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Redox behaviour of anti-tumor platinum (II) compounds (carboplatin) at solid electrodes

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Abstract: The electroactivity of a cytostatic complex of platinum (II) possessing no halide ligand (carboplatin) has been studied in aqueous media using platinum and carbon paste electrodes. Cyclic voltammetry has been conducted in order to elucidate the redox behaviour of carboplatin as a function of chloride concentration. Reduction was not observed although oxidation was detected. The nature of the compounds formed during the electro-oxidation was directly related to free chloride ions.

At the carbon paste electrode, the reduction of the oxidized species occurred in two steps with the formation of *cis*-platinum structures and a subsequent electrodeposition of platinum particles. The surface modification step at the carbon paste electrode has demonstrated electrocatalytic properties of the electrode towards platinum (II) complexes possessing halide ligands in their structures.

Electrodeposition of platinum ions at a platinum electrode surface as well as a judicious choice of working parameters allows quantitative determinations in the concentration range 3×10^{-4}–1×10^{-5}M.

Keywords: *Anti-tumor platinum (II) agents; carboplatin; cyclic voltammetry.*

Introduction

Continuing the work concerning the electroactivity of cytostatics [1–3] it has been attempted to extend the investigations toward studying platinum (II) derivatives used in cancer chemotherapy [4–6].

The literature concerning the electroanalytical chemistry of pharmacological compounds contains several works relating to the redox behaviour of platinum (II) complexes [7–13]. Most investigators have pointed out difficulties in studying the electrochemistry of these molecules. Indeed, voltammetric reactions generally proceed slowly at the electrode surface giving rise to rather spread out peaks.

This study is devoted to the redox behaviour of *cis*-diammine-1,1-cyclobutane-dicarboxylate platinum (II) (carboplatin, CBDCA) at platinum and carbon based electrodes.

Carboplatin is a newly developed molecule of the platinum anti-tumor series. The molecule has the platinum (II) metal ion in a square-planar coordination and is depicted

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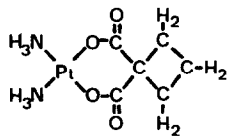
Figure 1
Schematic structure of carboplatin.



in Fig. 1. The cytostatic activity of platinum (II) complexes, such as carboplatin has been reported to be highly related to structure [14, 15].

In order to have anti-tumor activity platinum compounds should at least fulfil the following requirements: (a) presence of amine ligands (primary or secondary) in *cis* position and (b) the ligands X (chloride, sulphate, oxalate, malonate,...,) should be readily labile groups.

Experimental

Instrumentation

Voltammetric measurements were carried out with a Model 175 Universal programmer/Model 174 potentiostat (Princeton Applied Research) and a PAR RE 0074 recorder.

Measurements were performed using a conventional three electrode design at 20.0 ± 0.1°C. The reference electrode was a saturated calomel electrode (S.C.E.) and was placed into a PURLEY tube filled with the electrolyte used for the investigation. Glassy carbon was the auxiliary electrode. Working electrodes were: a carbon paste electrode (Metrohm EA 267) prepared from standard paste (Metrohm EA 207 C) of spectroscopic grade carbon powder and Uvasol liquid paraffin. The other working electrode was a platinum electrode (Radiometer P 101), geometric area = 0.16 cm^2.

The experiments were carried out at pH 6 and the pH of the solutions was measured using a Tacussel 60 pH-meter. All potentials are referred against a saturated calomel electrode (S.C.E.) Tacussel type C 10.

Reagents and solutions

The authors are indebted to the Bristol–Myers International Corp. (Brussels, Belgium) for the generous gift of carboplatin.

To prevent hydrolysis [16–17] and degradation of carboplatin in solution, care was taken to freshly prepare the solutions to be analysed by direct dissolution in double-distilled water. The measurements were carried out as soon as the solutions were ready.

Supporting electrolytes were either 0.1 M sodium perchlorate or 0.1 M sodium sulphate and were prepared with pure grade reagents (Merck PA). Traces of oxygen were removed from the solution to be analysed by passing purified nitrogen first through a filter OXISORB (Messer Griesheim) then through the cell.

