

Surface-enhanced Raman Spectroscopy of Benzimidazolic Fungicides: Benzimidazole and Thiabendazole

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Surface-enhanced Raman Scattering (SERS) spectroscopy is applied to the study of the adsorption of benzimidazolic fungicides benzimidazole (BIZ) and thiabendazole (TBZ) on silver mirrors. The influence of pH on the adsorption mechanism was investigated. In case of BIZ, two different adsorption mechanisms are deduced depending on the experimental conditions: *via* the π electrons of the ring in neutral conditions and through an ionic pairing of protonated nitrogen atom with the chloride adsorbed on the metal surface. The SERS spectra of TBZ revealed that most molecules were adsorbed on silver surface by the π electrons in neutral and acidic conditions but in acid conditions, some molecules were adsorbed *via* the sulfur and nitrogen atoms tilted slightly to the surface.

Key Words: Surface-enhanced Raman spectroscopy, Fungicide, Benzimidazole, Thiabendazole

Introduction

Thiabendazole (TBZ, 4-(1*H*-1,3-benzodiazol-2-yl)-1,3-thiazole) which belongs to the benzimidazole derivative, is a fungicide and parasiticide. It is known by the brand names Mintezol, Tresaderm, and Acrobetec. It is used primarily to control mold, blight, and other fungally caused diseases in fruits (e.g. oranges) and vegetables. TBZ is also used as a food additive, a preservative. For example, it is applied to bananas to ensure freshness, and is a common ingredient in the waxes applied to the skin of citrus fruits. Medicinally, thiabendazole is also a chelating agent, which means that it is used medicinally to bind metals in case of metal poisoning or antimony poisoning. As an antiparasitic, it is able to control roundworms, hookworms, and other helminth species which attack wild animals, livestock and humans.¹⁻³ Benzimidazole (BIZ, 1*H*-benzimidazole) also is known for commercial and biological importance as pharmaceuticals and fungicides. Despite its extensive use in the fields indicated above, it is surprising the fact that there is no complete vibrational assignment for the molecule in the scientific literatures. The only vibrational spectroscopic studies are limited to a brief report of the infrared spectrum.⁴⁻⁸

Surface-enhanced Raman spectroscopy (SERS) is a very useful technique in the analysis of the adsorption of molecules on metal surfaces. The enhancement of the Raman signal induced by metal nanoparticles, which is the basis of SERS spectroscopy, can be understood as a contribution from two different mechanisms: electromagnetic and charge-transfer mechanisms.⁹⁻¹² In the electromagnetic mechanism, the intensity of the surface plasmon resonance of the metal depends on many factors including the wavelength of the incident light and the morphology of the metal surface.¹⁰ The role of these nanoparticles is correlated with the mechanism involved in the SERS enhancement, being essential the formation of surface nanoparticles to induce a huge intensification of Raman emission through localized plasmon resonance in order to obtain high quality

SERS spectra.¹³⁻¹⁶ Recently, Saito *et al.*¹⁷ have shown that the use of silver mirror reaction produced a thin silver islands film on silicon wafer, which shows an order of magnitude superior surface enhancement property when compared to a conventional SERS substrate made by vacuum evaporation. Our group showed that the silver mirror substrates have an excellent surface enhancement on the Raman signal.^{18,19}

Therefore, in this paper, we studied the adsorption geometry of BIZ and TBZ on silver mirrors by SERS technique. The SERS study was done by testing the influence of pH. In order to assist in the interpretation of the Raman spectra, we have obtained preliminary assignments by means of the program HyperChem® (release 7.0). PM3 Hamiltonian was used to calculate the Raman frequencies of BIZ and TBZ in this program.

Experimental Section

Analytical-reagent grade BIZ, TBZ, silver nitrate, chloric acid, sodium hydroxide, d-glucose and ammonia water were purchased from Aldrich Co. and used without further purification. All solutions were prepared with triply distilled water and methanol. Silver mirror substrate was prepared by the Tollen's method, which is widely used for the identification of aldehyde.²⁰ A glass plate of 25 mm × 10 mm × 1 mm was put in a culture dish. A 10 mL of 5%-silver ammonia solution and 5 mL of 10%-sodium hydroxide solution were mixed in the culture dish. Then, 10 mL of 5%-D-glucose was added to the mixing solution. A few minutes later, the color of the solution turned to yellow, then dark brown. In the meanwhile, silver ions were reduced and deposited onto the glass plate to form a fine silver film called a silver mirror. After withdrawing the silver mirror from a culture dish, it was washed with distilled water and dried in room temperature. To make use of the prepared mirror, a 1 μ L of 1.0×10^{-3} M BIZ or TBZ solution was dropped onto it. After the evaporation of the solvents, the substrate was ready for SERS analysis. The pH of the solutions

was adjusted by injecting the necessary amounts of reagent grade HCl and NaOH solutions.

