Chemical immobilisation of humic acid on silica


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Abstract

Immobilisation of purified Aldrich humic acid (PAHA) on aminopropyl silica and glutaraldehyde-activated aminopropyl silica has been investigated. In general the humic acid is bound to the solid by both physical and chemical bonds. The physically adsorbed HA can be released to a large extent at high pH. To minimise physical adsorption, the samples are thoroughly washed using a 1 M sodium chloride solution of pH 10 and the free amino groups of the aminopropyl silica are end-capped. The amounts of PAHA bound, the charge density–pH curves, isoelectric points (ieps), FT-IR spectra and the stability–pH curves of the products are determined. The amounts of PAHA bound to the aminopropyl silica range from 42 to 72 mg HA (g SiO$_2$)$^{-1}$, depending on the method of preparation. The products have low ieps and are stable in a wide range of pH. The amount of PAHA bound on the glutaraldehyde-activated aminopropyl silica is 16 mg HA (g SiO$_2$)$^{-1}$ and the product has an iep value at pH 7.3, which is rather different from that of naturally occurring HA. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Humic substances are naturally occurring, biogenic, heterogeneous organic materials, that complex strongly with heavy metal ions and organic compounds [1]. In order to study their sorption characteristics, natural humics are isolated, purified and used in laboratory studies. However, these studies are often thwarted by the fact that it is difficult to separate the humics from the solution. A way to avoid this complication is the immobilisation of the humic substance to much larger inorganic or organic particles that can easily be separated from their suspension by sedimentation or centrifugation.

Humic acids (HAs) immobilised on solid particles can also be used as high capacity, selective sorbents for the recovery of trace amounts of metal ions and/or xenobiotic organics from solution. As examples of such applications, the work of Ho and Miller [2], Heitkamp and Wagener [3] and Seki et al. [4] can be mentioned. These authors adsorbed HAs on, respectively, haematite particles, anion exchange resins and alginate gels for the recovery of heavy metal ions. However, in all cases some adsorbed HA released into solutions
at higher pH and made regeneration and re-use difficult. Moreover, the HA released might be highly loaded with the toxin to be removed. Seki and Suzuki [5] also heated Aldrich HA at 330°C to obtain an insoluble humic-like sorbent. However, the humic particles obtained were too small to be packed in columns and/or fluid beds and the small particle size also complicated regeneration. Chen et al. [6] immobilised humic acids on cross-linked polystyrene resins and the products were stable and suited for regeneration, but large-scale production was not feasible.

The group of Szabo and coworkers [7–12] investigated the immobilisation of HAs on silica particles. An advantage of silica particles is that silica also frequently accompanies HAs in natural environments [13,14]. Moreover, silica and its derivatives have been used extensively in chromatography because of their excellent mechanical strength, well-controlled structural parameters (e.g., surface area, pore size and particle shape and size) and chemical stability. If HAs can be immobilised successfully on silica, a wide dissemination of applications may emerge. In most of the investigations by Szabo and coworkers [7–11], HAs were immobilised chemically on aminopropyl silicas or adsorbed directly on aminopropyl silica. Guczi et al. [12] used the phenolic groups of the HA for the chemical immobilisation, but the reactions used to prepare the diazotized silicas were troublesome [15] and the point of zero charge (pzc) of the immobilised-HA silica was high [16]. Such a high pzc is in strong contrast with the pH where HAs and silica have negligible charges. Unfortunately, the HA-silicas made by the chemical methods [7,9,12] released substantial amounts of organic carbons into solution over a wide range of pH values [16]. In spite of the observed drawbacks, Szabo and coworkers [7–12] concluded that the HA-silicas could be useful for the investigation of the binding properties of radio nuclides and organic pollutants to soil and sediment.

The aim of the present paper is to investigate a number of ways to immobilise HA on silica and to discover methods that lead to stable HA-silicas with low iso-electric points (i.e., in the design of the immobilisation methods, the use of carboxylic groups, that are abundant in humic substances [17], is most natural. In this paper, we investigate methods that make use of these carboxylic groups. In addition, the glutaraldehyde method and the physical adsorption method, used by Szabo and coworkers [9,11], are investigated.

