Spectroscopic Investigations of 316L Stainless Steel under Simulated Inflammatory Conditions for Implant Applications: The Effect of Tryptophan as Corrosion Inhibitor/Hydrophobicity Marker

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Abstract: In this paper, the conformational changes of tryptophan (Trp) on the corroded 316 L stainless steel (SS) surface obtained under simulated inflammatory conditions have been studied by Raman (RS) and Fourier-transform infrared (FT-IR) spectroscopy methods. The corrosion behavior and protective efficiency of the investigated samples were performed using the potentiodynamic polarization (PDP) technique in phosphate-buffered saline (PBS) solution acidified to pH 3.0 at 37 °C in the presence and absence of 10^{-2} M Trp, with different immersion times (2 h and 24 h). The amino acid is adsorbed onto the corroded SS surface mainly through the lone electron pair of the nitrogen atom of the indole ring, which adopts a more/less tilted orientation, and the protonated amine group. The visible differences in the intensity of the Fermi doublet upon adsorption of Trp onto the corroded SS surface, which is a sensitive marker of the local environment, suggested that a stronger hydrophobic environment is observed. This may result in an improvement of the corrosion resistance, after 2 h than 24 h of exposure time. The electrochemical results confirm this statement—the inhibition efficiency of Trp, acting as a mixed-type inhibitor, is made drastically higher after a short period of immersion.

Keywords: Raman spectroscopy (RS); Fourier-transform infrared spectroscopy (FT-IR); stainless steel (SS); tryptophan (Trp); corrosion

1. Introduction

In recent years, as witnessed in a number of publications, stainless steel (SS) has been widely used as a metallic biomaterial—i.e., as a significant component of biomedical implements (i.e., dental extraction forceps, thoracic retractors), treatments for orthopedic, dental, and cardiovascular implants [1–10]. However, SS implants are the most common use for bone fracture fixation [1]. SS materials are used for these applications because of their good strength, corrosion resistance, and good biocompatibility [2,4,9,10]. The very important aspect is the cost of SS materials, the lower price in comparison to titanium alloy-based implants, for example, makes it still competitive [9]. The corrosion resistance of this material is due to the formation of a thin oxide film on the surface (of approximately a few nanometers) [11]. It protects the material from corrosion, but it also creates a very good interface with the tissue [12]. However, when metallic materials are in contact with body fluids (which contain mixtures of inorganic and organic molecules, i.e., proteins), different reactions arise on the metallic surface and can cause corrosion [13]. Many factors...
can influence the corrosion process of a material—such as the presence of chloride ions and other ions, surface topography, pH, alloying elements, etc. [14]. For example, upon implantation, the local pH around the metallic biomaterial decreases what it causes by surrounding it with fibrin and chlorine ions, for example [14–16]. Furthermore, the decrease in the surrounding pH can be associated with infections [15,17] or cancer development, which results in the phenomenon of extracellular acidification observed around the cancer cells [18].

One of the effective options for improving corrosion resistivity is the modification of the surface of the material by using inhibitors, for example, organic molecules [19,20]. Amino acids are not only essential for the human body, but can also be used as corrosion inhibitors [20]. Their use has many advantages—i.e., amino acids are nontoxic, biogenic, soluble in aqueous media—and play an important role in many physiological processes, as well as in the formation of biofilms on the surface of the implant [14,21]. One of them, tryptophan (Trp), is an essential amino acid that needs to be acquired through the diet or supplements, a precursor of important metabolites—such as niacin, serotonin, melatonin, kynurenines [22,23]. Trp has been found to have a significant effect on bone and skeletal health [24,25]. Furthermore, Trp residues are found in many antimicrobial peptides [26].

An important aspect of the biocorrosion of metallic implants is the adsorption of proteins [14]. However, protein adsorption on implants is a very complex process, and its details are not yet satisfactorily understood with respect to the mode and process of protein adsorption on metallic surfaces [14,21]. The important factor in protein adsorption is the primary structure of the protein (the sequence of amino acids). The sequence of amino acids influences the protein–surface interaction [14]. To thoroughly analyze this process, detailed studies with amino acids should first be performed.

Amino acids have previously been used to improve the corrosion resistance of different materials—such as copper, mild steel, and stainless steel [20,27–33]. Moretti and Guidi [31] obtained a good inhibition efficiency of Trp in a short time of exposition and at the highest concentration (0.01 M) for copper corrosion under acidic conditions. Fu et al. [30] analyzed the effect of amino acids, among them Trp, on the corrosion process of mild steel in hydrochloric solution using electrochemical and theoretical methods and proposed that molecules adsorb on the metallic surface through the heteroatoms and the aromatic ring. Another research group proposed that Trp inhibits the corrosion process of the AA2024 alloy and presents the best inhibition efficiency in a 1 M HCl solution at 0.008 M i.e., IE = 87% [29].

