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Cobalt(II) Hematoporphyrin IX Immobilized in a Cellulose Acetate Niobium(V) Oxide Composite Membrane: Preparation and Oxygen Reduction Study

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A Hematoporfirina IX [ácido (8,13,-bis(1-hidroxietyl)-3,7,12,17-tetrametil-21-H-porfirina-2,18-dipropionico)] foi absorvida na superfície da membrana composta orgânica/inorgânica Cel/Nb₂O₅. A porfirina é fortemente aderida à superfície do Nb₂O₅ pela interação com o grupo carboxílico -COOH da porfirina e formação da ligação -COO-Nb. A metalação do anel porfirínico é feita mergulhando-se a membrana na solução de Co(II) por algumas horas. As bandas de absorção eletrônica Q indicaram a mudança de simetria de D_{2h} para D_{4h} pela metalação do anel porfirínico. A análise química demonstrou que a metalação ocorreu com rendimento de praticamente 100%. O oxigênio foi reduzido na superfície do eletrodo feito com o material a um potencial de pico de -390 mV em solução de KCl 1 M a pH 1, a uma temperatura de 298 K à pressão ambiente. Supõe-se que o processo de redução ocorre segundo o mecanismo de transferência de dois elétrons. Foi obtida uma resposta linear da corrente de pico catódico para concentrações do oxigênio dissolvido entre 1,5 e 15 ppm.

Hematoporphyrin IX [8,13,-bis(1-hydroxyethyl)-3,7,12,17-tetramethyl-21H-porphyrin-2,18-dipropionic acid] was adsorbed on the organic-inorganic composite membrane Cel/Nb₂O₅ surface. The porphyrin is strongly adhered on the Nb₂O₅ surface by reaction of the porphyrin CO₂H groups with Nb₂O₅ forming the -COO-Nb chemical bond. Metallation of the porphyrine ring was made very easily by immersing the membrane in Co(II) solution and allowing to rest for a few hours. The electronic absorption bands due to the Q bands indicated the symmetry change from D_{2h} to D_{4h} upon metallation of the porphyrin ring. The chemical analyses of the material confirmed that the metallation yield was practically 100%. Oxygen was reduced on a electrode surface made with the material under a cathodic peak potential of -390 mV, in 1 M KCl solution at pH 1, temperature of 298 K and at ambient pressure. The oxygen reduction was proposed to occur by a two electron transfer mechanism. A linear cathodic peak current response was obtained for dissolved oxygen concentrations between 1.5 and 15 ppm.

Keywords: *Co(II) hematoporphyrin IX, cellulose acetate membrane, cellulose-niobium(V) oxide composite membrane, oxygen catalytic reduction*

Introduction

Niobium pentoxide has been investigated with respect to its surface acid strength, ion exchange capacity, and use as specific sorbent for many metal ions¹⁻³. Since the bulk oxide is normally obtained as a fine untractable powder with poor mechanical resistance, it has been dispersed on a surface of silica gel⁴. However, the mineral matrix is rigid and for some applications where molding of the material is required it is not convenient. In a recent work, the obtention

and characterization of the composite membrane cellulose/oxide, Cel/Nb₂O₅, with high degree dispersion and homogeneity of the oxide particles was described⁵. The Lewis acid sites of the dispersed oxide were shown to be reasonably stable. It is known that these Lewis acid sites can immobilize an organic acid enabling the chemical species be tightly anchored to the metal oxide by the -COO-Nb bond formation and thus, avoiding leaching from the surface as the material is immersed in a solvent⁶.

In this work the immobilization procedures of hematoporphyrin IX [8,13,-bis(1-hydroxyethyl)-3,7,12,17-tetramethyl-21H-porphyrin-2,28-dipropionic acid], H₂(HMP), in cellulose/niobium(V) oxide composite membrane and further metallation with cobalt (II) ion are described. The nature of the cobalt ion was firstly investigated, since it has been described that the metal is readily oxidized in air⁷. A membrane of the resulting material was adhered on a platinum electrode surface and the study of electrocatalytic reduction of dissolved oxygen by the electroactive species is also reported in this work.

