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Membrane analytical test system for highly sensitive determination of Hg^{2+} ions in natural waters

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ABSTRACT. A membrane test system has been developed for sensitive and specific detection of Hg^{2+} ions in natural waters. The test system uses gold nanoparticles synthesized by citrate reduction and a nitrocellulose membrane with a band of the conjugate of mercaptosuccinic acid (MSA) and bovine serum albumin (BSA). In the presence of Hg^{2+} ions, they are reduced by citrate and adsorbed on the surface of gold nanoparticles. The resulting product moves along the membrane of the test strip under the action of capillary forces and specifically binds to the MSA-BSA conjugate with the formation of a visually detectable colored band. The analysis takes 20 minutes without requiring additional manipulations or instrumentation. Under optimized conditions, the detection limit for Hg^{2+} ions was 0.13 ng/mL. The efficiency of the proposed approach was confirmed by its approbation for the control of natural waters; Hg^{2+} recovery ranged from 70 to 120%.

Keywords: non-laboratory analysis, membrane tests, environmental monitoring, control of natural waters, determination of mercury ions

1. Introduction

Currently, mercury pollution of natural objects is becoming an essential problem, which has been significantly intensified due to urbanization and the increase in industrial waste volumes. The movement and accumulation of mercury in the food chains of ecosystems cause risks to human health. The high toxicity of mercury determines the need for its monitoring in environmental objects (Gworek et al., 2020). The maximum permissible concentrations of Hg^{2+} ions in drinking water have been established by various organizations and vary from 0.5 to 6 ng/mL (US EPA, 2009; GOST, 2011; WHO, 2017). Chromatographic and electrochemical methods are mainly used to detect such low concentrations (Saleh et al., 2020). However, their implementation requires stationary expensive equipment, which limits productivity and increases the cost of testing, significantly lengthening the time required for decision making. These limitations can be overcome only with the widespread introduction into the practice of highly sensitive, productive, and simple methods of non-laboratory testing.

Membrane test systems seem to be promising tools for the rapid and selective detection of various analytes, in particular, Hg^{2+} ions (Bhang and Patel, 2021). Membrane tests make assaying extremely easy: immersion of the test strip in the sample initiates all subsequent interactions and ensures obtaining the final result. Enzymes, aptamers, and antibodies are used in such tests as recognition molecules conjugated with marker nanoparticles. Chelating agents such as derivatives of ethylenediaminetetraacetic acid are effective additional means to bind metal ions. The possibility of Hg^{2+} ions detection through the formation of an amalgam with another metal, Ag or Au, was also shown, and the selectivity of Hg^{2+} detection in this way is ensured through its high affinity to gold nanoparticles (Mertens et al., 2011).

The principle implemented in this study consists of the interaction of mercury ions with gold nanoparticles (AuNPs) and citrate ions immobilized on their surface, followed by complexation with mercaptosuccinic acid (MSA) in the test zone of the membrane. Thus, contact of the test strip with the sample without any additional action leads to the appearance of a colored zone on the membrane.

2. Materials and methods

Aqueous solutions of metal ions (Hg^{2+}, Cd^{2+}, As^{3+}, Cu^{2+}, Pb^{2+}, Sn^{2+}, Sb^{3+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Fe^{3+}, and Pb^{2+}) were prepared by diluting the initial standards (Center for Standardization of

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Samples and Highly Pure Substances, Russia) with a concentration of 1 mg/mL.

AuNPs were synthesized by mixing 100 mL of 0.01% $HAuCl_4$ (Sigma-Aldrich, USA) with 1.5 mL of 1% sodium citrate (Sigma-Aldrich, USA) and incubating at 100°C for 15 min. 10% Tween-20 (10 µL per 1 mL of AuNPs) was added to the resulting product, cooled to room temperature, and incubated for 1 h. The resulting preparation was separated by precipitation at 8,000 g for 15 min and redissolved in deionized water.

The MSA-BSA conjugate was synthesized by adding 450 µL of MSA solution (4.4 mg/mL in citrate buffer, pH 4.0) and 10 mg of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (Sigma-Aldrich, USA) to 200 µL of an aqueous solution of BSA (10 mg/mL). The reaction mixture was incubated for 2 h at room temperature with continuous stirring. The resulting conjugate was dialyzed against 10 mM phosphate-buffered saline (PBS, pH 7.4) with a three-fold wash using Amicon-30 membrane (Merck Millipore, USA).

The test strips were made using the CNPH-90 nitrocellulose membrane as the working membrane, the PT-R7 glass fiber membrane as the membrane for the nanoparticle conjugate, and the AP045 adsorbing membrane as the final absorbent membrane. All membranes were manufactured by Advanced Microdevices, India. To form a test zone, the MSA-BSA conjugate (0.5 mg/mL in 10 mM PBS) was applied to the working membrane using IsoFlow automatic dispenser (Imagene Technology, USA). To cut the test strips 3.5 mm wide, an Index Cutter-1 automatic guillotine cutter (A-Point Technologies, USA) was used.

To carry out the testing, the edge of the test strip was immersed in the sample for 10 min and then placed to dry for 10 min on a horizontal surface. The CanoScan 9000F scanner (Canon, Japan) was used to register the assay results. The staining intensity of the test areas was calculated using the TotalLab TL120 program (Nonlinear Dynamics, UK).

