# Iron(III) Reduction by D-Galacturonic Acid. Part 3.† Influence of the Presence of Additional Metal Ions and of 2-Amino-2-deoxy-D-Gluconic Acid

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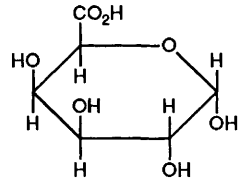
The effects of the presence of an aminosugar, namely 2-amino-2-deoxy-D-gluconic acid, on the reduction of iron(III) to iron(II) by D-galacturonic acid when copper(II), uranyl(VI), lead(II), nickel(II) or cadmium(II) ions are also present in the solution have been investigated. Lowering of the yield in iron(III) reduction has been found at any time in the cases of those metals whose addition to the system iron(III)–D-galacturonic acid favours iron(II) formation. Stoichiometries and stability constants of the complexes formed in the binary systems comprising one of the above-mentioned metal ions and the aminosugar ligand, as well as in the ternary systems with D-galacturonic acid as additional ligand, have been preliminarily determined by potentiometry. Relations between the compositions of the systems and the results of the kinetic tests have been suggested. The importance of similar reduction–complexation processes in the mobilization and consequent bioavailability of iron in biological systems, in particular at the root–soil interface, is emphasized.

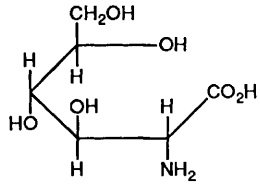
Uptake of metal ions by living organisms depends strongly on the presence of complexing agents in the environment. Redox processes are often coupled to these complexation reactions. In particular, complexing and reducing agents are formed in soil by biotransformation of the organic matter and by the activity of plant roots.[1–3] In this context, a fundamental role is played by uronic acids, which are present in both polymeric and monomeric form on root surfaces (mucigel) and on cell walls (apoplast). Uronic acid monomeric units form to a great extent from the corresponding polymers as a consequence of the biological activity of plants and the rhizosphere. Among the other organic ligands present in soil,[3–7] aminosugars are also involved in complex formation with essential and toxic elements.[8–12]

Iron mobilization constitutes a topic of particular importance in this field. In our previous studies on complexing and redox processes occurring in the iron(III)/iron(II)–D-galacturonic acid system we defined the nature of the complexes formed by the metal in the two different oxidation states, and established the stoichiometry of the iron(III) to iron(II) reduction.[13,14] We could also ascertain that the reducing species should be just iron(III)–D-galacturonate complexes, where a reducing aldehydic group is free as a consequence of the opening of the sugar molecule induced by the interaction of iron(III) both with the carboxylate group and with the ring oxygen, leading to a stable five-atom chelate centre. We also studied the effect of the addition to the binary system of a second metal ion, such as copper(II),[15] uranyl(VI), nickel(II), lead(II) or cadmium(II).[16] We verified that only those ions found to form particularly stable complexes with galacturonate ligand (chelate complexes) are also able to lead to a higher yield in iron(II).

In view of these considerations and of our previous findings, it seemed to us particularly interesting to study more complicated systems, looking for conditions which simulate biological environments more closely. The present paper reports the results of tests in which the yield of iron(II) from iron(III) was measured at different times in systems containing both D-galacturonic acid and 2-amino-2-deoxy-D-gluconic acid (glucosaminic acid), together with different metal ions, such as copper(II), uranyl(VI), nickel(II), cadmium(II) and lead(II). Taking advantage of our previous studies on the binary systems involving D-galacturonic acid and one of these metal ions,[15,16] as well as of investigations on corresponding binary systems with glucosaminic acid as ligand, reported here, the ternary systems consisting of both of these ligands and of any of the metal ions listed above could be defined. Helpful suggestions about the nature of the species involved in the iron(III) reduction could then be made.




