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Mechanism and stoichiometry of the redox reaction between iron(III) and caffeic acid

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Abstract

The stoichiometry of the redox reaction of caffeic acid with iron(III) was determined at pH 2.5. A linear increase in the yield of iron(II) was found with increasing iron(III) concentration until reached constant values when iron(III)/caffeic acid molar ratios were higher than 9. The reaction proceeds through two steps each having different rates, and involving intermediates with different redox activities. A mechanism of the redox reaction consistent with our results is proposed.

Introduction

The uptake of iron by plants depends considerably on the presence of complexing and reducing agents (Brown, 1969; Deiana et al., 1989; 1991a; 1991b; Mench et al., 1988).

In this context, a fundamental role is played by the phenolic substances that are released by the biological activity of the plants and the biodegradation of organic matter (Olsen et al., 1982; Whitehead et al., 1981; 1983). These enhance the availability of iron present in the soil solid phase in the forms of oxides, insoluble organic complexes, etc. (Lehmann et al., 1987; Olsen et al., 1982). Among these substances, caffeic acid (CAF) (Fig. 1) appears to be very active in the mobilization of iron, especially at the soil-root interface where it is present in high concentrations (Römheld and Marschner, 1983). Caffeic acid is able to reduce iron(III) and the redox process occurs principally in the free space and rhizosphere (Brown and Ambler, 1973; Olsen et al., 1981).

In studying the ionic mobilization mechanisms

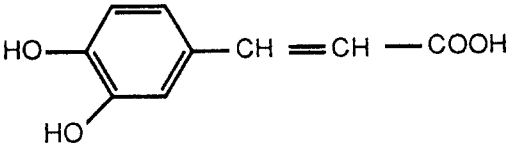


Fig. 1. Caffeic acid [3-(3,4-dihydroxyphenyl)-2-propenoic acid].

at the mucilaginous soil-root interface, we have synthesized a network of Ca-polygalacturonate with a fibrillar structure similar to that of real systems (Gessa and Deiana, 1990; 1991). Our results show that the fibrillar arrangement of the polymer collapses when iron(III) ions are substituted for calcium(II) ions (Gessa et al., 1991), and that biomolecules such as CAF are able to re-establish the porous system by reducing the reticulated iron(III) (Gessa et al., 1990).

Iron(II) appears to behave like calcium in that it interacts with the polymer to form outer-sphere complexes. This has important implications in plant nutrition because the porous structure of the interface makes easier the transfer of

nutrients towards the plasmalemma (Gessa et al., 1989; Gessa and Deiana, 1990).

Here we determine the mechanism and the stoichiometry of the redox reaction between iron(III) and caffeic acid in the aqueous phase.

Material and methods

Materials

High purity (Milli-Q, Millipore) water was used to prepare the solutions for kinetic studies. The ionic strength of the solutions was kept constant by 0.01 *M* $NaClO_4$ (Fluka). The iron solutions were standardized by titration with EDTA. The purity of the caffeic acid was checked by HPLC tests.

Instrumentation

Spectrophotometric measurements were carried out using a UV-visible Perkin Elmer Model Lambda 5 spectrophotometer. Analyses were performed using a HPLC Waters chromatograph in conjunction with a spectrophotometric detector 441 Model. A HC-ODS/PHA column (25 × 0.8 cm) supplied by Perkin Elmer was used.

Kinetics

Kinetic measurements were carried out at room temperature and involved monitoring the iron(II) and CAF contents. The iron(II) was determined in the form of the 1,10-phenantroline complex in a small volume of solution buffered by acetate at pH 4.5. The absorbance of the complex was measured at 510 nm. The HPLC tests were carried out using a H_2O-Acetonitrile-Acetic acid (78:20:2) mixture at a flow rate of 0.4 mL/min^{-1} at room temperature. Samples of 20 μL were applied to the column by means a 20 μL loop valve.

