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Proceeding Paper

Comparison of Different Formats for Immunochromatographic Detection of Surfactant Nonylphenol †

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Abstract: Immunochromatographic tests are of particular interest as tools for monitoring toxic environmental pollutants. In this regard, the aim of this study was to develop an immunochromatographic test system for the detection of surfactant nonylphenol in water. Two schemes of the assay were compared; they are characterized by detection limits of 1.1 and 0.4 μg/mL and recoveries of nonylphenol from spring water in the range of 78–113.7%.

Keywords: nonylphenol; endocrine disruptor; ecological monitoring; water sources; gold nanoparticles; immunochromatographic assay

1. Introduction

An urgent problem to this day remains the contamination of water sources with endocrine disruptors that have a negative impact on the reproductive functions of aquatic organisms and humans [1]. Nonylphenol, a persistent, bioaccumulative, and toxic nonionic surfactant, was found in wastewater, wastewater treatment plants, surface water, groundwater, and sediment at ng/mL or ng/g levels worldwide [2]. The entry of nonylphenol is caused by the degradation of nonionic surfactants (alkylphenol ethoxylates), which are widely used in the production of emulsifiers, laundry, and dish detergents [3]. In this regard, the widespread occurrence of nonylphenol in aqueous environments stimulates the development of accurate, rapid, and sensitive methods for its monitoring.

Current methods for detecting nonylphenol in water samples include high-performance liquid chromatography [4], gas chromatography–mass spectrometry [5], as well as combination of chromatography with other techniques [6,7]. However, these methods are associated with the need for sophisticated equipment and procedures for sample preparation and extraction, which limits the wide use of these approaches. As alternate solutions, sensors based on antibodies, molecularly imprinted polymers, or aptamers coupled with electrochemical [8,9] or optical readout [10–13] have been proposed. Among them, antibody-based assays, including enzyme-linked immunosorbent assay [14,15], fluorescence polarization immunoassay [16], or biacore biosensor [11], are usually more sensitive and more suitable for routine monitoring [17].

Therefore, this study focused on the immunochromatographic assay (ICA), which is of particular interest due to its rapidity, cost-effectiveness, high throughput, and simple sample preparation. So far as we know, at present, an antibody-based assay in the format of immunochromatography has not previously been proposed. Here, two schemes of ICA, namely the traditional competitive and inverted scheme were compared. Under optimal conditions, analytical characteristics were established for developed ICAs. As proof of the effectiveness of the developed ICA, it was used to analyze spiked spring water samples.

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2. Materials and Methods

2.1. Reagents and Materials

Nonylphenol (NPh, technical mixture of branched isomers), dimethylsulfoxide (DMSO), formaldehyde 37% *v*/*v* aqueous solution, gelatin, Tris, and Tween-20 were supplied by Sigma-Aldrich (St. Louis, MO, USA). Peroxidase-labeled goat anti-rabbit immunoglobulins were sourced from IMTEK (Moscow, Russia). Recombinant staphylococcal protein A and soybean trypsin inhibitor (STI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). In the current study, a rabbit antiserum obtained earlier [18,19] was used. The ready-to-use peroxidase substrate solution based on 3,3′,5,5′-tetramethylbenzidine (TMB) was provided by Immunotech (Moscow, Russia). Gold (III) chloride hydrate and sodium citrate were obtained from Fluka (St. Louis, MO, USA). Potassium dihydrogen phosphate, potassium carbonate, potassium hydroxide, sodium chloride, and hydrochloric acid were purchased from Chimmed (Moscow, Russia).

The nitrocellulose membrane CNPC with a 15 µm pore size, as well as sample pads GFB-R4, and GFB-R7L were provided by Advanced Microdevices (MDI, Ambala Cantt, India). Absorption pad CFSP223000A and macroporous CFCP203000 glass-fiber membrane were from Millipore (Bedford, MA, USA). Enzyme-linked immunosorbent assay (ELISA) was carried out in polystyrene 96-well high-bind 9018 microplates (Corning Costar, Tewksbury, MA, USA). All solutions were prepared with deionized water having a resistance < 18.6 MΩ·cm at 25 °C produced by Simplicity Water Purification System (Millipore, Bedford, MA, USA). All reagents were of analytical grade.

2.2. Preparation of Immunochromatographic (ICA) Components

Nonylphenol was conjugated with STI by the Mannich reaction as described in [18] to obtain NPh-STI preparation. Gold nanoparticles (AuNP) were prepared according to the Frens method [20]. To characterize the size and shape of the AuNP, transmission electron microscopy (TEM) images were acquired using the JEM CX-100 electron microscope (Jeol, Tokyo, Japan).

