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Binding of inorganic phosphate to dissolved metal humates

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Abstract

A cobalt-wire electrode was used to determine the concentration of free phosphate (P) in solutions containing a series of metal humates (MHA). The results were used in Scatchard plots to determine the stability constants of the dissolved MHA–P complexes formed. The constants were found to be high, with log *K* values in the range 4.8–6.0. Solutions containing MHA–P complexes were found to be relatively inert to the addition of phosphate-precipitating cations, indicating that MHA has a solubilizing effect on P and may enhance its mobility in the environment.

Keywords: Humic materials; Metal humates; Phosphate; Cobalt-wire electrode; Binding constant; Scatchard plot

1. Introduction

The availability of phosphates (P) for plant uptake is an issue of major concern in agriculture, especially in arid and semi-arid regions with calcareous soils. The view that these soils provide an infinite sink for P has frequently led to over-application of this nutrient, and P loss from agricultural soils has been implicated in the eutrophication of waterways. There are indications that the addition of organic materials, including manure, compost, and biosolids affects the sorption capacity and retention of the element. To predict the mobility of P, and the concomitant consequences to crop enhancement and water quality, it is important to have a detailed knowledge of the interactions between organic matter, metals, and phosphorus.

Sorption by direct association between P and natural organic matter (humic materials) – either in solution or at interfaces – is unlikely, because of the anionic character of both species at neutral pH. This is especially true for dissolved orthophosphates, which are electrostatically repelled by partially or fully deprotonated carboxyl groups on the humic polymers. Interactions between humic materials and P are, however, likely to be strongly influenced by the presence of organically complexed metals. In natural environments, humates invariably encounter metal ions and are virtually always found as metal complexes. These metals, including Ca, Mg, Zn, Fe, and Cd affect the morphology of humic aggregates in solution and at interfaces [1,2]. Recent work has revealed a definitive relationship between P sorption and organically complexed Fe and Mn [3]. It may be surmised that metals provide positively charged anchor sites for the attachment of P to humic polymers in both homogeneous and heterogeneous systems.

One of the prime parameters in a quantitative description of metal humate (MHA) interactions with phosphate is the stability constant, *K*, of the resulting complex. The measurement of *K* is, however, a challenging problem, since it is difficult to distinguish between free and associated phosphate in a humate solution. Most analytical techniques, even the mildest separation methods, affect the environment of the dissolved P and thereby the association dynamics.

The recent development of a practical ion-selective electrode for dissolved phosphate has provided a way to circumvent this problem. The device was first reported by Xiao et al. [4], and its response toward inorganic P was proposed by Meruva and Meyerhoff [5]. Due to the uncertain nature of the exact mechanism by which the electrode responds, a

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complete and applicable Nernst equation is yet to be developed. However, its analytical utility was demonstrated in a series of papers by De Marco and co-workers [6,7,8]. The electrode in question consists of high-purity cobalt wire, which produces a potentiometric response with a high specificity for $H_2PO_4^-$. It yields linear E versus log [P] plots with a reported slope of ca. 55 mV per decade change in activity [4]. The work described below shows that in MHA containing solutions, the electrode responded to free P only, and was unaffected by P associated with MHA. This made it possible to monitor the concentration of free P in solutions containing dissolved MHA, and hence determine the stability constants of complexes containing the two species.

2. Experimental

2.1. Chemicals

All studies were performed on leonardite humic acid (LHA), which was originally obtained as a crude blend under the trade name Agri-Plus from Horizon Ag-Products (Kennewick, WA, USA). This material was subjected to the isolation and purification procedure outlined by the International Humic Substance Society [9]. NaOH (Fisher, Pittsburgh, PA, USA), $CaCl_2$, $CdCl_2$ (EM Science, Cherry Hill, NJ, USA), $MgCl_2$, $FeCl_3$, KH_2PO_4 (JT Baker, Phillipsburgh, NJ, USA), $NH_2OH{\cdot}HCl$, 99.995% Co-wire (Alfa Aesar, Ward Hill, MA, USA), ascorbic acid, ammonium molybdate, potassium antimony tartrate (Acros, NJ, USA), and H_2SO_4 (Fisher, Pittsburgh, PA, USA) were all used as received.

