Electrooxidation of Hexacyanoferrate(II) Anions and Electroreduction of Oxygen in the Microfabricated Electrochemical Sensor-Array System

K. Pitman\textsuperscript{a}, J. Nerut\textsuperscript{b}, E. Lust\textsuperscript{b}, S. Franssila\textsuperscript{c}, M. Raud\textsuperscript{a}, and T. Kikas\textsuperscript{a}

\textsuperscript{a} Institute of Technology, Estonian University of Life Sciences, Fr.R.Kreutzwaldi 56/1, Tartu 51014, Estonia
\textsuperscript{b} Institute of Chemistry, University of Tartu, Ravila 14a, Tartu 50411, Estonia
\textsuperscript{c} Aalto University, Otakaari 1 B, Espoo 02150, Finland

The microfabrication technique was used to prepare a six-channel sensor-array chip. The sensor-array has been fabricated using borosilicate wafer containing Pt electrodes and connection pads and the silicon wafer with through holes, which were bonded with SU-8 using adhesion bonding method. To characterize the prepared sensor-array chips the cyclic voltammetry and electrochemical impedance experiments were carried out in a three-electrode electrochemical system. The electrooxidation of ferrocyanide and electroreduction of oxygen were studied. It was shown that the measured current density depends on the electrode potential sweep rate however, both linear and radial diffusion mass transfer components are important at high electrode potential scanning rate. The pseudo steady-state conditions were achieved at slow potential sweep rates (>0.01 V s\textsuperscript{-1}) and the electrodes behaved as the microelectrodes. The pseudo steady-state current density is proportional to the concentration of electroactive species. The recessed microdisc electrode model has been used to describe the system.

Introduction

The field of application of microelectrodes is very wide: from electroanalytical applications (detection of ultra-low quantities of analyte, biosensors, etc.), kinetic studies of very fast and complex reactions, to measurements in solutions of very low conductivity, etc. (1–3). Microelectrodes are very useful for electrochemical sensing in large part because it is possible to achieve (pseudo) steady-state quite quickly compared with larger electrodes (3,4). The microelectrodes could be made of different materials and the geometry of the electrode is limited only by the fantasy and the aim of user. Recessed microelectrodes are used for analytical purposes, e.g. for liquid chromatography (5). Often several microelectrodes are combined into sensor-arrays for simultaneous analysis of various components and kinetic parameters.

Many methods for microelectrode preparation have been proposed however, most of the array electrodes are normally fabricated using microphotolithography and screen-printing (6). Microfabrication is a high precision fabrication technology, which is suitable for preparing microelectrodes. It is based on deposition, lithography and etching steps using materials like silicon, polymers, metals and oxides that enable the fabrication of wells, reactors, channels, and electrodes with dimensions in the micrometer to millimeter
range. Microfabrication technology also enables batch-fabrication, which leads to production of inexpensive sensors and even, disposable, maintenance-free sensors (7).

Microelectrode arrays present an opportunity for the integration in ‘lab-on-a-chip’ devices, which can be used in a plethora of applications (8). Electrochemical microsensors and sensor-arrays enable to develop inexpensive, on-site and real time analytical methods for measuring various pollutants (9). Environmental monitoring and analysis is widely used field for application of sensor-arrays in samples such as drinking, ground, sea and industrial waters where reliable and robust analytical techniques with low detection limits are required (6). In addition, it has been shown that by applying various multidimensional data analysis methods to sensor-array data it is possible to extract more information from measurement data (7,10).

The previous paper was mainly dedicated to the fabrication of six-channel sensor-array chip (1). The chip was prepared using microfabrication technology and based on silicon and glass wafers and platinum electrodes. The sensor-array chip was designed for the future use for dissolved oxygen measurements in a biochemical oxygen demand (BOD) sensor array.

The aim of this work is to study the electro-oxidation of hexacyanoferrate(II) anions on the platinum microelectrode of the sensor-array in the 1.0 M KCl aqueous solution and use the same sensor-array for determination of oxygen concentration in the 0.5 M H₂SO₄ solution.

