

FTIR Study of Glyphosate–Copper Complexes

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Complexes of the herbicide glyphosate (GPS) and the heavy metal Cu were studied by infrared spectroscopy under controlled pH, in order to know the mechanisms involved in the formation of these complexes. In CuGPS⁻, the IR spectrum shows participation of the carboxylate and phosphonic moieties of the GPS molecule. The formation of the complex produces a lower symmetry in the phosphonate group because of loss of the resonance situation of PO₃²⁻ groups, with a subsequent split of their absorption bands. Carboxylate groups are participating by forming unidentate complexes. No conclusion is reached about the involvement of the amino group, but previous EPR findings indicate coordination of GPS to Cu via nitrogen. Consequently, glyphosate in this complex functions with a tridentate character by forming two chelate rings. A study of the CuGPSH species was not possible due to overlapping of its absorption bands with those of free GPS species.

KEYWORDS: Glyphosate; copper; complexes; infrared spectroscopy

INTRODUCTION

Glyphosate (GPS) is a widely used herbicide, the low phytotoxicity of which in soils is attributed to its strong adsorption to soil components, on which it is rapidly inactivated by microbial degradation (1). Adsorption in soils and sediments is considered to occur by the phosphonate group, which would explain the competition for GPS adsorption sites exerted by inorganic phosphate (2, 3). Moreover, this herbicide is able to form strong complexes with transition metals due to the presence of amino, carboxylic, and phosphonic moieties in its molecule, which each could coordinate separately to metal ions or in combination as a bidentate or tridentate ligand (4, 5). Morillo et al. (6) indicated that adsorption of glyphosate on soils and its release are dependent not only on the type of soil but also on whether there is any element in the soil solution or adsorbed on the soil capable of forming strong complexes with the herbicide. As a consequence, the strong adsorption reported for glyphosate on iron and aluminum oxides should be expected due to the high stability of Fe³⁺– and Al³⁺–glyphosate complexes (7, 8). Adsorption to organic matter is suspected to occur by complexation to metal–humic substances (9, 10). In homoionic montmorillonites, glyphosate is adsorbed only when it forms a neutrally or positively charged complex with the exchangeable cation (11).

On the other hand, glyphosate is involved in the biochemical alteration of various metabolic processes in plants and microorganisms. Its primary mode of action is the inhibition of the action of the EPSPS enzyme in the shikimate pathway, which is involved in the biosynthesis of essential aromatic amino acids (12). Although there is no good evidence of any other primary site of action, very rapid effects of glyphosate on photosynthesis

in some species are difficult to explain by an effect on EPSPS (13). In this concern, the effects of glyphosate on aromatic amino acid synthesis in *Escherichia coli* have been attributed to chelation of Co²⁺ and Mg²⁺, cofactors for enzymes in this pathway (14).

Because of the importance of metal chelation by glyphosate, we have aimed at elucidating which mechanisms are implicated in metal–glyphosate complexes by using vibrational spectroscopy (FTIR). We have focused on the metal copper due both to its agronomic importance, being frequently added as a fungicide together with this herbicide, and to the possibility to make further comparisons of our results with those from recent studies using different techniques (15, 16).

MATERIALS AND METHODS

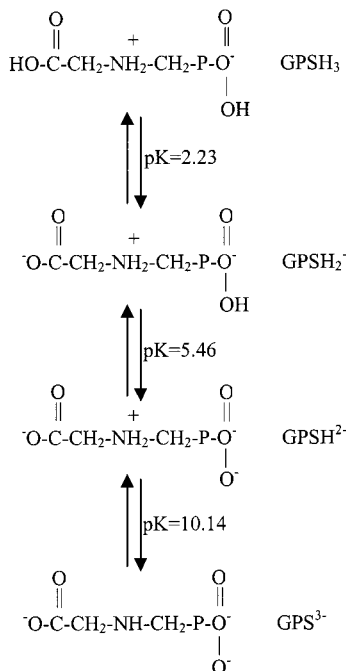
Glyphosate [*N*-(phosphonomethyl)glycine] was supplied by Monsanto Co. (S. Louis, MO). This pesticide is a nonselective, nonresidual herbicide with solubility in water of 12 g L⁻¹ at 25 °C. This herbicide is a weak acid with four acidity constants. However, only three of them can be measured due to the formation of a zwitterion structure between the amino and the phosphono groups in solution. The proposed dissociation diagram and protonation pK values (at 25 °C) are shown in **Scheme 1** (5).

