COMPARISON OF ELECTROCHEMICAL WITH OPTICAL BIOSENSORS

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SUMMARY

NAD^+/NADH und $NADP^+$/NADPH systems, which are important coenzymes, are studied with respect to their determination by electrochemical or optical methods relevant for biosensors. Concerning their sensitivity and linearity of the calibration curves, similar results were obtained, when the technique of Flow-Injection Analysis (FIA) was used in conjunction with an amperometric flow-through cell and when a fiberoptic device measuring the fluorescence of NAD(P)H was employed. In both cases the limit of detection was around 1 nmol/l and the calibration curves showed two linear ranges. As a consequence an optical glucose-sensor based on this technique resulted in a correspondingly high sensitivity compared to a classical needle type amperometric device based on the glucose-oxidase (GOD) method. Otherwise, the superb sensitivity achievable by the amperometric NADH oxidation could by used for a sensitive formaldehyde FIA method yielding a limit of detection in the sub-nmol/l range. In addition, a reagentless fiber optic lactate sensor based on lactate-dehydrogenase (LDH) and a reversible redox-dye is described and also a novel potentiometric immunosensor based on the principle of selectivity modulation of ion-selective membranes.

1. Introduction

A large variety of Biosensors are based on specific enzymatic reactions using NAD^+/NADH or $NADP^+$/NADPH for detection either by an electrochemical or an optical method. Therefore, this important coenzyme was chosen for a comparison study concerning the main features of both methods under identical conditions (same compound to be sensed, same level of commercially available instruments instead of sophisticated home-build devices, studied in the same not-biased working group with specialists for both techniques) in order to get a fair impression of the benefits and drawbacks of both biosensor principles. The coenzymes chosen stand for over 50 substrates [1,2] which are measurable by sensing the concentration of this coenzyme in the course of a corresponding enzymatic reaction (see Table 1 in the appendix). Therefore, the characteristic features of these coenzyme determinations should be transferable to the individual substrate cases.

Since FIA is also a widely used powerful technique in this field, this method should be included in this study. Only some selected examples of biosensors based on the above mentioned techniques can be given on the following chapters reflecting some recent research projects together with the "Gesellschaft für Chemo- und Biosensorik" at the University of Münster.

2. Compared optical and electrochemical NADH sensors

Fig. 1 shows the fiber-optical biosensor approach used in this work. The Y-formed fiber bundle, made from Quartz, had a diameter of 5 mm. At its end a nylon-net bearing the corresponding enzymes immobilized by the proper technique [3-5] could be placed (as shown in Fig. 1.). In case of the NADH detection the enzyme layer was omitted and the sensitive method of fluorimetry was used by stimulating the molecule with light of a wavelength of 365 nm and measuring the fluorescence emission of NADH at 465 nm. The Quartz fibers, which conduct both wavelengths were statistically distributed at the common open end of the fiber bundle.

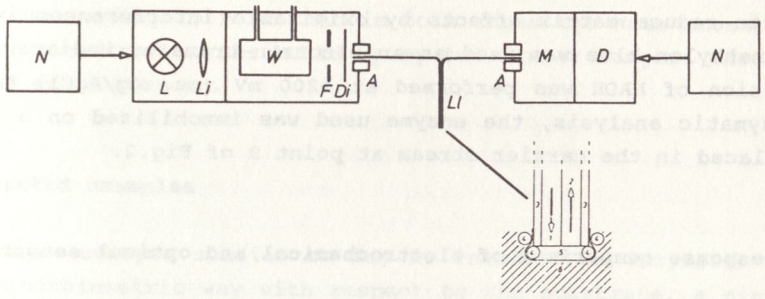


Fig.1: Construction of the fiber-optic sensors

N: Power-supply
L: Lamp
Li: Lens
W: IR-Filter
F: Filterholder
Di: Diffusor
Ll: Fiber-optic cable
A: Adapter
M: Monochromator
D: Detector

1: Fiber, connected with lamp
2: Fiber, connected with detector
3: Cladding
4: O-ring
5: Reaction chamber enclosed by a nylon-net [Reichelt] with immobilized enzyme
6: Sample solution

Fig.2 shows the FIA-based electrochemical approach to detect NADH by anodic oxidation at a gold working electrode in an amperometric wall-jet flow-through cell.

