

## ELECTROCHEMICAL STUDIES OF OXYGEN, HYDROGEN PEROXIDE, AND SUPEROXIDE ANION AT THIN METAL FILMS FOR THE DEVELOPMENT OF AMPEROMETRIC SENSORS

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**Abstract.** Electrocatalytic properties of thin films of gold and platinum evaporated onto a glassy carbon substrate were studied using the rotating disk electrode technique (RDE) and cyclic voltammetry (CV). The obtained kinetic parameters of oxygen reduction at thin films of gold and platinum electrodes are similar to the corresponding values at the bulk electrodes. An electrochemical method for the detection of the superoxide anion radical ( $O_2^-$ ) based on immobilized cytochrom *c* on the surface of a gold film is described.

**Key words:** electrochemical sensors, amperometry, voltammetry.

### INTRODUCTION

During the last decade much attention has been paid to the miniaturization of electrochemical sensors [1]. The miniaturized devices have several advantages over their macroscopic counterparts (e.g., reduced size, faster response, smaller sample volume). The application of carbon-based materials as a substrate for sensing elements allows us to fabricate inexpensive and disposable sensors [2]. The metallization of carbon substrates can be used for the improvement of sensor characteristics. Noble metals are widely used for the fabrication of thin films because of their electrocatalytic activity and chemical inertness.

The electrochemical reduction of oxygen has been extensively studied at noble metal electrodes because of its significance both from theoretical and practical aspects [3, 4]. The superoxide anion ( $O_2^-$ ) and hydrogen

peroxide are important intermediates of oxygen reduction at electrode surfaces. These species are also formed by partial reduction of oxygen in various biological systems and the determination of their concentration is of considerable interest.

In the present paper the electrochemical behaviour of  $O_2^-$ ,  $H_2O_2$ , and  $O_2$  at the thin film gold and platinum electrodes is discussed.

## EXPERIMENTAL

Electrochemical experiments were performed on a voltammetric system SVA-1 by using the rotating disk electrode technique (RDE) and cyclic voltammetry (CV). The rotation rate ( $\omega$ ) was varied from 360 to 4600 rpm.

The glassy carbon (GC) electrodes (0.41 cm in diameter) were cut from rods and mounted in a Teflon holder. The disk electrodes were polished to a mirror finish with 1.0 and 0.3 mm alumina (BDH) and then ultrasonicated in bidistilled water. Thin metal films were produced by vacuum evaporation at  $2 \times 10^{-6}$  torr. A tungsten spiral was used for film formation. Film thickness (ca. 50 nm) was measured by quartz crystal microbalance. The surface morphology of thin metal films was examined using scanning electron microscopy (JEOL JSM-35 CF).

All solutions were made in high purity 18 M $\Omega$  cm Milli Q water. The solutions 0.1 M KOH and 1.0 M KCl were prepared from reagents Pro Analyti (Merck). The solutions of  $H_2O_2$  were prepared in 0.066 M phosphate buffer. 1 M  $H_2O_2$  stock solution was used. Measurements were conducted in a three-electrode, three-compartment electrochemical cell. A Pt-wire counter electrode and a saturated calomel reference electrode were used. A conventional Clark-type oxygen sensor with thin film Au and Pt cathodes and a Ag/AgCl reference anode were constructed. A GC rod was imbedded into a Teflon sleeve and sealed with silicon rubber. The top surface of GC was coated with a noble metal film formed by vacuum evaporation. The outer surface of the sensor was covered with 20 mm thick polypropylene and the sensor's interior was filled with 1.0 M KCl.

The electrochemistry of immobilized cyt *c* was investigated in 10 mM phosphate buffer (pH 7) containing 10 mM KCl. The immobilization procedure was the same as that described in [5, 6]: 1) the thin film Au electrode was soaked into 10 mM N-acetyl-cysteine for 10 min and then rinsed thoroughly with bidistilled water, 2) the N-acetyl-cysteine modified gold film electrode was immersed into 10% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) solution for 20 min and was washed several times, and 3) the obtained electrode was incubated in 2 mM cyt *c* solution (pH 7) for 8 h at 4°C. The superoxide anion was generated using the xanthine/xanthine oxidase system. Xanthine, xanthine oxidase (grade III) and horseheart cytochrome *c* (type VI) were products of Sigma and used as received. All measurements were conducted at room temperature ( $23 \pm 1^\circ\text{C}$ ).

