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Redox-catalytic reduction of dioxygen by cobaltocene at a carbon paste electrode: application to trace analysis

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Abstract

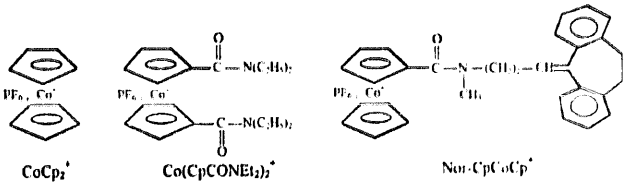
The redox-catalytic reduction of dioxygen at a carbon paste electrode took place when trace amounts of cobaltocenium salts substituted by electron-withdrawing groups were added. A potentiometric technique was set up, which allowed the detection of cobaltocenium salts down to ca. 1×10^{-7} M in a non-deaerated phosphate buffer solution (pH 7.4). The detection limit of a cobaltocenium-labeled model drug was ca. 2×10^{-7} M under conditions which mimic competitive immunoassays.

Keywords: Cobaltocenium; Dioxygen; Carbon paste electrode; Catalysis; Electrochemical assay

1. Introduction

Cobaltocenium ($CoCp_2^+$) and substituted cobaltocenium salts are reversibly reduced to cobaltocenes in both aqueous and non-aqueous electrolytes [1–4]. They are stable not only to oxygen but to a wide variety of reagents used in organic synthesis, and so cobaltocenium salts covalently attached to organic moities have been prepared for electrochemical applications. These applications include electrochemical recognition of anionic guest species by cobaltocenium amide receptors [5–8], photoelectrochemical generation of hydrogen by surface-bound cobaltocenium polymer [9], solid state diode comprising a cobaltocenium/ferrocene bilayer film [10], and electrochemical detection of traces by competitive immunoassay using cobaltocenium salts as redox labels [11–15]. The main feature of this latter method is that the substance, i.e. the antigen (Ag), to be assayed is covalently attached to the cobaltocenium redox label. The lower the detected limit quantity of cobaltocenium-labeled antigen (Ag–CpCoCp$^+$), the more sensitive the detection of the analysed substance (Ag) in unknown samples. We have shown in the case of several cobaltocenium-labeled drugs that detection of 1×10^{-8} M quantities of these substances in the bulk solution was possible by square-wave voltammetry at a Nafion®-coated glassy carbon electrode [12–14] or at a Nafion®-loaded carbon paste electrode [15]. The cationic labeled substances Ag–CpCoCp$^+$ accumulated for 5 min in Nafion®, a perfluorinated polymer, which possesses negatively charged sulfonate groups. This preconcentration improved the sensitivity of the immunoassay considerably.

In this paper, we show that the electrochemical reduction of dioxygen at a carbon paste electrode (CPE) proceeds at potentials more positive in the presence of trace amounts of cobaltocenium salts substituted by electron-withdrawing groups such as $Co(CpCONEt_2)_2^+$. A potentiometric technique was set up, which allowed the detection of this salt down to ca. 1×10^{-7} M in a non-deaerated phosphate buffer (PB) solution (pH 7.4). The technique was applied to the detection of nortriptyline (an antidepressant) labeled by cobaltocenium (Nor–CpCoCp$^+$), under conditions which mimic competitive immunoassays.



