

Methodological Approach to the Study of the Formation and Physicochemical Properties of Phosphate–Metal–Humic Complexes in Solution

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The aim of this work is to study the suitability of the complementary use of ultrafiltration (UF) and the interaction with an anion-exchange resin (AR) to characterize of phosphate–metal–humic complexes in solution. The results indicate that a methodological approach consisting of the validation and calibration of the AR method by the UF method and the further use of the AR method is suitable for characterizing phosphate–metal complexes. Such an approach has proven to be useful for calculating the phosphate maximum binding capacity of iron–humic complexes and stability constants. It might also be used to obtain valuable purified phosphate–metal–humic complexes for further structural characterization.

KEYWORDS: Humic substances; metal–humic complexes; phosphorus; phosphate; phospho-humic complexes; phosphate–metal–humic complexes; ultrafiltration; anion-exchange resin

INTRODUCTION

Numerous studies have reported the close relationship between phosphorus (P) bioavailability in soils and the concentration of soil organic matter (SOM) (1). Other studies have shown the beneficial effect of organic amendments (OA) containing significant concentrations of humic substances on both plant available P and plant P uptake in different soil types (2, 3). Although a number of studies have related this beneficial effect to the action of those fractions of organic acids with lower molecular weight derived from plant root exudation and SOM decomposition (3), other studies have shown that humic substances could also play an important role in this process (3).

Besides the possible action of the different fractions of SOM on soil P dynamics through the increase in soil microbial activity and the activity of soil enzymes (4), other more direct mechanisms could also be important. These include (i) the competition between specific acidic fractions of SOM, principally those of lower molecular weight, and phosphate (PO_4^{3-}), which inhibits PO_4^{3-} soil absorption (5); (ii) the modification of the precipitation pattern of calcium phosphates, which produces salt species with higher water solubility such as dicalcium phosphate dihydrate (6); (iii) the mobilization of free PO_4^{3-} from precipitated phosphates (iron, aluminum, or calcium phosphates) as a result of the formation of stable cation–humic complexes (7); and (iv) the mobilization of PO_4^{3-} through the possible formation of stable PO_4^{3-} –metal–humic complexes (8).

Many authors have proposed the formation of soluble or insoluble PO_4^{3-} –metal–humic complexes as one of the possible mechanisms of action of humic substances on soil P dynamics (8–12). In many cases, the hypothesis has been based on indirect results mainly related to the correlation among the PO_4^{3-} distribution in solution, certain metals (Fe or Al), and humic substances after fractionation of the solution using size exclusion chromatography (11, 13), the fixation of PO_4^{3-} in precipitated metal–humic complexes (12, 14, 15), or the variation in the ^{31}P NMR spectra obtained in the presence of humic substances and metal–humic complexes (3, 16). However, specific studies aimed at investigating the formation and physicochemical characteristics (stoichiometry, stability, and solubility) of these PO_4^{3-} –metal–humic complexes have not been carried out. This has been due to the lack of an appropriate methodology for measuring the variation of the free PO_4^{3-} /complexed PO_4^{3-} ratio in solution as a function of certain experimental conditions such as the humic substances/metal/ PO_4^{3-} ratio, the pH, or ionic strength (1). Recently, Riggle and von Wandruszka (17) reported the use of a special, noncommercial, selective Co electrode to evaluate the stability of the complexes formed by PO_4^{3-} with different metal–humic complexes in solution. These authors found both stability constant values in the same order as those of the metal–humic complexes ($\log K = 4\text{--}6$) (17) and no clear differences between the stability of PO_4^{3-} complexes formed by humic complexes with different metals. In this context the aim of our work has been to develop an alternative methodological approach that could allow us to measure the concentration of PO_4^{3-} that is retained by metal–humic complexes in solution. To this end we have explored the

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suitability of two complementary analytical techniques: ultrafiltration (UF) and the interaction with an anion-exchange resin (AR).

Theoretically, UF is a particularly suitable technique for separating free PO_4^{3-} from humic-bound PO_4^{3-} without interfering with the integrity of PO_4^{3-} -metal-humic complexes. However, this technique can be time-consuming and rather expensive. Conversely, AR may be faster and cheaper, but it can compete with metal-humic complexes for PO_4^{3-} , thus causing the possible hydrolysis of a fraction of the PO_4^{3-} -metal-humic substances complex. From this it can be seen that the complementary use of these two analytical techniques may facilitate the evaluation of the degree of hydrolysis of the PO_4^{3-} -metal-humic complexes due to the action of the anion-exchange resin from the data obtained using UF. In this way, we could use UF to validate AR for each specific PO_4^{3-} -metal-humic system and AR as a routine technique to investigate the interaction between PO_4^{3-} and the metal-humic complexes.