Metal-free LHA solutions (50 mg/L) were prepared by placing the material in water followed by the drop-wise addition of 0.1N NaOH to assist in dissolution. The solutions were stored at room temperature. MHA solutions (50 mg/L) were prepared by adding solid LHA to 10.0 mL of 0.005 M metal solutions and shaken for 2 h. The solid LHA was then filtered off, thoroughly rinsed, and dissolved in a similar manner as metal-free LHA. The metal content of the material was determined by flame AAS (Buck Scientific 200A). All aqueous solutions were prepared in doubly deionized water treated with a 0.22 μm Millipore filter system to a minimum resistivity of 16 MΩ cm. The pH of the MHA solutions was in the range 7.8–8.0.

2.2. Instrumentation

A 5 cm length of Co-wire (0.5 mm diameter) was used in conjunction with an Ag/AgCl reference electrode (Corning) and a Hanna Instruments 8519 pH/mV meter for potential measurements. Prior to use, the Co-wire was polished with 1200-grit emery paper and conditioned in 0.001 M $NH_2OH{\cdot}HCl$ for 20 min. A calibration curve was generated with standard KH_2PO_4 solutions before each use of the electrode with humates. Measurements were taken after a 30 s stabilization period, after which the potential maintained a constant value suggesting that equilibrium was attained. Sample solutions were prepared by adding a standard 0.10 M KH_2PO_4 solution in increments of 0.0020 mL to 25.0 mL of HA or MHA solutions under constant stirring. Potential measurements were taken after 30 s.

An Amicon 8050 stirred filter unit was used for ultrafiltration. Following filtration of 30.0 mL of a 50 mg/L MHA solution through a 500 MW membrane, the amount of residual free metal in the filtrate was determined by flame AAS.

Molecular absorption measurements were made with a Hitachi U-3000 Spectrophotometer at 720 nm. Incremental amounts of a known phosphate complexing agent (ammonium molybdate) were added to phosphate solutions of known concentration. Following equilibration, absorption measurements of each of the solutions were taken.

3. Results and discussion

The successful determination of stability constants of MHA–phosphate complexes depends on four issues:

1. the specificity of the Co-wire electrode for free phosphate — it should show no response toward phosphate complexed by MHA.
2. The presence of residual free metal — many metals form insoluble phosphates, and MHA should not release significant amounts of metal into the solution.
3. The need for a KHP buffer — prior work with Co-wire electrodes was largely carried out in KHP buffers, which would change the nature of the humate solution. The electrode should, therefore, operate in the absence of KHP over a suitable concentration range.
4. The response of the system to the presence of metal-free humate — this should be minimal.

To ascertain the specificity of the Co-wire electrode for free P, ammonium molybdate, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, was used. This compound forms the familiar blue ‘Mo–P’ complex with phosphate, which is routinely used to determine P in aqueous solution. (Note: this method could not be used in the present MHA–P study, since the formation of Mo–P interferes with MHA–P complexation.) Increasing amounts of ammonium phosphate were added to a series of identical aqueous P solutions, and the resulting solutions were monitored in terms of their absorbance at 720 nm and their Co-wire electrode potential. As expected, the former increased linearly with complex concentration, while the electrode response likewise underwent a linear change — both equally signaling a decrease in the free P concentration.

It should be pointed out that the original paper by Xia et al. [4] reported that the Co-wire electrode responded to ATP, ADP, and AMP, as well as free phosphate. Clearly, the adenosine phosphates are not ‘free P’, and there may appear to be disagreement between those findings and our observation that both Mo–P and MHA–P did not register at the electrode. This can be explained by noting that the organic phosphates are moderately strong acids — adenylic acid (AMP), for instance, has a pK_1 of 3.8. It is, therefore, reasonable to suggest that the reaction between CoO and orthophosphates at the electrode surface, as proposed by Meruva and Meyerhoff, [5] can happen with the adenosine derivatives as well. In contrast, no such reaction is possible with MHA–P, in which the phosphate is deprotonated and complexed through the metal.

The presence of residual free metal in solution upon MHA formation was examined by ultrafiltration. Following the formation and solubilization of MHA, the solution was filtered through a 500 MW cut-off membrane, which blocked the passage of essentially all humate species. The filtrate was analyzed by AA spectrometry and was found to be free of metal, down to the limit of detection for each metal. This indicates that virtually all metal ions were associated with humate and the concentration of those free in solution to capture P was negligibly small.