Cleaning and activation of the platinum electrode

Before every set of experiments the platinum electrode was cleaned by dipping it into diluted and boiling nitric acid and finally rinsed with double-distilled water.

Activation was essential in order to improve the electrode response and to yield reproducible current–potential curves. The following steps were applied:

Cyclic voltammetry (CV) experimentation (scan rate = 20 mV/s^{-1}) was performed over 10 min in order to deposit platinum particles (platinization) onto the electrode surface from a bath containing 1×10^{-3}M potassium hexachloroplatinate (IV) and 1 M sodium chloride. The potential scan range was performed between −0.5 and +1.0 V.

The prepared platinized platinum electrode was then carefully washed with double-distilled water.

Before each measurement adsorbed chloride was removed by polarization (CV) over six cycles in 0.5 M H_2SO_4 with potential ranges from 0 to +1.5 V (a rest period was then observed at +0.45 V for 2 min). After that, oxidation of the surface was effected by dipping the platinized electrode into a sulpho–chromic solution for 5 min. Finally the electrode was carefully rinsed with double-distilled water and with supporting electrolyte solution.

Preparation of the carbon paste electrode

The procedure, carried out on solutions containing the depolarizer, consisted of applying several CV cycles between −0.8 and +1.5 V for 15 min at a scan rate of 20 mV s^{-1}.

For quantitative determinations the measurements were carried out using the differential pulse technique voltammetry (parameters were: pulse amplitude = 25 mV, pulse repetition rate = 0.5 s, scan rate = 5 mV s^{-1}).

Calibration curves were obtained using the standard addition method. After each run the carbon paste electrode surface was renewed.

The activated platinum electrode was used with the same surface, just stirring the solution between each run.

Results and Discussion

Due to the influence of free chloride ions on the stability of platinum (II) complexes with readily labile groups [14, 16, 17] and on the kinetics of electrode reactions [8, 9], the study has been made in the absence and presence of increasing chloride ion concentrations.

Among the various carbon based electrodes tested (glassy carbon, graphite spray [18] and carbon–polyethylene [19]), in this particular case carbon paste (C.P.E.) was preferred.

Cyclic voltammetry studies at the C.P.E.

In the absence of chloride ions. The investigation of cathodic potentials with an initial potential of +0.5 V showed no detectable current corresponding to carboplatin reduction. On the oxidative side, with an initial potential of +0.5 V an anodic peak (Ep_a) was observed at very positive potentials near the cut-off potential (Fig. 2, curve a). In sodium sulphate solutions the peak occurred at slightly less positive potentials and was better defined than in sodium perchlorate due to a larger available potential range. The electro-oxidative process is diffusion controlled as indicated by the linearity of the relationship between peak current (Ip_a) and the square root of the potential scan rate ($v^{1/2}$) in the range 5–500 mV s^{-1}. The difference between peak potential and half-peak potential indicates a fairly slow process with a high degree of irreversibility (defined in terms of peak potential separation: Ep_a–Ep_c). Indeed changing the potential scan direction after Ep_a produced the appearance of two irreversible cathodic peaks at very

