METHODS

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APPLICATION OF GOLD NANOPARTICLES TO DETERMINE SPERMINE IN THE PRESENCE OF OTHER POLYAMINES

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The development of methods for the detection of polyamines in biological fluids is essential to improve early diagnosis and treatment of patients with prostate cancer. One of the promising areas is the use of noble metal nanoparticles. According to the literature data, there is no methodological approach have been developed to reliably distinguish spermine from other polyamines, in particular, from their acetylated forms and related compounds present in biological fluids. The paper presents the results of spectrophotometric determination of spermine both alone and in the presence of putrescine, spermidine or urea in the urine using gold nanoparticles. The results of the experiments proved that the developed method is suitable for the selective determination of spermine. It was shown that the presence of spermidine, putrescine, acetylated forms of polyamines or carbamide does not affect the results of the analysis.

K e y w o r d s: spectrophotometric method, selective determination of spermine, gold nanoparticles, carbamide, prostate cancer.

Biogenic polyamines are very valuable in the diagnosis of prostate cancer (PCa). Polyamines (PA), which include spermidine and spermine, are polycations with three or four amine groups. Almost all cells can produce polyamines, but their production is especially high in rapidly growing cells. Polyamine concentrations are often increased in the blood and urine of cancer patients, and these increased levels have been shown to correlate with poor prognosis. The increased blood and urinary polyamine levels are attributable to increased polyamine synthesis by cancer cells, since these elevations can be abolished by complete eradication of tumors by surgery or radio-chemotherapy. The capacity of cancer tissue to produce abundant polyamines likely contributes to cancer cells' enhanced growth rates because polyamines are indispensable for cellular growth [1].

The natural polyamines, spermidine, and spermine, are found in almost every living cell at high micromolar to low millimolar quantities. Polyamines are synthesized from arginine with arginase, converting arginine to ornithine, and ornithine decarboxylase (ODC) catalyzing ornithine decarboxylation to form putrescine, a polyamine precursor containing two amine groups.

Putrescine is the initial polyamine for the biosynthesis of spermidine and spermine, as well as methionine and ATP. Methionine, before being used for the biosynthesis of spermidine and spermine, interacts with ATP to form S-adenosyl-L-methionine (S-AM). Decarboxylation of S-AM is catalyzed by the enzyme S-adenosyl-L-methionine decarboxylase (S-AMDC) (EC 4.1.1.50).

Decarboxylated S-adenosyl-L-methionine provides aminopropyl groups for enzymatic biosynthesis of PA, which is carried out by two synthases: spermidine synthase (EC 2.5.16) and spermine synthase. Spermidine synthase catalyzes the formation of spermidine by attaching one aminopropyl group

to putrescine. Spermine synthase adds another similar group to spermidine, resulting in the formation of spermine [2]. Polyamines are involved in diverse functions as cell growth and differentiation, DNA synthesis and stability, regulation of transcription, ion channel regulation, and protein phosphorylation. Intracellular spermine and spermidine are degraded by spermidine/spermine N^1-acetyltransferase (SSAT) and N^1-acetylpolyamine oxidase (APAO). SSAT is a highly inducible enzyme, catalyzes the transfer of an acetyl group from acetyl-coenzyme A to the aminopropyl group of spermine and spermidine. APAO was previously described as polyamine oxidase but it preferentially catalyzes the oxidation of the N^1-acetylspermine and N^1-acetylspermidine produced by SSAT activity. This oxidation results in the production of H_2O_2, 3-acetoaminopropanal, and putrescine or spermidine, depending on the initial substrate. Mammalian spermine oxidase (SMO) is an inducible enzyme that specifically oxidizes spermine, with the production of H_2O_2, 3-aminopropanal (3AP) and spermidine. In addition to *de novo* synthesis and degradation, cellular polyamine concentrations are also regulated by transmembrane transport where cells take up polyamines from their surroundings or export them to the extracellular space [3].

Polyamine biosynthesis is up-regulated in actively growing cells, including cancer cells, therefore polyamine concentration as well as gene expression and activity of enzymes involved in polyamine biosynthesis, especially ODC, are higher in cancer tissues than in normal surrounding tissues. Numerous reports have shown that both blood and urine polyamine concentrations are often increased in cancer patients. A close correlation between blood polyamne levels and urinary polyamines has also been found in cancer patients. Moreover, these levels decrease after tumor eradication and increase after relapse, indicating that polyamines synthesized by cancer tissues are transferred to the blood circulation and kidney, where they are excreted into the urine [4].

Transformation of prostate cells is accompanied by typical changes in the activity of metabolic enzymes of PA and changes in their content [5-7].

