Spectrophotometric determination of epinephrine using new analytical systems based on label-free silver triangular nanoplates

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Abstract. Silver triangular nanoplates (AgTNPs) is a promising and still relatively poorly studied colorimetric probe for sensing various organic compounds. In particular, they undergo a change in their morphology when interacting with various catecholamines. This process is accompanied by a hypsochromic shift of the local surface plasmon resonance (LSPR) band of nanoparticles. The greatest spectral changes can be observed in the case of the interaction of AgTNPs with epinephrine which can be the basis for a sensitive method for its detection. It was found that the detection limit of epinephrine under the selected optimal conditions is equal to 3 μM, and the dynamic range is from 9 μM up to 50 μM. Selectivity of the proposed method for the epinephrine determination was evaluated as well. It was shown that the determination does not interfere with a 10-fold excess of vanillylmandelic acid and dopamine, and with a 1000-fold excess of common cations and anions. The proposed approach was successfully applied to the determination of epinephrine in a drug and a sample of artificial urine containing an epinephrine additive.

1. Introduction

It is well known that catecholamines such as dopamine, epinephrine and norepinephrine play a key role in the mechanisms of learning, memorization, emotions, sleep, as well as in psychomotor activity and neurohumoral regulation. The action of catecholamines is noticeable in stressful conditions when the adrenal medulla begins to intensively release epinephrine and norepinephrine to stimulate the central nervous system and increase the reaction rate for optimal response to the stimulus.

Epinephrine, also known as adrenaline, is the most important direct-acting hormone and medication. In addition to participating in homeostasis, epinephrine increases the strength and heart rate, innervates the vessels of the digestive system, bronchi and brain, as well as muscles. The action of epinephrine on the body is extensive and affects most of the major organs. Epinephrine narrows the vessels leading to the skin and digestive tract, and dilates the vessels supplying the muscles and brain, increases the strength and heart rate, and also lowers the sensitivity threshold of the senses in a
stressful situation. A change in the concentration of epinephrine in biological fluids (blood, urine, cerebrospinal fluid) can serve as a reliable indicator of a violation of homeostasis [1–3].

According to existing literature data, mainly HPLC/MS and various electrochemical methods are used to determine epinephrine. In chromatographic methods, high selectivity is achieved with respect to the components of the matrix. However, chromatographic methods often require an unreasonably large amount of various organic solvents. It is also worth noting the high cost of chromatographic equipment and the low speed of a single analysis. Electrochemical methods, despite their wide distribution and low cost of analytical equipment, are highly susceptible to matrix effects and often require complex preparation of electrodes [4].

In this way, the development of inexpensive and available analytical techniques that allow rapid screening of a large number of samples using simple methods or visual detection is one of the priority fields of modern analytical chemistry. From this point of view, the attention of many researchers is attracted to silver nanoparticles [5]. A large number of scientific papers are devoted to synthesis and application of the spherical silver nanoparticles as well as materials based on them [6–20]. Significantly fewer papers are devoted to the anisotropic silver nanoparticles, such as nanocubes [21], nanowires [22], nanodiscs [23], nanorods [24] and triangular nanoplates [25–30]. The unique optical properties of silver triangular nanoplates (AgTNPs) arise from the local surface plasmon resonance (LSPR) phenomenon resulting in an intense absorption band in the visible spectral region. High molar extinction coefficients of AgTNPs, as well as the ability to change LSPR band in the presence of substances that cause changes in the morphology of nanoparticles and in the state of their surface, allow us for considering AgTNPs as a distinct colorimetric reagent.

In this study, new analytical systems based on label-free silver triangular nanoplates were assessed to outline their possibilities as a colorimetric probe for spectrophotometric determination of epinephrine.