Hgal

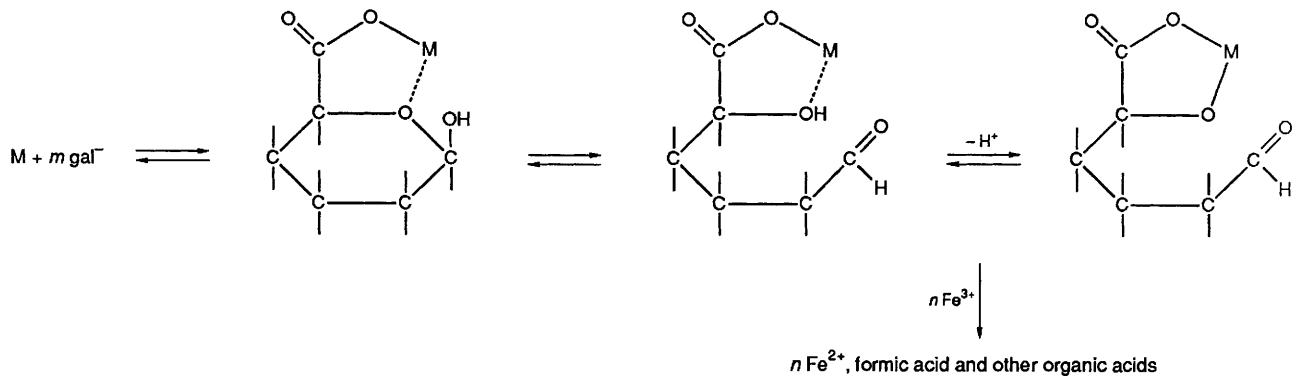



Hglu

## Experimental

*Materials.*—High-purity (Milli-Q Millipore) water was used to prepare the solutions for both potentiometric and kinetic studies. Sodium perchlorate monohydrate (Fluka) was recrystallized twice from cold and hot water; it was used in potentiometric and kinetic tests at 1 and 0.1 mol $dm^{-3}$ concentration, respectively. The titre of solutions of $Cu(ClO_4)_2$ (Fluka), $UO_2(NO_3)_2$ (Fluka), $Ni(NO_3)_2$ (C. Erba), $Cd(NO_3)_2$ (Fluka) and $Pb(NO_3)_2$ (J. T. Baker) was evaluated by following standard analytical procedures.[17] The solutions of D-galacturonic acid (Hgal) (Sigma) and of glucosaminic acid (Hglu) (Sigma) were standardized by acid–base titration with sodium

† Parts 1 and 2 are refs. 15 and 16, respectively.




