12 Hulmi, S.P., Forstrom, J., Sipponen, P., and Vilska, J., Mushroom poisoning by *C. speciosissimus*. A report of four cases. Duodecim, Helsinki *90* (1974) 1044.
13 Kurnsteiner, H., and Moser, M., Isolation of a leathal toxin from *Cortinarius orellanus*. Mycopathologia *74* (1981) 65.
14 Morris, H.R., Williams, D.H., and Ambler, R.P., Determination of the sequences of protein-derived peptides and peptide mixtures by mass spectrometry. Biochem. J. *125* (1971) 180.
15 Nieminen, L., Mottonen, M., and Heikkila, H., Damage caused by two Finnish mushrooms, *Cortinarius speciossisimus* and *Cortinarius gentilis* on the rat kidney. Z. Naturforsch. *30C* (1975) 668.
16 Sawyer, H., Neurohyophysial hormones. Pharmac. Rev. *13* (1961) 225.
17 Short, A.I.K., Watling, R., MacDonald, M.K., and Robson, J.S., Poisoning by *Cortinarius speciossisimus*. Lancet *2* (1980) 942.
18 Sieber, P., Riniker, B., Brugger, M., Kamber, B., and Rittel, W., Menschliches Calcitonin. VI. Die Synthese von Calcitonin M. Helv. chim. Acta *53* (1970) 2135.
19 Tebbett, I.R., Mechanism of action of Cortinarius toxins – a hypothesis, in preparation.
20 Tebbett, I.R., and Caddy, B., Analysis of Cortinarius mushrooms by HPLC. J. Chromat. *268* (1983) 535.
21 Tebbett, I.R., and Caddy, B., Analysis of Cortinarius toxins by reversed phase HPLC. J. Chromat., in press.
22 Tebbett, I.R., Kidd, C.B.M., Caddy, B., Robertson, J., and Tilstone, W.J., Trans. Br. Mycol. Soc., in press.
23 Testa, E., *Cortinarius orellanus* Fr. non Quelet. Indagini sulla tossicita dei funghi del genre Cortinarius. Rass. micol. tic. *2* (1970) 89.
24 Wieland, T., and Wieland, O., The toxic peptides of Amanita species, in: Microbial Toxins, vol. 8, p. 249. Eds S. Kadis, A. Ciegler and S.J. Ajl. Academic Press, New York 1972.
25 Woods, K.R., and Wang, K.T., Separation of dansyl-amino acids by polyamide layer chromatography. Biochim. biophys. Acta *133* (1967) 369.

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Polarographic activity of the antitumor drug cis-dichlorodiammineplatinum (II). The effect of hydrolysis and trans-isomerization of the drug[1]

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Summary. Electrochemical activities of cis-dichlorodiammineplatinum(II) (cis-DDP) and its trans isomer were studied by classical and differential pulse polarography (d.p.p.). It was shown that both isomers yielded a polarographic step or peak at about – 1.6 V (vs. Ag/AgCl), which corresponded to electroreduction of the complex and to catalytic hydrogen evolution. This signal was easily measurable with the aid of d.p.p. and was suitable for investigation of the extent of hydrolysis and trans-isomerization of cis-DDP leading to the formation of toxic products. The detection limit for determination of cis-DDP and its trans isomer by d.p.p. was 1×10^{-6} mol/l.

Introduction

Cis-dichlorodiammineplatinum(II) (cis-DDP) is a cytostatically active compound which is now widely used in combination therapy of different types of tumors[6,12,15]. However, the treatment is often accompanied by toxic effects, some of which could be ascribed to the action of platinum derivatives occurring in the drug sample as a consequence of improper handling or storage. It has been shown, for instance, that hydrolyzed cis-DDP is extremely toxic in vivo[17], even though it can be rapidly converted into the parent compound at the relatively high chloride ion concentration of the extracellular medium. Another process which could negatively affect the antitumor activity of the cis isomer is ultraviolet radiation-induced transformation into its trans-form (trans-DDP)[13], which is more toxic and cytostatically inactive[16].

Hydrolysis of cis-DDP as a function of chloride ion concentration, pH and temperature was studied by several authors[7,9,10,11,14], and the resulting products were identified. In a connection with investigation of the use of polarographic methods for the determination of platinum in biological materials[4,21], we tested the polarographic behavior of cis-DDP and found suitable conditions for simple, rapid and precise determination of the fractions of intact and hydrolyzed cis-DDP in solution and for the detection of trans-DDP in samples of cis-DDP.