Experimental

Preparations

About 10 g of cellulose acetate (Aldrich, 2.5 degree of acetylation) was added to a mixture containing 31 mL of glacial acetic acid and 71 mL of acetone. The mixture was vigorously stirred for 2 h and allowed to rest for 24 h after which a viscous syrup was formed. Freshly sublimed NbCl₅ (2 g) was added into 10 g of the cellulose acetate syrup and the mixture stirred under a nitrogen atmosphere until complete dissolution of the metal halide which occurred with evolution of gaseous HCl. The reaction flask was purged with nitrogen until almost all entrapped HCl was eliminated and could not be detected in the syrup. The test for Cl⁻ was carried out by adding AgNO₃ solution into a sample taken from the syrup and diluted with 0.1 M HNO₃ solution. This viscous syrup is named as solution A.

Films of the composite membranes were prepared by spreading the solution A over a glass plate and the thickness was controlled to 200 μm by using a spacer. The film was allowed to rest for 2 h open to room atmosphere in order to evaporate most of the solvents. To promote the metal hydrolysis, the glass plate was immersed in distilled water and the film was detached from the surface.

About 0.054 g (10⁻⁴ mol) of H₂(HMP) (Sigma) was dissolved in 50 mL of ethanol/water solution (9:1 v/v). The membrane of CeI/Nb₂O₅ with approximately 3 cm² was immersed in this solution and after 4 h it was washed and stored in bidistilled water protected against light. To metallate this membrane, it was immersed in 0.1 M CoCl₂ aqueous solution for 5 h with occasional shaking. The membrane was washed with water and dried at 333 K.

Chemical analyses

The quantity of incorporated metal oxide in the matrix was determined by igniting the material to 1173 K for 1 h and weighing the residue as Nb₂O₅.

In order to determine the amount of metallated hematoporphyrin incorporated in CeI/Nb₂O₅, 0.1 g of the membrane was immersed in concentrated sulphuric acid and gently heated until dissolution. To analyze the metal, the solution was carefully diluted to 100 mL and neutral-

ized with sodium bicarbonate aqueous solution (10% m/v) and measured on a atomic absorption spectrometer. The amount of porphyrin molecule was determined by nitrogen determination using the Kjeldhal method. The results of the elemental analyses can be summarized as follow: N = 152 ± 3 μmol g⁻¹ (corresponding to 38 μmol g⁻¹ of Hematoporphyrin); Co = 39.5 ± 0.8 μmol g⁻¹. The results indicate that porphyrin ring metallation was 100%.

Spectra measurements

The UV-Visible spectra of CeI/Nb₂O₅/HMP thin films, metallated and unmetallated, were obtained on a Beckman DU 640 spectrophotometer.

Electron spin resonance spectrum of the sample was obtained at 77 K, frequency modulated at 9.45 GHz. The equipment used was a Bruker 106 electron spin resonance spectrometer.

Electrochemical measurements

The electrode, consisting of a glass tube with a platinum disk fused at one end was immersed in the solution A, in which LiCl was previously dissolved (0.1 g per gram of solution A). The objective of adding this electrolyte in solution A was to obtain a porous membrane adhered on the platinum surface⁵. The solvent was evaporated in air and the film adhered electrode was washed with water. Adsorption of the porphyrin on the film was made by dipping the electrode in 10⁻⁴ M H₂(HMP) ethanol/water (9:1 v/v) solution, as described above. The metallation procedure was the same as that described above. The electrode was stored in a 1 M KCl solution.

Cyclic voltammetry study

The cyclic voltammetry studies were carried out in a cell with three electrodes: the saturated calomel electrode was the reference, the counter electrode was a platinum wire and the electrode prepared as above was the working electrode. The measurements were carried out on a PAR 273 A model instrument.

Dissolved oxygen

To measure the electrode response to dissolved oxygen, the cell was filled with 1 M KCl solution at pH 1 and saturated with pure oxygen at 298 ± 0.5 K. To change the concentration of the oxygen, argon was passed through the solution and the content of oxygen in each case determined by the Winkler method⁸.

Results and discussion

Organic compounds having carboxylate functional groups are immobilized on niobium oxide surface by a -COO-Nb bond formation⁶. Figure 1 illustrates the HMP and Co(HMP) immobilized on CeI/Nb₂O₅. The pK_a of the