2. Materials

HA in its sodium form was purchased from Aldrich (code: H1, 675-2). To purify the sample, 10 g of the HA was added to 1 l of NaOH solution with pH 11, stirred overnight and centrifuged to remove undissolved matter. To precipitate the HA fraction, the supernatant solution was brought to pH 2 with 1 M aqueous HCl, stirred for 24 h and centrifuged at 10 000 rev min⁻¹ for 30 min using a Beckman JA-20 centrifuge. The precipitate was rinsed with 0.01 M HCl several times to bring the HA in its proton form and to remove soluble fractions and inorganic salts. The HA obtained was freeze-dried for further use. Elemental analysis showed that the protonated Purified Aldrich HA (PAHA) contained C 55.08 %, H 4.25 % and N 0.72 %.

Non-porous Aerosil OX50 silica (specific surface area: 52 m² g⁻¹) was stirred in 0.4 % aqueous HF overnight to activate the surface and remove the impurities. Then the silica was washed thoroughly with water and transferred to a vacuum box where it was heated at 180°C under reduced pressure for 24 h and finally allowed to cool under reduced pressure for prompt use.

Toluene and N,N-dimethylformamide (DMF) were stored on molecular sieves (4 Å) to assure complete dryness. 3-Aminopropyltriethoxysilane (APTS), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), acetic anhydride and other reagents were analytical grade and used directly without further treatment.

3. Preparation methods

3.1. Aminopropyl silica

For the preparation of aminopropyl silica, according to the procedure of Vrancken and...
coworkers [18–20]. 20 g of the pre-treated silica was stirred at room temperature for 2 h with 2.1 1% v/v APTS/toluene solution, under a CaCl2 guard tube. The modified silica was centrifuged and transferred to a vacuum box where curing was performed for 20 h under reduced pressure at 150 °C. The final product will be indicated as APTS-SiO2.

3.2. Immobilisation of PAHA on the aminopropyl silica

3.2.1. Direct adsorption and curing at 120 °C

A 30 ml aqueous solution of 300 mg PAHA was prepared by slowly adding 0.1 M freshly prepared NaOH solution to the PAHA dispersed in water. Following Szabo and coworkers [9,11], the solution was added to a vessel containing 1.00 g of the cured aminopropyl silica and the volume of the system was adjusted to 50 ml and pH 7.5–8. The system was stirred for 20 h at room temperature. Before drying the sample was rinsed several times, first using 0.2 M NaCl (pH 7.5) and then with water of the same pH. The separation from the solution was achieved by centrifugation. In order to “stabilise” the adsorbed HA-silica, the dry product was cured at 120 °C for 5 h. After curing, the product was rinsed several times with 1 M NaCl (pH 10) to remove loosely bound PAHA and with 0.001 M HCl to protonate the HA. The product was dried and the free amino groups were end-capped according to the procedure described below. This product is denoted HA-SiO2(ADS-T).

3.2.2. Incubation with EDC at room temperature

In 30 ml of deionized water, 300 mg of PAHA was dissolved slowly by adding freshly prepared 0.1 M NaOH. The solution was added to a vessel which contained 1.00 g of the cured aminopropyl silica and the volume of the system was adjusted to 50 ml. Then 75 mg of EDC was added and the suspension stirred at room temperature for 2 h. Another 25 mg of EDC was added and the suspension was stirred at room temperature for 18 h. The pH of the suspension was about 10. Before drying, the sample was rinsed with 1 M NaCl (pH 10) and 0.001 M HCl. The steps for end-capping the free amino groups are described below. The final product is called HA-SiO2(EDC).

3.2.3. Immobilisation in DMF at 120 °C

In 30 ml of DMF, 300 mg of PAHA was suspended and the suspension was added to a vessel that contained 1.00 g of the cured aminopropyl silica. Another 20 ml of DMF was then added and the suspension was stirred and heated for 20 h at 120 °C to immobilise the HA. Then the HA-silica was rinsed repeatedly with DMF and centrifuged until the supernatant became colourless. End-capping of the free amino groups was done as described below. The final product is called HA-SiO2(DMF-T).

3.2.4. End-capping of the free amino groups

In order to end-cap the free amino groups and to obtain a stable product, the dry HA-silica was suspended in 50 ml of DMF that contained 5% acetic anhydride and was stirred for 8 h at room temperature. After the reaction, the product was centrifuged and rinsed successively with DMF, acetone, water of pH 10 and pure water to remove the released HA. Finally, the product was dried.