In this work we study the formation and stability of the complexes formed between PO_4^{3-} and iron (Fe)-humic complexes in solution at three different pH values: 4, 6, and 8. These pH values were selected to encompass the pH values corresponding to acid and alkaline soils. To consider the main physicochemical features of humic acids, one model humic acid obtained from leonardite was used in the experiments. Our main objective is to develop an analytical approach that allows PO_4^{3-} -metal-humic complexes to be studied at the laboratory level and under standardized experimental conditions. The adaptation of this methodology to the study of this type of complex in waters and soils will need further research.

In developing the methodology described here, we attempted without success ion chromatography, dialysis, and gel filtration techniques as alternative means for measurements of PO_4^{3-} -Fe-humic complexation.

MATERIALS AND METHODS

Elemental Analysis. P (as PO_4^{3-}) in solution was analyzed using both the green malachite method (18) and ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL).

The carbon (C) content in solution was analyzed using elemental analysis (LECO CHN 2000), and the Fe content in solution was determined using ICP-OES spectrometry.

Humic Sample and Preparation of the PO_4^{3-} -Fe-Humic Substances Complexes Used in the Experiments. The humic material selected as model humic acid and used in the different experiments was a humic acid extracted from leonardite (HA). HA was previously purified using the IHSS methodology without including the treatment with the HF-HCl mixture (19). The main composition of the purified HA was 58.47% C, 1.46% N, 2.69% H, 25.76% O, 0.01% P, 0.99% S, 1.02% Fe, and 0.95% Al. The concentration of the main acidic functional groups obtained using potentiometric analysis was 2.31 mmol g^{-1} of carboxylic groups and 0.62 mmol g^{-1} of phenolic groups.

Fe-HA complexes were prepared by dissolving 100 g of the humic material in 500 mL of pure water. pH was maintained around 10.5 with 10 M NaOH. The solution was stirred for 4 h and centrifuged for 15 min at 7000g. Fe (NO_3)₃·9H₂O (8.6 g) dissolved in 20 mL of water was slowly dropped into 200 mL of the supernatant containing HA under vigorous stirring and continuous pH control (8–9) with 10 M NaOH. The solution was diluted to 500 mL, stirred for 14 h, and then centrifuged for 15 min at 7000g to separate the Fe-HA complexes in solution from the precipitated Fe-HA complexes and iron hydroxides. Finally, the Fe and C contents in solution were analyzed as previously described. This reaction yielded ~80% of the total Fe introduced in the reaction as Fe complexed by HA, in solution.

PO_4^{3-} -Fe-HA complexes were prepared by adding an aliquot of a solution of 0.5 mM H₃PO₄ containing the necessary quantity of PO_4^{3-}

to achieve specific Fe/P molar ratios in solution to 15 mL of the Fe-HA complex solution. pH was adjusted to 4, 6, or 8 with 0.5 N HNO₃ or 0.5 N NaOH, and the solution was diluted up to 50 mL with pure water. Afterward, the solutions were stirred for 14 h at 22 °C. Once the reaction was over, the solutions were centrifuged for 15 min at 7000g. Finally, the total C, P, and Fe contents in solution were analyzed as described above. The concentration of P (as PO_4^{3-}) complexed by Fe-HA in solution was measured using AR and UF methodologies as described below.

Analysis of PO_4^{3-} Complexed by Fe-HA. Characterization of AR and UF Methodologies. AR Methodology. The OH⁻ anion-exchange resin Amberjet 4400 (Sigma) (1.8 mequiv g^{-1} of exchange capacity) conditioned in its HCO₃⁻ form was used in the experiments. The PO_4^{3-} fixation capacity of HCO₃⁻-conditioned resin was 30 $\mu\text{mol g}^{-1}$.

The methodology used to prepare the HCO₃⁻ anion resin (HCO₃-R) form is described below.

Fifty grams of the OH⁻ anion-exchange resin was stirred in 500 mL of a 1 M NaHCO₃ solution for 1 h. The suspension containing the resin was then vacuum filtered, washed with 750 mL of water, and air-dried for 10 min. The HCO₃-R obtained had a pH (1% in water) within the range of 7–8 and moisture between 40 and 50%.

