Chloride ion-dependent surface-enhanced Raman scattering study of biotin on the silver surface

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Abstract. In the present paper, the surface enhanced Raman scattering (SERS) technique was employed to study the SERS spectra of biotin molecules formed on the silver surface. The adsorption geometries of biotin molecules on the silver surface were analyzed based on the SERS data. It can be found that most vibration modes show a Raman shift in silver sol after the addition of sodium chloride solution. In addition, The Raman signals of biotin become weaker and weaker with the increase of the concentration of sodium chloride. This may be due to that the interaction between chloride ions and silver particles is stronger than the interaction between biotin molecules and silver particles. When the concentration of sodium chloride in silver colloid is higher than 0.05mol/L, superfluous chloride ions may form an absorption layer so that biotin can not be adsorbed on silver surface directly. The changes in intensity and profile shape in the SERS spectra suggest different adsorption behavior and surface-coverage of biotin on silver surface. The SERS spectra of biotin suggest that the contribution of the charge transfer mechanism to SERS may be dominant.

1. Introduction
Surface enhanced Raman scattering (SERS) effect was first observed by Fleischmann et al in pyridine adsorbed on the roughened silver electrode in 1974 [1]. SERS is a powerful and convenient analytical tool in structural analysis of materials, which can greatly enhance the weak normal Raman signals of molecules and materials.

Generally, there are two theories on SERS accepted widely. One is electromagnetic enhancement, and the other one is charge transfer enhancement [2, 3]. The first mechanism is based on the strong
enhancement of the electromagnetic field on the metal surface, as a consequence of the plasmon excitation induced by the incident radiation on a roughed surface. The second consists of a resonant Raman effect caused by a charge-transfer process from the metal to the adsorbate [4]. In most of the SERS systems, the electromagnetic and charge transfer enhancement happen simultaneously, and the ratio of their enhancement varies in different SERS systems.

In the course of SERS study, many works have focused on the influence of halides, mainly Cl\(^{-}\), in the adsorption and SERS effects [5, 6]. A number of works have reported that Cl\(^{-}\) anion is able to create a high degree of roughness in order to stabilize the surface active sites, attributed to the chemical mechanism of SERS enhancement [7, 8], as a large charge-transfer between the metal surface and the adsorbate may occur.

Biotin, which is also known as Vitamin H, is a water-soluble vitamin and generally classified as a B-complex vitamin. Biotin has always intrigued biochemists for it participates as a cofactor in gluconeogenesis, fatty acid synthesis and branched chain amino acid catabolism [9]. In addition, biotin regulates gene expression [10] and is involved in some systemic processes such as development [11] and metabolism [10]. It was reported that biotin deficiency or biotin excess affect reproductive functions [12].

In this study, the SERS spectrum of biotin in silver colloid was recorded under the treatment of low laser power and the adsorption geometries were analyzed based on the SERS data. The relation between adsorption and SERS of biotin on borohydride-reduced Ag colloids upon the addition of Cl\(^{-}\) was investigated. Through analysis of the SERS spectra, it is evident that the surface-coverage and adsorption orientation of biotin may be easily influenced by the added Cl\(^{-}\).

2. Experimental

2.1. Chemicals
The biotin in the solid form was purchased from Sigma-Aldrich Chemical Company (USA) with a stated purity of greater than 99.5% and it was used without any further purification. Silver nitrate and sodium borohydride were used as received. All aqueous solutions were prepared using doubly distilled water.

The colloidal silver nanoparticles were prepared by the chemical reduction of AgNO\(_3\) with NaBH\(_4\) according to the standard procedure [13]. Briefly, 8.5 mg AgNO\(_3\) powder dissolved in 50 mL doubly distilled water were added dropwise to 150 mL of ice-cold aqueous solution of 2×10\(^{-3}\) mol/L NaBH\(_4\) with vigorous stirring. The resulting yellowish colloidal solution was found to be stable over several days at room temperature and has a surface Plasmon absorption maximum measured at 392 nm (data not shown).

