Characterization of Ferricyanide-Humate Complexes by a Voltammetric Approach

L. Leita,1 A. Mori,1 M. De Nobili,2 G. Corso,1 I. Franco,2 and R.M. Cenci2

1Experimental Institute for Plant Nutrition, via Trieste 23, 34170 Gorizia, Italy; 2Department of Crop Production and Agricultural Technology, University of Udine; 3European Commission-Joint Research Institute, Environmental Institute, Soil and Waste Unit, Ispra, Italy

The industrial sites that deal with the production and/or use of cyanide in their processes often have contamination problems by cyanide in soils and water. The behavior of these hazardous compounds in soil and the interactions with soil components are mostly unknown. The toxicity of cyanide and its fate in soil are strongly influenced by the formation of complexes with iron, i.e., ferric — and ferrous cyanides (Fuller, W.H., 1994; Theis and West, 1988). These complexes may interact with the soil inorganic fraction containing ferrous or ferric iron, with consequent formation of stable precipitates (ferric ferrocyanide or ferrous ferricyanide, the Prussian Blue and the Turnbull Blue, respectively). However, no information is available on the capacity of humic substances to immobilize the cyano-complexes and thus to reduce their toxicity and the leaching, and the consequent risk of contamination of waterable.

In fact, one of the most significant properties of humic substances is their ability to interact with xenobiotics to form complexes of different solubility and chemical and biochemical stability.

In this paper we report the results obtained in a voltammetric investigation aimed to study the behavior of the ferricyanide/ferrocyanide redox couple as the indicator of redox system (Helburn and MacCarthy, 1994) in the absence and in the presence of humic fraction at nominal molecular weight lower than 5 kDa (HSLMW) extracted from a surface horizon of a Haplustert soil. The results obtained in these investigations, combined with the evidence gained by spectrophotometric measurements carried out in parallel, point out that ferrocyanide-HSLMW and ferricyanide-HSLMW complexes are more stable than the parent Fe-CN− ones. These complexes undergo electrochemical processes involving a slow homogeneous chemical reaction preceding the electron transfer step and causing their kinetic control (CE processes). The voltammetric measurements allowed the conditional constants K to be determined for ferric- and ferrous cyanide-HSLMW complexes, respectively: they turn out to be about three orders of magnitude higher than those for the ferricyanide and ferrocyanide complexes alone, respectively. From the data treatment we may hypothesize that two humate ligands, on average, are accommodated in the coordination sphere of both iron (III) and iron (II) in the corresponding complexes.

* Corresponding author. Liviana Leita, fax number: +39 0481 520208; E-mail: lleita@go.nettuno.it.

KEY WORDS: ferricyanide, humic fraction, complexation, electrochemistry.
INTRODUCTION

Cyanide is commonly used in several industrial plants, such as mining, metallurgical, and photographic industries, and in coal gasification plants. The soil of these sites often contain large amounts of cyanide, likely iron-cyanide complexes (Meeussen et al., 1992, 1994). The possible hazards for human health and the environment greatly depend on the toxicity of cyanides, closely related to their own complex chemistry, especially as regards the precipitation and dissolution equilibria in aquatic systems (Ghosh et al., 1999a, 1999b, 1999c). The behavior of these hazardous compounds in soil and the interactions with soil components are mostly unknown, especially concerning the ability of humic substances to immobilize the cyano-complexes in order to reduce the risk of contamination of the water table and to minimize the biological disturb of biotic compartment of soil (Leita et al., 1998a 1998b).

The metal complexation with fulvic and humic substances represents a subject of great nutritional and environmental importance. In fact, one of the most significant properties of these fairly ubiquitous species is their ability to interact with metal ions to form complexes characterized by different solubility as well as by chemical and biochemical stability. Humic substances play an important role in soil-forming processes (e.g., podzolization) and in the availability of essential or toxic elements to plants, as they affect immobilization and release equilibria of many cations in soil (Boyer et al., 1988, 1989; Chaney and Bell, 1987; Chen and Stevenson, 1986; Ritchie et al., 1982). In particular, the higher solubility of humic substances with a nominal molecular weight lower than 5 kDa makes them of considerable biological importance because they are involved in many processes that take place in the soil-plant system (Deiana et al., 1995) and contribute to the immobilization and release of xenobiotics, particularly heavy metals, in the environment (Tyler, 1981).

