Instrumental control of an enzyme immunoassay using ion-selective membrane electrodes and pulsed chronopotentiometry

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A new potentiometric enzyme immunosensor principle is evaluated for the detection of antigenantibody interactions at the cation responsive membrane surface without the need to add the enzyme substrate, which acts as the ion marker in the backside inner solution. This is accomplished by a triple pulse protocol to control the delivery of the enzyme substrate from the inner solution to the sample side using pulsed chronopotentiometry (Fig. 1).[1] First, a zero current measurement (I) gives a stable open circuit potential (OCP) of the system. The second step of the protocol consists of a controlled anodic current pulse (II) of defined magnitude and duration (i = 1µA and t = 1.5 s, in this case), which leads to a flux (J) of enzyme substrate (choline, Ch^+) through the ion-selective membrane (m). The anodic pulse is followed by a zero current measurement pulse (III). During this time, the previously extracted ions are consumed by the enzyme (choline oxidase, ChOx) after a sandwich enzyme immunoassay, resulting in different OCP measurements since the ion-selective membrane is responding to the Ch^+ concentration. At steady-state, the flux across the membrane is equal to the contribution from the flux across the aqueous diffusion layer in the sample side in addition to the enzyme reaction, which is described by the Michaelis-Menten kinetic model.

In the sandwich immunoassay, the target analyte to be measured is bound between two primary antibodies, each binding to a different epitope of the antigen. The monoclonal antibody on the surface of the electrode (capture antibody) captures the antigen from the sample; the polyclonal ChOx-labeled antibody (detection antibody) binds directly to a different site of the antigen. At equilibrium, the amount of bound ChOx labeled antigen-antibody complex is directly proportional to the concentration of antigen from the sample, resulting in different OCP (III) measurements.[2] The ion-selective membranes are surface-modified with an antibody using click chemistry between alkyne-PEG-NHS molecules and azide groups from the modified poly(vinyl chloride) of the membrane. The antibodies are covalently immobilized based on the interaction between NHS ester-activated and primary amines from the antibody structure. The surface modification is characterized by electrochemical impedance spectroscopy, fluorescence microscopy, and contact angle measurements.

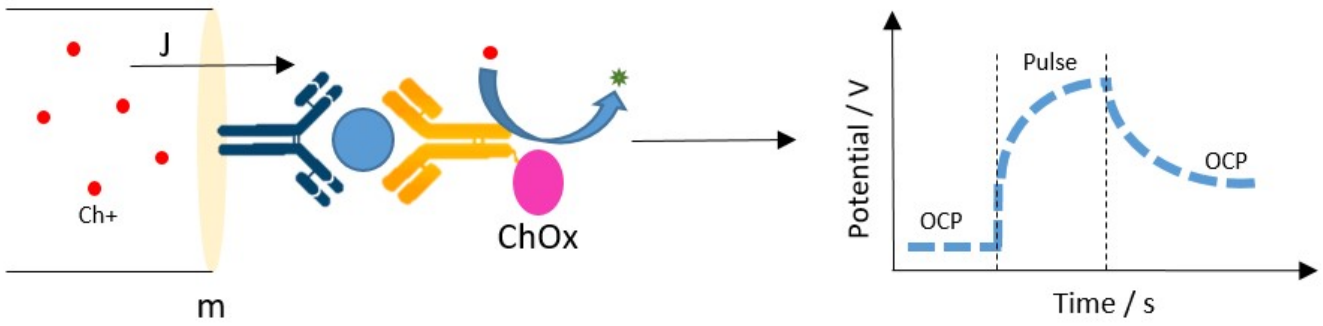

Figure 1. Schematic representation of the sensing principle.

[1] S.M. Mikhailov, A. Shvarev, E. Bakker, *J. Am. Chem. Soc*. **2004**, 34, 10548-10549.
[2] J.R. Crowther. The ELISA Guidebook. *Springer Protocols* **2009**, Volume 516.