3.2.5. Glutaraldehyde method

The method of Szabo and coworkers was also followed [9,11]. To activate the cured aminopropyl silica, 1.00 g of the solid was added to 20 ml of 5% glutaraldehyde solution. The activation procedure lasted for 5 h and then the red product was washed thoroughly with water. A 50 ml solution of 300 mg HA (pH 7) was freshly prepared and added to a vessel which contained the glutaraldehyde-activated aminopropyl silica. The pH of the suspension was ca. 7. The suspension was stirred at room temperature for 8 h to immobilise the HA. The excess aldehyde groups on the surface were end-capped using 0.1 M ethanolamine–HCl buffer of pH 9. The precipitate was rinsed repeatedly and centrifuged using a solution of 1 M NaCl at pH 10 and finally rinsed with water until the supernatant was colourless. The final product was dried and is denoted HA-SiO2(GA).
4. Physical measurements

4.1. Bound amount of HA

The amount of PAHA bound to the silica has been determined for each of the procedures directly after the preparation, after washing with the salt and after end-capping. For the determination, 1.0 ml of the total collected supernatant after each step was diluted (up to a known volume) to sufficiently low concentrations for the measurement of the absorbance at 254 nm on an Hitachi U-3210 spectrophotometer. The residual concentration of the HA could be obtained from the corresponding (same pH) linear calibration curve between the PAHA concentration and the absorbance. The calculated amount of free HA was subtracted from the amount initially present to obtain the amount of HA bound.

4.2. Iep determination

Ieps of intermediate and final products were determined by measuring the electrophoretic mobilities of the samples at pH values around the ieps. Before measurement, samples were placed in an ultrasonic bath for 30 s to ensure complete dispersion. Measurements were made in 0.01 M KCl using a Malvern Zetasizer 3. Mobilities were converted into zeta potentials using the Smoluchovski equation [21].

4.3. Potentiometric proton titration

Charge density–pH curves of the final products were determined at 25°C by potentiometric titration using an automatic titrator (Schott). For the measurements, an accurately weighed sample (ca. 0.8 g) was dispersed in 50 ml of 0.01 M KCl solution. Before the titration was started the suspension was titrated up to pH 3 and purged with argon for at least 2 h. Titrations were carried out from pH 3 to pH 11 and from pH 11 to pH 3, using 0.1 M KOH and 0.1 M HCl. To be able to calculate the charge densities, blank titrations, i.e. electrolyte titrations in the absence of the sample, were performed. Blank results were checked by comparing them with theoretical calculations.

Upon good agreement, a theoretical blank, with exactly the same volume and salt concentration as the suspension titration, can be calculated. This theoretical blank was subtracted from the titration data in the presence of the sample to calculate relative charge densities (milliequivalents per gram of SiO$_2$). The iep values were used to transform the relative charge densities into absolute charge densities by assuming that the pH of the iep corresponds with that of the pzc.

4.4. FT-IR analysis

The HA-silicas made by the different methods were rinsed with 0.001 M HCl several times to transform them into their protonated forms. The protonated samples were dried under reduced pressure at 50°C. The dried samples were mixed with KBr (spectroscopic grade, 1:100) and pressed into discs for FT-IR analysis. FT-IR spectra were recorded on a Perkin-Elmer 1725 spectrometer with an MCT detector. 200 scans were co-added with a resolution of 4 cm$^{-1}$. The area of the peaks between 1740 and 1690 cm$^{-1}$ and between 1950 and 1810 cm$^{-1}$ were calculated automatically using the software program in the accessory micro-computer.

4.5. Stability test of the immobilised HA-silicas

To determine the stability of an HA-silica, a series of tubes, each containing 0.2 g of sample dispersed in 5 ml 0.01 M aqueous KCl at different pH values, was shaken at room temperature for 4 h. The supernatants were separated from the precipitates by centrifugation and their pH values were adjusted to 10. Then the absorbance at 254 nm was measured and the concentrations of the HA released from the HA-silicas were calculated using the corresponding (same pH) linear calibration curves.