The most frequently used methods to investigate the inhibition process of amino acids are electrochemical, and less frequently, spectroscopic methods [13,34,35]. Vibrational spectroscopy is a powerful tool to monitor the corrosion and inhibition processes [28,34]. Raman and Fourier-transform infrared spectroscopic methods are usually quick, non-destructive, and do not require complicated sample preparation to obtain chemical complex information about the investigated sample. This is essential in the case of corrosion investigation [36]. It is noteworthy that Raman scattering and infrared absorption can be significantly enhanced for molecules adsorbed onto metallic surfaces [36,37]. The most commonly used surfaces are silver and gold (plasmon resonances in the visible and near-infrared region) [37]. Although, in the literature, stainless steel has also been shown to successfully improve the spectral signal [38,39]. The surface-enhanced effects are governed mainly by surface-enhanced Raman (SERS) and surface-enhanced infrared absorption spectroscopy (SEIRA). These techniques, as it was reported in our previous paper [28], are a promising tool for studying the conformational changes of potential inhibitors upon corrosion/inhibition process(es). Although the enhancement factor for SEIRA (up to $10^3$) is smaller than for SERS (usually up to $10^6$), the cross-section for infrared absorption is several times higher than the Raman cross-section [40,41].

Therefore, the above-mentioned spectroscopic methods have been used for a detailed investigation of the adsorption process of Trp, onto the corroded 316L SS surface obtained during the electrochemical tests under controlled conditions. The corrosion behavior
and protective efficiency of the investigated samples have been performed using the potentiodynamic polarization (PDP) technique in phosphate-buffered saline (PBS) solution acidified to pH 3 (e.g., simulated inflammatory conditions) at 37 °C with and without the addition of Trp after 2 h and 24 h time exposures. The characteristics of the morphology of the corroded sample surfaces, with and without Trp, have been investigated by scanning electron microscopy (SEM).

2. Materials and Methods

The samples were prepared from foil of AISI 316L stainless steel (SS) (Goodfellow Cambridge Limited, Huntingdon, Huntingdon, UK); the chemical composition of SS is listed in Table 1. The samples were machined with dimensions of 250 × 250 × 2 mm. The samples were polished with silicon carbide papers up to a 4000 grit finish. The surface was then mirror polished using 9, 3, and 1 µm diamond paste, washed with deionized water, and ultrasonically cleaned for 15 min in acetone.

| Table 1. Chemical composition of 316L SS (wt %). |
|-----------------|--|--|--|--|--|--|--|--|---|
| Steel | C | Si | Mn | Mo | Cr | Ni | P | S | Fe |
|-------|---|---|---|---|---|---|---|---|---|
| 316L  | 0.02 | 0.42 | 1.02 | 2.13 | 17.27 | 10.16 | 0.016 | 0.002 | Bal. |

The corrosion behavior of metallic samples was investigated in PBS solution (Sigma Aldrich, Poznań, Poland) pH 7.4 ± 0.01 acidified to a pH of 3.0 ± 0.01 by titration of 1M HCl (analytical grade reagent) without and with Trp (Sigma Aldrich, Poznań, Poland), concentration 10^{-2} M. Figure 1 presents the molecular structure of the investigated inhibitor [42]. The chemical composition of the PBS solution was 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride.

![Figure 1. Schematic molecular structure of Trp.](image)

Electrochemical measurements were carried out using a three-electrode electrochemical cell. SS samples were placed in a water-jacketed cell, connected to a heater (AJL Electronic, LW 502/S) as a working electrode. A graphite rod and an Ag/AgCl ([Cl^-] = 3 M) electrode were used as counter and reference electrodes, respectively. Electrochemical tests were performed with a Gamry potentiostat/galvanostat 600 (Gamry Instruments, Warminster, PA, USA). The potentiodynamic polarization (PDP) tests were carried out in a range of −0.1 V versus the corrosion potential (E_{corr}) to the potential value at which the current density exceeded 0.1 mA/cm^2 with a scan rate of 1 m/V, after holding the specimen for 2 and 24 h in the presence and absence of Trp. In each case, the experiment was performed three times. The potentiodynamic parameters were determined employing
the Echem Analyst 5.21 software. The values of the inhibition efficiency (IE\%) of Trp were calculated from the measured $i_{\text{corr}}$ values using the relationship [43]

$$\text{IE\%} = \frac{i_{\text{corr}} - i_{\text{inh corr}}}{i_{\text{inh corr}}} \times 100 \quad (1)$$

where $i_{\text{corr}}$ and $i_{\text{inh corr}}$ represent the values of the corrosion current densities without and with, the inhibitor, respectively. All electrochemical tests were performed at 37 °C temperature.

