AMPEROMETRIC DETERMINATION OF GLUCOSE WITH A CARBON PASTE BIOSENSOR

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A simple glucose biosensor has been developed by bulk modification of a carbon paste electrode with glucose oxidase as a biocomponent and manganese dioxide as a mediator. The sensor was employed as amperometric detector in a flow-injection system at 21 °C in 0.2 M phosphate buffer (pH 7.5). At an applied working potential of 0.48 V *vs* Ag/AgCl and a flow rate of 0.2 ml min^{-1}, the sensor exhibited well reproducible amperometric response to glucose. A linear relation between the peak current and the analyte concentration was found between 20 and 500 mg l^{-1} glucose with a detection limit (3σ) of 10.5 mg l^{-1} glucose. The sensor can be operated continuously for 12 h without any loss in the signal height and can be used for the determination of glucose in white wine samples.

Keywords: Glucose; Flow-injection analysis; Biosensors; Carbon paste electrodes; Oxidases; Amperometry.

Development of electrochemical biosensors is a fast-growing field in electroanalytical chemistry[1–3]. Due to the selectivity of biomolecules (enzymes) present in the recognition layer of a sensor, they can be specifically tailored for analytes present in a complex sample matrix, which makes them very interesting for a large number of industrial, environmental and clinical applications[2]. Among all enzymes employed, glucose oxidase (GOD) is most frequently used[3,4]. Glucose as a constituent of samples origi-

nating either from food industry or medicine has to be monitored quite often. The enzyme shows outstanding thermal stability, high selectivity to the substrate and is relatively inexpensive[5]. It catalyzes the oxidation of β-D-glucose to β-D-gluconolactone where, as a by-product, hydrogen peroxide (H_2O_2), an electrochemically active compound is formed. Biosensors can be operated without and with additional mediators[6]. The latter improve the analytical performance of the electrode; they increase the electron transfer between the electrode surface and the active site of the enzyme (often being located deep inside the protein structure) and reduce the overpotential of the electroactive analyte[7,8]. Glucose biosensors based on GOD with heterogeneous carbon transducers frequently contain various mediators, such as ferrocenes, nickelocene, tetrathiafulvalene, phthalocyanines, Os-based redox polymers, bis(bathophenanthroline)-copper, dispersed Ru, Rh and Ir, Pd, Pt, ruthenium dioxide, tetracyanoquinodimethane, Methylene Blue and Green, Meldola Blue, quinones and their derivatives, diaminodurene and 1-(dimethylamino)-4-morpholinobenzene[4].

In 1991, Taha reported on the amperometric determination of various hydrazines and of hydrogen peroxide at a glassy carbon electrode modified with a thin film of oxymanganese species using strongly alkaline solutions[9]. In our laboratory, we developed different carbon-paste- and carbon-ink-based MnO_2-bulk- and MnO_2-film-modified amperometric sensors for H_2O_2 that could be operated in ammonia buffer at pH 9.5 with very low detection limits (<1 μg l^{-1}) and remarkably wide linear ranges for more than five decades of concentration[10–13]. It was also proved that these types of sensors could successfully be used at physiological pH in phosphate buffer. In this paper, a MnO_2/GOD bulk-modified carbon-paste-based biosensor is presented.

EXPERIMENTAL

Apparatus

Hydrodynamic amperometry. Hydrodynamic amperometric measurements were performed with an electrochemical workstation (BAS 100B, Bioanalytical Systems Inc., West Lafayette (IN), U.S.A.). The cell compartment was a self-constructed electrode assembly made of acrylate glass[14], equipped with a glass titration vessel (6.1415.220, Metrohm, Herisau, Switzerland) and a platinum wire as the counter electrode. The Ag/AgCl reference electrode (6.1227.000, Metrohm) was in contact with solution *via* a salt bridge (1 M KCl) equipped with a Vycor frit. A magnetic stirrer and a Teflon-coated stirring bar (approx. 300 rpm) provided the convective transport. Data were evaluated using BAS 100W, version 2 software.