Experimental

The preparation of the sensor array microchips is described in our previous paper in detail (11). Shortly for fabrication silicon wafers (100 mm diameter and thickness of 500–525 µm) and borosilicate wafers (Pyrex glass with same dimensions as silicon wafers) were used. Chromium/platinum (10 nm/100 nm thick) were sputtered on a borosilicate wafer and patterned by wet etching method using aqua regia (3:1 HCl :HNO₃ 24°C, 4 min) and chromium etchant (solution of Ceric Ammonium Nitrate, 248, 10 sec). Silicon wafer was etched through using hot TMAH (tetramethylammonium hydroxide) solution and RIE (reactive ion etching) to form holes with slanting sidewalls for immobilization cavities. The hole depth, L, was 483 µm. The processed silicon and Pyrex wafers were bonded using adhesive bonding with SU-8 epoxy resin. The individual sensor-array chips were separated by dicing. The rectangular platinum working electrode with dimensions 700×400 µm were deposited in the center of the cavity.

The potentiostat Reference 600 (Gamry Instruments Inc) was used for cyclic voltammetry (CV) and electrochemical impedance (EIS) measurements. In the three-electrode configuration the working electrode was inner platinum band electrode, the counter electrode was a large Pt wire mesh separated from the working electrode compartment by a fritted glass membrane. The reference electrode was Ag|AgCl| aqueous saturated KCl (3). The CV data at fixed potentials: 0.0 and 0.7 V were measured at different potential sweep rates, v, from 5 to 1000 mV s⁻¹. The EIS data were obtained within ac frequencies, f, from 100 mHz to 0.1 MHz (12 points per decade) with 5 mV (rms) ac voltage amplitude. The glassware was treated with the hot (80 °C) concentrated...
sulfuric acid (95.0-98.0%, Sigma-Aldrich, puriss. p.a.) with small addition of hydrogen peroxide (30%, Merck KGaA, Perhydrol® for analysis) and rinsed carefully with Milli-Q+ (18.2 MΩ cm) water. All solutions were prepared using Milli-Q+ water.

Potassium ferrocyanide (K₄[Fe(CN)₆]) (chemically pure) was purchased from Реахим. Potassium chloride (KCl) (assay 100.0%) was purchased from Lach-Ner (Czech Republic). All the solutions were prepared from Milli-Q+ water. The concentration of potassium chloride was kept constant (1.0 M) and the concentration of potassium ferrocyanide was varied between 0.5 mM and 6 mM. Prior to the measurements, the solutions were deoxygenated with argon (99.9999%, AGA) during 15 minutes.

The oxygen reduction was studied in the 0.5 M H₂SO₄ solution at different partial pressures of oxygen. The 0.5 M H₂SO₄ solution was prepared from the concentrated sulfuric acid solution (≥ 95%, Fluka Analytical, TraceSELECT®, for trace analysis). Before measurements the electrolyte solution was saturated with argon or with mixture of argon and oxygen (99.9999%, AGA) during 15 minutes. The partial pressure of oxygen in the mixture of gases was varied from 0 kPa up to 98 kPa with five fixed compositions. The total pressure of gas was 101 kPa. The Brokhorst® mass flow controllers (EL-FLOW Select®) were used to prepare the gas mixtures.

The experiments were performed at room temperature (22±1°C). Three microchips were used. Parallel experiments were conducted and the data were statistically treated. It was found that different microelectrodes were electrochemically active and the reproducibility of EIS parameters and (pseudo) steady-state current densities was good. Within one measurement the time stability of cyclic voltammograms and EIS data was very good.

Results and Discussion

Purification and activation of the sensor-array

It was necessary to clean the platinum electrodes of the sensor-array before the measurements. Without the cleaning step it was impossible to get the electrical contact, i.e. the Pt electrode was covered with isolating film or the shape of the cyclic voltammogram in the sulfuric acid deviated considerably from the usual behaviour (3,12). The cleaning step consisted of two phases.

Firstly, the sensor-array was sonicated (Bandelin electronic RK 52 H, 60 W, 35 kHz) for 10 min in the acetone (>95%, APC Chemicals). After the sonication, the acetone was poured away and the array was dried in air. This procedure was performed only once with each chip. Continuous treatment with acetone led to decomposition of the sensor-array and the lower part of the array separated from the upper part.