Analytical grade CuCl₂ was obtained by Merck (Darmstadt, Germany).

A 0.5:0.5 mM Cu:GPS solution was prepared at pH 4.0 and 7.0. The pH was adjusted by using 0.01 M NaOH aliquots. In addition, a 0.5 mM GPS solution was prepared analogously at pH 4.0 and 7.0. These solutions were immediately freeze-dried, the FTIR spectra being obtained from the solid samples.

GPS species in these solutions were calculated by taking into account the pK values of protonation of GPS (**Scheme 1**) and the pK values of formation of GPS–Cu complexes (5). This calculation was carried out by a computer program developed by the authors, based upon the Newton method for solving polynomial *n*-degree equations.

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Scheme 1. Species of GPS Depending on the pH and Protonation Constants, from Motekaitis and Martell (5)**Table 1.** Cu^{2+} , GPS, and Cu–GPS Species in Solution (Molar) as a Function of pH in the Absence and Presence of Cu

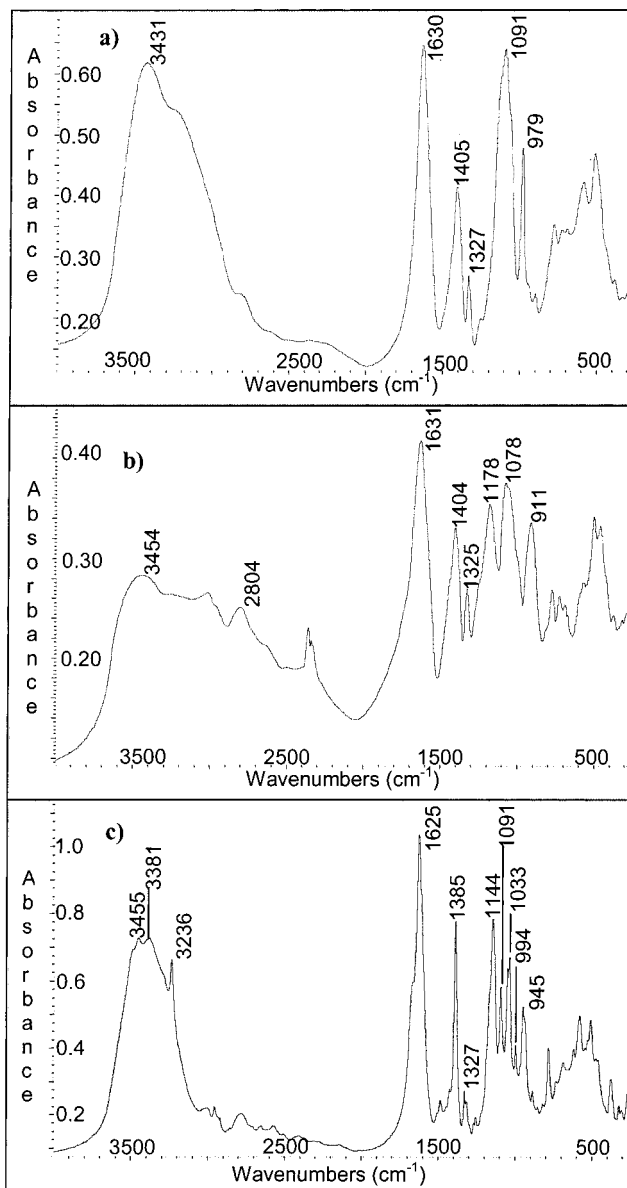
	GPS		Cu–GPS	
	pH 4.0	pH 7.0	pH 4.0	pH 7.0
Cu^{2+}			1.03×10^{-4}	9.1×10^{-7}
CuGPS^-			2.17×10^{-4}	4.98×10^{-4}
CuGPS_2^{4-}			6.53×10^{-12}	3.91×10^{-9}
CuGPSH			1.80×10^{-4}	4.14×10^{-7}
GPS^{3-}	1.19×10^{-10}	3.52×10^{-6}	2.47×10^{-12}	6.43×10^{-10}
GPSH^{2-}	1.65×10^{-4}	4.85×10^{-3}	3.40×10^{-6}	8.87×10^{-7}
GPSH_2^-	4.75×10^{-3}	1.39×10^{-4}	9.82×10^{-5}	2.56×10^{-8}
GPSH_3	8.08×10^{-5}	2.38×10^{-9}	1.67×10^{-6}	4.35×10^{-13}

