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Hydrolytic species of the ion *cis*-diaqua(ethylenediamine)palladium(II) complex and of *cis*-dichloro(ethylenediamine)palladium(II): fitting its equilibrium models in aqueous media with or without chloride ion

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Abstract

Thermodynamic data for equilibria involved in the overall hydrolytic process of the *cis*-$[Pd(en)(H_2O)_2]^{2+}$ complex in the presence or absence of chloride ion are reported. All expected hydrolytic species are taken into account to calculate their formation constants and to fit the equilibrium model. The formation constants ($\log \beta_{pqr}$) of aqua and/or hydroxo complexes were obtained from $E(H^+)$ data of alkalimetric titrations of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ solutions. The $\log \beta_{pqr}$ values of chloro-containing complexes were obtained from $E(H^+)$ and $E(Cl^-)$ data pairs, taking into account the above $\log \beta$ data as fixed values. All formation constants were fitted by SUPERQUAD calculations: $\log \beta_{pqr}$ for *cis*-aqua-hydroxo ($pqr = 10-1$, $-6.68(10)$), di-μ-hydroxo ($20-2$, $-7.758(4)$), *cis*-dihydroxo ($10-2$, $-14.523(4)$), *cis*-dichloro (120, 5.24(1)), *cis*-chloro-aqua (110, 3.18(1)) and *cis*-chloro-hydroxo ($11-1$, $-3.75(4)$) species for $I = 0.15$ mol dm^{-3} in $NaClO_4$ and $t = 37$ °C. This constant set allows good simulation of experimental titration curves and is used to obtain a variety of species distribution diagrams.

Keywords: Palladium complexes; Ethylenediamine complexes; Aqua complexes; Hydrolysis; Stability constants; Polynuclear complexes

1. Introduction

It is generally assumed that the hydrolytic products of the active antitumor complex *cis*-diamminedichloroplatinum(II) (cis-platin or cis-DDP) play an important role in the mechanistics of their antitumoral activity and renal toxicity. On this basis, several research groups have studied the hydrolytic reactions of cis-DDP [1–3] and related Pt(II) complexes [4–11] with antitumor and other biological properties. Some of these studies report kinetic data and equilibrium constants for one or several steps of the overall hydrolytic process [3] (hydrolysis, proton dissociation and dimerisation). However, the requirement of platinum(II) systems to be studied in both kinetic and thermodynamic senses prevents determination of the constants for all of the involved steps in similar conditions (methodology, concentration of the involved reagents, pH, etc.).

In order to save the inert kinetics of Pt(II) complexes and on the basis of the remarkable analogy of Pt(II) and Pd(II) coordination chemistry a variety of Pd(II) complexes have proved useful as models to obtain a reasonable view on the thermodynamic aspects of the hydrolytic reactions for closely related Pt(II) ones. Most of this research has been on the reactivity of Pd(II) complexes in aqueous solutions. In past years studies on the hydrolytic reactions of Pd(II) complexes [12–15], such as *cis*-dichloro(ethylenediamine)-palladium(II) (*cis*-$[Pd(en)Cl_2]$), have been of increasing interest because their solubility in water (4.3×10^{-3} M at 25 °C) [14] permits the use of potentiometric [4,14], spectrophotometric [13] or NMR [15] techniques. In such a context, the diaqua complex *cis*-$[Pd(en)(H_2O)_2]^{2+}$ seems a good model for the analogous *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. The *cis*-$[Pd(en)Cl_2]$ complex has two labile chloro ligands which in aqueous solution will be substituted by water molecules, followed by proton dissociation and perhaps oligomerisation processes in a stepwise manner. These processes are kinetically labile and overlapped depending on the pH, chloride and complex concentrations. Consequently the accurate fitting of the equilibrium model and the determination of their equilibrium constants require the use of rigorous methods which can treat all the overlapped steps simultaneously. In previous works [4,13–16] this treatment was not taken into account. The use of the proposed equilibrium models and constants to simulate the behaviour of *cis*-$[Pd(en)Cl_2]$

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in a variety of aqueous systems (for example, in plasma) may not be very successful in some cases.