To study the capacity of the conditioned anion-exchange resin to fix PO_4^{3-} as a function of both the PO_4^{3-} /HCO₃-R ratio and the presence of different concentrations of NO₃⁻, the following set of experiments was carried out: different amounts of HCO₃-R (0.3, 0.6, 0.9, and 1.2 g) were added to 10 mL of a solution of H₃PO₄ containing 50 mg L⁻¹ of P (0.5 mg of total P) and different concentrations of NO₃⁻. After 30 min (this time of equilibrium was selected on the basis of the results obtained in previous studies carried out with the objective of obtaining the minimum time required to fix free PO_4^{3-} in the resin as a function of PO_4^{3-} concentration and the amount of HCO₃-R; data not shown), the suspension was filtered and the P content in solution was analyzed. These studies were carried out at different pH values (4, 6, and 8). The pH was adjusted with 0.1 M NaOH or 0.1 M HNO₃. The NO₃⁻ concentrations in the solution used in the experiments, corresponding to *I* values of 10, 15, and 20 mM, were selected according to the range of *I* expected in the samples corresponding to the reactions.

To study the degree of fixation of HA and Fe-HA in HCO₃-R as a function of the time of contact between HCO₃-R and the solution, 0.9 g of HCO₃-R was added to 10 mL of a water solution containing a specific concentration of HA (~2250 mg L⁻¹ of C) selected according to the expected HA concentration in reactions. After time intervals of 15, 30, 60, 90, and 120 min, the suspension was filtered, and the retention of the humic material in HCO₃-R was analyzed by measuring the difference in absorbance at 400 nm between the HA mother solution and the HA solution treated with HCO₃-R. This same experiment was carried out using Fe-HA complexes.

To analyze the concentration of free and complexed PO_4^{3-} in the reactions between specific amounts of PO_4^{3-} and Fe-HA complexes (see above), the following methodology was used: 0.9 g of HCO₃-R was added to 10 mL of the reaction solution containing a total P content of ~50 mg L⁻¹. The pH in the determinations was 4, 6, or 8 depending on the study. After 30 min, the suspensions were filtered and the PO_4^{3-} content in solution was analyzed. The PO_4^{3-} complexed by Fe-HA was calculated by subtracting the P in solution in the reactions from the free P in solution in the blank. This blank (PO_4^{3-} and HCO₃-R only, in the same conditions of pH and ionic composition as in the reactions) indicates the amount of free PO_4^{3-} that is not fixed by HCO₃-R in these conditions of *I*. We assumed that the pattern of interaction between free PO_4^{3-} and HCO₃-R was not affected by the presence of Fe-HA and that the fraction of free PO_4^{3-} remaining in solution after the reaction was fixed by HCO₃-R (the fraction of free PO_4^{3-} that was not fixed by the resin is calculated using the blanks). The different analytical determinations were carried out with four replications.

UF Methodology. A Centricon YM-3, 3000 Da molecular weight cutoff (MWCO) (Millipore), was used in the experiments. The membranes were conditioned with pure water, immediately before every UF process, following the manufacturer's instructions.

To calculate the retention of PO_4^{3-} on the membrane in the experimental conditions of the real reactions, a curve representing the total free P (as PO_4^{3-}) in solution versus the total free P in the filtrate was obtained for each pH value. The range of PO_4^{3-} concentrations was selected to cover the PO_4^{3-} range included in the reactions (5–50 mg L^{-1}). The final pH in determinations was 4, 6, or 8 depending on the study (the same as in the reactions).

To calculate the degree of retention of HA and Fe–HA complexes by the UF membrane, solutions containing different concentrations of HA or Fe–HA were ultrafiltered under the same conditions of pH (4, 6, and 8) and I (0.01–0.02 M) as the reactions. The degree of membrane retention of HA and Fe–HA was calculated by measuring the change between the absorbance at 400 nm in the ultrafiltered samples and the non-ultrafiltered samples. The concentration of Fe in the filtrate was also measured as previously described.

UF determinations were carried out using the following methodology: 2 mL of the reaction containing free PO_4^{3-} and complexed PO_4^{3-} was ultrafiltered in the 3000 MWCO membranes (volume = 3 mL) by centrifugation at 7000g for 90 min. Once the centrifugation had finished, an aliquot of 0.1 mL was taken from the filtrate for the analytical determination of free P, and the real free P was calculated using the calibration curves previously obtained. The pH and I in UF were the same as in the reactions (pH 4, 6, or 8; and $I = 0.01$ – 0.02 M). The different analytical determinations were carried out with a minimum of four replications.

Other MWCOs (1000 Da and the consecutive use of 10000–1000 Da) were also studied in preliminary experiments (20). However, these alternatives were abandoned due to the high variability [coefficient of variation (CV) > 20%] in the values of PO_4^{3-} retention in membranes depending on the lot of manufacture of the UF (1000 MWCO) membranes used (data not shown).

