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Separation of fingerprint constituents using magnetic silica nanoparticles and direct on-particle SALDI-TOF-mass spectrometry

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ABSTRACT

Two types of amorphous, silica nanoparticles have been produced and used as surface assisting agents during laser desorption/ionisation time-of flight-mass spectrometry (SALDI-TOF-MS). The first is hydrophilic possessing surface aminopropyl groups and the second hydrophobic containing surface phenyl groups. Each particle type acts as a solid phase adsorbent, adsorbing analytes according to their charge and hydrophobicity. The adsorbed analytes can be directly analysed on the particles using SALDI-TOF-MS. Intrinsically magnetisable versions of the hydrophobic particles act as magnetic solid phase extraction (MSPE) materials which are used to selectively adsorb analytes within a mixture deposited onto a surface, transfer the adsorbed components using a magnetic wand and to deposit the particles at a site adjacent to that of the original mixture. Non-adsorbed components remain at the original site. The extracted and residual analytes are then directly analysed on the surface by SALDI-TOF-MS. Using fingerprints as an example of a complex biological matrix, this new approach has been used to separate polar (amino acids) and non-polar constituents (squalene and fatty acids) within latent fingerprints deposited on a surface and for their subsequent direct analysis on the surface by SALDI-TOF-MS. Alanine, ornithine, lysine and aspartic acid which were undetected or poorly detected prior to separation showed improved signal detection after separation.

1. Introduction

Magnetic solid phase extraction (MSPE) is an extraction procedure that uses magnetic or magnetisable particles as analyte-specific adsorbents. Particles are applied to the sample and separation is carried out by the application of an external magnetic field to remove the particles with adsorbed analyte(s) from non-adsorbed components within the sample. This rapid and simple extraction procedure has been used for various applications, such as the extraction of DNA [1–3], protein [3–5] and monitoring of environmental contaminants [6,7] from often complex sample matrices. The use of nano-sized particles in place of micro-sized particles as the absorbent material results in higher extraction rate and faster desorption time due to particles' increased surface area to volume ratio [8]. Carbon nanotubes have been used in SPE for the detection of organic pollutants [9] and nickel–aluminium nano structured double layered hydroxide particles for the monitoring of fluoride ions [10]. Examples of planar solid phase adsorbent systems coupled to MS are more limited. Silicon chips comprising areas (spots) coated with specific surface groups have been used mainly in proteomics. Components of a mixture are selectively adsorbed onto particular spots and the adsorbed analytes subsequently analysed by MS using surface enhanced laser desorption/ionisation MS (SELDI-MS) [11]. Matrix assisted LDI-MS (MALDI-MS) has been used following 2-D thin layer chromatography [12]. In both of these approaches, a matrix enhancing agent was added to the surface of the spots after chromatography/extraction to achieve successful MS.

A variety of solid phase materials have been used to assist the ionisation process on a surface during laser irradiation in LDI-MS. These include metals and metal oxides [13–15], carbon including activated carbon [16], graphite [17,18], carbon nanotubes [19,20], silicon [21,22] and silicate [23–25]. Nanoparticles made from zinc oxide [26] have been used for the analysis of low molecular weight organic compounds, and gold particles [27] for small molecules like glutathione. 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 laser desorption/ionisation (LDI) TOF-MS. This alternative method has been termed surface assisted-LDI-TOF-MS (SALDI-TOF-MS) [28–31]. SALDI-TOF-MS offers several advantages over organic matrices, such as ease of sample preparation, reduced background noise and high salt-tolerance [32].

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The aim of this paper is to investigate the use of silica nanoparticles as a combined MSPE and SALDI-TOF-MS enhancing agent for the simple direct analysis of amino acids within latent fingerprints. The MSPE absorbent which is also a SALDI enhancing agent eliminates the need for an elution step to release the trapped analyte from the particles prior to SALDI MS analysis. This reduces sample loss and preparation time. Chen and Chen attempted this for the analysis of peptides and proteins [33]. However, in their procedure, several rinse steps were required and the matrix additives citric acid and diammonium hydrogen citrate were added prior to MS analysis. Similarly, graphene has been used for the SPE and detection of small molecules by MS. In this case, magnetism was not incorporated in the SPE procedure, resulting in additional sonication and centrifugation steps required prior to MS analysis [34].

In earlier studies, we have used hydrophobic silica particles in SALDI-TOF-MS to identify drugs, nicotine and other residues and their metabolites within latent fingermarks [35–39]. To date it has not been possible to directly analyse hydrophilic constituents such as amino acids within such samples using these particles possibly due to the presence of hydrophobic constituents in excess which may suppress the ionisation of the minor more polar constituents. Analysis of amino acids with such fingerprints has been reported but this requires complex extraction and derivatisation prior to GC–MS [40]. For this study, latent fingerprints will be used as an example of a complex biological matrix. An intrinsically magnetisable form of the hydrophobic silica particles will be used as a suspension to selectively adsorb and concentrate components within a sample deposited directly on a planar surface. This will be followed by lateral separation and transportation of the particles to a new location using a magnet leaving non-adsorbed components at the original site. A suspension of non-magnetisable hydrophilic particles will be added to the non-adsorbed components and SALDI-TOF-MS will then be used to analyse components adsorbed to the magnetisable particles and non-magnetisable ones to ascertain the effectiveness of the separation process in overcoming suppression effects for non-polar constituents.