5. Results

5.1. Preparation of aminopropyl silica

The modification of silica with APTS is a complicated process [18–20]. It is generally accepted
that the majority of the silane-to-surface siloxane bonds are formed in the curing procedure. Upon curing, the amino group of APTS relinquishes its interaction with the silica surface and the APTS molecule turns from the original amino-down position towards an amino-up position. In preliminary experiments with the aminopropyl silica that had not been cured or inappropriately cured, we found that, even at low pH, a substantial release of organic carbons from the HA-silicas occurred because the APTS molecules were mainly physically adsorbed (rather than immobilised chemically) on the silica.

The APTS reaction was always executed in the absence of CO\textsubscript{2} gas [18–20]. If aminopropyl silica had a bright yellow colour, a contamination from CO\textsubscript{2} gas in the air had occurred [18] and the products were discarded because the contamination severely deteriorated the further reactions.

We found that the amount of HA bound on aminopropyl silica was highly dependent on the concentration of APTS used in the preparation of aminopropyl silica. The higher the APTS concentrations, the higher the loading of amino groups on the surface and the higher the adsorbed amount of the HA. However, according to Vansant et al. [18], high concentrations of APTS result in multilayer formation and non-specific coating of APTS. Therefore, in the final procedure, only 1% APTS was used in the preparation of aminopropyl silica.

The amount of APTS bound to the silica has been calculated using the data of the elemental analysis. Based on a value of 131 Da for the weighted average molar mass of the bound APTS fragments [18], a loading of 138 µmol g\textsuperscript{-1} is found. This corresponds with about 1.6 APTS molecules nm\textsuperscript{-2}, a value somewhat lower than reported in literature [18–20,22,23].

The measurement of the electrophoretic mobility as a function of pH revealed that the iep values of the pure silica and the aminopropyl silica were pH 3.8 and 9.8 respectively. The value for pure Aerosil OX50 silica is relatively high; in general, iep values of silicas are observed at pH ≤ 3 [13,21,24]. The high iep value for aminopropyl silica is due to the fact that the amino groups are positively charged at not too high pH values.

### 5.2. Chemical immobilisation of the HA on aminopropyl silica

For the different HA-silica products, the amount of PAHA bound, the iep values and IR intensity ratios are shown in Table 1. Also included are the amounts bound and iep values of some of the products before end-capping and washing with the 1 M NaCl (pH 10) solution. The results are described below.

#### 5.2.1. HA-SiO\textsubscript{2}(ADS.T)

It was found that HA could be rapidly and strongly adsorbed on the aminopropyl silica even at room temperature. However, the originally "black" colour of the sample became shallow brown (wet state) after extensive washing with 1 M NaCl (pH 10), indicating that most of the HA is physically adsorbed on the surface. Therefore, in subsequent preparations, a curing step was introduced.

The curing procedure at 120°C stabilised the adsorbed HA considerably. After the repeated rinse of the cured HA-SiO\textsubscript{2}(ADS.T) with 1 M NaCl (pH 10), the sample in the wet state was still described below.

<table>
<thead>
<tr>
<th>Method</th>
<th>ADS.T</th>
<th>EDC</th>
<th>DMF.T</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bound amount(^a)</td>
<td>65</td>
<td>107</td>
<td>124</td>
<td>60</td>
</tr>
<tr>
<td>Rel. amount bound</td>
<td>1</td>
<td>1.33</td>
<td>1.46</td>
<td>0.38</td>
</tr>
<tr>
<td>Iep (pH(^b))</td>
<td>2.5</td>
<td>2.6</td>
<td>2.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Rel. Iep</td>
<td>1</td>
<td>1.18</td>
<td>1.46</td>
<td>0.64</td>
</tr>
<tr>
<td>IR intensity ratio(^c)</td>
<td>1.12</td>
<td>1.32</td>
<td>1.64</td>
<td>0.72</td>
</tr>
<tr>
<td>Rel. IR ratio</td>
<td>1</td>
<td>1.33</td>
<td>1.67</td>
<td>—</td>
</tr>
<tr>
<td>Charge density (pH = 9)(^d)</td>
<td>0.09</td>
<td>0.12</td>
<td>0.15</td>
<td>—</td>
</tr>
<tr>
<td>Rel. charge density</td>
<td>1</td>
<td>1.33</td>
<td>1.67</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\) Before rinsing with 1 M NaCl (pH 10).  
\(^b\) Final product after end-capping.  
\(^c\) After curing and rinsing with 1 M NaCl (pH 10).
black. This result implies that the curing procedure leads to some chemical bonding between the HA and the amipropyl silica.