FTIR spectra (range $4000\text{--}250\text{ cm}^{-1}$) were obtained with 4 cm^{-1} resolution on a Nicolet 510P spectrophotometer. The samples were examined as KBr disks.

RESULTS AND DISCUSSION

Table 1 shows the calculated concentrations of Cu^{2+} , GPS, and Cu–GPS species in the solutions for which the infrared spectra were recorded after being freeze-dried. GPS remains mostly as GPSH^{2-} (97.1%) in the absence of Cu at pH 7, whereas the GPSH_2^- species becomes the more abundant (95.1%) when the pH is decreased to 4.0. Consequently, infrared information concerning the GPSH^{2-} and GPSH_2^- species can be obtained from these solutions after freeze-drying, and their FTIR spectra are shown in **Figures 1a,b**, respectively. The absorption bands of the functional groups in the GPS molecule and its assignments are listed in **Table 2** for the different species of GPS and Cu–GPS.

According to **Scheme 1**, the GPSH^{2-} species has completely deprotonated the phosphonate and carboxylic groups, whereas the nitrogen bears a positive charge. **Figure 1a** shows typical absorption bands of amino acids at 1630 and 1405 cm^{-1} corresponding to antisymmetrical and symmetrical stretching bands of ionized carboxylic groups. The presence of NH_2^+

**Figure 1.** FTIR spectra of 0.5 mM GPS solutions after freeze-drying at pH 4.0 (a) and pH 7.0 (b) and in the presence of a 0.5 mM Cu concentration at pH 7.0 (c).**Table 2.** Infrared Band Assignments (cm^{-1}) for GPS and Cu–GPS Species

assignment	GPS		Cu–GPS
	pH 4.0	pH 7.0	pH 7.0
$\nu(\text{OH})$	3454	3431	3236, 3381, 3455
hydrogen-bonded P–OH	2900–2500	2900–2700	
$\nu_{\text{asym}}(\text{COO}^-)$	1630	1630	1625
$\nu_{\text{sym}}(\text{COO}^-)$	1405	1405	1385
$\nu_{\text{asym}}(\text{P–O}^-), \text{PO}_2(\text{OH})^-$ group		1078	
$\nu_{\text{sym}}(\text{P–O}^-), \text{PO}_2(\text{OH})^-$ group		1178	
$\nu(\text{P–OH})$		911	
$\nu_{\text{asym}}(\text{P–O}^-), \text{PO}_3^{2-}$ group		1091	
$\nu_{\text{sym}}(\text{P–O}^-), \text{PO}_3^{2-}$ group		979	
$\nu(\text{P–O}^-), \text{PO}_2(\text{OCu})$ group			1144, 1091, 1033, 994
$\nu(\text{P–OCu})$			945

groups cannot be easily identified because the N–H deformation frequency is not detected due to overlapping with carboxylate bands.