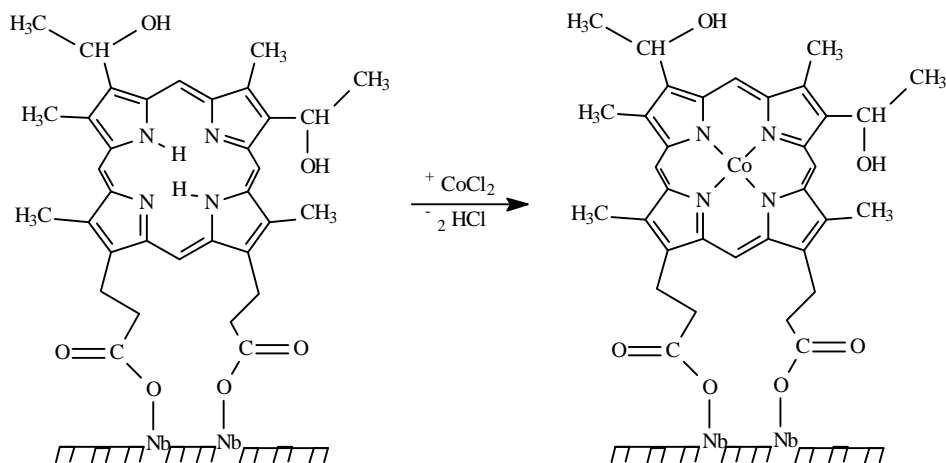


Figure 1. Metallation of the Hematoporphyrin IX immobilized on Cel/Nb₂O₅ with Co(II). The symmetry of the ring changes from D_{2h} to D_{4h} on metallation.

protonated imino nitrogens of the porphyrin ring are 3.0 and 6.1 in water⁹ and thus, insertion of the metal ion in the porphyrin ring is not difficult. The absorption spectra are shown in Fig. 2. The H₂(HMP) absorbs intensely at λ_{\max} 350 nm (Soret band, corresponding to the strongly allowed transition from a_{1u} to e_g energy levels) and more weakly between 470 and 630 nm (Q bands, corresponding to the transition from a_{2u} to e_g energy levels¹⁰) (Fig. 2A). Upon metallation of the porphyrin ring system, a change in the spectrum (Fig. 2B) is observed. For H₂(HMP) the Q bands (λ_{\max} in nm) are observed at 470, 530, 570 and 620 and, for Co(HMP) at 520 and 560. The decrease in the number of bands is assigned to the symmetry increase of the metal-

lated complex relative to the free immobilized porphyrin (Fig. 1) *i.e.*, the symmetry changed from D_{2h} in Cel/Nb₂O₅/HMP to D_{4h} in Cel/Nb₂O₅/Co(HMP). As the chemical analyses showed above, almost all porphyrin rings were metallated. The Soret band is broadened in the unmetallated ring due to dimerization of the porphyrin rings and it becomes narrower upon metal incorporation⁹.

ESR spectrum

It has been reported that the complex of cobalt (II) with hematoporphyrin is readily oxidized in air⁷. In order to certify that in the present case such oxidation can also occur, the esr spectrum of the Cel/Nb₂O₅/Co(HMP) was obtained at 77 K. The spectrum is shown in Fig. 3.

Under magnetic field below 3000 gauss, the g values are 2.573 and 2.315 which correspond to a Co^{II} species and the signal observed between 3200 and 3500 gauss (inserted figure in detail) is an indication of the existence of molecular oxygen from air coordinated to Co^{II}^{11,12}. The calculated parameters are: $g_{\parallel} = 1.973$ and $g_{\perp} = 2.042$; hyperfine coupling constants $A_{\parallel} = 14.24 \times 10^{-4} \text{ cm}^{-1}$ and $A_{\perp} = 15.83 \times 10^{-4} \text{ cm}^{-1}$.

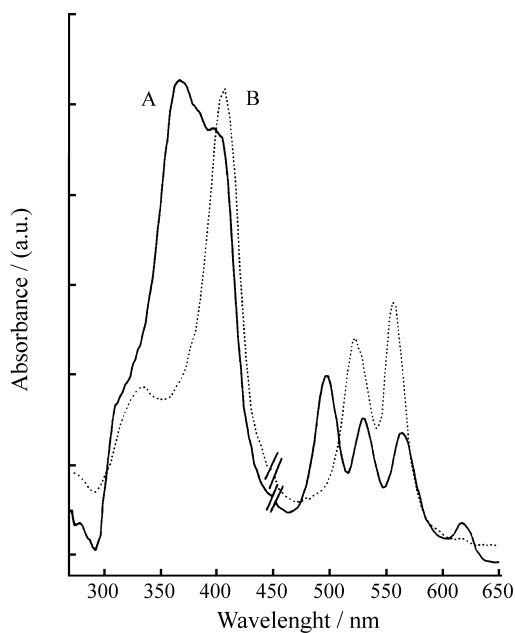


Figure 2. UV-Visible spectra of the membranes: A) Cel/Nb₂O₅/HMP, B) Cel/Nb₂O₅/Co(HMP). Between 450 and 650 nm the spectra were expanded since the intensities of Q bands are very weak in relation to the Soret bands.

