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Peculiarities of spectra of Raman scattering with glycine molecules adsorbed on the surface of metallic silver

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Received March 7, 2025
Revised March 18, 2025
Accepted March 19, 2025

The comparative study of spectra of Raman scattering with glycine molecules in aqueous solution (0.8 %) and on the surface of metallic silver, also in a solution, was conducted. The difference was found in the positions and relative intensity of the lines in these two cases. The results of the experiments made it possible to specify quality model representations of molecule interaction with the substrate.

Keywords: amino acids, glycine, raman light scattering, damping of oscillators.

DOI: 10.61011/PSS.2025.03.60885.47-25

1. Introduction

Amino acids — are a class of relatively small molecules being structural elements of larger and more complex molecules, such as DNA, providing for conduct of many processes in live organisms. The interest in the study of even such relatively simple structures is caused by the possibility to observe various effects, the use of different study methods and wide spectrum of potential applications.

One of the promising applications is development of selective sensors sensitive only to certain biomolecules. The most powerful example of such approach is development of solid-state sensors that enable detection of the presence of certain genes (DNA chip) [1], using hybridization with a substrate that is spatially prepared for the corresponding DNA sequences. Such systems make it possible to determine the presence of complicated structures at gene level. The sensors may use fluorescence of biomolecules [2], amplification of Raman scattering (RS) spectra on structured surfaces (SERS effect [3,4]), and other methods.

The above effect (SERS) is caused by excitation of surface plasmons in the subsystem of conducting electrons in the substrate material, preferably pre-nanostructured. Currently the effect is widely used in the methods to detect biomaterials at their extremely low concentration.

Besides, the recent trend to implement the elements of communication devices using organic molecules [5] requires to study the properties of such molecules, which were not of interest previously — such as interaction of such molecules with the solid body surface (with the potential substrate).

Biomolecular structures have hierarchy of complexity degrees in their organization (from amino acids to genes and proteins). At each level of complexity the biomolecular structures show unique properties, and many aspects of interaction between the biomolecules and inorganic surfaces still remain unclear [6–8]. This paper studies the biomolecules of the „lowest“ level — amino acids, and the effect of the inorganic surface at their oscillatory properties.

Glycine — is the smallest amino acid, which serves as a precursor of proteins and performs some biological functions. In paper [9] the RS spectroscopy made it possible to identify the peculiarities in the oscillating dynamics of monomers and dimers of glycine on colloidal particles in the aqueous solution. In our paper [10] we received the glycine spectra on different substrates, including dehydrated crystal films on a glass substrate. Previously we suggested [11,12] that the proximity of the substrate introduced certain damping of oscillations of the molecular dipoles, causing the reduction in the frequency of intrinsic oscillations. However, the available data was not sufficient for systemic comparison and detection of mechanisms of substrates' effect on the dynamics of the biomolecular film structures.

Initially the goal setting in this paper suggested the existence of certain difficulties. The effect of the substrate, on which the molecules were adsorbed, was detected by comparison of the spectra recorded from the substrate surface, with the spectra of molecules in the aqueous solution. At the same time it was evident that the adsorbed layer may not have significant thickness. The number of the adsorbed molecules should have been much smaller than their number in the focus of the microscope lens. With account thereof, silver was chosen as the adsorber material. It is known that it is silver that is the most effective material to implement the effect of the surface scattering amplification. The use of silver was intended to identify a signal from the localized molecules on the background of the signal from the free molecules in the solution at

