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Optical biosensors based on Prussian Blue films

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Optical biosensing schemes based on enzymatically modified inorganic/organic transparent films predominately composed of Prussian Blue are demonstrated. The composite film, which is non-electrochemically deposited on a non-conducting support, is used as an optical transducer for flow-through biosensors based on hydrolases and oxidases. Urease and glucose oxidase are utilized as model enzymes. Action of the urea biosensor is based on optical pH sensitivity of Prussian Blue indicator. The glucose biosensor is acting as first-generation optical biosensor based on *in situ* generated Prussian White transducer for hydrogen peroxide. These simple, single-pass transmission optical biosensors exhibit sensitivity in the millimolar range of concentration. The biosensors are very stable owing to presence of a poly(pyrrolylbenzoic acid) network in the composite material. This organic polymer plays a dual role as a binding agent for inorganic material and as a functionalized support for strong covalent immobilization of enzyme molecules.

Introduction

Inorganic films composed of Prussian Blue (PB) and its analogues are intensively studied owing to their unique electrochemical, electrocatalytic and electrochromic properties.[1] From the analytical point of view the films are mainly investigated as sensing and/or transducing elements of electrochemical sensors and biosensors. The electrocatalytic properties of metal hexacyanoferrates (MHCF) have been utilized in various electrochemical biosensors based on oxidases and dehydrogenases.

It is well known that PB film can act as an electrocatalyst for both the reduction and oxidation of hydrogen peroxide.[2] Electrochemical studies proved that the high-spin divalent iron ions present in the reduced film show catalytic properties towards the reduction. The electrocatalytic cathodic acitivity of PB modified electrodes towards hydrogen peroxide is nearly as high as the activity of a Pt electrode, whereas it is insensitive to O_2 reduction, in contrast to noble metals, which are not suitable for selective reduction of H_2O_2 in the presence of dissolved oxygen. Moreover, the catalytic efficiency of PB is comparable to that of biological catalysts. Studies of the kinetics of hydrogen peroxide reduction on PB electrodes[3] have shown that the bimolecular rate constant for this reaction is 3×10^3 L mol^{-1} s^{-1}. The activity of the natural enzyme peroxidase is of a similar order of magnitude to the reported electrocatalytic activity of PB. Owing to the high catalytic activity and selectivity, the PB-based electrocatalyst is denoted 'artificial peroxidase'.[3,4]

PB-based hydrogen peroxide sensors seem to be very promising for the construction of first-generation biosensors based on oxidases. Glucose oxidase has often been investigated as a model enzyme,[4–10] but there are no limits to the use of other oxidases for the preparation of PB-based amperometric biosensors. Utilization of D-amino acid oxidase, alcohol oxidase and glutamate oxidase permits the development of PB-based enzyme electrodes for the detection of D-alanine,[6] ethanol[7] and glutamate,[4,11] respectively. Amperometric biosensors based on glucose oxidase have been also prepared using other MHCF such as CrHCF,[12] CuHCF,[13] CoHCF[14] and NiHCF[15] electrodes. An enzyme biosensor for oxalate working in the same sensing mode has been developed by biomodification of a CrHCF film electrode with oxalate oxidase.[16]

Other biosensing schemes are based on the possibility of electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH) at MHCF-modified electrodes. Direct NADH oxidation at metal electrodes takes place at considerable overpotentials. MHCF-modified electrodes can cooperate with dehydrogenases that constitute a large class of redox enzymes using the redox cofactor and produce the reduced form NADH. NiHCF electrodes coated with a cross-linked enzyme layer of alcohol dehydrogenase permits the amperometric determination of alcohols by electrochemical detection of NADH generated in the course of enzymatic oxidation of analyte to aldehyde.[17,18] A similar biosensing scheme but with a gravimetric transducer has been demonstrated using PB film deposited on a quartz-crystal microbalance electrode and functionalized with the redox coenzyme.[19] This prototype of the transducer in connection with glucose-6-phosphate dehydrogenase has been utilized for the detection of glucose-6-phosphate.

