
**ENHANCEMENT FEATURES OF INFRA-RED
ABSORPTION BY α -GLY MOLECULES
IN THE SEIRA EFFECT**

O.M. FESENKO

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Institute of Physics, Nat. Acad. of Sci. of Ukraine
(46, Nauky Ave., Kyiv 03028, Ukraine; e-mail: fesenko@iop.kiev.ua)

Molecules of α -Gly are used as an example to demonstrate that the surface enhanced infrared absorption (SEIRA) effect is different for different molecular groups, with the enhancement being maximal for charged groups and groups with an unshared electron pair. The enhancement factor of IR absorption for multilayered films of α -Gly molecules deposited onto a gold substrate decreases, as the number of molecular layers increases, and can become 2 to 7 times lower for various molecular groups. It can be made 3 to 12 times higher for films obtained by the thermal sputtering of α -Gly molecules in vacuum onto a gold surface in comparison with the films deposited from an aqueous solution. If α -Gly and gold are sputtered simultaneously, a better resolution of the IR absorption bands of glycine is observed. It is shown that a decrease of the solution pH index to 2 gives rise to an increase of the enhancement factor of IR absorption by α -Gly by an order of magnitude, which allowed us to register overtones in thin films of glycine (250–275 nm in thickness).

1. Introduction

Effects of the intensification of optical processes in a vicinity of the nano-structured metallic surface are actively studied today for the optical transitions in adsorbed molecules (Raman scattering (RS) of light, luminescence, infra-red (IR) absorption) [1–3], as well as for processes independent of the presence of molecules on the metal surface (for example, the second harmonic generation) [4, 5]. The effect consists in a considerable enhancement of the efficiency of a process near a metallic surface. For instance, the effective cross-section of the surface enhanced Raman scattering (SERS) becomes 10^4 to 10^6 times larger [6–9], whereas the surface enhanced infrared absorption (SEIRA) by adsorbed molecules increases by a factor of 10 to 10^3 [1–3, 10–

12]. The interpretations of SEIRA and SERS effects are analogous. Both effects are based on two mechanisms. The *electromagnetic* mechanism consists in the enhancement of an external electromagnetic field in a vicinity of the rough metal surface owing to the interaction with surface or local plasmons [4, 13–24]. The *molecular* mechanism is associated with the growth of transition dipole moments and a modification of the polarizability of adsorbed molecules near the metal surface [15–18, 21, 22, 24–28]. The energy of incident photons excites optical transitions in adsorbed molecules, as well as surface (local) plasmon oscillations in islands or roughnesses on the metal surface. Plasmons are collective oscillations of the electron density. They are resonances, the frequency of which is governed by the surface shape, the free electron concentration, and the dielectric permittivity of the environment. Proceeding from the frequency dependence of the dielectric function for metals and considering the conditions, under which the enhancement is observed, it is possible to say about the nonzero electron density of states in the IR region, the excitation of those states with light, and the appearance of an additional local field. The energy is transferred from plasmon oscillations to adsorbed molecules, which gives rise to an enhancement of the absorption by the latter.

A theoretical interpretation of the enhancement effect was developed by V. Kosobukin in the general form. The effective cross-section for the process of interaction between light and a molecule adsorbed on a metal surface can be written down as follows [4, 19, 20]:

$$\sigma_{\alpha}(r, \omega) \sim \sigma_{\alpha}^{(0)} g(r, \omega)^2 h(r)^2, \quad (1)$$

where $\sigma_{\alpha}^{(0)}$ is the cross-section for a free molecule in the absence of a metal ($E = E_0$), $g(r, \omega)$ is the coefficient of electric field enhancement, and $h(r)$ is the coefficient of dipole moment enhancement for an adsorbed molecule. From the analysis of formula (1), one can see that the enhancement in the SEIRA effect has the electromagnetic, $g(r, \omega)$, and molecular, $h(r)$, components.

While studying the SEIRA effect, molecules of par-nitrobenzoic acid, which can be chemically adsorbed on a metal surface, are mainly used. All researches dealing with these molecules are concentrated on studying the SEIRA effect in the transmission or the attenuated total internal reflection geometry. The following question arises: Can an enhancement be reached for biological molecules, which are physically adsorbed on a rough metallic surface? Which factors influence the SEIRA effect? That is why the study of the SEIRA effect in this work was carried out with the use of glycine (α -Gly) molecules which are physically adsorbed on a rough gold surface. The glycine molecule was also selected, because it can be in either a monoionic or zwitterionic configuration (the latter being characterized by a larger electrical dipole moment) depending on the pH index. By changing the pH index of the solution, it is possible to modify the structure of Gly molecule and study the influence of the type of molecular groups on the amplification of the SEIRA effect.

