A New View on Chemistry of Solids in Solution—Cryo Energy-Filtered Transmission Electron Microscopy (Cryo-EFTEM) Imaging of Aggregating Palladium Colloids in Vitreous Ice

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Abstract: It is shown that by using cryo-transmission electron microscopy (cryo-TEM) it is possible to image the aggregation behaviour of nanoparticles while they are still in solution. This technique has allowed the study of the arrangement of colloidal palladium particles in solution by preparing the specimen by the plunge-freezing technique. This method of rapidly cooling the specimen avoids rearrangement of the particles during specimen preparation. The palladium particles were identified by energy-filtered cryo-TEM. The aggregation of particles in solution was studied as a function of pH and ionic strength. The results can be used as recommendations for colloidal solutions intended for deposition of single particles.

Keywords: aggregations • colloids • electron microscopy • energy-selected imaging • palladium

Introduction

The preparation of frozen amorphous thin films of solutions containing crystals, and subsequent imaging using cryo energy-filtered transmission electron microscopy (cryo-EF-TEM), opens the possibility to image the chemistry of solids interacting with liquids. The future possible areas of research include dynamics of crystal growth processes, adsorption mechanisms, ion exchange, and structure determination of solids in equilibrium with solutions. Standard transmission electron microscopy (TEM) specimens of particles are usually prepared by evaporating the particle-containing solvent after deposition on a carbon-coated copper grid. The arrangement of the deposited particles on the carbon support may not have any resemblance to that in solution. Here we show that the arrangement of nanoparticles in solution can be studied by means of low-electron dose cryo-EFTEM. The information content of the inelastically scattered electrons during cryo-EFTEM investigations can be used to record two-dimensional elemental distribution images from thin amorphous ice in less than a minute. This also opens the possibility to identify the solid particles in solution. This is shown here for the first time using a post-column AutoFilter GIF100 (Gatan Image Filter), allowing energy-filtered transmission electron microscopy (EFTEM)[1] to be used in a study of aggregation of colloids in solutions with different pH and ionic strength.

Results and Discussion

In order to prepare thin films of nanoparticles on supports by the principle of self-assembling colloids, it is essential to know their distribution in solution. For this purpose, a model system of water-soluble palladium colloids was selected. The synthesis of these colloids can be done in various ways.[2–4] Usually, a Pd salt reacts under increased temperature with a reducing agent and gives the appropriate sol. The Pd colloids used in the present case were covered with sodium sulfanilate as a protective ligand. With regard to the different experimental parameters such as the temperature and the chemical environment, the pH and the total ionic strength turned out to be most important for the arrangement of particles in solution. At extreme pH values an irreversible coagulation of ligand-protected colloids is observed. Whereas a decomposed colloid can be detected by eye, there is little knowledge of how the particles arrange in solution at different pH. For a realistic view of the arrangement of dissolved particles it is

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essential that any kind of reorganisation is avoided during specimen preparation. In order to prevent microstructural changes in the solution, the cryo-fixation has to take place on a time scale faster than the particle diffusion speed. This can be achieved by the use of the cryo-fixation techniques normally used for frozen aqueous samples in biological investigations.[5,6] The quantity of water-soluble colloid was kept constant, while the pH and the total ion concentration were varied. This led to several series of samples, which were subsequently all imaged by cryo-TEM.

In order to conclusively identify the Pd colloids, besides possible artefacts like cubic ice crystals and contamination on the surface of the amorphous ice, energy-filtered images and jump ratio maps were recorded. It was found that zero-loss images (Figure 1a and 1c), using a 10 eV slit and an exposure time of two seconds, could be recorded together with jump ratio maps (Figure 1b and 1d). The zero-loss image reveals shapes, sizes, structural defects and the distances between the agglomerated colloids. The jump ratio images (N-edge at 60 eV and M-edge at 335 eV) clearly identify the Pd colloids, even if the diffraction contrast causes some variation in intensity of the Pd signal. The distances between the local defects in the colloids reveal that the structural resolution is at least 2.5 nm in the jump ratio maps.

