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## Gold nanorods and their nanocomposites based on polyurethane foam for determination of catecholamines in biological fluids

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# Gold nanorods and their nanocomposites based on polyurethane foam for determination of catecholamines in biological fluids

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**Abstract.** Capability of gold nanorods and their nanocomposites based on polyurethane foam as spectrophotometric reagents for catecholamines determination was examined. Comparison of the analytical properties of the systems based on interaction of nanoparticles of different morphology with various reagents in the presence of catecholamines was carried out, gold nanorods-based system is characterized by the highest sensitivity. Applicability of gold nanorods and their nanocomposites for catecholamines determination in biological fluids (urine, serum) was proved by HPLC analysis. Catecholamines preconcentration by dynamic sorption on hypercrosslinked polystyrene and further elution with 6M acetic acid could serve to improve the sensitivity of the analysis.

## 1. Introduction

Nanoparticles (NPs) are widely used in chemical analysis for determination of various compounds. Special optical properties of NPs contribute a lot to their use as peculiar spectrophotometric reagents [1–5]. Correlation of the NPs surface plasmon resonance (SPR) spectrum with their state serves to determine compounds that can directly or indirectly change NPs characteristics. Non-spherical nanoparticles, e.g. gold nanorods (AuNRs) are of special interest as far as their SPR-spectrum is characterized by the presence of two SPR-bands in the visible spectral region – an analytical signal in this case could be based not only on the change of the SPR-maxima intensity but also on the mutual arrangement of maxima and the ratio of their intensities. Most of the analytical techniques are based on aggregation or AuNRs morphology changes, nevertheless, approaches based on formation of nanoparticles of “core-shell” type are also promising. Thus, an approach to catecholamines determination based on formation of silver layer on the AuNRs surface in the presence of catecholamines that leads to a hypsochromic shift of SPR-maxima was reported [6, 7].



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Along with NPs themselves their composites are of certain interest as far as immobilization of nanoparticles in solid matrices could stabilize them and contribute to their ease of use and analytical characteristics [8–11]. A promising material for such nanocomposites is polyurethane foam (PUF) that is characterized by sustainability, ease of use, low cost and absence of its own coloring [12–14].

An important modern task of analytical chemistry is determination of various biologically active compounds, catecholamines (CAs) among them. Such scientific interest to catecholamines, especially to natural epinephrine, norepinephrine and dopamine, is caused by their important vital functions – regulation of heart and psychomotor activity, processes of nervous system [15]. Timely detection of catecholamines abnormal concentrations serves to predict several diseases or to begin treatment at the illness initial stage. Use of nanoparticles could contribute to development of rapid and sensitive techniques of catecholamines determination.

## 2. Experimental

### 2.1. Materials

Hydrogen tetrachloroaurate (chemically pure) and silver nitrate (pure) were used for AuNRs synthesis. Sodium borohydride (analytical grade) and ascorbic acid (chemically pure) were used as reducing agents, cetyltrimethylammonium bromide (CTAB, analytical grade) was used as a stabilizer of AuNRs. Epinephrine (E), norepinephrine hydrochloride (NE) and dopamine hydrochloride (DA) (all of analytical grade) were used as subjects of the study. The substances stock solutions were prepared by dissolving their weighed portions in deionized water. Epinephrine was dissolved in 0.01 mol L<sup>-1</sup> HCl. The stock solutions were frozen and kept in a refrigerator. Working 0.2 mmol L<sup>-1</sup> solutions were prepared from the stock solutions immediately before use. Glycine buffer (pH 9.5) was prepared by addition of 74.8 mL of 0.1 mol L<sup>-1</sup> glycine (7.51 g of glycine and 5.85 g of NaCl in 1 L) to 25.2 mL of 0.1 mol L<sup>-1</sup> NaOH. Copper (II) chloride (chemically pure) was used for the comparative studies of various nanoparticles analytical characteristics. Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), octane-1-sulfonic acid sodium salt, acetonitrile (all of analytical grade) were used for preparation of chromatographic eluent. Acetic acid (analytical grade) was used for catecholamines solid-phase extraction.

Commercially available polyether-type polyurethane foam (PUF) was used as a matrix for nanocomposites. Tablets of PUF of 16 mm in diameter (20 ± 2 mg) were cut with a metal punch from an industrial sheet of the polymer. To remove impurities, the PUF tablets were placed in acetone and shaken for 10 minutes. The procedure was repeated twice, after which the tablets were dried under stream of air.

The extraction of catecholamines from biological fluids was carried out with the use of a microcolumn filled with hypercrosslinked polystyrene (Diapak P-3 cartridges, ZAO “BioChemMak ST), the particle size of the sorbent was 50 – 100 μm, the pore diameter was 10 – 1000 Å.

### 2.2. Instruments

Absorption spectra of solutions were recorded by SF-103 spectrophotometer (Akvilon, Russia). Diffuse reflection in the visible region was recorded by Eye-One Pro mini-spectrophotometer (X-Rite, USA).

