Humic acids as molecular assemblers in the surface-enhanced Raman scattering detection of polycyclic aromatic hydrocarbons

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Abstract

A humic acid (HA) extracted from lignite was successfully used in surface-enhanced Raman scattering SERS experiments as molecular occlusion assembler deposited onto a Ag colloidal surface to detect the polycyclic aromatic hydrocarbons PAHs chrysene and pyrene. Thus, this is the first time that HA compounds are employed in the design of metal nanoparticles-based sensors for pollutants detection. Chrysene (Chr) and pyrene (Pyr) were detected at low concentration. A charge transfer between HA and the analyte characterizes their interaction. The interaction resulted to be more significant in the case of the pyrene molecule. Extended Hückel calculations based on a molecular model for the interacting PAHs/HA/Ag system support the experimental conclusions. The PAHs–HA distance is about 3.5 Å and the most probable orientation for both analytes is plane parallel to the aromatic fragments of HA. An energy transfer, from the silver surface to HA and from the analyte to HA, is concluded.

Keywords: Humic acid; Assemblers; PAHs; SERS

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) has been consolidated as a powerful analytical technique in the last years. This is due to the giant electromagnetic enhancement induced by local plasmon resonance (LPR) [1], which may allow the detection of trace of analytes or even single molecules in the case of dyes [2,3]. Polycyclic aromatic hydrocarbons (PAHs) were detected in seawater at ng/L level using SERS [4]. To our knowledge, SERS spectra of these compounds in pure colloidal solutions have not been obtained. This situation changes by a functionalization of the metal surface with a host molecule. In the last time we have identified PAHs by using calixarenes [5,6] and carbon nanotubes [7] as molecular assemblies in SERS experiments.

Humic acids (HA) are able to interact with persistent organic pollutants POPs and PAHs due to their particular structural characteristics with the existence of hydrophobic cavities which display a high tendency in trapping environmental contaminants [8–15]. HA have been characterized in our laboratory by SERS [16–21]. In these studied we have demonstrated that the SERS intensity of HA depends on parameters such as the pH and the aromaticity degree. In particular, we have also demonstrated that the SERS intensity of HA decreases very much at pH between 5.0 and 7.0, and that the metal/HA interaction is mainly verified through carboxylate moieties and highly substituted phenols. These facts encouraged us to employ these natural compounds in the detection of pollutants involving the combination of advanced materials based on the HA-functionalized metal nanostructures and the

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enormous analytical powerful of the surface-enhanced optical spectroscopies. HA have been already employed in layer-by-layer functionalization systems for pollutant detection [22]. HA substances have been also employed in the fabrication of Au nanoparticles [23], and in the detection of the adsorbates 1-naphthylamine and pyridine [24]. These molecules are active in SERS without molecular assemblers or any functionalization of the metal surface. However, in this work we have employed HA for the detection of the non-active PAHs molecules. In addition, the SERS technique by using HA as assemblers has not been used so far in the detection of such pollutants.

The main objective of the present contribution is to use a humic acid (HA) as occlusion assembler for the detection of PAHs chrysene (Chr) and pyrene (Pyr) throughout the SERS technique. To complete the interpretation of the spectroscopic results we performed Extended Hückel calculations of molecular models in which chrysene and pyrene interact separately with the humic acid, the whole deposited on a silver surface. This work is the basis to use HA of different nature and origins and SERS, to detect POPs, analytes showing a much lower or negligible SERS activity due to their low tendency to be adsorbed onto the metal nanostructures usually employed for SERS.

2. Experimental

2.1. Sample preparation

The HA sample corresponds to a lignite (Li)—Standard Ward's-USA: TOC = 53.3 ± 2.1, pH 6.5, ash = 8.7 ± 0.1. Two grams of the sample was extracted under N_2 with 100 mL of 0.5 M NaOH [25]. The solution was acidified with 15% HCl until pH < 2 and centrifuged at 5.000 g for 20 min to separate the HA. The sample was dialyzed, using 1000 Da cut-off tube dialyzes (Cellu Sep H1-USA), against distilled water until a neutral pH was achieved and then freeze dried. Chrysene and pyrene were purchased from Aldrich and used as received.

Colloidal silver nanoparticles were prepared by using hydroxylamine hydrochloride as reducing agent [26]. These nanoparticles have the advantage of a more uniform distribution of size and shape together with the absence of interferences from remainder oxidation product. The adherence properties of these chloride-covered nanoparticles are better as concern their immobilization on glass giving rise to films by a simple deposition on the surface [27].