2. Experimental

2.1. Materials

Aqueous carbon black (CAB-O-JET® 300; provided as a 15%, w/v, pigment in water) was purchased from Cabot Carbon Ltd., Boston, MA, USA. The surface of these carbon black particles was provided in a carboxylated form by the suppliers. Tetraethyl orthosilicate (TEOS, 98%, Fluka), phenyltriethoxy orthosilicate (PTEOS 98%, GC), 3-aminopropyl triethoxysilane (APTES, 99%), ammonium hydroxide (28%), iron(II,III) oxide nanopowder, 2,5-dihydroxybenzoic acid, hydrochloric acid, amino acids, fatty acids and squalene (minimum 98%) were obtained from Sigma–Aldrich, St. Louis, MO, USA. All reagents were used as received. All solvents used were of HPLC grade.

2.2. Instrumentation

SALDI experiments were carried out on Shimadzu Biotech AXIMA TOF^{2TM} time-of-flight mass spectrometer (Kratos Analytical Ltd., Manchester, UK). The calibration mixture consisted of papaverine (1 mg/ml in methanol), reserpine (1 mg/ml in acetonitrile) and cesium iodide (10 mg/ml in deionised [DI] water) in the volume ratio 1:1:2 respectively. Calibration was carried out by spotting 1 μl of calibration mix onto a stainless steel MALDI plate which was left to air-dry under ambient conditions. Mass to charge values of 132.91 (Cs^+), 340.15 (papaverine + H)$^+$, 392.72 (Cs_2I^+) and 607.27 (reduced reserpine + H)$^+$ were used to calibrate the instrument in positive ion mode.

2.3. Synthesis of silica particles

2.3.1. Magnetic hydrophobic PTEOS derived particles (PTEOS-DP)

1 g of iron oxide was first pre-treated with HCl [41]. To a 50 ml tube, 1 g of the pre-treated iron oxide, 35 ml of ethanol, 5 ml DI water, 3 ml 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 min. The supernatant liquid was decanted off and 40 ml ethanol:water (50:50) mixture was added to the residue. The particles were then completely re-suspended using vortex mixing. This washing step was repeated 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.

2.3.2. Hydrophilic APTES derived particles (APTES-DP)

To a 50 ml tube, 35 ml of ethanol, 5 ml DI water, 3 ml carbon black suspension, 4 ml TEOS and 3.3 ml of ammonium hydroxide (28%) were added and the sealed tube rotated for 4 h after which the resulting silica colloidal dispersion was surface-functionalised by adding 0.4 ml of APTES. The mixture was rotated overnight to react when the contents were centrifuged at 4500 rpm for 5 min. The supernatant liquid was decanted off and 40 ml DI water was added. This was followed by vortexing of contents to achieve a suspension of particles. 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, v/v) then pure ethanol before drying in the oven at 40 °C.

2.4. Effect of particle concentration on MS signal intensity

A series of APTES-DP suspensions in pure ethanol was prepared in the ratio 1:2, 1:4, 1:6, 1:8 and 1:10 (v/v) particle:ethanol. To prepare a 1:2 (v/v) APTES-DP:ethanol suspension, 0.5 ml (which is approximately 0.065 g) of APTES-DP particles was placed in a micro-centrifuge tube with 0.5 ml of ethanol and vortexed to mix. By extracting 0.5 ml of the 1:2 (v/v) APTES-DP:ethanol suspension and adding it to a 0.5 ml centrifuge tube of ethanol, 1:4 (v/v) APTES-DP:ethanol suspension was prepared. This process was repeated to achieve the respective 1:6, 1:8 and 1:10 (v/v) APTES-DP:ethanol suspensions. The effect of particle concentration on SALDI efficacy was tested by analysing each prepared APTES-DP suspension separately with the amino acid, alanine. APTES-DP suspension of 1 μl was added to 1 μl of alanine (100 μg/ml in deionised water) and analysed by SALDI-TOF-MS in positive ion mode.

Similarly, PTEOS-DP suspensions in the ratio 1:2, 1:4, 1:6, 1:8 and 1:10 (v/v) particle:ethanol were prepared using the same method and tested against palmitic acid. The only difference was the weight of PTEOS-DP particles; 0.5 ml of PTEOS-DP weighed in at 0.160 g. Each PTEOS-DP suspension was individually tested by adding 1 μl of suspension to 1 μl of palmitic acid (100 μg/ml in ethanol) and analysed via SALDI-TOF-MS in positive ion mode.

