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Comparison of Au, Au–Pt, and Au–Ag nanoparticles as markers for immunochromatographic determination of nonylphenol

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Abstract

Gold spherical nanoparticles, gold-platinum nanoflowers, and gold-silver nanostars were obtained and compared as labels for immunochromatographic analysis. The nanoparticles were synthesized by chemical reduction from various precursors and then conjugated with staphylococcal protein A to be used in indirect immunochromatographic determination of nonylphenol. The results obtained were evaluated in terms of analytical characteristics and R^2 value, as well as the color intensity of the test band. According to the comparison results, it was revealed that the R^2 value varied from 0.82 for the gold-silver nanostars to 0.96 for the spherical gold nanoparticles. The working range of determined concentrations was from 2 to 100 μg/mL for unspherical and from 2 to 50 μg/mL – for spherical markers used; the analysis time was 20 min.

Keywords

gold nanoparticles
gold-platinum nanoflowers
gold-silver nanostars
immunochromatographic analysis
nonylphenol

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1. Introduction

Nanoparticles are widely used as labels in the development of analytical test systems [1]. Due to their unique properties – plasmon resonance, wide color range, the ability to immobilize proteins and other receptor molecules on their surface – they are used as markers in immunochromatography [2]. Synthesized metal particles without a polymer coating on the surface and without functionalization with specific active groups (carboxyl or amino) are able to enter into the process of conjugation by the physical adsorption method [3, 4].

The previous laboratory results have repeatedly confirmed the possibility of using various nanoparticles such as gold [5], magnetic [6], bimetallic [7], semiconductor [8], latex [9] etc., as labels in the analysis. Sometimes, when traditionally used gold nanoparticles were replaced, the sensitivity of the analysis increased [10]. However, in most cases, the sensitivity and the detection limit of the analyte were determined by the affinity of the antibodies, and, in case of insufficient sensitivity of the analysis, schemes for amplifying the analytical signal were offered [11, 12].

Nonylphenol (NP) is a surfactant widely used in everyday life as the main component of detergents. With contaminated wastewater, they enter water bodies and soil and spread through the food chains of ecosystems, which leads to a negative impact on human health [13]. The determination of surfactants in environmental objects is mainly carried out by instrumental chromatographic methods [14], which are characterized by laboriousness, high cost and significant analysis time.

An alternative approach is to use immunochemical methods, especially immunochromatographic analysis (ICA). It has undoubted advantages compared to other immunochemical methods – ease of analysis, short duration (no more than 20 min), and the possibility of visual detection of the analyte when using test systems in out-of-laboratory conditions [15].

The interest and purpose of this work was to compare three types of nanoparticles for the immunochromatographic determination of nonylphenol as the target analyte. To provide this investigation, conjugate hapten-protein, nanoparticles conjugated with protein A were synthesized and the antibodies previously obtained were used [16, 17]. Here, for small molecular weight analyte, a competitive determination scheme is used, in which analytes immobilized and free in the sample compete with each other for antibody binding sites. In this case, in the absence of analyte, the maximal intensity is observed. The increase in its concentration leads to the decrease in staining in the test zone.

2. Experimental part

2.1. Synthesis of a hapten-protein conjugate by the Mannich reaction

The technique was previously used for the synthesis of hapten-protein conjugates and the production of polyclonal rabbit antibodies [16]. For this purpose, 11.5 mg of nonylphenol (NP, hapten) was dissolved in 348 μL of dimethyl sulfoxide (DMSO) at a concentration of 33 mg/mL. A 11 mg of soybean trypsin inhibitor (STI, the carrier protein) was dissolved in 1 mL of 0.1 M carbonate buffer (pH = 10). Then 58 μL of nonylphenol solution was added to the protein solutions. Next, 100 μL of 35–37% formaldehyde was added to each eppendorf tube, followed by incubation under stirring for 30 min at room temperature (RT) and then for 5 days at 37 °C.

2.2. Synthesis of spherical gold (Au) nanoparticles

Spherical gold nanoparticles were prepared according to the typical citrate reduction process [18]. Briefly, the aqueous solution of $HAuCl_4$ (0.25 mM, 98.5 mL) was heated to boiling, and sodium citrate (0.03 M, 1.5 mL) was added with stirring. The mixture was allowed to stir for 15 min and was then cooled.