Metal films of Ag nanoparticles for micro-SERS measurements were prepared by immobilising the colloidal nanoparticles. An aqueous solution of humic acid was obtained by dissolving 1 mg of the material with 1 mL of water. Samples for SERS measurements were prepared by adding 10 μL of the HA solution to 1000 μL of the silver colloid. The pH of the mixture was adjusted to 7 by adding HCl and NaOH in aqueous solution. Previous to the immobilization, 10 μL of a 10^{-4} M of the analyte chrysene or pyrene solved in acetone was added to this mixture up to 10^{-6} M. Afterwards, the colloid was activated by addition of aqueous potassium nitrate up to a final concentration of 10^{-2} M. This activation is needed in order to increase the nanoparticles SERS activity by properly modifying the morphology of the particles. Then, 20 μL of the final suspension was deposited onto a glass cover slide and dried at room temperature. SERS spectra were obtained for HA, HA/Chr and HA/Pyr.

An aliquot of the original non-activated colloid was placed on a glass slide with a shallow groove, and then the cover glass slide containing the dried activated Ag nanoparticles was placed on the groove with the side containing the dried nanoparticles facing downwards so that the suspension is placed in the groove, scheme depicted in our previous work [5]. Only sample monolayers were assumed to contribute to the SERS spectra.

2.2. Spectral reproducibility

The SERS spectra obtained from different aggregates are reproducible in what concerns the SERS profiles, although the intensities vary due to the dependence on the aggregate morphology. This reproducibility was maintained from batch to batch.

2.3. Instrumentation

The SERS spectra were recorded with a Renishaw Raman Microscope System RM1000 equipped with the 514 nm laser line, an electrically refrigerated CCD camera, and a notch filter to eliminate the elastic scattering. The spectra shown here were obtained by using a 50× objective. The output laser power was 10.0 mW. Spectral resolution was 4 cm^{-1}. The spectral scanning conditions are chosen to avoid sample degradation. Spectra reported are single scans.

3. Theoretical study

Based on an earlier structure proposed by Schulten and Schnitzer [28], a humic acid model (HAM) was generated (Fig. 1). The structural model of the humic acid considered for
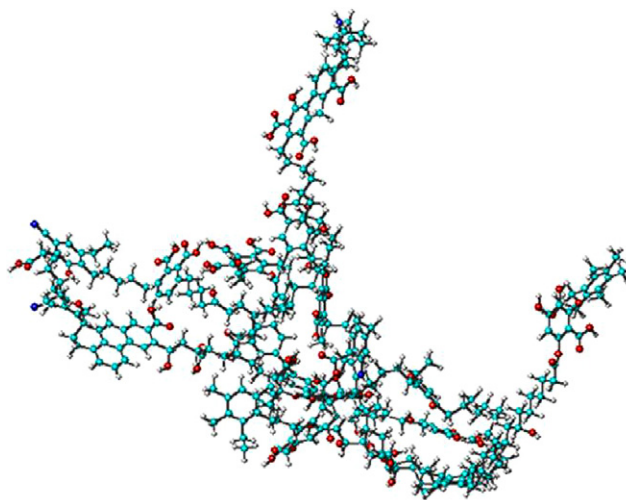

Fig. 1. Humic acid structural model.

the calculations corresponds to a standard and classical system generally assumed in many publications. The use of the basic molecular system is additionally supported by the fact that we worked with synthesized commercial species, also employed by several authors. Given that we expect normal bond lengths and angles we employed Molecular Mechanics (MM) to carry out a full geometry optimization of HAM. To identify possible binding sites for chrysene and pyrene, HAM's wavefunction and eigenvalue spectrum was obtained with the Extended Hückel method (EHT). The Hyperchem package of programs was used [29]. The strength of EHT is that it gives a good qualitative picture of the molecular orbitals (MOs). In fact, it was looking at the MOs that came from EHT that Woodward and Hoffman devised their rules. Recently, it was shown that, within the Hartree–Fock–Rüdenberg picture (HFR), EHT is compatible with the nonempirical Hartree–Fock method in Roothaan's form [30,31]. HFR thus explains why EHT turned out to be qualitatively successful. The combination of EHT with Molecular Mechanics (MM) was able to give, for example, a qualitative explanation of SERS results [7]. The same treatment was applied to Pyr and Chr.