2.5. Suppression of amino acid signal by addition of squalene

Each amino acid was tested separately. An amino acid solution of 1 μl (100 μg/ml in deionised water) was added to a well on the MALDI target plate and 1 μl of APTES-DP suspension was added to the same well and the mixture dried at ambient temperature of 20 °C prior to MS analysis in positive ion mode. This procedure was

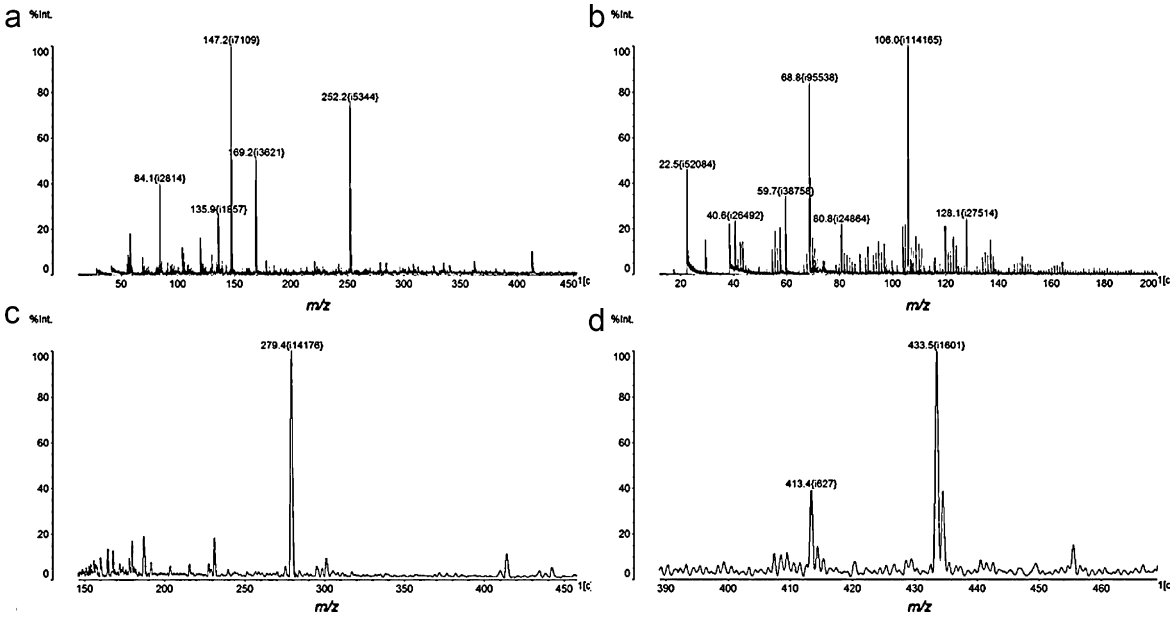

Fig. 1. Detection of amino and fatty acids using silica particles via SALDI-TOF-MS. (a) Lysine $[M+H]^+$ *m/z* 147 with APTES-DP. (b) Serine $[M+H]^+$ *m/z* 106 with APTES-DP. (c) Palmitic acid $[M+Na]^+$ *m/z* 279 with PTEOS-DP. (d) Squalene $[M+Na]^+$ *m/z* 433 with PTEOS-DP.

carried out 4 times for each of the 20 amino acids analysed. This represents the control group.

Similarly, in a second set of experiments, each amino acid was again tested separately. A mixture of 1 μl of amino acid solution and 1 μg of squalene solution was added to a well on the MALDI target plate and the mixture dried at ambient room temperature as described above. APTES-DP suspension (1 μl) was then added to the well and the suspension dried at ambient room temperate prior to MS analysis in positive ion mode. This was repeated four times for each amino acid and the results tabulated.

In a third set of experiments, a decreasing amount of squalene was added to a fixed amount of alanine. Squalene solutions of 1 mg/ml, 100 μg/ml, 10 μg/ml and 1 μg/ml were prepared in ethanol. Each squalene concentration was tested by adding 1 μl of the respective squalene solution to 1 μl of alanine (100 μg/ml) and the mixture left to dry at room temperature. Subsequently, APTES-DP suspension of 1 μl was added to the well and allowed to dry at room temperature prior to MS analysis in positive ion mode.

2.6. Separation and MS of a mixture of squalene and alanine

A mixture of squalene (dissolved in ethanol) and alanine (dissolved in distilled water), equivalent to 100 ng of each, was added as a solution to the surface of a stainless steel MALDI-TOF-MS plate (Shimadzu) and allowed to evaporate under ambient conditions. To this residue, a 3 μl suspension of magnetic hydrophobic PTEOS-DP was added. A commercial magnetic wand was used to move the particles in a circular motion over the surface for about 5 s. The wand was then slowly dragged to one side and the magnet retracted to deposit the particles on the surface adjacent to the original site but removed from it. The solutions were then allowed to evaporate under ambient conditions. The spot of particles on this side of the MALDI plate contained the separated hydrophobic components and was labelled as the new well. A 1 μl solution of 2,5-dihydroxybenzoic acid (10 mg/ml in 20:80, v/v, acetonitrile:water) was added to the original site of application of the mixture and the solvent allowed to evaporate under ambient conditions. The spot on this side of the plate containing the hydrophilic components was labelled as the original well. The plate was then analysed using a Shimadzu Axima TOF-MS system in positive mode at the new location on the surface where the particles were located and at the location of the original mixture.

In a largely similar process, a suspension of APTES-DP (1 μl) was used in place of DHB as an ionisation enhancing agent prior to MS at the original well. The plate was then subjected to MS analysis using a Shimadzu Axima TOF-MS system in positive ion mode at both the new and original well locations.

