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Enzyme-Based Electrochemical Sensors for Formaldehyde Detection*

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Three types of enzyme sensors selective for formaldehyde have been developed using potentiometric pH-sensitive field effect transistors, conductometric and amperometric planar electrodes as electrochemical transducers and highly purified alcohol oxidase from *Hansenula polymorpha* as a sensitive element. The linear dynamic range (in a semilogarithmic scale) of the sensor output signals corresponds to 5–300 mM formaldehyde for all types of biosensors. The operational stability of the biosensors was not less than 7 h. When stored at +4°C, the responses were stable for more than 60 days. Biosensors based on potentiometric and conductometric transducers have demonstrated a high selectivity to formaldehyde with no response to primary alcohols (including methanol) or glycerol and glucose. The possible causes of the unexpected high selectivity of these biosensors to formaldehyde are discussed. The influence of buffer type and concentration on the sensor responses to formaldehyde was investigated.

1. Introduction

Formaldehyde is considered to be one of the most important commercial chemicals, due to its high chemical activity and relative cheapness. Annual world production of formaldehyde reaches up to 10 million tons, half of this volume being utilised for the

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production of phenol-, urea- and melamine-formaldehyde resins which are widely used in manufacturing of building plates, plywood and lacquer materials.[1,2] Formaldehyde is also used in chemical synthesis, as an intermediate in the production of such consumer goods as detergents, soaps, shampoos, and as a sterilising agent in pharmacology and medicine. Some advanced technologies for potable water pre-treatment include an ozonation process that not only disinfects the water, but also removes iron and manganese. However, as a result of the ozonation, some toxic and mutagenic aldehydes are formed — in particular, acetaldehyde, benzaldehyde, propanal, glyoxal, methylglyoxal and, mostly, formaldehyde.[3,4] It is also important to note that formaldehyde is classified as a cytotoxin,[5] mutagen and possible human carcinogen.[6] Recently, formaldehyde has been described as one of the chemical mediators of apoptosis.

These considerations are sufficient to convince of the necessity of formaldehyde control in a broad range of applications including environmental pollution surveillance, the control of bio- and chemotechnological processes, and its use in medicine and sometimes even food.[7] Such control requires the development of simple, cheap, sensitive and selective methods for the analysis of this extremely toxic agent.

Popular methods for the detection of formaldehyde use reagents such as chromotropic acid or acetylacetone. The chromotropic acid method requires heating of the sample under strongly acidic conditions, which is undesirable in many applications. The acetylacetone method yields a yellow product and is less specific and sensitive. The reaction of formaldehyde with Purpald works under alkaline conditions at room temperature, and the sensitivity is superior to that of other methods. The colour development by this reagent, however, requires oxidation of the adduct with hydrogen peroxide, oxygen, or dilute periodate.[8]

A number of approaches using sensors for the detection and quantification of formaldehyde have been published. A voltammetric device[9] and chemosensors based on graphite paste electrodes modified by palladium have been proposed. Recently, a new polarographic method has been described for the determination of traces of formaldehyde by direct *in situ* analyte derivatization with (carboxymethyl)trimethyl ammonium chloride hydrazide (Girard T-reagent).[10] The disadvantage of this method is an expensive apparatus as well as the necessity of removing traces of oxygen from the assays by sparging with pure nitrogen. The amperometric biosensors,[11,12] a methylotrophic yeast-based potentiometric method[13] and an optical biosensor[14] have been suggested for the determination of formaldehyde concentration. However, several serious problems restrict the wide commercial application of these biosensors. In the case of formaldehyde dehydrogenase-based biosensors, difficulties arise from the necessary addition of a cofactor enabling formaldehyde conversion[11] and of electrochemical mediators.[12] For methylotrophic yeast-based biosensors, difficulties are caused by the significant dependence of the biosensor response on buffer capacity and their insufficient storage stability.[13] Moreover, chemo- as well as biosensor systems are often not very selective.

In this paper, three types of selective and stable sensor systems for formaldehyde detection based on conductometric and amperometric planar electrodes, potentiometric ion-sensitive field effect transistors (ISFETs) and enzyme alcohol oxidase (AOX) are described. The characteristics of the developed biosensors are discussed and compared.