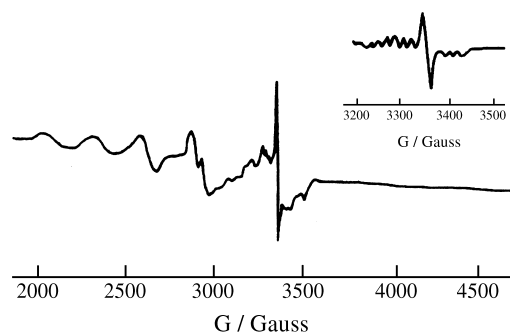


Figure 3. Electron spin resonance spectrum of Cel/Nb₂O₅/Co(HMP) membrane at 77 K.

Electrochemical properties

Figure 4 shows the pulse differential voltammetry of the metallated complex between -800 and 1000 mV under oxygen (Fig. 4A) and argon (Fig. 4B) atmospheres. Under oxygen atmosphere the anodic waves show the peak maxima of higher intensity at -390 mV and of a weaker peak at -190 mV (Fig. 4A) and, under argon atmosphere the intensity of these peaks are reversed (Fig. 4B). The observed peaks correspond to the $\text{Co}^{\text{I}} \rightarrow \text{Co}^{\text{II}}$ oxidation process of both complexes, the more negative peak being due to the oxygen axially coordinated complex species and the less negative peak to the uncoordinated complex species. Sweeping the potential to the more positive potential region, a single peak with maximum at 823 mV due to $\text{Co}^{\text{II}} \rightarrow \text{Co}^{\text{III}}$ oxidation process is observed. Reversing the sweeping direction, from the more positive to more negative potentials, similar results for $\text{Co}^{\text{II}} \rightarrow \text{Co}^{\text{I}}$ reduction process under oxygen (Fig. 4C) and argon (Fig. 4D) atmospheres are observed. The peak corresponding to $\text{Co}^{\text{III}} \rightarrow \text{Co}^{\text{II}}$ process was not observed.

The catalytic reduction of dissolved oxygen is very dependent on the solution pH in which the measurements

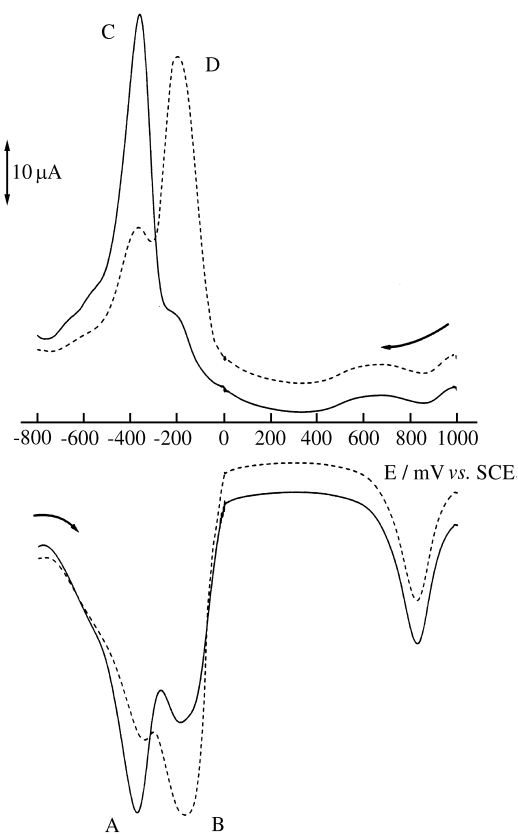


Figure 4. Differential pulse voltammetry curves of $\text{CeI}/\text{Nb}_2\text{O}_5/\text{CoHMP}$ electrode. Anodic sweeping: (A) under oxygen atmosphere, (B) under argon atmosphere; cathodic sweeping: (C) under oxygen atmosphere, (D) under argon atmosphere.

are carried out¹³⁻¹⁶. In the present case, the oxygen reduction at the electrode surface was studied in 1 M KCl solution and pHs between 1 and 6 at temperature of 298 K. Figure 5 shows the plot of current peak maxima against the solution pH. The current intensities with the maximum value at pH 1 exponentially decreased as the pH of the solutions was increased up to pH 6.