After completion of the electrochemical tests, ex-situ FT-IR and RS experiments were performed on the dried corroded samples with and without the presence of Trp. The FT-IR spectra were carried out in reflectance mode with a Nicolet iN10 MX spectrometer (Thermo Scientific, Madison, WI, USA) over the wavenumber range of 4000 cm$^{-1}$ to 800 cm$^{-1}$ with 128 scans at a resolution of 4 cm$^{-1}$. The spectrometer was equipped with a liquid nitrogen cooled MCT (Mercury–Cadmium–Telluride) detector and a permanently aligned 15× objective. FT-IR spectra were recorded from three different areas.

InVia Renishaw Raman spectrometer (Renishaw, Wotton-under-Edge, UK) equipped with a charge-coupled device (CCD) camera and the confocal microscope was used to measure the Raman scattering. The excitation wavelength was provided by the laser emitting at 532 nm 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 4000–50 cm$^{-1}$ spectral range. The spectra were measured from six points of the sample (four scans and 30 s of exposure time were enough to obtain a good signal-to-noise ratio). The 100-lens objective with a numerical aperture 0.9 was used. The Raman map was collected with a 1 µm step size (6075 acquisition, exposure time 1 s, laser power 5 mW, spectral range 1800–60 cm$^{-1}$).

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

Scanning electron microscopy (SEM) images were collected using a FEI VERSA 3D dual beam microscope (FEI Company, Hillsboro, OR, USA).

3. Results and Discussion
3.1. Electrochemical Results and Surface Characterization Using SEM and Raman Spectroscopy

Figure 2 and Table 2 show the polarization curve and the polarization parameters recorded in the presence and absence of $10^{-2}$ M Trp, after 2 and 24 h of immersion at open circuit potential. It is apparent from Figure 2 that for short immersion times (i.e., 2 h) the corrosion current density of the steel was significantly reduced after the addition of Trp to the tested solution from 13.59 to 4.89 µA cm$^{-2}$, reflecting an inhibition efficiency of 64%. The anodic branch of the sample exposed to the Trp-free solution shows a narrow activation peak before passivation, which is in contrast to the sample tested with Trp. At the active-passive region, the current density observed in the presence of Trp is lower than that observed for the blank solution—i.e., 6.95 and 10.93 µA cm$^{-2}$, respectively. The active domain corresponds to the metal dissolution (i.e., Fe in the matrix is oxidized to Fe$^{2+}$, Equation (2) [44]), and the lower current density observed for the metal in the presence of Trp is likely ascribed to its adsorption on the SS surface fully protecting its surface and hindering its dissolution before anodic passivation.

$$\text{Fe} \rightarrow \text{Fe}^{2+} + 2e^- \quad (2)$$
Figure 2. Potentiodynamic polarization curves of SS in PBS solutions with and without Trp (10^{-2} M) in aerated conditions at 37 °C and pH of 3.0.

Table 2. Polarization parameters of the SSs in PBS solution with and without Trp (10^{-2} M) for different immersion times.

| Solution   | \( E_{\text{corr}} \) (V) | \( i_{\text{corr}} \) (\( \mu \text{A cm}^{-2} \)) | \( E_{\text{pit}} \) | IE\% |
|------------|----------------------------|---------------------------------|-----------------|-------|
|            | 2 h                        |                                 |                 |       |
| Blank      | -0.449                     | 13.59                           | 0.480           | -     |
| Trp 10^{-2} M | -0.481                   | 4.89                            | 0.499           | 64    |
|            | 24 h                       |                                 |                 |       |
| Blank      | -0.526                     | 24.39                           | 0.426           | -     |
| Trp 10^{-2} M | -0.505                   | 21.06                           | 0.434           | ~1.4  |

Similar results were also reported by Talha et al. [13]. The authors studied the effect of bovine serum albumin on the corrosion behavior of 316L in PBS solution at pH = 7.4. From potentiodynamic measurements, they observed an inhibition efficiency of 23% and 32% after 1 h and 7 h of immersion, respectively. The higher value of IE% observed in this study after 2 h is likely due to the pH of the tested solutions. Inhibitors with amino groups in their structure have been reported to yield high values of IE% in acid environments [27,32,45]. In aqueous acid environments, the amino-inhibitor exists either as neutral molecules or in the form of polycation [27,32,45] according to the equation

\[
\text{Inh} + x\text{H}^+ \rightarrow [\text{InhH}_x]^{+}_{\text{sol}}
\]

where [InhH\(_X\)]\(^{+}\)\(_{\text{sol}}\) is the protonated inhibitor in the solution.

