Size-selective separation of gold nanoparticles using isoelectric focusing electrophoresis (IEF)

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Isoelectric focusing in a polyacrylamide pH gradient gel is used to analyze the size distribution of gold nanoparticles synthesized by a chemical route with mercaptosuccinic acid as a ligand. The isoelectric point of the nanoparticles is shown to be size dependent, allowing fractionation by electrophoresis. Each fraction has a narrow size distribution with a standard deviation lower than 0.4 nm.

Developing new techniques and routes of synthesis for tailoring and controlling the size and shape of small metal particles (SMPs) is necessary to enable their use for device fabrications. Many synthesis routes have been developed, but the most suitable remains the chemical reduction of metal salts.[1–3] The shape and size distribution can be controlled by choosing appropriate reaction conditions. The stability can be achieved through the attachment of thiols or the adsorption of charged species. The challenge is to obtain monodispersity and to avoid any aggregation of the colloids. Thioacid derivatives are known to be good candidates to generate a highly stable and disperse gold colloidal solution.[4–6] To produce SMPs with a well characterized size, a few methods have been described such as acid induced precipitation,[7] size exclusion chromatography,[8,9] reversed-phase high performance liquid chromatography,[10] Flow Field-Flow Fractionation (FFFF),[11] but new generally applicable methods are still in high demand.

A technique used extensively in biology to separate isoforms of proteins according to their isoelectric point (pI)[12] is Isoelectric Focusing (IEF) electrophoresis. In a typical IEF gel electrophoresis, the charged biomolecules (i.e. positively charged when $pH_{gel} <$ pI and negatively charged when $pH_{gel} >$ pI) migrate in an immobilized pH gradient (IPG) polyacrylamide gel. The pH range is generated by immobilines, weak acids or bases of defined pK_a values, covalently linked to polyacrylamide. Upon application of an electric field, molecules reach their point of zero charge (i.e. $pH_{gel} =$ pI). Already, gold and silver nanoparticles have been separated in a chromatographic column where the pH gradient was organized by carrier ampholytes.[13] It has been highlighted that the pI of the SMPs derivatised with carboxylic acid groups depends on the particle size. Consequently, separation of nanoparticles of different sizes could be achieved.

In this communication, we use the IEF technique for significantly narrowing the size distribution of a gold nanoparticle solution. Water soluble gold nanoparticles derivatised with a layer of mercaptosuccinic acid were prepared by classical chemical reduction using sodium borohydride as previously reported.[14]

Spherical nanoparticles are obtained with an average size of 1.7 nm and a standard deviation of 0.8 nm. The UV–VIS spectrum presents no surface plasmon resonance due to a high ratio of scattering of the conduction electrons at the surface of the particles, and TEM has been used for the size determination.

A solution (0.5 g L^{-1}) was loaded directly on an immobilized pH gradient (IPG) strip (non linear pH ranging from 3–10) at pH 8. At this basic pH, the SMPs are negatively charged and migrate to the anode until they reach a pH region corresponding to their pI. The voltage is ramped linearly from 0 to 1000 V within 2 hours. Well-defined bands are then visualized around pH 5 (Fig. 1).

The intensities of the bands reveal the concentration of the different sized nanoparticles. The distribution of the IEF spots shows a good correlation with the size distribution of the starting solution. Three major bands can be observed corresponding to sizes of 1.7(0.4) (band a), 3.3(0.4) (band b) and 4.9(0.3) nm (band c). It is observed that the more intense band at pH = 4.9 corresponds to the more concentrated form (i.e. 1.7 nm). By cutting the different bands of the IPG gel and extracting the colloids in an aqueous solution, it is possible to isolate the most important band and therefore to increase the monodispersity. Fig. 1 shows a pI shift from 4.9 to 5.5 for nanoparticles with sizes of 1.7 and 4.9 nm. The pI variation with the size of the nanoparticles shows a perfect linear shape ($R^2 = 0.9998$).

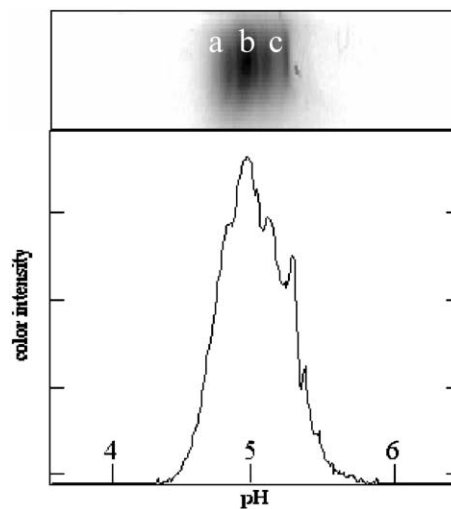An example of size distribution for band c is portrayed in Fig. 2.