**Scheme 1** M = $Fe^{3+}$, $Cu^{2+}$ or $UO_2^{2+}$

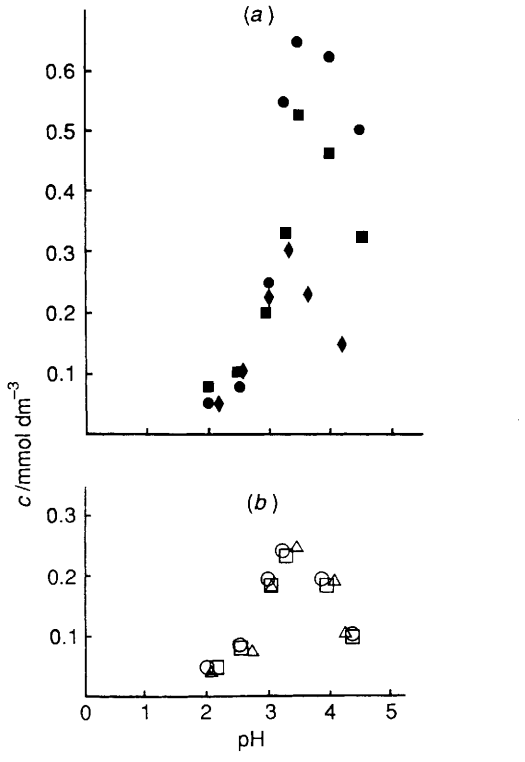



**Fig. 1** Iron(II) yield as a function of pH after 100 h. Initial concentrations: $Fe^{3+}$, 6.5 × $10^{-4}$; Hgal, 3.25 × $10^{-3}$; Hglu, 3.25 × $10^{-3}$; $Cu^{2+}$, $UO_2^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Cd^{2+}$, 6.5 × $10^{-4}$; $NaClO_4$, 0.1 mol $dm^{-3}$; 37 °C. (*a*) (■) Cu–Fe–gal–glu, (●) $UO_2$–Fe–gal–glu, (◆) Pb–Fe–gal–glu; (*b*) (○) Cd–Fe–gal–glu, (△) Ni–Fe–gal–glu, (□) Fe–gal–glu

hydroxide titrant solutions freshly prepared from Merck Titrisol ampoules; these hydroxide solutions were in their turn standardized with potassium hydrogenphthalate (Fluka). They were also used in the potentiometric acid–base titrations of binary and ternary systems (see below), as well as in the standardization of $HClO_4$ solutions. 1,10-Phenanthroline was a Baker product. 99.99% Nitrogen was used to deaerate solutions in both acid–base titrations and kinetic measurements.

*Potentiometric Titrations.*—Acid–base titrations were carried out at a temperature of 25.0 ± 0.1 °C at a fixed ionic strength for 1 mol $dm^{-3}$ $NaClO_4$. They were performed using a Metrohm model 655 Dosimat and a Metrohm pHM 84 Research pH-meter. Addition of the titrant solution and recording of pH values were carried out under the control of an Apple IIe personal computer with appropriate interfaces and software.

Glass electrode calibration, in terms of hydrogen-ion concentrations, was done according to ref. 18, by using the computer program MAGEC[19] to elaborate the results of a titration of 0.01 mol $dm^{-3}$ $HClO_4$ with standard 0.1 mol $dm^{-3}$ NaOH, at the specified ionic strength and temperature.

The formation constants were computed on the basis of acid–base titrations on solutions containing ligand-to-metal molar ratios in the range 1–5:1, the concentration of the metals varying between 4.7 × $10^{-4}$ and 4.4 × $10^{-3}$ mol $dm^{-3}$. These values were chosen to be equal to those in the kinetic tests. The data obtained were elaborated by the program SUPERQUAD,[20] while HALTAFALL[21] was used to compute the concentrations of the different species as a function of pH in the binary and ternary systems, as well as in those systems containing four different species.

*Kinetic Tests.*—These tests were carried out in the dark, at 37 °C. The extent of occurrence of the reaction was monitored on the basis of the iron(II) content, in the form of the 1,10-phenanthroline complex (absorption measurements in the visible region, at 510 nm).

## Results and Discussion

Scheme 1 shows the reaction sequence we have proposed to account for the reduction of iron(III) to iron(II) in systems containing D-galacturonic acid.[13,14] It is evident that those metals that are able to give such a kind of co-ordination with galacturonate anion are potential reductants. Study of the D-galacturonic acid–copper(II), –uranyl(VI), –nickel(II), –cadmium(II) and –lead(II) binary systems[15,16] allowed us to ascertain the formation of particularly stable copper and uranyl complexes, much more stable than the corresponding acetate adducts.[22] On the other hand, the complexes of nickel, cadmium and lead were more or less as stable as the corresponding acetate complexes. These findings have been taken by us as a strong indication that some of these metals are able to form chelate complexes with the D-galacturonate molecule, leading to reducing species similar to those reported in Scheme 1. Accordingly, the presence in the system of metal ions of the former listed group strongly enhances the yield in iron(II), indicating the formation of particularly powerful reductants, while with the metals of the latter group no significant change with respect to the simple iron(III)–D-galacturonate system can be observed.

Fig. 1(*a*) and (*b*) show the yield in iron reduction after 100 h, as a function of pH, in the systems containing initially iron(III), D-galacturonic acid and glucosaminic acid, together with copper(II), uranyl(VI), nickel(II), cadmium(II) or lead(II) ions. Fig. 2(*a*)–(*d*) show the amount of iron(II) formed in these systems at different pH values, as a function of time.