To obtain AuNP-protein A/NPh-STI, 1 mL of AuNP (OD_{525} = 1) was adjusted to pH 8.5 with 1% K_2CO_3, followed by the addition of 100 µL of protein A or NPh-STI solution to a final concentration of 12 µg/mL. The mixture was incubated at room temperature with stirring for 30 min. Then, 30 µL of 10% BSA was added and the mixture was incubated for another 30 min with stirring. To remove unbound components, the conjugate was centrifuged at 15,000× *g* 4 °C for 20 min. After removal of the supernatant, the pellet was diluted in 200 µL of phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and stored at 4 °C.

2.3. Immunochromatographic Procedure

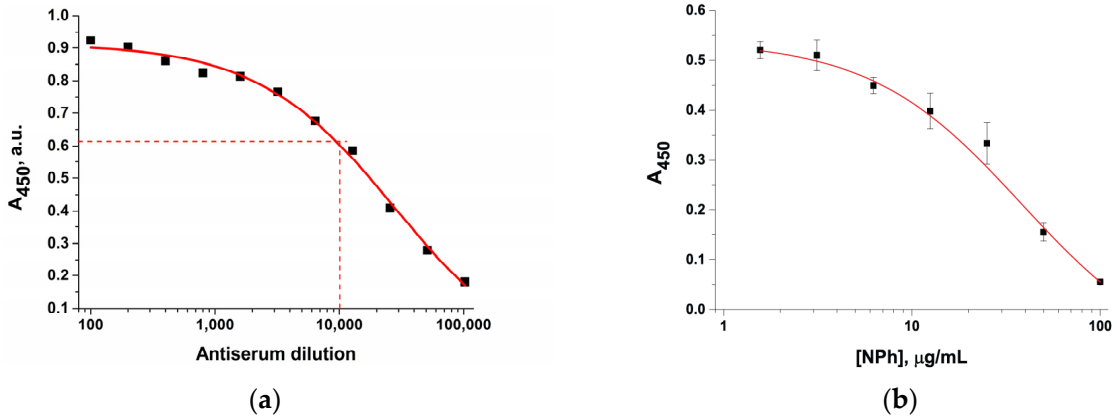
Test strips for ICA were assembled based on approaches described in [18]. Namely, 1 mg/mL of NPh-STI and 1 mg/mL of protein A, respectively, were applied to the test zone for the traditional and inverted ICA schemes. The traditional scheme was carried out as described earlier [18]. To implement the inverted scheme, the test strips were immersed in 100 µL of antiserum for 1 min followed by washing with PBS with 0.05% Tween (PBST) for 1 min. Then, the strips were dried for 10 min and placed in a mixture containing the NPh solutions and AuNP labeled NPh-STI conjugate in PBST for 2 min. To estimate the performance of ICA in real matrices, the test strips were immersed into 100 µL of spiked spring water samples. Next, the ICA strips were placed horizontally and dried for 5 min, followed by scanning with a CanoScan 9000F Mark II scanner (Canon, Japan) and processing by the TotalLab software Version 2.2 (Nonlinear Dynamics, Newcastle upon Tyne, UK).

3. Results and Discussion

3.1. Characterization of Au Nanoparticles and Testing of Antiserum

According to the TEM images, the AuNPs were spherical with an average size of 30.3 ± 1.6 nm (n = 106; the size distribution from 27.7 nm to 33.4) and a degree of ellipticity of 1.19 ± 0.15.

The results of ELISA titration of antiserum are shown in Figure 1a. According to the titration curve, a 10^4-fold dilution of antiserum was chosen for competitive ELISA. As follows from the calibration curve of the competitive ELISA (Figure 1b), the detection limit for NPh was 3.9 μg/mL.



(**a**) (**b**)

Figure 1. ELISA characterization of rabbit antiserum to NPh (n = 3): (**a**) binding of the antiserum to immobilized NPh-STI conjugate; (**b**) competitive assay for NPh.