Material and methods

Cis-DDP was obtained from Research Institute of Pure Chemicals, Lachema (Brno, Czechoslovakia). Trans-DDP was a generous gift of Professor B. Rosenberg. All cis-DDP and trans-DDP solutions analyzed in this work were prepared at low illumination intensity and stored in darkness at 28 ± 1 °C. They were used only after 6-day equilibration unless otherwise stated. The diaquodiammineplatinum(II) (cis-$[Pt(NH_3)_2(H_2O)_2]$ $(NO_3)_2$), was prepared by the addition of 1 equivalent of silver nitrate to cis-DDP. After standing overnight in the dark at 28 °C, the silver chloride formed was removed by filtration. Human albumin was obtained from Imuna (Šarišské Michalany, Czechoslovakia).

Polarographic measurements were performed with an EG&G PARC Polarographic Analyzer, Model 174A. A 3-electrode system was used, comprising an EG&G PARC Static Mercury Drop Electrode (SMDE) Model 303 (medium drop size), a spectrosopic graphite counter electrode and a silver/silver chloride (saturated KCl) reference electrode. All polarograms were recorded at 25 °C at a voltage scan rate of 0.002 V s^{-1}; drop time control of the Model 174A Polarographic Analyzer was set at 1.0 sec. Differential pulse polarographic (d.p.p.) curves were obtained with a pulse amplitude of −25 mV. The test solutions were always deoxygenated by purging with argon for about 6 min; an argon atmosphere was maintained in the cell throughout data acquisition. Compounds analyzed by polarography had to be dissolved in a medium with an ionic strength of at least 0.01 to attain sufficient conductivity of the tested solution. Therefore, sodium perchlorate was added to the sample to give an ionic strength of 0.01 immediately before initiating polarographic experiments with a platinum complex dissolved and equilibrated in distilled water. All polarographic measurements described below were carried out in sodium chloride or sodium perchlorate solutions, the pH of which was in the range 5.5–5.7. Spectra were measured at 25°C with a Beckman DU-8 recording spectrophotometer equipped with a thermostat-controlled cuvette holder.

Results

Polarography of cis-dichlorodiammineplatinum(II) and its hydrolyzed forms. With the aim of developing a method for the determination of intact cis-DDP, the polarographic behavior of this platinum complex was investigated in various media. Cis-DDP dissolved in 1.0 mol/l sodium chloride yielded on direct current (d.c.) polarograms a step with a halfstep potential ($E_{1/2}$) around −0.2 V (step P) (fig. 1). A maximum was superimposed on this step[5]. At potentials more negative than that of zero charge (p.z.c.) (around −0.5 V) the polarographic current decreased almost to that of the background electrolyte. Only at potentials around −1.5 V did the second step (step N) appear on d.c. polarograms, the height of which was directly proportional to the concentration of cis-DDP. At concentrations higher than 5×10^{-4} mol/l the step N was peak-shaped (fig. 1). The measurability of the current responsible for the origin of the step N was significantly improved if d.p.p. was applied (fig. 1). D.p.p. peak around −1.5 V (peak N) increased linearly with growing concentration of cis-DDP in the concentration range $1 \times 10^{-6} - 1 \times 10^{-3}$ mol/l (the correlation coefficient was higher than 0.99). The potential of peak N was shifted with increasing cis-DDP concentration to more negative values. For instance a cis-DDP concentration increase from 1×10^{-5} mol/l to 5×10^{-4} mol/l shifted the peak by 30 mV. The peak N was increased if hydrochloric acid was added to the solution of 1×10^{-5} mol/l cis-DDP in 1.0 mol/l sodium chloride. The presence of the acid at a concentration of 1×10^{-4} mol/l led to roughly a doubling of the peak N; the potential of this peak was simultaneously shifted by 60 mV to more positive values. Moreover, the height of the d.p.p. peak N of cis-DDP was seriously affected by addition of albumin. This protein, at a concentration of 0.01%, approximately doubled the height of peak N yielded by 5×10^{-5} mol/l cis-DDP in 0.1 mol/l sodium chloride. On the other hand addition of albumin to this solution of cis-DDP decreased the maximum superimposed on d.c. polarographic step P. D.p.p. behavior of cis-DDP was dependent on the concentration of chloride ions in the medium (fig. 2). The potential of peak N was shifted to more positive values with increasing concentration of sodium chloride. The peak N was lowered with decreasing concentration of sodium chloride at the salt concentrations lower than 0.1 mol/l. At higher concentrations of sodium chloride the height of peak N was independent of the salt concentration. A solution of 5×10^{-5} mol/l cis-DDP in 0.01–0.1 mol/l sodium perchlorate yielded no d.p.p. peak in the potential range from 0.0 to −1.7 V. Similarly no d.p.p. peak was yielded by cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ in the perchlorate media. If, however, cis-DDP was measured in these media at a concentration higher than 1×10^{-4} mol/l, d.p.p. peaks around −0.2 V and −1.5 V were observed (table 1). D.p.p. curves were also recorded for 2.5×10^{-3} mol/l cis-DDP dissolved in distilled water on the one hand and in 1.0 mol/l potassium chloride on the other. Both samples were incubated 6 days in the dark at 25°C and immediately before the d.p.p. analysis the solution of 1.0 mol/l sodium perchlorate was added up to a concen-