PB film has a deep blue color and its spectrum is compatible with low-cost near-infrared sources of light such as diode lasers and light emitting diodes. Taking into account the unique chemistry of this compound, substantial changes in its optical properties can be expected as a result of various processes of chemical recognition in which PB can be involved. This implies that PB films can play the role of a receptor part in optical sensors. Recently, optical pH-sensing[20,21] and redox-sensing[22] methods exploiting the chemical properties of PB films have been reported. This paper demonstrates optical biosensors based on specially prepared composite films of PB enzymatically modified with hydrolases and oxidases, using urease and glucose oxidase chosen as the respective model enzymes.

For the preparation of PB-based electrochemical biosensors, various enzyme immobilization methods have been investigated, including adsorption,[5] cross-linking with albumins using glutaral as bifunctional linking agent,[14–18] and entrapment in Nafion,[7,11] poly(vinyl alcohol)-based self-gelatinizable copolymer[8] or electrochemically deposited organic polymers.[10] The original method of immobilization is based on enzyme incorporation into the structure of the MHCF film during its electrochemical growth process (simultaneous codeposition of MHCF and enzyme on the surface of a bare electrode).[6,9,12] The lack of functional groups on PB polycrystals makes direct covalent linking of enzyme molecules impossible. The composite material reported in this paper as a PB-based optical sensor

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allows chemical attachment of enzyme molecules directly to the surface of the transducer.

Experimental

Materials

4-(Pyrrol-1-yl)benzoic acid (Pyr-BAc) was obtained from Aldrich. Urease (EC 3.5.1.5, type IX, from jack bean), glucose oxidase (GOx, EC 1.1.3.4, type II, from *Aspergillus niger*) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) were purchased from Sigma. All other organic and inorganic reagents were of analytical-reagent grade and were used without further purification. Experiments were carried out using solutions prepared with doubly distilled water. All solutions were prepared immediately prior to use, except for glucose standard solutions, which were left at room temperature for 24 h to mutorotate.

Deposition of Prussian Blue film

A dust-free polyester foil having one side protected with silicone rubber was used as a support. The composite PB film was deposited from 0.1 mol L^{-1} solution of $K_3Fe(CN)_6$ in 1.0 mol L^{-1} hydrochloric acid earlier saturated with Pyr-BAc. (**Caution**: toxic gaseous products such as hydrogen cyanide and chlorine are slowly released from the reaction mixture!) The foil was immersed in the reaction mixture exposed to ultraviolet (UV) radiation from a conventional UV lamp (Philips, 15 W). The films were obtained after 1 d of the deposition process. The foil with the deposited film was subsequently washed with 1.0 mol L^{-1} hydrochloric acid, 0.1 mol L^{-1} phosphate buffer (pH 7.0) and water. The silicone protecting layer was then removed and the sensor membrane was ready for the enzyme immobilization procedure.

Enzyme immobilization

A freshly prepared aqueous solution containing 15.0 mg mL^{-1} of urease or GOx and 6.0 mg mL^{-1} of EDAC was deposited on the surface of PB film and left overnight at ambient temperature. To remove an excess of unbound enzyme the resulting biofilm was left for 1 h in vigorously stirred 0.1 mol L^{-1} phosphate buffer (pH 7.0) and then stored in a refrigerator at 4 °C. For optical measurements the polyester foil with the enzymatically modified PB film was mounted so that it formed one wall of the flow-through cell.

Instrumentation and measurements

All optical measurements were carried out at a wavelength of 720 nm with a Shimadzu 2401/PC spectrophotometer. The measurements were performed using a simple flow injection manifold consisting of a Minipuls 3 peristaltic pump (Gilson Medical Electronics), rotary injection valve and flow-through optical cell (both laboratory made). The rectangular 12 × 12 × 40 mm (external dimensions) cell made of transparent polystyrene was adjusted for use with polyester foils coated with optical sensing film. The total effective volume of the flow-through cell was 0.15 mL. The injection volume used was 0.30 mL. The flow rates used for urea and glucose detection were 2.0 and 0.8 mL min^{-1}, respectively. The choice of flow rate and injection volume is a result of a compromise between the resulting sensitivity and response time. No reference cell was used. All spectrophotometric measurements were performed at room temperature without any special thermostatting.