Chromatographic determination of catecholamines was performed using a Tsvett Yauza chromatograph (NPO Khimavtomatika, Russia) with an amperometric detector (E = +0.8 V). The chromatographic column “Eclipse XDB-C18” (Agilent) and guard column Security Guard C18 were used. Eluent contained acetonitrile and 0.1% H<sub>3</sub>PO<sub>4</sub> in water (10:90, v/v) with addition of sodium 1-octanesulfonate (0.3 mmol L<sup>-1</sup>). The flow rate was 0.4 mL min<sup>-1</sup>. The eluent was degassed in a Bransonic 1510R-DTH ultrasonic bath (USA). Sample volume was 20 μL, injection was performed with a loop.

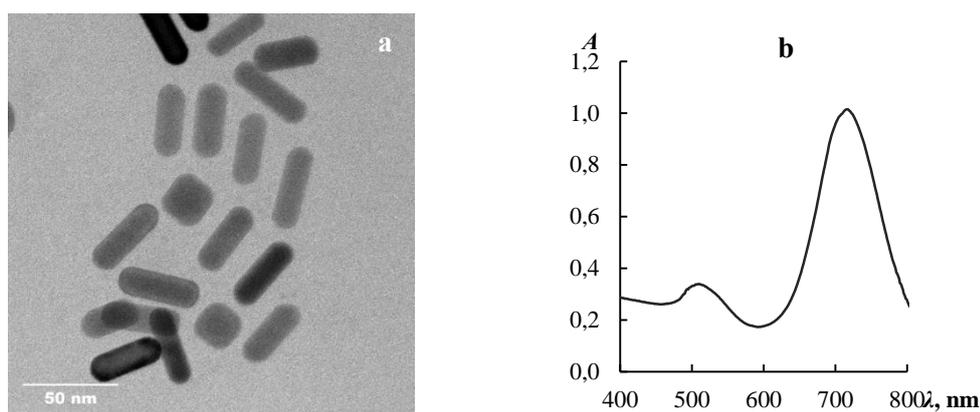
A concentrating microcolumn (l = 6 cm, d = 10 mm) and a vacuum unit for solid-phase extraction M6 (Manifold, Russia) were used for catecholamines preconcentration.

Deionized water was obtained from the purification system Millipore Simplicity (Millipore, Germany). A mechanical shaker and a magnetic stirrer were also used.

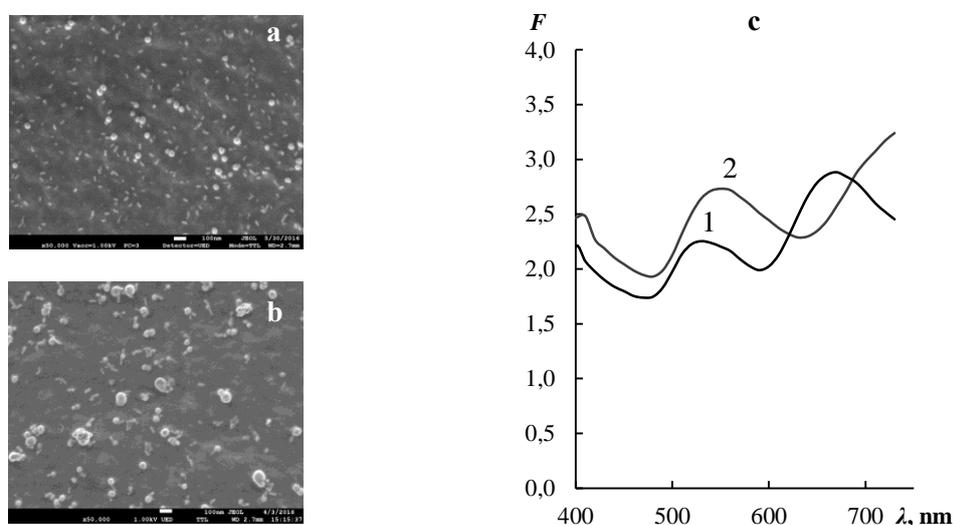
### 2.3. Synthesis of AuNRs and their nanocomposites based on polyurethane foam

AuNRs were synthesized according to the method previously reported by Nikoobakht and El-Sayed [16] with some modifications. A detailed scheme of the synthesis is described in [7]. The obtained nanoparticles are characterized by the presence of two SPR-bands in the visible spectral region, corresponding to transverse and longitudinal plasmon oscillations. TEM-image and typical absorption spectrum of the obtained AuNRs are presented in figure 1.

The nanocomposite material based on polyurethane foam and AuNRs was obtained by sorption modification of polyurethane foam [13]. Samples of the nanocomposite were modified with silver nitrate, this procedure is described in detail in [14]. SEM-images and typical diffuse reflectance spectra of the obtained nanocomposites before and after modifying are presented in figure 2.



