Spectroelectrochemical Investigation of the Interaction of Adenine with Pyridoxine at Physiological pH

Yasmin Roye, 1 Uche Udeochu, 1 Maraizu Ukaegbu, 2 and Jonathan Onuegbu 2

1Department of Natural Sciences, University of Maryland Eastern Shore, 1 Backbone Rd., Princess Anne, Maryland 21853, USA
2Department of Chemistry, Howard University, 525 College St. NW, Washington, DC 20059, USA

Correspondence should be addressed to Uche Udeochu; charlesudeochu@yahoo.com

Received 17 March 2019; Accepted 3 June 2019; Published 30 July 2019

Academic Editor: Rizwan Hasan Khan

Copyright © 2019 Yasmin Roye et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Spectroelectrochemical techniques were used to probe the interaction of adenine with pyridoxine at pH 7.0. Analysis of UV-visible absorption of the adenine-pyridoxine complex at 260 nm using the Lineweaver–Burk double reciprocal plot produced a linear graph indicating a 1:1 mode of interaction between the compounds and a binding constant of 29.1. Change in the background current and broadening of adenine and pyridoxine cyclic voltammetry (CV) oxidation peaks at 1.0 V and 0.8 V, respectively, compared to the CV of the individual compounds is indicative of an interaction. Raman shift of the coupled –C(11)H2-OH bending and in-plane N(7)-H mode at 1235 cm$^{-1}$ to 1215 cm$^{-1}$ of pyridoxine and the shift to the lower wavenumber of the adenine –N(10)H2 rocking band from 942 to 906 cm$^{-1}$ confirm that the adenine exocyclic amino group and its purine nitrogen atom N(7) interacts with pyridoxine O(12) via the formation of hydrogen bonds. Strong enhancement of surface-enhanced Raman spectroscopy (SERS) bands pertaining to adenine and the bathochromic shift of the normal Raman band due to the adenine ring breathing mode observed at 722 cm$^{-1}$ in the spectrum of adenine, to 732 cm$^{-1}$ in the SERS spectrum of aqueous adenine-pyridoxine indicates that the complex adsorbs onto the Ag nanoparticle surface with the adenine portion possessing a perpendicular orientation.

1. Introduction

The interaction of deoxyribonucleic acid (DNA) with small molecules has remained a subject of interest in many biochemical studies owing to its role in many cellular activities [1–3]. The obvious starting point for understanding the activity of DNA is the study of the components of its structure. Adenine (Figure 1(a)), one of the components of DNA, participates significantly in many life processes of the human body. It is involved in cellular respiration and protein synthesis, and its interaction with various molecules (as in its pairing with thymine within the DNA double helix and uracil in RNA) is essential for the transfer and expression of genetic materials and stabilization of the nucleic acid structure [4–7]. Furthermore, the level of adenine in the human body can serve as a marker for the body’s susceptibility to metabolic disorders and diseases. Therefore, understanding the kinetic characteristics, structure, and conformational behavior of adenine under physiological conditions especially in its interaction with small molecules is essential for understanding biochemical processes and drug discovery involving DNA nucleobases.

In recent years, there has been growing interest in characterizing how DNA interacts with small molecules using spectroelectrochemical techniques and this is with respect to establishing interaction models. Models relating to electrostatic interaction and hydrophobic binding within the minor groove and intercalation between stacked base pairs have been proposed for the interaction of small molecules with DNA [3, 8, 9]. Base groups on DNA exhibit supramolecular interaction with pyridoxine (Figure 1(b)) via the formation of hydrogen bonding. The choice of pyridoxine, a 4-methanol derivative of vitamin B6, as a small molecule for this interactional study is due to its presence as active ingredients in various food, dietary supplements, and drugs. The deficiency of pyridoxine in the human body has been...
linked to anemia, nerve damage, skin problems, and various neurological disorders [10, 11]. The ease of pyridoxine absorption within the small intestine makes it readily available for important roles in red blood cell regeneration, nervous and immune systems, and food regulatory functions. Pyridoxine is transformed to pyridoxal phosphate, a coenzyme for synthesis of amino acids, neurotransmitters, and sphingolipids in biological systems [12, 13].

Spectroelectrochemical methods have been shown to be sensitive for characterizing DNA interaction with small molecules [8, 14, 15] and for confirming DNA-pyridoxine interaction [8]. However, in this study, an in-depth investigation of the binding characteristics of adenine with pyridoxine using cyclic voltammetry, UV-visible spectra, and Raman spectroscopy is explored to gain better understanding on the binding characteristics between the two compounds at the molecular level. Adenine and pyridoxine are electrochemically active [8, 16–20]. The relevance of the Raman effect of adenine in DNA studies cannot be over emphasized because of the sensitivity of its vibrational modes in SERS studies [21, 22]. The SERS characteristic of pyridoxine adsorbed on Ag colloidal particles has been examined in aqueous solution, and the results have shown that pyridoxine exhibits a pH-dependent orientation on the Ag surface with a parallel or tilted orientation suggested as a more probable conformation [23]. In this work, changes in the UV-visible absorbance property of the adenine and pyridoxine system are used as the basis for determining the binding constant between the compounds at the physiological hydrogen ion condition. The interaction is investigated in aqueous solution using CV and Raman spectroscopy with the aim of identifying potential changes and vibrational modes associated with the interaction. Furthermore, SERS is used to probe the adenine-pyridoxine interaction on Ag nanoparticles to determine possible orientation of the complex on the surface. Experimental Raman and SERS data are analyzed with reference to computed fundamental and assigned bands of adenine and pyridoxine in literature.

2. Experimental

2.1. Chemicals. Adenine and pyridoxine were obtained from Sigma-Aldrich and used with no further purification. Sodium phosphate buffer solution prepared in deionized water was used to keep the pH value at 7.0, while buffer-electrolyte solution containing 0.1 M phosphate buffer and 5 mM NaCl supporting electrolyte at pH of 7.0 were prepared from analytical grade reagents and distilled deionized water for electrochemical measurements. A small amount of aqueous solution of adenine-pyridoxine was dropped on the Ag colloid that was prepared according to the procedure reported in an earlier study [24].