The customary KHP measuring medium, as well as electrode conditioning in KHP solutions, were re-examined for this study. It was found that the Co-wire electrode performed satisfactorily in KHP-free humate solutions. The inherent buffering ability of HA may contribute to the performance of the electrode in KHP-free solutions (the pH of these solutions was in the range 7.8–8.0, as noted under Section 2). Optimal results were obtained with electrodes that were cleaned with 1200-grit emery paper and then conditioned by soaking in 0.001 M $NH_2OH\cdot HCl$ for 20 min.

As noted above, minimal associative interactions between dissolved humate and phosphate are expected because of the anionic character of both species. To confirm this, a series of control measurements were carried out on solutions with a fixed P concentration and increasing amounts of metal-free added humate. Fig. 1 shows that the Co-wire-electrode response underwent little change in these solutions, indicating that the P remained unassociated in the presence of humate.

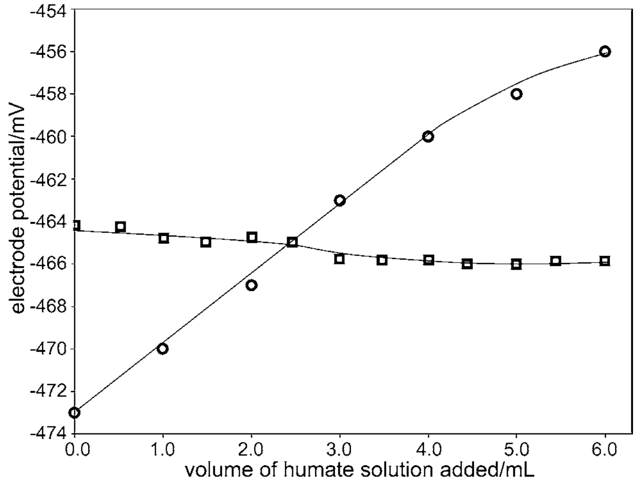

Fig. 1. Variation of Co-wire electrode potential with added humate solution. (☐) Metal-free humate; (○) Zn-HA; humate solutions: 50 mg/L; P solution: 25.0 mL, 1.48 × 10^{-4} M.

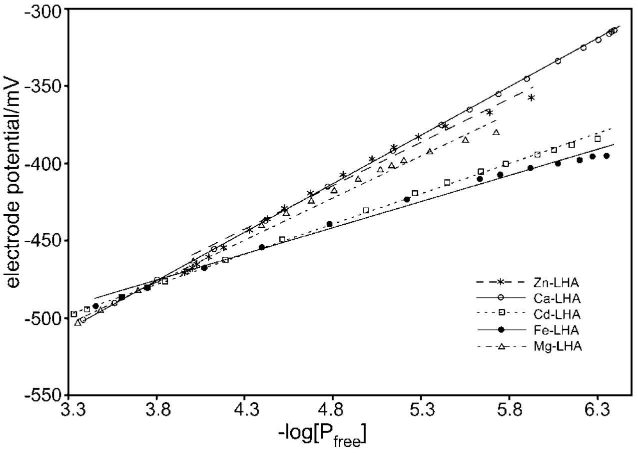

Fig. 2. Variation of the Co-wire electrode potential with free phosphate concentration in different MHA solutions.

The situation was markedly different with MHA additions, however. As shown for Zn-humate in Fig. 1, the addition of this MHA species to a phosphate solution led to an increase in the Co-electrode potential, indicating a progressive decrease in free phosphate.

As indicated under Section 2, sample solutions for the investigation of MHA–P complexation were prepared by adding increasing amounts of P to a MHA solution, and taking potential measurements at a time of 30 s after each addition. The electrode response for each of the MHA–P systems is shown in Fig. 2.

It can be seen in Fig. 2 that the curves diverged as the concentrations of P_{free} decreased. This can be rationalized by noting that at very small [P_{free}] values, the electrode potential was dictated by the MHA solution, i.e., the Co-wire became essentially an inert electrode. As the free-phosphate concentration increased, however, the Co–P interaction determined the potential, which varied in the prescribed manner as shown. At high [P_{free}] values, the potentials became, in effect, identical in all MHA solutions.

The potential of the Co electrode was found to drift slightly over extended periods — ca. 40 mV in 100 min, but this occurred in the same manner, both in the presence and absence of phosphate. This may be due to the effects of stirring and dissolved oxygen on the electrode as reported by Meruva and Meyerhoff. [5] There was, however, no indication of significant variations in the MHA-P potential after the 30 s period, eliminating the possibility of slow kinetics in P complexation by MHA.