The complexing capacity of humic substances has been studied extensively (Gregor and Powell, 1988; Gregor and Powell, 1989; Greter et al., 1979; Mota et al., 1996; Town and Powell, 1993; van den Berg et al., 1990; Fischer, 1986), whereas the reduction properties, as well as the redox mechanisms and the kinetic aspects, are scarcely reported (Greter et al., 1979). However, the characterization of metal-humic complexes is not simple from an analytical point of view in that humic substances have to be considered like macromolecular ligands characterized by multifunctionality, polyelectrolyte properties, and conformational factors that play a key role in binding metal ions (Senesi, 1990a; Senesi, 1990b). The use of spectral techniques like FTIR, ESR, NMR has improved the knowledge about metal-humic complexes (Senesi et al., 1986; Senesi, 1992). More recently, many investigations were carried out by electrochemical methods that are well suited for both the determination of total concentration and speciation of trace elements, owing to their high sensitivity and to the considerable relations existing between voltammetric signals and physico-chemical properties of the electroactive species.
Experimental

Extraction of Humic Fraction (HSL_{ww})

The humic substances were extracted from an air-dried sample taken from the surface horizon of an Haplumbrept soil (USDA) by following a standard procedure (De Nobili et al., 1987; De Nobili and Fornasier, 1996). A soil suspension, made by adding 10 ml of 0.1 M Na_2P_2O_7 (pH 7.1) per gram of the solid, was shaken for 1 h under nitrogen atmosphere at room temperature. It was then centrifuged at 4000 rpm for 30 min and its supernatant was first filtered on a Whatman WCN 0.45 μm membrane, and subsequently ultrafiltered through a YM5 membrane in an Amicon apparatus continuously supplied with 0.1 M Na_2P_2O_7 (pH 7.1), at a pressure of 4 atm and a flux of 100 ml h^{-1} with continuous stirring under nitrogen atmosphere. The collected fraction at nominal molecular weight <5 kDa (HSL_{ww}) was acidified to pH 2.0 using concentrated H_2SO_4 and loaded into a cross-linked polyvinylpyrrolidone (Aldrich) column (100 x 20 mm), where it was washed with distilled water in order to remove nonhumic organic compounds as well as the excess of pyrophosphate. Afterward, adsorbed humic material was eluted from the column with 0.5 M NaOH, and the resulting basic solution was treated with a H^+ loaded cationic-exchange resin (Amberlite IR 120) to remove the excess of sodium ions (Sequart et al., 1986). After adjusting the pH to 7 with 0.2 M NaOH, humic substances were frozen and lyophilized. The lyophilized powder was suspended before use in milli-Q water (w/v 2:1) and pH was adjusted to pH 7, using 1 M HClO_4.

All the chemicals employed were of analytical-reagent grade quality.

Apparatus and Voltammetric Procedure

A three-electrode polarographic analyzer (EG&G Research 264) connected to a 303 EG&G polarographic cell was employed. Voltammetric measurements were performed at a glassy carbon stationary electrode with a Pt wire as the counter electrode and a Ag/AgCl, sat. KCl as the reference electrode. The electroactive solution was 1 mM K_4[Fe(CN)₆], containing 0.1 M NaClO₄ as supporting electrolyte. The solution was adjusted to pH 7 with HClO₄ before measurements using a Crison Micro pH 2001 pH meter, since at neutral pH the interaction of humic substances with iron compounds is strongly favored and oxidative coupling, dimerization and tautomeration of phenoxyl radicals are avoided (Deiana et al., 1995). Potentiometric analyses under dark and light conditions were carried out in order
to check the photo-dissociation of iron-cyanide complex during measurements. Results obtained showed that iron-cyanide complexes solutions (as iron-cyanide in supporting electrolyte and in presence of HSL\textsubscript{MW}) were stable. Successive aliquots of humic fraction (HSL\textsubscript{MW}) were added to ferricyanide solution.

The interferences due to the adsorption of HSL\textsubscript{MW} on the working electrode were checked by voltammetric measurements of a 1 mM hydroquinone solution with or without HSL\textsubscript{MW} (quantified as 0.05 mg/ml of organic carbon).

The electrode was cleaned after each series of analysis by using alumina micropolish. A further electrochemical cleaning on the graphite active surface was performed on stirred solution, with a scanning excursion from 1.4 V to -0.4 V for 30 min. in 0.1 N H\textsubscript{2}SO\textsubscript{4}. The reliability of the working electrode was periodically checked by recording voltammograms on standard solutions of ferric cyanide (Helburn and Mac Carthy, 1994).

**UV-Visible Spectrophotometry**

UV-visible spectra were performed in parallel to the amperometric titrations. Spectra of 1 mM [Fe(CN)\textsubscript{6}]\textsuperscript{3−} solutions and 1 mM [Fe(CN)\textsubscript{6}]\textsuperscript{4−} solutions with humic fraction (HSL\textsubscript{MW}) were recorded at room temperature, between 600 and 200 nm, on a Varian Cary 1E UV-Visible spectrophotometer: 1-cm quartz sample cells were used. Distilled water or solutions containing HSL\textsubscript{MW} were used as blanks.