**Figure 1.** TEM-image (a) and a typical absorption spectrum (b) of AuNRs.



**Figure 2.** SEM-images (a, b) and typical diffuse reflectance spectra (c) of the nanocomposites before (a, c1) and after (b, c2) modifying with silver nitrate.

#### 2.4. Synthesis of spherical gold nanoparticles and their nanocomposites

Synthesis of spherical nanoparticles was performed similarly to AuNRs synthesis, the difference was the absence of silver nitrate that initiates formation of long nanoparticles. Previously the seeds were synthesized by adding 5.00 mL of 0.50 mmol L<sup>-1</sup> HAuCl<sub>4</sub>, 5.00 mL of 0.20 mol L<sup>-1</sup> CTAB aqueous solutions to a 250 mL round-bottom flask with stirring. Then, 0.60 mL of 0.010 mol L<sup>-1</sup> NaBH<sub>4</sub> solution cooled to 0–4 ° C was added dropwise. To synthesize spherical nanoparticles 2.25 mL of deionized water, 45 mL of 0.20 mol L<sup>-1</sup> CTAB, 45 mL of 1.0 mmol·L<sup>-1</sup> HAuCl<sub>4</sub> were added to a 250 mL round bottom flask with stirring. After this, 0.56 mL of 0.10 mol·L<sup>-1</sup> ascorbic acid was added to the solution dropwise, then 0.10 mL of the pre-synthesized seed solution was added. The resulting

solution was kept for 2 weeks, then centrifuged for 30 min at 8000 rpm, the supernatant was removed by decantation, NPs were washed with deionized water by dispersing the precipitate in it. Separation of the supernatant by centrifugation and redispersion of NPs in deionized water was repeated twice.

For the synthesis of nanocomposites, 2.0 mL of non-redispersed in deionized water spherical NPs were placed in a test-tube, 2 mmol of NaCl was added, the total volume was 5.0 mL. Then, a PUF tablet was added, the tube was shaken on a mechanical shaker for 30 minutes. The tablet was removed, dried between sheets of filter paper and placed into 5.0 mL of 1 mmol·L<sup>-1</sup> AgNO<sub>3</sub> solution. Modifying with silver nitrate was carried out for 10 min with stirring on a shaker. A tablet of the nanocomposite was removed from the solution and dried with filter paper.

### 2.5. General procedure

To study the interaction of colloidal solutions of AuNRs with catecholamines, 0.3 mL of AuNRs, 20 μL of 0.01 mol L<sup>-1</sup> AgNO<sub>3</sub>, 0.1 mL of glycine buffer solution and 0–0.5 mL of 25 μmol L<sup>-1</sup> catecholamines solution were added into a test-tube. The total volume of all components was 2.5 mL. The absorption spectra of the obtained mixtures were measured after 3 minutes. The position of the long-wavelength maximum was considered as the point at which the first derivative of the function A (λ) is zero.

Interaction of the nanocomposite modified with silver nitrate with catecholamines was studied by placing the nanocomposite into 5 mL of solution containing 1–100 μmol L<sup>-1</sup> catecholamines and 1 mL of glycine buffer solution. It was shaken on a mechanical shaker for 30 minutes. Then the nanocomposite was removed and dried between sheets of filter paper. The diffuse reflection coefficients (*R*) of the obtained nanocomposite were measured in a range of 400–730 nm with the 10 nm increment. Then, the values of the Kubelka-Munk function (*F*) were calculated from the formula:  $F = \frac{(1-R)^2}{2R}$ , here *R* is the diffuse reflection coefficient. The plotted spectra *F* versus λ were used to estimate changes in state of AuNRs in the phase of polyurethane foam. The exact position of the maximum was determined by calculation an arithmetic mean of two λ values of inflection points of the spectrum (zero-points of the *F*(λ) second derivative function).

To carry out solid-phase extraction of catecholamines in the dynamic mode, a concentrating microcolumn (l = 6 cm, d = 10 mm) packed with 0.02 g of hypercrosslinked polystyrene and a vacuum unit for solid-phase extraction M6 (Manifold, Russia) were used. Before use, the column was conditioned with 1 mL of acetonitrile and 10 mL of water. The rate of passing the solution through the column was 1.0 mL/min. Before desorption, the column was washed with 10 mL of water. Desorption of catecholamines was performed by 3 mL of 6 mol L<sup>-1</sup> acetic acid.

