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Investigation of Nickel(II)–Ascorbic Acid Complex Formation in Aqueous Solution Using Potentiometric Measurements, Optical Spectroscopy and ^{13}C NMR Spectroscopy

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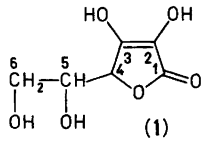
Potentiometric titrations, giving 205 experimental points, have been carried out for the system Ni(II)–ascorbic acid in 2M $NaNO_3$. The following equilibria and equilibrium constants were found to fit the experimental data in the range $-6.3 < \log h < -1.2$ at 25 °C:

$HAsc^- + H^+ \rightleftarrows H_2Asc$
$Ni^{2+} + HAsc^- \rightleftarrows NiHAsc^+$
$Ni^{2+} + HAsc^- \rightleftarrows NiAsc^0 + H^+$

$\beta_{101} \pm 3\sigma = (1.27 \pm 0.03) \times 10^4\ M^{-1}$
$\beta_{011} \pm 3\sigma = 1.5 \pm 0.4\ M^{-1}$
$\beta_{\bar{1}11} \pm 3\sigma = (1.9 \pm 0.4) \times 10^{-6}$

Based on this equilibrium model which has been extrapolated to $\log h = -8.5$, the neutral complex $NiAsc^0$ has been further characterized with optical spectroscopy and carbon-13 NMR. The visible–near IR spectrum corresponding to the $d-d$ transitions of the $NiAsc^0$ complex in a 1 M solution of ascorbic acid containing 0.1 M Ni^{2+} at $\log h = -7.5$ has been observed. These measurements give evidence for an octahedral surrounding in the $NiAsc^0$ complex. Measurements of the paramagnetic contribution to the carbon-13 spin-lattice relaxation times (T_{1P}) of 0.8 M ascorbic acid containing 2.5 mM Ni^{2+} at $\log h = -8.5$ have also been performed. The T_{1P} data show that Ni^{2+} is bidentally chelated to ascorbate with a symmetric binding through the oxygens at carbons 2 and 3.

Interactions of L-ascorbic acid (1) with metal ions are known to play an important role in many biological systems.[1,2] Nevertheless, relatively little is known about the details of the complexation of ascorbic acid with metal ions, particularly regarding the structure and equilibria of such complexes. Careful potentiometric studies of equilibria have earlier been performed at this laboratory for the cases of cadmium(II), calcium(II) and iron(II) complexes with ascorbic acid.[3–6] Equilibrium constants for a number of metal complexes $MeAsc^0$ and $MeHAsc^+$, have been published.[7–9] UV–visible spectra for ascorbic acid complexes of numerous metal ions have been reported.[10,11] The kinetics of oxidation in the presence of cupric and ferric ions has also been investigated.[12,13] ^{13}C NMR shifts have been published for aqueous solutions of ascorbic acid as a function of pH in the absence of metals.[14]



(1)

In this paper a potentiometric study of the complex formation between ascorbic acid and Ni^{2+} in 2M $NaNO_3$ is reported. The equilibria thus obtained allow for determination of the pH range at which the different complexes exist as predominant species for a given solution. The visible–near IR spectrum corresponding to the nickel(II)-d-d transitions of a 1M ascorbic acid solution containing 0.1 M Ni^{2+} at pH 7.5, which allows an unambigiuous determination of the symmetry of the nickel(II)–ascorbate complex, is also presented. Furthermore,

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the paramagnetic contribution to the observed ^{13}C spin-lattice relaxation rates of all six carbons of 0.8 M ascorbic acid at pH 8.5, caused by the presence of 2.5 mM Ni^{2+}, is reported. These measurements give information on the position of the nickelous ion relative the different carbon atoms of the complexing ligand. Taken together, the data thus obtained from the three independent methods make possible a comprehensive characterization of the most abundant complex between L-ascorbic acid and Ni^{2+} at weakly basic pH and low metal concentration.

EXPERIMENTAL

Chemicals. Analytical grade L-ascorbic acid from Merck, Darmstadt, was used and samples were prepared by weighing. The samples containing Ni^{2+} used in the spectroscopic measurements were prepared by weighing analytical grade $NiSO_4 \times 6H_2O$ from Merck, Darmstadt. For the potentiometric measurements analytical reagent $NiNO_3 \times 6H_2O$ was used and the solutions were prepared by weighing. The concentration of Ni^{2+} was analyzed by using a cation exchanger (Amberlite IR-20). The results from repeated determinations yielded an Ni^{2+} content of 0.2005 ± 0.00001 M.

