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Chemical Availability and Reactivity of Functional Groups grafted to Magnetic Nanoparticles monitored In situ by ATR-IR Spectroscopy

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Characterization of organic functional groups grafted to magnetic nanoparticles often causes considerable difficulty because of lack of information concerning their accessibility and chemical reactivity. Classical solid-state techniques only allow gaining qualitative information and the determination of upper bounds of the total loading of organic moieties. To overcome this limitation we applied attenuated total reflection (ATR) infrared spectroscopy in a specially designed mechanically stirred in situ batch reactor-cell. Using the example of iron oxide magnetic nanoparticles embedded in a silica matrix that have been functionalized by grafting aminopropyl groups, we elucidated the accessibility and reactivity of amino groups. Reactivity tests using the transformation of the amine to benzaldimine revealed that less than 40% of the introduced amino groups reacted to the corresponding benzaldimine indicating that the theoretical loading provides only an upper bound of active functional groups. The reactivity of these groups has been followed by observing the transformation of the amine to the corresponding benzaldimine, revealing population density, accessibility, and reactivity of these groups. The present in situ technique facilitates the monitoring of the transformation of grafted functional groups and simultaneously provides quantitative and structural information when the material is suspended in a solvent system.

1. Introduction

A key issue for the efficient use of surface-modified magnetic nanoparticles in biomedical and chemical applications is the characterization of functional groups grafted on the surface of the particles. Functionalized iron oxide (magnetite or maghemite) nanoparticles have many different innovative applications both in biomedicine and chemistry.[1−5] The interest shown for this material depends on several factors: (i) it can be easily separated from a reaction mixture or guided to a target by means of an external magnetic field; (ii) because of their small size, magnetite particles possess exceptional size-related magnetic properties known as superparamagnetism; they do not show a permanent magnetic moment, but can be readily magnetized upon exposure to an external magnetic field; and (iii) it is simple to prepare in the gram scale.

Some of the best known potential applications in the fields of medicine and biology are cancer therapy by hyperthermia,[6] target-specific diagnostic imaging,[7−10] separation of biological molecules,[11] and targeted drug delivery.[12,13]

In chemistry, magnetic particles with surface functional groups are studied in relation to the preparation of magnetically separable catalysts[14−17] as well as for the scavenging of undesirable chemicals.[18] In particular, the

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anchoring of expensive homogeneous catalysts to the pending groups of magnetic nanoparticles was shown to be a feasible approach to develop new heterogeneous recyclable catalysts.[15]

The functionalization of a solid material by means of organic molecules allows the powerful combination of the solid-state properties of the carrier to the chemical properties of the functional groups that are attached to it. Efficient use of such materials requires the understanding of the chemical behavior and availability of organic moieties which are covalently bound to the solid phase. Thus transferring our knowledge of organic chemistry to the chemistry of mixed organic−inorganic materials critically depends on techniques that allow the study of the chemical behavior of the material.

Optical ellipsometry and UV−vis have been proposed over a decade ago[19] and again recently[20] as tools for the study of functional groups grafted on nanoparticles and suspended in a solvent, whereas only very recently has high-resolution magic angle spinning (HRMAS) NMR been interestingly applied to the study of functionalized magnetic nanoparticles[21] in suspension. Nonetheless functionalized magnetic nanoparticles are still in most cases characterized as solid phases using infrared or Raman spectroscopy, elemental chemical analysis, or thermal gravimetric analysis,[22−26] thus showing the lack of a simple and powerful technique to analyze surface functionalization of magnetic nanoparticles in a solvent or suspension. Solid-state techniques allow in the best cases for the identification of the presence of organic functionalities, but provide no information on their actual behavior in the environment where they will finally be used, generally as a suspension. The widespread analysis of functionalized nanoparticles as solids originates from the fact that they consist only of a few weight units percent of organic moieties, whereas the bulk of the weight and volume (usually over 90%) consist of solid inorganic carrier. Characterization of the solid can at most give an upper bound for the amount of organic moieties actually available on nanoparticles. Such moieties may be unable to behave as free functionalities because of limited accessibility, sterical hindrance, or partial incorporation in the solid matrix. Furthermore typical loadings are of the order of mmol of functional groups per gram of carrier, and the extent of the influence of this bulk upon chemical reactivity cannot be evaluated without a technique able to monitor the transformation of functional groups while the reaction is occurring.