2.2. Spectroscopic Measurements. Solutions of adenine and pyridoxine of different concentrations meant for UV-visible measurements were prepared from stock solutions of adenine (0.01 mol/L) and pyridoxine (0.01 mol/L) prepared in the sodium phosphate buffer solution. Samples were scanned between 200 and 800 nm using a UV-visible light DU 800 Spectrophotometer equipped with a quartz cuvette of 1 cm path length. The maximum absorbance of adenine was plotted against various concentrations (1.00 × 10⁻³ M–6.00 × 10⁻⁷ M) at pH 7.0 at fixed wavelength. For the determination of the binding constant of adenine-pyridoxine, the Lineweaver–Burk double reciprocal plot, which is a linear transformation of the Michaelis–Menten equation, is especially useful. By using a method similar to what was used by Charak et al. [25], the concentration of adenine was kept constant (1 × 10⁻⁷ M) with the concentration of added pyridoxine varied between 4.00 × 10⁻³ and 4.00 × 10⁻⁴ M. The data obtained were treated according to the following equations:

Adenine + pyridoxine ⇌ adenine pyridoxine

\[ K = \frac{\text{adenine} - \text{pyridoxine}}{\text{adenine uncomplexed} \times \text{pyridoxine uncomplexed}} \]

\[ \frac{A_0}{A - A_0} = \frac{\epsilon_A}{(\epsilon_{AP})(K)[\text{pyridoxine}]} + \frac{\epsilon_A}{\epsilon_{AP}} \]

where \( A_0 \) is the absorbance of adenine at its absorption maxima, in the absence of pyridoxine, \( A \) is the absorbance of adenine observed after adding different concentrations of pyridoxine to it, and \( A \) and \( AP \) are the molar extinction coefficients of adenine and adenine-pyridoxine, respectively. By plotting \( 1/(A - A_0) \) versus \( 1/[	ext{pyridoxine}] \) and obtaining a linear graph, a 1:1 stoichiometry between the two compounds is justified. The binding constant \( (K) \) for the interaction is deduced from the ratio of the intercept to the slope.
2.3. Electrochemical Measurements. For the CV, stock solutions of adenine (1.0×10⁻³ mol/L) and pyridoxine (1×10⁻² mol/L) were prepared using the pH 7 electrolyte-buffer solution. Electrochemical measurements were performed on a Princeton potentiostat/galvanostat Model 263 A. A three-electrode system incorporating a 1.5 mm-diameter glassy carbon working electrode, platinum wire counter-electrode, and a standard calomel electrode (SCE) reference was employed. The glassy carbon electrode (GCE) was polished with alumina oxide (particle size 0.3 μm) before every electrochemical assay, and for every GCE cleaning sequence, the electrode was immersed in solutions of distilled deionized water and sonicated for 5 minutes.

2.4. Raman and SERS Measurements. Raman spectra of saturated solutions of adenine, pyridoxine hydrochloride, and adenine-pyridoxine and the SERS of 1.0×10⁻³ M adenine-pyridoxine in the sodium phosphate buffer were recorded using a DeltaNU micro-Raman microscope having 633 nm laser line and a laser power of 200 mW. SERS measurements of adenine-pyridoxine were accomplished by first dropping 1.0×10⁻³ M solution of adenine-pyridoxine on Ag nanoparticles and then recording with the micro-Raman instrument. The 633 nm laser line of an Ar ion laser was employed for the acquisition of data. The resolution of the instrument is 2 cm⁻¹, and measurements were performed at room temperature. Ag nanoparticles were prepared according to the procedure reported in literature [24, 26].

3. Results and Discussion

3.1. UV-Visible Spectral Studies on the Adenine-Pyridoxine Complex. The UV-visible absorption spectra of adenine, pyridoxine, and the adenine-pyridoxine complex were measured to preliminarily investigate the extent of the interaction of adenine with pyridoxine. In the UV-visible spectrum of adenine shown in Figure 2, there is a presence of an absorption peak at 260 nm. In literature, the presence of this peak in the UV-visible spectrum of DNA has been attributed to presence of adenine and guanine [27]. Beer’s plot of absorbance versus concentration of adenine at λ_{max} yielded a linear graph (R² = 0.9818) with a molar absorptivity of 7.710×10⁻⁴ M⁻¹ cm⁻¹ (Figure 3). The UV-visible absorption spectrum of pyridoxine between 240 and 280 nm has been reported since this is the wavelength range of interest for the double reciprocal plot. However, in this study, it was found that pyridoxine absorbs at 252 and 323 nm as reported in literature [28]. The spectrum of the pyridoxine absorption band at 252 nm is shown in Figure 4. The absorption of UV-visible light by pyridoxine increases with concentration, and Beer’s plot of pyridoxine at 252 nm shows that the molar absorptivity of pyridoxine is 6.800×10⁻⁴ Lmol⁻¹ cm⁻¹.

UV-visible spectra recorded for adenine-pyridoxine (Figure 5) show that adenine absorbance at 260 nm increases with pyridoxine concentration. Performing a double reciprocal plot using absorbance maxima values of adenine, that is, at wavelength of 260 nm as shown in Figure 6, generates a linear graph with R² = 0.9138. The linearity of the graph indicates that the binding of adenine to pyridoxine is characterized by a 1:1 stoichiometric relationship. Computing the ratio of intercept to slope as determined from Figure 6, the binding constant is estimated as 29.1. This value

![Figure 2: UV-visible absorption spectrum of aqueous solution of adenine at pH 7.0: (a) 1×10⁻³ M; (b) 2×10⁻³ M; (c) 3×10⁻³ M.](image1)

![Figure 3: Plot of absorbance versus concentration of aqueous solution of adenine at 260 nm, pH 7.0, and R² = 0.9818.](image2)

![Figure 4: UV-visible absorption spectrum of aqueous solution of pyridoxine at pH 7.0. The lowest to the highest spectrum corresponds with (a) 4.0×10⁻⁵ M, (b) 5.0×10⁻⁵ M, (c) 2.0×10⁻⁶ M, (d) 3.0×10⁻⁴ M, and (e) 4.0×10⁻⁴ M pyridoxine.](image3)
suggests a moderate interaction between adenine and pyridoxine.