Raman and SERS spectra were recorded on a Nicolet Almega XR spectrophotometer in combination with a Thermo Scientific DXR Raman microscope by using radiation at 780 nm from diode laser for excitation. The laser power and confocal pinhole size were 100 mW and 25 μm , respectively. The images of the Scanning electron microscope (SEM) were obtained with a Hitachi S-4200.

Results and Discussion

As shown in Figure 1, the molecules of BIZ and TBZ are planar structures. Generally, the lone-pair electron and aromatic π system were easily bound to a metal surface. Hence, we assumed that BIZ and TBZ could be adsorbed on metal surface by the sulfur, the nitrogen atom or the π electrons of the ring. The SEM image of silver mirror substrate prepared by Tollen's method is shown in Figure 2.

Figure 3 shows the surface-enhanced Raman spectrum of BIZ adsorbed on silver mirror(a) and the ordinary Raman spectrum of BIZ in solid state(b) and 0.1 M solution(c). It is obvious that there are great-enhanced effects in the SER spectrum of BIZ on the roughened silver surface. The stronger Raman bands at 553, 782 and 1012 cm^{-1} in SERS are assigned to $\delta(\text{N-H})_{\text{oop}}$, $\delta(\text{C-H})_{\text{oop}}$ and $\delta(\text{C-C-C})_{\text{oop}}$, respectively. The weaker band at 1279 cm^{-1} is assigned ν_{ring} and $\delta(\text{C-H})_{\text{ip}}$. The band frequencies and their assignments based on literature and calculation are given in Table 1.^{21,22} To perform the PM3 calculations, we focused on the vibrational modes of BIZ. Figure 4 presents a diagram of the normal mode frequency of 553, 782, 1012 and 1363 cm^{-1} . As is well known that if the adsorption occurs *via* donation of π -system electrons, the plane of the aromatic ring would lie parallel to the surface and the totally symmetric in-plane stretching modes would be expected to show little or no surface enhancement, and certain out-of-plane vibrations would then show enhancement as per allowance of the symmetry. This line of approach has been successfully applied by many investigators.^{23,24} Besides, the C-H stretching band at 3072 cm^{-1} in ordinary Raman spectrum of BIZ became weaker in SERS remarkably. Therefore, it is thought that BIZ is adsorbed on to the silver surface with its molecular plane lying flat, as is shown in Figure 6a.

In Figure 5, the SER spectra of BIZ at pH 2.0, 7.0 and 12.0 are shown in the wavenumber range 200 ~ 1800 cm^{-1} and nearby 3000 cm^{-1} . The spectral pattern of the acidic solution spectrum differs from that of the neutral and basic solutions. The significant change in acidic condition is the intensity increase of the 628, 942, and 1174 cm^{-1} modes. Also, the 1353, 1513 and 1624 cm^{-1} modes are enhanced. The discussion about the orientation of the BIZ molecules to the silver surface has been made by applying the results of "surface selection rules" in the in-plane and out-of-plane vibrations. Therefore, according to these observations mentioned above in Figure 5, it is thought that BIZ is adsorbed on to the silver surface *via* the nitrogen atom with its molecular plane standing perpendicularly. These absorptions are possible by the formation of a strong ionic pair a nitrogen atom with the anion (in this case chloride) previously

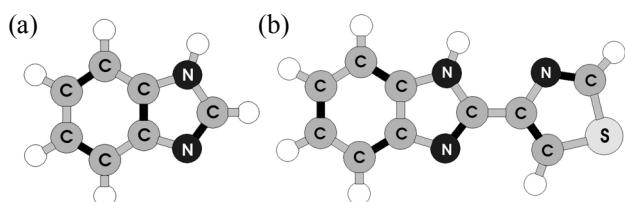


Figure 1. Chemical structure of the fungicides: (a) benzimidazole and (b) thiabendazole.

