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## Supercritical carbon dioxide versus toluene as reaction media in silica functionalisation: Synthesis and characterisation of bonded aminopropyl silica intermediate.

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### Highlights

- Supercritical CO<sub>2</sub> a competitive alternative reaction medium to organic solvents.
- Synthesis of aminopropyl bonded silica (APS) intermediate for LC applications.
- Higher %C loading/conversion efficiency for APS using supercritical-CO<sub>2</sub> protocol compared to toluene.
- Change in surface charge of silica from negative to positive for APS, confirmed by zeta potential measurements.

### Abstract

This research reports supercritical carbon dioxide versus toluene as reaction media in silica functionalisation for use in liquid chromatography. Bonded aminopropyl silica (APS) intermediates were prepared when porous silica particles (Exsil-pure, 3 $\mu$ m) were reacted with 3-aminopropyltriethoxysilane (3-APTES) or N,N-dimethylaminopropyltrimethoxysilane (DMAPTMS) using supercritical carbon dioxide (sc-CO<sub>2</sub>) and toluene as reaction media. Covalent bonding to silica was confirmed using elemental microanalysis (CHN), thermogravimetric analysis (TGA), zeta potential ( $\xi$ ), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, scanning electron microscopy (SEM) and solid-state nuclear magnetic resonance (CP/MAS NMR) spectroscopy. The results demonstrate that under sc-CO<sub>2</sub> conditions of 100 $^{\circ}$ C/414 bar in a substantial reduced time of 3 h, the surface coverage of APS (evaluated from %C obtained from elemental analysis) prepared with APTES (%C: 8.03, 5.26  $\mu$ mol/m<sup>2</sup>) or DMAPTES (%C: 5.12, 4.58  $\mu$ mol/m<sup>2</sup>) is somewhat higher when compared to organic based reactions under reflux in toluene at a temperature of 110 $^{\circ}$ C in 24 h with APTES (%C: 7.33, 4.71  $\mu$ mol/m<sup>2</sup>) and DMAPTMS (%C: 4.93, 4.38  $\mu$ mol/m<sup>2</sup>). Zeta potential measurements revealed a change in electrostatic surface charge from negative values for bare Exsil-pure silica to positive for functionalised APS materials indicating successful immobilization of the aminosilane onto the surface of silica.

**Keywords:** Chemical functionalisation; Supercritical CO<sub>2</sub>; Aminopropyl bonded silica (APS); bi-functional bonding; Surface coverage/conversion efficiency.

### 1. Introduction

The chemical functionalisation of silica particles and surfaces is an active research area owing to flexibility in pore structure and universal acceptance of these materials in a variety of applications compared to other metal oxides such as alumina and titania [1]. Silica particle surface area, accessibility and pore size has a direct influence on percentage carbon loading and functionalisation efficiency. The higher the surface area and pore access, the more available sites for ligand immobilization. The type and functionality of silane ligand (chloro, or alkoxy) used in the generation of silica bonded phases determines the reaction mechanism and the corresponding type and

quantitative amount of surface bound species. Solid state NMR spectroscopy is particularly valuable in elucidating the surface bonded species. Reactions involving monofunctional silanes with silica provide a  $^{29}\text{Si}$  CP/MAS NMR single resonance peak centered at +13ppm. Multifunctional silanes (di-,tri) will provide more than one linkage of siloxane attachment to a highly crosslinked siloxanes between silanes and silica surface silanols [2].  $^{29}\text{Si}$  CP/MAS NMR spectra of di-functional modified silica surface will yield resonances between -4ppm and -22ppm of the silane, and trifunctional modified silica will have resonances of  $\text{T}^1$  (-46 ppm),  $\text{T}^1$  (-50ppm) and  $\text{T}^2$  (-56ppm),  $\text{T}^3 + \text{T}^3$  (-59ppm) in addition to signals at -92ppm ( $\text{Q}^2$ , geminal silanols), -101ppm ( $\text{Q}^3$ , free silanols) and -110ppm ( $\text{Q}^4$ , siloxanes) [2].

Amino functionalised silicas, if not used directly as the stationary phase, are often used as intermediates for further modification of bonded stationary phases in chromatography [3, 4]. Other uses of aminopropyl silica (APS) cited in the literature are in the area of catalysis [5, 6], drug delivery [7], solid-phase extraction [8], chemical sensors[9] and in nanomaterials [10, 11].

Aminopropyltriethoxysilane (APTES) is of particular importance due to its bi-directional character, the ethoxy groups reacting with hydroxyl groups on the surface of silica forming siloxanes (Si-O-Si) linkages, with the amino group extending from the surface as a linker for the attachment of other ligands or molecules via nucleophilic substitution reaction. The amino group is further reacted by formation of amide or amine, and by alkylation in addition with nucleophilic aromatic substitution of suitable activated rings. The formation of amides can be achieved with acid chlorides or anhydrides. The directional property has led to the popularity of APTES as a silanating ligand. Albert *et al.* have described the chemical species of APS gel formed with APTES as ligand [12], while Tripp and White examined the nature of APS species by reacting silica with the mono-alkoxysilane, aminopropyldimethylethoxysilane (APDMES)[13].

Current methods of preparing APS intermediate phase primarily involve the use of conventional organic solvents, requiring disposal or recycling. For example, Erden *et al.* [8] activated silica with acetic acid for 1h, then proceeded to reflux with APTES in toluene for 24 h. Ray *et al.* [3] used 24 h to immobilized APTMS onto silica surface under reflux. In both examples, additional organic solvents were employed for purification procedures. Alternative functionalisation strategies such as solvent-free vapour phase and supercritical fluid silanisation that avoid organic solvents at high temperature would be desirable. Solvent-free vapour uses dry argon or nitrogen while supercritical fluid functionalisation can utilise  $\text{sc-CO}_2$  as the reaction medium. However, the former can require purification/drying of functionalised materials [14, 15] involving the use of organic solvents. Materials functionalised in  $\text{sc-CO}_2$  can be conveniently flushed with  $\text{sc-CO}_2$  after reaction is complete [16, 17, 18,

19, 20, 21, 22] and the medium constitutes a more benign/efficient method of silanisation compared to solvent-free vapour protocol and conventional organic solvent based methods. These features can allow direct introduction of the functionalised material into a spectroscopic or chromatographic system without a further purification step [19, 20, 23]. Conventional based methods of functionalising silica can span several hours or even days at higher temperatures [3, 8]. Moreover, organic based synthetic methods are often prone to polymerization [24, 25] leading to higher carbon loading than expected.