There are data presented in the literature, concerning the presence of differences in the metabolism of PA in PCa not only in comparison with normal tissue and benign prostate tumors, but also with malignant tumors of other localizations [5, 8, 9].

PCa is characterized by an increase in spermidine synthase (SDSY) activity, upregulation of SSAT and spermine oxidase (SMOX) and a sharp decrease in spermine levels, which is apparently caused by increased both SSAT and SMOX activity [8, 10-13].

The consequence of a sharp decrease in spermine in PCa tissue results in a decrease of spermine levels in biological fluids, in particular in urine. There are many works devoted to the study of the possibility of using spermine as a marker of PCa [9, 14-17]. But the evaluation of spermine, as a rule, was carried out using complex and expensive methods. In order to use spermine as a marker of PCa in a clinical laboratory, it is necessary to develop a simple, low cost and fast method for its determination.

A promising direction in the early diagnosis of cancer is the use of gold nanoparticles [18]. Among various organic and inorganic nanoparticles, gold has unique optical surface plasmon resonance (SPR) properties, thanks to which they have attracted the attention of researchers, especially in the biological and medical fields. Due to the optical properties, which are based on the phenomenon SPR, gold nanoparticles can be used in ultrasensitive methods for the diagnostics of cancer. SPR is the process by which gold electrons resonate in response to radiation, causing them to absorb and scatter light. Gold nanoparticles (AuNPs) can be given different sizes and shapes, as a result of which the maximum shift of the localized SPR can vary from 520 to 800-1200 nm [19]. The most common methods that use AuNPs for the diagnosis of cancer are spectrometric, colorimetric, fluorimetric, as well as methods of magnetic resonance and biosensor analysis. Fluorimetric methods are usually more sensitive than colorimetric, but the latter do not require complex equipment [20].

We investigated the possibility of using stabilized colloidal gold nanoparticles (AuNPs) to detect spermine in specially prepared solutions. Among the biologically important polyamines are putrescine, spermidine and spermine. Spermine can be considered as a promising biochemical marker for the differential diagnostics of human prostate cancer. In particular, its concentration in the urine of patients with prostate cancer is probably lower than that in patients with benign prostate tumors and healthy men [21]. This may allow to perform a timely test of the subject without the use of invasive diagnostic methods. An obstacle to the introduction of this

approach is the cross-influence of other PA on the results of analytical measurements of spermine content. Thus, the problem is the lack of clinically adapted selective methods for the determination of spermine in biological fluids, in particular in urine, in the presence of concomitant PA (in particular spermidine). We noted that other widely-used methods such as enzyme-linked immunosorbent assay or immunofluorescence analysis, high-performance liquid chromatography, capillary electrophoresis are time-consuming and high-priced [22, 23].

Aggregate instability is inherent in metal nanoparticles (NPs) in colloidal solution. Therefore, in the process of synthesis of NPs, the stabilizers in the form of surfactants (sodium dodecyl sulfate, cetyltrimethylammonium bromide, tetramethylammonium bromide) and polymers (polyethyleneglycol, polystyrenesulfonate, poly-L-glutamic acid, etc.) are usually used [24], which increase the stability of the system owing to hydrophilic-hydrophobic interactions. Another approach to the stabilization of NPs is the use of substances that provide an electric potential on the surface of metal particles, which prevents their interaction with each other by repelling equal charges. Molecules of such substances usually contain groups that are able to form donor-acceptor bonds, in particular carboxyl and amino groups. The synthesis of AuNPs using sodium citrate is a classic example of this approach [25].

Among other properties of nanoparticles, they are able to promote apoptosis of tumor cells [26].

Nowadays the most common methods for the detection of polyamines in blood and urine are high performance liquid and gas chromatography, enzyme-linked immunosorbent assay, mass spectrometry. Although they are widely used, they have a number of disadvantages such as a long period of analysis, high price and the possibility of cross reactivity with metabolites of blood or urine.

The aim of our work was to develop a spectrophotometric method for the selective determination of spermine using AuNPs and prove the possibility of its use in practice.

Materials and Methods

Spectrophotometric studies of the interaction of AuNPs with biologically important PA (putrescine, spermidine, spermine) were performed. Colloidal gold, synthesized and kindly provided by the staff of the Institute of Surface Chemistry of the National Academy of Sciences of Ukraine was stabilized with sodium citrate [21]. The concentration of Au in the colloidal solution was $3 \cdot 10^{-4}$ M, the maximum band of localized surface plasmon resonance (lSPR) corresponded to λ = 526 nm. The size of AuNPs, determined by the method of registration of diffuse light scattering (DLS), was in the range of 13-25 nm (Fig. 1).

