ELECTROCHEMICAL INVESTIGATIONS OF THE NICKEL(II)–PENICILL-AMINE SYSTEM. INERT AND LABILE COMPLEXES DETECTED BY POLAROGRAPHY

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The effect of penicillamine on nickel diffusion current demonstrates that NiL_2^{2-} is a kinetically inert complex whereas NiL is labile ($L^{2-} = (CH_3)_2C(S^-)CH(NH_2)COO^-$). The difference in kinetic stability between the analogous bis-ligand complexes of Ni(II) with either penicillamine or cysteine is tentatively explained taking into account their structures. It is suggested that nickel detoxication by chelate ligands depends on the inert character rather than on the thermodynamic stability of the relevant nickel complex.

Key words: Amino acids; Chelates; Penicillamine; Nickel complexes; Nickel toxicology; Electrochemistry.

D-Penicillamine (Pen), $(CH_3)_2C(SH)CH(NH_2)COOH$, is the main product of penicillin decomposition[1]. It forms very stable complexes with various transition metal ions[2] and this property is employed for the drug treatment of the Wilson disease, heavy metal intoxication, rheumatoid arthritis and cystinuria, where, however, serious side effects could occur owing to complexing capacity[3].

The interest in the complexes of Ni^{2+} with Pen has several reasons. Apparently such complexes are involved in the mechanism of nickel(II) detoxication by Pen[4]. On the other hand, the electrochemistry of nickel complexes with α-aminothiols could offer useful insights on the function of nickel center in hydrogenases[5,6]. In this regard, cysteine (Cys) and its derivatives in particular were employed in previous investigations[5] owing to the occurrence of this amino acid in natural proteins. As the number of Pen-based complexes is limited by the steric effect of additional methyl groups, a much more reliable description of the complex equilibria in the Ni^{2+}–Pen system is avail-

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able[7–15]. It allows a more accurate interpretation of electrochemical data. Finally, nickel reduction catalyzed by Pen was recently investigated by cathodic stripping voltammetry leading to a sensitive method for the determination of Pen at concentrations down to 10^{-9} mol l^{-1} (ref.[16]). Further electrochemical investigations in the Ni^{2+}–Pen system bring theoretical ground to this method.

   This paper decribes the influence of Pen complexation on the nickel diffusion current in connection with the kinetic stability of relevant complexes. Investigation of other electrochemical processes occurring in this system is under progress and will be published in forthcoming papers.

EXPERIMENTAL

The DC and AC polarographic measurements were performed in a thermostated cell at 25 ±0.1 °C by means of a Radelkis OH-105 polarograph. The dropping mercury electrode was characterized by the drop-time of 4.94 s; flow-rate of 1.509 mg s^{-1} in 1 M KCl at 0.00 V *vs* SCE and by the mercury column height of 66.2 cm. The reported potential values refer to the SCE. Solution was deoxygenated by bubbling of pure hydrogen.

   The reagents used NiCl$_2$ (Riedel de Haen), D-penicillamine (Fluka) and other chemicals were of analytical grade and were used without further purification. The stock solution of 0.05 M NiCl$_2$ was standardized by amperometric titration with EDTA. Fresh Pen solutions were prepared daily.

   In most of the experiments the supporting electrolyte was a buffer system prepared from Na$_2$HPO$_4$·12 H$_2$O and CH$_3$COONa·3 H$_2$O (0.024 mol l^{-1} of each in the test solution) with proper additions of 1 M perchloric acid according to ref.[17]. A borax buffer pH > 8 was prepared by mixing appropriate volumes of 0.05 M borax and 0.1 M hydrochloric acid.

RESULTS

Electrode Processes in the Ni(II)–Pen System

According to curve *1* in Fig. 1, the reduction of nickel ion in the phosphate–acetate buffer is irreversible in the region of the wave A with a half-wave potential of –1.03 V and the apparent transfer coefficient of 0.35. Pen in the absence of Ni^{2+} gives only the anodic wave B (Fig. 1, curve *2*) attributed to the formation of sparingly soluble mercury thiolate[18]. In the presence of Ni^{2+} (curve *3*) the wave shifts by about 0.05 V towards more positive potentials and at the same time its limiting current decreases. These effects are in an agreement with the observation of anodic oxidation of mercury to insoluble compounds[19] and are ascribed to the decrease in the concentration of free Pen due to the formation of Ni^{2+}–Pen complexes. Additionally, the catalytic nickel prewave (C) and the catalytic hydrogen prewave (D) are also formed. The assignment of waves C and D is made by the analogy with Cys under similar conditions[17,20–23]. In contrast to the Cys system however, the limiting current of nickel diffusion wave (A) on curve *3* becomes lower compared to the value recorded in the absence of the ligand (curve *1*).

