ELECTROCHEMICAL INVESTIGATIONS OF THE NICKEL(II)–PENICILLAMINE SYSTEM. 3. A STUDY OF THE CATALYTIC HYDROGEN PREWAVE IN CONNECTION WITH STRUCTURE OF NICKEL(II)–PENICILLAMINE COMPLEXES

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The catalytic hydrogen evolution on the dropping mercury electrode in the presence of Ni(II) and D-penicillamine (Pen) at pH around 6 yields a catalytic hydrogen prewave (CHP) with $E_{1/2}$ = –1.21 V *vs* SCE. This wave is similar to the CHP produced by selenocysteine and cysteine described previously. The occurrence of the CHP depends on the formation of the mono(D-penicillamine-*N*,*S*)nickel(II) complex whereas bis(D-penicillamine-*N*,*S*)nickel(II) complex is inert and has no influence on the CHP electrode process. Although the analogous bis(cysteine) complex is labile, there is strong evidence that it does not take part directly in the CHP process in the Ni(II)–cysteine system. The actual proton donor in the CHP electrode process is the hydronium ion and not the acid component of the buffer. A tentative reaction mechanism was formulated with emphasis on the state of the intermediate hydrogen species. The characteristic pH, nickel ion involvement and the structure of the ligand make the CHP process an attractive model for hydrogen redox reactions catalyzed by [NiFe] hydrogenase.

Key words: Electrocatalysis; Hydrogen evolution; Nickel complexes; Penicillamine; Polarography; Catalytic hydrogen prewave; Nickel hydrogenase; Amino acids.

Previous papers in this series[1,2] dealt with D-penicillamine (Pen) effects on nickel ion reduction on a dropping mercury electrode. The interest in this topic was mainly stimulated by the structural analogy of Pen ($(CH_3)_2C(SH)CH(NH_3^+)COO^-$, H_2L) with the natural amino acid cysteine ($CH_2(SH)CH(NH_3^+)COO^-$) which is well known as a catalyst for reduction of both cobalt(II) (ref.[3]) and nickel[4,5] ions.

As already pointed out[1,2], the principal merit of the Ni(II)–Pen system is the simple coordination scheme, including only two complex species[6a,6b]:

[NiL] (log β_{11} = 10.749) and $[NiL_2]^{2-}$ (log β_{12} = 22.886) (ref.[6a]). This enables accurate evaluation of the species concentrations in order to make further correlation with polarographic data. By contrast, cysteine displays a more intricate behavior, forming several polynuclear complexes with nickel[6a,7]. Moreover, the composition of the mononuclear species is ambiguous; the occurrence of both [NiL] and $[NiLH]^+$ forms was suggested[6a]. As a result, the coordination scheme is rather involved in this case, preventing reliable assessment of the species concentrations.

The direct correlation of polarographic data with the species concentration proved that, despite of identity of binding sites in Pen and cysteine and close values of the formation constants[6a], the relevant bis(ligand)-nickel(II) complexes, $[NiL_2]^{2-}$, behave in a completely different way in the cathodic potential region. Whereas the cysteine complex dissociates very fast, thus enabling the reduction of the Ni^{2+} ion in the hydrated state, the Pen counterpart is chemically inert and Pen actually acts as a masking agent for Ni(II) (ref.[1]). This peculiar distinction results from the different spatial configuration of Pen and cysteine nickel complexes and, as suggested earlier[1], it may form the basis for understanding the detoxification effect of Pen in nickel intoxication. Moreover, it was demonstrated that the [NiL] species is the actual electron acceptor in the nickel(II) reduction process catalyzed by Pen (ref.[2]). This electrode process yields a catalytic nickel prewave which shows much analogy with that produced by cysteine and its derivatives[4,5]. Therefore, investigation of the Ni(II)–Pen system enables a better understanding of the mechanism of Ni(II) reduction catalyzed by amino thiols, including cysteine and related compounds. Besides purely mechanistic aspects, this electrode reaction arose recently a revived analytical interest as a detection process in indirect cathodic stripping voltammetry of cysteine[8a] and related compounds[8a–8d], including Pen (ref.[9]).

One of the most prominent electrochemical reactions involving cysteine and some of its derivatives and analogues is the catalytic reduction of hydrogen ion in the presence of either cobalt[10–12] or nickel[13] ions in slightly alkaline solutions. The relevant polarographic pattern is termed as the Brdička wave after the name of the discoverer.

This paper focuses on a different kind of catalytic hydrogen evolution process, producing the so-called catalytic hydrogen prewave (CHP). This electrode process was first detected for systems containing selenocysteine and the nickel(II) ion[14a,14b]. Selenocysteine shows the particular property of not being able to produce the catalytic Brdička wave, despite of its structural analogy with cysteine and the ability to catalyze the reduction of both Co(II) (ref.[14c]), Ni(II) (ref.[14d]) and hydrogen ion (in the form of a pre-

sodium wave[14e], *i.e.*, in the absence of any transition metal ion). This difference was tentatively accounted for by the different acid dissociation constants for SH and SeH groups[14a]. It was further shown that the catalytic hydrogen prewave also occurs in the presence of cysteine[5,15] and related compounds[16], including cysteine-containing peptides[17]. Moreover, it was suggested that this electrode process shows some interesting analogies with the hydrogen oxidation/reduction process catalyzed by nickel hydrogenase[18]. In this connection, it is worth mentioning that hydrogenases are enzymes which catalyze the reversible two-electron oxidation of molecular hydrogen $H_2 \rightleftarrows 2\ H^+ + 2\ e^-$. These enzymes play a central role in hydrogen metabolism, which is essential for many microorganisms of biotechnological interest, such as methanogenic, acetogenic, nitrogen fixing, photosynthetic and sulfate-reducing bacteria[19]. The discovery of nickel occurrence in some hydrogenases and other enzymes lead to the emergence of biomimetic chemistry of nickel as a new topic in the frame of bioinorganic chemistry[20]. A distinctive contribution to this field is brought about by electrochemical investigation of hydrogen evolution catalyzed by nickel complexes with cysteine-like ligands in the physiological pH range. In this respect, the CHP shows the most promising prospects[20].