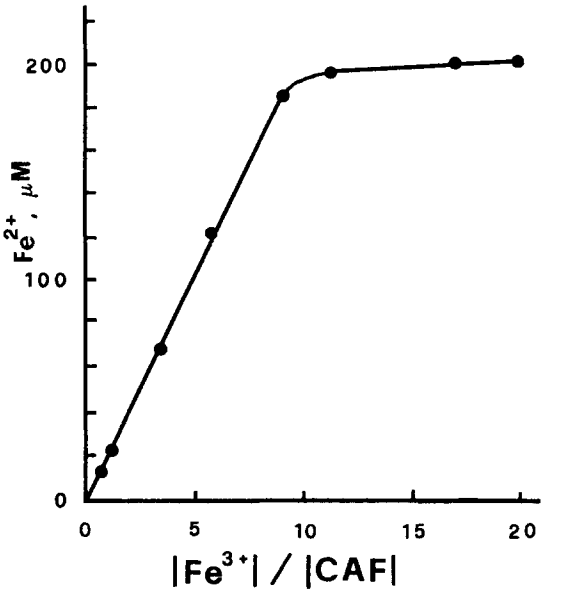
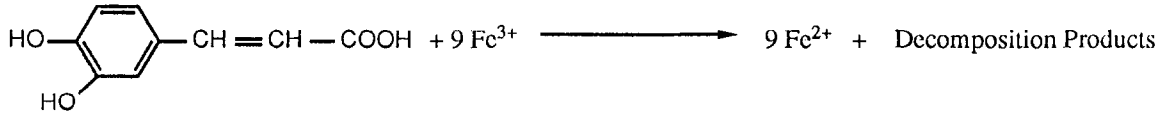


Fig. 2. Yield in iron(II) after 8 h as a function of the iron(III)/CAF molar ratio. (22 μM CAF).

Results and discussion

Our first goal was to determine the stoichiometry of the redox reaction in the CAF-iron(III) binary system. For this purpose, numerous reaction systems at pH 2.5 were prepared by adding different amounts of iron(III) to the 22.3 μM CAF solution. The iron(II) obtained as a function of the iron(III)/CAF molar ratio is reported in Figure 2. The yield of iron(II) linearly increased as iron(III) concentration increased tending to a constant value for iron(III)/CAF molar ratios higher than 9. Therefore, in our analytical conditions, a maximum of 9 electrons were released from one molecule of CAF.

The redox kinetics of the different systems (Fig. 3) suggest that the reaction proceeds through two steps; the first is faster and involves 5 electrons, the second 4 electrons. On the basis of these data the following stoichiometry was established:



Scheme 1.