2. Materials and methods of research

2.1. Reagents and instruments

The following reagents were used: L-epinephrine (Acros Organics, 99%), silver nitrate (PZTsM-Vtormet, analytical grade), sodium citrate (Sigma, ≥99.5%), poly(N-vinyl-2-pyrrolidone) (Acros Organics, M.W. 58000 g mol⁻¹, 99%), hydrogen peroxide (Sigma-Aldrich, 30 wt. % in H₂O, ACS), sodium borohydride (Acros Organics, 99%), sodium hydroxide (Reachim, pure grade) and acetic acid (IREA 2000, pure grade). Working solutions of these substances were prepared by dissolving their weighed portions or aliquots and dilution in deionized water obtained with the Millipore Simplicity water purification system (Merck Millipore, USA).

Absorption spectra of AgTNPs in the wavelength range 400–1100 nm were recorded in standard quartz cuvettes with an optical path length of 1 cm using the SF-103 spectrophotometer (Aquilon, Russia). Stirring of solutions was carried out using the Ekros PE-6100 magnetic mixer (Ekros, Russia). pH was measured using the Ekspert 001 pH-meter (Ekoniks Ekspert, Russia).

Chromatographic determination of epinephrine in samples was carried out using the Tsvet-Yauza high-performance liquid chromatograph (Khimavtomatika, Russia) with an amperometric detector (E = 1.2 V). The Luna C18 chromatographic column (Phenomenex, USA) was used as a stationary phase. A mixture consisting of degassed acetonitrile (50% vol.) and degassed deionized water (50% vol.) was used as a mobile phase. Flow rate of the mobile phase was equal to 0.4 mL min⁻¹. The injected volume of samples was equal to 20 μL.

TEM images of AgTNPs were recorded using the Libra 200 transmission electron microscope (Carl Zeiss, Germany) with a thermal field emission cathode at the accelerating voltage of 200 kV, the limit of information in a bright field transmission microscopy registration mode is better than 0.1 nm. Dispersions of the AgTNPs samples in hexane/heptane were deposited onto a copper grid support with a formvar film covered by amorphous carbon Formvar®/Carbon Reinforced Copper Grids 3440C-MB.
(SPI, USA). Measurements of ζ-potentials of AgTNPs were carried out by the dynamic light scattering method using the Zetasizer Nano ZS (Malvern Instruments, UK) with non-invasive backscattering optics NIBS.

2.2. Preparation of label-free silver triangular nanoplates
Preparation of AgTNPs was performed as described by G.S. Metraux and C.A. Mirkin [30] with minor modifications. Glassware used was pre-washed with freshly prepared nitrohydrochloric acid, thoroughly rinsed with distilled water and dried. A 0.5 mL aliquot of 0.01 mol L⁻¹ silver nitrate aqueous solution was diluted with 4.3 mL of deionized water. Then 2.3 mL of 1% sodium citrate aqueous solution, 0.6 mL of 20 g L⁻¹ poly(N-vinyl-2-pyrrolidone) aqueous solution and 1.2 mL of 3% hydrogen peroxide aqueous solution were successively added under vigorous stirring. Then 1.0 mL of freshly prepared 35.0 mmol L⁻¹ sodium borohydride aqueous solution was dropwisely added to the solution under stirring. Mixture got pale yellow-green color, which half an hour later abruptly changed to intense emerald-green and then to blue-violet. The stirring was stopped. The as-prepared AgTNPs colloidal solution was stored at room temperature. The calculated final concentration of AgTNPs in the solution was 56 μg mL⁻¹ (0.52 mmol L⁻¹ in terms of silver atoms). A TEM image of synthesized AgTNPs is present in Fig. 1a.

![Figure 1. TEM images of silver triangular nanoplates before (a) and after (b) interaction with L-epinephrine.](image)

2.3. Sample preparation
The medicinal form of the «Epinephrine» drug (solution for infusions) was 200 times diluted with deionized water. For the determination, an aliquot portion of this solution was taken.