Samples for SERS measurement were prepared by adding 2 mL saturated biotin solutions to 1.5 mL silver sol. In the final step, 0.05 mL water or aqueous NaCl solutions of different concentrations were added in order to characterize the influence of Cl\(^{-}\). The final concentration of biotin in all mixed systems was 5×10\(^{-5}\) mol/L. After the addition of different concentrations of aqueous NaCl, the final concentrations of Cl\(^{-}\) in the mixed systems were 0.01, 0.04 and 0.05 mol/L, respectively.

The final solution was sealed in a capillary with one hour incubation for the SERS measurement. In
the measurement of SERS on silver surface, a droplet (0.02 mL) of incubated solution was evenly dropped on a clean glass slide at room temperature.

2.2. Instrumentation
Raman spectra were acquired using a triple grating Raman micro-spectrometer (Acton Spectro @2300i, Princeton Acton, USA) equipped with a Ar ion laser (\(\lambda = 514.5\) nm) and a liquid nitrogen cooled coupled charge device (CCD) detector. The spectral resolution was set at 2 cm\(^{-1}\) and the laser power focused on the sample was about 5 mW.

3. Results and Discussion

3.1. Molecular geometry
Figure 1 shows the chemical structure of biotin. The molecule contains a bicyclic ring system whose mirror symmetry is broken by a valeric acid side chain. The top ring of the bicyclic system is an ureido ring, and the bottom ring containing sulfur atom, is a tetrahydrothiophene ring. Biotin further possesses three contiguous stereocenters on the tetrahydrothiophene ring in the all-cis configuration [14].

![Figure 1. Chemical structure of biotin.](image)

3.2. SERS of biotin in silver sol
Figure 2 displays the Normal Raman spectroscopy of biotin in aqueous saturated solution, in which the Raman bands are sparse and the intensity is low, especially in the higher wavenumber range. Figure 3 shows the SERS of biotin in silver colloid. The Raman bands assignments of the NRS and SERS in silver colloid are given in Table 1. In making assignments, several former literatures have been used [14-18].

The density functional theory (DFT) and Hartree-Fock calculational frequencies of biotin performed by Emami et al [14] suggest that O-H in-plane bending and O-H out-of-plane bending vibrations mixes with C-C stretching and C-H out-of-plane bending vibrational modes, respectively. Our experimental bands of 1318 cm\(^{-1}\) and 795 cm\(^{-1}\) in the NRS show excellent agreement with theoretically values, respectively. The strong band at 1635 cm\(^{-1}\) which generally appears around 1662 cm\(^{-1}\) comes from the ureido ring stretching of biotin [18].

The relative intensities of the SERS spectra are expected to differ significantly from that of the NRS owing to specific surface selection rules [19]. The surface selection rule suggests that when a
molecule adsorbed flat on the silver surface, its out-of-plane vibrational modes will be more enhanced than its in-plane vibrational modes, and vice versa when it is adsorbed perpendicular to the surface [19, 20]. Further, vibrations involving atoms that are close to the silver surface will be enhanced. When the wavenumber difference between Raman bands in normal and SERS spectra is not more than 5 cm\(^{-1}\), the molecular plane is expected to be perpendicular to the silver surface [21].

**Figure 2.** Normal Raman spectrum of biotin in aqueous saturated solution.

**Figure 3.** SERS spectrum of biotin in silver colloid.

In the SERS spectrum of the title compound, prominent bands are observed at 1592, 1275, 1210, 814, 672, 230 and 142 cm\(^{-1}\). The ureido ring stretching vibration mode is observed as a very strong band at 1592 cm\(^{-1}\) in the SERS spectrum which is at 1551 cm\(^{-1}\) in the Raman spectra. It suggests that the ureido ring is not perpendicular to the silver surface. The medium band at 814 cm\(^{-1}\) in the SERS spectrum and the weak band at 795 cm\(^{-1}\) in the NRS have been attributed to the C-H out-of-plane
deformation. The weak band at 1007 cm\(^{-1}\) in the SERS spectrum has been attributed to the C-H in-plane bending vibrations. The comparison of the above two different C-H vibrational modes suggests that the molecular of biotin is parallel to the silver surface. Further, the C-H out-of-plane bending is obtained in the SERS spectrum at 1490 cm\(^{-1}\) and the absence of this vibration here in the NRS indicates again the parallel orientation of the molecule at the metal surface.