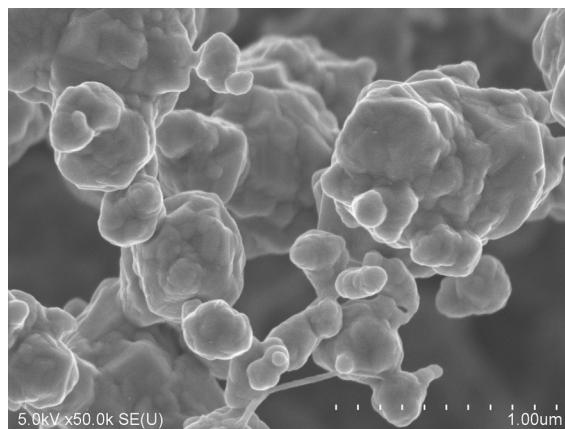


Figure 2. The SEM image of silver mirror.

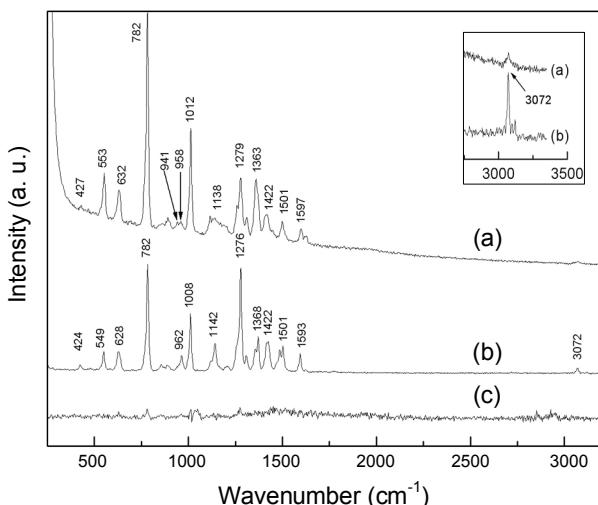


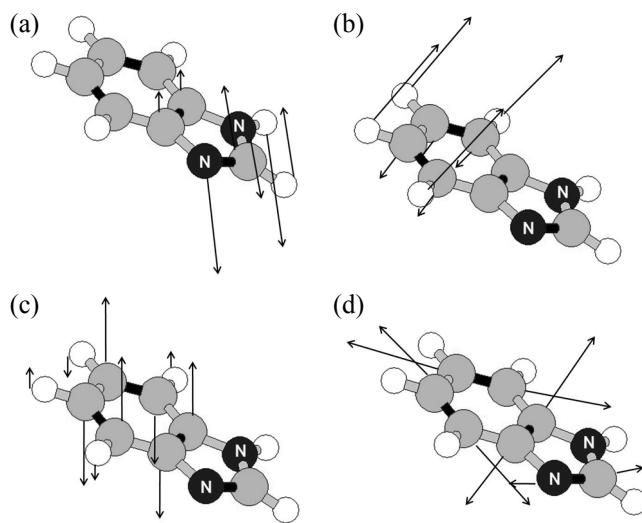
Figure 3. (a) SERS of BIZ (1.0×10^{-3} M) and ordinary Raman spectra of (b) solid state and (c) 0.1 M BIZ solution in methanol.

adsorbed onto the metal, as also observed for other cationic species.²⁵⁻²⁷ The interaction of the protonated N atom of BIZ with Cl^- seems to be so strong that a CT occurs between the chloride and BIZ in acidic conditions.²⁸ When the H_2SO_4 or HNO_3 solution was used instead of HCl, the SERS spectrum was not enhanced. But the SERS spectrum of the BIZ added to the H_2SO_4 and KCl solution was similar to Figure 5(a). These experimental results support the formation of a strong ionic pair. The enhancement of the band at 3072 cm^{-1} (Figure 5, inset)