Cao *et al.* studied reactions of organosilanes with silica surfaces in carbon dioxide [26]. Robson and co-workers prepared/endcapped silica bonded phase in sc-CO<sub>2</sub> [24], while Yarita *et al.* endcapped an octadecyl silica (ODS) previously prepared in organic solvent using sc-CO<sub>2</sub> as a silylation medium [27]. Ashu-Arrah *et al.* prepared bonded mercaptopropyl silica (MPS) [17], silica hydride (Si-H) intermediates [16] and phenyl and pentafluorophenyl silica phases [18, 19] using sc-CO<sub>2</sub> as the reaction medium.

Furthermore, Cao *et al.* [26] and Tripp and Combes [28] demonstrated that sc-CO<sub>2</sub> absorbed moisture from silica while performing silanisation thus acting as a desiccant, a feature which further differentiates sc-CO<sub>2</sub> from organic solvents. Ashu-Arrah *et al.* [16] and McCool and Tripp [28] observed that inaccessible silica silanols are accessible in sc-CO<sub>2</sub>. Tripp and co-workers investigated the reaction of silica with organosilanes in sc-CO<sub>2</sub> using infrared spectroscopy [29, 30] while Gu and Tripp highlighted the effectiveness of silica functionalisation in sc-CO<sub>2</sub> by studying the reaction of silanes with metal oxides such as titania and alumina [31].

Ashu-Arrah *et al.* demonstrated superior surface coverage for sc-CO<sub>2</sub> generated silica hydride intermediate (using triethoxysilane, TES and dimethylmethoxysilane, DMMS) compared to conventional organic solvent based method [16]. The relatively higher surface coverage of SiH from TES compared to DMMS generated in sc-CO<sub>2</sub> is attributed to the trialkoxy moiety of the TES that favours siloxane crosslinkage, forming polymeric surface attachments to yield a higher ligand density than the monomeric DMMS ligand. Furthermore, silica hydride (SiH) conversion efficiency (estimated from <sup>29</sup>Si CP/MAS NMR analysis) of ca. 42.4% achieved for SiH prepared from DMMS using sc-CO<sub>2</sub> is superior to a 33.3% efficiency obtained in dioxane [16]. In this research, the authors examine the preparation of bonded aminopropyl silica (APS) intermediate under supercritical CO<sub>2</sub> conditions and compare the results with APS prepared using toluene as reaction medium. It has been demonstrated that polymeric C<sub>18</sub> bonded stationary phases prepared using different synthetic route have different chromatographic properties [32]. The extend of bonding of APTES and DMAPTMS onto the silica surface was examined by elemental microanalysis (CHN), thermo-gravimetric analysis (TGA), zeta

potential ( $\xi$ ), scanning electron microscopy (SEM), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy and where available solid-state nuclear magnetic resonance ( $^{29}\text{Si}$  and  $^{13}\text{C}$  CP/MAS NMR) spectroscopy.

## 2. Experimental

### 2.1. Materials

Exsil-Pure porous silica ( $3\mu\text{m}$ ,  $223\text{m}^2/\text{g}$ ,  $120\text{\AA}$ ) was purchased from Alltech/Exmere (Lancashire, UK), while 3-aminopropyltriethoxysilane (3-APTES), N,N-dimethyl aminopropyltrimethoxysilane (DMAPTMS), triethylamine, 99% (TEA), acetonitrile, methanol (Chromosolv HPLC grade) and anhydrous toluene (99.8% analytical grade) were obtained from Sigma-Aldrich (Tallaght-Dublin, Ireland). All water was distilled and ionised on a Milli-Q water purification system (Millipore, Milford, Mass, USA) to a resistivity of  $18.2\text{ M}\Omega\cdot\text{cm}$ .  $\text{CO}_2$  supplied in cylinder was obtained from Irish Oxygen (Cork, Ireland) with  $> 99.9\%$   $\text{CO}_2$  purity, a moisture content  $< 50\text{ ppmv}$  and  $< 10\text{ ppmv}$  methanol. All reagents were used as supplied except porous Exsil-Pure silica particles that were pre-treated by drying in an oven at  $120^\circ\text{C}$  overnight to remove surface bound moisture. These trace amounts of water and methanol present in the  $\text{CO}_2$  are too low to influence the critical parameter settings chosen in this work to maintain supercritical conditions.

### 2.2. Instrumentation

Reaction in supercritical  $\text{CO}_2$  was carried out using a stainless steel pressure cell with a total volume of  $25\text{ml}$ , serial no 120773-4 fitted with  $0.625$  sapphire windows at either end of the cell, coupled with an Isco model 260D syringe pump obtained from Thartech Inc. (USA) used to establish and maintain  $\text{CO}_2$  pressure. Temperature was controlled within  $\pm 1^\circ\text{C}$  using an electrical heating tape monitored with a K thermocouple (Type: NiCr-Ni) placed in the reaction cell. The K thermocouple was purchased from Sigma-Aldrich (Dublin, Ireland). Gentle agitation of the cell content was accomplished by stirring with a Teflon coated magnetic stirrer bar in the cell and a magnetic stirrer plate underneath the cell. The magnetic stirring plate (Midi-MR 1 digital IKAMAG) was purchased from Ika Werke GmbH & Co. KG (Germany).

### 2.3. Synthetic protocol for bonded aminopropyl silica (APS) stationary phase

#### 2.3.1. Synthesis of aminopropyl silica intermediate in $\text{sc-CO}_2$

The procedures for the supercritical  $\text{CO}_2$  functionalisation of silica intermediates [16, 17] and stationary phases [18, 19] has been described previously. Thus for  $1.0\text{g}$  of dried porous silica,  $1.2\text{g}$  of 3-APTES or DMAPTMS was used. Porous silica ( $1.0\text{g}$ ) and  $1.2\text{g}$  of APTES or DMAPTMS are charged into

a 25mL stainless steel reaction cell containing a magnetic stirring bar. The cell was sealed and wrapped with an electrical heating tape and placed right on top of the magnetic stirrer. The magnetic stirrer was switched on to ensure gentle agitation of the cell contents ( $600 \pm 2$  rpm), which was visually verified by looking through the sapphire windows at either end of the reaction cell. The temperature of the reaction cell was raised beyond the critical temperature of  $\text{CO}_2$  ( $31.2^\circ\text{C}$ ) when above the critical pressure. Once the temperature of the reaction cell was above the critical temperature of  $\text{CO}_2$  ( $31.2^\circ\text{C}$ ), all inlet valves were opened and pressurized  $\text{CO}_2$  allowed to flow into the reaction cell under constant static conditions at  $100^\circ\text{C}$ , to maintain 414 bar for 3 h [16]. Following this reaction time of 3 h, the magnetic stirrer plate was switched off to allow the cell content to settle for  $\sim 20$  min, followed by dynamic extraction for 30 min with ca. 120mL of fresh sc- $\text{CO}_2$  flushed through the cell at a rate of ca. 8mL/min at constant conditions of  $100^\circ\text{C}$  and 414 bar. The cell was then depressurized, vented, cooled, opened and the bonded aminopropyl silica (APS) phase removed as a white flowing powder. The APS phases were oven dried (thermal or post-curing) overnight at  $120^\circ\text{C}$  as this is regarded as a final strengthening of the already immobilized silane species by condensation [33]. The supercritical  $\text{CO}_2$  generated APS was labelled as sc-APS to differentiate from the org-APS synthesis in organic based method described below with toluene as solvent.

