

Article

In Situ and Ex Situ Raman Studies of Cysteine's Behavior on a Titanium Surface in Buffer Solution

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Abstract: In this paper, surface-enhanced Raman spectroscopy (SERS) was used to investigate the adsorption process of cysteine (Cys). Studies were carried out in the presence of phosphate-buffered saline solution (PBS), at pH 7.4, and acidified to pH 5, 3, and 1, on the surface of Ti for implant application. In situ SERS spectra obtained for the Cys/Ti solution system, after 24 h of immersion time, indicated that the buffer solution strongly influences the adsorption behavior of Cys on the Ti surface. This results in a decrease in Cys adsorption on the Ti surface, in the range of pH 7.4 to 3. The strong interaction between a sulfur atom of Cys and a Ti surface was observed only at pH = 1, under strongly acidic conditions. In contrast, ex situ SERS spectra recorded for the same samples but in a dried Cys/Ti system show a completely different behavior of Cys on the Ti surface. Formation of a disulfide (S-S) bond has occurred as a result of the dimerization or aggregation of Cys molecules on the Ti surface. Detailed analysis of the adsorption behavior of Cys on the Ti surface can be very important in the preparation of bioactive materials (i.e., coated by organic layers).

Keywords: titanium (Ti); cysteine (Cys); buffer solution; adsorption; Raman spectroscopy



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1. Introduction

Cysteine (Cys) is a sulfur-containing endogenous amino acid that acts in a wide range of biological functions, i.e., protein synthesis, enzyme structure, detoxification cellular systems, and metabolism [1,2]. It is a metabolic precursor for cystine (thiol groups (SH) of two Cys molecules form a disulfide bond (S-S)), glutathione (γ-glutamyl-cysteinyl-glycine) [2,3]. The concentration gives important information about the physiological functions of the human body, it can be an indicator of health conditions [4]. Both deficiencies and high concentrations of this plasma amino acid are harmful and can contribute to various diseases such as edema, liver damage and Alzheimer's disease, respectively [1,4–6]. Cys has many important applications in cosmetic, pharmaceutical, and food branches of industries such as antioxidants and medicine [7,8].

In addition, Cys is a popular stabilization agent for metallic structures [9]. The Cys-protected structure presents good optical properties and biocompatibility for biomedical applications [10,11], as well as in the area of studies of protein interactions with metallic nanostructures [12]. A wide range of experimental and theoretical studies have shown that Cys is strongly adsorbed onto different metal surfaces, such as Ag [9], Au [9,13], Cu [9], and metal oxides, i.e., TiO₂ [14–16].

Commercially, pure titanium (Ti; grades 1–4) and its alloys are widely used for medical applications, such as orthopedic devices [17], and dental implants [18], due to their very good biocompatibility, chemical stability, and mechanical properties [19]. Titanium has excellent corrosion resistance due to the oxide layer formed (mainly TiO₂) on its surface [19,20].

The adsorption of proteins on implanted materials is regarded as the first biologic response that occurs after implantation. The metallic surface of the biomaterial is coated with proteins from the surrounding environment and forms a variable protein layer [21,22].

It is a very complex process, which depends on many parameters such as roughness, hydrophobicity/hydrophilicity of the surface, protein molecular structure (i.e., amino acid chain, charge), and solution (pH, concentration, temperature) [22]. Cys is the amino acid that is present in many plasma proteins, for example, serum albumin, which has a high Cys content [23]. Additionally, thiol groups can be used for protein immobilization due to the formation of disulfide bonds with Cys amino acids [24]. Furthermore, the very important aspect that appears after implantation is the decrease of local pH around the biomaterials, which is caused by surrounding it with, i.e., chloride ions [25]. In addition, the bacterial colonization and adhesion of bacterial biofilms provide pH alterations and create a localized acidic condition, causing damage to the surface oxidation layer of metallic implants [26]. The application of oral drugs (acidic chemicals) can strongly influence Ti dental implants due to changes that occur in the implant environments [27].

Raman spectroscopy is a great tool for investigating the molecular structure of various species in a wide variety of applications [28,29]. It is quick, non-destructive, and insensitive to the water method. Raman spectra give a fingerprint of a molecule and can be analyzed for molecules in different states [30,31]. Raman scattering can be drastically enhanced for molecules adsorbed or in close proximity to the metallic surface, resulting in a very sensitive surface-enhanced Raman spectroscopy technique [32–36]. Vibration fingerprints depend on many parameters, i.e., that is, the molecular environment (pH of the buffer solution) [37,38] and properties of the metallic surface [39]. This sensitive technique has been used in many in situ studies to determine the composition of different adsorbate layers in an aqueous solution [36]. The greatest enhancement factor can be obtained for noble metals such as Ag, and Au [9]. The SERS signal intensities obtained on Ti structures (TiO₂ surfaces), compared to the above-mentioned metals, are rather lower [40].