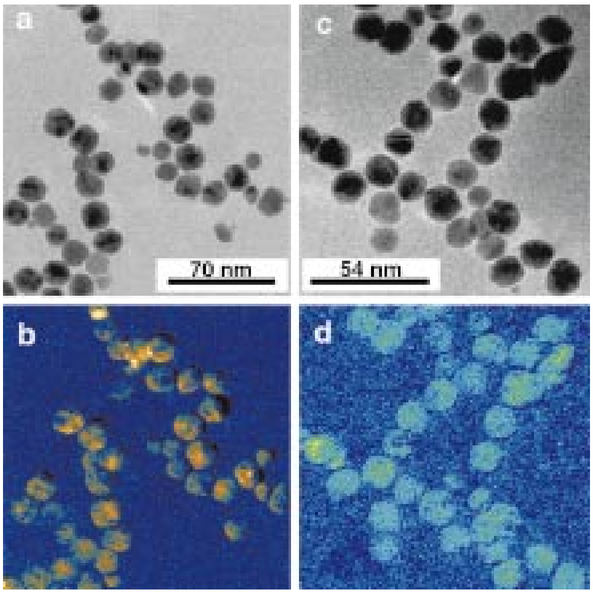

Figure 1. Zero-loss images (a and c) of aggregates consisting of 20 nm Pd colloids in amorphous ice. Pd-jump ratio maps of corresponding areas (b and d) are recorded at the ionization edges $N_{2,3}$ (60 eV) and $M_{4,5}$ (335 eV).

The statistical evaluation of the images was done by counting the number of particles per agglomerate. For reasons of visual perception, it is often not possible to count the quantity of nuclei that forms big aggregates. Therefore, aggregates consisting of more than two particles were summed in one last category. From these data we calculated the relative figures, that is related to the total number of agglomerates, as the size of the evaluated areas in each image series varied. Thus, it is possible to derive clear tendencies from the obtained data. The evaluation leads to histograms showing the number of particles per agglomerate, the relative quantity of agglomerates as a function of pH or the ionic strength, respectively. They give information about the increase and decrease of agglomerate sizes depending on the different sets of parameters. The data series were sorted according to equal ionic strength at different pH or, the other way round, according to a constant pH at varying ionic strength. Three pH values of 4.2 (pure colloid in water), 7 and 9 were chosen. The solutions were prepared with conductivities of 1.9 mS, 2.3 mS and 3.3 mS, corresponding to the different ionic strength. Ionic strength higher than 3.3 mS results in unstable solutions which show fast precipitation within a few hours. This means, it is not possible to distinguish between such agglomerates, which are the obvious sign of an initial decomposition of the colloid, and those which represent a stable equilibrium state of particle arrangement.

In an acidic regime, the sulfonate residue of the sulfanilate ligand pointing towards the solvent is protonated. The colloidal particles, with their ligand shell, become more and more electrically neutral with decreasing pH. Following the same principle, the electrostatic double layer (EDL), which separates the particles, also becomes thinner.[7] With an increase in surface charge, the Pd colloids should have a tendency to separate more to single particles at higher pH values. This is indeed supported by the images of frozen hydrate solutions of different pH at constant ionic strength. Some typical images and the corresponding histogram are shown in Figure 2. It is obvious, that the fraction of single particles increases with the pH, while the fraction of bigger aggregates decreases. Due to the higher pH, and the growing EDL, the increase of single particles can be up to 20%, but it is lower in solutions with higher ionic strength. The reason is that the increasing ion concentration, reduces the EDL, and this acts contrary to the effect of a high pH.

In order to monitor the influence of the ionic strength on the stability of single Pd colloids, a series of experiments at

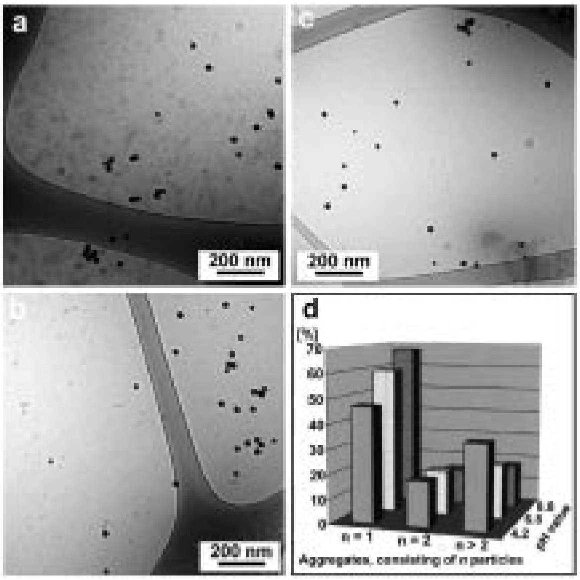

Figure 2. The typical distribution of aggregates, cryo-TEM imaged, in solutions with different pH; a) 4.2, b) 6.8 and c) 8.8. The histogram (d) summarizes the relative distribution at the different pH values and at constant ionic strength (the conductivity is 2.3 mS).