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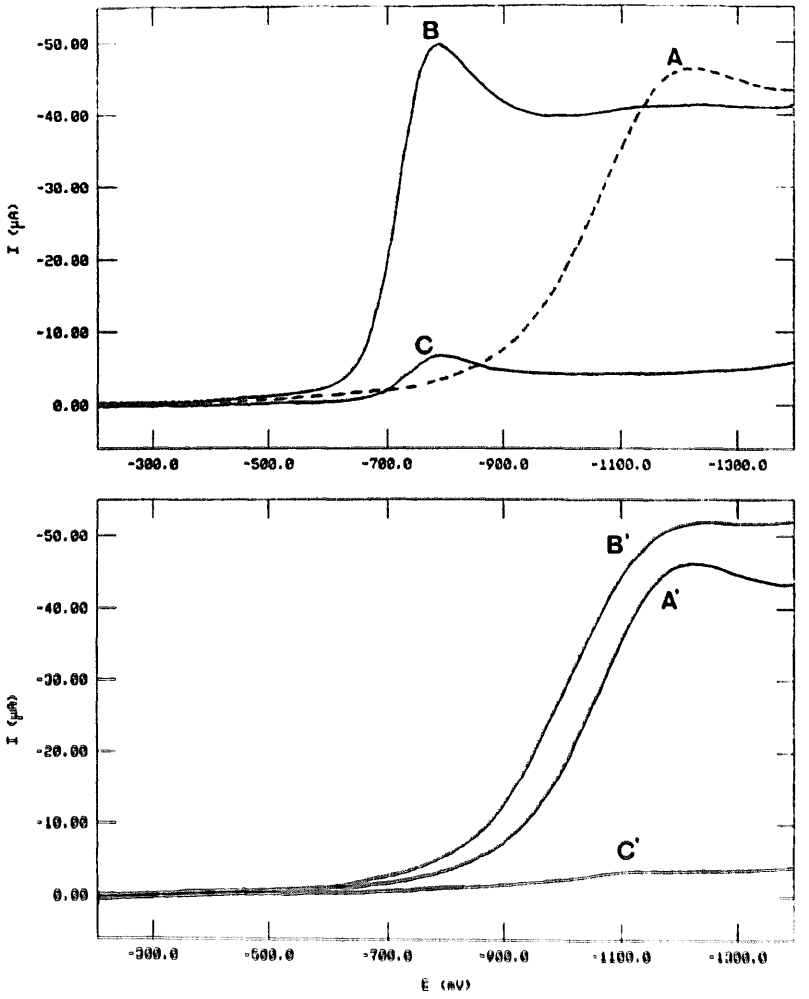

Fig. 1. Voltammetric curves (scan rate 500 mV s^{-1}) on a stationary CPE in a non-deaerated (curves A, A', B, B') and a deaerated (curves C, C') PB solution (pH 7.4) in the absence (curves A, A') and presence of 3.8×10^{-5} M $Co(CpCONEt_2)_2^+$ (curves B, C) and 4.4×10^{-5} M $CoCp_2^+$ (curves B', C')

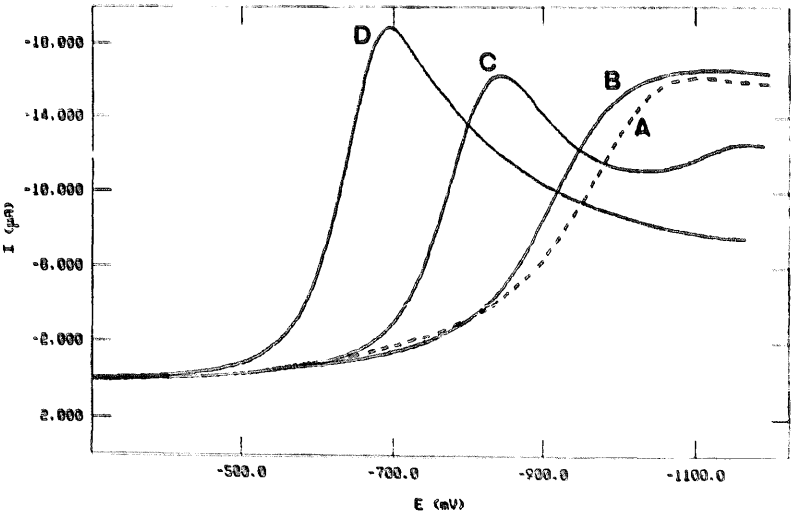

Fig. 2. Voltammetric curves (scan rate 50 mV s^{-1}) on a stationary CPE in a non-deaerated PB solution (pH 7.4) containing Nor-CpCoCp$^+$ at 0 M (curve A), 1.2×10^{-7} M (curve B), 5.8×10^{-7} M (curve C), and 8.7×10^{-6} M (curve D).

2. Results and discussion

Addition of trace amounts of the diamide salt $Co(CpCONEt_2)_2^+$ (3.8×10^{-5} M) to a non-deaerated PB solution (pH 7.4) facilitated the reduction of dioxygen, as shown in the upper part of Fig. 1. The voltammetric curve at a stationary CPE was shifted to more positive potentials and the peak potential E_p moved from −1.22 V (curve A) to −0.78 V (curve B) whereas the peak current I_p increased only slightly. A peak of low amplitude and with $E_p = -0.78$ V was associated with the reduction of $Co(CpCONEt_2)_2^+$ in a deaerated solution (curve C – note that some residual catalytic current was left, although the solution was carefully deaerated by argon). The reduction of dioxygen proceeded by redox catalysis [16] and led most probably to hydrogen peroxide according to reactions (1) and (2).