Results and discussion

Sensing films

The optical sensitivity of the reported films exploits the unique spectrochemical properties of PB. Changes in the intensity of its characteristic blue color are treated as analytical signals. The PB films were chemically deposited on transparent plastic foil. In a strongly acidic environment the hexacyanoferrate complex slowly decomposes forming the film of so-called 'insoluble' PB, $Fe_4[Fe(CN)_6]_3$. The process of the film deposition is initiated and accelerated by UV irradiation. In acidic solution in the presence of oxidant, oxidative polymerization of Pyr-BAc also takes place, leading to the formation of mixed inorganic–organic composite material. The mechanism of the non-electrochemical deposition of the composite material on a non-conducting support has been investigated and discussed previously.[22]

The thin sensing film is composed of PB in a poly-(pyrrolylbenzoic acid) network. The content of the *N*-substituted polypyrrole in the composite is very low, not determinable by elemental analysis, but its presence can be proved by IR spectroscopy. The presence of organic polymer in this composite material is crucial for the physical properties of the film. In contrast to pure PB films, the composite film is homogeneous and robust and adheres strongly to the support. In comparison with PB films prepared by electrochemical means, where many cracks occur, the quality of the composite film is considerably improved. Traces of the organic polymer play the role of a binding agent for the inorganic material. A uniform and homogeneous structure of the film was observed using scanning electron microscopy. The sensing film is robust and exhibits very high stability. Only by hard mechanical scratching can the material be removed from the foil. PB, the main component of the composite causing its optical properties, is almost insoluble. Therefore, disadvantages typical for chromoionophore-based sensors, such as sensing membrane swelling or leaching out of active components, do not exist in this case. The material is very stable with respect to its physical, optical and chemical properties. Neither 1 year of measurements nor 3 years of storage under ambient conditions caused any changes in its parameters.

On the surface of the composite films of PB and *N*-substituted polypyrroles, reactive groups are present and the materials are acceptable as ready-to-use supports for enzyme immobilization.[23] The carboxy groups of the organic component (PPyr-BAc) allow the chemical binding of biomolecules to the film surface using a simple one-step carbodiimide method. The method leads to the formation of a monomolecular layer of enzyme molecules bound covalently directly to the surface of the PB film, as shown in Fig. 1. Owing to the lack of any intrinsic absorption near 700 nm, the biolayer does not change the absorbance of the film. After enzyme immobilization, the films are still transparent and show no sign of turbidity. In contrast to turbid enzyme membranes utilizing pre-activated supports, the extremely thin monomolecular enzyme layers are fully transparent, which is particularly advantageous in the case of optical measurements in transmission mode. These covalently linked enzyme molecules form a stable layer exhibiting high biocatalytic activity. No additional membranes, neither protecting nor anti-interference, were used. There are no limitations in substrate transport to the biolayer as the 'naked' enzyme molecules are exposed to analyte solution. PB films prepared in such a way, which are enzymatically sensitized with urease and glucose oxidase, have been used for further investigations. High efficiency of the immobilization procedure provides additional (besides results of morphology and IR studies of the film) indirect evidence that the sensing material is really composed of organic polymer introducing reactive groups on the surface of the film. Utilization of the same

enzyme immobilization protocol with pure PB films (without PPyr-BAc) gave negative results. In this case, only low, unstable and short-time biocatalytic activity, caused by non-specific adsorption of enzyme molecules, was observed.