Sorption pre-concentration of epinephrine from a sample of artificial urine containing epinephrine supplement was carried out in a dynamic mode. A cartridge filled with 20 mg of hypercrosslinked polystyrene was pre-conditioned with 3 mL of acetonitrile and washed with 10 mL of deionized water. Then 30 mL of artificial urine containing epinephrine was passed through the cartridge at an average rate of 1 mL min⁻¹. After that, the cartridge was washed with 10 mL of deionized water. Epinephrine was eluted with 3.00 mL of 0.1 M acetic acid. For the determination, an aliquot portion of this solution was taken.

3. Results and discussion
3.1. Interaction of silver triangular nanoplates with epinephrine

The study of the interaction of AgTNPs with epinephrine was carried out according to the following procedure. First of all, 1.55 mL of AgTNPs solution was added into polypropylene test-tubes. After that, certain amounts of L-epinephrine solution were injected. The final volume was adjusted to 5.0 mL with an acetate buffer solution (CH₃COOH/CH₃COONa) with pH 4.5. After 15 minutes, UV-Vis absorption spectra of nanoparticles were recorded.

One can see that there were a large number of silver nanodiscs and silver nanospheres presented on a TEM image of nanoparticles after the interaction (Fig. 1b). We propose that a change in the morphology of AgTNPs when interacting with epinephrine takes place. The value of the electrokinetic potential of AgTNPs after interaction with epinephrine changed from −27 mV to −6 mV which indicates a significant loss of nanoparticles stability.

It was also found that strong hypsochromic shift of the LSPR band of AgTNPs after interaction with L-epinephrine can be observed in the recorded absorption spectra (Fig. 2). The shift value of AgTNPs LSPR band maximum (Δλ) monotonically increases with increasing concentration of the analyte so that it can be considered as the analytical signal. To achieve maximum sensitivity of the AgTNPs-based analytical systems for epinephrine, it was necessary to study the effect of various factors and select the optimal values of experimental parameters that provide us with the largest value of Δλ.

3.1.1. Optimization of AgTNPs concentration. The effect of AgTNPs concentration upon the analytical signal was studied. It was found that the sensitivity of epinephrine determination increases with increasing AgTNPs concentration in the range 0–0.32 mM Ag. At the same time, a measurement error of the analytical signal increases sympathetically. For further experiments, AgTNPs concentration was chosen equal to 0.16 mM Ag which corresponds to the middle of the above-mentioned concentration range.

3.1.2. Optimization of pH value. To study the effect of pH upon the analytical signal, a certain amount of L-epinephrine was added into the test-tubes. 1.55 mL of AgTNPs was further injected, and the solution volume was adjusted to 5.0 mL with aqueous solutions of 0.1 M CH₃COOH and 0.1 M NaOH in various ratios. After 15 minutes, UV-Vis absorption spectra of nanoparticles were recorded.

Figure 2. UV-Vis absorption spectra and photo of silver triangular nanoplates after interaction with L-epinephrine. c(epinephrine) = 40 μM (1), 30 μM (2), 20 μM (3), 10 μM (4), 0 (5); c(AgTNPs) = 0.13 mM Ag; pH 4.5; t = 15 min.
At pH < 4, the shape of nanoparticles changes from triangular nanoplates to nanodisks or nanospheres [31]. This fact was indirectly evidenced by the shift of the LSPR band maximum to shorter wavelengths. For this reason, the above-mentioned pH range was not studied in detail. The dependence of the analytical signal on the pH > 4 values is shown in Fig. 3. We assume that reactivity of AgTNPs increases with a decrease of pH value. This contributes to the occurrence of an interaction between AgTNPs and epinephrine. One can see that the maximum shift of the LSPR band can be observed at pH 4.3. Nonetheless, this value is very close to the stability boundary of colloidal AgTNPs solution. Moreover, the reproducibility of the analytical signal at pH 4.3 was not rather good. Therefore, pH 4.5 was chosen for further experiments. This pH provides us with a sufficiently high analytical signal and, at the same time, good reproducibility of the results.

