

Effect of nanostructured gold surface on the SEIRA spectra of nucleic acid, Albumin, α -Glycine and Guanine

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This Paper is dedicated to Professor Georg Zundel on the occasion of his seventy fifth birthday

Influences of nanostructured gold and colloidal gold surfaces on spectra of DNA, albumin and some of their building blocks- α -glycine and guanine in the SEIRA (Surface Enhanced InfraRed Absorption) experiment in reflectance-absorbance mode are presented and discussed. Experimental factor of enhancement for studied samples within 0.1 mkg-0.5 mg (film thickness from 4 nm to 385 nm) was equal to 1.3-20 for different vibrations. A monolayer of albumin on gold substrate was clearly deposited. We have found that plated gold does not practically influence the conformational state of DNA and albumin in comparison with those on CaF₂ substrate. Shift in most of the bands on the gold does not exceed 2-3 cm⁻¹. In contrast, colloidal gold could change the conformation state of DNA, inducing the B to A conformation with elements of Z-form. Nanostructured gold surface influences the vibrational modes of guanine and α -glycine more than those for complex biopolymers. It seems to be connected with donor-acceptor properties of different groups to gold. © Anita Publications. All rights reserved.

1 Introduction

During the last decade spectroscopy of enhanced optical signals from molecules located or attached to metal surface or metal particles is effectively used for biological, electrochemical, surface, sensor and other applications¹⁻⁷. The enhancement of optical process by a factor of 10²...10¹¹ near rough metal surface (Au, Ag, Fe, etc.) has been already known for twenty years for both optical transitions in adsorbed molecules and the processes, which do not depend on the presence of molecules on metal surface⁴. These processes are surface-enhanced Raman scattering (SERS), surface enhanced infrared absorption (SEIRA), metal-enhanced fluorescence, second harmonics generation, etc. The effect consists in essential increase of the intensity of transition (e.g. the effective cross-section increases by a factor of 10⁵...10¹¹ for Raman scattering and 10...10⁴ for IR absorption and fluorescence) or efficiency of the process near metal surface. For the first time an enhancement of infrared signal from molecules chemisorbed on Au and Ag surface by a factor up to 10³ has been registered by Harstein and colleagues in 1980². However, this publication has passed practically insensible because of discovery of SERS with giant enhancement factor equal to 10⁶ at the same time³⁻⁵. Only in the beginning of the 1990's scientists came back to studying enhanced infrared effect. The main reason for this was the very low limit of molecules that could be detected by IR spectroscopy, The other advantages of this method, when compared to SERS, include its non-destructive behaviour, higher signal-noise ratio and less distortion of the spectra). In 1991 Osawa and Ikeda⁶ named the effect SEIRA by analogy with SERS. The explanation of the effect is not simple and includes several mechanisms, namely: i) the increase of the electromagnetic field near a rough metal surface or island metal films, or so called electrodynamic mechanism; ii) the increase of dipole transition moment of adsorbed molecules, or chemical mechanism^{1,3-5}. A great contribution in the theoretical explanation of surface enhanced effects was made by Kosobukin^{4,8}.

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However, along with doubtless SEIRA advantages (high sensitivity of the method, detection of monolayer film or less amount of substance up to $10 \text{ pg}^{1,9}$, a possibility for determination of orientation of the molecular groups, enhancement of intensity of some bands in $10\text{-}10^3$ times, much improved observation of the bands due to surface selection rules) the method has some disadvantages. One of them is metal surface influence on SEIRA spectra in comparison with conventional FTIR spectra. Indeed, high enhancement is achieved when absorbed molecules are situated closely to the metal surface^{1,6}. Usually these distances are about tens of angstroms or less. Naturally, the question arises how gold influences the structure of molecules, what type of interaction exists between adsorbed molecules and gold, whether SEIRA could be used in trace analysis. Here we present a study of gold surface influence upon vibration modes of DNA, albumin and several of their building blocks, α -glycine (Gly) and guanine (G) deposited on rough gold and colloidal gold surfaces.

2 Experimental

2.1 Materials

Gold thin layers for SEIRA experiments were obtained by vacuum deposition of 99.999 pure Au upon glass supports (TF-1 glass, 20x20 mm) via an intermediate adhesive Cr layer¹⁰. Before Au deposition, glass surface was cleaned by $\text{NH}_4\text{OH}:\text{H}_2\text{O}_2:\text{H}_2\text{O}$ and $\text{HCl}:\text{H}_2\text{O}_2:\text{H}_2\text{O}$ solution subsequently, both 1:2:2 by volume concentration during 5 minutes at boiling temperature. Then it was rinsed in double distilled water and dried in a flow of pure nitrogen. The gold was evaporated from molybdenum heater and deposited at a rate of $10\text{-}15 \text{ \AA} / \text{s}$ on room temperature substrate. The thickness of gold surface was within $150\text{-}250 \text{ \AA}$ in different experiments. The Cr interlayer does not exceed $10\text{-}15 \text{ \AA}$. Size of roughness for gold film was about 50 \AA (Fig.2).

Glycine (Gly), guanine (G) and sodium salt of calf-thymus DNA were obtained from Serva, bovine serum albumin (BSA) was obtained from Fluka and Sigma.

The samples for FTIR and SEIRA experiments were prepared by deposition of substances from $0.25\text{...}10 \text{ mg/ml}$ Gly aqueous solutions, 0.5 mg/ml G aqueous solution, 1 mg/ml DNA aqueous solution, $1\text{...}5 \text{ mg/ml}$ albumin aqueous solution on gold or CaF_2 substrates.

The films of Gly prepared through thermal vapor evaporation at the rate of $6.4 \text{ \AA} / \text{s}$ at pressure of about $5 \times 10^{-7} \text{ Torr}$ on gold/ SiO_2 and CaF_2 were also studied. The thicknesses of the films were measured by interferometry method.

Colloidal gold nanoparticles of $15\text{-}400 \text{ \AA}$ size were produced by reduction of Au from HAuCl_4 with citrate, concentration 12.1 mg/ml . Then they were mixed with sodium salt of calf-thymus DNA (highly polymerised) aqueous $5 \cdot 10^{-3} \text{ M}$ solution, kept in refrigerator for 24 hours, precipitated on gold substrate as well as on CaF_2 and dried lyophilically. We prepared the samples mixing 10 \mu l of colloidal gold per the 10 \mu l of DNA solution. As a reference the same DNA aqueous solution without colloidal gold was used. BSA aqueous solution of $1\text{...}5 \text{ mg/ml}$ concentration was mixed with colloidal gold, stirred and then precipitated on gold substrates.

2.2 FTIR and SEIRA spectroscopy

IR spectra were collected in the $380\text{-}5300 \text{ cm}^{-1}$ region with IFS-66 Bruker instrument in conventional transmittance mode for all substances (Gly, G, DNA, BSA) on CaF_2 substrate and in reflectance-absorbance (RA) mode on gold substrate (Fig.1). The reflectance attachment used in the experiment has the light incidence angle close to 16.5° ⁷. Spectra evaluation was performed with Opus 2.2 software. The position of the bands was estimated by the method of second derivative and/or standard method. Accuracy of frequency values in the spectra was equal to 0.5 cm^{-1} and of absorption values - 0.0005 a.u.