In this work, the simultaneous thermal deposition of Gly molecules and gold atoms is applied for the first time, and the influence of the deposition technique and the thickness of a Gly molecular film adsorbed on the gold substrate on the SEIRA effect is studied. Such researches are important for the verification of available theories concerning the SEIRA effect, as well as for its further practical application in IR spectroscopy, biology, and medicine.

2. Materials and Methods

We used an α -Gly powder from SERVA, without its additional purification. Substrates for SEIRA experiments were fabricated by sputtering 99.999-% pure gold in vacuum onto the glass substrate (glass TF-1) $20 \times 20 \text{ mm}^2$ in size. An intermediate layer of chrome was preliminarily deposited to ensure the adhesion between gold and the glass surface. Before the sputtering of gold, the glass surface was cleaned by the solutions $\text{NH}_4\text{OH}:\text{H}_2\text{O}_2:\text{H}_2\text{O}$ and $\text{HCl}:\text{H}_2\text{O}_2:\text{H}_2\text{O}$ (both with the volume concentration ratio 1:2:2) for 5 min at a boiling temperature. Afterward, the surface was washed out in bidistilled water and dried up in the pure nitrogen environment. Gold

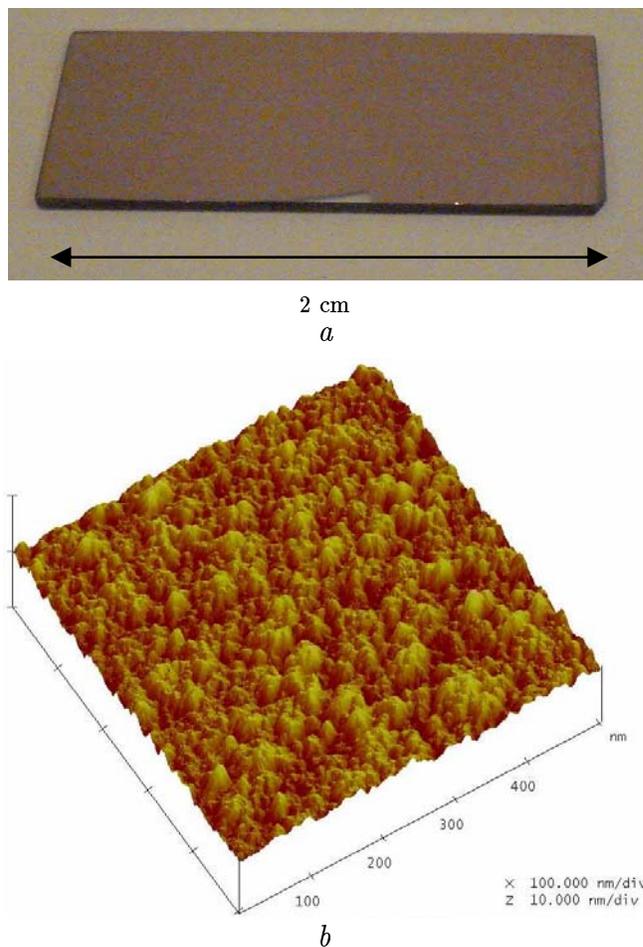


Fig. 1. (a) Appearance of the substrate with a gold-covered surface. (b) Gold surface image reconstructed using the data of atomic force microscopy

was deposited at a rate of 1–1.5 nm/s at room temperature. In the course of the deposition of gold and chrome, the thicknesses of corresponding films were monitored with the help of a quartz resonator. The film thickness was characterized in terms of the so-called “mass” thickness, which is equally suitable for both continuous and island films. The thickness of the intermediate chrome layer did not exceed 50 \AA . The thickness of the gold film was 250 \AA in our experiments. The appearance and the image of the gold surface reconstructed with the use of an atomic-force microscope are exhibited in Fig. 1.

