

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

A. Adriaens, M. De Keersmaecker, A. Verstraete, J. Garrevoet, L. Van De Voorde, L. Vincze

Department of Analytical Chemistry, Ghent University, Krijgslaan 281-S12, B-9000 Ghent, 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, under investigation is gelatin, an example of a physically cross-linked hydrogel.

The enzyme containing biolayers, 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 40 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 ionization chamber readout 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 a minimum 12 h at room temperature. After rinsing these modified electrodes (MH|Au) with deionized water, the immobilization of the enzyme horse heart cytochrome *c* (HHC) in a layer of gelatin (gelatin A and gelatin B) was performed by means of the drop drying technique or the spin coating technique. The modified electrodes (HHC|Gel|MH|Au) were dried in air and stored at 4 $^{\circ}\text{C}$ prior to starting the micro-XRF experiment.

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

In this work we focused on the distribution of the iron (Fe) containing protein horse heart cytochrome *c* in a layer of gelatin and is considered as a continuation of ongoing research [1].

In a first experiment we tested the distribution of HHC in two types of gelatin. One can distinguish between type A gelatin (GelA) and type B gelatin (GelB). The first one is obtained by acidic hydrolysis of collagen, and has a net positive charge at physiological pH (due to an iso-electric point of ~ 8), while GelB results from alkaline treatment of collagen and is characterized by a negative charge at pH 7 (iso-electric point ~ 5). Due to their different net charges one has the opportunity to choose between the two types, depending on the iso-electric point of the enzyme to be encapsulated. The question we aimed to see answered is whether both gelatins are able to entrap the enzyme and whether the charge of the gelatin has any effect on the entrapment.

A second set of experiments focused on the immobilization technique: drop drying versus spin coating (2000 RPM, 1 minute). For both techniques we waited at least 2 hours to dry the electrode before the XRF measurements. In the latter case the deposited layers are expected to be much thinner. The question here is whether the method also has an influence on the homogeneity of HHC onto the surface and the reproducibility of the deposition.

Finally the last set of experiments consisted of comparing between high and low concentration of the used gelatin (5 w/v % and 10 w/v % in the HEPES buffer solution). Here we aimed to obtain information about the amount of enzyme immobilized on the surface using both deposition techniques.

References

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