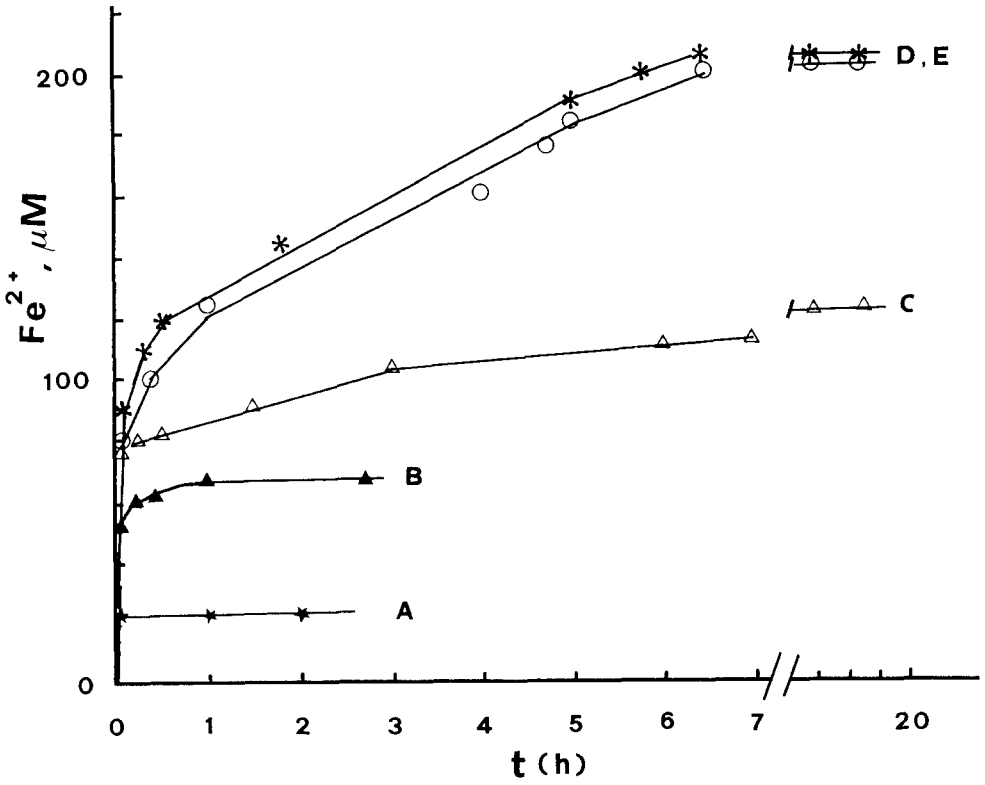


Fig. 3. Iron(II) concentrations (μM) measured at different times in iron(III)-CAF systems. Starting conditions: 22.3 μM CAF; 0.01 M $NaClO_4$; pH = 2.5; Curve A: 25.26 μM iron(III)=; Curve B: 76.02 μM iron(III); Curve C: 127 μM iron(III); Curve D: 253.78 μM iron (III); Curve E: 381.01 μM iron(III).

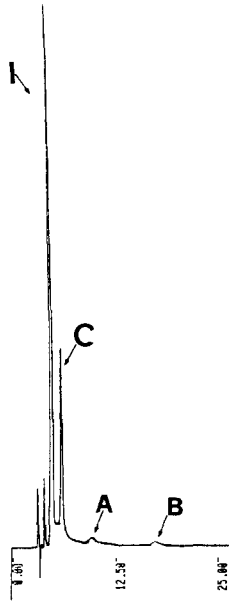


Fig. 4. Chromatogram relative to the [iron(III)]/[CAF] system = 3.4 after 3 min of reaction. I, A and B intermediate compounds; C: caffeic acid. Rt: I = 4.6; C = 6.1; A = 10.4; B = 17.6.

An unstable compound appears to be the principal product of the first step. Indeed, after a few minutes the chromatograms show the presence of a species I characterized by a retention time of 4.9 min. This gradually decreased while other compounds were formed (Fig. 4).

The distribution of iron(II), CAF and I determined on the different iron(III)/CAF systems after 3 minutes of reaction are reported in Table 1. The number of electrons involved in this step can also be calculated. At an iron(III)/CAF

Table 1. Distribution of iron(II), CAF and I as a function of the iron(III)/CAF molar ratio after 3 minutes of reaction

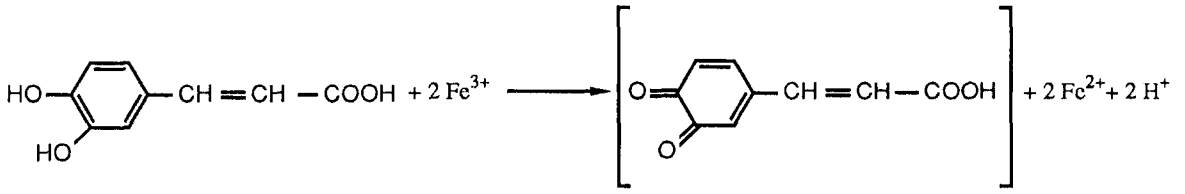
[Fe(III)]/[CAF]	[a][CAF] μM	[Fe(II)] μM	I[b]	n° e
1.13	11.00	23	1.3	2.02
3.40	11.90	52	13.3	4.90
5.68	7.76	76	18.5	5.20
11.35	4.33	80	15.3	4.65
17.04	2.36	90	17.0	4.60

[a][CAF] = 22.36 μM; no e^- = [Fe(II)/CAF].
[b]I = [I] expressed in chromatogram peak high (cm).

molar ratio equal to 1, the iron(III) was totally reduced while 50% of the CAF was consumed. In this case only 2 electrons were transferred and the probable formation of diquinonic species has been suggested (Laird, 1979; Olsen et al., 1982).

two quinonic groups could break giving the unstable intermediate I. Three electrons would be involved in this transformation.

The I product easily decomposed and gives rise mainly to two compounds (A and B) (Fig.



Scheme 2.

A small amount of I was also detected. In the other systems at higher iron(III)/CAF molar ratios, the concentration of the I species increased considerably. It appears to be the result of the interaction between iron(III) and diquinone. The carbon-carbon bond between the

5). This degradation was also detectable when iron(III) was completely reduced suggesting that it may take place with a dismutation mechanism.

