

Research article

Comparative spectral behavior of tryptophan in Au/SiO₂ and Cu/SiO₂ nanocomposites

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Abstract

The peculiarities of fluorescence and Raman spectra of amino acid tryptophan (Trp) on the surface of dispersed silica containing gold (Au/SiO₂) and copper (Cu/SiO₂) nanoparticles (NPs) have been studied. IR- and UV-spectra of Cu-containing systems revealed that Cu NPs coexist with copper oxides in solution and on the surface of SiO₂.

The fluorescence of Trp is efficiently quenched in the presence of gold and copper NPs both in solution and on the SiO₂ surface due to charge transfer processes between Trp and metal NPs. The intensities of the vibration bands of benzene and pyrrole rings as well as carboxyl and amino groups of Trp in Raman spectra in the presence of Au NPs are greatly enhanced. Whereas in the presence of Cu NPs the enhanced vibration of Trp occurred for carboxyl groups and benzene ring adsorbed on Cu-OH groups resulted from Cu NPs oxidation.
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Keywords: gold, copper, nanoparticles, tryptophan, enhanced Raman spectra, fluorescence quenching.

Introduction

Gold and silver nanoparticles (NPs) are a special group of materials with unique optical, electrical, catalytic properties and great potential applications in biology and medicine [1, 2]. Gold NPs are promising supports for drug delivery due to their easy functionalization in practice by any ligands and antibodies and low toxicity to normal

cells, making them also suitable for use as biosensors in medical diagnostic and therapeutic applications [3-7]. Nanosized particles of copper have also high catalytic and bactericidal activities that play a key role in the metabolism of all living organisms, starting from simple cells [8, 9].

Recently, the ability of nanosized particles to enhance low signals of photosensitive molecules in fluorescence (Fl) and Raman spectra especially attracts attention because of the need to increase the sensitivity of molecular markers for diagnostic purposes. Theoretical calculations suggested the enhancement of the fluorescence intensity of photosensitive molecules located in the vicinity of the NPs in tens and hundreds times, that was the basis for a new class of biosensors. It is known that quenching of fluorophore emission occurs in contact with metals because of the electron or energy transfer between molecule and metal [10]. Theoretical analysis on fluorescence quenching and enhancement by metal NPs was performed in [11]. The changes in the fluorescence of molecule located near a metal were caused by the plasmon field of the particle, and the nature of metal. The fluorescence enhancement rate is the combined effect of the enhancement of the excitation decay rate and the change in the quantum yield, both influenced by the plasmon field. The field intensity decreases rapidly with the distance from the surface for all sizes, at that the rate of decrease is greater for the smaller size [11].

At the same time, the intensity enhancement in the Fl or Raman spectra of fluorophore molecules in the presence of the metal NPs in a heterogeneous environment, where both components are adsorbed on the developed surface of the support, almost have not been studied. Heterogeneous composites based on silica modified with small amount of Au, Ag or Cu NPs in presence of biomolecules (aminoacids, vitamins etc) can be the basis for a new class of multifunctional nanomaterials because of their stability, biocompatibility, long-term storage, but at the same time high light-scattering ability of dispersed SiO₂ greatly complicates the interpretation of the spectra. Au and Cu NPs are of different resistance to oxidation and thus the varying stability. Thus the interaction of Au and Cu NPs with biomolecules strongly depends on their chemical stability in time and in particular on the formation of the oxide phase of metal NPs. In this work we studied the peculiarities of Fl and Raman spectra of amino acid tryptophan adsorbed on dispersed silica containing gold (Au/SiO₂) and copper (Cu/SiO₂) NPs.

Materials and Method

Cu NPs synthesis in solution and on the SiO₂ surface. The reduction of copper ions in solution by Trp did not occur, even when heated to 80⁰C, in contrast to the case with gold ions. Copper ions (Cu²⁺) were reduced to Cu NPs (Cu⁰) using NaBH₄ in presence of iodine ions as stabilizers similar to the procedure described in [12] followed by addition of Trp solution after the appearance the SPR band of Cu NPs at 560 nm. Concentrations of Cu and tryptophan in the resulting solution used were C(Cu) = C(Trp) = 10⁻³M. Cu NPs stabilized by Trp were adsorbed on the surface of dispersed silica. Samples marked as Cu-Trp/SiO₂ were dried at 80°C about 8 hours. The intensity of the absorption band of Trp at 280 nm was not changed in time.