Fig. 1 Picture and concentration profiles of a colloidal solution of 1.7 nm MSA–gold nanoparticles (0.5 g L^{-1}) after in gel IEF (pH gradient 3–10, 2 h from 0 to 1000 V).
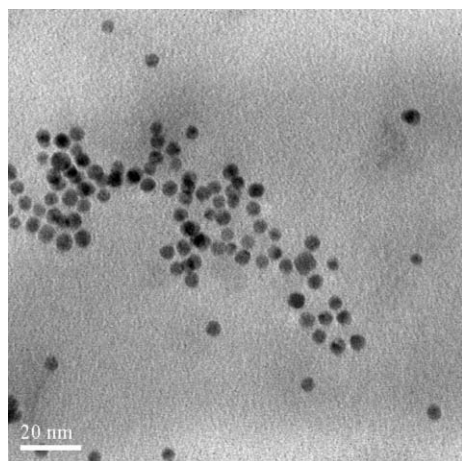
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Fig. 2 The TEM image (with a reference scale of 20 nm) is shown for the highest nanoparticle size (*i.e.* 4.9 nm). An average size of 4.9 $\pm$ 0.3 has been estimated.

This IEF separation process depends on the degree of dissociation of the MSA molecules and on the number attached to the nanoparticle. It must be remembered that MSA is a dicarboxylic acid with two equilibrium constants $K_1 = 6.45 \times 10^{-5}$ and $K_2 = 2.29 \times 10^{-6}$. The degree of dissociation has been estimated to vary with pH using the following eqn. [1]:

$$\alpha = \frac{2K_1K_2 + (K_1 + K_2)x}{2(K_1 + x)(K_2 + x)} \qquad (1)$$

where $x = [H^+]$.

When the nanoparticles are introduced on the gel at pH = 8, the dissociation degree is 0.99 and the surfaces of the nanoparticles are fully negatively charged. The particles can migrate to the anode. By decreasing the pH, the dissociation coefficient decreases to 0 at pH = pI.

The number of MSA molecules bound to the gold depends on its size. By increasing the diameter from 1.7 to 4.9 nm, the number of molecules attached to the gold surface varies from 60 to 495 molecules. This value was estimated considering that the maximum packing density of a thiol is in the order of 0.214 nm².[14] This parameter depends strongly on the size and the type of thiol at the surface of the nanoparticles. It has been reported that for a size of 2 nm, the occupation area per MSA molecule was estimated to be about 0.156 nm².[5]

For this reason, estimating the number of MSA molecules in a unit volume of colloidal solution with sizes of 1.7, 3.3 and 4.9 nm shows a considerable increase from 1.4×10^{14} to 2.5×10^{16} cm^{-3}. In that way, the modification of the occupation area for thiol molecules with the nanoparticle size induces the variation of the charge–charge repulsion between MSA molecules and the observed shift of the apparent pK_a at the surface of the nanoparticles. This significant variation of MSA molecules and thus of the number of free protons modifies the zeta potential. Previous work has demonstrated that decreasing the density of carboxylic acid groups at the surface of a metal lowers the surface acidity.[15]

In summary, IEF has been used to separate nanoparticles with respect to their size. From an initial solution of SMPs having a distribution of 1.7 $\pm$ 0.8 nm, three narrow size distributions have been obtained with average sizes of 1.7, 3.3 and 4.9 nm. The possibility to change the surface charge of the nanoparticles by changing the number density of thiol molecules at the surface of the gold nanoparticles allows a net separation of the particles depending on their size. Compared to other techniques of fractionation such as size-exclusion chromatography used for the separation of gold nanoparticles, the IEF technique provides better size distribution, reaching a standard deviation of about 6% for a colloidal dispersion with an average size of 4.9 nm. Using liquid chromatography, Wilcoxon *et al.* obtained a standard deviation of 9.5% for a dispersion with an average size of 4.2 nm.[8] In that way, IEF is an interesting technique for separating charged nanoparticles with respect to their size and estimating the particle surface charge through the pI.

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