Scanning X-ray micro-fluorescence study of gelatin as a matrix for the immobilization of redox enzymes

A. Verstraete, T. Schoonjans, K. De Wael, L. Van de Voorde, M. De Keersmaecker, 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)*

Introduction

The work presented in this paper contributes to a research project in which electrode surfaces are modified with redox enzymes using improved immobilization techniques, such as their incorporation into a biocompatible matrix. Such systems have their importance, amongst other fields, in the development of bioelectrochemical devices (e.g. biosensors). The embedding matrix, currently under investigation at this moment, is gelatin, an example of a physically cross-linked hydrogel.

The enzyme containing bio layers, which are deposited onto the electrode surfaces, are studied by performing scanning micro-XRF experiments at beamline L of the Doris III storage ring. These measurements are based on the excitation of the metal ion present in the enzyme. By constructing elemental maps based on the scanning micro-XRF measurements, emphasis is placed on the investigation of the uniformity or homogeneity of the deposited film on a 20 µm scale. In addition, a comparison with a pure standard foil of the element under study provides quantitative information of the surface coverage. The obtained results were normalized with the ionisation chamber readiout in order to construct the elemental maps and to perform the calculations with the fundamental parameter method.

Experimental procedure

Gold electrodes (BASi, UK) of 1.6 mm diameter were modified according to the following procedure. In a first step, the gold surface was modified with a self-assembled monolayer (SAM) of 6-mercapto-hexanol by immersing the electrode in a water solution containing 1 mmol.L^{-1} 6-mercaptohexanol (MH) for ca. 18 h at room temperature. After rinsing these modified electrodes (MH|Au) with water, the immobilization of the enzyme under investigation was performed by means of the drop drying technique. In this technique, a mixed solution (7 µL), containing gelatin and an enzyme solution (in a 5:2 ratio), is deposited on the MH|Au surface. Different concentrations of the enzyme were studied, while the used gelatin solution was always 5 w/v%. The modified electrodes (enzyme|Gel|MH|Au) are exposed to air and stored at 4 °C prior to starting the micro-XRF experiment.

Immobilization of horse heart cytochrome *c* into a gelatin matrix

In a first series of micro-XRF experiments, we focussed on the distribution of the iron (Fe) containing protein horse heart cytochrome *c* (HHC) in a layer of gelatin. The electrochemical behaviour of this system has been published in [1]. The studied HHC concentrations were 0.143, 0.100 and 0.050 mmol.L^{-1}. This work is a continuation of earlier work in which a different immobilisation method was examined for HHC [1]. Thorough processing of the measured data revealed an experimental difficulty concerning unexpectedly strong elastic scattering intensities, resulting in a high total detected count rate (and high dead time) by the silicon drift detector used in the experimental set-up. The problem was revealed by studying the individual XRF spectra across the map (20 µm x 20 µm), which clearly showed too high detector dead time values, reaching in some cases up to 70 %. The unexpected extra elastic peak intensity most likely originates from diffraction effects from the gold surface at specific excitation energies for Fe under conventional 45-45º excitation/detection geometry. Due to the observed high count rates/dead times at specific beam positions, standard dead time correction algorithms can not be applied during these data processing and internal normalization techniques will be necessary.

Immobilization of alcohol dehydrogenase into a gelatin matrix

During a second experiment, the immobilization of the zinc (Zn) containing enzyme alcohol dehydrogenase (ADH) in gelatin was investigated. The ADH concentrations under study were 0.500 and 0.125 mmol.L^{-1}.
In order to avoid the above-mentioned dead time problem, a new measuring strategy was applied according to the following procedure. Prior to each scanning experiment, a fast scan was made to check two important factors: the maximum detector dead time and the maximum total count rate during the fast scan. In case of observing too high count rates during the pre-scanning step (resulting in dead times > 30 %), the incoming beam intensity was reduced until the appropriate conditions (dead time < 30 %, total maximum count rate < 35000 counts/s) were attained. The data processing indicated that this extra optimization strategy was a suitable solution to the aforementioned problem. The results of the samples with the highest ADH concentration are shown below.

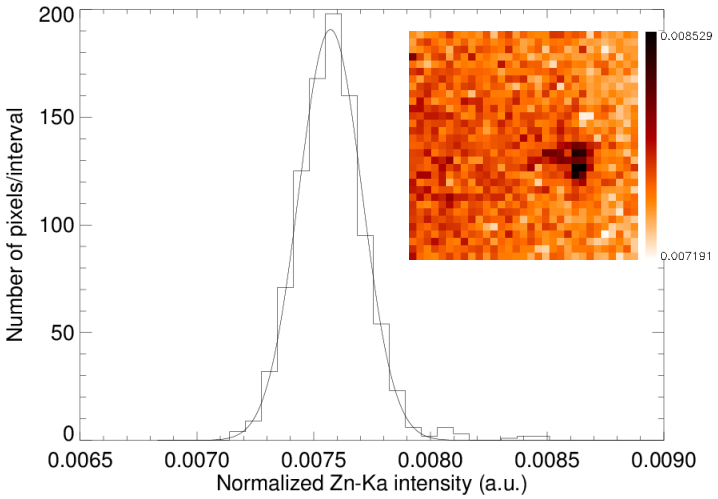

Figure 1: Zn-K_α histogram and micro-XRF map (both normalized with ionisation chamber) of the ADH(0.5 mM)|Gel|MH|Au sample (a)

Sample	Total Zn intensity		Mean of histogram	
	Normalized Zn-K_α intensity	Surface concen-tration (nmol Zn.cm^{-2})	Normalized Zn-K_α intensity	Surface concen-tration (nmol Zn.cm^{-2})
(a)	0.00761	49.6	0.00751	50.2
(b)	0.00775	50.6	0.00744	49.8
(c)	0.00783	51.1	0.00775	51.8

Table 1: Normalized Zn-K_α intensity and surface concentration values, based on the total Zn intensity and the mean of the histogram of the ADH(0.5 mM)|Gel|MH|Au samples

Figure 1 represents a typical Zn-K_α intensity histogram and the corresponding map (both normalized with ionisation chamber) of a ADH|Gel|MH|Au samples with 0.5 mmol.L^{-1} being the concentration of the ADH solution. The results of the ADH(0.5 mM)|Gel|MH|Au samples, based on the total Zn intensity and the mean of the obtained histogram, are shown in Table 1. Blanc correction with a Gel|MH|Au (no enzyme present) was not necessary since the Zn intensity of this sample is ~ 0 counts.s^{-1}. There is not only a good agreement between the three samples, suggesting a reproducible modification method, but also between both methods, which indicates a rather high homogeneity of the measured samples.

References

[1] K. De Wael K., S. De Belder, S. Van Vlierberghe, G. Van Steenberge, P. Dubruel, A. Adriaens, Talanta **82**, 1980 (2010)

[2] K. De Wael, H. Buschop, S. Van Vlierberghe, P. Dubruel, B. Vekemans, E. Schacht, L. Vincze, A. Adriaens, Surface and Interface Analysis **41 (5)**, 389 (2010).

Acknowledgements

This work was supported by Hasylab within the initiative “ELISA: EU Support of Access to Synchrotrons/FELs in Europe”.
The Flemish Institute for Technological Research (VITO, Belgium) and the Research Foundation-Flanders (FWO, Belgium) are acknowledged for the Ph.D. funding granted to Annelies Verstraete.