1. Introduction

In this overview it is intended to show with two examples, how the application of a combination of powerful characterisation techniques can improve our chemical and microstructural knowledge of nanomaterials.

On one hand microscopy techniques will be used: Classical transmission electron microscopy (TEM), high resolution TEM (HRTEM) and electron diffraction (ED) will give the structural information. Energy filtered TEM (EFTEM) is giving compositional maps\(^1\) with a very high lateral resolution (1 nm).

On the other hand the use of spectroscopic techniques sensible to chemical composition and chemical state will be also emphasized: X-ray photoelectron spectroscopy (XPS) for surface analysis and X-ray absorption spectroscopy (XAS) for bulk analysis. In the case of XAS also structural information can be obtained for amorphous and nanocrystalline materials.\(^2\) Special attention will be paid to the use of electron energy loss spectroscopy (EELS) in the TEM microscope what is a good tool for connecting spectroscopy and microscopy. In this case information on structure, chemical composition and chemical state could be obtained\(^3\) with a very high lateral resolution, conventionally up to 1 nm resolution.

Two examples of metal particles nanostructures will be described in this overview to illustrate this study: i) Nickel nanoparticles embedded in an amorphous carbon matrix for the combined use of TEM, ED, EELS, XPS and XAS analysis. ii) Gold nanoparticles functionalised with neoglycoconjugates molecules to illustrate the combined use of TEM and EFTEM analysis.

2. Experimental

Nickel nanoparticles encapsulated in carbon were obtained by a sonication method. A 0.02 kmol m\(^{-3}\) solution of Ni(COD)\(_2\) (COD: cyclo-octadiene) in toluene was sonicated for 5 hours under an Ar pressure of 1.5 \(\times\) \(10^5\) Pa. The obtained powder (as-prepared sample) was also heated at 773 K for 20 hours under a flow of pure Ar (heated sample). For further details and characterisation of these samples see Ref. 4) and 5).

The gold glyconanoparticles were obtained by “in situ” functionalisation of gold nanoparticles following the procedure of Brust \textit{et al.}\(^6\) for the synthesis of monolayer protected gold nanoclusters. For further details and characterisation of these samples see Ref. 7) and 8).

The microstructural characterisation and the microanalysis were performed using a variety of microscopes and associated techniques that are described bellow. The TEM samples were dispersed in ethanol by ultrasound and dropped on a copper grid coated with a carbon film.

(1) PHILIPS CM200 with a supertwin objective lens, working at 200 kV with a LaB\(_6\) filament and \(\pm 45^\circ\) tilt side-entry specimen holder (point resolution = 0.24 nm). The instrument is equipped with a heating holder, an EDX detector (EDAX) and a PEELS spectrometer (GATAN 766-2K).

(2) JEOL 2010 FEG operating at 200 kV, probed size between 0.5 and 2.4 nm, point resolution ca. 0.19 nm. The instrument is equipped with an EDX detector (Oxford Link) and a PEELS spectrometer (GATAN 766-2K).

(3) ZEIS CEM 902 with a Castaign-Henry magnetic-sector/electrostatic-mirror and a Kontron image analysis system\(^9\) to produce energy-filtered images. EELS spectra at the C-K, O-K and Ni-L\(_{2,3}\) edges were

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recorded in the diffraction mode. A low-loss spectrum was also recorded with each edge in the same illuminated area and using the same experimental conditions. After the subtraction of the background with a standard power-law function, the spectra were deconvoluted for plural scattering with the Fourier ratio method. All these treatments were performed within the EL/P program (Gatan).

In order to collect energy-filtered images, and to obtain elemental mapping and EELS spectra from small areas, the method called “Imaging-spectrum” was used. By this method a series of images (256 × 256 pixels) are recorded with a given energy step between each image. Once the series was recorded, it is possible to select an area of interest of a single pixel, the computation process reads the grey level for each selected pixel of each image and plots a curve, energy loss vs. grey level, resulting in the corresponding EELS spectrum of the selected area. Elemental maps can be also obtained by choice of appropriate images and background calculation procedures.

XAS spectra were recorded at the beam line BM29 in the ESRF storage ring in Grenoble running at 6 GeV, with an average current of 165 mA. The detection of the XAS absorption coefficient was in the transmission mode for pressed pellets of the samples diluted in boron nitride. Spectra were recorded at the Ni K-edge at ca. 8330 eV. The EXAFS (extended X-ray absorption fine structure) oscillations were analysed with the software package developed by Bonin et al. The coordination numbers (N), distances (R) and Debye Waller factors (σ) were extracted by a least-squares fitting procedure that uses the theoretical phases and amplitudes proposed by Rehr and coworkers previously calibrated with the appropriate reference (Ni foil).

X-ray photoelectron spectroscopy analysis was carried out in a VG210 ESCALAB instrument operated with AlKα radiation in the ΔE = 50 eV constant mode.

