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RESONANCE PROPERTIES OF THE SOLUTION OF QUERCETIN STABILIZED SILVER NANOPARTICLES IN A NUTRIENT MEDIUM

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Changes in the light absorption spectrum when mixing colloids of Ag nanoparticles with a diameter of 7 nm in a quercetin shell with a nutrient medium were studied in the present article. Colloids of silver nanoparticles were prepared by chemical reduction of $AgNO_3$ silver salt with sodium tetrahydroborate ($NaBH_4$) in an aqueous solution. Quercetin is a flavonoid of plant origin. It was chosen to stabilize nanoparticles due to its capability to form complexes with metals. The quercetin shell is capable to preserve the bactericidal effect of silver NPs on bacteria and weaken their toxic effect on healthy cells of the human body. The absorption spectra of solutions from which nanoparticle colloids were synthesized were used to control the synthesis result. The Luria-Bertani nutrient medium was studied in the work. Absorption spectra of the nutrient medium and nanoparticle colloids were again obtained immediately before mixing. Then, the nutrient medium and the nanoparticle colloid were mixed in volume proportion 1:1, and the absorption spectrum of the mixture was mesured. The absorption spectrum of the mixture did not reproduce a simple overlay of the nanoparticle colloid spectrum on the absorption spectrum of the nutrient medium. To describe the experimental spectra, a colloid of stabilized silver nanoparticles, a nutrient medium, and a mixture of a colloid and a nutrient medium were considered by nanocomposites of various organic and inorganic nanoparticles in a liquid. As a result, experimental absorption spectra were theoretically approximated by related to these nanoparticles elementary oscillators. The error of the discrepancy between experimental and simulated spectra did not exceed 3%. Analysis of the complex spectra of the mixture of the nanoparticle colloid and the nutrient medium has shown that the frequency of the localized plasmon resonance in the nanoparticles most likely does not change. It means that for studying the effect of nanoparticles on biological objects (microbes or viruses), the wavelength of external irradiation must be chosen equal to the wavelength of LPR in the colloid.

***Keywords**: Absorption spectrum, Ag nanoparticles, quercetin, Luria-Bertani nutrient medium, plasmon resonance*

INTRODUCTION

In recent decades, nanoparticles have become the basis of technologies in many areas of human activity. In particular, nanoparticles are attractive for use in the medical industry [1], pharmaceuticals [2], production of packaging materials [3, 4] and other industries aimed at combating infectious diseases. Nanoparticle preparations show antiviral and antimicrobial effects [5, 6]. There is an intensive search for the creation of nanoparticles of different chemical compositions and ensuring the stability of their properties when added to various environments.

There are two views on the mechanisms of antiviral and antimicrobial action of nanoparticles. The first one is based on the chemical interaction between atoms (ions) of metals and bio-objects [7–9]. Another view is based on the physical (field) effect of nanoparticles on the envelopes of viruses and microbes [10] at the first stage of interaction between the nanoparticle and the surface of the virus/microbe envelope - physical adsorption and already at the second stage - chemical adsorption – chemical interactions occur. Silver nanoparticles have broad-spectrum [11] antimicrobial potential and can act at low concentrations, which reduces potential toxicity for humans and the environment. During the manufacture of nanoparticles, control over the synthesis result is carried out by measuring the optical absorption spectra of the solution. When using nanoparticle colloids, they are added into various solutions. The condition of the solutions obtained after mixing is also monitored by measuring the optical absorption spectra. As it is known, the antimicrobial potential of silver nanoparticles increases when the mixture is irradiated with light with a wavelength close to the conditions of localized plasmon resonance (LPR) of silver nanoparticles. At the same time, the experimental light absorption curves in the mixture very often differ significantly from the absorption curves of the mixture components. Therefore, the study of optical absorption spectra of solutions, modelling of spectra and prediction of changes in physical properties confirms the relevance of the research. Thus, the aim of this study is a detailed analysis of the experimental light absorption curves.

