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Amperometric biosensor for formic acid in air

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Abstract

The possibility of developing a simple, inexpensive and specific personal passive ”real-time” air sampler based on biosensor technology was investigated. Formic acid was used as a model substance. The sensor is based on the enzymatic reaction between formic acid and formate dehydrogenase with NAD^+ as a cofactor and Meldola’s blue as mediator. An effective way to immobilise the enzyme, cofactor and Meldola’s blue on screen-printed electrodes was found to be in a mixture of glycerol and phosphate buffer covered with a gas-permeable membrane. When the sensor was introduced into an atmosphere containing formic acid, it gave a distinct and rapid amperometric response.

Keywords: *biosensor, air monitoring, formic acid, gas-permeable membrane, glycerol.*

Introduction

Personal exposure monitoring is an important way of establishing if governmentally determined guidelines are followed in regard to chemical exposure. Companies are also in need of more efficient methods for internal control and quality assurance. Sampling and analysis methods must be facilitated to encourage more measurements to be performed in the workplace. Methods used for personal exposure measurements are usually based on a two-step procedure; sampling followed by analysis. The sampling step can either be performed by using pumps or by using diffusive sampling devices, where the diffusive samplers have the advantage of being less invasive to the worker's performance during sampling [1-5]. A general disadvantage for these methods is mainly that they are time consuming since the samples usually have to be sent to a laboratory for analysis. Commonly used real-time instruments, on the other hand, have short feedback time, but lack the selectivity of laboratory instruments such as chromatographic systems.

These problems can be avoided by using biosensors in real-time monitoring devices. Although the biological element of the biosensors is sensitive to temperature and humidity, such sensors can be constructed with high specificity and sensitivity. The principal focus for biosensor development to date has been biomedical applications, but a number of studies have been reported for air monitoring [6].

In this study, formic acid was chosen as a model substance. It has been measured in various locations such as atmosphere [7], museum cabinets [8], farmers' exposure during silage making [9] and exhaust emissions [7,10]. When formic acid is sampled it is usually trapped as formate using potassium hydroxide on filters but standard adsorbent tubes have also been used [9,11]. Formic acid has been analysed both with and without prior derivatisation. When

the sample is derivatised prior to analysis, standard GC and HPLC methods are generally used and when the sample is not derivatised ion chromatography is usually preferred. As formic acid was chosen as the model substance, formate dehydrogenase (FDH) was chosen as a suitable enzyme. Formate reacts with FDH in presence of a co-factor, nicotinamide adenine dinucleotide (NAD^+), which is reduced in the process, producing NADH [12]. Amperometry was chosen to measure the amount of NADH that was reoxidised. To accelerate this process Meldola's blue was introduced as a mediator in the system. Meldola's blue requires lower oxidation potential than NADH, which also decreases the risk of interference.

In this paper, we describe initial studies for the construction of a diffusive sampling device, based on biosensor technology, for monitoring personal exposure to formic acid. The aim of this piece of work was to ascertain how the biological system could be attached to the screen-printed electrodes to produce a biosensor for air monitoring.

Experimental

Biosensor

Electrodes

The electrodes used in the electrochemical experiments were manufactured by depositing carbon paste on polyester sheets using the technique of screen-printing. Screen-printing is now a well established method which allows inexpensive electrodes to be manufactured easily and disposed of after use.

The working, counter and reference electrodes were printed (Fig. 1A) on the polyester sheets (Melinex MSTS725, Cadillac Plastic Limited, Swindon, UK) with a carbon paste (I45 ink, MCA, Cambridgeshire, UK). The sheets were then left over night to dry under ambient

conditions. The next step was to print the surface of the reference electrode. This surface consisted of a Ag/AgCl paste (Ag/AgCl ink C20R15, MCA, Cambridgeshire, UK) which was printed on top of one of the carbon paste electrodes (Fig. 1B). Since the Ag/AgCl layer is sensitive to light, the sheets were hereafter stored in darkness. When the Ag/AgCl layer had dried an insulation layer (242-SB ink, ESL Europe, Reading, UK) was printed (Fig. 1C). This ink was dried in an oven at 120°C for two hours. The design is such that only the electrode areas and contact pads are left exposed. After this layer was printed, the sensors were cut to their final size, which was approximately 15 x 50 mm (Fig. 1D). All screen printing was carried out with a DEK 248 screen-printer (DEK, Weymouth, UK).