To the flat-bottomed wells of the plate (96 ×) was added 80 µl of colloidal solution of AuNPs ($3 \cdot 10^{-4}$ M) and their ζ-potential corresponded to -27 – -30 mV. Furthermore, to the solution of AuNPs was added 10 µl of the corresponding PA and 10 µl of H_2O. Water was added as an aliquot of an additional factor whose effect on the aggregation of AuNPs in the interaction with PA was investigated in some variants of experiments (in this case it was urea or urine of patients and healthy men). Thus, the final concentration of colloidal gold was $2.4 \cdot 10^{-4}$ M, and the final concentration of urea was equal to its average content in the daily urine of healthy men $6.7 \cdot 10^{-3}$ M [27]. The sample was mixed thoroughly with a sampler and immediately placed on a reader Synergy HT (USA), where the absorption spectra in the range of 350-800 nm were measured with a period of 2 nm. Measurements of a series of samples lasted from 5 to 8 min and were repeated three times for each of them.

In some cases, PA caused the aggregation of colloidal gold nanoparticles. This caused a noticeable changes in the color of the AuNPs/PA system sample from pink to blue. At the same time, the drift of the lSPR band into the long-wavelength red region of the spectrum was recorded spectrophotometrically.

Aqueous solutions of the studied PA did not have light absorption bands in the visible part of the

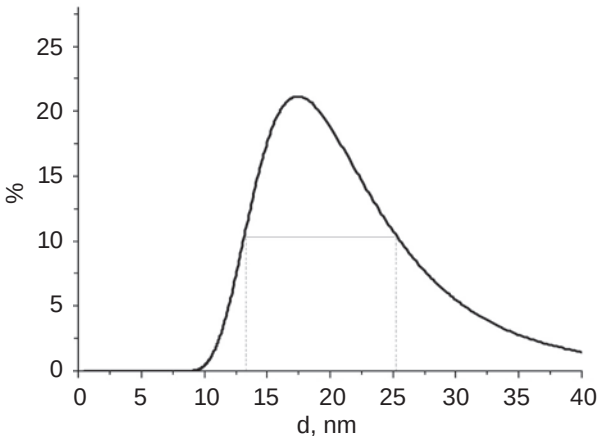

Fig. 1. Distribution by size of colloidal gold nanoparticles (AuNPs) according to DLS

spectrum, so the original band of the AuNPs/PA system always corresponded to the lSPR of colloidal gold, and in all cases its maximum λ = 526 nm. Regarding the obtained data, the corresponding symbols were introduced: the symbol λ_{max1} denotes the wavelength corresponding to the maximum of the original band of gold lSPR (526 nm) both in the case of its further shift to the long-wavelength part of the spectrum, and the wavelength equal to the maximum of a single band conditions of absence of its shift (also 526 nm). The symbol λ_{max2} indicates the wavelength corresponding to the maximum of the shifted band lSPR. The same applied to the values of the optical density of the samples for these λ_{max1} and λ_{max2}, it was denoted as I_{max1} and I_{max2}, respectively.

Urine samples of healthy men (10 samples) and patients with PCa cancer of II-III stage (20 samples) were studied by the ELISA method. We used morning urine as samples for research. Furthermore, we analyzed samples of urine by the proposed method, among which were samples of 3 healthy men and 3 patients with PCa of stage III. All patients were treated in the Department of Plastic and Reconstructive Oncourology of the National Cancer Institute of the Ministry of Health of Ukraine, their average age was 65.5 ± 2.5 years, the age of healthy men in the control group was 61.5 ± 4.5 years. Patients were examined according to the standards of diagnosis and treatment of patients approved by the orders of the Ministry of Health of Ukraine, the stage of PCa was determined according to the International Classification of Malignant Neoplasms TNM 7 (2009). According to the information given by the Department of Plastic and Reconstructive Oncourology all patients had given their written agreement for use of their material for scientific purposes. The research was approved by the Bioethical Committee of IEPOR of NAS of Ukraine (protocol No. 3, approved on 24th of September 2021).

Statistical processing of the obtained results was performed using Microsoft Excel.

Results and Discussion

The research results are presented in Tables 1-8 and Fig. 2-4. It is observed that only a very high concentration of putrescine (1 mM) can cause aggregation of colloidal gold nanoparticles, which does not occur at concentrations up to 10 μM (Table 1).

High and ultra-high concentrations of spermidine (5 μM - 1 mM) to a greater extent than putrescine at the same values, cause aggregation of colloidal gold nanoparticles and, as a consequence, the shift of the lSPR band in long-wavelength region (Table 2).