$$2Co(CpCONEt_2)_2^+ + 2e^- \rightleftarrows 2Co(CpCONEt_2)_2 \quad (1)$$

$$2Co(CpCONEt_2)_2 + O_2 + 2H^+ \rightarrow 2Co(CpCONEt_2)_2^+ + H_2O_2 \quad (2)$$

The reduction peak of dioxygen was only slightly shifted when unsubstituted cobaltocenium $CoCp_2^+$ (4.4×10^{-5} M) was added (compare curves A′ and B′ in the bottom part of Fig. 1). The peak potential E_p of $CoCp_2^+$ in a deaerated solution (curve C, $E_p = -1.10$ V) is more negative than in the preceding case ($E_p = -0.78$ V for $Co(CpCONEt_2)_2^+$), owing to the absence of electron-withdrawing substituents, and so the cathodic reduction of $CoCp_2^+$ and dioxygen occurred simultaneously.

The peak potential of Nor–CpCoCp$^+$ was the same as in the case of $Co(CpCONEt_2)_2^+$, i.e. $E_p = -0.78$ V, and so this salt was also a good mediator for the indirect reduction of dioxygen. Voltammograms B–D in Fig. 2 correspond to the reduction of dioxygen with increasing amounts of Nor–CpCoCp$^+$. As expected for a catalytic process, the higher the Nor–CpCoCp$^+$ concentration, the more positive the E_p values. A plot of the potential values E_I measured at a fixed current versus the concentration provides a potentiometric calibration curve for Nor–CpCoCp$^+$. Curve A in Fig. 3 shows the calibration curve for a current of 14 μA. The potential shift was not significant below 1×10^{-7} M ($E_I = -1.01$ V) in the absence of Nor–CpCoCp$^+$ and the plot was linear between 1×10^{-7} and 6×10^{-6} M with a positive slope of 180 mV per decade. This slope is ca. three times as high as the slope of a Nernstian potentiometric curve for a reversible one-electron process (59 mV per decade).

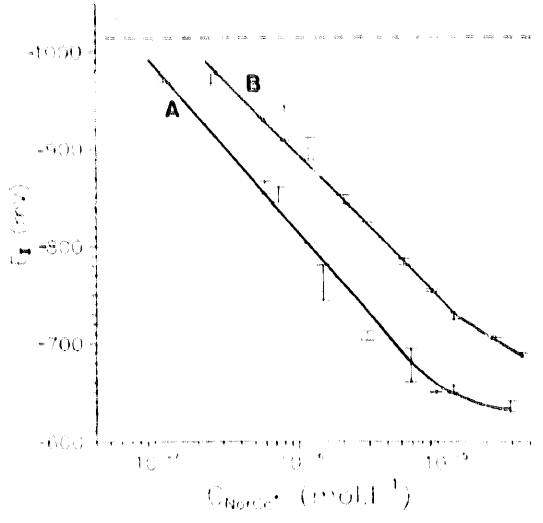

Fig. 3. Variations of the potential E_I measured at a constant current (14 μA) with the concentration of Nor–CpCoCp$^+$ under the conditions of Fig. 2 (curve A) and after the extraction step described in the Experimental section (curve B). Error bars represent the standard deviation for two measurements. The dotted line indicates the E_I value in the absence of Nor–CpCoCp$^+$.

Table 1
Shifts ΔE_x of E_x for the reduction of dioxygen mediated by Nor–CpCoCp$^+$ (2.3×10^{-6} M) at different pH values

pH	1.1	5.7	7.4	8.2	13
ΔE_I (mV) ($I = 14$ μA)	60	214	216	178	215

The reduction of dioxygen mediated by Nor–CpCoCp$^+$ (2.3×10^{-6} M) was examined at several pH values and the largest shift for E_I (ΔE_I) was observed for a phosphate buffer of pH 7.4 (Table 1). This buffer was selected for further study, since immunoassays are usually carried out at physiological pH 7.4.

It is worth noting that the catalytic process was not observed at a glassy carbon electrode (GCE), since the reduction peak of dioxygen was not shifted when Nor–CpCoCp$^+$ (9.6×10^{-6} M) was added (compare voltammograms A and B in Fig. 4). The direct reduction of dioxygen occurred much more readily at a GCE ($E_p = -0.64$ V for curve A) than at a CPE ($E_p = -1.10$ V under the same experimental conditions, see curve A in Fig. 2), and so the cathodic reductions of dioxygen and Nor–CpCoCp$^+$ are consecutive at a GCE.