In our laboratories we have developed a potentiometric methodology to study the hydrolytic reactions of the *cis*-dichloro(diaminoacid)palladium(II) complexes and related compounds in poor and rich chloride ion aqueous systems [17]. This paper reports the application of such a procedure to the model compound *cis*-$[Pd(en)Cl_2]$ in aqueous solution with or without Cl^- ions. Our results are compared with others reported previously.

2. Experimental

2.1. Chemicals

To synthesize *cis*-$[Pd(en)Cl_2]$ a solution of ethylenediamine (4 mmol) in 150 ml of 0.05 M HCl was added dropwise to a warm solution of K_2PdCl_4 (4 mmol) in 25 ml of 0.1 M HCl. The mixture was stirred and heated (60 °C) for 1 h. When the resulting clear solution was cooled, yellow needles were formed. This product (first fraction) was then filtered, washed with acetone and air-dried. The filtrate was evaporated at room temperature for two days until well-shaped yellow–orange crystals were obtained. These crystals (second fraction) were then filtered, washed with acetone and air-dried. The two obtained fractions were identified as the desired product *cis*-$[Pd(en)Cl_2]$. *Anal.* Calc. for $PdC_2H_8N_2Cl_2$: C, 10.12; H, 3.40; N, 11.80. Found: C, 10.22; H, 3.29; N, 11.66%. The IR spectra (KBr pellet) are in accordance with that reported in the literature [18].

Other required products and reagents for the potentiometric study were purchased from Aldrich, Sigma or Merck and were used without further purification.

2.2. Potentiometric study

All solutions were prepared with CO_2-free doubly distilled and freshly boiled water. To prepare the aqueous solution of the diaqua–palladium(II) complex, an accurate amount of dichloro–palladium(II) complex was dissolved in the smallest volume of water and treated with two equivalents of a freshly prepared and standardised $AgClO_4$ aqueous solution, with topaz stained glass material in a darkroom. The AgCl precipitate was filtered off and the resulting clear yellow solution of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ was checked by potentiometric, spectrophotometric and other analytical methods, in order to verify the practical absence of undesired chemical species (Cl^-, Ag^+, $[Pd(en)_2]^{2+}$). Appropiate amounts of this starting solution were added to the neccesary stock solution of $NaClO_4$ and water to obtain the tested solutions of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ ($I = 0.15$ mol dm^{-3} in $NaClO_4$). The ionic strength of the titrant reagents (NaOH or NaCl) was also adjusted. The composition of the tested solutions for calculations are given in Table 1.

2.3. Measurements

All titrations were performed at 37.00 ± 0.05 °C by circulating thermostated water into the appropriate reaction cell and a slow and constant stream of N_2 (presaturated with $NaClO_4$ 0.15 mol dm^{-3}) flowed over the tested solutions.

Experimental e.m.f. data were obtained with Crison 2002 digital (pH/mV)-meters equipped with Ag/AgCl reference electrodes (Ingold 373-90-WTE-ISE-S7) and a glass electrode (Ingold 10-401-3664) or a chloride ion selective electrode (Ingold 15-213-3000), respectively. The reference electrodes allow the use of an intermediate electrolyte chamber (0.15 mol dm^{-3} $NaClO_4$) which was replaced daily.

The addition of titrants was manually controlled with a Metrohm Dosimat 665 Titroprocessor with a 5.0 ml burette (±0.002 ml). After the reading (e.m.f.) was stabilised successive additions of titrant(s) were made. The standard electrode potential $E°(H^+)$, the concentration of the NaOH titrant and K_w values were checked before and after each experiment by titration of a known amount (50 ml) of $HClO_4$ ~ 10^{-3} mol dm^{-3} in $NaClO_4$ 0.15 mol dm^{-3} [17]. An analogous calibration procedure of this potentiometric system was reported by Leporati [19]. The value of K_w (4.57(1) × 10^{-14}) is in agreement with the literature [20]. In experiments requiring the simultaneous measurement of $E(H^+)$ and $E(Cl^-)$, we have furthermore checked, before and after, the $E°(Cl^-)$ and the response slope (Q) of the chloride electrode. For this purpose an NaOH (0.1 mol dm^{-3})/NaCl (0.1 mol dm^{-3})/$NaClO_4$ (0.15 mol dm^{-3}) solution was used as titrant reagent to the $HClO_4$ solution. The response