2. Materials and Methods

2.1 *Materials*

Alcohol oxidase (EC 1.1.3.13) from a wild strain of methylotrophic yeast *H. polymorpha* was produced by the method developed by Gibson.[15] Bovine serum albumin (BSA) was purchased from Sigma (USA). Aqueous solutions (25% w/v) of glutaraldehyde (GA), inositol and paraformaldehyde were obtained from Serva (Germany). DEAE-dextran was obtained from Pharmacia Ltd. (UK). All other chemicals were of analytical grade.

2.2 *Enzyme immobilisation*

A biologically active membrane on the transducer surface was formed by a method of protein cross-linking in saturated GA vapour.[16] Highly purified AOX (20% w/v) and BSA (10% w/v) were dissolved in 10 mM K-,Na-phosphate buffer, pH 7.7. These solutions were mixed in a defined proportion, then inositol and DEAE-dextran were added. The mixture containing 10% (w/v) highly purified AOX, 5% (w/v) BSA, 1% (w/v) DEAE-dextran and 5% (w/v) inositol was deposited in dropwise on the sensitive surface of a sensitive measuring element. The mixture containing 15% (w/v) BSA, 1% (w/v) DEAE-dextran and 5% (w/v) inositol was placed on a reference element. DEAE-dextran and inositol were added to stabilise the enzyme activity.[17]

2.3 *Sensor design and measurements*

The ion sensitive field effect transistors (Fig. 1(a)) were fabricated at the Research Institute of Microdevices (Kiev, Ukraine). The potentiometric sensor chip contains two identical Si_3N_4-ISFETs, the design and operation mode of which have been previously described.[18] The ISFETs were operated at a constant source current and drain-source voltage mode (I_s = 100 μA, V_{ds} = 1 V). The bare substrate of the sensor chip was used as a quasi-reference electrode.

The planar electrodes (Fig. 1(b)) consist of two identical pairs of gold interdigitated electrodes fabricated by vacuum deposition on a ceramic substrate (sintered aluminum oxide, thickness 0.5 mm, dimensions 5×40 mm).[19] The sensitive area of each electrode pair was about 1×1.5 mm. These electrodes were used as transducers for conductometric[19] or amperometric[20] measurements. In the case of amperometric biosensors a three-electrode cell was used. The working electrode was potentiostated at +650 mV versus the reference Ag/AgCl electrode potential.

Measurements were conducted in daylight at room temperature (20–23°C) in a glass cell (2 ml volume) filled with K-,Na-phosphate buffer, pH 7.7, or tris-HCl buffer, pH 7.7. The biosensors were immersed in a vigorously stirred sample solution. After baseline stabilisation, formaldehyde was added to the vessel. The differential output signal between the measuring and reference sensitive elements was registered with the laboratory devices manufactured in-house, and the steady-state response of the biosensors was plotted as a function of formaldehyde concentration.