Study of the Stability of PO_4^{3-} –Fe–HA Complexes. The pH-dependent stability of PO_4^{3-} –Fe–HA complexes in solution was estimated using an apparent stability constant (K) calculated according to the Scatchard method (19):

$$(P_{\text{TC}}/\text{MBA})/P_{\text{TF}} = K - (P_{\text{TC}}/\text{MBA})K \text{ or } \theta/P_{\text{TF}} = K - \theta K$$

P_{TF} is the total free P in the equilibrium, P_{TC} is the total complexed P, and θ is the sites bound/maximum metal binding capacity (MBA) (P_{TC}/MBA) ratio, where K was obtained from the plot of θ/P_{TF} versus θ .

MBA was estimated by extrapolating the straight-line segment corresponding to binding at high PO_4^{3-} ion saturation in a P_{TC} versus $P_{\text{TC}}/P_{\text{TF}}$ plot. Only results corresponding to PO_4^{3-} /Fe–HA reaction ratios without precipitation of HA were considered in the calculation of K .

To obtain P_{TC} and P_{TF} values, the reaction of a specific concentration of the Fe–HA complex with different P (as PO_4^{3-}) concentrations (in the range of 2–50 mg L^{-1} of P) was carried out as described above for the preparation of PO_4^{3-} –Fe–HA complexes. The analytical determinations were carried out with three replications. The regression analysis carried out to obtain MBA and log K values was made using the software Excel (Windows XP).

Statistical Analysis. The statistical studies were carried out with the software SPSS v 13.

RESULTS AND DISCUSSION

Potential Suitability of AR To Evaluate P Complexation in Humic Systems. The results showing the capacity of different amounts of $\text{HCO}_3\text{-R}$ to fix free PO_4^{3-} (0.5 mg of total P in 10 mL), over 30 min, at pH 6 and in the presence of different NO_3^- concentrations are presented in Figure 1. As can be seen in this figure, $\text{HCO}_3\text{-R}$ amounts equal to or higher than 0.9 g were capable of fixing nearly the 100% of free PO_4^{3-} in 30 min. Similar results were obtained when the study was carried out at pH 4 and 8 (data not shown). Under these conditions the $\text{PO}_4^{3-}/\text{HCO}_3\text{-R}$ proportion selected to discriminate free PO_4^{3-} from complexed PO_4^{3-} in solution, in 30 min, was 1 mg of P (as PO_4^{3-})/1.8 g $\text{HCO}_3\text{-R}$ (50% of water content). This

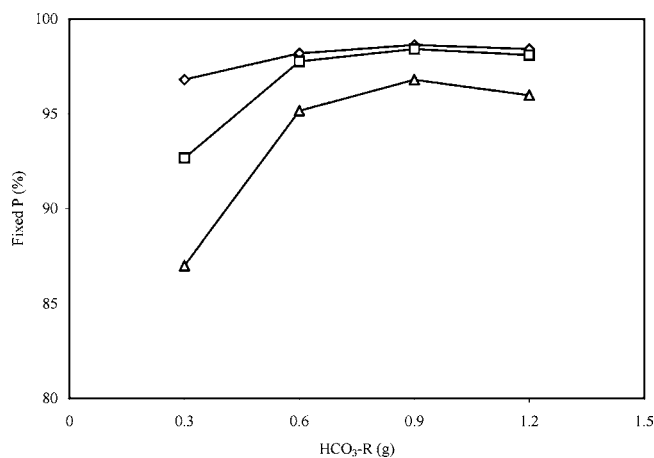


Figure 1. PO_4^{3-} fixation in $\text{HCO}_3\text{-R}$, in 30 min at pH 6, in the presence of different concentrations of nitrate in the solution: (◇) 10 mM NaNO_3 ; (□) 15 mM NaNO_3 ; (△) 20 mM NaNO_3 .