The experimental factors of enhancement were determined as a ratio of peak intensity of the bands for substances on gold surface in reflectance mode to those on CaF_2 substrate in conventional geometry. Calculated enhancement factors were determined as coefficients that are proportional to $g(\omega_r) \sim |\epsilon'|/\epsilon''$. According to Kosobukin⁴ the coefficient of enhancement is determined by the dielectric function of the metal. Based on the experimental data for our gold films¹¹ and supposition about close amount of dielectric constants for gold and nanostructured gold film, we calculated the coefficient proportional to enhancement factor. In further we will name it as factor of enhancement; however, they will differ from real factor of enhancement by constant. In general case the dielectric constants of nanostructured gold film could not be fitted by amount that is valid for gold. We used this simplification only for comparison data.

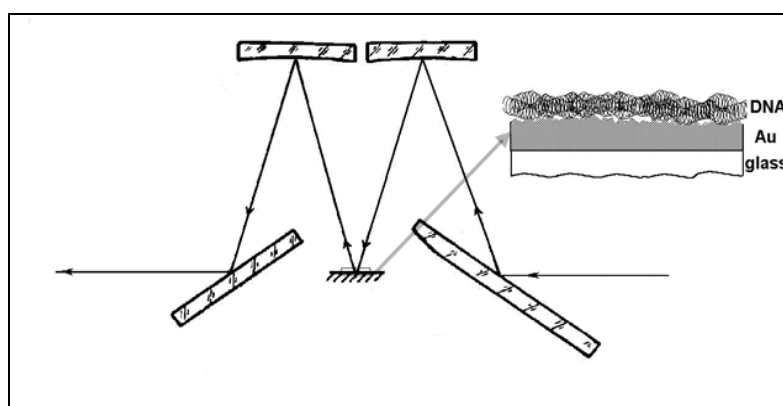


Fig.1. Scheme of SEIRA unit used in the experiment .

Principal component analysis was applied to SEIRA spectra of DNA. One principal component was chosen as the ratio of intensity of the phosphate asymmetrical band (at 1239 cm^{-1}) to the intensity of the maximum in the $3400\text{--}2300\text{ cm}^{-1}$ region, which is assigned to OH stretching vibration. According to M. Shie¹² this component multiplied by 5.25 characterizes the number of water molecules per nucleotide. The second principal component was the ratio of the intensities at 1712 cm^{-1} and 1700 cm^{-1} that is a characteristic of the conformation state of DNA relating to A, B or Z-form.

2.3 AFM imaging

The images of Au surface, used as a substrate for SEIRA (Fig.2 a, b), and of colloidal gold particles (Fig.3a, b), were obtained by atomic force microscopy (AFM). We used the tapping mode under AFM imaging with a commercial Nanoscope IIIa (Digital Instrument, Santa Barbara, CA). Tapping force mode scans were performed using commercially available AFM tips (silicon nitride). The scanning frequency was approximately 1 Hz in all experiments.

3 Results and Discussion

3.1 Theoretical background of the SEIRA effect

The interpretation of the effect of enhancement is presented in general form by Kosobukin⁸. The theoretical description of the effect is connected with the enhancement of the external electric field due to excitation of local (surface) plasmon vibrations at essential curvature of rough surface or on metal particles. In such systems, according to Kosobukin⁸, the electric field in the point of space r could be presented as:

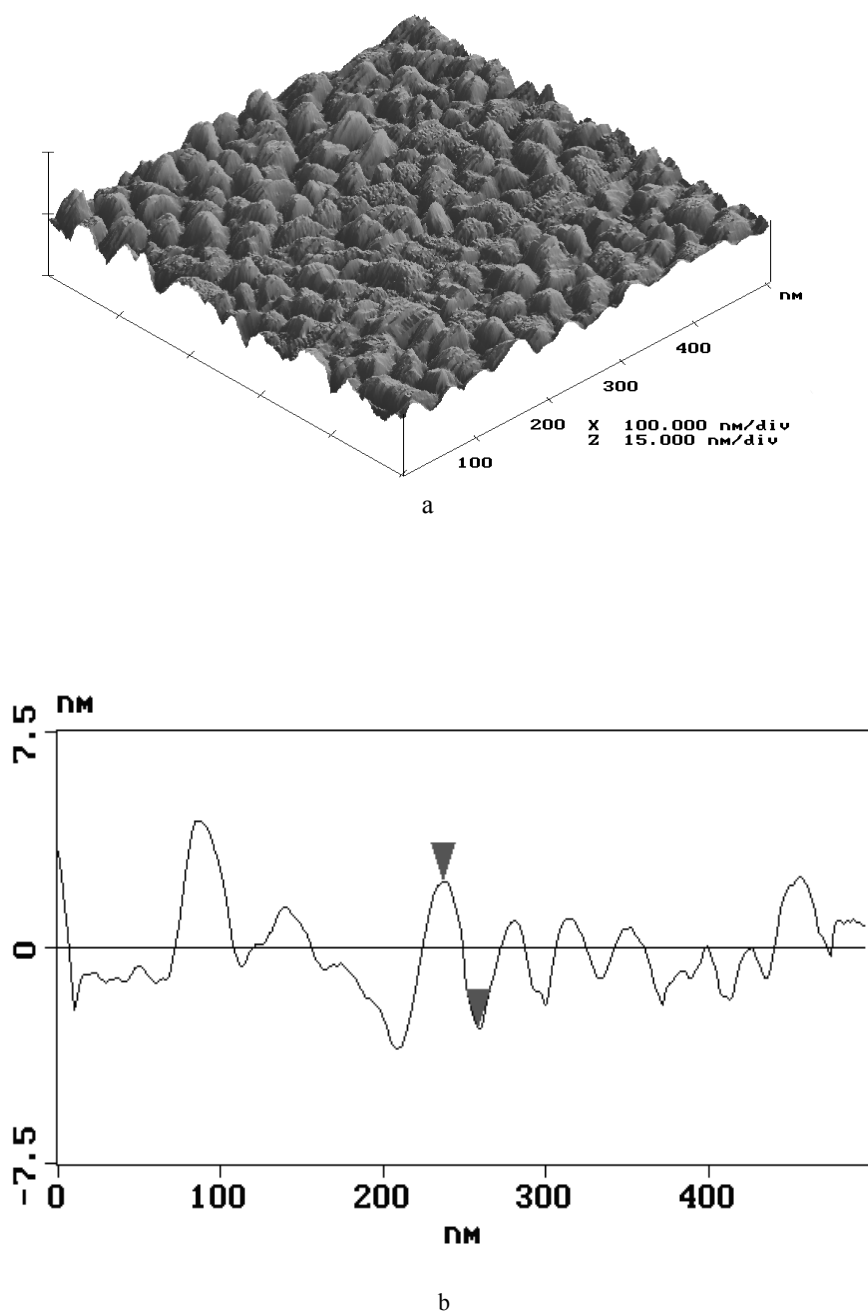


Fig. 2. a - AFM image of Au surface. Scale in X-direction is 100 nm/div and in Y-direction is 15 nm/div. Surface roughness is 3-7 nm; b - AFM-profile of Au surface after deposition.