Apparently, the distinct characteristics of the CHP *vs* the Brdička wave are not appropriately recognized as demonstrated, for example, by terming the CHP as "Brdička prewave" in a recent review[21], although none of the original papers dealing with this topic[5,15–18] did use such a designation. In order to prevent any further confusion, it is important to point out the fundamental differences between the CHP and the well-known Brdička hydrogen catalytic wave. To this end, the main characteristics of the above processes are summarized and compared in Table I. The data in this table rely on experimental results for cysteine and related compounds. No detailed data on the Brdička wave produced by Pen are available, although this process was proposed long ago for the determination of penicillin after hydrolysis to Pen (ref.[22]).

This work relies on the well-characterized complexation scheme in the Ni(II)–Pen system and aims at identifying the complex compound directly involved in the CHP electrode process. To this end, polarographic data were correlated with the species concentrations calculated using relevant equilibrium constants[6a]. Further, structural and kinetic data were used to formulate a reaction mechanism for hydrogen evolution, with main emphasis on the composition and chemical state of intermediate hydrogen forms. Since the CHP induced by Pen shows basically the same characteristics as that

caused by cysteine itself, it was possible to draw some general conclusions which are valid for cysteine and its derivatives as well.

EXPERIMENTAL

Polarograms were recorded using conventional, three-electrode equipment with a saturated calomel electrode (SCE) as reference and a platinum plate as auxiliary electrode, in a thermostated cell (25 °C). The background electrolyte consisted of CH_3COONa and Na_2HPO_4 (0.025 mol l^{-1} each) with appropriate addition of $HClO_4$ for pH adjustment[15d]. Other experimental details have been given elsewhere[1].

TABLE I
A comparison of characteristic features of the CHP (refs[5,14a,14b,15–18]) and Brdička wave[10–13]

Parameter	Catalytic hydrogen prewave	Brdička wave
Shape	In accord with the equation of an irreversible wave, with a distinct limiting current.	Maximum-shaped; no distinct limiting current occurs.
Characteristic potential	$E_{1/2}$ between –1.2 and –1.3 V *vs* SCE; slightly dependent on the ligand structure. For a given ligand, $E_{1/2}$ is almost independent of pH and ligand concentration.	Maximum potential at about –1.6 V *vs* SCE.
Ligand structure	The occurence of vicinal SH and NH_2 functions is a rule.	Besides the SH function, a second coordinating group (*e.g.*, NH_2, COO^-, SH) must be present to form a five-membered chelate ring with Co(II). Exceptions: some nonsulfur ligands (ref.[10]).
Metal ion effect	Typical behaviour is observed in the presence of Ni(II). Co(II) induces analogous patterns, but additional processes occur and make the polarogram more complex (refs.[15f])	Both Co(II) and Ni(II) give the Brdička wave, but, under the same conditions, the wave current is higher for Co(II).
Optimum pH range	Around 7 (acetate or acetate-phosphate buffer); the wave vanishes at pH > 8 and pH < 5.	Around 9.5 (ammonia or borate buffer).
Interface effects	No ligand adsorption detected in the potential region of the wave.	No direct proof for adsorption. It is presumed that the shape of the wave is due to the effect of the electrode potential on the surface concentration of the ligand.

RESULTS

Main Characteristics of the Catalytic Hydrogen Prewave

A typical polarogram for the system under investigation is shown in Fig. 1. As it was previously demonstrated[1], wave A is due to the diffusion-controlled reduction of hydrated nickel ion either as present in the bulk of the solution or resulting from the fast dissociation of the [NiL] complex. The maximum-shaped wave C is produced by the catalytic reduction of Ni(II) with the [NiL] species as the electron acceptor[2]. The catalytic character of this process results from the regeneration of the [NiL] species by the reaction of an excess of hydrated Ni(II) with ligand molecules released by the reduction process.

The particular point this paper is dealing with, is the wave D in Fig. 1. It has the usual shape of a steady-state irreversible wave ($E_{1/2}$ = –1.21 V; αn = 0.43), as demonstrated by the logarithmic plot shown in the inset to Fig. 1. This is one of the most striking differences between the CHP and the bell-shaped Brdička wave. The above values are very close to those reported for the CHP produced by ethyl cysteinate[16] ($E_{1/2}$ = –1.22 V; αn = 0.40) or cysteinylglycine[17a] ($E_{1/2}$ = –1.18 V; αn = 0.42) and are not essentially different from those found for cysteine itself[5,15d] ($E_{1/2}$ = –1.25 V; αn = 0.56). Such