In the present paper, we applied in situ SERS spectroscopy to monitor the Cys behavior on the Ti surface in real-time, after 24 h of immersion in the solution of PBS, at pH 7.4 (physiological conditions), and in the acidic solution at pH 5, 3, and 1. To better understand the adsorption behavior of Cys on the Ti surface, ex situ SERS spectroscopy measurements obtained for dried samples in the same conditions were provided. Despite the many investigations conducted on biomolecules immobilized on different metallic surfaces [33–35,41], there is still a lack of studies in this area. Detailed analysis of the adsorption process of Cys on the Ti surface, in the presence of buffer solution, in a different state, under control conditions, can be very important in the preparation of bioactive materials (i.e., coated by organic layers).

2. Materials and Methods

The samples were prepared from a Ti grade 2 sheet (Wolften, Wrocław, Poland) (chemical analysis: 0.02% C; 0.002% H; 0.05% Fe; 0.12% O; balance Ti). Samples were machined with dimensions of 200 mm × 200 mm × 2 mm. After that, the samples were polished with silicon carbide papers up to a 2400 grit finish. The surface of Ti was then washed with deionized water and ultrasonically cleaned for 20 min in ethanol.

Cys (Cys, 10^{−1} M, Sigma Aldrich, Poznań, Poland) reference Raman spectra in PBS (Sigma Aldrich, Poland) solutions at pH 7.4, 5, 3, and 1 and for the same dried samples were recorded. The pH value was adjusted by titration of HCl (analytical grade reagent) using a pH meter. The PBS composition of the solution at pH 7.4 was the following: 0.0027 M potassium chloride, 0.137 M sodium chloride and 0.01 M phosphate buffer.

The adsorption behavior of Cys (10^{−2} M) onto the Ti surface was investigated in PBS solution at pH 7.4, 5, 3, and 1. In situ SERS measurements were recorded after 24 h of immersion of Ti samples in Cys solution at controlled pH. Ex situ SERS measurements were performed for samples prepared under the same conditions as described above, but in a dried system (drying temperature 37 °C).

An InVia Renishaw Raman spectrometer (Renishaw, Wotton-under-Edge, UK) with a CCD camera and a confocal microscope was applied to measure Raman scattering. The excitation wavelength was provided by the laser emitting at 633 nm (mode Regular)

with 1800 grooves/mm grating. The output power of the laser at the sample was set at approximately 5 mW. The spectra presented were collected with a spectral resolution of 1 cm^{-1} and in the spectral range of 1530 to 360 cm^{-1} . The in situ spectra maps were collected with a $1\text{ }\mu\text{m}$ step size (2640 acquisitions, exposure time 1 s, laser power 5 mW, spectral range 1530 – 400 cm^{-1}). The 100-lens long Leica objective was used. The ex situ spectra were recorded by the same Raman system from 8 different points of the sample (spectral resolution 1 cm^{-1} , 1 scan, exposure time 10 s, laser power 5 mW, spectral range 1530 – 400 cm^{-1}).

Interpretation and analysis of the recorded spectra were performed using the OMNIC (9.0 version) and WIRE 5.2 software.

3. Results and Discussion

3.1. In Situ Raman Measurements

The reference Raman spectra recorded for the solution of Cys (10^{-1} M/PBS) at pH = 1, 3, 5, and 7.4 are shown in Figure 1A. The proposed band assignments were based on experimental and theoretical vibrational data from the literature on the molecular structure of free Cys [31,42–44] in different states and adsorbed on different metallic surfaces, such as Au, Ag, Cu [9,13,39,45,46], FeS_2 [47] and TiO_2 [14–16]. Table 1 presents the wavenumber and band assignments of characteristic vibrational modes of the investigated samples. It should be noted that the preparation of samples for measurements has a significant impact on the spectral information obtained. Parameters such as the sample's condition [35], type of solvent [48], buffer solutions [49], and pH [30,33,50] can influence the recorded spectra.