### *2.3.2. Synthesis of aminopropyl silica intermediate in toluene*

To compare aminopropyl silica prepared in sc- $\text{CO}_2$  with conventional organic solvent based method, reaction in toluene was carried out in a 2-neck 100mL round bottom flask equipped with a reflux condenser. 1.0g of pre-dried (overnight oven drying at  $120^\circ\text{C}$ ) porous silica was placed in a 2-neck 100mL round flask with 10ml of anhydrous toluene as solvent, a magnetic stirring bar and 500 $\mu\text{L}$  of deionised water, stirred and heated for 10 min at  $110^\circ\text{C}$  under reflux. Then 1.2g of APTES or DMAPTMS and  $\sim 1\%$  TEA (as catalyst) was added quickly and the mixture was maintained under reflux for 24 h at  $110^\circ\text{C}$  under  $\text{N}_2$  atmosphere. The content of the 2-neck 100mL round bottom flask was cooled at room temperature, and separated by vacuum filtration. The bonded aminopropyl silica (APS) phases were washed with 10mL of anhydrous toluene (three times) and rinsed with 10mL of methanol (three times). The resulting white powder of APS was oven dried overnight at  $120^\circ\text{C}$  and labelled as org-APS to differentiate from sc-APS.

## *2.4. Characterisation of bonded APS generated in sc- $\text{CO}_2$ and toluene*

### *2.4.1. Percentage carbon*

Elemental analysis of bonded APS was performed using a CE 440 elemental analyzer fully automated (Exeter Analytical Inc., North Chelmsford, Mass, USA) with thermal conductivity detection at the Microanalytical Laboratory, Chemistry Department, UCC (Cork, Ireland).

#### 2.4.2. Thermogravimetric analysis

Thermogravimetric analysis (TGA) experiments were performed on a Star<sup>e</sup> TGA/ DSC instrument, from Mettler-Toledo AG, Switzerland, over a temperature range from 30-900°C at a heating rate of 40°C/min under a nitrogen flow rate of ~30 mL/min on an alumina 70µL pan. 5-10mg of bonded APS phases were placed on a pre-tared alumina pan and the weight recorded. The samples were heated using a linear gradient under an inert atmosphere of nitrogen. Data was analysed using Star<sup>e</sup> Excellence software.

#### 2.4.3. Zeta potential ( $\xi$ ) analysis

The zeta potential measurement was performed using a Zetasizer nano ZS model: ZEN3600 and serial number: MAL1030324, equipped with a MPT-2 multipurpose titrator operated using a DTS (nano) software ver.5.10 (Malvern Instruments, Worcestershire UK). 0.01% (w/w) (recommended for in-house zeta measurement) or ~0.001g samples were dispersed in 10 mL methanol and acetonitrile, and sonicated in a water bath for 12 min. Measurement were performed immediately after removing the suspension from the bath. A zeta dip cell was filled with samples and data analysed using a DTS (nano) software ver.5.10.

#### 2.4.4. Scanning electron microscopy (SEM)

The external surface morphology of bonded APS phase and bare Exsil-pure silica particles were performed on a JSM-5510 SEM (FEI Company Europe) at 20 kV at the Bioscience Institute, University College Cork, Ireland.

#### 2.4.5. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy

DRIFT spectra of APS and bare Exsil-pure silica were performed on a Perkin-Elmer Spectrum Two FT-IR spectrometer (Chalfont Road, UK) with a FT-IR attachment (Pike Technologies, USA). Pre-dried KBr was ground to a fine powder using a mortar and pestle. A sample holder (width: 6 mm; depth: 2 mm) was filled with KBr, the surface levelled and subsequently used to record the background spectra. Bonded APS phase was then mixed with KBr at a ratio of 1:30, and further ground to a fine powder and a sample holder was filled and the surface levelled. All spectra were run from 4000cm<sup>-1</sup> to 400 cm<sup>-1</sup> at a scan rate of 16s<sup>-1</sup> and a resolution of 4 cm<sup>-1</sup>.



#### 2.4.6. Solid-state nuclear magnetic resonance spectroscopy

<sup>29</sup>Si and <sup>13</sup>C NMR spectroscopy measurements were performed at the Institute of Organic Chemistry, Tübingen, Germany, on Bruker ASX 300 and DSX 200 spectrometers (Bruker, Rheinstetten, Germany) using cross polarization and magic-angle spinning (CP/MAS). The <sup>13</sup>C CP/MAS and <sup>29</sup>Si CP/MAS NMR were recorded at 75.5MHz and at 59.6MHz respectively, with a contact time of 3 ms for <sup>13</sup>C and 5 ms for the <sup>29</sup>Si nucleus, and a recycle delay time of 1s. Representative samples of ~250 mg were spun at 4 KHz using 7 mm double bearing ZnO<sub>2</sub> rotors (for <sup>13</sup>C: ~80 mg in 4 mm rotors at a spinning rate of 10 kHz). The line broadening used was about 30Hz and the spectra width was about 20 kHz. All chemical shifts were referenced to trimethylsilyl ester of octameric silica (Q<sub>8</sub>M<sub>8</sub>) and glycine respectively. Spectrum processing was performed using Bruker TOPSPIN 2.0 software.

### 3. Results and Discussion

#### 3.1. Elemental analysis, surface coverage and conversion efficiencies

Surface coverage and %C loading are reliable means of assessing the extent of a functionalisation method [1, 34]. As illustrated in Table 1, the observed increases in the CHN values for APS prepared in sc-CO<sub>2</sub> and toluene as reaction media compared with non-functionalised Exsil silica (%C: 0.02, %H: 0.32) are evidence of successful functionalisation of silica with APTES and DMAPTMS. The C/N value of sc-APS (i.e. C/N: 3.34 using APTES and 4.57 using DMAPTMS) is found to be greater than the theoretical value of the 3-amino propyl group but lower in the case of org-APS (i.e. C/N: 2.92 and 3.92) indicating extensive hydrolysis of the ethoxy and methoxy groups of APTES and DMAPTMS during the silanisation process.

**Table 1.** Elemental analysis values of APS intermediate prepared from 3-APTES and N, N-DMAPTMS) using sc-CO<sub>2</sub> (100°C/414 bar/3 h) and toluene (110°C/24 h) as reaction medium.

