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Characterization and stability of gold nanoparticles depending on their surface chemistry: Contribution of capillary zone electrophoresis to a quality control☆

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ABSTRACT

Four kinds of gold nanoparticles (AuNP) quite similar in terms of gold core size (*ca.* 5 nm) and shape (spherical) but differing by their surface chemistry (either negatively, or positively charged, or neutral) were synthesized. They were analyzed using both the classical physicochemical approach (spectrophotometry, dynamic light scattering coupled or not to electrophoresis and transmission electron microscopy) and capillary zone electrophoresis equipped with photodiode array detection. The results obtained by both methodologies (related to Surface Plasmon Band-maximal absorbance wavelength-, and zeta potential and electrophoretic mobilities) were well correlated. Moreover, taking advantage of the separation method, the sample heterogeneity was evaluated and an impurity profile was extracted. This allowed setting some specifications which were then applied on the one hand to a batch-to-batch survey to declare NP as conform or not after production and on the other hand to a stability study.

1. Introduction

Nanoparticle development is an extensively growing research field especially for cancer diagnosis and treatment and among them gold nanoparticles (AuNP) are widely studied [1]. Indeed, they are characterized by several properties that have led two products to enter clinical trials (CYT-6091 [2] and Auroshell (ClinicalTrials.gov identifier: NCT01679470)). They are biocompatible, easily synthesized and can be functionalized by a wide range of active pharmaceutical ingredients, and their specific optical properties (Surface Plasmon Band) confer an interesting capacity to behave as biosensors or diagnostic agents [1,3].

However, now that they are translated from bench to bedside and in order to ensure patient safety, a rigorous quality control to frame minimal batch-to-batch variation should be defined. A physicochemical characterization is always performed using classical methodologies to define the nano-objects in terms of size, shape (light scattering, electron or atomic force microscopies, ...) and chemical composition (atomic spectroscopies, electroanalytical techniques, ...), but this approach does not take into account the profile of impurities and population polydispersity and still suffers from some limitations [4]. As a result, various separative methods have already been applied to NP analyzes: size exclusion chromatography [5], high performance liquid chromatography [6], flow field fractionation [7], and gel electrophoresis [8]. Capillary electrophoresis is also suitable for nanoparticle analyzes [9]. Focusing on AuNP, they were separated according to their size [10–12] and shape [13] with a high performance level obtained in separation resolution as well as in number of theoretical plates [14]. This method was also used to investigate their surface chemistry [10,15] and as a tool to monitor the nano-object stability (flocculation or aggregation) [16]. Therefore, capillary zone electrophoresis (CZE) has already been suggested as an appropriate method devoted to the quality control [9,13] of inorganic nanoparticles.

In this study, four kinds of AuNP were synthesized varying their surface chemistry in terms of charge (negatively charged using citrate ions, AuNP-Cit, neutral using a triblock copolymer polyethylene oxide-*b*-polypropylene oxide-*b*-polyethylene oxide, AuNP-COP, and positively charged using cetyltrimethylammonium, AuNP-CTA), as well as in terms of ligand binding energy (low energy with the previous cited ones *vs.* covalent binding using dihydrolipoic acid AuNP@DHLA). These AuNP were quite similar in terms of size (*ca.* 5 nm for the gold core) and shape (spherical). The aim of this work was to establish a quality control devoted to batch-to-batch NP production and to study their stability using the classical physicochemical approach usually applied

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to nanomedicines (*i.e.* UV–vis spectral properties, dynamic light scattering (DLS) coupled or not to electrophoresis (zeta potential, ζ), and transmission electron microscopy (TEM)) in parallel to CZE equipped with a diode array detection (DAD). As these nano-objects represent the mostly AuNP used in the literature, this work is the first, to the best of our knowledge, to evaluate a wide panel of quite similar AuNP only differing by their surface charge, and using the same methodology.

2. Materials and methods

2.1. Chemicals

All reagents and solvents were of analytical grade and used without further purification. Sodium borohydride, sodium citrate, α-dihydrolipoic acid, tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), Pluronic F-127, HCl 1 M and NaOH 1 M were supplied by Sigma Aldrich; boric acid and NaOH were purchased from Riedel-de-Haën and cetyltrimethylammonium bromide (CTAB) came from Prolabo. Phosphate-buffered saline (PBS) was prepared as follows: $[Na_2HPO_4] = 6.48 \times 10^{-3}$ M, $[KH_2PO_4] = 1.47 \times 10^{-3}$ M, $[NaCl] = 137.93 \times 10^{-3}$ M, and $[KCl] = 2.68 \times 10^{-3}$ M, final pH was adjusted to 7.4. Ultrapure water (>18.2 mΩ cm) was used for the preparation of all solutions.

2.2. Gold nanoparticle synthesis and surface chemistry modification

Citrate stabilized AuNP (AuNP-Cit) and α-dihydrolipoic acid capped AuNP (AuNP@DHLA) were synthetized in aqueous solution according to previous works [17–19]. Briefly, 1 mL of $AuCl_4^-$ solution (10 mg/mL) was added into 90 mL of water and 2 mL of sodium citrate 55 mM was added. The solution was stirred for 1 min then 1 mL of $NaBH_4$ 19.5 mM was added and the solution was stirred for 5 min under nitrogen. The resulting suspension was immediately stored in the dark at 4 °C. Based on AuNP-Cit, three kinds of NP were prepared varying their surface chemistry.