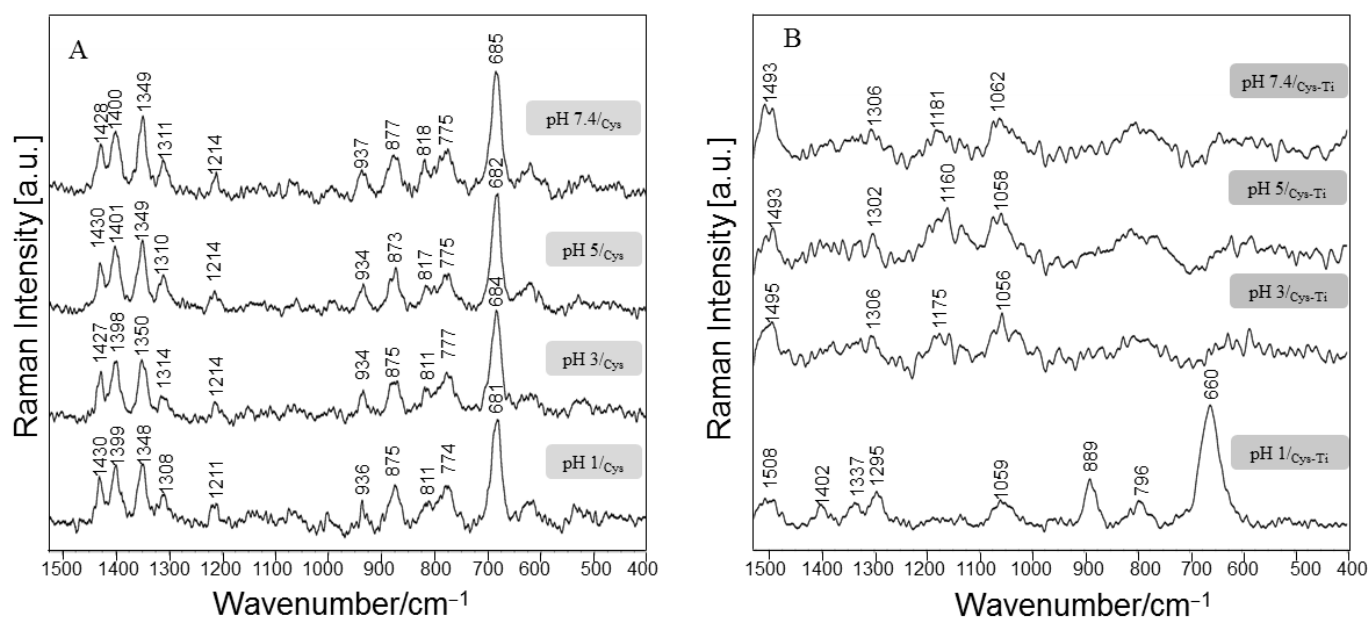


Figure 1. Raman spectra of Cys (10^{-1} M/PBS) recorded at different pH in the solution state (A) and in situ SERS spectra of Cys (10^{-2} M/PBS) recorded at different pH, after 24 h of exposure to the Ti surface in the solution system (B).

The reference Raman spectra for Cys (10^{-1} M/PBS) were obtained in solution at a pH from 1 to 7.4 and do not differ significantly from each other. However, a slight shift between the wavenumbers of bands is observed, i.e., at $\sim 1430\text{ cm}^{-1}$, $\sim 1399\text{ cm}^{-1}$, $\sim 1348\text{ cm}^{-1}$, $\sim 875\text{ cm}^{-1}$, $\sim 681\text{ cm}^{-1}$ upon pH changes (see Table 1 for appropriate bands assigned). At pH = 1 (below $\text{pK}_{a1} = 1.96$) the Cys is positively charged, due to the amine group (NH_3^+). At a pH higher than pK_{a1} and lower than pK_{a2} ($\text{pK}_{a2} = 8.18$), in the case of Raman spectra obtained at pH = 3, 5, and 7.4, Cys exists in the zwitterionic form ($\text{COO}^-/\text{NH}_3^+$) [16].

Table 1. Proposed band assignments for Raman spectra for Cys in solution and solid states at pH = 1, 3, 5, and 7.4 and recorded in the Cys/Ti in situ and ex situ system.