Stock solution of $NaNO_3$ (p.a. from Merck, Darmstadt) was prepared by weighing. Stock solutions of NaOH were prepared as described earlier.[15] All solutions used in this study were prepared from distilled, de-aerated water saturated with argon.

Potentiometric measurements. The potentiometric titrations were carried out with a fully automated computer-controlled system developed at this laboratory.[16] The reference electrode used was: 2M $NaNO_3$|1.99 M $NaNO_3$, 0.01M NaCl saturated with AgCl|AgCl, Ag. The glass electrode was an Ingold 201. A Wilhelm saltbridge was used. The temperature was kept constant at 25.00 ± 0.02 °C with an oil bath thermostat. During the measurements, a stream of argon gas was passed through the solution for stirring and for maintaining an inert atmosphere.

Optical spectra. A solution of 1M ascorbic acid containing 0.1 M Ni^{2+} at pH = 7.5 was prepared by titration with NaOH. The preparation was carried out under an atmosphere of argon. pH was measured with a Radiometer PHA 925. The visible – near IR electron spectrum was recorded at 298 K on a Cary 17 spectrometer. An ascorbic acid solution of the same concentration and with the same pH was used as reference. The spectra remained unchanged for several hours and no precipitation of $Ni(OH)_2$ was observed.

NMR-measurements. In order to obtain solutions stable enough for the very time-consuming T_1-measurements, special precautions were undertaken to obtain samples in a reduced anaerobic state. An acidic (pH 2) solution containing an appropriate amount of ascorbic acid and Ni^{2+} was prepared and was reduced by passing a stream of hydrogen gas through it for at least one hour. Platinum foil was used as a catalyst. The solution was then titrated with NaOH. Hydrogen and argon were bubbled through the solution during the whole titration. Both the diamagnetic and the paramagnetic solutions were 0.8 M ascorbic acid at pH 8.5; the paramagnetic solution contained in addition 2.5 mM Ni^{2+}. The sample was then degassed with several freeze-pump-thaw cycles and sealed off *in vacuo*. Prepared in this way, the samples and their spectra remained unchanged during the period of study. In order to exclude the possibility of any competitive coordination from NO_3^- at the low Ni^{2+} concentration used, the samples were prepared without $NaNO_3$ medium.

Natural abundance, ^1H noise-decoupled ^{13}C NMR spectra were obtained in the Fourier transform mode with a Varian XL 100 spectrometer operating at 25.2 MHz ^{13}C resonance frequency (magnetic field of 2.35 Tesla). Measurements were made using 12 mm NMR tubes with 10 mm tubes containing the sample and with D_2O placed in the annulus between the 10 mm tube and the 12 mm tube for field-frequency stabilization. The chemical shift was measured relative to *p*-dioxane which was placed in the 12 mm tube as external standard. Spin-lattice relaxation times were measured using the fast inversion – recovery method[17] and a non-linear three parameter fitting of line intensities.[18] T_{1P}^{-1}, the paramagnetic contribution to the observed spin-lattice relaxation rate was obtained as the difference between the relaxation rates measured in the paramagnetic and the diamagnetic solutions: $T_{1P}^{-1} = T_{1,obs}^{-1} - (T_1^0)$. The contribution to T_{1P}^{-1} from the outer sphere relaxation mechanism was assumed to be negligible. Each T_1 was measured at least twice, with mean values of T_1 reported. The 1σ standard deviations were 2–6 % in each measurement except for carbons 1 and 4 in the paramagnetic solutions, where the standard deviations were 12 %. The temperature, controlled with the Varian variable temperature unit, was measured in a 0.8 M ascorbic acid solution at pH = 8.5.

RESULTS

Potentiometric measurements. For a thorough description of the symbols used and the general procedure in the data analysis see Ulmgren and

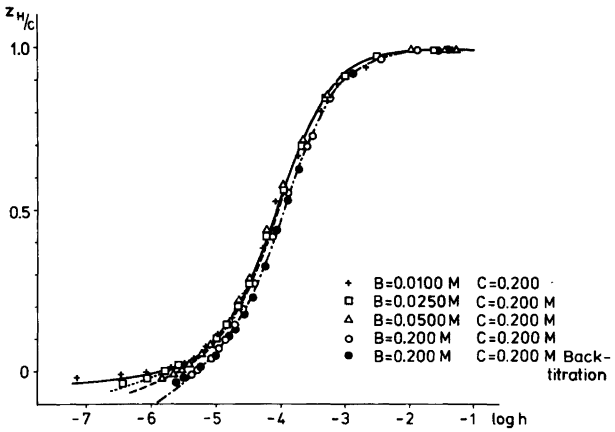

Fig. 1. $Z_{H/C}$ (the average number of H^+ bound per C) as a function of log *h*. The curves have been calculated with the final equilibrium constants using the program HALTAFALL.[20]

