Complexation of calcium ions by humic and fulvic acids

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Complexe formation between humic matter and ions in soil can increase the solubility of several nutrients and consequently their biodisponibility. To give a better insight about such complexation, humic and fulvic acid were extracted from farmyard manure and their ability to complexe calcium ions was characterized experimentally through simultaneous potentiometric and conductimetric titration with NaOH and Ca(OH)$_2$. Complexation power was assessed by pH Stat-conductimetric titration. Results shows that complexation of calcium by humic and fulvic acid was detected only at slightly acid, neutral and basic pH and complexation power was favored by increasing pH. The amount of protons released following calcium complexation by humic and fulvic acid was determined for each pH studied and the nature of functional groups involved in complexation was discussed.

Introduction

Humus content is one of the most important factor determining the productivity of soil because of multiples effects of humic substances such as release of plant nutrients, improvement of soil physical and biological conditions, and increasing the efficiency of fertilizer [1,2]. This last effect have been largely attributed to the ability of humic substances to control the solubility and biodisponibility of several chemical element via complexation process [3,4]. In calcareous soils, calcium is a reactive and ubiquitously concentrated cation witch can decrease the biodisponibility of several micronutrients (e.g. Fe^{++}, Zn^{++}, Cu^{++}) as well as main nutrients (P) through the formation of insoluble calcium phosphates. Thus, calcium complexation by humic matter can play a decisive effect on phosphorus dynamic by increasing apatite solubilization [5,6] and limiting phosphorus sorption and fixation [7,8]. It is also found that calcium complexing power of humic substances is well correlated with improved phosphate nutrition in calcareous soils [9]. Such improvement is however still difficult to interpret since

humic substances can increase phosphorus solubility also as a result of competitive adsorption on phosphorus fixation sites [10]. Further, positive effect of calcium complexation on phosphorus seems to be dependent on soil pH [11]. To give a deeper insight about such effect the investigation reported here is a preliminary study to characterize the complexation of calcium by humic substances using potentiometric and conductimetric titration.

Materials and methods

Preparation of humic and fulvic acids

Humic matter was extracted from composted farmyard manure using KOH 0.1N (1/50: W/V) by agitation at 400 t/min, for 16 H. After centrifugation for 30 min at 12 000 t/min, the supernatant was carefully recovered, filtered through 0.45 μm membrane and then adjusted to pH 2 by HCl 1N. The solution obtained was allowed to coagulate for 24 H at 4 °C then it was centrifuged as above. Fulvic acid (FA) was recovered directly and humic acid (HA) was washed twice with deionised water and then dissolved in NaOH 0.01N. Excess cation were eliminated by repeated dialysis using a Spectra Port type C MWCO 100 membrane. Humic and fulvic solutions were then brought to one liter with deionised water. Final pH were 7.8 and 3.7, for humic and fulvic acid respectively. Humic matter content was determined by oxidation with KMnO^4 0.1 N.

Calcium complexation

Citric acid was chosen as reference because his reaction with calcium is well known. Titration was performed with 100 mL of citric acid 0.25 mM, humic acid (25 mg/L) and fulvic acid (25 mg/L) initially adjusted to pH 3.5 with HCl 0.1N. Each acid was then neutralized stepwise using NaOH and Ca(OH)$_2$ at 0.040 N under N_2 bubbling. Titration speed of 50 μL /30 seconds was chosen so that the pH and the conductivity becomes stable after each increment of base added. The end of titration is indicated by a sharp increase of conductivity due to execess of OH$^-$ ion. Calcium complexation was also monitored at constant pH over a wide range of pH values: 4.5 to 9.5. Samples of 25 mg humic

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acid or fulvic acid were adjusted to a starting pH and brought to a volume of 100 mL. For each pH studied, calcium was added by increments of 50 µL of $CaCl_2$ 0.2N using Eppendorf pipette while the pH was kept constant during titration by an automatic addition of $Ca(OH)_2$. The end of titration is indicated by a change in the slope of conductimetric curve witch becomes parallel to conductimetric curve of deionised water used as control.

For all titrations and complexation experiments a titrator Mettler DL 21 was used. Its vessel is equipped with four ports: a pH combination electrode, a conductivimeter electrode, a N_2 bubbling tube and a port which could be opened for addition of reagents ($CaCl_2$ in calcium complexation). The conductivimeter used was a Mettler YSI 32. Agitation was controled by the titrator.

