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Magnetic Iron Oxide Nanoparticle Functionalization: Isocyanate Moiety as a Suitable Monodentate Anchoring Group

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Supporting Information

ABSTRACT: A new strategy for anchoring organic molecules onto superparamagnetic iron oxide nanoparticles (SPIONs) using isocyanate containing linkers has been realized. This functional group easily and efficiently reacts with the hydroxyl residues of the nanoparticle surface, leading to the formation of a stable carbamate bond, as confirmed by means of spectroscopic and analytical data.

Nanoparticles (NPs) are chemical materials of great scientific interest because they can represent a bridge in terms of reactivity and properties between bulk materials and molecules or structures at the atomic level.[1]

Superparamagnetic iron oxide nanoparticles (SPIONs),[2] in particular those with diameters less than 20 nm, have a size comparable to that of biologically important objects and are therefore particularly appealing and promising for a wide range of biomedical applications, such as cell labeling, imaging, tracking, and as carriers.[3] Their small dimension is also responsible for their unique magnetic properties because the size of the nanoparticles coincides with the size of a magnetic domain in bulk magnetic materials.[4] In addition, they have chemically tailorable physical properties, unusual and useful target binding features, and joint to structural robustness.[5]

The application of SPIONs in biomedecine or catalysis usually requires the nanoparticle to be coated with appropriate organic or bioorganic molecules. However, the successful conjugation of organic molecules onto NPs depends on a proper surface modification, and the functionalization strategies frequently use a bifunctional linker as a spacer between the NP and the molecule. One of the two functional groups of the linker is used to support it on the NP, while the other one is used to bind the organic molecule through different types of reactions such as condensations, cycloadditions, or Michael conjugate additions.[6] Taking into account that naked SPIONs expose on their surface hydroxyl groups or iron atoms, the general strategies followed to load organic molecules involve direct passive noncovalent adsorption on the outer particle surface (for instance, by a carboxylate moiety)[7] or the formation of a more stable covalent bond by using, usually, a trialkoxysilane group.[8] As far as we know, no other methods are reported to form a covalent bond between a naked NP and an organic molecule, and therefore, complementary and alternative surface modifications are highly desirable in order to broaden the scope and potentiality of the NP−organic molecule assembly.

In this paper, we describe the use of the isocyanate moiety as a suitable functional group for the anchoring of organic molecules to SPIONs of maghemite (γ-Fe_2O_3); as a result of this interaction, a stable carbamate-like bond between the isocyanate and the hydroxyl groups of the nanoparticle is formed. To the best of our knowledge, no other examples of functionalization of SPIONs with such a functional group has so far been reported.[9]

We have prepared a small library of new nanoconjugates **1**−**6** (Figure 1) by reacting SPIONs with the commercially available isocyanates **7**−**11** or with the isocyanate **12** which can be easily synthesized.[10]

In particular (Scheme 1), the proper isocyanate was added to a slurry of commercially available naked maghemite (γ-Fe_2O_3, average diameter of 10 ± 2 nm) nanoparticles in dry toluene, under sonication at 60 °C for 4 h. The resulting suspension was then centrifuged at 5000 revolutions/min for 10 min; the supernatant solution was eliminated, and the solid residue was washed with fresh toluene and diethyl ether affording the desired nanoconjugates **1**−**6**. Functionalized SPIONs **1**−**6** were characterized by means of elemental analysis, infrared, and ^{1}H and ^{13}C high-resolution magic angle spinning (HR-MAS)

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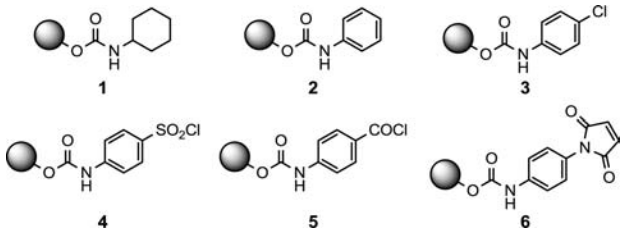
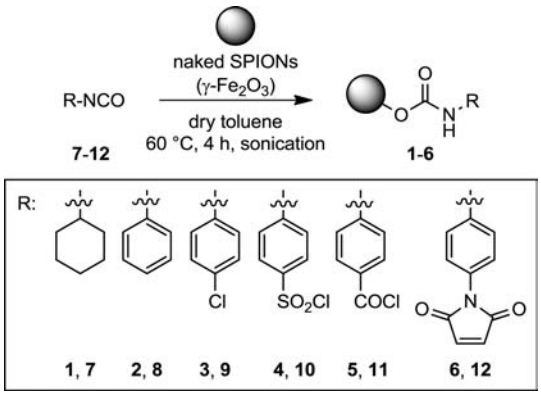


Figure 1. Synthesized nanoconjugates **1**–**6**.

Scheme 1. Procedure for the Synthesis of Nanoconjugates 1–6



NMR spectroscopy, which confirmed the presence of the linker on the nanoparticle. Furthermore, all of the spectroscopic characterizations evidenced the presence of the carbamate bond and the absence of the isocyanate group.