2.7. Separation and MS of a latent fingerprint

A donor was requested to briefly rub his/her fingers across his/her forehead without prior washing of their hands. One of the donor's fingers was then pressed onto a clean stainless steel MALDI target plate to deposit a fingerprint. A 400 μl suspension of magnetisable PTEOS-DP in ethanol was pipetted onto the print. The suspension covering the print area was stirred as before. After which, using the magnetic wand, the hydrophobic magnetic particles were slowly pulled to one side of the print. DHB or APTES-DP was added to the wells on the original area of the print as described above. Both solvents were allowed to dry and the new and original wells analysed as before.

3. Results and discussion

3.1. Amino and fatty acid analysis

In an earlier SALDI-TOF-MS study with carbon black-doped silica particles, amino acids analysed were best detected in positive ion mode and squalene was not detected in negative mode [42]. A recent review also reported that lipids were more easily detected and with greater sensitivity in positive mode than in negative mode [43]. Hence, all MS analysis for this paper was conducted in positive ion mode.

Using APTES-DP, amino acids were mainly detected as $[M+H]^+$ ions as shown in Fig. 1(a) and (b). In contrast, PTEOS-DP proved more suitable for the detection of fatty acids and ions were mainly detected as $[M+Na]^+$ ions as shown in Fig. 1(c) and (d). These observations can be explained by a proposed mechanism [42] which involves the initial adsorption of the analyte onto the particle surface. Ionic interactions enable the analyte to remain close

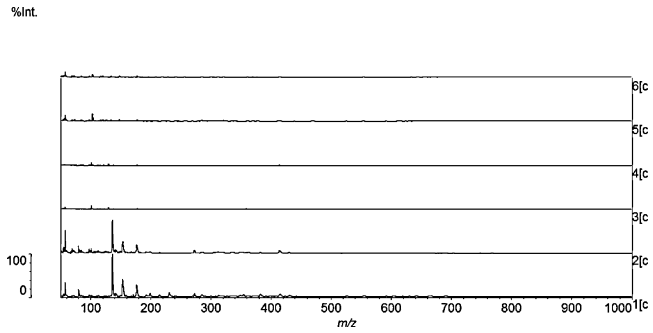

Fig. 2. Background signals of matrices at same laser power of 100 units. It can be observed that there is considerable background peaks from DHB as compared to the particle matrices, particularly up to *m*/*z* 500. Spectra 1 and 2: DHB blank. Spectra 3 and 4: PTEOS-DP blank. Spectra 5 and 6: APTES-DP blank.

to the particle surface. This is followed by the formation of primary ions via adsorption of laser irradiation by carboxyphenyl residues attached to the carbon black dopant. Proton or electron donation occurs between ionised particle surface groups and analyte molecules on the surface during secondary processes. The APTES-DP used were washed to pH 5 and their effectiveness as a LDI-enhancing agent may be derived from the full protonation of both carboxylic acid and amino residues on the particles following this treatment which may facilitate analyte–particle binding interactions and subsequent proton donation on laser irradiation [42]. The ionised analyte is then released from the surface via thermal effects and electrostatic attraction towards the detector. On the other hand, in the absence of proton donors/acceptors as with PTEOS-DP, the primary ions are released from the particles during desorption and form cationic adducts in the gas phase above the surface. Similar results were observed when hydrophobic graphene was used as a surface assisting material for the analysis of small molecules by MS [34]. Using graphene, amino acids and lipids were all observed as sodiated ions, which can be also explained using this proposed mechanism. Peaks arising from the silica particles were also observed but these matrix-derived peaks were far fewer and of lower intensities than peaks seen with DHB particularly in the range 50–500 mass units as shown in Fig. 2.

3.2. Effect of particle concentration

It is well understood that nanoparticle concentration is a factor that affects the sensitivity of detection [44]. There has to be a balance between increasing the nanoparticle concentration to increase laser absorbance and decreasing the nanoparticle concentration to increase the analyte to nanoparticle distribution ratio. APTES-DP and PTEOS-DP concentrations of between 1:2 and 1:10 (v/v) particle:ethanol were prepared and respectively tested with alanine and palmitic acid. As shown in Fig. 3(a), the preferred dilution for optimal signal performance of APTES-DP with alanine was 1:2 (v/v) particle:ethanol dilution (equivalent to 0.13 g/ml). In contrast, as shown in Fig. 3(b), optimal concentration for PTEOS-DP with palmitic acid was 1:10 (v/v) particle:ethanol dilution (equivalent to 0.02 g/ml). Concentration of PTEOS-DP suspension required for optimal performance is approximately 6 times lesser than APTES-DP. At the highest PTEOS-DP concentration of 1:2 (v/v) particle:ethanol dilution (0.32 g/ml), there was no signal detected for palmitic acid. This similar observation has been reported in other studies whereby the increase in the concentration of nanoparticles beyond a certain level of concentration resulted in a decrease in signal detection [45,46].

Table 1
Intensity of amino acid signal with and without squalene, $n = 4$.