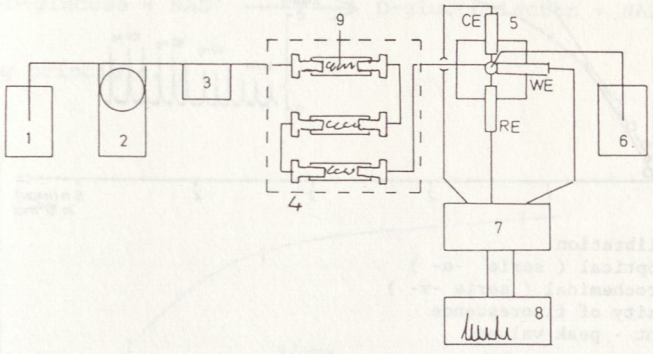


Fig.2: Electrochemical FIA - set-up

1: Stock solution
2: Pump
3: Injection valve
4: Mixture space
5: Electrochemical detector (wall-jet), Metrohm EA 1096
6: Waste
7: VA-Detector, Metrohm E 611
8: Recorder
9: Nylon-net with immobilized enzyme

In order to reduce matrix effects by oxidizable interferences in real samples, methylen blue was used as an electron-transfer mediator. Thus the oxidation of NADH was performed at +200 mV (vs. Ag/AgCl).In case of an enzymatic analysis, the enzyme used was immobilized on a nylon-net and placed in the carrier stream at point 9 of Fig.2.

3. NADH-response comparison of electrochemical and optical sensors

The response of both types of biosensors towards the primary molecule NADH is documented in Fig. 3.

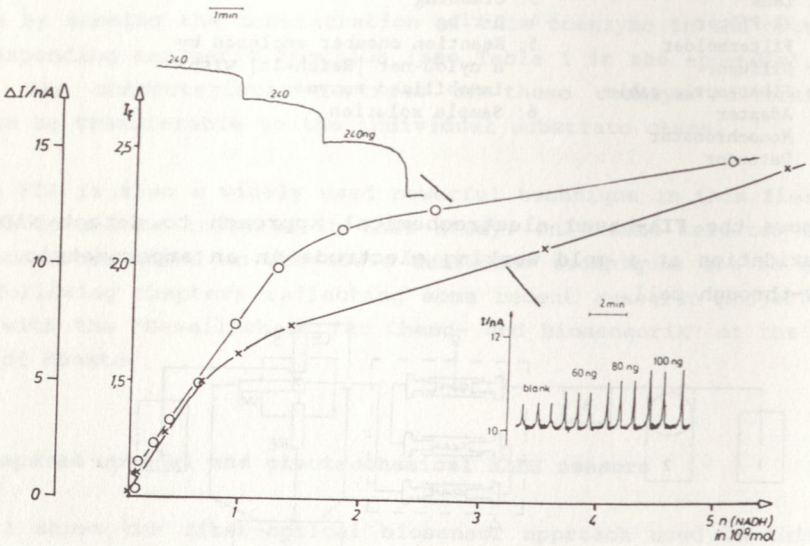


Fig.3: NADH - calibration
a) Fiberoptical (serie -o-)
b) Electrochemical (serie -x-)
I_f: Intensity of fluorescence
ΔI: Current - peak values

It is evident from Fig. 3 that both techniques yield similar sensitivities, limits of detection and show similar base-line drift in case of the addition of pure NADH. It is impossible to make general remarks in the case of real samples with complex matrices since both techniques are influenced by parameters which are not known a priori, e.g. other substances than NADH may be oxidized or other components yield a fluorescence or can function as a quenching reagent.

It is interesting to note that in both cases the same measuring range at low concentrations is build up by two linear ranges. The origin of this is still unknown.

4. Selected examples

Since the coenzyme studied so far is involved in enzymatic reactions in a stoichiometric way with respect to the substrate, a similar sensitivity as shown in Fig. 3 should also be achievable with corresponding substrates. Two examples, an optical glucose and an amperometric formaldehyde sensor, will be presented here.