The substrate with a rough gold surface was partitioned into two parts. One part of the substrate was used for the deposition of α -Gly molecules from an aqueous solution 1 mg/ml in concentration (a 200- μl droplet was used for this purpose); then, it was dried up lyophilically. The α -Gly powder was thermally sputtered in

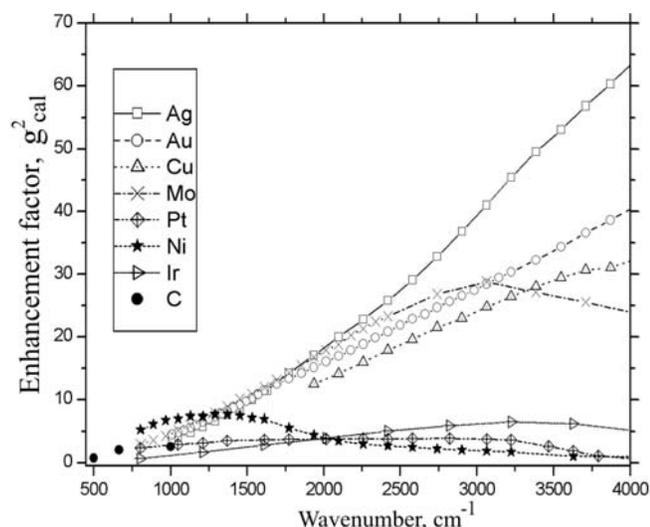


Fig. 2. Estimations of the factor of electric field enhancement near surfaces of various metals and carbon

vacuum onto the other part of the substrate at a rate of 1–1.5 nm/s and at a temperature of 250–255 °C. The effective thickness of the glycine film was measured with an atomic force microscope and an MII-4 Linnik microinterferometer. It did not exceed 385 ± 5 nm in both the deposited and adsorbed specimens.

The SEIRA effect was studied with the help of a serial Bruker IFS-66 Fourier-transform spectrometer (Germany) in the range from 400 to 4000 cm^{-1} and in the reflection and transmission geometries. The spectra were treated taking advantage of the Opus-5.5 (in the Bruker IFS-66 complex) and OMNIC software programs.

3. Results and Their Discussion

Using α -Gly molecules as an example, the manifestation of two basic mechanisms—namely, *electromagnetic* and *molecular* ones—resulting in the enhancement of SEIRA effect has been experimentally studied.

The enhancement of an electromagnetic field depends on the shape and the dimensions of metal film inhomogeneities, as well as on the dielectric properties of a metal and the environment. However, it is mainly determined by the dielectric permittivity of the metal ε : $g(\omega) \sim |\varepsilon'|/\varepsilon''$. Therefore, the coefficient of electromagnetic field enhancement in the SEIRA effect was calculated as a ratio between the real and imaginary parts of the dielectric permittivity of a metal at the corresponding frequency, $g_{\text{cal}} = |\varepsilon'(\omega)|/\varepsilon''(\omega)$, using the experimental values for the optical constants n and κ in the metal. Earlier [29, 30], we calculated the coefficients of electric

field enhancement for the following metals: Ag, Au, Cu, Mo, Pt, Ni, and Ir (Fig. 2). The results of calculations showed that, among them, Au-, Ag-, and Cu-surfaces are the most effective enhancing surfaces in the IR range. In addition, gold is a chemically inert element, which determined its ultimate choice for our further researches.

The experimental coefficient of IR absorption enhancement in the SEIRA effect was determined as a ratio between the integrated intensity of the absorption band of a corresponding molecular group on the metal surface to the intensity of the absorption band of this molecular group on a neutral substrate, CaF_2 : $g_{\text{exp}} = I_m(\omega)/I_{\text{CaF}_2}(\omega)$.

