# Absorption and Circular Dichroic Spectral Studies on Complexes of Nickel(II) with α-Amino Acids[1]

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**Abstract:** A characteristic circular dichroic spectrum is demonstrated for the 1:1 chelate of Ni(II) formed with the L-amino acids, alanine, serine, valine, arginine, ornithine, and glutamic acid. This chelate, which involves the carboxyl and the α-amino groups, becomes detectable between pH 3 and 4, as acidity is decreased. With higher ratios of amino acid to Ni(II), evidence is seen for formation of a bis chelate, starting at about pH 7. No sign is seen of a tris chelate. The 1:1 chelates of asparagine or aspartic acid with Ni(II) have a dichroic spectrum different from the one above, which is attributed to terdentate chelation. Solutions of asparagine and Ni(II) with ratios of ligand to metal ion 2:1 or greater apparently revert to α-amino chelates like the normal amino acids, above pH 8–9.

Prior studies[2-4] of the interaction of rare earth ions with optically active ligands have shown the power of combined absorption and circular dichroic (CD) spectral data to illuminate both the chemistry and spectroscopy of these systems. This report extends such studies to the interaction of some α-amino acids with a 3d transition element ion, Ni(II).

## Experimental Section

Solutions for spectral observation were prepared as follows. The required weights of amino acid were dissolved in a minimal volume of water, 1 ml of stock 1 *F* $NiCl_2$ was added, and this was followed by the amount of 5 *F* or concentrated $NH_3$ solution required to yield the desired pH. The volume was then generally made to 6 ml, so that the final Ni(II) concentration was about 0.16 *F*. To follow the same solution with change of pH, after recording the initial spectra, additional drops of concentrated or 5 *F* $NH_3$ were added. In some series, NaOH was added before the solutions were adjusted to volume, to give precise stoichiometries. The concentration region used was chosen to facilitate forming and measuring complexes with weak dichroism against a fairly strong absorption background. This concentration range in turn imposed some solubility limitations on the amino acid systems and solution compositions that could be investigated.

The following L-α-amino acids were used as supplied by Nutritional Biochemical Corp.: alanine, valine, serine, arginine (both free base and monohydrochloride), ornithine monohydrochloride, asparagine, aspartic acid, and glutamic acid.

The Durrum–Jasco ORD/UV/CD-5 instrument was used to record the CD and absorption spectra, as in prior investigations.[2-4] For the above solutions, a 10-mm cell was generally used for the CD measurement, and a 5-mm cell (sometimes 1 mm was required) for the absorption measurements.

A few absorption spectral observations at wavelengths longer than 700 nm were made with the Cary Model 14 spectrophotometer.

## Results

Aqueous Ni(II) shows absorption maxima at 393 and 656 nm in the spectral region under investigation (*ca.* 250–700 nm). The sharper absorption at 393 nm is some 2.9 times as intense as the broader one with maximum at 656 nm. With increasing concentrations of ammonia, both peaks intensify and move to shorter wavelength, so that at pH 9 they approximate 373 and 613 nm, with intensity ratio about 1.4, and at pH 11, 365 and 597 nm, with a slightly higher intensity ratio, about 1.5. The spectral effects of chelation of the nickel by the amino acids were qualitatively and semiquantitatively the same as those for substitution of coordinated water by ammonia, though there might be differences in the absorptivity values, which were not investigated.

In general, in the presence of the amino acids alanine, valine, serine, ornithine, arginine, or glutamic acid, in 1 to 4:1 mole ratio, there is no significant change of the Ni(II) spectrum at pH 3 from that in the absence of amino acid, and there is no significant CD detectable at the absorptions. At pH 4 a shift in absorption shows, in the direction indicated above, which progresses further with increase of pH, the change continuing into the alkaline region. In the pH 4–6 range, and sometimes considerably higher, all the systems show what is, aside from minor quantitative differences, essentially the same CD spectral pattern (Figure 1). The intensity, for a given amino acid and stoichiometry, might increase somewhat with the increase in pH, particularly in the initial stages.