In a typical cyclic voltammetry experiment, the cyclic voltammogram curves of $\text{CeI}/\text{Nb}_2\text{O}_5/\text{Co(HMP)}$ in the presence of oxygen (Fig. 6A) and when the solution was purged with argon (Fig. 6B) are shown. The peak that remains at about -450 mV (Fig. 6B) is presumably due to the oxygen molecule coordinated to Co(II) ^{11,12} which is difficult to eliminate during purging of the system with argon. Fig. 6C shows the curve obtained for the electrode made with $\text{CeI}/\text{Nb}_2\text{O}_5$ in the presence of oxygen (blank test). In this case, a peak with small current intensity is observed at -400 mV, the origin of which has not clear explanation. However, it disappeared on purging the electrochemical cell with argon. Anyway, the observed peak at -390 mV (Fig. 6A) is considerably enhanced in the presence of O_2 and it is assigned to the oxygen electrocatalytic reduction at the modified electrode surface. On the Pt electrode surface under similar conditions, oxygen is reduced at nearly -570 mV¹⁷.

The stability of the electrode was tested by cycling the potential between -200 and -500 mV many times. Leaching of the electroactive species was not observed and the intensity of the peak current remained constant during the experiment. In another experiment, in order to test electrode response to dissolved oxygen, the electrochemical cell was alternately purged with pure argon and pure oxygen at a fixed potential of -390 mV and constant temperature of 298 K and pH 1. Bubbling oxygen into the cell (O_2^{in} , Fig. 7) or argon (Ar^{in} , Fig. 7) the current intensities increased

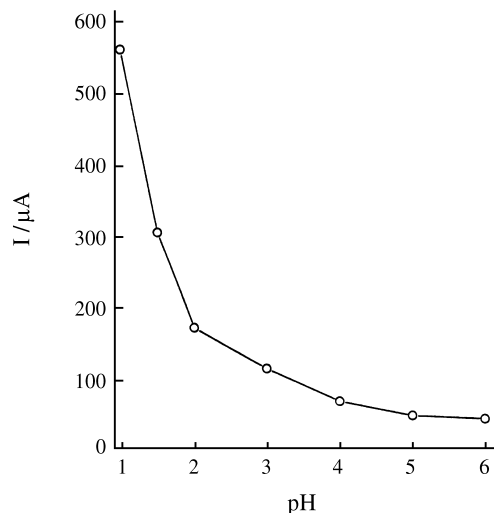


Figure 5. Electroreduction peak current intensities of $\text{CeI}/\text{Nb}_2\text{O}_5/\text{Co(HMP)}$ electrode in 1 M KCl solutions at different pHs.

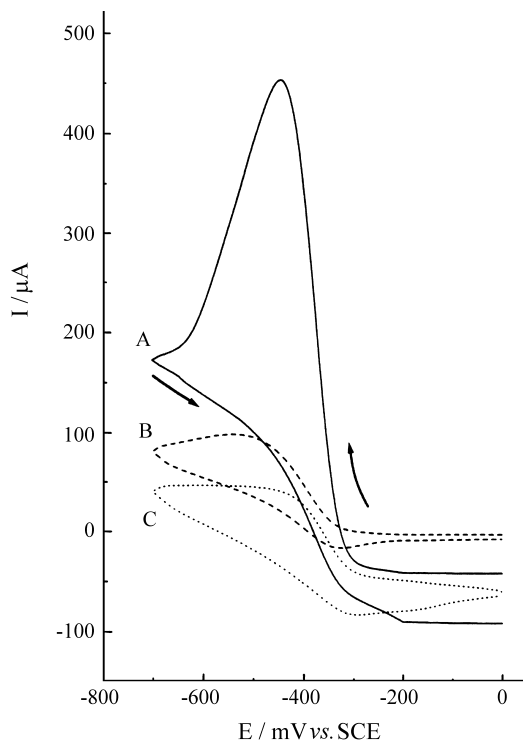


Figure 6. Cyclic voltammograms of Cel/Nb₂O₅/Co(HMP) electrode in 1 M KCl solution at pH 1 and scan rate of 20 mV s⁻¹: A) in dissolved oxygen solution, B) in solution purged with argon, C) unmetallated Cel/Nb₂O₅/HMP in dissolved oxygen solution measured in similar conditions.