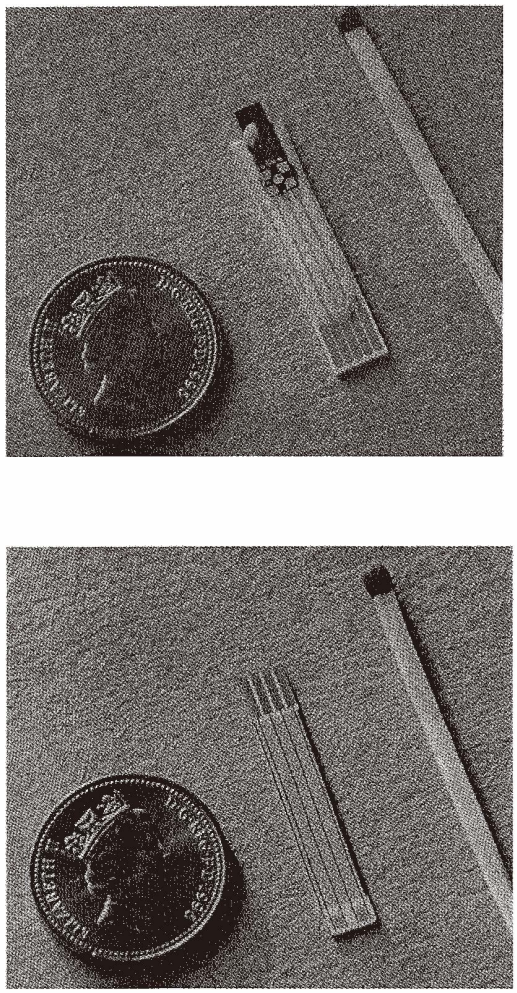


Fig. 1. General view of ion-sensitive field effect transistors (A) and planar electrodes (B).

3. Results and Discussion

The AOX from *H. polymorpha* is a suitable component for solid phase biosensors since its catalytic activity is virtually independent of pH over the range of 6–10, is rather high at 40–50°C and does not require the addition of any external cofactors.[17] The biorecognition element (AOX) immobilised on the transducer surface catalyses the oxidation of formaldehyde according to the scheme:

$$CH_2O + O_2 + H_2O \xrightarrow[AOX]{} HCOOH + H_2O_2 \qquad (1)$$

$$HCOOH \rightarrow HCOO^- + H^+$$

The calibration curves (Fig. 2) were linear (in a semilogarithmic scale) within the range of formaldehyde concentration from 5 to 300 mM for all types of electrochemical biosensors.

The results presented in Fig. 3 show that the output signal of the potentiometric and conductometric sensor systems was suppressed by increasing buffer concentrations but not as strongly as in the case of the glucose ENFET or the formaldehyde intact cell-ISFET described earlier.[13,21] Such a reduction of the influence of buffer concentration on the response of biosensors can perhaps be explained by taking into account the properties of a multicomponent enzyme membrane. The positively charged groups of DEAE-dextran inside the enzyme membranes can create a barrier against the diffusion of positively-charged ions and effectively bind the negatively-charged ions. In the case of phosphate buffer, the buffer-mediated mechanism of proton diffusion out of an enzyme layer is substantially lower.

When formaldehyde was tested in Tris-HCl buffer, we observed a very interesting phenomenon for the potentiometric and conductometric biosensors developed; the response and sensitivity to formaldehyde in this buffer were substantially higher than that in the phosphate buffer (Fig. 4). The chemical nature of this effect seems to be the reactions (2) and (3) of formaldehyde with the amino group of tris(hydroxymethyl)aminomethane with production of a hydroxymethylamine and azomethine derivatives, leading to the release of free protons:

$$(HOCH_2)_3CNH_3^+ + CH_2O \longleftrightarrow (HOCH_2)_3CNHCH_2OH + H^+ \quad (2)$$

$$(HOCH_2)_3CNH_3^+ + CH_2O \longleftrightarrow (HOCH_2)_3CN{=}CH_2 + H_2O + H^+ \quad (3)$$

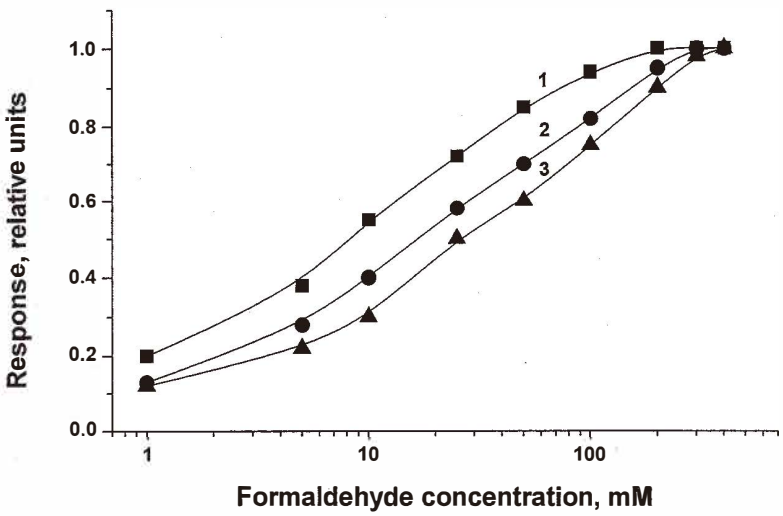


Fig. 2. Calibration curves (in semilogarithmic scale) for formaldehyde specific amperometric (1), conductometric (2) and potentiometric (3) biosensors as measured in a 10 mM phosphate buffer, pH 7.7.