3.2. Electrochemical Study of Adenine-Pyridoxine Interaction. Figure 7 presents the CV electrochemical activity of adenine (1.0 × 10^{-3} M) in the sodium chloride electrolyte/sodium phosphate buffer (pH 7.0) on the glassy carbon electrode (GCE). In the forward scan at a scan rate of 0.05 V/s, there is a one-step anodic peak at 1.0 V (versus SCE) corresponding with the oxidation of the hydroxyl group on adenine. The absence of CV peaks in the reverse sweep is an indication that the oxidation of adenine is irreversible. The CV recorded at different potential scan rates (0.05–0.7 V/s) shows an increase in the peak current with scan rate, and by plotting the peak current against the square root of the scan rate, a linear graph was generated. (inset in Figure 7, $R^2$ = 0.9979). This is typical of a diffusion-controlled electrochemical oxidation.

A broad irreversible oxidation peak at 0.8 V (versus SCE) is a characteristic of the CV of pyridoxine (2.0 × 10^{-2} M) in sodium chloride electrolyte-sodium phosphate buffer (pH 7.0) on GCE (Figure 8). A linear plot (inset in Figure 8, $R^2$ = 0.9979) of peak current versus square root of scan rate showed that the oxidation of pyridoxine is diffusion controlled. Other authors observed similar electrochemical behavior for pyridoxine on carbon electrodes stating that it is oxidized via a one-electron diffusion-controlled process [29–32].

Further investigation of the adenine-pyridoxine interaction at pH 7.0 performed by preparing a solution containing adenine and pyridoxine (1.0 × 10^{-3} M and 1.0 × 10^{-2} M, respectively) on GCE at 0.1 V/s (Figure 9) showed peaks associated with the individual compounds were present in the CV of the complex. Very noticeable is the imprecise nature and broadness of the adenine and pyridoxine peaks. Also obvious is the slight shift to the higher potential of the adenine peak at 1.2 V from 1.0 V.

3.3. Raman Study of the Interaction of Adenine with Pyridoxine. Figure 10(a) shows the Raman spectrum of adenine at 633 nm excitation. The Raman vibrational assignment of adenine vibrational modes has been reported in the literature [21, 33]. In this study, the observed bands in the Raman spectrum of adenine are assigned according to literature report and are presented in Table 1. The four most prominent bands in the Raman spectrum of adenine are 722, 1250, 1335, and 1485 cm\(^{-1}\). The Raman band at 1485 cm\(^{-1}\) is assigned to the N7-C8 stretching coupled to C8-H bending modes. The vibrational motions that contribute the most to the different normal modes of the purine ring in the structure of adenine are observed at 1128, 1250, and 1335 cm\(^{-1}\). The Raman band at 1335 cm\(^{-1}\) is assigned to the C8-N9 and C4-N3 stretching coupled to the N9-H and C2-H bending modes, respectively. Also, the band at 1250 cm\(^{-1}\) is assigned to the in-plane N7-C8 stretching coupled to the C8-H and N9-H bending mode. The Raman band at 1165 cm\(^{-1}\) has been assigned to the in-plane N10H\(_2\) rocking mode coupled to the C6-N10, N3-C4, and C4-N9 stretching modes [21, 33]. A strong Raman band observed at 1128 cm\(^{-1}\) which
The Raman spectrum of aqueous pyridoxine reveals noticeable changes, and this is shown in Figure 10(c). Examining the Raman spectrum of adenine-pyridoxine solution, there is essentially a sharp decrease in the relative intensity of the Raman bands at 690, 751, 1235, 1361, 1452, and 1630 cm\(^{-1}\) when compared to their counterparts in the Raman spectrum of pyridoxine. These bands are very strong in the Raman spectrum of pyridoxine but very weak in the spectrum of adenine-pyridoxine solution. In a study that focused on understanding the binding characteristics of adenine, Otto et al. reported that vibrations originating from molecular groups located close to interacting surfaces are the strongly enhanced Raman modes [37]. Therefore, the decrease in the relative intensity of the mentioned vibration modes (690, 751, 1235, 1297, and 1327 cm\(^{-1}\)) suggests that groups associated with these modes in pyridoxine are less involved in the interaction with adenine. Also obvious is the enhancement of the band at 590 cm\(^{-1}\) in the Raman spectrum of adenine-pyridoxine, when compared to its counterpart band in the Raman spectrum of pyridoxine. This band is assigned to the C-C-C-C torsion bending modes of the pyridoxine structure.

Shifts in the position of bands at 900 and 1084 cm\(^{-1}\) were observed in the Raman spectrum of adenine-pyridoxine solution. The band at 1084 cm\(^{-1}\) in the Raman spectrum of adenine-pyridoxine solution is assigned to \(\nu\) (C11–O12) stretching coupled to deformation of C11–H and C-O-H. The Raman band at 1084 cm\(^{-1}\) is observed in the Raman spectrum of pyridoxine at 1091 cm\(^{-1}\), with the red shift suggesting interaction between the two compounds.

It is expected that the interaction between the two molecules will result in shifts or enhancement of Raman bands in the spectrum of the interacting molecules. The electromagnetic enhancement model suggests that vibrational modes that involve a large change in polarizability of bonds that are perpendicular to surfaces are enhanced the most [38]. The spectral region of 350–1700 cm\(^{-1}\) in the Raman spectrum of adenine-pyridoxine solution shows that there is an increase in the relative intensity of the Raman bands at 624, 770, 1250, 1334, and 1484 cm\(^{-1}\). The increase in intensity suggests that the adenine bands associated with these vibrations are more involved in the intermolecular interaction with pyridoxine [37].

Adenine interacts with molecules preferentially using the nitrogen atom on position 7 (N7) in the adenine structure [39] and the exocyclic amino group (NH\(_2\)) [22], and the most intense lines in its spectrum are associated with atoms in closest proximity to the SERS surface. In Watanabe’s report, the presence of the strong Raman band at 1334 cm\(^{-1}\) in the SERS spectrum of adenine was used to characterize the adsorption of adenine to a surface via the nitrogen atom on the position 7 of the adenine structure. Since this is a

![Figure 8](image-url)

**Figure 8:** CV of 2.0 \(\times\) 10\(^{-2}\) M pyridoxine on the glassy carbon electrode in the 0.1 M phosphate buffer/5 mM NaCl supporting electrolyte at pH 7.0. Scan rate: (a) 0.05 V/s; (b) 0.1 V/s; (c) 0.2 V/s; (d) 0.3 V/s; (e) 0.4 V/s; (f) 0.5 mV/s; (g) 0.6 V/s.