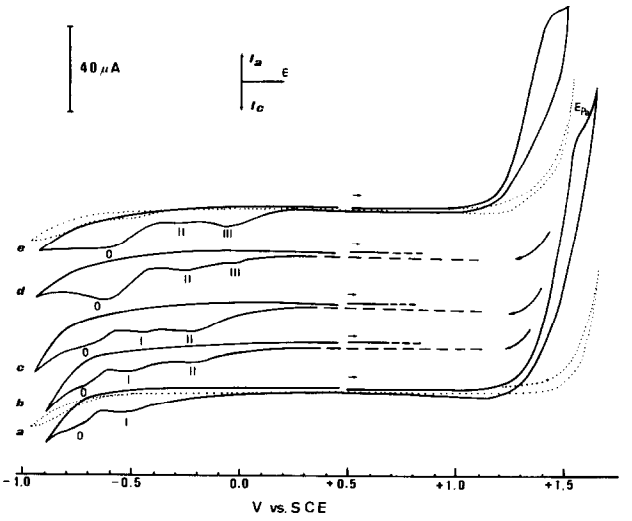


Figure 2
Cyclic voltammograms of 1×10^{-3}M carboplatin in 0.1 M $NaClO_4$ at the C.P.E. Scan rate = 20 mV s^{-1}, starting potential = +0.5 V, initial anodic scan. (a) $Cl^- = 0$, (b) $Cl^- = 10^{-3}$M, (c) $Cl^- = 10^{-2}$M, (d) $Cl^- = 10^{-1}$M and (e) $Cl^- = 1$ M. Surface renewed after each cycling, dotted lines = supporting electrolyte.

negative potentials: Ep_c (I) and Ep_c (0). The heights of these peaks were small compared to Ip_a, this may be attributed to a slow charge-transfer process and to a loss of oxidative products by diffusion away from the electrode surface.

Continued cycling between +1.5 and −0.8 V led to the formation of a new anodic peak Ep_a^* (1) and the appearance of a spread out reduction current which covered the whole range of negative potentials. Maintaining the potential at Ep_c (0), it was possible to see evolution of bubbles at the C.P.E. surface. These phenomena, which were due to a surface modification process were more pronounced in sodium perchlorate than in sodium sulphate.

In the presence of chloride ions. Starting at +0.5 V no cathodic peak corresponding to carboplatin reduction was observed. However, initiating the sweep in the anodic direction oxidation (peak Ep_a) was observed. When the concentration of chloride ions was increased the peak tended to be more rounded and when the concentration exceeded 1×10^{-2}M oxidation occurred more readily but the intensity of the peak was diminished. However, when the scan direction after Ep_a was reversed the influence of chloride was well marked and new reduction peaks appeared as illustrated in Fig. 2. At 1×10^{-3}M, a second reduction peak Ep_c (II) developed whose intensity increased with an increase in chloride ions till 1×10^{-2}M, then diminished to be replaced by a new peak Ep_c (III). Simultaneously Ep_c (I) diminished and was no longer visible at 1×10^{-1}M chloride. Peak Ep_c (0) was continuously present with increasing intensity until 1×10^{-1}M chloride ions after which it diminished (Fig. 2).

Cyclic voltammograms realized as a function of the switching potential ($E\lambda$) showed the irreversible nature of peaks Ep_c (I), Ep_c (II) and Ep_c (III) in the range of scan rates 5–500 mV s^{-1}.

Referring to Fig. 3, the voltammograms of 1×10^{-3}M carboplatin during four successive cycles on the same surface are shown. Changing the sweep direction at $E\lambda_1$, $E\lambda_2$ and

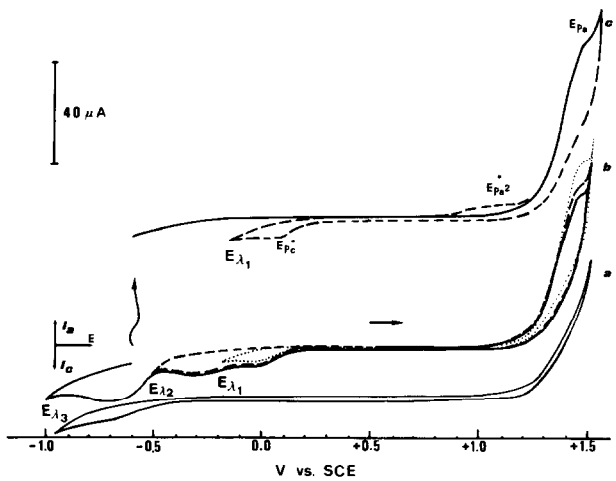


Figure 3
Cyclic voltammograms of 1×10^{-3}M carboplatin in 0.1 M $NaClO_4$ and 1 M NaCl at the C.P.E. as a function of inversion potential $E\lambda$. Scan rate = 20 mV s^{-1}, starting potential = +0.5 V, initial anodic scan. Several cycles on the same surface. (a) supporting electrolyte, (b) (...) first cycle, (– – –) second cycle, (——) third cycle and (c) fourth cycle.