Band Assignments	pH = 1		pH = 3		pH = 5		pH = 7.4	
	Raman Solution/Raman Dried	In-Situ SERS/Ex-Situ SERS	Raman Solution/Raman Dried	In-Situ SERS/Ex-Situ SERS	Raman Solution/Raman Dried	In-Situ SERS/Ex-Situ SERS	Raman Solution/Raman Dried	In-Situ SERS/Ex-Situ SERS
NH ₃ ⁺ bend.	—/—	1508/—	—	1495/—	—	1493/—	—	1493/—
CH ₂ bend.	1430/1424	—/—	1427/1424	—/—	1430/1423	—	1428/1425	—/—
COO sym. stretch.	1399/1398	1402/—	1398/1398	—/1390	1401/1398	—/1393	1400/1398	—/1392
COO stretch./CCH bend.	1348/1343	1337/—	1350/1344	—/1341	1349/1344	—/1342	1349/1344	—/1341
CH ₂ wag./CCH bend.	1308/1292	1295/—	1314/1293	1306/—	1310/1293	1302/—	1311/1293	1306/—
CH ₂ bend./HCN bend./phosphate anions	1211/1199	—	1214/1199	1175/1137	1214/1199	1160/—	1214/1199	1181/—
C _α N stretch./phosphate anions	1074/1067	1059/—	1064/1066	1056/—	1060/1066	1058/—	1072/1066	1062/—
NCH stretch.	—/1005	—	—/1006	—	—/1002	—	—/1004	—
HCN bend.	936/940	—	934/941	—	934/940	—	937/940	—
NH ₃ ⁺ rock./NC _α stretch.	875/867	889/—	875/868	—	873/867	—/876	877/869	—/878
CH ₂ wag./C _α N stretch.	811/822	796/784	811/822	—	817/826	—	818/822	—
S-H bend.	774/772	—	777/773	—	775/772	—	775/772	—
C _β S stretch.	681/692	660/—	684/692	—	682/692	—	685/692	—
C _β S stretch.	—/639	—/635	—/639	—/676	—/639	646/679	—/639	—/672
COO wag.	614/—	—	620/—	—/607	620/—	620/615	620/—	—/—
CH ₂ —CH—N bend.	—/535	—	—/535	—	—/535	—	—/535	—
S-S stretch./CCN bend.	—	—/496	—	—/496	—	—/496	—/498	—/496
CCC bend./CH ₂ —CH—SH bend	—/441	—/469	—/441	—	—/441	—	—/441	—

Abbreviations: $\bar{\nu}$ —wavenumber; bend.—bending; sym. stretch.—symmetric stretching; stretch.—stretching; rock.—rocking, wag.—wagging.

The Raman spectra of free Cys are dominated by characteristic modes due to molecular fragments of CS, COO, NH₃⁺, and CN/CH (see Table 1 for detailed assignments). The most intense band (the comparable relative intensity among the spectrum) appeared at $\sim 681\text{ cm}^{-1}$ (full-width at half-maximum, fwhm = 23 cm^{-1}) and is characteristic of the C_βS stretching bond. Another band at $\sim 774\text{ cm}^{-1}$ is due to thiol bending vibration modes. In Raman spectra, in the region between $\sim 1500\text{ cm}^{-1}$ and $\sim 1200\text{ cm}^{-1}$, the main carboxyl symmetric stretching bands of free Cys are located (see Table 1, for appropriate band assignments). The bands in the wavenumber range between $\sim 1200\text{ cm}^{-1}$ and $\sim 800\text{ cm}^{-1}$ can be assigned to the vibration bands of amine ($\sim 875\text{ cm}^{-1}$ (NH₃⁺ rocking)) and CN ($\sim 1211\text{ cm}^{-1}$ (HCN bending), $\sim 1074\text{ cm}^{-1}$ (C_αN stretching), $\sim 1001\text{ cm}^{-1}$ (NCH stretching), $\sim 936\text{ cm}^{-1}$ (HCN bending)) vibration bands (see Table 1) [9,37].

The in situ SERS spectrum obtained for a Cys (10^{-2} M) sample at pH 1.0 in solution adsorbed onto the Ti surface obtained after 24 h of exposure presents significant differences in the relative intensity, position, and width of wavenumber corresponding to the Raman of the non-adsorbed Cys. The largest change in the SERS spectrum for Cys adsorbed on the Ti surface (solution/pH = 1) is the appearance of the strong C_βS stretching band at 660 cm^{-1} . The position of this band is sensitive to the torsion angle N-C_αC_βS and provides information about the rotational configuration of the Cys during adsorption onto the metallic surface, as reported by Yao and Hang [13] and Rodríguez-Zamora et al. [9,45]. This spectral characteristic shows a greater enhancement, a significant red-shift ($\Delta\bar{\nu} = 21\text{ cm}^{-1}$), and a broadening ($\Delta\text{fwhm} = 22\text{ cm}^{-1}$) compared to those observed in the Raman spectrum of the free Cys (Figure 1A). Spectral data reported for different metallic structures (Ag, Au,

Cu) indicated that the red-shift of the $C_{\beta}S$ stretching band of Cys confirms a structural modification of the molecular structure of the amino acid in the adsorption process [9,46]. It can be suggested that Cys (solution/pH = 1) strongly interacted with the surface of Ti via the thiol group.