Table 1
Data of the solutions used in the potentiometric study for log β_{pqr} SUPERQUAD calculations of the chloride-free hydroxo species (a) and of chloro-containing ones (b)

Solution	$\lvert[Pd(en)(H_2O)_2]^{2+}\rvert_o$	$\lvert OH^-\rvert_o$	$\lvert Cl^-\rvert_o$	V	pH range	pCl range
(a-1)	0.22803			50	4.95 [a]–10.58	
(a-2)	0.11440			50	4.97 [b]–10.51	
(a-3)	0.11118			100	4.95 [c]–9.92	
(b-1)	0.14736	0.05045	0.29473	50	5.92–6.48	2.42–1.98
(b-2)	0.14736	0.02522	0.29473	50	5.53–6.14	2.53–2.00
(b-3)	0.08842	0.02522	0.17684	50	5.67–6.29	2.68–2.11

$\lvert\ \rvert_o$ = initial amount (mmol); V = initial volume titrated (ml); [a],[b],[c] = the corresponding initial pH values were 3.76, 3.96, and 4.23, respectively.

of the Cl^- electrode was of the straight line type in the range 10^{-4} to 10^{-2} M of Cl^- ion having a slope (Q) of -60.66 in agreement with the theoretical value of -61.51.

2.4. Calculations

The equilibrium model and the corresponding hydroxo and/or chloro complexes formation constants ($\log \beta_{pqr}$) can be defined by the general equilbrium (1) (charges omitted for simplicity):

$$p\text{Pd(en)}(H_2O)_2 + qCl \pm rH \rightleftarrows (\text{Pd(en)})_pCl_q(H_2O)_2H_{\pm r} \quad (1)$$

where $q=0$ for chloride-free species, $pqr=100$ corresponds to *cis*-$[\text{Pd(en)}(H_2O)_2]^{2+}$ and for example, $pqr=10-2$ indicates *cis*-$[\text{Pd(en)}(OH)_2]$. This equilibrium can also serve to describe other reactions involving chloro and dichloro species (with $q=1$ and 2, respectively). The equilibrium constants ($\log \beta_{pqr}$) for the hydroxo species were obtained from 287 $E(H^+)$ data of chloride-free solutions. Then, these constants (fixed values) and 245 data pairs of $E(H^+)$ and $E(Cl^-)$ were used to obtain the $\log \beta_{pqr}$ of chloro-containing species. All these equilibrium constants were refined by rigorous least-squares calculations using the SUPERQUAD program [21]. The calculations were performed with $\sigma_v=0.002$ ml, $\sigma_E=0.1$ mV for the H^+ electrode and $\sigma_E=0.2$ mV for the Cl^- electrode. The corresponding fitted values of $\log \beta_{pqr}$ were used to simulate alkalimetric titrations of *cis*-$[\text{Pd(en)}(H_2O)_2](ClO_4)_2$ and *cis*-$[\text{Pd(en)}Cl_2]$ solutions. Also a variety of distribution diagrams were obtained.

3. Results and discussion

3.1. Potentiometric titrations

Three representative alkalimetric titration curves of *cis*-$[\text{Pd(en)}(H_2O)_2](ClO_4)_2$ and *cis*-$[\text{Pd(en)}Cl_2]$ with similar concentrations are shown in Fig. 1. Both products exhibit a behaviour corresponding to two weak different acidities, with one inflection in the titration curves to a (eq. base/mole Pd(II)) = 1. In chloride-free solutions, we can expect that the diaqua complex ($pqr=100$) reacts with OH^- ions to give hydroxo species. However, the assumption that only mononuclear hydroxo ($pqr=10-1$) and dihydroxo ($pqr=10-2$) complexes are formed is not reasonable. Suggestions have been made about the formation of a di-μ-hydroxo dinuclear species $[\text{Pd(en)}(OH)_2\text{Pd(en)}]^{2+}$ ($pqr=20-2$) during the first step ($0 \le a \le 1$) of the diaqua complex hydrolysis [4,13a,14]. In accordance with such a proposal is the remarkable difference in the two acidities of the diaqua species (100). On the other hand, the assumption that the first hydrolytic step of the diaqua complex gives only the dinuclear species ($20-2$) does not seem acceptable. In this regard, it is interesting to note that 'apparent' pK_1 and