For the electrochemical experiments, the screen-printed sensors were connected to an electrochemical analyser (a µAutolab type II, from Eco Chemie in Utrecht, the Netherlands) by a connector manufactured from an IDC edge connector (Maplin, Milton Keynes, UK) and standard IDC cables. The µAutolab was controlled by a Digital Celebris 590 PC with GPES software (Eco Chemie, Utrecht, the Netherlands).

Seventeen electrodes were used to investigate the variability of the electrochemical response of the electrodes. The variability was measured in a stirred solution containing phosphate buffer (0.1 M, pH 7.0 with 0.1 M KCl) and 5.3×10^{-5} M Meldola's blue (hemi salt, 90 % dye, Sigma, Sweden). The electrodes were connected to the electrochemical analyser and immersed in the solution. A potential of -50 mV versus the internally printed Ag/AgCl reference electrode was applied. After 500 seconds, a 10 µl aliquot of a 2 µg/µl solution of NADH (99 %, Sigma, Sweden) was added and the peak heights measured.

Enzymes system and immobilisation

Initial enzyme immobilisation experiments were performed using alginate gel, SOL-GEL [13] and glycerol solution, respectively. The sol-gel method was discarded because the transparent silica gel did not adhere properly to the surface of the screen-printed electrodes and hence cracked. The alginate gel was prepared by mixing approximately 20 µl of a 4 % sodium alginate solution, dissolved in a phosphate buffer (0.05 M, pH 7.0 with 0.1 M KCl), with 7 µl of a 20 U/ml solution of FDH (from yeast, Fluka, Sweden), 2 µl of a 40 mg/ml solution of NAD^+ (98 %, Sigma, Sweden) and 1 µl of a 20 mg/ml solution of Meldola's blue. Approximately 10 µl of the gel was placed on an electrode and the electrode was then dipped in 0.1 M $CaCl_2$ to complete the gel. The glycerol solution used to immobilise the enzyme, cofactor and mediator on the screen-printed electrodes consisted of a mixture of glycerol and phosphate buffer. Glycerol has been used previously in a biosensor for analysing phenol in air and proved to be very efficient in this application, mainly because of its ability to retain water and its ability to concentrate phenol in the gel [14]. The glycerol solution consisted of 80 % (w/w) glycerol (99 %, Sigma,UK) and 20 % (w/w) phosphate buffer (0.1 M, pH 7.2 with 0.1 M KCl). An aliquot (20 µl) of the solution was mixed with 7 µl of a 20 U/ml solution of FDH, 2 µl of a 40 mg/ml solution of NAD^+ and 1 µl of a 20 mg/ml solution of Meldola's Blue. An aliquot (10 µl) of this mixture was applied to the screen-printed sensor to cover all three electrodes. The comparison was performed by measuring the gaseous formic acid above an equilibrated 0.012 M solution of formic acid using the two different types of immobilisation method. In the initial comparison of alginate gel and glycerol solution, glycerol was found to have a quicker and larger response to formic acid in air. Glycerol was therefore chosen as the immobilisation medium for the following experiments.

Membranes.

To ensure that the glycerol solution was secured on the electrodes, gas permeable membranes were placed on top of the glycerol solution. Six types of membrane, all acquired from Millipore (Sundbyberg, Sweden) were investigated (Table 1).

The membranes were cut into squares of approximately 14 x 14 mm. Aliquots of 10 µl of the glycerol solution were placed on the electrodes and the membranes were attached to the electrodes by applying small amounts of cyanoacrylate glue (Loctite 420, Loctite Sweden AB, Gothenburg, Sweden) to the edges of the membrane squares. The electrodes were then connected to the electrochemical analyser and exposed to formic acid in an exposure chamber. The potential was -50 mV versus the internally printed Ag/AgCl reference electrode and the current at steady state was measured.

In an investigation of storage stability, 42 electrodes, using the LS5 membrane, were prepared as above. The storage was performed by placing 18 electrodes each in 4ºC and -15ºC. After storing the electrodes for 1, 2 and 3 days, 6 electrodes from each storage condition were analysed each day. The analyses were performed by exposing the electrodes to formic acid in air and measuring the current at steady state.