It is obvious that the effects illustrated by curve *3* are the consequences of nickel complexation by Pen. Straightforward correlation between the electrochemical data and the complexes occurrence can be made by means of the wave A.

The Effect of Pen Concentration

The effect of the gradual increase in Pen concentration is depicted in Fig. 2. Accordingly, the rise in Pen concentration induces the depression of the nickel diffusion current (I_{Ni}) until its complete decay in the presence of an excess of ligand (curve *5*). This effect cannot be attributed to the inhibition by Pen adsorption. Neither electrocapillary curves or alternating current data indicated significant changes in the double layer structure in presence of Pen at potentials negative from –1.00 V. Also, the conversion of nickel ion into a species reducible in the range of the prewave C cannot be responsible for the depression of Ni^{2+} current either. There are no relevant correlations between the prewave and main wave currents and, in addition to it, the catalytic prewave also vanishes in presence of an excess of Pen (Fig. 2). Finally, there are no reasons for ascribing the above effect to the shift of nickel reduction in the region of the wave D which shows all the characteristics of the catalytic hydrogen prewave[17,20–23]. Like the analogous wave produced by Cys (ref.[17]), the wave D occurs in a limited pH range around 7 and has the shape of the irreversible polarographic wave with $E_{1/2}$ of about –1.22 V and
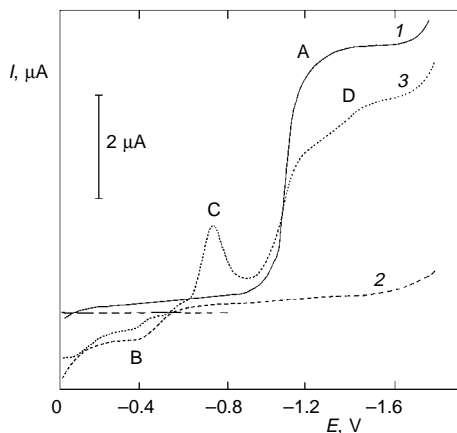

FIG. 1

Typical polarograms recorded in the Ni^{2+}–Pen system. Phosphate–acetate buffer pH 6.64. *1* 1 mM Ni^{2+}; *2* 0.7 mM Pen; *3* 1 mM Ni^{2+} and 0.7 mM Pen. A nickel diffusion wave; B anodic wave of Pen; C catalytic nickel prewave; D catalytic hydrogen prewave
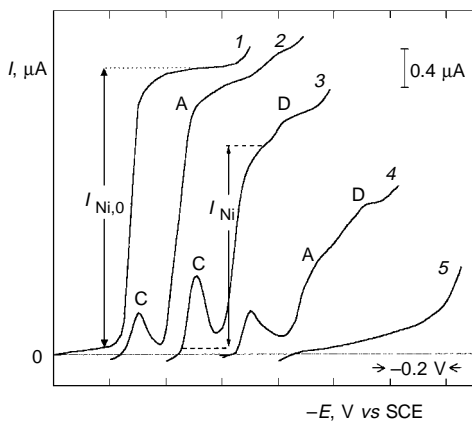
FIG. 2

Effect of Pen concentration on the nickel waves at pH 6.52 in the phosphate–acetate buffer and 0.6 mM Ni^{2+}. Concentration of Pen in mmol l^{-1}: *1* 0; *2* 0.12; *3* 0.44; *4* 0.87; *5* 1.4. Initial potential –0.400 V

αn = 0.45. On the other hand, the wave D does not appear in presence of an excess of Pen (Fig. 2, curve *5*) proving that it is not due to the simple reduction of a nickel complex.

It is therefore obvious that a non-reducible nickel complex is formed in presence of Pen. The remaining reducible species are reduced either in the region of the wave A or C. Doubtless, the species reduced in the region of the wave A in presence of Pen is still the hydrated nickel ion, as follows from the value of the half wave potential. Further investigation in this paper aims to identify the non-reducible species and attempts to provide an explanation for their inert character.