Figure 2A shows the map of the distribution of the $C_{\beta}S$ stretching band at 660 cm^{-1} on the Ti surface and selected in situ SERS spectra recorded from three different positions (Figure 2B). The obtained map of the distribution of the Cys on the Ti's surface confirms the strong Cys adsorption on the metallic surface. In a strongly acidic environment (pH = 1), the cationic form of Cys is the predominant species. Under these conditions, the protonated form of Cys can electrostatically adsorb onto the chloride-covered metallic surface. The chloride ions can promote the physical adsorption of cations on the metallic surface and, in addition, the Cys molecules can be adsorbed on the metallic surface due to the formation of linkage between the lone pair of electrons of the S atom and the Ti surface.

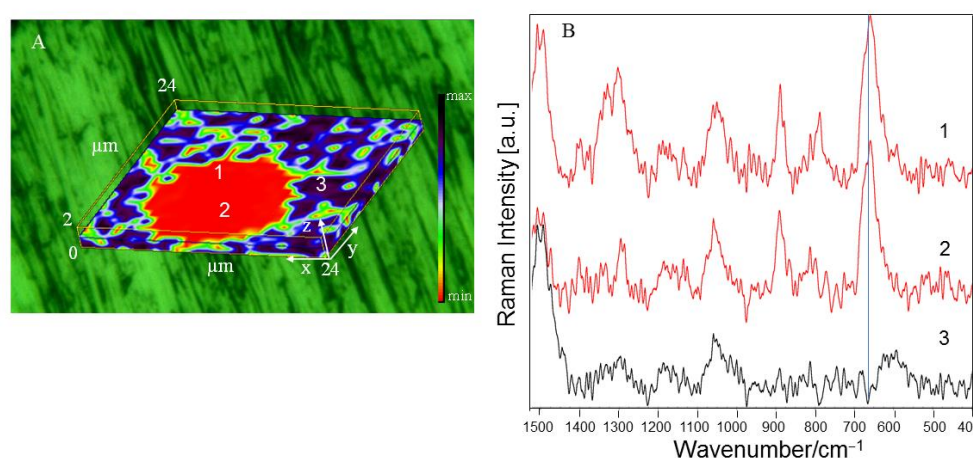


Figure 2. The intensity map of the SERS bands at 660 cm^{-1} (Cys (10^{-2} M /PBS, pH = 1.0) (A). In situ SERS spectra of Cys (10^{-2} M /PBS) recorded at pH = 1, after 24 h of exposure to the Ti surface in the solution system from points 1, 2, and 3, marked spots in A (B).

In the case of Cys, the existence of three different isomeric configuration forms is possible, allowing for three conformations due to its torsion angle ($N-C_{\alpha}C_{\beta}S$) [9,13,45]. The above-mentioned isomeric rotational conformers/rotamers are named by the atom or functional group that is in antiposition the SH group (P_N : the amine (NH_3^+) group in the antiposition and the COO group in the gauche position; P_H —the hydrogen (H) atom is in the antiposition and COO and NH_3^+ groups are in the gauche position; P_C —COO group in the antiposition and NH_3^+ group in the gauche position) [9,13,45].

The $C_{\beta}S$ stretching band for the P_N conformer is located at a higher wavenumber (as proposed by Yao and Huang [13]) than for the P_C and P_H conformers. Based on these data, the position of the $C_{\beta}S$ band at 660 cm^{-1} excluded the formation of the P_N conformer during the adsorption of Cys on the Ti surface in the solution state. The blue-shift of a band at 889 cm^{-1} due to NH_3^+ rocking and NC_{α} stretching vibration compared to free Cys (see Figure 1A) can be explained as the reduction of the $N-C_{\alpha}C_{\beta}S$ dihedral angle during the adsorption process of Cys molecule on the Ti surface, followed by Rodríguez-Zamora et al. [9]. This observation indicates that the NH_3^+ group is close to the negatively charged metallic surface (high concentration of chloride anions on the Ti surface), but it does not adsorb directly on the metallic surface. Furthermore, the significant reduction in the relative intensity of characteristic bands due to the COO symmetric stretching band appearing at 1402 cm^{-1} compared to the Raman spectrum of Cys indicated that this molecular fragment is at some distance from the Ti surface. Based on these spectral changes, it can be proposed that Cys at pH = 1 in solution is adsorbed mainly by the sulfur atom with the carboxyl group some distance from the Ti surface under acidic conditions. We can suggest that the Cys conformer that stabilizes on the Ti surface is mainly P_C (COO group in antiposition

with respect to the S atom). Figure 3 shows the proposed adsorption process of Cys in the solution on the Ti surface at pH = 1.