With respect to the phosphonate group of GPS in **Figure 1a**, the absorption bands at 1091 and 979 cm^{-1} should be assigned to the antisymmetrical and symmetrical stretching modes of P–O bonds, respectively. The intensity of the antisymmetrical absorption band is considerably higher than that of the symmetrical mode in accord with infrared studies by Bellamy (17).

Barja and Dos Santos (18) reported similar assignments for an AT-FTIR study of GPS in aqueous solutions. Despite the FTIR spectra being recorded in different media, the absorption bands obtained in both states and their frequencies are similar, although a broadening of the absorption bands in aqueous solutions in comparison to those in solid state is noticed. These authors considered the band at 1327 cm^{-1} due to stretching of an alkyl–P vibration, but in agreement with Bellamy (17), we attributed it to a typical band present in most of amino acids at $\sim 1300 \text{ cm}^{-1}$.

The spectrum of GPS at pH 4.0 is shown in **Figure 1b**. The changes of the absorption bands of GPS with respect to those in **Figure 1a** are due to the presence of the species GPSH_2^- . In **Figure 1b**, new bands at 1178, 1078, and 911 cm^{-1} appear instead of those at 1091 and 979 cm^{-1} . Shoval and Yariv (19) reported the ranges of 1160–1180 and 1070–1080 cm^{-1} for the asymmetric and symmetric stretching modes of P–O bonds in the phosphonate group of GPS, due to the resonance between P–O and P–OH forms involved in H-bonding. Barja and Dos Santos (18) also gave similar assignments to the bands at 1188 and 1082 cm^{-1} . We also assigned the bands at 1178 and 1078 cm^{-1} to the vibration modes of the P–O bonds in GPSH_2^- .

The band at 911 cm^{-1} can be assigned to P–O stretching band of the P–OH system. Bellamy (17) reported a relatively large frequency range for this absorption (from 1040 to 909 cm^{-1}) because the frequency varies with the inductive properties of the substituents. Barja and Afonso (18) also attributed a band at 917 cm^{-1} to the P–OH group, although Shoval and Yariv (19) and Piccolo and Celano (20) assigned this absorption band to CCNC vibrations.

The region between 2900 and 2500 cm^{-1} shows a complex of many broad bands that correspond to strongly hydrogen-bonded P–OH groups.

Infrared spectra of Cu–GPS complexes can be studied by using a 1:1 Cu:GPS ratio. As shown in **Table 1**, CuGPS^- is the most abundant species at pH 7.0, being 99.6% of the total GPS added. However, at pH 4.0, the percent of CuGPSH increases to 36.0%, whereas the percents of the CuGPS^- and GPSH_2^- species amount to 43.3 and 19.6%, respectively, of the total GPS. A larger amount of CuGPSH species by a larger decrease in pH is not feasible due to a concomitant increase in the percents of noncomplexed GPS species instead. The concentration of the CuGPS_2^{4-} species is very low (10^{-9} – 10^{-12}) in the whole range of pH studied.

The spectrum of Cu–GPS at a 1:1 molar ratio and pH 7.0 is shown in **Figure 1c**. The CuGPS^- spectrum is much sharper than those of GPSH_2^- and GPSH^- in **Figure 1a,b**, respectively, and displays more discrete absorption in the entire range. Spectra of GPS in **Figure 1a,b** have broader absorptions over the 3000–2000 cm^{-1} region, indicative of hydrogen-bonded acid hydroxyls from P–OH group, which are less probable in the presence of Cu.

An important difference of this spectrum from those in **Figure 1a,b** is the shift of the stretching bands of carboxylate groups to lower frequencies (1625 and 1385 cm^{-1} instead of 1630 and 1405 cm^{-1}). According to Boyd et al. (21), the separation of the vibrations corresponding to the antisymmetrical and symmetrical modes of carboxylate groups is related to its mode of

complexation with metals: unidentate complex, chelated ion, or uncomplexed. In our case, the difference obtained between these two vibration modes (240 cm^{-1}) is in agreement with a unidentate complex.