![Figure 9](image-url)

**Figure 9:** CV of a solution of 1.0 \(\times\) 10\(^{-3}\) M adenine + 1.0 \(\times\) 10\(^{-2}\) M pyridoxine on the glassy carbon electrode in the 0.1 M phosphate buffer/5 mM NaCl supporting electrolyte at pH 7.0. Scan rate is 100 mV/s.
solution-based study, pyridoxine is thought to produce a surface-like effect that enhances the vibrational mode associated with the Raman band at 1334 cm\(^{-1}\), which is the most intense band in the Raman spectrum of adenine-pyridoxine. This enhancement highlights the involvement of the endocyclic nitrogen atom (N7) of adenine in its interaction with pyridoxine.

Furthermore, Cinta et al. [23] reported the Raman and SERS of aqueous pyridoxine in the pH range of 1 to 10. In their study, it was observed that there were changes in the relative intensities of the SERS bands at 978, 1240, and 1330 cm\(^{-1}\) in the basic pH region and vibrational modes arising from ring deprotonation at 1240 and 1330 cm\(^{-1}\). The vibrational modes at 1240 and 1330 cm\(^{-1}\) have been assigned

---

**Figure 10:** (a) Raman spectrum of aqueous solution of adenine at pH 7.0 (laser output: 633 nm, \(P = 200\) mW). (b) Raman spectrum of aqueous solution of pyridoxine at pH 7.0 (laser output: 633 nm, \(P = 200\) mW). (c) Raman spectrum of aqueous solution of adenine-pyridoxine at pH 7.0 (laser output: 633 nm, \(P = 200\) mW).
Table 1: Experimental Raman shift values (cm\(^{-1}\)) for adenine in solution.

| Experimental Raman (cm\(^{-1}\)) | B3LYP/6-31G (d, p) | Experimental Raman values (cm\(^{-1}\)) B3LYP/6-31G (d, p) | This work, Raman values (cm\(^{-1}\)) | Vibrational assignment [21] |
|----------------------------------|-------------------|-------------------------------------------------|----------------------------------|---------------------------|
| 1639 1641                        | 1674 1665         | 1680                                            | sciss NH2, \(\nu\) (C6-N10), \(\nu\) (C5-C6) |
| 1612 1617                        | 1613 1643         | 1610                                            | \(\nu\) (N3-C4), \(\nu\) (N1-C6), \(\nu\) (C5-N7), \(\nu\) (N7-C8), \(\delta\) (N9-H) |
| 1599 1584                        | 1597 1613         | 1597                                            | sciss NH2                      |
| 1482 1502                        | 1483 1524         | 1485                                            | \(\nu\) (N7-C8), \(\delta\) (C8-H), sciss NH2 |
| 1474 1487                        | 1463 1510         | 1420                                            | \(\nu\) (C4-N9), (C4-C5), (C6-N10), (N7-C8), \(\delta\) (C2-H) |
| 1419 1416                        | 1419 1441         | 1373                                            | \(\delta\) (C2-H, N9-H), \(\nu\) (C8-N9, C4-N9) |
| 1389 1400                        | 1372 1423         | 1335                                            | \(\delta\) (C2-H, C8-H, N9-H), \(\nu\) (C6-N1, C8-N9, N3-C4) |
| 1345 1350                        | 1333 1372         | 1335                                            | \(\nu\) (C5-N7, N1-C2), \(\delta\) (C2-H, C8-H), |
| 1328 1342                        | 1333 1365         | 1308                                            | \(\nu\) (C2-N3, N1-C2, C5-C6, C5-N7) |
| 1290 1317                        | 1308 1341         | 1308                                            | \(\delta\) (C8-H, N9-H), \(\nu\) (N7-C8) |
| 1240 1250                        | 1248 1272         | 1250                                            | rock NH2, \(\nu\) (C5-N7, N1-C2, C2-N3) |
| 1228                             | 1234 1246         | 1234                                            | \(\delta\) (C8-H, N10-H11), \(\nu\) (C4-N9, N3-C4, C6-N10) |
| 1127                             | 1129 1148         | 1165                                            | \(\nu\) (C8-N9), \(\delta\) (N9-H, C8-H), |
| 1032                             | 1065 1085         | 1128                                            | rock NH2                       |
| 1005                             | 1000 1025         | 1025                                            | wag (C2-H) o.p. |
| 958                              | 953 974           | 942                                             | def R5                         |
| 927                              | 925 942           | 942                                             | def R5, R5 (\(\nu\) C5-N7) |
| 887                              | 882 899           | 899                                             | def R6, R5 (\(\nu\) C5-N7) wag (C8-H) o.p. |
| 848                              | 831 849           | 849                                             | wag (C8-H) o.p. |
| 802                              | 793 805           | 773                                             | def R6 (wag C4-C5-C6), wag (C8-H) o.p. |
| 717                              | 713 726           | 722                                             | Ring breath whole molecule (distorted) |
| 672                              | 672 685           | 685                                             | def R5, R6 (\(\tau\) C4-C5-C6, wag N3-C4-N9) o.p. |
| 655                              | 658 666           | 666                                             | def R5 (wag C5-N7-C8), wag C8-H, N9-H,) o.p. |
| 610                              | 607 618           | 622                                             | def R6 and R5                  |
| 566                              | 568 576           | 560                                             | wag (C2-H, N9-H) o.p. |
| 528                              | 528 540           | 533                                             | \(\tau\) (NH2) o.p. |
| 521                              | 514 533           | 533                                             | def R6--i.p./o.p. |
| 513                              | 504 518           | 474                                             | wag (N9-H), def R6, R5--i.p./o.p. |
| 298                              | 300 375           | 375                                             | wag (N9-H), \(\tau\) (NH2) o.p. |