The Nature of the Rate-Determining Step in the Region of Wave A

Since several complexes are present in the system under investigation, it is important to assess whether chemical equilibria are disturbed by the electrochemical reaction occurring in the region of the wave A. To this end, the effect of the temperature and the mercury column pressure on the limiting current of the wave A have been studied under various conditions.

The temperature coefficient, defined in ref.[24] as $\omega = 100(\Delta I_{Ni}/I_{Ni}\Delta T)$ was found to be 1.6% K^{-1} in the absence of Pen which is in accord with the typical value for diffusion currents. The temperature coefficient determined at various Pen concentrations $(2–5 . 10^{-4}$ mol $l^{-1})$ in the temperature region of 15–40 °C varies between 1.4 and 1.5% K^{-1}, indicating the diffusion control of the wave A even in the presence of the ligand. The same conclusion results from the study of effects of the mercury pressure (column height, h) at various nickel and Pen concentrations. Under all the investigated conditions the I_{Ni} *vs* $h^{1/2}$ plot is represented by straight lines crossing the origin of both axes. The slope values (in μA $cm^{-1/2}$) at pH 6.52 for various nickel and Pen concentrations (in mmol l^{-1}), respectively, are as follows: 0.073 (0.12; 0); 0.046 (0.12; 0.10); 0.310 (0.65; 0.50); 0.057 (0.56; 1.00). Taking into account the above results, the wave A recorded in the presence of Pen is further approached as a typical diffusion wave and the experimental data are analysed in terms of the Ilkovi equation.

The above results demonstrate that the non-reducible nickel complex is also chemically inert. Indeed, although the reduction of free nickel ion in the region of the wave A does disturb the complexation equilibria, this wave does not show any characteristic that could be assigned to the conversion of the non-reducible form into the reducible one.

Composition of the Non-Reducible Complex

In order to find the metal/ligand ratio in the non-reducible complex, the effect of Pen concentration on the limiting current of wave A was studied in both the phosphate–acetate (Fig. 3) and borax (Fig. 4) buffers at several pH values and various nickel concentra-

tions (c_{Ni}). Under all the investigated conditions, the limiting current decreases linearly with growing Pen concentration as a consequence of gradual conversion of nickel into the non-reducible form. Intercepts of lines in Figs 3 and 4 with the abscissa axis give the Pen concentration required for the completion of the reaction ($c_{Pen,0}$). The ratio c_{Pen0}/c_{Ni} gives the ligand/metal ratio in the non-reducible complex. The values of the $c_{Pen,0}/c_{Ni}$ ratio determined from the plots in Fig. 3 are as follows: 2.01 (curve *1*), 2.08 (curve *2*) and 2.10 (curve *3*). Analogously, the following values results from Fig. 4: 2.12 (curve *1*), 2.02 (curve *2*) and 2.05 (curve *3*).

These results indicated that the ligand/metal ratio in the non-reducible species is 2/1, in accord with the stoichiometry of the NiL_2^{2-} complex previously detected by other methods[7,8,10–15] (L^{2-} = $(CH_3)_2C(S^-)CH(NH_2)COO^-$). However, there is not a convincing proof about whether or not NiL, which is a minority species, also takes part in the electrode process of the wave A. The next section present some additional tests aiming to elucidate this question.

FIG. 3
Effect of Pen concentration on nickel diffusion current in the phosphate–acetate buffer at pH 6.52. Concentration of Ni^{2+} in mmol l^{-1}: *1* 0.59; *2* 0.57; *3* 0.20
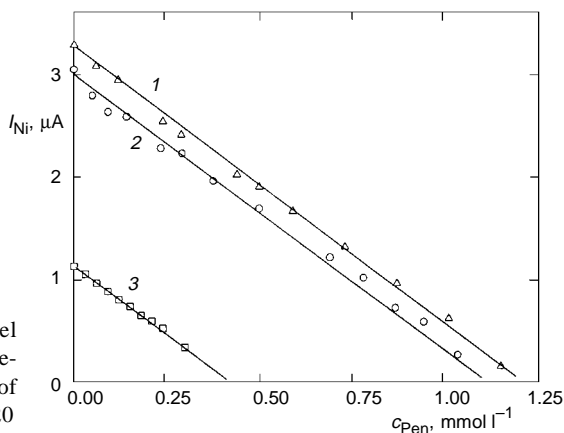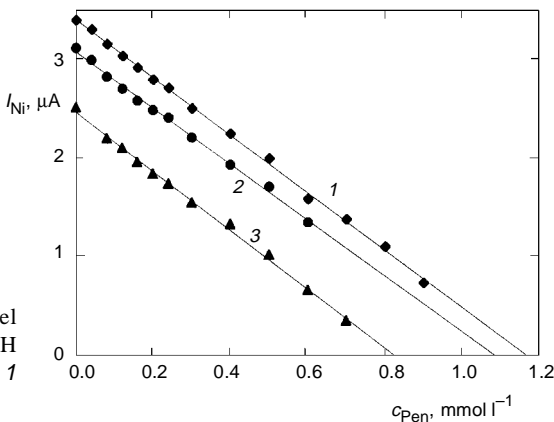