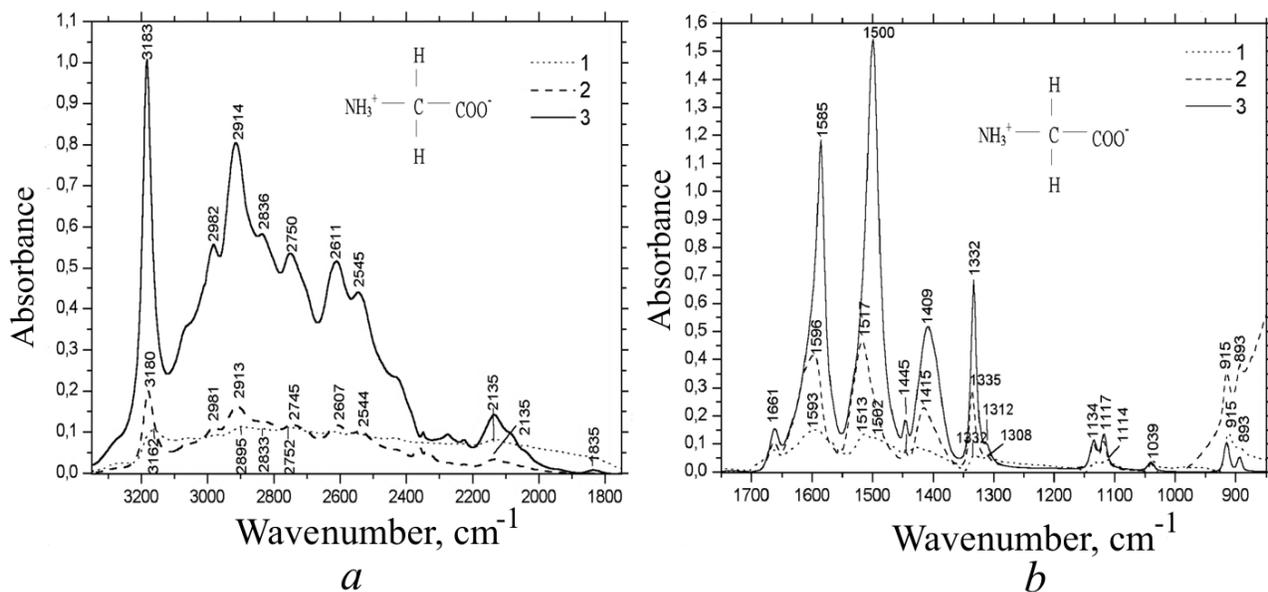
First, the influence of the technique used for the deposition of α -Gly molecules onto gold on the SEIRA effect magnitude was examined. For this purpose, we compared the IR absorptions for films of α -Gly molecules deposited from the aqueous solution and thermally sputtered in vacuum on CaF_2 and gold surfaces with a nanoroughness of about 13 nm. Having compared the spectra of the IR absorption by α -Gly molecules deposited from the solution and thermally sputtered, one may assert that glycine molecules do not decay at the thermal sputtering (Fig. 3).

In the case of the zwitterionic configuration of α -Gly molecules deposited from the solution onto the specimen surface, the IR absorption spectrum demonstrated the low-frequency shifts of the valence NH_3^+ (from 3169 to 3162 cm^{-1}) and asymmetric COO^- (from 1606 to 1593 cm^{-1}) vibrations and the high-frequency shifts of deformation NH_3^+ (from 1661 to 1663 cm^{-1}) and symmetric COO^- (from 1419 to 1427 cm^{-1}) vibrations (see Table). The assumption can be made that, in this case, gold affects the donor-acceptor properties of NH_3^+ and COO^- molecular groups of glycine. The neighbor α -Gly molecules in a film are known to be bound by means of intermolecular H-bonds of the N–H...O type, where the distance between two electronegative atoms is more than 3 Å [32, 33]. It is also known that an intermediate layer of water molecules from the solution is formed between gold and α -Gly molecules. Therefore, for specimens deposited from the solution, the interaction between α -Gly molecules in the film is higher than that between α -Gly molecules and gold. It can be a reason for a weak enhancement of the infra-red absorption for α -Gly molecules deposited from the solution onto a rough gold surface.

In the course of the thermal sputtering in vacuum, α -Gly molecules were managed to be oriented to some extent on the gold surface (the results of our quantum-chemical calculations showed that the distance between

Enhancement of the IR absorption coefficient for α -Gly molecules deposited by various techniques

α -Gly adsorbed on CaF_2 , frequency, cm^{-1}	α -Gly adsorbed on Au, frequency, cm^{-1}	Enhancement factor		α -Gly sputtered on Au, frequency, cm^{-1}	Enhancement factor		Vibration type
		Exp.	Cal.		Exp.	Cal.	
3169	3162	2.3	36.0	3183	10.2	37.2	NH sym. val.
2917	2895	2.6	31.4	2914	7.1	31.4	CH val.
2752	2752	3.9	28.1	2750	4.8	28.1	NH sym. val.
2130	2135	5.9	16.8	2135	1.7	17.6	2-nd order band
1663	1661	2.5	10.2	1661	2.0	10.3	NH_3^+ asym. def.
1606	1593	2.1	9.6	1585	7.9	9.6	COO^- sym. val.
1520	1513	2.2	9.0	1500	12.6	8.4	NH_3^+ sym. def.
1446	1445	4.5	7.8	1445	2.4	7.7	CH def.
1419	1427	4.1	7.3	1409	5.7	7.8	COO^- sym. val.
1335	1332	1.8	6.8	1332	11.7	6.8	NH def., CH_2 def.
1134	1133	3	4.8	1134	3.2	4.8	CH_2 def., NH_3^+ def.
1116	1114	3.6	4.4	1117	3.0	4.4	CCN def.