ATR infrared spectroscopy has been shown to be a powerful technique to study intermolecular interactions, the reaction between pending groups attached to a solid and organic molecules, and the immobilization of cations on solids.[27−31] In such cases, the ATR-IR technique was used to investigate a thin film (few micrometers) of a functionalized powder material (silica) that was deposited on the internal reflection element (IRE). Furthermore, it has been used to monitor homogeneous[32−34] and heterogeneous reactions[35−38] in batch reactors and in the design of mesoporous zeolites.[39]

In the present manuscript, we describe how we were able to apply ATR-IR spectroscopy using a homemade batch reactor cell to study the chemical behavior of magnetic functionalized nanoparticles under real conditions, i.e., under vigorous stirring and in the concomitant presence of a reactant.

The present investigation focuses on the differences between the characterization of the solid material and the determination of its chemical reactivity. It is shown that in situ ATR-IR spectroscopy can be used (i) to improve the quantification of chemically available organic moieties present on particle surfaces, and (ii) to follow the transformation of immobilized organic functionalities while it is occurring, therefore allowing comparison between homogeneous and heterogeneous reactions.

2. Experimental Section

In this section, some general aspects of the experiments performed for the present investigation are described, whereas details of the original experimental setups and of the techniques used for determining the chemical reactivity of the material are described extensively in the next section.

2.1. Sample Preparation and Characterization. The preparation and characterization of the SiO_2/Fe_3O_4 composite material, consisting of iron oxide nanoparticles (Fe_3O_4 or γ-Fe_2O_3) embedded in a silica matrix, with a Si:Fe atomic ratio of 14:1, was described in detail elsewhere.[40] For the functionalization of the silica-iron oxide material 4 g of SiO_2/Fe_3O_4 and 3 g (17 mmol) of aminopropyltrimethoxysilane (APTS) were allowed to react in 70 mL of toluene under reflux conditions at 120 °C and under an Ar atmosphere for 17 h. The sample was

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then washed with 40 mL of toluene and 10 mL of a 1:1 diethylether and methanol solution. The functionalized material (APTS-SiO_2/Fe_3O_4) was finally dried for 2 h at 60 °C.

X-ray diffraction patterns were recorded using a Siemens D5000 powder X-ray diffractometer using Cu Kα-radiation in the range of $15° < 2\theta < 65°$ and a step size of 0.01°.

The specific surface area of the materials was determined by nitrogen adsorption at 77 K applying the BET model in the p/p_0 range of 0.05−0.3. Before adsorption the samples were dried under vacuum at 80 °C for 2 h.

ATR-IR spectra of the solid dry material were recorded in the range of 4000−400 cm^{-1} with a FT-IR spectrometer (Bruker Optics, Vertex 70) equipped with a commercial ZnSe ATR mirror unit (MVP, Harrick).

Combined thermal gravimetric analysis-mass spectroscopy (TGA-MS) experiments were carried out nonisothermally in 20 vol % O_2, balance He, with a constant heating rate of 10 K/min on a Netzsch STA 409 simultaneous thermal analyzer. The sample masses corresponded to approximately 30 mg. The gases evolved during the reaction were monitored online using an OmniStar (Pfeiffer) quadrupole mass spectrometer connected to the thermal[32] analyzer by a heated capillary (approximately 200 °C).

2.2. ATR-IR Reactor Cell. In situ ATR-IR measurements were performed with a home-built batch reactor cell (Figure 1). The cell is composed of a horizontal ZnSe internal reflection element (Crystran Ltd., 30°, 50 mm x 20 mm x 2 mm) serving as the infrared waveguide, the cell body (stainless steel) and a heatable support incorporating the optics (two Al mirrors, Newport). The cylindrical cell body (internal volume, 17.3 mL) was fixed on the support and was sealed against the ZnSe crystal using a Viton O-ring. The cell cap was equipped with a mechanical axial flow impeller (diameter = 2.15 cm, Büchi Glas Uster AG) allowing stirring speeds up to 3000 rpm, which was sealed against the cell body using a second Viton O-ring. Moreover, injection ports were mounted to allow admittance of gases and injection of liquids and a thermocouple was fixed through the cap to measure the temperature of the liquid inside the cell. The support of the cell was mounted within the sample compartment of an Equinox 55 spectrometer (Bruker Optics) equipped with a MCT detector cooled with liquid nitrogen and was flushed with dry air in order to avoid absorption from atmospheric CO_2 and water. The background of the spectrum was taken while mechanically stirring 1 g of amino-functionalized material in 10 mL of toluene at 1000 rpm. Subsequently, spectral acquisition started and benzaldehyde (0.1 mL) was added to the stirred reaction mixture. Spectra were collected at room temperature by coadding 100 scans at 4 cm^{-1} resolution and are reported in absorbance units.

