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The electrokinetic characterization of gold nanoparticles, functionalized with cationic functional groups, and its' interaction with DNA

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ABSTRACT

Gold nanoparticles have attracted strong biomedical interest for drug delivery due to their low toxic nature, surface plasmon resonance and capability of increasing the stability of the payload. However, gene transfection represents another important biological application. Considering that cellular barriers keep enclosed their secret to deliver genes using nanoparticles, an important step can be achieved by studying the functionalization of nanoparticles with DNA. In the present contribution the synthesis of nanoparticles consisting of a gold core coated with one or more layers of amino acid (L-lysine), and cationic polyelectrolytes (poly-ethyleneimine and poly-L-lysine) is reported. All nanoparticles were subjected to dynamic light scattering, electrophoretic mobility measurements, UV–vis optical spectrophotometry analysis and transmission electron microscopy imaging. In addition, the adsorption of DNA plasmid (pSGS) with linear and supercoiled configurations was studied for those gold nanoparticles under the most suitable surface modifications. Preliminary results showed that the gold nanoparticles functionalized with poly-ethyleneimine and poly-L-lysine, respectively, and bound to linear DNA configurations, present in absolute value a higher electrophoretic mobility irrespective of the pH of the media, compared to the supercoiled and nicked configuration. The findings from this study suggest that poly-ethyleneimine and poly-L-lysine functionalized gold nanoparticles are biocompatible and may be promising in the chemical design and future optimization of nanostructures for biomedical applications such as gene and drug delivery.

1. Introduction

Gold nanoparticles (AuNPs) have sparked much interest in the fields of chemistry, physics, biology, medicine and biomaterial sciences. They are bioinert, non-toxic and readily synthesized [3] and their small size allows them to penetrate cells and tissues, making their application in medicine far reaching. They have become one of the most interesting sensing materials because of their unique size and shape dependent optical properties, high extinction coefficient and super-quenching capability. AuNPs hold the promise for efficient gene or drug delivery into cells by absorbing and resonantly scattering visible and near-infrared light upon excitation of their surface plasmon-oscillation [14]. AuNPs do not have target recognition abilities necessary for selective binding of analytes of interest. In order to selectively detect analytes of interest antibodies, short-chain organic acids, proteins or DNA molecules have been used to modify the surface properties of AuNPs to provide target recognition sites [20]. These molecules can be incorporated into AuNPs through electrostatic attraction or covalent bonding. It is an effective way to enhance specificity and efficacy of nanoparticle based delivery systems [17]. For them to be successfully utilized in medical applications their fundamental properties such as the forces that are responsible for nanoparticle-drug stabilization must be understood.

AuNPs are also currently being used as delivery vehicles to transport siRNA into cells for efficient knockdown of target genes

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without significant cytotoxicity. They demonstrated high stability and effective transfection in multiple myeloma cells ([8] and [2]). These particles can also be carriers of peptides and proteins. They present strategies for the treatment of genetic as well as acquired diseases. The release of gold nanoparticles is vitally important for effective therapy. This release could be triggered by internal (e.g. gluthathione), pH or external stimuli such as light [6].

Electrokinetic characterization was carried out first, because in order to design a carrier, one needs to know the features and properties of the system that one is working with. These include hydrophobicity/hydrophilicity, size and most importantly charge. It is important to note that each time the surface of a nanoparticle is functionalized, any one of these (or all three at the same time) properties may change. Adsorption on the surface commonly results in a modification of the charge. Therefore electrophoretic mobility (U_e) studies were carried out to determine and optimize the preparation conditions of the final surface charge. Complementary to this feature, dynamic light scattering was also performed in order to confirm the surface modification of the nanoparticle with an external agent. Size and surface charge of DNA complexes are two important factors in determining the efficiency of cellular uptake. Although there is a common theme in all vectors, each vector has to be suitably tailored and optimized to meet the needs of a specific application.

This was seen when attaching a functional group such as lysine to a nanoparticle, the p*K*a values (2.2, 8.5, and 10.28) had to be considered in order to determine at which pH this amino acid needed to be prepared. Depending on the physical orientation of its side chains, lysine may form bridges with the citrate ions enclosing the AuNP and resulting in aggregation. This may account for the negative or decreased electrophoretic mobility observed. Lysine contains 1 carboxylic group and 2 amine groups so a fully protonated lysine molecule has a +2 net charge. The two ε-amino groups have p*K*a's of 9.0 and 10.2 respectively which are significantly higher.