varying ionic strength and constant pH were carried out. The relative quantity of single particles (pH 4.2 is shown in Figure 3 as an example) is similar at lower ionic strength, whereas at high ionic strength a significant decrease can be observed. At first sight, the data for a conductivity of 3.3 mS suggest an initial decomposition process. Observation of the sample during the following days, however, revealed no sign of precipitation. As expected, an increase in large aggregates can be observed. This is also true for the samples when the conductivity is raised from 1.9 mS to 2.3 mS and even more when the conductivity is raised from 2.3 mS to 3.3 mS. For each individual pH value the amount of single particles decreases with increasing ionic strength. Moreover, the cryo-TEM images show that even in solutions with low electrolyte concentration the colloids are not distributed homogeneously as single particles. The distribution includes all sizes of agglomerates, but single particles clearly form the majority, which is the greater, the lower the ion concentration.

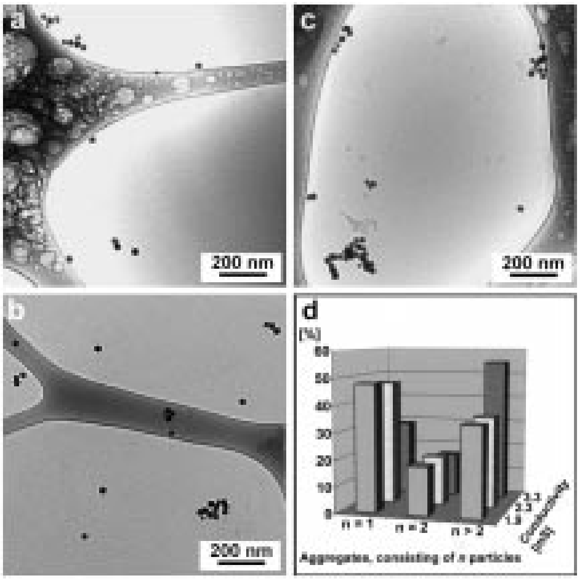

Figure 3. The typical distribution of Pd-colloid aggregates in solutions with different ionic strength, but constant pH (4.2). The cryo-TEM images are recorded from solutions with the conductivities a) 1.9 mS, b) 2.3 mS and c) 3.3 mS. The histogram (d) summarizes the relative distribution at different ionic strength.

Surprisingly, the influence of the pH value on the sulfonic acid residue of the ligand turned out to be very weak. This conclusion can be drawn from the fact, that the relative number of single colloids in solutions increases by 14% between pH 4.2 and 8.8, whereas the influence of the total concentration of ions (the ionic strength) causes a 20% relative decrease of single particles between 1.9 and 3.3 mS. The ionic strength most likely affects the configuration of the ligand shell to a greater extent than the pH. For applications, such as the deposition of monomolecular colloidal films on supports, only diluted, alkaline solutions of this type of colloid are suitable. For other particle/ligand combinations we can derive that the lower the acidic strength of the protective ligand, the more the pH has to be considered. For weakly acidic ligands the increase of single particles with increasing pH is stronger than that of strong acids. For strong, entirely dissociating acids or ligands that are not influenced by the pH, the size of agglomerates and their distribution depends entirely on the total ionic concentration.

As a conclusion, we can say that the investigated Pd colloids always occur as a mixture of single nuclei and aggregates in solution. Although it is possible to increase the number of single particles by lowering the ionic strength and raising the pH, there are still agglomerates consisting of two and more nuclei. With this information it is possible to determine the most favourable deposition conditions for our Pd/ligand combination and to derive recommendations for other metal/ligand systems. Our future work will focus on growth and decomposition phenomena of nanoparticles in solution and the description of possible reorientation and rearrangement of metal atoms and ligands during these processes.