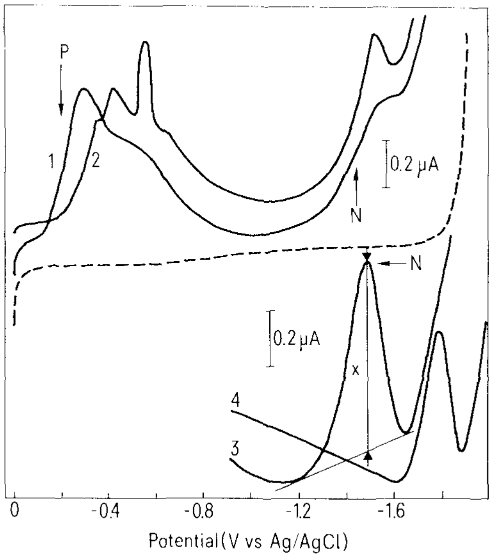

Figure 1. Polarograms of cis-DDP in 1.0 mol/l sodium chloride (curves 1–3) and in 0.01 mol/l sodium perchlorate (curve 4). D.c. polarography: curve 1, 1×10^{-4} mol/l cis-DDP; curve 2, 1×10^{-3} mol/l cis-DDP (this curve was recorded at 10 × lower sensitivity of the polarographic analyzer than indicated); Differential pulse polarography: curves 3 and 4, 5×10^{-5} mol/l cis-DDP; (– – – – –) background electrolyte curve for curve 1. The value x was taken to represent the height of d.p.p. peak N.

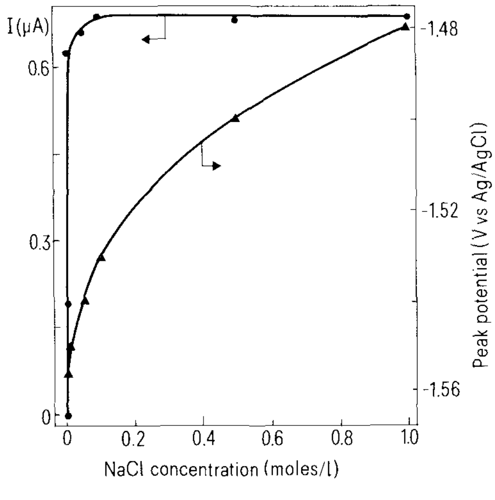

Figure 2. Dependence of the height (●) and potential (△) of d.p.p. peak N yielded by 5×10^{-5} mol/l cis-DDP on sodium chloride concentration.

Table 1. Comparison of polarographic and spectrophotometrical data obtained for solutions of 7.40 × 10^{-4} mol/l cis-DDP equilibrated in media containing various concentrations of sodium chloride

NaCl Concentration	h(μA)[a]	A_{300}[b]	% Intact drug Polarographic measurements[c]	Absorbance measurements[d]
0	2.66[e]	0.0632	26	30
0.005	5.74[e]	0.0772	56	59
0.01	6.97	0.0839	68	73
0.05	9.02	0.0889	88	83
0.1	10.15	0.0942	99	94
0.5	10.25	0.0972	100	100
1.0	10.25	0.0972	100	100

[a] The height of d.p.p. peak at −1.5 V; [b] Absorbance at 300 nm; [c] The percentage of intact cis-DDP was calculated using equation (1) of this paper (see Results); [d] The percentage of intact cis-DDP was calculated using equation (1) of the paper of Greene et al.[7]; [e] The solution of 1.0 M sodium perchlorate was added to the solution of cis-DDP immediately before DDP measurement so that the resulting concentration of the perchlorate was 0.01 mol/l. The values of h were corrected for the change in cis-DDP concentration induced by this addition.