In the final preparation, the sample was tested after the immobilisation and first washing step, after curing and rinsing with the alkaline salt solution, and after end-capping and washing. In the stability tests, some of the adsorbed HA (without curing) was released into the solutions with pH > 5 (see Fig. 1, curve 1). At pH 11, about 3% of the HA was released. After curing, the stability test shows (see Fig. 1, curve 2) that at pH 11 less than 1% of the HA is released. The best stability is achieved after end-capping of the free amino groups. After this step, physically adsorbed HA still remaining on the surface could easily be washed out and the final product was stable between pH 3 and 11 (see Fig. 1, curve 3). At pH 11 the release of HA is less than 0.1%.

The iep of the product before curing and extensive washing was 2.5 (see Fig. 2), showing that the HA screens the amino groups rather effectively. The amount of HA bound is 65 mg g\(^{-1}\). After curing and washing the amount bound is reduced; this raises the iep to 5.4 (Fig. 2). The end-capping procedure removes the free NH\(_2\) groups and results in a lowering of the iep of the product to 2.8.

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5.2.2. HA-SiO\(_2\)(EDC)

Incubation of HA and silica with EDC is based on the procedure used for enzyme immobilisation. It leads to the formation of amide bonds between amino groups and the carboxylic groups at room temperature [25, 26]. The method works well and a bound amount of 107 mg g\(^{-1}\) is found in the first step, but part of the HA is also physisorbed on the surface. Rinsing with 1 M NaCl (pH 10) and end-capping of the free amino groups gives a stable product with 56 mg HA bound to 1 g of silica. The iep values and the results of the stability tests after the different steps are comparable with HA-SiO\(_2\)(ADS.T), see Table 1.

5.2.3. HA-SiO\(_2\)(DMF.T)

In general, at elevated temperatures, a dewatering reaction between carboxyl groups and amino groups may occur in an anhydrous medium like DMF [27]. The method indeed works well for the immobilisation of HA. Before the washing with the alkaline salt solution, the amount of HA bound is 124 mg g\(^{-1}\) and the iep equals 2.3. However, also in this case, a considerable amount of HA is adsorbed by electrostatic attraction. After removal of the physically adsorbed HA by washing with 1 M NaCl,
(pH 10) and the end-capping of the free amino groups, the amount bound is reduced to 72 mg g\(^{-1}\) (see Table 1). The wet product still has a black colour and the iep is 2.6. The results of the stability test are very similar to those of HA-SiO\(_2\) (ADS.T).

### 5.2.4. HA-SiO\(_2\) (GA)

The colour of glutaraldehyde-activated amino-propyl silica is red because of the formation of Schiff’s bases between bonded APTS and glutaraldehyde [25, 26]. The activation of the aminopropyl silica with glutaraldehyde leads to an iep at pH 8.8. The glutaraldehyde method relies on the reaction between aldehyde groups on the glutaraldehyde-activated aminopropyl silica and amino groups of the HA. At the first stage, about the same amount of HA adheres to the surface as with HA-SiO\(_2\) (ADS.T), but the iep is considerably higher (Table 1). However, most of the HA is physically bound. About 75% of the adsorbed HA can be easily washed out with 1 M NaCl (pH 10). The release of HA raises the iep from 5.3 to 7.2.

5.3. FT-IR analysis

The FT-IR spectra of the pure silica and its derivatives are shown in Fig. 3. The spectral range below 1300 cm\(^{-1}\) is not shown because strong peaks in the range between 1250 and 1000 cm\(^{-1}\), assigned to Si–O–Si asymmetric stretching vibrations, and the range between 900 and 750 cm\(^{-1}\), assigned to Si–OH bending from the silica itself [18, 29], mask almost all the other information.

Spectrum A in Fig. 3 is of the pure silica. The broad peak centred at 3500 cm\(^{-1}\) can be assigned to the Si–OH stretching vibration and the weak shoulder between 1950 cm\(^{-1}\) and 1810 cm\(^{-1}\) can be assigned to Si–O–Si vibration [18, 29]. The peak at 1640 cm\(^{-1}\) is possibly due to the OH bending vibration from adsorbed water.