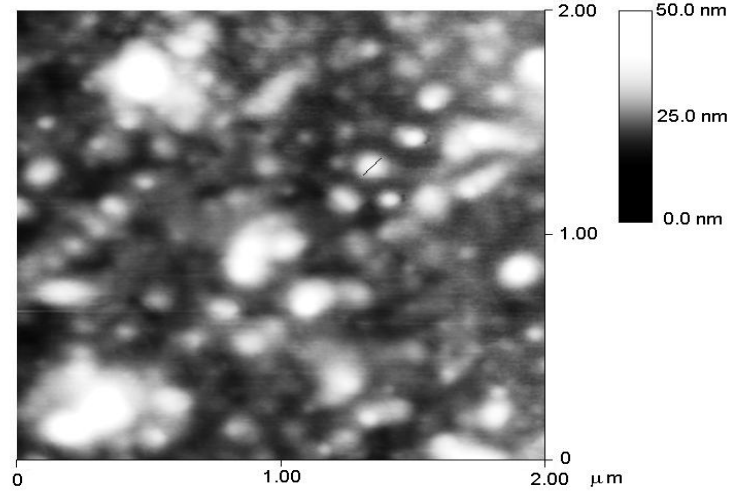


Fig. 3. 2-D AFM images of colloidal gold on Au/SiO₂ substrate.

$$E(r,t) = \exp(-i\omega t)[E_0(\omega) + E_1(r,\omega)], \quad (1)$$

Where ω - frequency, t - time. The additional term $E_1(r,\omega)$ is connected with the excitation of local (surface) plasmon vibrations and has a local character, i.e. decreases asymptotically. Then the enhancement of the external field could be presented as:

$$E_\alpha(r,\omega) = g_{\alpha\beta}(r,\omega)E_{0\beta}(\omega), \quad (2)$$

Where $g_{\alpha\beta}(r,\omega)$ - enhancement coefficient.

The effective cross-section of the process of light interaction with a molecule adsorbed on metal surface could be expressed as:

$$\sigma_\alpha(r,\omega) = \frac{16\pi^2\omega_{fi}}{cE_0^2} |\langle f|H(r,\omega)|i\rangle|^2 \delta[h(\omega-\omega_{fi})] \sim \sigma_\alpha^{(0)} |g(r,\omega)|^2 |h(r)|^2, \quad (3)$$

where $\sigma_\alpha^{(0)}$ - cross-section in the absence of metal ($d = d_0$, $E = E_0$); $i \rightarrow f$ - optical transition from i state (with the energy ε_i) to f state; $cE_0^2/8\pi$ - density of an incident energy flux, c - light velocity; $\langle f|H(r,\omega)|i\rangle$ - matrix elements of Hamiltonian of molecule interaction with a field; $h(r)$ - enhancement coefficient of dipole moment of adsorbed molecule.

The energy of incident photons induces optical transitions in adsorbed molecules and local plasmon vibrations in metal islands. It is known that in the islands the component of electric field vector, which is directed perpendicularly to the curvature of metal surface, possesses nonzero value at the distances of tens of nanometers beyond the metal surface frontier, i.e. in the regions of adsorbed molecules' location. In other words, plasmon

vibrations excite molecules adsorbed on metal surface that lead to their additional absorption. The frequencies of plasmon vibrations are determined by electron concentration, dielectric constants of environments and metal surface curvature. These frequencies are located in UV and visible spectral region. In IR region that corresponds to the wing of plasmon resonance the density of plasmon states is insignificant for non-modified surfaces, though their amount could be considerable. But in most cases the SEIRA enhancement does not exceed a factor of 100.

3.2 SEIRA spectroscopy of DNA on gold

Earlier we had concluded that gold substrate did not practically influence the vibration modes and macromolecular conformations of DNA^{7,13}. However, we found some minor distinctive features of DNA on gold substrate. We registered the factor of enhancement of 3-7 for different molecular groups; the mass of substance was equal to 1...10 µg. In this case the shift of many vibrations was around 2-3 cm⁻¹ for DNA on gold in comparison with DNA on CaF₂, shift of complex stretching OH-NH-CH band was 5-10 cm⁻¹ (Fig.4), conformation of the molecules in CaF₂ and DNA was the same and close to A-form. The last fact could be explained by the statement that the majority of the spectral markers for Na-DNA on the gold substrate are close to the markers of A-form¹³ for DNA of 65% humidity. The data presented for DNA on gold substrate are 1092 (1095 - in brackets data for A-DNA-form according to Schrader¹⁴), 1240 (1240), 1277 (1275), 1370 (1375), 1419 (1418), 1701 (1705) cm⁻¹. The same is valid for B-form of DNA of 95% humidity¹⁵ and principal component analysis gives a possibility to visualise it¹⁵.

Analysing the sugar region of DNA spectra, namely the region lower 1000 cm⁻¹, we must assume that any substrate can strongly influence the sugar conformation of DNA, even in the case when other structural components are not perturbed. In our case, we observed that the gold substrate induces conformational changes of the sugars' residuals if they come closely to gold surface. It is well-known that sugars in A-DNA have characteristic features at 805 cm⁻¹ and 860 cm⁻¹ as well as for B-DNA - at 832 cm⁻¹. The band observed at 834 and 832 cm⁻¹ for A and B-form DNA¹³, respectively, appears presumably due to reformation of intermolecular H-bonding (NH, OH and CH groups) near to the gold substrate¹⁶ as the result of DNA bending at gold peaks. It is also possible that water molecules take active part in this process. As usually we observed a band near 830 cm⁻¹ for A-DNA on BaF₂ substrate. Therefore, this feature is not due to gold substrate but for reformation of bonds near the interface.

A similar process was reported earlier by Gaigeot et al.¹⁷ in connection with non-coincidence of the data obtained with inelastic scattering of nucleic acid blocks, in solid state and calculations with density functional theory for the spectral region under 900 cm⁻¹. It has been shown that intermolecular H-bonds were formed in the film involving N₁-H and N₃-H groups of the bases, giving a strong band at the 830 cm⁻¹.

3.3 Effect of colloidal gold on DNA

Colloidal gold interacts with DNA changing DNA conformation. Drastic changes in vibration mode position, half-width and intensity in SEIRA spectra of DNA-colloidal gold in comparison with FTIR spectra of DNA have been observed (Fig. 5).

