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SYNTHESIS OF MONODISPERSE GOLD NANOSPHERES FOR THEIR USE IN DNA HYBRIDIZATION BIOSENSORS

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Aim. In this work, we describe a reliable and straightforward method of synthesis of monodisperse spherical gold nanoparticles (AuNPs) for DNA labeling in hybridization biosensors based on surface plasmon resonance (SPR) spectrometry.

Materials and Methods. Several samples of colloid AuNPs were prepared by citrate reduction of gold (III) chloride and studied with dynamic light scattering to determine their distribution by diameter. The samples with the least variance of AuNP diameters (8–33 nm) were designated for labeling of DNA probes used to amplify the signal of the SPR-based hybridization DNA biosensor.

Results. The experiments showed the possibility of the synthesis of precision AuNPs from gold (III) chloride through control of the duration of their boiling. An optimal variation of the method of synthesis of such AuNPs was proposed. AuNPs synthesized by the described method were instrumental in achieving a substantial specific increase in biosensor signal.

Conclusions. The proposed method can be used to produce precision citrate-capped AuNPs suitable for use in a wide range of biosensors.

Keywords: hybridization DNA biosensors, surface plasmon resonance, gold nanoparticles, Turkevich synthesis, AuCl3.

Gold nanoparticles (AuNPs) of many sizes, shapes, and surface modifications are currently used in biotechnology [1]. The significant structural and functional diversity of AuNPs contributes to the booming field of their application, but at the same time makes it hard for a researcher without a substantial background in materials science, wave physics, and biochemistry to predict their physical and biological properties. Such properties are hard to verify experimentally due to the particular sensitivity of AuNPs to the conditions of their synthesis; in other words, the production of two identical batches of AuNPs using currently established protocols is an arduous task. Scarcity of our knowledge about the health effects of AuNPs is but one consequence of such uncertainty [2]. Therefore, establishing novel, precise methods of synthesis of AuNPs, as well as clarification of existing ones, is a priority task in any AuNP-related research.

The AuNPs studied here are intended for use as a colloidal part of a DNA hybridization biosensor system based on surface plasmon resonance (SPR) spectrometry [3]. Given that such an application demands that AuNPs are low in diameter, monodisperse, resistant to aggregation, and fit for modification of their surface with thiolated DNA probes, we settled on their synthesis by citrate reduction [4,5]. In this technique, boiling is the most crucial step of the synthesis during which Au ions are incorporated into growing nanoparticles. However, few citrate reduction protocols specify their duration, which can lead to discrepancies between the results of different researchers who use

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the same guidelines. Therefore, it was decided to dedicate this research to the determination of the influence of the boiling of AuNPs during their synthesis on their size.

Additionally, previous research indicated the presence of sub-4 nm fractions in the AuNPs synthesized by citrate reduction of $HAuCl_4$ [5]. Current evidence points toward potential genotoxicity of such nanoparticles, suggesting maximum caution during work with them [2]. Due to this, it was decided to explore the possibility of the synthesis of precision AuNPs by citrate reduction of AuCl3 instead of $HAuCl_4$.

Aim. The primary aim of this work was to thoroughly describe a method of precision synthesis of AuNPs for biosensor modification. The additional objective of our research consisted of demonstrating the possibility of the use of $AuCl_3$ as the precursor of gold nanoparticles synthesized by citrate reduction (instead of the usual $HAuCl_4$).

Methods. The general method of synthesis of AuNPs is similar to the one in our previous research [3], with the volume of precursor solution halved (down to 11 mL), $HAuCl_4$ substituted for $AuCl_3$, and without reflux. Keeping in mind the desired simplicity and safety of the procedure, the synthesis must take place under a fume hood to minimize the risk of inhalation of nanoparticles. Here, the boiling of AuNP precursor solutions was achieved with the MSH-300 magnetic stirrer manufactured by Biosan. All synthesized AuNP samples were analyzed with a photon correlation spectroscopy system of the Malvern Zetasizer Nano series to obtain their size distribution. Here, these size distributions are presented graphically with mean values and standard deviations.

Lastly, the synthesized AuNPs were modified with probe DNA oligonucleotides and applied for signal enhancement of the SPR biosensor according to the earlier established methods [3].

Results and discussion. At the beginning of our research, the synthesis of several batches of AuNPs was conducted. A few seconds after 1 mL of 38.8 mM $Na_3C_6H_5O_7$ is injected into 10 mL of boiling 1 mM $AuCl_3$, the color of the solution changes to black. This indicates the start of AuNP growth, which goes on for 80–130 seconds (for different samples) before the colloid gradually assumes a bright red color. In different samples, boiling was manually stopped after one of three events:

- The solution starts changing color from black to red.
- Solution entirely changes color from black to red;
- Solution fully changes color from black to red and keeps being boiled for 30 seconds.

Several samples of AuNPs were prepared using the described method of synthesis with different durations of boiling. It was noted that boiling of solutions after they assume a bright red color leads to the formation of a visible black precipitate on the inner surface of the synthetic vessel (Fig. 1).

The most probable cause for this phenomenon consists of the aggregation of AuNPs on the glass surface due to the dissociation of protective citrate ions from AuNP surfaces under high temperature. Since AuNP aggregation is irreversible, the presence of such aggregates in the colloid would effectively contaminate the sample, rendering it unusable for biosensor modification.

Nextly, the size distribution of prepared samples was investigated. The results of these measurements are present in Fig. 2.