<sup>a</sup> Reference: Erdem *et al.*[8]

<sup>b</sup> Reference: Ray *et al.*[3]

<sup>c</sup> Reference: [4]

-, Value not available

The surface coverages of bonded APS intermediate was calculated using %C derived from elemental analysis, assuming bifunctional attachment and applying Barendsen-de-Galan equation [34, 35, 36]. A surface coverage of 5.26 μmol/m<sup>2</sup> (%C: 8.03) for APS prepared with APTES and DMAPTMS (4.58 μmol/m<sup>2</sup>, %C: 5.12) using sc-CO<sub>2</sub> corresponding to a conversion efficiency of 65.8% and 57.3% respectively of surface silanols based on a silanol concentration of 8.0 ± 1.0 μmol/m<sup>2</sup> of bare silica. These values are higher than 4.71 μmol/m<sup>2</sup>, APTES (%C: 7.33) and 4.38 μmol/m<sup>2</sup>, DMAPTMS (%C: 4.93) corresponding to a conversion efficiency of 58.9% and 54.8% respectively obtained in toluene using

same silica type at a reaction temperature of 110°C for 24 h (Table 2). These results are in good agreement with C/N values, which is attributed to extensive hydrolysis of the ethoxy and methoxy groups of APTES and DMAPTMS in addition to subsequent silanol condensation or cross-linking reaction, coupled with the enhanced mass transfer kinetics (lower viscosity and high diffusivity) and solvating power (density) of sc-CO<sub>2</sub> enabling aminosilane to penetrate silica silanols and pores that are inaccessible in organic solvent [16, 28]. As illustrated in Table 2, the surface coverages of APS prepared in this research with APTES ligand (5.26 μmol/m<sup>2</sup> using sc-CO<sub>2</sub> and 4.71 μmol/m<sup>2</sup> in toluene) is higher than 4.67 μmol/m<sup>2</sup> reported by Buszewski *et al.*[37] but somewhat lower than 6.74 μmol/m<sup>2</sup> reported by Ray *et al.* [3]. The surface coverage of APS from DMAPTMS (4.58 μmol/m<sup>2</sup> in sc-CO<sub>2</sub>, and 4.38 μmol/m<sup>2</sup> in toluene) is higher than 2.8 μmol/m<sup>2</sup> reported by Bocian *et al.*[4]. Differences due to the use of silica with different physiochemical properties from different manufacturer, synthetic route, reaction solvents and aminosilane type are evident as highlighted in Table 2. For example, Buszewski *et al.* used Kromasil silica (100 Å, 5 μm, 310 m<sup>2</sup>/g), Ray *et al.* used aminopropyltrimethoxysilane (APTMS) and YMC silica (SIL-120-S5, 5 μm) while Bocian *et al.* used APTMS and Kromasil silica (100 Å, 5 μm, 313 m<sup>2</sup>/g).

**Table 2.** Surface coverages of APS prepared with APTES and DMAPTMS using sc-CO<sub>2</sub> and toluene as reaction medium.

$$^a \text{ Surface coverage calculated based on de Galan equation: } \alpha (\mu\text{mol}/\text{m}^{-2}) = \frac{10^6 \times \%C}{S_{BET} (100Cn_c - \%CM_r)}$$

<sup>b</sup> Reference: [37]

<sup>c</sup> Reference: [3]

<sup>d</sup> Reference: [4]

### 3.2. Thermogravimetric analysis (TGA)

The TGA weight loss observed from 220 to 880°C range (Fig. 1) corresponds to the decomposition of the chemically immobilized aminopropyl groups attached on the surface of silica. As illustrated in Fig.1A, a weight loss of 12% and 7% at 490-500°C correspond to the decomposition of aminopropyl groups (i.e. APTES) of APS using sc-CO<sub>2</sub> and toluene respectively as reaction medium. TGA of APS prepared with DMAPTES in toluene shows a weight loss of 10% at 500°C (Fig. 1B). The increase TGA weight loss of 12% observed for APS prepared in sc-CO<sub>2</sub> clearly indicate an increased or strengthening

of bound aminopropyl groups following thermal treatment, which is attributed to the hydrolysis of the unreacted ethoxy groups of APTES directly with residual silanols and Si–O–Si linkages resulting in structural rearrangement of chemically bound aminopropyl groups. A small TGA weight loss 2-3% at 100°C corresponds to adsorbed moisture while weight loss at temperature above 650°C is attributed to the condensation of surface silanols liberating siloxanes and water. While TGA shows the thermal decomposition, the derivative (DTA) shows how rapid or slow the rate of decomposition is occurring, and is used to identify the desorption of different species [19].

**Fig. 1A.** TGA overlay with varying loading of aminopropyl groups of bonded APS intermediate phase prepared with 3-APTES using sc-CO<sub>2</sub> and toluene as reaction medium.

**Fig. 1B.** TGA/DTA of bonded APS intermediate phase prepared with DMAPTMS in toluene showing 3 distinct decomposition steps.

### 3.3. Zeta potential ( $\zeta$ ) analysis

Zeta potential measurements were performed four times each for bare silica and bonded APS intermediate phase and calculated average presented. As illustrated in Table 3, the negative zeta potential values (at pH 7-8) observed on bare silica is attributed to surface charges from silanol groups in the dissociated form (SiO<sup>-</sup>).

**Table 3.** Zeta Potentials (mV) for Exsil-pure silica and functionalized APS prepared from APTES

And these values are more negative in acetonitrile than in methanol partly due to the physiochemical properties of the dispersants (Table 4). A change in electrostatic charges from negative to positive for bonded APS confirmed successful immobilization of 3-APTES onto the surface of silica, and is consistent with cited results in the literature [10]. The more positive  $\zeta$  values observed for supercritical CO<sub>2</sub> generated APS intermediate phase (sc-APS) compared to organic solvent based method in both methanol and acetonitrile indicate greater amino groups (-NH<sub>3</sub><sup>+</sup>) immobilization on the surface of silica as supported with higher carbon loading/surface coverages (Table 1 and 2). The difference in positive zeta potentials observed in methanol and acetonitrile is likely due to differences in dipole moment as shown in Table 4 coupled with differences in solvation of bonded APS. The high zeta potentials thus indicate that the APS particles have a strong surface charge and the repulsive force between particles is less susceptible to aggregation (also confirmed by SEM, Fig. 2) and therefore more stable. This

stability is in good agreement with TGA (Fig. 1) as the organic groups do not detach from the surface of silica at temperature lower than  $\sim 490$  to  $500^\circ\text{C}$ .

