TEOS-, APTES- and TPSA-derived particles (DPs) by XPS
Table 2	Signal intensity ^{ab} of carbon black-doped powders as ionizing agents for various analytes
Table 3	Limits of detection ($S/N > 3$) for the analytes tested using SALDI- and MALDI-TOF-MS

List of Figures

- Fig. 1** a) Functional chemical groups on the surface of the different silica particles: particle (A) formed from mixture of PTEOS and TEOS will contain a mixture of phenyl and (B) groups (silicate, hydroxyl and ethyl groups); particle (B) formed from tetraethoxysilane (TEOS) starting product will contain groups from (B) (silicate, hydroxyl and ethyl groups); particle (C) formed from 3-(triethoxysilyl)propylsuccinic anhydride (TPSA) will contain succinic acid groups and those from (B); particle (D) formed from *N*-(trimethoxysilylpropyl)ethylene-diamine triacetic acid (EDTA) will contain EDTA groups and those from (B); particle (E) formed from 3-aminopropyltriethoxysilane (APTES) will contain amine groups and those from (B); particle (F) formed from *p*-aminophenyltrimethoxysilane (APhTES) will contain *p*-aminophenyl groups and those from (B). (b) Chemical groups covalently attached to carbon black networks; (i) underivatised carbon black without any surface functional groups; (ii) phenyl functional groups on carbon black network; (iii) 2-carboxyphenyl functional groups on carbon black network; (iv) 3-carboxyphenyl functional groups on carbon black network; (v) 4-carboxyphenyl functional group on carbon black network.
- Fig. 2** (a) Surface enhanced Raman spectroscopy of TEOS-, APTES- and TPSA-derived particles; peaks are observed at 3299.4 cm^{-1} and 3455.7 cm^{-1} for APTES-derived undoped powder showing -NH stretch. These peaks are absent in the TEOS-derived undoped powder. A peak can be distinctly seen at 2920.9 cm^{-1} for TPSA-derived powder, suggesting the presence of carboxylic acid-derived C–H and O–H bonds. (b) XPS C1s Spectra for TPSA-derived particles; C–C/C–H is observed at 285 eV and C=O/O–C–O at 288 eV indicating the presence of carboxylate side groups.
- Fig. 3** Representative positive SALDI-TOF mass spectra of the analytes: (a) alanine $[\text{M} + 1]^+ = 90$ (matrix E: *S/N* 211; C: *S/N* 20; A: *S/N* 8), $[\text{M} + \text{Na}]^+ = 112$ (matrix E: *S/N* 124; C: *S/N* 66; A: *S/N* 115); (b) lysine $[\text{M} + 1]^+ = 147$ (matrix E: *S/N* 219; C: *S/N* 23; A: *S/N* 6), $[\text{M} + \text{Na}]^+ = 169$ (matrix E: *S/N* 116; C: *S/N* 11; A: *S/N* 5); (c) phenylalanine $[\text{M} + 1]^+ = 166$ (matrix E: *S/N* 246; C: *S/N* 44; A: *S/N* 12), $[\text{M} + \text{Na}]^+ = 188$ (matrix E: *S/N* 148; C: *S/N* 5; A: *S/N* 4); (d) squalene $[\text{M} + \text{Na}]^+ = 433$ (matrix E: *S/N* 2; C: *S/N* 0; A: *S/N* 22); upper spectrum is obtained using particle matrix E (APTES-DP-CB), middle spectrum is obtained using C (TPSA-DP-CB) and lower spectrum using A (PTEOS-DP-CB).

Fig. 4 Modified carbon black incorporated APTES and PTEOS derived particles for amino acid analysis.

Fig. 5 SALDI-TOF mass spectra of fatty acids and metabolites: (a) palmitic acid $[M + Na]^+ = 279$ (matrix E: *S/N* 76; A: *S/N* 589), $[M + K]^+ = 295$ (matrix E: *S/N* 10; A: *S/N* 48); (b) oleic acid $[M + Na]^+ = 305$ (matrix E: *S/N* 177; A: *S/N* 447), $[M + K]^+ = 321$ (matrix E: *S/N* 55; A: *S/N* 84); (c) creatine $[M + 1]^+ = 132$ (matrix E: *S/N* 142; A: *S/N* 23), $[M + Na]^+ = 154$ (matrix E: *S/N* 106; A: *S/N* 64), $[M + K]^+ = 170$ (matrix E: *S/N* 106; A: *S/N* 15); (d) urocanic acid $[M + 1]^+ = 139$ (matrix E: *S/N* 250; A: *S/N* 44), $[M + Na]^+ = 161$ (matrix E: *S/N* 157; A: *S/N* 17), $[M + K]^+ = 177$ (matrix E: *S/N* 251; A: *S/N* 11); upper spectrum is obtained using particle matrix A (PTEOS-DPs–CB) and lower spectrum using E (APTES-DPs–CB).

Fig. 6 Mass spectra of creatine and oleic acid: (a) creatine $[M + 1]^+ = 132$, $[M + Na]^+ = 154$, $[M + K]^+ = 170$; upper spectrum is obtained using DHB and lower spectrum using particle matrix E (APTES-DP–CB). (b) oleic acid $[M + Na]^+ = 305$, $[M + K]^+ = 321$; upper spectrum is obtained using DHB and lower spectrum using particle matrix A (PTEOS-DP–CB).

