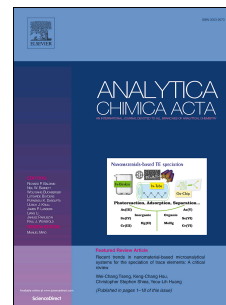


Accepted Manuscript

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PII: S0003-2670(19)30414-3

DOI: <https://doi.org/10.1016/j.aca.2019.04.006>

Reference: ACA 236695

To appear in: *Analytica Chimica Acta*

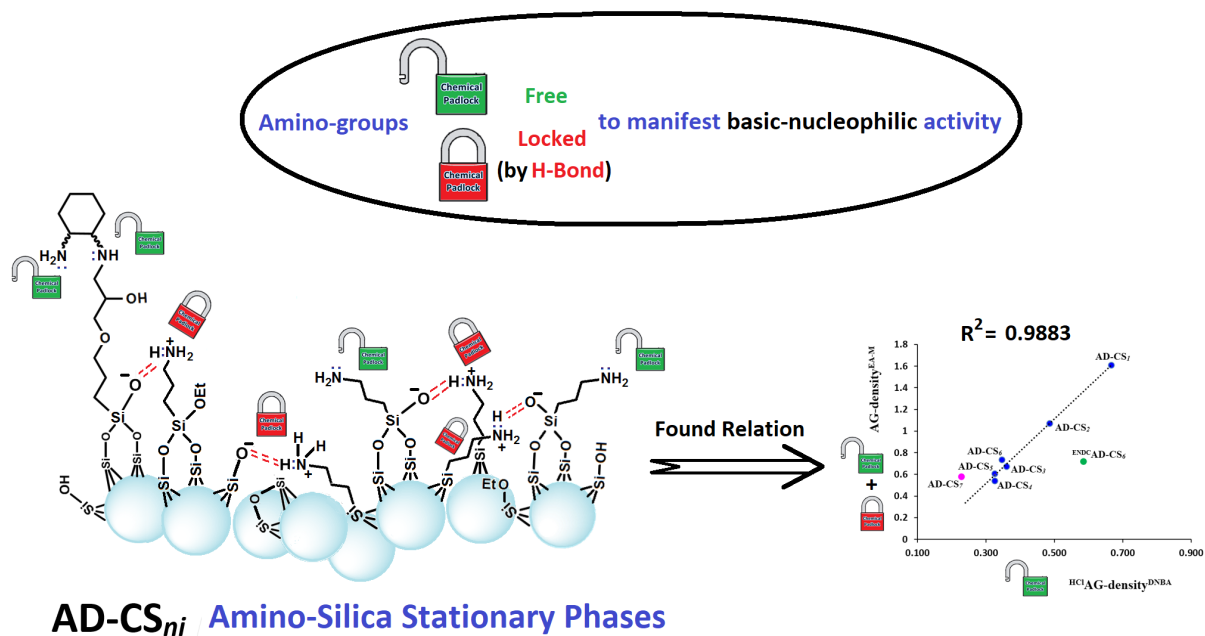
Received Date: 9 December 2018

Revised Date: 4 April 2019

Accepted Date: 6 April 2019

Please cite this article as: G. Manzi, F. Buonsenso, O.H. Ismail, A. Ciogli, G. Siani, M. Pierini, Modular and Conservative Procedure for the Quantification of Amino Functionalities Bonded to Solid Porous Matrices, *Analytica Chimica Acta*, <https://doi.org/10.1016/j.aca.2019.04.006>.

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Modular and Conservative Procedure for the Quantification of Amino Functionalities Bonded to Solid Porous Matrices

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KEYWORDS Amino-silica characterization; solids for CO₂ capture; amino-groups dosage on silica; basic stationary-phases; basic silica-selectors.

ABSTRACT: Nowadays solid materials in which amino groups are linked to silica matrices through alkyl chains of different length (C18, C8, C4) are successfully employed in CO₂ capture and storage technologies, as well as in a variety of chromatographic applications. In particular, their use as stationary phases finds remarkable success in performing HILIC separations and, in general, in the effective resolution of important compound classes (e.g. mixtures of mono- or

oligo-saccharides). In this study an original and operationally simple procedure designed to quantify the density of basic groups (typically amino groups) chemically bonded to the surface of porous solids, which also allows a full recovery of the analyzed material, is presented. The method is based on the preventive acid-base reaction of the basic groups linked to the solid by 3,5-dinitrobenzoic acid (DNBA). The quantification of the basic functionalities is then performed by an UV-spectrophotometric retro-titration of the thus salified solid matrix (or, alternatively, by HPLC approach), resorting to a preventive either acid or basic displacement of DNBA from the matrix. The uncertainty of the density measurements is assessed by 13%.

1. INTRODUCTION

Amino-functionalized solid materials are widely employed in a variety of applications, from CO₂ capture and storage technologies to separations technology. The development of efficient CO₂ capture and storage technologies to control CO₂ emissions has received a great deal of attention as the increased atmospheric concentration of carbon dioxide is considered the main responsible for the global warming and climate change. Moreover, stored CO₂ is a useful carbon source that can be recycled as C1 building block in many chemical processes. In such a contest, amino-functionalized solid materials are emerging as promising CO₂ sorbents, due to their ability in forming strong covalent bonds with CO₂ molecules. [1-8] For this purpose, a number of porous materials, in most of which, porous silica was profitably used as the solid support, has been functionalized with basic nitrogen groups, such as -NH₂, -NHR, -NR₂, amino alcohols or amino-terminated dendrimers. [9-12]

Amino-functionalized solid materials play an important role also as essential components of stationary phases in chromatographic techniques. [12-30] Indeed, amino phases with

aminopropyl ligands as functional groups, are among the most widely used HILIC (Hydrophilic Interaction Liquid Chromatography) phases for carbohydrate separation. [17-19]

Aminopropylated silica gel is largely employed to prepare polysaccharide-based chiral stationary phases for separation of enantiomers by HPLC techniques and for studying the kinetic aspects involved in the eventual interconversion between the resolved chiral species by dynamic-HPLC determinations. [23-30] Indeed, polysaccharides, as cellulose, amylose or chitin, can be either strongly adsorbed on the surface of the amino phase, through the formation of a widespread number of hydrogen bonds, or covalently linked to the surface, through very stable amide-bonds.

However, in the considered amino stationary phases, the perfect coating of the matrix with the selector is far from guaranteed, and the unreacted amino groups can interfere acting as promoter (or inhibitor) agents of secondary reactions, as it happens in the case of on-column bimolecular enantiomerizations due to tautomeric equilibria catalysed by bases. [31,32]

In such a context, the quantification of the surface concentration of amino groups appears to be of remarkable importance to define the potential properties of the final stationary phase and to rationalize some of the observed chromatographic outcomes.

X-ray photoelectron spectroscopy (X-ray-M), [33] contact angle [34] and atomic force microscopy (CAM-M and AFM-M, respectively) [35] have been used to characterize matrix surfaces, although these methods work well only on flat surfaces. Determinations based on elemental analysis (EA-M) [36] provide affordable information on the absolute percentage of nitrogen present in the analysed samples, from which the desired surface density of amino groups can be easily obtained. A different approach that exploits the nucleophilic properties of amino groups, is that related to the reaction of these basic frameworks with ninhydrin (NHD-M), which leads to the formation of colored organic compounds. [37] This method was initially

developed to detect incomplete coupling reaction in the Merrifield solid-phase synthesis and revealed a sensitivity of 5 mol/g. According to this method, the nitrogen atom of the amine is extracted by ninhydrin and incorporated in the purple complex in solution. The absorption of the complex is measured by UV-visible spectrometry and the concentration is calculated from a calibration curve obtained using hexylamine as the source of amino groups. Although this method potentially works well, it has some limitations. First of all, the analysed portion of silica is destroyed and, considering the need to repeat the test at least three times, the method is quite expensive, both in terms of cost and time needed to produce great quantities of material when the synthesis is made on a laboratory scale. Moreover, the ninhydrin test requires quite long times for analysis and, finally, it is not useful in the presence of tertiary amino groups.