This could cause some obstacles to the selective determination of spermine, but it is impossible to encounter such high concentrations of spermidine in biological fluids (Fig. 2). For all tested spermine concentrations, a shift of the lSPR band of colloidal gold to the long-wavelength part of the spectrum was detected. The ratio I_{max2}/I_{max1} for the system AuNPs/spermine in its values goes to the “plateau” in the

Table 1. Interaction of colloidal gold nanoparticles with putrescine (Put)

Concentration of Put, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	–	0.134 ± 0.029	–	–	–
$5 \cdot 10^{-6}$	526	–	0.162 ± 0.036	–	–	–
10^{-5}	526	–	0.158 ± 0.032	–	–	–
10^{-3}	526	684	0.124 ± 0.025	0.147 ± 0.028	1.19	+ 158

Table 2. Interaction of colloidal gold nanoparticles with spermidine (Spd)

Concentration of Spd, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	–	0.150 ± 0.035	–	–	–
$5 \cdot 10^{-6}$	526	664	0.124 ± 0.028	0.140 ± 0.034	1.13	+ 138
10^{-5}	526	674	0.132 ± 0.032	0.157 ± 0.036	1.19	+ 148
10^{-3}	526	702	0.119 ± 0.022	0.146 ± 0.032	1.23	+ 176

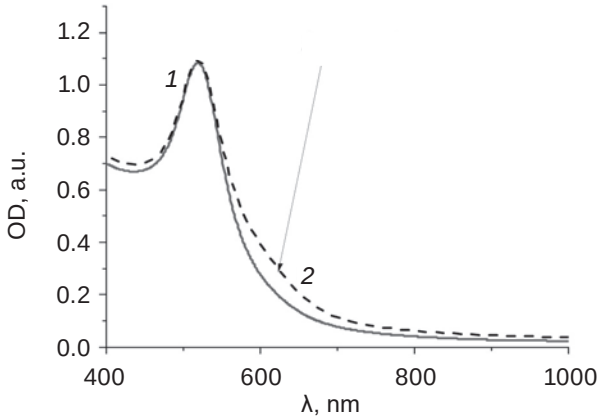

Fig. 2. Absorption spectra of AuNPs (curve 1) and AuNPs/spermidine 7.2 μM systems (curve 2)

range of spermine concentrations from 100 nM to 5 μM (Table 3, Fig. 3). Thus, the selective determination of spermine in biological fluids in the presence of spermidine or putrescine by colorimetric parameters of a colloidal solution of gold nanoparticles can be successfully performed if the content of spermine and associated PA does not exceed 100 nM (Tables 1-3), at least several hundred nM. This fully corresponds to the conditions of diagnostic determination of the level of spermine in the urine of patients at risk of prostate cancer, because the expected values of the concentration of PA in it fluctuate below 100 nM (Fig. 4).

With the exception of the main biogenic PA, some interest invokes their acetylated forms, which occur during catabolism under the influence of spermidine/spermine acetyltransferase (SSAT) [28, 29]. Always present in biological fluids, they could influence the results of selective determination of spermine, which has diagnostic value on its own. Further research is devoted to issue mentioned above (Tables 4-6). In the first stage of the research, experiments were performed with polyamines acetylated by the first nitrogen atom, even in the case of spermine and spermidine, for a more correct comparison with the acetylated diamine putrescine.

It is shown that neither N-AcPut nor N^1-AcSpd is able to interfere with the determination of spermine. Although, the acetylated form of putrescine (N-AcPut) and the acetylated form of spermidine (N^1-AcSpd) nevertheless promotes emergence of a new maximum of a lSPR band, but at concentrations higher than 100 nM (Table 4, 5), acetylated spermine (N^1-AcSpn) causes a maximum drift of the lSPR

Table 3. Interaction of colloidal gold nanoparticles with spermine (Spn)

Concentration of Spn, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	650	0.124 ± 0.030	0.135 ± 0.032	1.09	+ 124
$5{\cdot}10^{-6}$	526	680	0.114 ± 0.025	0.134 ± 0.032	1.18	+ 154
10^{-5}	526	680	0.116 ± 0.020	0.140 ± 0.034	1.21	+ 154
10^{-3}	526	698	0.115 ± 0.025	0.137 ± 0.035	1.19	+ 172