## RESULTS AND DISCUSSION

The real surface area of the electrodes was determined from the quantity of the charge under the hydrogen desorption peaks (for Pt film) and under the oxide formation peaks (for Au film). The roughness factor was about 5 for both thin film electrodes. The SEM micrographs indicate a complete coverage of the substrate surface by a metal layer.

RDE measurements were conducted in order to obtain data on the electrocatalytic properties of the thin film electrodes towards oxygen reduction. The kinetic parameters of oxygen reduction at the thin film gold and platinum electrodes were similar to those at the corresponding bulk electrodes. Two distinct linear Tafel slope regions for oxygen reduction were obtained for both the thin film and bulk platinum electrodes in alkaline electrolyte. The intersection point of the Tafel lines nearly coincides with the onset potential of Pt surface oxidation [7]. The exchange current density ( $i_0$ ) of oxygen reduction was estimated by extrapolating the Tafel lines to the reversible potential of the  $O_2/OH^-$  couple. The Table lists the kinetic parameters for oxygen reduction at platinum surfaces.

Kinetic parameters for oxygen reduction at thin Pt film and bulk Pt in 0.1 M KOH solution.

$$P_{O_2} = 1 \text{ atm}$$

Electrode	Tafel slope, mV/dec	$E_{1/2}$ , V; vs. Hg/HgO	$i_0 \cdot 10^{10}$ , A/cm <sup>2</sup>
thin Pt film	63±3; 238±20	-0.14	4.1
bulk Pt	61±2; 230±12	-0.16	2.3

The voltammetry curves for oxygen reduction at thin film Au and Pt electrodes in 1.0 M KCl solution are presented in Fig. 1. For the Pt film a single wave was observed with a well-defined diffusion-limited current plateau in the potential range from -0.3 to 0.8 V. As the electrode rotation rate increases, the width of the limiting current region becomes narrower, and the plateau is not ideally flat. The RDE data were analysed using the Koutecky-Levich (K-L) method (by plotting  $i^{-1}$  vs.  $\omega^{-1/2}$ ). From the slope of the K-L plots the number of transferred electrons per oxygen molecule was found to be close to four at the potentials of the limiting current region for both electrodes. However, there is a large variation of literature data for the diffusion coefficient of oxygen. It should be mentioned that the extrapolation of the straight lines  $i^{-1}$  vs.  $\omega^{-1/2}$  to the infinite rotation rate yields a nonzero intercept, which indicates that a step other than transportation is slow. Probably, this step is  $O_2$  adsorption at the electrode [8].

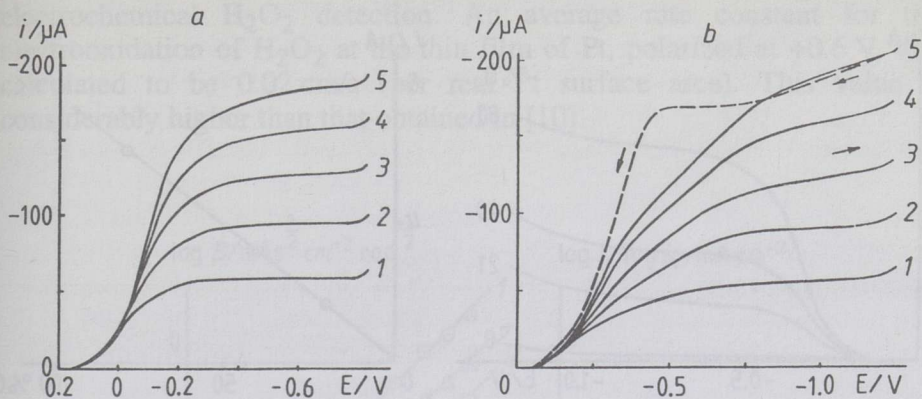


Fig. 1. Polarization curves for oxygen reduction in air-saturated 1.0 M KCl solution at  $\omega$  values of 360 (1); 960 (2); 1900 (3); 3100 (4); and 4600 (5) rpm. *a*, thin Pt film; *b*, thin Au film.

The thin film of gold shows two oxygen reduction waves on  $i,E$ -curves. The region of limited diffusion currents is shifted toward more negative potentials ( $-0.7\text{ V} > E > -1.2\text{ V}$ ) than that for the thin film Pt. The electrochemical behaviour of gold films toward oxygen reduction is dependent on electrode pretreatment [9]. Therefore, Fig. 1*b* shows just a set of data for a given type of electrode preconditioning. It is interesting to note that the polarization curves for  $\text{H}_2\text{O}_2$  reduction at the thin film of gold exhibit a limiting current region nearly in the same potential range as those for  $\text{O}_2$  reduction.