In an acid environment, the surface of the steel is positively charged [27,32,45] which leads to the chloride ions present in the solution being electrostatically adsorbed on its surface.

\[
\text{Cl}_2^{-\text{sol}} + \text{H}_2\text{O}_{\text{ads}} \rightarrow \text{Cl}_{\text{ads}}^{-} + \text{H}_2\text{O}_{\text{sol}}
\]

As a result of this process, the charge at solution/interface changes from positive to negative and therefore assists the adsorption of the inhibitor. The spectroscopy analysis reported in this study (see Section 3.2.) showing that Trp is mainly adsorbed on the metal surface via the amino groups suppressing the hydrogen evolution and the dissolution of the metal.

It can be seen from Figure 2 that the corrosion potential in the presence of Trp is slightly shifted to a more negative value, compared to the one observed in the blank solution. It is generally assumed that after the addition of the inhibitor, if the corrosion potential shifts less
than 85 mV, with respect to the corrosion potential of the blank solution, the inhibitor can be regarded as a mixed-type inhibitor [46]. In this study, the shift between the two corrosion potentials is only 22 mV, indicating that Trp acts as a mixed-type inhibitor. However, it is worth mentioning that previous studies on the corrosion effect of Trp in strong acid solution and different metals (e.g., copper, low carbon steel alloys) found that corrosion acted more as a cathodic inhibitor than an anodic inhibitor [27,31,33]. The value of pitting potential \( (E_{\text{pit}}) \) observed in the presence of Trp is slightly higher than the one observed for the blank solution of circa 20 mV (i.e., 0.499 and 0.480 V). The RS spectrum collected from corrosion pits (Figure 3, inset e) indicated that the observed spectral feature corresponds mainly to the mixture of iron hydroxides \((\delta-\text{FeOOH}—1320 \text{ cm}^{-1}; \text{Fe(OH)}_2—408 \text{ cm}^{-1})\) and iron \((\text{FeO and } \gamma—\text{Fe}_2\text{O}_3—655 \text{ cm}^{-1}; \text{FeO and/or } \gamma—\text{Fe}_2\text{O}_3—289 \text{ cm}^{-1}, \alpha—\text{Fe}_2\text{O}_3—223 \text{ cm}^{-1})\) and chromium \((\alpha—\text{Cr}_2\text{O}_3—550 \text{ cm}^{-1})\) oxides which are characteristic for corrosion pits [47,48]. SEM analysis (Figure 3a,b) carried out after the polarization measurements showed that the sample exposed to Trp displays a much smoother surface compared to the Trp-free solution.

As time increases, the corrosion resistance of the steel drops significantly, with and without the inhibitor. In the presence of Trp, the corrosion current density is slightly lower than the one observed in the blank solution (i.e., 33.81 and 42.81 93 \( \mu \text{A cm}^{-2} \), respectively). The polarization curves show a wider active domain and the passivation width is reduced with respect to the ones observed at short immersion times. The pitting potential is around 60 mV lower at higher immersion times, reflecting an increase in the number of pits on the metal surface as shown in Figure 3c,d. Talha and co-author [13] also reported a significant drop in IE% after higher immersion times. After 24 h the authors reported that the tested inhibitor (e.g., bovine serum albumin) even had a detrimental effect and increased the corrosion rate of the metal. The literature reported [49–51] that the decrease in IE% after higher immersion times in acid solutions is very common and may be ascribed to the instability of the adsorptive film or/and the desorption of the adsorbed inhibitor from the metal surface due to the weak physical interactions between the inhibitor and the electrode surface.

### 3.2. Spectroscopic Results of the Surface Adsorption Geometry of Tryptophan on Corroded 316L Stainless Steel

Before analyzing the adsorption geometry of Trp on the corroded SS surface, the RS and FT-IR spectra of nonadsorbed Trp were interpreted. The reference RS and FT-IR spectra of the dried Trp solution \( (10^{-2} \text{ M Trp/PBS, pH 3.0}) \) are shown in Figures 4a and 5a, respectively. The proposed band assignments were based on many theoretical and experimental data from the literature on the molecular structure of Trp and Trp derivatives.
obtained under various conditions [42,52–66]. As mentioned in our previous paper [28], the preparation of samples for experiments may affect the information obtained from spectral patterns (i.e., the state of the sample condition, pH of the surrounding environment). Therefore, the same sample preparation procedure was applied in all experiments. Table 3 summarizes the wavenumber and proposed band assignments with full width at half maximum (FWHM) of the selected vibrational modes of the studied samples.

![Figure 4](image1.png)

**Figure 4.** (a) RS spectrum of Trp (10^{-2} M/PBS/pH 3); (b) SERS spectra of Trp (10^{-2} M), 2 h exposure time; and (c) Trp (10^{-2} M), 24 h exposure time adsorbed onto the corroded SS surface. Measurement conditions: spectral range, 1800–400 cm^{-1}; laser line, 532 nm (d) Inset d: the map of intensity ratio (I_{1357}/I_{1338}).