**Table 4.** Physicochemical properties of methanol and acetonitrile used in zeta potential analysis at  $25^\circ\text{C}$  <sup>a</sup>

<sup>a</sup> Data from DTS (nano) software ver.510

<sup>b</sup> Reference:[38]

### 3.4. Scanning electron microscopy (SEM)

Examinations of the surface morphology of bonded APS intermediate phase prepared in  $\text{sc-CO}_2$  and toluene by SEM (Fig. 2) clearly shows no evidence of aggregation.

**Fig. 2.** SEM micrographs of bare silica and APS phase generated from APTES using  $\text{sc-CO}_2$  and toluene as reaction medium.

### 3.5. Diffuse reflectance infrared fourier transform (DRIFT) spectroscopy

DRIFT analysis clearly confirms the immobilization of 3-APTES onto the surface of silica (Fig.3) through comparing the spectra of bare silica and supercritical  $\text{CO}_2$  generated APS (sc-APS). The appearance of new peaks at  $3300\text{cm}^{-1}$ ,  $2932\text{cm}^{-1}$ ,  $2877\text{cm}^{-1}$ ,  $1557\text{cm}^{-1}$  and  $1327\text{cm}^{-1}$  are evident corresponding to the aminopropyl characteristic vibrations. As illustrated in Fig.3, the adsorption peaks of  $\text{CH}_2$  at  $2932\text{cm}^{-1}$  and  $2877\text{cm}^{-1}$  is due to the propyl alkyl chain of 3-APTES. The intense peak at  $1557\text{cm}^{-1}$  is attributed to the bending vibrations of amino groups ( $\text{NH}_2$ ), and the broad N-H band at  $3300\text{cm}^{-1}$  is due to asymmetric/symmetric stretching of hydrogen bonded  $\text{NH}_2$  groups. The low intensity peak at  $1327\text{cm}^{-1}$  is assigned to the C-N stretching vibration. A similar spectrum (not shown here) was also obtained for APS prepared in toluene.

**Fig. 3.** DRIFT spectra of APS (APTES) generated in  $\text{sc-CO}_2$  at conditions of  $100^\circ\text{C}/414\text{bar}/3\text{ h}$ .

Moreover, the spectrum of the sc-APS revealed a decreased in the intensity of the peak at  $3661\text{cm}^{-1}$  attributed to the single/geminal hydroxyl and hydrogen hydroxyl (O-H), compared to a broad peak at  $3669\text{-}3648\text{cm}^{-1}$  in the case of bare silica. The peak at  $2338\text{cm}^{-1}$  is attributed to residual adsorbed  $\text{CO}_2$  on the surface of silica due to the use of  $\text{sc-CO}_2$  as solvent, consistent with cited results in the literature

[17]. The band at  $1650\text{cm}^{-1}$  for both bare silica and modified APS is ascribed to physisorbed water, and/or bending vibration of water, while the strong signals observed at  $1114\text{-}1013\text{cm}^{-1}$  and  $803\text{cm}^{-1}$  are due to Si–O–Si vibrations [16, 17].

### 3.6. Solid-state nuclear magnetic resonance spectroscopy

The  $^{29}\text{Si}$  CP/MAS spectrum of bonded aminopropyl silica (APS) stationary phase prepared with DMAPTMS in  $\text{sc-CO}_2$  shows resonances at  $-66\text{ppm}$  and  $-69\text{ppm}$  assigned to  $\text{T}^2$  and  $\text{T}^3$  species respectively attributed to covalent bonding of ligand onto the surface of silica. The signals at  $-101\text{ppm}$  and  $-110\text{ppm}$  are associated with vicinal or free silanols ( $\text{Q}^3$ ) and siloxanes ( $\text{Q}^4$ ) typical of native silica [2] respectively. The absent of the monodentate species ( $\text{T}^1$ ) and the most reactive geminal silanols ( $\text{Q}^2$ , which normally appeared at  $-92\text{ppm}$ ) is attributed to extensive hydrolysis and silanol condensation or cross-linkage resulting in higher surface coverage and efficiency ( $5.24\mu\text{mol}/\text{m}^2$ ). The relative low intensity of  $\text{Q}^3$  and absent or significant reduction of  $\text{Q}^2$  and  $\text{T}^1$  are indicative of a lower degree of residual silanols activity following silanisation in  $\text{sc-CO}_2$ .

**Fig. 4.**  $^{29}\text{Si}$  CP/MAS NMR spectrum of APS prepared from DMAPTMS using  $\text{sc-CO}_2$  as reaction medium.

The  $^{13}\text{C}$  CP/MAS NMR spectrum revealed all peaks associated with the carbon atoms of the DMAPTMS ligands with the exception of the methoxy carbon atoms (Fig. 5). The peak at  $13\text{ppm}$  correspond to the carbon atom (C-1) directly attached to silica, while peaks at  $23\text{ppm}$  and  $65\text{ppm}$  correspond to the second (C-2) and third (C-3) methyl carbons of the propyl chain respectively. The peak at  $36\text{ppm}$  is attributed to the carbon atoms (C-4) directly attached to the nitrogen atom of DMAPTMS ligand. The absent of a peak associated with carbon atoms of the methoxy groups ( $-\text{OCH}_3$ ) is further evidence of extensive hydrolysis and silanols condensation or cross-linkage using  $\text{sc-CO}_2$  as a reaction solvent coupled with post-curing at  $120^\circ\text{C}$  and consistent with cited results in literature[39].

**Fig. 5.**  $^{13}\text{C}$  CP/MAS NMR spectrum of APS prepared from DMAPTMS using  $\text{sc-CO}_2$  as reaction medium.

#### 4. Conclusions

Supercritical carbon dioxide (sc-CO<sub>2</sub>) has been demonstrated as a competitive alternative medium for the synthesis of aminopropyl bonded silica (APS) compared to conventional organic solvents (e.g. toluene) based methods. Using sc-CO<sub>2</sub> conditions of 100°C, 414 bar and 3 h, surface coverages and efficiencies of APS prepared with APTES (5.26 μmol/m<sup>2</sup>, 65.75%) and DMAPTMS (4.58 μmol/m<sup>2</sup>, 57.25%) are higher but comparable to conventional organic solvents based methods with APTES (4.71 μmol/m<sup>2</sup>, 58.88%) and DMAPTMS (4.38 μmol/m<sup>2</sup>, 54.75%) at a temperature of 110°C in 24 h. It is expected that the effect on the chromatographic performance of higher surface ligand density obtained with the sc-CO<sub>2</sub> protocol is a decrease in secondary interactions particularly of basic analytes due to lower degree of residual silanol activity.