3. Results and Discussions

3.1 Nickel nanoparticles encapsulated in carbon.

Figure 1 shows that the as-prepared sample is formed by spherical amorphous particles of ca. 100-150 nm. The composition of these particles was determined by EDX showing the presence of nickel, carbon and oxygen with the nickel phase highly dispersed in a carbon matrix. A TEM study of the heated sample (Fig. 2) shows, however the formation of small nanocrystallites inside the spherical particles. While some spherical particles show an average size for the nanocrystals of 2-3 nm (particles A) others showed a size of 10-20 nm (particles B) for these nanocrystals. The selected area electron diffraction pattern can be indexed to metallic cubic nickel with no evidence of nickel oxide or carbide crystals formation. The high resolution TEM micrograph in Fig. 2 shows the small metallic nickel crystallites and the amorphous carbon matrix. A lattice distance is indexed in nickel nanoparticles along the [111] zone axis.

To obtain more information about the oxidation state of the nickel phase, the XPS spectra for C1s, Ni2p, O1s and NiLVV Auger peaks were recorded for the as-prepared and heated samples (Fig. 3). The main Ni peaks in the as-prepared material are the Ni2p1/2 peak at 855.7 and the Ni2p3/2 at 873.4 eV, as well as their corresponding shake-up resonances at 861.4 and 880.0 eV. These peaks are assigned to oxidised nickel in a dispersed phase. The XPS technique probes mostly the surface of the particles, and it is expected that a highly dispersed nickel phase mixed with carbon and exposed to air will appear oxidised at the surface. On the other hand, the heating of the sample reveals a new peak at 853.0 eV, in addition to the 2p1/2 855.7 eV peak and the shake-up resonance at 861.4 eV. This additional peak and its spin-orbit component at 870.4 eV fit very well the previously reported results for zero valent metallic nickel. Same results can be concluded from the Auger peaks. The detection of metallic nickel at the surface of the samples indicates clearly the reduction of Ni⁰⁺ species when the original sample is heated in argon at 773 K. Also an important decrease in the intensity of the O1s peak is observed (see Fig. 2) upon heating. At that point the reduction of Ni²⁺ in the presence of carbon during heating can be postulated as the reaction producing the nickel nanocrystals. Further exposition of the heated sample to air may produce the partial oxidation of nickel nanocrystals.

In order to achieve a quantification of the oxidation degree of the samples, X-ray absorption spectra (XAS) were recorded at the Ni K edge. By this technique chemical information, as well as structural data relating to the short range order of nanocrystalline and amorphous materials can be obtained. Figure 4 shows the XANES region of the XAS spectra for both samples (as-prepared and heated) and for a metallic nickel foil and NiO sample included as reference. The first region in the spectra corresponds to a low energy
shoulder before the edge threshold at ca. 8334 eV. This shoulder is usually attributed to the 1s-3d transition which in perfect O_h symmetry is strictly dipole-forbidden. However, owing to the mixing of p- and d-like states this feature is visible here. The intensity of this peak is strongly dependent on the oxidation state and local structure of the nickel atoms, as demonstrated in Fig. 4. The second region of the spectra corresponds to the sharp rise for the excitation of the 1s electron into ionization. In the third region, a few eV above the threshold (8349 eV), the final states of the photoelectron should be described in terms of unoccupied bands close to the Fermi level. The intensity of this feature (white line) is also strongly dependent from the oxidation state of nickel, being very intense for oxidised nickel atoms.

By comparison of the Ni-K edge XANES spectra for the as-prepared and heated samples in Fig. 4, we observe a higher intensity of the feature at 8334 eV and a lower intensity of the feature at 8349 eV for the heated sample as compared to the as-prepared sample. In this sense the heated sample appears to contain nickel in an oxidised state that is well dispersed in the carbon matrix, as can be deduced from the TEM/EDX analysis previously described. The heating treatment leads to the reduction of Ni^{2+} species in the presence of carbon, as evident from the decrease in the white line intensity of this sample. This reduction may lead to the formation of the metallic nickel nanocrystalline particles detected by TEM. To quantify the oxidation degree of the heated sample, we have carried out a linear combination of the XANES spectra for the reference materials (Ni foil and NiO sample). The best fitting curve was obtained for 42% Ni and 58% NiO.

The Fourier transforms of the EXAFS oscillations are shown in Fig. 5. Although a fitting procedure is needed to determine the exact position of the maxima, some conclusions can already be obtained by glancing at this figure. The heated sample show similar information about the coordination spheres to that observed in the nickel foil reference, the peaks being nearly coincident, although the intensities are smaller. This indicates the formation of very small nickel nanocrystallites so that a perfect long range structure could not be obtained. The information for the as-prepared sample is clearly different in terms of the coordination spheres, which is clearly visible in the first two peaks.