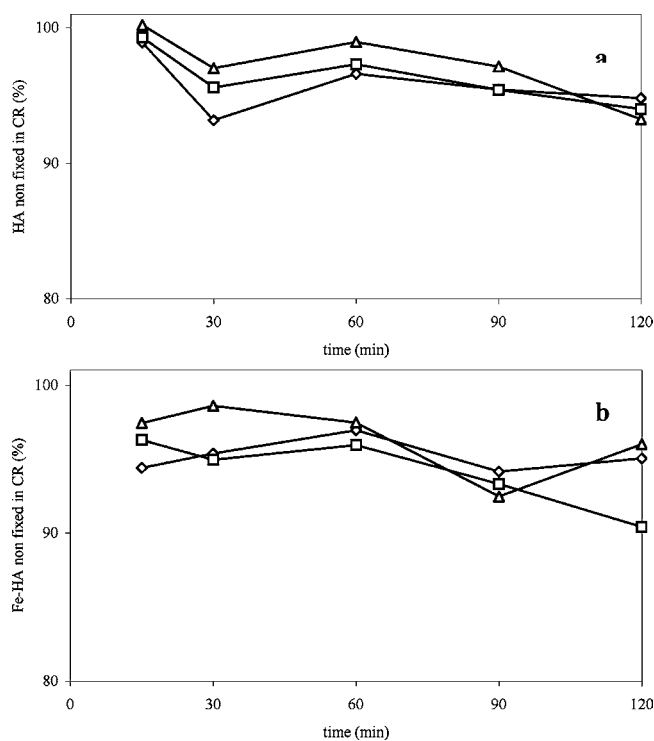


Figure 2. Nonfixed HA (a) and Fe–HA (b) in $\text{HCO}_3\text{-R}$ at different pH values: (◇) pH 4; (□) pH 6; (△) pH 8.

parameter, which constituted the minimum amount of $\text{HCO}_3\text{-R}$ to ensure an almost complete retention in $\text{HCO}_3\text{-R}$ of free PO_4^{3-} , is very important to reduce the possible hydrolysis of PO_4^{3-} –Fe–HA complexes by the competition of $\text{HCO}_3\text{-R}$ and humic complexes for PO_4^{3-} . Likewise, the presence of very low but significant amounts of nonfixed free PO_4^{3-} in $\text{HCO}_3\text{-R}$ underscored the importance of using corresponding blanks (solutions containing only $\text{HCO}_3\text{-R}$, PO_4^{3-} , and NO_3^-) in the determinations. In fact, as can be seen in Figure 1, the NO_3^- concentration affected PO_4^{3-} fixation on $\text{HCO}_3\text{-R}$. This result was expected, because NO_3^- competed with PO_4^{3-} for the resin binding sites.

As for the potential interference of HA in PO_4^{3-} retention on $\text{HCO}_3\text{-R}$ due to HA absorption in the resin, our results indicated that the degree of retention of HA in $\text{HCO}_3\text{-R}$ varied over time, thus suggesting the presence of a certain adsorption–desorption process (Figure 2). In any case, HA fixation on $\text{HCO}_3\text{-R}$ was <8% at different times during the study (Figure

Table 1. Total Complexed P (P_{TC}) in Reactions with Fe–HA at Different pH Values, As Measured Using Ultrafiltration (UF) or the Interaction with an Anion-Exchange Resin (AR)

pH	C_T^a (mg/L)	Fe_C^b (mM)	P_{TC} (AR)		P_{TC} (UF)		P_{TC}/Fe_C (AR)	P_{TC}/Fe_C (UF)
			\bar{x} (mM)	CV ^c (%)	\bar{x} (mM)	CV (%)		
4	2279	4.036	0.738	5.6	0.818	4.0	0.183	0.203
6	2295	4.065	0.688	8.8	0.693	7.4	0.169	0.171
8	2220	3.932	0.540	0.9	0.459	1.1	0.137	0.117

^a Total carbon. ^b Complexed Fe in Fe–HA. ^c Coefficient of variation ($n = 4$).

2a). Similar results were obtained in relation to the degree of retention of Fe–HA complexes in HCO_3^- -R (**Figure 2b**). We obtained no HA samples free of complexed metals. Thus, after the isolation–purification process, the Fe and Al contents in HA remained around 1%. Previous studies carried out in our laboratory showed that PO_4^{3-} complexation in HA was, in any case, very low ($\sim 4.8 \times 10^{-6}$ mmol/mg of C) (20). However, any possible interference of HA in the fixation of free PO_4^{3-} on HCO_3^- -R will be detected in the comparison between the results obtained using AR and UF methods.

In consequence, these results indicated that the use of HCO_3^- -R to obtain the fractions of free PO_4^{3-} and complexed PO_4^{3-} in solution in the presence of HA and Fe–HA complexes is potentially suitable. Furthermore, the optimum conditions to carry out this analysis included a PO_4^{3-} (milligrams of P)/ HCO_3^- -R (grams) ratio of 1:1.8 for a time of contact between HCO_3^- -R and solution of 30 min.

The suitability of the AR method was further evaluated by comparison with UF methodology as described below.