Amino acid	Intensity without squalene ± SD	Intensity with squalene ± SD
Ala	51,375 ± 11,501	1514 ± 2140
Arg	150,574 ± 2027	90,779 ± 26,475
Asn	53,980 ± 3490	9815 ± 5409
Asp	81,453 ± 24,123	4443 ± 2618
Cys	4834 ± 1056	0 ± 0
Glu	44,856 ± 11,541	3497 ± 532
Gln	59,982 ± 11,154	3917 ± 1451
Gly	17,100 ± 14,897	0 ± 0
His	86,042 ± 53,061	18,822 ± 15,591
Ile	21,968 ± 1638	0 ± 0
Leu	18,195 ± 9989	12,277 ± 6312
Lys	50,477 ± 3372	10,987 ± 4166
Met	17,725 ± 7721	7539 ± 2587
Phe	21,298 ± 91	2264 ± 3201
Pro	76,745 ± 1495	5197 ± 2984
Ser	4677 ± 260	0 ± 0
Thr	30,127 ± 11,737	6336 ± 177
Trp	18,240 ± 2394	6387 ± 2685
Tyr	23,981 ± 4450	0 ± 0
Val	15,056 ± 255	2828 ± 351

3.3. Suppression of signal by addition of squalene

Squalene was chosen in this experiment to represent one of the most abundant hydrophobic compounds found in fresh latent fingerprints [47,48]. The amount of squalene added (1 μg) corresponded to the minimum amount of squalene that is commonly found in latent fingerprints [49]. Averaged intensity values for the amino acid signal in positive ion mode are shown in Table 1. When squalene was added, signals from all 20 amino acids were reduced by approximately half to 34 times. Five amino acids (Cys, Gly, Ile, Ser and Tyr) registered no signal in the presence of squalene. The lower level of detection for these 5 amino acids with APTES-DP, even without the addition of squalene, may have attributed to the absence

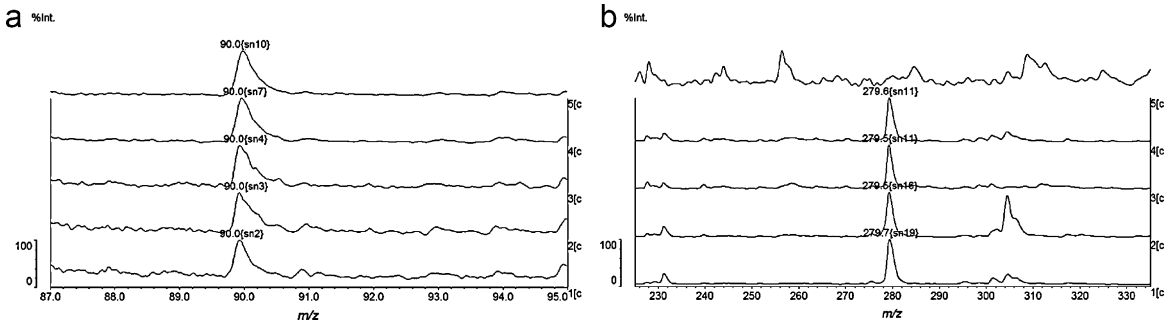

Fig. 3. Effect of particle (APTES-DP and PTEOS-DP) concentration on signal intensity. (a) 100 ng of alanine analysed with increasing concentrations of APTES-DP suspensions from bottom (spectrum 1; sn 2) to top spectrum (spectrum 5; sn 10). (b) 100 ng of palmitic acid analysed with increasing concentrations of PTEOS-DP suspensions from bottom (spectrum 1; sn 19) to top spectrum (spectrum 5; sn 0).

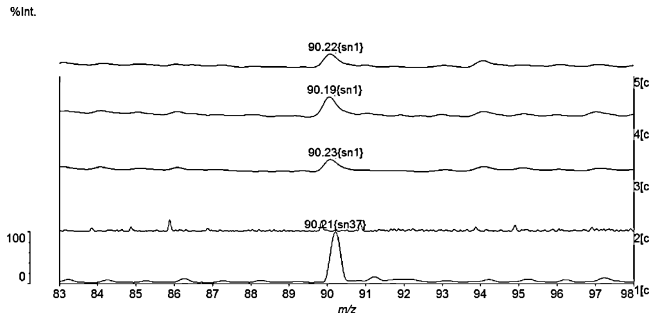

Fig. 4. Suppression of alanine detection in mixtures regardless of amount of squalene added. Spectrum 1: 100 ng alanine + APTES-DP. Spectrum 2: 100 ng alanine + 1 μg squalene + APTES-DP. Spectrum 3: 100 ng alanine + 100 ng squalene + APTES-DP. Spectrum 4: 100 ng alanine + 10 ng squalene + APTES-DP. Spectrum 5: 100 ng alanine + 1 ng squalene + APTES-DP.

of signals after the addition of squalene. The results demonstrate that suggested lipophilic components like squalene in fingerprints will reduce or completely suppress signals of amino acids within the marks. When the concentration of alanine was maintained at 100 ng and concentration of squalene was reduced in steps from 1 μg to 1 ng, a drop in signal was still observed (Fig. 4). It may be possible that when a mixture of alanine and squalene is placed in the same well, due to differences in density and upon repartition within the droplet, a layer of squalene will be on the surface atop of alanine. Hence upon laser irradiation, less alanine is exposed on the surface for MS analysis, resulting in a reduction or complete suppression of signal.