3. Results and Discussion

3.1. Conceptual Approach. As mentioned in the introduction, solid-state characterization methods have serious limits when applied to mixed organic−inorganic materials such as functionalized nanoparticles. The fundamental information on the chemical behavior of organic moieties is basically lost. Our approach comprised the following steps: (i) preparation of functionalized nanoparticles; (ii) characterization with standard solid state methods, and (iii) in situ ATR-IR characterization with a tailored homemade reactor cell to evidence the set of new information that this technique can offer as compared to solid-state techniques.

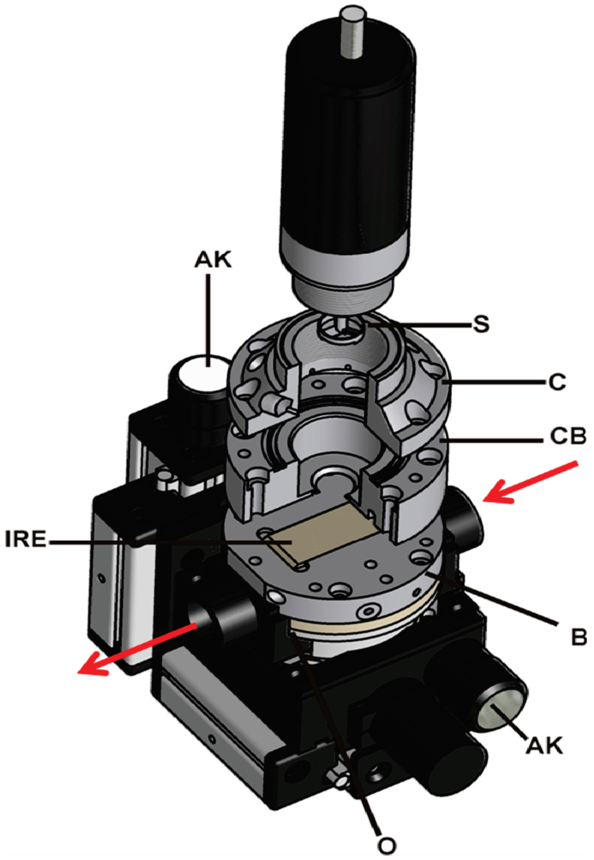

Figure 1. Schematic view of the in situ reactor cell which is mounted within the sample compartment of the FT-IR spectrometer. S, mechanical stirrer; C, stainless steel cap; CB, stainless steel cell body; IRE, ZnSe internal reflection element; B, heatable base; O, optics; AK, horizontal and vertical adjusting knobs. The red arrows represent the optical path of the IR radiation.

3.2. Qualitative Characterization of Functionalized and Unfunctionalized Nanoparticles Using Standard Methods. Figure 2 shows the XRD-diffraction pattern of (a) the pristine iron oxide nanoparticles, (b) the SiO_2/Fe_3O_4 sample, and (c) APTS-SiO_2/Fe_3O_4. Iron oxide nanoparticles (approximately 10 nm by TEM[40]) possess the diffraction patterns of the magnetic spinel structure magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), which are indistinguishable by XRD (Figure 2a).

After silica coating, the structure is maintained and an additional broad reflection appears at low 2θ values that originates from the amorphous silica matrix (Figure 2b). The additional functionalization with APTS does not induce any structural changes of the bulk material and the same reflections as for the unfunctionalized sample are observed (Figure 2c).

In contrast, the textural properties of the material are considerably changed by the presence of the functional groups and the specific surface area decreases from 95 m^2/g for SiO_2/Fe_3O_4 to 45 m^2/g for APTS-SiO_2/Fe_3O_4. Because the additional weight of the amino functional groups corresponds to only 7.7 wt % (see the next section), the large decrease in the specific surface area (more than 50%) is evidently caused by a real surface modification. Indeed, the pending groups can partly