However the I transformation was promoted by the presence of iron(III) as suggested by Figure 6 showing that its kinetics is faster in the

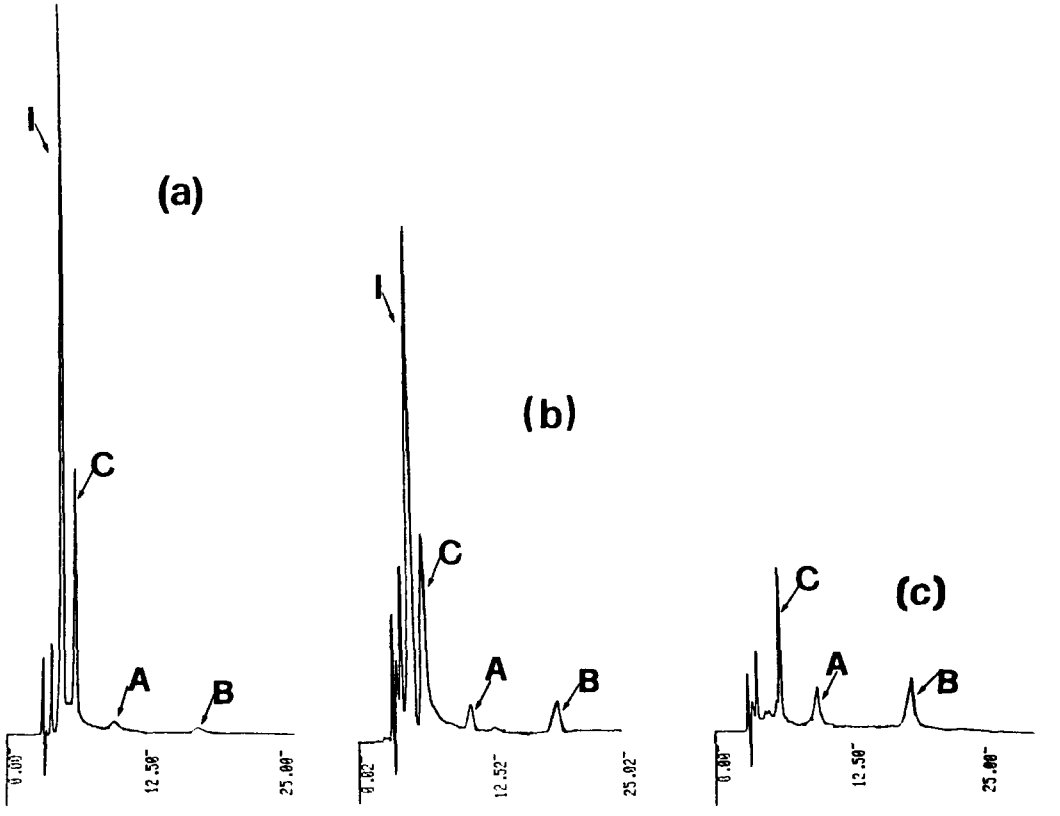


Fig. 5. Chromatograms relative to the [iron(III)]/[CAF] = 3.4 after 3 (**a**), 30 (**b**) and 150 (**c**) min, respectively. I, **A** and **B** intermediate compounds. Rt see Figure 4.