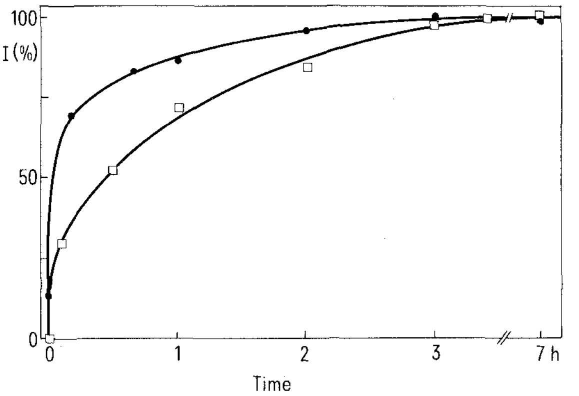

Figure 3. Time dependence of changes induced by addition of chloride ions in height of d.p.p. peak N for a hydrolyzed cis-DDP solution. The initial solution was prepared by dissolving cis-DDP at a concentration of 1.08 × 10^{-4} mol/l (●) and 1.08 × 10^{-5} mol/l (□) in 0.01 mol/l sodium perchlorate. After aging the solution (see Material and methods) chloride ion concentration was increased to 0.3 mol/l by the addition of 4.0 mol/l sodium chloride so that the concentration of platinum in the samples subjected to d.p.p. measurement was 1.0 × 10^{-4} mol/l (●) and 1.0 × 10^{-5} mol/l (□). The peak height of 100% corresponded to 1.38 μA for 1 × 10^{-4} mol/l cis-DDP and to 0.14 μA for 1 × 10^{-5} mol/l cis-DDP.

tration of 0.01 mol/l. The d.p.p. peak N of the sample of cis-DDP in 1.0 mol/l potassium chloride was roughly 3 times higher than that of cis-DDP incubated in distilled water.

As mentioned in the preceding paragraph, the solutions of 5 × 10^{-5} mol/l cis-DDP and cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ in 0.01–0.1 mol/l sodium perchlorate yielded no d.p.p. peak at potentials ranging from 0.1 V to − 1.7 V. Only at potentials of about −1.8 V did the solutions of cis-DDP and its diaquo-form (prepared by the addition of silver nitrate) in 0.01 mol/l sodium perchlorate yield a new d.p.p. peak (fig. 1). It was, however, interesting that solutions of cis-DDP or cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ incubated in distilled water to which sodium perchlorate was added immediately before the d.p.p. measurement were d.p.p. inactive. These solutions yielded a peak at − 1.8 V only after incubation in the presence of sodium perchlorate for at least 1 day (in the dark at 28 °C). If sodium chloride was added to the solution of 1 × 10^{-5} mol/l cis-DDP in 0.01 mol/l sodium perchlorate in such a way that the concentration of chloride ions was 0.3 mol/l, the d.p.p. peak N appeared again (around the potential of −1.5 V). This peak increased with the time of incubation at 28 °C, while its potential was not changed. After 4 h the peak reached its maximum height, which did not change in the course of further incubation (fig. 3). The height and potential of the peak N corresponding to the limiting part of the time dependence were identical to the parameters of peak N yielded by cis-DDP dissolved in the same concentration directly in 0.3 mol/l sodium chloride. A similar result was obtained if cis-DDP was incubated in a medium containing chloride ions at a concentration of 1.0 mol/l. The d.p.p. peak N increased more rapidly, however, and it reached its maximum height after only 2 h (table 2).

The equilibrium between cis-DDP and products of its hydrolysis was also investigated by means of absorption spectrophotometry. The absorption spectrum of cis-DDP dissolved in distilled water or in 0.01 mol/l sodium perchlorate exhibited a maximum at 268 nm (fig. 4), characteristic of fully hydrolyzed cis-DDP[7]. After 1-week incubation in the presence of increasing concentrations of sodium chloride, this maximum gradually decreased, and a new maximum at 300 nm appeared. The set of absorption spectra exhibited isosbestic points at 273, 324 and 360 nm. At sodium chloride concentrations higher than 0.1 mol/l the spectrum already remained constant, the position of the near-ultraviolet maximum (300 nm) and its molar extinction coefficient (130 l mol^{-1} cm^{-1}) being characteristic of intact cis-DDP. Similar spectral changes were observed in a kinetic experiment when sodium chloride was added at a concentration of 4.0 mol/l to cis-DDP dissolved in 0.01 mol/l sodium perchlorate, to obtain a concentration of chloride ions of 1.0 mol/l. After 60 min the maximum at 268 nm practically disappeared and the peak at 300 nm reached a maximum value which, similarly to the height of the d.p.p. peak, did not change with further incubation (table 2).