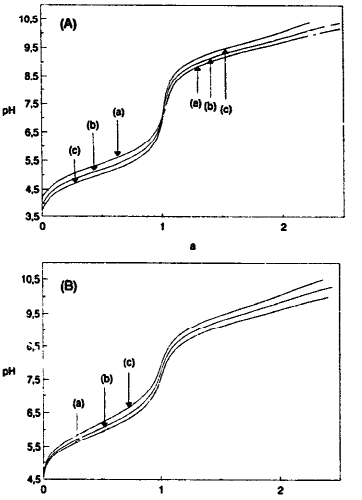

Fig. 1. Alkalimetric titrations of *cis*-$[\text{Pd(en)}(H_2O)_2]^{2+}$ (A) and *cis*-$[\text{Pd(en)}Cl_2]$ (B) solutions with NaOH ~0.1 M: (a) 1.112, (b) 2.288, (c) 4.561 mM.

pK_2 values (which are approximately the pH values at $a=0.5$ and 1.5, respectively, in the curves of Fig. 1(A)) decrease and increase, respectively, as the total molar concentration of the diaqua complex increases (Fig. 1(a)–(c)). Such 'decreasing or increasing' trends of these pK_a values strongly suggest that: (i) the first hydrolytic step gives more than one hydroxo species with an OH/Pd ratio of 1/1 (because the pK_1 value does not remain a 'constant' value for various complex concentrations); (ii) the first hydrolytic step involves, at least, the formation of one (or more) oligomeric μ-hydroxo species. Thus the second hydrolytic step can represent the reaction of the mono-hydroxo complex ($10-1$) as well as depolymerisation processes of the polynuclear complexes (having OH/Pd ratio 1/1) with OH^- ions to give only the mononuclear dihydroxo species ($10-2$). The 'apparent' pK_a values obtained from potentiometric curves of Fig. 1(A) suggest that the formation of poly-μ-hydroxo complexes is favoured as the total molar concentration of the diaqua complex increases, whereas the formation of the dihydroxo species ($10-2$) will be inhibited in the referred second step. The oligomerisation of *cis*-aqua-hydroxo–Pd(II) and –Pt(II) complexes is well known because the water is a good leaving group in complexes of both metal ions. In this connection, the chloro ligand is a leaving group as poor as the aqua one. Consequently, the titration curves of *cis*-$[\text{Pd(en)}Cl_2]$ (Fig. 1(B)) show the expected increase of 'apparent' pK_1 and pK_2 values as the total concentration of

Table 2
Formation constants (log β_{pqr}) of hydroxo and/or chloro complexes related with *cis*-diaqua(ethylenediamine)palladium(II) and *cis*-dichloro-(ethylenediamine)palladium(II). $I=0.15$ mol dm^{-3} ($NaClO_4$), $t=37$ °C

Complex [a]	pqr	log β	
Aqua-hydroxo	10−1	−6.68(10) [c]	
Di-μ-hydroxo	20−2	−7.758(4) [c]	$Z=287$ [b], $\sigma=3.87$, $\chi^2=11.68$
Dihydroxo	10−2	−14.523(2)	
Dichloro	120	5.24(1)	
Chloro-aqua	110	3.18(1)	$Z=245$ [b], $\sigma=1.76$, $\chi^2=5.93$
Chloro-hydroxo	11−1	−3.75(4)	

[a] Univalent ligands bounded to one ($p=1$) or two ($p=2$) Pd(en) chelate moieties.
[b] Z= Total number of experimental data points used in the refinement.
[c] Log K_D = log β_{20-2} − 2log β_{10-1} = 5.602.