**RESULTS AND DISCUSSION**

The evaluation of interferences at the electrode surfaces is one of the main aspects to be considered when polyelectrolytes have to be analyzed by electrochemical techniques. Therefore, we carried out two series of CV measurements in order to check the reliability of the analytical response. We did measurementes in absence and in presence of humic fraction (HSL\textsubscript{MW}) on the reversible redox couple quinone/hydroquinone, to verify if and how HSL\textsubscript{MW} might be adsorbed onto the surface of the glassy carbon electrode. The quinone/hydroquinone redox system has been chosen because humic acids are known to have quinonoid moieties. Results indicated that any complexation process between quinone/hydroquinone-HSL\textsubscript{MW} has been avoided.

In Figure 1a are shown the voltammograms recorded at pH 7 in absence of HSL\textsubscript{MW}, after successive additions of 0.10 ml of a 25 mM hydroquinone solution. The addition of hydroquinone caused an anodic current peak at +0.42 V, and corresponding cathodic peak at +0.18 V. Both peak currents proportionally increased with hydroquinone concentration. The same measurements were performed in the presence of humic fraction (HSL\textsubscript{MW}) (Figure 1b). As voltammetric responses are similar in both cases, no interaction should occur between HSL\textsubscript{MW}
Voltammetric current/potential curves relative to quinon/hydroquinone redox couple in absence (Figure 1a) and in presence (Figure 1b) of HSLuv.
and the surface of the working electrode. The same results were also obtained at pH 3.5 and pH 12.5. The pH of electroactive solution did not change significantly (ΔpH 0.02) during voltammetric investigation, probably because of the buffer capacity of humic fraction (Stevenson, 1994).

Cyclic voltammograms recorded on ferricyanide solutions at pH 7 displayed a well-defined forward peak at +0.160 V (FeIII-CN/FeII-CN reduction), associated to a backward peak at +0.230 V (FeII-CN/FeIII-CN oxidation). As shown in Figure 2, a significant depression of both coupled i p's, together with an appreciable cathodic shift of the FeIII-CN reduction peak concomitant to a symmetrical anodic shift for the associated FeII-CN oxidation peak, were observed just after the first addition of HSL MW (25 μg of organic carbon). In particular, the ΔE p value (calculated as E pF - E pC relative to the FeIII-CN/FeII-CN redox couple) increased from 65 mV to 105 mV.

Subsequent stepwise additions of HSL MW caused further depression of both i pF and i pC and a concomitant further shift of peak potentials relative to both the associated peaks, whose ΔE p attained a value of 470 mV after addition of 1.4 mg of organic carbon. No further change of these cathodic and anodic waves was observed after supplemental additions of organic carbon (up to 2 mg) to the electroactive solution. Because the cyanide interaction with humic substances are pH dependent, we measured the pH values at the beginning and at the end of the electrochemical analyses. We found that the pH of electroactive solutions unchanged, indicating the buffer capacity of humic substances (Stevenson, 1994).

These results suggested the formation of ferro-ferricyanide - HSL MW complexes and were confirmed by UV-Vis spectra recorded in parallel to voltammetric measurements (Figure 3). The original spectrum exhibited in aqueous solution by the ferricyanide complex was in fact converted into that displayed by ferricyanide ion in the presence of humic species alone just after the first addition of HSL MW. As shown in Figure 3, these spectra are nearly coincident in the region 250 to 500 nm, where three smooth absorption bands were observed in both cases at 263 nm, 303 nm (this last accompanied by two shoulders at 280 nm and 320 nm) and at 417 nm, but they differed markedly at wavelengths lower than 250 nm in that the ferricyanide complex displays two intensive and sharp charge transfer bands at 200 nm and 225 nm (Sharpe, 1976), which nearly disappeared in the spectrum recorded for the ferricyanide-HSL MW system.

A possible explanation for the complexation process of ferricyanide by humic fraction (HSL MW) can be the formation of products by the involvement -COOH, phenolic, enolic, and alcoholic -OH or -NH2 moieties of HSL MW and cyanide ligands present in the inner coordination sphere of iron. In order to verify this hypothesis, another series of CV scans of ferricyanide and ferricyanide-HSL MW systems were performed at pH 3.5 and 12.5, respectively. The voltammograms recorded at acid pH (3.5) showed a progressive peak potential shift after additions of HSL MW, with a
Cyclic voltammograms recorded on a 10^{-2} M [Fe(CN)_6]^{3-}, 0.1 M NaClO_4 aqueous solution containing the following increasing amounts of added HSL_{MW} (expressed as mg of organic carbon): (a) 0, (b) 25, (c) 50, (d) 100, (e) 150, (f) 250, (g) 450, (h) 600, (i) 950, (l) 1400.