Experimental Section

The conductance was measured by using a Metrohm immersion cell (EA608) attached to a Metrohm E527 conductometer. The wetting properties of the microscope grid were enhanced by means of a BALTEC SCD005 glow discharge device. The Philips CM120 BioTWIN Cryo, here used for cryo-TEM, was equipped with a specially designed objective lens, with a focal length of 6 mm and a structural resolution of 4 Å, enabling a higher image contrast to be achieved than in a conventional 120 kV microscope. The specimens were mounted in a cryo-holder (Oxford CT3500), cooled with liquid nitrogen to keep the temperature below $-180\,^{\circ}C$. To keep beam damages at a minimum, samples were imaged under low-dose conditions, about 20 electrons $nm^{-2}s^{-1}$. The images were recorded with a Gatan 791 cooled multiscan CCD camera. The experimental set up for recording elemental maps with EFTEM, using the post-column AutoFilter GIF100 (Gatan Image Filter), was very much dictated by electron beam sensitivity of the amorphous ice. Short exposure time and low-dose conditions were the most important points to follow. Since the three-window method for elemental mapping requires the recording of at least three images or more commonly six, this method is not very suitable. The two-window method (jump ratio imaging) was applied instead. The jump ratio imaging method produces maps by dividing the ionization edge image by a pre-edge image. The images were processed with Gatan's Digital Micrograph software. The synthesis of the colloid was reported before with slight differences.[8]

Citrate stock solution: The solution was prepared from tri-sodium citrate dihydrate (200 g, 0.68 mol) dissolved in deionized water (1 L).

Palladium stock solution: Palladium(II) chloride (6.6 g, 37 mmol) was dissolved in 1M hydrochloric acid (800 mL) and diluted to 1 L with deionized water.

Preparation of 20 nm Pd colloid: The citrate stock solution (60 mL) and the palladium stock solution (15 mL) were added to deionized water (3 L). This solution was vigorously stirred and heated to reflux for 24 h. The initial yellow color turned into typical colloidal-brown. The solution contained unstabilized colloidal palladium (60 mg). The solution was allowed to cool down to room temperature and sodium *p*-sulfanilate dihydrate (0.5 g, 2.56 mmol) was added. After the mixture was stirred for half an hour, most of the solvent was removed in weak vacuum by means of a rotary evaporator ($T < 40\,^{\circ}C$). With recognizable coagulation the colloid was centrifuged and dried at room temperature. The remaining powder could be redispersed in water or methanol. TEM images show a narrow size distribution and an average particle diameter of 20 nm.

Specimen preparation: The colloid concentration was $0.2\,mg\,mL^{-1}$. The series with a pH of 6.8 and 8.8 were prepared by using commercial buffer solutions (pH 7 and 9). Any differences in ionic strength were measured with a conductometer and were compensated by addition of appropriate quantities of sodium sulfate. All components of the solution mixtures were prepared in advance except for the colloid. After the particles were added, the solutions were mixed intensively and used for the plunging immediately. The specimens were prepared on perforated carbon films supported on copper grids. In order to give a large amorphous film of ice, the insufficient wetting properties of the grids were enhanced by glow discharging. After the treatment the carbon film was negatively charged and the hydrophilic character was enhanced. Each experiment was carried out under controlled humidity and temperature. The grids were wetted in a temperature range of 25 – 32 °C and at close to 100 % humidity. The grids were rapidly cooled by plunging into liquid ethane close to its freezing point (– 171 °C). Such rapid cooling caused the water to vitrify, and thus prevented microstructural changes by ice crystallization. These thin films (2 – 100 nm) were kept under liquid nitrogen and transferred into the electron microscope, keeping the temperature as low as possible ($T <$ – 180 °C). After each plunging experiment the conductivity of the solutions was measured to prove the constant ionic strength in each series.

Cryo-EFTEM: For the experiments a 0.1 mm condenser aperture and a 0.07 mm objective aperture were used. The width of the energy window was adjusted depending on the shape of the ionization edge and its energy loss. The width was 5 – 10 eV at losses up to 100 eV and 20 – 30 eV between 100 and 500 eV. Higher energies were difficult to analyze because of the weak signal giving too long exposure times. Longer exposure times than 20 – 30 s per image were very difficult to perform. In cases of edge profiles with delayed intensity the post-edge images were usually recorded at its maxima.

Statistics: The three pH values used (4.2, 6.8 and 8.8) as well as the three ionic strengths (1.9, 2.3 and 3.3 mS) led to a series of nine plunging mixtures. In each experiment at least two grids were prepared for cryo-TEM investigations as described above. From these grids 233 images of various areas were taken. All recorded images were evaluated separately leading to a total number of 4586 different aggregates. The data which belong to the same parameter set (pH, ionic strength) were collected, converted into relative figures and visualized in histograms.

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