Wahlberg.[19] The complex formation studied can be written in the general notation (1).

$$pH + qB + rC \rightleftarrows H_pB_qC_r \qquad (1)$$

The associated equilibrium constant is denoted β_{pqr}. We define $\beta_{100}=\beta_{010}=\beta_{001}=1$. In this study $H \equiv H^+$, $B \equiv Ni^{2+}$ and $C \equiv HAsc^-$. With *H,B,C* we understand the total concentrations of H,B,C, respectively.

Five different titrations were carried out with $C=0.200$ M and with *B* ranging from 0.200 to 0.010 M, in the pH range $1.25<pH<8.0$. Two titrations with no Ni^{2+} present were carried out. The titrations gave a total of 205 experimental points. In Fig. 1, part of the set of titrations is shown plotted as $Z_{H/C}$ (the average number of H^+ bound per C) as a function of log *h* ($h=[H^+]$, the concentration of free H^+). The titrations were terminated when equilibrium was unattainable within reasonable time. This instability was caused by a precipitation, presumably of $Ni(OH)_2(s)$.

Fitting of the experimental data was done using the least squares program LETAGROP[21] minimizing $U=\sum (Z_{calc}-Z)^2$. First the data for $\log h > -4.5$ were treated and then the complex formation in the range $\log h < -4.5$ was treated with the constants obtained in the first fit. For the acid range it was assumed that the complexes formed were of the type $Ni_q(HAsc)_r$, while in the neutral range it was assumed that species having the general formula $Ni_q(HAsc)_rH_p$ also existed. Points affected by precipitation were not used in the data fitting. Therefore we obtained data points in a limited concentration range. This in turn means that the model as stated below is poorer verified in the region $\log h < -6.3$ where the number of data points (12) were few (see Fig. 1).

The resulting reactions and constants which we propose to be valid in 2M $NaNO_3$ medium at 25 °C, are given in eqn. (2).

$$\begin{aligned} &HAsc^- + H^+ \rightleftarrows H_2Asc \\ &Ni^{2+} + HAsc^- \rightleftarrows NiHAsc^+ \\ &Ni^{2+} + HAsc^- \rightleftarrows NiAsc^0 + H^+ \end{aligned} \qquad (2)$$

$$\begin{aligned} &\beta_{101} \pm 3\sigma = (1.27 \pm 0.03) \times 10^4\ M^{-1} \\ &\beta_{011} \pm 3\sigma = 1.5 \pm 0.4\ M^{-1} \\ &\beta_{\bar{1}11} \pm 3\sigma = (1.9 \pm 0.4) \times 10^{-6} \end{aligned}$$

The values $\sigma(Z)=0.006$ (acidic range) and $\sigma(Z)=0.008$ give an opinion of the good quality of the fit. It is noteworthy that $NiAsc_2^{2-}$ or $Ni(HAsc)_2^0$ could not be detected in the concentration range studied. Complexes with the stoichiometry $MAsc_2^{2-}$ or $M(HAsc)_2^0$ were not found for $M=Ca^{2+}$, Cd^{2+} or Fe^{2+} in solutions with *C/B* up to 20.[3-6] Even if these facts do not exclude the presence of such complexes at higher pH and *C/B* ratio, they indicate that complexes of this type are of minor importance.

Equilibrium constants for the equilibrium $Ni^{2+} + Asc^{2-} \rightleftarrows NiAsc$ are reported by Pfeilsticker[7] ($\log K \approx 5$, ionic medium not reported) and Dubey

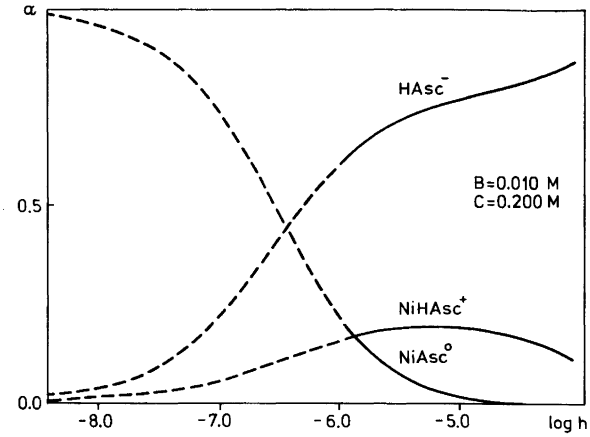

Fig. 2a. The distribution of Ni^{2+} on different species as a function of log *h*. $B=0.01$ M and $C=0.2$ M. The calculations have been performed using the program HALTAFALL, with the final equilibrium constants. The broken curves refer to the concentration range outside the experimental region.