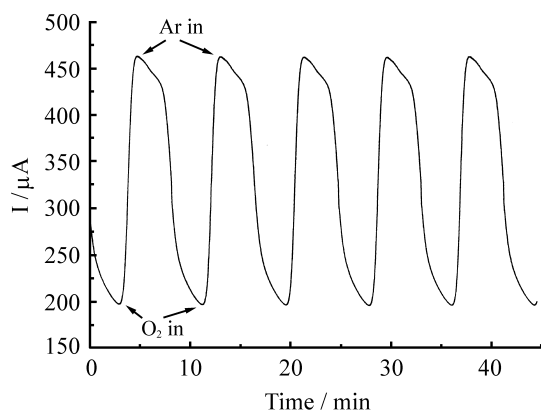


Figure 7. Cathodic peak currents in 1 M KCl solution at pH 1 for alternated cycles in presence of oxygen (O₂ⁱⁿ) and purged with argon (Arⁱⁿ) measured at -390 mV.

for O₂ⁱⁿ and decreased for Arⁱⁿ alternately in five successive cycles, the maxima and the minima remaining practically constant. Furthermore, the electrode response towards dissolved oxygen concentrations was also tested. The experiment was carried out measuring the cathodic peak current of the cyclic voltammograms for a given dissolved oxygen concentration in the cell. Figure 8 shows the cathodic peak current intensity against dissolved oxygen concentration,

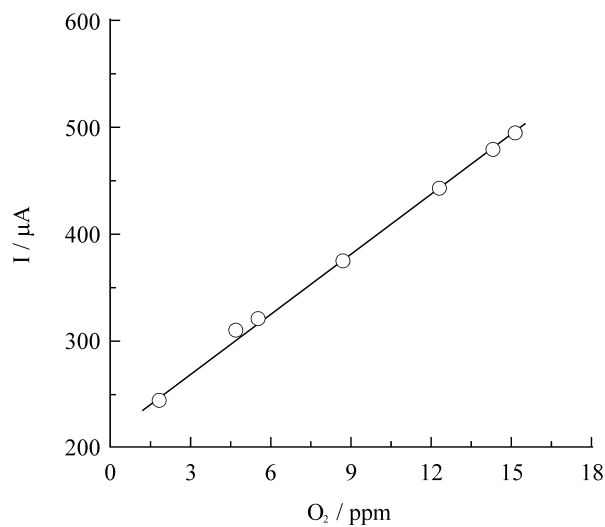
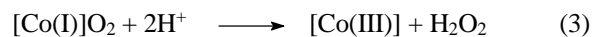
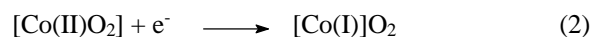


Figure 8. Cathodic peak current intensities against concentration of dissolved oxygen concentration. Experimental conditions: 298 K, 1 M KCl solution at pH 1, ambient pressure, and electrode of Cel/Nb₂O₅/Co(HMP)

where a linear correlation between 1.5 and 15 ppm was observed.

Although the untreated cellulose acetate membranes normally present high rejection coefficient for electrolytes, the efficient electrochemical process at the electrode surface-solution interface is related with the facility under which the membrane is wet when immersed in the electrolyte solution¹⁸ and also the presence of Nb₂O₅ in the matrix which increases its hydrophilic character.

The probable mechanism of oxygen reduction is that of a two electron transfer process¹³ as described for a similar system in an homogeneous solution, according to the following suggested mechanism^{14,15}:



The electroreduction of O₂ by Co(II) porphyrin starts with oxygen coordination to the metal ion (Eq. 1)¹⁹ followed by further electrochemical reduction at the electrode surface (Eq. 2). The transference of two electrons by cobalt(I) ion to O₂ occurs at the third stage of the reaction (Eq. 3). As the last stage, Co(III) is electrochemically reduced to Co(II) and the cycle starts with coordinative adsorption of O₂.

Conclusions

Cobalt(II) ion is quantitatively incorporated to the hematoporphyrin IX ring immobilized on the Cel/Nb₂O₅ surface. The complex is not leached from the surface even at high supporting electrolyte solution since it is immobilized

into the matrix surface by the –COO–Nb chemical bond. The cobalt-porphyrin acts as the active site oxygen reduction, presumably by a two-electron transfer mechanism.

Considering the relatively good chemical stability, the linear response of the peak current against the dissolved oxygen concentration at atmospheric pressure and ambient temperature, this metalloporphyrin-modified electrode system appears to be potentially useful for developing a new oxygen sensor.

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19. An alternative mechanism suggests that the reaction starts with the electroreduction, $\text{Co(II)} \rightarrow \text{Co(I)}$, and the molecular oxygen is coordinatively adsorbed on $[\text{Co(I)}]$ species.

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