The greatest changes for DNA – colloidal gold system were observed for OH-NH-CH band, namely, an increase of the half-width of OH and NH stretching bands ca. 250 cm⁻¹ and their high frequency shift of about 100 cm⁻¹. We observed the following: a decrease in the intensity of the phosphate bands at 1104 cm⁻¹ (symmetric stretch) 2.4-fold and at 1240 cm⁻¹ (asymmetric stretch) 2-fold; a decrease in the intensity of the base bands at 1800-1550 cm⁻¹ (1.3-folds), an increase of half-width of phosphate bands about 35-40 cm⁻¹ for asymmetric phosphate; a high frequency shift of asymmetric phosphate from 1240 to 1244 cm⁻¹, appearance of shoulder at 1222-1215 cm⁻¹; a high frequency shift of symmetric phosphate from 1093 to 1104 cm⁻¹; and, a decrease of shoulder band of symmetric phosphate at 1050 – 1070 cm⁻¹. The DNA with colloidal gold does have features both A, B and Z-form. Indeed, canonical forms of DNA are present here also, especially B and Z-forms (930 cm⁻¹) and

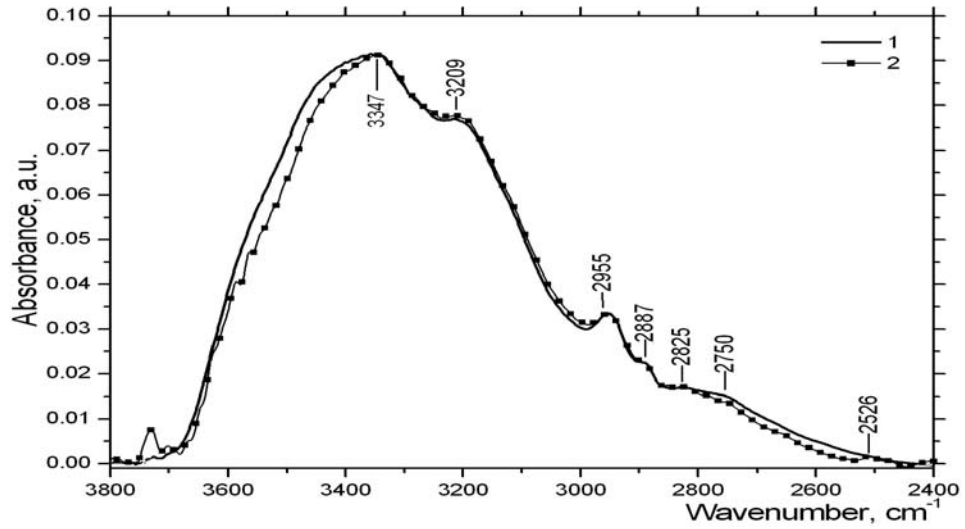


Fig. 4. SEIRA spectra of DNA on gold substrate in comparison with A-form of DNA on CaF_2 substrate in the $3800\text{-}2400\text{ cm}^{-1}$.

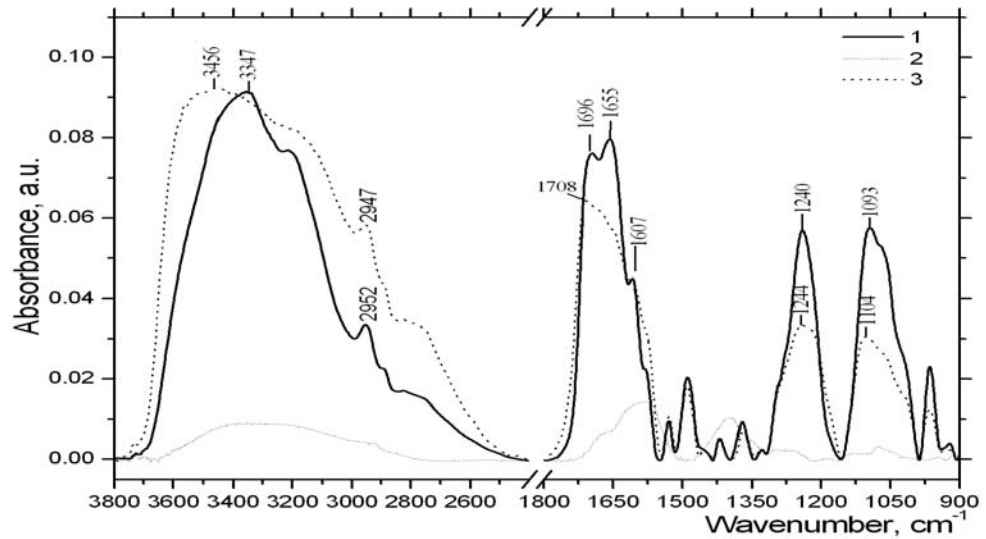


Fig.5. SEIRA spectra of reference DNA (1), colloidal gold (2) and DNA-colloidal gold system on Au substrate in the region of $3800\text{-}900\text{ cm}^{-1}$.

reduction of A-form (860 cm^{-1}). The intensity of the band at 960 cm^{-1} decreased about 1.5 to 2-fold. Principal component analysis was applied for the conversion of the different spectra in separate points. Such presentation (Fig.6), shows that the points corresponded to canonical DNA (A and B form) and DNA –colloidal gold have different region of localization in principal component plane. In this plane the DNA-colloidal gold is close to A-B-form with elements of Z-form, however, our experimental condition induces A-form.