Secondly, the array was cleaned in the hot (50 °C) concentrated sulfuric acid with small addition of hydrogen peroxide (30 wt%). The array was heated up in the sulfuric acid and left to stand overnight. Next morning the array was rinsed with Milli-Q+ water. Thereafter the array was treated in boiling water from three to five times. Between the
boiling steps the array was rinsed with Milli-Q+ water. The same cleaning method was applied before each measurement.

After the cleaning of the array, the platinum electrodes were electrochemically activated in the 0.5 M H₂SO₄ solution (12). The electrode potential was swept between −0.18 V and 1.20 V 50-100 times (500 mV s⁻¹). The activation process is illustrated in Fig. 1. During the activation process, the current density |j| increased in the region of hydrogen adsorption and desorption (from −0.18 V to 0.20 V), and platinum oxidation and reduction (from 0.40 V to 1.20 V) regions. The cycling was performed until the cyclic voltammogram was invariant within mentioned potential region. The experiments were performed only with the electrodes that had good electric contact and showed similar characteristics in the 0.5 M sulfuric acid solution.

Electrochemical Experiments

Cyclic voltammetry in 1 M KCl and 0.5 M H₂SO₄ solutions. All sensor-array chip electrodes were characterised in the 0.5 M H₂SO₄ solution and in the 1 M KCl solution. The cyclic voltammograms are presented in Fig. 2. The current density is proportional to the sweep rate (inset in Fig. 2a) i.e. the mainly capacitive processes are taking place at the Pt | 1 M KCl interface. There is slight increase of current density |j| at potentials more negative than −0.05 V that could be caused by the slow hydrogen evolution. At potential more positive than 0.60 V, the increase of current density might be induced by the oxidation of the platinum surface i.e. formation of the oxide layer.

For the sulfuric acid solution, the cyclic voltammetry data (Fig. 2b) are presented as capacitance, C, vs. potential. The capacitance is calculated using equation \( C = j/v \). The shape of curves for the Pt | 0.5 M H₂SO₄ interface at different potential sweep rates is typical for polycrystalline platinum (12,13). The hydrogen adsorption/desorption region (below 0.15 V) is very well defined. At potentials more positive than 0.60 V (anodic

Figure 1. The electrochemical activation of platinum working electrode in the 0.5 M H₂SO₄ solution. The potential sweep rate was 500 mV s⁻¹ (50 cycles). The increase of cycle number is indicated with arrows.
scan) the surface oxides are formed which are reduced during cathodic scan. Between these two regions, there is so-called electrical double layer (edl) area within this region. The capacitance is almost independent of potential sweep rate except for lowest sweep rates at potentials more negative −0.10 V. The increase in capacitance is caused by the start of hydrogen evolution at platinum electrode in acid solution.

The real (or working) surface area of platinum electrode, \( A_{\text{EL}} \), has been calculated using method in Refs (12,14–16). The area under the hydrogen adsorption-desorption peaks was integrated to get the total charge, \( q \). The charge, \( q_{\text{edl}} \), consumed for charging and discharging of the edl in the region of the peaks was estimated from the edl capacitance at 0.2 V and it was assumed to be the same in the region of peaks. The edl charge was subtracted from the total charge to obtain the charge, \( q_{\text{Pt-H}} \), used for the formation of the monolayer of adsorbed hydrogen atoms Pt-H on the surface of platinum. T. Biegler et al. (17) evaluated that the charge required to adsorb/desorb a monolayer of hydrogen atoms on the smooth polycrystalline Pt electrode is equal to 2.1 C m\(^{-2}\). Using this approximation, it is possible to estimate the real surface area of the platinum electrode as (14):

\[
A_{\text{EL}} = \frac{q_{\text{Pt-H}}}{2.1 \text{ C m}^{-2}} S, \tag{1}
\]

where \( S \) is the geometric area of the electrode. The results of the calculations of \( q_{\text{Pt-H}} \) are presented as inset in Fig. 2b. At slow sweep rates the charge is about 13% higher compared to the value at higher sweep rates. This is caused by the hydrogen evolution
and these sweep rates are left out from the analysis. The real surface area of the platinum electrode were estimated as 0.104±0.023 mm$^2$.