Results and discussion

Potentiometric and conductimetric titration

For citric acid, conductimetric and potentiometric variations were similar up to pH 4.5. They were determined by the neutralization of H^+ and its replacement by Na^+ or Ca^{++} of low equivalent conductivity (Fig. 1). From pH 4.5 the complexation of calcium by citric acid is shown by a release of H^+ as increase of pH was slowed compared to that of NaOH titration. The absence of any conductivity increase in the case of $Ca(OH)_2$ titration suggests that added calcium was complexed. Further, a slight steady decrease of conductivity was observed around pH 4.8 and 6.2. It could be attributed to dissociation and neutralization of the first and the second COOH functional groups of citric acid, respectively. Beyond pH 9 (D') the sharp increase in conductivity is due to OH^- excess as complexation and neutralization were completed. The amount of NaOH and $Ca(OH)_2$ consumed by citric acid at the end of neutralization are equal (D and D'): 80 µeq H^+. Total acidity of citric acid solution used is 75 µeq H^+. This difference could be attributed to the error in determining the end point. Titration of HA and FA are shown on the same figure. The bevaviour of those acids is close to that of citric acid. Until pH 4.5, potentiometric and conductimetric variations are the same with the two bases. They suggest the absence of calcium complexation. However, as titration progresses differences appear as there was a release of protons in the case of $Ca(OH)_2$ titration. Also, the conductivity increases only slightly with comparison to NaOH titration. This suggest that a part of added calcium was complexed. Similar results were obtained by Arai and Kumada [12] and Brun et al. [13]. Beyond pH 10.5 (D') the complexation becomes negligible and there is a sharp increase in conductivity. For NaOH titration the fast increase of conductivity was unregistered at pH 10 (D). It is equivalent to the end of titration. The amount of $Ca(OH)_2$ consumed was superior to that of NaOH (point D' via D). Hence, complexation of calcium by humic and fulvic acids should release some acids. According to Aplincourt et al. [14], Piccolo and Stevenson
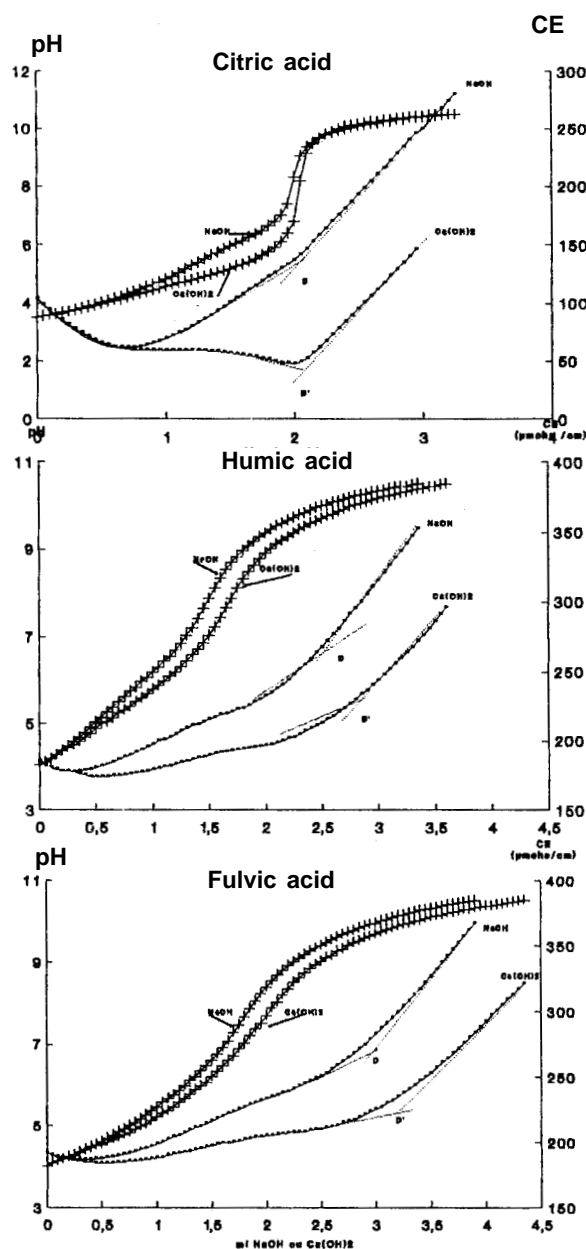

Figure 1. Potentiometric and conductimetric curves of citric acid, humic and fulvic acids.

[15] and Khalili [16], complexation of calcium with humic substances induce some structural modifications which could bring about some new functional groups and consequently an additional acidity. This hypothesis agrees with our finding. Total acidity based on the amount of $Ca(OH)_2$ consumed correspond to 444 and 550 m eq H^+/100g humic matter for humic and fulvic acid respectively.