![Figure 5](image2.png)

**Figure 5.** (a) FT-IR spectrum of Trp (10^{-2} M/PBS/pH 3.0); (b) SEIRA spectra of Trp (10^{-2} M), 2 h of exposure time; and (c) Trp (10^{-2} M), 24 h of exposure time adsorbed onto the corroded SS surface. Measurement conditions: spectral range, 1800-1000 cm^{-1}, MCT detector. Inset (d): the schematic orientation of Trp onto the SS in a corrosive environment.

The RS spectrum of the nonadsorbed molecule (Figure 4a) under acidic conditions (PBS/pH 3.0) is dominated by the characteristic modes of the indole (Ind) moiety (pyrrole (P) and benzene (B) rings). The most characteristic RS bands are observed at 1575 cm^{-1} [\nu(CC)_{ind}], 1552 cm^{-1} [\nu(CC)_{ind(P)} + \delta(CH)_{ind(P)} \text{ i.p.}], 1424 cm^{-1} [\delta(HN)_{ind(P)} \text{ i.p.}], 1360 cm^{-1} [\nu(CC)_{ind} (doublet component)\text{ i.p.}], 1009 cm^{-1} [\nu(CC)_{ind(B)} \text{ breath.}], and the most intense at 757 cm^{-1} [\nu(CC)_{ind breath.}] (see Table 3). In analogy, in the FT-IR spectrum of (Figure 5a), indole ring vibrations can also be noticed (1616, 1541, 1490, 1458, 1357, 1339, 1234 and 1009 cm^{-1}).

The SERS and SEIRA spectra (see Figures 4 and 5) obtained for dried samples of Trp (10^{-2} M Trp/PBS, pH 3.0) adsorbed onto the corroded SS surface obtained after PDP tests after 2 and 24 h exposure times, present clear differences in the relative intensity, position, and width of wavenumber corresponding to the RS and FT-IR spectra of Trp, respectively.
One of the most visible differences appears in the intensity ratio of the two bands at 1357 and 1338 cm\(^{-1}\) in the SERS spectrum obtained for the Trp with a concentration of 10\(^{-2}\) M adsorbed on the corroded SS surface after 2 h of immersion time (Figure 4b) compared to the RS spectrum of the free molecule. It can be observed that the band at 1357 cm\(^{-1}\) becomes more intense than the band at 1338 cm\(^{-1}\) onto the corroded SS surface. The Raman modes of the Trp functional group are very sensitive to, i.e., the conformation and hydrophobicity of the environment surrounding the indole ring [52,63]. The Trp doublet in the 1360–1338 cm\(^{-1}\) spectral range has been reported to arise from the Fermi resonance between the fundamental in-plane N–C stretching band and combinations of the indole ring out-of-plane vibrations [52,62,66].

The intensity ratio of the above-mentioned doublet in Raman, as well as in the SERS spectra, is believed to be a sensitive probe of the local environment of the indole ring [52,62]. The intensity ratio of the high-wavenumber band to the low-wavenumber band of the Fermi doublet (I\(_{1360}/I_{1339}\)) is a marker of the hydrophobicity of the environment [52,62]. In the SERS spectrum obtained for Trp adsorbed on the corroded metallic surface after 2 h (see Figure 4b), it is observed that the band at 1357 cm\(^{-1}\) is the most enhanced band (the comparable relative intensity among the spectrum) and becomes dominant compared to the component at 1338 cm\(^{-1}\) (I\(_{1357}/I_{1338} = 1.8\)) which can indicate the presence of a hydrophobic surrounding during the adsorption process of Trp onto the metallic surface under acidic conditions obtained after PDP tests (Section 3.1). Interestingly, the band at 1357 cm\(^{-1}\) becomes narrower, and the bandwidth described as FWHM is smaller compared to the Raman spectrum of the nonadsorbed molecule (\(\Delta\text{FWHM} = -7\text{ cm}^{-1}\)). The intensity map I\(_{1357}/I_{1338}\) (Figure 4, inset d) confirms that the 1357 cm\(^{-1}\) band dominates on the corroded surface and the above-mentioned ratio reaches high values. The color legend in the present map indicates high intensity in red and low intensity in blue. This observation may be a result of the formation of a strong hydrophobic microenvironment in close proximity to the Trp ring. The presence of hydrophobic properties of the metallic surface can provide an improvement in corrosion resistance [67–69]. It should be noted that the inhibition efficiency after 2 h of immersion was 64% and decreased drastically after a longer immersion time (24 h, IE about 1.4%, see Table 2).