As it is visible from the graph, prolonged boiling of AuNP colloid leads to substantial increase in mean diameter of AuNPs, potentially resulting in appearance of particles that are far from nanosized (>100 nm in diameter). Of all studied AuNPs, the ones in which boiling was stopped the earliest were determined to be the least polydisperse. They were subsequently selected for modification with thiolated oligonucleotides to test the effectiveness of synthesized AuNPs in enhancement of SPR signal. The sensor responses of SPR-based DNA biosensor to injection of complementary and noncomplementary oligonucleotides labeled with monodisperse AuNPs are demonstrated below (Fig. 3).

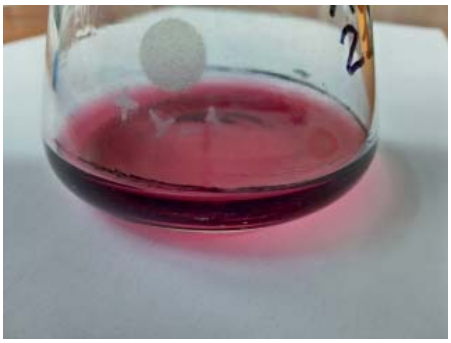

Fig. 1. **Black precipitate becomes visible above the meniscus in excessively boiled AuNPs**

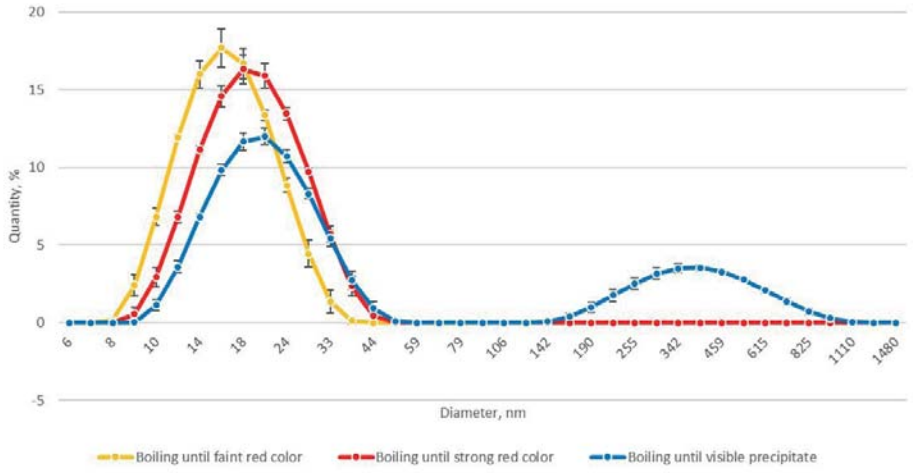

Fig. 2. **Size distribution of AuNP samples synthesized with different duration of boiling.** X axis scale is logarithmic

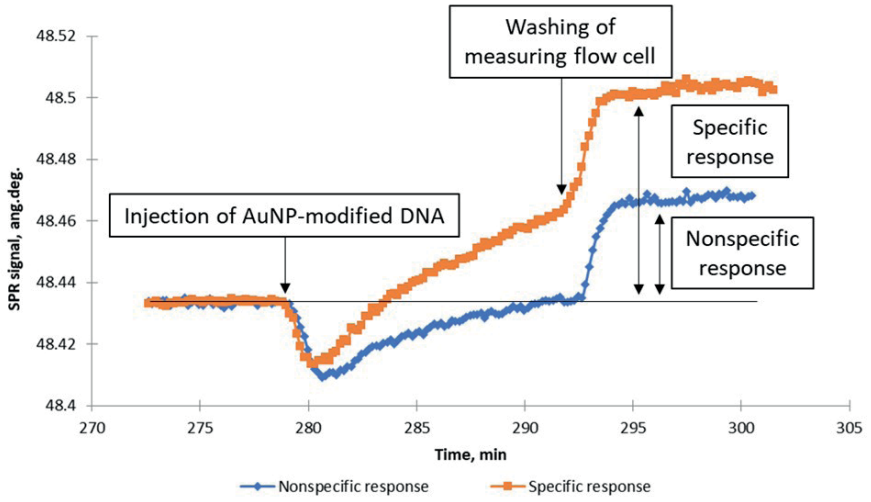

Fig. 3. **Nonspecific and specific responses of the hybridization SPR biosensor to injection of 450 pM DNA labeled with AuNPs**

It can be seen that the level of nonspecific and specific response to AuNP-modified 450 pM DNA is in line with the previously published results, which indicates successful reproduction of AuNPs with particular properties. The limit of detection (LOD) of DNA by SPR biosensor without AuNPs is 50 nM [3], while the newly synthesized AuNPs allow for the lowering of the LOD by several orders of magnitude (to 450 pM).

Conclusions. In this work, a novel perspective method for the synthesis of gold nanoparticles was proposed. It has been shown that the lowest polydispersity of such AuNPs can be achieved through early discontinuation of heating during their synthesis.

The AuNPs synthesized by citrate reduction of AuCl3 were successfully utilized as a colloidal part of a DNA hybridization biosensor system based on SPR spectrometry. As the exact replication of the results of synthesis is dependent on the perception of color of the colloid, it may be suggested that the experimental setup be supplemented with a real-time spectrophotometer.

Authors' contribution

SMS was engaged in the main research work and creation of the original text of theses; KIS was engaged in consultations on biomolecular electronics; LAM was engaged in consultations on AuNP

physics and dynamic light scattering; DSV — helping in planning experiments; SOO — editing theses, supervision.

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