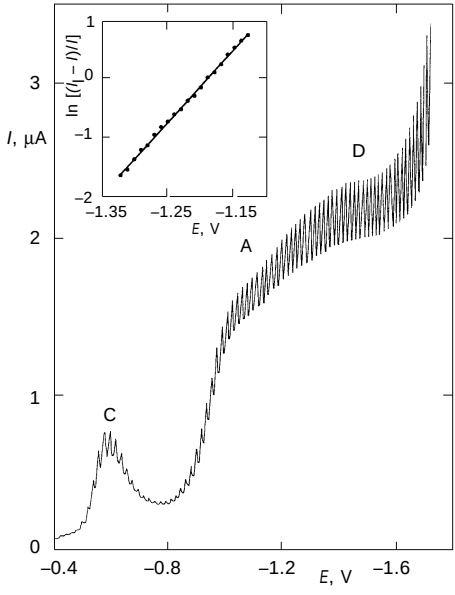


FIG. 1
Polarographic waves in the Ni(II)–Pen system. Conditions: pH 6.90; [Ni(II)] 0.5 mmol l^{-1}; [Pen] 0.5 mmol l^{-1}; initial potential –0.4 V. Inset: logarithmic analysis of the catalytic hydrogen prewave (wave D)

analogy makes it possible to assign the wave D to the catalytic hydrogen evolution in the form of the CHP. The strongest support to this assumption is the identity of the complexing sites in both cysteine and Pen. Further resemblance of the wave D with the CHP produced by cysteine and its derivatives will be pointed out in next sections. It is clear that the CHP (wave D) is due to some nickel complexes with Pen as an α-aminothiol ligand, as it happens in the case of cysteine as well. Although the nature of the binding sites is the deciding factor, some additional structural details in ligand molecule bring about slight differences in the CHP parameters, as demonstrated by the above data. These differences are the consequences of some secondary aspects like the molecule orientation at the interface due to steric factors or to hydrophobic properties of some parts of the molecule.

Adsorption effects in the Ni(II)–Pen system were investigated previously by alternating current polarography and the method of drop time–potential curves[2]. Both methods show no significant adsorption in the potential region of the CHP, in accord with the results in the Ni(II)–cysteine system[5,15d].

Effect of D-*Penicillamine Concentration*

The effect of Pen concentration at constant total nickel concentration, $[Ni(II)]_{tot}$, is shown in Fig. 2 as a function of the $[Pen]/[Ni(II)]_{tot}$ ratio for both the CHP current (Fig. 2a) and the species concentrations (Figs 2b, 2c).

As it results from Fig. 2a, curves *1* and *2*, the CHP current shows a maximum when the $[Pen]/[Ni(II)]_{tot}$ ratio is close to unity and tends to vanish when Pen is in excess. A comparison of the curves in Fig. 2a with species concentrations (Figs 2b and 2c) clearly proves that the current variation parallels the change in [NiL] concentration. The fall of current at $[Pen]/[Ni(II)]_{tot} > 1$ is due to the gradual conversion of nickel ion into the inert species $[NiL_2]^{2-}$. Therefore, it may be concluded that the single complex species involved in the catalytic hydrogen evolution of the CHP type is [NiL]. However, an attempt at correlating the CHP current with [NiL] concentration at constant $[Ni(II)]_{tot}$ leads to intricate patterns, proving that some kinetic factors have to be taken into account in order to explain the effect of Pen. It is also clear that the occurrence of the ligand (H_2L) in the free state has no favorable effect on the CHP. On the contrary, under such circumstances nickel ion is masked in the form of the $[NiL_2]^{2-}$ complex and any cathodic electrode process in the system is prevented. This effect is also demonstrated by the polarograms in ref.[1], Fig. 2.

A main characteristics of the typical Brdička wave is the very high ratio of the catalytic current to the expected diffusion current for the complex species supposed to take part in this electrode process[10a]. This is a decisive proof for the catalytic character of the electrode process. That is why the curve *3* in Fig. 2a was included, showing the $I_{CHP}/I_{d,[NiL]}$ ratio, where $I_{d,[NiL]}$ is the expected diffusion current of the [NiL] species. $I_{d,[NiL]}$ was calculated as the product of [NiL] concentration and the Ilkovič constant for the free Ni^{2+} ion (5.15 μA mmol^{-1} l, ref.[1]). The value thus calculated is slightly higher than the actual one due to the lower diffusion coefficient of the complex but is still sufficiently accurate for the purpose of this comparison. It results from Fig. 2a, curve *3* that the $I_{CHP}/I_{d,[NiL]}$ ratio is high enough to contradict the assumption that the CHP is actually due to a simple electron transfer to a site activated by the previous reduction of the nickel ion in the

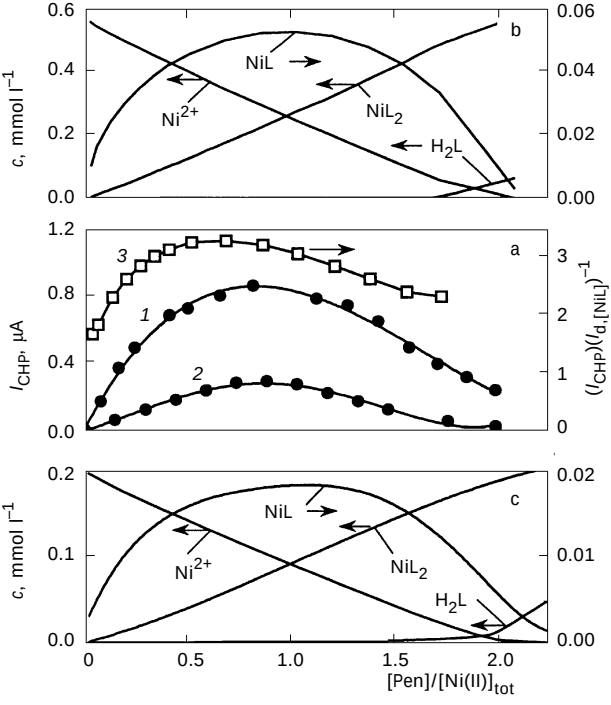


FIG. 2

Effect of Pen concentration at pH 6.5 and constant total nickel concentration. a CHP limiting current. $[Ni(II)]_{tot}$ (in mmol l^{-1}): *1* 0.57, *2* 0.2, *3* the CHP current/[NiL] diffusion current ratio for $[Ni(II)]_{tot}$ 0.57 mmol l^{-1}. b and c Nickel species distribution for $[Ni(II)]_{tot}$ 0.57 and 0.2 mmol l^{-1}, respectively