The results obtained specifically at a carbon paste electrode led us to examine whether the sensitive determination of Nor–CpCoCp$^+$ could be carried out at a CPE under the conditions of a competitive homogeneous immunoassay [12–15]. When such conditions are involved, a mixture of an unknown amount of antigen (Ag) and a constant amount of cobaltocenium-labeled antigen (Ag–CpCoCp$^+$) is allowed to compete for a limited and constant concentration of antiserum, i.e. antibody binding sites (Ab) contained in serum, and so the reaction mixture upon equilibrium consists of an unbound phase (unbound Ag and Ag–CpCoCp$^+$) and a bound phase (Ab/Ag and Ab/Ag–CpCoCp$^+$ complexes). The redox label loses its electrochemical activ-

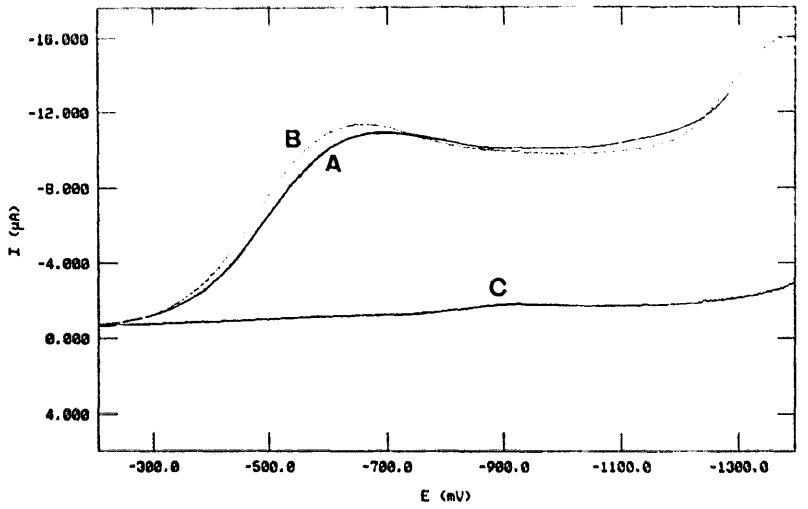

Fig. 4. Voltammetric curves (scan rate 50 mV s^{-1}) on a stationary GCE in a non-deaerated (curves A, B) and deaerated (curve C) PB solution (pH 7.4) in the absence (curve A) and the presence (curves B, C) of 9.6×10^{-6} M Nor-CpCoCp$^+$.

ity when it is included in the Ab/Ag–CpCoCp$^+$ complex [12–15]. Therefore, the electrochemical detection of the amount of Ag–CpCoCp$^+$ present in the unbound phase allows the determination of the quantity of analysed substance (Ag), using a calibration curve plotted for standardized amounts of Ag–CpCoCp$^+$ and Ag. In brief, the determination of Ag–CpCoCp$^+$ is carried out in the presence of antigen and serum.

The indirect reduction of dioxygen mediated by Nor–CpCoCp$^+$ was studied in the presence of nortriptyline, and then rabbit normal serum (RNS) to mimic the immunoassay conditions. The addition of increasing amounts of nortriptyline (from 1×10^{-6} to 1×10^{-5} M) to a non-deaerated solution of Nor–CpCoCp$^+$ (7.5×10^{-6} M) did not modify significantly the voltammetric curves. Conversely, the presence of serum strongly inhibited the catalytic process, as shown in Fig. 5 where peaks B and B′ were obtained in the presence of Nor–CpCoCp$^+$ (5.9×10^{-6} M) and after addition and incubation of RNS (1%) in the case of peak B′. Peak B′ was shifted positively to a lesser extent than peak B. The direct reduction of dioxygen was not disturbed significantly by serum (compare peaks A and A′ in Fig. 5). Fig. 6 shows that the catalytic peak (curve B) almost merged with the direct reduction peak of dioxygen (curve A) above 3% RNS content. These results show that the serum constituents (amines, acids, proteins, inorganic cations, lipids) interfere in the overall cat-