and Parven[8] (log $K=5.6$, ionic strength 0.06 M). To compare these constants with $\beta_{\bar{1}11}$ we have used the value of $\log(\beta_{\bar{1}01}) = -11.1$ reported by Wahlberg and Ulmgren (for 2M NaCl).[15] The values agree reasonably well, whereas the value of $\log(\beta_{011}) = 1.05$ (extrapolated to zero ionic strength) obtained by Veselinovic and Susic[9] differ considerably from the value found in this work. Fig. 2a illustrates the distribution of Ni^{2+} for one of the titrations. The dashed curves indicate the log *h* range in which the equilibrium model is not verified experimentally.

In Fig. 2b the distribution of Ni^{2+} on different species for $B=0.10$ M and $C=1.0$ M is shown. The concentrations correspond to those used in the electron spectrum study (see below). At pH 7.5, where the visible–near IR spectrum was recorded, the complex $NiAsc^0$ dominates. Fig. 2c shows the corresponding curves for $B=2.5\times10^{-3}$ M and $C=0.8$ M, which were used in the ^{13}C NMR measurements (see below). The ^{13}C NMR measurements were made at pH 8.5, at which pH the equilibrium data indicate that all Ni^{2+} forms the complex $NiAsc^0$. A point of caution when drawing conclusions from Fig. 2 is that the potentiometric measurements were carried out in a medium of 2M $NaNO_3$, whereas the spectroscopic measure-

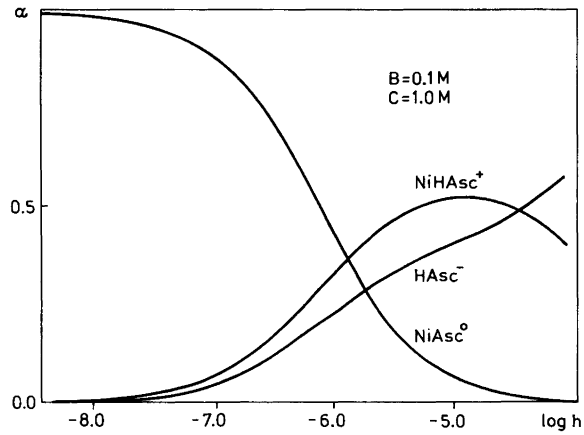

Fig. 2b. The distribution of Ni^{2+} on different species as a function of log *h*. $B=0.1$ M and $C=1.0$ M.

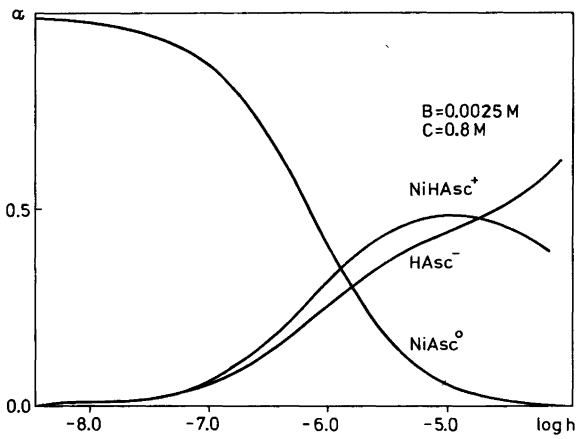

Fig. 2c. The distribution of Ni^{2+} on different species as a function of log *h*. $B = 2.5 \times 10^{-3}$ M and $C = 0.8$ M.

ments were performed without ionic medium (see below).

d-d *transition spectrum.* Absorption bands for the electron transitions of octahedral nickel(II) are usually very weak, because they are symmetry forbidden.[22] The case of tetrahedral complexes is quite different due to the fact that the transitions are no longer symmetry forbidden (ε max $10^2 - 10^3$), and the main visible bands move to longer wavelengths.