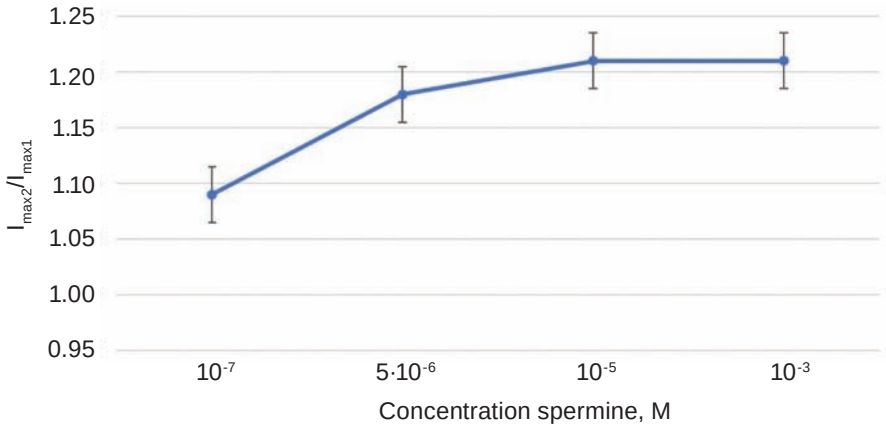

Fig. 3. Dependence of I_{max2}/I_{max1} on concentrations of PA in the AuNPs/spermine system

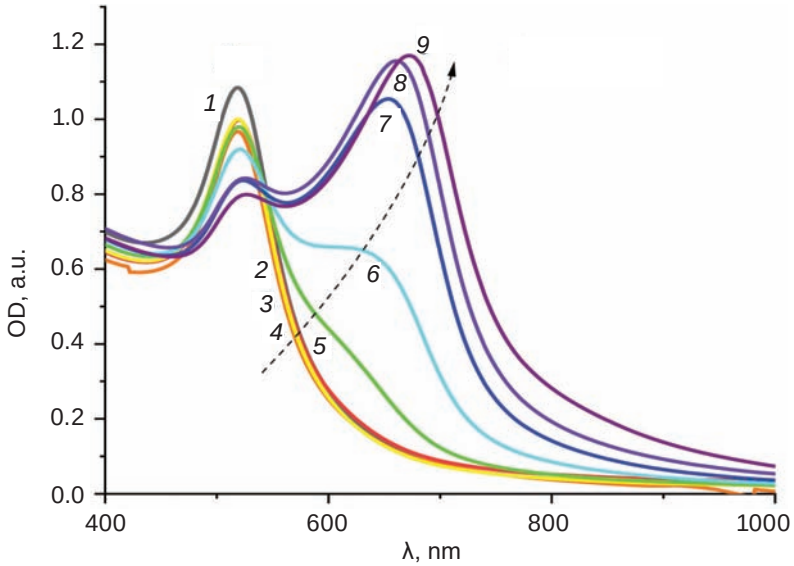

Fig. 4. Absorption spectra of AuNPs (curve 1) and AuNPs/spermine: 9, 36, 90, 450, 600, 690 and 900 nM – curves 2-9, respectively

band only at concentrations above 5 μM. Therefore, N^1-AcSpn differs in some way from non-acetylated spermine and cannot interfere with the selective detection of the N^1-AcSpn (Tables 1, 6).

It should be noted that the non-acetylated putrescine at a very high concentration (1 mM) caused a drift of the lSPR band of colloidal gold in the long-wavelength part of the spectrum (Table 1). In contrast, N-AcPut at all concentrations studied, up to 1 mM inclusively, did not cause such a shift (Table 4).

Acetylated spermidine (N^1-AcSpd) caused a drift of the colloidal gold lSPR band only at ultra high concentrations (1 mM), which did not occur at lower levels of its concentration (Table 5).

Acetylated spermine (N^1-AcSpn) caused a shift of the lSPR band of colloidal gold, starting from a concentration of 5 μM and above. It did not cause aggregation of colloidal gold nanoparticles at a concentration of 100 nM, as well as at lower concentrations (Table 6).

Almost complete correlation of spectrophotometric characteristics and values of the ratio I_{max2}/I_{max1} for concentrations of N^1-AcSpn at 10 μM and 1 mM indicates that the process of aggregation of colloidal gold nanoparticles in its interaction with acetylated spermine reaches a "plateau" in the range between 5 and 10 μM.

Therefore, acetylated forms of PA, N^1-AcSpd and N^1-AcSpn in particular, are also able to cause drift of the lSPR colloidal gold band in the long-wavelength part of the spectrum due to the aggregation of its nanoparticles through PA "bridge". However, it seems that this process is directly proportional to the number of positively charged acetyl groups and the length of the carbon structure of the PA molecule. For example, acetylated diamine N-AcPut did not cause a shift in the band of colloidal gold (Table 4), in contrast to unacetylated putrescine, which at a concentration of 1 mM provoked its drift at 158 nm (Table 1). The results of our research show that the longer carbon chain of aliphatic PA molecules, the lower their concentrations cause the phenomenon of aggregation of colloidal gold nanoparticles, which can be registered by shifting its lSPR band (Tables 1-6).