With the higher ratios of ligand to Ni(II), at pH values 7 and higher, a change in the CD pattern may take place. With 3:1 or higher arginine–Ni systems, between pH 7 and 8 there is an abrupt and almost complete conversion to the second CD spectrum (Figure 1). At the 2:1 ratio the change is initiated at pH 8, and though strengthened is still only partial at pH 9, while the 1:1 system at pH 9 shows no sign of the second pattern. Ornithine at 1:1 ratio and pH 9 does show that some of the second form is present, but only at 3:1 ratio does it come to dominate the spectrum from pH 8 up. Serine shows an influence of the second form in alkaline solution of high pH even at the 1:1 ratio, but even at 4:1 ratio the conversion is not as complete as with the basic amino acids at lower ratio. Alanine at pH 9, in 3:1 or 4:1 ratio, shows the effect strongly, the conversion with 4:1 ratio apparently being further than for the serine. However, alanine and valine in 1:1 ratio fail to show any signs of the second form. This second spectrum, when free of the first, probably consists of a negative band slightly displaced from the short-wavelength absorption peak, and a second, somewhat more intense one also slightly displaced from the longer wavelength peak, with indications of additional negative CD contribution probably centered at wavelengths longer than 700 nm.

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) L. I. Katzin, *Inorg. Chem.*, 7, 1183 (1968).

(3) L. I. Katzin and E. Gulyas, *ibid.*, 7, 2442 (1968).

(4) L. I. Katzin, *ibid.*, 8, 1649 (1969).

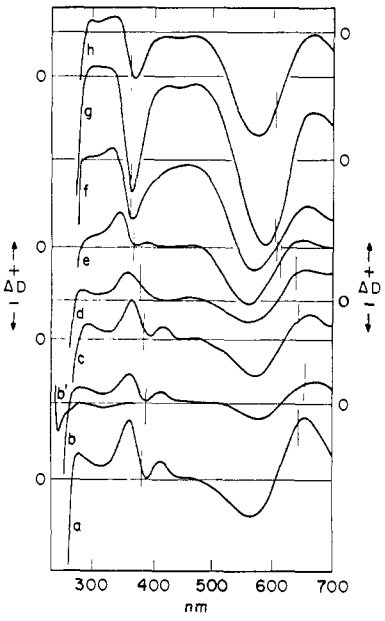


Figure 1. CD spectra of Ni(II)–amino acid systems. Vertical bars indicate positions of maxima in the absorption spectra of the given solutions: (b′, b, a) valine–Ni(II), 1:1, at pH 3, 5, and 6, respectively; (c) arginine, 1:1, pH 6; (d) glutamic acid, 1:1, pH 6; (e) alanine, 2:1, pH 8; (f) alanine, 3:1, pH 8; (g) arginine, 3:1, pH 7; and (h) ornithine, 3:1, pH 7.

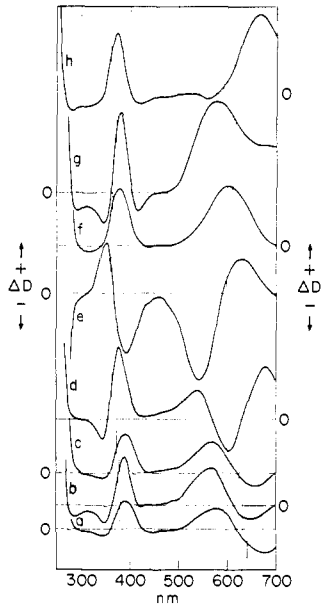


Figure 2. CD spectra of Ni(II) with asparagine and aspartic acid. Vertical bars indicate positions of maxima in the absorption spectra of the given solutions: (a) asparagine–Ni(II), 1:1, pH 4; (b) aspartic acid, 1:1, pH 6; (c) asparagine, 1:1, pH 8; (d) aspartic acid, 1:1, pH 9; (e) asparagine, 3:1, pH 8; (f) asparagine, 2:1, pH 7; (g) aspartic acid, 2:1, pH 6; and (h) aspartic acid, pH 10.