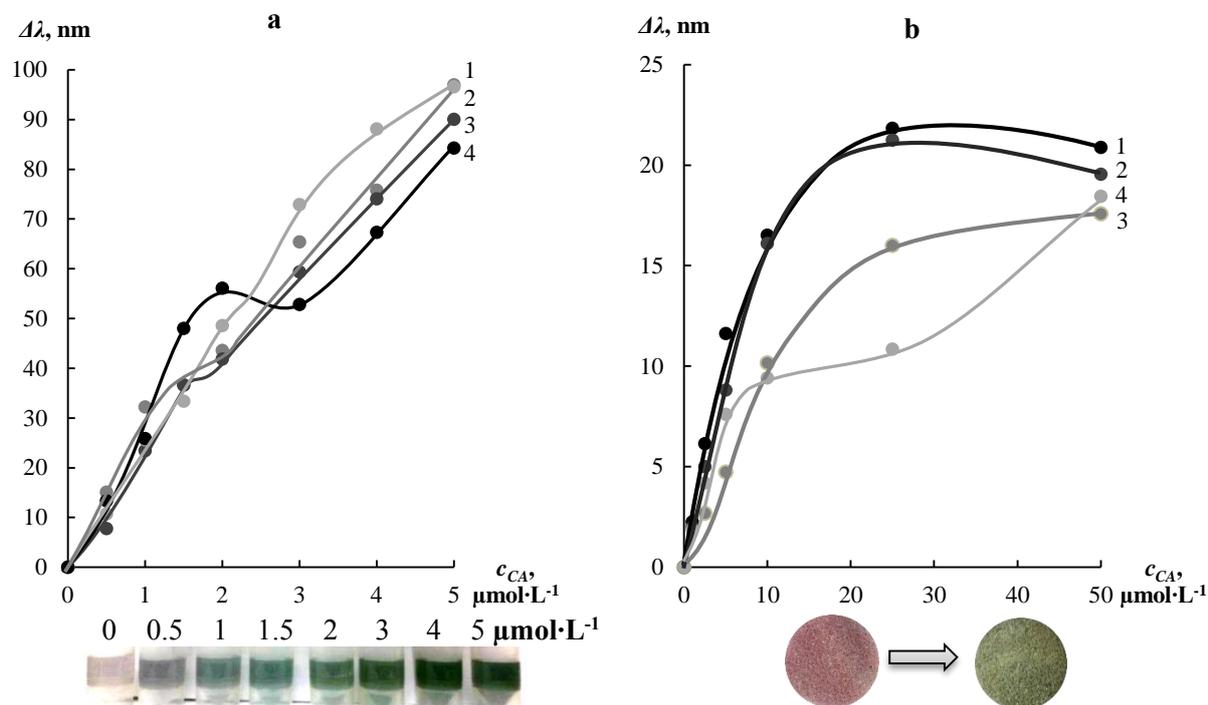
## 3. Results and discussion

### 3.1. Interaction of catecholamines with AgNO<sub>3</sub> in AuNRs colloid solutions and nanocomposites

As it has been described before [7, 14, 17], the interaction of catecholamines with silver ions in the presence of AuNRs or the nanocomposite is accompanied by a hypsochromic shift of the SPR bands and an increase of their intensity. These effects are visually detected as change of color of the AuNRs solution from pale purple to bright green; the nanocomposites color changes from pale purple to grey. The shift of the maxima (Δλ) can be considered as a characteristic of completeness of the interaction.

The scheme of the interaction includes oxidation of the catechol group by silver ions with formation of the ortho-quinonoid structures and subsequent deposition of metallic silver onto the AuNRs surface.

Dependencies of the shift of maxima on concentration of individual epinephrine, norepinephrine, dopamine and their equimolar mixture are represented in figure 3. Linear parts of these dependencies can be used as calibration graphs for determination of catecholamines. Some analytical characteristics of the determination are presented in table 1. Determination of catecholamines with the use of colloidal solutions of AuNRs is characterized by higher sensitivity in comparison to the nanocomposites. Nevertheless, analysis with the use of nanocomposites is characterized by larger linear range and higher selectivity in the presence of inorganic ions that could be ascribed to the matrix effects.



**Figure 3.** Dependencies of the shift of maxima of AuNRs solutions (a) and nanocomposites (b) on concentration of individual epinephrine (1), dopamine (2), norepinephrine (3) and their equimolar mixture (4) and photos of the obtained solutions and composites.

**Table 1.** Analytical characteristics of the catecholamines determination ( $n = 3$ ,  $P = 0.95$ ).

		dopamine	norepinephrine	epinephrine	equimolar mixture
With the use of AuNRs colloidal solution	LOD, $\mu\text{mol}\cdot\text{L}^{-1}$	0.08	0.1	0.1	0.1
	Linear range, $\mu\text{mol}\cdot\text{L}^{-1}$	0.2 – 5.0	0.3 – 5.0	0.3 – 5.0	0.3 – 5.0
With the use of nanocomposites	LOD, $\mu\text{mol}\cdot\text{L}^{-1}$	0.3	0.3	0.4	0.3
	Linear range, $\mu\text{mol}\cdot\text{L}^{-1}$	0.9 – 25	1.0 – 25	1.1 – 25	0.9 – 25