3.4. Separation and MS of alanine and squalene in mixtures

The results suggest that it is possible to overcome signal suppression by squalene on the detection of amino acids in fingerprints by using magnetic PTEOS-DPs. These hydrophobic particles act as a solid phase extraction medium for selective adsorption of hydrophobic compounds. Its intrinsic magnetic properties can be then used to separate adsorbed components from non-adsorbed ones and deposit them at a new site adjacent to that of the original sample on the surface. The separated compounds can then be analysed by MS in general following conventional extraction of adsorbed analytes or directly via SALDI-TOF-MS in situ on the surface of the carbon black-doped silica nanoparticles. In this experiment, squalene was used to represent a major hydrophobic component commonly seen in latent fingerprints and alanine was used as a representation of an amino acid also found in fingerprints. The effectiveness of this approach when applied to a mixture of squalene and alanine is shown in Fig. 5(a) where alanine was not observed in the original well prior to separation due to MS suppression by the major hydrophobic constituents within the prints. After the majority of such hydrophobic components were removed and upon the addition of DHB, alanine was detected at the original site of sample application and none observed in associated with the hydrophobic particles at the new site. As seen in Fig. 5(b), only the peak due to the sodium adduct of squalene at was observed m/z 433 at the new site and no squalene was observed in the original site.

A repeat of this experiment was carried out to test the suitability of using APTES-DP in place of DHB. In a similar fashion, the MS of the residual hydrophobic components left at the original site of the deposited mixture is shown in Fig. 6(a) which shows a peak for alanine at m/z 90 and no trace of squalene at m/z 433. The ability to detect alanine using APTES-DP demonstrated that APTES-DP was a plausible replacement for DHB as a SALDI-enhancing agent for amino acids like alanine. With reference to Fig. 6(b), only the peak

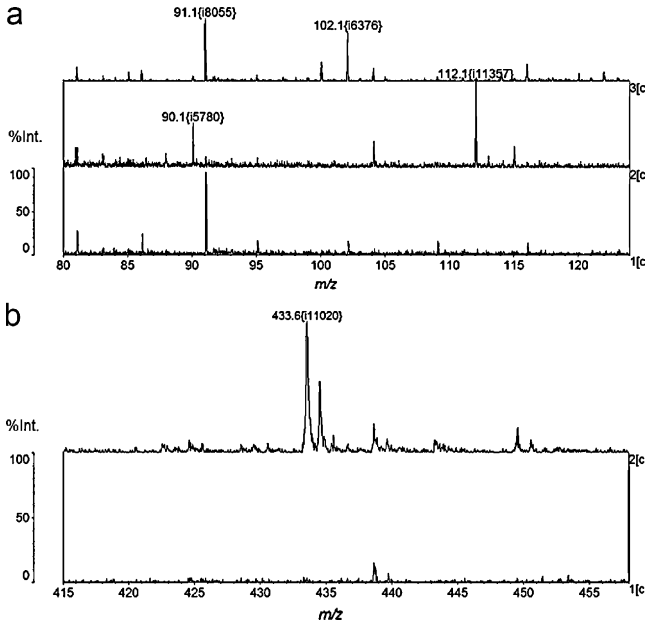

Fig. 5. Effect of separation of a mixture of 100 ng of alanine and squalene using magnetisable PTEOS-DP and DHB for MS analysis. (a) Alanine $[M+H]^+ = m/z$ 90, $[M+Na]^+ = m/z$ 112. Lower spectrum: Original well prior to separation, no alanine was observed. Middle spectrum: After separation, original well with added DHB, alanine was detected. Upper spectrum: After separation, hydrophobic well with magnetic hydrophobic particles, no alanine was observed. (b) Squalene $[M+Na]^+ = m/z$ 433. Lower spectrum: After separation, original well with added DHB, no squalene was observed. Upper spectrum: After separation, hydrophobic well with magnetic hydrophobic particles, squalene was detected.

due to the sodium adduct of squalene was observed in the new hydrophobic well at m/z 433, without any trace of alanine. Squalene was not observed in the original hydrophilic well. These results demonstrate selective and efficient adsorption of squalene onto the magnetisable hydrophobic particles. The highly polar alanine does not bind to these particles and remains mostly at the original site, whereas the squalene adsorbed onto the magnetisable particles is removed from this site as the particles are dragged to the new site on the surface.

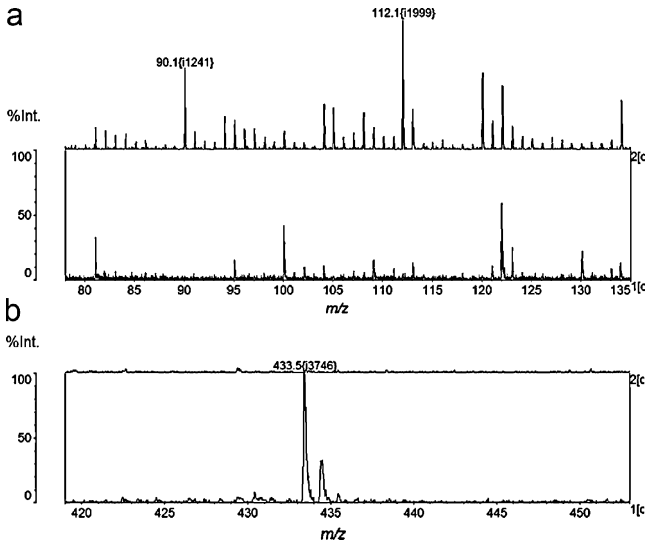

Fig. 6. Effect of separation of a mixture of 100 ng of alanine and squalene using magnetisable PTEOS-DP and APTES-DP for MS analysis. (a) Alanine $[M+H]^+ = m/z$ 90, $[M+Na]^+ = m/z$ 112. (b) Squalene $[M+Na]^+ = m/z$ 433. Lower spectra: After separation, hydrophobic well with magnetic PTEOS-DP. Upper spectra: After separation, original well with added APTES-DP.