Au NPs synthesis in solution and on the SiO₂ surface. Colloidal solutions of gold NPs were prepared by reduction of tetrachlorauric acid using Trp as reducing / stabilizing agent. Components interacted in a molar ratio v(Au³⁺):v(Trp) = 1:1 similar to that given in [13], but without additional heating of the reactants. Concentrations of gold and tryptophan in the resulting solution used were C(Au) = C(Trp) = 10⁻³M. Heterogeneous composites Trp/Au/SiO₂ were obtained in two ways:

i. Complex of gold NPs with tryptophan (Au-Trp) was quantitatively adsorbed from colloidal solution on the surface of SiO₂ calcined at 500°C, C(Au) = C(Trp) = 10⁻⁵ mol/g (Au 0,2 % mass) (sample marked as Au-Trp/SiO₂) similar to that described by us in [14] and modified in this work;

ii. Gold NPs on SiO₂ surface (Au-SiO₂, 0,2 % Au mass) were synthesized by the thermal reduction at 500°C of tetraauric acid previously adsorbed on the silica surface. After that the Trp solution was adsorbed on Au-SiO₂ surface (sample marked as Trp/Au-SiO₂).

The absorption and diffuse reflectance spectra of Trp/M/SiO₂ (M-metal) samples were recorded using a spectrophotometer Perkin-Elmer Lambda 35. The morphology of the samples was studied on a LEO 1530 electron microscope. IR spectra were recorded on Nicolet NEXUS FTIR spectrometer in the frequency range 400-4000 cm⁻¹. Fluorescence spectra were recorded on spectrofluorimeter Cary Eclipse (Varian). Measurements of Raman spectra were carried out by Raman spectrometry microscope Renishaw InVia. The source of radiation served argon laser with a wavelength of 488 nm and a power of 900 mW.

Results and Discussion

Morphology and IR-spectra of Cu/SiO₂ composites. Electron-microscopic image of Cu/SiO₂ composite is shown in Fig.1. The average diameter of Cu NPs calculated with a program Image J is 2-3 nm.

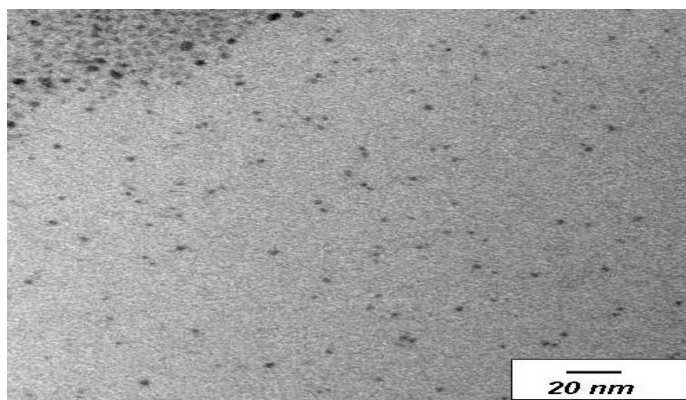


Figure 1: TEM image of Cu/SiO₂ composite, Cu 0,5 %. Scale bare is 20 nm.

Identification of vibration bands of Cu/SiO₂ was made according to data of IR spectra given in [15-19]. All spectra were registered 48 hrs after preparation. IR spectra are contained the Cu – O vibration bands belonging to oxide and hydroxide form of copper, i.e. CuO (470 cm⁻¹, 414 cm⁻¹) and Cu(OH)₂ (479 cm⁻¹) (Fig. 2). The formation of hydrated copper ions CuOH⁺ (as a surface-active particles) and their adsorption on the surface of copper oxide was discussed in [20]. The final product observed in our experiment is yellow precipitate of Cu(OH)₂.

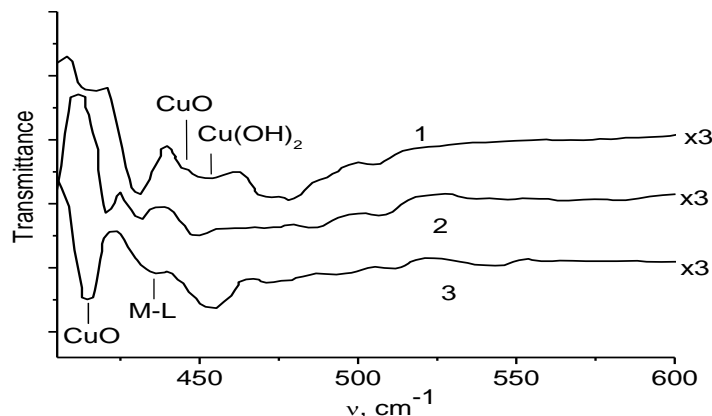


Figure 2: IR spectra of nanocomposites 1 - Cu/SiO₂, 2 - Trp/SiO₂, 3 – Trp/Cu/SiO₂ with molar ratio $\nu(\text{Cu}):\nu(\text{Trp}) = 1:1$.