Flow-injection system. The flow-injection system consisted of a high-performance liquid chromatographic (HPLC) pump (Model 510, Waters, Milford (MA), U.S.A.), a sample injec-

tion valve (U6K, Waters), and a thin-layer electrochemical cell (CC5, BAS). Teflon spacers (MF-1047, MF-1048, BAS) were used to adjust the thickness of the flow-through cell to 0.2 mm. The surface area of the working electrode was 0.45 cm^2. An Ag/AgCl electrode (3 M KCl, model RE-1, BAS) served as the reference. The counter electrode was the back plate of the cell, made of stainless steel with an active surface area of *ca* 1.4 cm^2. The currents obtained were recorded with the electrochemical workstation BAS 100B and evaluated with the software given above.

Reagents and Solutions

Deionized water was distilled twice in a quartz still and then purified using an ion-exchange system (Nanopure, Barnstead). Phosphate buffer (0.1 mol l^{-1}, pH 7.5) was prepared by mixing aqueous solutions (0.1 mol l^{-1}) of sodium dihydrogenphosphate (Fluka, Buchs, Switzerland) and disodium hydrogenphosphate (Merck, Darmstadt, Germany) to achieve the desired pH.

L-Ascorbic acid was purchased from Fluka, glucose oxidase (EC 1.1.3.4) from Sigma–Aldrich (St. Louis (MO), U.S.A.). All other compounds used were of AR grade (Merck).

Working Electrode

Hydrodynamic amperometry. A Teflon rod (outer diameter 11 mm) with a hole at one end (7 mm in diameter, 3 mm deep) for the carbon paste filling served as working electrode body. Electrical contact was made with a platinum wire through the center of the rod. Unmodified carbon paste was prepared by adding 1.58 g paraffin oil (Uvasol, Merck) to 5.00 g of spectral carbon powder (RWB, Ringsdorff-Werke, Bad Godesberg, Germany). MnO_2/GOD bulk-modified carbon pastes were prepared by substituting 3.8% of the carbon powder by manganese dioxide and the corresponding amounts of carbon by GOD, respectively, and then by adding the paraffin oil. The mixture was homogenized intimately and was allowed to stand at least for 24 h. The modified paste was packed into the hole of the electrode and smoothened with a PTFE plate.

Flow-injection analysis. The bulk-modified paste was filled into the electrode holes of the working electrode of the thin-layer cell and smoothened with a PTFE spatula. After assembling, the amperometric cell was flushed with deaerated phosphate buffer. The electrodes were activated once a day by injecting five times 250 µl of an aqueous solution containing 10 mg l^{-1} Mn^{2+} (as $MnCl_2$) at an applied working potential of 0.48 V and a flow rate of 0.2 ml min^{-1}. After such treatment, the sensor exhibited an increased amperometric response to glucose which became stable after about ten injections of 250 µl of a 500 mg l^{-1} analyte solution.

Procedures

Hydrodynamic amperograms were carried out in stirred measured solutions at an applied potential of 0.48 V *vs* Ag/AgCl. Flow-injection analysis (FIA) was performed with an applied potential of 0.48 V. The typical flow rate was 0.2 ml min^{-1}, the injection volume 250 µl. The responses were evaluated using their peak heights.

Analysis of Samples

Two samples of Austrian white wines (Neuburger "lieblich", Winzerhaus GmbH, A-1110 Wien, and Österreichischer Landwein, Tscheppe, A-8010 Graz) were purchased in a local shop and stored at 4 °C in a refrigerator. Before the FIA amperometric determination of the glucose content, appropriate volumes of the samples were diluted with 0.1 M phosphate buffer (pH 7.5) in a ratio of 1 : 100 and 1 : 20. The solutions were then analyzed without any further treatment. Determinations were made using the external calibration method by injecting 250 μl of the samples. The responses were evaluated using their peak heights.