Table I. Results of calcium complexation (a: meq Ca^{++} complexed by 100 g of humic matter, b: mole H$^+$ released for each mole Ca^{++} complexed).

			pH		
	5.5	6.5	7.5	8.5	9.5
Humic acid					
a	99 ± 6	127 ± 6	145 ± 6	165 ± 6	175 ± 6
b	0.52 ± 0.04	0.52 ± 0.03	0.42 ± 0.01	0.36 ± 0.01	0.27 ± 0.01
Fulvic acid					
a	85 ± 6	131 ± 6	140 ± 6	168 ± 6	178 ± 6
b	0.52 ± 0.04	0.41 ± 0.02	0.33 ± 0.02	0.23 ± 0.01	0.29 ± 0.01

pH- Stat- Complexation

Results of calcium complexation at fixed pH for humic and fulvic acid are shown in figures 2 and 3, respectively. Except for pH 4.5 all curves show a first region where conductivity increases slightly and a second region where conductivity variations are equal to those of deionised water which suggest that complexation has been completed. Release of protons was also limited to the first region. The amount of calcium complexed and protons released are given by table I. Values obtained reflect pH dependency of calcium complexation by humic and fulvic acid: complexation was negligible at pH 4.5 but it increases as pH increases. Similar results were found by others authors [5,17,18]. The amount of released protons decreases as pH increases. The highest amount found was 0.56 mole H$^+$/ mole of calcium and it is however less than the theoretical value of 2 moles H$^+$ for each mole of calcium complexed. So, complexation of calcium by humic and fulvic acid should involve mainly strong acidic groups. The existence of such functional groups was confirmed by the first derivative of potentiometric curve obtained with NaOH for humic and fulvic acid (Fig. 4). In fact, the first pKa is around 4 (pKa1). It corresponds to strong COOH groups. The second pKa around 6.2 is attributed to week COOH groups. A possible OH phenolic groups is however not clearly present here as it is difficult to found by direct titration.

For fulvic acid the shape of curve suggests the existence of several functional groups with close pKa.

In all case, for the range of pH studied, the protons released are attributed to dissociation of week COOH and to very week COOH and phenolic OH groups. Further, strong acidic COOH should be quantitatively the most important but, as results shows, formation of Ca-humic and Ca-fulvic complex are not possible at acidic pH probably due to higher competitively of H$^+$ compared to that of Ca^{++} for dissociated COO$^-$ [19]. Stability of calcium humate and calcium fulvate at higher pH is also still difficult to interpret. It is believed that configuration changes in HA and FA occurs at basic pH and allows stability of the complexes formed between humic acid, fulvic acid and bivalent cations [14].
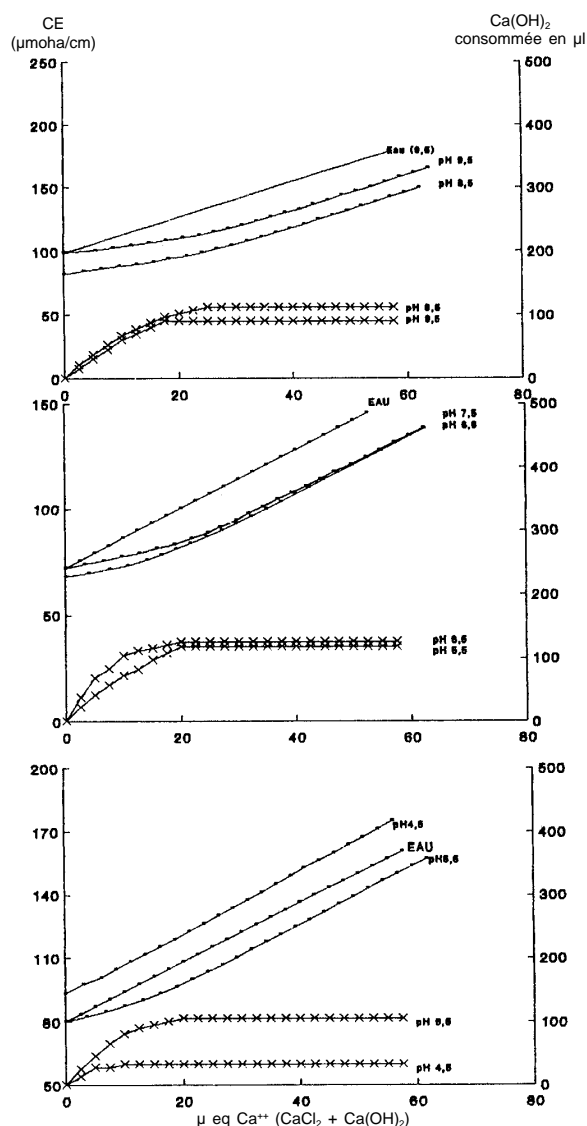