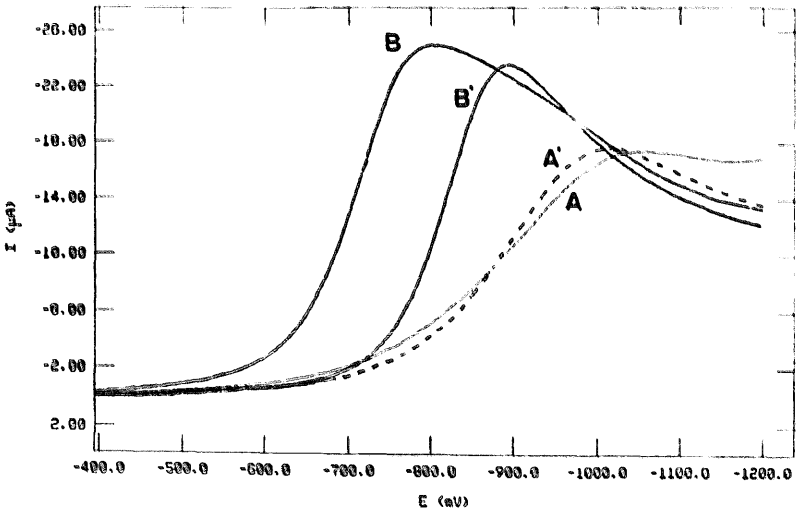

Fig. 5. Voltammetric curves (scan rate 50 mV s^{-1}) on a stationary CPE in a non-deaerated PB solution (pH 7.4) in the absence (curves A, B) and the presence (curves A′, B′) of 1% RNS. For curves B and B′ Nor–CpCoCp$^+$ (5.9×10^{-6} M) was added.

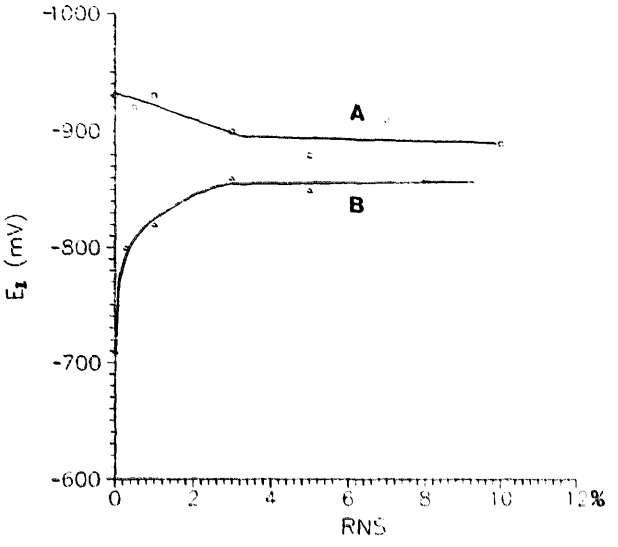

Fig. 6. Variations of the potential E_1 measured at a constant current (14 μA) with the RNS content (v/v) under the conditions of Fig. 5. Curves A and B correspond to the direct and catalytic reduction of dioxygen respectively.

alytic process. We have previously shown [12,17] that interactions between serum proteins and cobaltocenium salts are usually negligible, which suggests that the bulk reaction (2) would be less disturbed by serum than the cathodic reduction of Nor–CpCoCp$^+$ (1). A comparison of the cyclic voltammograms in Fig. 7 shows that the reversibility of the cobaltocenium/cobaltocene redox couple contained in Nor–CpCoCp$^+$ was partially lost when serum was present. Indeed, the anodic peak current on the reverse anodic sweep dropped from 3.3 μA (curve A) to 1.7 μA (curve B) and the peak potential separation increased from 45 to 64 mV (the shoulder observed at ca. −0.6 V in the absence of serum was related to residual dioxygen). The heterogeneous electron exchange (1) might be impeded by serum proteins adsorbed on the CPE surface. Clearly, the direct potentiometric detection of Nor–CpCoCp$^+$ was not possible under conditions which mimic homogeneous competitive immunoassays.

However, an additional extraction step by methylene dichloride after incubation allowed the catalytic effect of Nor–CpCoCp$^+$ to be recovered partially but reproducibly. The potentiometric calibration curve (B) in Fig. 3 corresponds to these new experimental conditions when ca. 10% of RNS was initially present. The plot is linear in the concentration range 3×10^{-7}–1×10^{-5} M, the corresponding slope is ca. 150 mV per decade and the detection limit is ca. 2×10^{-7} M.