\(\nu\) = stretching; \(\delta\) = bending; \(\tau\) = torsion; rock = rocking; def = deformation; sciss = scissoring; wag = wagging; R5 = five-membered ring; R6 = six-membered ring; breath = breathing; i.p. = in plane; o.p. = out plane.
**Table 2: Raman shift values (cm$^{-1}$) for aqueous solution of pyridoxine.**

| Experimental Raman values (cm$^{-1}$) [23] | IR (ab initio) [23] | Experimental Raman values (cm$^{-1}$) [35] | DFT/6-311++G (d, p) [35] | This work, Raman values (cm$^{-1}$) | Vibrational assignment [21, 35] |
|------------------------------------------|--------------------|------------------------------------------|--------------------------|-------------------------------|--------------------------------|
| 1646                                     | 1618               | 1611                                     | 1645                     | ν (C-C)/ring stretch         |
| 1630                                     | 1600               | 1565                                     | 1630                     | ν (C-C), δ (H15–C10–H16), δ (C9–N7–H20) |
| 1520                                     | 1542               | 1501                                     | 1545                     | δ (C-H ring) + ν (C-C)       |
| 1451                                     | 1465               | 1460                                     | 1459                     | δ (C4–O8–H13) + δ (C-C-O) + νas (CH3), δ (C3–C9–H19) |
| 1389                                     | 1378               | 1376                                     | 1385                     | ν (C1–C2, C2–C4) + δ (CH3)  |
| 1360                                     | 1351               | 1362                                     | 1361                     | δ (C11–O12–H23) + δ (C-O-H) + (CH2) twist |
| 1326                                     | 1325               | 1316                                     | 1327                     | δ (C9–N7–H20) + δ (C3–C9–H19) + δ (C-H) |
| 1295                                     | 1288               | 1280                                     | 1297                     | ν i.p. + ring τ + δ (H17–C1–O6), ν (C-N) |
| 1234                                     | 1256               | 1236                                     | 1235                     | ν (C5–N7)/(C-C) + τ (C5–CH3) + δ (C10–H) |
| 1219                                     | 1178               | 1178                                     | 1091                     | δ (C-O-H)/(C-C-H), δ (N–H) i.p. |
| 1054                                     | 1061               | 1054                                     | 1052                     | Ring stretch + τ (H17–C1–O6–H24) + ν (C-C-O) |
| 966                                      | 972                | 986                                      | 967                      | δ (N–H) o.p. + δ (C-C) + ν (C-O) |
|                                           |                    | 928                                      | 904                      | τ (C-C-N)                   |
|                                           |                    |                                           | 895                      | τ (C-C-N-H)                 |
| 753                                      | 756                | 751                                      | 751                      | τ (H19–C9–N7–H20) + δ (C6–C1–O6) + ν (C1–C2–C3) |
| 692                                      | 655                | 695                                      | 706                      | τ (C2–C3–C9–H19) + stretch quadrant (C2–C4–C5–N7) + τ (C3–C2–C4–C5) |
|                                           |                    |                                           | 585                      | τ (C-C-C-N)                 |
| 533                                      | 539                | 519                                      | 520                      | δ (OH–C4–C5–CH3) + τ (H19–C9–N7–H20), ν (C-C) + ν (C-O) |
| 477                                      | 453                | 482                                      | 494                      | τ (H19–C9–N7–H20) + δ (C-C-O)/(C-C-H) |
| 403                                      | 404                | 391                                      | 411                      | δ (H17–C1–O6–H24) + τ (H21–C11–O12–H23), ν (C-O) |

ν = stretch; δ = bending; τ = torsion; twist = twisting; i.p. = in plane; o.p. = out plane.
to ring stretching modes of pyridoxine. Cinta et al. [23] also observed the deprotonation of the N-ring atom leading to a SERS response typical of pyridine derivatives. In this study, a difference is observed in the relative intensity of bands at 1235 and 1327 cm\(^{-1}\) in the Raman spectrum of pyridoxine when compared to corresponding bands (1250 and 1334 cm\(^{-1}\)) in the spectrum of adenine-pyridoxine solution. A very strong band at 1334 cm\(^{-1}\) in the spectrum of adenine-pyridoxine solution suggests a strong interaction between the adenine and pyridoxine molecule via the N7 atom, as shown in Figure 11(a).

Otto et al. observed that the vibrational modes at 626, 960, 1028, and 1194 cm\(^{-1}\), linked to the motion of C6-NH2, gave rise to strong contributions in the SERS spectrum of adenine, leading them to conclude that adenine was bound to the surface via the exocyclic amino group [22]. In the Raman spectrum of adenine-pyridoxine solution, it is evident that the bands at 624, 940, 1024, 1128, and 1167 cm\(^{-1}\) are enhanced when examined with respect to their equivalents (622, 942, 1025, 1128, and 1165 cm\(^{-1}\)) in the Raman spectrum of adenine. This suggests that adenine is coordinated to pyridoxine via the exocyclic amino group (Figure 11(a)). This is not surprising because adenine can undergo protonation at the endocyclic nitrogen atoms N1, N3, and N7 [40]. Considering that the adenine exocyclic amino group has the least sterically hindered nitrogen atom, this portion of the ring is likely the preferred site for intermolecular interactions. Furthermore, the delocalization of electrons on the exocyclic amino group N10 into the heterocyclic ring due to the resonance effect confers a quaternary structure on the nitrogen, thereby increasing the possibility of having an intermolecular interaction between it and pyridoxine O12.

### 3.4. SERS Spectrum of Adenine-Pyridoxine on Ag Nanoparticles.

The SERS spectrum adenine-pyridoxine is characterized by the presence of strong SERS bands at 732 and 1332 cm\(^{-1}\). The in-plane breathing mode at 733 cm\(^{-1}\) as reported in literature is the most prominent band in the SERS of adenine adsorbed onto the Ag surface [21, 33]. This band that occurs at 732 cm\(^{-1}\) in this study is also observed as the most prominent band in the SERS spectrum of adenine-pyridoxine.