Alpha-dihydrolipoic acid capped AuNP [20] (AuNP@DHLA) were prepared using 600 μmol of α-dihydrolipoic acid in 10 mL of 0.5 M NaOH solution added to 25 mL of freshly prepared AuNP-Cit. The reaction mixture was stirred under inert atmosphere for 24 h at room temperature (20–23 °C). A dialysis against PBS for 48 h using a bag (regenerated cellulose with a cut-off of 6–8 kDa (Roth® France)) was performed. The dialysis medium was changed to fresh PBS after 24 h.

Pluronic F-127 stabilized AuNP (AuNP-COP) were prepared by mixing 5 mL of 90 nM AuNP-Cit with 5 mL 0.4 μM Pluronic F-127 in water. The reaction mixture was stirred under inert atmosphere at room temperature for 4 h to perform the ligand exchange.

Cetyltrimethylammonium stabilized AuNP (AuNP-CTA) were prepared by mixing 5 mL of 90 nM AuNP-Cit with 5 mL 10 mM CTAB in water. The reaction mixture was stirred under inert atmosphere at room temperature for 4 h.

The final concentration reaches 90 nM for each kind of AuNP obtained and they were stored in the dark at 4 °C.

2.3. AuNP characterization

A UV–vis spectrophotometer (UV-1800 Shimadzu) was used for spectra recordings and absorbance measurements. The hydrodynamic diameter (Dh) and ζ of AuNP were measured at 20 ± 1 °C using a Zetasizer Nano ZS (Malvern Instruments, UK). All Dh values are volume averaged, based on three independent measurements realized on at least three AuNP batches. Zeta potential measurements were carried out in ultrapure water (implying preliminary dialysis of AuNP@DHLA for 4 h). All surface charges are mean values based on three independent measurements realized on three AuNP batches. Transmission electron microscopy images were recorded using a Philips CM 200 instrument with a LaB6 cathode operating at 200 kV. Gold NP suspensions were deposited onto a 400 mesh carbon film copper grids. The average diameter of the gold core (Dc) was calculated for each AuNP sample by counting *ca.* 200 individual particles from the TEM images.

2.4. Capillary zone electrophoresis

Capillary Zone Electrophoresis measurements were carried out using a P/ACE MDQ CE system (Beckman Coulter) equipped with a diode array detector (DAD, λ from 200 to 600 nm). Measurements were performed at 25 ± 1 °C using a 40 cm bare silica capillary (effective length: 34 cm). For anionic and neutral AuNP species, the background electrolyte (BGE) was a 25 mM boric acid/sodium borate buffer pH 8.5 adjusted with 40% (m/V) NaOH. AuNP detection was performed under 12 kV (normal polarity, cathodic electroosmotic flow, EOF). For the cationic AuNP-CTA, a reverse polarity method was used (−12 kV), the BGE was changed to 25 mM sodium borate buffer pH 8.5 containing 0.5 mM of CTAB (below critical micellar concentration (CMC) *i.e.* 1.0 mM). 90 nM of AuNP were injected hydrodynamically (20 mbar for 20 s). The capillary was rinsed with NaOH 1.0 M (2 min), ultrapure water (2 min) and BGE (2 min) before each injection. The EOF was determined using the neutral marker, benzyl alcohol, (migration time = 2.06 ± 0.03 min, EOF = $9.1 \pm 0.1 \times 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ (n = 5)). AuNP@DHLA suspensions were dialyzed against water for 4 h before injection.

Each injection was made in triplicate and the obtained peaks were identified by their relative migration time (RMT):

$$RMT = {}^{T_m}/_{T_{mneutralmarker}}$$

in which T_m is the migration time of the peak of interest and $T_{mneutralmarker}$ is the migration time of the neutral species.

3. Results and discussion

3.1. Gold nanoparticle characterization

3.1.1. Classical approach

Results of AuNP characterization performed by the classical physicochemical approach as well as by CZE are presented in Table 1, Figs. 1 and 2. Visible spectra of AuNP (Fig. 1 and Table 1) highlighted the Surface Plasmon Band (λ_{max}) which is specific of these nano-objects. Surface Plasmon Band of AuNP is due to "the collective oscillation of the conducting electrons of metal NP when their frequency matches that of the incident electro-magnetic radiations" [21]. It mainly depends on NP size and aggregation, shape, core/corona composition and suspension medium (interparticle distance, dielectric constant) [21]. After ligand exchange or grafting from AuNP-Cit to prepare to other kinds of particles, a red shift was observed (increase of λ_{max}), which reflected the surface chemistry modification. The images obtained by TEM (Fig. 1) showed well-individualized AuNP whatever the considered surface chemistry without the presence of any aggregate. The values of Dc (corresponding to the gold core) for AuNP-Cit, AuNP@DHLA, AuNP-COP and AuNP-CTA were similar (5.0 ± 0.8, 4.9 ± 0.7, 5.2 ± 0.7 and 6.4 ± 1.2 nm, respectively). The values of Dh (including gold core, surface chemistry and some hydration layers) were larger than Dc (Table 1), as expected. The overall results (λ_{max}, Dh and Dc) were in accordance with previously reported studies [18,22,23]. Surface potential values of prepared AuNP corresponded well to the charge conferred by capping of citrate anions or cetyltrimethylammonium cations or neutral copolymer or by grafting of dihydrolipoate anion (Table 1). Concerning AuNP-CTA, ligand exchange may not be fully