Asparagine–Ni(II) at 1:1 ratio shows a slight CD at pH 3, which at about pH 6 is nearly at full intensity, changing little more to pH 8. However, this CD pattern (see Figure 2) is sharply different from the spectral patterns above, in sign and intensity relations. The 2:1 system in the pH range 5–8 gives a pattern that is obviously different again, in that the positive–negative CD pair in the 600-nm region for the 1:1 system becomes a single broad positive peak. About pH 9 a change commences, with the CD coming to resemble the high-ratio CD for many of the other amino acids in the same pH region. The same CD spectra are seen with 3:1 and 4:1 ratios of ligand to Ni(II), with the transitions taking place at slightly lower pH values, and with some increase in the CD intensity. The absorption spectra show no obvious differences from those for comparable solutions with the other acids.

Aspartic acid–Ni(II) solutions in 1:1 ratio, pH 4–6, show CD spectra equivalent to those of the corresponding systems with asparagine (Figure 2). At higher pH, particularly pH 9 and above, in the presence of $NH_3$, the movement of the absorption peak to shorter wavelength, bringing more of the CD pattern below the 700-nm instrumental limit, shows that there are three CD components in the 600-nm region absorption. (The 1:1 asparagine–Ni system shows the same phenomenon.) With 2:1 ratio, in the interval pH 4–8, the CD in the 600-nm region becomes a single broad positive peak, as in the case of asparagine. The CD in the 400-nm region resembles more an intensification rather than a definite alteration of the pattern for the 1:1 system; the negative component now visible at 410–415 nm probably is present weakly in the 1:1 pattern also. Higher pH values seem only to shift the long-wavelength positive peak to gradually longer wavelengths, with perhaps a small increase in intensity. Increase of ligand ratio to 3:1 produces no essential changes, except at pH 10.

For 0.04 *M* 1:1 valine–Ni(II) complex, at pH 6, which may be slightly stronger spectrally than some of the other complexes, the apparent $|\Delta\epsilon|$ values for the 360-, 570-, and 660-nm CD extrema are $5.2 \times 10^{-3}$, $1.7 \times 10^{-3}$, and $3 \times 10^{-3}$, respectively, while the apparent $|\Delta\epsilon/\epsilon|$ values are $0.6 \times 10^{-3}$, $1.2 \times 10^{-3}$, and $0.9 \times 10^{-3}$, for the same components. The $\Delta\epsilon/\epsilon$ values are comparable with those for most of the spectral components of the Pr(III)–amino acid complexes,[3] but both are lower than the values for some components of the Eu(III) complex spectrum.[4]

## Discussion

The absorption spectrum of Ni(II) in the octahedral environment of MgO has been analyzed by Low.[5] Later work in the solid state has added some fine structural details, but the basic picture for octahedral Ni(II) remains a $\Gamma_2(^3F)$ ground state, with upper levels $\Gamma_5(^3F)$ at 8600 $cm^{-1}$, $\Gamma_3(^1D)$ at 13,400 $cm^{-1}$ (746 nm), $\Gamma_4(^3F)$ at 14,800 $cm^{-1}$ (676 nm), $\Gamma_5(^1D)$ at 21,550 $cm^{-1}$ (464 nm), $\Gamma_4(^3P)$ at 24,500 $cm^{-1}$ (408 nm), $\Gamma_1(^1G)$ at 25,950 $cm^{-1}$ (385 nm), $\Gamma_4(^1G)$ at 28,300 $cm^{-1}$ (353 nm), and a $\Gamma_3$, $\Gamma_5(^1G)$ doublet at 34,500 $cm^{-1}$ (290 nm). Application of this analysis to the absorption of aqueous $NiCl_2$ is direct (Figure 3), but for $Ni(H_2O)_6^{2+}$ the wavelength peak of the $\Gamma_4(^3F)$ is seen to be 656 nm, not 676 nm. Analysis of the absorption of the components in the 400-nm region, using the du Pont gaussian curve analyzer, indicates that some of these also are shifted to higher energy than for the MgO values. In the framework of the crystal-field approach, this corresponds to a slightly higher value of the parameter *Dq* for the aqueous solution than for MgO. An earlier theoretical interpreta-

(5) W. Low, *Phys. Rev.*, **109**, 257 (1958).