### 3.2. Comparison of the analytical characteristics of the catecholamines determination using various nanosystems

Epinephrine was used to study the interaction of catecholamines with silver nitrate, chloroauric acid and copper (II) chloride as oxidizing agents in the presence of colloid solutions of AuNRs and spherical gold NPs, spectra of the obtained solutions are presented in figure 4. In case of AuNRs and silver nitrate, as it was described above, hypsochromic shift of both maxima is observed (figure 4 a). In case of the interaction of catecholamines with chloroauric acid in the presence of AuNRs, a bathochromic shift of the SPR maxima of AuNRs is observed, which is presumably caused by the growth of AuNRs in the longitudinal direction as a result of the predominant reduction of chloroauric acid at the end faces of AuNRs (figure 4 b). If copper (II) chloride is used as an oxidizing agent, no significant changes in the absorption spectra of AuNRs occur (figure 4 c). Varying the amount of CA (1–4  $\mu\text{mol L}^{-1}$ ) and copper chloride (8–160  $\mu\text{mol L}^{-1}$ ) does not lead to any spectral changes. It is likely that under the given conditions, the formal potential of CA oxidation is insufficient for the reduction of Cu (II); in addition, the formation of complex compounds of Cu (II) with CA is possible, that causes decrease of the reduction potential of copper.

Along with AuNRs, spherical NPs were studied as spectrophotometric reagents for the determination of catecholamines. As can be seen from figure 4 d, as a result of the interaction, a hypsochromic shift of the SPR-band of the nanoparticles occurs, this is caused by formation of a silver shell on the nanoparticles surface. The obtained dependence of  $\Delta\lambda$  on epinephrine concentration is shown in figure 5. It is seen that in the case of the interaction of catecholamines with  $\text{AgNO}_3$  in the presence of NPs, lower  $\Delta\lambda$  values are observed than in the presence of AuNRs (figure 3 a, table 2). The same interaction has been studied under heating. To do this, the reaction mixtures were kept in a water bath at  $70^\circ\text{C}$  for 25 min, then cooled for 5 min in ice water, and the absorption spectra were recorded. Heating contributes to an increase of the analytical signal, but the  $\Delta\lambda$  values do not exceed the values obtained in the system with AuNRs, the color change of the solutions is less contrast (figure 5 a). The determination range is also narrower because of the impossibility of fixing the exact position of the SPR maximum at high catecholamine contents.

Comparison of the catecholamines interaction with the nanocomposites based on AuNRs and spherical NPs has been also carried out. Figure 5 b shows the dependence of a hypsochromic shift of the SPR maximum of spherical NP-based nanocomposites on the epinephrine concentration. The initial section of this curve was used as a calibration graph. Table 3 summarizes analytical characteristics of the epinephrine determination using nanocomposites based on spherical NPs and AuNRs. According to the data, the sensitivity of the catecholamines determination using nanocomposites based on spherical nanoparticles is noticeably worse than the sensitivity of the technique based on AuNRs nanocomposites. This fact is associated with poor reproducibility of the SPR maximum position in the control experiment.

All these data prove that the most applicable nanosystem for catecholamines determination is that based on AuNRs/AuNRs-nanocomposites and silver nitrate, these systems should be used for the catecholamines determination in real samples.

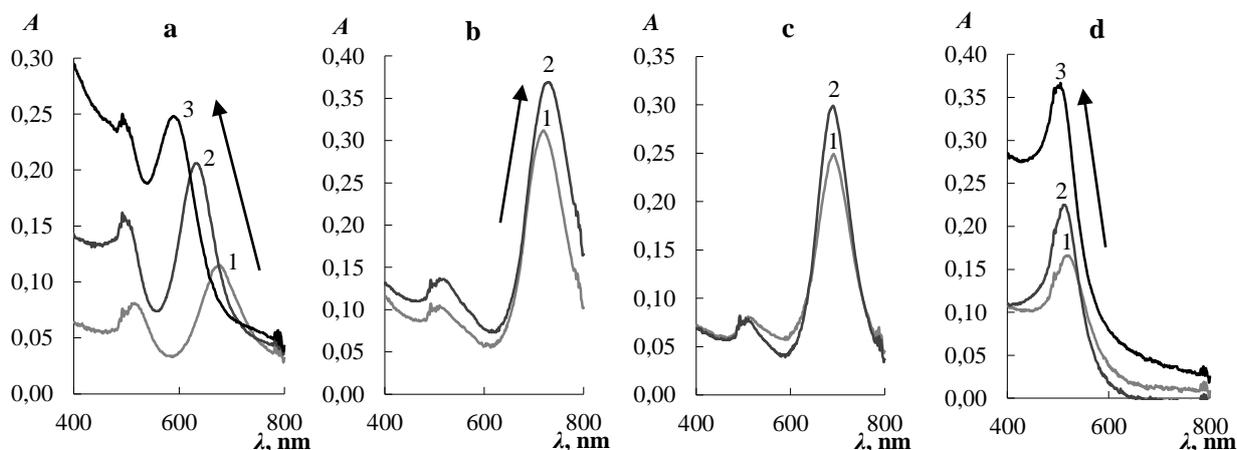
**Table 2.** Characteristics of the epinephrine determination with the use of various nanoanalytical systems ( $n = 3$ ,  $P = 0.95$ ).