3.2. Traditional and Inverted Schemes of ICA for NP Determination

Two formats of immunochromatography, namely traditional competitive and inverted schemes, were considered and compared in terms of the minimum detectable concentrations of the analyte. Previously, Sotnikov et al. [21] showed an increase in sensitivity achieved by implementing an inverted analysis scheme and proved the universality of this approach for the determination of low-molecular-weight analytes. The principle of competitive ICA (traditional scheme) is schematically presented in Figure 2a and consists of the competition between the NPh-STI conjugate immobilized in the test zone and the NPh in the sample for binding to specific antibodies in the serum. The number of antigen–antibody complexes formed in the test zone is visualized by passing protein A labeled with AuNP along the test strip. An inverted ICA scheme is shown in Figure 2b and involves the binding of serum antibodies to pre-impregnated protein A in a test zone. After washing the test strip from unbound components of the antiserum, the competition between the NPh in the sample and the NPh-STI conjugate labeled with AuNP for binding to specific antibodies follows. For both schemes, with an increase in of NPh concentration in the sample, the coloration of the test zone decreases up to complete disappearance.

3.3. Optimization of ICA in Traditional and Inverted Schemes

A number of conditions have been optimized to achieve the lowest limit of detection of NPh. First, three types of sample membranes were considered—GFB-R4 and pretreated GFB-R7L, as well as the glass-fiber pad. As can be seen in Figure 3a, the most intense coloration of the test zone in both schemes developed when using a glass-fiber sample pad. Next, the effect of two detergents, namely Tween-20 and Tween-80, on the analytical signal in the absence of NPh in the sample was considered. As follows from Figure 3b, a higher signal was achieved when Tween-20 was added to the running buffer (PBS). Finally, the concentration of the AuNP labeled conjugate was chosen, which provides a detectable signal (Figure 3c). The addition of the AuNP-protein A with an OD_{525} of 0.4 to the sample provided the highest signal while increasing OD_{525} and resulted in background coloration of the strip, indicating an excess of the reagent. As a result, the optimal conditions for the

traditional and inverted ICA schemes were established as follows: glass-fiber sample pad; running buffer containing 1% Tween-20; detecting probe with OD_{525} = 0.4.

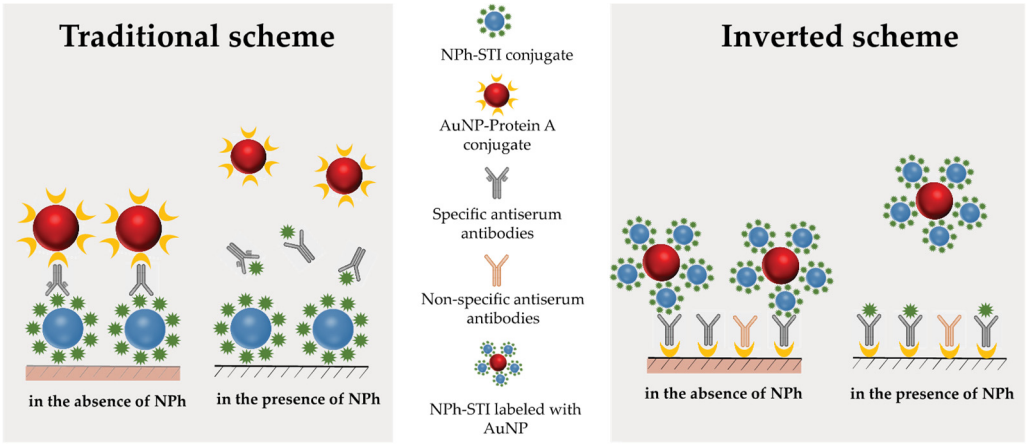


Figure 2. Traditional (**a**) and inverted (**b**) formats of ICA for NPh detection.