In this study, more vibrational information on the interaction of adenine with pyridoxine is gained by examining the SERS spectrum of adenine-pyridoxine adsorbed onto the surface of Ag nanoparticles. The SERS spectrum of adenine-pyridoxine is shown in Figure 12, and vibrational assignments are presented in Table 3. The SERS spectrum of adenine-pyridoxine is well resolved, and it shows a noticeable change in the intensity of the bands when compared with the normal Raman bands of the complex. There are shifts in the SERS bands of adenine-pyridoxine at 1463, 1033, 962, 732, and 455 cm\(^{-1}\). The SERS band at 732 cm\(^{-1}\) is observed in the Raman spectrum of adenine-pyridoxine solution at 722 cm\(^{-1}\). There is also the enhancement of bands at 455, 732, 962, 1033, 1332, and 1463 cm\(^{-1}\) in the SERS spectrum of adenine-pyridoxine when compared to the corresponding normal Raman bands of adenine-pyridoxine (1484, 1334, 1024, 940, 722, and 474 cm\(^{-1}\)). This suggests that these vibration modes more related to the adsorption of adenine-pyridoxine on the Ag nanoparticles. The SERS band at 1332 cm\(^{-1}\) is assigned to the C-N stretching mode of adenine [21]. This SERS band (1332 cm\(^{-1}\)) is relatively enhanced when compared to its counterpart Raman band at 1335 cm\(^{-1}\). The stronger enhancement of SERS bands at 1332 and 732 cm\(^{-1}\) strongly suggests a perpendicular orientation of the adenine-pyrimidine-purine ring portion of the complex close to the Ag nanoparticle surface. Furthermore, the SERS spectrum of adenine-pyridoxine shows a pattern of enhancement of other vibrational modes pertaining to adenine, at 1171, 1120, 1033, 962, and 623 cm\(^{-1}\), as was observed by Otto et al. [22].

In conclusion, spectroelectrochemical analyses show evidence of adenine interaction with pyridoxine. Analyses of UV-visible data using the Lineweaver–Burk double reciprocal plot reveal that adenine interacts with pyridoxine in

![Figure 11: Proposed chemical structures of adenine-pyridoxine interaction.](image-url)
a 1:1 mode with a binding constant of 29.1. The broadening of the CV oxidation peak of pyridoxine and the shift to a higher potential of the adenine anodic peak provides evidence supporting the interaction between the two compounds. Additional evidences obtained from experimental and theoretical analysis of Raman data showed that adenine interacts with pyridoxine O(12) using its exocyclic amino and purine nitrogen N(7) groups. Shifts of spectral peaks to the lower wavenumber especially with the pyridoxine hydrochloride \(-\text{CH}_2\text{-OH}\) bending and in-plane \(-\text{N-H}\) mode at

![Figure 12: SERS spectrum of aqueous solution of adenine-pyridoxine at pH 7.0 (laser output: 633 nm, \(P = 200\) mW).](image_url)

**Table 3: Experimental Raman and SERS shift values (cm\(^{-1}\)) for aqueous solution of adenine-pyridoxine.**

| Experimental Raman values (cm\(^{-1}\)) | SERS values (cm\(^{-1}\)) | Vibrational assignment [34, 35] |
|----------------------------------------|--------------------------|---------------------------------|
| 1680                                   | 1662                     | sciss NH2, \(\nu\) (C-N), \(\nu\) (C-C)/ring stretch |
| 1610                                   | 1626                     | \(\nu\) (N-C), \(\nu\) (C-C), \(\delta\) (H-C-H), \(\delta\) (C-N-H) |
| 1597                                   | 1589                     | sciss NH2, \(\nu\) R6 (C=C)     |
|                                        | 1518                     | \(\delta\) (C-H ring) + \(\nu\) (C-C) |
| 1485                                   | 1463                     | \(\nu\) (N-C), \(\delta\) (C-H), sciss NH2 |
| 1420                                   | 1402                     | \(\delta\) (C-H), \(\nu\) (C-C) + \(\delta\) (CH3) |
| 1373                                   | 1372                     | \(\delta\) (C-H, N-H), \(\nu\) (C-N) and \(\delta\) (C-O-H) + \(\delta\) (C-O-H) + (CH2) twist |
| 1335                                   | 1332                     | \(\delta\) (C-H, C8-H, N9-H), \(\nu\) (C6-N1, C8-N9, N3-C4) |
| 1335                                   | 1335                     | \(\delta\) (C-N-H) + \(\delta\) (C-C-H) + \(\delta\) (C-H) i.p., ring \(\tau\) + \(\delta\) |
| 1308                                   | 1245                     | \(\nu\) (C-N), \(\nu\) (C-C)    |
| 1250                                   | 1245                     | \(\delta\) (C-H, N-H) i.p., \(\nu\) (N7-C8), \(\delta\) (C-O-H)/(C-C-H) |
| 1234                                   | 1171                     | rock NH2, \(\nu\) (C-N, N-C)    |
| 1165                                   | 1120                     | \(\delta\) (C-H, N-H), \(\nu\) (C-N, N-C) |
| 1128                                   | 1033                     | \(\nu\) (C8-N9), \(\delta\) (N9-H, C8-H), |
|                                        | 982                      | rock NH2, ring stretch, \(\tau\) (H-C-O-H) + \(\nu\) (C-C-O) |
|                                        |                          | wag (C-H) o.p., \(\delta\) (N-H) o.p. + \(\delta\) (C-C), \(\nu\) (C-O) |
| 942                                    | 962, 942 sh.             |                                                                 |
| 893                                    | 882                      | def R5                          |
| 773                                    | 800                      | def R6 (wag C-C-C), wag (C-H) o.p. |
| 722                                    | 732                      | Ring breath whole molecule (distorted) |
|                                        |                          | def R6 and R5, \(\tau\) (C-C-C-H) + stretch quadrant (C-C-C-N), \(\tau\) (C-C-C-C) |
| 622                                    | 623                      | def R6 (wag C-C-C), wag (C-H) o.p. |
|                                        |                          | \(\delta\) (C-N-H), \(\nu\) (C-C) + \(\delta\) (C-O) |
| 560                                    | 591                      | wag (C2-H, N9-H) o.p.           |
| 533                                    | 532                      | def R6—i.p./o.p.                |
| 533                                    | 532                      | wag (N-H), def R6, \(\delta\) (OH–C–C–CH3) + \(\tau\) (H–C–N–H), \(\nu\) (C-C) + \(\nu\) (C-O) |
| 474                                    | 455                      | wag (N-H), \(\tau\) (NH2) o.p.  |
| 375                                    | 377                      | def R6, R5 wag (N-C-N-C, C-N-N-C), wag (C-N) o.p. |