[NiL] complex. Only the occurrence of a regenerative chemical reaction, which is typical of the catalytic waves, accounts for the values of the $I_{CHP}/I_{d,[NiL]}$ ratio. It must be noted that the very low stability of the intermediate zero-valent nickel complex acting as the actual catalyst[5,14b,15e] may be one of the reasons for the rather low values of the $I_{CHP}/I_{d,[NiL]}$ ratio.

It is also interesting to compare the Pen effect on the CHP current with that of cysteine[5,15d] or ethyl cysteinate[16]. No maximum occurs on the I_{CHP}–ligand concentration curves for the last two compounds. Conversely, a steady increase in current may be observed, with a trend to level off at a high ligand excess. The different behavior can be accounted for by the labile character of the bis(ligand)Ni(II) complex in the cases of both cysteine and its ester. This does not bring about some substantial difference in the reaction mechanism, as demonstrated by the value of the half-wave potentials for the CHP. The single important distinction is the availability of a higher amount of Ni(II), partly present in the labile $[NiL_2]^{2-}$ form.

Effect of Nickel Ion Concentration

The effect of nickel ion concentration is presented in Fig. 3, where both the CHP current (Fig. 3a) and species concentrations (Fig. 3b) are plotted as functions of the $[Ni(II)]_{tot}/[Pen]$ ratio at constant Pen concentrations. It results from Fig. 3a that the CHP current assumes significant values for $[Ni(II)]_{tot}/[Pen] > 0.5$ only. This limit is just the combining ratio in the $[NiL_2]^{2-}$ complex and, as demonstrated by Fig. 3b, this is the single complex species in the solution below the above limit. It is obvious that the $[NiL_2]^{2-}$ form is not involved in any way in the CHP reaction, in accord with its inert character[1]. On the other hand, curve *1* in Fig. 3a matches the [NiL] concentration curve in Fig. 3b (which was plotted for the same conditions), this suggesting the involvement of the last complex in the electrode process. A direct proof for this assumption is provided by the inset to Fig. 3a, that demonstrates the linear relationship between the CHP current and [NiL] concentration, with a common correlation line for two different Pen concentrations: 0.2 mmol l^{-1} (empty circles) and 0.5 mmol l^{-1} (full circles). It is noteworthy that the presence of the free ligand (mostly existing in the H_2L form in this pH range) is not required for the occurrence of the CHP, as it results from Fig. 3b which demonstrates a negligible concentration of this form in the region of the significant current values (*i.e.*, for $[Ni(II)]_{tot}/[Pen] > 0.5$). Finally, it must be noted that the presence of the hydrated nickel ion (Ni^{2+}) does not seem to play a particular role in the electrode process, as the steady

increase in the Ni^{2+} concentration (Fig. 3b) is not accompanied by a similar trend in the variation of the current at $[Ni(II)]_{tot}/[Pen] > 0.5$ (Fig. 3a).

The above data demonstrate that the effect of nickel ion concentration in the Ni(II)–Pen system does not show the usual trend noticed in the case of selenocysteine[14a], cysteine[5,15d], cysteinyl dipeptides[18] or ethyl cysteinate[16]. A monotonous increase in the CHP current with Ni(II) concentration was reported for all the above compounds. Such a trend can be also displayed by the Pen system as long as an excess of Ni(II) permits the formation of the [NiL] species. If this condition is not fulfilled, the nickel ion is actually masked in the form of an inert species, $[NiL_2]^{2-}$. That is why the effect of EDTA, which is a typical masking agent for Ni^{2+}, was also investigated. An amperometric titration of a Ni^{2+} solution with EDTA (in the absence of Pen) clearly demonstrates that the Ni(II)–EDTA complex is electrochemically inactive under the experimental conditions used in this work. Consequently,

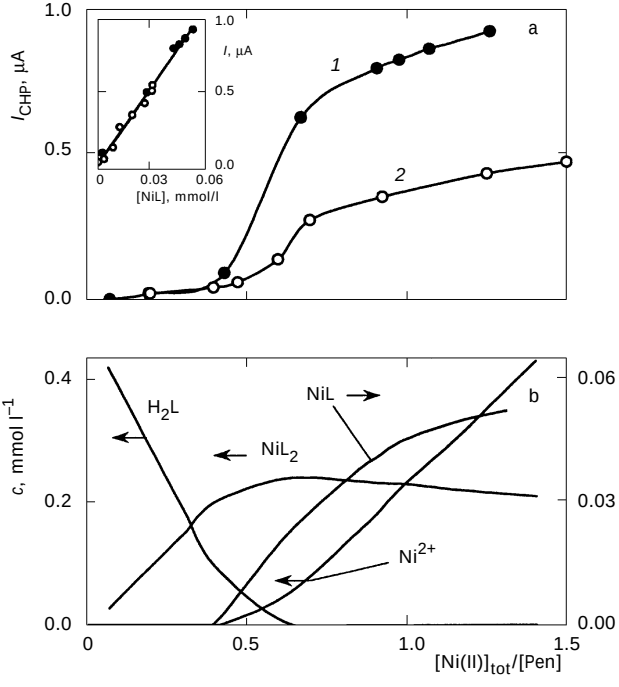


FIG. 3

Effect of nickel ion concentration at pH 6.5 and constant Pen concentration. a CHP current *vs* $[Ni(II)]_{tot}/[Pen]$ ratio. [Pen] (in mmol l^{-1}): *1* 0.5, *2* 0.2. b Species concentrations for [Pen] 0.5 mmol l^{-1}. Inset: CHP current *vs* calculated [NiL] concentration under the same conditions as for curve *1* (full circles) and curve *2* (empty circles)