2.3. Synthesis of gold-platinum (Au–Pt) nanoflowers

The synthesis of gold-platinum nanoflowers was carried out according to the following procedure [19]. $HAuCl_4$ (0.13 M, 100 μL) was added to bidistilled water (50 mL) and heated to boiling with stirring. Sodium citrate (0.03 M, 800 μL) was then added rapidly, and the color of the reaction mixture changed from pale yellow to golden red within a few minutes. For the growth of gold-platinum nanoflowers, ascorbic acid (0.1 M, 1 mL) and sodium hexachloroplatinate (18 mM, 1.25 mL) were successively added to the resulting boiling solution and left for 25 min. All freshly prepared solutions of gold nanoparticles were stored at 4 °C for 3 months.

2.4. Synthesis of gold-silver (Au–Ag) nanostars

Gold nanostars were synthesized using seed-mediated growth [20]. At the first stage, sodium citrate (0.03 M, 15 mL) was added to the boiling aqueous solution of $HAuCl_4$ (0.25 mM, 98.5 mL) to prepare seeds. The reaction mixture was allowed to stir for 15 minutes, after which it was cooled to room temperature. To obtain gold nanostars, hydrochloric acid (1 M, 10 μL), $HAuCl_4$ (0.25 mM, 10mL) and the seed solution (100 μL) were mixed in a 20 mL flask, followed by stirring at about 700 rpm at room temperature. Then, silver nitrate (2 mM, 100 μL) and ascorbic acid (100 mM, 50 μL) were rapidly added to the boiling mixture. The solution was stirred for 30 sec until the color changed to blue.

2.5. Conjugation of nanoparticles with staphylococcal protein A

Protein A was dissolved in deionized water to a concentration of 1 mg/ml. The synthesis was carried out at the rate of 10 μg of protein per 1 ml of nanoparticle sol. Then, prepared Au nanoparticles were pre-adjusted to pH of 8.0 by 0.2 M K_2CO_3. Freshly prepared Au–Pt nanoparticles (pH 2.5) and Au–Ag nanoparticles (pH 3.6) were adjusted with 1 M NaOH to reach the pH 7.9. For conjugate synthesis, 1 mL of nanoparticles was dropped into the glass vessel containing protein A for 45 min at RT. Then, 25 μL of 10% BSA in Milli-Q water was added to stabilize each suspension for 15 min. During the synthesis, conjugates were stirred at Shaker IntelliMixer (ELMI, Riga, Latvia).

The obtained conjugate was separated from unbound protein A and excess of BSA by centrifugation at 9,500 g at +4 °C for 15 min with the use of Amicon 30 kDa centrifuge tubes and then stored in 10 mM borate buffer, pH 8.0.

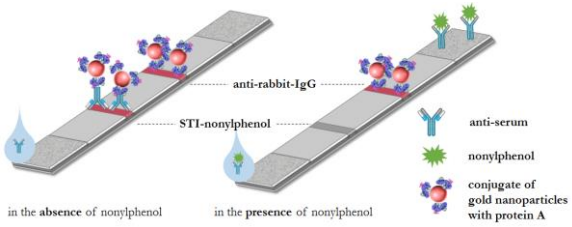
Characterization of the conjugates was provided using a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan) and a JEM CX-100 electron microscope (JEOL, Tokyo, Japan).

2.6. Assembly of the immunochromatographic test system

The lateral flow strip was prepared using the nitrocellulose working membrane, the porous cellulose absorbent membrane, and the glass-fiber membrane for sample adsorbtion. STI-NP (2 mg/mL) and goat-anti-rabbit antibody (0.5 mg/mL) were immobilized on the working nitrocellulose CNPC 15 membrane (0.1 μL per 1 mm) to form the test and the control lines, respectively. Then the multimembrane composite was assembled, dried overnight at RT and cut onto the test strips with a width of 3.5 mm with the use of a guillotine cutter (IndexCutter, USA).

3. Results and Discussion

This work was based on the principle of indirect competitive analysis, which was as follows. Rabbit polyclonal antibodies against nonylphenol previously obtained in the laboratory were used as the receptor molecule in ICA. [16, 17]. The conjugate of nonylphenol with the carrier protein (STI) was immobilized on the working membrane of the test strip to form a test zone (Scheme 1). To determine nonylphenol, the following scheme was used when the stages of interactions were separated: (i) the formation of an antigen-antibody complex in the test zone, and then (ii) the use of a conjugate of nanoparticles with protein A. This was necessary to minimize conjugate consumption and reduce the number of non-specific interactions, since rabbit antiserum contains not only specific antibodies to nonylphenol, but also a host of other immunoglobulins.