Figure 2. Conductimetric and Ca(OH)$_2$ consumption curves of humic acid at different pH.
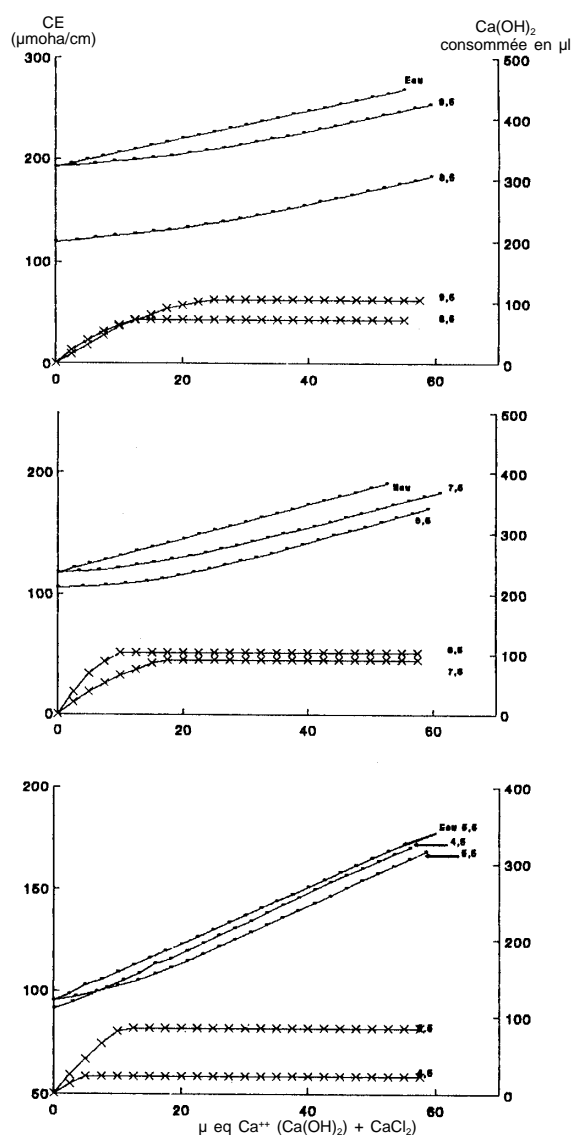
Figure 3. Conductimetric and Ca(OH)$_2$ consumption curves of fulvic acid at different pH.

Figure 4. Firts derivative of NaOH potentiometric curve.

Conclusion

Potentiometric and conductimetric titrations provides an acceptable and easy method to study calcium complexation by humic matter. Results found gives evidences for Ca-humic and Ca-fulvic complex formation at different pH: slightly acid, neutral and basic pH. Both strong acidic COOH and week COOH are involved in the formation of such complexes. The stability of the complexes is pH dependent. It deserve further interest as the mechanisms involved are still limited to hypothesis.
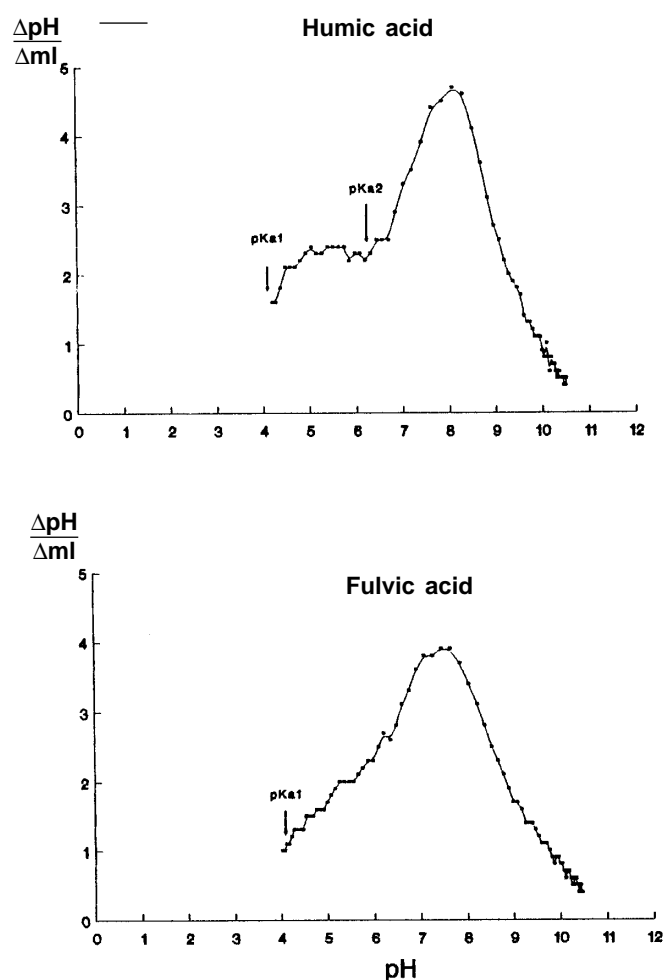
Acknowledgments

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