

APPLICATION OF GOLD AND SILVER NANOPARTICLES FOR SELECTIVE ASSAY OF SPERMINE IN MIXTURE WITH SPERMININE

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Aim: To assess the applicability of the novel technique based on the detection of spermine in solutions by spectrophotometric method using gold and silver colloidal nanoparticles. **Materials and Methods:** Colloidal solution of gold nanoparticles were synthesized by chemical reduction of tetrachlorauric acid with trisodium citrate. Colloidal solution of silver nanoparticles was obtained by chemical reduction of silver nitrate with tryptophan. The absorption spectra of gold/silver metal colloids and their mixtures with polyamines were recorded. **Results:** The increase of spermine concentration in solution caused the change in the intensity of the band of localized surface plasmon resonance that was not affected by the excess of spermidine. The color shift in colloidal gold due to its aggregation with spermine was registered spectrophotometrically. **Conclusion:** The principal possibility of selective quantification of spermine in the presence of spermidine in extremely high concentration using colloidal gold has been shown. This method can be used to assay selectively spermine in biological fluids.

Key Words: spermine, spermidine, gold, colloid, spectrophotometry.

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Aliphatic polyamines (PA), namely putrescine (put), spermidine (spd) and spermine (spn) play an important role in cell proliferation, growth and differentiation. The interest in PA in oncology is explained by the fact that the growth of malignant tumors is accompanied by enhanced PA synthesis and accumulation in cells. For many malignant tumors, a significant increase in the synthesis and constitutive level of PA was found. Nevertheless, in tumors of various histogenesis, the processes of synthesis and catabolism of PA, as well as the content of individual PAs, are significantly different. Since spd is involved in the processes of growth and proliferation, and spn in the processes of differentiation, differences in the concentration of these PA may be one of the reasons for the differences in the proliferative activity of tumor cells, the growth rate and aggressiveness of tumors.

The quantification of spn in biological fluids may be important from diagnostic point of view. Nevertheless, cross-impact of other PAs may distort the results of analytical measurements of spn content. Thus, the problem is to develop selective methods for determining exactly the specified PA in biological fluids, particularly in urine, and adapting them to the conditions of the clinical laboratory. Among the time-consuming and costly methods we should mention enzyme-linked immunosorbent assay or immunofluorescence analysis, high-performance liquid chromatography, capillary electrophoresis, etc. [1, 2]. However, in 2013, colorimetry has been successfully used to achieve

the stated goal [2]. The essence of the proposed approach was the selective spectrophotometric registration of the color change of colloidal solution of gold nanoparticles (AuNPs) due to their aggregation in the process of interaction with spn. Moreover, none of the other biogenic PA such as put, cadaverine, spd, or other related compounds, affects significantly such interaction, if AuNPs 15–20 nm in size were synthesized as described in [2].

Metal nanoparticles (NPs) in colloids are unstable. Therefore, in the process of NPs synthesis different stabilizers like surfactants (sodium dodecyl sulfate, cetyltrimethylammonium bromide, tetramethylammonium bromide) and polymers (polyethylene glycol, polystyrenesulfonate, poly-L-glutamic acid, etc.) are generally used to increase the stability of colloidal systems due to hydrophilic-hydrophobic interactions. Another approach for the stabilization of NPs is the use of substances that provide the electrical potential on the surface of the metal particles that prevents their interaction due to repulsion of charged particles. Molecules of such substances typically contain groups, as carboxyl and/or amino groups, capable to form donor-acceptor bonds with metal. Synthesis of AuNPs using sodium citrate is a classical example of this approach [3].

The purpose of the presented experiments was to develop a method for the determination of spn using noble metal NPs, in particular gold and silver. Two types of NPs were used in the study: AuNPs, synthesized and stabilized in colloidal solutions in the presence of sodium citrate, and, for comparison, silver NPs (AgNPs), obtained in the presence of amino acid tryptophan.

MATERIALS AND METHODS

Gold nanoparticles preparation. AuNPs were synthesized by chemical reduction of tetrachlorauric

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Abbreviations used: AgNPs – silver nanoparticles; AuNPs – gold nanoparticles; DLS – dynamic light scattering; LSPR – localized surface plasmon resonance; NPs – nanoparticles; PA – polyamines; put – putrescine; spd – spermidine; spn – spermine.

acid (HAuCl_4 , Merck, Germany) with trisodium citrate, $\text{Na}_3\text{C}_6\text{H}_9\text{O}_9$, in aqueous medium. HAuCl_4 was added into boiling solution of trisodium citrate, stirred while boiling during 5 min, then cooled at room temperature. The molar concentration of gold in colloids was $C(\text{Au}) = 3 \times 10^{-4} \text{ M}$, while the molar ratios of initial reagents were $v(\text{Au}):v(\text{Na}_3\text{C}_6\text{H}_9\text{O}_9) = 1:4$ and $1:2$. Prepared samples of gold colloids were indicated as “AuNPs (I)” and “AuNPs (II)”, respectively.