The fitting analysis of the EXAFS oscillations leads to the data included in Table 1. The first peak appears, according to Table 1, at ca. 0.205 nm and corresponds to Ni-O distances typical of oxidised nickel. Another peak corresponding to Ni-Ni distances at 0.302-0.305 nm has been ascribed to the second coordination sphere around nickel in nickel oxide. These two peaks are present in the as-prepared sample and diminish strongly with intensity for the heated sample as expected upon reduction of the oxidised nickel to the metal. In addition, the heated sample shows a strong peak in the Fourier transform curve at 0.248 nm that corresponds to Ni-Ni distances for metallic nickel. Additionally Ni-C distances may be also present in the first peak at ca. 0.205 nm.

With the purpose of analysing the composition of the areas around and within the small Ni nanoparticles, a FEG-TEM microscope was used (with a probe size of <1 nm). Figure 6
shows a high magnification micrograph corresponding to the heated sample in an area of a spherical particle. The EELS spectra at the C K-edge and Ni L\textsubscript{2,3}-edges were recorded both outside (Fig. 6 left) and inside (Fig. 6 right) a small Ni particle. The results indicate that nickel atoms form the nanocrystallites.

3.2 Gold nanoparticles functionalised with neoglycoconjugates

In a previous paper we have described the synthesis of water soluble gold nanoparticles (Le\textsuperscript{x}-Au and Lac-Au) functionalised with a monolayer of 11-thioacetateundecanol derivatised neoglycoconjugates of two biologically significant oligosaccharides: the lactose disaccharide (Lac; Gal\textsubscript{1}\textsubscript{1}C\textsubscript{12}\textsubscript{1}O-R) and the trisaccharide Le\textsuperscript{x} antigen (Le\textsuperscript{x}; Galβ(1 → 4)[Fucα(1 → 3)]Glc-NACβ1-OR).\textsuperscript{7} In that paper we showed how these tailored globular carbohydrate models (see Fig. 7) can be used to investigate in solution a
novel mechanism of cell adhesion through carbohydrate-carbohydrate interactions. It was found that the Le^x antigen molecules show a specific carbohydrate-carbohydrate interaction that is not present in the case of the lactose disaccharide. The Lex specific interaction is mediated by Ca^{2+} ions and can be used to control the formation of different nanostructures like isolated nanoparticles or aggregates of nanoparticles with different sizes.

In Fig. 8 we show the TEM analysis of different solutions containing the Le^x-Au sample. Figure 8a shows a typical micrograph obtained from the sample in pure aqueous solution (0.1 mg/mL), together with the corresponding particle size distribution histogram. Similar images were obtained for the Lac-Au sample. The mean diameter of the gold clusters was evaluated to be 2 nm. In Fig. 8b the micrograph shows the microstructure for the same Le^x-Au sample in aqueous solution in the presence of 10 mol·m^{-3} Ca^{2+} ions. The specific Le^x-Le^x interaction is producing the aggregation of the nanoparticles. Solutions containing increasing amounts of the glyconanoparticles (0.3 and 0.9 mg·mL^{-1}) with the same amount of Ca^{2+} were also investigated, as shown in Figs. 8c and d. An important increase in the size of the aggregates is observed, which indicates a nucleation-and-growth mechanism. For low concentrations (0.1 mg·mL^{-1}) the growth ceases at aggregate sizes of around 8-30 nm with almost no isolated nanoparticles remaining. At higher concentrations (0.3 and 0.9 mg·mL^{-1}) aggregate sizes in the range 12-40 and 40-150 nm, respectively, were observed together with remaining isolated nanoparticles.

The use of the EFTEM technique for the characterisation of metal particle nanostructures will be illustrated in Fig. 9, were the role of Ca^{2+} ions for promoting the carbohydrate-carbohydrate interactions is also demonstrated. The image obtained with the elastically scattered electrons (zero loss image, Fig. 9 left) shows the aggregates for a 0.1 mg·mL^{-1} Le^x-Au sample in 10 mM CaCl_2. The Ca map image (obtained at the Ca L_{2,3} edge), given by Fig. 9 right, shows the formation of the aggregates in the Ca-rich regions (white contrast in Fig. 9 right). It is important to reemphasize that aggregates were not detected for the Lac-Au sample. Both Lac and Le^x saccharide molecules are neutral, so that the aggregation in the presence of Ca^{2+} ions is driven by specific carbohydrate-carbohydrate interactions.
4. Conclusions

In the present paper we have shown that in order to achieve a full characterisation of metal particles nanostructures, a good approach is the combined use of different techniques: (a) Microstructural analysis techniques like TEM, HRTEM and EFTEM with associated EDX and EELS. (b) Surface analysis techniques like XPS and Auger spectroscopy. (c) X-Ray absorption spectroscopy techniques like XANES and EXAFS sensitive to local structure and chemical state.

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