\(\nu = \) stretching; \(\delta = \) bending; \(\tau = \) torsion; \(\text{rock} = \) rocking; \(\text{def} = \) deformation; \(\text{sciss} = \) scissoring; \(\text{wag} = \) wagging; \(\text{R5} = \) five-membered ring; \(\text{R6} = \) six-membered ring; \(\text{breath} = \) breathing; i.p. = in plane; o.p. = out plane; sh. = shoulder.
1235 cm$^{-1}$ and adenine -NH$_2$ rocking band at 942 cm$^{-1}$ confirm that there is the formation of hydrogen bonds as the dominant intermolecular force between the two compounds. Shifts in the SERS bands of adenine-pyridoxine, particularly the band at 732 cm$^{-1}$, observed in the Raman spectrum of adenine-pyridoxine solution at 722 cm$^{-1}$ and strong enhancement of SERS bands pertaining to adenine suggest that the complex interacts with the Ag nanoparticle surface possessing a perpendicularly orientation, with the adenine moiety closest to the surface. The results obtained in this study validate the utility of spectroelectrochemical techniques in investigating the interaction of small molecules like pyridoxine with nucleobases and for understanding how various parts of DNA interact with vitamins on substrates in biological systems.

Data Availability
The authors will provide supplementary research data if requested.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Acknowledgments
The authors thank the University of Maryland Eastern Shore, Department of Natural Sciences, and Louis Stokes Alliances for Minority Participation program for, respectively, providing funds for the purchase of reagents and support of the undergraduate student who participated in this project. The authors also wish to appreciate the research group of Professor Charles Hosten of Howard University, Department of Chemistry, for granting access for the use of the CV and Raman instrument for analysis.

References
[1] M. Aleksić and V. Kapetanović, “An overview of the optical and electrochemical methods for detection of DNA-drug interactions,” Acta Chimica Slovenica, vol. 61, no. 3, pp. 555–573, 2014.
[2] B. H. Geierstanger and D. E. Wemmer, “Complexes of the minor groove of DNA,” Annual Review of Biophysics and Biomolecular Structure, vol. 24, no. 1, pp. 463–493, 1995.
[3] H. Li, W.-J. Mei, Z. Xu, D.-W. Pang, L.-N. Ji, and Z.-H. Lin, “Electrochemistry of a novel monoruthenated porphyrin and its interaction with DNA,” Journal of Electroanalytical Chemistry, vol. 600, no. 2, pp. 243–250, 2007.
[4] D. E. Metzler, “Vitamins and coenzymes,” in Encyclopedia of Physical Science and Technology, pp. 509–528, Academic Press, Cambridge, MA, USA, 2003.
[5] P.-O. Löwdin, “Proton tunneling in DNA and its biological implications,” Reviews of Modern Physics, vol. 35, no. 3, pp. 724–732, 1963.
[6] P. O. Lowdin, “Quantum genetics and the aperiodic,” Advances in Quantum Chemistry, vol. 2, pp. 213–360, 1966.
[7] S. W. Ebbinghaus, “Site-selective DNA binding drugs,” Chemistry & Biology, vol. 10, no. 10, pp. 895–897, 2003.
[8] S.-Q. Liu, M.-L. Cao, and S.-L. Dong, “Electrochemical and ultraviolet-visible spectroscopic studies on the interaction of deoxyribonucleic acid with vitamin B6,” Bioelectrochemistry, vol. 74, no. 1, pp. 164–169, 2008.
[9] T. R. Krugh, “Drug-DNA interactions,” Current Opinion in Structural Biology, vol. 4, no. 3, pp. 351–364, 1994.
[10] G. Arena, R. Purrello, E. Rizzarelli, A. Gianguzza, and L. Pellerito, “Thermodynamics of hydroxazo complex formation of dialkyltin(IV) ions in aqueous solution,” Journal of the Chemical Society, Dalton Transactions, no. 5, pp. 773–777, 1989.
[11] D. A. Bender, “Non-nutritional uses of vitamin B6,” British Journal of Nutrition, vol. 81, no. 1, pp. 7–20, 1999.
[12] R. Casasnovas, A. Saltà, J. Frau, J. Donoso, and F. Muñoz, “Theoretical study on the distribution of atomic charges in the Schiff bases of 3-hydroxy pyridine-4-aldehyde and alamine: the effect of the protonation state of the pyridine and imine nitrogen atoms,” Chemical Physics, vol. 355, no. 2-3, pp. 149–156, 2009.
[13] S. Dakshinamurti and K. Dakshinamurti, “Antihypertensive and neuroprotective actions of pyridoxine and its derivatives,” Canadian Journal of Physiology and Pharmacology, vol. 93, no. 12, pp. 1083–1090, 2015.
[14] E. Paleček, M. Fojita, F. Jelen, and V. Vetterl, “Electrochemical analysis of nucleic acids,” in The Encyclopedia of Electrochemistry, Bioelectrochemistry, 9, A. J. Bard and M. Stratmann, Eds., pp. 365–429, Wiley-VCH Verlag, Weinheim, Germany, 2002.
[15] A. M. Oliveira-Brett, M. Vivian, I. R. Fernandes, and J. A. P. Diede, “Electrochemical detection of in situ adriamycin oxidative damage to DNA,” Talanta, vol. 56, no. 5, pp. 959–970, 2002.
[16] A. M. Oliveira-Brett, J. A. P. Diede, L. A. Silva, and V. C. Diculescu, “Voltammetric Determination of all DNA nucleotides,” Analytical Biochemistry, vol. 332, no. 2, pp. 321–329, 2004.
[17] M. L. Wang, Y. Y. Zhang, Q. J. Xie, and S. Z. Yao, “In situ FT-IR spectroelectrochemical study of electrooxidation of pyridoxol on a gold electrode,” Electrochimica Acta, vol. 51, no. 6, pp. 1059–1068, 2005.
[18] T. Pineda, J. M. Sevilla, A. J. Román, and M. Blázquez, “Electrooxidation of pyridoxal (PL) on a polycrystalline gold electrode in alkaline solutions,” Journal of Electroanalytical Chemistry, vol. 492, no. 1, pp. 38–45, 2000.
[19] W. Qu, K. Wu, and S. Hu, “Voltammetric determination of pyridoxine (vitamin B6) by use of a chemically-modified glassy carbon electrode,” Journal of Pharmaceutical and Biomedical Analysis, vol. 36, no. 3, pp. 631–635, 2004.
[20] R. C. Barthus, L. H. Mazo, and R. J. Poppi, “Simultaneous determination of vitamins C, B6 and PP in pharmaceutics using differential pulse voltammetry with a glassy carbon electrode and multivariate calibration tools,” Journal of Pharmaceutical and Biomedical Analysis, vol. 38, no. 1, pp. 94–99, 2005.
[21] B. Giese and D. McNaughton, “Surface-enhanced Raman spectroscopic and density functional theory study of adenine adsorption to silver surfaces,” Journal of Physical Chemistry B, vol. 106, no. 1, pp. 101–112, 2002.
[22] C. Otto, F. F. M. De Mul, A. Huizinga, and J. Greve, “Surface enhanced Raman scattering of derivatives of adenine: the importance of the external amino group in adenine for surface binding,” Journal of Physical Chemistry, vol. 92, no. 5, pp. 1239–1244, 1988.
[23] S. Cinta, C. Morari, E. Vogel et al., “Vibrational studies of B6 vitamin,” Vibrational Spectroscopy, vol. 19, no. 2, pp. 329–334, 1999.
[24] V. Ramakrishnan, N. Krishnamurthy, M. Gurunathan, and V. J. P. Srivatsavoy, “SERS studies of some α-amino-anthraquinones in silver sol,” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 46, no. 11, pp. 1615–1619, 1990.