RESULTS AND DISCUSSION

Hydrodynamic amperometry. Carbon paste bulk-modified with 3.8% manganese dioxide powder was found to be very sensitive to hydrogen peroxide[10]. In order to prove if a MnO_2/GOD bulk-modified sensor can be used as an amperometric detector for glucose and to evaluate the optimum concentration of glucose oxidase in the electrode material, the corresponding amounts of GOD were directly admixed to the paste. The resulting sensors were tested with glucose solutions as analyte by hydrodynamic amperometry applying a potential of 0.46 V. Electrodes containing 3–5% of the enzyme exhibited an optimum response to the analyte. A typical amperogram for a 3.8% GOD bulk-modified CPE is shown in Fig. 1. With each addition of 50 mg l^{-1} glucose, the sensor gave an additional response

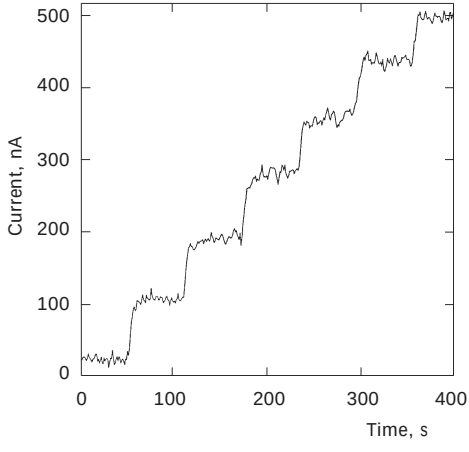
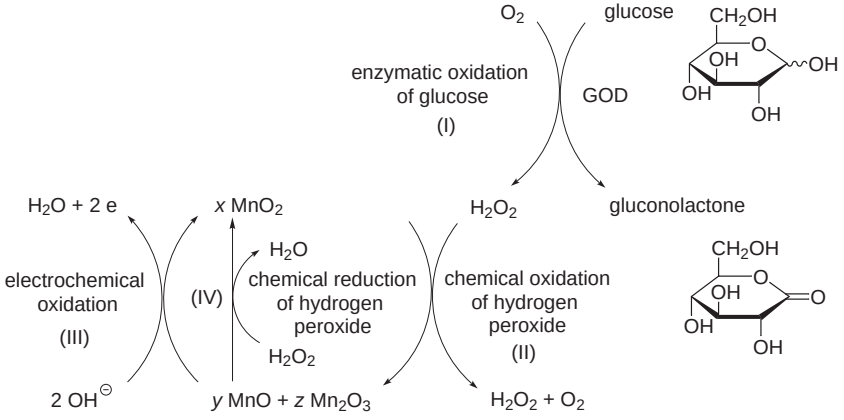


FIG. 1
Hydrodynamic amperogram of H_2O_2 with a GOD/MnO_2 (3.8% each) bulk-modified CPE. Operation potential 0.46 V *vs* Ag/AgCl, supporting electrolyte 0.1 M phosphate buffer (pH 7.5), temperature of the measured solution 28 °C, addition of 50 mg l^{-1} glucose every 60 s, stirring speed *ca* 300 rpm

of about 80 nA which clearly confirmed that the double modified CPE could be exploited as an amperometric detector in FIA. Scheme 1 demonstrates the enzymatic, chemical and electrochemical reactions occuring in the recognition layer of the sensor.



SCHEME 1

Glucose is enzymatically oxidized with molecular oxygen forming gluconolactone and hydrogen peroxide (I). The latter reacts with MnO_2 producing manganese species at lower oxidation states (II) which can be electrochemically reoxidized to MnO_2 (III). The oxidative current flow can be directly related to the glucose concentration. Besides this rapid electrochemical process, a kinetically slower chemical reoxidation of MnO/Mn_2O_3 with H_2O_2 can be observed (IV). Thus, manganese dioxide does not act as a mediator to restore the enzyme itself, but acts on one product of the enzymatic reaction, hydrogen peroxide.