The use of the spectrophotometric method for the determination of spermine could be hindered by carbamide (Carb), which is always present in the urine at a fairly high concentration. To shed light on this issue, experiments were performed in which carbamide was introduced into the AuNPs/PA system at a final concentration corresponding to its content in the daily urine of adult men, namely $6.7 \cdot 10^{-3}$ M. As shown in Table 7, the carbamide did not affect the process of aggregation of AuNPs in their interaction with PA (Tables 1-3, 7).

Table 8 presents the results of determining the concentration of spermine in the urine samples of healthy men and patients with PCa, obtained by replacing in the test system AuNPs/spermine polyamine solution with an aliquot of native urine. For comparison, similar data were obtained by the method of

Table 4. Interaction of colloidal gold nanoparticles with acetylated form of putrescine (N-AcPut)

Concentration of N-AcPut, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	–	0.126 ± 0.020	–	–	–
$5 \cdot 10^{-6}$	526	–	0.169 ± 0.036	–	–	–
10^{-5}	526	–	0.194 ± 0.038	–	–	–
10^{-3}	526	–	0.196 ± 0.034	–	–	–

Table 5. Interaction of colloidal gold nanoparticles with acetylated form of spermidine (N^1-AcSpd)

Concentration of N^1-AcSpd, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	–	0.152 ± 0.032	–	–	–
$5 \cdot 10^{-6}$	526	–	0.163 ± 0.038	–	–	–
10^{-5}	526	–	0.197 ± 0.036	–	–	–
10^{-3}	526	738	0.152 ± 0.034	0.193 ± 0.035	1.27	+ 212

Table 6. Interaction of colloidal gold nanoparticles with acetylated form of spermine (N^1-AcSpn)

Concentration of N^1-AcSpn, M	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
10^{-7}	526	–	0.165 ± 0.034	–	–	–
$5 \cdot 10^{-6}$	526	670	0.129 ± 0.026	0.139 ± 0.030	1.08	+ 144
10^{-5}	526	1744	0.142 ± 0.032	0.187 ± 0.038	1.32	+ 218
10^{-3}	526	752	0.143 ± 0.032	0.183 ± 0.035	1.28	+ 226

Table 7. The role of carbamide (Carb) in the process of aggregation of colloidal gold (Au) nanoparticles in their interaction with polyamines (PA)

Test-system	λ_{max1}, nm	λ_{max2}, nm	I_{max1}, o. u.	I_{max2}, o. u.	I_{max2}/I_{max1}	$\lambda_{max2} - \lambda_{max1}$, nm
Au	526	–	0.206 ± 0.005	–	–	–
Au + Carb	526	–	0.207 ± 0.003	–	–	–
Au + PA ($5 \cdot 10^{-6}$ M)						
Au + Put	526	–	0.196 ± 0.039	–	–	–
Au + Spd	526	640	0.169 ± 0.035	0.183 ± 0.038	1.08	+ 115
Au + Spn	526	650	0.145 ± 0.032	0.179 ± 0.036	1.23	+ 125
Au + PA ($5 \cdot 10^{-6}$ M) + Carb						
Au + Put + Carb	526	–	0.192 ± 0.038	–	–	–
Au + Spd + Carb	526	650	0.157 ± 0.034	0.185 ± 0.035	1.18	+ 125
Au + Spn + Carb	526	676	0.141 ± 0.032	0.177 ± 0.036	1.26	+ 154

Table 8. Determination of Spn content in the urine of healthy men and patients with PCa (stage II-III)

System AuNPs/ urine	I_{526}	I_{680}	I_{680}/I_{526}	Spn (M), graphic determination	Spn (ng/ml), ELISA
Healthy men	0.204 ± 0.050	0.245 ± 0.046	1.20	$8 \cdot 10^{-6}$	162.5 ± 59.3
Patients with PCa	0.176 ± 0.052	0.198 ± 0.049	1.13	$\approx 1 \cdot 10^{-6}$	13.8 ± 7.2

ELISA. The concentration of spermine, calculated from the calibration curve (Fig. 3), is lower in the urine samples of patients with PCa, compared with the concentration of spermine in the urine samples of healthy men. The content of spermine in the urine of sick and healthy men, determined by analytical methods, shows the same trend and confirms the legitimacy of spectrophotometric/colorimetric method of selective determination of spermine in the presence.

The data, given in Tables 1-6 allow us to declare that other polyamines (spermidine, putrescine), including their acetylated forms do not affect a selective detection of spermine, if their concentration does not exceed 500 nM. On the other hand, spermine can be presented in urine at concentration lesser than 100 nM (Fig. 3).