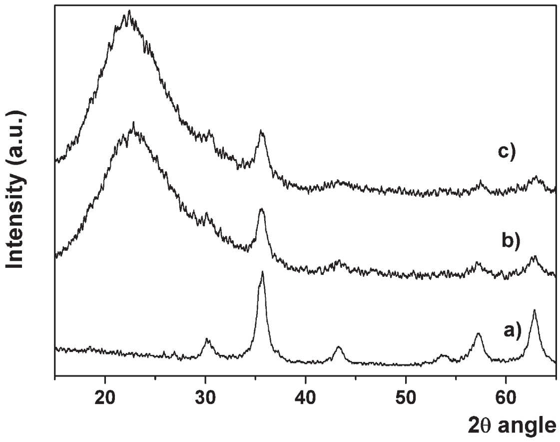

Figure 2. XRD pattern of (a) iron oxide, magnetite (Fe_3O_4), or maghemite (γ-Fe_2O_3), (b) SiO_2/Fe_3O_4, and (c) APTS- SiO_2/Fe_3O_4.

inhibit the adsorption of N_2 on the material and decrease the accessible surface area.

Figure 3 shows the IR-spectra of APTS-SiO_2/Fe_3O_4 and liquid APTS. In the modified solid the bands of the asymmetric and symmetric stretching modes of CH_2 groups at 2940 and 2858 cm^{-1}, respectively, indicate the presence of the aminopropyl chain attached to SiO_2/Fe_3O_4.[41] The signal at 1573 cm^{-1} can be assigned to the NH_2 bending vibration, which confirms the presence of amino terminal groups on the modified solid.[41] Typically, these vibrations are used to verify that the functionalization of the material with amino pending groups occurred successfully. In contrast to APTS, the two bands at 3360 and 3300 cm^{-1} of the N−H asymmetric stretching are too weak to be observed on APTS-SiO_2/Fe_3O_4.[42]

3.3. Quantitative Identification of Immobilized Functional Groups with Standard Methods and ATR-IR. The total amount of nitrogen atoms is determined using microelemental analysis. Under the approximation that all the N atoms contribute to functional groups (in our case an amine), the total amount of amino functional groups determined by microelemental analysis corresponds to 1.5 mmol/g. This approximation is often found in the literature concerning the preparation of functionalized nanoparticles.[42,43] This value does not necessarily correspond to the number of pending groups that are actually free to react. In fact, a part of the functional groups may not be accessible to other molecules because of steric reasons or might be trapped into the silica matrix. The determined value of 1.5 mmol/g therefore constitutes an upper bound to the possible loading in terms of chemically available amino groups.[20]

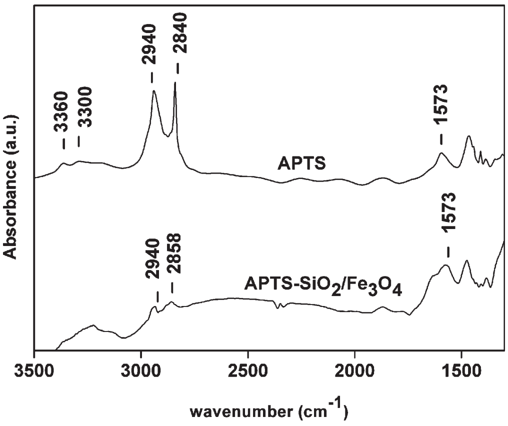

Figure 3. ATR-IR spectra of APTS (top) and of the solid APTS-SiO_2/Fe_3O_4 (bottom).

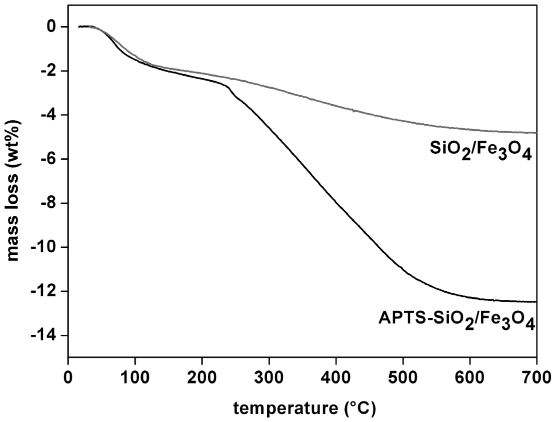

Figure 4. Mass changes versus temperature for SiO_2/Fe_3O_4 and for APTS-SiO_2/Fe_3O_4 recorded during heating in 20 vol % O_2, balance He. Conditions: heating rate 10 °C/min.

Figure 4 shows the thermogravimetric curves (TGA-MS) of SiO_2/Fe_3O_4 and of APTS-SiO_2/Fe_3O_4 in 20 vol % O_2. Both samples show an initial small mass loss at temperatures below 200 °C due to the desorption of adsorbed water and CO_2 as observed from mass spectrometry (m/z = 18, m/z = 44). At temperatures above 200 °C, the two samples show very different thermogravimetric curves due to the decomposition of the functional groups in APTS-SiO_2/Fe_3O_4. The difference in mass loss between the two samples, which is assigned only to the pending groups, corresponds to 7.7 wt %.