$E\lambda_3$ (cycles 1, 2 and 3) produced no new anodic peak beside Ep_a. However, scanning the potential till $E\lambda_3$ induced a drastic surface modification which was observed ulteriorly. Indeed during the fourth and subsequent cycles (Fig. 3, curve c, dashed lines) when reversing the sweep at $E\lambda_1$ or $E\lambda_2$, formation of a new anodic peak [Ep_a^* (2)], corresponding to the reoxidation of reductants generated during peaks Ep_c (II) or Ep_c (III), appeared. However, peak Ep_a^* (2) was never detected when reversing at $E\lambda_3$, only a small anodic peak Ep_a^* (1) was detected. Peak Ep_a^* (1) was no longer detected at chloride concentrations higher than 1×10^{-2}M. Intensity of peaks Ep_a^* (1) and Ep_a^* (2) gradually increased while cycling, attaining a limiting value after 15 min.

In Fig. 4, are reported the curves obtained with a modified C.P.E. of a solution containing 1×10^{-3}M carboplatin and 1 M chloride. Initiating the potential scan at −0.8 V no oxidation peak was detected during the first cycle between −0.8 and +1.0 V (curve a, dashed line) but a broad cathodic current was observed around −0.4 V. Extending the second cycle between −0.8 and +1.4 V peak Ep_a was detected and on the reverse cathodic sweep the corresponding peak Ep_c^* at +0.10 V, as well as an increase of the broad current at −0.4 V was observed. Reversing at Ep_c^* (Fig. 3, dotted lines) continuously led to the formation of the anodic peak Ep_a^* (2) whose intensity was higher than Ep_c^*: $Ip_a^*/Ip_c^* > 1$.

Results and behaviour obtained at the platinum electrode

It is well known that cyclic voltammetric experiments conducted with platinum electrodes require a rigourous control of the surface state in order to improve the electrode response and to obtain highly reproducible current–potential curves. For instance, during the studies of the redox behaviour of platinum (II) complexes at platinum electrodes Hubbard *et al.* [8, 20] have pointed out the catalytic effect of adsorbed chloride on the kinetics of the electro-oxidation of these molecules.

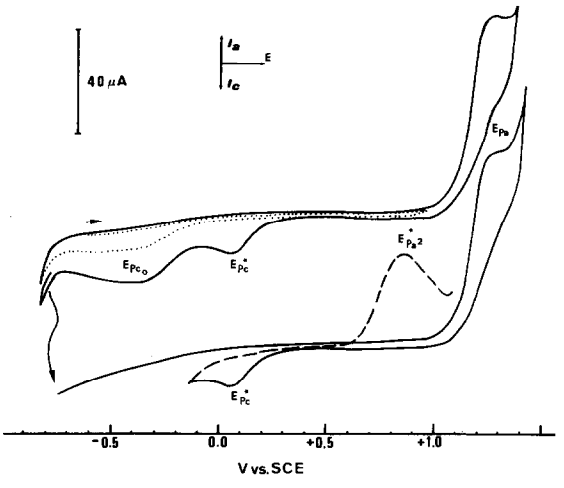


Figure 4
Cyclic voltammograms of 1×10^{-3}M carboplatin in 0.1 M $NaClO_4$ and 1 M NaCl at the modified C.P.E. as a function of inversion potential. Scan rate = 20 mV s^{-1}, starting potential = −0.8 V, initial anodic scan. Several cycles on the same surface. (...) first cycle, (——) second and third cycles and (– – –) fourth anodic scan.

At a smooth platinum electrode, cycling (CV) in presence of carboplatin and chloride ions improves the electrode response (Fig. 5), a phenomenon attributable to a surface activation process [21–24].

At the activated electrode in the absence of chloride ions, peak Ep_a^* occurred at potentials corresponding to oxide formation at the surface and on the reverse sweep, one peak was observed at the potential of the reduction of the oxide film. In the presence of chloride, oxide film formation was inhibited [25, 26] resulting in well-defined and reproducible current–potential curves. The reversibility of the reactions also increased and oxidation occurred more readily. Reversing the sweep after Ep_a^* produced the appearance of one or two cathodic peaks (Fig. 6).