FIG. 4
Effect of Pen concentration on nickel diffusion current in the borax buffer; pH and Ni^{2+} concentration in mmol l^{-1}: *1* 8.00, 0.54; *2* 8.10, 0.4; *3* 8.50, 0.54

Nickel Species Distribution; Effects on Nickel Diffusion Current

In the pH range covered in this work, the carboxyl group of Pen is ionized ($pK_1 < 2.5$, ref.[8]) and the subsequent protonation equilibria involve four different species. Although the values of the microconstants are available[25], complex equilibria are usually dealt with by assuming that one single intermediate species (HL^-) occurs. This symbol stands for both forms with the labile proton bound to either sulfur or nitrogen[7-15]. The values of the ionization macroconstants measured by potentiometric titration are $pK_{a1} = 8.032$ and $pK_{a2} = 10.679$ (ref.[7]).

It is generally accepted that Pen forms only mononuclear species in contrary to Cys which gives polynuclear complexes with nickel[7,26]. It is in a consequence of the steric hindrance by the additional methyl groups. With one single exception[9], it is assumed that the complex species occuring in the Ni^{2+}–Pen system are NiL and NiL_2^{2-}. The formation constants for the above mentioned species reported by various authors[7,8,10-15] agree fairly well. The data reported by Perrin and Sayce[7] can be assumed as typical (log $\beta_{110} = 10.749$; log $\beta_{120} = 22.886$) and were used in the present work to calculate the species distribution under various conditions. A different model advanced in ref.[9] assumes the occurence of ML_2^{2-} and ML_2H^- species (log $\beta_{120} = 22.92$, log $\beta_{121} = 27.06$). It appears that the β_{120} constant has almost the same value in both models.

As shown in Fig. 5a, the rise in pH at constant Pen and Ni^{2+} concentrations leads to the decrease of the nickel diffusion current. This behaviour is obviously due to the shift of the complex equilibria towards the formation of the non-reducible NiL_2^{2-} species. In order to confirm this assumption the difference between the diffusion current in absence of the complexation ($I_{Ni,0}$) and the current measured at various pH values in
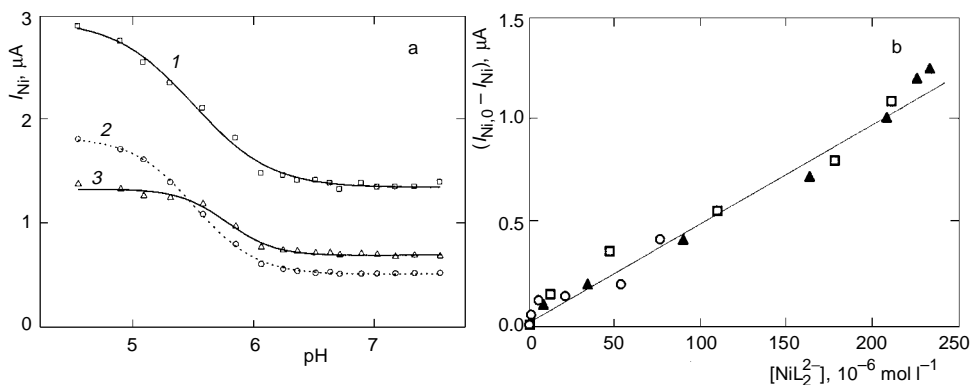

Fig. 5

Influence of pH on the Ni^{2+} diffusion current (I_{Ni}) in phosphate–acetate buffer at following Ni^{2+} and Pen concentrations in mmol l^{-1}: *1* 0.50, 0.50; *2* 0.31, 0.50; *3* 0.22, 0.20. a pH dependence of I_{Ni}; b dependence of ($I_{Ni,0} - I_{Ni}$) on NiL_2^{2-} concentration calculated from data plotted in Fig. 5a: ▲ curve *1*, ❑ curve *2*, ❍ curve *3*

presence of Pen (I_{Ni}) was plotted in Fig. 5b as a function of the NiL_2^{2-} concentration calculated from available equilibrium data[7]. Owing to the interference of the catalytic hydrogen evolution, the data at pH > 6 are less reliable and were omitted. Small systematic errors can also occur at higher pH due to the formation of complex species $Ni(HPO_4)$, with log K = 2.08 (ref.[27]). This is not a major drawback as the most important pH effect occurs at lower pH values.