MATERIALS AND PREPARATIONS

Nanoparticles preparations. Colloids of silver nanoparticles were prepared by chemical reduction of $AgNO_3$ silver salt with sodium tetrahydroborate ($NaBH_4$) in an aqueous solution [12]. The recovery of Ag^+ ions to Ag^0 occurred while monitoring the optical absorption spectra of the solution. Stabilizing the colloid and avoiding the oxidation of silver atoms at the nanoparticle surface, quercetin solution was added to the colloid after the observation in the absorption spectrum of a stable plasmon resonance band of silver nanoparticles. Quercetin is a flavonoid of a plant origin. It is poorly soluble in water. Therefore, NaOH was added to quercetin when obtaining its aqueous solution, bringing it to pH = 6.5. Quercetin was chosen for nanoparticle stabilization due to its capability to form complexes with metals and beneficial properties (anti-inflammatory, anti-tumour, antioxidant, *etc.*). Thus, the quercetin shell is capable to preserve the bactericidal effect of silver NPs on bacteria and weaken their toxic effect on healthy cells of the human body [13]. The molar ratio of silver to quercetin was 1:1. It was chosen to obtain the most stable colloids and minimize the desorption of silver ions from the nanoparticle surface. Absorption spectra of the solutions were monitored at the synthesis stages using a Perkin-Elmer Lambda 35 spectrophotometer.

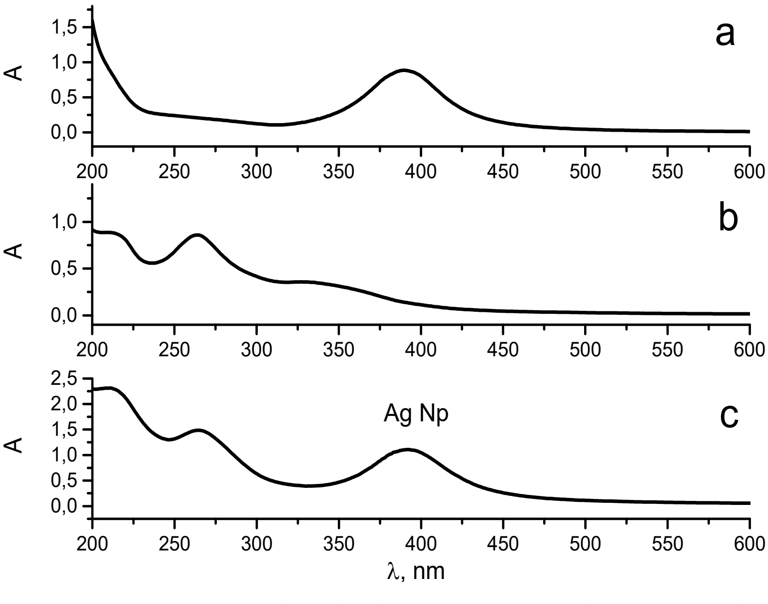

Fig. 1. Absorption spectra: *a* – Ag nanoparticle colloid; *b* – quercetin solution; *c* – colloid of Ag nanoparticles stabilized by quercetin (Ag : Quer = 1 : 1)

As it is known, the position of the LPR band maximum depends on the size and shape of the formed nanoparticles [14]. The average linear dimension of the obtained Ag NPs is 7 nm, and their shape is almost spherical. The prepared Ag NP colloids were used for the biological part of the experiments. Therefore, it is important to determine the resonance properties of mixtures of colloids and nutrient medium in which bio-objects - bacteria are grown and stored.

Nutrient Medium. For the cultivation of bacterial cultures, here was studied the Luria-Bertani nutrient medium [15, 16] with the following composition: peptone – 10.0 g; yeast extract – 5.0 g; NaCl – 10.0 g; pH – (at 25 °C) 7.0±0.2. In a volumetric flask with a volume of 1.0 l, all components were dissolved alternately in 500 ml of distilled water. Each component was added after the complete dissolution of the previous one. In case of necessity, the pH (7.0) was adjusted with a 0.1 M HCl solution or a 0.1 M NaOH solution. The resulting medium was poured into 5–6 ml biological test tubes under non-sterile conditions. After that, tubes with the nutrient medium were sterilized at 0.75 atm for 15 minutes.

Creating a Mixture. The investigated mixture was created by mixing equal volumes of the nutrient medium solution and the silver nanoparticle colloid with a particle size of 7 nm. An amount of the nutrient medium was poured into a quartz cuvette for studies of optical density of liquids. Then, near the same amount of colloid of silver nanoparticles was added to this cuvette. After that, the cuvette was shaken to mix the solutions into a homogeneous mixture.