Urea biosensor

Owing to its protolytic properties PB can play the role of an optical pH-indicator.[20] The effect of pH is explained in terms of a decomposition of PB by hydroxyl ions. The optical pH sensitivity of PB films is associated with pH-dependent equilibria between fully isocyano hexacoordinated and partially hydrated high-spin Fe(III) ions in the film. The reversibility of the sensing system depends on complex protolytic processes. In acidic solutions (pH < 3) protolysis of PB is negligible. With increasing pH, reversible hydrolysis of PB is observed according to

$$Fe_4[Fe(CN)_6]_3 + 3H_2O \rightleftharpoons Fe(OH)_3 + 3FeFe(CN)_6^- + 3H^+ \quad (1)$$

This protolytic process is completely reversible, because it is not associated with destruction of the zeolitic lattice of the PB film. Optical measurements of pH in range up to 8 are connected with the indicating process. In more alkaline solutions further decomposition of PB takes place:

$$FeFe(CN)_6^- + 3OH^- \rightarrow Fe(OH)_3 + Fe(CN)_6^{4-} \quad (2)$$

This process is irreversible owing to destruction of the zeolitic structure of PB and escape of hexacyanoferrate(II) ions out of the film. In conclusion, PB films allow the sensitive and reversible evaluation of pH in the physiological range. This implies that the film can serve as an optical transducer for pH-based biosensors.

Urease catalyses the hydrolysis of urea. The protolytic products of the enzymatic reaction increase the pH of the sensor's environment and decrease the absorbance of the pH-sensitive PB film. Therefore, the analytical signal depends on urea concentration. Typical calibration of the flow-through urea biosensor and the corresponding calibration graph are shown in the Fig. 2. Over a wide range of analyte concentration the calibration curves have a sigmoidal shape predicted by the kinetic model of pH-based enzyme biosensors.[24] All analytical characteristics of the biosensor are in good agreement with this model and they are similar to those reported for many potentiometric and optic biosensors working in the same sensing mode. An increase in buffer concentration causes both an increase in the detection limit and a decrease in sensitivity. The larger buffer capacity has a dumping effect on the pH changes within the enzyme layer and as a result it suppresses the sensitivity of the biosensor. On the other hand, measurements in buffers of low capacity are more time consuming owing to the necessity for a long time for return to the baseline absorbance after each contact with samples. Generally, the response time is relatively short and depends strongly on analyte concentration (Fig. 2). Satisfactory sensitivity and detection limit were found with a carrier phosphate buffer of pH 7.0 owing to both high activity of the immobilized enzyme and high sensitivity of the pH transducer. pH values lower than 5.5 dramatically decrease the catalytic activity of urease. An increase in pH limits the available pH changes caused by products of the enzymatic reaction. Moreover, operating at higher pH is connected with a risk of the film destruction owing to the irreversible reaction (2).

The urea biosensor exhibits good stability and lifetime. The operational stability of the biosensor was examined under flow injection conditions. After 40 injections of urea standards no decay in sensitivity was observed. Reproducible absorbance signals of 0.0189 ± 0.0009 and 0.0933 ± 0.0008 were measured with 2.0 and 10.0 mM urea, respectively (n = 20 for each concentration). The biosensors were examined for their operational and storage lifetimes. The sensitivity of the biosensor decreases by 15–20% after 3 weeks of daily usage. After 2 months of dry storage at 4 °C, the sensitivity of the biosensor did not change. Longer examination of the lifetimes was not performed. The experiments indicated that the (bio)films are stable and can be applied for the mass production of biosensors.

The main drawback of the urea biosensor, common to all pH-based enzyme sensors, is a strong effect of the buffer capacity of samples on the analytical signal.[24] This inconvenience limits the utility of the sensors for some kinds of analytical determinations. Despite this disadvantage, the PB-based urea biosensor can find various practical applications. The biosensor is useful for the analysis of samples having a low buffer capacity. Alkali metal cations, non-buffering anions and complexing ligands, commonly existing in real samples, do not influence its response. Consequently, the biosensor can be dedicated to some

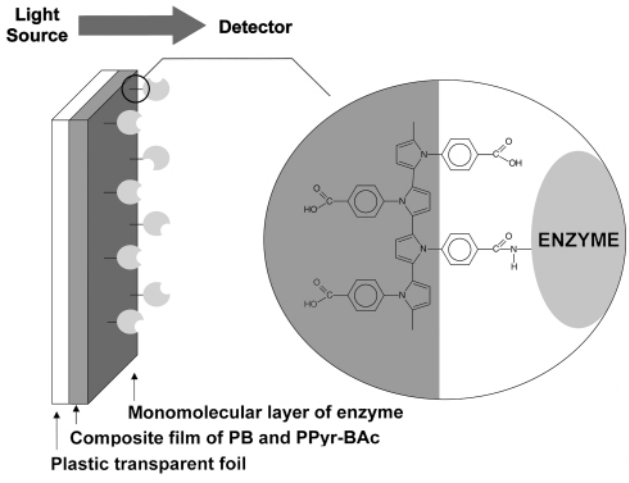

Fig. 1 Biosensing film.

