CHALLENGES IN PRINTED BIOSENSOR PROCESSING

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Introduction

Biosensors offer a unique operating system, the so called "induced-fit" concept, which might constitute a key to innovative and pioneering technologies. The integration of a bio-active receptor molecule into a sensor allows the quantification of a specific substance. Biosensors are featured by good selectivity, high sensitivity, uncomplicated handling, and easy sample preparation.

However, their industrial scale production remains a great challenge. Various parameters have a significant impact on the functionality of the biosensor during the coating and drying process, thereby easily leading to a loss of the catalytic activity of the enzyme, and thus of the main sensing component. The factors range from the formulation step, in which composition, pH, and shearing play an important role, to the coating and drying step, which is characterized by the employment of elevated temperatures or additives that are used to improve coating results. Therefore, the aim of this research is to address the mentioned impacts by applying a developed procedure to assess the enzymatic activity of the active component in solution and coated films.

[1] Unpublished. ISCST shall not be responsible for statements or opinions contained in papers or printed in its publications.

Experimental

The experiments are based on a model system which can be applied for glucose processing biosensors.

Materials

As sensing component, the novel and pioneering enzyme FAD-dependent glucose dehydrogenase (FAD-GDH) is chosen. The dye 2,6-dichlorophenolindophenol (DCPIP) is used to observe the kinetics of the enzyme catalyzed reduction, thereby indicating the activity of the main component. For the assessment of the enzymatic activity in the coating solution, FAD-GDH is dissolved in phosphate buffer solution; when investigating the catalytic activity of the immobilized enzyme in the final coating, a PEDOT:PSS-PVA composite matrix is used, devoted to future biosensor generations.

Methods

An assay explicitly developed for this is used to determine the reaction rate of FAD-GDH spectrophotometrically. The residual activity is defined by normalizing the measured reaction rate of the exposed enzyme to the native one (cp. Equation 1).

$$Residual\ activity = \frac{reaction\ rate_{exposed\ enzyme}}{reaction\ rate_{native\ enzyme}} \cdot 100\% \quad (1)$$

In order to assess the parameters influencing the enzymatic activity, additives such as ethanol or isopropanol, which are sometimes used to improve wetting behavior of coating solutions, were added to the enzyme solution. Furthermore, FAD-GDH in solution was exposed to elevated temperatures or sheared at different shear rates each time with varying durations to imitate impacts that occur during the coating process.

For a profound understanding of impacts while drying, the enzyme was physically immobilized into a PEDOT:PSS-PVA polymer matrix. Therefore, one coating solution was prepared and, subsequently, coated on a substrate with a knife coater and dried under defined conditions. The films were either prepared at constant coating and drying conditions when investigating the influence of composition or with a constant composition when studying the impact of drying conditions, for example, the drying temperature. A two-stage procedure was proposed to evaluate the catalytic activity in the film. At first, the enzyme was released from the film; secondly, the obtained solution was analyzed by two methods: Lowry [1] and catalytic activity assays [2]. The former assay is a standard procedure to determine the amount of released enzyme; the latter was developed for this purpose as reported in literature.

Results and discussion

Elevated temperatures which are needed for the coating and drying of films are a dangerous factor for the structure and activity of the enzyme. A study of temperature influence on the residual activity of enzyme in solution shows that a rapid inactivation takes place above 40°C (cp. figure Figure *1* (A) on the left). When shortening the exposure time to 5 min, a reasonable catalytic activity can be retained.

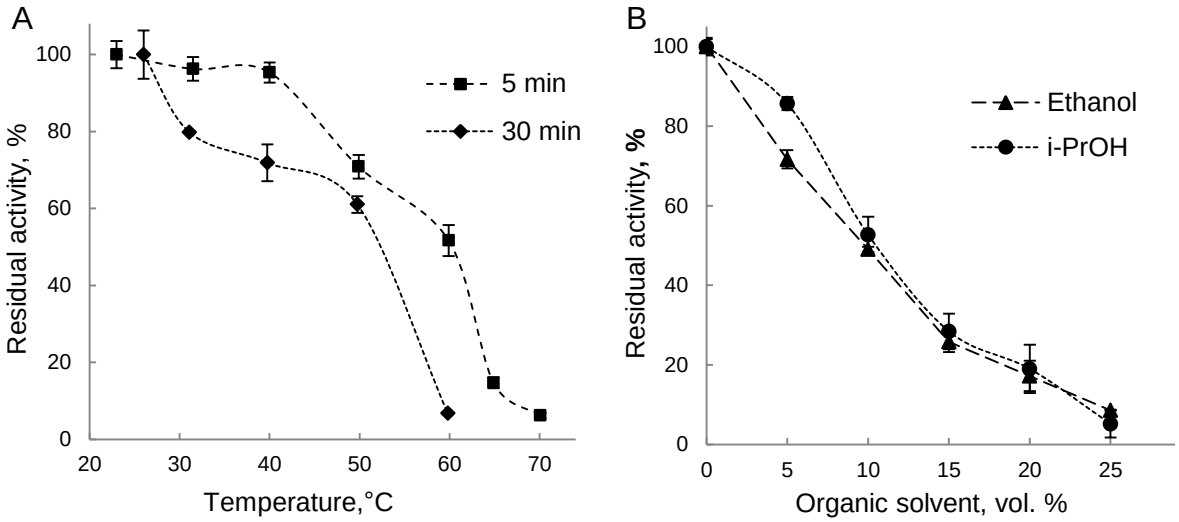

Figure 1: Influence of temperature and time (A) and of the amount of organic solvent (B) on the catalytic activity of FAD-GDH in model solution (1 nM FAD-GDH in 50 mM PBS, pH 6.5) (n=3, P=0.95).

A dramatic decrease of the catalytic activity of FAD-GDH is observed with an increase of the amount of additives, in particular ethanol and isopropanol, in solution (cp. figure Figure *1* (B) on the right). A greater inactivation of the enzyme is shown with the addition of 5 vol.% of ethanol than with the addition of the same amount of isopropanol. However, at higher contents of organic solvents, no difference between these two alcohols is found. Interestingly, when investigating the influence of shearing, the residual activity unexpectedly increased with rising shear rates although shear force might lead to alternations in the structure of the enzyme. However, at higher shear loads a slight decrease is observed.

Finally, with regard to the prepared films, a strong dependency of the residual activity on the pH value of the coating solution as well as on its composition is recorded. It is found that the FAD-GDH benefits from a high share of enzyme which desensitizes it to other impacts in the film, such as low buffer salt content or pH, respectively. On the contrary, the amount of polymeric component decreases with increasing FAD-GDH content leading to a poorer processability of the coating. The impact of elevated temperature during processing will also be evaluated. As priorly explained, a decrease of catalytic activity of the enzyme immobilized in the film is expected with harsher drying conditions as well.

To conclude, these results should be seen as a fundamental investigation that puts the obtained findings in context with the coating process and is able to point out the main challenges when processing biosensors.

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