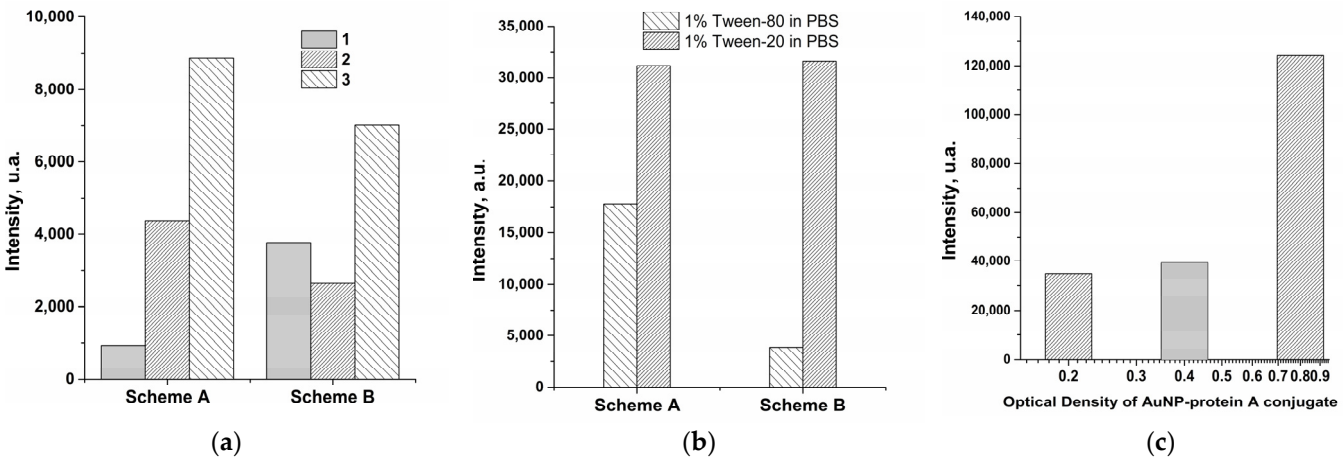
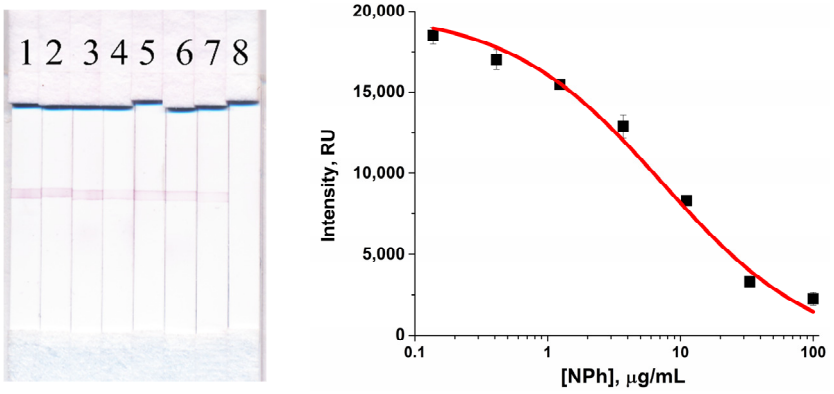


Figure 3. Choice of ICA conditions: (**a**) The coloration intensity of the test zone when using GFB-R4 pad (1), GFB-R7L pad (2), and glass-fiber pad (3). (**b**) Effect of detergents in running buffer (PBS) on color intensity, the absence of NPh. (**c**) The intensity of the test zone of the traditional competitive ICA when using the AuNP-protein A conjugate with a concentration corresponding to OD_{525} of 0.4 (1), 0.2 (2) and 0.8 (3).

3.4. Comparison of Traditional and Inverted ICA Schemes for NPh

The performance of the developed ICAs was evaluated using three-fold serial dilutions of NPh standards from a concentration of 100 μg/mL. The limit of detection of NP calculated as three standard deviations of the blank sample was 1.1 μg/mL for the traditional scheme. The images of test strips and calibration curve of NPh in inverted scheme obtained under optimized conditions are shown in Figure 4. The limit of detection for inverted scheme was calculated to be 0.4 μg/mL. The time required to complete the analysis for both schemes was 20 min. The gain in the values of the detection limit is explained by the sorption capacity of the nitrocellulose membrane; therefore, the number of bound protein A will also be proportionally larger [21]. This is of fundamental importance due to the excess content of total immunoglobulins in addition to specific antibodies in antiserum, which can also bind to protein A.



(**a**) (**b**)

Figure 4. Inverted scheme of ICA: (**a**) scanned image of test strips after application standard solutions of NPh of the following concentrations (1–8): 0; 0.1; 0.4; 1.2; 3.7; 11; 33; 100 μg/mL (**a**); calibration curve of NPs detection (n = 3) (**b**).

To evaluate the efficiency of Scheme B, spring water samples with HPLC-confirmed initial absence of NPh were spiked with different concentrations of NPh and tested. The recovery values (Table 1), which ranged from 78 to 113.7%, confirm the reliability and practicality of the inverted scheme.

Table 1. Recoveries of NPh from spiked spring water samples (n = 3).

NPh Spiked (μg/mL)	NPh Measured (μg/mL)	Recovery (%)
0.5	0.39 ± 0.04	78
1	0.87 ± 0.11	87
3	3.41 ± 0.07	113.7

4. Conclusions

Immunochromatographic test systems for the detection of nonylphenol based on traditional competitive and inverted immunoassay formats were first developed. A comparison of the achieved analytical characteristics showed a 2.75-fold decrease in the detection limit for the inverted scheme. The assay duration for both schemes was 20 min. The reliability of the inverted scheme was verified by testing spiked samples.

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