Interpretations of cyclic voltammetric measurements at the C.P.E. indicated a fairly poor electrochemical activity of the platinum derivative. Indeed, in the negative potential range, the reduction of the molecule was not observed and in the anodic range oxidation was highly irreversible with a peak near the cut-off potential. The oxidation products were reduced at fairly negative potentials in two steps, the last one giving rise to concomitant gas evolution. The presence of chloride in the solution markedly modified the nature of the oxidation products as suspected by the appearance of additional cathodic peaks. A systematic study of the shape of the CV curves as a function of $E\lambda$ shows the irreversible nature of the processes at the carbon paste surface and suggests that the species were reduced in a common final electronic step: Ep_c (0). During this step, a modification of the C.P.E. occurred, attributable to electrodeposition of platinum particles, giving rise to hydrogen evolution [27] and to the appearance of new anodic peaks: Ep_a^* (1) and Ep_a^* (2). Peak Ep_a^* (1) corresponds to the oxidation of the platinum particles deposited, as proved by cycling the modified C.P.E. in a supporting electrolyte in the absence of carboplatin. Moreover the formation of Ep_a^* (1) was inhibited in the presence of chloride ions.

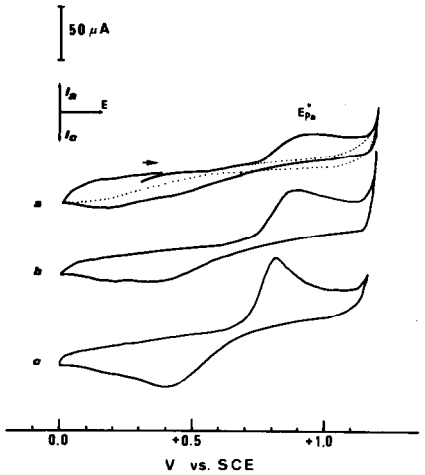


Figure 5
Cyclic voltammograms of 1×10^{-3}M carboplatin in 0.1 M Na_2SO_4 and 1 M NaCl at the smooth platinum electrode as a function of cycling. Scan rate = 20 mV s^{-1}, starting potential = +0.3 V, initial anodic scan. Several cycles on the same surface, dotted lines = supporting electrolyte. (a) first cycle, (b) fourth cycle and (c) sixth cycle.

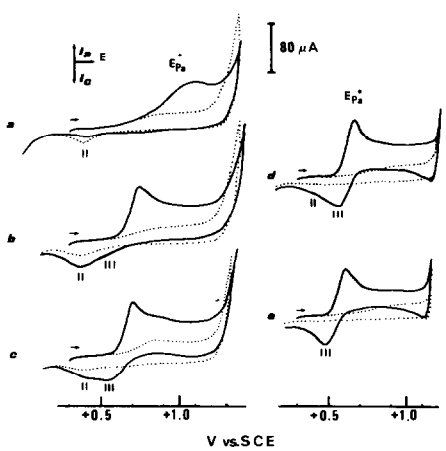


Figure 6
Cyclic voltammograms of 1×10^{-3}M carboplatin in 0.1 M Na_2SO_4 at the activated platinum electrode as a function of Cl^-. Scan rate = 20 mV s^{-1}, starting potential = +0.3 V, initial anodic scan, dotted lines = supporting elecrolyte. (a) Cl^- = 0, (b) Cl^- = 10^{-3}M, (c) Cl^- = 10^{-2}M, (d) Cl^- = 10^{-1}M and (e) Cl^- = 1 M.

Because of the presence of electrodeposited platinum particles, the catalytic reoxidation of the reductants generated may be observed at peaks Ep_c (II) and Ep_c (III).