The SERS spectrum obtained for Trp onto the corroded SS surface after 24 h immersion time presents the same correlation between bands due to the Fermi resonance, however, the ratio (I\(_{1359}/I_{1338} = 1.4\)) reaches the values smaller than in the case for the result obtained after 2 h. In addition, significant decreases in the relative intensity of these bands are observed after a longer time of exposition in the aggressive environment. The observation of the subtle changes in the recorded spectra suggested that Trp seems to be a sensitive marker of the hydrophobic properties of the SS surface.

Furthermore, the appearance of a band around 1552 cm\(^{-1}\) (Figure 4a) is classified by Takeuchi et al. [52] and Niaura et al. [63] as a sensitive marker of the side chain conformation. The position of this band strongly depends on the torsional angle (\(\chi^{2,1}\)) about the C\(_2\)C\(_3\)C\(_{\beta}\)–C\(_{\alpha}\) linkage of the Trp side chain (see Figure 1). Takeuchi et al. [70] proposed that the upshift of this band indicates an increase in the \(\chi^{2,1}\) angle, which implies that the C\(_{\alpha}\) atom can be moved away from the C\(_2\) atom. It is worth noting that, in the SERS spectrum of adsorbed Trp on the corroded SS surface after 2 h, the band at 1556 cm\(^{-1}\) exhibits a higher relative intensity, blue-shift (\(\Delta\nu = 4\text{ cm}^{-1}\)) and a narrower shape (\(\Delta\text{FWHM} = -3\text{ cm}^{-1}\)) compared to the Raman spectrum of the free molecule.

Interestingly, a completely different direction of changes of the band at 1550 cm\(^{-1}\) is visible in the SERS spectrum of Trp on the corroded SS surface after 24 h of immersion. This spectral characteristic shows significant enhancement (the most intense band in the spectrum), slight red-shift (\(\Delta\nu = 2\text{ cm}^{-1}\)), and broadening (\(\Delta\text{FWHM} = 2\text{ cm}^{-1}\)) compared to those observed in the normal RS spectrum of the nonadsorbed molecule. These changes can be ascribed to the decreased C\(_2\)C\(_3\)C\(_{\beta}\)–C\(_{\alpha}\) torsional angle and the stronger interaction between the nitrogen pyrrole of the indole ring for the sample obtained after the PDP test performed after 24 h. The changes in the \(\chi^{2,1}\) angle can be provided by a stronger interaction
between the protonated amine group and the corroded SS surface. The appearance of broad vibration modes at 1643, 1617 and 926 cm\(^{-1}\) can confirm this statement (see Table 3 for appropriate band assignments).

Based on the clear changes in the band that appear below 880 cm\(^{-1}\) during the adsorption process of Trp on the corroded SS surface (Figure 4b,c) with respect to the ordinary RS spectrum, it can be concluded that there are hydrogen bonding interactions between the indole ring and the metallic surface [52,53,62–64]. This spectral feature, which can be assigned to the NH group deformation, exhibits a higher relative intensity, red-shift (\(\Delta\nu = 3—1\) cm\(^{-1}\)), and substantial broadening (\(\Delta\text{FWHM} = 10—16\) cm\(^{-1}\)) compared to those observed in the normal RS spectrum. These changes indicate the strong interaction at the NH site of the pyrrole ring. Takeuchi et al. [53] proposed that the downshift of this band indicated an increasing strength of the H-bonding interaction. Furthermore, the appearance of a medium intensity band at ~1423 cm\(^{-1}\) (more strongly enhanced than in the ordinary Raman spectrum), corresponding to the indole ring NH group, confirms that the pyrrole ring interacted with the corroded surface of the SS. It should be noted that the SERS spectrum obtained for samples prepared after 24 h of time exposure exhibits substantial broadening (\(\Delta\text{FWHM} = 9\) cm\(^{-1}\)) compared to those observed in the normal RS spectrum of the nonadsorbed molecule and the SERS spectrum recorded for Trp on the corroded surface of the SS obtained after 2 h. The NH moiety of the indole ring is a proton donor, which provides the formation of strong hydrogen bonds with the corroded SS in the presence of chloride ions.