Comparison of these figures with analogous plots obtained in the absence of glucosaminic acid[16] evidentiates that in the case of copper(II) and uranyl(VI) additional metal ions the yield in iron reduction at any time is lower, so that equilibrium conditions are attained by the system at a longer time. No

**Table 1** Stability constants of the M–gal, M–glu and M–gal–glu systems (M = $Fe^{3+}$, $Cu^{2+}$, $UO_2^{2+}$, $Pb^{2+}$, $Cd^{2+}$ or $Ni^{2+}$), as determined by potentiometric titrations (25.0 + 0.1 °C; $I$ = 1.0 mol $dm^{-3}$, $NaClO_4$). The values in parentheses are the standard deviations on the last significant figures

| | log β | | | | | |
|---|---|---|---|---|---|---|
| Species | $Fe^{3+}$ | $Cu^{2+}$ | $UO_2^{2+}$ | $Pb^{2+}$ | $Cd^{2+}$ | $Ni^{2+}$ |
| M(gal) | | 3.39(9) [a] | | 2.50(3) [b] | 1.52(5) [b] | 1.04(2) [b] |
| $M(gal)_2$ | | 5.99(1) [a] | 6.19(2) [b] | | | |
| $M(gal)_3$ | 8.51(6) [c] | | | 6.30(7) [b] | | |
| $M(gal)H_{-1}$ | | −2.60(2) [a] | | | | |
| $M(gal)_2H_{-2}$ | | | −2.03(4) [b] | | | |
| $M(gal)_3H_{-2}$ | 1.54(5) [c] | | | | | |
| $M(gal)_3H_{-3}$ | −2.03(5) [c] | | −4.72(11) [b] | | | |
| M(glu) | | 7.72(1) | 7.01(2) | 5.08(1) | 4.69(1) | 6.54(3) |
| $M(glu)_2$ | | 14.39(1) | 13.36(2) | 9.53(1) | 9.39(1) | 12.33(2) |
| $M(glu)_2H_{-1}$ | | 4.88(1) | | 1.28(1) | 0.97(1) | 4.99(3) |
| $M(glu)_2H_{-2}$ | | −5.12(1) | | −6.84(1) | | −2.65(2) |
| $M(glu)_2H_{-3}$ | | | | −16.34(2) | | −11.86(3) |
| $M_2(glu)$ | | | | | 7.48(3) | |
| M(gal)(glu) | | 11.81(5) | 11.43(12) | 7.69(4) | 7.63(9) | 9.48(4) |
| M(gal)(glu)$H_{-1}$ | | 7.02(6) | 7.40(8) | | | 2.99(9) |
| $M_2$(gal)(glu) | | | | | 11.98(7) | |

The overall charge of the complexes has been omitted for clarity. log $\beta_{Hgal}$ = 3.15(1) [a]; log $\beta_{Hglu}$ = 9.19(3); log $\beta_{H_2glu^+}$ = 11.76(5). [a] From ref. 15. [b] From ref. 16. [c] From ref. 14.