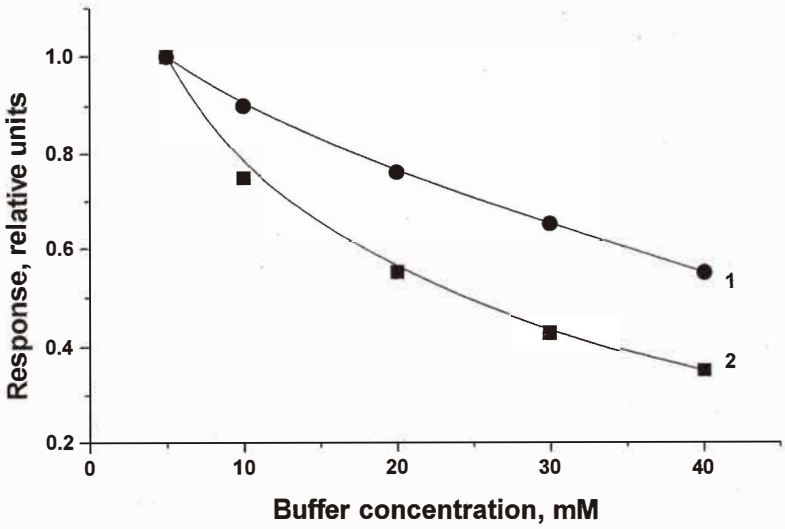


Fig. 3. Dependence of the output of the formaldehyde specific conductometric (1) and potentiometric (2) biosensors on the buffer concentration of the sample as measured in phosphate buffer, pH 7.7, at a formaldehyde concentration of 50 mM.

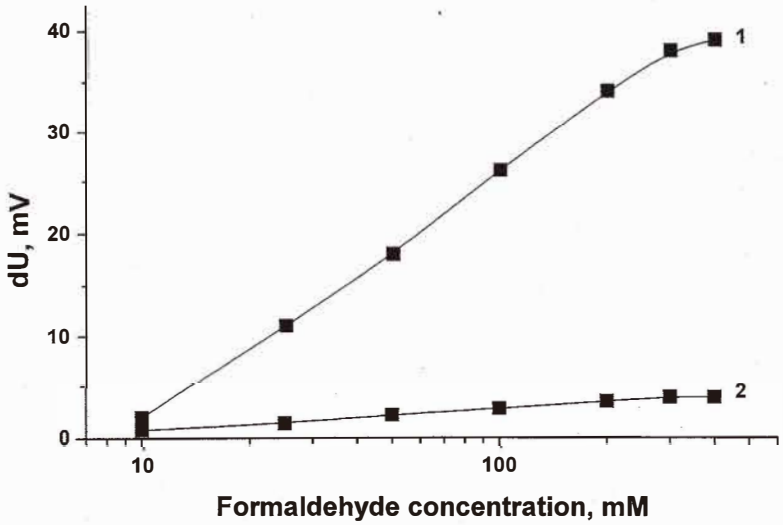
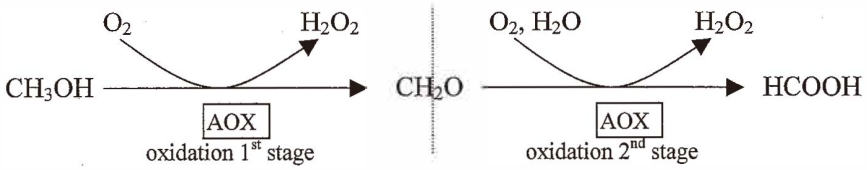


Fig. 4. Calibration curves (in semilogarithmic scale) for formaldehyde determination. Biosensor based on ISFETs. Steady-state responses were measured in 10 mM tris-HCl buffer, pH 7.7 (1) and 10 mM phosphate buffer, pH 7.7 (2). The same results were also obtained for biosensors based on conductometric transducers.