The presence of a current plateau on  $i,E$ -curves is favourable for the development of amperometric sensors with good performance characteristics. In order to achieve a stable output of the oxygen sensor, it is desirable that there should be no accumulation of electroactive intermediates of oxygen reduction (e.g.  $\text{H}_2\text{O}_2$ ) in the sensor's internal solution. To study the applicability of thin metal films for electrochemical sensors, a Clark-type oxygen sensor was fabricated. A specially designed holder was used for membrane attachment giving it a reliable fixation.

The linearity of the oxygen sensor response was tested in  $\text{O}_2\text{-N}_2$  gas mixtures. Current-potential curves for the oxygen sensor with the thin film gold cathode are given in Fig. 2. A horizontal current plateau extends to more positive potentials compared with the polarization curves in Fig. 1*b*. This indicates that in the case of a reduced oxygen flow the electrode process is fast enough, involving four-electron reduction of oxygen. By applying a constant voltage between the electrodes (0.8 V), a linear relationship of the output current vs. oxygen concentration was observed (Fig. 3). This is an indication that the overall process is under diffusion control. The response time of the sensor on step change in  $\text{O}_2$  concentration (21-0%) was within 15-20 s.

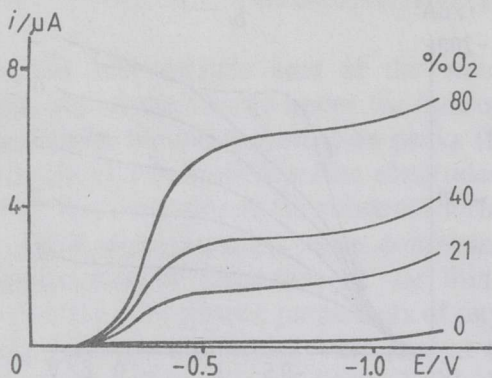


Fig. 2. Voltammograms for oxygen sensor with a thin film Au cathode in  $O_2-N_2$  gas mixtures.  $v = 0.5$  mV/s.

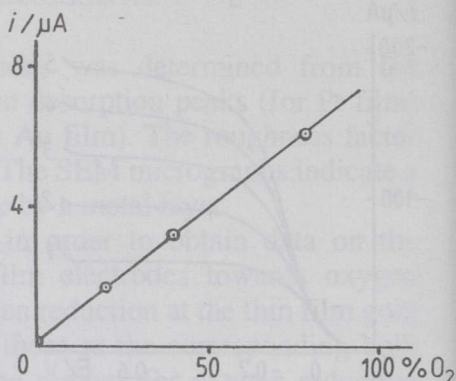
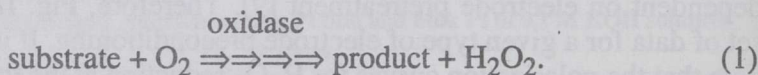
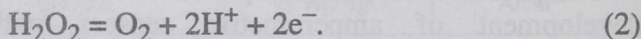


Fig.3. Calibration graph for oxygen sensor.

A large group of oxidase-based biosensors is based on the detection of hydrogen peroxide, whose production is directly related to the concentration of the substrate in the test media.  $H_2O_2$  is formed enzymatically according to the equation:



The rate of the hydrogen peroxide production is measured electrochemically by poisoning the electrode at a suitable anodic potential:



Owing to a high potential applied (which is required to ensure fast  $H_2O_2$  oxidation) the electrodes are prepared of noble metals. Platinum is used most frequently as an indicating electrode in  $H_2O_2$ -based biosensors, because of its high electrocatalytic activity towards  $H_2O_2$  oxidation.

The miniaturization of electrodes is a challenging goal in designing biosensors. The sensitivity of the thin film Pt on GC was studied by holding the electrode at a constant potential and by varying the  $H_2O_2$  content in the solution. The potential at which measurements were conducted was chosen to be +0.6 V. At this potential the electrode surface is highly oxidized, and it is necessary to stabilize the oxide layers for a long time. The  $H_2O_2$  concentration was changed by adding dropwise different aliquots of the  $H_2O_2$  stock solution. The range of the concentrations studied was within  $10^{-5}$ – $2 \times 10^{-2}$  M. The kinetic current density ( $i_k$ ) and the Levich parameter  $B$  were obtained from the K–L plots. Figure 4 shows the plot of  $\log B$  and  $\log i_k$  on the  $H_2O_2$  concentration. Both dependences were nearly linear with the slope values of 0.96 and 1.03, respectively. The proportionality of the oxidation current vs. hydrogen peroxide concentration provides the basis of the use of the thin film Pt for

electrochemical  $\text{H}_2\text{O}_2$  detection. An average rate constant for the electrooxidation of  $\text{H}_2\text{O}_2$  at the thin film of Pt, polarized at +0.6 V, was calculated to be 0.02 cm/s (per real Pt surface area). This value is considerably higher than that obtained in [10].