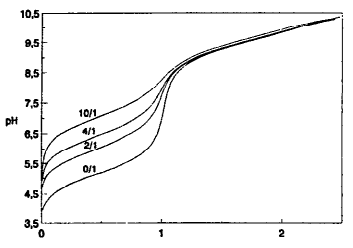

Fig. 2. Alkalimetric titrations with NaOH of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ solution (2.288 mM) (0/1) and several mixed solutions of Cl^-/*cis*-$[Pd(en)(H_2O)_2]^{2+}$ having molar ratios of 2/1, 4/1 and 10/1.

this complex in solution increases. In addition, for a given concentration of the diaqua complex (100), the titration curves of Fig. 2 reveal that the increasing chloride ion concentration promotes only an increase of the 'apparent' pK_1 value. This behaviour is qualitatively explained as a consequence of the inhibition effect of chloro complex formation upon the several hydrolytic reactions involved in the referred 'first step'. To obtain a more informative view of the hydrolytic reactions of *cis*-diaqua(ethylenediamine)palladium(II) in aqueous solutions with or without chloride ions, we have carried out a variety of potentiometric experiments to fit the corresponding complexation model and formation constants (log β_{pqr}). This study will provide valuable information about the abundance of each one of the species involved in a variety of conditions (pH, $[Cl^-]$, [Pd(II)-drug]) such as those of the biochemical or biological probes.

3.2. *Equilibrium constants*

Starting from solutions of the *cis*-$[Pd(en)Cl_2]$ complex, with addition of NaOH (only) or NaCl (only) or both titrant reagents, SUPERQUAD calculations with the $E(H^+)$ and/or $E(Cl^-)$ potentiometric data results were unable to fit a coherent complexation model and a formation constant set for both expected hydroxo and chloro complexes. However, on the basis of previous approaches [4,14] used to study the hydrolysis of *cis*-$[Pd(en)Cl_2]$ and other related complexes [12,13] we have successfully applied a strategy in two steps. First, the hydroxo complex formation constants (log β_{pqr}) were obtained from $E(H^+)$ potentiometric data of alkalimetric titrations with chloride-free *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ solutions. In a second step, we use these constants as fixed values to fit the chloro complex formation constants from $E(H^+)$ and $E(Cl^-)$ data pairs simultaneously obtained in titrations with chloride ion of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ solutions partially hydrolysed with NaOH. Experimental data of the solutions used for calculations are shown in Table 1. The results obtained in the treatment of these data by the SUPERQUAD program are shown in Table 2. In the experimental conditions of the present work, the hydrolysis model fitted for *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ in chloride free-solutions involves the formation of two mononuclear complexes (aqua-hydroxo *cis*-$[Pd(en)(H_2O)(OH)]^+$ ($pqr=10-1$) and dihydroxo *cis*-$[Pd(en)(OH)_2]$ ($pqr=10-2$)) as well as one dinuclear di-μ-hydroxo complex $[Pd_2(en)_2(OH)_2]^{2+}$ ($pqr=20-2$). In this sense, it is interesting to note that higher oligomers (e.g. trinuclear μ-hydroxo complexes) can be expected in more concentrated solutions. Indeed evidence of such polynuclear species has been obtained for analogous Pd(II) and Pt(II) systems [15]. In the present work, starting from solutions of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ added to NaOH, the titration with chloride ion produces the referred hydroxo complexes as well as dichloro *cis*-$[Pd(en)Cl_2]$ ($pqr=120$), chloro-aqua *cis*-$[Pd(en)Cl(H_2O)]^+$ ($pqr=110$) and chloro-hydroxo *cis*-$[Pd(en)Cl(OH)]$ ($pqr=11-1$) complexes. It is interesting to note that other hypothetical di- or polynuclear species (i.e. of the Pd(en)(OH)Pd(en) type, $[Pd(en)OH]_n$ ($n>2$) [15,16] etc.) were rejected from the equilibrium model. Our results for the three hydroxo complexes (with $\sigma\sim3$ and $\chi^2<12.6$ which represents a confidence level >95%) [19] give a good simulation of the experimental alkalimetric titration data points for a representative solution of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ (Fig. 3). In addition, the complete set of formation constants yield a simulated titration curve for *cis*-$[Pd(en)Cl_2]$ in good agreement with the corresponding experimental one.