Zeta potential measurements shows a change in electrostatic charges of silanol groups from negative values for bare Exsil-pure silica to positive for bonded APS due to immobilization of amino groups (-NH<sub>3</sub><sup>+</sup>) onto the surface of silica. Moreover, zeta potentials become more positive as carbon loading increases and the values are higher in methanol than in acetonitrile probably due to differences in dipole moment and dielectric constant.

<sup>29</sup>Si-CP/MAS NMR of APS prepared from DMAPTMS using sc-CO<sub>2</sub> as a reaction medium revealed the present of bound organic moiety on the surface of silica confirming successful functionalisation. The <sup>12</sup>C-CP/MAS NMR spectrum shows the propyl carbons of used ligand couple with absent of the methoxy groups (-OCH<sub>3</sub>) indicating extensive hydrolysis and silanols condensation, further confirming effective sc-CO<sub>2</sub> functionalisation.

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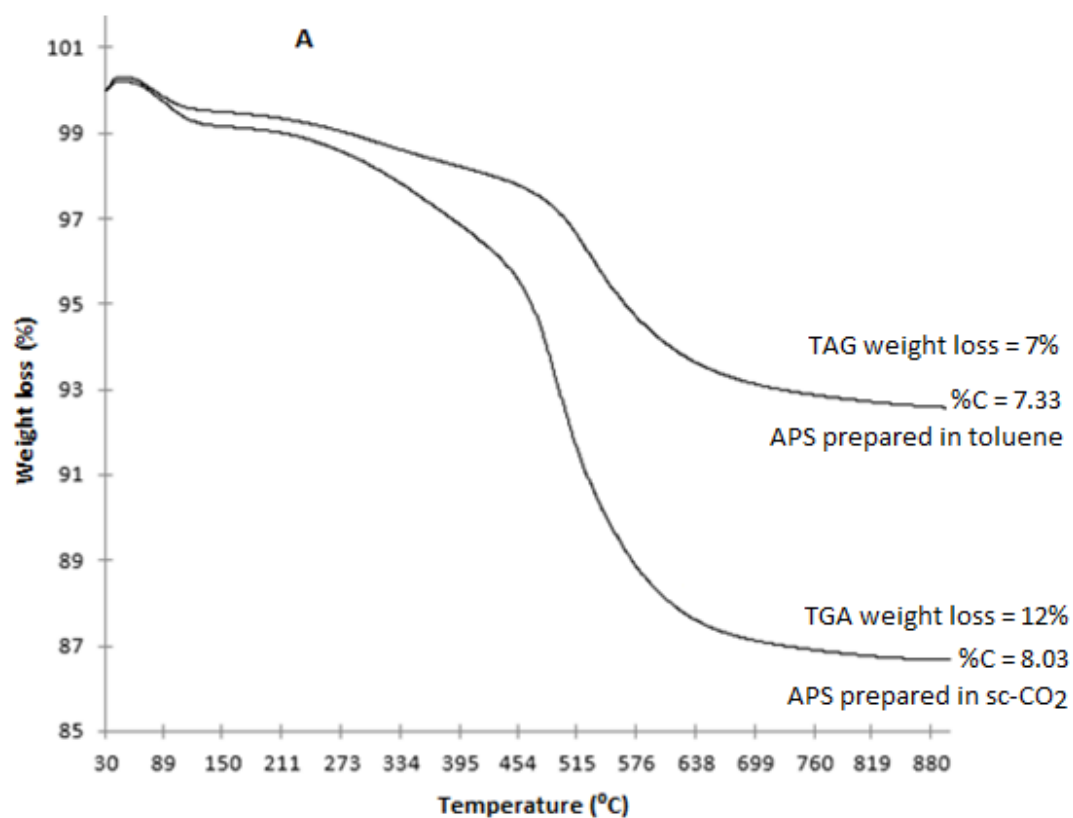
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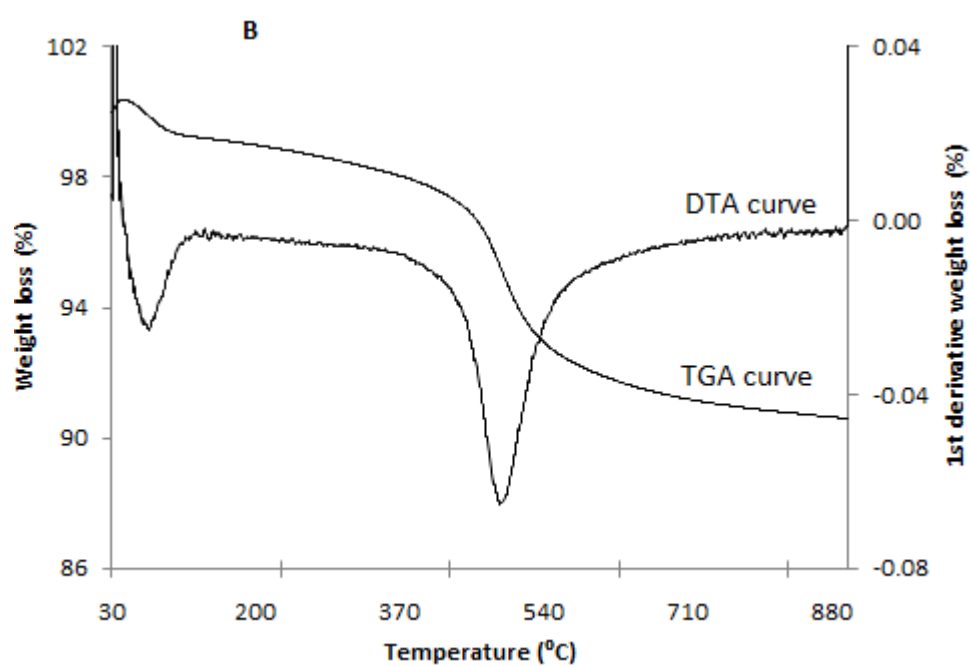
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Figure<sup>1</sup>; Caption