In further analysis, the current densities measured in the base electrolytes were subtracted from the current densities measured in the solution containing also $K_4[Fe(CN)_6]$ or oxygen to get the current density of the electrode reaction, $j_c$.

**Electrooxidation of ferrocyanide.** In the first set of experiments, the ferricyanide/ferrocyanide redox couple was used to test the electrochemical behaviour of the sensor-array chip. The data of the CV measurements are presented in Fig. 3. The oxidation of $[Fe(CN)_6]^{4-}$-anions starts at 0.22 V:

$$[Fe(CN)_6]^{4-} = [Fe(CN)_6]^{3-} + e^-, \tag{2}$$

which is in the region of the formal reduction potential of the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox couple in 1 M KCl ($E^{0'} = 0.286$ V vs. Ag|AgCl|sat. KCl (18,19)). Clear diffusion limited current plateaus were reached for low concentrations of $[Fe(CN)_6]^{3-}$ anions ($c \leq 3$ mM). However, for highest concentrations clear diffusion limited current plateaus were not achieved. There is no reduction peak of the $[Fe(CN)_6]^{3-}$ anions formed at the surface in the reverse scan (Fig. 3a) (even at the highest sweep rate) indicating that the contribution of the radial diffusion is dominant in the system under study. Although complete stationarity is not achieved as, there is hysteresis between cathodic and anodic scans. The extent of hysteresis is somewhat more pronounced at highest sweep rate although the oxidation peak is not formed. The hysteresis also increases with increasing concentration of ferrocyanide.

![Figure 3](image_url)

Figure 3. The dependence of the corrected current density, $j_c$, on the electrode potential, $E$, (a) at different electrode potential sweep rates, $v$, (noted in figure) for the 1.0 M KCl + 5 mM $K_4[Fe(CN)_6]$ solution saturated with argon and (b) in the 1.0 M KCl solution saturated with argon with different additions of $K_4[Fe(CN)_6]$ (noted in figure) at $v = 0.01$ V s$^{-1}$. Inset in part (a): $j_c$ vs. $c(K_4[Fe(CN)_6])$ for different sweep rates and in part (b): $j_c$ vs. $v$ for different additions of $K_4[Fe(CN)_6]$ at 0.75 V.
Inset of Fig. 3a shows at 0.75 V linear dependence of the corrected current density on the concentration of $K_4[Fe(CN)_6]$ at all sweep rates. These dependences are linear at all sweep rates and the intercept is zero in agreement with that expected for the first order reaction. At higher concentrations of ferrocyanide $c > 6 \text{ mM}$, there is negative deviation from the linearity.

The peak current vs. $\nu^{1/2}$ dependencies are expected to be linear for reactions where the semi-infinite linear diffusion at planar electrode takes place (3). At constant concentration of the $[Fe(CN)_6]^{4-}$ anions, dependences of $j_c$ (at 0.75 V) on $\nu^{1/2}$ are nonlinear, the ordinates differ from zero and depend systematically on the reactant concentration (inset in Fig. 3b). Thus, the mass transport is dominated by the radial component of diffusion and the electrodes behave as microelectrodes. In these conditions the recessed microdisk electrode model developed by A.M. Bond et al. (20) describes the behavior of experimental current density. The stationary current density, $j_{ss}$, could be calculated using equation:

$$j_{ss} = \frac{4\pi n F c D r^2}{S(4L + \pi r)},$$

where $n$ is the number of electrons transferred in electrode reaction, $F$ is the Faraday constant (96485 C mol$^{-1}$), $c$ is the bulk concentration of the reactant, $D$ is the diffusion coefficient of the reactant and $r$ is the radius of the disk. The theoretical slope of $j_{ss}$ vs. $c$ in these conditions is 0.11 A m$^{-2}$ mM$^{-1}$ if $n = 1$, $D([Fe(CN)_6]^{4-}) = 8.07 \cdot 10^{-6}$ cm$^2$ s$^{-1}$ (in 1 M KCl) (19) and $r$ is calculated from the surface area of the electrode. The experimental current densities at 0.75 V were extrapolated to sweep rate $\nu = 0$ and used to calculate the experimental slope in same conditions. The experimental value 0.16 A m$^{-2}$ mM$^{-1}$ is in relatively good agreement with the theoretical calculated value. The slope value is greatly overestimated with models using other geometries (10 mV s$^{-1}$): microdisk (5 times), microband (50 times), and infinite plane (6 times) and therefore these models cannot be used (11).