To overcome many of these operative limits, a simple and quick protocol to determine the density of basic groups linked to the surface of a solid is reported in this work. Although, in principle, this protocol could be applied on any kind of solid material, the particular importance of siliceous structures for the above mentioned wide variety of applications, prompted us to develop the method by employing, as test samples, matrices of silica powder derivatized with amino groups and linked to the surface through short alkyl spacers ($\text{SiO}_2\text{-R-NH}_2$). A large number of information are available in literature on the properties possessed by silica porous matrices which prevalently concern size, morphology and chemical structure characterizing the surface of these materials in particulate form. [38] Among these properties, of particular relevance and practical utility for our purpose are density, typology and acid character of the silanol groups ($\equiv\text{Si-OH}$) distributed on the surface. Thus, it is significant to mention:

1) the surface density of $\equiv\text{Si-OH}$ groups that, in an amorphous silica, is considered almost like a physicochemical constant (known as Kiselev-Zhuravlev constant), with the value ranging from 4.2 to 5.7 silanol sites/nm²; [39,40]

2) the two main typologies of $\equiv\text{Si-OH}$ groups, commonly denoted by the acronyms Q3 and Q2, which identify the cases of one and two silanols attached to the silicon center, respectively; [41,42]

3) the very different acid strength that characterizes the two Q3 and Q2 typologies of silanols, the first ones having a pKa value between 2 and 4.5, while the others, much less acidic, with pKa values close to 8.5. [43,44]

According to the above silica-properties, it is reasonable to expect that amino-silica materials, typically featured by a degree of derivatization of the silanols much lower than the total available on the original matrix (frequently, a loading less than 50% of the total $\equiv\text{Si-OH}$ groups, regardless of the particular bound selector [45-48]), are not able to express the full potential basic/nucleophilic activity of their amino sites, because of the establishment of acid-base and/or H-bond interactions between amino and residual $\equiv\text{Si-OH}$ groups in a non-negligible extent. The methods used to quantify amino sites covalently bonded on silica could be distinguished in two typologies:

a) methods able to reveal the total amount of amino moieties linked on the matrix, regardless of whether the relevant nitrogen atoms are able or not to manifest their basic/nucleophilic properties (i.e. methods that allow the evaluation of all the amino groups, without distinguishing between those having free or engaged lone pairs). The X-ray-M, CAM-M, EA-M and AFM-M methods can be traced back to this typology;

b) methods that exclude from the final quantification the amino groups unable to express their basic/nucleophilic activity, for example those involved in acid-base interactions with close silanol groups. The NHD-M approach based on ninhydrin can be traced back to this typology.

In the latter case, if the final application for which the material has been designed is expressly based on the exploitation of the basic/nucleophilic properties possessed by its amino groups, the data obtained through these procedures will provide information of primary and irreplaceable importance.

As it will be widely clarified in the “Results and Discussion” section, in the case of amino-silica materials, our analytical protocol is able to meet at the same time both types (points a and b) of quantitative information.

2. EXPERIMENTAL SECTION.

2.1 MATERIALS AND METHODS

2.1.1 Materials

Silica Gel 60 (480-540 m²/g, 40-63 μm), LiChroprep Si 60 (480 – 540 m²/g, 15-25 μm) and LiChrosorb Si-60 (500 m²/g, 10μm) were provided by Merck Millipore (Darmstadt, Germany). (3-Aminopropyl)trimethoxysilane (APTMS), (3-glycidyloxypropyl)trimethoxysilane (GOPTMS), tetraethylenpentamine (TEPA), 1-(trimethylsilyl)imidazole (TMSI), 3,5-dinitrobenzoic acid (DNBA) at 99% of purity, potassium bromide FTIR grade (KBr), triethanolamine (TEA), (±)1,2-diaminocyclohexane (DACH), acetonitrile (ACN), dichloromethane (DCM), methanol (MeOH), chloroform, water HPLC grade, dry toluene,

hydrochloric acid reagent grade 37% (HCl), trifluoroacetic acid (TFA) were purchased from Sigma Aldrich. HCl solutions 1 and 0.1 M were prepared by dilution of HCl 37% with MilliQ water.

2.1.2 Instruments.

A Jasco 430 Fourier transform FTIR spectrometer with a resolution of 4 cm^{-1} , Jasco Europe, Milan, Italy. A Jasco V570 UV/Vis/NIR spectrometer, Mary's Ct, Easton, MD 21601, US.

2.1.3 Synthesis of 2-aminopropyl silica gel (AD-CS₁₋₆)

The 2-aminopropyl silica derivatives, AD-CS₁₋₆, were prepared according to the methods described in literature [45]. Typically, a slurry of 3.0 g of previously dried (high vacuum pump, T=120°, 1 h; P= 0.1 mbar) silica gel in 60 ml of dry toluene was prepared in inert atmosphere. The (3-aminopropyl)triethoxysilane was then added (1.5 ml, 6.6 mmol) and the mixture was heated to reflux for 4 h. Modified silica gels were isolated by filtration, washed with 30 ml portions of toluene, MeOH, and DCM and dried until constant weigh (90 °C, 0.1 mbar, 1 h). Modified amino-silica were characterized by FT-IR (KBr pellet and ATR) and (C,H,N) elemental analysis. FT-IR (KBr): 2931, 2854 cm^{-1} . Found elemental analysis and silica specifications are listed in Table 1.

Table 1. Physico-chemical data of the starting silicas and carbon, hydrogen and nitrogen loadings data obtained after their derivatizations, relevant to the AD-CS₁₋₆ samples.

Amino-silica	Starting silica	Elemental analysis			Loaded selector mmol/g _{matrix} (based on nitrogen percentage)	Silanols mmol/g _{silica}
		%C	%H	%N		
AD-CS ₁	Silica Gel 60 (480-540 m ² /g, 40-63 μm)	6.65	1.78	2.25	1.61	3.9 ÷ 4.4
AD-CS ₂	LiChrosorb Si 60 (500 m ² /g, 10 μm)	4.52	1.26	1.50	1.07	4.1
AD-CS ₃	LiChroprep Si 60 (480 – 540 m ² /g, 15-25 μm)	2.95	0.77	0.94	0.67	3.9 ÷ 4.4
AD-CS ₄	LiChroprep Si 60 (480 – 540 m ² /g, 15-25 μm)	2.30	0.73	0.76	0.54	3.9 ÷ 4.4
AD-CS ₅	LiChroprep Si 60 (480 – 540 m ² /g, 15-25 μm)	2.52	0.75	0.85	0.61	3.9 ÷ 4.4
AD-CS ₆	LiChroprep Si 60 (480 – 540 m ² /g, 15-25 μm)	4.28	1.17	1.03	0.74	3.9 ÷ 4.4

2.1.4 AD-CS₆ chemical endcapping (^{ENDC}AD-CS₆)

The unreacted silanol groups of 2-aminopropyl derivatized silica, AD-CS₆, were subsequently end capped as described in reference [49]. Briefly, N-trimethylsilylimidazole (TMSI, 0.9 ml, 2.7 mmol) was added to a slurry of AD-CS₆ in dry toluene (1.5 g in 30 ml), at room temperature. The mixture was heated to reflux for 4 h. The modified silica gel was isolated by filtration, washed with toluene, MeOH, MeOH + TEA 1 %, MeOH and DCM and dried under vacuum until constant weigh. FTIR (KBr): 2931, 2854 cm⁻¹.

Elemental analysis of C,H,N provided the following percentages : 5.58 %C, 1.48 %H, 1.01 %N corresponding to 0.72 mmol of amino groups per gram of silica (based on nitrogen percentage).

2.1.5 Synthesis of cyclohexyl-1,2-diamine silica gel (AD-CS₇)

Silica was modified as previously reported [50]. A slurry of 10.2 g of silica gel (LiChrosorb Si-60 10 μm , 500 m^2/g) and 4.3 g of (+/-) 1,2-diaminocyclohexane ($\text{C}_6\text{H}_{14}\text{N}_2$, Mw 114.19) in MeOH (45 ml) was heated to reflux under argon atmosphere and continuous stirring. A solution of glycidoxypropyltrimethoxysilane ($\text{C}_9\text{H}_{20}\text{SiO}_5$, Mw 236.34, d: 1.07 g/ml) in MeOH (1.5 ml in 30 ml) was added dropwise during 1 h. The mixture was heated to reflux for ~ 8 h. After cooling to r.t., modified silica gel was isolated by filtration, washed with MeOH, DCM (100 ml each) and dried in vacuum (0.1mmHg) at $T=50^\circ\text{C}$ to constant weight.

FTIR (KBr): 2938, 2862, 1878, 1622, 1456, 1088 cm^{-1} .

Elemental analysis of C,H,N provided the following percentages: 5.13 %C; 1.10%H, 0.81% N, corresponding to 0.29 mmol of derivatizing framework, and to 0.58 mmol of amino groups per g of silica (based on nitrogen percentage).

2.1.6 Acid-base reaction of $\text{SiO}_2\text{-R-NH}_2$ amino groups with 3,5-dinitrobenzoic acid: preparation of $\text{AD-CS}_{ni}^+\text{DNB}^-$ silicas.

Depending on the amount of derivatized silica to be reacted with the 3,5-dinitrobenzoic acid (DNBA), the adopted procedure must be distinguished in two independent approaches, listed below as type A and B.

Approach A. An amount ranging from 100 to 900 mg of the synthesized amino-silica derivatives AD-CS_{ni} is reacted with DNBA by dispersing it in a water solution of 3,5-dinitrobenzoic acid 2.3×10^{-3} M under constant stirring, at room temperature, for 30 minutes, respecting a silica-mass/DNBA-solution ratio of 1 mg/ml. The so-treated silica powder ($\text{AD-CS}_{ni}^+\text{DNB}^-$) is recovered by filtration, and then washed in two steps, first with water, and next with methanol. Finally, the $\text{AD-CS}_{ni}^+\text{DNB}^-$ silica is exhaustively dried, under reduced

pressure (40°C, 0.1 mmHg), until constant weight. FTIR (KBr), shows new characteristic absorbing bands close to 1635, 1547, 1385 and 1356 cm^{-1} .