the effect of EDTA in the presence of Pen is expressed by the dependence of the CHP current on the "free" Ni(II) concentration, $[Ni(II)]_{free}$, *i.e.*, the concentration of nickel ion not bound into the inert EDTA complex, at a constant total nickel concentration (Fig. 4, curve *1*). Like in Fig. 3a, the CHP current produced by Pen may be detected only if the $[Ni(II)]_{free}$/[Pen] ratio is higher than 0.5. It appears that Pen cannot substitute EDTA in the EDTA–Ni(II) complex and only Ni(II) not bound to EDTA is able to take part in the CHP process.

As shown previously[1], nickel diffusion current ($I_{d,Ni}$, wave A in Fig. 1) is directly proportional to the sum of Ni^{2+} and [NiL] concentrations and is, in this way, a measure of the "reactive" nickel concentration in the system. The inset to Fig. 4 presents the relationship between the CHP current and nickel diffusion current when the "reactive" nickel concentration was varied in two ways: either by changing the total nickel concentration (empty circles) or by increasing gradually the EDTA concentration at a constant total nickel concentration of 0.25 mmol l^{-1} (full circles). In the latter case, a fraction of the total nickel content is masked in the form of both $[NiL_2]^{2-}$ and Ni(II)–EDTA complexes. However, current values rest on the same curve under both the above circumstances, proving again that Pen is not able to displace EDTA in the Ni(II)–EDTA complex.

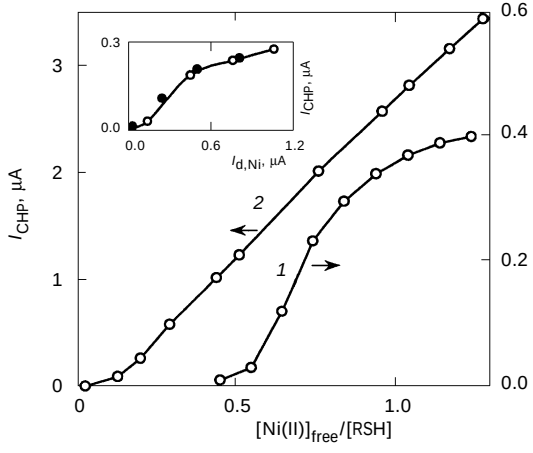


FIG. 4

CHP current as a function of the "free" nickel ion concentration varied by EDTA additions. RSH stands for both Pen and cysteine. Conditions: pH 7.17; [Pen] (*1*) or [cysteine] (*2*) 0.5 mmol l^{-1}; $[Ni(II)]_{tot}$ 0.25 mmol l^{-1}. Inset: CHP current as a function of nickel diffusion current varied either by changing $[Ni(II)]_{tot}$ from 0 to 0.25 mmol l^{-1} (empty circles) or by adding EDTA to a solution of 0.25 mmol l^{-1} Ni(II) (full circles). Conditions: pH 7.18; [Pen] 0.2 mmol l^{-1}

In order to compare the catalytic activity of Pen and cysteine nickel complexes, curve *2* in Fig. 4 shows the effect of "free" nickel ion on the CHP produced by cysteine, under the same conditions as in the case of Pen (curve *1* in Fig. 4). Three main differences are evident. First, the absence of any masking effect of cysteine makes the CHP current to assume significant values for $[Ni(II)]_{free}$/[cysteine] much lower than the combining ratio in the pertinent $[NiL_2]^{2-}$ complex. Second, the CHP current produced by cysteine steadily increases with $[Ni(II)]_{free}$, proving that the labile bis(L-cysteine-*N*,*S*)-nickel(II) complex can provide, by dissociation, the nickel ion required in the catalytic process. Finally, it must be noted that, under similar conditions, the CHP current due to cysteine is higher than that due to Pen.

Effect of pH

Complex equilibria involving Pen, which is a protonizable ligand, depend on solution pH. On the other hand, the reaction rate for the catalytic hydrogen evolution may depend on hydrogen ion concentration and also on concentrations of available proton donors. Consequently, as shown in Fig. 5a, the CHP current induced by Pen can be detected only in a narrow interval centred at pH 5.5. This value is roughly one pH unit lower than the maximum-current pH for cysteine and its derivatives[5,15d,15e,16,17]. Such difference may be put in accord with the slightly higher formation constants for the Ni(II)–Pen complexes as compared with the similar cysteine species[6a].

In order to assess the contribution of various species to the pH effect on the CHP current, species concentrations were calculated as functions of pH and plotted in Fig. 5b under the same conditions as for curve *1* in Fig. 5a. Species distribution for other Ni(II) and Pen concentrations does not show any salient difference.