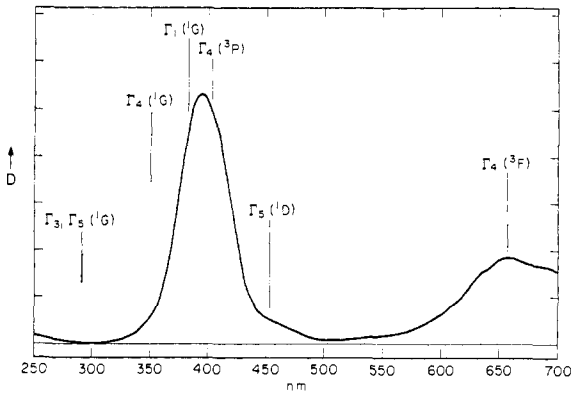


Figure 3. Absorption spectrum of $NiCl_2$ in water.

tion[6] of the solution spectra had suggested that for aqueous Ni(II) the upper level for the 650-nm transition should be labeled $\Gamma_3(^1D)$, and that the $\Gamma_4(^3F)$ upper state was represented by the 740-nm absorption. Only with higher values of *Dq*, as for the hexaammine or the "trisglycine" complexes of Ni(II),[6] would the order of the levels correspond with Low's for the MgO environment. Since, however, it has been shown that the $\Gamma_4(^3F)$ lies higher for MgO, and the value of *Dq* seems higher in the hexaquo complex, this order must hold for the aqueous solution also.

If one assumes that a 1:1 chelate corresponds to a primary environment for Ni(II) of $C_{2v}$ symmetry, the octahedral parent $\Gamma_4$ states should split[7] into two, a $\Gamma_3$ and a $\Gamma_4$. With the ground state being $\Gamma_2$, both of these should have magnetic moments, and therefore reasonably should be optically active. A $\Gamma_3$ upper state in octahedral symmetry should also split into two under $C_{2v}$, but only one component should have a magnetic moment. The $\Gamma_1$ level remains unchanged, and should also possess a magnetic moment.

At first glance, the CD pattern in the 400-nm region of the 1:1 complexes in Figure 1 suggests a single CD each for the transitions to the $\Gamma_1(^1G)$ and the $\Gamma_4(^3P)$ upper states. Closer inspection shows that the spacing between these two positive peaks is much too great (about 50 nm rather than 20–25 nm in the $NiAq_6^{2+}$ absorption spectrum). This, with the shape of the curves in the interval (*e.g.*, the almost flat long-wavelength side of the more intense CD), and the anomalously long wavelength of the smaller CD, suggests that one has a strong single CD of the $\Gamma_1$ state, while the $\Gamma_4$ state is split into two components, one with negative and one with positive CD. The appearance of the traces then reflects in part the superposition of the relatively weaker negative CD on the strong positive of the $\Gamma_1$ state. This would be consistent with the fact that the transition to the $\Gamma_4$ upper state in the 600-nm region also shows a positive–negative CD pair, with the same relationship in wavelength order.

Nothing can be said from these data about the spectrally weak $\Gamma_4(^1G)$ or $\Gamma_5(^1D)$ transitions since, as the $\Delta\epsilon/\epsilon$ ratio is not significantly greater than for the strong peaks, the dichroism cannot be identified. The breadth of the CD attributed to the $\Gamma_1(^1G)$ upper state might suggest a possible contribution from the neighboring $\Gamma_4(^1G)$, but the latter's position in water (about 350 nm) is moved to shorter wavelength in exact correspondence to the increase in *Dq* (*cf.* ref 5). It, however, conceivably could account for the apparent tendency of the CD between 300 and 350 nm to lie off the base line.

The entrance of a second chelating group is most likely to occur *trans* to the first, to give an effective $D_{2h}$ field around the Ni(II). For this configuration[7] the ground state becomes an A state, as do also the $\Gamma_1$ and $\Gamma_3$ states. The $\Gamma_4$ and $\Gamma_5$ octahedral states each give rise to three B states, the transitions to each of which will have a magnetic moment and will therefore be expected to show a dichroism. The CD patterns of the second kind, shown by the 3:1 solutions at pH 7 and 8 (Figure 1), can be interpreted as a mixture of a species with components having a single sign of CD, associated in wavelength with the original $\Gamma_4$ upper states, and some residual 1:1 species. No resolution of the CD components of the same sign is seen, so definitive relation to the expectation is not possible.

