Materials Letters 65 (2011) 1197–1200

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

# Preparation of stable Pd nanoparticles with tunable size for multiple immunolabeling in biomedicine

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ARTICLE INFO

*Article history:*
Received 15 December 2010
Accepted 17 January 2011
Available online 25 January 2011

*Keywords:*
Palladium nanoparticles
Nanocrystalline materials
Immunolabeling

ABSTRACT

Stable Pd nanoparticles (PdNP) with a tunable size (3–15 nm) were synthesized by controlled chemical reduction of $PdCl_2$ with sodium citrate in water. The morphology of PdNP was characterized by transmission electron microscopy, while their stability in solution was verified by quasi-elastic light scattering and small-angle X-ray scattering. Intensive stirring of reacting mixture played a vital role in achieving reproducible particle sizes. Controlled changes of pH and initial concentrations were employed in fine-tuning particle size distributions. Finally, 10 nm PdNP were conjugated with goat anti-mouse IgG antibody as proved by electrophoresis (SDS–PAGE) and used for ultrastructural immunolabeling, which confirmed suitability of PdNP for multiple immunolabeling in biomedicine.



## 1. Introduction

Metallic nanoparticles combine large surface and specific chemical properties with unique electronic and optical properties. Consequently, they have been used in a broad range of fields such as catalysis, photonics, surface-enhanced Raman spectroscopy, and biological labeling [1]. Such nanoparticles conjugated to antibodies are of great interest in life sciences for detection of biomolecules in TEM (immunolabeling) [2]. Gold nanoparticles with sizes 5–15 nm are commercially available and widely used for the immunolabeling [3,4]; however, a broader range of tags is needed for multiple targets. We suggest that stable palladium nanoparticles (PdNP) could be used in parallel with Au nanoparticles for multiple immunolabeling of biological structures as they can be differentiated in energy-filtered TEM (EFTEM) or in a TEM microscope equipped with a detector for energy-dispersive analysis of X-rays (TEM/EDX).

Classical ways of synthesis of stable PdNP in water (colloids, hydrosols) were described by Turkevich et al. [5] as early as in 1970. Then, a number of authors synthesized Pd and Au nanoparticles for various purposes (e.g., references 6–8). Recently, Lim et al. [9] summarized various ways of controlling the shape of PdNP in hydrosols. In this study, we present a simple and reproducible synthesis of PdNP with a tunable size within 3–15 nm range; they can be conjugated with antibodies used for multiple ultrastructural immunolabeling in biomedicine.

## 2. Experimental

### 2.1. Materials and reagents

Palladium chloride ($PdCl_2$, >99.999%), sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, >99.0%), and sodium hydroxide (NaOH, p.a.) were purchased from Sigma-Aldrich; goat anti-mouse IgG antibody from Invitrogen; $K_2CO_3$ and $NaH_2PO_4$ buffers from Amresco; polyethylene glycol (PEG; MW 20000) from SERVA Electrophoresis; and other reagents were from Sigma.

### 2.2. Synthesis of Pd nanoparticles

PdNP were synthesized as reported by Turkevich et al. [5] with a few important modifications. The following three aqueous solutions were prepared: (i) $9.3 \times 10^{-4}$ M solution of $PdCl_2$ (0.033 g of anhydrous $PdCl_2$ in 4 mL of 1N HCl, filled with $H_2O$ up to 200 mL), (ii) 1% water solution of sodium citrate, and (iii) 0.05 % water solution of NaOH. The base colloid, Pd10 (particle size ~10 nm), was prepared as follows: 7.5 mL of $PdCl_2$ solution, 15 mL of citrate solution, and 52.5 of deionized water were mixed in a 250 mL Erlenmeyer flask on a heated magnetic stirrer and refluxed under intensive stirring (400 rpm, 6 h). Colloids with smaller nanoparticles, Pd06 (~6 nm) and Pd04 (~4 nm), were prepared like the base colloid Pd10, but the pH of the original solution (pH≈6.25) was increased before the

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doi:10.1016/j.matlet.2011.01.046