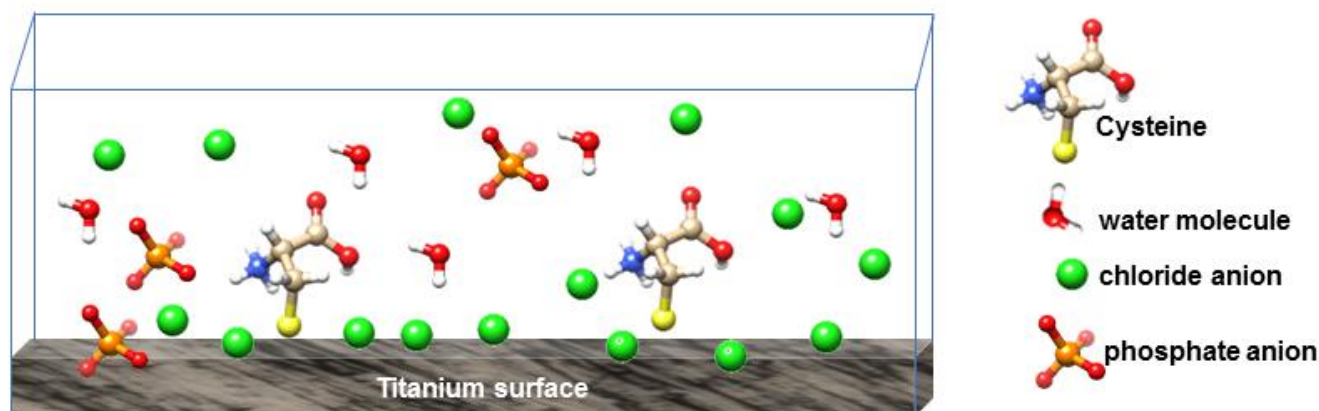


Figure 3. Proposed adsorption behavior of Cys in the solution system on the Ti surface in an acidic environment (pH = 1).

For in situ SERS spectra obtained for the sample immersed in the presence of Cys and PBS solution at pH 3, 5, and 7.4 in PBS solution (see Figure 1B), the characteristic bands due to Cys are not enhanced, or very weak. The characteristic band at 660 cm^{-1} is not enhanced. The in situ SERS spectra (Figure 1B) obtained under these conditions drastically differ from the respective reference Raman spectra of Cys at the same pH (Figure 1A). At pH 3, 5, and 7.4 the appearance of bands at $\sim 1175\text{ cm}^{-1}$ and $\sim 1056\text{ cm}^{-1}$ can be mainly assigned to vibrations due to phosphate groups from the buffer solution [49,51,52]. Moulton et al. [51] and Loreto et al. [52] have studied the influence of PBS on the adsorption behavior of different proteins (immunoglobulin, myoglobin) on TiO_2 films and reported that the buffer is strongly adsorbed on the metallic surface. They proposed that the use of PBS has an influence on the adsorption process and can inhibit the adsorption of proteins. In addition, Wang et al. [49] proposed that SERS substrates immersed in PBS solutions were found to be unstable in low buffer solutions (pH = 3) and neutral pH (=7). In the case of our system, the drastic weakness of the bands due to the molecular Cys species in the range of pH from 3 to 7.4, together with the appearance of bands of phosphate anions, can confirm this statement. The buffer species interacted with the Ti surface, leading to a competing interaction with the Ti surface, as has also been reported by Loreto et al. on commercial mesoporous TiO_2 [52].

3.2. Ex Situ Raman Measurements

Figure 4A presents the Raman spectra obtained for dried samples of Cys ($10^{-1}\text{ M/PBS/pH 1, 3, 5, and 7.4}$). As was claimed in Chapter 3.1., the preparation of the sample strongly influences the obtained spectral information. The Raman spectra for Cys obtained in the solid state (Figure 4A) differ from those obtained in the solution state (Figure 1A). The most intense band due to the C_βS stretching bond appeared at 639 cm^{-1} (fwhm = 10 cm^{-1}); in the solution state, this band has been red-shifted and appears at $\sim 681\text{ cm}^{-1}$. These Raman spectra, similar to the Raman spectra obtained in the solution system, only slightly differ in the pH range from 1 to 7.4. The most important difference in the spectral pattern appears at pH 7.4; in the Raman spectrum, we observed band at 498 cm^{-1} , which can be assigned to the stretching vibration of the disulfide (S-S) bond [42]. The appearance of this spectral band can suggest that the dimerization or aggregation of Cys is occurring at neutral pH [39,42,53].