Scheme 1. Composition of lateral flow test strip and the main components for analysis.

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Therefore, a washing step in 50 mM phosphate-buffered saline solution with 0.05% Tween-20 (PBST) was added to remove excess antiserum and third-party proteins before the interaction with conjugate of nanoparticles.

First, 50 μL solutions of nonylphenol with known concentrations were added to the wells of the microplate, then 50 μL rabbit antiserum diluted in phosphate-buffered saline containing 1% Tween-20 (PBST*) was added. After the reaction mixture of nonylphenol and rabbit antiserum was incubated for 10 seconds, the test strip was immersed into the solution and initiated the movement of liquid along the pores of the membranes under the action of capillary forces. As soon as the entire liquid front passed over the surface of the working membrane, the test strips were immersed into 100 μL PBST to remove excess amount of antiserum. Then, after 20 seconds, the test strips were transferred to the wells of a microplate with solution of developing conjugate of nanoparticles with protein A in 100 μL PBST* for signal accumulation.

Bonding in the test zone reflects competition. In the absence of nonylphenol in the sample, a colored band formed in the test zone, indicating the formation of a complex with followed composition: (STI-nonylphenol)-antibody-(protein A-nanoparticle). With an increase in the concentration of nonylphenol in the sample, the intensity of staining in the test zone decreased.

In this work, the series of nanoparticles were obtained by chemical oxidation-reduction of various precursors and used as a label. In total, three preparations of different composition (Au, Au–Pt and Au–Ag), size and shape (nanospheres, nanoflowers, nanostars) were synthesized.

The preparations obtained by the chemical syntheses were characterized by spectrophotometry (Figure 1, 1A, 2A, 3A) and transmission electron microscopy (TEM) (Figure 1, 1B, 2B, 3B). The first method made it possible to characterize the spectra of adsorbtion nanoparticles in the synthesis medium. For analysis of nanoparticles by TEM, they were deposited on preliminarily prepared meshes with polyvinylformal precipitated in chloroform. Micrographs were obtained using a JEM-100C electron microscope (Jeol, Japan) and analyzed using the Image Tool program (Health Science Center at the University of Texas at San Antonio, USA). This software allowed to measure the average size of nanoparticles and calculate the effective diameter with the coefficient of ellipticity. Thus, for preparations of Au, Au–Pt, and Au–Ag nanoparticles, the average diameters were 31.7±4.4, 63.4±5.6, and 53.2±9.4 nm, respectively. The ellipticity coefficient for all preparations did not exceed 1.3. The absorption spectra of the obtained sols showed that various methods of synthesis made it possible to obtain preparations with different optical properties, and their absorption peaks were located in different ranges (Figure 1, 1A, 2A, 3A).

Under the selected interaction conditions, calibration curves for the determination of nonylphenol in a buffer solution were obtained (Figure 1, 1C–D, 2C–D, 3C–D). The intensity of staining in the test zone differed depending on the type of nanoparticles used as a label. In all experiments, a 1:4000 dilution of antiserum was used, which allowed obtaining a reliable analytical signal and minimizing non-specific staining in the test zone when nonylphenol was introduced (Table 1).

Table 1 Choosing of antiserum dilution for immunochromatographic analysis with the use of spherical Au nanoparticles conjugated with protein A.

Parameter	Antiserum dilution			
	1:1000	1:2000	1:4000	1:8000
Intensity in the test zone, a.u.	20030	20185	13741	4862
Intensity in the test zone in the presence of NP, a.u.	12694	11575	4949	2357