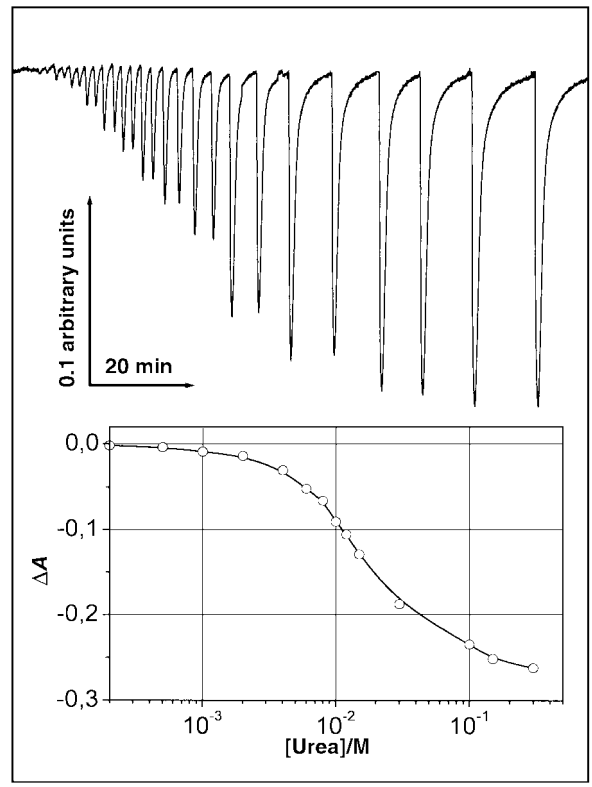

Fig. 2 Wide range calibration of the urea biosensor (top) and corresponding calibration plot (bottom). Measurements performed in 10 mM phosphate buffer (pH 7.0).

pharmaceutical and biomedical applications such as the analysis of urine samples and saline extracts from pharmaceutical and cosmetic ointments containing urea.[25] Fig. 3 shows the applicability of the urea biosensor for such analyses. The calibration graph of the urea biosensor over a narrow range of concentration (2.0–12.0 mM) is linear and the frequency of measurements varied from 15 to 30 sample injections per hour depending on the analyte concentration. The recorded analytical signals for real samples are stable and reproducible. Results of the analyses are fully comparable to those obtained by the kinetic bienzymatic spectrophotometric method recommended for the determination of urea in complex samples.[26] As can be seen in the inset in Fig. 3, the correlation between the results obtained for real samples was excellent. Similarly, good results have been obtained in post-dialysate fluid analysis. The proposed bioanalytical system is useful for on-line monitoring and control of hemodialysis therapy. Real, clinical evaluation of this optical biomonitor is being investigated and results of these biomedical studies will be presented in a separate paper.[27]

Glucose biosensor

Glucose detection utilizing a pH-based biosensing scheme similar to that demonstrated for urea is possible because gluconic acid, the final product of the enzymatic oxidation, causes a decrease in pH and an increase in absorbance of PB film. However, the performance of such a sensing system was not satisfactory with respect to sensitivity and dynamics of the analytical response. Glucose biosensing based on the redox properties of PB film seemed to be more promising.

The enzymatically modified PB film shows no sensitivity towards glucose in a redox-based sensing scheme. This result confirms that optical monitoring of the biocatalytic process exploiting the redox properties of the film is impossible, as hydrogen peroxide can neither oxidize nor reduce PB. Moreover, the potential utility of PB as a mediator in second-generation optical biosensors, being able to reoxidize the active site of glucose oxidase, has been excluded.