Silica modified with APTS (spectrum B) shows two additional shoulders at 2924 cm\(^{-1}\) and 2875 cm\(^{-1}\) assigned to the asymmetric and symmetric stretch vibrations of CH\(_2\) and CH\(_3\). It reveals the successful immobilisation of APTS molecules on the silica surface.

The spectra of the HA-silicas ADS.T, EDC, DMF.T and GA are shown in Fig. 3(C–F) respectively. The spectrum of PAHA is shown in Fig. 3(G). In the spectra of the HA-silicas, some groups from HA, silica, APTS molecules and the chemical bonds formed in the immobilisation procedures all make contributions to the peaks at ca. 3500 cm\(^{-1}\) (assigned to the Si–OH and C–OH stretching vibrations), 3320 cm\(^{-1}\) (assigned to the stretch vibrations of H-bonded OH and NH groups), 2924 cm\(^{-1}\) (assigned to the asymmetric stretch vibrations of CH\(_2\) and CH\(_3\)), 2875 cm\(^{-1}\)
(assigned to the symmetric stretch vibration) and 1640 cm\(^{-1}\) (assigned to the amide band, the water bending vibration, the aromatic C=O stretch, the H-bonded carbonyl groups and the asymmetric COO stretch). Thus these peaks are of little value for further analysis. More useful are the peaks in the range between 1740 and 1690 cm\(^{-1}\) that can be mainly assigned to the C=O stretch vibration of the carboxyl groups of the PAHA. The relative intensity ratio of these peaks and the shoulder in the range between 1950 and 1810 cm\(^{-1}\), assigned to Si-O-Si vibration, reveals the relative amount of bound HA. The results are listed in Table 1 as IR intensity ratio. The relative IR intensity ratios, using HA-SiO\(_2\)(ADS.T) as standard, can be compared with the ratios of the bound amounts (see Table 1). In view of the error in these calculations the agreement is reasonable.

5.4. Charge density–pH isotherms

The effect of pH on the charge densities of Aerosil OX50 and its derivatives, measured at 0.01 M KCl are shown in Figs. 4–6.

5.4.1. SiO\(_2\)

The charge density–pH curve of the pure silica (see Fig. 4) shows that substantial charging only occurs at pH values larger than about 6. The result is in good agreement with existing literature data [30–33].

5.4.2. APTS-SiO\(_2\)

The charge density–pH isotherm of the amino-propyl silica is rather different from that of pure silica (Fig. 4). The iep (pzc) of APTS-SiO\(_2\) is located at a high pH value (9.8). In the pH range of the titration, the amino groups always contribute positive charges, whereas the silanol groups...
contribute negative charges. Over most of the pH range investigated the charge of the aminopropyl groups dominates. Nevertheless, it is noted that the charge difference between pH 3 and 11 is very similar for APTS-SiO₂ and for pure silica.

5.4.3. HA-SiO₂/(ADS.T)/(EDC)/(DMF.T)

The charge density–pH curves of the HA-silicas made by the ADS.T and the DMF.T methods have similar shapes and iep (pzc) values (see Fig. 5). The main difference is the magnitude of the charge density. However, the ratios of the charge densities at pH 9, taking HA-SiO₂ (ADS.T) as standard, correspond very well with the bound HA ratios (Table 1). The negative charge density of the HA-SiO₂(EDC) increases relatively sharply at high pH (>9). This is probably due to the fact that this method leaves more free silanol groups on the surface than the ADS.T and DMF.T methods. The fact that the samples have low iep values, makes their behaviour similar to that of pure HA, which reaches its iep asymptotically at low pH values. Assuming that the charge density in the range pH 3–9 is entirely due to bound PAHA allows us to compare this charge increment with that of “free” PAHA. For the bound PAHA we find 2.1 meq g⁻¹ HA, whereas for free PAHA a value of 3.5 meq g⁻¹ HA is found. This suggests that some 40% of the charged groups are involved in the immobilisation step.

5.4.4. HA-SiO₂(GA)

The charge density–pH isotherm of the HA-SiO₂(GA) sample is shown in Fig. 6. For comparison, the surface charge–pH isotherm of the blank SiO₂(GA) sample is also shown. Owing to the small amount of bound PAHA, and the possibility that ethanolamine may have blocked part of the carboxylic groups from the HA, the difference between the two curves is hardly dependent on the pH. However, the difference between the two ieps clearly indicates the presence of the bound HA.