As observed, the CD that appears for 1:1 amino acid–Ni(II) systems in the pH 4 region intensifies and continues to high pH. There seems to be no reason to question its identification with a 1:1 chelate. The primary carboxyl p*K* for all of the amino acids used is less[8a] than 2.4, so the carboxylate is available for cation–anion interaction[8b] even at pH 3, though negligible chelate formation occurs until about pH 4. Since the acid ionization of the $-NH_3^+$ has a p*K* generally[8] about 9.6, the facts suggest that after initial carboxyl–metal ion interaction the Ni(II) forces deprotonation of the $-NH_3^+$ group to yield the chelate. Chemical and absorption spectral studies by others[9,10] are in accord with the assignment of the 1:1 composition to the complex in the region below pH 7.

The second CD pattern, which comes into significance at pH 7 and above, and with higher ligand–Ni(II) ratios, logically is to be ascribed to a bis chelate, as indicated above. As we have suggested, this probably is a *trans* or equatorial chelate. The higher pH of formation than for the 1:1 complex implies further that the proton-displacing influence of the Ni(II) toward a second $-NH_3^+$ group is greatly diminished after it is already involved in one chelate complex, so that geometry is not the only consideration. The 1:1 serine–Ni(II) system at high pH tends to show a little influence of the bis-chelate form, which may be ascribed to consequences of coordination competition from hydroxyl ions. Our results give no indication (see also ref 11) that tris chelate[6,12,13] may be expected to form under conditions readily attained. This would be particularly true if the disposition of the ligands in the bis chelate is equatorial, as we have discussed.

The CD spectral pattern for the 1:1 aspartic acid–Ni(II) system at pH 9, with three definite components in the 600-nm region, is highly suggestive. If the ligand were bidentate, whether with *cis* or *trans* attachment to the Ni(II), the symmetry would remain effectively $C_{2v}$, as for the acid-region chelates. Tridentate chelation,

(6) C. K. Jørgensen, *Acta Chem. Scand.*, 9, 1362 (1955).
(7) E. B. Wilson, Jr., J. C. Decius, and P. C. Cross, "Molecular Vibrations," McGraw-Hill Book Co., Inc., New York, N. Y., 1955.
(8) (a) D. M. Greenberg, "Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1951; (b) C. W. Childs and D. D. Perrin, *J. Chem. Soc.*, 1039 (1969).
(9) S. Pelletier, *J. Chim. Phys.*, 57, 287, 295, 301, 306 (1960).
(10) C. B. Monk, *Trans. Faraday Soc.*, 47, 297 (1951).
(11) J. H. Ritsma, G. A. Wiegers, and F. Jellinek, *Rec. Trav. Chim. Pays-Bas*, 84, 1577 (1965).
(12) G. R. Brubaker and D. H. Busch, *Inorg. Chem.*, 5, 2110 (1966).
(13) R. P. Martin and R. A. Paris, *Bull. Soc. Chim. Fr.*, 3170 (1964).

including two positions *trans* to each other, would not alter the symmetry. Tridentate chelation of three positions *cis* to each other, however, would leave only a plane of symmetry, through the coordinated amino group. Under this $C_s$ symmetry, the octahedral $\Gamma_4$ state will yield three components, transitions to all of which will have magnetic moments, and presumably therefore, optical activity. The model of aspartic acid is adaptable to coordination to three *cis* positions, but not so readily to the arrangement in which the two carboxyls are *trans*.