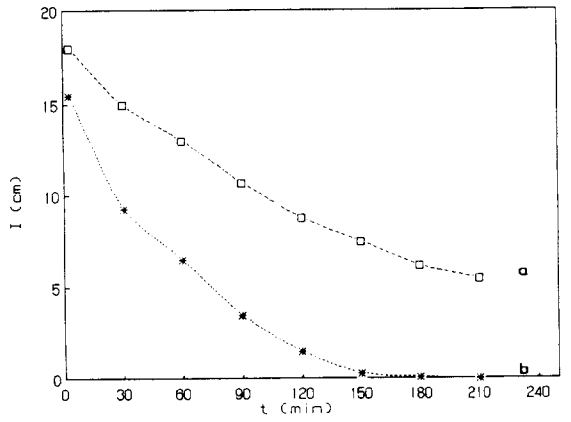
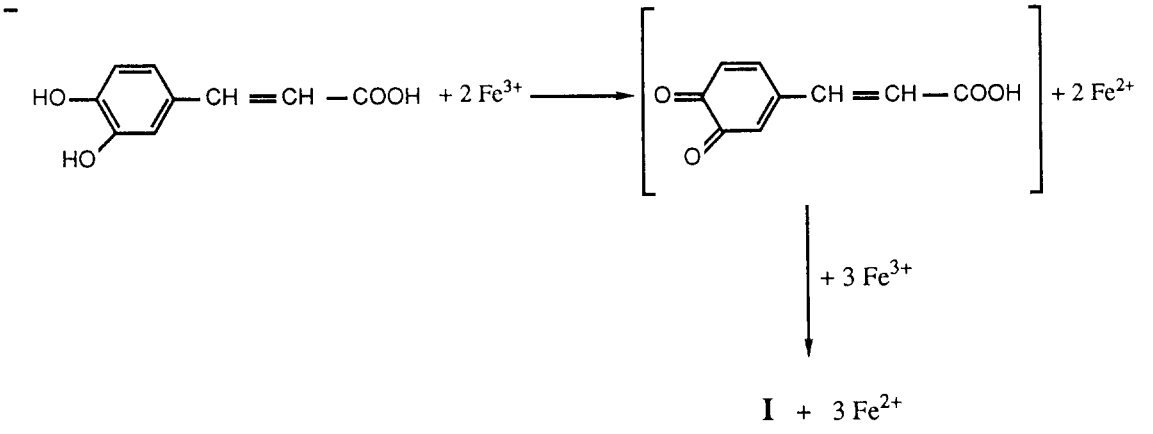


Fig. 6. I, expressed as peak height (cm), in function of the reaction time; (a) [iron(III)]/[CAF] = 5.68; (b)[iron(III)]/[CAF] = 11.32.

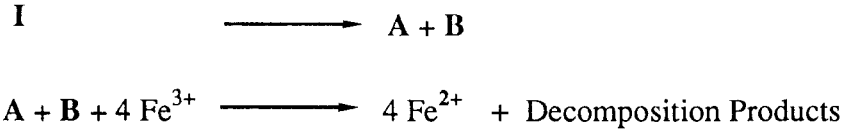
systems at higher iron(III)/CAF molar ratios. The influence of the metal on the transformation rate of I is connected with the second slow step of the reaction which characterized the system with iron(III)/CAF molar ratios higher than 5 (Fig. 3) and may be due to the redox activity of the degradation compounds A and B. In fact in the presence of iron(III), that is at iron(III)/CAF molar ratios higher than 5, A and B were involved in further redox reactions, their concentrations decreased and the decomposition of I was promoted.

The chemical activity of A and B is clearly indicated by the chromatograms reported in Figure 7, which show their disappearance and the formation of other compounds. Four electrons were associated with the redox activity of A and B. The reactivity of A and B was confirmed by spectrophotometric tests (Fig. 8). Three absorption bands at 205, 245 and 320 nm characterized the UV spectra of our systems. During the first minute of the reaction a fast decrease in the caffeic acid absorption band at 320 nm and an increase in the peak at 245 nm was noticeable. This peak, which was attributable to the unstable intermediate slowly decreased in intensity whereas the peak at 205 nm became more intense. This latter peak rapidly decreased for the systems at iron(III)/CAF molar ratios higher than 5 and thus must be due to the A and B species. On the basis of our results the following comprehensive redox process can be proposed.

Fast Step of the Reaction



Slow Step of the Reaction



Scheme 3

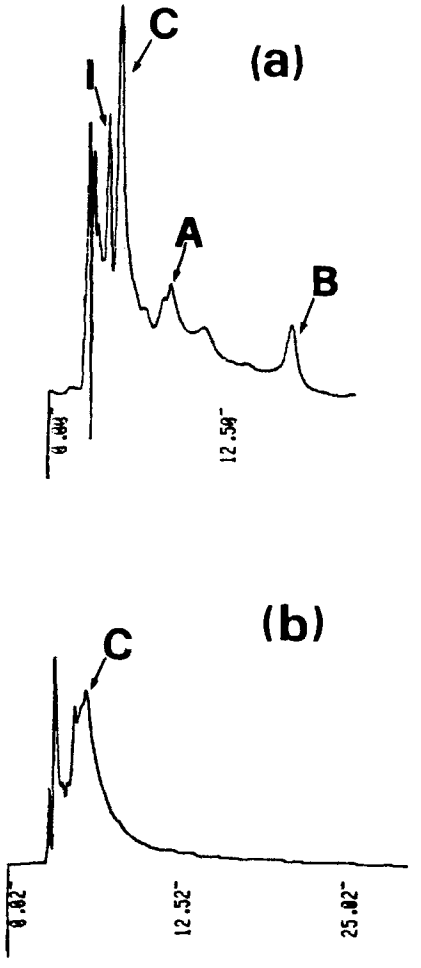


Fig. 7. Chromatograms relative to the [iron(III)]/[CAF] = 5.6 (**a**) and [iron(III)]/[CAF] = 11.32 (**b**) systems, after 3 and 6 h, respectively.