New weak vibration at 436 cm⁻¹ in the IR spectrum of Trp/Cu -SiO₂ sample was observed, that was absent in the Trp/SiO₂ and Cu/SiO₂ IR spectra. According to [21] this vibration belongs to the metal-ligand coordination bond. The large number of NH₂, NH₃, CH₂ vibration bands related to Trp are in the region of much stronger vibrations of Si-O-Si and water adsorbed on the silica surface. Thus it is difficult to make proper attribution of the vibration frequencies based only on IR-spectroscopy measurements. To clarify the peculiarities of Trp interaction with Cu NPs the UV and Raman spectra were investigated.

UV-absorption spectra of Trp/Cu NPs in solution and on SiO₂ surface. The position of Cu NPs SPR band at 567 nm undergoes slight hypsochromic shift during the process of copper ions reduction and accumulation of Cu NPs (Fig. 3. a). This occurs perhaps because of gradual oxidation of the surface of Cu NPs with a further formation of the oxide layer and, consequently, decrease the size of the metallic core. Thus it should be stated that the use of the term Cu NPs in this paper is quite conditional and relative, because of coexisting the Cu NPs, Cu(I) and Cu(II) oxides and copper ions both in solution and on the SiO₂ surface. The states of copper in these systems will be noted when discussed.

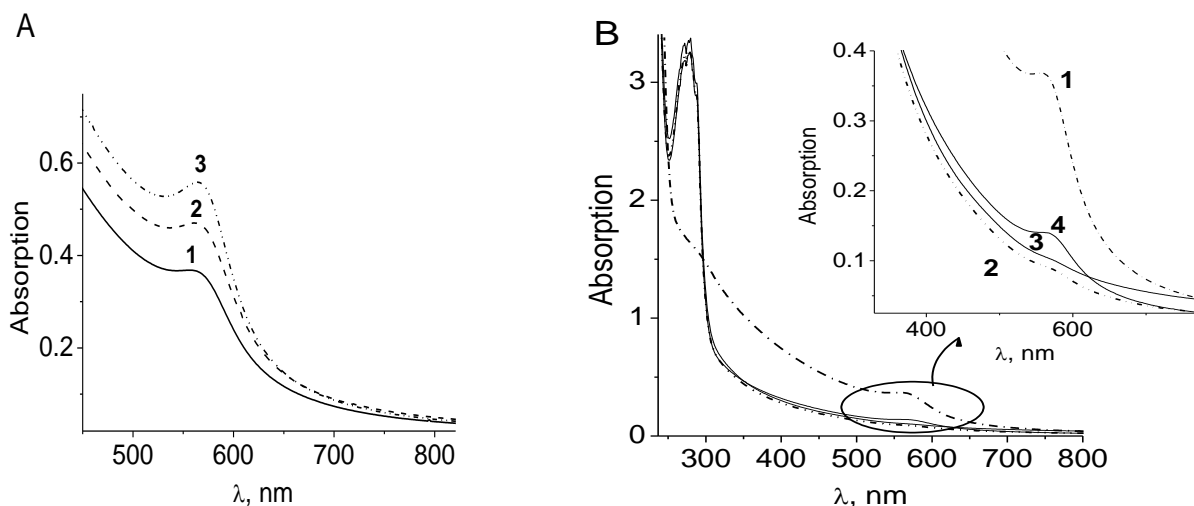


Figure 3: Changes in absorption spectra of Cu NPs aqueous solution in 15 (1), 60 (2), 140 (3) min of preparation (a); absorption spectra of Cu NPs without Trp (1) and in 15 (2), 60 (3), 140 (4) min after Trp addition, the molar ratio $\nu(\text{Cu}):\nu(\text{Trp}) = 1:5$ (b).