Approach B. An amount of amino-silica derivative of type AD-CS_{ni} ranging from 10 to 30 mg is dispersed in 10 ml of a water solution of 3,5-dinitrobenzoic acid (DNBA) 2.3×10^{-3} M, and then the solution is left under constant stirring, at room temperature, for 10 minutes. After its quantitative recover by centrifugation, the dispersion/centrifugation steps are repeated two more times. Finally, the obtained powder of AD-CS_{ni}⁺DNB⁻ silica is washed with water and separated from the supernatant through centrifugation, repeating these steps for three times. The wet sample is then directly initiated to the subsequent step of DNB⁻ displacement, used for the desired amino-groups quantitation.

2.1.7 Displacement of DNB⁻ ions from AD-CS_{ni}⁺DNB⁻ silicas by treatment with an acid solution of HCl and UV-visible quantitation of the obtained DNBA species.

An appropriate amount of amino-silica salt AD-CS_{ni}⁺DNB⁻ is dispersed under stirring, at room temperature, in a water solution of HCl 1M, always respecting a silica-mass/HCl-solution ratio in the range 2 - 6 mg/ml. Next, the supernatant is recovered by centrifugation and transferred into a graduated flask, while the isolated silica is dispersed into a HCl 1M solution. The whole procedure is repeated for other two times, collecting all the recovered supernatants into the same graduated flask. Finally, the content of the graduated flask is brought to volume with HCl 1M solution and the absorbance of the released DNBA is measured at 229 nm by UV visible spectrophotometry. The quantitation of DNBA is then obtained from a calibration curve created

with ten standard solutions of DNBA (2×10^{-4} M \div 1×10^{-5} M) in HCl 0.1 M (Figure S1 of Supplementary Material, SM).

2.1.8 Displacement of DNB⁻ ions from AD-CS_{ni}⁺DNB⁻ silicas by treatment with a basic solution of TEPA and relevant UV-visible quantitation of the obtained H:TEPA⁺DNB⁻ species.

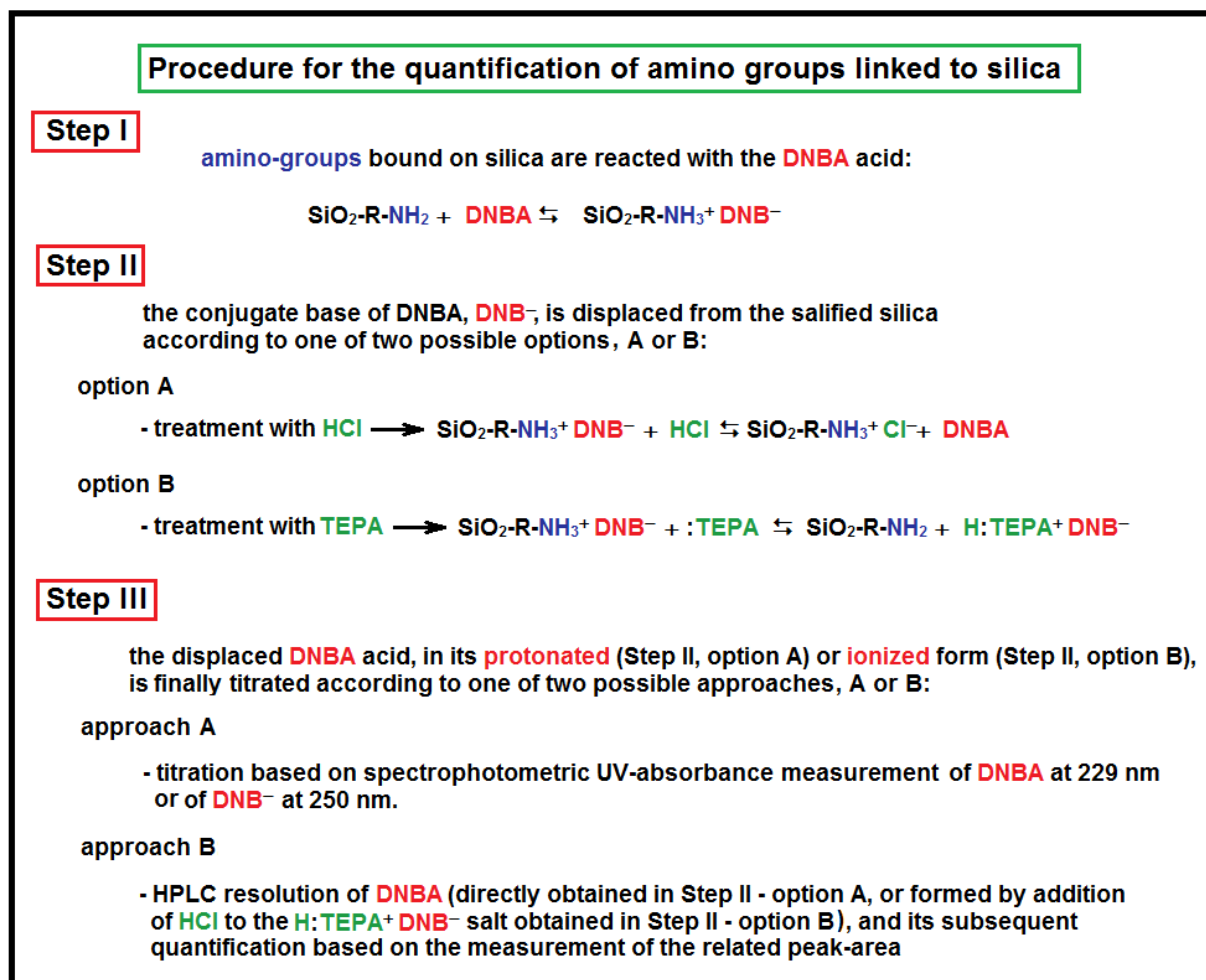
An appropriate amount of amino-silica salt AD-CS_{ni}⁺DNB⁻ in the range 10-30 mg is dispersed under stirring, at room temperature, in 5 ml of a 0.1 M solution of tetraethylenpentamine, TEPA in acetonitrile (ACN) (molar mass: 189.31 g/mol; 4.735 g dissolved in 250 ml of ACN). Next, the supernatant, containing the H:TEPA⁺DNB⁻ salt, is recovered by centrifugation and transferred into a 20 ml graduated flask, while the isolated silica is again dispersed into 5 ml of the 0.1M TEPA solution. This procedure is carried out for three times, collecting all the recovered supernatants into the same 20 ml graduated flask. Finally, the content of the graduated flask is brought to volume with 0.1M TEPA solution, and the absorbance at 250 nm of the H:TEPA⁺DNB⁻ species is registered by UV visible spectrophotometry, after dilution with methanol (ACN:MeOH 1:2). The quantitation of the released H:TEPA⁺DNB⁻ salt is then performed by referring the recorded absorbance to the relevant calibration curve obtained from a standard solution of TEPA in ACN, that was used for the preparation of six diluted solutions with concentrations ranging from 8.3×10^{-5} M to 8.3×10^{-6} M, respecting the solvent ratio ACN/MeOH 1:2 (Figure S1 of SI).

2.1.9 Quantification of amino-group densities within the AD-CS_I and AD-CS₅ silicas by NHD-M method.

A 0.35% (w/v) ninhydrin solution in absolute ethanol was freshly prepared. A variable amount (in the range 6-30 mg) of dried amino-functionalized AD-CS₁ or AD-CS₅ silica samples is dispersed in 10 ml of ninhydrin solution in a capped vial, placed in an ultrasonicator bath for 30 min. Next, the vial is transferred in a water bath at 65 °C for 2 hours under constant stirring, in order to guarantee a complete reaction between ninhydrin and amino-groups of the AD-CS_{ni} silicas (see Figure S4 of SM for the change suffered over time by the UV-visible spectra recorded for the supernatant of the dispersion with AD-CS₁ as function of time). After cooling to room temperature, the colored supernatant is recovered from the dispersion by filtration and then analysed by UV-visible spectrophotometry to quantify the density of amino-groups reacted with ninhydrin, making reference to a proper calibration curve, obtained from four solutions of hexylamine (concentration range 0.19 ÷ 0.76 mM), prepared by diluting a standard solution of hexylamine 7.6 mM in ethanol with appropriate amounts of the 0.35% (w/v) ninhydrin solution. The standard solutions were placed in a water bath at 65 °C for 2 hours before the UV-visible spectra registration at 585 nm for each sample.

3. RESULTS AND DISCUSSION

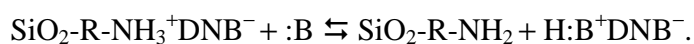
As stressed in the “Introduction” section, the aim of the present study was to propose a new procedure able to allow an accurate quantification of the basic sites chemically bound on the surface of solid porous matrices that, with respect to the methods already available in the field, was simpler, faster and non-destructive of the analysed sample. Overall, the conceived approach consists in a back-titration protocol to be carried out in three-steps (Scheme 1).



Scheme 1. Flowchart of the procedure adopted to quantify amino-groups linked to silica.