We found that the enzymatically modified Prussian White (PW) film is able to detect the product of the enzyme reaction (H_2O_2), forming an optical biosensing scheme similar to that reported for first-generation electrochemical biosensors. The film of PW (reduced form of PB) is mechanically stable, however, as it is easily oxidized. Therefore, the PB film with covalently bound molecules of glucose oxidase was chemically reduced *in situ* to the PW form. For this purpose ascorbate was chosen as a strong reducing agent, but not dangerous for the enzyme. Colorless PW film is easily oxidized by enzymatically generated hydrogen peroxide to the PB form that can be optically detected. The biosensing system is depicted in Fig. 4.

The freshly prepared sensing film is composed of 'insoluble' PB.[20,22] Optical experiments exploiting the redox properties of PB with such a form of the film were poorly reproducible. Prior to measurements, the film should be converted into so-called 'soluble' form [$KFeFe(CN)_6$] by conditioning it in neutral buffer solution containing a high level of potassium ions. In the course of the conditioning potassium ions are incorporated into the zeolitic structure of PB, according to the following reaction:

$$Fe_4[Fe(CN)_6]_3 + 3K^+ + 3H_2O \rightarrow 3KFeFe(CN)_6 + Fe(OH)_3 + 3H^+ \quad (3)$$

This ion-exchange process is not associated with the destruction of the zeolitic lattice of PB. The process causes a small decrease in absorbance of the film. Conversion of the 'insoluble' PB into 'soluble' form was confirmed by elemental analysis and energy dispersive X-ray fluorescence spectrometry and by the shift of the maximum of the film absorption from 720 to 708 nm. A greater shift caused by the conversion was reported earlier for electrochemically deposited pure PB films.[28] This effect is negligible for the reported analytical application, as the absorption band of PB is very wide. The conditioned films seem to work reproducibly in the optical biosensing scheme based on the redox properties of PB.

Fig. 5 shows a typical calibration of the biosensor in the sub-millimolar range of glucose concentration. The calibration plot is linear from 0.1 up to 1.0 mM glucose. Both the response and regeneration times are relatively short, allowing frequent

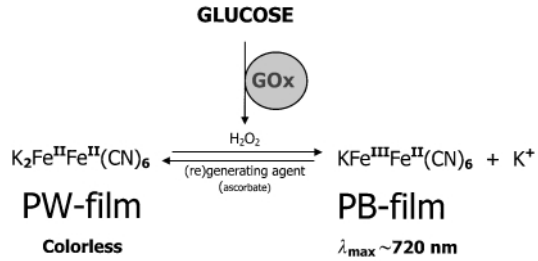

Fig. 4 Scheme of optical biosensing of glucose with GOx/PB/PW film.

