



Metrological protocols for reaching reliable and SI-traceable size results for multi-modal and complex-shaped reference nanoparticles

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nPSize01 / bimodal gold:

Citrate stabilised colloidal gold NP suspensions with diameter of approximately 30 nm and 60 nm and dispersed in water were purchased from a commercial supplier, BBI solutions Ltd. (UK). The two suspensions were further processed as received, by mixing at ~ 1:1 ratio (particle number concentration based), ampouled using 5 mL amber glass vials and packaged at LGC (Luckenwalde, Germany). The vials were sterilised using Co⁶⁰ gamma-irradiation with a minimum dose of 35 kGy. Homogeneity and stability in terms of the number concentration of the two size fractions were tested with spICP-MS (frequency method) by LGC NMI (Teddington, UK) to ensure the material meets the set requirements and remains stable throughout its shelf-life. The value of the number concentration of the colloid gold particles was determined by LGC (Teddington, UK) with spICP-MS (frequency method) [1] and was $(1.88 \pm 0.24) \cdot 10^{13} \text{ kg}^{-1}$ and $(1.93 \pm 0.24) \cdot 10^{13} \text{ kg}^{-1}$ for the 30 nm and 60 nm fractions, respectively.

nPSize12/ bimodal silica:

nPSize12/ bimodal silica: two batches of colloidal silica NPs suspension in water were synthesized by CEA following a well-known approach [2] with nominal particle diameters of either 30 nm or 60 nm, PSD (Particle Size Distribution) varying from <10% to ~20%. The concentration (number of particles per mL) for each batch of 30 nm ($3.34 \cdot 10^{12} \text{ nps/mL}$) and 60 nm ($4.02 \cdot 10^{12} \text{ nps/mL}$) were measured using the CEA laboratory SAXS instrument (Xeuss 2) and protocol described in ref. 3. nPSize12 was obtained following gentle mixing of the suspensions to obtain a ratio of 90% of 30 nm and 10 % of 60 nm. At each step of the ampouling process, storage and during the characterization campaign,

the stability of the samples was monitored by regularly measuring the sizes, PSD, concentration and ratios with the same SAXS protocol.

nPSize03 / bipyramidal titania:

TiO₂ anatase bipyramids with high percentage of {101} facets exposed were synthesized by hydrothermal treatment of Ti(IV)-triethanolamine complex aqueous solution at 493 K, as described in detail in the literature [4-5]. Briefly, the precursor used for the hydrothermal synthesis is a complex of Ti(IV) with triethanolamine with molar ratio 1:2 ([Ti(Teoah)₂]). The synthesis of the TiO₂ nanoparticles was carried at the following conditions:

- Precursor concentration: 65 mM;
- Triethanolamine: 40 mM;
- Initial pH: 10;
- Temperature: 220°C;
- Time: 50 hours

In general, after dissolution of the required mass of [Ti(Teoah)₂] and of the triethanolamine in MilliQ ultrapure water, pH is adjusted with 0.1 M NaOH, as required. The solution is N₂ purged for at least 5 minutes in order to eliminate O₂ before sealing the hydrothermal reactor. The autoclave is heated to 40 °C for 30 minutes, then to the set temperature for the treatment (±1°C) with a ramp of 1 °C min⁻¹. The final temperature is kept constant for 50 hours. The reactor is then cooled in air.

nPSize04 / Gold nanocubes:

Mono-crystalline Au nanocubes were prepared by colloidal chemistry, according to a two-step approach, composed of a seeding process and a growth process [6]. The seed solution was prepared by adding an ice-cold NaBH₄ solution (0.42 ml, 10mM) into an aqueous solution composed of a mixture of HAuCl₄ (92µl, 10mM) and CTAB (7ml, 100mM). The growth solution was prepared by the successive addition of three aqueous solutions i.e. CTAB (9ml, 22mM), HAuCl₄ (0.250ml, 10mM) and ascorbic acid (3ml, 38mM). Next, 20µl of a water-diluted seed solution (1:50) was injected into the growth solution. Purified solutions of nanocubes were obtained by centrifugation twice with water redispersion.

Cetyltrimethylammonium bromide (CTAB ≥ 98%), chloroauric acid (HAuCl₄·3H₂O), sodium borohydride (NaBH₄, 99%), and ascorbic acid (99+%) were purchased from Sigma and used as received. Deionized water is used for all experiments.

nPSize07 / Gold nanorods:

Material: Cetyltrimethylammoniumbromide (CTAB, 99%) was purchased from GBiosciences. Tetrachloroauric(III) acid (HAuCl₄, extra pure), silver nitrate (AgNO₃, extra pure), sodium borohydride (NaBH₄, 98%), L-ascorbic acid were obtained from Sigma without further purification. All the glassware was cleaned with aqua regia and washed with distilled water before conducting the experiments.

Protocol: The synthesis of gold nanorods (GNRs) follows a seeded growth protocol in water with first, the synthesis of preformed seeds and secondly, their growth in a growth solution [7]. 300µL of cold NaBH₄ (0.01M) was added to a solution made of CTAB (4.6 mM, 0.1M) and HAuCl₄ (25µL, 0.05 M) water solutions at 30°C. The growth solution contains CTAB (0.1M), HAuCl₄ (0.5 mM), AgNO₃ (0.06 mM) and ascorbic acid (0.75 mM) as a mild reducing agent. 120 µl of the CTAB capped seeds suspension was added in the growth solution. The final solution was kept for 2 h at 30°C, then the GNRs were purified by centrifugation (3500 rpm, r = 4.5 cm, 20 min) twice with water redispersion.

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