Polarography of trans-dichlorodiammineplatinum(II). Similarly to cis-DDP, in the medium of 1.0 M sodium

Table 2. Changes induced in polarographic and spectrophotometrical behavior of a hydrolyzed cis-DDP solution by addition of chloride ions, plotted against time[a]

Time (min)	h (μA)[b]	A_{300}[b]	% Intact drug[b] Polarographic measurement	Absorbance measurement
0	2.29	0.0537	26	29
3		0.0783		87
6	7.83	0.0797	89	91
10	8.00	0.0803	91	92
17	8.27	0.0820	94	96
32	8.44	0.0827	96	98
90	8.70		99	
120	8.80	0.0833	100	100
240	8.80	0.0833	100	100

[a] The initial solution was prepared by dissolving cis-DDP at a concentration of 8.53 × 10^{-4} mol/l in 0.01 mol/l sodium perchlorate. After aging the solution (see Material and methods) chloride ion concentration was increased to 1.0 mol/l by the addition of 4.0 mol/l sodium chloride so that the concentration of elementary platinum in the sample subjected to polarographic and absorbance measurements was 6.4 × 10^{-4} mol/l; [b] The meaning of symbols and the calculation of % intact drug are the same as in table 1.

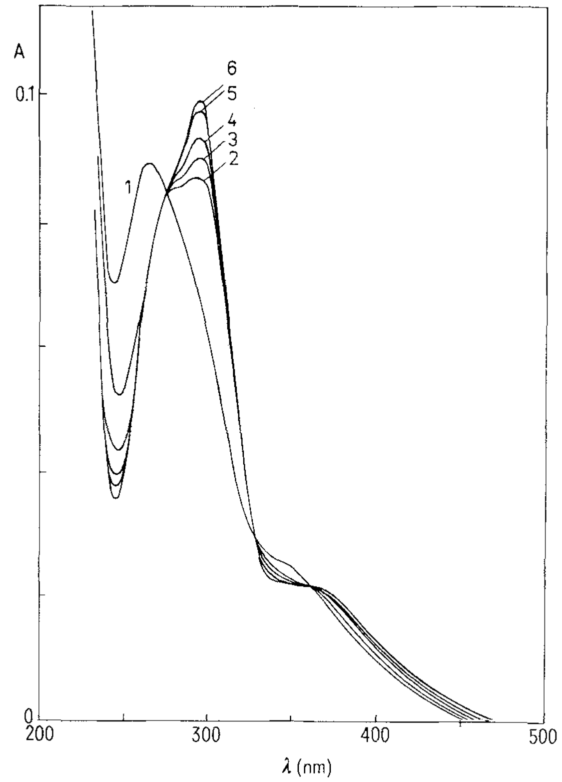

Figure 4. Absorption spectra of 7.48×10^{-4} mol/l cis-DDP equilibrated in media containing various concentrations of sodium chloride: 1, 0 mol/l; 2, 0.01 mol/l; 3, 0.05 mol/l; 4, 0.1 mol/l; 5, 0.3 mol/l; 6, 0.5 1.0 mol/l.

chloride trans-DDP yielded a step with $E_{1/2}$ in the vicinity of −0.2 V (step P) on d.c. sampled polarograms (fig. 5a). At trans-DDP concentrations higher than 5×10^{-5} mol/l a maximum peaking at about −0.3 V was superimposed on the step P. Contrary to the behavior of cis-DDP, the polarographic current yielded by trans-DDP at potentials more negative than p.z.c. was considerably higher than that yielded by the background electrolyte. At potentials of about −1.6 V trans-DDP dissolved in 1.0 mol/l sodium chloride gave a second step (step N) on d.c. polarograms, similarly to cis-DDP. However, in contrast to cis-DDP, the trans isomer yielded 2 d.c. polarographic steps even in the medium of sodium perchlorate (fig. 5b). The more positive step P had $E_{1/2}$ at about −0.03 V in a medium of 0.01 mol/l sodium perchlorate and no superimposed maximum appeared even at trans-DDP concentrations higher than 1×10^{-4} mol/l. $E_{1/2}$ of the step N was about 30 mV more negative than $E_{1/2}$ of trans-DDP in 1.0 mol/l sodium chloride (fig. 5b). Similarly to cis-DDP behavior, the polarographic current corresponding to the step N increased after the addition of hydrochloric acid and was considerably more easily measurable by means of d.p.p. (fig. 5).