Potential Suitability of UF To Evaluate P Complexation in Humic Systems. The degree of retention of HA in the UF membrane (3000 MWCO) was between 93 and 94% for all of the concentrations of the humic material studied. In the case of Fe–HA complexes this retention degree was $\sim 96\%$. This difference between AH and Fe–AH complexes may be due to the possible aggregation induced by Fe complexation (19). It is also noteworthy that Fe, either free or complexed, was not detected in the filtrate. As a consequence, the P present in the filtrate would correspond to free (noncomplexed) PO_4^{3-} . These results indicate that this UF membrane is, in principle, well suited to the role it plays in this analytical approach, at least for the model humic acid selected for this study.

The curves relating the concentration of free PO_4^{3-} in the solution before UF to PO_4^{3-} concentration in the filtrate presented a CV of the determinations ($n = 5$) of $< 10\%$ in all cases, and the correlation coefficient (R^2) was > 0.99 ($p < 0.01$) at all pH values. Likewise, the retention of PO_4^{3-} in the UF membrane decreased in line with the increase in free PO_4^{3-} in solution.

Consequently, these results indicate that the use of the UF membrane of 3000 MWCO is potentially suitable for separating the fraction of bound PO_4^{3-} from free PO_4^{3-} in our experimental systems and conditions.

Analysis of Complexed PO_4^{3-} in Fe–HA Using AR and UF Methodologies. The results for the degree of complexation of PO_4^{3-} in Fe–HA complexes as a function of pH (4, 6, and 8) are presented in **Table 1**. Agreement between the UF and AR results were reasonable. Differences between both methods were lower than 15% when calculated in relation to the AR method. Likewise, the results from the two methods did not deviate systematically, thus suggesting that the differences could be due to experimental error. In fact, we found no significant differences ($p = 0.01$) between the means for pH 4 and 6 (one-way ANOVA test). In the case of pH 8 there were significant

differences between the two methods for $p = 0.05$. In addition, the repeatability of both techniques was satisfactory, as both CV values were $< 10\%$ ($n = 4$). These results indicate that the interaction of PO_4^{3-} –Fe–HA complexes with HCO_3^- -R did not lead to a significant hydrolysis of the complexes under our experimental conditions. In contrast, previous studies carried out in our laboratory showed that the use of the anion-exchange resin in its OH^- form led to a significant hydrolysis of the complexes (data not shown).

Another finding was that PO_4^{3-} complexation varied as a function of pH. Thus, the fraction of PO_4^{3-} complexed in Fe–HA decreased in line with the alkalization of pH (**Table 1**). This result confirmed preliminary studies carried out in our laboratory (20), indicating less PO_4^{3-} complexation as the pH increases. It could be related to both the characteristics of stability of Fe–HA complex and the different ionic species of PO_4^{3-} (HPO_4^{2-} at pH 8 and $H_2PO_4^-$ at pH 4) depending on the pH, which could also affect the pattern of interaction in the complexation process.

In any case, the two methods clearly showed the capacity of Fe–HA complexes to bind PO_4^{3-} under the conditions of this experiment (**Table 1**). Likewise, the involvement of HA-complexed Fe is also supported by previous studies showing that PO_4^{3-} complexation by HA (HA contained $\sim 1\%$ of Fe and Al) was very low (20). These results, showing the formation of stable PO_4^{3-} –Fe–humic complexes in solution, corroborate other results obtained using less conclusive techniques indicating the ability of Fe–humic complexes to fix PO_4^{3-} in experiments carried out under different experimental conditions (8, 10–15, 17).

Likewise, our results indicated that the stoichiometry PO_4^{3-}/Fe in PO_4^{3-} –Fe–HA complexes varied between 0.11 and 0.20 depending on the pH (**Table 1**). These values, indicating that only a relatively small fraction of complexed Fe was involved in PO_4^{3-} complexation, suggest that restricted (specific) populations of Fe–HA sites might be involved in PO_4^{3-} complexation. Likewise, a possible influence of the speciation technique on the results cannot be ruled out.