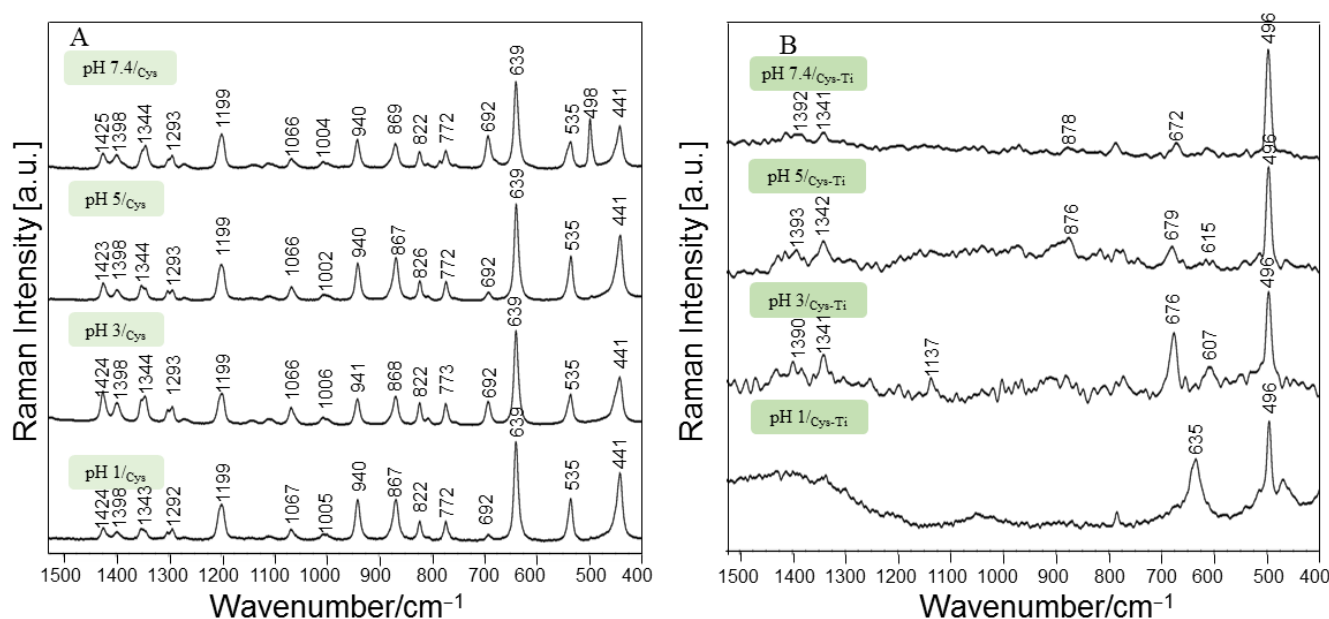


Figure 4. Raman spectra of Cys (10^{-1} M/PBS) recorded at different pH values in the solid state (A) and ex situ SERS spectra of Cys (10^{-2} M/PBS) recorded at different pH, after 24 h of exposure on the Ti surface in the dried system (B).

The SERS spectra obtained for dried samples of Cys (10^{-2} M/PBS) at pH of 1 to 7.4 adsorbed on the Ti surface show significant differences in the relative intensity, position and width of the wavenumber corresponding to the Raman spectra of free Cys.

As mentioned above (see Section 3.1.), the position of the band due to the $C_{\beta}S$ molecular species of Cys is very sensitive to conformational changes of the molecule and gives key information about the adsorbate behavior on the metallic surface. In the SERS spectrum obtained for Cys, adsorbed on the Ti surface in an acidic medium (pH = 1), the reduction in relative intensity, slight red-shift ($\Delta\bar{\nu} = 4 \text{ cm}^{-1}$), and broadening ($\Delta\text{fwhm} = 7 \text{ cm}^{-1}$) of the band at 635 cm^{-1} ($C_{\beta}S$ bond) compared to the Raman spectrum suggest that the sulfur atom is adsorbed on the Ti surface. Upon increase of pH, at pH = 3, 5, and 7.4, a weakening of the relative intensity of the $C_{\beta}S$ band is also observed compared to the normal Raman spectra. However, unlike pH = 1, as the pH increases, there is a significant blue-shift ($\Delta\bar{\nu}_{\text{pH}=3} = 37 \text{ cm}^{-1}$; $\Delta\bar{\nu}_{\text{pH}=5} = 41 \text{ cm}^{-1}$, and $\Delta\bar{\nu}_{\text{pH}=7.4} = 33 \text{ cm}^{-1}$) of this band. These spectral changes can suggest the rearrangement of adsorbate molecules on the Ti surface.