D.p.p. peak of trans-DDP was directly proportional to its concentration in both media, sodium chloride and sodium perchlorate. The lower limit of analytical utility of d.p.p. for the determination of trans-DDP is approximately 1×10^{-6} mol/l. The addition of albumin to a solution of trans-DDP led to an increase of the peak N in the same way as in the case of cis-DDP. Similarly, the addition of the protein decreased the maximum on the step P yielded by trans-DDP in media containing chloride ions. The reproducibility of polarographic measurements of solutions of cis-DDP and trans-DDP deteriorated if these solutions were in contact with mercury for a longer time. It was therefore necessary to keep this contact as short as possible. For instance, if a normal dropping mercury electrode (DME) (not SMDE) is used, it is recommended to deoxygenate the solution in a separate vessel with a subsequent short deoxygenation in the polarographic cell in the presence of DME. Similarly, if the polarographic record has to be repeated, this should be done with a new identical solution of the platinum compound. Other details of our polarographic measurements have been published elsewhere[4,21]. It follows from the above polarographic properties of the 2 platinum complexes that it is possible to find conditions under which the d.p.p. behavior of cis-DDP and trans-DDP is quite different: in the medium of 0.01 mol/l sodium perchlorate 5×10^{-5} mol/l cis-DDP is d.p.p.-inactive, whereas trans-DDP yields a distinct peak N (fig. 5b). This fact offers an opportunity of determining the content of the trans isomer in preparations of cis-DDP. We recorded d.p.p. curves of solutions containing 5×10^{-5} mol/l cis-DDP and 0.05–1×10^{-5} mol/l trans-DDP in 0.01 mol/l sodium perchlorate (the solutions were incubated in darkness at 28 °C for 6 days). The solutions which contained trans-DDP in concentrations higher than 2.5×10^{-6} mol/l yielded a peak in the vicinity of: 1.54 V: the height of this peak was directly proportional to trans-DDP concentration in the analyzed solutions.

Assay methods for cis-DDP stability. On the basis of our results described above 2 methods for the assay of cis-DDP stability based on the polarographic activity of this drug are proposed:

1. *Decomposition of cis-DDP involving displacement of the chloride ligands.* The method can be used for analysis of solutions of cis-DDP in distilled water or for aqueous solutions of cis-DDP containing chloride or perchlorate ions (or both) at a concentration of at least 10^{-2} mol/l. If solutions of cis-DDP in distilled water are to be analyzed, sodium perchlorate must be added, as mentioned above. The standard sample is prepared by dissolving solid cis-DDP in 1.0 mol/l sodium chloride. The concentration of cis-DDP in the standard sample is adjusted in such a way that the concentration of elementary platinum is identical to that of the sample to be analyzed. The solution is then incubated in the dark for at least 24 h. D.p.p. curves (initial potential −1.00 V, voltage scan direction negative) are recorded for the standard and unknown samples. The content of intact cis-DDP in percent (Y) is calculated from the equation

$$Y(\%) = 100 \frac{h_t}{h_{100}}$$

were h_t is the height of d.p.p. peak at −1.4 to −1.6 V recorded for the unknown sample and h_{100} is the height of the same peak recorded for the standard sample.

2. *Trans-isomerization of cis-DDP.* The method can be used for determination of the trans isomer in solutions of cis-DDP in distilled water or in 0.01 mol/l sodium

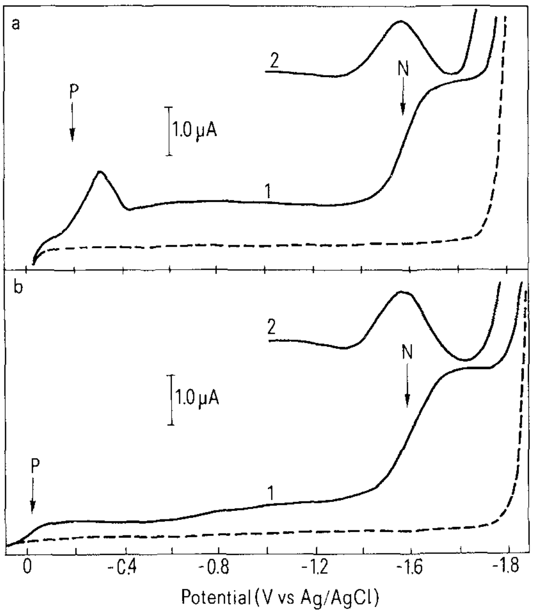

Figure 5. Polarograms of trans-DDP in 1.0 mol/l sodium chloride (a) and 0.01 mol/l sodium perchlorate (b). Curves 1) d.c. polarograms of 1×10^{-4} mol/l trans-DDP, curves 2) d.p.p. curves of 5.0×10^{-5} mol/l trans-DDP, (– – – – –) d.c. polarographic background electrolyte curves.

perchlorate. If solutions of cis-DDP in distilled water are to be analyzed sodium perchlorate must be added as specified above. The concentration of a cis-DDP sample to be analyzed for trans-DDP content is adjusted to a value corresponding to 5×10^{-5} mol/l of elementary platinum. The d.p.p. curve is recorded for this sample under the same conditions as in the case of the preceding method. If a peak appears on the d.p.p. curve around −1.6 V, the content of trans-DDP is determined by the standard addition method. A solution of 1×10^{-3} mol/l trans-DDP in 0.01 mol/l sodium perchlorate can be used as the standard. Before use the standard solution has to be equilibrated at least 1 day in the dark at 28 °C.