To conclude, the detection limits of cobaltocenium salts substituted by electron-withdrawing groups are enhanced considerably when these salts are reduced at a carbon paste electrode immersed in a non-deaerated neutral buffer solution. Moreover, we have shown in the case of Nor–CpCoCp$^+$ that a potentiometric technique can allow the detection of trace amounts of a cobaltocenium-labeled antigen in a PB solution (pH 7.4) containing serum, insofar as an additional extraction step follows the incubation step. Therefore, this technique can be envisaged for competitive homogeneous immunoassays.

3. Experimental section

Cobaltocenium hexafluorophosphate ($CoCp_2^+;PF_6^-$) was provided by Strem Chemicals. The synthesis of $Co(CpCONEt_2)_2^+$ and Nor–CpCoCp$^+$ were described in Refs. [18] and [11] respectively. Rabbit normal serum was purchased from Sigma. Most of the experiments

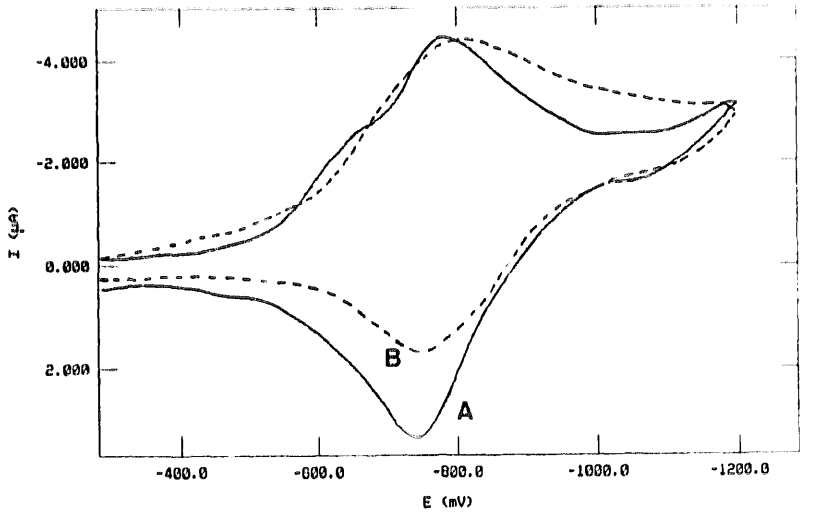

Fig. 7. Cyclic voltammetric curves (scan rate 50 V s^{-1}) on a CPE in 500 μl of deaerated PB solution (pH 7.4) containing Nor–CpCoCp$^+$ (7.2×10^{-5} M) before (curve A) and after (curve B) addition of 50 μl RNS.

were performed in phosphate buffer pH 7.4 (PB; 4.35 mM NaH_2PO_4, 15.1 mM Na_2HPO_4, and 50 mM NaCl) and the solutions were not deaerated by argon unless stated otherwise. Also used were solutions of pH 1.1 (50 mM NaCl and 0.1 M H_2SO_4), pH 5.7 (50 mM NaCl, 93 mM KH_2PO_4, and 6.7 mM NaOH), pH 8.2 (50 mM NaCl, 52 mM KH_2PO_4, and 48 mM NaOH), and pH 13 (50 mM NaCl and 0.1 M NaOH). All reagents were analytical grade, and water was deionized and twice distilled.

Carbon paste was prepared by hand-mixing 125 mg of graphite powder with 104 mg of silicon oil, and was then introduced and pressed into a plastic tip with an internal diameter of 3 mm. The surface of the CPE so obtained was polished on a Teflon sheet until a smooth surface was achieved.

An EG&G PAR 273 potentiostat interfaced to an IBM XT 286 computer system with PAR M270 software was used for linear and cyclic voltammetry. Electrochemical experiments were carried out at 25 °C in a one-compartment glass cell with a working volume of 1 ml. A platinum wire counter electrode and an Ag/AgCl (50 mM NaCl) reference electrode were introduced into the cell in addition to the CPE.

3.1. Calibration curve of Nor–CpCoCp$^+$ in the presence of RNS

A 1 h incubation at 35 °C of Nor–CpCoCp$^+$ in a mixture of 0.9 ml PB (pH 7.4) and 0.1 ml RNS was followed by addition of 1 ml methylene dichloride. The mixture was gently stirred to avoid emulsion, and then 0.5 ml of the CH_2Cl_2 phase was pipetted out and introduced into the electrochemical cell where it was allowed to evaporate to dryness by air streaming. Finally, 0.5 ml of PB (pH 7.4) was added before recording the voltammetric curve.

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