Simultaneous characterization of protein coated iron oxide nanoparticles with nuclear inelastic scattering and atomic force microscopy

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Abstract Bovine serum albumin coated magnetic iron oxide nanoparticles (IONPs), which were synthesized using a co-precipitation method with $^{57}$Fe have been subject to a combined study using atomic force microscopy (AFM) and nuclear inelastic scattering (NIS). The obtained partial density of vibrational states (pDOS) shows evidence for lattice stiffening and a pronounced mode at 23 meV compared to thin film magnetite at room temperature.

Keywords Nuclear inelastic scattering · Iron oxide nanoparticles · Atomic force microscopy

1 Introduction

Magnetic iron oxide nanoparticles (IONPs) are interesting for medical as well as for industrial application, as they show superparamagnetism in small nanoparticles (<30 nm) and thus can be separated easily in a magnetic field gradient [1]. Neverthe-
less there are still challenges to obtain biocompatible, water-soluble, superparamagnetic, long-term stable nanoparticles. A promising way for the production of such nanoparticles is the coating of iron oxide nanoparticles with proteins like bovine serum albumin (BSA) after their synthesis via the coprecipitation method [2]. The IONPs produced with this method consist mainly of maghemite, as earlier findings showed [3].

As the properties of nanoparticles depend strongly on their size and shape, the combined usage of atomic force microscopy (AFM) and nuclear inelastic scattering (NIS) is promising to characterize nanoparticles and their size-dependent dynamics properties.

A possibility to make simultaneous NIS and AFM measurements has been recently introduced at P01, PETRA III, Hamburg in 2012 [4]. Here we present as a first application of this new sample environment a combined AFM/NIS study of BSA-coated $^{57}\text{Fe}$ IONPs.

## 2 Materials and methods

Nanoparticles were synthesized by co-precipitation using Massart’s method [2], with 1 part $^{57}\text{FeCl}_2$ and 2 parts $^{56}\text{FeCl}_3$. Bovine serum albumin was purchased from Carl Roth GmbH and BSA-coated IONPs were prepared according to the procedure described in [3]. A drop of protein coated IONPs was deposited on an especially thinned silicon support and dried on air before further measurements (see Fig. 1) the preparation of which is described in [4].

AFM pictures were obtained in contact mode with a Nanos AFM head attached to a Senterra Raman-Microscope (Bruker) using PR-CO10 cantilevers with a tip radius of 7 nm. The microscope was installed as described in [4], in a way that allowed simultaneous measuring of the topography with AFM and taking NIS data by placing the focused synchrotron beam on the sample next to the cantilever. The spot where the NIS data were taken and the area of the AFM area were in close proximity. AFM data processing was done with SPIP (Image Metrology) and Gwyddion (Czech Metrology Institute), including line correction and mean plane subtraction.

NIS data were recorded at room temperature at the Dynamics Beamline P01 at PETRA III, DESY under experiment number I-20110655. PETRA III was operated in 40 bunch mode with a bunch spacing of 192 ns. Two sets of monochromators, a high heat-load monochromator followed by a high resolution monochromator provided a photon flux of about $10^{10}$ photons per second in a bandwidth of 1 meV around the $^{57}\text{Fe}$ resonance energy of 14.4125 keV. The data were taken at room temperature within an energy range from $-40$ to 60 meV with respect to the elastic line in steps of 0.25 meV. The measuring time was 4 s per scan point. The detector was an avalanche
Fig. 2  pDOS of the BSA-coated IONPs measured at room temperature (a). For comparison, the pDOS of magnetite thin-film at room temperature (b) and 120 K (c) taken from [4] and the pDOS of magnetite / maghemite nano powder at room temperature (d) taken from [5] are shown.

The partial density of vibrational states (pDOS) obtained from the NIS data is shown in Fig. 2a. The first band shows a splitting, resulting in two broad peaks at 18 meV and 23 meV, with shoulders at 13.5 meV, 16.5 meV and 19.5 meV. A second broad band is seen from 35–50 meV. The maximum of the broad peak of the higher energy is at about 44 meV.

The pDOS shows a notable similarity in the low energy range from 15 meV to 25 meV to the pDOS taken from [5] (see Fig. 2c) for magnetite thin-films at much lower temperatures (~120 K). For a better comparison, also the pDOS for magnetite thin-films at room-temperature taken from [5] (see Fig. 2b) and for nanoparticles consisting of mixtures of maghemite and magnetite, prepared by inert gas condensation taken from [6] (see Fig. 2d) are shown. The peak at 23 meV, presumably a transverse optical mode, which was proposed to be related to that below the Verwey-Transition (= 120 K) [5, 7] is significantly stronger than that at room temperature in thin film magnetite. This could also be due to a maghemite mode (as seen on Fig. 2d), but the iron oxide nano powders from [6] don’t show the splitting of the low energy band. Remarkably, thin film magnetite showed a similar, but smaller shift of this peak at cooler temperatures (~120 K), which was discussed.
to be due to lattice stiffening [5]. The high energy part from 35 meV to 50 meV is also shifted to higher energies (about 8 meV) compared to previous measurements at room temperature [5, 6]. In our measurements, this could be interpreted as lattice stiffening due to internal lattice strain, changing the lattice dynamics in nanoparticles [8] in a way that would resemble dynamics in bulk magnetite at lower temperatures.

Figure 3 shows the AFM height picture (a) with two cross sections that are shown in (b) and (c), as well as a 3D view of the height profile (d). The data show a surface composed of small peaks with a height of 5–12 nm and several higher peaks. As the total concentration of the iron ions was about 70 μmol/ml in the suspension and therefore is also high in the resulting nanoparticle suspension, and because the silicon substrate itself has a rms roughness of below 1 nm, it is assumed that the small peaks seen on the image represent a layer of aggregated nanoparticles and the larger peaks are higher aggregates of stacked nanoparticles. Assuming a dense packing of the spherical nanoparticles, only the upper half of the nanoparticles that can be reached by the tip would be seen in the AFM picture, resulting in particle diameters of more than 10–24 nm. As the z-resolution (<1 nm) is better than the x and y resolution
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(∼20 nm for 10 μm scan size), only the z-range was used to estimate the particle size, and it is concluded that the larger peaks of about 100 nm on the surface were no single nanoparticles, but stacks of agglomerated nanoparticles, as the average size determined by TEM was 12 nm [3], and the BSA coating could be seen as a shadow of approximately 30 nm.

In conclusion we were able to demonstrate that a parallel measuring of a nanoparticle covered sample and NIS data with synchrotron radiation is possible. The minimal resolvable nanoparticle size depends upon the tip geometry, so a size determination is only possible for nanoparticles with a particle size that is bigger than the tip radius, but to check the surface coverage and the roughness of the sample surface, the technique is a very helpful tool.

Another conclusion we were able to draw from the results was that the NIS data of the iron-oxide nanoparticles differed significantly from that of thin-film magnetite. A shift in a mode at 23 meV, which we were able to see at room temperature was the most evident change, and a general shift to higher energies compared to magnetite at room temperature could be seen, leading to the assumption that the lattice of the nanoparticles has a higher rigidity and the dynamics in the lattice resemble those of bulk material at lower temperatures.

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References