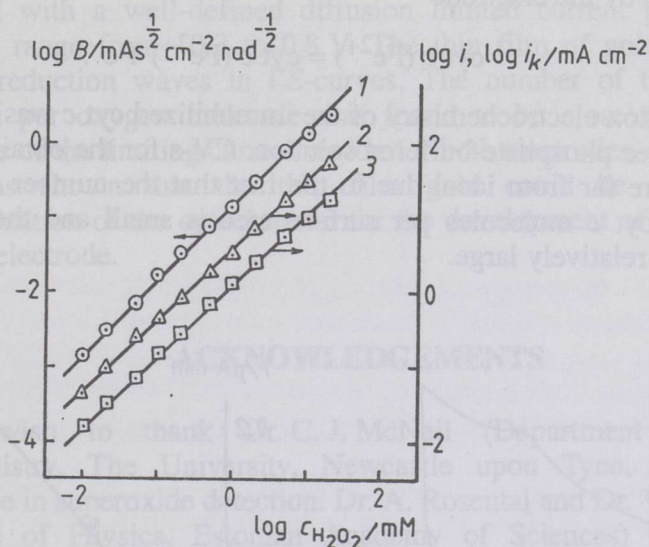
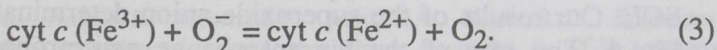


Fig. 4. The dependences of  $\log B$  (1),  $\log i_k$  (2), and  $\log i$  (3) on  $\text{H}_2\text{O}_2$  concentration for  $\text{H}_2\text{O}_2$  oxidation on thin Pt film in 0.066 M phosphate buffer, pH 7, containing 0.1 M NaCl;  $E = +0.6$  V.

The electrocatalytic behaviour of a thin film gold electrode on  $\text{H}_2\text{O}_2$  reactions is quite different from that of the thin film of Pt. The thin Au film exhibits a remarkable activity towards  $\text{H}_2\text{O}_2$  oxidation at high pH values. At neutral pH values both the reduction and oxidation processes of  $\text{H}_2\text{O}_2$  proceed at a high overpotential on a gold electrode. At more anodic potentials than +1.0 V, the Au film starts to deteriorate. Therefore, a gold electrode cannot be used as an indicating electrode for  $\text{H}_2\text{O}_2$  detection at neutral pH values.

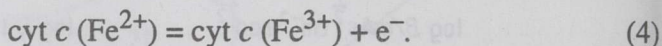
The superoxide anion radical ( $\text{O}_2^-$ ) belongs to reactive oxygen species and may damage organisms if it exceeds the level at which the organisms are able to provide defense. The determination of superoxide ion in vivo is a topical problem in clinical medicine. For the purpose of superoxide detection an electrochemical method can be used [5, 6].

It has been known earlier that  $\text{O}_2^-$  reacts with ferricyt *c*:



For the development of a superoxide-specific electrode, cytochrome *c* was immobilized covalently on the surface of a gold film. Recently, a method based on cyt *c* attachment at the self-assembled monolayer modified gold electrode via carbodiimide coupling was proposed in the literature

[5, 6, 11]. The surface modifier is not electroactive at the applied potentials, but it must provide a favourable orientation of *cyt c* molecules, which makes possible a fast electron transport from the redox protein to the electrode. The principle of the determination of superoxide ion is the following:  $O_2^-$  reduces ferricyt *c* to ferrocyt *c* (Eq. 3) and the latter is reoxidized by the electrode



The redox electrochemistry of the immobilized *cyt c* was studied in an oxygen-free phosphate-buffered solution. CV-s for the obtained electrode (Fig. 5) are far from ideal due to the fact that the number of covalently attached *cyt c* molecules per surface area is small and the background current is relatively large.