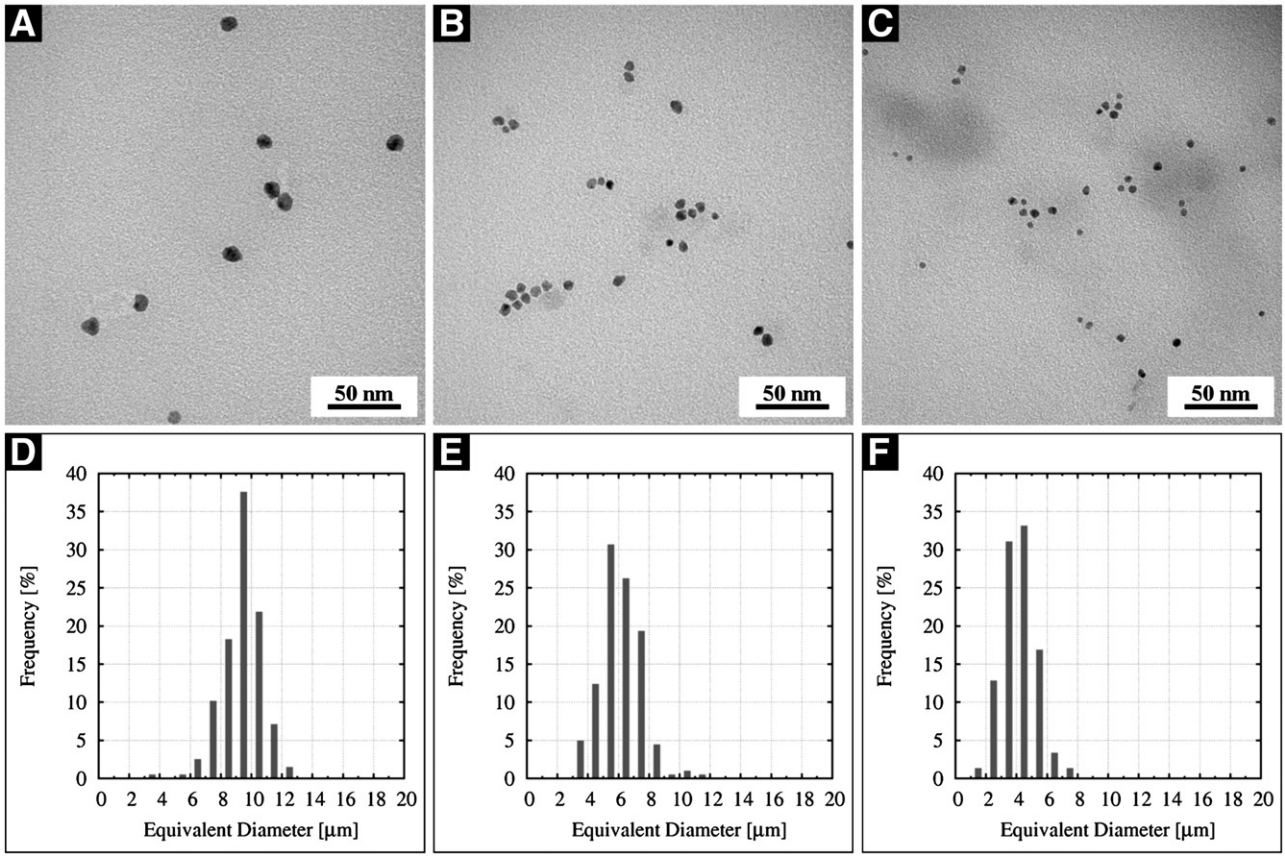


**Fig. 1.** TEM micrographs (A–C) and particle size distributions (D–F) of colloids Pd10, Pd06, and Pd04.

refluxing (using 0.05% NaOH); the increase was 0.2 for Pd06 (pH≈6.45) and 0.4 for Pd04 (pH≈6.65). Colloids with larger nanoparticles, Pd12 (~12 nm) and Pd14 (~14 nm), were prepared like the base colloid Pd10, but the concentration of *both* $PdCl_2$ and citrate solution was increased; the increase in concentrations was 3× and 6× for Pd12 and Pd14, respectively.

### 2.3. Characterization of Pd nanoparticles

Size distributions of PdNP were determined by image analysis of TEM micrographs (TEM microscope; Tecnai G2 Spirit Twin; NIS-Elements software). Stability of PdNP solutions was monitored by quasi-elastic light scattering (QELS) and small-angle X-ray scattering (SAXS) as described elsewhere [10].

### 2.4. Immunolabeling with Pd nanoparticles

PdNP colloid and antibody solution were adjusted to pH 8.5 using 0.1 M $K_2CO_3$. Antibody solution was added to the colloid and mixed intensively for 2–5 min. A sub-saturation amount of antibody was used as measured by SDS–PAGE electrophoresis (50 μg of goat anti mouse (GAM) IgG mixed with 1 mL of PdNP colloid gave optimal results). To prevent aggregation and non-specific binding, 0.25% PEG was added, and the solution was stirred for additional 5 min followed by an overnight incubation at 4 °C. In order to remove unbound antibodies, centrifugation at 15000*g* was used (1 mL volumes, 1 h, 4 °C). After discarding the supernatant, the pellet was re-suspended in 1% PEG solution in 0.01M $Na_3PO_4$, pH 7–8, and stored at 4 °C. The amount of conjugated IgG was evaluated on 10% SDS–PAGE (Mini PROTEAN, Bio-Rad, USA) after dissociating the IgG from the nanoparticles by boiling 1 min in a sample buffer (50 mM Tris–HCl, 10% glycerol, 2% SDS, 100 mM dithiothreitol, and 0.01% bromophenol blue, pH 6.8). The gels were stained with quantitative stain PageBlue (Fermentas), and band intensity was measured on Odyssey infrared imaging system (LI-COR Biosciences, USA). For calibration, concentration series of free GAM IgG were run on the same gel. These Pd-GAM IgG nanoparticles were then used in parallel with the commercially available Au-DAM IgG (DAM12; Jackson ImmunoResearch) for immunolabeling on ultrathin sections of HeLa cell embedded in LR White resin as described previously [11].

## 3. Results and discussion

The main objective of this work was to prepare stable PdNP in size range 5–15 nm, which could be employed in multiple immunolabeling (section 1, references 3,4). Our experiments were based on the previously described synthesis of stable palladium hydrosols (reference 5, section 2.2). Nevertheless, the original procedure was modified in order to (i) increase the reproducibility of the syntheses and (ii) produce particles within the desired size range.

### 3.1. Reproducibility of the syntheses

In the case of citrate Pd hydrosols, the intensity of stirring was found to be a crucial factor influencing reproducibility. The best results were obtained with 75 mL solution refluxed on a heated magnetic stirrer at 400 rpm. Numerous experiments confirmed that the increase in volumes or less intensive mixing drastically reduced the reproducibility from the point of view of average particle size. The second important factor was concentration: the base colloid Pd10 (particle size ~10 nm) was prepared using half concentrations than described in the original procedure [5].