Under the assumption that all the methoxy groups were eliminated during grafting of APTS to the silica and that only the organic part of the linker contributes to the mass loss this value corresponds to 1.3 mmol/g, in fairly good agreement with the value obtained from the elemental analysis of nitrogen (1.5 mmol/g). As in the case of microelemental analysis, TGA-MS allows for an estimation of the upper bound of the total amount of functional groups, whereas no information is provided about their accessibility.[20]

The functionalized APTS-SiO_2/Fe_3O_4 nanoparticles will ultimately be used as a source of immobilized amines, and it is therefore of great relevance to determine the amount of aminic groups that can actually react as such in a chemical reaction. This value can be called "loading of chemically available functional groups", to differentiate it from the total loading that also comprises chemically unavailable organic moieties. We addressed this problem by performing a reaction between the APTS-SiO_2/Fe_3O_4

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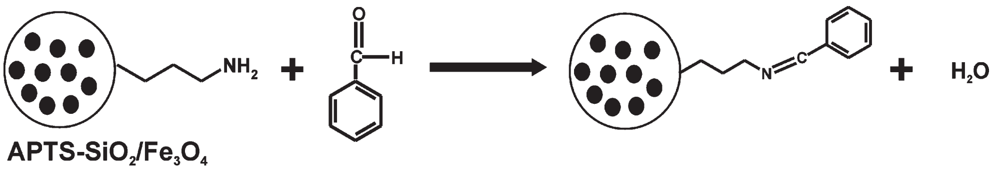

Figure 5. Condensation reaction between ATPS-SiO_2/Fe_3O_4 and benzaldehyde to form immobilized benzaldimine.

and a known amount of benzaldehyde to form the corresponding benzaldimine (Figure 5). Because benzaldehyde is clearly detectable with infrared spectroscopy by means of the intense C═O stretching band, we decided to quantify the amount of APTS-SiO_2/Fe_3O_4 by measuring the amount of benzaldehyde that had not reacted. For this purpose we proceeded in three steps: (i) First a calibration curve was established using solutions of benzaldehyde at different concentrations in toluene. In this curve, the integral of the carbonyl signal corresponds to a given concentration of benzaldehyde. Using the calibration curve, it was possible to quantify the amount of benzaldehyde in the reactor cell by integration of the C═O stretching band. (ii) In the second step, the amount of physisorbed benzaldehyde was determined by contacting a known amount of benzaldehyde with SiO_2/Fe_3O_4, the unfunctionalized material. This amount was 0.25 mmol/g. In this case, the loss of benzaldehyde compared to the introduced amount was attributed only to adsorption on the surface of the solid. (iii) In the final step, a known amount of benzaldehyde was reacted with 0.5 g of functionalized material APTS-SiO_2/Fe_3O_4 in 5 mL of toluene under vigorous stirring, and the reaction was allowed to proceed for 2 h. The aldehyde reacted readily with the immobilized amino group to form an imine that is easily recognizable from the stretch of the C═N bond of the benzaldimine group at ca. 1645 cm^{-1} (Figure 6).

The benzaldimino-functionalized SiO_2/Fe_3O_4 was magnetically separated from the liquid solution and the excess benzaldehyde was quantitatively determined by ATR-IR spectroscopy using the calibration curve determined in step i. The resulting loading of amino groups corresponded to (0.80 ± 0.08) mmol/g. From this value, we had to subtract the determined amount of aldehyde that was adsorbed on the support, as determined in step ii, thus resulting in a loading of chemically available amino groups on APTS-SiO_2/Fe_3O_4 of approximately (0.55 ± 0.08) mmol/g. This value was obtained under the assumption that the adsorption properties of benzaldehyde on SiO_2/Fe_3O_4 and APTS-SiO_2/Fe_3O_4 are comparable.

It is striking that the number of chemically available functional group is less than 40% of those determined by measuring the content of atomic nitrogen of the material. The large deviation between the two methods indicates that chemical analysis or thermal gravimetry can be used only to determine an upper bound of the amount of amino moieties that are actually available for reaction.