A comparison of curve *1* in Fig. 5a with the nickel species distribution in Fig. 5b clearly demonstrates that the rising part on the current–pH curve corresponds to the pH range of the increase in [NiL] concentration. However, at pH > 5.5, all nickel species have constant concentrations and such trend cannot account for the drop in the CHP current in this region. Furthermore, the drop in the free ligand (H_2L) concentration may not be responsible for the shape of the current–pH curve. This assertion is supported by Fig. 3 which demonstrates no correlation between the presence of the free ligand and the occurrence of the CHP. Moreover, the kind of pH dependence shown in Fig. 5a was also demonstrated for selenocysteine[14a], cysteine[5,15d] and cysteine derivatives[16–18] in buffer systems of various compositions. Consequently, the fall in the CHP current can be ascribed neither

to particular characteristics of the Ni(II)–Pen complexes nor to the buffer composition. The single sound alternative is to assume that the decline in the CHP current at higher pH values is due to a strong decrease in the H_3O^+ concentration (labelled by H^+ in Fig. 5b). One or more proton donors should be involved in the catalytic hydrogen evolution process and data in Fig. 5 suggest that the single one is actually the hydronium ion, which is the strongest acid in the system. Besides this one, the system also contains $H_2PO_4^-$ and HPO_4^{2-} ions as weak acids. If the former, as a stronger acid, is assumed to be the proton donor, then the maximum on the current–pH curves should be shifted to a pH value close to the $pK_{a,2}$ value for phosphoric acid, *i.e.*, around 7. Besides, if HPO_4^{2-} were also able to act as a proton donor, the marked decrease in the CHP current at higher pH values would not be possible. Moreover, the same kind of pH dependence was found for selenocysteine[14a] and cysteine[5] in the acetate buffer, although the pK_a for acetic acid is distant from the CHP characteristic pH range. Finally, the

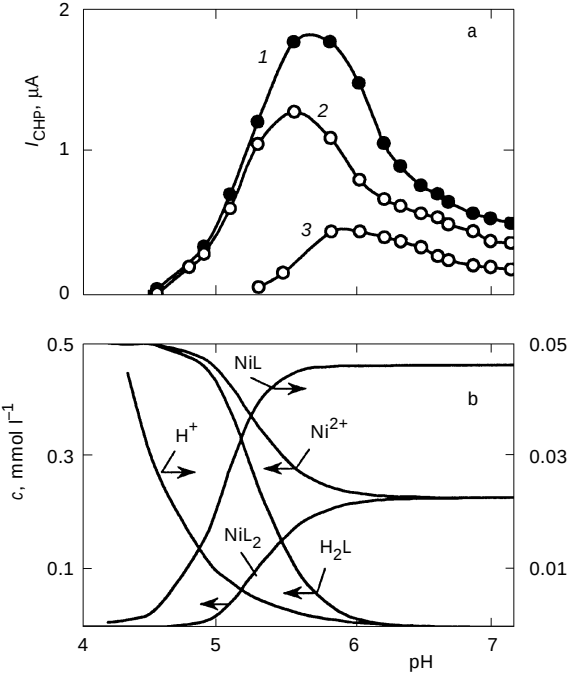


FIG. 5

pH effect on the CHP limiting current (a) and the species concentrations (b). a Pen and nickel concentrations (in mmol l^{-1}): *1* 0.5, 0.5; *2* 0.5, 0.3; *3* 0.2, 0.2. b Same as for curve *1*

shape of the curves in Fig. 5a cannot be assigned to a decrease in the buffer capacity because it is high enough for the buffer system employed[15d].

Therefore, the shape of the current–pH curves in Fig. 5a is the result of two opposite effects of pH increase: the rise in the [NiL] concentration and the simultaneous decline in that of hydronium ion. Accordingly, the decline on the current–pH curve starts when the H_3O^+ concentration becomes much lower than that of the [NiL] complex (Fig. 5).

Effect of Mass Transfer Processes

The most obvious test for the effect of mass transfer on the overall reaction rate in polarography is the influence of mercury pressure, expressed by the mercury column height, *h*. A rise in this parameter leads to a higher mass transport velocity owing to the faster expansion of the mercury drop. Additionally, when using a free-dropping capillary electrode, the drop time also depends on *h* and, in this way, surface coverage by the adsorption of reactants, products or inhibitors occurs accordingly. As already demonstrated for this system[2], adsorption is absent in the potential region of the CHP. It remains therefore to make interpretation of the *h* influence in terms of mass transfer effects only.

As a characteristic feature, hydrogen evolution process catalyzed by nickel or cobalt complexes occurs in the potential region corresponding to the limiting current of the metal ion. Reduction of the metal ion and catalytic hydrogen evolution give two distinct components of the total current under such circumstances. As a rule, it is implicitly assumed that the two components are independent and the catalytic component is measured from the level of the metal ion diffusion current. Actually, it is difficult to define a better procedure, but the correctness of this approach may be equivocal when performing kinetic interpretation of some data, as it happens in the case of the effect of mercury pressure.

In this respect, the most critical issue may be an essential change in the mechanism of the metal ion reduction in the potential region of the catalytic process. Such a change is well documented for the cobalt-bovine serum albumine[23] and cobalt–cysteine[24] systems in an ammonia buffer, *i.e.*, under typical conditions for the occurrence of the Brdička wave. In this example, the actual catalyst may be a zero-charge metal ion complex produced by the reduction of the available metal(II) complexes (hereinafter precursor complexes). The rate of generation of such a complex surely depends on the mass transport rate for the precursor compound and makes questionable the above-mentioned hypothesis on current additivity. It may

be mentioned in this context that the concept of catalysis by a product of electrochemical conversion of a precursor was also accepted in the field of the pre-sodium type process (*i.e.*, hydrogen evolution catalyzed by organic compounds)[25].