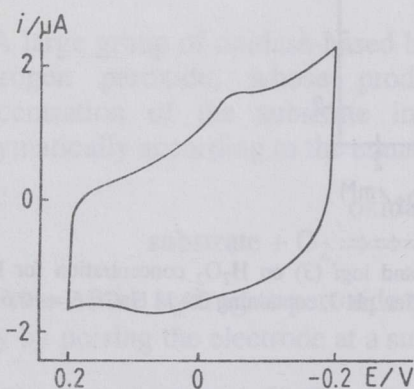


Fig. 5. Cyclic voltammograms for the *cyt c* immobilized thin film Au electrode in 10 mM phosphate buffer, pH 7. Sweep rate 50 mV/s.

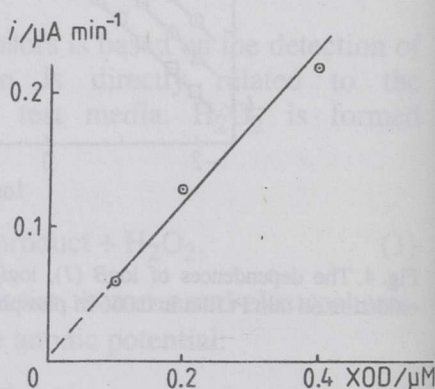
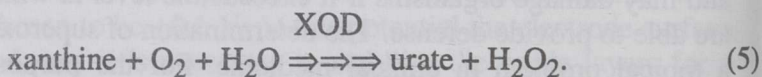


Fig. 6. The dependence of the rate of current change on XOD concentration in 20 mM phosphate buffer (pH 7.2) containing 0.1 M NaCl. Xanthine 0.5 mM.

The *cyt c* modified gold film electrode was tested *in vitro* by using a xanthine/xanthine oxidase system for superoxide generation:



$O_2^-$  is formed as an intermediate of reaction (5). The ferrocyt *c* reoxidation current was measured by poisoning the electrode at +50 mV vs. SCE. Our results of the superoxide anion determination are presented in Fig. 6. The rate of the current change was proportional to the XOD concentration. The slope value was  $3.3 \text{ mA} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot \text{mM}^{-1}$ , which is higher than in [5] due to an increased surface area.

Further research is needed for the improvement of *cyt c* immobilization methods.

## CONCLUSIONS

Using the rotating disk electrode technique and cyclic voltammetry, it was shown that the kinetic parameters of the reduction at thin films of gold and platinum evaporated onto a glassy carbon substrate are similar to those at the corresponding bulk electrodes. For the Pt film a single wave was observed with a well-defined diffusion limited current plateau in the potential range from  $-0.3$  to  $0.8$  V. The thin film of gold showed two oxygen reduction waves in  $i,E$ -curves. The number of the transferred electrons per oxygen molecule was found to be close to four at the potentials of the limiting current region for both electrodes.

It was shown that the thin film gold could be used for the immobilization of the cytochrom *c* for the development of a superoxide-specific electrode.

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## **ÕHUKESTEL METALLKATETEL TOIMUVATE HAPNIKU, VESINIKPEROKSIIDI JA SUPEROKSIIDIOONI ELEKTROKEEMILISTE REAKTSIOONIDE UURIMINE AMPEROMEETRILISTE SENSORITE VÄLJATÖÖTAMISEKS**

Kaido TAMMEVESKI, Toomas TENNO

On uuritud õhukeste metallkatete (Au ja Pt) elektrokatalüütilisi omadusi. Metallkatted valmistati vaakumis aurustamisel klaassüsinikalusele. Elektrokeemilised mõõtmised tehti pöörleva ketaselektroodi meetodil. Jälgiti hapniku redutseerumist, vesinikperoksiidi oksüdeerumist ning superoksiidiooni elektrokeemilist detekteerimist tsütokroom *c*-ga modifitseeritud kuldelektroodil.

## **ИССЛЕДОВАНИЕ ЭЛЕКТРОХИМИЧЕСКИХ РЕАКЦИЙ КИСЛОРОДА, ПЕРЕКИСИ ВОДОРОДА И СУПЕРОКСИДИОНА НА ТОНКИХ МЕТАЛЛОПОКРЫТИЯХ ДЛЯ АМПЕРОМЕТРИЧЕСКИХ СЕНСОРОВ**

Кайдо ТАММЕВЕСКИ, Тоомас ТЕННО

Исследованы электрокаталитические свойства тонкослойных металлопокрытий из золота и платины, полученных методом вакуумного напыления. Электрохимические исследования выполнены методом вращающегося дискового электрода. Установлено, что электрокаталитические свойства тонкослойных покрытий из золота и платины достаточно мало отличаются от соответствующих свойств цельнометаллических электродов. Показана возможность использования тонкослойного золота при конструировании чувствительного к супероксидиону электрода для сенсора супероксидиона.