In the first step, the free amino groups of $\text{SiO}_2\text{-R-NH}_2$ are submitted to acid-base reaction by suspending the powder in an aqueous solution of an organic acid with appropriate acidic strength and absorbance in the near ultraviolet range. We selected the DNBA acid ($\text{pK}_a=2.7$, for the related UV spectrum see Figure 1), so that hereafter our method will be referenced using the acronym DNBA-M. Indeed, DNBA has been previously used to generate charge transfer complexes with a variety of aromatic amines, for which some parameters as stoichiometry, formation constant and molar extinction coefficient were effectively determined by an UV-spectrophotometric approach. [51-55]

In the second step, the anion DNB^- (i.e. the conjugate base of DNBA) of the generated amino-silica salt (hereafter generically symbolized as $\text{SiO}_2\text{-R-NH}_3^+\text{DNB}^-$) is displaced from the solid according two possible options: $\text{SiO}_2\text{-R-NH}_3^+\text{DNB}^-$ can be dispersed in 1) a water solution of HCl, which, being much more acidic than DNBA, causes the release of DNBA from the amino-silica salt in its un-ionized form or 2) in an acetonitrile solution containing a non UV-absorbing base (:B), able to quantitatively deprotonate the $\text{SiO}_2\text{-R-NH}_3^+$ cation, according to the following double exchange reaction:



As the base :B, we chose tetraethylenpentamine, TEPA ($\text{pKa}_1 = 9.68$; $\text{pKa}_2 = 9.10$; $\text{pKa}_3 = 8.08$; $\text{pKa}_4 = 4.72$; $\text{pKa}_5 = 2.98$).

Thus, in the case of treatment with acid, a supernatant containing the UV-absorbing DNBA species is recovered, while, in the case of treatment with base, a supernatant with the UV-absorbing anion DNB^- is obtained. Finally, in the third step, the amount of DNBA or DNB^- species is quantified by UV spectrophotometric measurements, using an appropriate calibration curve. The ratios millimoles of DNBA or DNB^- / related mass of amino-silica from which they were displaced, provide the desired amino-group densities, symbolized from now on as $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ or $^{\text{TEPA}}\text{AG-density}^{\text{DNBA}}$, depending on whether the DNBA-displacement was performed with HCl or with TEPA, respectively. In Figure 1 the UV spectra of DNBA in its neuter and ionised form are reported. The spectra show the absorbance peaks at 229 nm for DNBA and at 250 for the DNB^- , which has been used to obtain the respective calibration curves (Figure S1, of SM).

In addition to the spectrophotometric approach, an alternative quantification of the DNBA acid or of its conjugate base DNB^- has been obtained by reverse phase chromatography (in-depth details about are given in SM).

The DNBA acid (that, in the case of the TEPA treatment is obtained by subsequent protonation of the DNB^- anion with addition of HCl) has been effectively isolated from the supernatant (examples of chromatograms are reported in Figure S2 of SM). Its dosage can then be obtained through measurement of the area subtended by the related peak, referring to a proper calibration curve, (Figure S3 and Table S1 of SM). However, for reasons of greater practicality, all the AG-density^{DNBA} density data reported and discussed in the text have been obtained by the spectrophotometric approach. It is important to notice that, concomitant with the step of DNBA or DNB^- release, the starting amino-silica material is restored in its $\text{SiO}_2\text{-R-NH}_3^+$ cationic or $\text{SiO}_2\text{-R-NH}_2$ neuter form, which, in turn, can be advantageously reused. In particular, thanks to the very mild conditions involved, the final back-titration step of the amino-silica salt promoted by TEPA base in a non-aqueous solvent could be advantageously exploited for quantitative determinations of basic sites bonded on stationary phases already packed in chromatographic columns. This should make possible to establish direct quantitative relations between the basic activity of these sites and chromatographic behaviours specifically related to their presence. For the development of the method, seven batch-samples of silica (denoted CS_{ni} , with ni ranging from 1 to 7), three of which different in particle size, pore size and surface area (details about their characteristics are reported in Table 1), have been amino-derivatized, and hereafter symbolized with the abbreviation AD- CS_{ni} .

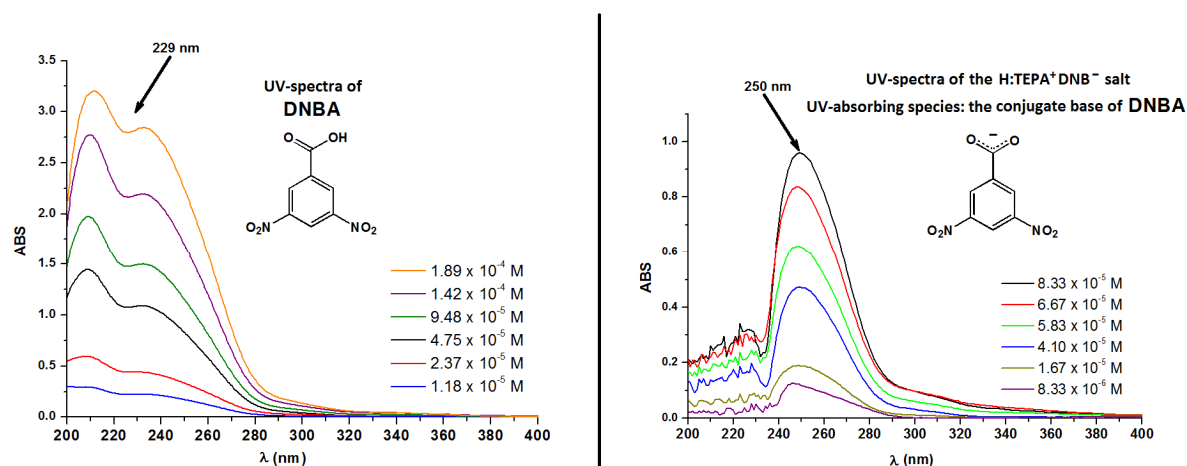
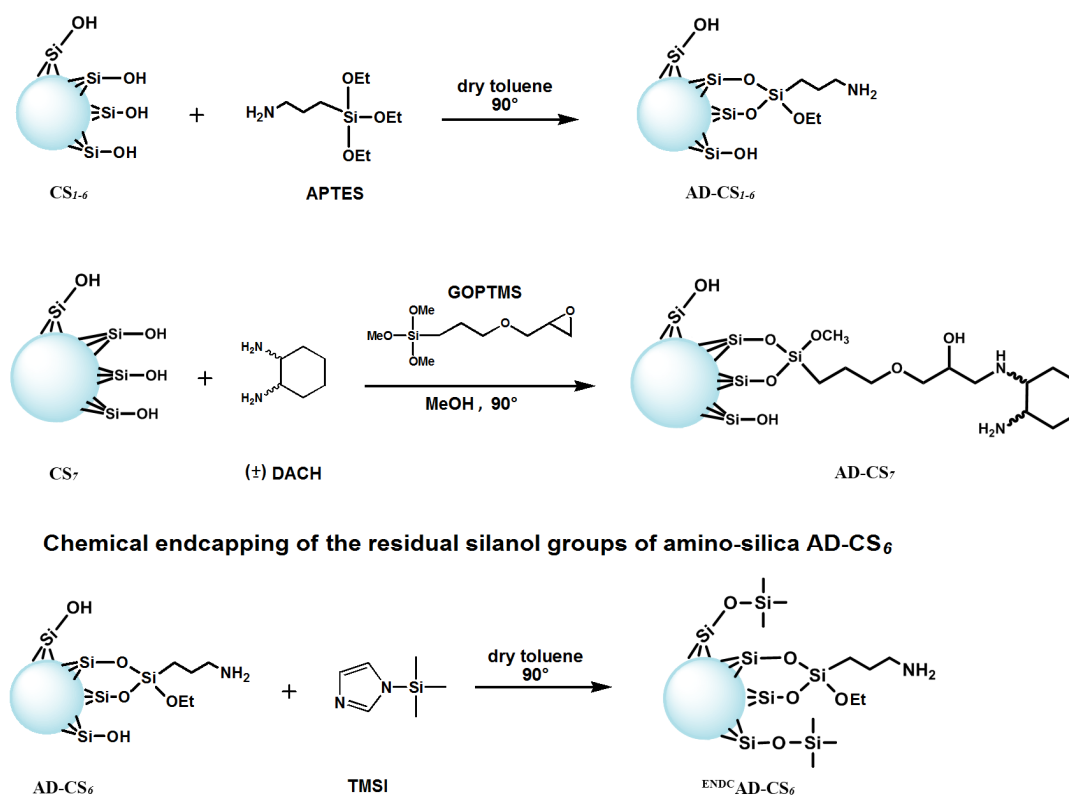


Figure 1. UV spectra of DNBA in its both neuter (left side) and ionised form (right side), dissolved within an HCl aqueous solution and TEPA ACN/MeOH 1:2 solution, respectively. The wavelengths selected for the preparation of the calibration curves used for the UV-quantitation of DNBA and of its conjugated base DNB^- were 229 nm and 250 nm, respectively.

In details, samples derivatized from CS_7 to CS_6 silica were obtained by reaction with APTMS, while the sample AD- CS_7 has been prepared in one step through reaction between silica-powder, (\pm)-cyclohexane-1,2-diamine and glycidoxypropyltrimethoxysilane, this latter used as anchor and spacer of the amino-selector on the silica surface (Scheme 2, see also experimental section).



Scheme 2. Schematic representation of amino derivatization of CS_{ni} silica samples. First and second line: preparation of AD-CS₁₋₆ amino-propyl silica and AD-CS₇ cyclohexyl-1,2-diamine silica, respectively. Third line: end-capping procedure of residual silanols of AD-CS₆, which supplies the ^{ENDC}AD-CS₆ silica.