Absorption Spectra of the Mixture and Its Mixing Components. The optical density of solutions was studied on a UV-1800 spectrophotometer. Fig. 2 shows the optical density curve of the colloid of nanoparticles (Fig. 2 *a*), nutrient medium (Fig. 2 *b*) and mixture of nutrient medium and colloid of silver nanoparticles (Fig. 2 *c*). Curve 1 (solid line) has a visible optical density maximum of 1.12 at the irradiation wavelength of 400 nm. When moving away from this maximum, the optical density of this colloid is small, and it is in the range of 0.1–0.5 units, and only when the wavelength of light approaches ~ 200 nm there is a slight increase in the optical density. The optical density of the nanoparticle colloid at 200 nm was 0.98 units. The curve obtained from the nutrient medium in the wavelength range of 200–500 nm has three well-marked optical density maxima: the first with an optical density of 3.11 at a wavelength of 244 nm, the second with an optical density of 2.71 at a wavelength of 354 nm, the third with an optical density of 2.58 at a wavelength of 395 nm. The optical density of the nutrient medium in the interval between the first and second maxima monotonically decreases from 3.0 (with increasing wavelength) of light to 2.69 around the second maximum. Therefore, the solution of the nutrient medium had a higher optical density compared to the colloid of silver nanoparticles over the entire range of light wavelengths. It is well demonstrated in Fig. 2.

The light absorption spectrum of the mixture (Fig. 2 *c*) in the wavelength range of 200–360 nm almost repeats the contours of the absorption spectrum of the nutrient medium. In this interval of wavelengths, two maxima are observed in the mixture. The wavelengths of the first two maxima in the nutrient medium are almost the same. Thus, the first maximum for the mixture is observed at a light wavelength of 235 nm. It is a slightly shorter wavelength than the first maximum of the optical density in the nutrient medium (244 nm). The wavelengths of the second maxima of the optical density coincided in both media. The maxima of the mixture have a slightly lower optical density: the first peak has an optical density of 2.88, and the second has 2.43. Such discrepancies in the optical density are not difficult to explain because of the addition of the component with a lower optical density to the solution. Almost the same wavelengths of the maxima of the nutrient medium, for the interval 200–360 nm, clearly indicate that when the components are mixed, there are no significant changes in the components of the resulting solution. When the wavelength of light increases, we notice that the light absorption spectrum of the mixture does not repeat either the absorption spectrum of the nutrient medium or the absorption spectrum of the nanoparticles. In particular, the absorption spectrum of the colloid of nanoparticles had a maximum of 400 nm, the absorption spectrum of the nutrient medium had a maximum of 395 nm, and the maximum in the absorption spectrum of the mixture was 375 nm. In addition, the optical density of this maximum is 2.13. It most likely indicates a sufficiently strong influence of the nutrient medium on the formation of the surface plasmon resonance of the nanoparticle, which leads to a shift in the resonance frequency. Thus, this wavelength can be used acting as external radiation to study the effect of nanoparticles on biological objects (bacteria). Indeed, with a simple superimposition of the spectra, the absorption spectrum of the mixture would have a maximum close to 400 nm. In the experiment, we got a different result.