Gold nanoparticles preparation. AgNPs was obtained by chemical reduction of silver nitrate (AgNO_3 , Merck, Germany) with amino acid tryptophan (Trp, SC12-20120713, China). The metal concentration in final solutions was $C(\text{Ag}) = 1 \times 10^{-4} \text{ M}$, while the molar ratio of initial reagents was $\text{Ag}:\text{Trp} = 1:2$. The initial solution of Trp was adjusted to $\text{pH} = 10$ with 1N NaOH and heated to boiling, followed by injection of AgNO_3 solution as described in [4].

Particle size distribution function was studied by a laser correlation spectrometer Zeta Sizer Nano S (Malvern, UK) equipped with a correlator (Multi Computing Correlator Type 7032 CE) by the dynamic light scattering (DLS). The helium-neon laser LGN-111 was used with the output power of 25 mW and wavelength of 633 nm to irradiate the suspension. The registration and statistical processing of the scattered laser light at 173° from the suspension were performed triply for 120 s at 25°C . The resulting autocorrelation function

was treated with standard computer programs PCS-Size mode v.1.61.

The absorption spectra of metal colloids and PA/Au(Ag)NPs systems were recorded in the UV-visible region by a spectrophotometer Lambda 35 (Perkin-Elmer, USA) in 1 cm quartz cells. Spectra of Au(Ag)NPs/spn(spnd) systems were recorded immediately after mixing of reagents.

RESULTS AND DISCUSSION

AuNPs (I) and AuNPs (II) have characteristic red color, which is reflected in the absorption spectra as a band of localized surface plasmon resonance (LSPR) in the visible range with a maximum of $\lambda_{\text{max}} = 519\text{--}520 \text{ nm}$. The average size of AuNPs, determined by the DLS method as a projection of the half-width of the particle size distribution function curve, was 8–14 and 13–25 nm, respectively (Fig. 1).

Changes in the spectral characteristics of LSPR bands of nanoscale gold reflect the processes occurring in the PA/Au NPs system.

Absorption spectra of the mixture of AuNPs (I) colloid with spn and spd solutions are presented in Fig. 2, a, b. Characteristics of LSPR bands in the system depending on PA concentration are listed in the Table.

There were noticeable changes the absorption spectra with the increase of spn concentration from 9 to 900 nM, accompanied by a clear color change

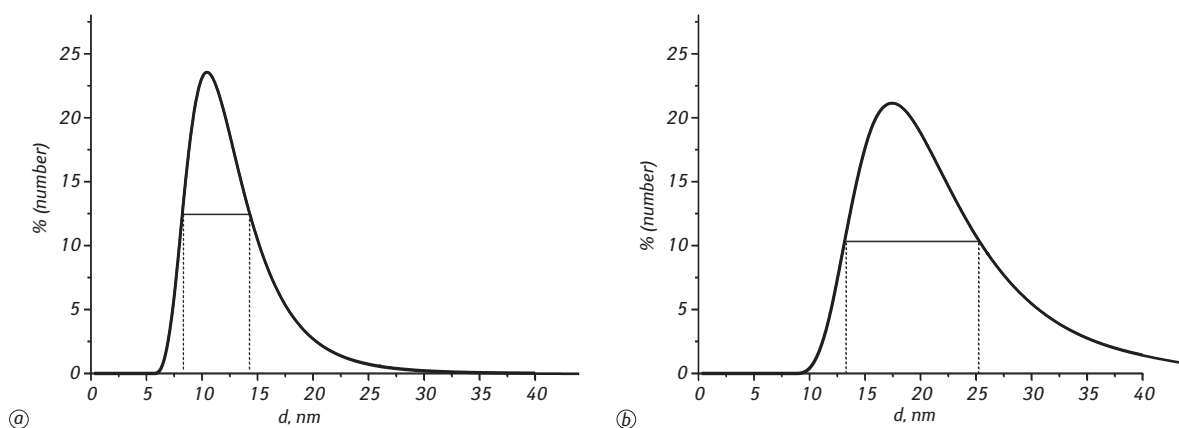


Fig. 1. Size distribution of gold nanoparticles in colloids according to DLS data: AuNPs (I) — a, AuNPs (II) — b