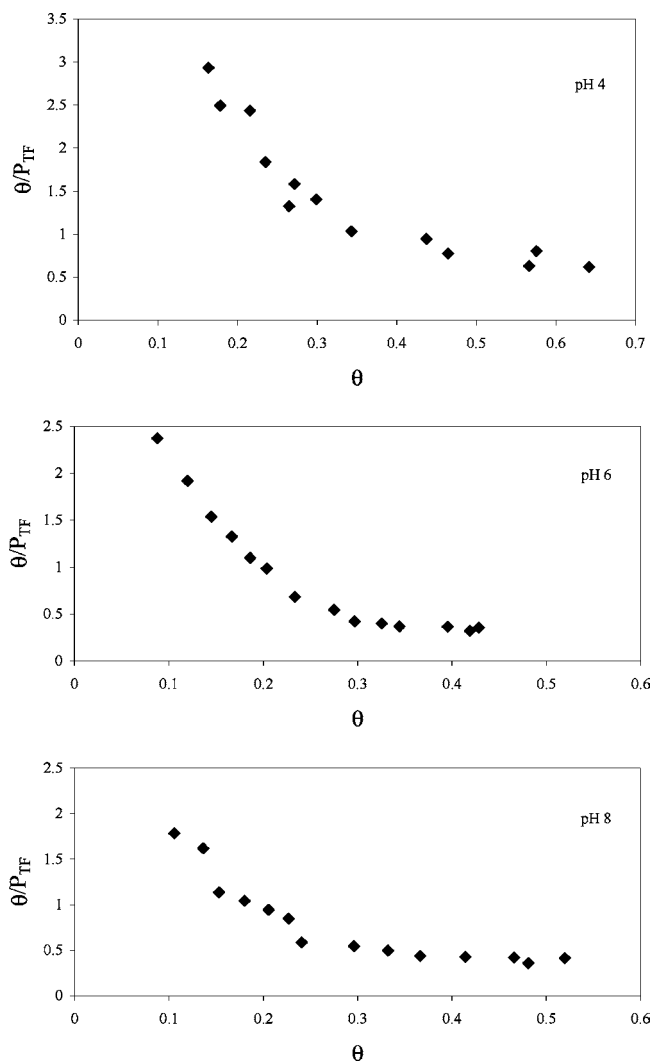
Other possible speciation techniques were also considered in the study, but the results are not presented here because they were not positive. Ion chromatography proved to be unsuitable as the use of this technique in the presence of high concentrations of humic substances is highly problematic due to the degradation of the anion column. This had serious negative effects on the reproducibility of the chromatograms.

Dialysis was also considered. We used different types of dialysis membranes considering different molecular weight cutoffs (500 and 1000 Da). However, this technique was abandoned by two reasons. First, the chemical retention of PO_4^{3-} in the membrane was high ($> 20\%$ of the total added), and this caused a very high variability in the data. Second, the period of time that was necessary to reach the equilibrium was very long (> 10 days on average).

Table 2. PO₄³⁻–MBA^a and Log K Values Corresponding to the Different Classes of Binding Sites in Fe–HA

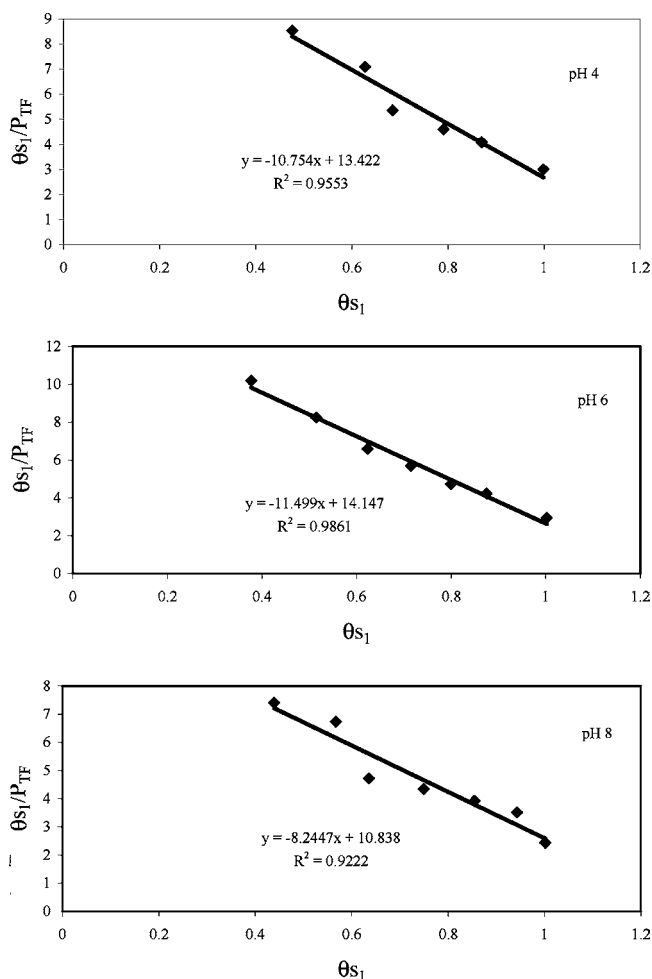
binding site	MBA (mM)			Log K		
	pH 4	pH 6	pH 8	pH 4	pH 6	pH 8
S ₁	0.202 ± 0.011	0.177 ± 0.005	0.108 ± 0.008	1.03 ± 0.07	1.06 ± 0.21	0.92 ± 0.03
S ₂	0.386 ± 0.029	0.593 ± 0.017	0.341 ± 0.018	0.14 ± 0.27	-0.08 ± 0.23	-0.78 ± 0.81
total	0.588 ± 0.054	0.770 ± 0.150	0.449 ± 0.055			

^a MBA values are referred to 4 mM of HA complexed Fe (mean ± SE; n = 3).