Table 1 contains observed wavelengths and extinction coefficients for a solution of 1M ascorbic acid with 0.1 M Ni^{2+} at pH 7.5 (in which, according to Fig. 2b, approximately all Ni^{2+} is in the $NiAsc^0$ complex) and the same data, reported earlier for the octahedral $Ni(H_2O)_6^{2+}$ complex.[23] The extinction coefficients have thus been calculated under the assumption that the concentration of the $NiAsc^0$ complex is 0.1 M. For reasons discussed above, it is clear that these values should be regarded as approximations. The nickel(II)-ascorbate spectrum displays a great similarity to that of $Ni(H_2O)_6^{2+}$ but the bands are somewhat stronger and broader, however still very weak. These observations are in agreement with a pseudooctahedral complex, with symmetry C_{2v} or lower.[24] Secondly, it can also be noted that all the bands are displaced to longer wavelengths, which shows that the ligands in the $NiAsc^0$ complex provide a weaker ligand field (the average environmental rule[25]) as compared to $Ni(H_2O)_6^{2+}$.[26]

Table 1. Observed $d-d$ transition wavelengths and extinction coefficients for 0.1 M Ni^{2+} with 1 M ascorbic acid at pH 7.5 and the corresponding data for the $Ni(H_2O)_6^{2+}$ complex.

$NiAsc^0$		$Ni(H_2O)_6^{2+}$	
λ	ε	λ	ε
–[a]	–	395	5.2
690	4.1	658	1.9
740	3.8	724	2.1
1210	3.9	1176	2.0

[a] Not measurable due to overlap from ascorbic acid transition.

^{13}C *NMR data.* Nuclear spin-lattice relaxation time (T_1) measurements have been widely used to study the structure of paramagnetic metal complexes in solution.[27] The paramagnetic contribution T_{1P}^{-1}, to the observed spin-lattice relaxation rate of a nucleus in a paramagnetic complex arises primarily from a dipolar interaction between the nuclear spin and the unpaired electron spin. If the electron spin is treated as point dipole centred on the metal atom[28,29] and if the fast exchange condition hold,[30] then T_{1P}^{-1} is given by eqn. (3) (neglecting the outer sphere mechanism); K is a

$$T_{1P}^{-1} = KP_M/r_i^6 f(\tau_C) \qquad (3)$$

proportionality constant common for all nuclei of the same kind in the ligand. P_M is the ratio of bound to free ligands, r_i is the distance between the metal ion and the nucleus i under consideration and $f(\tau_C)$ is a function of the rate of modulation of the

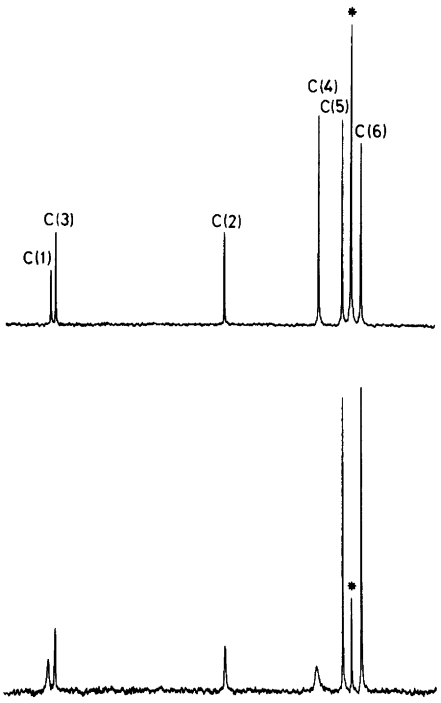

Fig. 3. Carbon-13 NMR spectra at 2.35 Tesla of 0.8 M solutions of ascorbic acid in water at pH 8.5 and 317 K, with *p*-dioxane as external standard (marked with an asterisk). The solution corresponding to the lower spectrum contains, in addition, 2.5×10^{-3} M Ni^{2+}.

electron–nuclear dipolar interaction. For one given complex, P_M and $f(\tau_C)$ remain constant. The different carbon-13 T_{1P}^{-1} in the ligand can therefore provide a measure of relative distances to the paramagnetic center. In addition to the effect on T_1, the unpaired electron spin of the metal ion also affects the spin–spin relaxation rate T_2^{-1} which is proportional to the width of the observed line, and the chemical shift of the nucleus under consideration.

Fig. 3 shows the spectrum of 0.8 M ascorbic acid at pH 8.5 and 317 K, and the corresponding spectrum of the same solution containing in addition 2.5×10^{-3} M Ni^{2+}.