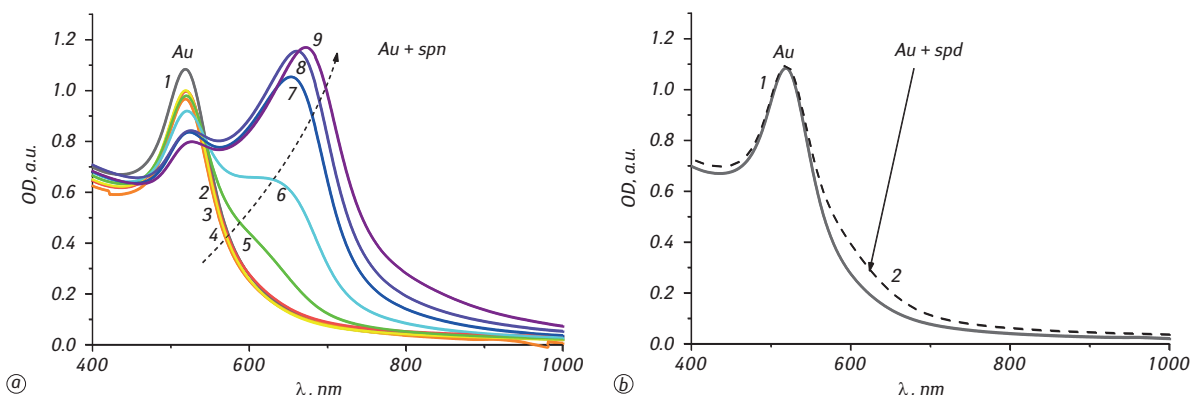


Fig. 2. Absorption spectra of AuNPs (I) interacting with spn (a): curve 1 — control solution of AuNPs (I), 2 — AuNPs/spn systems with spn content of 9; 3 — 36; 4 — 90; 5 — 450; 6 — 600; 7 — 690; 8 — 750; 9 — 900 nM, and AuNPs (I) interacting with spd (b): curve 1 — control solution of AuNPs (I), 2 — AuNPs/spd system with spd content of 7140 nM

from red to violet. Namely, a new LSPR band of gold appeared in a long-wavelength region. The position of band maximum was red-shifted followed by the increase of its intensity.

The rise of a new LSPR band of gold is associated with the formation of associates (aggregates) of NPs interacting with PA. Interparticle interaction through spn as a bridge lead to hybridization, splitting, and shifting of from the plasmon energies, that is reflected as a shift of the absorption band maximum of the system in the long-wavelength region.

In particular, starting from spn concentration of 450 nM, a new LSPR band with has a pronounced maximum in the region $\lambda_{\max 2} = 654\text{--}672$ nm. Its intensity $I_{\max 2}$ reaches the plateau after the concentration of 750 nM. At the same time, the maximum of the main LSPR band, located at $\lambda_{\max 1} = 519$ nm, inherent to individual AuNPs, did not change its position within the spn concentrations up to 90 nM, and gradually shifted to 527 nm with the increase of polyamine content up to 900 nM.

Given the objectives of this study, it is very important that in the two-component mixture of colloidal gold and a large amount of spd (final concentration 7140 nM), the optical density of the sample at $\lambda_{\max 2} = 672$ nm was extremely small and was equal to 0.17 relative units (see Fig. 2, *b*). Unlike the absorption spectra of spn systems, the absorption spectrum of the AuNPs/spd-system (see Fig. 2, *b*) strongly suggests that even an excessively high spd concentration (7140 nM) does not cause AuNPs aggregation.

A similar phenomenon was shown in Fig. 3, which presents experiments performed using a colloidal solution of AuNPs (II). The size of these NPs varied from 13 to 25 nm (see Fig. 1, *b*), resulting in slightly different spectral profile of the AuNPs/polyamine samples. The maximum of the LSPR band of AuNPs (II) was located at 523 nm and did not change position in the mixture of AuNPs (II) and spn at a final concentration of 9 nM (Fig. 3, *a*). The increase of the spn concentration to 5000 nM also caused the change of sample color from pink to violet. In this case the positions of band maxima were slightly red-shifted compared to those for

the system with AuNPs (I), namely $\lambda_{\max 1} = 527$ nm and $\lambda_{\max 2} = 705$ nm.