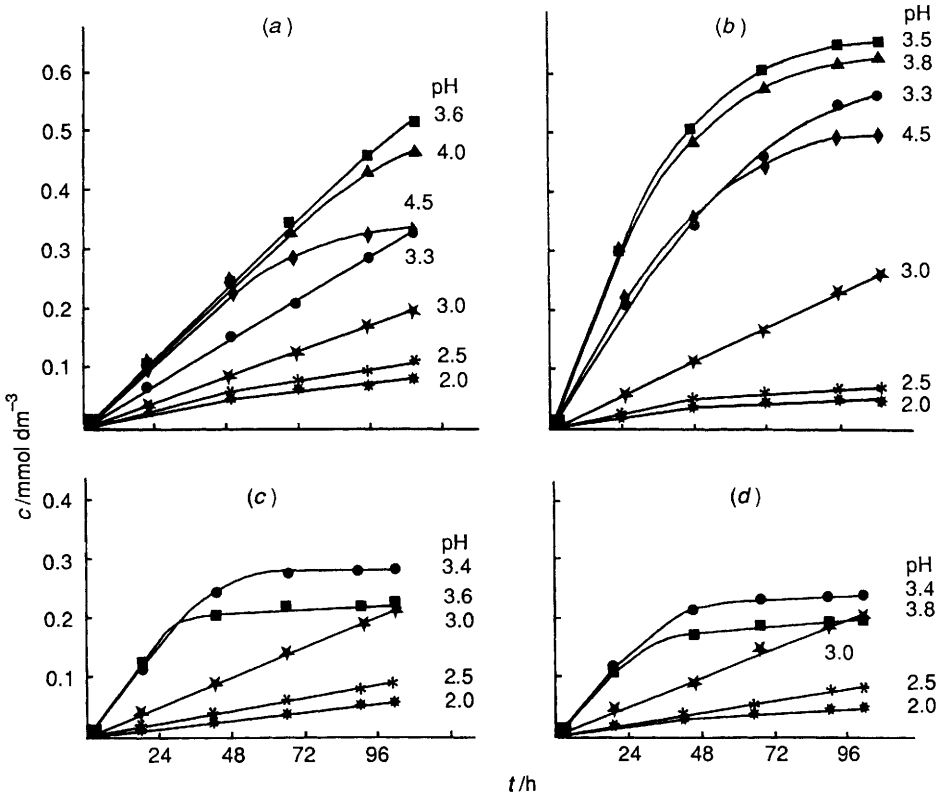



**Fig. 2** Iron(II) formation plots. Conditions as in Fig. 1. (*a*) Cu–Fe–gal–glu, (*b*) $UO_2$–Fe–gal–glu, (*c*) Pb–Fe–gal–glu, (*d*) Ni–Fe–gal–glu, Cd–Fe–gal–glu or Fe–gal–glu

significant change is evidentiated for nickel(II), cadmium(II) and lead(II) ions, as well as in the case when no additional metal ion is present in the system. The data reported in Table 1, concerning stoichiometries and formation constants for the complexes formed by iron(III), copper(II), uranyl(VI), nickel(II), cadmium(II) and lead(II) with D-galacturonic acid and with glucosaminic acid in binary systems, as well as by the same metals with both ligands in ternary systems, can be helpful in accounting for the results of the iron reduction tests. A first remark is that iron(III) does not form any stable complex with glucosaminic acid; hence, possible changes in the behaviour of the systems studied which are induced by the addition of this ligand cannot be ascribed to the iron itself. On the contrary, wide series of glucosaminate complexes are formed by any of the other metal ions: particularly stable adducts are formed by copper(II), uranyl(VI) and nickel(II). Ternary complexes are also formed, of highest stability in the case of copper(II) and uranyl(VI).

On these bases, an explanation for the effect of the addition of glucosaminic acid on the yield in iron reduction at any time is given by a lower formation of galacturonate complexes, which have been identified as the reducing agents with respect to