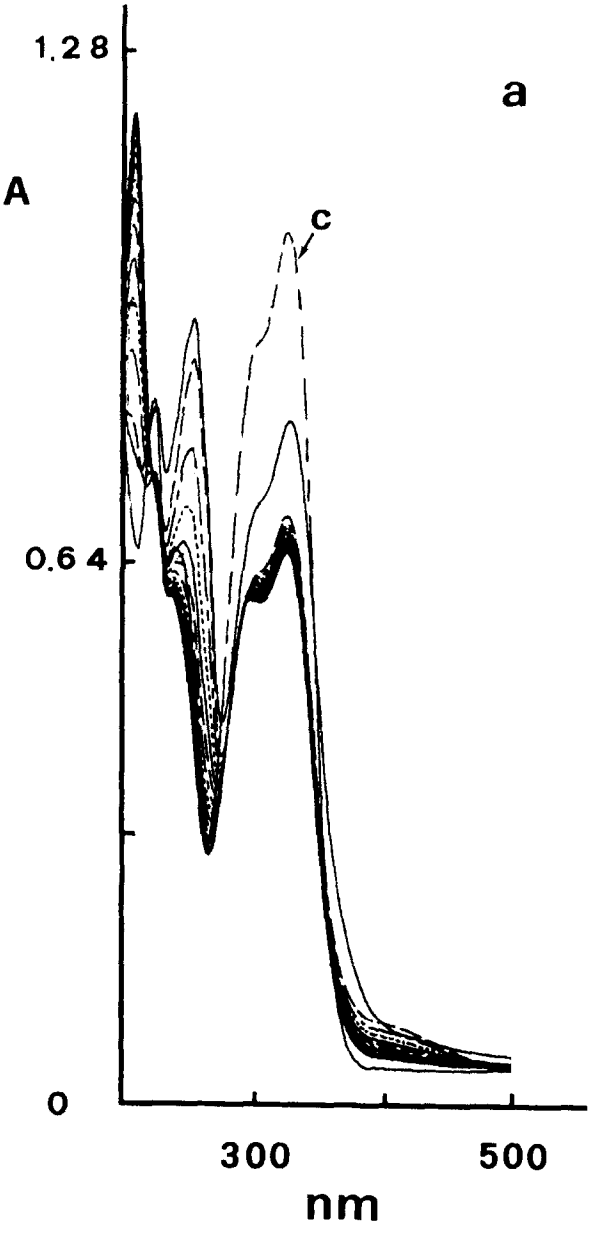


Fig. 8. Ultraviolet-Visibile spectra of the reaction solution at pH = 2.5 during oxidation of CAF by iron(III). Initial conditions: 22.36 μM CAF; pH = 2.5 (0.01 M $NaClO_4$). **a**: [iron(III)]/[CAF] = 1.13; **b**: [iron(III)]/[CAF] = 3.4; **c**: [iron(III)]/[CAF] = 5.68. The cycle time was 10 min; curve c refer to 22.36 μM CAF/0.01 M sodium perchlorate, pH = 2.5, A: absorbance.

The complexity of the mechanism and the variety of species involved in the redox reactions which regulate the uptake of iron by plants suggested to apply to simple systems and structure models resembling the natural root mucilages (Gessa and Deiana, 1990; 1992). Thus, the results reported here about the mechanism and and stoichiometry the iron reduction by caffeic acid constitute the first step in the comprehension of the mechanisms of iron reduction in the biological systems.