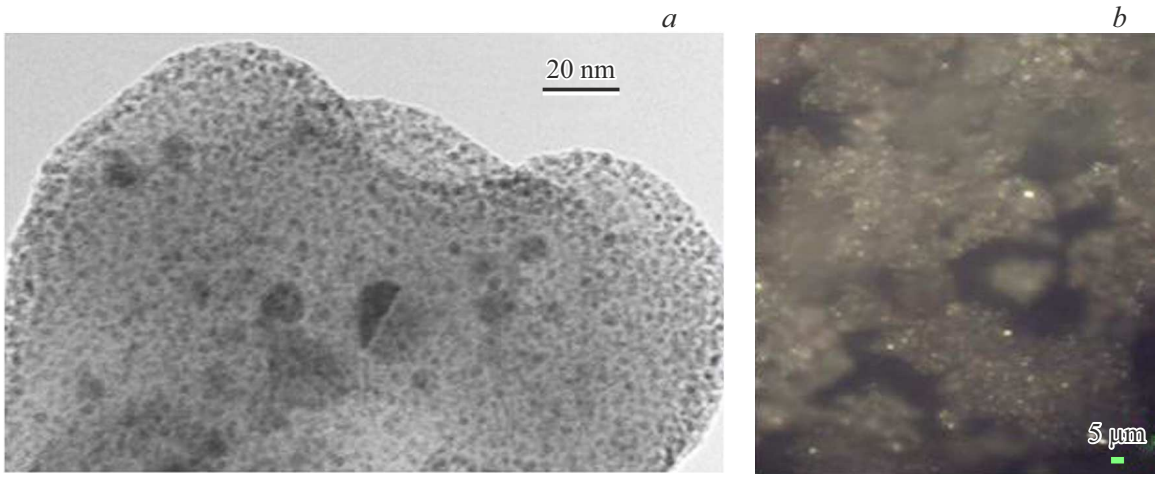

Figure 1. On the right — optical image of silver powder grains (in the solution). Scale bar at the bottom below — 5 μm. On the left — a structure of an individual grain in nanoscale.

the expense of scattering amplification with the molecules directly adjacent to the surface.

Besides, the spectra of the dissolved solutions, in contrast to the spectra of crystalline specimens, show relatively small quantity of intensive lines [13,14]. The spectra of crystalline films of amino acids that we obtained previously [10], could not have been used as reference ones, since in a crystal (a molecular one) the molecules are located in a different environment. This limited the selection of objects (bands in the spectra), in respect to which the comparative analysis may be conducted. Data of paper [10] and various published data of other authors (see, for example [13,14]) were used by us to relate the lines with the oscillation modes of glycine molecules.

2. Experimental technique and objects of research

Nonpolarized RS spectra (Raman spectra) were recorded using modular micro-Raman spectrometer HORIBA-Jobin-Yvon MRS320 with excitation by light of He–Ne-laser ($l_{ex} = 632.81$ nm) with capacity of 1–2 mW. Recording was done in the back scattering mode through OLIMPUS BX41 microscope lens. The monochromator wavelength was corrected using tabular lines of the gas discharge in the isotopically pure neon. Experiments were performed at room temperature.

Glycine was contained in the aqueous solution with weight concentration of 0.8 %. Low concentration was selected to minimize the potential aggregation of the studied material molecules.

As it was specified previously, metallic silver was used as the adsorber to identify the signal of adsorbed molecules. Silver in the experiments was both in the form of a metal plate with roughness of about 1 μm and in the form of a nanostructured powder with visible grain size of about several microns, and with grain nanostructure of below 10 nm (Figure 1). In the last case (during spectra registration from the powder surface) the spectra contained multiple lines. For control, under the same conditions, spectra were also received from Ag-powder, without addition of glycine. This made it possible to exclude the lines not related to glycine from consideration.

The authors are not able to estimate the value of the implemented amplification coefficient based on the results of this paper. However, the observation in the recorded spectra of the lines with the modified positions makes it possible to assume that to some extent the SERS effect contributes to the obtained results.

Besides, as expected, the lines in the RS spectra of glycine on the surface were not elementary usually, but demonstrated structure. The glycine lines were not broken down by components. For the same reason the authors believed it was proper to fix the position of the lines integrally, with the precision of not better than a reciprocal centimeter.

Further in the text of this paper everywhere where the molecules are mentioned on the metallic silver surface it is assumed that the metal is submerged into 0.8 % solution of glycine in water.

3. Results of the study

RS spectra of molecules in the volume of the solution and molecules adsorbed on the metallic silver surface were recorded in the field of frequencies 80–1800 cm^{-1}.

In the area of 80–700 cm^{-1} there were no intensive lines observed, which could have been confidently referred to scattering with glycine molecules; probably, they were imposed upon the interferential maxima of the scattered excitation light, also recorded by the set.