Simultaneous addition of spn/spd mixture, at final concentrations of 90 and 7140 nM respectively, into the colloidal solution of AuNPs (II) did not cause changes in the profile of the absorption spectrum evidencing that in the presence of such a large amount of spd, the maximum $\lambda_{\max 2}$ was slightly shifted to 722 nm (Fig. 3, *b*).

These data open the prospect of using a spectrophotometric method for spn determining in the presence of spd in the urine of patients for diagnostic/prognostic purpose.

With the aim to determine the spn concentration in the AuNPs/PA system a calibration curve was plotted on the basis of the results obtained for AuNPs (I).

In case of AuNPs (I), the position of the maximum $\lambda_{\max 1}$ remained almost unchanged and was equal to 519–520 nm, quite consistent with the literature. At the same time, $\lambda_{\max 2}$ was shifted in the visible range, so the intensity in the band maximum was determined for each specific spectrum individually, that corresponded to $\lambda_{\max 2} = 654\text{--}672$ nm (Table, see Fig. 2).

The second approach for the calculation is described in [2], where the authors measured the absorbance of samples containing colloidal gold at fixed reference wavelengths, in particular, $\lambda_{\max 1} = 520$ and $\lambda_{\max 2} = 610$ nm.

Both approaches were used for the calculation: 1) intensities of LSPR bands were defined in their maxima ($I_{\max 2}/I_{\max 1}$); 2) the intensities of LSPR bands were defined at fixed position, namely at $\lambda_{\max 1} = 520$ and $\lambda_{\max 2} = 620$ nm (I_{620}/I_{520}).

Table. Characteristics of surface plasmon band maxima of AuNPs (I) in systems with polyamines

Content	$C_{\text{polyamine}}$, nM	$\lambda_{\max 1}$	$I_{\max 1}$	$\lambda_{\max 2}$	$I_{\max 2}$
Au	-	519	1.08	-	-
Au + spn	9	519	0.99	-	-
Au + spn	36	519	0.97	-	-
Au + spn	90	519	0.99	-	-
Au + spn	450	520	0.98	620 shoulder	0.37
Au + spn	600	521	0.92	620 shoulder	0.66
Au + spn	690	524	0.84	654	1.05
Au + spn	750	525	0.84	661	1.16
Au + spn	900	527	0.80	672	1.17
Au + spd	7140	519	1.09	620 shoulder	0.31

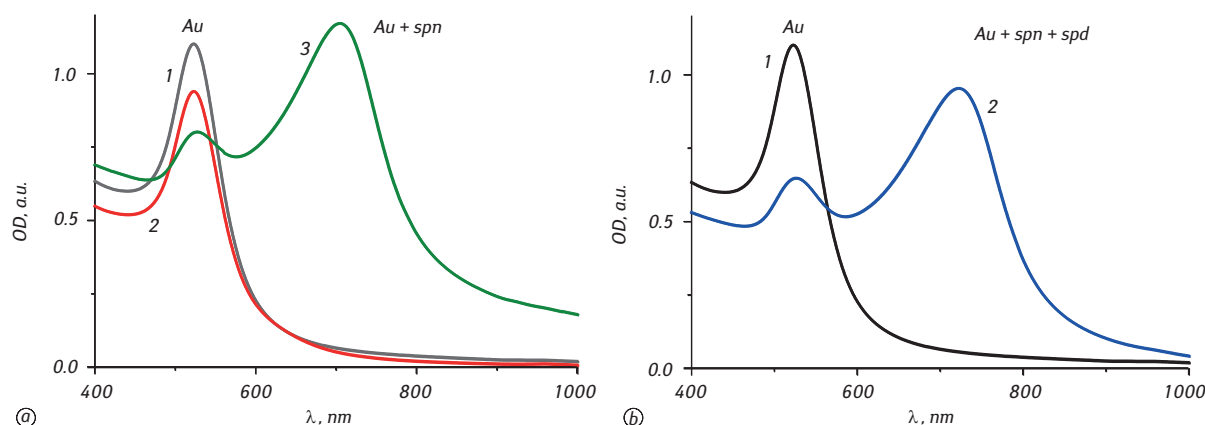


Fig. 3. Absorption spectra of AuNPs (II) interacting with spn (*a*): curve 1 — control solution of AuNPs (II), 2 — AuNPs/spn systems with spn content of 9; 3 — 5000 nM, and AuNPs (II) interacting with mixture spn + spd (*b*): curve 1 — control solution of AuNPs (II), 2 — AuNPs/PA system with 90 + 7140 nM of spn and spd in accordance