Each amino-derivatized AD-CS_{ni} silica has been effectively reacted with acid by suspending it in a water solution of DNBA (namely, AD-CS_{ni}⁺DNB⁻ in the text). The differences, visible by comparing the FTIR spectra of silicas recorded before and after their reaction with DNBA, confirmed the success of the salt formation. Indeed, by inspection of the spectra, reported in Figures 2 and S5 of SM, the appearance of absorbing bands strictly close to those characteristic of asymmetric (1635 cm⁻¹) and symmetric (1385 cm⁻¹) stretching bonds of the carboxylate group,

as well as of the asymmetric (1547 cm^{-1}) and symmetric (1356 cm^{-1}) stretching bonds of the nitro moieties can be observed. In addition, FTIR spectra of the silica samples recovered after treatment of $\text{AD-CS}_{ni}^{+}\text{DNB}^{-}$ with HCl solution (see next subsection for discussion) have also confirmed the effective release of DNBA (the exemplificative case of silica CS_I is reported in Figure 2), witnessed by the disappearance of all the absorbing bands typical of the $\text{AD-CS}_{ni}^{+}\text{DNB}^{-}$ salts.

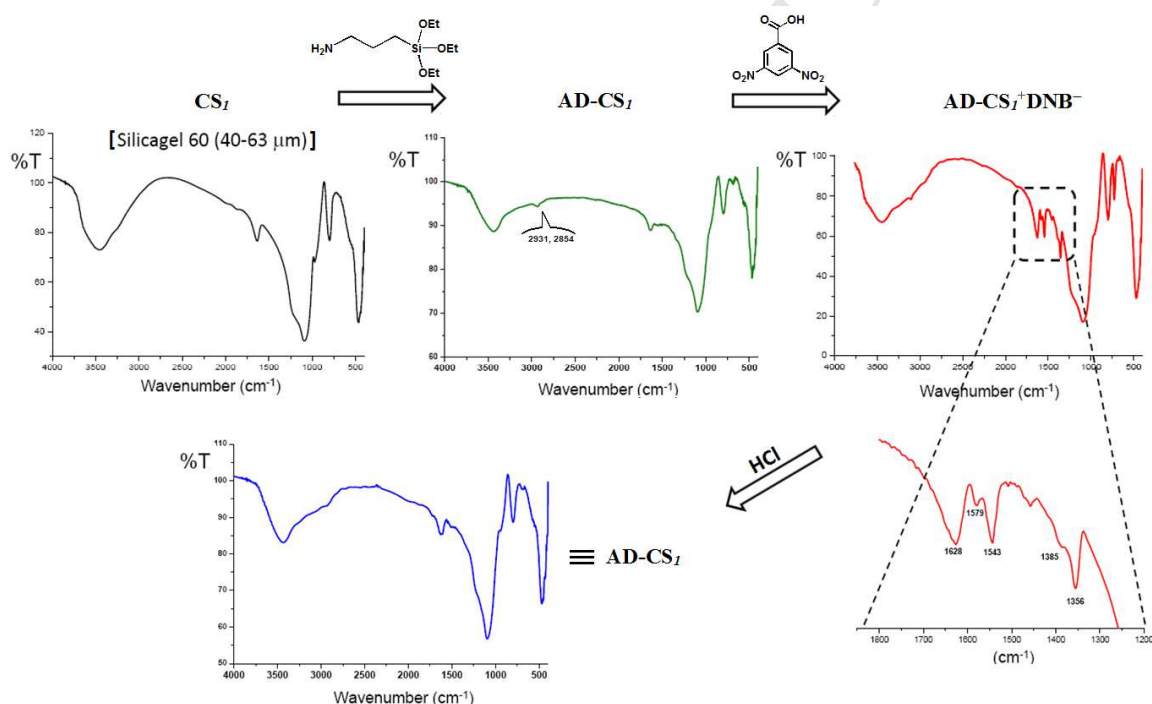


Figure 2. FTIR spectra monitoring the progressive structural modification underwent by the silica sample CS_I (selected as case-example): i) covalent aminopropyl-derivatization to form AD-CS_I ; ii) reaction of AD-CS_I with DNBA to form $\text{AD-CS}_{ni}^{+}\text{DNB}^{-}$; iii) displacement of DNBA from $\text{AD-CS}_{ni}^{+}\text{DNB}^{-}$ by dispersion in HCl solution and regeneration of AD-CS_I .

3.1 Displacement of DNBA from AD-CS_{ni}⁺DNB⁻ silicas by dispersion within an aqueous solution of HCl.

By adopting, as final step of the whole procedure, the DNBA displacement with HCl, all the AD-CS_{ni} samples have been submitted to estimation of the pertinent amount of amino-groups, AGs, bonded on 1 gram of derivatized silica (i.e. a density expressed as mmol-AGs / g-AD-CS_{ni}). In order to confer statistical significance to the performed assessments, for each AD-CS_{ni} sample the amino-groups density determination was repeated at least three time, (seventeen measurements in the case of silica AD-CS₁). The obtained quantitative results are collected in Table 2.

Table 2. Collection of single AG-density data measured by means of the EA-M, DNBA-M and NHD-M methods on the AD-CS₁₋₇ silica samples and of the related statistical quantities.

AGD = ^{HCl} AG-density ^{DNBA} , AG-density ^{EA} or AG-density ^{NHD} (mmol/g)																	
	AD-CS ₁		AD-CS ₂		AD-CS ₃		AD-CS ₄		AD-CS ₅		AD-CS ₆		AD-CS ₇		ENDC AD-CS ₆		
	mg	AGD	mg	AGD	mg	AGD	mg	AGD	mg	AGD	mg	AGD	mg	AGD	mg	AGD	
^{HCl} AG-density ^{DNBA}	1	31.6	0.73	57.6	0.49	69.5	0.37	67.1	0.32	68.4	0.30	24.6	0.34	71.7	0.21	12.6	0.52
	2	19.4	0.75	37.6	0.49	57.8	0.36	67.6	0.30	59.3	0.30	13.2	0.37	51.9	0.23	8.5	0.55
	3	11.0	0.82	20.1	0.48	41.5	0.35	47.6	0.32	40.2	0.34	32.1	0.33	30.8	0.22	6.2	0.69
	4	42.5	0.65			19.0	0.37	29.0	0.32	19.6	0.29			11.9	0.25		
	5	31.6	0.73					9.0	0.33	20.4	0.37						
	6	120.3	0.63					24.5	0.35	21.8	0.35						
	7	100.3	0.63					22.4	0.33								
	8	74.5	0.64					25.0	0.34								
	9	50.5	0.61					27.1	0.33								
	10	26.2	0.65														
	11	9.9	0.64														
	12	59.3	0.55														
	13	99.9	0.51														
	14	19.5	0.55														
	15	49.2	0.73														
	16	37.2	0.76														

	17	20.5	0.77														
Standard deviation			0.09		0.00(3)		0.010		0.01		0.03		0.02		0.02		0.09
Average value of ${}^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$			0.67		0.49		0.36		0.33		0.33		0.35		0.23		0.59
AG-density^{EA}			1.61		1.07		0.67		0.54		0.61		0.74		0.58		0.72
AG-density^{NHD}	1	8.6	0.76							6.3	0.38						
	2	28.0	0.73							13.4	0.49						
Average value of AG-density^{NHD}			0.75								0.44						

From the ${}^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ values measured for the AD-CS₁ sample (i.e. the one analysed with the greater number of determinations), it can be noted that they are dispersed according to a gaussian distribution ($R^2=0.9852$, Figure 3), with a standard deviation, σ , of 0.09, that corresponds to a maximum uncertainty of the measure amounting to $\pm 13\%$ of the average value (i.e., of the best estimation obtained for ${}^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$, Figure 3).

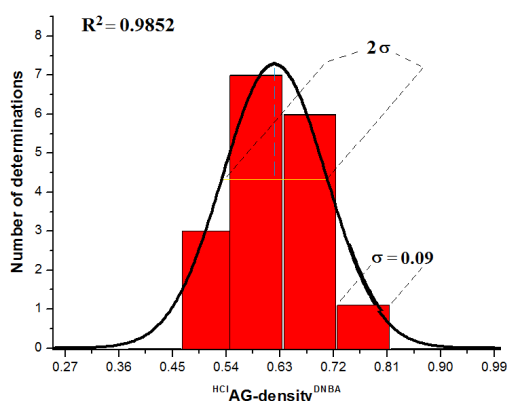


Figure 3 Gaussian distribution of the seventeen ${}^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ values measured for the AD-CS₁ sample, clustered within intervals corresponding to their calculated standard deviation, $\sigma = 0.09$.