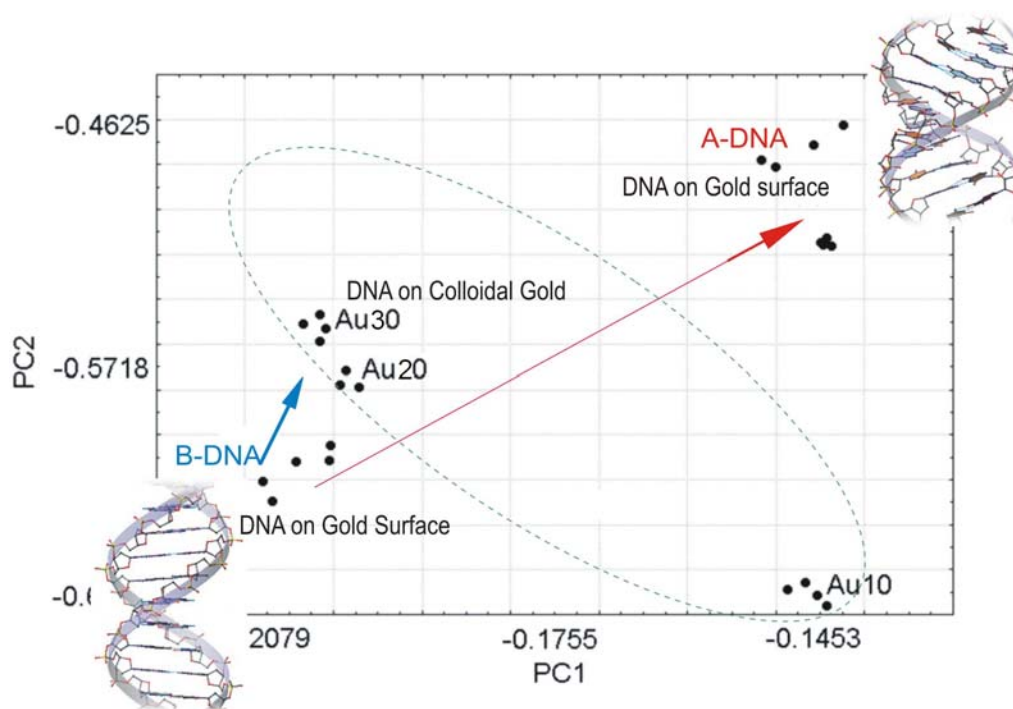


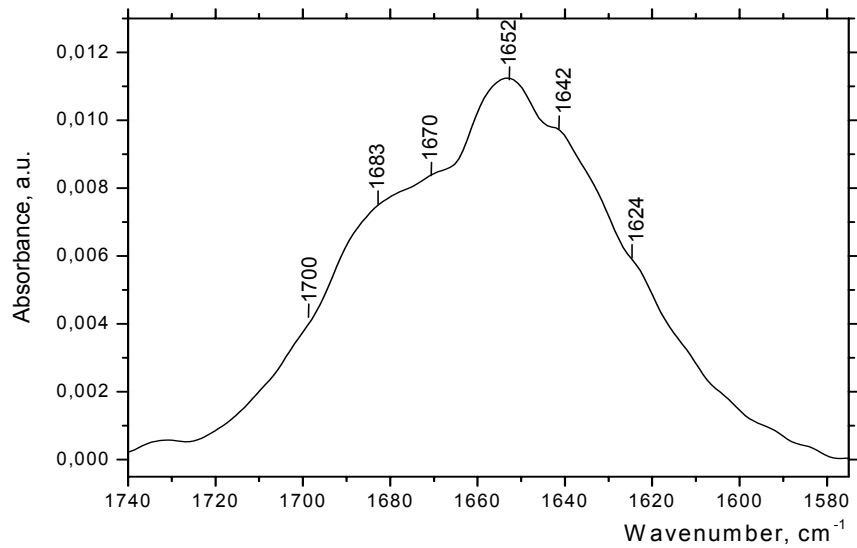
Fig.6. Principal component analysis of DNA of A, B DNA canonical forms assigned by A, B accordingly and DNA with colloidal gold of 10 μl , 20 ml and 30 ml per 10 ml DNA of $5 \cdot 10^{-3}$ M, assigned to Au10, Au20, Au30, accordingly.

3.4. BSA on gold and colloidal gold

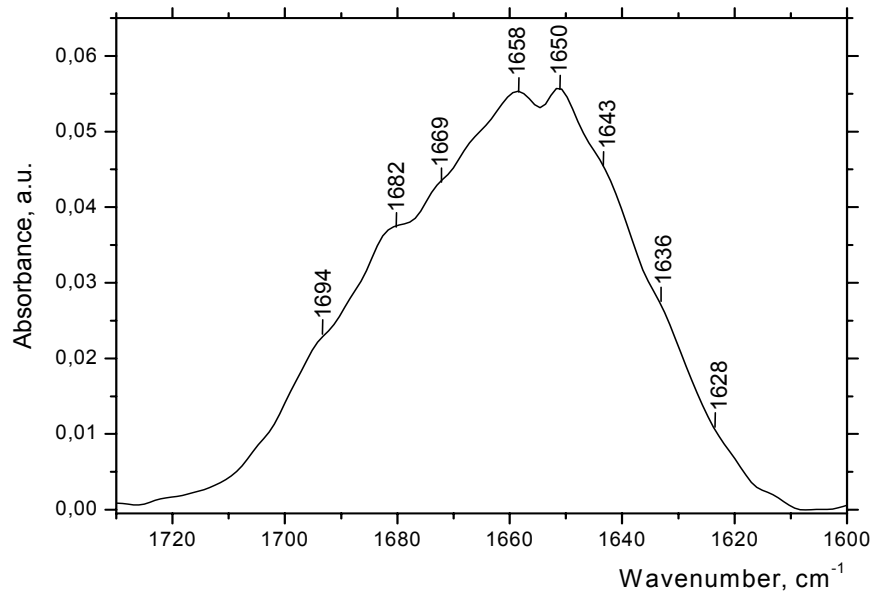
We have studied the conformational state of BSA on gold surface and on colloidal gold particles by SEIRA spectroscopy.

The SEIRA method has allowed deposition of a monolayer film of BSA on gold surface. The spectra of monolayer were compared with the spectra of thick BSA film on gold substrate. Some specific differences in the spectra of monolayer and thick film were observed. These changes are connected with the rearrangement of contributions of the absorption bands attributed to the various types of protein conformation, i.e. α -helix, β -sheet, unordered coil, turns (Fig. 7). Namely, the following positions of absorption bands were noted in the spectra of monolayer (in brackets the frequency for thick film is shown): α -helix - 1652 (1650, 1658); β -sheet - 1642 (1643),

1670 (1669, 1636); unordered coil – 1700 (1694), 1683 (1682); turns – 1624 (1626) cm^{-1} . It is impossible to obtain and register a monolayer film on CaF_2 substrate.



a



b

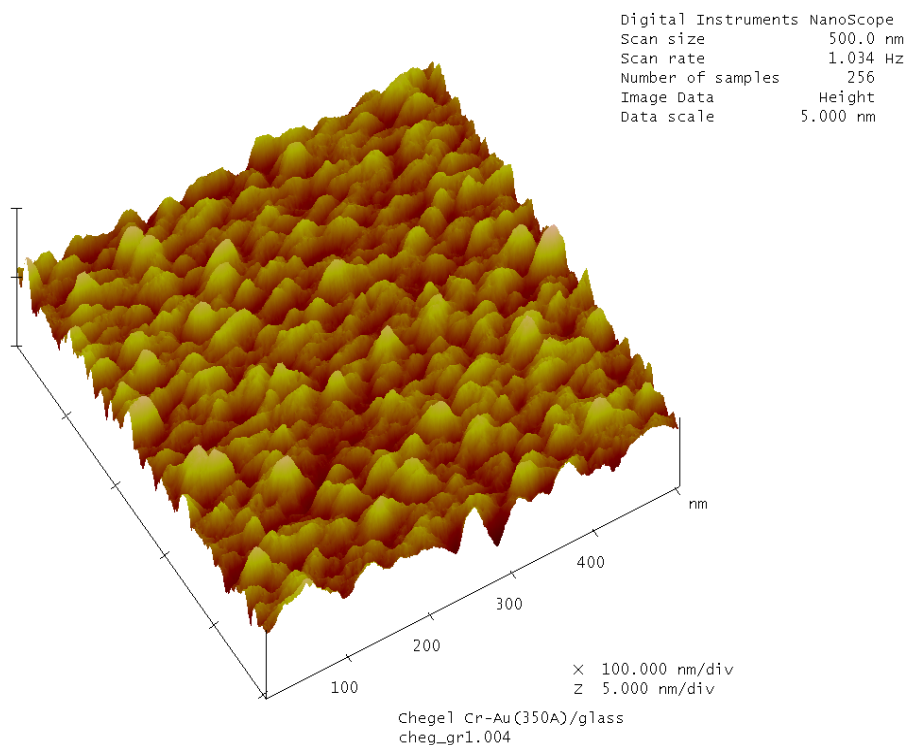
Fig.7. SEIRA spectra of BSA monolayer (a) and thick layer (b) in the region Amid I band (1750-1600 cm^{-1}).