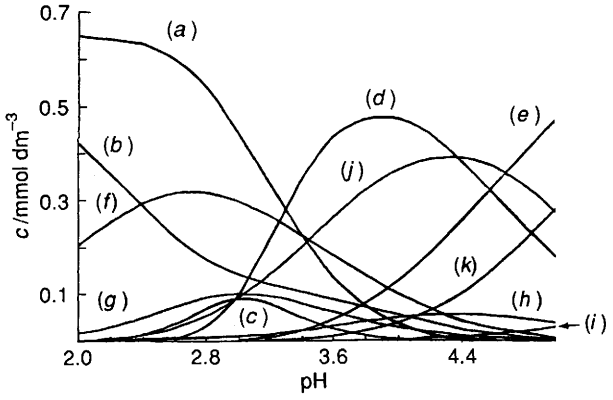



**Fig. 3** Concentrations of the species present in the system Fe–Cu–gal–glu as a function of pH. Protonated and deprotonated free ligands are always present in excess (not shown). The species $[Cu(gal)H_{-1}]$, $[Cu(glu)_2H_{-1}]^-$ and $[Cu(glu)_2H_{-2}]^{2-}$ are present in a very low concentration, so that the relevant plots are indistinguishable from the abscissa. Overall concentrations: $Fe^{3+}$, $6.5 \times 10^{-4}$; $Cu^{2+}$, $6.5 \times 10^{-4}$; Hgal, $3.25 \times 10^{-3}$; Hglu, $3.25 \times 10^{-3}$ mol dm$^{-3}$. Species: (*a*) $Fe^{3+}$, (*b*) $Cu^{2+}$, (*c*) $[Fe(gal)_3]$, (*d*) $[Fe(gal)_3H_{-2}]^{2-}$, (*e*) $[Fe(gal)_3H_{-3}]^{3-}$, (*f*) $[Cu(gal)]^+$, (*g*) $[Cu(gal)_2]$, (*h*) $[Cu(glu)]^+$, (*i*) $[Cu(glu)_2]$, (*j*) [Cu(gal)(glu)] and (*k*) $[Cu(gal)(glu)H_{-1}]^-$

**Table 2** Relative concentrations of the species present at pH 3.4 in the systems iron(III)–copper(II)–D-galacturonic acid and iron(III)–copper(II)–D-galacturonic acid–glucosaminic acid, respectively (25.0 ± 0.1 °C, 1 mol dm$^{-3}$ $NaClO_4$)

| Species* | % | Species | % |
|---|---|---|---|
| (*a*) Fe–Cu–gal System | | | |
| $Fe^{3+}$ | 17.99 | $Fe(gal)_3H_{-3}$ | 1.78 |
| $Cu^{2+}$ | 12.79 | Cu(gal) | 27.51 |
| $Fe(gal)_3$ | 3.88 | $Cu(gal)_2$ | 9.66 |
| $Fe(gal)_3H_{-2}$ | 26.30 | $Cu(gal)H_{-1}$ | 0.07 |
| (*b*) Fe–Cu–gal–glu System | | | |
| $Fe^{3+}$ | 17.54 | $Cu(gal)H_{-1}$ | 0.05 |
| $Cu^{2+}$ | 8.12 | Cu(glu) | 1.82 |
| $Fe(gal)_3$ | 3.94 | $Cu(glu)_2$ | 0.04 |
| $Fe(gal)_3H_{-2}$ | 26.68 | $Cu(glu)_2H_{-1}$ | 0.00 |
| $Fe(gal)_3H_{-3}$ | 1.81 | $Cu(glu)_2H_{-2}$ | 0.00 |
| Cu(gal) | 17.63 | Cu(gal)(glu) | 15.73 |
| $Cu(gal)_2$ | 6.23 | $Cu(gal)(glu)H_{-1}$ | 0.42 |

* Overall charges of complexes are omitted for clarity.

iron(III),[15,16] due to the competitive formation of either glucosaminic or mixed glucosaminic–galacturonic complexes. It is obvious that no effect is to be expected in any case for those metals that have been found not to induce significant changes in the yield of iron reduction by D-galacturonic acid.