Table 2
Averaged signal-to-noise values of fatty acids, squalene and amino acid detection in real fingerprints by MS using magnetic PTEOS-DP and DHB, $n = 4$.

Fatty acids	m/z	Before separation (S/N)	After separation (S/N)
Common fatty acids reported in fingerprints			
Nanoic	181 $[M+Na]^+$	54	52
Dodecanoic	223 $[M+Na]^+$	75	82
Myristic	251 $[M+Na]^+$	51	45
Palmitoleic	277 $[M+Na]^+$	37	13
Palmitic	279 $[M+Na]^+$	283	300
Oleic	305 $[M+Na]^+$	749	171
Stearic	307 $[M+Na]^+$	11	18
Squalene	433 $[M+Na]^+$	93	48
Amino acids	**m/z**	**Before separation (S/N)**	**After separation (S/N)**
Common amino acids reported in fingerprints			
Glycine	76 $[M+H]^+$	0	0
Alanine	90 $[M+H]^+$	11	72
Serine	106 $[M+H]^+$	0	15
Ornithine	133 $[M+H]^+$	0	128
Aspartic acid	134 $[M+H]^+$	20	84
Lysine	147 $[M+H]^+$	44	72

3.5. Separation and MS of a latent fingerprint using magnetic PTEOS-DP and DHB

Separation experiments were carried out 4 times in positive ion mode. Prior to and after the separation process, the fingerprints were analysed by MS for the commonly reported fatty acids and amino acids found in fingerprints [48]. Signal to noise values for most fatty acids did not differ considerably before and after separation. The more significant changes included squalene, palmitoleic and oleic acid where a reduction in signal was recorded after separation as shown in Table 2. Oleic acid recorded the largest decrease in signal, with a reduction to approximately 1/4 that of before separation. The reduced intensity values of fatty acids were possibly due to incomplete uptake of all the hydrophobic components onto the magnetic PTEOS-DP due to the large excess of lipids present in the fingerprint.

It was observed that the separation process improved the detection of the amino acids. The efficacy of the separation process is evident as shown in Table 2 since on average, before separation, only lysine was significantly detected whereas after the separation process and upon the addition of DHB in the hydrophilic well, 4 amino acids were observed, namely alanine, ornithine, lysine and aspartic acid. The averaged intensity value of lysine also improved after separation. Although glycine and serine were reported as 2 of the major amino acids found in fingerprints [48], neither was significantly detected after separation. Referring to Table 1, glycine and serine were 2 of the 5 amino acids completely suppressed by the addition of squalene. It was possible that the suppression threshold for glycine and serine was much lower than compared to alanine, ornithine, lysine and aspartic acid. A more thorough separation of the hydrophobic components from the original site of the latent fingerprint may be required for the detection of glycine and serine.