The paramagnetic effect on the spin–spin relaxation times T_2 of the carbon nuclei, manifested in an increased linewidth, is demonstrated in the lower spectrum. It is noteworthy that the effect is largest for carbons C(4) and C(1). This observation can be explained with help of the paramagnetic shifts

Table 2. Spin-lattice relaxation times (T_1) and shifts (δ) for 0.8 M ascorbic acid at pH 8.5 and 317 K.

Carbon atom	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
T_1(s)	63.7	24.7	26.0	1.96	1.86	1.19
δ(ppm)[a]	178.1	114.1	176.2	79.3	70.6	63.7

[a] Calculated with $\delta_{TMS} - \delta_{dioxan} = 67.4$ ppm.

given in Table 3. The paramagnetic shift, which is proportional to the electron–nuclear hyperfine coupling constant,[27c] is considerably larger for carbons C(4) and C(1). This implies that at least for these two carbons, the paramagnetic linewidth is dominated by the scalar interaction.[27c] T_2 measurements can thus not provide any information on the structure of the nickel(II)-ascorbate complex. To get structural information one must therefore turn to T_1 measurements.

The carbon-13 spin lattice relaxation times and chemical shifts for the diamagnetic sample of 0.8 M ascorbic acid at pH 8.5 and 317 K are listed in Table 2. The relaxation times for the non-protonated carbons are long, which makes them very suitable as probes for interactions with a paramagnetic metal ion. The chemical shifts agree well with the data reported by Berger.[14]

In Table 3, T_{1P}^{-1}, the paramagnetic contribution to the observed spin-lattice relaxation rates and the paramagnetic shifts, $\Delta\nu_p$, of the six carbons in ascorbic acid for the sample corresponding to the lower spectrum in Fig. 3, are given. According to the equilibrium data, at the concentrations and pH of the sample treated in Table 3, practically all nickelous ions form the complex $NiAsc^0$. Furthermore, measurements of the paramagnetic shifts as a function of temperature, showed that above 313 K the conditions of fast exchange hold (this fact is the motivation for the choice of temperature in the

Table 3. Paramagnetic spin-lattice relaxation rates (T_{1P}^{-1}) and shifts ($\Delta\nu_P$) for 0.8 M ascorbic acid with 2.5 mM Ni^{2+} at pH 8.5 and 317 K.

Carbon atom	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
T_{1P}^{-1}(s^{-1})	0.83	1.47	1.41	0.99	0.18	0.14
$\Delta\nu_P$(s^{-1})	13	−10	−5	21	−1	0

^{13}C NMR measurements). Consequently the T_{1P}^{-1} values can be interpreted in terms of eqn. (1). The fact that the quotient $T_{1P,i}^{-1}/T_{1P,j}^{-1}$ is nearly unity for carbons C(1)/C(4) and C(2)/C(3) clearly shows that nickel(II) binds in a symmetric manner to oxygens 2 and 3 of ascorbate.

It should, however, be noted that the relatively fast relaxation rates for carbons C(4) and C(1) as compared to C(2) and C(3), together with the large paramagnetic shifts for these carbons (which indicates that there is a considerable amount of delocalized electron spin density at the positions of these two carbons), imply that the point-dipole approximation inherent in eqn. (3) may not be strictly valid.[31,32]

Lastly it may also be noted that any presence of the complex $Ni(Asc)_2^{2-}$ would not alter the conclusions drawn from the spectroscopic measurements. However, it is not possible from the optical and NMR data alone to conclude the number of ascorbate molecules bound to Ni(II).

CONCLUSIONS

Based on the equilibrium measurements, it was concluded that the neutral complex $NiAsc^0$ was predominant in the solutions which were characterized by electron spectrum measurements and ^{13}C NMR.

The nickel(II) – ascorbate *d-d* transition spectrum showed that the complex has a pseudooctahedral symmetry while the ^{13}C relaxation data gave clear evidence for a bidental chelated complex with binding through oxygens 2 and 3. We thus propose the structure in Fig. 4 for the $NiAsc^0$ complex in aqueous solution.

The present study clearly shows the usefulness of a combination of potentiometric measurements, optical spectroscopy and ^{13}C NMR spectroscopy for studying paramagnetic metal complexes in aqueous solution. In order to characterize more completely the interaction of L-ascorbic acid with Ni^{2+}, the kinetics of the ligand exchange process ought to be studied with variable temperature ^{13}C NMR.

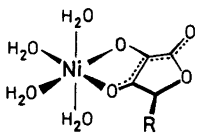

Fig. 4. Proposed structure of the $NiAsc^0$ complex in aqueous solution.

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