According to the standard procedure, the CHP current was plotted as a function of $h^{1/2}$ in Fig. 6 for three different values of the $[Pen]/[Ni(II)]_{tot}$ ratio. Because $[NiL_2]^{2-}$ is inert[1], it can be disregarded when interpreting the $h^{1/2}$ effect. Line *1* demonstrates the absence of any mass transfer effect for $[Pen]/[Ni(II)]_{tot} = 0.4$. Under these conditions 76% of the nickel content is present as the hydrated ion and about 7.1% as [NiL]. Lines *2* and *3*, plotted for $[Pen]/[Ni(II)]_{tot}$ equal to 1 and 2, respectively, show a unusual pattern, which can be interpreted, at first glance, as a consequence of a coupled control by both the mass transport processes and some chemical reaction. The percentages of Ni^{2+} and [NiL] are 46 and 9.2, respectively, for line *2*, and 0.9 and 1.9 for line *3*. For all the three lines in Fig. 6, the concentration of the free ligand is negligible. It appears therefore that the drop in Ni^{2+} concentration is the main factor dictating the shape of the current–$h^{1/2}$ relationship, and a pure kinetic control occurs when a high excess of non-complexed nickel ion is present. Interestingly, it was also noticed that the CHP current is independent of *h* in the Ni(II)–selenocysteine system at a high excess of Ni(II) (ref.[14a]). On the other hand, cyclic voltammetry on the HMDE in the potential region of the CHP produced a pattern typical of electrode processes with reactant regeneration by a rate-determining chemical reaction[15e,18]. All the above observations point to the clear kinetic control of the CHP process under certain circumstances, although this cannot be a general rule and the overall kinetics may change in dependence on some experimental parameters.

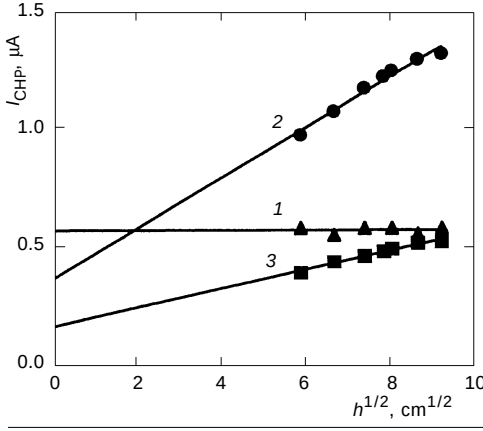


FIG. 6
Effect of the mercury column height on the CHP current. Conditions: pH 6.52; [Ni(II)] 0.5 mmol l^{-1}; [Pen] (in mmol l^{-1}): *1* 0.2, *2* 0.5, *3* 1.0

DISCUSSION

Data in the previous sections demonstrate that the occurrence of the CHP in the presence of Ni(II) and Pen is associated with the formation of the [NiL] complex compound. The bis(D-penicillamine-*N*,*S*)nickel(II) complex proved to be completely inert with regard to any cathodic process involving complex species in this system. Taking into account the close analogy of catalytic reactions in the presence of either Pen on one hand and selenocysteine, cysteine and its derivatives on the other, it is reasonable to assume that a [NiL]-type complex is responsible for the emergence of the CHP in the presence of all these compounds. The key difference between Pen and other cysteine derivatives is the labile character of the $[NiL_2]^{2-}$ species in the last cases. Its rapid dissociation during the reduction of the Ni(II) centre provides an additional amount of [NiL] that may take part in catalytic processes.

Coordination in the [NiL] complex should occur *via* the amino and sulfur groups with a minor (if any) contribution of the carboxyl group, as demonstrates the small difference between the formation constants for the mono(ligand)nickel(II) complexes of Pen ($\log \beta_1 = 11.20$) and its methyl ester ($\log \beta_1 = 9.95$) at 22 °C in the presence of 0.1 M KNO_3 (ref.[6b]). Masking of either the NH_2 or SH group removes the catalytic activity of cysteine in the CHP electrode process[16]. Furthermore, data in ref.[2] and in this paper clearly demonstrate that the same nickel complex is responsible for the occurrence of both the catalytic nickel prewave[2] and the CHP.

The CHP reaction mechanism formulated previously[18] and reproduced, in an adapted form, as Fig. 43 in ref.[20], involves the hydride ion coordinated to the low-valence nickel centre. Protonation of a metal centre as an intermediary step in electrochemical hydrogen evolution was pointed out long time ago[26], whereas more recent papers demonstrate the occurrence of the hydride intermediate coordinated to the electron-rich Fe(0) (ref.[27a]) or Co(I) (ref.[27b]) porphyrin centre. It was also proved that a Ni(I) complex, [Ni(psnet)] (psnet = bis[5-(diphenylphosphino)-3-thiapentanyl]amine) reacts with Brønsted acids to yield H_2 in a homogeneous reaction involving H^- ligand as an intermediate state[28]. The hypothesis of the coordinating hydride intermediate was also put forward to explain the catalytic hydrogen evolution in cathodic stripping voltammetry of SH^- ion in the presence of Co(II) (ref.[29]) . In an indirect way, the quoted papers bring about some support to the proposed mechanism for the CHP process[18,20] but cannot provide it with an ultimate confirmation.