**Figure 3.** Scatchard analysis of the interaction of PO₄³⁻ with Fe–HA at different pH values.

Finally, gel filtration chromatography using Sephadex with different pore sizes was also attempted. However, good separations between free PO₄³⁻ and bound PO₄³⁻ were not achieved in our experimental conditions, because depending on the pore size used, the elution pattern of free PO₄³⁻ coincided with that of the PO₄³⁻–Fe–humic complexes to some extent.

MBA and Stability of PO₄³⁻–Fe–HA Complexes. The results for the Scatchard analysis of the data of the PO₄³⁻/Fe–HA interaction over the whole range of P are presented in **Figure 3**. As can be observed, the presence of two different slopes in the plots of θ/P_{TF} versus θ for the different pH values indicates the possible presence of two populations of binding sites with different stabilities: one corresponding to low values of complexed PO₄³⁻ (lower values of total θ) with higher stability (S₁) and another corresponding to high values of complexed PO₄³⁻ (higher values of total θ) with lower stability

**Figure 4.** Calculation of stability constants for S₁ binding sites ($\log K_{S_1}$) at different pH values.

(S₂). This feature of the Scatchard plot has also been obtained for other metal–humic complexes and probably reflects the heterogeneity in the chemical nature of the binding sites (19). MBA and log K values for these two different populations are presented in **Table 2**. $\log K_{S_1}$ calculation is presented in **Figure 4**.

As can be observed in **Table 2**, MBA values corresponding to S₁ varied in line with pH alkalization. The stability, however, was quite similar between pH 4 and 6 but lower at pH 8. As discussed above, these results may be related to the possible variation of Fe–HA stability as a function of pH that could also affect the integrity and characteristics of PO₄³⁻ binding sites, as well as to the variation in PO₄³⁻ ionic species as a function of pH. As for S₂, MBA values also varied with pH but differently. Thus, there was no difference between pH 4 and 8, whereas MBA was higher at pH 6. In this case, however, considering the very low stability of this interaction (**Table 2**), the real occurrence in this case of stable PO₄³⁻/Fe–HA

interactions other than mere ionic or sorption phenomena may be dubious.

As for log K values (1.2–0.9 for S_1 depending on pH), these were significantly lower than those reported by Riggle and von Wandruszka (17) (log $K = 4$ –6) for this same type of interaction. Such differences may be due to the different analytical techniques used to measure the different fractions of PO_4^{3-} in the two studies. More importantly, differences may be due to the specific population of binding sites determined in both studies as a function of the speciation techniques used to determine the fraction of complexed PO_4^{3-} . Thus, the results presented by Riggle and von Wandruszka (17) might correspond to a very small family of binding sites, principally those related to a very low occupation and therefore to those binding sites with higher stability. This hypothesis is supported by the fact that these authors found only one class of binding site based on the Scatchard results. In this sense, MBA values corresponding to the log K values reported by Riggle and von Wandruszka (17) will be of great interest in the evaluation of the real significance of these stability values. In contrast, in our methodology we consider the complete range of binding sites, those related to high occupation (S_2) as well as those related to low occupation (S_1).

On the other hand, the log K values obtained in our study were also significantly lower than those obtained for metal–humic complexes (log K around 4–6 depending on the speciation method) (19). This result was expected to some extent, taking into account the secondary character of the PO_4^{3-} /Fe–HA interaction.

Nevertheless, although our results indicate that the formation of PO_4^{3-} –Fe–HA complexes may play a role in P soil dynamics, the values of stability and MBA obtained also indicate that their importance (their real significance) may well be very different depending on soil characteristics.

ABBREVIATIONS USED

Al, aluminum; AR, anion-exchange resin methodology; HCO_3^- -R, bicarbonate conditioned anion-exchange resin; Fe, iron; Fe–HA, iron humic complexes used in the study; HA, humic acid used in the study; P, phosphorus; PO_4^{3-} , phosphate; PO_4^{3-} –Fe–HA, phosphate–iron–humic complexes; MBA, maximum binding ability; MWCO, molecular weight cutoff; SOM, soil organic matter; OA, organic amendments; UF, ultrafiltration.

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