**Table 1.** Frequencies and assignments of Normal Raman and SERS bands of biotin.

| NRS (cm\(^{-1}\)) | SERS (cm\(^{-1}\)) | Assignment | References |
|-------------------|-------------------|------------|------------|
| 137 vs            | 142 vs            | CCCC torsion | [17]       |
| 230 vs            | 248 m             | C-S-C out-of-plane deformation, Ag-O stretching | [15,16] |
| 485 m             | 488 vw            |            |            |
| 559 m             | 552 vw            | O-H out-of-plane deformation | [17]       |
| 627 vw            | 621 vw            | N-H in-plane bending | [14]       |
|                   | 672 s             | N-H wagging | [14]       |
| 795 w             | 814 m             | C-H out-of-plane deformation | [16]       |
|                   | 848 m             | C-H out-of-plane bending | [14]       |
| 848 m             | 1007 vw           |            |            |
| 1089 vs           | 1099 s            | C-O stretching | [14]       |
| 1210 vs           | 1275 vs           | C-O stretching, C-H in-plane bending, C-C stretching, O-H in-plane bending | [16,17] |
| 1318 m            | 1399 s            | C-H out-of-plane bending, CH-CH\(_2\) deformation, C-C stretching | [16,17] |
| 1490 m            | 1592 vs           | CH\(_2\) stretching | [14]       |
| 1551 s            | 1635 s            | Ureido ring stretching | [18]       |

Abbreviations: vw, very weak; w, weak; m, medium; s, strong; vs, very strong.

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The presence of CH\(_2\) mode at 1399 cm\(^{-1}\) in the SERS spectrum indicates the closeness of CH\(_2\) group with metal surface. Emami et al [14] suggested that the vibrations of -CH\(_2\)- group (scissoring, wagging, twisting and rocking) contribute to several normal modes in the low frequency region and the wagging, twisting and rocking modes are distributed in a wide range. The experimental band at 1210 cm\(^{-1}\) supports this argument. The strong band at 1275 cm\(^{-1}\) is originated by the C-O stretching which is one of the characteristic carboxylic group vibrations. Also medium C-O stretching is found at 1099 cm\(^{-1}\) in the SERS spectrum with a blue shift of 10 cm\(^{-1}\) compared with this band in NRS. The bands at 672 cm\(^{-1}\) and 621 cm\(^{-1}\) are probably originated by N-H wagging and N-H in plane bending, respectively. The Raman band at 559 cm\(^{-1}\) in NRS assigned to O-H out-of-plane deformation vibration mode appears at 552 cm\(^{-1}\) in the SERS spectrum with a shift of 7 cm\(^{-1}\). This pronounced shift in the spectrum of the hydrogen-bonded species demonstrates that the OH group vibrations are likely to be
most sensitive to the environment. The appearance of a strong band at 230 cm\(^{-1}\) in the SERS spectrum in colloid ascribed to the Ag-O stretching indicates that the biotin molecule is adsorbed on the silver surface in colloid by the chemisorption of oxygen atom. Also, the band at 230 cm\(^{-1}\) is strengthened by the C-S-C out-of-plane deformation mode resulting from the tetrahydrothiophene ring vibration. According to the surface selection rule, it is supposed that the C-O and N-H vibration modes have strong interaction with the silver surface. The tetrahydrothiophene ring and the ureido ring of biotin are close to the silver particle surface in the adsorption geometry.

3.3. Chloride effect on SERS of biotin

Figure 4 displays the SERS spectra of biotin after addition of different concentrations of Cl\(^-\). The presence of Cl\(^-\) shows the following main effects: (1) decrease in the relative intensity of most SERS bands; (2) the spectral profile changes with shifts and splitting of selected bands; (3) decrease of a Ag-adsorbate bending band below 200 cm\(^{-1}\); and (4) the elimination of bands appearing at 488, 1007, 1055, 1399 and 1490 cm\(^{-1}\).