Fig. 7 (a) Proposed mechanism for the electrostatic binding of amino acids to APTES modified, carbon black incorporated particle E; the silica surface groups such as the amino group shown, assists in anchoring the amino acid to the surface *via* electrostatic interactions. The adsorbed amino acid is placed in close proximity to carboxyphenyl groups attached to the carbon black network so that on laser irradiation, primary ionisation of the adsorbed amino acid can take place *via* the carboxyphenyl group. This is followed by acceptance or donation of protons from surface residues to the amino acid followed by thermal desorption of the secondary ionised amino acid. (b) Proposed mechanism for the hydrophobic binding of squalene to carbon black incorporated hydrophobic particle A; squalene is anchored onto the surface through hydrophobic bonding between phenyl residues attached to surface silyl groups. This places the adsorbed molecule in close proximity to carboxyphenyl groups attached to the carbon black network so that on laser irradiation, primary ionisation of the squalene molecule can take place followed by thermal desorption when cationisation takes place in the gas phase.

	N (%)	O (%)	Si (%)	C (%)	C-C/ C-H (%)	C=O/ O-C-O (%)
TEOS-DPs	0.0	63.8	32.3	3.9	100.0	0.0
APTES-DPs	3.1	50.3	30.3	16.3	100.0	0.0
TPSA-DPs	0.4	54.2	30.1	15.3	86.2	13.8

Table 1

Particle designation	Surface modifying agent	Analyte	Analyte (M_r)	$[M + H]^+/[2M]^+$ intensity	$[M + Na]^+$ intensity	$[M + K]^+$ intensity	$[M - H]^+$ intensity
A	PTEOS (hydrophobic)	Alanine	89	—	—+	—	+
		Lysine	146	—	—+	—	++
		Glutamic acid	147	—	—+	—	+
		Phenylalanine	165	—	—+	—	+
		Tyrosine ^c	181	—/++	—	—	—+
		Squalene	309	—	++	—+	—
B	TEOS	Alanine	89	—+	+	—	+++
		Glutamic acid	147	—	+	—	+++
		Lysine	146	+	+	—	+++
		Phenylalanine	165	—	++	—	+++
		Tyrosine	181	—/++	++	—	+++
		Codeine	299	—	+	—	—
		Squalene	309	—	++	—+	—
		Alanine	89	+++	++	++	++++
C	TPSA	Lysine	146	++	++	++	++++
		Glutamic acid	147	+++	++	—+	++++
		Phenylalanine	165	++	++	+	++++
		Tyrosine	181	++/++	+	—+	+++
		Squalene	309	—	+	—	—
		Alanine	89	+	++	—+	++
		Lysine	146	—+	++	—	++++
		Glutamic acid	147	++	++	—+	+++
D	EDTA	Phenylalanine	165	—+	++	—+	+++
		Tyrosine	181	++/—	++	—+	+++
		Squalene	309	—	+	—	—
		Alanine	89	++	+	—	—
		Lysine	146	++	++	—	++
		Glutamic acid	147	++	++	—	++
		Phenylalanine	165	++	+	—	+
		Tyrosine	181	++/++	+	—	+
E	APTES	Squalene	309	—	—	—	—
		Alanine	89	—	—+	—	—+
		Lysine	146	++	+	—+	++
		Glutamic acid	147	++	+	—	++
		Phenylalanine	165	++	+	—	+
		Tyrosine	181	++/++	+	—	+
F	APhTES	Squalene	309	—	—	—	—
		Alanine	89	—	—+	—	—+
		Lysine	146	++	+	—+	++
		Glutamic acid	147	++	+	—	++
		Phenylalanine	165	++	—+	—	++
		Tyrosine	181	++/—	—+	—	+
		Squalene	309	—	—	—	—

^a Key for signal intensities: +++++ very strong with intensity >100K mV; +++ strong with intensity 50K to 100K mV; ++ intense with intensity 10K to 50K mV; + good with intensity 5K to 10K mV; —+ weak with intensity 1.5K to 5K mV; —very weak with intensity <1.5K mV or no signals. ^b Signal intensity of analyte tested is one of several factors used to determine efficacy of the powder. Other factors include presence of interfering peaks due to blank particles and signal intensity of background noise present in spectra. ^c Tyrosine analysed in positive mode is detected as a monomer ($M + H$)⁺ and as a dimer ($2M$)⁺. Intensities are indicated as monomer/dimer.

Table 2

Enhancing agent	Method used		
	MALDI-MS (DHB)	SALDI-MS (APTES-DP-CB)	SALDI-MS (PTEOS-DP-CB)
Alanine	1 ng	10 ng	—
Lysine	10 ng	10 ng	—
Glutamic acid	100 ng	10 ng	—
Tyrosine	1 ng	10 ng	—
Phenylalanine	1 ng	10 ng	—
Squalene	1 ng	—	10 ng
Oleic acid	100 ng	—	10 ng
Palmitic	100 ng	—	100 ng
Creatine	1 ng	10 ng	—
Urocanic acid	10 ng	10 ng	—

Table 3

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7