Discussion

The more positive polarographic step P yielded by the nonhydrolyzed cis-DDP (fig. 1) corresponds to the electroreduction of this complex[5,19,20]. A decrease in the limiting polarographic current observed at potentials more negative than about −0.5 V is connected with the fact that cis-DDP has a dipole moment[11,19,20]. It has been suggested that a change in the electrode charge induces a change in the orientation of adsorbed molecules of cis-DDP, which is unfavorable for the electron transfer[11,20]. However, at potentials more negative than about −1.3 V the planar structure of the complex becomes distorted and a direct transfer of electrons from the electrode to the platinum central atom occurs again. The more negative step N analytically exploited in this study might thus correspond to the renewed reduction of cis-DDP.

However, in a contrast to the step P the step N increases with increasing concentration of hydrogen ions in the medium. Thus in a medium containing 5×10^{-4} mol/l hydrochloric acid it is several times higher than the reduction step P. Moreover the step N is also yielded by the trans isomer (fig. 5), which has no dipole moment[11,20], and on whose polarograms no decrease of the limiting current at potentials more negative than − 0.5 V[5,19] (fig. 5) was therefore observed. Thus, for trans-DDP one cannot expect any renewal of its reduction. It does not therefore seem probable that the appearance of the step N of cis-DDP (fig. 1) is connected only with the electroreduction of the complex.

The dependence of the magnitude of the polarographic current corresponding to the step N on hydrogen ion concentration, the fact that the step N may have the shape of a maximum, and the effect of albumin addition on this step[3] make it conceivable that the polarographic activity of both cis-DDP and trans-DDP in the vicinity of −1.5 V (fig. 1) reflects catalytic evolution of hydrogen. This conclusion is also in accord with results of coulometric measurements performed on a large mercury pool electrode with solutions of cis-DDP and trans-DDP[20], which showed that catalytic hydrogen evolution starts at potentials even more negative than about −1.3 V. We propose that at the potentials corresponding to the step N both cis-DDP and trans-DDP are reduced to a lower oxidation state with formation of catalytically active centers at the surface of the mercury electrode containing platinum atoms. Since the hydrogen overvoltage on platinum is much smaller than on mercury, hydrogen evolution is easier at the platinum centers formed than at the mercury surface. A similar mechanism has been proposed for the explanation of the formation of catalytic steps of some other platinum complexes[3,9,18]. Considering the fact that at potentials more negative than W1.3 V the reduction of cis-DDP is renewed, it is possible to suppose that, besides the catalytic current, the reduction current also participates in the appearance of the step (peak) N.

A comparison of the results of polarographic and spectrophotometric measurements with samples of cis-DDP incubated in different media (tables 1 and 2) indicates that electrochemical reduction of nonhydrolyzed intact cis-DDP is responsible for the appearance of the polarographic step N (or the d.p.p. peak N). This conclusion is in accord with the fact that totally hydrolyzed cis-DDP, i.e. samples with concentrations lower than 5×10^{-5} mol/l incubated either in distilled water or in sodium perchlorate, does not yield the d.p.p. peak N. Moreover, a sample of 2.5×10^{-3} mol/l cis-DDP dissolved in distilled water, which, according to the results of Reishus and Martin[14] contains only 33.2% of intact cis-DDP, 12.4% of the totally hydrolyzed complex and 54.4% of the monochloro-form yielded a d.p.p. peak N approximately 3 times lower than cis-DDP at the same concentration, but dissolved in 1.0 mol/l potassium chloride; it can be assumed that in the latter sample practically all cis-DDP was intact. The latter result thus yields a proof that neither totally hydrolyzed cis-DDP nor its monochloroform give the polarographic step or d.p.p. peak N in the vicinity of W1.54 V. The method for determination of the extent of cis-DDP hydrolysis proposed in this communication (see Results) is based on the fact that the peak N is yielded only by the intact complex. The advantage of the described method lies mainly in its usability for analysis of samples containing cis-DDP in a relatively low concentration (1×10^{-5} mol/l) and in the fact that, in addition to kinetic measurements, it can be easily used for the determination of fractions of intact cis-DDP and its hydrolytic products under equilibrium conditions in different types of biological experiments.

We have also found different d.p.p. behavior of cis-DDP dissolved in distilled water and in 0.01 mol/l sodium perchlorate. This result indicates that the exchange of chloride ligands leads to different products in these 2 media. The trans isomer yields a polarographic signal in the vicinity of W1.6 V independently of the content of chloride ions in the medium (fig. 5). This property prevents the exploitation of polarography for determination of the extent of hydrolysis of this platinum complex. On the other hand, the different behavior of cis-DDP and trans-DDP in 0.01 mol/l sodium perchlorate made it possible to propose a d.p.p. method for determination of the extent of trans-isomerization in samples of cis-DDP (see Results). Considering the limit of analytical usability of d.p.p. for determination of trans-DDP in the presence of cis-DDP, which is 1×10^{-6} mol/l, the method described can be used only when the content of trans isomer in samples of cis-DDP exceeds 5%.