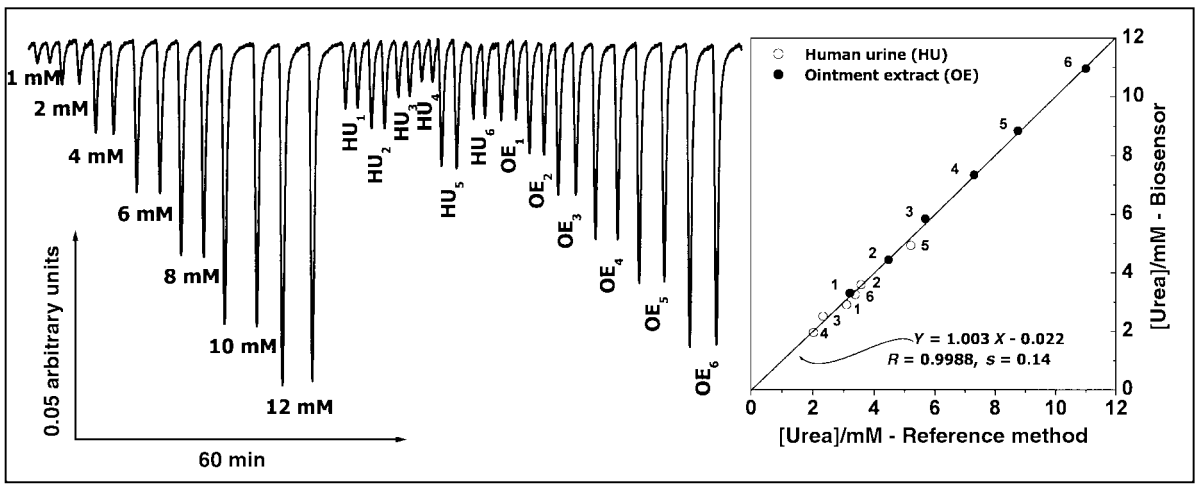

Fig. 3 Recording of typical measurements with the urea biosensor that includes calibration (urea concentrations are given) and measurements of real samples (human urine, HU_1–HU_6, and saline extracts from pharmaceutical ointments, OE_1–OE_6). For urea extraction from non-water-wettable ointments (Xerials, Laboratories SVR Evry) chloroform and the carrier buffer spiked with NaCl (10%) were used.[25] Samples of human urine from healthy persons were diluted 100-fold with the carrier buffer before injection.[25] Correlation between results of analysis obtained with a reference method[26] and with the biosensor is shown in the inset. Measurements performed in 10 mM phosphate buffer (pH 7.0).

injections of up to 15 samples per hour. Although alkali metal cations did not affect the response of the biosensor, a high content of potassium ions is recommended, as it causes shortening of the time necessary for resetting the film to the baseline absorbance. The optimum pH for glucose determination was found to be between 5 and 6. More acidic solutions cause a decrease in the biocatalytic activity of glucose oxidase. On the other hand, in neutral solutions having a low buffer capacity the films were slowly damaged by hydroxyl ions generated in the course of hydrogen peroxide reduction by PW.

The operational stability of the glucose biosensors was examined under flow injection conditions for two concentrations of the analyte. After a series of injections of 0.4 and 1.0 mM glucose standards, no decay of the analytical signals was observed and reproducibilities of the signals were 0.0237 ± 0.0007 and 0.0488 ± 0.0010 absorbance, respectively (results for 23 injections of each standard). The operational lifetime of the biosensor was assessed by discontinuous measurements. The sensitivity decreased within the first 4 days. Over the next 4 weeks the responses remained highly stable and reproducible. After 1 month of usage (1–4 h daily) the sensitivity of the biosensor had decreased by only 5–10% of the initial value. The storage lifetime of the (bio)film was also excellent. After 3 months of dry storage in a refrigerator (+4 °C), the sensitivity of the biosensing film did not drop.

Primary experiments showed that the biosensor could be used successfully for the determination of glucose in samples of infusion fluid. Electrolytes, other sugars and amino acids present in such samples do not interfere. Reducing agents, especially ascorbate are known to be the major interfering compounds for the majority of electrochemical biosensors based on PB electrodes.[4–11] In this work vitamin C is a key substance responsible for the operation of the glucose biosensor. This reducing regeneration agent present at relatively high concentration in the carrier buffer suppresses effects from redox species present in injected samples. The agent masks other reductants. No effects of ascorbate and cysteine at concentrations comparable to the glucose level in injected samples were observed. Obviously the sensitivity of the optical biosensor is lower than those reported for electrochemical biosensors, but still useful for the selective determination of glucose in real samples. As can be seen from Table 1, the bioanalytical system has been used successfully for the analysis of real samples (juices and wines) containing high levels of vitamin C and other reducing species. Also in the analysis of artificial serum samples the results obtained were in agreement with the declared values. Detailed studies of the application of the glucose biosensor in pharmaceutical, food and clinical analysis are in progress.