Conclusions. According to the results of experimental data, it was determined that the developed spectrophotometric method with use of synthesized colloidal gold nanoparticles is suitable for the selective determination of spermine in the urine of patients with prostate cancer, insensitve to other polyamines (putrescine, spermidine) and urine metabolites (carbamide) because their concentration does not exceeds 100–500 nM and could be used in future practice as an additional tool alongside with such traditional methods as PSA test, biopsy and others.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЗАСТОСУВАННЯ НАНОЧАСТИНОК ЗОЛОТА ДЛЯ ВИЗНАЧЕННЯ СПЕРМІНУ В ПРИСУТНОСТІ ІНШИХ ПОЛІАМІНІВ

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Розробка методів визначення поліамінів у біологічних клітинах необхідна для вдосконалення ранньої діагностики та лікування у хворих на рак передміхурової залози. Застосування наночастинок благородних металів є одним із перспективних напрямів у діагностиці захворювань. Згідно з даними літератури, досі не було розроблено методу, який би дозволив достовірно відрізнити спермін від інших поліамінів, а також їх ацетильованих форм і інших молекул, присутніх у біологічних рідинах. Зокрема, недостатньо вивчено вплив таких вторинних метаболітів, як сечовина, що присутня у сечі здорових людей і хворих на рак передміхурової залози. Розроблений метод демонструє спектрофотометричне визначення за допомогою наночастинок золота власне сперміну, а також сперміну в комбінації з іншими поліамінами, такими як путресцин і спермідин, або сечовиною. Показано, що метод можна застосовувати для селективного визначення сперміну, а наявність спермідину та путресцину істотно не впливає на результати досліджень. Більше того, не було отримано жодних доказів впливу ацетильованих форм поліамінів або сечовини на визначення сперміну.

Ключові слова: спектрофотометричний метод, селективне визначення сперміну, наночастинки золота, карбамід, рак простати.

References

1. Soda K. The mechanisms by which polyamines accelerate tumor spread. *J Exp Clin Cancer Res.* 2011; 30(1): 95.
2. Berdinskikh NK, Zaletok SP. Polyamines and tumor growth. Naukova dumka, 1987. 141 p. (In Russian).
3. Löser C, Fölsch UR, Paprotny C, Creutzfeldt W. Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum, and urine of 50 patients with colorectal cancer. *Cancer.* 1990; 65(4): 958-966.
4. Kubota S, Okada M, Yoshimoto M, Murata N, Yamasaki Z, Wada T, Imahori K, Ohsawa N, Takaku F. Urinary polyamines as a tumor marker. *Cancer Detect Prev.* 1985; 8(1-2): 189-192.
5. Shukla-Dave A, Castillo-Martin M, Chen M, Lobo J, Gladoun N, Collazo-Lorduy A, Khan FM, Ponomarev V, Yi Z, Zhang W, Pandolfi PP, Hricak H, Cordon-Cardo C. Ornithine decarboxylase is sufficient for prostate tumorigenesis via androgen receptor signaling. *Am J Pathol*. 2016; 186(12): 3131-3145.
6. Huang W, Eickhoff JC, Mehraein-Ghomi F, Church DR, Wilding G, Basu HS. Expression of spermidine/spermine N(1) -acetyl transferase (SSAT) in human prostate tissues is related to prostate cancer progression and metastasis. *Prostate*. 2015; 75(11): 1150-1159.
7. Zabala-Letona A, Arruabarrena-Aristorena A, Martín-Martín N, Fernandez-Ruiz S, Sutherland JD, Clasquin M, Tomas-Cortazar J, Jimenez J, Torres I, Quang P. et al. mTORC1-dependent AMD1 regulation sustains polyamine metabolism in prostate cancer. *Nature*. 2017; 547(7661): 109-113.
8. Tessem MB, Bertilsson H, Angelsen A, Bathen TF, Drabløs F, Rye MB. A Balanced tissue composition reveals new metabolic and gene expression markers in prostate cancer. *PLoS One*. 2016; 11(4): e0153727.
9. Andersen MK, Giskeødegård GF, Tessem MB. Metabolic alterations in tissues and biofluids of prostate cancer patients. *Curr Opin Endocr Metab Res.* 2020; 10: 23-28.
10. Goodwin AC, Jadallah S, Toubaji A, Lecksell K, Hicks JL, Kowalski J, Bova GS, De Marzo AM, Netto GJ, Casero RA Jr. Increased spermine oxidase expression in human prostate cancer and prostatic intraepithelial neoplasia tissues. *Prostate*. 2008; 68(7): 766-772.
11. Affronti HC, Rowsam AM, Pellerite AJ, Rosario SR, Long MD, Jacobi JJ, Bianchi-Smiraglia A, Boerlin CS, Gillard BM, Karasik E, Foster BA, Moser M, Wilton JH, Attwood K, Nikiforov MA, Azabdaftari G, Pili R, Phillips JG, Casero RA Jr, Smiraglia DJ. Pharmacological polyamine catabolism upregulation with methionine salvage pathway inhibition as an effective prostate cancer therapy. *Nat Commun*. 2020; 11(1): 52.
12. Sun L, Yang J, Qin Y, Wang Y, Wu H, Zhou Y, Cao C. Discovery and antitumor evaluation of novel inhibitors of spermine oxidase. *J Enzyme Inhib Med Chem*. 2019; 34(1): 1140-1151.
13. Bentrad VV, Zaletok SP, Klenov OO, Vitruk YuV, Stakhovsky E.O. Gene expression of polyamine metabolism proteins in human prostate cancer and benign prostatic hyperplasia. *Oncology*. 2020; 22(1-2): 32-35.
14. Lynch MJ, Nicholson JK. Proton MRS of human prostatic fluid: correlations between citrate, spermine, and myo-inositol levels and changes with disease. *Prostate*. 1997; 30(4): 248-255.
15. Serkova NJ, Gamito EJ, Jones RH, O'Donnell C, Brown JL, Green S, Sullivan H, Hedlund T, Crawford ED. The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer in human expressed prostatic secretions. *Prostate*. 2008; 68(6): 620-628.
16. Tsoi TH, Chan CF, Chan WL, Chiu KF, Wong WT, Ng CF, Wong KL. Urinary polyamines: a pilot study on their roles as prostate cancer detection biomarkers. *PLoS One*. 2016; 11(9): e0162217.
17. Kdadra M, Höckner S, Leung H, Kremer W, Schiffer E. Metabolomics Biomarkers of Prostate Cancer: A Systematic Review. *Diagnostics (Basel)*. 2019; 9(1): 21.
18. Akshaya K, Arthi C, Pavithra AJ , Poovizhi P, Antinate SS, Hikku GS, Jeyasubramanian K, Murugesan R. Bioconjugated gold nanoparticles as an efficient colorimetric sensor for cancer diagnostics. *Photodiagnosis Photodyn Ther*. 2020; 30: 101699.