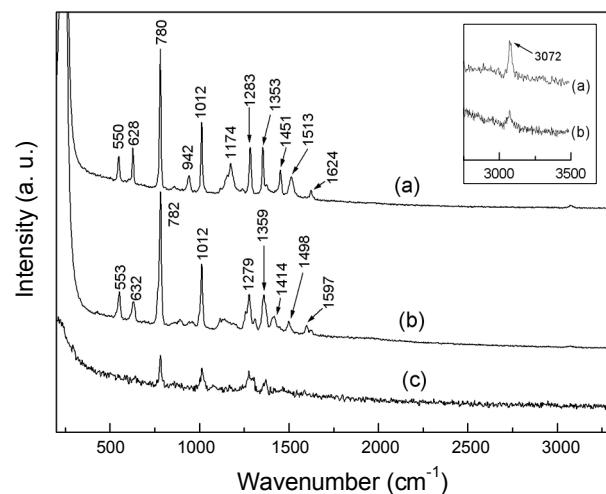
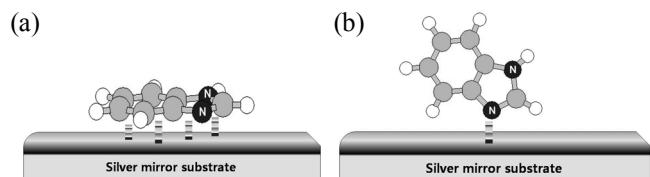
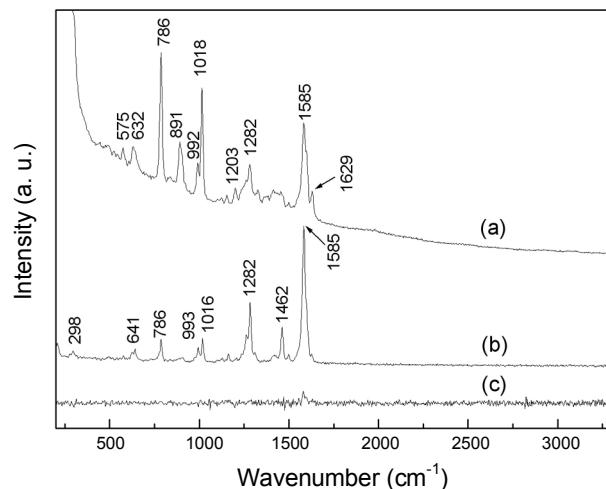
Table 1. Wavenumbers (cm^{-1}) and assignments corresponding to the calculated and experimental spectra of BIZ.

Calcul- ation	^a Solid Raman	^a SERS (pH = 7)	^a SERS (pH = 2)	^b Assignment
425	424(w)	427(vw)	-	$\delta(\text{C-C-C})\text{ip}$
556	549(m)	553(m)	550(m)	$\delta(\text{N-H})\text{oop}$, $\delta(\text{N-C-N})\text{oop}$
611	628(m)	632(m)	628(m)	$\delta(\text{C-C-C})\text{ip}$
771	782(vs)	782(vs)	780(vs)	$\delta(\text{C-H})\text{oop}$
951	-	-	942(m)	C-C ring breathing
956	962(w)	958(vw)	-	$\delta(\text{C-H})\text{oop}$
1000	1008(s)	1012(s)	1012(s)	$\delta(\text{C-C-C})\text{oop}$
1151	1142(m)	1138(w)	-	$\delta(\text{C-H})\text{ip}$
1151	-	-	1174(m)	$\delta(\text{C-H})\text{ip}$
1291	1276(s)	1279(m)	1283(s)	$\nu_{\text{ring}} + \delta(\text{C-H})\text{ip}$
1291	1309(sh)	1309(sh)	-	$\delta(\text{C-H})\text{ip}$
1408	1368(m)	1363(m)	1353(s)	$\nu(\text{C=C})$
1425	1422(m)	1422(m)	-	$\nu(\text{C=C})$
1495	1484(sh)	-	1451(w)	$\delta(\text{N-H})\text{ip}$
1495	1501(w)	1501(w)	1513(w)	$\nu(\text{C=C})$
1607	1593(w)	1597(w)	-	$\nu(\text{C=C})$
1639	1622(vw)	1627(vw)	1624(w)	$\nu(\text{C=N})$
3080	3072(w)	3072(vw)	-	$\nu(\text{C-H})$
3084	3118(vw)	-	-	$\nu(\text{N-H})$

^as = strong; m = medium; w = weak; vw = very; vs = very strong; sh = shoulder; ^bv = stretching; δ = bending; ip = in plane; oop = out of plane.

**Figure 4.** Vibrational modes of (a) 553, (b) 782, (c) 1012 and (d) 1363 cm^{-1} for BIZ. The arrows represent the relative displacement of the nuclei for that specific vibrational mode.

spectra), which is assigned to the stretching mode of C-H, further supports the adsorption orientation as shown in Figure 6b. The SERS spectrum of BIZ revealed little enhancement at pH = 12.0 as shown in Figure 5c, since the adsorption of molecules is supposed to be hindered by hydroxide ions previously adsorbed on to metal. It would rather similar to the ordinary Raman spectrum of 0.1 M BIZ solutions. In Figure 6, the plausible adsorp-

**Figure 5.** SERS spectra of 1.0×10^{-3} M benzimidazole on silver mirror at (a) pH = 2.0, (b) pH = 7.0, and (c) pH = 12.0.**Figure 6.** The plausible adsorption orientation of BIZ onto the silver mirror surface in (a) neutral and (b) acidic conditions.**Figure 7.** (a) SERS of TBZ (1.0×10^{-3} M) and ordinary Raman spectra of (b) solid state and (c) 0.1 M TBZ solution in methanol.

tion orientations of BIZ onto the silver mirror surface in neutral and acidic conditions were presented.