2. Materials and methods

Water (Milli-Q Academic, Millipore, France) was used in the preparation of all suspensions. Gold(III) chloride trihydrate, trisodium citrate dehydrate, ethanol, L-lysine, polyethyleneamine (PEI branched, 25 kDa) and poly-L-lysine (PLL) were purchased from Sigma Aldrich. Citric acid was from Pancreac (Spain). All chemicals were of analytical quality. DNA plasmid pSGS was amplified at Hospital Universitario San Cecilio, Granada. The following instruments were used. Nanosight Nanoparticle Tracking Analysis (NTA) Version 2.2 Build 0377, Zetasizer Nanoseries Nano-ZS Model ZEN 3600 (Malvern Instruments, U.K), Eppendorf Centrifuge 5418, NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, USA), Hettich Zentrifugen Mikro ZZ0R, Gel electrophoresis apparatus, pH meter, Perkin Elmer Lambda 20 UV–vis spectrophotometer, Transmission electron microscope Jeol (T 1010), iTEM Soft Imaging Systems (SIS) MegaView III side-mounted 3 megapixel digital camera.

2.1. Synthesis of colloidal gold nanoparticles by citrate reduction

0.45×10^{-3} M of an aqueous colloidal gold solution was prepared by $HAuCl_4$ reduction with sodium citrate using the method of [18]. A solution consisting of 0.11 mL gold colloidal in 25 mL water was boiled for 15 min and thereafter 1 mL of 1% sodium citrate was added rapidly to the vortex of the solution. On addition of the citrate into the boiling tetrachloroaurate solution the colour changed from pale yellow to dark blue within the first 3 min and then ruby red after 15 min of reaction time. It was boiled for a further 5 min on the heating mantle, thereafter removed and left to cool down to room temperature. It was stored in a dark bottle.

2.2. UV–vis optical spectrophotometry analysis

The qualitative analysis in particle uniformity and stability of each gold nanoparticle was followed up by examining the absorption spectra of the individual nanoparticles and to confirm in agreement with literature that the expected SPR absorption of the nano-sized gold particles occurs at 534 nm. UV–vis absorption measurements were conducted and the spectra recorded by measuring dilute samples in a quartz cell with a path length of 1 cm.

2.3. TEM and size analysis

The morphology of gold nanoparticles was observed by TEM. The zeta potentials and mean sizes of the gold nanoparticles were analyzed using a Zetasizer. The zeta potential measurements were performed in triplicate at pH 7.

2.4. Functionalization with amino acid and cationic poly-electrolytes

Different concentrations of the amino acid lysine (0.01, 0.1, 5, 10, 100, 200 and 500 mg/mL) and cationic poly-electrolytes; poly-L-lysine (1, 5, 10 and 100 mg/mL) and poly-ethyleneimine (1, 10 and 100 mg/mL) were prepared by dissolving the required amount into water according to the desired pH. Adsorption was carried out by slowly adding the gold colloidal solution dropwise to each amino acid and poly-electrolyte solution under constant and moderate vortexing. They were washed three times to remove any free polyelectrolytes that remained in solution.

2.5. Electrokinetic characterization

Zeta potential is a powerful tool that is used to analyse the electrostatic forces within the bulk solution and on the surface of the nanoparticles and nanocomplexes, respectively [15]. One drop of the gold suspension coated by the respective amino acid and poly-electrolyte as described in Section 2.4, was added to 10 mL of an aqueous solution of respective pH 3–9 and desired ionic strength. After 24 h of incubation, the pH was re-adjusted and the electrophoretic mobility was measured. Citric acid and sodium hydroxide were used to adjust the pH.

2.6. Ionic strength studies

Ionic strength studies were performed to determine the compactness of the adsorbed layer. To achieve this we investigated the effect of electrolyte concentrations on the U_e. Essentially in the presence of layers the mobility should tend towards zero because of the electric double layer compression at high ionic strength. The dispersion medium, sodium chloride, was prepared in different ionic salt concentrations of (1×10^{-4}; 1×10^{-3}; 2.5×10^{-3}; 5×10^{-3}; 7.5×10^{-3}; 1×10^{-2}; 2.5×10^{-2}; 5×10^{-2} mg/mL).

3. Results and discussion

3.1. Gold nanoparticle synthesis by citrate reduction

The transition in colour of the AuNPs from pale yellow to ruby red confirmed the formation of citrate-capped gold nanoparticles [18]. When the pale yellow colour fades it signifies the formation of the gold atoms which nucleate to form dark red nanowires. The