Concerning FTIR, for all of the nanoconjugates **1**–**6** the strong Fe–O absorption from about 650 to 500 cm^{-1} is clearly visible, while the typical strong band of isocyanate group stretching (generally from 2300 to 2250 cm^{-1}) is absent.

On the contrary, all of the acquired spectra for **1**–**6** show a peak in the range 1699–1626 cm^{-1} due to C=O stretching of the new carbamate group, originating from the interaction of the isocyanate moiety with the OH functions on the SPION surface (see Table S5, Supporting Information). The presence of iron justifies the found frequencies which are at lower energy if compared with classical organic carbamates.[11] Moreover, all the vibrational peaks related to the presence of the organic residue (cyclohexyl, phenyl, or maleimidophenyl rings), are also present. Thus, FTIR spectroscopy was crucial to point out that the interaction between the isocyanate function and nanoparticles results in the formation of a carbamate bond.

Nanoconjugates **1**–**3** and **6** were also characterized by HR-MAS NMR spectroscopy.[12] Indeed, it was already shown by some of us[13] that this technique could be a powerful tool not only facing the chemical shift anisotropy problems, but also reducing the artifacts related to the presence of the superparamagnetic iron oxide core. The important result is the elaboration of nicely resolved NMR spectra with well structured signals which allow the structural characterization of different organic ligands bound to SPIONs.

Nanoconstruct **2** was chosen as a representative compound, and ^{1}H and ^{13}C NMR spectra are shown for structure description (Figures 2 and 3, respectively).

In the ^{1}H NMR spectrum (Figure 2) the NH signal is clearly visible. Its chemical shift value (8.6 ppm) is consistent with the chemical structure of a carbamate bond. In the ^{13}C NMR

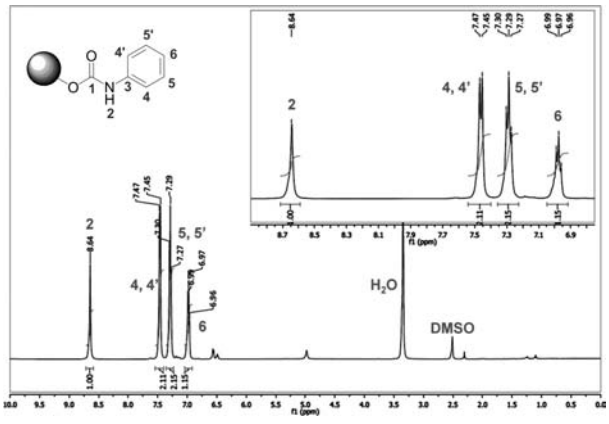


Figure 2. ^{1}H HR-MAS NMR spectrum of nanoconjugate **2**.

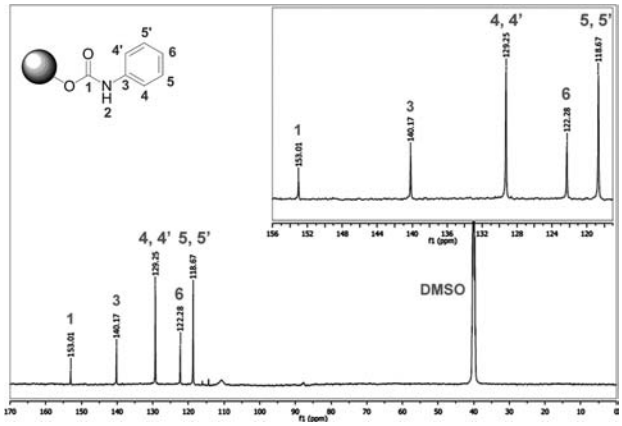


Figure 3. ^{13}C HR-MAS NMR spectrum of nanoconjugate **2**.

spectrum (Figure 3) the signal at 153.0 ppm is clearly associated with the carbamate C=O quaternary group and also chemical shift values of aromatics protons and carbons are in agreement with the proposed chemical structure.

In order to verify the possibility of modulating the loading of the isocyanates onto the SPIONs, three sets of experiments were performed, using increasing amounts of compounds **7**–**12** (0.2, 0.4, or 0.8 mmol respectively) in reaction with 100 mg of naked SPIONs. The elemental analysis of nanoconjugates **1**–**6** obtained from these experiments allowed us to determine the loading of the functionalized SPIONs (Table 1), which ranged between 14% and 52%, depending on the amount of isocyanate used (see also Tables S1–S3, Supporting Information).

It is worthy to note that, as the molar ratio of the NCO increases, the final loading also increases with a good linearity thus proving that it is possible to tune the amount of the organic molecule anchored onto the SPION surface (Figures S1–S3, Supporting Information).