Thus, it can be assumed that, in first approximation, this value also represents the upper limit of uncertainty featuring the measurements of AG-density performed through our DNBA-M approach. Afterwards, the necessary new step of the study was focused on the validation of the method. This was pursued by comparing the $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ values, obtained employing the DNBA-M method on all the AD-CS_{ni} samples, with those determined by using another independent procedure. The choice fell on the EA-M method, which is commonly appreciated for the known good quality of the results it is able to provide in the field. Therefore, all the AD-CS_{ni} samples were also submitted to EA-M determinations, and the obtained AG-density results, symbolized in this case as AG-density^{EA} (values collected in Table 2), have been plotted versus the correspondent $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ data. The resulting plot (see Figure 4) evidenced a very good linear correlation when the regression analysis was limited to the silica samples based on the same typology of amino-selector (i.e. the AD-CS₁₋₆ silicas):

$$\text{AG-density}^{\text{EA}} = 2.9731 \times ^{\text{HCl}}\text{AG-density}^{\text{DNBA}} - 0.3718 \quad (1) \quad (R^2 = 0.9883)$$

The correlation quality underwent, instead, a modest decrease if also the silica derivatized with 1,2-diaminocyclohexane is included within the analysis ($R^2 = 0.9372$). This difference may be reasonably justified as due to a dissimilar freedom of movement and steric hindrance that the amino molecular framework of AD-CS₇ is able to manifest in establishing interactions with silanols when compared to the more flexible aminopropyl arm of the AD-CS₁₋₆ silicas.

Nevertheless, with respect to the AG-density^{EA} values, the $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ data resulted systematically underestimated by an average factor of 2.0 ± 0.3 (2.1 ± 0.3 when also AD-CS₇ is included within the calculation), suggesting that the DNBA-M approach is able to detect just a partial quote of the whole AGs bonded to silica. This is not an unexpected result, in fact, our DNBA-M method, for the principle on which it is based, can only allow the dosage of amino-

groups with lone pairs free by any possible chemical interaction with residual $\equiv\text{Si-OH}$ groups of the matrix, while EA-M is not affected by this limit, since it merely takes into account the nitrogen percentage characterizing the sample. To obtain a direct confirmation of the correctness of such an interpretation, we performed an experiment expressly designed for this purpose.

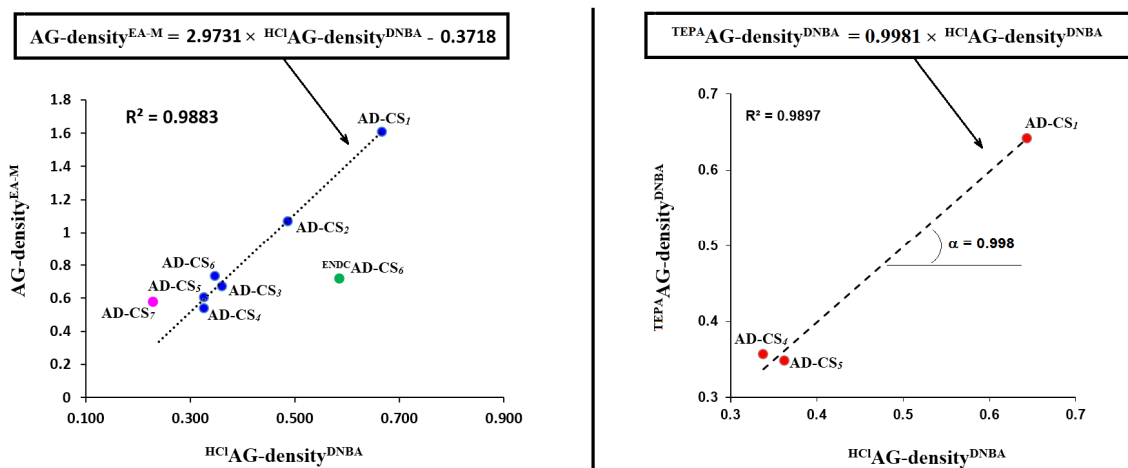


Figure 4. Left side: linear correlation found between $\text{HClAG-density}^{\text{DNBA}}$ and $\text{AG-density}^{\text{EA-M}}$ density values determined for the AD-CS₁₋₆ silica samples (see Table 1). Magenta and green full points (not included in correlation) are relevant to the AD-CS₇ and ENDC-AD-CS_6 silicas, respectively,. Right side: plot of the correspondence existing between $\text{HClAG-density}^{\text{DNBA}}$ and $\text{TEPAAG-density}^{\text{DNBA}}$ values.

A proper amount of AD-CS₆ silica has been submitted to chemical endcapping of its residual silanol groups by reaction with 1-(trimethylsilyl)-1H-imidazole (TMSI), giving rise to the derivatized silica sample (ENDC-AD-CS_6). It was expected that, compared to the case of AD-CS₆, the acid-base reaction of ENDC-AD-CS_6 silica with DNBA (i.e. the $\text{ENDC-AD-CS}_6^+\text{DNB}^-$ solid salt) could involve a much larger number of amino-groups, because free by interaction with

endcapped $\equiv\text{Si-OH}$ groups. This was exactly what we found when samples of $^{\text{ENDC}}\text{AD-CS}_6^+\text{DNB}^-$ have been submitted to $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$ dosage. In particular, with respect to what found for AD-CS_6 , the increase of $^{\text{HCl}}\text{AG-density}^{\text{DNBA}}$, due to the performed silanol-endcapping, was of 78% (see about Figure 4). Nevertheless, the absolute amount of AG detected for $^{\text{ENDC}}\text{AD-CS}_6$ through the EA-M method was still 19% superior to the one obtained by means of the DNBA-M approach, indicating that a little amount of silanols still lie unreacted on the surface of $^{\text{ENDC}}\text{AD-CS}_6$. To achieve an independent and definitive confirmation about the correctness of our analysis, two samples have been selected (the AD-CS_1 and AD-CS_5 silicas) among the seven considered AD-CS_{ni} samples and submitted to AG-density measurement by means of the standard NHD-M ninhydrin method. The obtained $\text{AG-density}^{\text{NHD}}$ values have been collected in Table 2. The reason that prompted us to perform this additional test is that, similar to what already stressed about the DNBA-M procedure, also the approach based on ninhydrin should be able to dose only AGs endowed with lone pairs free by chemical interaction with silanol sites, since these are essential to allow the nucleophilic attack on ninhydrin required by the reaction. The obtained $\text{AG-density}^{\text{NHD}}$ values have then been compared with those obtained by means of both the EA-M and DNBA-M methods, (see Table 2). As expected, such a comparison clearly indicated that, analogously to the results afforded by the DNBA-M measurements, also the AG-density values obtained by the NHD-M procedure are significant lesser than those coming from EA-M determinations, in this case by an average factor of 1.8 ± 0.4 . This confirms that also the procedure based on ninhydrin cannot afford AG-density values inclusive of nitrogen atoms strongly interacting with $\equiv\text{Si-OH}$ groups.

Therefore, all data support us to affirm that, in the case of solids of siliceous nature, the proposed DNBA-M method is able to provide, in a direct way, affordable quantitative information about the density with which amino-groups, still active in their capacity to express basic/nucleophilic

properties, are distributed on the surface of the silica matrix. At the same time, by resorting to the very good linear correlation found between AG-density^{EA-M} and ^{HCl}AG-density^{DNBA} data, expressed by equation (1), the DNBA-M method can also provide reliable information (within 11% of error) on the absolute AG-density of the analysed samples endowed with aminopropyl selector, just making reference to their already determined ^{HCl}AG-density^{DNBA} amounts.

3.2 Displacement of H:TEPA⁺DNB⁻ from AD-CS_{ni}⁺DNB⁻ silicas by dispersion within an acetonitrile solution of TEPA.

Once established the validity of the proposed DNBA-M method and considering the substantial conservative effect towards the analysed samples that the procedure demonstrated, we focused the attention on the possibility to further improve the already mild operative conditions characterizing the approach. In fact, although the final stage of the proposed procedure requires short times of contact of the AD-CS_{ni}⁺DNB⁻ samples with the strong acid HCl for the displacement of DNBA, this condition could not be acceptable in some possible extensions of application of the method. Thus, as a practical example, a suitable reduction of the potential chemical aggressiveness attributable to the terminal step of the method could allow its use for gaining direct information about the amounts of basic sites distributed on the surface of stationary phases (SPs) already packed in chromatographic columns. In fact, it is well known that these sites may be responsible for a wide variability of effects on both retention times and selectivity concerning selectands submitted to chromatographic resolution. For this purpose, it has been verified the possibility to quantitatively promote the displacement of the DNB⁻ anion from the AD-CS_{ni}⁺DNB⁻ powders by dispersing them in an acetonitrile solution of TEPA. This non-aggressive operative condition would avoid any possible degradation reaction of the silica

matrix due to hydrolysis that, instead, could be triggered out in the aqueous and strongly acidic environment required by the DNBA-displacement promoted by HCl. The test was performed on a subset of three AD-CS_{ni} samples, the amino-silicas AD-CS₁, AD-CS₄ and AD-CS₅. Details about the step of DNB⁻ displacement from the AD-CS_{ni}⁺DNB⁻ samples promoted with TEPA, the UV-absorbance measurements of the recovered supernatant solutions containing the H:TEPA⁺DNB⁻ salt and the construction of the used calibration curve (Figure S1 of SM), needed for the final AG-density quantitation, are reported in Experimental Section. The obtained results confirm that full coherence between ^{HCl}AG-density^{DNBA} and ^{TEPA}AG-density^{DNBA} values exist, when carried out starting from a same batch of AD-CS_{ni}⁺DNB⁻ salt for each of the three analysed AD-CS_{ni} silica. More specifically, the percentage differences found between the determined ^{HCl}AG-density^{DNBA} and ^{TEPA}AG-density^{DNBA} values is very little (0.6 % for AD-CS₁, 5.7 % for AD-CS₄ and 3.8 % for AD-CS₅, Table 3 and Figure 4).