 Fig. 3. IR absorption spectra of α -Gly films deposited from the solution onto an Au/SiO_2 substrate (1) and thermally sputtered in vacuum onto CaF_2 (2) and Au/SiO_2 (3) substrates measured in the $3300\text{--}1800\text{ cm}^{-1}$ (a) and $1800\text{--}800\text{ cm}^{-1}$ spectral ranges (b) [31]

the nearest glycine and gold atoms in vacuum amounts to $2\text{--}2.5\text{ \AA}$ [34, 35]), which resulted in that the absorption can be enhanced by a factor of 2 to 13 (Table). While analyzing the data presented in Table for the experimental enhancement factor, a conclusion can be drawn that the absorption of different molecular groups is enhanced differently, whereas the theoretical value of enhancement factor monotonously increases with the light frequency. This fact testifies that, besides the electromagnetic mechanism, there exists another one, which depends on the type of molecular groups and their spatial arrangement with respect to the nano-structured gold

surface. After the thermal sputtering, the absorption of α -Gly molecules in the range $3300\text{--}1300\text{ cm}^{-1}$ increases, although the same molecules, but deposited from the solution, demonstrate a growth of the IR absorption in the range $1300\text{--}500\text{ cm}^{-1}$.

In this work, the influence of the thickness of a film of molecules adsorbed onto gold on the SEIRA effect was also studied. To make the enhancement stronger, molecules must be arranged as closer to the metal surface as possible. This was done by sputtering α -Gly and gold almost simultaneously on a neutral CaF_2 substrate. A glycine powder was thermally sputtered in

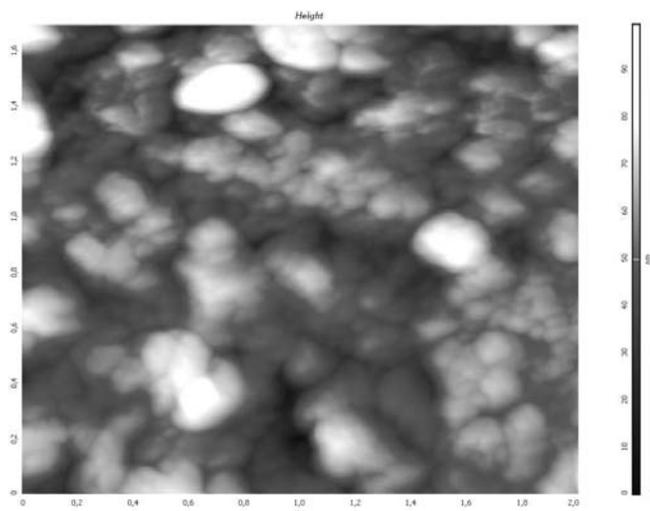
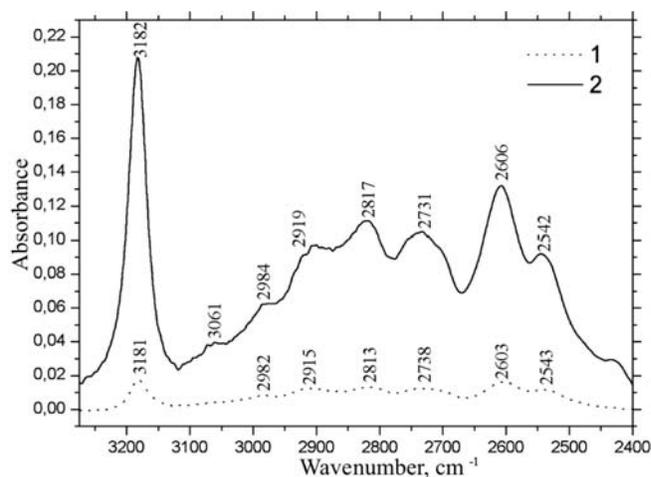