4.1 Optical and electrochemical glucose sensors

Fig. 4 shows the glucose response of an optical glucose sensor based on Fig. 1 with the immobilized enzymes glucose dehydrogenase (GDH) and mutarotase (approximatly 10 units per nylon-net) [3-5] using the enzymatic reaction:

$$\beta\text{-D-glucose} + NAD^+ \xrightarrow{GDH} \text{D-gluconolacton} + NADH + H^+$$

as sensing principle.

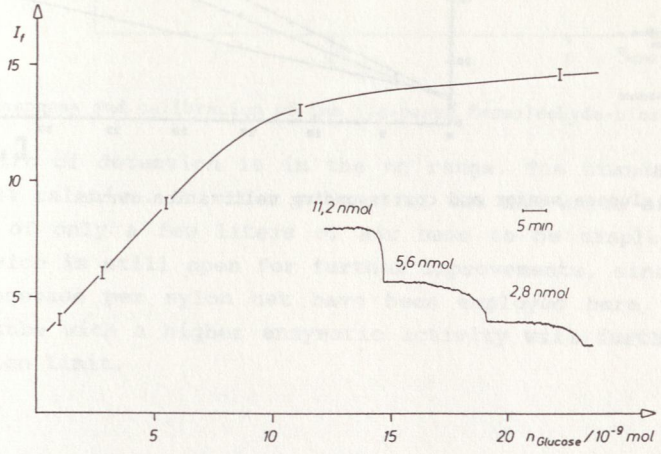


Fig. 4: Calibration and response of an enzymatic glucose opto-sensor
0.12 mol/l phosphate buffer, pH 7.6
2.2 mmol/l NAD^+

As in the case of NADH, the calibration curve of the optical sensor showed two linear ranges, the sensitivity and limit of detection correspond to Fig. 3. Such a high sensitivity allows a substantial dilution of a physiological sample which may be beneficial for diminishing the effect of interfering substances as there are: clogging of the sensor surface by protein-layers, lyase activities destroying the enzyme activity etc.

Compared to this sensitivity the developed needle type amperometric glucose sensor shown in Fig.5 [6], which is based on the classical enzymatic reaction using glucose oxidase (GOD):

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{GOD}} \text{D-gluconolacton} + H_2O_2$$

resulted in a much lower sensitivity by sensing the H_2O_2 although the physiological range fits the linear response range. Both glucose sensors show a similar response time of some minutes, since the complex diffusional processes are independent of the sensing method.

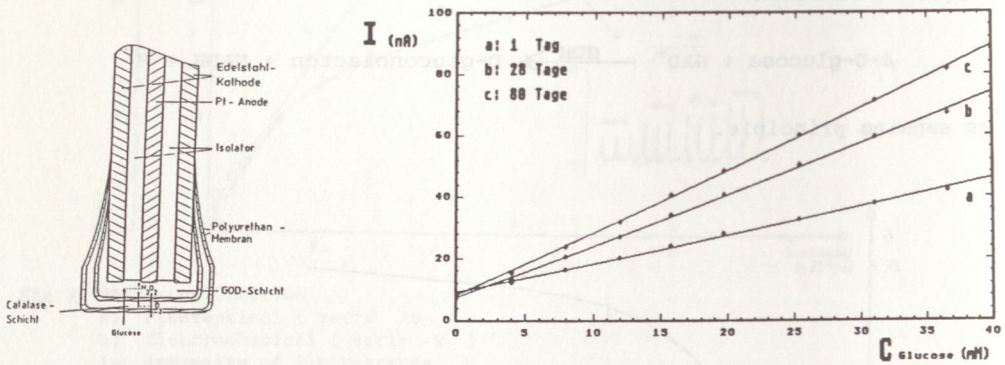


Fig. 5: Amperometric glucose sensor and corresponding calibration curves

4.2 Sensitive formaldehyde determination by enzymatic electrochemical FIA

A specific enzymatic method for a fast determination of formaldehyde without a separation step is based on the reaction:

$$HCHO + NAD^+ + H_2O \xrightarrow{FDH} HCOOH + NADH + H^+$$

If the FIA arrangement of Fig. 2 is supplied with nylon-nets with the immobilized enzyme formaldehyde dehydrogenase (FDH) a fast and extremly sensitive HCHO response shown in Fig. 6 is obtained.