Changes in the SERS spectral pattern of the vibration mode, due to the Trp ring compared to the Raman spectrum of the free molecule (see Figure 4a), indicated that the reorientation of the indole ring is observed upon adsorption onto the corroded metallic surface. In general, there are two mechanisms that provide a large enhancement of the Raman signals: an electromagnetic mechanism (the molecule is adsorbed or is located in close proximity to the metal surface), and a chemical mechanism (the molecule binds chemically to the substrate) [71]. Based on the electromagnetic surface selection rules, the comparison of the relative intensities of bands at ~1007 cm\(^{-1}\) and ~755 cm\(^{-1}\) can be a probe of the indole ring orientation on the metallic surface [62,72]. The first vibrational mode is due to the breathing mode of the indole benzene ring and the second arises from the in-phase breathing mode of the indole ring, respectively (see Table 3) [52,62,72]. Significant reduction in the relative intensity of the band at ~755 cm\(^{-1}\) and slight red-shift (\(\Delta\nu = 1—2\) cm\(^{-1}\)) compared to the Raman spectrum suggested that the indole ring of Trp adopts a more or less tilted orientation with respect to the corroded SS surface. However, for the SERS spectrum of Trp on the corroded SS surface obtained after 24 h, a slightly stronger interaction between the indole ring (\(\Delta\text{FWHM}_{1008} = 9\) cm\(^{-1}\)/\(\Delta\text{FWHM}_{756} = 2\) cm\(^{-1}\)) can be proposed.

The SEIRA signals present (Figure 5b,c) the noticeable changes in the 1700–1300 cm\(^{-1}\) spectral region in comparison to the FT-IR spectrum of the free molecule (see Table 3 for detailed band assignment). It is worth noting, that the separation of two bands with maxima at 1666 and 1591 cm\(^{-1}\) (in the FT-IR spectrum a broad band with a maximum at 1616 cm\(^{-1}\)) is observed (see Figure 5a and Table 3). These spectral features can be correlated with the \(\delta_{\text{as}}(\text{NH}_3^+\)) and \(\nu(\text{CC})_{\text{Ind}}\) group oscillations, respectively.

The appearance of a separated band at 1666 cm\(^{-1}\) may be evidence that the protonated amine group is close to the corroded metallic surface of the SS. It should be emphasized that, in general, the amine groups are not strongly enhanced in the Raman spectra in the 1700–500 cm\(^{-1}\) region [73], which can be an explanation as to why the bands of the terminal amine group, although visible in the SERS spectra, are not the dominant spectral signals (see Figure 4b,c).
Table 3. Proposed band assignments and FWHM for RS, FT-IR, SERS, and SEIRA spectra of Trp (10⁻² M) adsorbed onto the corroded SS surface.

| Band Assignments | RS       | FWHM (cm⁻¹) | FT-IR    | FWHM (cm⁻¹) | SERS 2 h/(24 h) | FWHM (cm⁻¹) | SEIRA 2 h/(24 h) | FWHM (cm⁻¹) |
|------------------|----------|-------------|----------|-------------|----------------|-------------|-----------------|-------------|
|                  | ν(C=O)   | 1721 w      | 7        | 1717 w      | 51             |             | 8 (24)          | 1666 s/(1666 s) | 16/(17)     |
|                  | δ as(NH₃⁺) |             |          |             |                |             | 1654 vυ/(1643 vυ) |             |             |
|                  | ν(CC)₇₈   | 1575 m      | 10       | 1579 m      | 11 (9)         |             | 1575 m/(1574 m) | 11 (9)      |             |
|                  | δ(CH) ind | 1552 m      | 15       | 1541 w      | 14             |             | 1556 s/(1550 vs) | 12 (17)     |             |
|                  | ν(CC) ind | 1487 w      | 8        | 1490 m      | 32             |             | 1486 w/(1486 w) | 12 (22)     |             |
|                  | δ(CH) ind | 1457 m      | 9        | 1458 m      | 8              |             | 1456 w/(1456 w) | 12 (19)     |             |
|                  | δ(CH) ind | 1424 m      | 12       | 1411 m      | 15             |             | 1423 m/(1424 m) | 12 (21)     |             |
|                  | ν(CC) ind | 1360 m      | 17       | 1357 sh     | 10             |             | 1357 vs/(1359 s) | 10 (15)     | 1356 s/(1355 s) | 15 (17)     |
|                  | δ(CH) ind | 1339 m      | 13       | 1339 m      | 27             |             | 1338 m/(1338 m) | 11 (22)     |             |
|                  | δ(CH+CH₂) | 1231 m      | 12       | 1234 w      | 19             |             | 1232 w/(1228 w) | 10 (23)     |             |
|                  | δ(CH) ind | 1208 w      | 12       | 1208 w      | 12             |             | 1208 w/(−/−)   | 12 (−/−)    |             |
|                  | ρ(CH) ind | 1132 w      | 13       | 1156 w      | 12             |             | 1153 w/(1154 w) | 20 (23)     |             |
|                  | δ(CH+CH₂) | 1118 w      | 13       | 1125 w      | 12             |             | 1125 w/(1125 w) | 20 (23)     |             |
|                  | δ(NH₃⁺)  | 1074 vυ     | 9        | 1096 w      | 16             |             | 1078 vs/(1078 vυ) | 15 (22)   | 1099 w/(1098 w) | 31 (12)     |
|                  | ν(CC) ind | 1009 s      | 8        | 1009 w      | 8              |             | 1007 s/(1008 s) | 9 (10)      | 1007 sh/(1008 vυ) | 10 (9)      |
|                  | ρ(CH) ind | 896 vυ      | 18       | 876 w       | 6              |              | 837 m/(875 m)  | 16 (22)     |             |
|                  | δ(CO) ind | 862 w       | 10       | 843 vυ      | 23             |              | 838 m/(838 w)  | 23 (23)     |             |
|                  | δ(CH) ind | 757 vs      | 7        | 755 s       | 16              |              | 755 s/(756 s)  | 10 (16)     |             |
|                  | ν(CC) ind | 701 w       | 6        | 705 w       | 13              |              | 705 w/(708 w)  | 13 (24)     |             |
|                  | δ(O) ind  | 572 w       | 8        | 624 w       | 18              |              | 624 w/(625 w)  | 18 (23)     |             |