Table 3
Formation constants (log β_{pqr}) [a] of hydroxo and/or chloro complexes related with *cis*-diaqua(ethylenediamine)palladium(II) [b–e] and with *cis*-diaqua-(diaminosuccinate diethyl ester)palladium(II)

Complex	*pqr*	Pd(en) derivatives				Pd(Et_2dasa) derivatives
		This work	Ref. [14] [c]	Ref. [13a] [d]	Ref. [4] or [16] [e]	Ref. [17] [b]
Aqua-hydroxo	10−1	−6.68(10)	−5.6		−6.2	−5.25
Di-μ-hydroxo	20−2	−7.758(4)		−8.25	−8.33	−6.55
Dihydroxo	10−2	−14.523(2)		−15.43		
Dichloro	120	5.24(1)	5.71			5.86
Chloro-aqua	110	3.18(1)	3.60			3.65
Chloro-hydroxo	11−1	−3.75(4)	−3.75			−2.86

[a] The constant values reported in the literature have been redefined in accordance with the corresponding definitions of β_{pqr} used in this work (see equilibrium (1)).
[b] I = 0.15 M ($NaClO_4$), 37 °C.
[c] I = 0.1 M ($NaClO_4$), 25 °C.
[d] I = 1 M ($NaClO_4$), 25 °C.
[e] I = 0.2 M (KNO_3), 23 °C.

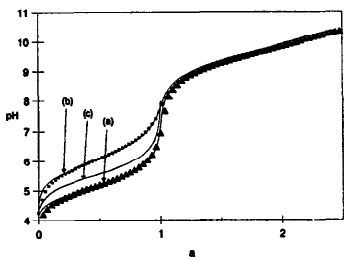

Fig. 3. Experimental potentiometric data points for *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (2.288 mM) (a) and $[Pd(en)Cl_2]$ (2.947 mM) (b) solutions and the corresponding simulated titrations (unbroken lines) with NaOH (0.1021 and 0.1009 M, respectively) added. V_0 = 50 ml. Curve (c) is the simulated titration for the experimental conditions of (a) with Martin's data [16].

The formation constant values obtained in this work agree reasonably well with the corresponding data previously reported by other researchers [13a,14,16] as well as for other related diamino–palladium(II) complexes such as *cis*-diaqua(diaminosuccinate diethyl ester)palladium(II), *cis*-$[Pd(Et_2dasa)(H_2O)_2]^{2+}$ and its hydroxo and/or chloro species [17] (see Table 3). However, the dimerisation constant (log K_D = 5.60) for the equilibrium:

$$2[Pd(en)(H_2O)(OH)]^+ \rightleftarrows 2H_2O + [(en)Pd(\mu\text{-}OH)_2Pd(en)]^{2+}$$

obtained in this study from log β_{20-2} = −7.758 and log β_{10-1} = −6.68 is higher than the value (log K_D = 3.70) reported by Martin [16] for I = 0.02 mol dm^{-3} (KNO_3) and 23 °C. The observed difference (Δ log K_D = 1.90) seems too important to be due to the different conditions of temperature and ionic strength. In addition to the influence of temperature and ionic strength the low value obtained for log K_D by Martin can be attributed to the nature of the background electrolyte (KNO_3) which should produce nitrato complexes with the chelate moiety Pd(en). Accordingly, the simulation of the alkalimetric titration of *cis*-$[Pd(en)(H_2O)]^{2+}$ in the range $0 < a \leq 1$, made with the values of log β proposed by the Martin [16] model, falls between that reported in this work for the diaqua (100) and dichloro (120) complexes (see Fig. 3). The lowering order of the potentiometric curves in Fig. 3 is that of the decreasing ability of the ligands $Cl^- < NO_3^- < H_2O$ for Pd(II).