It can be concluded that polarography mainly offers the possibility of relatively very sensitive and rapid determination of the extent of hydrolysis of the antitumor drug cis-DDP. Since this information is of a great importance from the point of view of the biological effect of cis-DDP[10], the use of polarography in this sphere of biological research can be expected in the future.

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3 Alexander, P.W., Hoh, R., and Smythe, L.E., Trace determination of platinum-I. A catalytic method using pulse polarography. Talanta *24* (1977) 543–548.

4 Brabec, V., Vrána, O., and Kleinwächter, V., Determination of platinum in biological material by differential pulse polarography: analysis in urine, plasma and tissue following sample combustion. Colln Czech. Chem. Commun. *48* (1983) 2903–2908.

5 Chakravarty, B., and Banerjea, D., Polarographic behaviour of some platinum(II) and palladium(II) complexes. J. inorg. nucl. Chem. *16* (1961) 288–295.

6 Drobnik, J., Antitumor activity of platinum complexes. Cancer Chemother. Pharmac. *10* (1983) 145–149.

7 Greene, R.M., Chatterji, D.C., Hiranaka, P.K., and Gallelli, J.F., Stability of cisplatin in aqueous solution. Am. J. Hosp. Pharm. *36* (1979) 38–43.

8 Kolthoff, I.M., and Lingane, J.J., Polarography, vol. 2, 2nd edn, pp. 238–298. Interscience Publ., New York 1952.

9 Lee, K.W., and Martin, D.S., Cis-dichlorodiamminoplatinum(II) aquation equilibria and isotopic exchange of chlorine ligands with free chloride and tetrachloroplatinate(II). Inorg. chim. Acta *17* (1976) 105–110.

10 Lippard, S.J., New chemistry of an old molecule: cis-/$Pt(NH_3)_2Cl_2$. Science *218* (1982) 1075–1082.

11 Llopis, J.F., and Colom, F., Platinum, in: Encyclopedia of Electrochemistry of the Elements, vol. 6, pp. 169–275. Ed. A.J. Bard. M. Dekker, Inc., New York 1976.

12 Marcelis, A.T.M., and Reedijk, J., Binding of platinum compounds to nucleic acids with respect to the anti-tumor activity of cis-diamminedichloroplatinum(II) and derivatives. Recl Trav. chim. Pays-Bas Belg. *102* (1983) 121–125.

13 Nikolaev, A.V., Peschevickij, B.I., Beljaev, A.V., and Benediktov, A.B., *Isomerization of cis-dichlorodiammineplatinum(II)* in aqueous solution. Dokl. Akad. Nauk SSSR *230* (1976) 1118–1121 (in Russian).

14 Reishus, J.W., and Martin, D.S. Jr, Cis-dichlorodiammineplatinum(II). Acid hydrolysis and isotopic exchange of the chloride ligands. J. Am. chem. Soc. *83* (1961) 2457–2462.

15 Roberts, J.J., and Thomson, A.J., The mechanism of action of antitumor platinum compounds, in: Progress in Nucleic Acid Research and Molecular Biology, vol. 22, pp. 71–133. Ed. W.E. Cohn. Academic Press, New York 1979.

16 Rosenberg, B., Antitumor activity of platinum coordination complexes. Cancer Chemother. Rep. *59* (1975) 589–598.

17 Rosenberg, B., Platinum complex-DNA interactions and anticancer activity. Biochimie *60* (1978) 859–867.

18 Slendyk, I., Polarographic studies with the dropping mercury cathode. Part XXVI. The decrease of hydrogen overpotential effected by traces of platinum. Colln Czech Chem. Commun. *4* (1932) 335–350.

19 Sundholm, G., On the anomalous polarographic reduction of certain anions VI. An investigation of the polarographic reduction of cis- and trans-dichlorodiammineplatinum(II) and some other inorganic platinum(II) complexes. Suomen Kemistilehti *341* (1968) 306–314.

20 Sundholm, G., On the electrochemical reduction of inorganic platinum(II) complexes on mercury. Commentat. Physico-math. *34* (1968) 39–47.

21 Vrána, O., Kleinwächter, V., and Brabec, V., Determination of platinum in urine by differential pulse polarography. Talanta *30* (1983) 288–290.

0014-4754/84/050446-06$1.50 + 0.20/0