The stability constant, K, of the MHA–P complex was determined by progressively adding P to the MHA solution using the Scatchard plot method. [10] This technique, familiar from protein chemistry, [11] has been used for the determination of metal humate stability constants [12,13] and is based on the Scatchard equation:

$$\frac{\nu}{[P_{free}]} = nK - \nu K \qquad (1)$$

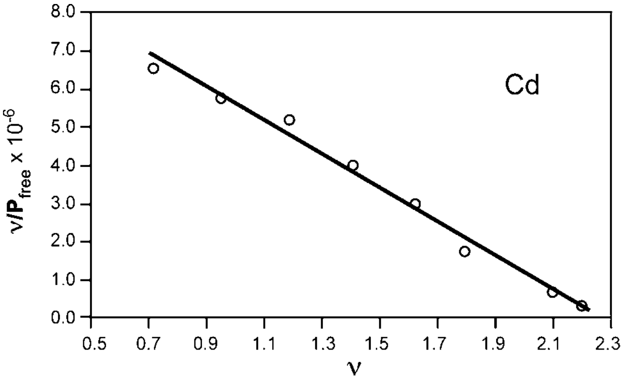

Fig. 3. Scatchard plot for Cd-LHA association with P.

Here ν is the concentration ratio of bound-P to metal humate (taken as the metal ion concentration), and n the total number of reactive sites. A plot of $\nu/[P_{free}]$ versus ν yields K as the slope. When Scatchard plots are used to determine stability constants of metal humate complexes, they tend to deviate from linearity because of the range of metal binding sites present in the humic polymer. Typically, such plots have two distinct slopes — one pertinent to strong binding sites, and one to weak binding sites. In the present study, no clear evidence was found for more than one Scatchard slope and only a single stability constant was determined for each system. The measurement of higher K-values, which may exist at very low P concentrations, was precluded by the sensitivity limit of the Co-wire electrode. The limit of detection for P_{free} in MHA solutions was found to be approximately 6×10^{-7} M, which made it impossible to determine its concentration at very small P_{free}/P_{bound} ratios. An example of a Scatchard plot for Cd–HA complexation with P is shown in Fig. 3.

The use of the metal ion concentration in place of the ligand concentration is dictated by the lack of an established humate structure, which means that they cannot be dissolved at a known molarity. Moreover, the procedure is justified by the fact that metal-free humate does not associate with P, implying that MHA coordinates P through its metal anchor site only. The approach is consistent with the one normally taken in the determination of metal humate stability constants, where the carboxyl group concentration is assumed to represent the ligand concentration. The stability constants for various MHA species with P are listed in Table 1.

The MHA–P stability constants were found to be high, with values in the order of those generally reported for metal humate interactions. This indicates that metal humates, in contrast to their metal-free counterparts, can provide strong binding sites for phosphates in the environment.

Table 1
Stability constants of MHA–P complexes

MHA	log K
Ca-LHA	5.84
Mg-LHA	5.92
Zn-LHA	4.87
Fe-LHA	5.84
Cd-LHA	5.64

Since the MHA species used in this study were all in solution, it is of interest to determine whether the corresponding P complexes would remain dissolved in the presence of a relatively high concentration of a precipitating cation. This was investigated for the case of the P complex of Fe-LHA in the presence of increasing amounts of Fe^{3+}. A 50 mg/L Fe-LHA solution (25.0 mL) containing 1.3×10^{-5} mol/L of complexed Fe and 2.0×10^{-4} mol/L of associated P was compared to a similar aqueous solution containing only P. In the latter, ferric phosphate was seen to precipitate after 0.075 μmol of Fe^{3+} was added, but this did not occur in the Fe-LHA solution. The latter solution required the addition 0.23 μmol of Fe^{3+} before a visible precipitate was formed. This ca. three-fold increase in the iron concentration necessary for precipitation was probably due to a combination of effects: (i) sequestration of the added Fe^{3+} by humate; and (ii) unavailability of associated P for precipitation with free Fe^{3+}. In any event, the result suggests that orthophosphate is appreciably solubilized in the presence of dissolved metal humates. This can have a significant effect on the mobility of phosphates in natural waters.

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