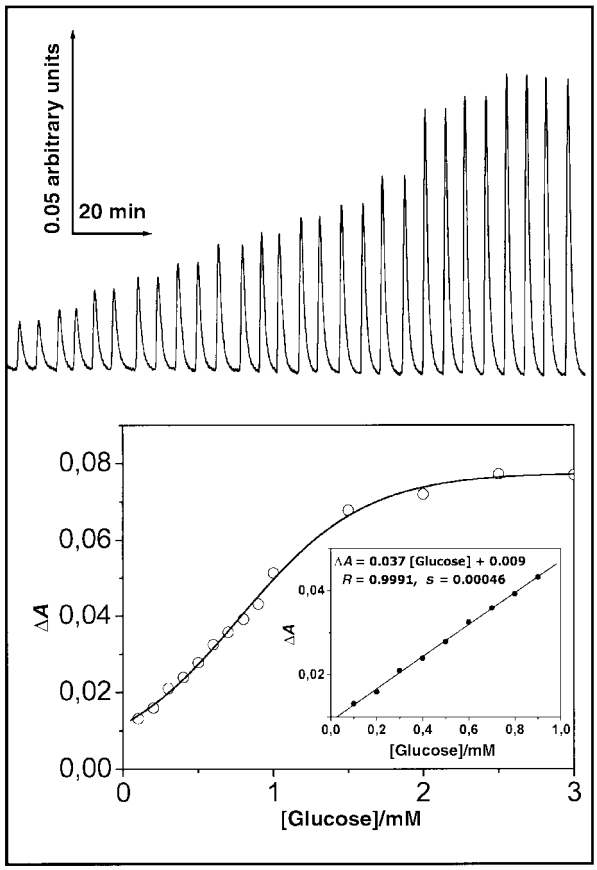

Fig. 5 Calibration of the glucose biosensor (top) and corresponding calibration plot (bottom) with indicated linear part (inset). Measurements performed in 0.2M acetate buffer (pH 5.0) spiked with 10 mM ascorbic acid (regeneration agent).

In contrast to pH-based biosensing with a PB film that exploits an indicating scheme,[30] detection of enzymatically generated hydrogen peroxide by PW film is a typical stoichiometric process.[30] H_2O_2 irreversibly oxidizes PW to the PB form. The changes in absorbance measured by the biosensor are related to the integrated amount of glucose in contact with the biosensing film, rather than to its concentration [as in the case of the indicating scheme of (bio)sensing]. The amount of PW in the sensor film decreases over the time of contact and a steady-state signal cannot be reached. After contact with glucose, this partially oxidized biofilm should be reset to the initial state using a reductant (here ascorbate). The flow-through glucose biosensor is based on integration of reaction and detection leading to sensing of the analyte.[31] Utilization of the biosensing scheme in flow injection conditions permits both optical detection and regeneration of the sensing (bio)film. In other words, the presented flow injection system allows the application of a non-indicating, stoichiometric scheme of (bio)recognition for multiple and reversible optical (bio)sensing of the analyte.[30,31]

Conclusion

As an alternative to many MHCF-based biosensors based on electrochemical detection reported in the literature,[4–19] optical PB-based biosensors are demonstrated here. In biosensor development two kinds of problem have to be addressed, namely stable immobilization of the enzyme and high sensitivity of the transducing system. Our specially prepared PB composite films are robust, stable and effective as supports for covalent immobilization of biomolecules, and they can act as optical transducers for biosensors based on hydrolases and oxidases. The presented prototypes of absorbance biosensors exhibit long operational and storage lifetimes. They work in the conventional transmission mode of optical measurements and therefore exhibit relatively low sensitivity. However, the (bio)films reported here seem to be useful as (bio)sensing

Table 1 Glucose content (g L^{-1}) in selected juices and wines. Determination for each sample was repeated three times using both methods

Sample	GOx/PB/PW-based biosensor	Reference method[29]
Apple juice	24.3 ± 0.2	24.2 ± 1.0
Grape juice	17.1 ± 0.2	16.9 ± 0.6
White wine (sweet)	24.9 ± 0.5	24.9 ± 0.5
White wine (dry)	8.4 ± 0.2	7.9 ± 0.7
Red wine (sweet)	14.5 ± 0.1	14.3 ± 1.2
Red wine (dry)	8.1 ± 0.2	7.8 ± 0.6

materials for more advanced optical devices such as attenuated total reflection or evanescent-wave sensors that are expected to exhibit much better sensitivity.[32]

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