In the area of 700–1100 cm^{-1} the line 893 cm^{-1} prevailed. The position of this line was not different between the spectra of the molecules in the solution and the spectra of molecules on the metallic silver surface (Figure 2). It should be noted that in crystalline films this line had a different position 889 cm^{-1}. This line had the same position in the case when Ag-powder soaked with the solution stayed in the air for a while. We assume that in this case the dissolvent (water) managed to evaporate, and glycine could crystalize between the granules. The difference in the position of the line in the solution and in the crystalline film is not surprising, since in the crystal (in this case molecular) the molecule is in the dense environment differing from the one available in the solution.

The spectrum of molecules in the solution of this spectral area also clearly shows the line $\sim 864\,cm^{-1}$. In the spectrum recorded from the silver surface, there is no such line.

The spectra of the glycine solution on the surface of the nanostructured silver powder are much more complicated to analyze for several reasons. Firstly, the spectra contain múltiple lines, including the lines observed in the spectrum of Ag-powder without glycine. Therefore, it is possible to review the results only for some of the lines, the genesis of which may be assumed with a significant degree of confidence, omitting a part of the observed lines. The line that we assume to be corresponding to the glycine line in the solution, when recorded from the powder surface (Figure 2) is a doublet of a narrow intensive line ($w = 889\,cm^{-1}$, its full width at half maximum of the line $FWHM = 6\,cm^{-1}$) and a heterogeneous satellite of much lower intensity (3–4 times) with maximum of about 915 cm^{-1} and with considerable $FWHM = 20–30\,cm^{-1}$. In the area of 1200–1800 cm^{-1} (Figure 3) several glycine lines and a wide band of water molecules oscillation $\sim 1630\,cm^{-1}$ are reliably identified. Note additionally the asymmetric shift of the band that belongs to the oscillation of the water molecules 1630 cm^{-1} (OH-bending). High-energy edge of the band practically coincides for the spectra recorded from the volume of the solution and from the surface of the silver plate. The edge from the side of lower energies, on the contrary, differs noticeably in these two cases: when recorded from the silver plate surface, it is shifted to lower energies. A similar effect was observed by us for the water molecules oscillation band 3300 cm^{-1} (OH-stretch) [11,12]. The potential similarity of the line shifting mechanisms will be discussed further.

The lines that belong to glycine vary in a different manner when recorded from the surface of the metallic silver. Thus, line 1406 cm^{-1} does not change the position and reduces in intensity only slightly. At the same time the shape of the line 1322 cm^{-1} changes significantly. Its intensity in the spectrum taken from the metal surface decreases approximately 2.5 times, and from the Stokes side in the area of 1283–1293 cm^{-1} a noticeable trapezoidal band appears. It is natural to assume that the line 1322 cm^{-1} that maintained its position belongs to the molecules in the volume, and the components of the trapezoidal band are

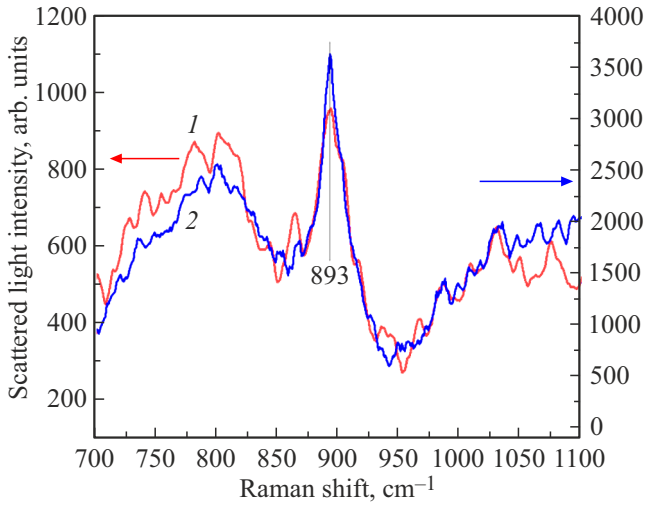

Figure 2. RS spectra of glycine molecules in 0.8 % aqueous solution (*1*) and on the metallic silver surface (*2*) in area 700–1100 cm^{-1}.