Abbreviations: ν—wavenumber; w—weak; vυ—very weak; m—medium; s—strong; vs—very strong; sh—shadow; r—stretching; δ—deformation; s, as—symmetric and asymmetric; ρ b—bending; ρ r—rocking; ρ w—wagging; i.p.—in plane, oop—out of plane, breath.—breathing; ind—I—indole moiety, Ind(P)—pyrrole in the indole moiety, Ind(B)—benzene ring in the indole moiety, F—origin of doublet due to Fermi resonance.

In a way that is analogous to the SERS spectra (Figure 4b,c), among the SEIRA spectra (Figure 5b,c), the characteristic vibration modes due to the oscillations of the indole ring are noticed. These spectral features are observed at 1591 cm⁻¹, ~1415 cm⁻¹, and ~1356 cm⁻¹ (see Table 3 for proposed band assignments). In addition, the significant red-shift of band at 1591 cm⁻¹ can be a suggestion that the strong interaction between the indole ring (via NH group) and the corroded metallic surface affects the adsorption process of Trp. The above-mentioned observation allows us to conclude that the adsorption between the corroded SS surface can occur through the lone electron pair of the nitrogen atom of the indole ring which adopts a more/less tilted orientation to the surface and the protonated amine group. Figure 5, inset d, presents the schematic orientation of Trp on the corroded SS surface.

4. Conclusions

This study presents the comprehensive investigation behavior of Trp on the corroded 316L stainless steel obtained under controlled conditions (PBS/pH 3.0/37°C). The presence of Trp in the aggressive solution has a strong influence on the metallic surface. The electrochemical results indicated that the amino acid which acts as a mixed-type bioinhibitor has shown good inhibiting properties for SS in an acidic solution. The corrosion inhibition efficiency has been found to be 64% after 2 h of immersion. However, the longer time of exposure to corrosive solution results in a drastically decrease in IE%. SEM images of the corroded surface with the presence of 10⁻² M Trp obtained after 2 and 24 h of exposure times confirm these observations. The addition of Trp in the corrosive solution led to a smaller visible amount of pitting on the surface after 2 h than that found after 24 h.
Spectroscopic results obtained by complementary RS and FT-IR methods indicate that Trp is strongly adsorbed on the corroded SS surface. Visible changes in the relative intensity, width, and wavenumber of Trp bands upon adsorption on the corroded SS surface compared to ordinary RS and FT-IR spectra have been observed. It has been shown that the SS surface enhanced the spectral signal. In an acid environment, at pH 3.0 (for Trp, pK1 and pK2 are 2.38 and 9.34, respectively), the terminal amine group of Trp is protonated, which results in the adsorption process of this terminal fragment onto the metallic surface in the presence of chloride ions. In addition, Trp strongly interacts with the corroded metallic surface of the NH moiety of the indole ring (adopts a more/less tilted orientation), and the hydrogen bond formation is observed. Trp adsorbed onto the corroded SS surface after 2 h and 24 h of exposure times. However, the significant changes in Fermi doublet (I~1360 / I~1339) which appear to be a good marker of hydrophobicity of the surrounding environment, appear for Trp adsorbed onto the corroded SS surface during the time of exposure in an aggressive environment. The improvement in corrosion resistance of metallic surfaces may be due to the existence of stronger hydrophobic properties of the SS surface after 2 h than after 24 h, which reflects in the enhancement of the band at 1357 cm−1 on the corroded surface after 2 h of exposure. However, the instability of the adsorptive film of the adsorbed inhibitor on the SS surface after a longer time of immersion cannot be excluded. The complex electrochemical and spectroscopic analysis of the behavior of Trp on the surface of the SS allowed for a better understanding of the amino acid interaction under simulated inflammatory conditions.

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