This result is in good agreement with other methods used recently to quantify the accessible functional groups on modified silica surfaces. Hodgkins et al. showed that the amount of accessible functional groups in a surface modified mesoporous silica containing magnetite corresponded to only 56% of the total amount, underlining the importance of using specific quantification methods for accessible functional groups.[44] Similarly, Rosenholm and Linden show a considerable discrepancy between the total and the accessible number of functional groups for a series of amino-modified mesoporous silicas.[20] They determined the accessible organic groups by imine formation between the amino functionalized material and 4-nitrobenzaldehyde. The imino group on the surface of the silica was then hydrolyzed and the regenerated nitrobenzaldehyde was determined by UV−vis spectroscopy. These results show that specific techniques sensitive to the accessible functional groups are necessary for the determination of the number of functional groups that are responsible for the reactivity of the material.

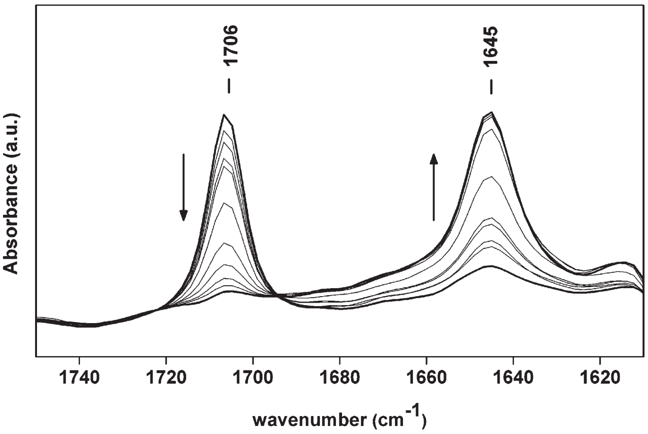

Figure 6. In situ ATR-IR spectra of APTS-SiO_2/Fe_3O_4 reacting with benzaldehyde in toluene measured in the batch reactor cell. Conditions: 25 °C, 1 g APTS-SiO_2/Fe_3O_4 /10 mL toluene, 1000 rpm.

3.4. Monitoring of the Reactivity of Immobilized Functional Groups. In the previous section we have demonstrated that accurate quantification of chemically available functional groups attached to a solid can be determined spectroscopically. The same approach can be used to follow the test reaction shown in Figure 5, a condensation between the aminopropyl group attached to the surface of the magnetic solid material and benzaldehyde to form the corresponding benzaldimine. This was done by monitoring the integral of fingerprint signals of the reactants and of the products. In the present case, we have chosen to monitor the stretching vibration of the carbonyl moiety of the benzaldehyde and that of the immobilized imine product. It should be underlined that to the best of our knowledge no other technique allows monitoring in such detail the

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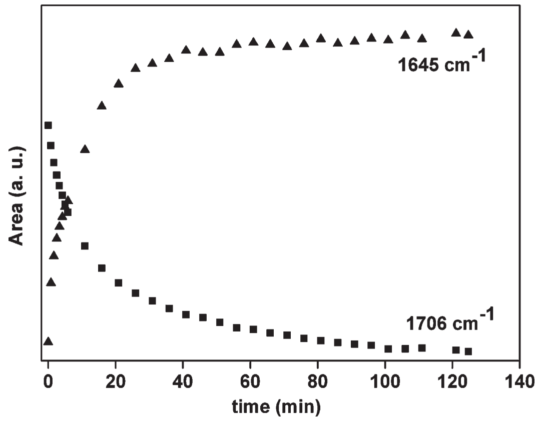

Figure 7. Rate of formation of the immobilized benzaldimine on SiO_2/Fe_3O_4 (triangles) and of disappearing benzaldehyde (squares) in solution. Conditions: 25 °C, 1 g APTS-SiO_2/Fe_3O_4 /10 mL toluene, 1000 rpm.

reactivity of immobilized functional groups in situ. The ATR-IR spectra of the reaction mixture containing APTS-SiO_2/Fe_3O_4 and benzaldehyde in the range 1750−1610 cm^{-1} are shown in Figure 6. The band at 1706 cm^{-1} is clearly assigned to the C=O stretching vibration of the benzaldehyde in solution. During reaction this band decreases until complete disappearance after approximately 2 h. The prominent band at 1645 cm^{-1} shows the opposite trend and increases during reaction. This signal corresponds to the ν(C=N) mode of the benzaldimine product. It should be stressed that this signal belongs to a functional group attached to the solid that is stirred during the measurement. Hence the reactivity of the amino-functional group grafted to the solid is directly monitored in situ under real reaction conditions. The signal at 1645 cm^{-1} corresponds to that fraction of functionalized powder that owing to the vigorous stirring circulates through the small volume (few micrometer thickness) above the ZnSe crystal monitored by the infrared radiation, as defined by the penetration depth.[45]

The formation of the benzaldimine group immobilized on the magnetic SiO_2/Fe_3O_4 can be quantitatively followed by integrating the signals at 1645 cm^{-1} (fingerprint of benzaldimine) and 1706 cm^{-1} (fingerprint of benzaldehyde), as shown in Figure 7. The former decreases at the same rate as the latter increases.