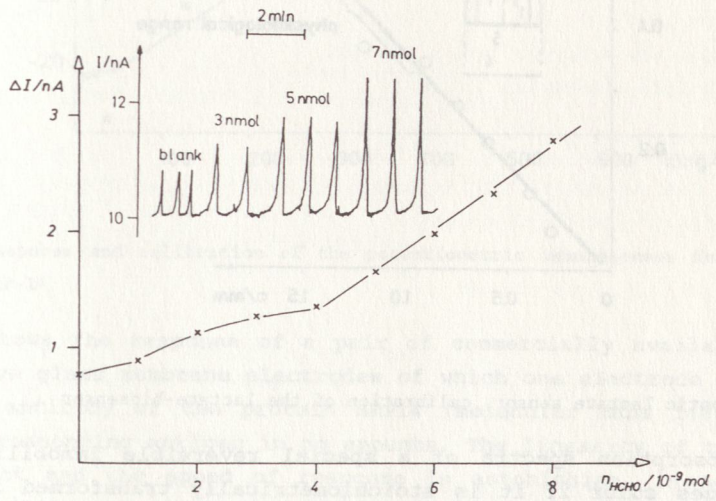
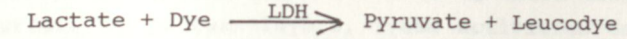


Fig.6: Response and calibration of the FIA-based formaldehyde-biosensor

The limit of detection is in the ng range. The standard deviation is about 3% relative. In order to control the MAK value of 1 ppm a minute amount of only a few liters of air have to be sampled in this case. The device is still open for further improvements, since only 15 units dehydrogenase per nylon net have been employed here. Using a longer nylon tube with a higher enzymatic activity will further decrease the detection limit.

4.3 Optical reagentless biosensor for lactate

Fig. 7 shows a fiber optical arrangement used to follow the enzymatic reaction using lactatedehydrogenase (LDH):



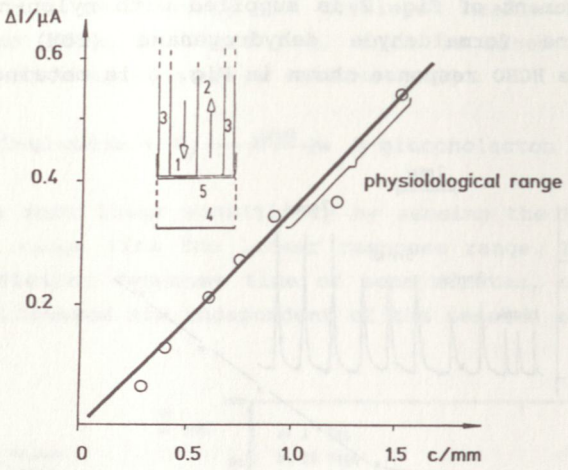


Fig.7: Fiberoptic lactate sensor, calibration of the lactate-biosensor

via the absorption spectra of a special reversible immobilized dye which changes color if it is stoichiometrically transformed into its leucoform [7]. Since the latter is reoxidized by oxygen, a sensor can be constructed which does not need any reagent for the measurement as it is the case in all above mentioned NAD^+ based sensors. The response of this optical lactate sensor is shown in form of the calibration curve in Fig.7. The response times are in the range of minutes.

4.4 Potentiometric immunosensor

The theoretical approach of ion-selective membranes considering the ion-exchange kinetics at the phase boundary, mainly developed by our working group, predicts a potentiometric signal in case an immunological interfacial reaction under certain measuring conditions, where mixed potentials are established, takes place [8].