![Figure 3. The shift value of AgTNPs LSPR band maximum depending on pH in solutions containing 20 μM epinephrine. c(AgTNPs) = 0.16 mM Ag, t = 15 min.](image)

3.1.3. Optimization of the interaction time. The effect of the interaction time upon the analytical signal was studied. A certain amount of epinephrine was introduced into polypropylene test-tubes, and 1.55 mL of AgTNPs solution was added. The final volume was adjusted to 5.0 mL with an acetate buffer solution with pH 4.5. After 1, 2.5, 5, 10, 15, 20, 25 and 30 minutes, UV-Vis absorption spectra of nanoparticles were recorded.

The dependence of the analytical signal on the interaction time is shown in Fig. 4. One can see that during the first minute after the addition of AgTNPs a fast shift of the LSPR band is observed, and during the next 14 minutes, its slow shift continues. There is an almost linear time shift of the nanoparticles LSPR band during this time period. We assume that such behaviour of the analytical system is connected with the fact that the angles of AgTNPs have the largest excess surface energy and lose faceting in the first place. Further slow changes are likely to occur with the participation of silver atoms located on the edges of AgTNPs. Starting from 15 minutes, the LSPR band shift is terminated. Therefore, this time value was used in further experiments.
3.2. Spectrophotometric determination of epinephrine

3.2.1. Analytical performance. The observed spectral changes during the interaction with AgTNPs can be used as a basis for spectrophotometric determination of epinephrine. As shown above, the hypsochromic shift value ($\Delta \lambda$, nm) of the LSPR band can be considered as the analytical signal. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated as $3s_0/k$ and $9s_0/k$ respectively; here, $s_0$ is the standard deviation of LSPR band maximum for the blank experiment and $k$ is the slope of a calibration curve for epinephrine: $\Delta \lambda$, nm = $k \times c$(epinephrine), μM. It was found that LOD for epinephrine under the selected optimal conditions is equal to 3 μM, and the dynamic determination range is from 9 μM up to 50 μM. The calculated RSD value for 30 μM epinephrine is equal to 4%.

3.2.2. Selectivity studies. Selectivity of the proposed method for epinephrine determination was evaluated. It was found that the determination is not interfered by a 10-fold excess of vanillylmandelic acid and dopamine as well as with a 4000-fold excess of CH$_3$COO$^-$, Na$^+$ and K$^+$, and 1000-fold excess of NO$_3^-$, Mg$^{2+}$, Ca$^{2+}$, Al$^{3+}$ and Pb$^{2+}$.

3.2.3. Application. To show applicability of the proposed method to analysis of real samples, the determination of epinephrine in the «Epinephrine» drug (solution for infusions) and a sample of artificial urine containing an epinephrine additive was performed after appropriate dilution of the samples. To confirm the accuracy of the epinephrine determination the studied samples were also analyzed by an independent method which was the reversed-phase high-performance liquid chromatography with amperometric detection ($E = 1.2$ V). The results are shown in Table 1. In all cases, the content of epinephrine found using the proposed method coincided with the content obtained by the independent method and the data declared by the manufacturers which proves good accuracy of the determination using AgTNPs. It should be stressed that the analysis of these samples could be done with a minimum sample preparation.

4. Conclusion
As shown in the present article, label-free silver triangular nanoplates are a promising colorimetric probe for spectrophotometric determination of epinephrine. It has been proposed that a change in morphology of AgTNPs when interacting with epinephrine takes place. It was shown that the hypsochromic shift value of the local surface plasmon resonance band of nanoparticles can be considered as the analytical signal. The detection limit of epinephrine under the selected optimal
conditions is equal to 3 μM, and the dynamic determination range is from 9 μM up to 50 μM. The determination of epinephrine is not interfered by a 10-fold excess of vanillylmandelic acid and dopamine, and 1000-fold excess of common cations and anions. Other advantages of the approach are simplicity, rapidity, good analytical performance, availability of the equipment and ease of the test-method implementation.