![Figure 4](image)

**Figure 4.** Effect of Cl\(^-\) in the SERS of biotin with the final concentration of Cl\(^-\) in the mixed systems is: (a) 0.01 mol/L; (b) 0.02 mol/L; (c) 0.04 mol/L; (d) 0.05 mol/L.

The effect of Cl\(^-\) on the SERS intensity is not well understood, since there are some adsorbates whose SERS signals are positively enhanced by the halide [22], while others are negatively decreased by it [23]. It is believed that these differences can be attributed to the nature of the adsorbate and the double contribution of the halide on both the EM and CT mechanisms, thus making difficult the
analysis of Cl\(^-\) effect. The SERS intensity quenching observed may be attributed to a surface-coverage restriction imposed by the co-adsorption of the halide [23], effectively competing with biotin for adsorption sites on the metal surface. When the concentration of sodium chloride in silver colloid is higher than 0.05mol/L, superfluous chloride ions may form an absorption layer so that biotin can not be adsorbed on silver surface directly.

The above phenomena may be attributed to the changes in adsorption orientation and surface-coverage [24]. In addition, these changes are assumed to be correlated with the aggregation of Ag sol in the absence of Cl\(^-\). A correlation between the UV–Vis absorption spectra and the morphologies observed in the TEM micrographs indicates that Cl\(^-\) induces changes in the particle sizes with the formation of both bigger and smaller particles due to the aggregation and diffusion of the initial particles in the presence of the Cl\(^-\) [25]. Due to the aggregation effect, large average radii of new particles leads to a decrease of the available surface and, a resultant increase of the adsorbate/surface ratio. It may be concluded that the SERS spectra obtained after adding Cl\(^-\) may be due to multilayer surface-coverage. In this case, the gradual decrease of SERS intensity with increasing Cl\(^-\) can account for the strong contribution of the CT mechanism, where absorbed molecule always shows weak SERS intensity at dense surface coverage [25].

On the basis of the increase of the adsorbate/surface ratio in the presence of halide, it may be deduced that the negative effect of surface concentration induced by the co-adsorption of halide, which competes with biotin for the adsorption on the surface, is compensated by the multilayer surface coverage and the aggregation of Ag sol created by the Cl\(^-\). The intensity, frequency and shape of the bands may also change due to the adsorbate reorientation.

After addition of Cl\(^-\) to the biotin-Ag system, the adsorption intensity and spectral profile differ greatly from that of both aqueous biotin and biotin-Ag system, in the absence of Cl\(^-\), such that one cannot consider the deviations to be attributed to simple desorption of biotin from the silver surface. The effect of Cl\(^-\) is also noted in the <300 cm\(^{-1}\) region. Figure 4 shows the SERS of biotin with different concentration of Cl\(^-\). The existence of an intense band attributed to Ag-adsorbate stretching vibration at 230 cm\(^{-1}\), in the absence of Cl\(^-\), demonstrates the adsorption of biotin on the metal, which may be produced by the electron transition between biotin and silver surface. With the increase of the concentration of Cl\(^-\), this band is strengthened, indicating the existence of adsorption competition between Cl\(^-\) and biotin and the increased possibility of CT interaction between adsorbate and metal. The changes in intensity and profile shape in the SERS spectra suggest different adsorption behavior and surface-coverage of biotin on silver surface.

4. Conclusion
The NRS and surface-enhanced Raman scattering spectra of biotin were obtained with very low laser power and the assignment of the vibrational wavenumbers was accomplished with the help of several former reports. The adsorption geometries of biotin molecules were analyzed based on the SERS data.

The Cl\(^-\) effect studies of the SERS spectra of biotin on silver surface suggests the existence of a first-layer effect and changes of adsorption behavior in the presence of Cl\(^-\). The changes of SERS intensity and spectral profile in presence of Cl\(^-\) are governed by the CT mechanism and co-adsorption of anions and molecules.
SERS is a powerful and convenient analytical tool for the investigation of the molecular structure information of biotin and its interaction with metal surface.

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