Figure 5b shows that data obtained under different conditions (curves *1–3* in Fig. 5a) fit to the same straight line crossing the coordinate axis close to the origin. The residuals of the correlation in Fig. 5b are normally distributed (according to the Ryan–Joiner test) with the average value $-4 . 10^{-5}$ μA and the standard deviation 0.06. The slope of this line (4.92 μA per mmol l^{-1}) does not differ too much from the Ilkovi constant found in the absence of Pen (5.15 μA per mmol l^{-1}). No attempts was made to perform the same test in borax buffer due to uncertainties regarding the composition and stability of nickel–polyborate complexes[28].

An analogous treatment was used for the data collected at variable Ni^{2+} concentrations (Fig. 6). This shows that the current is negligibly small as long as the Ni^{2+}/Pen ratio does not exceed 2/1. The data in Fig. 6 were analysed by plotting the diffusion current (I_{Ni}) as a function of computed concentration of reducible species. It was found that the data on both curves in Fig. 6 fit the same straight line with the regression equation: $I_{Ni} = 0.038 + 5.33 ([Ni^{2+}] + [NiL])$ where I_{Ni} is in μA and the concentration in mmol l^{-1}. The residuals for this correlation are normally distributed (Ryan–Joiner test) with the average $4.2 . 10^{-5}$ μA and the standard deviation 0.05 μA (*i.e.* approximately 2.5% of the maximum current value in Fig. 6). The intercept of the above regression line is negligible, indicating the Ilkovi -type dependence of I_{Ni} on the concentration of reducible species and its slope, 5.33 μA $mmol^{-1}$ l of concentration, is very close to the value of the Ilkovi constant for free Ni^{2+} ion as measured in the

Fig. 6
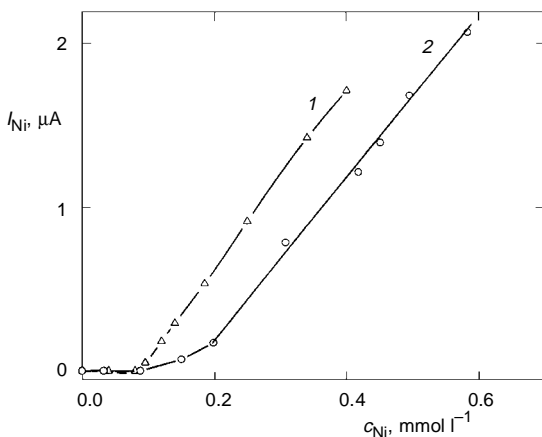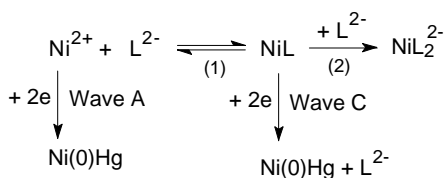Effect of Ni^{2+} concentration on the diffusion current I_{Ni} at pH 6.52 and Pen concentration in mmol l^{-1}: *1* 0.2; *2* 0.5

absence of Pen ($5.15\ \mu A\ mmol^{-1}\ l$). Alternatively, the plot I_{Ni} *vs* $[Ni^{2+}]$ is also linear but the slope value of 6.20 is anomalously high compared with the Ilkovi constant of Ni^{2+}.

The results of various tests presented in this section are summarized in Table I. Each kind of correlation included in this table is linear, with a negligible intercept. Consequently, the direct proportionality between I_{Ni} and concentrations of considered species is not sufficient to decide the proper model. However, a comparison of slopes with the Ilkovi constant for Ni^{2+} clearly demonstrates that the non-reducible species is NiL_2^{2-} whereas both Ni^{2+} and NiL species take part in the diffusion-driven process preceding the electron transfer in the region of the wave A.