Characteristic	Analytical system			
	AuNRs, $\text{AgNO}_3$	AuNRs, $\text{HAuCl}_4$	AuNRs, $\text{CuCl}_2$	Spherical NPs, $\text{AgNO}_3$
$\Delta\lambda$ in the presence of $2 \mu\text{mol L}^{-1}$ epinephrine	43 74*	-9.6	0	6.5 16
LOD, $\mu\text{mol L}^{-1}$	0.1 0.1*	0.1	Determination is impossible	0.2 0.1*
Linear range, $\mu\text{mol L}^{-1}$	0.3 – 5.0 0.4 – 5.0*	0.4 – 4.0		0.6 – 5.0 0.4 – 3.0*

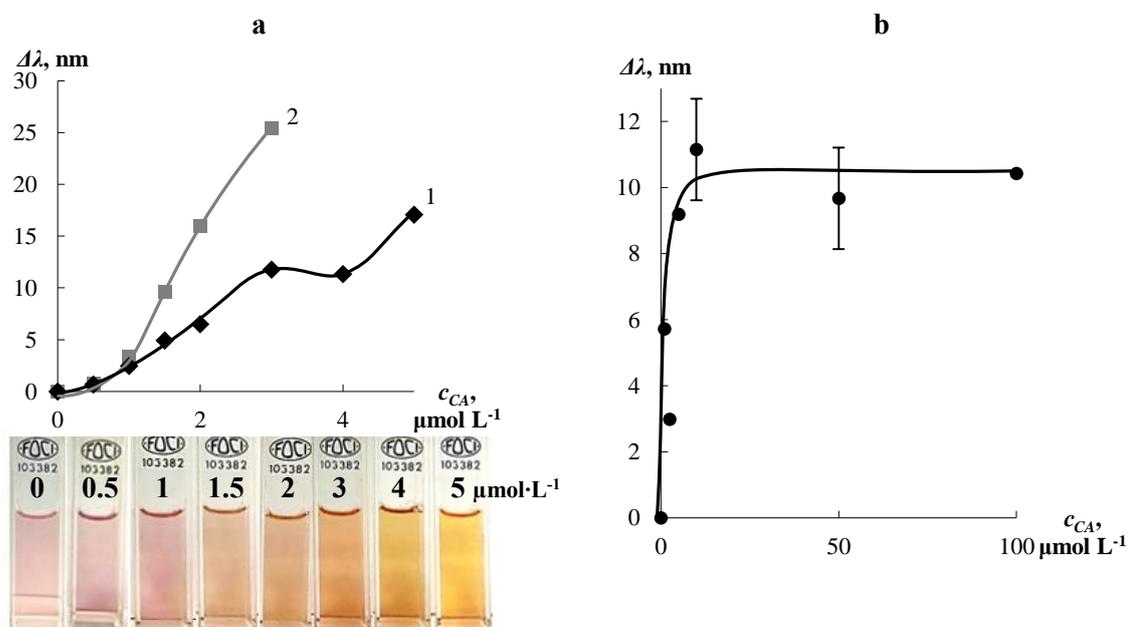
\* Determination was carried out at  $70^\circ\text{C}$

**Table 3.** Analytical characteristics of the epinephrine determination using nanocomposites based on AuNRs and spherical NPs ( $n = 5$ ).

Characteristic	Nanocomposites are based on	
	AuNRs	Spherical NP
LOD, $\mu\text{mol}\cdot\text{L}^{-1}$	0.4	3
Linear range, $\mu\text{mol}\cdot\text{L}^{-1}$	1.1 – 25.0	–



**Figure 4.** Absorption spectra of AuNRs (a–c) and spherical NPs (d) in the presence of  $80 \mu\text{mol L}^{-1}$   $\text{AgNO}_3$  (a, d),  $\text{HAuCl}_4$  (b),  $\text{CuCl}_2$  (c) and 0 (1) / 2 (2)  $\mu\text{mol L}^{-1}$  epinephrine registered after the reaction carried out at  $25^\circ\text{C}$  (1, 2) and  $70^\circ\text{C}$  (3).



**Figure 5.** Dependencies of the SPR maximum shift of colloid solutions (a) and nanocomposites (b) of spherical NPs on epinephrine concentration after interaction at  $25^\circ\text{C}$  (1) and  $70^\circ\text{C}$  (2); and photos of the solutions obtained at  $70^\circ\text{C}$  ( $c_{\text{CA}} = 0\text{--}5 \mu\text{mol L}^{-1}$  (a),  $c_{\text{CA}} = 0\text{--}100 \mu\text{mol L}^{-1}$  (b),  $c_{\text{AgNO}_3} = 80 \mu\text{mol L}^{-1}$  (a), glycine buffer solution, pH 9).