An interesting aspect we observed during this study was the chemoselectivity of the reaction between SPIONs and the isocyanates **10** and **11** bearing a second functional group in principle reactive toward hydroxyl moieties, namely the chlorosulphonyl and acyl chloride. In fact, the attainment of nanoconstructs **4** and **5** assesses the preferred isocyanate–nanoparticle OH interaction with respect to the one with SO_2Cl and COCl. Only two examples have been reported in which a chemoselective reaction of compound **10** with alcohols was performed at room temperature, and in one case the dibutyltinlaurate was used as a catalyst.[14] In our case, such a

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Table 1. Organic Loading Efficiency in Nanoconjugates 1−6 at increasing isocyanate/SPION ratio

nanoconjugate	RNCO	loading[a] (%) entry A[b]	entry B[b]	entry C[b]
1	**7**	13.83	26.69	51.47
2	**8**	15.36	26.55	43.80
3	**9**	16.62	29.83	40.33
4	**10**	17.54	19.74	21.75
5	**11**	22.17	25.74	35.01
6	**12**	14.42	31.07	51.81

[a]Loading was determined by elemental analysis as total percentage of the organic residue anchored onto SPIONs (see the Supporting Information for more details). [b]Entry A, B and C correspond to the experiments perfomed with 0.2, 0.4, and 0.8 mmol of RNCO/100 mg of SPIONs, respectively.

selectivity is maintained even performing the reaction at 60 °C under sonication, and is verified also with the benzoyl chloride **11**. The lacking of reactivity of sulphonyl and acyl chloride groups with SPIONs came from two experiments we carried out, in which tosyl and benzoyl chloride were treated with SPIONs in the previously described reaction conditions: in both cases no loading of the organic molecule was observed on the NP surface. The possibility of exploiting the SO_2Cl and COCl groups present on the nanoparticles **4** and **5** for further functionalization with an organic molecule was investigated by performing a test reaction between them and the *n*-butylamine. From these reactions we isolated the new nanoparticles **13** and **14** (Figure 4), whose structure was confirmed by means of elemental analysis and FTIR spectroscopy.

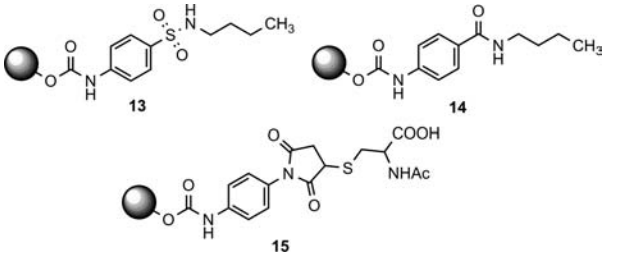


Figure 4. Nanoconjugates **13**−**15** synthesized exploiting the reactivity of the second additional functional groups of **4**−**6**.

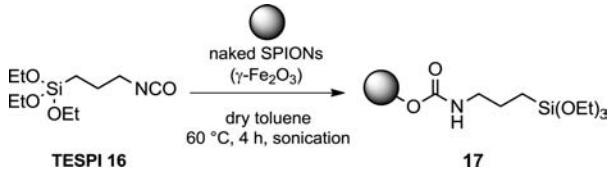
Moreover, when the surface was coated with a Michael acceptor unit, as in the case of nanoparticle **6**, the reaction with *N*-acetylcysteine gave the corresponding Michael adduct **15**. (Figure 4, see also the Supporting Information).

Even more interesting was the selectivity we found in the reaction between the triethoxysilylpropylisocyanate (TESPI) **16** and the naked SPIONs: surprisingly, in this case the isocyanate moiety of **16** proved to be more reactive than the triethoxysilane one, and, as a result, we obtained the corresponding nanoconstruct **17** coated with triethoxysilyl groups (Scheme 2).

Even in this case, the elemental analysis and FTIR performed on **17** confirmed, without any doubt, the formation of the carbamate bond between the NP and the TESPI as well as the presence of the unreacted triethoxysilane moiety.

In conclusion, we have established a new methodology for the straightforward covalent anchoring of organic molecules onto SPIONs through the use of linkers containing an isocyanate group, which easily and efficiently reacts with the

Scheme 2. Synthesis of Nanoconjugate 17



hydroxyl residues of the nanoparticle surface, leading to the formation of a stable carbamate bond. When the linker is endowed with a second functional group such as a sulphonyl chloride, acyl chloride or triethoxysilane, an unexpected and advantageous chemoselectivity is observed: in these cases, the isocyanate moiety reacts with the SPION providing nanoparticles covered with sulphonyl, acyl or triethoxysilane groups as chemical handles suitable for further conjugation with organic molecules. We have also assessed that the loading can be easily tuned by varying the ratio between the linker and SPION. HR-MAS NMR spectroscopy proves, once again, a very powerful tool for the structural characterization of different organic ligands bound to SPIONs.

We strongly believe that this new surface modification method could represent a general tool for the hierarchical building of a covalent organic molecule-SPION assembly, not only for the obtainment of nanoconstructs for biomedical applications but, more in general, for achieving modified magnetic nanoparticles functionalized with specific components such as for example catalytic species. Further studies in this area, such as the use of this methodology for the peptide nucleic acids anchoring on nanoparticles or the utilization of bis-isocyanate linkers, are under active investigation and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis of **1**−**6**, **13**−**15**, and **17**, determination of loading, and infrared and HR-MAS NMR spectroscopic studies. This material is available free of charge via Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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