[25] S. Charak, M. Shandilya, G. Tyagi, and R. Mehrotra, “Spectroscopic and molecular docking studies on chlorambucil interaction with DNA,” *International Journal of Biological Macromolecules*, vol. 51, no. 4, pp. 406–411, 2012.

[26] S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, and D. Dash, “Characterization of enhanced antibacterial effects of novel silver nanoparticles,” *Nanotechnology*, vol. 18, no. 22, article 225103, 2007.

[27] N. Li, Y. Ma, C. Yang, L. Guo, and X. Yang, “Interaction of anticancer drug mitoxantrone with DNA analyzed by electrochemical and spectroscopic methods,” *Biophysical Chemistry*, vol. 116, no. 3, pp. 199–205, 2005.

[28] F. Manouchehri, Y. Izadmanesh, E. Aghaei, and J. B. Ghasemi, “Experimental, computational and chemometrics studies of BSA-vitamin B6 interaction by UV-vis, FT-IR, fluorescence spectroscopy, molecular dynamics simulation and hard-soft modeling methods,” *Bioorganic Chemistry*, vol. 68, pp. 124–136, 2016.

[29] M. F. S. Tixeira, G. Marino, E. R. Dockal, and E. T. G. Cavalheiro, “Voltammetric determination of pyridoxine (vitamin B6) at a carbon paste electrode modified with vanadyl(IV)-salen complex,” *Analytica Chimica Acta*, vol. 508, no. 1, pp. 79–85, 2004.

[30] H.-Y. Gu, A.-M. Yu, and H.-Y. Chen, “Electrochemical behavior and simultaneous determination of vitamin B2, B6, and C at electrochemically pretreated glassy carbon electrode,” *Analytical Letters*, vol. 34, no. 13, pp. 2361–2374, 2001.

[31] Y. Wu and F. Song, “Voltammetric investigation of vitamin B6 at a glassy carbon electrode and its application in determination,” *Bulletin of the Korean Chemical Society*, vol. 29, no. 1, pp. 38–42, 2008.

[32] G. Chen, X. Ding, Z. Cao, and J. Ye, “Determination of melatonin and pyridoxine in pharmaceutical preparations for health-caring purposes by capillary electrophoresis with electrochemical detection,” *Analytica Chimica Acta*, vol. 408, no. 1-2, pp. 249–256, 2000.

[33] F. Madzharova, Z. Heiner, M. Gühlke, and J. Kneipp, “Surface-enhanced hyper-Raman spectra of adenine, guanine, cytosine, thymine, and uracil,” *Journal of Physical Chemistry C*, vol. 120, no. 28, pp. 15415–15423, 2016.

[34] M. J. Nowak, L. Lapinski, J. S. Kwiatkowski, and J. Leszczyński, “Molecular structure and infrared spectra of adenine. Experimental matrix isolation and density functional theory study of adenine 15N isotopomers,” *μT_e Journal of Physical Chemistry*, vol. 100, no. 9, pp. 3527–3534, 1996.

[35] B. Atak-Bulbul and S. Akyuz, “Ab initio density functional theory calculations on pyridoxine and its water clusters,” *Asian Journal of Chemistry*, vol. 26, pp. S299–S304, 2014.

[36] M. Srivastava, P. Rani, N. P. Singh, and R. A. Yadav, “Experimental and theoretical studies of vibrational spectrum and molecular structure and related properties of pyridoxine (vitamin B6),” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 120, pp. 274–286, 2014.

[37] C. Otto, T. J. J. van den Tweel, F. F. M. de Mul, and J. Greve, “Surface-enhanced Raman spectroscopy of DNA bases,” *Journal of Raman Spectroscopy*, vol. 17, no. 3, pp. 289–298, 1986.

[38] J. A. Creighton, “Surface Raman electromagnetic enhancement factors for molecules at the surface of small isolated metal spheres: the determination of adsorbate orientation from SERS relative intensities,” *Surface Science*, vol. 124, no. 1, pp. 209–219, 1983.

[39] T. Watanabe, O. Kawasaki, H. Katoh, K. Honda, Y. Nishimura, and M. Tsuboi, “SERS study of molecular adsorption: some nucleic acid bases on Ag electrodes,” *Surface Science*, vol. 158, no. 1–3, pp. 341–351, 1985.

[40] J. E. Sponer, J. Leszczynski, F. Glahé, B. Lippet, and J. Sponer, “Protonation of platinated adenine nucleobases: gas phase vs condensed phase picture,” *Inorganic Chemistry*, vol. 40, no. 14, pp. 3269–3278, 2001.