To test that a truly heterogeneous reaction occurred and that no pending groups leached from the solid during the reaction, the solid was magnetically separated from the solution after 2 h of reaction and washed several times with toluene. The reaction solution was analyzed by gas chromatography and did not show any presence of benzaldimine.

The magnetically separated and washed material was analyzed by ATR-IR spectroscopy of the solid. Figure 8 shows the resulting spectrum compared to that of solid APTS-SiO_2/Fe_3O_4. Both show a strong contribution from the ν(Si−O−Si) vibration at 1075 cm^{-1} since the materials mainly consist of SiO_2. The spectrum of the functionalized solid after reaction with benzaldehyde

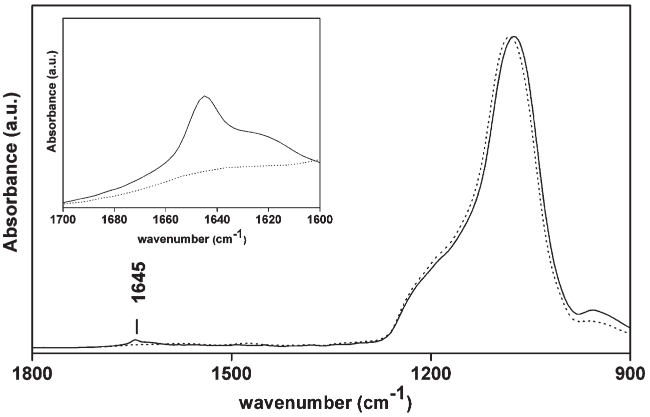

Figure 8. ATR-IR spectra of dry pristine APTS-SiO_2/Fe_3O_4 (dotted curve) and of the corresponding dry solid after reaction with benzaldehyde. The two spectra were normalized to the maximum intensity of the band at 1075 cm^{-1}. The inset shows an enlargement in the region 1700−1600 cm^{-1}.

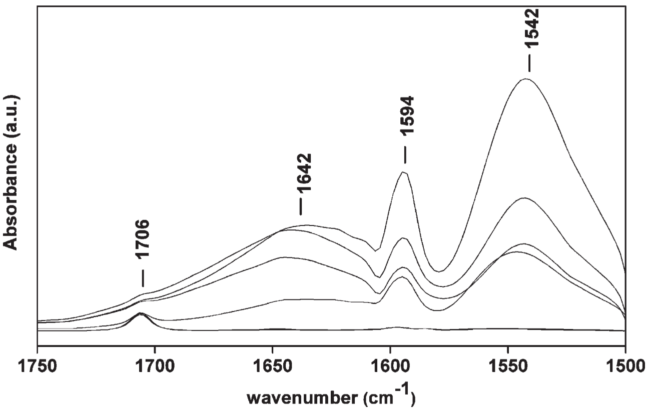

Figure 9. ATR-IR spectra of propylamine reacting with benzaldehyde in toluene with formation of H_2O. Conditions: 100 μL benzaldehyde, 66 μL propylamine, 10 mL toluene, 25 °C, 1000 rpm.

additionally shows a small band of the imine at 1645 cm^{-1} that is not present in the pristine amino-functionalized sample. These results give the evidence that formation of benzaldimine observed by ATR-IR spectroscopy in the batch reactor cell takes place exclusively at the functionalized solid surface and that no leaching occurs. The preceding results show that ATR-IR spectroscopy coupled with a batch reactor system allows the direct study of reactions taking place at functionalized nanoparticles.

For a matter of comparison, the homogeneous reaction between benzaldehyde and propylamine could also be followed with the ATR-IR batch reactor cell (Figure 9). If the reaction is performed in the absence of a water scavenger, a broad signal corresponding to the bending mode of H_2O is observed at ~1642 cm^{-1} that covers the signal of the imine. In fact, during the reaction, water is formed and deposits on the ZnSe crystal, disturbing the spectrum. Additionally, three strong bands at 1594, 1542, and 1386 cm^{-1} are observed that are due to carboxylates of the benzoic acid, which is present as an impurity of benzaldehyde and dissolves in the water droplet.