### 3.3. Catecholamines determination in biological fluids

Previously, it was reported that the use of AuNRs is appropriate to total catecholamines determination, as far as the sensitivity coefficients in the calibration graph equations differ slightly [17]. Determination of total catecholamines is relevant for diagnosis of such diseases as Alzheimer's disease, Addison's disease, pheochromocytoma – illnesses that cause change of the one of the catecholamines content or simultaneous increase/decrease of several catecholamines content [18–20]. In this work applicability of AuNRs colloid solutions and their nanocomposites for catecholamines determination in biological fluids (urine, serum) was examined.

### 3.3.1. Determination of the total catecholamine content without preconcentration

Urine samples were analyzed using the standard addition method by introducing urine samples and catecholamines mixture (epinephrine : norepinephrine : dopamine = 1:7:32) to the reaction mixture, the additives of catecholamines in case of AuNRs solutions were 0/1/2/3  $\mu\text{mol L}^{-1}$ , in case of nanocomposites – 0/5/15  $\mu\text{mol L}^{-1}$ .

It was found out that introduction of 1.0 mL of urine into the reaction mixture containing AuNRs solution leads to aggregation of nanorods, determination of catecholamines in these conditions is impossible. Introduction of 0.25 mL of urine into the reaction mixture does not violate the stability of the colloidal system; registration of the analytical signal is possible. It was found that 3 min is not enough for the reaction: the  $\Delta\lambda$  values of some solutions were negative, in other solutions the  $\Delta\lambda$  values were significantly lower than the corresponding data for aqueous solutions in the absence of urine. The maximum  $\Delta\lambda$  values were reached after 1.5 h after mixing the reagents.

Nanorods immobilized on PUF are characterized by greater stability in comparison to the colloidal system: introduction of 3 mL of urine into the reaction mixture does not cause AuNRs aggregation, CAs determination is possible. Good agreement between the data obtained for the same urine sample using both colloidal solutions and using the nanocomposites, as well as the results of chromatographic analysis (table 4), indicates good accuracy of the proposed methods.

As can be seen from the results of HPLC analysis, the ratio of catecholamines contents in the studied samples does not correspond to the average catecholamines ratio in human daily urine (1:7:32) [15]. This can be explained by that the samples were collected in separate short time intervals. Since it was found that the concentration ratio of catecholamines in the samples was not 1:7:32, in further experiments equimolar catecholamine mixtures were used as additives.

**Table 4.** Results of total catecholamines determination ( $\mu\text{mol L}^{-1}$ ) in urine samples with the use of AuNRs colloid solutions, nanocomposites and HPLC

Analysis method	Sample 1	Sample 2
With the use of AuNRs colloid solutions		14.0 $\pm$ 0.7* (RSD 6%)
With the use AuNRs nanocomposites	10.2 $\pm$ 0.9 (RSD 9%) 9.9 $\pm$ 0.4* (RSD 5%)	14.5 $\pm$ 0.9 (RSD 5%) 14 $\pm$ 1* (RSD 9%)
HPLC	10 $\pm$ 3 (RSD 20%) E: 7 $\pm$ 3 NE: 1.4 $\pm$ 0.2 DA: 0.9 $\pm$ 0.7	14 $\pm$ 5* (RSD 20%) E: 10 $\pm$ 2 NE: 1 $\pm$ 1 DA: 3 $\pm$ 4

\* The analyzed samples were diluted with deionized water

To evaluate the possibility of catecholamines determination in blood serum the spiked samples were used. To do this, 0.10 mL of serum was introduced into the reaction mixture in the case of using AuNRs solutions and 0.25 mL of serum in case of nanocomposites. Preliminarily, the dependences of  $\Delta\lambda$  on the total concentration of catecholamines in the system were measured, the initial sections of which were used as calibration plots. According to the results presented in table 5, the developed techniques are applicable for determination of catecholamines in the reaction mixture containing blood serum at the levels of 0.5 and 5  $\mu\text{mol L}^{-1}$  in case of AuNRs colloidal solutions or their nanocomposites, respectively. However, the sensitivity of the proposed methods in some cases is insufficient for analysis at a clinically significant levels, therefore the development of methods for pre-concentration of catecholamines is required.

**Table 5.** Results of total catecholamines determination in blood serum with the use of AuNRs colloid solutions and their nanocomposites (n = 3, P = 0.95).

Analysis method	Added, $\mu\text{mol L}^{-1}$	Found, $\mu\text{mol L}^{-1}$	RSD, %
With the use of AuNRs colloid solutions	0.5	0.5 $\pm$ 0.1	8
With the use AuNRs nanocomposites	5	4.8 $\pm$ 0.3	2

### 3.3.2. Determination of the total catecholamines content after their solid-phase extraction with the use of hypercrosslinked polystyrene

As can be seen from the data presented in tables 4, 5, the developed methods allow reliable determination of CAs if their total content is in the region of 0.5–15  $\mu\text{mol L}^{-1}$ . Since in some cases the content of CAs in biological fluids is lower than this value, it is important to study the possibility of increasing the sensitivity of the developed determination techniques by pre-concentration of analytes. A promising option is solid-phase extraction (SPE) of CAs with the use of hypercrosslinked polystyrene, followed by their elution with a small volume of a solvent. It is well-known [21–23] that this sorbent is universal in relation to various organic compounds and, due to its microporous structure, allows to separate these compounds from the matrix high molecular weight substances. Therefore, this sorbent can be used to reduce the matrix effect.