Binding sites for Pyr and Chr were identified as follows. Keeping fixed HAM's geometry, several molecules of Pyr (and Chr) were incorporated. Using MM, we carried out a full geometry optimization of the whole system until Pyr (Chr) molecules reached an equilibrium position close to some areas of the HAM. An analysis of HAM wavefunction of these areas and of the orbital diagram for the isolated species was carried out.

4. Results and discussion

4.1. SERS of the humic acid

The SERS spectrum of lignite humic acid, in Fig. 2(b), displays two typical strong and large bands at about 1375 and 1600 cm^{-1}, which correspond to polycondensed aromatic moieties existing in the sample. In general, the SERS spectrum of humic substances changes when varying the pH [16–18]. This spectral behaviour by pH effect is interpreted in terms of conformational changes adopted by the molecules. At neutral pH the molecule adopts a nearly globular structure and the SERS signal is lower [18], while a pH increasing causes a decurling of the humic acid [10] which may low their affinity towards PAHs. Thus, we have carried out our experiments at neutral conditions in order to avoid the interference from the host molecule and to preserve the hydrophobic cavities naturally existing in the native HA conformation.

4.2. SERS of the HA/PAHs systems

The SERS spectrum of the HA/Chr and HA/Pyr molecular systems are displayed in Figs. 2 and 3. These spectra are dominated by both the spectral shape of HA, large bands at about 1380 and 1600 cm^{-1}, and the chrysene and pyrene bands, which are not seen in the absence of HA. Thus, the presence of the Chr and Pyr bands at concentrations around 10^{-6} M is an
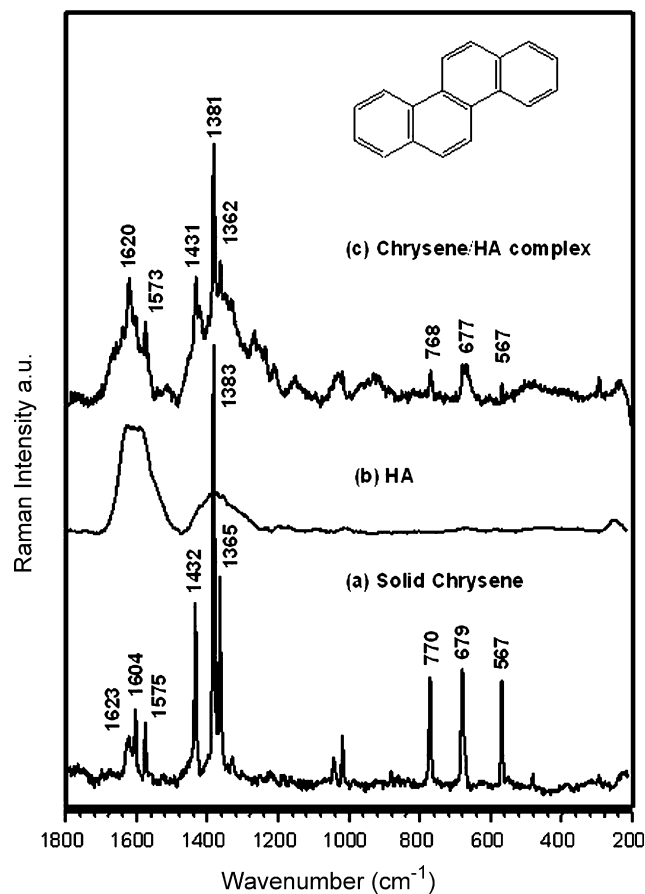

Fig. 2. (a) Raman spectrum of chrysene solid, (b) SERS spectrum of the humic acid HA (10^{-6} M) and (c) SERS spectrum of chrysene (10^{-6} M).

unequivocal argument that Chr and Pyr display a SERS effect when interacts with the HA assembler.

Most of Chr bands are located at about the same frequency than in the solid by keeping similar relative intensities (Fig. 2), which suggests that the HA/Chr interaction is rather weak with a van der Waals type character. The spectral differences between the SERS and the solid spectra, mainly in the relative intensity of bands below 1000 cm^{-1}, could be related to a preferential molecular orientation of chrysene interacting with HA.