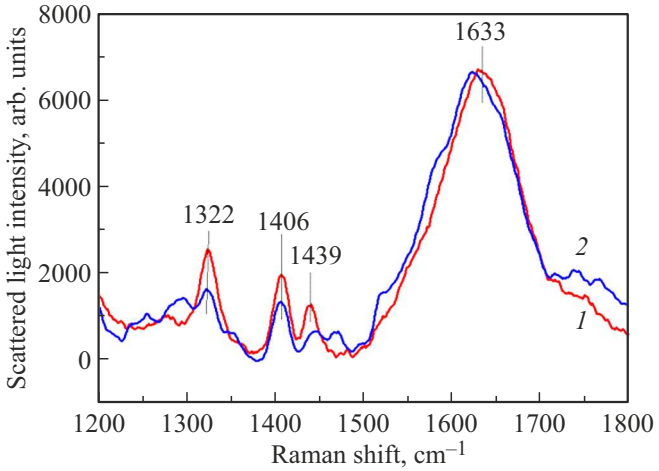

Figure 3. RS spectra of glycine molecules in 0.8 % aqueous solution (*1*) and on the metallic silver surface (*2*) in area 1200–1800 cm^{-1}. Positions of the lines in the spectra are specified for the case of glycine in the solution volume. The spectra are normalized in terms of intensity by peak 1633 cm^{-1}.

caused by the oscillations of the molecules exposed to the effect of the silver substrate surface.

The line 1439 cm^{-1} of the initial spectrum is exposed to even stronger effect. In the place of this line in case of the spectrum recorded from the metallic silver surface, doublet 1444, 1468 cm^{-1} is recorded.

In the area of 1250–1750 cm^{-1} a wide band is observed in the spectra on the surface of Ag-powder. The same wide bell-shaped band was available in the glycine-free powder. Various additional maxima were observed in this band in various points of the specimen. It may be assumed that this is due to the size-dependent plasma resonances in the silver powder grains. The band of water oscillations $\sim 1600\,cm^{-1}$ may also contribute to the specified wide band.

In the spectra in the area of $1200-1800\,cm^{-1}$ the lines 1314, 1390, $1456\,cm^{-1}$ may belong to glycine. The line $1314\,cm^{-1}$ may confidently be assumed such. In the spectrum of crystalline films of glycine this line corresponds to line $1318\,cm^{-1}$. In certain points of the specimen (Ag-powder under the glycine solution) the line $1314\,cm^{-1}$ prevails.

On top of the bell-shaped band there is a pronounced relatively wide maximum $1390\,cm^{-1}$ (3–4 times wider than line $1314\,cm^{-1}$). Probably, it corresponds to lines $1406/1404\,cm^{-1}$ in the spectra for the glycine molecules in water and in its crystalline film, accordingly.

The maximum $1456\,cm^{-1}$ observed in these spectra has width close to the width of the line $1390\,cm^{-1}$. It is difficult to relate this maximum to any specific line in other spectra, since the crystalline film near $1456\,cm^{-1}$ contains several narrow closely located weak lines.

Near the band of water oscillations $3300\,cm^{-1}$ (OH-stretch) (the spectra are not shown) the glycine in the solution volume demonstrates the band $2965\,cm^{-1}$. On the silver powder the line has the maximum at $2957\,cm^{-1}$.

Let us repeat it once again that the description of the results of the experiments with glycine on the surface of Ag-powder for the above reasons is not very reliable, but in general these experiments confirm the trends specific for the glycine spectra on the surface of the silver plate — change of the positions of the lines or splitting, and their shift towards the lower energies.

4. Discussion of results

The results of the conducted experiments demonstrate noticeable effect of the used substrate on the RS spectra with the molecules of the glycine amino acid.

At the same time, the existence of the effect of scattering amplification by the surface as such assumes interaction of the oscillatory degrees of freedom of any objects with the surface elements, which must be somehow reflected in the properties of the oscillatory systems — shift of the intrinsic frequencies of oscillations. In this case such oscillatory systems are the intrinsic oscillations of the molecules.