Comparing these processes with the reduction and oxidation peaks of *cis*-platinum on C.P.E. [28] and upon consideration of the works of R. B. Martin [29] concerning the mole fraction of Pt (II) versus chloride and those of A. T. Hubbard [8] relative to the electro-oxidation of platinum (II) complexes, the redox behaviour of carboplatin at the C.P.E. may be interpreted. From the data obtained and discussed above, the presence of three oxidation products may be deduced: structure I in the absence of chloride, structures I and II between 1×10^{-3} and 1×10^{-2}M chloride, and structures II and III between 1×10^{-2} and 1 M chloride. Taking into account that perchlorate and sulphate are noncomplexing ions [15] and assuming that the oxidation of the compound involves a single two-electron step to platinum (IV) the following structures are suggested, the number and respective concentration ratio of these forms being directly related to chloride concentration.

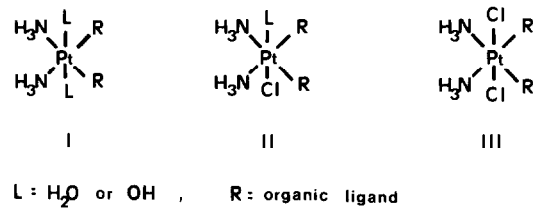


Figure 7
Schematic structures of the three oxidation products.

The reduction of these oxidized forms of carboplatin, which may occur in step Ep_c (I), Ep_c (II) or Ep_c (III) gives rise to further new products. Indeed neither does Ep_a^* (2) correspond to the catalytic oxidation of carboplatin nor Ep_c (0) to the reduction of the species. For these reasons and due to the fact that the organic ligand is a better leaving group than NH_3 [15, 16] and taking into account the redox behaviour of *cis*-platinum at the C.P.E., it is proposed that reduction of structures I, II and III gives rise to the following products (Fig. 8) with elimination of the organic ligand.

Compounds II′ and III′ having one or two halide ligands are catalytically oxidizable [Ep_a^* (2)] due to the in-plane bridging effect [8], contrary to compound I whose reoxidation is not detected at the modified C.P.E. The further reduction of structures I′, II′ or III′ involves the common step Ep_c (0) giving rise to platinum electrodeposition, which is similar to the behaviour of *cis*-platinum at the C.P.E. [28].

The study of carboplatin at a smooth platinum electrode confirmed the high degree of irreversibility of the redox behaviour of platinum (II) complexes possessing no halide ligands [8, 9]. Addition of free chloride ions in the solution slightly improved the processes due to the axial bridging effect [8]. Similar to what happened at the C.P.E., reduction at the platinum electrode was not detected, but oxidation and subsequent reduction gave rise to *cis*-platinum structures as mentioned above. They may be further reduced with platinum electrodeposition and appearance of the corresponding catalytic properties. The formation of one or two reduction peaks after Ep_a^* as a function of chloride ions, confirmed the presence of species II and III. Reduction of species I was not detected due to the irreversible nature of the process which was masked by a rapid hydrogen evolution at the platinized platinum electrode. Similarly with the results obtained at the C.P.E. we may conclude that at 1×10^{-3}M chloride species II appears whose concentration increases till 1×10^{-2}M chloride, then diminishes on behalf of species III (the most abundant form in 1 M chloride solution).

As indicated above, a judicious choice of working parameters permits the electro-oxidative quantitative determination of carboplatin at carbon paste and platinum electrodes. Reproducibility of peak intensity measurements (n = 10) calculated at the

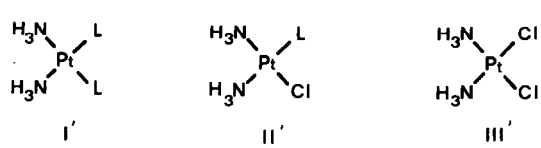


Figure 8
Schematic structures of the three reduction products.

C.P.E. and at the activated platinum electrode gave coefficients of variation of 1.1 and 1.9%, respectively. In chloride media a linear relationship was obtained for peak intensity (Ip_a) as a function of carboplatin concentration in the range 3×10^{-4}–1×10^{-5}M at both electrodes with correlation coefficients between 0.999 and 0.994. The detection limit was 8×10^{-6}M at both electrodes.

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