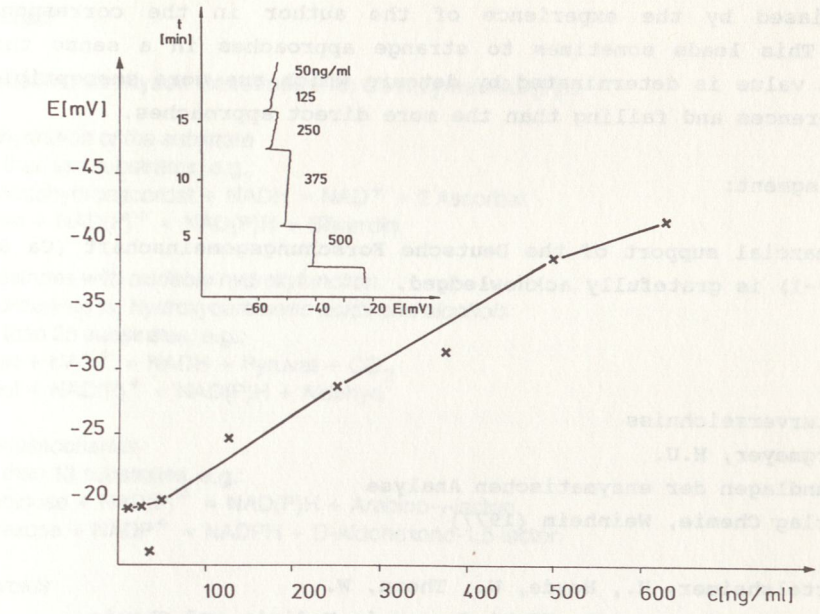


Fig.8: Response and calibration of the potentiometric immunosensor for the protein MRP-14

Fig.8 shows the response of a pair of commercially available sodium-selective glass membrane electrodes of which one electrode was covered by the antibody of the protein MRP14 (molecular mass 14000) towards the corresponding antigen in ng amounts. The linearity of the calibration plot and the speed of response is astonishing, in addition the reversibility (about 5 min: glycine/HCL, pH 2.5). This immunosensor worked for about 3 months with only minor decrease of the slope of the calibration curve. Using the technique of standard addition, extremly simple and fast analyses of a large variety of substances are possible.

5. Concluding remarks

We have summarized in this article several of our recent research projects to demonstrate that there is no general answer to the question how the physical transducer behind the more important specific recognition layer should work. Many attempts to do so in the literature

seem biased by the experience of the author in the corresponding field. This leads sometimes to strange approaches in a sense that a certain value is determinated by detours which are more susceptible to interferences and failing than the more direct approaches.

Acknowlegment:

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8) Patent pending

Appendix:

Enzymatical Catalysed Reactions with Coenzyme NAD(P)H

1) Dehydration of the substrate
more than ten substrates, e.g.:
2 Monodehydroascorbat + NADH = NAD^+ + 2 Ascorbat
Bilirubin + $NAD(P)^+$ = NAD(P)H + Biliverdin

2) Substrates with oxidable hydroxyfunction
a) Hydroxyketons, Hydroxycarboxylic acids and alcohols
more than 25 substrates, e.g.:
L-Malat + NAD^+ = NADH + Pyruvat + CO_2
Alcohol + $NAD(P)^+$ = NAD(P)H + Aldehyd

b) Monosaccharids
more than 13 substrates, e.g.:
D-Arabinose + $NAD(P)^+$ = NAD(P)H + Arabino-γ-lacton
Aldohexose + $NADP^+$ = NADPH + D-Aldohexono-1,5-lacton

c) Steroids
e.g.:
Oestradiol + $NADP^+$ = NADPH + Oestron
Cortisol + $NADP^+$ = NADPH + 21-Dehydrocortisol

d) Heterocycles
e.g.:
4-Hydroxy-L-prolin + NAD^+ = NADH + 4-Oxoprolin
Indollactat + NAD^+ = NADH + Indolpyruvat

3) Substrats with oxidable aldehyd-function
more than 11 substrates, e.g.:
Retinal + H_2O + NAD^+ = NADH + Retinoat
Arylaldehyd + ADP + P_i + H_2O + $NADP^+$ = NADPH + Carbonsäure + ATP

4) Oxidation of amino-function
e.g.:
L-Leucin + H_2O + NAD^+ = NADH + 2-Oxo-4-methylpentanoat + NH_3

5) Oxidation at aromatic cycle
e.g.:
Nicotinat + H_2O + $NADP^+$ = NADPH + 6-Hydroxynicotinat

6) Oxidation at metalatom
e.g.:
2 Ferricytochrom C_2 + NADH = NAD^+ + 2 Ferrocytochrom C_2