DISCUSSION

As follows from the experimental results in this paper, the main chemical and electrochemical processes involving nickel ion in the presence of Pen can be represented by Scheme 1.

$$Ni^{2+} + L^{2-} \underset{(1)}{\overset{}{\rightleftharpoons}} NiL \xrightarrow[(2)]{+\ L^{2-}} NiL_2^{2-}$$

$$+ 2e \downarrow \text{Wave A} \qquad\qquad + 2e \downarrow \text{Wave C}$$

$$Ni(0)Hg \qquad\qquad\qquad Ni(0)Hg + L^{2-}$$

SCHEME 1

Accordingly, the species Ni^{2+} and NiL are in equilibrium whereas the back conversion of the species NiL_2^{2-} is extremely slow and the corresponding complexation step (2) can be considered as irreversible. Consequently, Pen displays a masking effect on the nickel ion. Conversely, the species NiL is electrochemically active as its direct reduction occurs in the region of the wave C by a catalytic mechanism. This involves the regeneration of the reactant by the reaction of the nickel ion with ligand molecules released after the electron uptake. The occurrence of the regeneration is proved by the fact that the wave C current is much higher than the diffusion current of the species NiL computed by means of the Ilkovic constant for Ni^{2+}, although this gives a slight overestimation of the result.

The irreversible wave A correspond to the reduction of free Ni^{2+} ion even in presence of Pen, as it follows from values of characteristic potentials. Data in Table I demonstrate that the transport of nickel towards the electrode surface occurs by the diffusion of both free nickel ion and the labile NiL complex. The last one dissociates inside the diffusion layer where the concentration of free nickel ion is brought below the equilibrium value due to the reduction process.

A comparison with the behaviour of the analogous complexes of Cys reveals a striking difference as far as the reactivity of the NiL_2^{2-} species is concerned. The masking

effect of Pen on the nickel ion is similar to that produced by EDTA despite the overwhelming difference in chemical structure. As the effect of Pen is not dependent on the buffer composition, it cannot be assigned to the occurrence of some mixed complexes. Conversely, under similar conditions Cys does not influence the limiting current of nickel ion although both the catalytic prewaves of nickel and hydrogen occur[17,20–23]. Similar behaviour was observed in the case of various cysteine analogues, for example selenocysteine[29,30], various cysteinyl-dipeptides[31,32] and cysteine ethyl ester[33]. An important effect of Cys on the nickel diffusion current was still noticed in the ammonia buffer[34]. There are, nevertheless, some peculiar features in this case. First, the nickel diffusion current is not completely suppressed even in the presence of a high Cys/Ni^{2+} concentration ratio (up to 10). Conversely, the amount of reducible nickel species corresponds to, at least, one half of the total amount of nickel in the solution. Second, it is not possible to find a well defined relationship between the Cys effect and the stoichiometry of complex species, although the data in ref.[34] suggest that the metal/ligand ratio in non-reducible species is 1/2. It is interesting that the decrease in the nickel diffusion current parallels the increase in the Brdicka wave current. Hence it can be suggested that this process is related to the occurrence of a nickel–Cys complex of the above mentioned ratio. However, the masking effect of Cys does not occur at pH < 8 and in absence of a secondary ligand originating from the buffer composition, such as ammonia.

The difference in reactivity between the analogous Pen and Cys NiL_2^{2-}-type complexes cannot be explained in terms of the thermodynamic stability because the difference between the formation constants is only about 2 logarithmic units (ref.[7]). A kinetic interpretation based on the differences in the structure of complexes is therefore attempted.

TABLE I
Testing of various hypothesis about the nature of the reducible and non-reducible complex species in the Ni^{2+}–Pen system. Slope is in μA per mmol l^{-1}

Hypothesis	Correlation	Slope	Source of data
	$I_{Ni,0}$ vs $[Ni^{2+}]$ (in absence of Pen)	5.15	independent measurements
1. Both Ni^{2+} and NiL are reduced	I_{Ni} vs $([Ni^{2+}] + [NiL])$	5.30	Fig. 7
2. Only Ni^{2+} ion is reduced	I_{Ni} vs $[Ni^{2+}]$ (in presence of Pen)	6.20	Fig. 7
3. NiL_2^{2-} is the single non-reducible species	$(I_{Ni,0} - I_{Ni})$ vs $[NiL_2^{2-}]$	4.92	Fig. 5
4. Both NiL_2^{2-} and NiL are non-reducible	$(I_{Ni,0} - I_N)$ vs $([NiL_2^{2-}] + [NiL])$	3.56	Fig. 5