Fig. 4. AFM image of an α -Gly film sputtered onto nanostructured Au

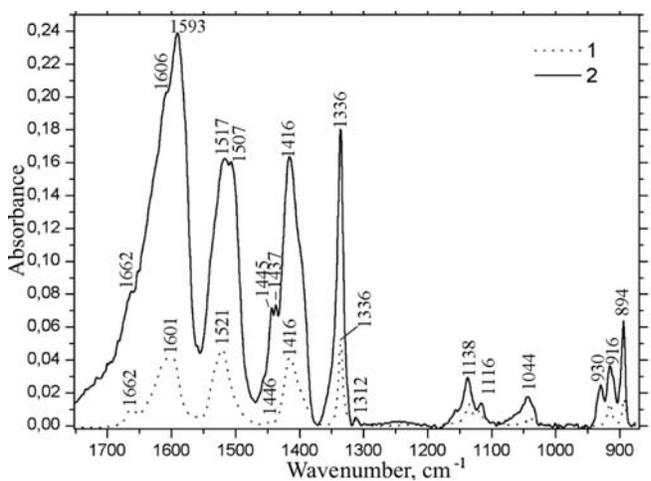
a vacuum installation from a copper furnace simultaneously onto two CaF_2 substrates separated by a glass plate. Under one of the CaF_2 substrates, a tungsten thread with some amount of gold was arranged. The glass plate separating the substrates was so fixed that gold arrived at only one of the CaF_2 substrates. As a result, we obtained two specimens with identical amounts of sputtered α -Gly on gold. The difference consisted in that one of the glycine films contained nanostructures of gold. From the analysis of the AFM image obtained for this glycine film on nano-structured gold (Fig. 4), it becomes evident that the film has a granular structure. The granular size varied from 10 to 100 nm.

The simultaneous sputtering gave rise to a better resolution of α -Gly absorption bands. In particular, the band at 1437 cm^{-1} , which is observed for α -Gly molecules in the gaseous phase, revealed itself. The enhancement of CH deformation vibrations at 1445 cm^{-1} by a factor of 22 was also observed (Fig. 5). This fact can testify that the glycine film is penetrated by gold nanostructures, so that a composite film of Au and α -Gly molecules is formed.

The α -Gly molecules in the condensed phase are in the zwitterionic configuration. However, at the thermal sputtering, they form a film, in which the zwitterionic and molecular (neutral) forms coexist. Despite that the films of α -Gly molecules sputtered onto gold had a thickness of $3855 \pm 5\text{ nm}$, we managed to register an enhancement of the IR absorption by α -Gly molecules by a factor from 10 to 13. Therefore, we may assume that the absorption enhance-



a

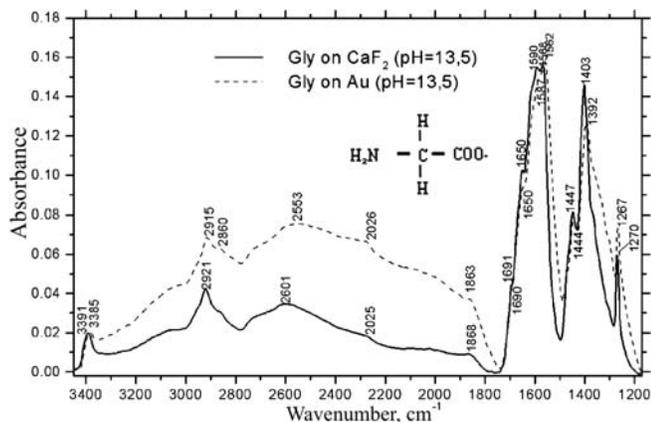


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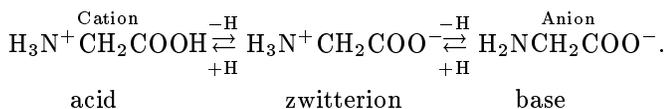
Fig. 5. IR absorption spectra of an α -Gly film thermally sputtered in vacuum onto a CaF_2 substrate (1) and the SEIRA spectrum of α -Gly simultaneously sputtered with gold onto a CaF_2 substrate (2) in the spectral ranges of $3300\text{--}2400$ (a) and $1750\text{--}850\text{ cm}^{-1}$ (b)

ment takes place not only for molecular layers located close to gold (at $10\text{--}50\text{ nm}$), but for the distant ones as well. In other words, there exist both short- and long-range enhancement components. This enables the SEIRA effect to be used for sensitive spectroscopic researches of both monolayers and multilayered films.