The influence of colloidal gold on BSA has been also studied. In some cases SEIRA spectra of BSA-colloidal gold system showed an evident shift of Amid I band from 1651 to 1588 cm^{-1} that is caused by overlap of the spectra of protein and colloidal gold¹⁸. It should be taken into account in studies of BSA with colloidal gold.

3.5. SEIRA spectra of guanine on gold substrate

Surface metal structure and geometry influence the factor of enhancement. Thus, we have analyzed two surfaces of different geometry to study the surface influence on enhancement factor of guanine (Fig. 8). Glass plates with the sputtered gold layer with the thickness of 350 Å (substrate *a*) and 170 Å (substrate *b*) were used. The roughness of the surface *a* was arranged almost uniformly on the substrate surface and was close to 19 Å. Then substrate *a* was annealed at 250°C to obtain another surface geometry and size of heterogeneities and named as substrate *b*. This resulted in their non-uniform distribution on the surface of substrates and their aggregation into separate groups. The height of some aggregates reached 40 Å, though the average roughness of substrates *b* was close to 10 Å. The annealing also caused the decrease of the average radius of the horizontal cross-section of the roughness and of their quantity in comparison with the substrate *a*.

To study the surface geometry influence on the enhancement effect, the samples of aqueous solution of guanine ($\text{C}_5\text{H}_5\text{ON}_5$) with the concentration of 0.5 g/l were deposited on the substrates *a*, *b* and on CaF_2 and then dried lyophilically. The samples were deposited in such a way to obtain the layers of the same size and thickness. When comparing the surface geometry influence, it was ascertained that the substrate *a* causes a greater enhancement than the substrate *b* does (Fig. 9). This fact could be explained by gold roughness influence on factor of enhancement. Indeed, more roughness and more homogeneity of gold surface (Fig. 8a) cause to increase of factor of enhancement (Fig. 9) in comparison with substrate *b* (2 time less roughness and more heterogeneity).



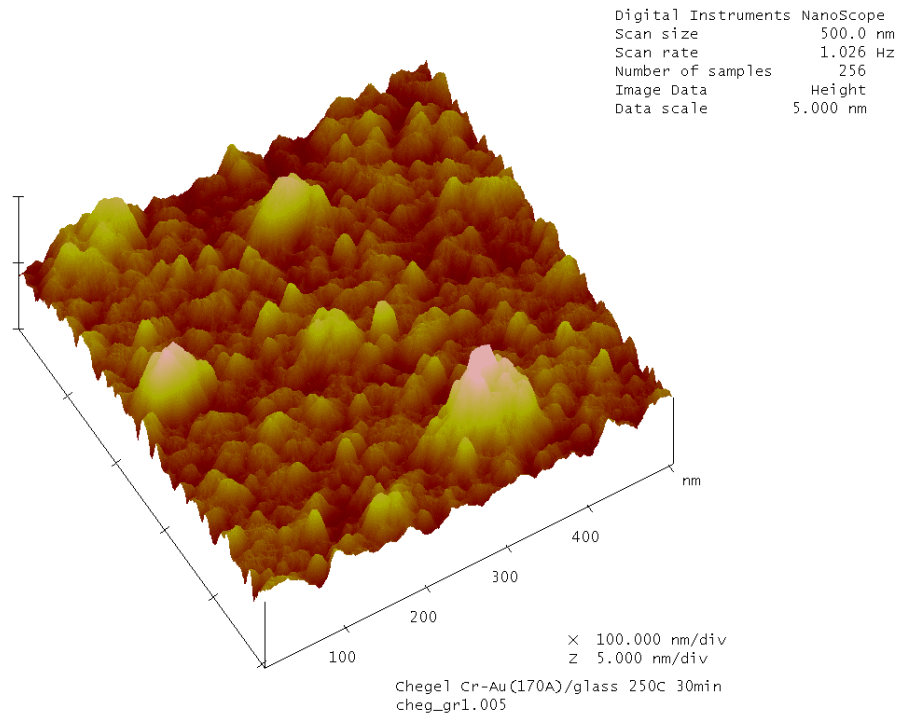


Fig. 8. AFM images of gold surface in SEIRA experiment (a) and after annealing under 250 °C (b).

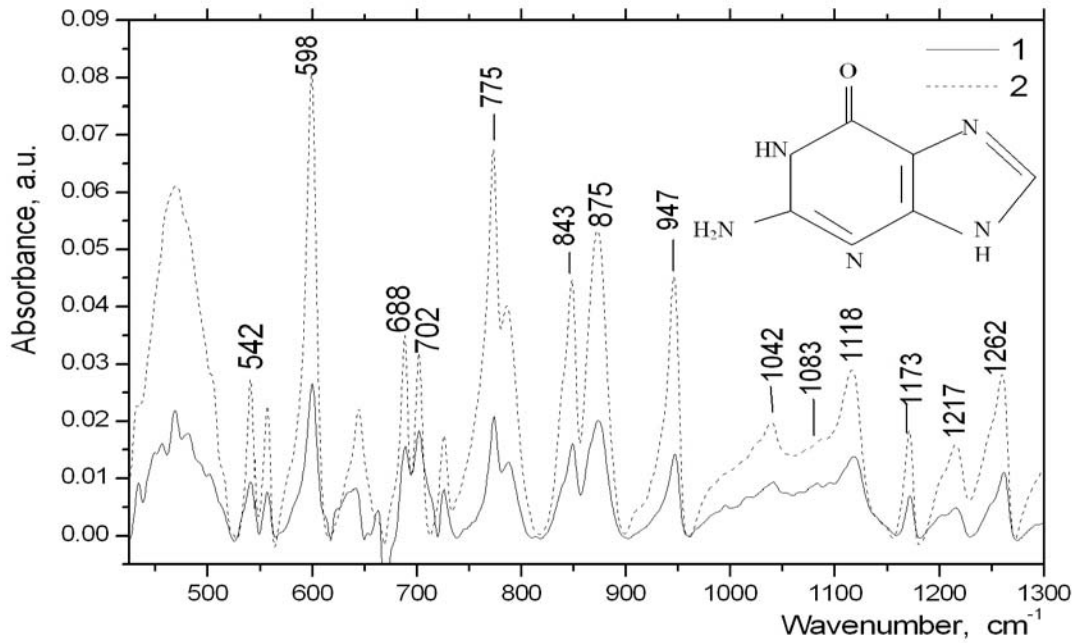


Fig. 9. SEIRA spectra of guanine on gold substrate-b (1) and gold substrate-a (2).