Table 3. AG-density values (determined starting from a same batch of AD-CS_{ni}⁺DNB⁻ salt) obtained by displacement of the DNBA acid with both HCl and TEPA treatments.

AG-density values determined through DNBA-M procedure (mmol/g)						
	AD-CS ₁		AD-CS ₄		AD-CS ₅	
	mg	^{HCl} AG-density ^{DNBA}	mg	^{HCl} AG-density ^{DNBA}	mg	^{HCl} AG-density ^{DNBA}
1	26.2	0.65	24.5	0.35	20.4	0.37
2	9.9	0.64	22.4	0.33	21.8	0.35
Average value of ^{HCl}AG-density^{DNBA} (mmol/g)	0.64		0.34		0.36	
	mg	^{TEPA} AG-density ^{DNBA}	mg	^{TEPA} AG-density ^{DNBA}	mg	^{TEPA} AG-density ^{DNBA}
1	14.2	0.64	21.0	0.35	21.0	0.34

2	11.9	0.64	20.8	0.35	22.9	0.33
3	25.2	0.64	19.7	0.37	21.1	0.34
4					21.7	0.38
Average value of TEPA AG-density^{DNBA} (mmol/g)	0.64		0.36		0.35	

4. CONCLUSION

The aim of this work was the development of an original and non-destructive analytical procedure (the DNBA-M method) to quantify the density of basic sites chemically bonded to the surface of solid porous matrices (AG-density values). The study was performed on amino-silica matrices specifically synthesized at the purpose, but it is expected the approach can be employed to analyze any other typology of solid matrices containing basic-sites. The goal was achieved by splitting the overall procedure in three steps:

- i)* an initial, quantitative acid-base reaction of the basic sites of the matrix with the UV-absorbing acid DNBA;
- ii)* the displacement of DNBA from the solid salt generated in the previous step by dispersing it in an acid or basic solution;
- iii)* the final titration (performed by UV-absorbance measurement) of the DNBA displaced in its neuter or deprotonated form, contained in the supernatant recovered in the second step of the procedure. As a possible alternative, the final titration could also be performed by integration of the area subtended by the chromatographic peak of the displaced DNBA, isolated by HPLC in its protonated form.

The uncertainty of the density measurements has been assessed by 13%. In comparison with the other analytical approaches commonly employed at the same purpose, the proposed method introduces several advantages that can be summarized in the following points:

- 1) all the steps of the procedure are operationally simple and fast to be performed, with a modest instrumental requirement, consisting in the availability of a common UV-visible spectrophotometer;
- 2) in order to prevent/avoid the trigger of any possible degradation reaction of the regenerated solid matrix, in the third stage of the procedure the choice of the acid or basic character of the solution used for the displacement of DNBA can be rationally based on the particular chemical nature characterizing the structure of the analyzed sample;
- 3) each sample submitted to the determination is completely recovered at the end of the procedure, and therefore available to be reused, without limitations;
- 4) in the case of analyses performed on amino-silica matrices, each determination carried out through the DNBA-M method can afford two kind of AG-density values: the amount of amino-functionalities with free lone pairs, and therefore able to express the basic/nucleophilic activity typical of these sites, and the amino-groups chemically quiescent, because already involved in acid-base reaction with silanol groups of the matrix. With respect to determinations based on elemental analysis, the total amount of amino groups, resulting from the sum of the above two typologies, can be estimated with an error of no more than 11%. In the present study, the found amount of amino-groups endowed with lone pairs “locked” by acid-base reaction with close silanols achieve the average value of about 51% in the case of the aminopropil-silica samples, while the value of about 40% in the case of the cyclohexanediamine-silica sample. AD-CS₇;

5) according to the principle on which DNBA-M is based, the applicability of the method is not precluded for the evaluation of AG-density values related to tertiary amino groups, as happens if the chosen approach is that based on the ninhydrin;

6) on the base of what quoted in the above points 3) and 4), the here proposed procedure can also be considered convenient under an economic point of view. Indeed, it allows to characterize solid matrices without any recourse to more specialized, but relatively expensive, external analyses (X-ray-M, CAM-M, AFM-M or EA-M), as well as to make possible a complete recycle of the investigated materials, breaking down any waste.

By adopting the particularly mild conditions required by the DNBA-M method when the final step is carried out with a basic solution of TEPA in acetonitrile, the new procedure could be usefully exploited for a direct characterization of some typology of chromatographic columns, as those conceived for HILIC applications, as well as those that manifest behaviours specifically connectable to the presence of basic-sites distributed on their stationary phase (e.g. the catalytic promotion "on-column" of dynamic equilibria). This will be precisely the topic we aim to address in a related study to be developed in the close future.

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ACKNOWLEDGMENTS

This work was conducted with financial support from Sapienza University of Rome, Italy (DR n 3210/16 of 16/12/2016 and DR n 2936/17 of 20/11/2017).

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at...

