Scanning X-ray micro-fluorescence study of spincoated gold-electrodes modified with cytochrome *c*

H. Buschop, K. De Wael, A. Verstraete, T. Schoonjans, B. Vekemans, L. Vincze, A. Adriaens*

Department of Analytical Chemistry, Ghent University, Krijgslaan 281-S12, B-9000 Ghent, Belgium
**Postdoctoral Fellow of the Research Foundation – Flanders (Belgium)*

The present works aims at the characterization of immobilized horse heart cytochrome c (HHC) onto a mercaptoethylamine (MEA) modified gold electrode. The immobilization occurs by means of cross-linking HHC with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), after which the cross-linked HHC film is deposited onto the MEA modified gold surface. MEA is used to ensure a covalent bonding between the protein and the gold surface. Modified electrodes, such as the one described above, can form the basis in the development of new kinds of biosensors or bioreactors. In previous measurements a thick layer (45 µm) of immobilized proteins was characterised [1]. In this work measurements were performed on spincoated electrodes, which results in a thinner layer of the immobilized protein on the electrode surface.

The formation of the above-mentioned films was done according to the following procedure. Gold electrodes (Basi) of 1.6 mm diameter were immersed into a water solution containing 5 mmol L^{-1} mercaptoethylamine (MEA) for 24 hours at room temperature. The modified gold electrodes were consequently rinsed with water to remove physically adsorbed MEA.

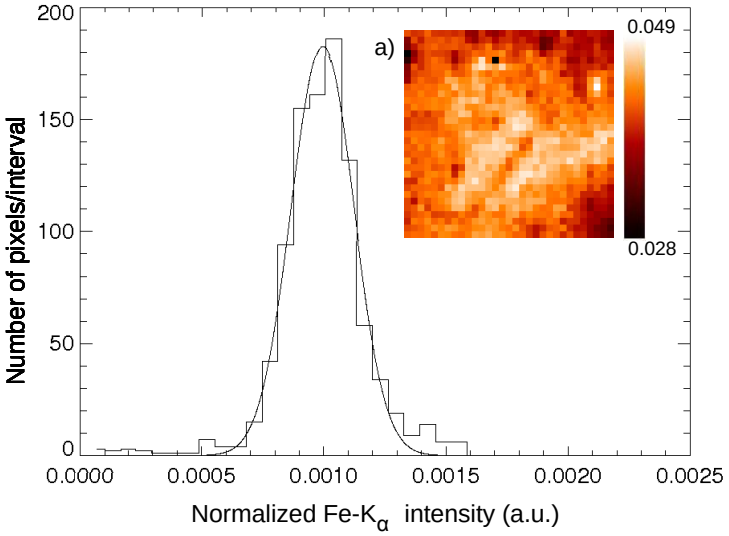

Figure 1. Derived Fe-K_α histogram (normalized with ionisation chamber) and (a) typical micro-XRF map (normalized with gold-M lines) from a 3.75 mmol L^{-1} spincoated HHC thin film.

In what follows these modified electrodes are denoted as MEA|Au. To immobilize HHC onto MEA|Au, a drop of 20 µL, containing 3.75 mmol L^{-1} of HHC and 0.6 mol L^{-1} EDC solution was brought onto the surface. The electrode was then spincoated at 3500 RPM for 5 minutes. Afterwards the electrodes were exposed to the air for 2 hours at room temperature. Finally, the electrodes were washed in a HEPES buffer solution. In what follows they are denoted as HHC|EDC|MEA|Au.

Micro SR-XRF measurements were performed on the above-mentioned samples at beam line L to provide experimental evidence of the existence of the HHC layer and to determine the surface concentration of the formed film. With these values an estimation of the thickness of the immobilised film can be made. The distribution of the iron concentration (and therefore also the HHC concentration) on a microscopic level was derived by recording 961 individual spectra (representing a 31 × 31 SR-XRF map), each of which corresponds to concentration

values from microscopic regions on the 20 µm scale. The mappings of the performed measurements were normalized with the gold-M-lines which can be used as an internal standard. For the calculations with the fundamental parameter method and the histogram the measurements were normalised with the ionisation chamber. The concentrations were derived by using a Fe-foil of 125 µm as a standard. Figure 1 shows a histogram and a mapping derived from the measurements.

Preliminary results are shown in Table 1. The surface concentration for an immobilised film with a HHC concentration of 3.75 mM in the droplet is approximately 7.02 nmol cm^{-2}. This value is corrected for the iron concentration on a MEA|Au electrode (1.6 nmol cm^{-2}) and is obtained with the fundamental parameter method. Table 1 also shows the value calculated by means of the histogram and the heterogeneity factor $\delta_{het,r}$ [2]. The concentrations derived with both techniques are more or less equal. The total set of experiments included samples with a HHC concentration range of 1.5 mmol L^{-1} and 3.75 mmol L^{-1}.

Table 1. Surface concentration values calculated from the XRF measurements and corrected with a measurement of a MEA|Au electrode

Sample	Total Fe intensity / counts s^{-1}	Surface concen-tration / nmol cm^{-2} (blanc correction)	Mean (histogram)	Surface concen-tration / nmol cm^{-2} (blanc correction)	$\delta_{het,r}$ (%)
HHC(3.75mM)|EDC|MEA|Au	443	6.7	0.032	6.3	52
HHC(3.75mM)|EDC|MEA|Au	421	7.3	0.036	7.3	30
HHC(3.75mM)|EDC|MEA|Au	373	7.0	0.027	6.6	44

When taking into account that a monolayer of HHC has a surface concentration of 3.37×10^{-11} mol cm^{-2} [3] and that HHC is a globular protein with a diameter of 2.5 nm [4], a thickness of approximately 500 nm can be calculated.

References

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