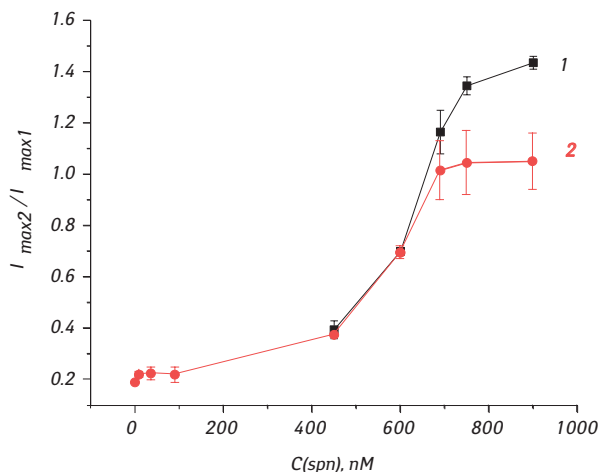


Fig. 4. The spn calibration curves (dependence of the ratio of absorption intensities $I_{\max 2}/I_{\max 1}$ of the LSPR band maxima $\lambda_{\max 2}$ and $\lambda_{\max 1}$ on the spn content), where curve 1 was calculated using intensities of LSPR bands defined in their maxima ($I_{\max 2}/I_{\max 1}$), curve 2 was calculated using intensities of LSPR bands at fixed position, namely at $\lambda_{\max 1} = 520$ and $\lambda_{\max 2} = 620$ nm (I_{620}/I_{520}).

The dependence of the ratio of LSPR band intensities, $I_{\max 2}/I_{\max 1}$, on spn content is shown in Fig. 4.

The spectral profile and absorption intensity $I_{\max 1}$ of plasmon resonance band of gold with $\lambda_{\max 1} = 519$ –520 nm for the first three samples, containing 9, 36, and 90 nM spn, practically coincided with those for the actual colloidal gold, as shown in Fig. 2. Since these spectra do not yet have a second peak in the red region inherent in AuNPs with a higher degree of aggregation, it was not possible to determine the ratio of absorption intensity $I_{\max 2}/I_{\max 1}$ for the indicated concentrations of spn using the first approach.

For comparison, similar experiments can be performed with a colloidal solution of another noble metal, namely, AgNPs. There are different experimental procedures for the synthesis of nanosized silver [4, 5]. In present study, AgNPs (1×10^{-4} M) stabilized with tryptophan (Trp) prepared as described in [6] were used. The average size of the NPs in this system, according to DLS data, was 3–5 nm (Fig. 5).

Fig. 6 shows the absorption spectra of AgNPs stabilized with Trp, with a maximum of the LSPR band at $\lambda_{\max 1} = 416$ nm (curve 1). A sharp decrease in the absorption intensity of the main LSPR band of Ag NPs occurred in the AgNPs/spn system, accompanied with a slight shift of $\lambda_{\max 1}$ to 405–410 nm (Fig. 6, a). There was a tendency for a new peak to appear in the orange-red spectral region. However, the latter does not preserve its constant position and for the spn concentration of 690 nM is at $\lambda_{\max 2} = 569$ nm, for 750 nM — at 627 nm, and at 1800 nM the maximum of the new LSPR band disappears at all. In the spectrum of the AgNPs/spd system (Fig. 6, b), the presence of two pronounced absorption maxima for spd at a concentration of 450 nM were detected: $\lambda_{\max 1} = 408$ nm and $\lambda_{\max 2} = 629$ nm. It is possible that colloidal silver can also be used to detect spd itself. This issue should be clarified in further studies.