The layer of copper oxide was formed on the surface of non-stable Cu NPs and it became a source of Cu ions. Addition of Trp to the Cu NPs colloid was accompanied by significant decrease of the absorption intensity of the Trp band at 280 nm (Fig. 3. b). This fact is an indirect confirmation of Trp interaction with copper ions and Cu NPs. At the same time, the decrease of the SPR band intensity of Cu NPs in presence of Trp on Fig. 3. b in compared to those on Fig. 3.a occurred probably due to the complex formation between copper ion and Trp and thus the removal of the part of Cu ions from the process of Cu NPs formation. The maximum of absorption band of Trp complex with copper ions is very close to the SPR band position of Cu NPs. The authors [18] ascribed the electronic absorption band of Cu ions with Trp at about 612 nm to the Cu(Trp)₂ chelate, that is similar to the spectrum observed in this work. Direct evidence for the interaction of Trp with copper can give the fluorescence spectra.

Fluorescence spectra of Trp in the presence of Cu NPs. The fluorescence spectrum of Trp in aqueous solution is represented by a broad structureless band with maximum at 360 nm; the excitation spectrum has a maximum near

290 nm. On the SiO₂ the main emission maximum of adsorbed Trp was blue-shifted to 334 nm compared with that in solution due to the protonation of amino group of the Trp by weakly acidic OH-groups of silica surface[14] that is typical for the electronic spectra of aromatic molecules containing amino group in acid medium [22]. The intensity of Trp fluorescence in solution is dramatically entirely quenched in the presence of the Cu NPs (Fig. 4.).

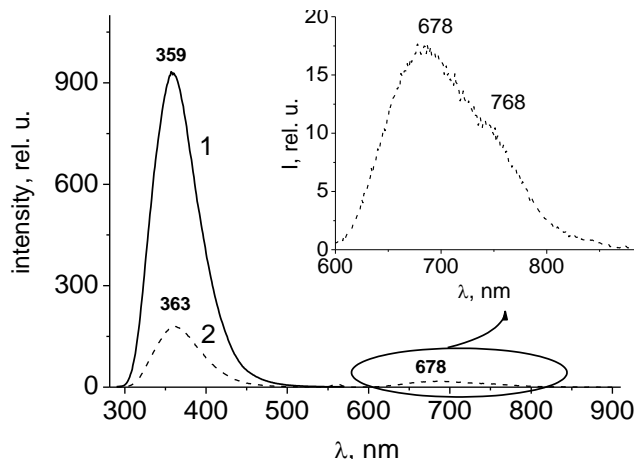


Figure 4: Fluorescence spectra of Trp in solution (1) and in presence of Cu NPs after 80 min of interaction (2), molar ratio was $\nu(\text{Cu}):\nu(\text{Trp}) = 1:1$.

Conversion of Trp fluorescence intensity on the concentration of the Cu NPs calculated by the Stern-Folmer equation gives a non-linear plot (Fig. 5). It is known that the fluorophore can be quenched due to the i) collision, and ii) complex formation with the quencher. Character of Fig. 5 suggests the presence of both mechanisms of interaction of Trp with copper in solution. Thus, in contrast to the appearance of fluorescence and excitation bands of the Trp/Au NPs CTC [14], complex Trp/CuNPs does not fluoresce.

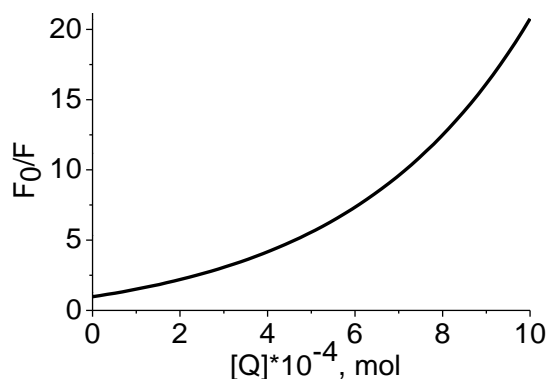


Figure 5: Dependence of Trp relative fluorescence intensity of on the Cu NPs concentration in solution, where F_0 is the fluorescence intensity of Trp without Cu, $F - I_n$ in the presence of Cu NPs, Q - concentration of Cu NPs.

The emission of Trp adsorbed on Cu/SiO₂ powder is completely quenched. This gives the reason to believe that Trp molecules on the surface of Cu/SiO₂ are localized near Cu NPs.

As it was mentioned above, instability and fast oxidation of copper nanoparticles is accompanied by formation of an oxide layer on the surface of the nanoparticle. This layer contains Cu-OH groups, that determines the spectral

behavior of adsorbed Trp. It should be suggested that a broad low-intensity band at 600-800 nm with a maximum at about 680 nm can be attributed to the fluorescence of copper oxide shell. The authors of [23, 24] observed emission at 657 nm, that was attributed to CuO NPs. It should be noted that Trp molecules adsorbed on the SiO₂ surface outside the sphere of influence of electromagnetic field of the Cu NPs also contribute to the IR, electronic and Raman spectra that complicates their interpretation.