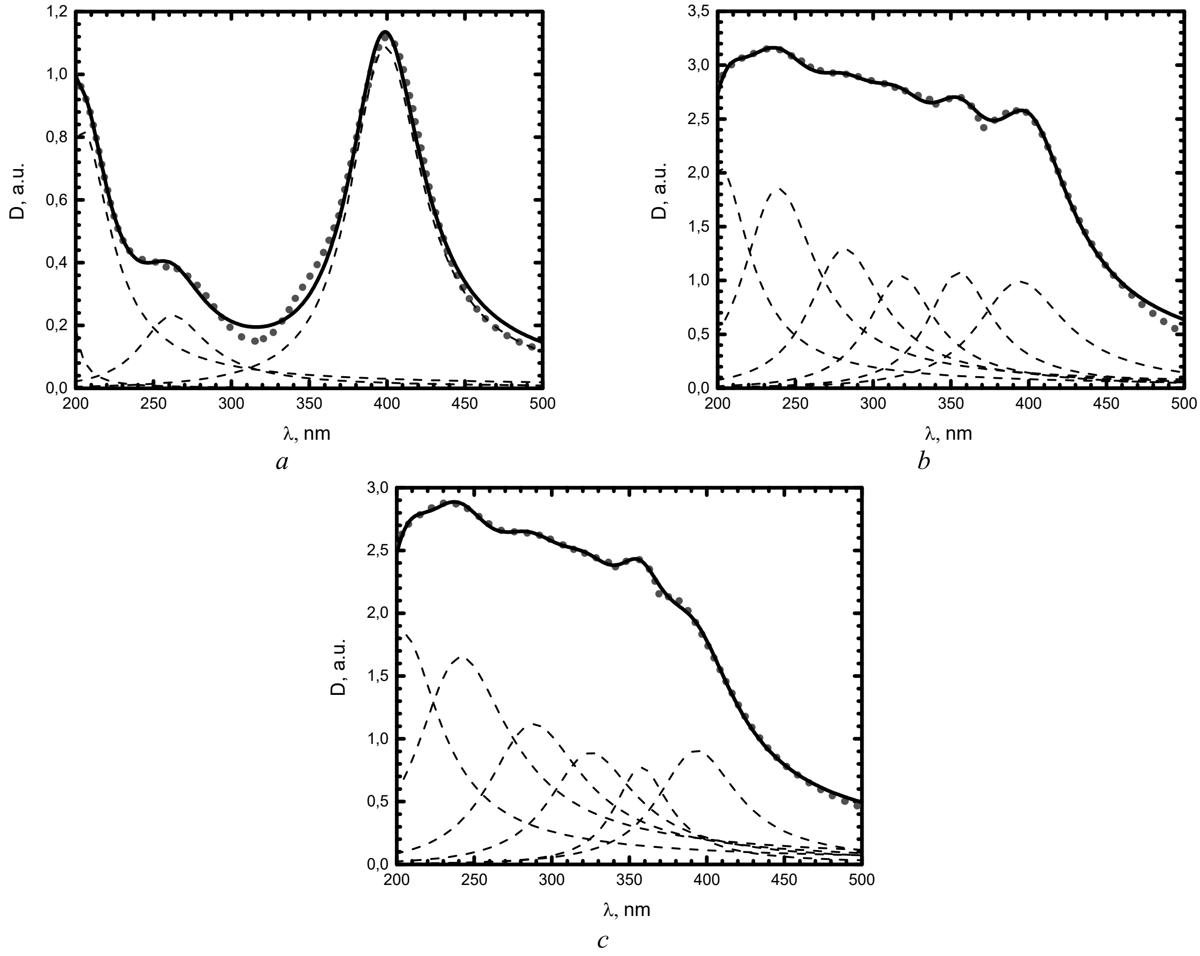

Fig. 2. Optical density of the solutions investigated in the work. Black solid curves correspond to theoretical calculations, dots show experimental results, thin dashed lines - optical densities of oscillators simulating solutions $D(\lambda)$

Let us suppose that in the entire wavelength range of 200–500 nm, after mixing the liquids, we obtained a mixture with optical characteristics being a simple sum of the optic characteristics of its components. The weight contribution of components (the nutrient medium and the colloid of nanoparticles) to the optical density of the mixture cannot be the same, even when equal-volume parts are mixed. The optical density of the nutrient medium is greater than that of the colloid of nanoparticles, and its contribution to the optical density of the mixture should be higher. Let the weight contribution to the optical density of the colloid mixture be *X*. In this case, the weight contribution to the optical density of the nutrient medium will be (1-*X*). With simple addition, one can obtain

$$D_c = \left(1 - X\right) D_p + X \cdot D_k \ , \qquad (1)$$

where D_c is the optical density of the mixture, D_p is the optic density of the nutrient medium; $D_к$ is the optical density of the nanoparticle colloid. The equation (1) and the absorption spectra obtained from the experiment were used to calculate the weight contribution of the colloid of nanoparticles to the optical density of the mixture. Numerical values were obtained, based on which the dependence of the weight contribution X of the colloid of silver nanoparticles on the wavelength of light was constructed (Fig. 3).

From Fig. 3 one can see that in the wavelength range of 200–360 nm, the optical density of the mixture can be well described in the approximation of a simple addition of the spectra, taking into account the weight contributions of the mixture components. The weight contribution of *X* colloid of silver nanoparticles to the optical density of the mixture when mixed with an equal volume of the nutrient medium almost does not change. In the light wavelength range of 360–500 nm, the weight contribution X of the colloid of silver nanoparticles firstly increases to 0.62 up to the light wavelength of 412 nm, then decreases to the values from which the growth began. Interestingly, the maximum weight contribution corresponds neither to the wavelength of the maximum optical density of the colloid of nanoparticles, to the wavelength of the maximum optical density of the nutrient medium, nor to the wavelength of the maximum optical density of the mixture. Therefore, over the entire range of light lengths (200–500 nm), the mixture spectrum cannot be described by a simple overlay of the spectra of its components. Thus, there is an absolute need to find a theoretical model to explain the observed phenomenon.