The distribution diagram (concentration *vs.* pH) of the species present in the system comprising both organic ligands together with iron(III) and copper(II) ions in amounts corresponding to those of the kinetic tests is shown in Fig. 3. It is representative only of the situation in solution at the start of the redox process, and it must be stressed that quite different situations are encountered as the reaction goes on. We could ascertain that different smaller molecules, which are potential ligands, form from D-galacturonic acid,[13,23] together with iron(II), which also forms stable galacturonate complexes.[14] This and similar plots for the other ions studied can however be useful for a first indication of the nature of the species involved in the reduction. From the results found in the work reported here, as well as in our previous papers on this subject,[13–16] reasonable explanations for the trend reported in Figs. 1 and 2 can be advanced. Table 2 reports data from Fig. 3, at pH 3.4, a value near that for the maximum yield in iron reduction. The percentages of the different species present in the copper(II)–iron(III)–D-galacturonic acid–glucosaminic acid system, when compared with those in the copper(II)–iron(III)–D-galacturonic acid system, should, to a first approximation, account for the decrease in the iron reduction yield due to the additional presence in the solution of glucosaminic acid. In the former case the percentages of the complexes $[Cu(gal)]^+$ and $[Cu(gal)_2]$, that have been identified as the reducing species responsible for the increase in iron reduction yield upon addition of copper(II) to the iron(III)–D-galacturonic acid systems,[15] are lower; however, the concentrations of copper(II)–glucosaminic acid complexes are negligible at these pH, while the extent of formation of the ternary complex [Cu(gal)(glu)] almost equals the decrease in galacturonate complexes. Hence, it must be assumed that [Cu(gal)(glu)] is a much less powerful reductant than $[Cu(gal)]^+$ and $[Cu(gal)_2]$. The relatively high concentration found for [Cu(gal)(glu)] and, at pH > 4, for $[Cu(gal)(glu)H_{-1}]^-$ species deserves some comments. It can be explained by considering the results obtained on the copper(II)–glucosaminic acid, –D-galacturonic acid and –D-galacturonic acid–glucosaminic acid systems. From the data in Table 1, it can be inferred that in any case the pH range of predominance of galacturonate or glucosaminate complexes in the relevant binary systems is, obviously, strongly affected by the $pK_a$ values of the corresponding protonated ligand, the complexes with the much weaker glucosaminic acid being stable at higher pH values. The weak acidic character of Hglu with respect to Hgal accounts for the formation in the ternary system of the ternary complexes [Cu(gal)(glu)] and $[Cu(gal)(glu)H_{-1}]^-$ rather than $[Cu(glu)]^+$ and $[Cu(glu)_2]$, in spite of the higher values of the formation constants of the Cu–glu complexes with respect to those of the Cu–gal. Furthermore, the particularly strong interaction between $Cu^{2+}$ and deprotonated galacturonate ligand[15] is the reason for the higher stability of $[Cu(gal)(glu)H_{-1}]^-$ compared with $[Cu(glu)_2]$.

Similar arguments hold in the case of uranyl(VI), where the most interesting change in passing from the uranyl(VI)–iron(III)–D-galacturonic acid to the uranyl(VI)–iron(III)–D-galacturonic acid–glucosaminic acid system lies, at the pH of interest, in the decrease in the $[UO_2(gal)_2]$ concentration, in favour of the $[UO_2(gal)(glu)]$ternary complex. Although the experimental data we have collected until now do not allow us to suggest a reliable co-ordination pattern for the ternary species, it should be assumed that complexation with the aminosugar molecule does not allow the metal to co-ordinate the galacturonate ligand according to Scheme 1, and hence to open the sugar ring with consequent formation of a free aldehydic reducing group. A study better to understand this key point, *i.e.* to clarify the co-ordination mode inside these complexes in solution, based on the application of different spectroscopic techniques is in progress.

The results found suggest that widely diffused biomolecules such as aminosugars can inhibit in part the reduction of iron by D-galacturonic acid. The effect of different strong complexing agents on the iron complexation–reduction process, as well as the influence on these reactions of the presence of additional complexing sites in the sugar ring of the uronic acid, is being studied in our laboratories. Our attention is also devoted to the study of the transport of metal ions through the root–soil interface, which is strongly conditioned by the electronic charge of the diffusing species. As a first step we have synthesized a polygalacturonate membrane that is a suitable model for this interface.[24,25] The results in the present paper also constitute a valuable starting point for the study of the transport of iron and other metal ions, both in the form of free ions and of the different complex species, through this synthetic membrane.

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