corresponding decrease of the peak currents, until the voltammogram shape showed a constant sigmoidal pattern (Figure 4a). No significant change of peak potentials occurred at pH 12.5 (Figure 4b) after additions of HSL_{MW} to the ferricyanide solution and the decrease of the peak currents was smaller than those shown at lower pH. At pH 12.5 most of binding groups of humic fraction were deprotonated, thus the interaction with [Fe(CN)_6]^{3-} by hydrogen bonding was most likely suppressed. Therefore, a possible explanation could be due to the formation of a hydrogen bond between ferricyanide and HSL_{MW}. In addition, the dynamics of complexes formation might be controlled by kinetic process.

This statement concerning the kinetic control for both the associated electrochemical processes was confirmed by verifying that their normalized peak current, i_p/v^{1/2}, did not remain constant with increasing scan rates, as expected for diffusion controlled process, but it decreased appreciably as shown in Figure 5, which refers to measurements performed on the reduction peak recorded in the presence of different excess amounts of HSL_{MW} at different scan rates in the range 20 to 200 mV s^{-1}.

A further confirmation of the kinetic control in the electrochemical processes involving iron-cyanide-humate complexes was the agreement between experimen-
FIGURE 3

UV-vis spectra: (a) $[\text{Fe(CN)}_6]^{3-}$ vs. $HSL_{\text{Mn}}$ solutions at the beginning of the amperometric titration; (b) $[\text{Fe(CN)}_6]^{3-}$ - $HSL_{\text{Mn}}$ vs. $HSL_{\text{Mn}}$ at the half titration point; $[\text{Fe(CN)}_6]^{3-}$ - $HSL_{\text{Mn}}$ vs. $HSL_{\text{Mn}}$ at the final titration point.
FIGURE 4
Voltammetric current/potential curves relative to iron-cyanide complexation process by HSL$_{Me}$ on $[\text{Fe(CN)}_6]^{3+}$ solutions in absence of HSL$_{Me}$ (1), after the first addition of HSL$_{Me}$ (2), at half titration point (3), and at the end of the experiment (4), recorded at pH 3.5 (Figure 4a) and pH 12.5 (Figure 4b).
Normalized peak current recorded with increasing scan rates on a 1 mM [Fe(CN)₆]³⁻, 0.1 M NaClO₄ aqueous solution added with the following different amounts of HSL_MW (expressed as organic carbon): □ 50 μg; ▽ 600 μg; ○ 1100 μg.
tual results and theoretical expectation from Equation 1, applicable to kinetically controlled processes (Yu and Ji, 1993).

Equation (1):

\[ \Delta E_p = E_p^* - E_p \approx 0.059 \log \left( \frac{i_d}{i_{ML}} \right) + 1 \]

where \( E_p \) is the potential shift caused by the ligand addition, \( i_d \) is the peak current in the absence of the added ligand, \( HSL_{MW} \), \( i_{ML} \) is the peak current in the presence of excess ligand, \( n \) is the average number of ligands in the complex, \( L \) is the ligand concentration expressed as mg L\(^{-1}\) of organic carbon in the humic fraction (HSL\(_{MW}\)), \( z \) is the number of electrons involved in the electrochemical process (\( z = 1 \) in our case), and \( K \) is the conditional constant.

We calculated the conditional formation constant \( K \) for HSL\(_{MW}\)-ferric and HSL\(_{MW}\)-ferro-cyanide complexes by plotting \( \Delta E_p = 0.059 \log \left( \frac{i_d}{i_{ML}} \right) \) vs \( \log L \) (per log \( L = 0 \), relative to the cathodic and associated anodic processes (Figure 6). We found a linear regression for both ferro and ferricyanide-HSL\(_{MW}\) and the values of \( K \) were about \( 10^3 \). In addition, the number of ligand groups (n)

**FIGURE 6**

Plot relative to Eq. 1 allowing the determination of stoichiometry and stability constants for both the Fe(CN)\(_3^+\)-HSL\(_{MW}\) (*) and Fe(CN)\(_3^-\)-HSL\(_{MW}\) (o) complex.
was 2.3, and we emphasize that the n values found must be considered with care because of the complex nature of humate ligands.

In conclusion, results obtained indicated that humic substances, in particular the fraction
at nominal molecular weight lower than 5 kDa should be recommended among natural resources as important factors in remediation of cyanide-contaminated soil, and thus for environmental protection.

ACKNOWLEDGMENTS

The authors are very grateful to Prof. G. Bontempelli for important suggestions, and for continuous support and patience.

REFERENCES