Several SERS bands in the spectrum of pyrene in Fig. 3 display spectral modifications related with both frequency shifts and relative intensity variations. The νCC aromatic bands above 1450 cm^{-1} shifts to higher energy by keeping a similar intensity compared to the spectrum of the solid. Due to the interaction the strongest νCC band at 1402 cm^{-1} in the solid shifts to 1379 cm^{-1}. The νCC/δCH band at 1240 cm^{-1} maintains the frequency but drastically decreases its relative intensity due to the same effect. Spectral modifications in the region of the δCH bands and an intensity decrease of the out of plane bending ρCH vibrations below 1000 cm^{-1}, appearing at 591, 456 and 408 cm^{-1} are observed. These changes can be attributed to a specific molecular orientation caused by the interaction with the HA. The general frequency shift to lower and higher frequencies is unambiguously associated to a short
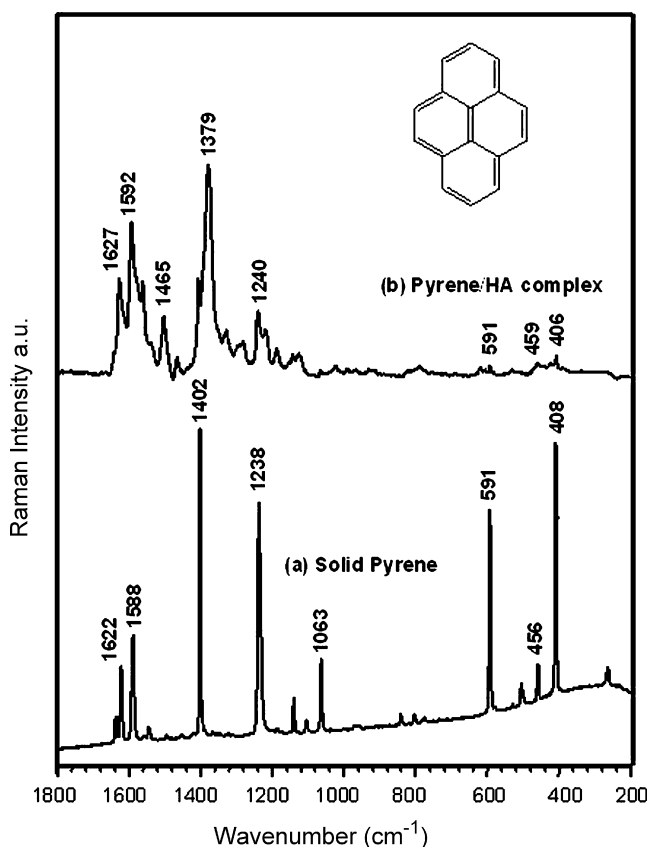
Fig. 3. (a) Raman spectrum of pyrene solid and (b) SERS spectrum of pyrene (10^{-6} M).
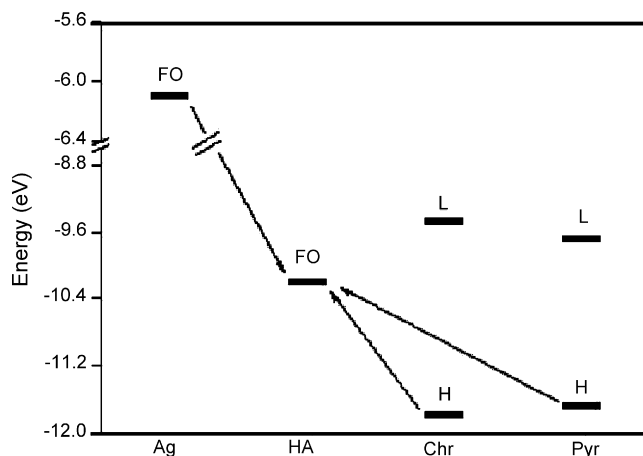

Fig. 4. Molecular orbital diagram for Ag, humic acid HA, chrysene Chr and pyrene Pyr (FO refers to frontier orbitals, H the HOMO, and L to the LUMO).

distance HA/Pyr interaction which causes a π-electronic redistribution of the pyrene molecule. Thus, a face-to-face interaction of pyrene with aromatic fragments of the humic acid molecule is proposed.

The spectral differences between the solid and SERS spectra of both analytes, suggest a different interaction degree of each species with HA, being the Pyr molecule located closest to HA. On the other hand, the changes observed in the SERS spectrum of HA/Pyr complex are stronger than those seen in the case of the interaction of Pyr with calix[4]arene hosts [5,6]. This fact is interpreted as due to the higher aromatic availability in the case of HA, due to its higher flexibility, in comparison to the rigidity of calix[4]arene hosts.