3. Results and discussion

The scheme for the colorimetric determination of Hg^{2+} ions is shown in Fig. 1. Citrate ions located on the AuNPs surface ensure the reduction of Hg^{2+} to Hg^0 (Ojea-Jiménez et al., 2012). In the presence of Hg^{2+}, reduced elemental mercury is deposited on the AuNPs' surface due to its high affinity to gold. Additionally, gold atoms act as catalysts of the amalgamation process. Due to this, the mercury-modified nanoparticles, passing along the test zone with the liquid flow, bind to the thiol groups of the immobilized MSA-BSA conjugate. In the absence of Hg^{2+} ions, AuNPs stabilized with Tween-20 detergent migrate along the membrane, passing the test zone.

In the course of the work, the optimal conditions for Hg^{2+} ions detection were established: the concentration of the MSA-BSA conjugate applied to the test zone was 0.5 mg/mL; medium for the standard (calibration) solutions preparation – citrate buffer, pH 4.0; concentration of detergent Tween-20 – 1%; color development time – 10 min.

Solutions of Hg^{2+} ions with concentrations of 0.1 to 100 ng/mL were tested under the given optimal conditions. The intensity of the test zone is correlated with the concentration of Hg^{2+} ions. Fig. 2 shows the calibration curve for the detection of Hg^{2+} ions in the range from 0 to 25 ng/mL, which a linear approximation with high accuracy describes. The detection limit for Hg^{2+} ions calculated by the 3σ method was 0.13 ng/mL (0.64 nM).

The selectivity of the proposed technique was characterized using solutions of other toxic metal ions (Pb^{2+}, Ag^+, Ba^{2+}, Co^{2+}, Sb^{3+}, Cd^{2+}, Sn^{4+}, Fe^{3+}, Cu^{2+}, As^{3+}, and Ni^{2+}) and cations that are present in large amounts in natural waters (Ca^{2+} and Mg^{2+}). Even with a 100-fold excess of alternative cations, the specific signal exceeds the nonspecific one more than 9 times, demonstrating the high selectivity of the developed test system with respect to Hg^{2+} ions.

To evaluate a practical application, water samples (waterfall and spring) with added known concentrations of Hg^{2+} ions were analyzed. Photos of the test strips after sample analysis are shown in Fig. 3. The recovery of Hg^{2+} detection was within the acceptable limits of 113-120% and 70-100% for spring water and waterfall, respectively. These results confirm the suitability of the developed test system for the detection of Hg^{2+} ions in natural waters.

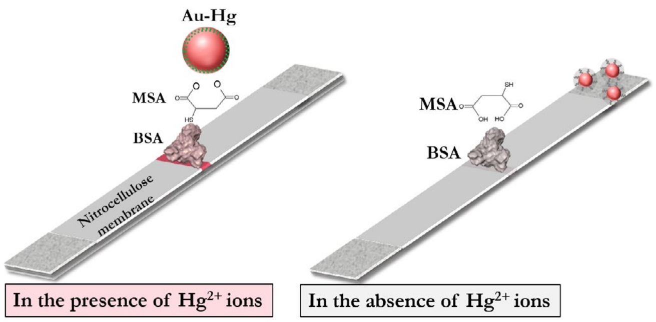

Fig.1. The proposed principle of operation of the test-system for the Hg^{2+} ions detection.

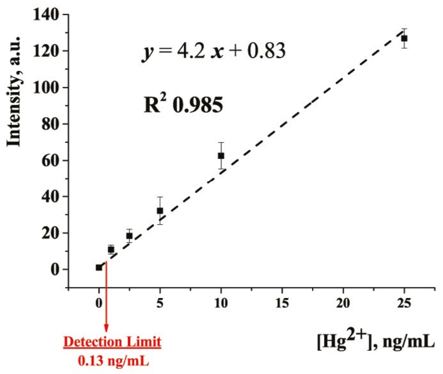

Fig.2. Calibration curve of Hg^{2+} ions.

4. Conclusions

We have proposed a membrane analytical test-system for selective and highly sensitive determination of Hg^{2+} ions. This technique combines the maximum simplicity of analysis, the clarity, and reliability of its results. Analytical characteristics were determined under the optimized conditions, demonstrating the prospects of the developed approach for Hg^{2+} ions determination in natural waters. Moreover, comparison with conventional analytical methods has revealed that the proposed approach is simple to perform, more economical, and exhibits high sensitivity and excellent selectivity.

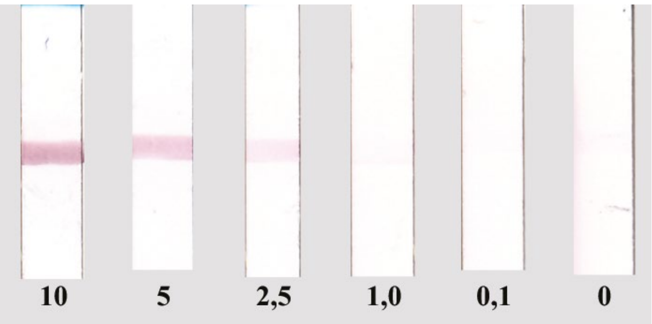

Fig.3. Results of testing spring water samples with different concentrations of Hg^{2+} ions (ng/mL).

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Conflict of interest

The authors declare no conflict of interest.

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