New changes are also observed for the antisymmetric and symmetric stretching modes of the phosphonate group, indicating its involvement in Cu–GPS complexation. The formation of the CuGPS^- species produces a loss of the resonance situation of PO_3^{2-} groups, because one of the oxygen atoms is probably strongly bonded to Cu. As a consequence, this lack of symmetry in the phosphonate group produces a split of the absorption bands of the stretching modes into four new bands. At the level of this study, we cannot predict which bands belong to the asymmetric and symmetric modes of vibration of the P–O group.

The spectrum in **Figure 1c** is more comparable to that in **Figure 1b** than in **Figure 1a**, due to the presence of a PO_2^- group both in the species GPSH_2^- and in CuGPS^- . The band at 911 cm^{-1} in **Figure 1b**, which was assigned to the P–OH group, disappears, and, instead, a new band appears at $\sim 945 \text{ cm}^{-1}$ due to the P–OCu group. This confirms our previous assignment of the band at 911 cm^{-1} to P–OH.

In **Figure 1c**, we cannot observe absorption bands corresponding to the secondary amine group of GPS due to their overlapping with carboxylate bands. In this case, the use of another technique such as electron spin resonance spectroscopy (EPR) could provide valuable information about N involvement in the formation of this complex. Undabeytia et al. (22) reported spectral parameters for CuGPS^- that were consistent with 1N3O coordination in the equatorial ligand positions by comparison with a modified Peisach–Blumberg plot. The coordination via nitrogen would be analogous to that in copper–glycine complexes (23). McBride (24) also considered a square-planar geometry complex where copper was coordinated to nonprotonated amine N, phosphonate, and carboxylate moieties and an additional water molecule. This is consistent with the sharp absorption band at 3236 cm^{-1} observed in CuGPS^- (**Figure 1c**) and not in GPS at pH 7.0 (**Figure 1a**), which suggests the presence of a hydrate similar to that found in a salt hydrate (25). In addition, the bands at 3455 and 3381 cm^{-1} could also be hydrate OH stretching bands. These latter features are not due to the presence of a basic copper salt. Calculations not shown indicate that free Cu concentrations were undersaturated at the selected pH as compared to those for precipitation of basic copper salts according to the constants given by Lindsay (26).

Dubin et al. (15) established by using EXAFS spectroscopy that glyphosate functions as a tridentate ligand in the complex CuGPS^- via amine nitrogen, carboxylic, and phosphonate oxygens. These results are consistent with the binding mechanisms proposed in the present study and with those reported by Daniele et al. (16), who also suggested for CuGPS^- a tridentate character, with the formation of two chelate rings, consistent with a coordination by one amino group and two negatively charged donor groups.

Concerning the CuGPSH species, Motekaitis and Martell (5) argued that this bidentate complex is protonated on the amine group. However, McBride (24) considered direct bonding between nonprotonated nitrogen and Cu. This author suggested that the displaced proton for the $\text{CuGPSH} \rightarrow \text{CuGPS}^-$ reaction should have come from a more acidic group rather than a protonated amine. As stated previously, molecular information concerning CuGPSH species is not possible to obtain from the infrared spectrum at pH 4.0 (not shown) due to its overlapping

with that of the GPSH_2^- species, which amounts to ~19% of total GPS (**Table 1**).

The behavior of glyphosate in soils and water is strongly dependent on the formation of metal–glyphosate complexes. pH has a strong impact on the mobilization of this herbicide, by affecting the soil surface properties and its speciation in solution. The assignment of the absorption bands of the current work can prove useful for a further study of the interaction mechanisms between glyphosate and metal ions with soil constituents.

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