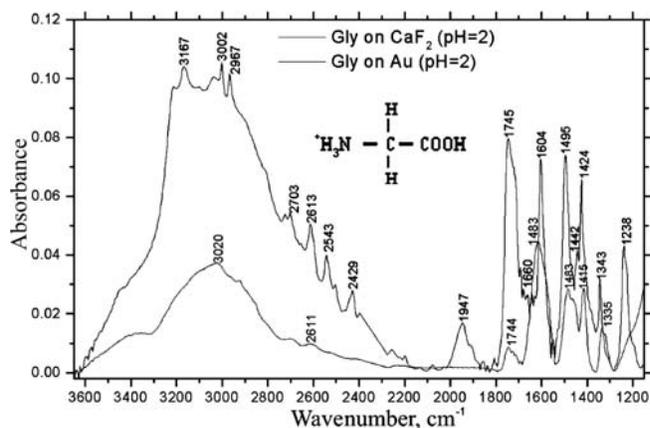
α -Gly molecules were also used by us to study the molecular mechanism of IR absorption enhancement in the SEIRA effect. This molecule contains two atomic groups possessing opposite properties: a carboxyl group with acid properties and an amino group with base ones. At the neutral pH index, the α -Gly molecule is in


 Fig. 6. SEIRA spectra for α -Gly at pH = 13.5

the zwitterionic configuration, but transforms into the monoionic one, as pH increases or decreases. All amino acids behave simultaneously as acids (donors of protons) and bases (acceptors of protons) at pH = 4 ÷ 9:



At pH = 13.5, a shift from 3391 to 3385 cm^{-1} and a broadening of the valence vibration band for the NH_2 group and a shift of the COO^- -band toward the low-frequency range were observed in the gold-substrate case (Fig. 6). However, no absorption enhancement was observed for COO^- and other groups (Fig. 6). In this case, we may suppose that gold affects the “acceptor” properties of α -Gly molecules. Specifically, gold governs the orientation of α -Gly molecules on the surface, attracts the electron shells of COO^- and NH_2 molecular groups, and influences the process of proton transfer from one molecular group to another between α -Gly molecules in the film. At pH = 2, α -Gly molecules on gold demonstrate a ten-fold increase in the intensity of the COOH valence vibration band at 1745 cm^{-1} , as well as an enhancement of H-bond valence (at 3167 cm^{-1}) and NH_3^+ deformation (at 1604 cm^{-1}) vibrations. The presence of the 1745- cm^{-1} band, which is related to the $\text{C}=\text{O}$ valence vibration in the COOH group, evidences the existence of α -Gly cations in the film (Fig. 7). The growth of pH leads to the disappearance of this band. We may suppose that, at pH = 2, there are some α -Gly molecules in the zwitterionic configuration on gold. This supposition is confirmed by a band at 1424 cm^{-1} , which is related to the symmetric valence vibration of the COO^- group. We managed to register overtones in the interval 2200–2800 cm^{-1} for a thin (250–275 nm) α -Gly film on


 Fig. 7. SEIRA spectra for α -Gly on Au (upper curve) and CaF_2 (lower curve) at pH = 2

gold (Fig. 7). Usually, those overtones can be registered only in thick single crystals. Therefore, the assumption can be made that gold affects the “donor” properties of α -Gly molecules at low pH and the reorganization of hydrogen bonds. Hence, the IR-absorption enhancement factor is governed by the charge state of the molecule and the presence of charged groups in the solution.

At varying pH, gold can influence the “donor–acceptor” properties of α -Gly molecules, which may result in the formation of a specific bond between gold and α -Gly molecules. From the analysis of our data, it is possible to draw conclusion that this interaction is weak. The maximum shift of frequencies, which we observed for α -Gly molecules on gold (for the valence N–H bond), was about 10–25 cm^{-1} . Hence, the interaction between the molecular groups and gold is lower in comparison with the intermolecular one. In works [36, 37], a possibility was indicated that nonconventional specific “anchor” hydrogen bonds $\text{N–H} \dots \text{Au}$ and $\text{O–H} \dots \text{Au}$ may appear. In this case, the absorption bands become shifted: from 199 cm^{-1} to 221 cm^{-1} for N–H and from 453 cm^{-1} to 520 cm^{-1} for O–H .

The formation of copper salts with a specific blue shade is typical of amino acids. These substances are internal complex salts: the atom Cu in them is bound not only with oxygen atoms of amino groups, but also with nitrogen ones. In the course of our experiments, when α -Gly molecules were deposited onto the Cu surface, the specimen color got a blue shade, and new bands emerged in the absorption spectrum (Fig. 8), which testified that complexes of α -Gly with Cu had formed.