The structures of Cys and Pen bis-ligand complexes with nickel(II) have been determined by X-ray diffraction[35–37]. Both of them have a square planar structure but, despite of the similarity of coordinating sites, there are still some important differences, as shown in Fig. 7. First, the two ligands are in the *cis* configuration in the Pen complex whereas the *trans* arrangement occurs in the Cys analogue. Second, the carboxyl groups in the Cys complex have an axial orientation relative to the coordination plane. Conversely, in the Pen complex carboxyl groups are assumed to be in an equatorial conformation. This is evidently due to the steric effect of the additional methyl groups in Pen. These substituents bring about a hydrophobic character to the complex molecule and, in addition, shift the hydrophilic carboxyl groups far from the coordination center. These structural characteristics allow the interpretation of the complexes reactivity in terms of the general rules for the substitution reaction at nickel square planar complexes[38]. As the decomposition of the complex starts with axial binding of a water molecule, this is promoted by a high local water activity in the case of Cys complex caused by carboxyl groups (Fig. 7a). This interpretation is supported by X-ray diffraction data evidencing the simultaneous presence of several water molecules and counter-cations (*e.g.* K^+) in contact with the carboxyl groups. On the contrary, the position of the carboxyl groups in the Pen complex (Fig. 7b) does not stimulate the approach of the water molecule to the nickel ion and the hydrophobic character of the methyl groups even makes this step more difficult.

During the next step the breaking of a nickel–nitrogen bond occurs rather than the cleavage of the stronger sulfur–nickel one. The subsequent breaking of the second nitrogen–nickel bond is thereafter facilitated by the *trans* effect in the case of Cys complex. *Trans* effect should be much less effective in the case of Pen because the sulfur ccordinand in the *trans* position forms a much stronger chemical bond as compared to nitrogen. Consequently, the splitting of the second coordinate bond may proceed much more slowly in the case of Pen complex.

It can be concluded that a combined effect of polar group orientation, coordinating group position and absence of steric hindrance makes $[Ni(Cys^-)_2]^{2-}$ less kinetically stable than its Pen analogue despite the small difference in thermodynamic stability.
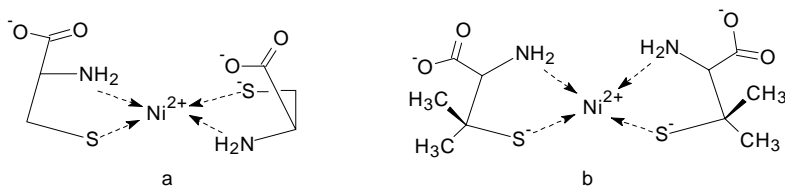

FIG. 7
Structure of bis-ligand Ni(II) complexes of Cys (a) and Pen (b). Adapted from refs[35–37]

This conclusion suggests that the kinetic factors could play a decisive role in the mechanism of nickel(II) detoxication by chelating agents explaining thus the lower detoxifying effect of Cys compared with Pen[4], despite of small difference in the stability constants. On the other hand, this also could explain strong detoxifying effect of polyamino carboxylic acids[4] which yield much weaker nickel complexes compared to both Cys and Pen. Kinetically inert nickel complex appears therefore as more efficient excretion form compared to the species that are thermodynamically stable but kinetically labile. This could be an attractive explanation for the paradoxical conclusion of a recent paper[39] stating that no definite relationship exists between the structure of the chelating agents and their ability to counteract the toxic effects of nickel.

The above interpretation could also have some relevance for the reactivity of nickel hydrogenase. It is assumed that dihydrogen splitting by this enzyme occurs *via* forming hydride ion intermediate bonded to the nickel ion[5,6]. As previously demonstrated, some subtle differences in vicinal group polarity and orientation could bring about important effects in nickel center reactivity.

Finally, it is worth noting the potential analytical applications of the inert NiL_2^{2-} complex for masking either Pen or Ni^{2+}.