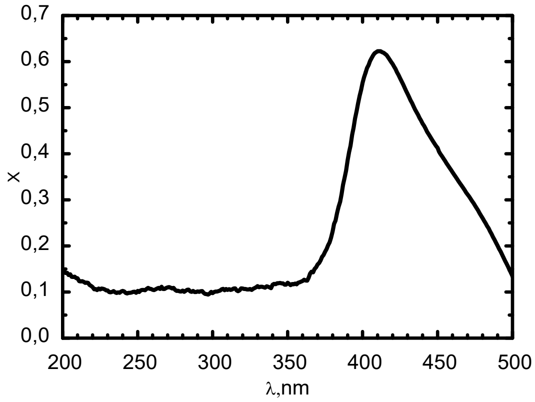

Fig. 3. The dependence of coefficient X on the wavelength (see (1))

THEORETICAL MODELING OF ABSORPTION SPECTRA AND DISCUSSION

Theoretical modelling. For a theoretical explanation of the optical properties of a colloidal solution of stabilized silver nanoparticles, a nutrient medium, and a mixture of a colloidal solution and a nutrient medium, each solution can be imagined as a nanocomposite of various organic and inorganic nanoparticles in a liquid. The properties of these nanoparticles can be approximated by a single-oscillator model

$$\varepsilon_i(\omega) = \frac{f_i}{\omega_i^2 - \omega^2 - i\omega\Gamma_i}\,. \tag{2}$$

Since the absorption in a nanoparticle $A(\omega) = \frac{4\pi}{\lambda}\frac{\operatorname{Im}\varepsilon_i(\omega)}{\sqrt{\operatorname{Re}\varepsilon_i(\omega) + |\varepsilon_i(\omega)|}} : \operatorname{Im}\varepsilon_i(\omega)$, the optical density of a solution will be

$$D(\omega) = \sum_i \frac{f_i\omega\Gamma_i}{\left(\omega_i^2 - \omega^2\right)^2 + \left(\omega\Gamma_i\right)^2}\,. \tag{3}$$

The optical density (3) is the sum of lorentzians. It has *3N* parameters, where *N* is a number of oscillators (2). Parameters f_i , Γ_i , ω_i and *N* were chosen, to close the theoretically calculated $D(\omega)$ to the experimental curve $D_{\exp}(\omega)$, that is

$$F(f_1,\ldots,f_N,\omega_1,\ldots,\omega_N,\Gamma_1,\ldots,\Gamma_N) =$$

$$= \int_{\omega_0}^{\omega_1} \left|D(\omega) - D_{\exp}(\omega)\right| d\omega \to \min\,. \tag{4}$$

Problem (4) is inverse and non-linear, so the pattern search was used for its solution [17, 18]. The final optimal theoretical spectral curves are presented in Fig. 2. In particular, the solid black curve in Fig. 2 *a* represents the theoretical curve of optical density in a colloidal solution. It is the result of the interaction of four elementary oscillators, the absorption curves of which are also plotted with thin dashed lines in Fig. 2 *a*. For comparison, experimental values of the optical density of the colloidal solution of silver nanoparticles stabilized by quercetin are also plotted there by dots. Fig. 2 *b*, *c* show the results of similar calculations for a nutrient medium and a mixture of a colloidal solution with a nutrient medium. In both cases, the optimal approximation requires the use of six oscillators.