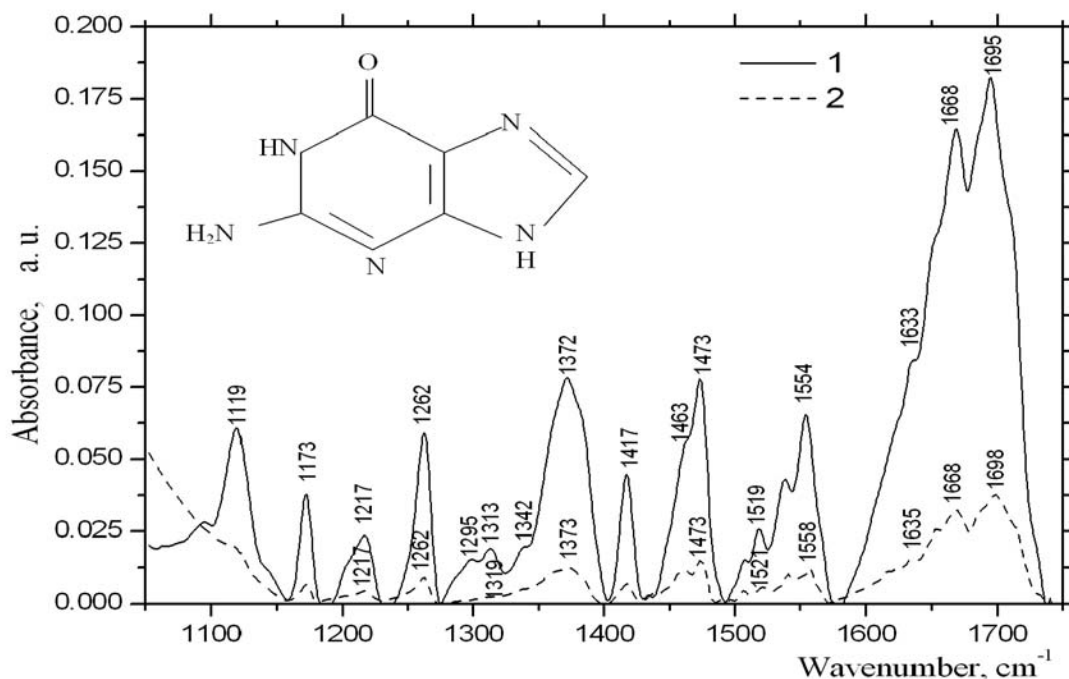


Fig.10. SEIRA spectra of guanine on gold substrate-a (1), on CaF_2 (2)

In Table 1 we present the experimental and calculated factor of enhancement for various guanine molecular groups adsorbed onto gold substrate and CaF_2 (Fig. 10). The experimental frequencies of guanine from our experiment and calculated by Pelmenchikov et al.¹⁹ are also summarized in Table 1. The most enhancement is observed for ring vibrations- CNH and NCH. We suppose that the plane of ring of guanine is perpendicular to plane of gold or inclined at small angle to normal to gold surface.

Table 1. The enhancement factor for guanine in comparison with calculation data.

Guanine on CaF_2 , band position, cm^{-1}	Guanine on Au, band position, cm^{-1}	Factor of enhancement		Assignment ^{17,19}
		Experiment g^2	Calculation g^2	
1698	1695	4.9	10.9	def NH, strCN
1635	1633	5.0	10.2	def NH, strCN
1558	1554	5.4	9.2	strCN
1473	1473	5.6	8.3	strCN, def CNH
1462	1463	4.6	8.2	strCN, str CC
1417	1417	6.2	7.7	def CNH, str CN
1373	1372	6.0	7.2	str CN, str CC, def CNH
1342	1342	3.1	6.9	def NCH
1319	1313	7.3	6.6	def NCH
1118	1119	3.1	4.8	def CNH, str CN

3.4 Gold surface influences the vibration modes of α -Gly

We have studied vibration modes of α -Gly deposited from aqueous solution on nanostructured rough gold surface (Fig.11) and thermal evaporated on the same surface (Fig.12).

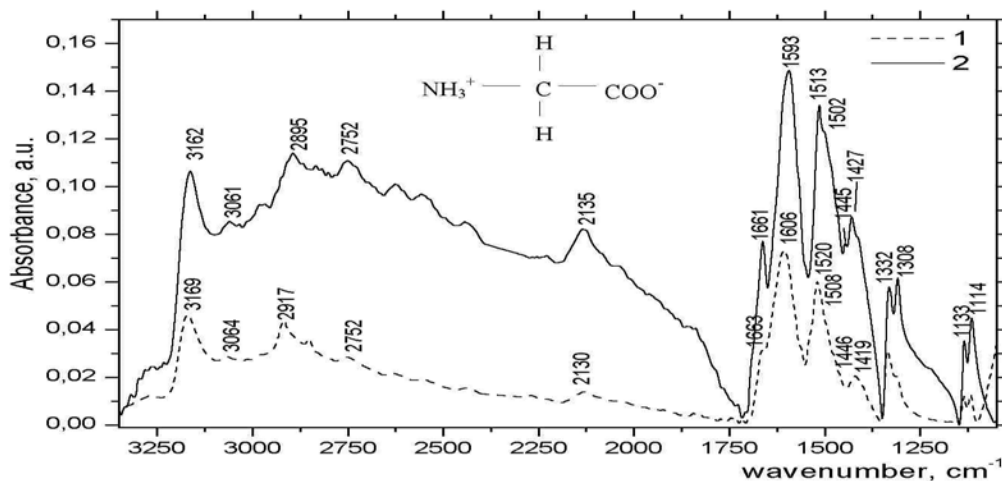


Fig. 11. SEIRA spectra of α -Gly: 1 — on CaF_2 , 2 — on gold substrate. The samples were deposited on substrates by drop of 200 μl from 2.5 mg/ml Gly aqueous solution.

Table 2. The experimental enhancement factor for α -Gly in comparison with calculation data.