REFERENCES

 1. Greenstein J. P., Winitz M.: *Chemistry of the Amino Acids*, Vol. 3, p. 2644. Wiley, New York 1961.
 2. Gergely A., Sovago I. in: *Metal Ions in Biological Systems* (H. Siegel, Ed.), Vol. 9, p. 77. Dekker, New York 1979.
 3. Hoppe A., Denneberg T., Frank A., Kagedals B., Petersson L. R.: *J. Vet. Pharmacol. Ther.* **1993**, *16*, 93.
 4. Jones M. M., Basinger M. A., Weaver A. D.: *J. Inorg. Nucl. Chem.* **1981**, *43*, 1705.
 5. Banica F. G.: *Bull. Soc. Chim. Fr.* **1991**, *128*, 697.
 6. Halcrow M. A., Christou G.: *Chem. Rev.* **1994**, *94*, 2421.
 7. Perrin D. D., Sayce J. G.: *J. Chem. Soc. A* **1968**, 53.
 8. Sugiura Y., Yokoyama A., Tanaka H.: *Chem. Pharm. Bull.* **1970**, *18*, 693.
 9. Sovago I., Gergely A., Harman B., Kiss T.: *J. Inorg. Nucl. Chem.* **1979**, *41*, 1629.
10. Laurie S. H., Prime D. H., Sarkar B.: *Can. J. Chem.* **1979**, *57*, 1411.
11. Hakkinen P., Lajunen K.: *Finn. Chem. Lett.* **1987**, *14*, 7.
12. Tiwari B. B., Singh R. K. P., Yadava K. L.: *J. Chromatogr.* **1991**, *542*, 537.
13. Nair M. S., Arsu P. T., Pillaian M. S., Natarajan C.: *J. Chem. Soc., Dalton Trans*. **1993**, 917.
14. Nair M. S., Arsu P. T., Pillaian M. S., Natarajan C.: *Transition Met. Chem. (London)* **1995**, *20*, 132.
15. Nair M. S., Arsu P. T., Mansoor S. S., Shenbagavalli P., Neelakantan M. A.: *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.* **1995**, *34*, 365.
16. Ion A., Banica F. G., Fogg A. G., Kozlowski H.: *Electroanalysis* **1996**, *8*, 40.
17. Banica F. G., Diacu E.: *Rev. Roum. Chim.* **1990**, *35*, 9.
18. Brezina M., Zuman P.: *Die Polarographie in der Medizin, Biochemie und Pharmazie*, p. 429. Akademische Verlagsgesellschaft, Leipzig 1956.
19. Heyrovsky J., Kuta J.: *Principles of Polarography*, p. 172. Academic Press, New York 1966.

20. Calusaru A., Banica F. G.: *J. Electroanal. Chem.* **1973**, *47*, 190; 554.
21. Banica F. G., Calusaru A.: *J. Electroanal. Chem.* **1983**, *145*, 389.
22. Banica F. G., Calusaru A.: *J. Electroanal. Chem.* **1983**, *158*, 47.
23. Banica F. G., Diacu E.: *Collect. Czech. Chem. Commun.* **1991**, *56*, 140.
24. Ref.[19], p. 88.
25. Wilson E. W. Jr., Martin R. M.: *Arch. Biochem. Biophys.* **1971**, *142*, 445.
26. Kozlowski H., Decock-Le Reverend B., Ficheux D., Loucheux C., Sovago I.: *J. Inorg. Biochem.* **1987**, *29*, 187.
27. Kotrly S., Sucha L.: *Handbook of Chemical Equilibria in Analytical Chemistry*, p. 118. Ellis Horwood, Chichester 1985.
28. Ref.[27], p. 111.
29. Calusaru A., Voicu V.: *J. Electroanal. Chem.* **1971**, *32*, 427.
30. Calusaru A., Voicu V.: *J. Electroanal. Chem.* **1973**, *43*, 257.
31. Banica F. G.: *J. Electroanal. Chem.* **1984**, *171*, 351.
32. Banica F. G.: *Talanta* **1985**, *32*, 1145.
33. Banica F. G., Florea M., Moraru M.: *J. Electroanal. Chem.* **1990**, *285*, 281.
34. Kuik M., Basinski A., Zalewska B.: *Rocz. Chem.* **1968**, *42*, 855.
35. Baidya N., Ndreu D., Olmstead M. M., Mascharak P. K.: *Inorg. Chem.* **1991**, *30*, 2448.
36. Baidya N., Olmstead M. M., Mascharak P. K.: *Inorg. Chem.* **1991**, *30*, 3967.
37. Muller A., Johannes K. U., Straube M., Krickemeyer E., Bogge H.: *Z. Anorg. Allg. Chem.* **1993**, *619*, 1037.
38. Basolo F., Pearson R. G.: *Mechanisms of Inorganic Reactions*, p. 417. Wiley, New York 1967.
39. Tandon S. K., Singh S., Jain V. K., Prasad S.: *Fundam. Appl. Toxicol.* **1996**, *31*, 141.