4.3. Theoretical results

Fig. 4 displays part of the molecular orbital diagram of the isolated systems. In a previous work it was shown that a planar Ag surface has the Fermi level located at −6.14 eV [7]. From the figure is observed that the lowest empty molecular orbital (LUMO) of the humic acid model has an energy of −10.15 eV, which indicates that the electron flow goes from Ag to the HAM. This implies that the HAM areas in which the LUMO and the higher unoccupied MOs lay, interacts with the Ag surface. This, in turn, hinders this area of HAM for the interaction with the analytes. The effect of the injection of electrons from the Ag colloidal particles to HAM does not

propagate to the whole molecule due to the great quantity of saturated bonds separating the aromatic portions of HAM.

Chrysene and pyrene interact with five sites of HAM. The inspection of HAM's wavefunction shows that they are π-electron rich sites. Therefore, Pyr and Chr interact with these aromatic moieties through a π-π slipped-stack mechanism. The interaction is van der Waals type (distances of 3.4–3.7 Å between partners), with a very small electron transfer from the analytes to the HAM. Pyr donates more electrons than Chr; which is compatible with the MO diagram of Fig. 4. Thus, Pyr/HAM interaction is more intense than in the case of chrysene.

All these results are pictorially summarized in Fig. 5. Letters A–E indicate the areas in which the analyte/HAM interaction occurs. The location of the first two unoccupied MOs of HAM is shown in green. These MOs are separated by 0.01 eV. The Ag colloids were placed close to the empty MO's only to indicate the Ag–HAM interaction areas. Then, in our HAM, area B is probably forbidden to interact with the analytes when HAM is placed on a silver particle.
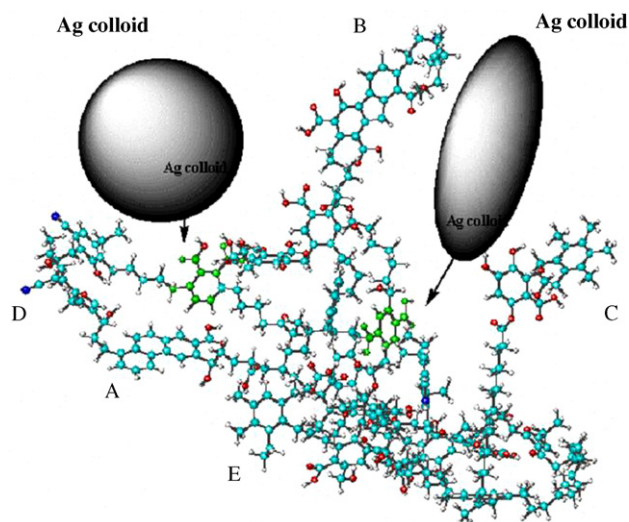

Fig. 5. Pictorial representation of the results.

The above results suggest that the areas available for Chr or Pyr to interact with humic acids are regulated by two main factors. The first factor is the location of the HA's empty MOs defining the position of HA respect to the Ag colloids. This, in turn, defines the forbidden area for the analytes. The second factor is the quantity and location of the alkyl residues which could hinder the Chr/HA or Pyr/HA interaction.

Both molecules can interact with all exposed π-electron rich sites. Nevertheless, since pyrene's volume is smaller than chrysene (12% less), for small cavities with π-electron rich moieties in the HA structure, the local geometry could permit the interaction only with Pyr molecules. Thus, for a fixed HA geometry, Pyr should have more interacting sites than Chr.

5. Conclusions

A lignite humic acid sample was successfully used to trap humic acids in solution. The resulting adduct deposited onto a silver colloidal surface displays an intense SERS spectrum where it is possible the identification and detection the analytes chrysene or pyrene at low concentrations. A charge transfer between pyrene and the assembler is inferred. The HA/Pyr interaction resulted to be more energetic than in the case of the chrysene molecule. Theoretical results based on a molecular model for the PAHs/HA/Ag surface system agree with the experimental results. The charge transfer flows from the surface to the HA and from the analyte to HA. The averaged distance between chrysene and pyrene and HA is about 3.5 Å and the most probable orientation of the analytes is plane parallel to the aromatic moieties of HA. In summary, the present results suggest that HA could be used as molecular occlusion assemblers to detect PAHs due to their high aromatic availability provided by their enormous flexibility.