One of the reasons for such shift has a universal nature for all oscillatory systems. One may assume that the close presence of the substrate would damp the oscillators, and this in its turn will automatically reduce their frequency. As specified, previously such effect was found by us for the oscillation of the OH group in the water molecule on band $3300\,cm^{-1}$ [11,12]. Based on the results of this paper, one may say that such effect of the conducting substrate (shift of the lines towards the lower energies) is in average true for a glycine molecule, too.

It is especially interesting that the effect of the metal substrate manifests itself differently in various lines of the spectrum. We refer this to the fact that the molecules are not isotropic objects and may be localized near the surface in the differing orientations. The specific position in process of localization should depend on the geometry of the surface in the point of localization and on the electronic configuration of the molecule, mainly on how the charge density is distributed.

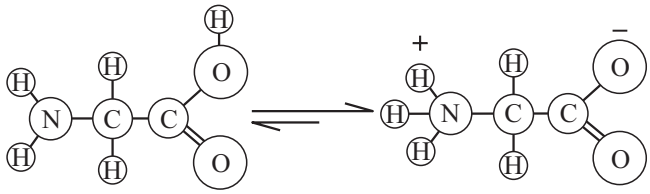

Figure 4. On the left - schematic image of glycine molecule, corresponding to gross formula; on the right — a molecule in the form of an amphion. The natural equilibrium is shifted towards the amphion.

Certain conservative assumptions may be made for the case of the glycine molecules. Being the simplest of the molecules of known amino acids, glycine is nevertheless a rather complex object. First of all, glycine under natural conditions is preferably in the form of an amphion [15]. In case of glycine it means that one of their protons in the carboxyl group is transferred to the amino group, so that the molecule, remaining in general electrically neutral, acquires a significant dipole moment (Figure 4). The dipole moment value of glycine in the form of an amphion is estimated from 11.9 to 15.7 D [16] (here D — debye — is a unit of dipole moment).

It is natural to assume that in process of adsorption the charged ends of the molecule must induce the appearance of charges of „reflection“ in the conducting metal, and therefore the molecule will be exposed to attraction from the side of the „reflected“ dipole. In its turn, this decreases the energy of the molecules in the state with a high dipole moment and additionally shifts the equilibrium towards the amphion. This makes it possible to assume that the adsorbed molecules are located primarily with the orientation of the electric dipole along the plane, with the fixed charged ends.

We will take that into account to consider the nature of the lines in the spectra. The lines discussed above were published and interpreted previously. Focusing on paper [17] one may assume that the line $893\,cm^{-1}$ meets the approved torsional oscillations of two groups: NH_2 and CH_2. The absence of the shift of this line may be attributed to the fact that the group CH_2 (middle atom of carbon) is not fixed, and the possibility to twist the molecule remains. Besides, since the line is narrow, the specified oscillation is relatively good and is hardly subjected to the environmental effects.

In paper [17] the line $1334\,cm^{-1}$ is also attributed to this type of oscillations. However, in our case, as it was specified above, a part of the line was converted to a wide maximum with lower energies.

The line $1406\,cm^{-1}$ does not differ in the spectra of the solution and the ones obtained from the surface of

Ag-powder. In article [17] this line is attributed (not a full match, it is 1410 cm^{-1} there) to „scissoring“ vibration of group CH_2. According to our assumption, during adsorption by Coulomb forces, the end groups are fixed, while CH_2 group (with the central carbon atom of the molecule) remains relatively free. As we can see, in our spectra this line does not change when transitioning from the spectrum of the solution to the spectra of the molecules on the surface. This confirms the correctness of the approach. More detailed analysis of the results requires additional studies.

5. Conclusion

The RS spectra of the glycine molecules in the aqueous solution and on the surface of metallic silver (also in the solution) were studied in the spectral area of 80−1800 cm^{-1}. The comparative analysis was done on the position of the most intensive lines in the spectra. The assumption was made that the main interaction determining the position of these molecules in process of adsorption was the Coulombic attraction between the charged end groups and the induced dipole of „reflection“ in the conducting metal.

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by M.Verenikina