6. Discussion

HA-silicas are suited as new sorbents when they can meet several criteria. First, the amount of humic substance bound on the silica should be sufficiently high to ensure that the adsorption capacity of the product is high. Second, the product should be stable for use in a wide range of conditions. Third, regeneration of the product should be reasonably simple.

When HA-silicas are intended to be used as a model of natural HA-coated silica matrices, as done in Refs. [7–12], they should meet two further criteria. First, the immobilisation reactions should be mild and result in only a slight modification of the humic substance. To achieve this, only a limited number of HA segments should be chemically bound and the layer should be homogeneous. Second, the final products should have iep values close to that of natural HA.

Marko-Varga et al. [34] found that humic substances could be adsorbed strongly on aminopropyl silica from aqueous solution. Szabo and coworkers [9,11] used this method to prepare physisorbed HA-silica for further research. We followed the recipe from Szabo and coworkers [9,11] for the preparation of HA-SiO₂(ADS.T), but no buffer has been used in our experiments since humic substances themselves act as buffer. The present results show that the adsorbed HA-silica is not sufficiently stable at high pH and/or high salt concentrations. Thus, an additional curing step at elevated temperatures is introduced to ensure that the HA-silica has a good stability. A similar heating step is also used for the preparation of HA-SiO₂(DMF.T). It is not unreasonable to expect that curing of HA-SiO₂ (ADS) and direct heating of HA and APTS-SiO₂ in DMF result in the formation of some chemical bonding, most probably from the dewatering reactions between the amino groups and the carboxylic groups [27]. However, heating may also result in the removal of some aliphatic fragments and some carbohydrate and amino acid residues [35]. In addition, dewatering reactions between carboxylic groups and phenolic groups in the HA, as well as between carboxylic groups from the HA and silanol groups from the silica surface may also occur at elevated temperature [27]. Thus, additional modifications of humic substances during heating cannot be excluded. In general, the EDC
method also result in the formation of the amide bonds between carboxylic groups and amino groups. Since the EDC method is executed at low or room temperature, it does not have the possible disadvantages of a high temperature treatment. It should be noted that when an HA contains a high amount of amino groups, inter- and intra-molecular reactions may occur and these reactions will also modify the HA. Since there is only a low nitrogen content in PAHA, these undesirable reactions can only occur to a limited extent with PAHA.

The final end-capping step is needed for all three methods to ensure that the iep is low. The reactions that prepare the aminopropyl silica and end-cap the free amino groups are executed in toluene and DMF respectively. This was done to get the well-defined products. However, both of the reactions can also be executed in aqueous media [18,36]. Therefore, it might be feasible to prepare HA-silicas on a large scale without the use of organic solvents.

Szabo and coworkers [7,9,11] and Koerdel et al. [28] have prepared HA-silicas with the glutaraldehyde method. Following their method we found that a lot of HA is physisorbed. After extensive washing only a low content of chemically immobilised HA resides on the surface of HA-SiO$_2$(GA). We found this not only with PAHA, but also with several soil HAs (Shitara Blank, Kuragari, Sanage from Ref. [37]) with high nitrogen content. With HA-SiO$_2$(GA) the immobilised amount of PAHA is not enough to compensate the positive charge from Schiff bases and thus the iep value of the final product is located at high pH. This iep is rather different from that of free HA. Therefore, it is not suited as a model to simulate HA in natural environments. The product is also less attractive as a sorbent because of the low amount of immobilised humic.

7. Conclusions

The present investigation of four methods to prepare HA-silica lead to the following conclusions.

1. The adsorption and curing method (ADS$_T$), the heating in DMF method (DMF$_T$) and the EDC method are well suited for chemical immobilisation of humic substances on aminopropyl silica.

2. Without further steps these procedures also lead to a substantial amount of electrostatically bound humic substances.

3. End-capping of the free amino groups is essential to eliminate physical adsorption of the humics and to obtain stable products with low ieps.

4. The products obtained with all three methods are promising sorbents with an HA character.

5. The glutaraldehyde method is not well-suited for the production of sorbents with an HA character, because the immobilised amount of HA is low and the iep of the products is rather different from that of free HAs.

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References