Testing the biosensor

The biosensor constructed as described above, was tested in an exposure chamber with known concentrations of formic acid in the atmosphere. The formic acid concentrations were set to 1.9, 3.7 and 5.6 mg/m^3, which is near the Swedish threshold limit value for formic acid. The Swedish National Board of Occupational Safety and Health has set the limit value at 5 mg/m^3 [15] and the limit value stated by the American Conference of Governmental Industrial Hygienists has been set at 9.4 mg/m^3 [16]. The biosensor was introduced in the chamber

before the formic acid was added to give it time to equilibrate. The amperometric analysis, controlled from the µAutolab, was then started by applying the potential (-50 mV versus the internally printed Ag/AgCl reference electrode). When the signal had reached a steady state (approximately 3 minutes) the micro-injection pump was started and the formic acid atmosphere, at 1.9 mg/m^3, was generated in the exposure chamber. After 20 minutes, the concentration of the formic acid atmosphere was increased to 3.7 mg/m^3 and after another 20 minutes the concentration was increased to 5.6 mg/m^3.

Generation of formic acid atmosphere

To ensure better control of the formic acid atmosphere during sampling, standard atmospheres were generated using a purpose-built generation system (Fig. 2). A solution of 40 mg/ml formic acid, diluted from concentrated formic acid (98-100 %, Riedel-de Haën, Seelze, Germany) was used to generate standard atmospheres. A micro-injection pump (CMA/100, Carnegie Medicin, Stockholm, Sweden) with a 5 ml syringe (model 1005, Hamilton, Nevada, USA) slowly injected the formic acid solution into a nebuliser (Meinhard nebulizer TR-30-A3, J E Meinhard Associates Inc., California, USA) and compressed air was led through the nebuliser to facilitate evaporation of the formic acid. The air mixture was firstly diluted in the mixing chamber with compressed dry air and secondly diluted with humidified air giving the required atmosphere in the exposure chamber. The temperature and relative humidity was measured at the exhaust of the exposure chamber, with an HMI 14a R.H. & T indicator (Vaisala, Helsinki, Finland). Similar generation systems have been used to generate other compounds [3,4,17]. The relative humidity was set to 50 % throughout all the experiments, by regulating the airflow through a moisturising outfit consisting of three, water-filled, dispersion bottles. The concentration was adjusted by changing the injection speed of the micro-injection pump.

Results and discussion

Variability of the electrode response

The relative standard deviation of the electrochemical response of the screen-printed electrodes was 13 % (n=17). The experiments were performed over four days and each day new solutions were made. No difference was observed in the size of the signal with time, either between or within groups.

System for immobilisation of the enzyme

When the enzyme system was immobilised in glycerol the response was larger and quicker compared to immobilisation in alginate gel (Fig. 3). The alginate gel has a less permeable structure and this probably caused the difference in response. For this reason, glycerol was chosen as the immobilisation media.

Choice of membrane

Of the six membrane were investigated, the LS5 membrane gave a stable response for over 60 minutes and the relative standard deviation of steady state current between six electrodes was 15 %. The AA08 membrane was not suitable for this application and produced no signal, probably because of the ability of the membrane to absorb the glycerol solution into the membrane. Electrodes with the fluoropore membrane (FG02, FH05, FA1 and FS3) all produced larger responses than electrodes with the LS5 membrane, but they also exhibited larger variation in the signal within each group of electrodes. Fig. 4A shows the experiments performed with the LS5 membrane and Fig. 4B shows the experiments performed with the FA1 membrane as an example of the fluoropore membrane type. All fluoropore membranes gave a similar response as the FA1 membrane, with larger response but much greater variation in the signal. Some of the electrodes with fluoropore membrane did not maintain a

steady response for 60 minutes as can be seen in Fig. 4B. Although the response was lower for the electrodes with the LS5 membrane, this membrane was chosen for further investigation since the stability within the group of electrodes was better.

Linearity of sensor response

When the biosensors were exposed to formic acid concentrations between 1.9 and 5.6 mg/m^3 the biosensors responded well to the increase in concentration in the test atmosphere, as can be seen in Fig. 5. A fast response time was achieved, which indicates that the device could be used as a real-time monitor. Six experiments were performed using the same conditions and all the responses showed the same pattern. One of the biosensors, however, did have approximately 17 % lower response. This is illustrated in Fig. 6 by the three points well below the calibration curve. It also shows a linear correlation between the formic acid concentrations and the responses.