19. Singh P, Pandit S, Mokkapati VRSS, Garg A, Ravikumar V, Mijakovic I. Gold Nanoparticles in Diagnostics and Therapeutics for Human Cancer. *Int J Mol Sci*. 2018; 19(7): 1979.
20. António M, Vitorino R, Daniel-da-Silva AL. Gold nanoparticles-based assays for biodetection in urine. *Talanta*. 2021; 230: 122345.
21. Zaletok SP, Klenov OO, Gogol SV, Bentrad VV, Stakhovsky EO, Vitruk YuU, Grechko BO. Blood and urine polyamines as new diagnostic markers of prostate cancer. *Oncology*. 2019; 21(3): 219-223. (In Ukrainian).
22. Fletcher JT, Bruck BS. Spermine detection via metal-mediated ethynylarene 'turn-on' fluorescence signaling. *Sens Actuators B Chem*. 2015; 207(Pt A): 843-848.
23. Jornet-Martínez N, González-Béjar M, Moliner-Martínez Y, Campíns-Falcó P, Pérez-Prieto J. Sensitive and selective plasmonic assay for spermine as biomarker in human urine. *Anal Chem*. 2014; 86(3): 1347-1351.
24. Checkman IS, Pryskoka AO. Nanogold and nanocoatings: state scientific investigation prospects in medical application. *Ukr Med J*. 2010; 2(76): 37-43. (In Ukrainian).
25. Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, Plech A. Turkevich method for gold nanoparticle synthesis revisited. *J Phys Chem B*. 2006; 110(32): 15700-15707.
26. Katifelis H, Lyberopoulou A, Mukha I, Vityuk N, Grodzyuk G, Theodoropoulos GE, Efstathopoulos EP, Gazouli M. Ag/Au bimetallic nanoparticles induce apoptosis in human cancer cell lines via P53, CASPASE-3 and BAX/BCL-2 pathways. *Artif Cells Nanomed Biotechnol*. 2018; 46(sup3): S389-S398.
27. Suzuki I, Ogawa M, Seino K, Nogawa M, Naito H, Yamakoshi K I, Tanaka S. Reagentless Estimation of Urea and Creatinine Concentrations Using Near-Infrared Spectroscopy for Spot Urine Test of Urea-to-Creatinine Ratio. *Adv Biomed Eng*. 2018; 7: 72-81.
28. Casero RA Jr, Murray Stewart T, Pegg AE. Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer*. 2018; 18(11): 681-695.
29. Bae DH, Lane DJR, Jansson PJ, Richardson DR. The old and new biochemistry of polyamines. *Biochim Biophys Acta Gen Subj*. 2018; 1862(9): 2053-2068.