Flow-injection analysis. For FIA, the modified electrode was used as an amperometric detector in a thin-layer cell with the supporting electrolyte as a carrier. A representative amperogram is shown in Fig. 2. The GOD/MnO_2 double bulk-modified CPE responds reproducibly to dynamic changes in the H_2O_2 concentration. In the absence of GOD or of MnO_2, no response was observed upon injection of the analyte. Due to the kinetically slow enzymatic oxidation of glucose, a slow flow-rate of 0.2 ml min^{-1} was chosen leading to about 10 min for a sample run. Evaluation of the responses is best made by using the heights of the peaks. A slight increase (*ca* 8%) in the electrode response to the analyte was observed when the temperature was increased from 20 to 30 °C. This is in good agreement with the results published by Pravda *et al.* who have found that a GOD-modified CPE showed a

maximum response to glucose at about 70 °C and that the signal increase in the range from 20 to 30 °C was relatively small[15]. However, keeping the temperature of the measured solution within the described range (±2 °C) the response of the electrode can be considered to be constant.

A critical parameter for the amperometric response is the operation potential. Figure 3 displays the dependences of the signal height and of the background current upon the working potential within 0.6 and 0.2 V. As can be seen, the amperometric responses of the analyte and of the background currents have entirely different character. With decreasing working potential, an increasing amperometric response to the analyte can be observed. In contrast, the background currents drop linearly with decreasing potential. If potentials are more negative than 0.46 V, the background currents correspond to the reduction of Mn(IV). Due to the formation of soluble manganese species at lower oxidation states, the electrode starts to "bleed"[16] and hence a drastic decrease in the reproducibility of results of the sensor can be observed. The optimum analyte signal with respect to the baseline current lies between 0.46 and 0.5 V; a value of 0.48 V was chosen as an optimum for the subsequent measurements. It should be mentioned that the Ag/AgCl reference electrode provides a stable reference potential in the phosphate-containing bulk solution, but that potential shifts with respect to true potentials may occur due to the absence of chloride.

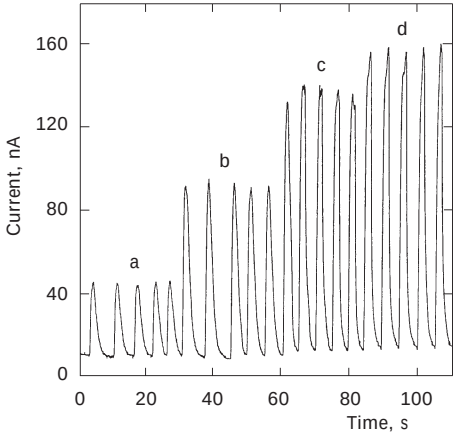


FIG. 2

Dependence of the FIA peak current on the concentration of glucose using a GOD/MnO_2 bulk-modified carbon paste electrode. Flow rate 0.2 ml min^{-1}, operation potential 0.48 V *vs* Ag/AgCl, 0.1 M phosphate buffer (pH 7.5), injection volume 250 µl, temperature of the measured solution 23 °C; 5 injections of 200 (a), 500 (b), 800 (c), 1 000 (d) ppm glucose

The volume of injected sample investigated (25–250 μl) also influences the resulting amperometric response (Fig. 4). The current increases markedly with increasing the volume up to 100 μl and then just gradually. A volume of 100 μl still allows to detect at least 80% of the electrode response obtained by injections of 250 μl.

The flow rate has a significant influence on the height of the amperometric signal (Fig. 5). With increasing flow rate, the sensor response decreases strongly, which indicates that the relatively slow enzymatic reaction plays a major role in the reaction sequence proceeding in the recognition layer. Flow rates below 0.2 ml min^{-1} lead to increased amperometric signals but are not very convenient, because they bring about relatively long analysis times. A flow rate of 0.2 ml min^{-1} was found to be reasonable for amperometric glucose measurements.