Figure 7 shows the surface-enhanced Raman spectrum of TBZ adsorbed on silver mirror (a) and the ordinary Raman spectrum of TBZ in solid state (b) and 0.1 M solution (c). The ordinary Raman spectrum of TBZ solution also cannot be obtained as the same as BIZ. The most significant change in SER spec-

Table 2. Wavenumbers (cm^{-1}) and assignments corresponding to the calculated and experimental spectra of TBZ.

Calculation	^a Solid Raman	^a SERS (pH=7)	^a SERS (pH=3)	^b Assignment
298	298(w)	-	-	$\delta(\text{C}-\text{S}-\text{C})\text{ip}$
578	-	575(w)	573(w)	$\delta(\text{C}-\text{C}-\text{C})\text{ip}$
635	641(w)	632(w)	641(w)	$\delta(\text{C}-\text{C}-\text{C})\text{ip}$, $\delta(\text{S}-\text{C}-\text{N})\text{ip}$,
771	786(m)	786(s)	781(s)	$\delta(\text{C}-\text{H})\text{oop}$
839	-	891(m)	889(m)	$\delta(\text{C}-\text{C}-\text{S})\text{oop}$, $\delta(\text{C}-\text{H})\text{oop}$
988	993(sh)	992(sh)	987(m)	$\nu(\text{C}-\text{S})$
1010	1016(m)	1018(s)	1015(s)	$\delta(\text{C}-\text{H})\text{oop}$
1194	-	1203(w)	1203(w)	$\delta(\text{C}-\text{H})\text{ip}$
1297	1282(s)	1282(m)	1282(w)	ν_{ring}
1315			1313(w)	$\nu(\text{C}=\text{C})$
1362			1360(w)	$\nu(\text{C}=\text{C})$
1434		1415(vw)	1432(w)	ν_{ring}
1451	1462(m)	1458(vw)	1470(w)	$\nu(\text{C}=\text{N})$
1584	1585(vs)	1585(s)	1601(m)	$\nu_{\text{ring}} + \nu(\text{C}=\text{N})$
1639		1629(sh)	1631(m)	$\nu(\text{C}=\text{N})$
3077			3086(vw)	$\nu(\text{C}-\text{H})$

^as = strong; m = medium; w = weak; vw = very; vs = very strong; sh = shoulder; ^b ν = stretching; δ = bending; ip = in plane; oop = out of plane.

trum is the strong intensity decrease of the band 1585, 1462 and 1282 cm^{-1} . These are attributed to totally ring stretching vibrations as also confirmed by comparison with assignments made for similar structural pesticides.^{22,29} While, the strong bands of intensity appearing at 786, 1018 and 891 cm^{-1} in the SERS spectrum may be assigned to C-H out-of-plane bending modes and C-C-S out-of-plane bending mode, respectively. The band frequencies and their assignments based on literature and calculation are given in Table 2.^{21,30} As shown in Figure 1, TBZ has delocalized π electrons in the molecule of planar structure, since the benzimidazole and thiazole rings are not allowed to rotate. Therefore, according to the SERS selection rule, the strong intensities of these out-of-plane vibration modes in the SERS suggest the parallel orientation of TBZ molecules to the silver surface as shown in Figure 10a. Figure 8 presents diagrams of the normal mode vibration of 786, 891, 1018 and 1585 cm^{-1} which are remarkable peaks in the SERS.