Discussion of the Results of the Simulation. Numerical calculations within the proposed model allowed us to obtain the optimal number of oscillators for each spectrum obtained in the experiments. The deviation errors in numerical approximations and in experiments did not exceed 3%. Thus, the absorption spectrum of nanoparticle colloids was approximated by four oscillators with wavelengths corresponded to 398, 261, 204, and 192 nm. Such wavelengths also agree well with the spectra shown in Fig. 1. The absorption spectrum of the nutrient medium was approximated by six oscillators, the wavelengths of which were: 392, 357, 323, 285, 239, and 203 nm. It was also possible to approximate the absorption spectrum of the mixture due to six oscillators with wavelengths corresponding to 393, 354, 317, 280, 237, and 201 nm. It is seen that the values of the wavelengths of the oscillators, thanks to which the absorption spectra of the numerical experiments in the nutrient medium and the mixture succeeded, are almost the same. But they slightly shifted towards shorter wavelengths in the case of the mixture. The oscillators with the longest wavelengths are some exceptions to this trend. Namely, for an oscillator with a wavelength of 392 nm in a nutrient medium, when nanoparticles were added to the colloid solution, the wavelength was increased by 1 nm (393 nm). In this wavelength range for the nanoparticle colloid, we had an oscillator with a wavelength of 398 nm. The change in the wavelength of the elementary oscillator, which corresponds to the LPR wavelength in the colloid for the mixture, by a small amount (1 nm) allows us to state that the addition of the colloid to the nutrient medium has little effect on the frequency of oscillations of the localized plasmon on the nanoparticle.

CONCLUSIONS

The analysis of the spectral curves has shown that the absorption spectrum of the mixture in the wavelength range of 360–500 nm is not a simple sum of the spectra of the colloidal solution of nanoparticles and the nutrient medium. The wavelength of the localized plasmon resonance of silver nanoparticles is also in this range of wavelengths. Despite the fact that the experimental curves differed significantly, analysis based on a simple model of oscillators has shown that the spectra of the mixture are decomposed into the same components that are present in the decompositions of the colloidal solution of nanoparticles and the nutrient medium. The wavelengths of the peaks have practically not changed. Changes in the absorption spectrum of the mixture occurred due to a change in the intensity of the oscillators.

As a result, we can state that the analysis of the complex spectra of the mixture of nanoparticle colloid and nutrient medium has shown that the LPR frequency in the nanoparticles does not change (change within 1 nm). It means that in order to study the effect of nanoparticles on biological objects (microbes or viruses) under the conditions of plasmon resonance, the wavelength of external irradiation must be chosen equal to the wavelength of LPR in the colloid solution.

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Резонансні властивості суміші поживного середовища і колоїду наночастинок срібла, стабілізованих кверцетином

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У статті досліджено зміни спектра поглинання світла при змішуванні колоїдів наночастинок Ag діаметром 7 нм в кверцетиновій оболонці з поживним середовищем. Колоїди наночастинок срібла готували методом хімічного відновлення солі срібла $AgNO_3$ тетрагідроборатом натрію ($NaBH_4$) у водному розчині. Кверцетин є флавоноїдом рослинного походження. Його було обрано для стабілізації наночастинок через здатність утворювати комплекси з металами. Оболонка з кверцетину здатна зберегти бактерицидний вплив НЧ срібла на бактерії і послабляти їхню токсичну дію на здорові клітину організму людини. Спектри поглинання розчинів з яких відбувавсь синтез колоїдів наночастинок, використали для контроля результату синтезу. В роботі досліджували поживне середовище Luria-Bertani. Спектри поглинання поживного середовища, колоїдів наночастинок знов були отримані безпосередньо перед змішуванням. Потім отримали суміш 1:1 об'ємів поживного середовища з колоїдом наночастинок і дослідили спектр поглинання суміші. Цей спектр не відтворив просте накладання спектра колоїду наночастинок на спектр поглинання поживного середовища. Для опису експериментальних спектрів колоїдний розчин стабілізованих наночастинок срібла, живильне середовище та суміш колоїду та живильного середовища розглядали як нанокомпозити різних органічних і неорганічних наночастинок у рідині. В результаті експериментальні спектри поглинання були теоретично наближені пов'язаними з цими наночастинками елементарними осциляторами. Похибка розбіжності експериментальних і модельних спектрів не перевищила 3 %. Аналіз складних спектрів суміші колоїду наночастинок та поживного середовища показав, що частота локалізованого плазмонного резонансу в наночастинках майже не змінюється. Це означає, що для вивчення впливу наночастинок на біологічні об'єкти (мікроби або віруси) довжину хвилі зовнішнього опромінення треба вибирати такою, що дорівнює довжині хвилі ЛПР у колоїді.

***Ключові слова**: Спектри поглинання, наночастинки срібла, кверцетин, поживне середовище Luria-Bertani, плазмонний резонанс*

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