Linear range, detection limit and reproducibility. A linear relation between signal and concentration was ascertained in an interval of 20–500 mg l^{-1} glucose (i [nA] = 1.717 c [mg l^{-1}] + 26.262; r^2 = 0.998). At concentrations higher than 500 mg l^{-1}, a slight deviation from linearity was observed. The detection limit (given as 3σ-values calculated from 3 injections of 250 μl H_2O_2 at a concentration of 20 μg l^{-1}) was determined to be 1.3 mg l^{-1}. The reproducibility was determined as 5% relative standard deviation (5 injec-

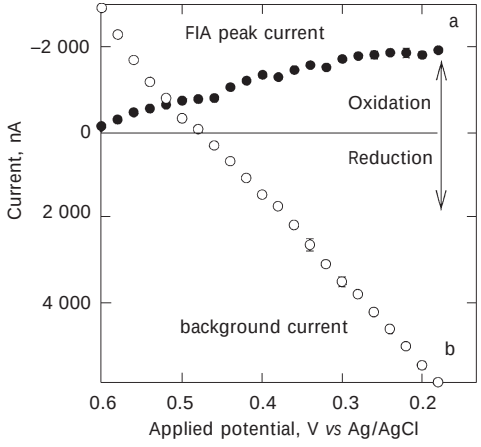


FIG. 3

Dependence of the FIA current response on the applied working potential using a GOD/MnO_2 bulk-modified (3.8% each) electrode, temperature 25 °C, flow rate 1.5 ml min^{-1}, injection volume 100 μl, 0.1 M phosphate buffer (pH 7.5), glucose concentrations 500 mg l^{-1}. Peak height of the current response (a, ●), background current (b, ❍)

tions, 100 mg l^{-1} glucose). Analytical performance of the sensor described here is compared with some other amperometric glucose biosensors based on glucose oxidase and heterogeneous carbon transducers (see Table I). The data on the analytical performance of various amperometric glucose

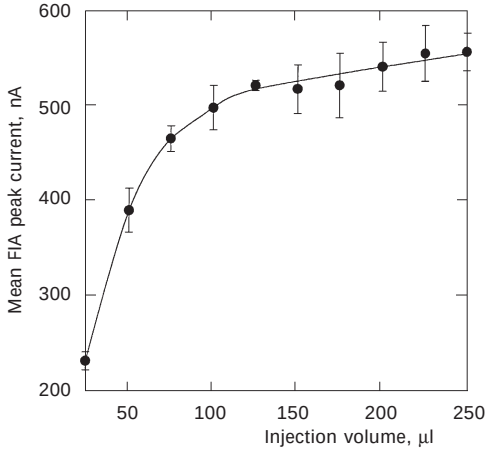


FIG. 4

Dependence of the FIA peak current on the volume of injected sample using a GOD/MnO_2 bulk-modified (3.8% each) electrode, temperature 25 °C, operation potential 0.48 V, flow rate 0.6 ml min^{-1}, 0.1 M phosphate buffer, glucose concentration 500 µg l^{-1}

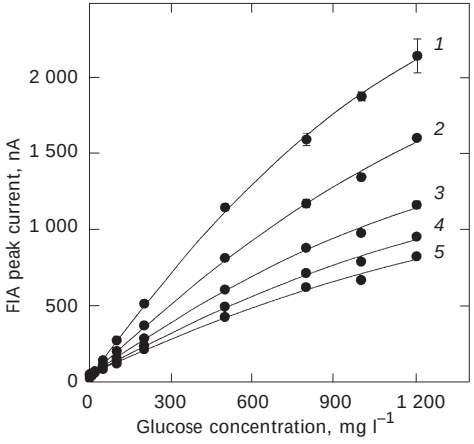


FIG. 5

Dependence of the FIA peak current on the glucose concentration at various flow rates using a GOD/MnO_2 bulk-modified (3.8% each) electrode, temperature 21 °C, operation potential 0.48 V, injection volume 250 µl, 0.1 M phosphate buffer; flow rate 0.2 (*1*), 0.5 (*2*), 1.0 (*3*), 1.5 (*4*), 2.0 (*5*) ml min^{-1}