Figure 8 demonstrates that the chemical adsorption of α -Gly on copper (upper curve) is accompanied by the disappearance of the NH_3^+ sym. def. vibration band

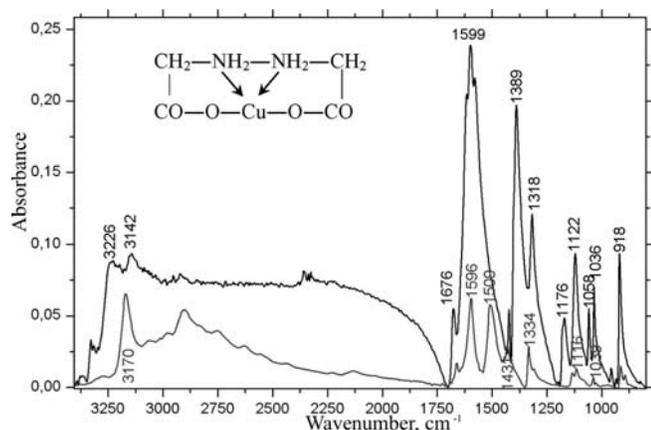


Fig. 8. SEIRA spectrum for Gly on copper (upper curve) and gold (lower curve)

at 1500 cm^{-1} and the emergence of new bands at 1389 and 1176 cm^{-1} , which can be related to the complexes formed of α -Gly and Cu. In addition, owing to the chemical adsorption of α -Gly molecules onto the Cu surface, the enhancement factor of IR absorption grows in the SEIRA effect. In particular, the asymmetric COO^- valence vibration at 1599 cm^{-1} becomes enhanced by a factor of five, and the CCN deformation vibration at 918 cm^{-1} by a factor of six in comparison with the absorption of α -Gly molecules deposited onto the rough Au surface (lower curve). Hence, our results testify that the formation of a chemical bond gives rise to a growth of the enhancement factor in the SEIRA effect.

4. Conclusions

In this work, the following dependences of the SEIRA enhancement on the molecular layer thickness, the IR transition frequency, pH of a solution, and the deposition technique of the α -Gly layer and gold were revealed:

1. For different molecular groups, the absorption enhancements are different. The most enhanced is the absorption by charged groups and groups with an unseparated electron pair.
2. The enhancement factor of the IR absorption for multilayered films of α -Gly molecules on gold decreases, as the number of molecular layers increases, by a factor of 2 to 7 for various molecular groups. It can be made 3 to 12 times as high for films obtained by the thermal sputtering of α -Gly molecules in vacuum onto the gold surface in comparison with the α -Gly films deposited from the aqueous solution.

3. If α -Gly and gold are sputtered simultaneously, the absorption bands of glycine demonstrate a better resolution.

4. The change of pH of the solution leads to a variation of the enhancement factor for the IR absorption by α -Gly molecules. At the decrease of pH to 2, the enhancement factor of the α -Gly absorption was an order of magnitude higher, which allowed overtones in the glycine films 250–275 nm in thickness to be registered.

5. At the adsorption of α -Gly onto a copper surface, the formation of a chemical bond was observed, with the enhancement factor in the SEIRA effect growing by a factor of five.

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ОСОБЛИВОСТІ ПІДСИЛЕННЯ ІЧ-ПОГЛИНАННЯ МОЛЕКУЛ α -GLY В ЕФЕКТІ SEIRA

О.М. Фесенко

Резюме

На прикладі молекул α -Gly показано, що в ефекті SEIRA молекулярні групи підсилюються по-різному, найкраще підсилюються заряджені та з неподіленою парою електронів групи. Коефіцієнт підсилення ІЧ-поглинання для багат шарових плівок молекул α -Gly, осаджених на золото, зменшується зі збільшенням кількості шарів молекул і досягає 2–7 рази для різних молекулярних груп. Його можна збільшити у 3–12 разів для плівок, отриманих термічним вакуумним напиленням молекул α -Gly на поверхню золота порівняно з плівками, осадженими з водного розчину. При одночасному термічному напиленні α -Gly і золота спостерігається краще розділення смуг поглинання в ІЧ-спектрах. Показано, що при зниженні рН розчину до 2 відбувається підвищення коефіцієнта підсилення ІЧ-поглинання α -Gly на порядок, що дозволило зареєструвати обертони в тонких плівках гліцину (товщиною 250–275 нм).