**Electroreduction of oxygen.** The sensitivity of the microchip array was also tested towards oxygen reduction (Fig. 4). The oxygen reduction reaction on the platinum electrode is irreversible and the four-electron process prevails in acid solution (21):

$$O_2 + 4H^+ + 4e^- = 2H_2O$$

As the oxygen electroreduction on the platinum electrode is irreversible, there is no anodic peak during the reverse scan. The hysteresis between anodic and cathodic scans is very small (Fig. 4b) and the clear limiting current plateaus have been achieved, compared with ferrocyanide. At potentials more negative than $-0.1$ V, the corrected current densities $|j_c|$ started to decrease because the two electron reduction pathway becomes also important and hydrogen peroxide is additionally formed on the electrode (21,22):

$$O_2 + 2H^+ + 2e^- = H_2O_2.$$  

The limiting current density is proportional to partial pressure of oxygen at all sweep rates used (inset in Fig. 4a) and the intercept is very close to zero. As in case of ferrocyanide, the corrected current density at 0 V is not proportional to $\nu^{1/2}$ (inset in
Figure 3b). The propagation of the diffusion layer, \( \delta \), in time, \( t \), could be described by simple formula (3,4):

\[
\delta = \sqrt{\pi D t} .
\]  

[6]

In case of oxygen reduction, the diffusion layer thickness becomes comparable with the critical dimension of the electrode more quickly and the radial diffusion becomes more important than linear diffusion at higher sweep rate as the diffusion coefficient of oxygen \( D(O_2) = 18 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1} \) (in 0.5 M H_2SO_4) (13) is more than two times higher compared to ferrocyanide. The diffusion limited current density \( j_c \) for solution saturated with oxygen \( c(O_2) = 1.13 \text{ mM} \) in 0.5 M H_2SO_4 (13,23)) is 10 times higher if \( \nu = 10 \text{ mV s}^{-1} \) and 13 times higher at \( \nu = 1000 \text{ mV s}^{-1} \) higher compared to \( j_c \) at 0.75 V in 1 mM K_4[Fe(CN)_6]. This could be explained using Eq. 3: the difference in the factor \( nDc \) for oxygen and ferrocyanide is ten times. As at slower sweep rate the system is closer to pseudo steady-state, the agreement is also better. The theoretical and experimental slope values of \( j_c \) (at 0.1 V) vs. \( c \) dependencies are \(-0.97 \) and \(-1.18 \text{ A m}^{-2} \text{ mM}^{-1} \), respectively. The consistency is reasonable, although for both substances the theoretical values are underestimated. The oxygen reduction process conditions are closer to the pseudo steady-state mode as the diffusion coefficient of oxygen is higher. To get better understanding of the system behavior the simulation should be performed using the right geometry of the working electrode (24).

Electrochemical impedance spectroscopy measurements. The data of the EIS measurements in 1 M KCl solution without and with addition of ferrocyanide are
presented in Figs. 5-6. The shape of Nyquist plots (Fig. 5) depends on the electrode potential and concentration of the potassium ferrocyanide in 1.0 M KCl solution. The electrolyte resistance, $R_{el}$, (between the reference electrode and working electrode) is calculated from the extrapolation of real part of impedance to infinity ($R_{el} = Z'(f \to \infty)$). The electrolyte resistance is equal to 0.9 Ω cm$^2$ and is independent of potential and concentration of electroactive species (insets in Fig. 5). There are two minima in the $\theta$ vs. log$f$ plots at ac frequencies 10 Hz and 10 kHz (Fig. 6), respectively. Two processes with different time constants, which differ by three orders of magnitude, govern the adsorption. A. K. Ahuja et al. (25) also observed two minima in the Bode phase angle plot and the deepening of high frequency minimum becomes with decreasing of the radius of microdisk electrode in the surface inactive electrolyte. The appearance of the high frequency minimum was explained by the step-by-step replacement of “solution resistance regime” with “the area driven capacitive regime” i.e. the $RC$ time constant decreases (25). There are also two linear areas with the same slope in the Bode magnitude plot in 1 M KCl (Fig. 6a) – the transition region is between 100 and 1000 Hz. The appearance of the low frequency maximum could be explained by the increase of relative importance of the secondary current distribution at lower frequencies (25).