References

[1] R. Aroca, R. Surface-enhanced, Vibrational Spectroscopy, John Wiley & Sons, Chichester, 2006.
[2] S. Nie, S.R. Emory, Science 275 (1997) 1102.
[3] K. Kneipp, H. Kneipp, I. Itzkan, R.R. Dasari, M.S. Feld, Chem. Rev. 99 (1999) 2957.
[4] H. Schmidt, N.B. Ha, J. Pfannkuche, H. Amann, H.-D. Kronfeldt, G. Kowalewska, Mar. Pollut. Bull. 49 (2004) 229.
[5] P. Leyton, S. Sanchez-Cortes, M.M. Campos Vallette, C. Domingo, J.V. García-Ramos, C. Saitz, Appl. Spectrosc. 59 (2005) 1009.
[6] P. Leyton, S. Sanchez-Cortes, J.V. García-Ramos, C. Domingo, M.M. Campos-Vallette, C. Saitz, R.E. Clavijo, J. Phys. Chem. B 108 (2004) 17484.
[7] P. Leyton, J.S. Gómez-Jeria, S. Sanchez-Cortes, C. Domingo, M.M. Campos-Vallette, J. Phys. Chem. B 110 (2006) 6470.
[8] K.S. McCain, J.M. Harris, Anal. Chem. 75 (2003) 3616.
[9] J.F. McCarthy, B.D. Jimenez, Environ. Sci. Technol. 19 (1985) 1072.
[10] P. Larsen, L. Carlsson, Chemosphere 34 (1997) 817.
[11] M. Terashima, S. Tanaka, M. Fukushima, J. Environ. Qual. 32 (2003) 591.
[12] Y.-P. Chin, G.R. Aiken, K. Danielsen, Environ. Sci. Technol. 31 (1997) 1630.
[13] R.R. Engebretsonand, R. von Wandruszka, Environ. Sci. Technol. 33 (1999) 4299.
[14] M.U. Kumke, F.H. Frimmel, F. Ariese, C. Gooijer, Environ. Sci. Technol. 34 (2000) 3818.
[15] Y. Laor, C.H. Kolkov, R. Armon, Environ. Sci. Technol. 36 (2002) 1054.
[16] O. Francioso, S. Sanchez-Cortes, V. Tugnoli, C. Ciavatta, L. Sitti, C. Gessa, Appl. Spectrosc. 50 (1996) 1165.
[17] O. Francioso, S. Sanchez-Cortes, V. Tugnoli, C. Ciavatta, C. Gessa, Appl. Spectrosc. 55 (1998) 270.
[18] S. Sanchez-Cortes, O. Francioso, C. Ciavatta, J.V. Garcia-Ramos, C. Gessa, J. Colloid Interf. Sci. 198 (1998) 308.
[19] O. Francioso, S. Sanchez-Cortes, V. Tugnoli, C. Marzadori, C. Ciavatta, J. Mol. Struct. 565–566 (2001) 481.
[20] O. Francioso, S. Sanchez-Cortes, D. Casarini, J.V. García-Ramos, C. Ciavatta, C. Gessa, J. Mol. Struct. 609 (2002) 137.
[21] S. Sanchez-Cortes, G. Corrado, O.E. Trubetskaya, O.A. Trubetskoj, B. Hermosin, C. Saiz-Jimenez, Appl. Spectrosc. 60 (2006) 40.
[22] F.N. Crespilho, V. Zucolotto, J.R. Siquiera, C.J.L. Constantino, F.C. Nart, O.N. Oliveira Jr., Environ. Sci. Technol. 39 (2005) 5385.
[23] D.S. dos Santos, R. Alvarez-Puebla, O.N. Oliveira, R. Aroca C, J. Mater. Chem. 29 (2005) 3045–3049.
[24] R. Alvarez-Puebla, D.S. dos Santos, R. Aroca, Analyst 132 (2007) 1210.
[25] C. Ciavatta, M. Govi, G. Bonoretti, C. Gessa, Fert. Res. 44 (1996) 225.
[26] N. Leopold, B. Lendl, J. Phys. Chem. B 107 (2003) 5723.
[27] M.V. Cañamares, S. Sanchez-Cortes, J.D. Gómez-Varga, C. Domingo, J.V. García-Ramos, Langmuir 18 (2005) 8546.
[28] H.R. Schulten, M. Schitzer, Soil Sci. 162 (1997) 115.
[29] Hyperchem, Hypercube Inc., Gainesville, 2002, FL, USA.
[30] W. Koch, Int. J. Quant. Chem. 76 (2000) 148.
[31] W. Koch, B. Frey, J.F. Sanchez, T. Scior, Z. Naturforsch. 58A (2003) 756.