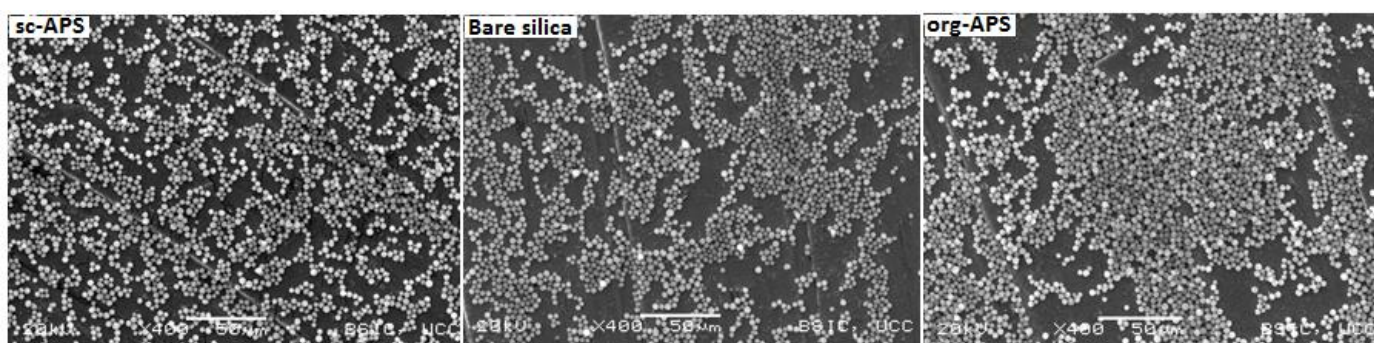
Figr-1List of figures



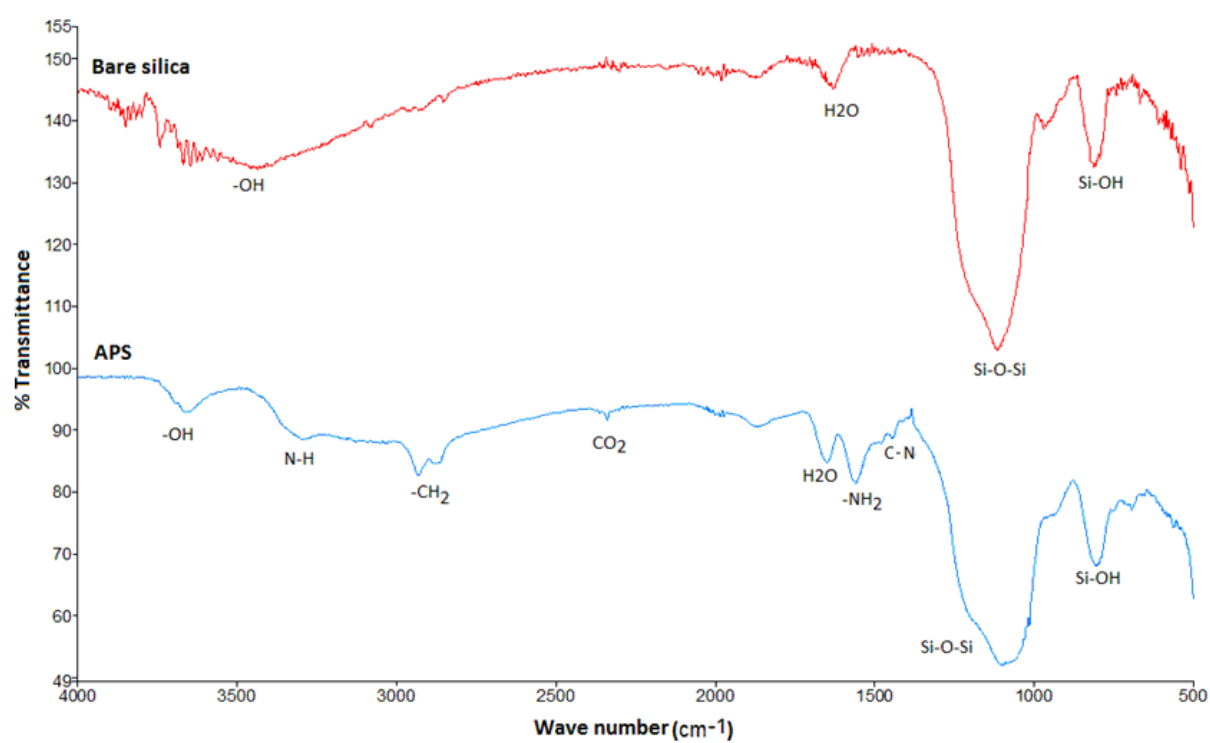
**Fig. 1A.** TGA overlay with varying loading of aminopropyl groups of bonded APS intermediate phase prepared with 3-APTES using sc-CO<sub>2</sub> and toluene as reaction medium.



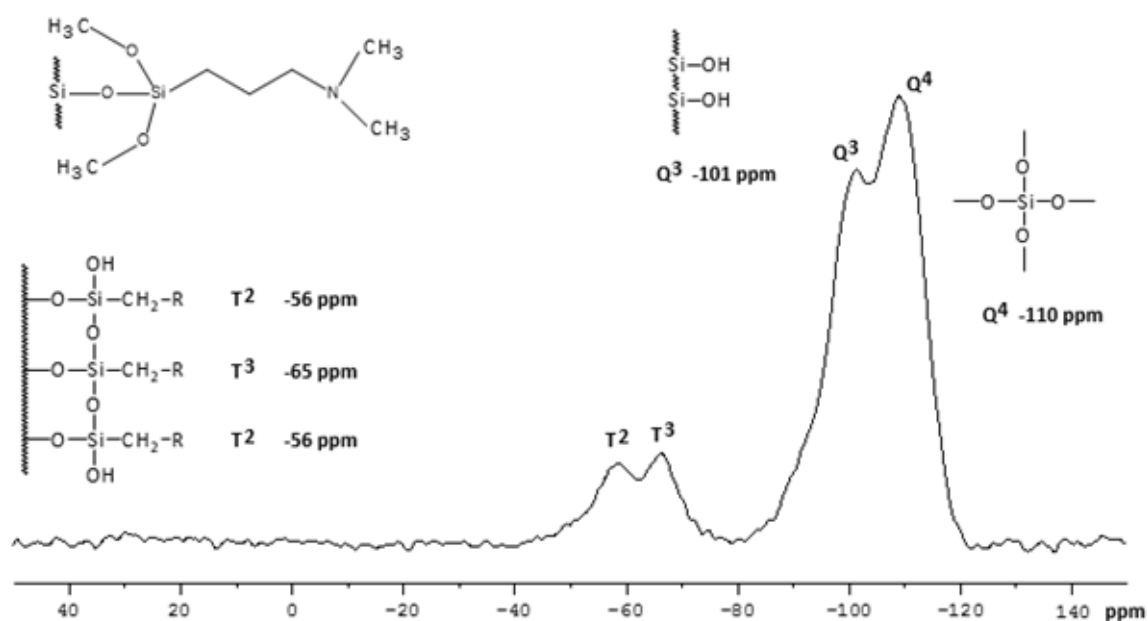
**Fig. 1B.** TGA/DTA of bonded APS intermediate phase prepared with DMAPTMS in toluene showing 3 distinct decomposition steps.