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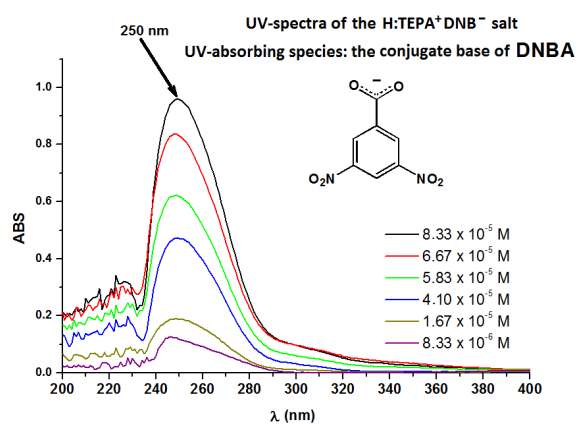
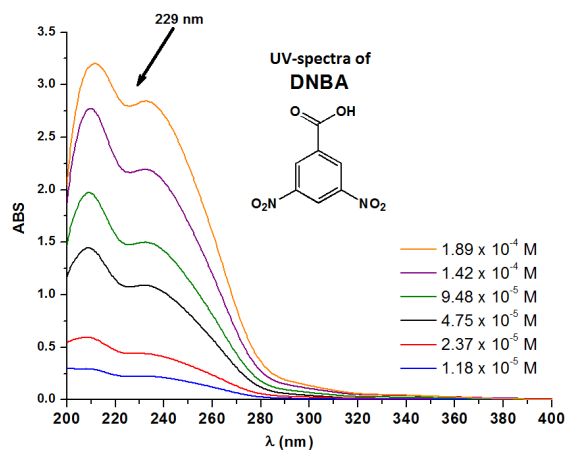
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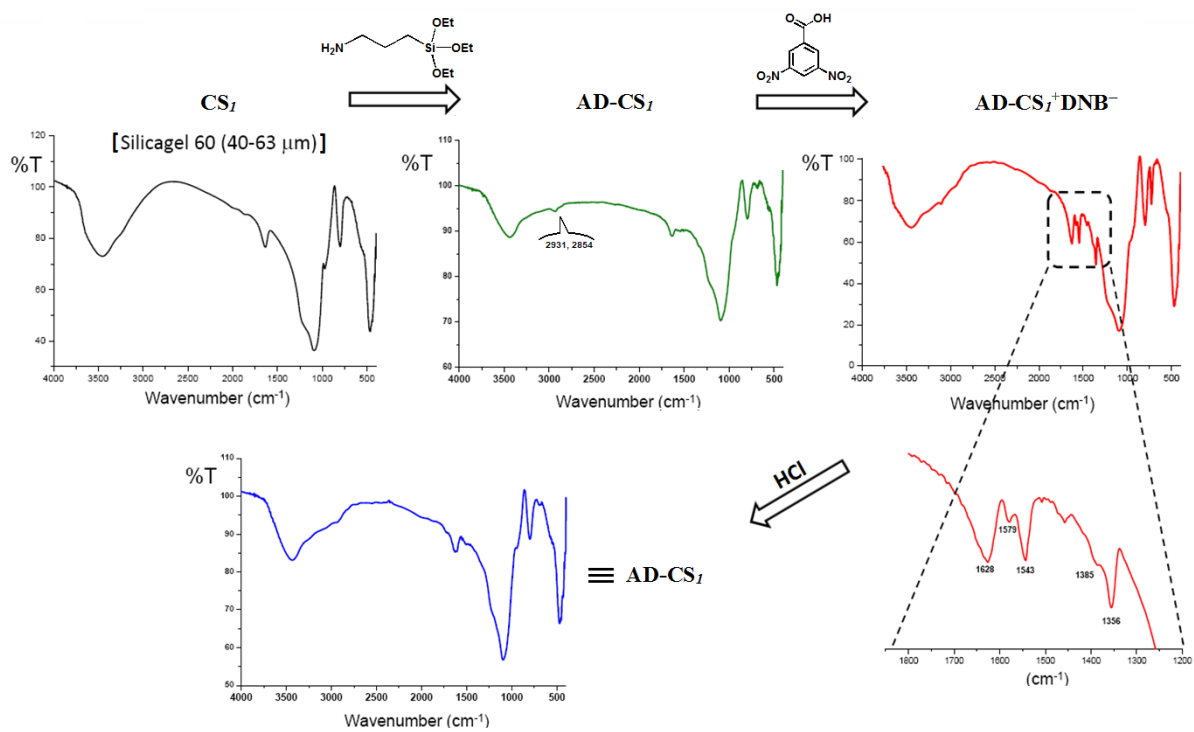
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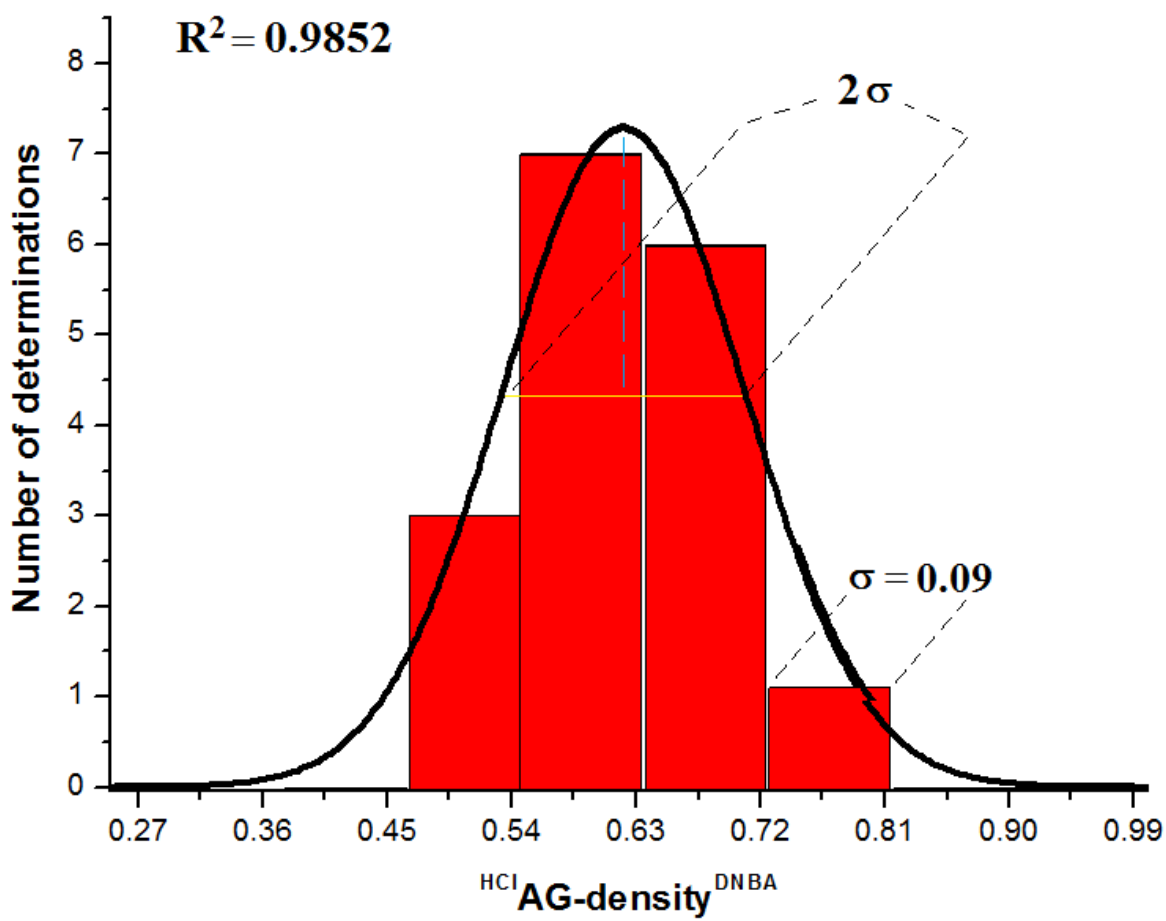
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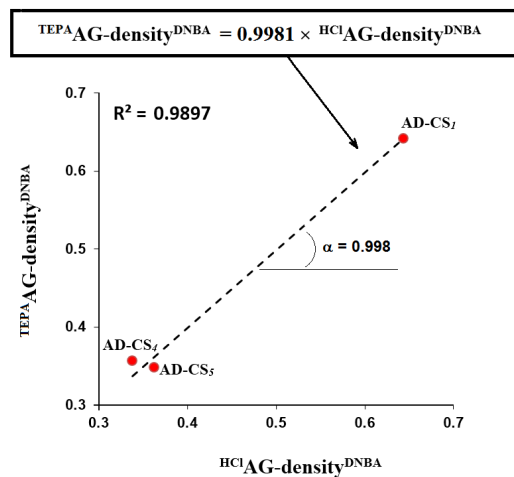
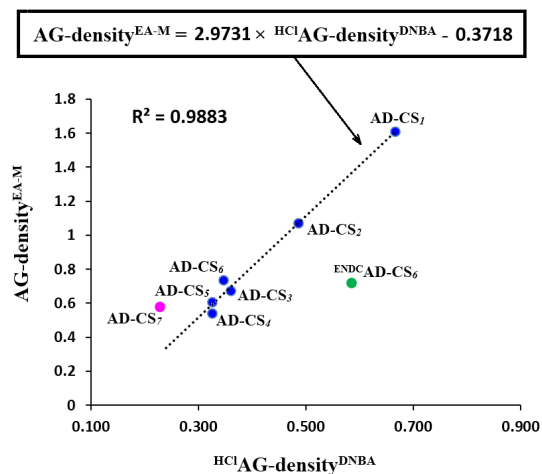




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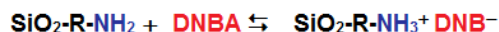


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Procedure for the quantification of amino groups linked to silica

Step I

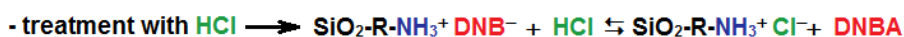
amino-groups bound on silica are reacted with the DNBA acid:



Step II

the conjugate base of DNBA, DNB^- , is displaced from the salified silica according to one of two possible options, A or B:

option A



option B



Step III

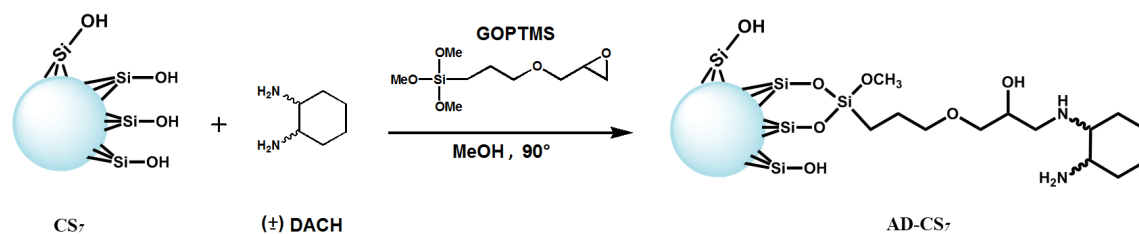
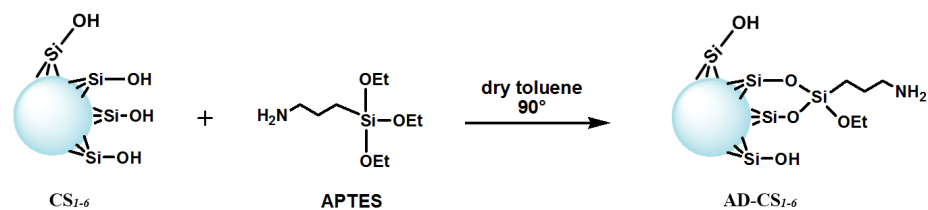
the displaced DNBA acid, in its protonated (Step II, option A) or ionized form (Step II, option B), is finally titrated according to one of two possible approaches, A or B:

approach A

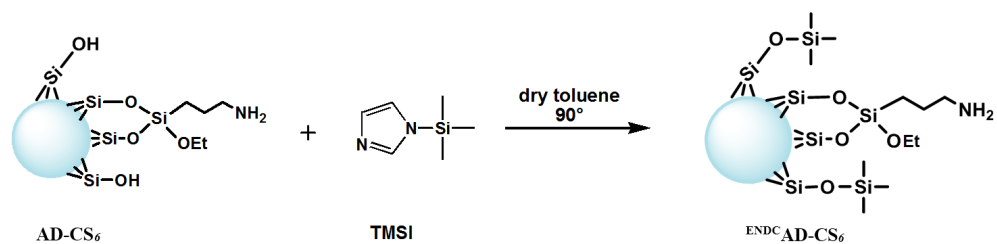
- titration based on spectrophotometric UV-absorbance measurement of DNBA at 229 nm or of DNB^- at 250 nm.

approach B

- HPLC resolution of DNBA (directly obtained in Step II - option A, or formed by addition of HCl to the $\text{H:TEPA}^+ \text{DNB}^-$ salt obtained in Step II - option B), and its subsequent quantification based on the measurement of the related peak-area



Chemical endcapping of the residual silanol groups of amino-silica AD-CS₆



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A new method was developed to quantify the density of basic sites on solid matrices.

The method is simple, fast and not destructive towards the analyzed material.

Amino-groups free or reacted with acid silanols of silica can be discriminated.

The method is not precluded for the quantification of tertiary amino-groups.

The method could be used to quantify basic sites of matrices packed in HPLC columns.

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