To analyze urine samples, they were 10 times or 1.2 times diluted and 0, 0.5 and 1  $\mu\text{mol L}^{-1}$  or 0, 5 and 10  $\mu\text{mol L}^{-1}$  CAs equimolar mixtures were added. Total volumes of the samples with the additives were 10.0 mL and 15.0 mL respectively. The mixtures were passed through a microcolumn packed with hypercrosslinked polystyrene. Then, CAs were eluted by 3 mL of 6 mol  $\text{L}^{-1}$  acetic acid. Before the CAs determination with the use of AuNRs, solution of 10 mol  $\text{L}^{-1}$  NaOH was added to the eluates in order to provide pH 8. In the first case, the determination by the standard addition method is possible using colloidal solutions of AuNRs and HPLC. The data obtained are presented in table 6. Since the content of CAs in the eluates without additives was below LOD of the procedure using nanocomposites, analysis by this method was not applicable. The second variant of CAs preconcentration (1.2 times dilution of urine samples) turned out to be appropriate for the analysis with the use of nanocomposites. This is confirmed by a good agreement with the results of the analysis using AuNRs solutions and HPLC (table 6). The LODs for total CAs for these samples in the case of AuNRs solutions are below the normal CA content. In the case of nanocomposites, they correspond to the lowest values of the normal content.

Use of the spiked samples proved that catecholamines determination in blood serum after their SPE on hypercrosslinked polystyrene is also possible. The solutions contained 1.0 mL of serum and various additives of the equimolar mixture of CAs. SPE technique was the similar to analysis of urine samples. The data obtained are presented in table 7. Since the content of CAs in the blood was below LOD of the developed technique, this experiment shows only the potential possibility of using AuNRs to determine CAs in blood serum.

SPE of catecholamines from biological fluids allows to reduce the influence of external components of the matrix, which is especially evident in the case of HPLC determination. Also it increases sensitivity of the determination due to preconcentration, as was demonstrated by the analysis of urine samples.

**Table 6.** Results of total catecholamines determination ( $\mu\text{mol L}^{-1}$ ) in urine samples after solid-phase extraction with the use of hypercrosslinked polystyrene

Analysis method	Sample 1	Sample 2
With the use of AuNRs colloid solutions	1.50 $\pm$ 0.05 <sup>a</sup> (RSD 1%)	3.0 $\pm$ 0.1 <sup>a</sup> (RSD 2%)
With the use of AuNRs nanocomposites	–	3 $\pm$ 1 <sup>b</sup> (RSD 20%)
HPLC	1.5 $\pm$ 0.4 <sup>a</sup> (RSD 10%)	E: 0.83 $\pm$ 0.07 NE: 0.1 $\pm$ 0.3 DA: 0.6 $\pm$ 0.2
		3.7 $\pm$ 0.7 <sup>a</sup> (RSD 8%)
		E: 0.7 $\pm$ 0.5 NE: 0.06 $\pm$ 0.04 DA: 2.9 $\pm$ 0.5

Composition of the solutions:

<sup>a</sup> 1.0 mL of urine, 0/0.5/1  $\mu\text{mol L}^{-1}$  additive of catecholamines equimolar mixture, V = 10.0 mL

<sup>b</sup> 12.5 mL of urine, 0/5/10  $\mu\text{mol L}^{-1}$  additive of catecholamines equimolar mixture, V = 15.0 mL

**Table 7.** Results of total catecholamines determination ( $\mu\text{mol L}^{-1}$ ) in serum after solid-phase extraction with the use of hypercrosslinked polystyrene (n = 3, P = 0.95)

Analysis method	Added, $\mu\text{mol L}^{-1}$	Found, $\mu\text{mol L}^{-1}$	RSD, %
With the use of AuNRs colloid solutions	0.5	0.5 $\pm$ 0.1	8
With the use of AuNRs nanocomposites	2.5	2.1 $\pm$ 0.3	6

#### 4. Conclusions

Gold nanorods colloid solutions and their nanocomposites proved to be appropriate spectrophotometric reagents for catecholamines determination. Determination is based on measuring hypsochromic shift of the SPR-maxima as a result of “core-shell” nanorods formation caused by covering AuNRs surface by silver shell, because of silver reduction from silver nitrate by catecholamines. Comparison of this system with other nanoanalytical systems has showed that use of AuNRs and silver nitrate is characterized by the best analytical characteristics. The developed techniques for catecholamines determination with the use of AuNRs solutions and the nanocomposites could be applied to analysis of urine and serum samples. As an approach to enhance the analysis sensitivity, solid-phase extraction of catecholamines with the use of a microcolumn packed with hypercrosslinked polystyrene could be applied.

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