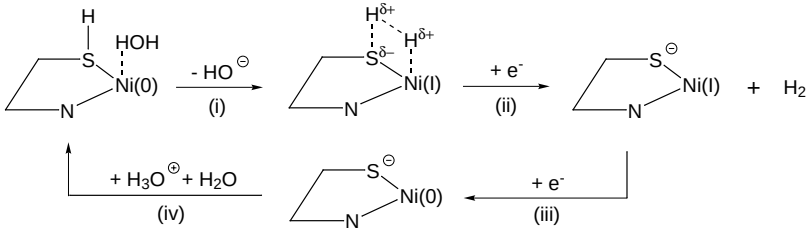
It must be pointed out that, in the above-mentioned reactions, the electron-rich metal centre plays the double role of electron donor and support for the intermediate hydrogen form. This implies the occurrence of hydrogen evolution at a specific potential for the formation of the low valence metal atom by the reduction of a stable form[26,27,29]. In the case of the CHP, none of the characteristic potentials of the metal ion reduction (waves A and C) does fit the half-wave potential for the catalytic hydrogen evolution. A similar behavior was found for a nickel macrocyclic complex catalyst[30] and was accounted for by the initial electron transfer to the ligand itself to form an anion-radical. Such a mechanism is less probable with a cysteine-like ligand not containing empty orbitals that could accommodate the electron, at least temporarily.

The main conclusions emerging from this investigation can be summarized as follows: (i) the occurrence of the CHP is due to the [NiL] complex, with no intervention of $[NiL_2]^{2-}$ and no essential role of Ni^{2+}; (ii) the presence of hydronium as a proton donor is essential; (iii) the overall reaction rate is not dependent on mass transfer processes provided a large excess of Ni^{2+} is available. In addition, it is useful to remember the large isotope effect associated with the CHP, *i.e.*, a decrease in the CHP current when deuterium is substituted for the labile proton (including the use of deuterium oxide as solvent)[15b]. The isotope effect points to the occurrence of a slow proton transfer reaction. But the protonation of a Brønsted base (including an electronegative element with a lone electron pair) is extremely fast and it remains to assume that the proton transfer involves another kind of proton acceptor[31]. In this case, the proton acceptor could be Ni(0) which may use the added electron pair in the d_{z^2} orbital for hydrogen bonding with hydronium ion. The occurrence of hydrogen bonds with a metal centre as the proton acceptor is largely documented in organometallic chemistry[32]. In the case of the Ni–Pen complex, formation of hydrogen bond may occur after fast reorientation of a neighboring water molecule located in the axial position. An additional charge to the nickel centre may also induce the protonization of the thiol functionality, leading finally to the formation of a series of unstable intermediates tentatively depicted in Scheme 1. This reaction scheme includes another unfamiliar feature, the H···H hydrogen bond, which was already demonstrated for a series of complex compounds[33]. The mechanism in Scheme 1 is basically consistent with that previously formulated[18] and includes, in the second step, a coordinating $H^{\delta+}\cdots H^{\delta+}$ form. It reminds the H_2^+ intermediate from the Heyrovský mechanism of the catalytic hydrogen currents[12a]. The main amendment to the previous mechanism consists in the localization of the proton at sulfur and

not at the nitrogen site, as it was inferred initially[18]. This alternative assumes a neutral rather than negative sulfur atom and is more consistent with the fact that the nickel–sulfur bond is preserved despite the excess of negative charge resulting after the reduction of the Ni(II) centre.

In order to account for the absence of a pH effect on the CHP half-wave potential, it must be accepted that the reaction (iii) in Scheme 1 is the potential-determining step and every proton-involving reaction (jointly formulated as the step (iv)) proceeds after the step (iii). For the same reason, the step (ii) must be assumed to be very fast. The pH effect on the CHP limiting current is the consequence of the proton participation in the steps (i) and (iv) whereas the deuterium isotope effect noticed in the case of cysteine[15a] may be the consequence of charge redistribution and a new H–H bond formation in both steps (i) and (ii).

The above conclusions deserve some comments in connection with the recent results on the structure of *Desulfovibrio gigas* [NiL] hydrogenase[19b]. The active site in this enzyme contains not only a nickel atom but also an iron atom bound to the former by two sulfur bridges (provided by cysteine residues) and a non-protein, still unidentified ligand. Two additional cysteine residues bind to nickel through the S-functional groups, giving a distorted square pyramidal structure. It is possible now to formulate some more elaborate assumptions on the reaction mechanism, although a definitive option demands more investigation. The presence of iron in the active site suggests that at least a part of the electron-transfer processes involved in hydrogen redox reaction could occur at this site[19b]. In this respect, it is assumed that studies on mononuclear model compounds may provide useful results concerning hydrogen binding and activation since nickel ion is presumably the catalytic site in [NiFe] hydrogenases[19]. Such conclusions are very encouraging for the future prospects of the field of hydrogen



SCHEME 1
Proposed reaction mechanism for catalytic hydrogen ion reduction in the CHP electrode process

electrocatalysis by nickel complexes with cysteine-like ligands. Clearly, among the various kinds of catalytic hydrogen currents investigated so far, the CHP-type process is the best match for the hydrogenase-catalyzed redox chemistry of hydrogen.

SYMBOLS AND ACRONYMS

CHP	catalytic hydrogen prewave
h	mercury column height, cm
HMDE	hanging mercury drop electrode
$I_{d,Ni}$	nickel diffusion current
$I_{d,[NiL]}$	expected diffusion current of the [NiL] complex, calculated using the Ilkovič equation
I_{CHP}	CHP limiting current
Pen	D-penicillamine
$[Ni(II)]_{tot}$	total nickel ion concentration
$[Ni(II)]_{free}$	"free" nickel ion concentration, *i.e.*, $[Ni(II)]_{tot}$–[EDTA]
SCE	saturated calomel electrode

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