From the reported research it was possible to draw the following conclusions:

a) One molecule of caffeic acid is able to reduce nine atoms of iron(III) to iron(II).

b) The reaction proceeds through two main steps: the first one is very rapid and involves an electron transfer of five electrons from the organic molecule to iron(III), the second one

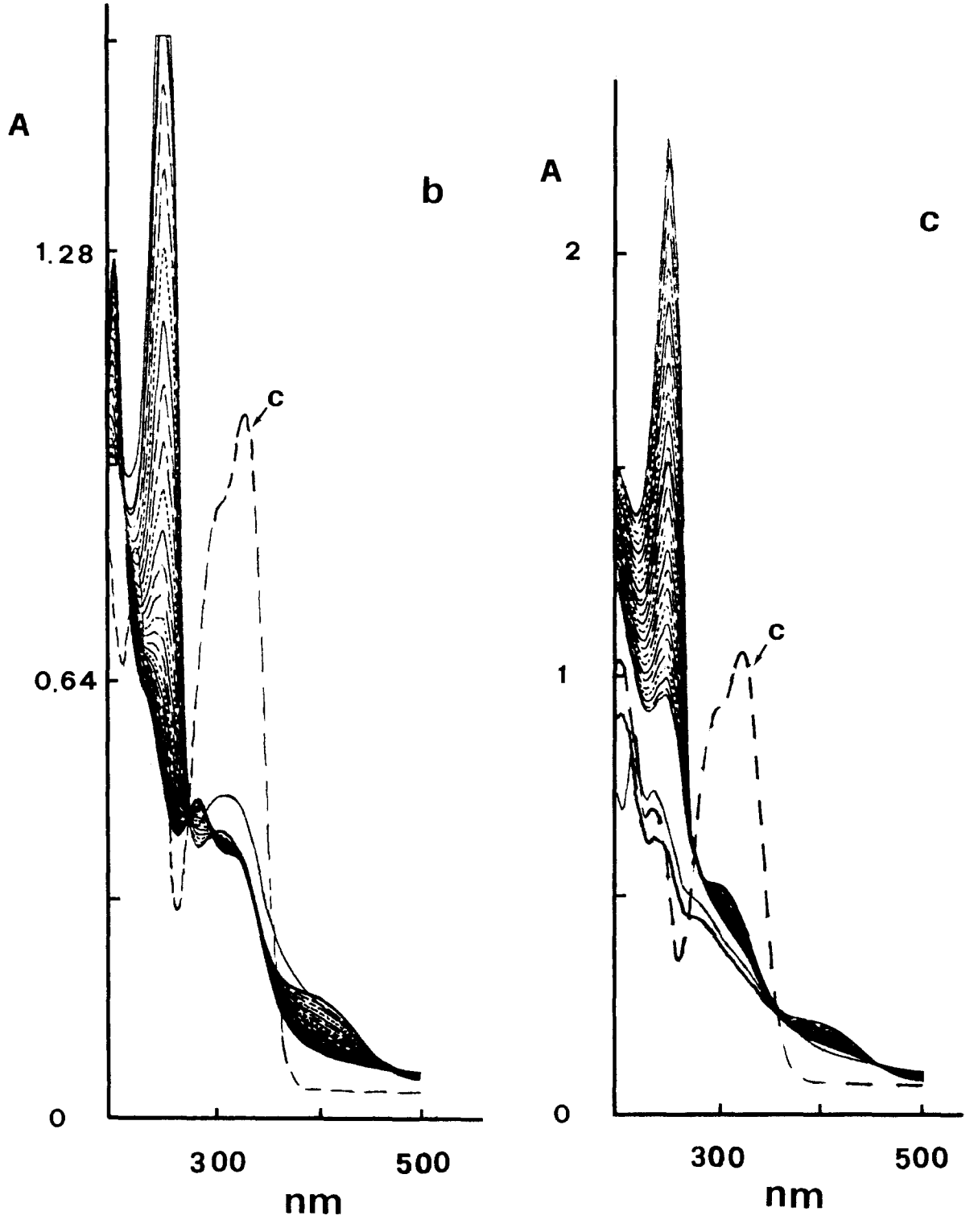


Fig. 8. continued

is slower and involves the transfer of four electrons.

These facts induce us to guess that the phenolic substances released by the plants and naturally present in the soils are able to reduce a relatively large amount of iron(III) which become available for the plant roots.

Researches about the influence of the pH of the system and of the presence of some organic and inorganic species, as well as about the identification of the reduction products, are in progress and will be the object of a later paper.

Acknowledgements

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