Storage stability

Fig. 7 shows that the performance of the biosensors was not maintained after storage. The amperometric response decreased by 50 % after only one days storage at -15ºC. Since storage stability is an important feature, this has to be improved in the future development of the biosensor. When the biosensors were stored at 4ºC the response decreased by 80 % after one days storage.

Conclusions and future work

This study has shown, for the first time, that there is potential for a simple, inexpensive and specific personal passive ”real-time” sampler based on biosensor technology, for measurement of formic acid in air. The enzyme system can be immobilised in glycerol and kept in place using a gas-permeable membrane producing electrodes with a stable and linear

response. However, further development is required to create a device with sufficient sensitivity, response time and storage stability for practical application.

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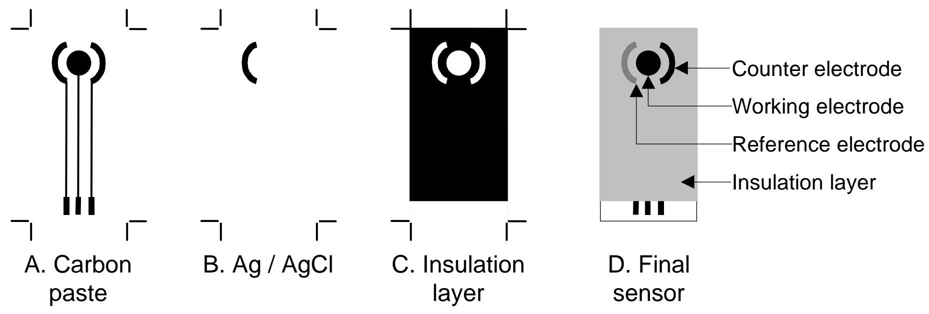


Fig. 1.

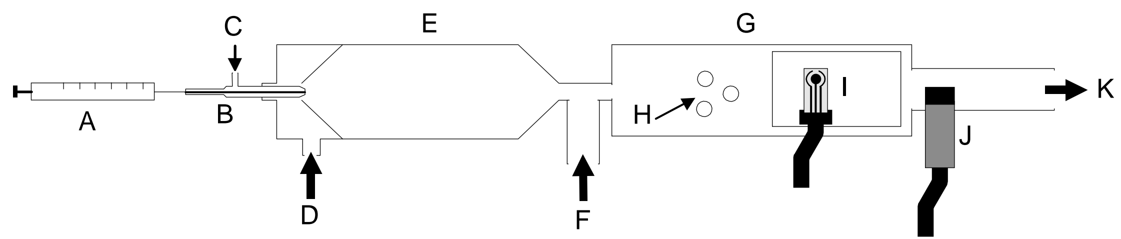


Fig. 2.

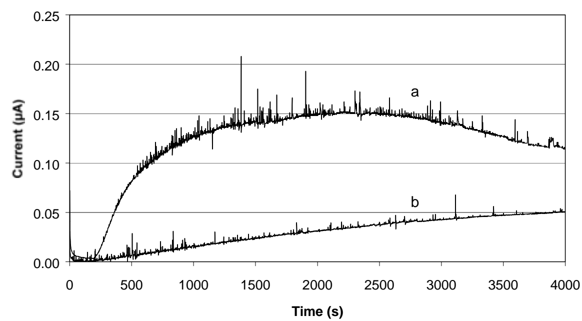
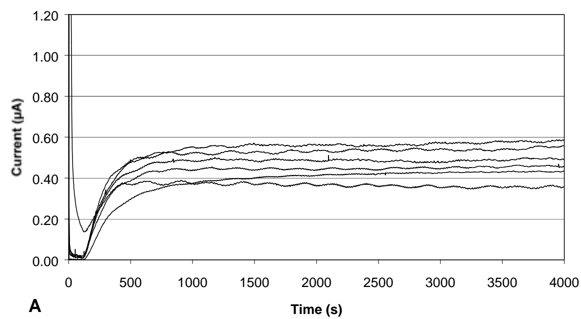
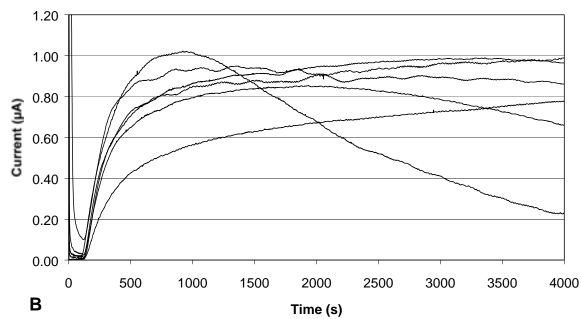


Fig. 3.