Raman spectra of Trp adsorbed on Cu-SiO₂. In the Raman spectrum of Trp on Cu/SiO₂ the intensity enhancement of stretching vibrations of the carboxyl group and the C=C vibrations of the benzene ring are clearly visible (Fig. 6). Analysis of the normal modes of vibration for the molecule showed that this involves stretching and librational vibrations of the benzene and pyrrole rings (about 1480 cm⁻¹). Probably the Raman spectra reflect mainly the interaction of Trp with OH groups, covering the surface of the Cu NPs.

Increasing content of Cu NPs on the silica surface significantly affects the Raman spectrum of Trp. In the Trp/Cu/SiO₂ spectrum (C(Cu) = 0,1 % molar, Cu: Tr = 1:1) there are vibration bands of OH groups associated with copper ions at the 450, 527 and 698 cm⁻¹ [27]. Thus, to obtain the direct evidence of the interaction of Trp molecules with copper NPs, it should be considered a set of results obtained by IR-, UV- and Raman spectroscopy.

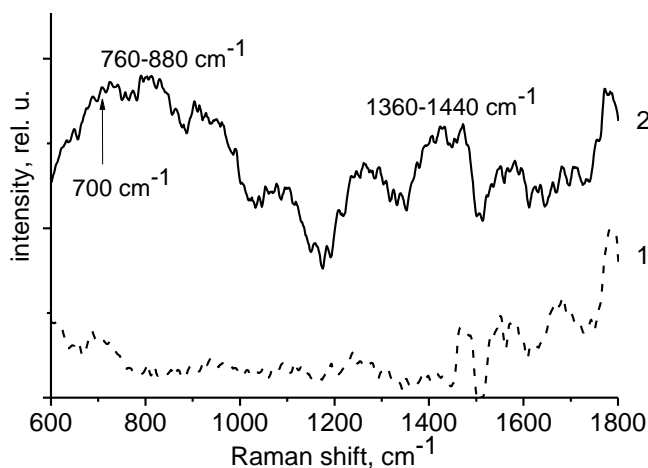


Figure 6: Raman spectra of tryptophan on SiO₂ (1) and Cu/SiO₂ (2). Molar ratio of Cu:Trp = 1:1, Cu 0,1% molar, C(Trp) = 10⁻⁵ mol/g.

In the Raman spectra of Trp/Cu-SiO₂ samples all three badly defined vibration bands of CuO: 320, 347 and 622 cm⁻¹ (Ag, Bg (1) and Bg (2), respectively) are presented. Identification was made according to the data given in [15, 25]. As it was mentioned above, in the IR spectrum the low-intensity band at 436 cm⁻¹ was registered, belonging to the complex of copper with Trp. In the Raman spectra of Trp/Cu-SiO₂ this band overlaps with broad bands at 330-520 cm⁻¹, that are the superposition of H₂O and CuO deformation vibrations. According to [18], the vibration bands at 401, 423 and 470 cm⁻¹, belonging to Cu-N bond, coincided in Raman and IR spectra and were expressed very weakly. Overall, the interaction mechanism in the Trp/Cu-SiO₂ system includes adsorption of Trp on OH groups of silica, OH-groups of copper oxide, formation of Cu(Trp)₂ with residual copper ions in solution and long-range electron transfer from Trp directly to Cu NPs, vibration bands of which may be partially overlapped. According to the data in [26], interaction of Trp with metal nanoparticles occurs via the unshared electron pair on the nitrogen atom, and is a result of the formation of a complex between the metal and the π -electrons of the indole ring of Trp [26].

Tryptophan fluorescence spectra of Au NPs in solution and on the Au-SiO₂ surface presented and discussed by us in [14]. FI spectrum of Trp-Au in solution is more informative, than that for Trp-Cu. For both metals fluorescence of Trp was quenched, being stronger in the case of Cu NPs. Electron transfer from an excited Trp to a gold nanoparticle was accompanied by appearance of new emission ($\lambda_{\text{max}} = 450 \text{ nm}$) and excitation ($\lambda_{\text{max}} = 373 \text{ nm}$) bands due to charge transfer complex (CTC) formation in the ground state of the system. The nature of the interaction between Trp and the gold nanoparticles was discussed in the theoretical paper [28]. The authors, based on theoretical calculations, offered formation of metal–molecule hybrid orbitals, including *d*-orbital of gold and molecular orbitals of Trp, i.e. the amino, carboxyl groups and indole ring, having a mixed character. Nonradiative energy transfer from excited Trp to such a hybrid state is accompanied by quenching of Trp fluorescence.