Therefore, we demonstrated the principal possibility of assaying up to $1 \mu\text{M}$ spn in the presence

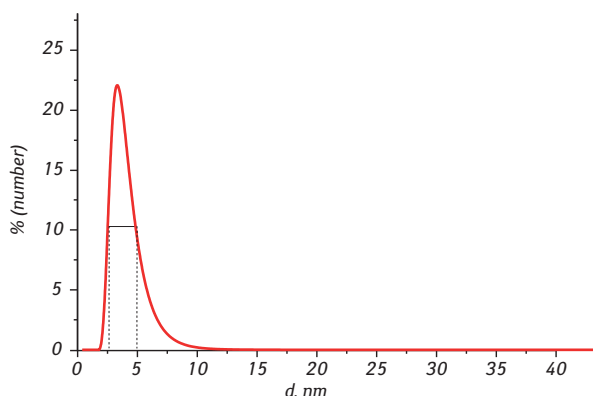


Fig. 5. Size distribution of colloidal silver nanoparticles according to DLS data

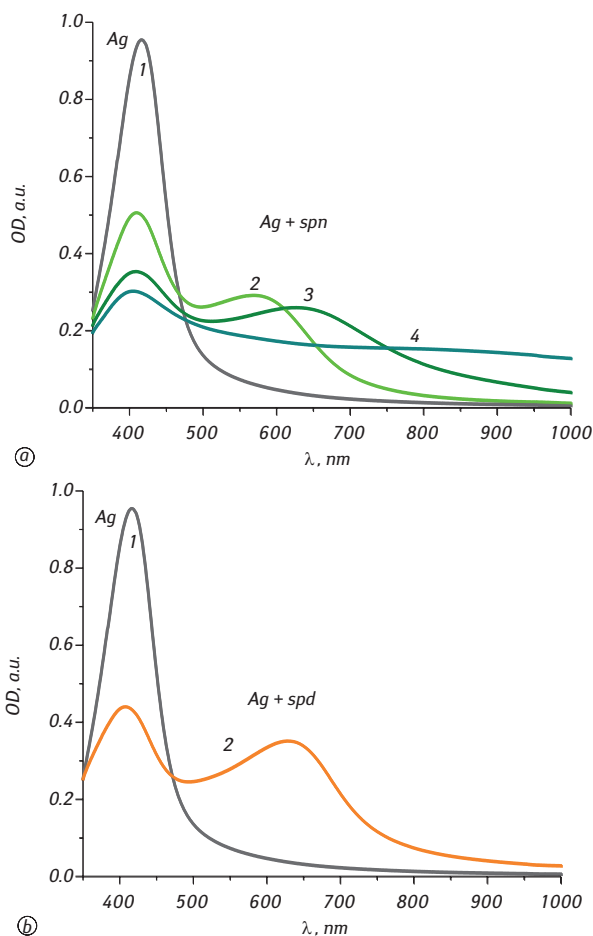


Fig. 6. Absorption spectra of AgNPs interacting with spn (a): curve 1 — AgNPs control solution, 2 — AgNPs/spn system with spn content of 690; 3 — 750; 4 — 1800 nM, and AgNPs interacting with spd (b): curve 1 — AgNPs control solution, 2 — AgNPs/spd system with spd content of 450 nM

of extremely high concentrations of spd using colloidal gold stabilized with sodium citrate. Nevertheless, tryptophan-stabilized colloidal silver does not seem to be suitable for determining spn in the presence of spd by the proposed method.

This approach may be useful for diagnostic purposes since urine concentration of spn could be considered as a putative auxiliary non-invasive biochemical marker for prostate cancer detection [7, 8]. The clinical options of this technique should be further examined.

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ЗАСТОСУВАННЯ НАНОЧАСТИНОК ЗОЛОТА І СРІБЛА ДЛЯ СЕЛЕКТИВНОГО ВИЗНАЧЕННЯ СПЕРМІНУ В СУМІШІ ЗІ СПЕРМІДИНОМ

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Мета: Оцінити придатність нового методу, заснованого на спектроколориметричному визначенні сперміну в розчині із застосуванням колоїдних частинок золота та срібла.

Матеріали та методи: Колоїдний розчин наночастинок золота синтезували хімічним відновленням з тетрахлорауринової кислоти з цитратом натрію. Колоїдний розчин наночастинок срібла одержували хімічним відновленням з нітрату срібла з триптофаном. Реєстрували спектри поглинання колоїдних розчинів золота та срібла та їх сумішей з поліамінами. **Результати:** З підвищенням концентрації сперміну в розчині змінюється інтенсивність смуги локального поверхневого плазмонного резонансу. Надлишок спермідину в розчині не впливає на цей процес. Зсув колюру колоїдних частинок золота через їх агрегацію із сперміном реєструється спектрофотометрично. **Висновки:** Показана принципова можливість селективного визначення сперміну за наявності надлишку спермідину з використанням колоїдного золота. Метод може знайти своє застосування для вибіркового визначення сперміну в біологічних рідинах.

Ключові слова: спермін, спермідин, золото, колоїд, спектрофотометрія.