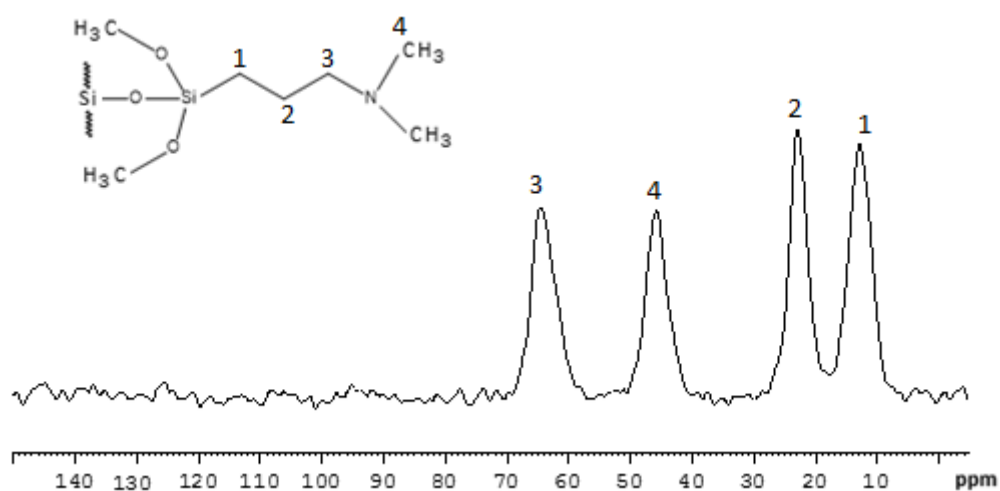
**Fig. 2.** SEM micrographs of bare silica and APS phase generated from APTES using sc-CO<sub>2</sub> and toluene as reaction medium.



**Fig. 3.** DRIFT spectra of APS (APTES) generated in sc-CO<sub>2</sub> at conditions of 100°C/414bar/3 h.



**Fig. 4.**  $^{29}\text{Si}$  CP/MAS NMR spectrum of APS prepared from DMAPTMS using  $\text{sc-CO}_2$  as reaction medium.



**Fig. 5.**  $^{13}\text{C}$  CP/MAS NMR spectrum of APS prepared from DMAPTMS using  $\text{sc-CO}_2$  as reaction medium.

## List of tables

**Table 1.** Elemental analysis values of APS intermediate prepared from 3-APTES and N, N-DMAPTMS) using sc-CO<sub>2</sub> (100°C/414bar/3 h) and toluene (110°C/24 h) as reaction medium.

Property of silica used	Silanisation process	%C, %H, %N, C/N
Exsil-Pure silica, 3 µm, 120 Å, 223 m <sup>2</sup> /g	APTES in sc-CO <sub>2</sub> , 3 h /100°C/ 414 bar	8.03, 1.76, 2.36, 3.40
Exsil-Pure silica, 3 µm, 120 Å, 223 m <sup>2</sup> /g	APTES in toluene, 24 h /110°C	7.33, 1.64, 2.51, 2.92
Exsil-Pure silica, 3 µm, 120 Å, 223 m <sup>2</sup> /g	DMAPTMS in sc-CO <sub>2</sub> , 3h/100°C/414 bar	5.12, 1.17, 1.12, 4.57
Exsil-Pure silica, 3 µm, 120 Å, 223 m <sup>2</sup> /g	DMAPTMS in toluene, 24 h/110°C	4.93, 1.81, 1.01. 3.92
Porous silica of particle diameter 0.2-0.5 nm	APTES in toluene, 24 h /110°C <sup>a</sup>	6.16, 1.85, 2.17, -
YMC silica (SIL-120-S5), 5 µm	APTMS in toluene, 24 h <sup>b</sup>	6.54, 2.09, 2.25, 2.90
Kromasil 100 Å, 5µm, 313 m <sup>2</sup> /g	APTMS in toluene, 12 h /120°C <sup>c</sup>	2.83, 1.31, 1.25, -

<sup>a</sup> Reference: Erdem *et al.*[8]<sup>b</sup> Reference: Ray *et al.*[3]<sup>c</sup> Reference: [4]

-, Value not available

**Table 2.** Surface coverages and TGA mass loss (%w/w) of APS prepared with APTES and DMAPTMS using sc-CO<sub>2</sub> and toluene as reaction medium.

Properties of silica used	Silanisation process	%C	TGA mass loss	$\alpha$ (µmol/m <sup>2</sup> ) <sup>a</sup>
Exsil-Pure silica, 3µm, 120Å, 223m <sup>2</sup> /g	APTES in sc-CO <sub>2</sub> , 3 h/100°C/414 bar	8.03	12% from 220 - 880°C	5.26
Exsil-Pure silica, 3µm, 120Å, 223m <sup>2</sup> /g	APTES in toluene, 24 h/110°C	7.33	7% from 220 - 880°C	4.71
Exsil-Pure silica, 3µm, 120Å, 223m <sup>2</sup> /g	DMAPTMS in sc-CO <sub>2</sub> , 3 h/100°C/414 bar	5.12	-	4.58
Exsil-Pure silica, 3µm, 120Å, 223m <sup>2</sup> /g	DMAPTMS in toluene, 24 h/ 110°C	4.93	10% from 220 - 880°C	4.38
Kromasil 100Å, 5µm, 310 m <sup>2</sup> /g	APTES in toluene, 12 h, 100°C <sup>b</sup>	4.47	-	4.67
Kromasil 100Å, 5µm, 313 m <sup>2</sup> /g	APTMS in toluene, 12 h, 120°C <sup>c</sup>	2.83	-	2.8
YMC silica (SIL-120-S5), 5µm	APTMS in toluene, 24 h <sup>d</sup>	6.54	8.07% from 200 -800°C	6.74

<sup>a</sup> Surface coverage calculated based on de Galan equation:  $\alpha(\mu\text{mol}/\text{m}^2) = \frac{10^6 \times \%C}{S_{BET}(100Cn_c - \%CM_r)}$

<sup>b</sup> Reference: [36]

<sup>c</sup> Reference: [4]

<sup>d</sup> Reference: [3]

-, Data not available

**Table 3.** Zeta Potential (mV) for bare silica and functionalized APS prepared from APTES

Phase	Bare silica		sc-APS		org-APS	
	Methanol	Acetonitrile	Methanol	Acetonitrile	Methanol	Acetonitrile
Average $\xi$	-24.3	-34.9	+45.8	+28.8	+33.0	+24.7

**Table 4.** Physicochemical properties of methanol and acetonitrile used in zeta potential analysis at 25°C <sup>a</sup>

Properties	Symbol	Methanol	Acetonitrile
Refractive index (RI)	n	1.326	1.345
Viscosity (cP)	$\zeta$	0.5476	0.400
Dielectric constant	$\epsilon_r$	33.0	37.5
Dipole moment (D) <sup>b</sup>	$\mu$	1.854	3.184

<sup>a</sup> Data from DTS (nano) software ver.510

<sup>b</sup> Reference:[38]