Raman spectra of Trp on Au/SiO₂ surface. The spectral behavior of the Trp in presence of Au NPs on SiO₂ is easier to control than in the case of Cu/SiO₂ due to the stability of gold to oxidation, stability of the composite Trp/Au/SiO₂ over time and the possibility of two-way control (FI and Raman).

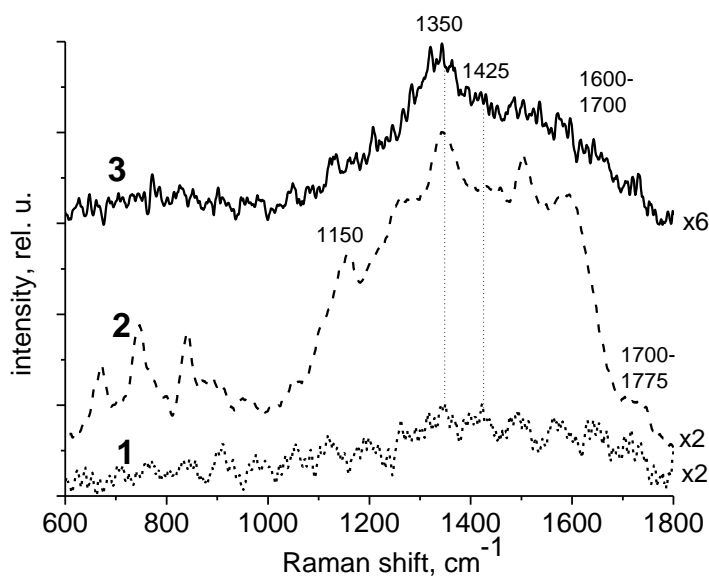


Figure 7: Raman spectra of Trp/SiO₂ (1), complex Au-Trp/SiO₂ (2), Trp/Au-SiO₂ (3). C(Trp) = 10⁻⁵ mol/g, Au 0,02 % mass.

The stable complex of Trp with Au NPs was formed that clearly demonstrated Raman spectra as in solution [14], as on the SiO₂ surface (Fig. 7, sp. 2). Bending vibrations of benzene and pyrrole rings, asymmetric stretch of the COO–group of Trp are enhanced in presence of Au NPs in this complex. In case of adsorption of Trp on Au-SiO₂ (Fig. 7. sp. 3) we did not register the formation of complex compound between Trp and Au. In Raman spectra the stretching vibrations of CC in the carboxyl group and the benzene ring of Trp are enhanced.

There are two types of interaction Trp with Au NPs: 1) CTC and coordination through the amino group and 2) chelate complex of free molecules of Trp with NPs by COO–group. Indicated variants become evident from the Raman spectra of Trp in the presence of gold NPs localized on the silica surface. Enhanced vibration bands of indole ring of tryptophan were detected around 1500 cm⁻¹ for complex Trp-Au. Intense Trp-Au signal at 1150 cm⁻¹ corresponded to OH- vibration in the carboxyl group, and at around 1720-1770 cm⁻¹ to C=O vibration. Contrary to this, for tryptophan, adsorbed on the surface of SiO₂, and Au-SiO₂, there were observed signals of protonated aminogroup at 1425 cm⁻¹ and 1620-1680 cm⁻¹. In both cases Trp/Au-SiO₂ and Trp-Au/SiO₂, the strongest vibration was one of C atom in C-COO (about 1350 cm⁻¹).

Conclusion

The fluorescence, Raman and IR- spectra of amino acid tryptophan (Trp) in the presence of gold and copper NPs either in solution and on the SiO₂ surface indicated a direct chemical interaction of Au NPs and Cu/CuO NPs with adsorbed Trp, due to charge transfer processes and formation of complex compounds NP-Trp. The fluorescence of Trp is efficiently quenched in the presence of gold and copper NPs, the Raman spectra of Trp in the presence of NPs are greatly enhanced, especially in case of Au NPs with specific spectral characteristics, that makes it possible to control interaction of NPs with biological objects by measuring optical and/or Raman spectra and use them as biomarkers. It should be noted that the major obstacle when the analysis of fluorescence and Raman spectra of Trp on the Cu/SiO₂ and Au/SiO₂ powders is strong light scattering by dispersed silica, that complicates their interpretation.

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