The inference of tridentate chelation is backed by chemical evidence. If 1 molar equiv of aspartic acid is added to a solution about 0.16 *M* in $NiCl_2$, 80–90% of it remains undissolved. Slow addition of 1 *N* NaOH gives a clear solution when 1 equiv of base has been added, and the pH is about 3.8. A weak CD is seen, but the absorption is essentially that of the aqueous Ni(II). With further addition of NaOH the CD intensifies. At 0.75 equiv of additional NaOH the pH is still not quite 5.0, and only with another 0.25 equiv of base does the pH pass the neutral point. The absorption also shifts during this neutralization. With 1:1 Ni(II)–asparagine, 0.75 equiv of NaOH is required to bring the mixture to about pH 5, and another 0.25 equiv to reach pH 7. The absorption spectral change is not distinguishable from that for the aspartic acid. For both of these systems, therefore, the removal of the zwitterion proton from the $-NH_3^+$ is an accompaniment of the chemical change producing the characteristic CD pattern, and the CD difference from the simple amino acids must be due to binding additionally the carboxylate or the amide group, respectively, of these four-carbon species. One could conceive of a bidentate aspartate complex using the amino group and the carboxyl $\beta$ to it, but a corresponding complex involving the amide and not the carboxylate seems most unlikely, while the CD spectral evidence indicates the aspartic acid and the asparagine complexes should correspond. Deprotonation of the amide groups apparently does not occur.

With higher ligand ratios than for the 1:1 systems, both asparagine and aspartic acid systems show new CD patterns which may be taken as characterizing 2:1 chelates (Figure 2). In the lower pH region, to neutrality, the two ligands give CD spectra differing only in detail, most noticeably in the 400-nm region. From pH 8–9 up, the asparagine–Ni(II) systems show a radical CD alteration, to a pattern essentially indistinguishable from the mixture of 1:1 and 2:1 patterns characteristic of the $\alpha$-chelate systems. The aspartic acid–Ni(II) spectra are not unaffected at corresponding pH values, but are more recognizably related to the CD spectra at the lower pH values.

Additional differentiation can be seen by following the sign of the strong CD below 250 nm, whose initial portion only is seen in the curves (Figures 1 and 2). In solutions of the amino acids without nickel present, this CD is positive through the whole pH range.[14] In the normal amino acid–Ni(II) systems, it is positive at pH 3, before any significant complex CD appears, with often a small dip at about 250 nm for high ligand ratios (see Figure 1b′), indicating a very small amount of chelate may have formed. From pH 4 on (*cf.* Figure 1b), when the complex CD becomes definitely recognizable, this short-wavelength CD is negative, a change thus correlated with bidentate chelation through the $\alpha$-amino group. For the 1:1 asparagine–Ni(II) system, this CD remains positive through the whole pH range. In the 2:1 system, it undergoes reversal at approximately pH 10, and in the 3:1 and 4:1 systems it is negative from as low as pH 8. None of the aspartic acid–Ni(II) systems shows the change in sign of the short-wavelength CD to negative.

The whole behavior is consistent with a rearrangement of the asparagine 2:1 complex to one exhibiting at least in part the characteristics of the normal $\alpha$-chelation. That the 2:1 complex, at pH below that for the change, is a bis chelate, is shown by a simple titration experiment. Two equivalents of NaOH is required to bring the 2:1 asparagine–Ni mixture to about pH 7. The equivalent rearrangement does not take place with the 1:1 complex even at pH 9.75, implying that the amide group competes successfully with only one ligand against that concentration of hydroxide. In the 2:1 ratio systems, from pH 9 up, and in the higher ratio systems from pH 8 up, one must conclude that some displacement occurs, but it cannot be said if it is only one amide group, or one on each ligand. The corresponding carboxyl of aspartic acid, which is negatively charged, is apparently sufficiently strongly held to prevent a corresponding displacment.

In the case of the basic amino acids with terminal amino groups, the dissociation pH of the terminal $-NH_3^+$ group is so high ($pK = 10.5$–$11$)[8] that additional coordination of this group does not come into consideration below (say) pH 9, and we have seen no indications for this in CD spectral changes. It has been deduced,[12] on the basis of numerical absorption spectral parameters, and invoking Jørgensen's average-environment approximation,[15] that a basic amino acid may be held simply through the two amino groups, with the carboxyl released. This is contrary to our data on the basic acids, and to the implications of the aspartic acid–asparagine systems.

(14) L. I. Katzin and E. Gulyas, *J. Amer. Chem. Soc.*, **90**, 247 (1968).
(15) C. K. Jørgensen, *Acta Chem. Scand.*, **10**, 887 (1956).