Furthermore, the appearance of a band at 496 cm^{-1} (see Figure 4B, pH = 1), characteristic of the disulfide (S-S) bond, may indicate that the Cys molecules undergo dimerization upon adsorption on the Ti surface, resulting in cystine formation [42]. Jing and Fang [39] proposed that the Ag surface activated by the co-adsorption of Cl^{-} anions provided the formation of cystine molecules. Furthermore, Rodríguez-Zamora et al. [9] suggested that adsorbed Cys on the Ag surface propitiated the formation of the stretching vibration belonging to the S-S bond. The dimerization of some Cys molecules was also observed by Marti et al. [54] upon adsorption on the Cu surface. Upon an increase in pH, the strengthened relative intensity of the band at 497 cm^{-1} (Figure 4B) indicates that the formation of the Cys dimer on the Ti surface cannot be excluded. The increase in pH enhances the dimerization of Cys, as claimed by Shkirskiy et al. [55].

Based on the changes in vibration modes that occur at $\sim 1390 \text{ cm}^{-1}$ and $\sim 1341 \text{ cm}^{-1}$ upon the Cys adsorption process on the Ti surface (Figure 4B, pH = 3–7.4) relative to the ordinary Raman spectra, it can be suggested that the COO group or groups (the formation of cystine) are also at some distance from the metallic surface. These bands are blue-shifted compared to those observed in the normal Raman spectrum (see Figure 4A). As was claimed by i.e., Rodríguez-Zamora et al. [9] and López-Tobar et al. [56], the above-

mentioned changes of spectral patterns can indicate that carboxyl groups assist in the adsorption process of Cys/cystine on the Ti surface.

The proposed orientation of the investigated molecule on the Ti surface in the dried system is presented in Figure 5. An interaction occurring via S-S bonds being tilted to the Ti surface and interacting via lone pair of electrons without disulfide bridge cleavage can be suggested.

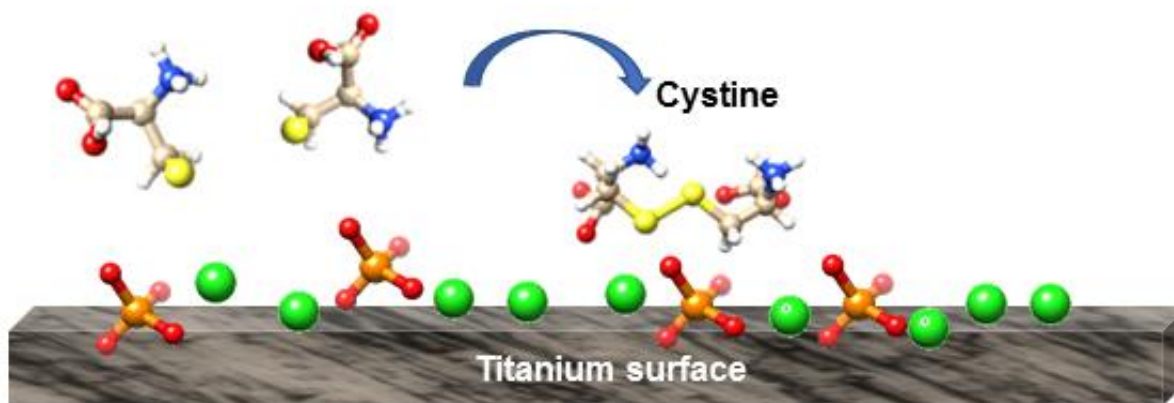


Figure 5. Proposed adsorption behavior of the investigated molecule (Cys) in the dried system on the Ti surface.

4. Conclusions

In this work, the adsorption behavior of Cys in two different systems is presented. Raman spectroscopy was applied to obtain information about Cys at different pH levels (from pH = 7.4 to pH = 1, acidified by HCl), in the presence of PBS solutions on the Ti surface. The reference Raman spectra for free Cys were recorded under the same conditions.

In situ SERS spectra for Cys (10^{-2} M) obtained after 24 h of exposure time in an acid solution (pH = 1) on the surface of Ti indicated that the amino acid was strongly adsorbed mainly through an electron pair of the sulfur atom. Upon an increase of pH, from pH 3 to 7.4, after 24 h of exposure, the presence of the PBS solution in the investigated system drastically decreases the adsorption of Cys on the Ti surface. These findings are visible through weakening of the bands due to Cys groups, together with enhancements of the spectral signal from phosphate anions.

The ex situ SERS results, which were obtained for the same samples but in a dried system, present a completely different spectral response. The appearance of the strong band due to the disulfide (S-S) bond suggested that Cys undergoes dimerization to cystine and interacts with the Ti surface. The comparison of in situ and ex situ spectroscopic results gives new information about amino acid behavior on the Ti surface under controlled conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

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