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Table 1. Determination of L-epinephrine in samples (n = 3, P = 0.95).

| Sample                                      | Declared epinephrine content | Found epinephrine content     |
|---------------------------------------------|------------------------------|--------------------------------|
| «Epinephrine» drug (solution for infusions) | 1 g L⁻¹                       | (1.11 ± 0.05) g L⁻¹, (1.09 ± 0.01) g L⁻¹ |
| Model solutions prepared on the basis of artificial urine* with addition of 20 μM epinephrine | —                            | (19 ± 2) μM, (20.3 ± 0.2) μM |

*Composition of artificial urine: 22 g L⁻¹ urea, 5.2 g L⁻¹ sodium chloride, 1.5 g L⁻¹ creatinine, 1.08 g L⁻¹ sodium dihydrogen phosphate dihydrate, 1 g L⁻¹ sodium azide, 0.97 g L⁻¹ sodium hydrogen phosphate monohydrate, 10 mg L⁻¹ tetrazine.

References
[1] Djelić N, Radaković M, Borozan S, Dimirijević-Srećković V, Pajović N, Vejnović B, Borozan N, Bankoglu E, Stopper H and Stanimirović Z 2019 Oxidative stress and DNA damage in peripheral blood mononuclear cells from normal, obese, prediabetic and diabetic persons exposed to adrenaline in vitro Mutat. Res. Genet. Toxicol. Environ. Mutagen. 843 pp 81–9
[2] Whiting M and Doogue M 2009 Advances in biochemical screening for pheochromocytoma using biogenic amines Clin. Biochem. Rev. 30 pp 3 – 17
[3] Y-Hassan S 2019 Plasma epinephrine level and its causal link to Takotsubo syndrome revisited: critical review with a diverse conclusion Cardiovasc. Revasc. Med. 20(10) pp 907–14
[4] Tsunoda M 2006 Recent advances in methods for the analysis of catecholamines and their metabolites Anal. Bioanal. Chem. 386(3) pp 506–14
[5] Gorbunova M, Gutorova S, Berseneva D, Apyari V, Zaitsev V, Dmitrienko S and Zolotov Y 2019 Spectroscopic methods for determination of catecholamines: A mini-review Appl. Spectrosc. Rev. 54(8) pp 631–52
[6] Terenteva E, Apyari V, Kochuk E, Dmitrienko S and Zolotov Y 2017 Use of silver nanoparticles in spectrophotometry J. Anal. Chem. 72 pp 1138–54
[7] Siripattanakul-Ratpukdi S and Fürhacker M 2014 Review: issues of silver nanoparticles in engineered environmental treatment systems Water. Air. Soil. Pollut. 225 pp 1939–57
[8] Fan M, Andrade G and Brolo A 2011 A review on the fabrication of substrates for surface enhanced Raman spectroscopy and their applications in analytical chemistry Anal. Chim. Acta. 693 pp 7–25
[9] Oliveira E, Núñez C, Santos H, Fernández-Lodeiro J, Fernández-Lodeiro A, Capelo J and Lodeiro C 2015 Revisiting the use of gold and silver functionalised nanoparticles as
colorimetric and fluorometric chemosensors for metal ions Sensor. Actuat. B. 212 pp 297–328

[10] Beyene H, Werkneh A, Bezabh H and Ambaye T 2017 Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review Sustain. Mater. Technol. 13 pp 18–23

[11] Ravindran A, Chandran P and Khan S 2013 Biofunctionalized silver nanoparticles: advances and prospects Colloids. Surf. B. 105 pp 342–52

[12] Tran Q, Nguyen V and Le A 2013 Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives Adv. Nat. Sci. Nanosci. Nanotechnol. 4 p 033001