Glycine adsorbed on CaF_2 , (cm^{-1})	Glycine adsorbed on Au, (cm^{-1})	Factor of enhancement		Glycine evaporated on gold (cm^{-1})	Factor of enhancement Experiment g^2 Assignment		Assignment ²⁰
		Exp. g^2	Cal. g^2		Exp. g^2	Cal. g^2	
3169	3162	2.3	36.0	3183	10.2	37.2	NH sym str.
3064	3061	2.9	34.8	2982	6.6	33.2	CH str
2752	2752	3.9	28.1	2750	4.8	28.1	NH sym str.
2917	2895	2.6	31.4	2914	7.1	31.4	CH str
2130	2135	5.9	16.8	2134	1.7	17.6	Second order tones
1663	1661	2.5	10.2	1661	2.0	10.24	NH_3^+ as def
1606	1593	2.1	9.6	1585	7.9	9.61	COO^- as str
1520	1513	2.2	9.0	1499	12.6	8.41	NH_3^+ sym def
1419	1427	4.1	7.3	1409	5.7	7.8	COO^- sym str
1446	1445	4.5	7.8	1446	2.4	7.7	CH def sym
1335	1332	1.8	6.8	1333	11.7	6.8	CH_2 def, NH bend
1310	1308	1.9	6.3	1312	1.6	6.7	CH_2 def, NH bend
1134	1133	3	4.8	1134	3.2	4.8	CH_2 bend, NH_3^+ rock
1116	1114	3.6	4.4	1117	3.0	4.4	CCN bend

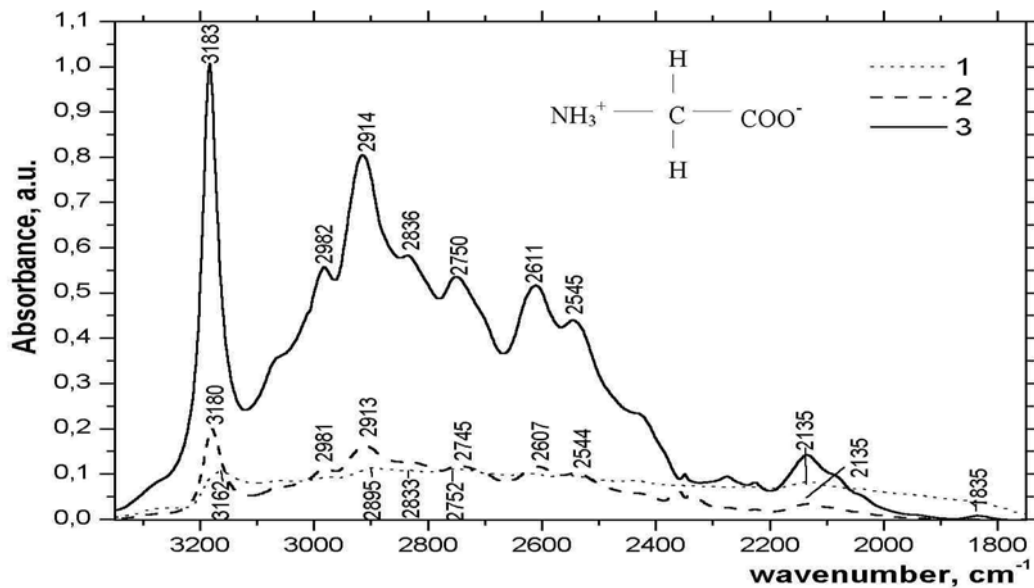


Fig. 12 a. SEIRA spectra α -Gly films of 385 nm thickness obtained by deposition from solution on gold/SiO₂ substrate (1) and thermal evaporation on gold/SiO₂ (2) and CaF₂ (3) substrates in the region of 3300-1750 cm^{-1} .

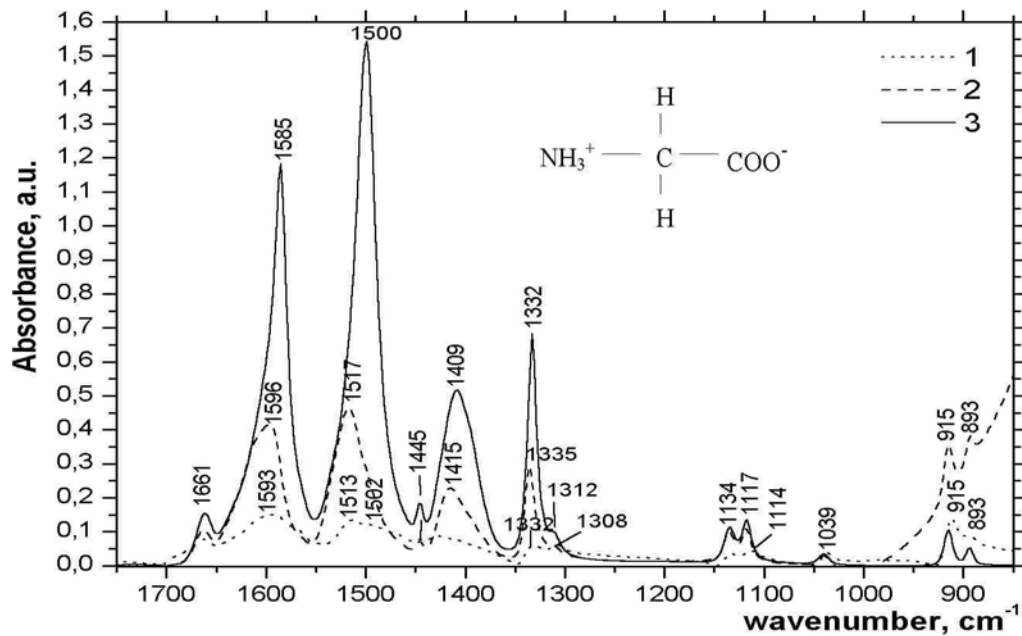


Fig. 12 b. SEIRA spectra α -Gly films from of 385 nm thickness obtained by deposition from solution on gold/SiO₂ substrate (1) and thermal evaporation on gold/SiO₂ (2) and CaF₂ (3) substrates in the region of 1750-800 cm^{-1} .

In the case of zwitterionic form of Gly at pH 6 low-frequency shifts of H-bound stretching NH_3^+ (from 3169 to 3162 cm^{-1}), asymmetric COO^- (from 1606 to 1593 cm^{-1}), symmetric COO^- (from 1427 to 1419 cm^{-1}) vibrations take place as well as a high-frequency shift of deformation NH_3^+ vibration from 1661 to 1663 cm^{-1} . We suppose that in this case gold shows both donor and acceptor properties for NH_3^+ and COO^- moieties, respectively. If it is so, the Gly molecule should be turned to the gold by both moieties. Due to the fact that they are located very close to each other, namely 2-3 Å, their mutual influences could be compensated. We suggest that this is a reason for small enhancement in the spectra of Gly deposited from solution on gold in different experiments. Only under vacuum deposition could we induce certain orientation of Gly molecule on gold surface and reach more enhancement of vibrations in the SEIRA experiment. Interestingly, thermally deposited Gly shows more enhancement in the 3300-1300 cm^{-1} region in comparison with the drop deposited Gly. However, the vibrations in the region of 500-1300 cm^{-1} region are more enhanced for drop deposited Gly in all cases. It is possible to conclude that intermolecular interaction increases in Gly drop deposited and that Gly on gold shows different molecular orientation and as well as conformation in comparison with those on CaF_2 .

4 Conclusions

Gold surface influences the SEIRA spectra of simple biological molecules more than those for complex biological molecules as DNA and BSA. A monolayer of BSA on gold substrate was registered. Gold substrate practically does not influence the conformational state of DNA and albumin. However, colloidal gold could cause the change in conformational state of DNA. We propose that more enhancement influence on the SEIRA spectra of simple biological molecules are connected with close distance of this molecules to gold peaks and donor-acceptor interaction of molecular groups (NH_3^+ , COO^- , CNH) with gold surface.

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