After addition of the ferrocyanide to the 1 M KCl solution, the Nyquist and Bode plots do not change remarkably at 0 V (Figs 5a and 6a) as at this potential ferrocyanide does not oxidize (Fig. 3). Some changes in the impedance could be explained by the alternation of the edl capacitance induced by the adsorption of ferrocyanide. The situation is different at 0.7 V. The depressed semicircle is formed as the concentration of the ferrocyanide in the solution increases (Fig. 5a). The Bode plot reveals that at low frequencies ($f<100$ Hz) the phase angle $-\phi$ and the impedance $Z$ decreases with the increasing concentration of ferrocyanide. The apparent polarization resistance, $R_p$, could be calculated from the extrapolation of the real part of the impedance to zero frequency ($R_p = Z'(f \to 0)$). The apparent polarization resistance at 0.7 V (inset in Fig. 6b) decreases.

Figure 5. The dependencies of the imaginary part, $Z''$, vs. real part, $Z'$, of the impedance in the 1.0 M KCl solution with different additions of $K_4[Fe(CN)_6]$ (noted in figure) at the electrode potentials: (a) 0 V and (b) 0.7 V.
with increasing of the concentration of ferrocyanide. The $R_p^{-1}$ vs. $c(K_4[Fe(CN)_6])$ dependence is linear as the $j_c$ (at 0.7 V) vs. $c(K_4[Fe(CN)_6])$ dependences, however the EIS data are measured in stationary state. The interpretation of EIS data at 0.7 V using physical models is challenging task. The mixed charge transfer kinetics: faradaic charge transfer, mass transfer and adsorption limited steps are determining the electrochemical behavior of the system under study. A. Lasia (26) modelled semi-infinite external spherical diffusion and found that, contrary to the semi-infinite linear diffusion, where the Warburg impedance approaches infinity as $f \to 0$, in the case of spherical diffusion the impedance goes to a real constant as $f \to 0$. This means that the current passes in the system as it occurs for microelectrodes in stationary state.

Summary

The sensor-array has been fabricated using borosilicate wafer containing Pt electrodes and connection pads and the silicon wafer with through holes, which were bonded with SU-8 using adhesion bonding method.

The sensor-array was sensitive towards ferrocyanide and oxygen. The calibration graphics were linear for both reactants (oxygen and ferrocyanide) used.

It was shown that the measured current density depends on the electrode potential sweep rate and both linear and radial diffusion mass transfer components are important at high electrode potential scan rates. For slow potential sweep rates ($\nu \leq 0.01$ V s$^{-1}$) the pseudo steady-state has been achieved and the electrodes behaved as the microelectrodes. The recessed microdisk model worked out by Bond et al. was used to describe the behavior of pseudo steady-state current densities. The consistency of theory with experiment was very good for oxygen electroreduction because the pseudo-steady state

![Image](image.png)
was achieved more readily for this system at slow sweep rates. For ferrocyanide oxidation slower sweep rates have to be used to accomplish better agreement.

The EIS was used to describe the system performance in 1 M KCl solution with and without addition of ferrocyanide. For the system with addition of ferrocyanide the depressed semicircle was formed in the Nyquist plot in the region of diffusion limited current densities. The polarization resistance decreased systematically with increasing concentration of ferrocyanide.

Based on the electrochemical characterization data collected, it can be concluded that the produced sensor array is suitable for future research including BOD biosensor-array construction.

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