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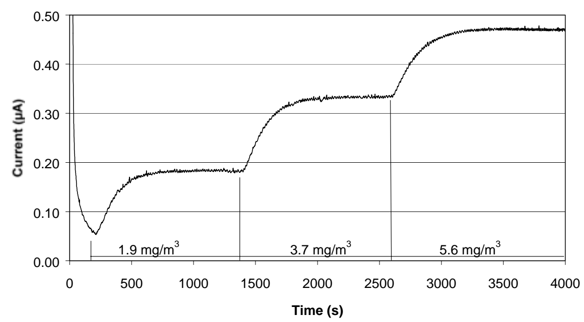

Fig. 5.

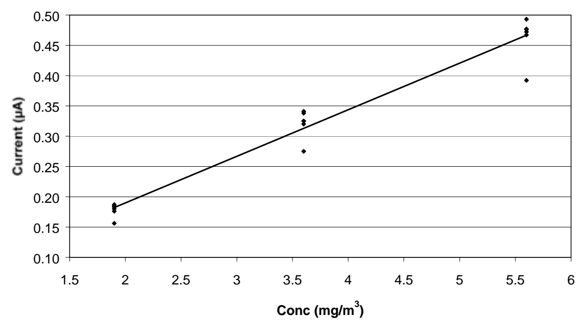

Fig. 6.

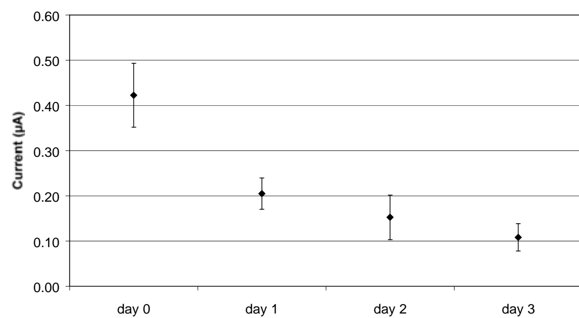

Figure 7.

Table 1.

Membrane code	Membrane type	Pore size (µm)
AA08	MF millipore	0.8
FG02	Fluoropore	0.2
FH05	Fluoropore	0.5
FA1	Fluoropore	1.0
FS3	Fluoropore	3.0
LS5	Mitex	5.0

Fig. 1. The disposable sensors were constructed by screen-printing pastes in different layers on polyester sheets. Black areas in A, B and C shows the layer printed in each step.

Fig. 2. Generation system for production of test atmosphere. A: Micro-injection pump. B: Nebuliser. C: Air inlet to nebuliser (0.4 l/min). D: Dry compressed air (4 l/min). E: Mixing chamber. F: Compressed humidified air for dilution (60 l/min). G: Exposure chamber. H: Outlets for sampling with sampling tubes. I: Opening in the exposure chamber for sampling with the biosensor. J: Meter for relative humidity and temperature. K: Outlet.

Fig. 3. FDH, NAD+ and Meldola's blue immobilised, on screen-printed electrodes, in glycerol (a) and alginate gel (b) and exposed to formic acid in air.

Fig. 4. Comparison of the membranes used to secure the glycerol solution on the electrodes performed by exposing them to formic acid in air and measuring the amperometric response at steady state. A) LS5 membrane and B) FA1 membrane.

Fig. 5. Amperometric response of a biosensor when exposed to 1.9, 3.7 and 5.6 mg/m^3 of formic acid in air.

Fig. 6. The correlation between the formic acid concentrations and the amperometric response for six experiments. Same conditions as Fig. 5

Figure 7. Determination of the storage stability at -15ºC. Analyses were performed after 0, 1, 2 and 3 days storage. Analytical conditions same as Fig. 4.

Table 1. Membranes investigated.

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