TABLE I
Mediators and analytical performance of glucose biosensors based on glucose oxidase and carbon transducers

Mediator	Linear range mg l^{-1}	Detection limit, mg l^{-1} (estimated as)	Samples	Ref.
Ferrocenes, tetrathiafulvalene	200–47 000		wine	17
Horseradish peroxidase, 1,1′-dimethylferrocene	up to 90	3.6 (5 S/N)	soft drinks	18
Pt	1.8–600		juices, soft drinks, wine, beer, milk, butter milk	19
Os-Based polymer	up to 10 800	9 for non cross-linked, 18 for cross-linked sensors (2 S/N)		20
Ferrocene	18–144		serum	21
Ir	up to 1 100			22
Ferrocene	18–1 800	18 (5 S/N)		23
Cobalt octaethoxyphtalocyanine	up to 720	0.54 (3 S/N)		24
1-(Dimethylamino)-4-morpholinobenzene, 1-[ethyl(methyl)amino]-4-morpholinobenzene	0.36–2.9			25
Meldola Blue	up to 4 500			26
Ubiquinone (CoQ_{10})	36–720			27
Poly(etheramine quinone)s	1.8–36			28
Ferrocene	up to 540	1.8 (S/N)		29
Rhodinized carbon	up to 900	18		30
Pd (dispersed)	up to 2 500	27 (3 S/N)		31
Ru (dispersed)	up to 270	36 (3 S/N)		32
Mediatorless, operated at +0.9 V *vs* Ag/AgCl	180–3 600 for 10 µl, 18–900 for 50 µl injection volume		soft drinks	33
1-(Dimethylamino)-4-morpholinobenzene	180–2 900			34
Surface-activated CPE	3 600–12 600		Monitrol®	35
Mediatorless, operated at +0.9 V *vs* Ag/AgCl	18–450	9 (3 S/N)	milk	36
Pt, tetrathiafulvalene (TTF)	up to 180 for Pt-containing inks, 180–540 for TTF	0.18 (3 S/N) for Pt-containing inks	red wine, fruit juices	37

TABLE I
(*Continued*)

Mediator	Linear range mg l^{-1}	Detection limit, mg l^{-1} (estimated as)	Samples	Ref.
Pt-containing paste, TTF	up to 2 700 for Pt, 360 for TTF			38
Metallocenes	up to 180			39
Rhodinized carbon	18–4 500			40
Platinized carbon	up to 1 440	3.6 (3 S/N)		41
Ferrocyanide	up to 4 500		whole blood	42
Ru (dispersed)	up to 540	1.8 (S/N)		43
Cobalt phthalocyanine	180–3 500	180		44
Pt	up to 180	0.180		45
Pt or TTF	1.8–630 for Pt, 180–540 for TTF		fruit juices	46
Methylene Green			serum	47
Rhodinized carbon			beer , lactic fermentation solutions	48
Manganese dioxide	20–500	1.3 (3σ)	wines	this work

biosensors show that best results have mostly been achieved with dispersed noble metal mediators. However, it seems that such sensors are less convenient for practical use because of the costs of the modifiers and, in many cases, rather complicated construction. Other mediators provide comparable performance of the sensors but are significantly more expensive than that described here. Another advantage appears to be the fact that manganese(IV) oxide is a relatively harmless compound compared with other substances used as mediators.