In other to compare the adsorption geometry according to the pH, we obtained the SER spectra of TBZ at pH 2.0, 7.0 and 12.0 as shown in Figure 9. The spectral patterns of TBZ in acidic and neutral solutions were similar. Only the bands of 987 and 1631 cm^{-1} in the acidic SERS are enhanced a little which may be assigned to $\nu(\text{C}-\text{S})$ and $\nu(\text{C}=\text{N})$, respectively. Also, the C=C and C=N stretching modes are splitted slightly in 1200~1500 cm^{-1} region. Therefore, most molecules were also adsorbed on surface *via* the delocalized π electrons in planar rings and some molecules were adsorbed *via* a sulfur and nitrogen atoms tilted slightly to the silver surface, in acidic solutions. The enhancement of the band at 3086 cm^{-1} (Figure 9, inset spectra), which is assigned to the stretching mode of C-H, further supports this adsorption orientation. The plausible adsorption orientations of TBZ in neutral and acidic conditions were presented in Figure

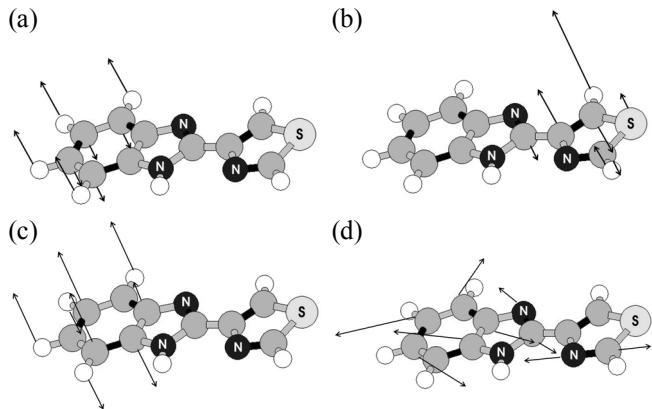


Figure 8. Vibrational modes of (a) 786, (b) 891, (c) 1018 and (d) 1585 cm^{-1} for TBZ. The arrows represent the relative displacement of the nuclei for that specific vibrational mode.

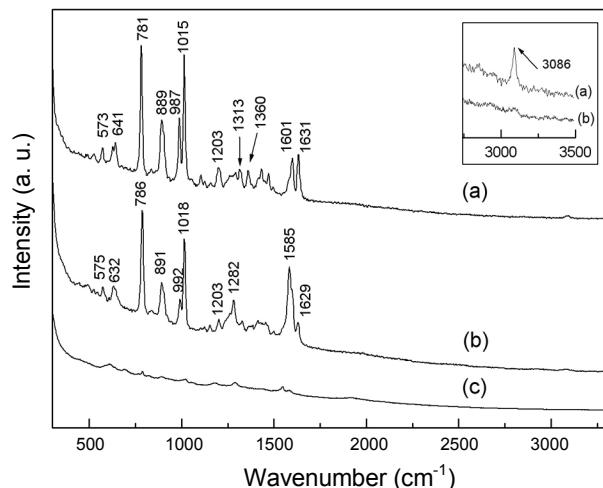


Figure 9. SERS spectra of 1.0×10^{-3} M TBZ on silver mirror at (a) pH = 2.0, (b) pH = 7.0, and (c) pH = 12.0.

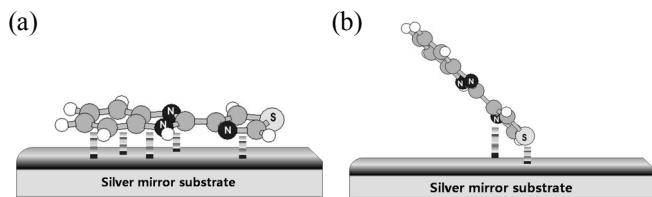


Figure 10. The plausible adsorption orientation of TBZ onto the silver mirror surface in neutral and acidic conditions.

10. The SERS spectrum of the basic solution of TBZ could not be enhanced as the same reason as BIZ.

Conclusion

SERS spectroscopy allows for the achievement of vibrational spectra of 1.0×10^{-3} M BIZ and TBZ fungicide solutions. In SERS spectroscopy, there is no necessary preprocessing of

sample solutions, therefore this technique could be employed for the detection of such fungicides in the environments. We have measured the SER spectra of BIZ and TBZ adsorbed on silver mirror substrates. In case of BIZ, completely different adsorption mechanisms are possible depending on the pH conditions. In neutral conditions, the adsorption of BIZ occurs *via* delocalized π electrons parallel to the surface and in acidic conditions, the interaction of the protonated N atom with chloride ion lead to perpendicular adsorption to the surface. The adsorptions of TBZ have similar mechanisms. Most adsorptions occur parallel to the surface *via* the delocalized π electrons in planar rings but some adsorptions are slightly tilted *via* the sulfur and nitrogen atoms in acidic conditions. In basic conditions, the SER spectra of both of BIZ and TBZ could not be obtained since the adsorption of molecules is supposed to be hindered by hydroxide ions.

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