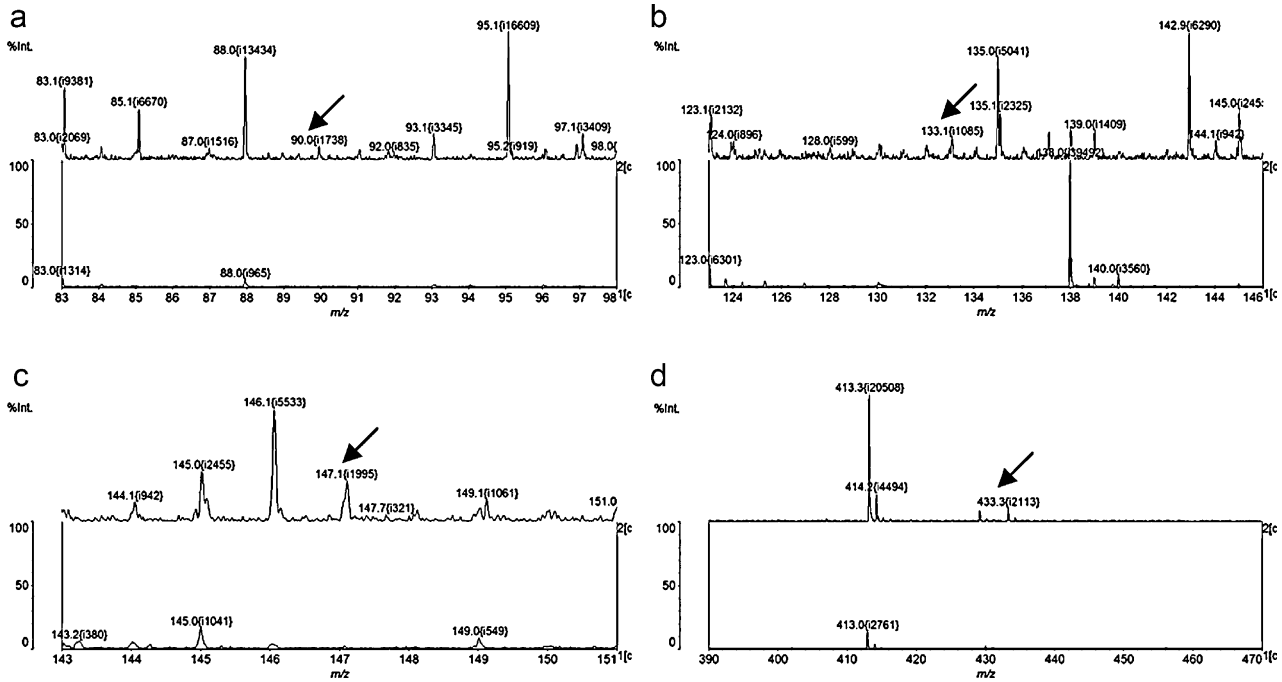

Fig. 7. Magnetic PTEOS-DP added to a real fingerprint to separate the hydrophobic components from the hydrophilic components prior to MS analysis. (a) After separation, alanine $[M+H]^+$ m/z 90 in original site with added APTES-DP. (b) After separation, ornithine $[M+H]^+$ m/z 133 in original site with added APTES-DP. (c) After separation, lysine $[M+H]^+$ m/z 147 in original well site with added APTES-DP. (d) After separation, squalene $[M+Na]^+$ m/z 433 in new well site with added magnetic PTEOS-DP. Upper spectra: Fingerprint with silica particles. Lower spectra: Silica particle blanks.

In an attempt to resolve this, a second separation was carried out and the concentration of PTEOS-DP particles added increased but glycine and serine were still insignificantly undetected.

3.6. Separation and MS of a latent fingerprint using magnetic PTEOS-DP and non-magnetic APTES-DP

It was demonstrated that this technique could be used successfully to separate and detect amino acids and squalene in an actual deposited fingerprint. In this experiment, APTES-DP was used in place of DHB at the original site of print deposition. Fig. 7(a)–(c) shows the spectra of the 3 amino acids detected after separation with peaks at m/z 90 which is representative of alanine $[M+H]^+$ at m/z 133 which is representative of ornithine $[M+H]^+$ and at m/z 147 which is representative of lysine $[M+H]^+$. Fig. 7(d) shows the presence of squalene in the hydrophobic well. Using APTES-DP, only 3 out of the 6 listed major amino acids found in fingerprints were detected. In comparison, although APTES-DP provided a reduction in matrix background peaks, DHB proved more sensitive in detection.

In addition, it was observed that selective adsorption of squalene in a fingerprint occurs onto hydrophobic silica particles while amino acids in the print does not adsorb onto these hydrophobic particles and remains in the original well. This separation process minimised the suppression effects of hydrophobic constituents on amino acid ionisation in the LDI-MS process enabling their detection and that of hydrophobic constituents within fingerprints.

4. Conclusion

This paper introduces a novel application of amorphous silica nanoparticles as solid phase extraction mediums. By introducing an iron oxide core, these particles are transformed into magnetisable SPE materials which enable them to be moved laterally over a planar surface so that separated components can be analysed directly by SALDI-TOF-MS while still adsorbed on the particles.

We have shown that separation and concentration of biochemical molecules within a complex biological matrix such as a fingerprint can be easily carried out on a planar surface. Magnetisable PTEOS-DPs were used to demonstrate this application. In the separation process, suppression effects from the hydrophobic constituents such as fatty acids and squalene were reduced allowing the subsequent detection of trace amount of amino acids by SALDI-TOF-MS with minimal sample processing. It is assumed that the aqueous alcohol (1:2, v/v) solvent used to apply the suspension of particles to the sample dissolves both hydrophobic and hydrophilic constituents within the matrix and that the swirling action induced by the magnetic wand assists this process. Other solvents and combinations may be required for complete dissolution of other disparate components with fingerprints such as urea, uric acid, creatine and wax esters. Chen and Chen [33] described the use of negatively charged silanized magnetic particles in suspension for the adsorption/enrichment of small proteins and their trypsin-catalysed hydrolysis products. Their method required a 1 h incubation step followed by rinse steps whereas the corresponding process described herein required about 5 s. Also, they did not use a complex bio-matrix whereas our study has been used to isolate traces of amino acids within an excess of hydrophobic material within a complex biological matrix. The proposed procedure is cost effective and quick, paving the way for the discovery of hydrophilic biochemical components by SALDI-TOF-MS whose presence could have been masked by hydrophobic components within fingerprints.

Further studies will be carried out to identify why not all the reported amino acids in fingerprint material were detected by the reported method and to ascertain whether other polar metabolites can be detected in fingerprints and other biological matrices using this method. This may require use of alternative hydrophilic silica particles or mixtures of hydrophilic particles. This could provide a simple reagent for separating and then identifying new biological entities in a range of complex biological matrices using on-particle SALDI-TOF-MS.

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