Interferences. Alkali, alkaline earth metals (as of sulfates, chlorides, nitrates or tartrates) did not affect the amperometric glucose response. A notable additional amperometric current could be registered in the presence of L-ascorbic acid and/or uric acid in the sample matrix. Preliminary studies employing sensors with protective membranes, such as cellulose acetate, Nafion or polypyrrole films have shown, that the response of the interferants is partly diminished and is accompanied by a decreased sensitivity of the sensor to glucose.

Analysis of wine samples. The new sensor described here can be used for the determination of glucose in wine. Two types of the beverage were chosen that should represent a more and a less sweet type. Using the external calibration method, the concentrations of glucose in Österreichischer Landwein and in Neuburger "lieblich" were found to be 0.24 ± 0.03 and 0.95 ± 0.04%, respectively. The results were in a very good agreement with those of standard methods using HPLC, 0.23 ± 0.01 and 0.97 ± 0.03%.

CONCLUSION

A new MnO_2/GOD-containing carbon-paste amperometric biosensor shows a promising analytical performance and is applicable to analyses of practical samples such as wines. Compared with other amperometric bioelectrodes hitherto recommended for the determination of glucose, the biosensor presented here appears to offer some advantages. In particular, it can be easily prepared when using an inexpensive, chemically stable, and practically harmless mediator.

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REFERENCES

1. Janata J., Josowicz M., Devaney D. M.: *Anal. Chem.* **1994**, *66*, 207.
2. Pandey P. C., Glazier S., Weetall H. H.: *Anal. Biochem.* **1993**, *214*, 233.
3. Gorton L.: *Electroanalysis (N. Y.)* **1995**, *7*, 23.
4. Kalcher K., Schachl K., Švancara I., Vytřas K., Alemu H.: *Sci. Pap. Univ. Pardubice, Ser. A* **1997**, *3*, 57.
5. Wang J., Liu J., Cepra G.: *Anal. Chem.* **1997**, *69*, 3124.
6. Schmidt H. L., Schuhmann W.: *Biosens. Bioelectron.* **1996**, *11*, 127.
7. Hecht H. J., Kalisz H. M., Hendle J., Schmid R. D., Schomburg D.: *J. Mol. Biol.* **1993**, *229*, 153.
8. Wang J., Chen L., Liu J.: *Electroanalysis (N. Y.)* **1997**, *9*, 298.
9. Taha Z., Wang J.: *Electroanalysis (N. Y.)* **1991**, *3*, 215.
10. Schachl K., Alemu H., Kalcher K., Ježková J., Švancara I., Vytřas K.: *Analyst (Amsterdam)* **1997**, *122*, 985.
11. Schachl K., Alemu H., Kalcher K., Ježková J., Švancara I., Vytřas K.: *Anal. Lett.* **1997**, *30*, 2655.