Reaction (2) is well known for primary and secondary amines.[1] The possibility of reaction (3) is clear, since it is a basic reaction for the synthesis of azomethine dyes, but in our case the formation of a double bond on the nitrogen from the molecule of tris(hydroxymethyl)aminomethane seems energetically unfavourable.

It was shown that only potentiometric and conductometric biosensors have demonstrated a high selectivity to formaldehyde with no response to methanol, ethanol, glucose or glycerol (Table 1). But in the case of the equimolar mixture of methanol and formaldehyde, sensor responses were decreased. In the case of methanol detection, this result is unusual as it is the preferred substrate of most AOXs, as it is directly oxidised to formaldehyde, as shown in the first stage of reaction (4):



(4)

It would be expected that the formaldehyde (CH_2O) produced would then follow the normal oxidation step (second part of reaction (4)) to give a signal from the potentiometric and conductometric biosensors. The absence of a measurable response to methanol may be explained as follows:

1) The rate of methanol oxidation in the AOX reaction is much higher than that of formaldehyde.[22] This should cause competitive inhibition of the subsequent stage of formaldehyde oxidation by methanol. Results obtained on a mixture of equimolar concentrations of methanol and formaldehyde provide evidence for a competition between these substrates; the response of potentiometric and conductometric biosensors in the mixture of CH_2O and CH_3OH drops to 65% of formaldehyde response (Table 1).

Table 1
Results of the selectivity test for potentiometric (P), conductometric (C) and amperometric (A) biosensors.

Analytes	Relative value of the sensor responses (%)		
	P-sensor	C-sensor	A-sensor
Formaldehyde	100	100	6
Methanol	0	0	100
Ethanol	0	0	100
Formaldehyde/Methanol	65	65	23
Glycerol	0	0	0
Glucose	0	0	0

All measurements were performed in 10 mM phosphate buffer, pH 7.7, at the analyte concentration of 10 mM. Maximal response of each biosensor to the one from detected analytes was taken as 100% and other responses were calculated in relation to the maximal one.

2) Effective oxidation of methanol is likely to result in local oxygen depletion in the bioactive zone limiting the oxygen available for formaldehyde oxidation; this has been seen to occur in a trigliceride assay system using glycerol-3-phosphate oxidase.[23]

3) Formaldehyde produced from methanol can diffuse from the bioactive zone back into the bulk solution without oxidation due to the low affiinity of CH_2O relative to immobilised AOX (apparent K_m is about 300 mM).

4) Formaldehyde produced from methanol is likely to bind covalently with NH_2-groups of AOX, thus reducing the effective concentration of CH_2O in the bioactive zone and the possibility for its further oxidation. The spontaneous reaction of formaldehyde with amino groups of proteins is well documented[24] and has been discussed above for other amines.

All these factors may result in a decrease in the concentration of formic acid produced from methanol in bioactive membranes to a level less than the sensitivity of the potentiometric and conductometric biosensors described and therefore no response to methanol is apparent.

In the case of amperometric biosensor (Table 1) one can see that there was no selectivity to formaldehyde. This result is attributed to the determination of H_2O_2 by the amperometric biosensor and therefore oxidation reactions of methanol, ethanol or formaldehyde are detected (see reaction (4)).

It is also worth noting that all biosensors developed are not specific to formaldehyde (see Table 1, responses in the mixture of methanol and formaldehyde).

All electrochemical biosensor responses were reproducible, and repetitive measurements with the same membrane can be successfully performed after 2 min washing in buffer solution. The relative standard deviation of the output signal was 2% compared with 10% for biosensors containing membranes deposited *de novo*.

A test of operational stability demonstrated that the steady-state responses of all biosensors did not decrease for at least 7 h (approximately 70 measurements). The responses remain stable for more than two months for all types of biosensors used.

4. Conclusion

All types of formaldehyde specific biosensors developed have demonstrated similar main analytical characteristics that allow their areas of application to be extended, but better selectivity towards formaldehyde has been achieved using potentiometric and conductometric transducers.

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