[13] Shenashen M, El-Safty S and Elshehy E 2014 Synthesis, morphological control, and properties of silver nanoparticles in potential applications Part. Part. Syst. Charact. 31 pp 293–316

[14] Wei L, Lu J, Xu H, Patel A, Chen Z and Chen G 2015 Silver nanoparticles: synthesis, properties, and therapeutic applications Drug. Discov. Today. 20 pp 595–601

[15] Vilela D, González M and Escarpa A 2012 Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: chemical creativity behind the assay. A review Anal. Chim. Acta. 751 pp 24–43

[16] Liang A, Liu Q, Wen G and Jiang Z 2012 The surface-plasmon-resonance effect of nanogold/silver and its analytical applications Trends. Anal. Chem. 37 pp 32–47

[17] Haider A and Kang I 2015 Preparation of silver nanoparticles and their industrial and biomedical applications: a comprehensive review Adv. Mater. Sci. Eng. 2015 p 165257

[18] Rycenga M, Cobley C, Zeng J, Li W, Moran C, Zhang Q, Qin D and Xia Y 2011 Controlling the synthesis and assembly of silver nanostructures for plasmonic applications Chem. Rev. 111 pp 3669–712

[19] Cobley C, Skrabalak S, Campbell D and Xia Y 2009 Shape-controlled synthesis of silver nanoparticles for plasmonic and sensing applications Plasmonics. 4 pp 171–9.

[20] Li M, Cushing S and Wu N 2015 Plasmon-enhanced optical sensors: a review Analyst. 140 pp 386–406

[21] Personick M, Langille M, Zhang J, Wu J, Li S and Mirkin C 2013 Plasmon mediated synthesis of silver cubes with unusual twinning structures using short wavelength excitation Small. 9 pp 1947–53

[22] Han S and Lee J 2012 Synthesis of length-controlled polyvalent silver nanowire-DNA conjugates for sensitive and selective detection of DNA targets Langmuir. 28 pp 828–32

[23] Kim B and Lee J 2015 One-pot photochemical synthesis of silver nanodisks using a conventional metal-halide lamp Mater. Chem. Phys. 149 pp 678–85

[24] Zhang J, Langille M and Mirkin C 2011 Synthesis of silver nanorods by low energy excitation of spherical plasmonic seeds Nano. Lett. 11 pp 2495–8

[25] Zhang Q, Li N, Goebl J, Lu Z and Yin Y 2011 A systematic study of the synthesis of silver nanoparticles: is citrate a «magic» reagent? J. Am. Chem. Soc. 133 pp 18931–9

[26] Tang B, Xu S, Hou X, Li J, Sun L, Xu W and Wang X 2013 Shape evolution of silver nanoparticles through heating and photoinduction ACS. Appl. Mater. Interfaces. 5 pp 646–53

[27] Furletov A, Apyari V, Garshhev A, Volkov P, Tolmacheva V and Dmitrienko S 2018 Sorption of triangular silver nanoparticles on polyurethane foam Rus. J. Phys. Chem. A. 92 pp 357–60

[28] Furletov A, Apyari V, Garshhev A, Dmitrienko S and Zolotov Y 2017 Triangular silver nanoparticles as a spectrophotometric reagent for the determination of mercury(II) J. Anal. Chem. 72 pp 1203–7

[29] Apyari V, Gorbunova M, Shevchenko A, Furletov A, Volkov P, Garshhev A, Dmitrienko S and Zolotov Y 2018 Towards highly selective detection using metal nanoparticles: a case of silver triangular nanoparticles and chlorine Talanta. 176 pp 406–11

[30] Metraux G and Mirkin C 2005 Rapid thermal synthesis of silver nanoprisms with chemically tailorable thickness Adv. Mater. 17 pp 412–5

[31] Millstone J, Hurst S, Metraux G, Cutler J and Mirkin C 2009 Colloidal gold and silver triangular nanoprisms Small. 5 pp 646–64