12. Schachl K., Alemu H., Kalcher K., Ježková J., Švancara I., Vytřas K.: *Sci. Pap. Univ. Pardubice, Ser. A* **1997**, *3*, 41.
13. Schachl K., Alemu H., Kalcher K., Moderegger H., Švancara I., Vytřas K.: *Fresenius' J. Anal. Chem.* **1998**, *369*, 194.
14. Kalcher K.: *Fresenius Z. Anal. Chem.* **1986**, *323*, 238.
15. Pravda M., Adeyoyu O., Iwuoha E. I., Vos J. G., Smyth M. R., Vytřas K.: *Electroanalysis (N. Y.)* **1995**, *7*, 619.
16. Kalcher K., Wang J., Kauffmann J.-M., Švancara I., Vytřas K., Neuhold C., Yang Z. P.: *Electroanalysis (N. Y.)* **1995**, *7*, 5.
17. Švorc J., Miertuš S., Katrlík J., Streďanský M.: *Anal. Chem.* **1997**, *69*, 2086.
18. Yabuki S., Mizutani F.: *Biosens. Bioelectron.* **1995**, *10*, 353.
19. Schmidt A., Rohm I., Rüger P., Weise W., Bilitewski U.: *Fresenius' J. Anal. Chem.* **1994**, 349, 607.
20. Csöregi E., Laurell T., Katakis I., Heller A., Gorton L.: *Mikrochim. Acta* **1995**, *121*, 31.
21. Milagres B. G., Kubota L. T., Oliveira Neto G.: *Electroanalysis (N. Y.)* **1996**, *8*, 489.
22. Wang J., Rivas G., Chicharro M.: *Electroanalysis (N. Y.)* **1996**, *8*, 434.
23. Mizutani F., Yabuki S., Katsura T.: *ACS Symp. Ser.* **1994**, *556*, 41.
24. Mizutani F., Yabuki S., Iijima S.: *Anal. Chim. Acta* **1995**, *300*, 59.
25. Kulys J., Hansen H. E.: *Anal. Chim. Acta* **1995**, *303*, 285.
26. Kulys J., Hansen H. E., Buch-Rasmussen T., Wang J., Ozsoz M.: *Anal. Chim. Acta* **1994**, *288*, 193.
27. Kawakami M., Uriuda N., Koya H., Gondo S.: *Anal. Lett.* **1995**, *28*, 1555.
28. Lan H. L., Kaku T., Karan H. I., Okamoto Y.: *ACS Symp. Ser.* **1994**, *556*, 124.
29. Gasparini R., Scapra M., Vianello F., Mondovi B., Rigo A.: *Anal. Chim. Acta* **1994**, *294*, 299.
30. Wang J., Chen L., Liu J., Lu F.: *Electroanalysis (N. Y.)* **1996**, *8*, 1127.
31. Wang J., Chen Q. A.: *Analyst (Amsterdam)* **1994**, *119*, 1849.
32. Wang J., Chen Q. A., Pedrero M., Pingarron J. M.: *Anal. Chim. Acta* **1995**, *300*, 111.
33. Zhang X. N., Rechnitz G. A.: *Electroanalysis (N. Y.)* **1994**, *6*, 361.
34. Kulys J., Hansen H. E.: *Biosens. Bioelectron.* **1994**, *9*, 491.
35. Motta N., Guadalupe A. R.: *Anal. Chem.* **1994**, *66*, 566.
36. Katsu T., Zhang X. N., Rechnitz G. A.: *Talanta* **1994**, *41*, 843.
37. Bilitewski U., Chemnitius G. C., Rüger P., Schmid R. D.: *Sens. Actuators, B* **1992**, *7*, 351.
38. Bilitewski U., Rüger P., Schmid R. D.: *Biosens. Bioelectron.* **1991**, *6*, 369.
39. Atanasov P., Kaisheva A., Gamburzev S., Iliev I.: *Sens. Actuators, B* **1992**, *8*, 59.
40. White S. F., Tothill I. E., Newman J. D., Turner A. P. F.: *Anal. Chim. Acta* **1996**, *321*, 165.
41. Cardosi M. F., Birch S. W.: *Anal. Chim. Acta* **1993**, *276*, 69.
42. Marcinkeviciene J., Kulys J.: *Biosens. Bioelectron.* **1993**, *8*, 209.
43. Wang J., Pamidi P. V. A., Park D. S.: *Electroanalysis (N. Y.)* **1997**, *9*, 52.
44. Gilmartin M. A. T., Hart J. P., Patton D. T.: *Analyst (Amsterdam)* **1995**, 120, 1973.
45. Rüger P., Bilitewski U., Schmid R. D.: *Sens. Actuators, B* **1991**, *4*, 267.
46. Bilitewski U., Jäger A., Rüger P., Weise W.: *Sens. Actuators, B* **1993**, *15–16*, 113.
47. Kulys J., Wang L., Hansen H. E., Buch-Rasmussen T., Wang J., Ozsoz M.: *Electroanalysis (N. Y.)* **1995**, *7*, 92.
48. Tothill I. E., Newmann J. D., White S. F., Turner A. P. F.: *Enzyme Microb. Technol.* **1997**, *20*, 590.