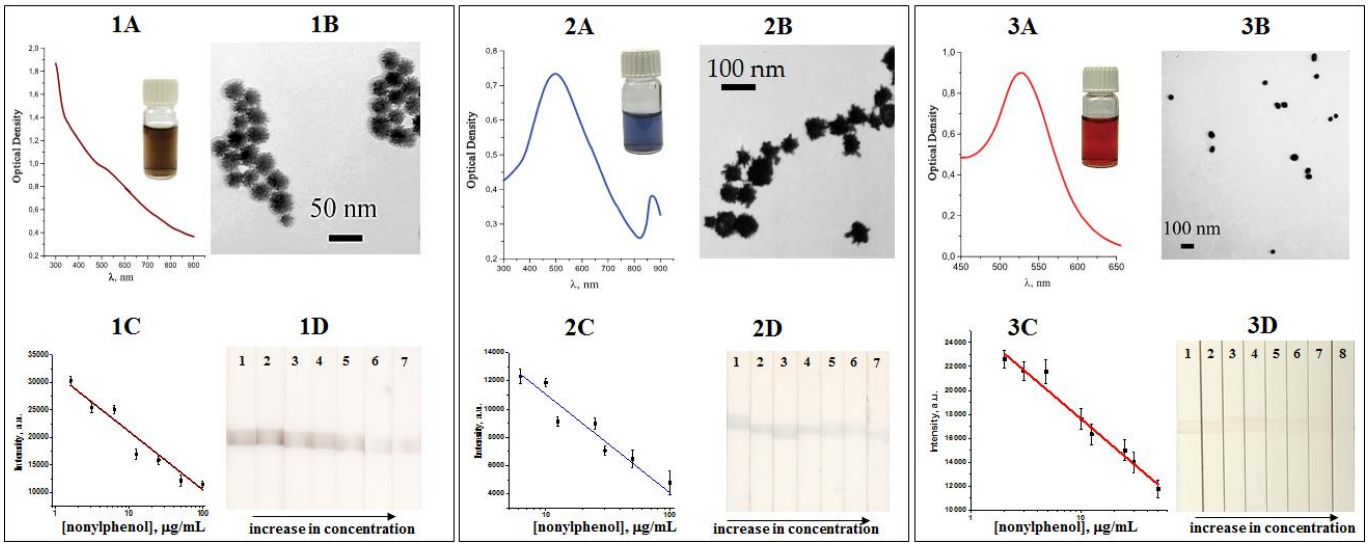

Figure 1 Absorption spectrum (1A, 2A, 3A), TEM images (1B, 2B, 3B), calibration curves (1C, 2C, 3C) and digital images of test strips (1D, 2D, 3D) for Au–Pt nanoflowers (1), Au–Ag nanostars (2) and Au spherical nanoparticles (3). The concentrations of nonylphenol were: for figures 1C–D: 1.6, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL for test strips 1–7; for figures 2C–D: 6.25, 10, 12.5, 25, 30, 50 and 100 μg/mL for test strips 1–7; for figures 3C–D: 2, 3, 4.8, 10, 12.5, 25, 30 and 50 μg/mL for test strips 1–8, n = 3.

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4. Limitations

The presented data demonstrate possibilities to realize immunochromatographic test systems for nonylphenol detection using different nanosized labels. Actually, a lot of preparations were stated as possible alternatives to traditionally used spherical gold nanoparticles, but detailed comparisons of several labels using the same analyte and the same antibody preparations are very limited. The obtained data demonstrate similar working ranges for the compared labels, and reasons for their choice are based mainly on linearity and sensitivity. Probably, this similarity of working ranges is associated with competitive assay format, where affinity of immune interactions becomes limiting factor (in contrast to sandwich ICA of large molecules with strong impact of label's properties on the final analytical parameters). But, overall conclusion about sensitivity, working ranges and linearity for different labels cannot be made only on the basis of the given data and needs additional consideration.

However, further application of the given test systems to different kinds of real samples contaminated by nonylphenol could include additional factors. Matrices of the tested substances may have influence on the rate of the lateral flow along the test strips and the levels of non-specific binding. Due to this, the obtained data of comparative characterization of different labels cannot be simply transferred to real sample testing. Additional characterization of these labels will be necessary as a basis for further conclusion about the best tests for introduction into practice.

5. Conclusions

As part of this work, three types of nanoparticles were obtained and compared – Au nanospheres, Au–Pt nanoflowers, Au–Ag nanostars for their use as a marker in the immunochromatographic determination of nonylphenol. They were conjugated with staphylococcal protein A and used in the last stage of indirect competitive interaction. By the value of the coefficient R^2, it was possible to identify the preferred ones for use as a label. Therefore, in the series (Au) nanoparticles – (Au–Pt) nanoflowers – (Au–Ag) nanostars there is a decrease in the R^2 value, as well as the analytical signal and the sensitivity of the nonylphenol determination.

• Supplementary materials

No supplementary materials are available.

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• Conflict of interest

The authors declare no conflict of interest.

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