For a better understanding of the hydrolysis model reported here for *cis*-$[Pd(en)(H_2O)_2]^{2+}$ in the absence or presence of chloride ion, appropriate species-distribution diagrams have been obtained. In chloride-free solutions of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ at representative concentrations 1 mM–1 μM (Fig. 4) a mixture of cationic mono-hydroxo (10−1) and di-μ-hydroxo (20−2) species is produced at pH > 4, the latter being more abundant as the concentration of the diaquo complex increases. Because the dilution favours the dissociation, in a 1 μM solution of the diaqua complex the mononuclear complex (10−1) becomes more abundant than the dinuclear one (Fig. 4(b)). On the other hand, it has been suggested that the hydrolysis of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (and other Pd(II) or Pt(II) related complexes) produces a lowering of the pH values observed in their aqueous solutions (i.e. pH ~ 2 have been reported for mM concentrations) [15]. In the course of our work we have observed that the solutions ~ 1 mM of the diaqua complex (*pqr* = 100) give such low pH values if they are obtained by reaction of stoichiometric amounts of $AgClO_4$ and samples of *cis*-$[Pd(en)Cl_2]$ complex having a small quantity of $[Pd(en)_2][PdCl_4]$ (by-product). The complex $[Pd(en)_2]^{2+}$ (λ_{max} = 295 nm) is easily distinguished from *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (λ_{max} = 345 nm) and $[Pd(en)Cl_2]$ (λ_{max} = 360 nm) in the electronic spectrum of a given solution [14,22,23]. The reaction of the referred by-product with $AgClO_4$ will be:

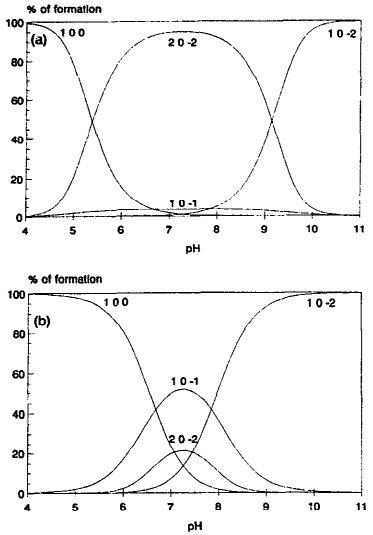

Fig. 4. Distribution diagrams of aqua and hydroxo species in solution of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ as a function of pH and complex concentration: (a) 1 mM; (b) 1 μM. Species indicated as *pqr* codes.

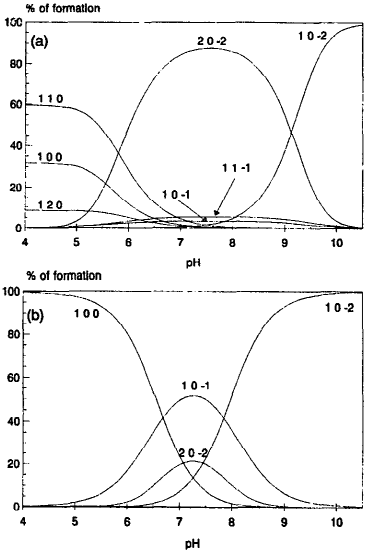

Fig. 5. Distribution diagrams of aqua, hydroxo and chloro species in solution of *cis*-$[Pd(en)Cl_2]$ as a function of pH and complex concentration: (a) 1 mM; (b) 1 μM.

$$[Pd(en)_2][PdCl_4] + 4AgClO_4 \rightleftarrows [Pd(en)_2]^{2+} + [Pd(H_2O)_4]^{2+} + 4ClO_4^- + 4AgCl\downarrow$$

because the cation $[Pd(en)_2]^{2+}$ is identified in the UV–Vis spectrum of the remaining clear solution. The above reaction also produces the ion tetraaquapalladium(II), $[Pd(H_2O)_4]^{2+}$, from which hydrolysis we can expect anomalous low initial pH values. The synthesis of $[Pd(en)Cl_2]$ proposed in this paper avoids the co-precipitation of such a by-product.