This effect is not observed when the amino-functionalized material is used, indicating that APTS-SiO_2/Fe_3O_4 acts as a water scavenger, thus naturally promoting

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(45) Harrick, N. J., *Internal Reflection Spectroscopy*; Interscience Publishers: New York, 1967.

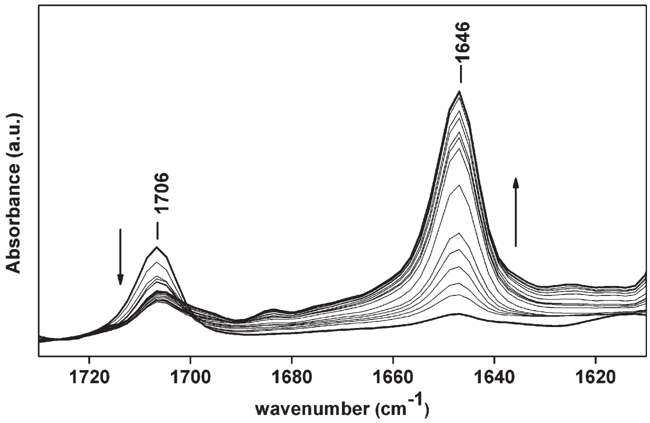

Figure 10. ATR-IR spectra of propylamine reacting with benzaldehyde in presence of SiO_2/Fe_3O_4 as water scavenger. Conditions: 100 μL of benzaldehyde, 66 μL of propylamine, 1 g of SiO_2/Fe_3O_4, 10 mL of toluene, 25 °C, 1000 rpm.

condensation reactions. Hence, to monitor the homogeneous reaction using ATR-IR spectroscopy, we added 1 g of unfunctionalized SiO_2/Fe_3O_4 to the reaction mixture to act as water scavenger (Figure 10). In this case, signals of water were not observed and the formation of the benzaldimine could be followed in situ by the increase of the ν(C=N) mode at 1646 cm^{-1} and by the disappearance of the C=O stretching vibration at 1706 cm^{-1}.

The experiments here presented were repeated by using a lower amount of APTS-SiO_2/Fe_3O_4 (0.2 and 0.1 g, respectively, in 10 mL of solvent) in order to test the sensitivity of the measurement and in both cases the above results could be neatly reproduced.

The technique presented here could provide access to the in situ analysis of a much wider scope of functional groups, including functional groups immobilized on nonmagnetic nanoparticles, which have been shown to be applicable in the catalysis of complex reactions.[46,47]

4. Conclusions

Amino-functionalized silica-coated magnetite nanoparticles (APTS-SiO_2/Fe_3O_4) were prepared and characterized with the conventional techniques (TG-MS, microelemental analysis, FT-IR spectroscopy) applied to study surface-modified magnetic materials. The same material was also studied using ATR-IR spectroscopy in a batch reactor cell developed ad hoc for magnetic nanoparticles. This technique was used both to quantify the extent to which functionalization of the nanoparticles had occurred in terms of chemically available organic groups, and to follow their reactivity toward imine formation in situ.

A great discrepancy was observed between the estimation of the total amount of functional groups determined by the classical solid-state methods and the real number of pending groups chemically available for reaction, because not all the organic material attached to the solid was accessible for reactivity. We showed that ATR-IR spectroscopy has the great potential to study the reactivity of these pending groups. Compared to previously applied techniques that at best allow qualitative determination of the presence of pending groups, here the real reactivity of these groups was observed in situ. Both the formation of a new bond directly at the functional groups as well as the disappearance of the reactant in liquid solution could be monitored at the same time. With this method, we provided a tool to characterize the potential reactivity of mixed organic–inorganic nanoparticles, under real reaction conditions for chemical applications. This technique can greatly contribute to the understanding of the organic chemistry of functional groups immobilized on nanosized inorganic structures and to their application in real world problems such as catalysis and biomedicine.

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(46) Al-Hashimi, M.; Qazi, A.; Sullivan, A. C.; Wilson, J. R. H. *J. Mol. Catal. A: Chem.* **2007**, *278*(1−2), 160–164.

(47) Al-Hashimi, M.; Sullivan, A. C.; Wilson, J. R. H. *J. Mol. Catal. A: Chem.* **2007**, *273*(1−2), 298–302.