In an aqueous solution 1 mM of *cis*-$[Pd(en)Cl_2]$ (120) ($4 < pH < 5$) we can expect chloro ligand substitution by water to give 62% of chloro-aqua (110) and 33% of diaqua (100) complexes in the presence of only 9% of the dichloro species (Fig. 5(a)). The hydrolysis of these three complexes at $pH > 5$ mainly yields the di-μ-hydroxo complex (20−2) in equilibrium with lower amounts of mononuclear aqua-hydroxo complex (10−1) and chloro-hydroxo complex (11−1). In alkaline solution the above three hydroxo complexes give the dihydroxo one (10−2). As expected the hydrolysis of a $[Pd(en)Cl_2]$ aqueous solution begins at a higher pH value than that of $[Pd(en)(H_2O)_2]^{2+}$ aqueous solution, the total molar concentration of the complex being equal. On the other hand, because dilution favours the dissociation processes, Fig. 5(b) shows that a 1 μM solution of *cis*-$[Pd(en)Cl_2]$ (120) indeed contains the diaqua complex (100) and the first hydrolytic step yields monor uclear aqua-hydroxo (10−1) in a higher proportion than the di-μ-hydroxo one (20−2). The significance of the di-μ-hydroxo complex in the hydrolysis model fitted for *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(en)Cl_2]$ makes the relative amounts formed of each species to be dependent on the pH as well as on the chloride ion and/or the complex concentration.

In a practical sense, we can take *cis*-$[Pd(en)Cl_2]$ as an appropriate palladium(II) model for cis-platin (the chelating role of the en ligand prevents *cis* → *trans* isomerisation around the Pd(II) atom). Because the Cl^- concentration remains constant but quite different in blood plasma (104 mM) and in the cell (4 mM), it seems instructive to discuss briefly the distribution species of the studied compound at physiological pH 7.4 as a function of the $pCl = -\log [Cl^-]$ for representative total complex concentrations, 1 mM and 1 μM (Fig. 6). From these diagrams we note the following observations. (i) At physiological pH 7.4, the studied systems become dependent on $[Cl^-]$ and the total complex concentration, the latter factor being more noteworthy at low $[Cl^-]$ (high pCl). (ii) At plasma chloride ion concentration ($pCl = 0.98$) and total complex concentrations of 1 mM or 1 μM, the distribution of species is virtually the same, with dominance of the neutral complexes *cis*-dichloro (120) and *cis*-chloro-hydroxo (11−1), the latter in lower proportions. (iii) On lowering the $[Cl^-]$ the neutral dichloro complex (120) gives the neutral chloro-hydroxo (11−1) and the

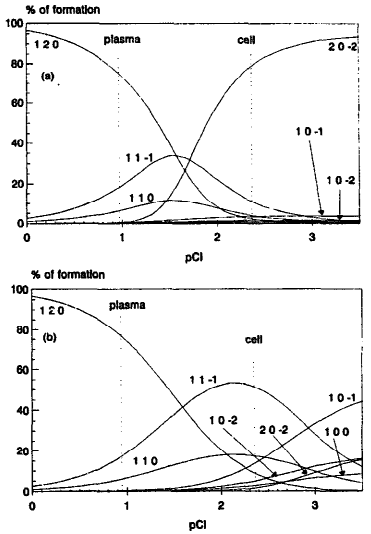

Fig. 6. Distribution of species (indicated by *pqr* codes) in solutions of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ 1 mM (a) or 1 μM (b) at pH = 7.4 as a function of chloride ion concentration ($pCl = -\log[Cl^-]$). Dotted lines correspond to plasma (104 mM) and inside cell (4 mM) Cl^- ion concentrations.

cationic chloro-aqua (110) complexes, but these complexes give different species at lower $[Cl^-]$ depending on the total complex concentration. (iv) In cells (pH 7.4, pCl 2.4) the main species is cationic (20−2) at 1 mM total complex concentration or is neutral (11−1) with a lower proportion of other cationic species (110 and 10−1) at 1 μM total complex concentration.

In conclusion, our results offer a new potentiometric procedure to fit the hydrolytic model and the corresponding formation constants of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(en)Cl_2]$ which gives simulation curves in good agreement with the experimental ones. In addition to a variety of mononuclear species, only a dinuclear di-μ-hydroxo complex is involved in the model. The studied hydrolytic systems are dependent on the pH as well as on the chloride ion concentration and/or the total complex concentration. Regarding *cis*-$[Pd(en)Cl_2]$ as a model compound for cis-platin, in physiological plasma the main species are *cis*-dichloro (120) and *cis*-chloro-hydroxo (11−1), whereas inside cells the main hydrolytic products are the cationic di-μ-hydroxo complex (20−2) or the neutral *cis*-chloro-hydroxo complex (11−1) at 1 mM or 1 μM total molar complex concentrations, respectively.

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